

LONDON
SCHOOL of
HYGIENE
& TROPICAL
MEDICINE



LSHTM Research Online

McCarthy, AJ; Stabler, RA; Taylor, PW; (2018) Genome-Wide Identification by Transposon Insertion Sequencing of Escherichia coli K1 Genes Essential for in vitro Growth, Gastrointestinal Colonizing Capacity and Survival in Serum. Journal of bacteriology. ISSN 0021-9193 DOI: <https://doi.org/10.1128/JB.00698-17>

Downloaded from: <http://researchonline.lshtm.ac.uk/id/eprint/4646167/>

DOI: <https://doi.org/10.1128/JB.00698-17>

Usage Guidelines:

Please refer to usage guidelines at <https://researchonline.lshtm.ac.uk/policies.html> or alternatively contact researchonline@lshtm.ac.uk.

Available under license: <http://creativecommons.org/licenses/by-nc-nd/2.5/>

<https://researchonline.lshtm.ac.uk>

Genome-Wide Identification by Transposon Insertion Sequencing of *Escherichia coli* K1 Genes Essential for *in vitro* Growth, Gastrointestinal Colonizing Capacity and Survival in Serum

Alex J. McCarthy, Richard A. Stabler, Peter W. Taylor

LEGENDS FOR SUPPLEMENTAL TABLES

Table S1. Tab 1: Genes identified by TraDIS as essential for growth of *E. coli* A192PP in Luria-Bertani (LB) broth. Systematic ID, gene identifier in annotated A192PP genome (1); strand, strand location of coding DNA sequence (CDS); gene, predicted gene annotation; size, size of CDS (bp); function, predicted function; pvalue_essential, value of essentiality determined from gamma distribution; K12, essential for growth of *E. coli* K12 MG1655 (2); EC958, essential for growth of *E. coli* ST131 urinary isolate (3); KEGG_no, KEGG orthology number; KEGG_description, KEGG predicted function; ko_no, KEGG pathway number; ko_description, KEGG pathway description; EC_no, Enzyme Commission number (EC number) for enzyme classification. Tab 2: KEGG pathways enriched for, or depleted of, *E. coli* A192PP essential genes. KEGG pathway, KEGG pathway description; whole, total number of CDS in the *E. coli* A192PP genome for each category; Whole%, percentage of CDS for each category in the *E. coli* A192PP genome; Essential, number of CDS defined as essential by TraDIS; Essential%, percentage of CDS for each category; Dif%, Essential% minus whole%; %genome, ratio Essential:Whole (D:B) X 100.

Table S2. *E. coli* K1 A192PP genes required for GI colonization. GeneID, A192PP genome systematic gene number; Norm_in, normalised read depth in input pool; Norm_MSI, normalised read depth in from TraDIS library recovered from the middle section of the small intestine (MSI) 4 h after initiation of colonization; log₂FoldChange, log₂ (Norm_out/Norm_in); * indicates number approaching negative infinity due to division of zero reads in output pool; pval, p-value; Gene, predicted gene name; Function, manually curated gene function; PROKKA function, automated functional annotation using an *E. coli* custom library.

Table S3. *E. coli* K1 A192PP genes required for survival in human serum. GeneID, A192PP genome systematic gene number; Function, manually curated gene function; PROKKA function, automated functional annotation using an *E. coli* custom library. Log₂-fold change value and a *p* value for each mutant of each gene are provided.

References

1. McCarthy AJ, Negus D, Martin P, Pechincha C, Oswald E, Stabler RA, Taylor PW. 2016. Pathoadaptive mutations of *Escherichia coli* K1 in experimental neonatal systemic infection. PLoS One 11:e0166793.
2. Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H. 2006. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol Syst Biol 2:2006.0008.
3. Phan MD, Peters KM, Sarkar S, Lukowski SW, Allsopp LP, Gomes Moriel D, Achard ME, Totsika M, Marshall VM, Upton M, Beatson SA, Schembri MA. 2013. The serum resistome of a globally disseminated multidrug resistant uropathogenic *Escherichia coli* clone. PLoS Genet 9:e1003834.

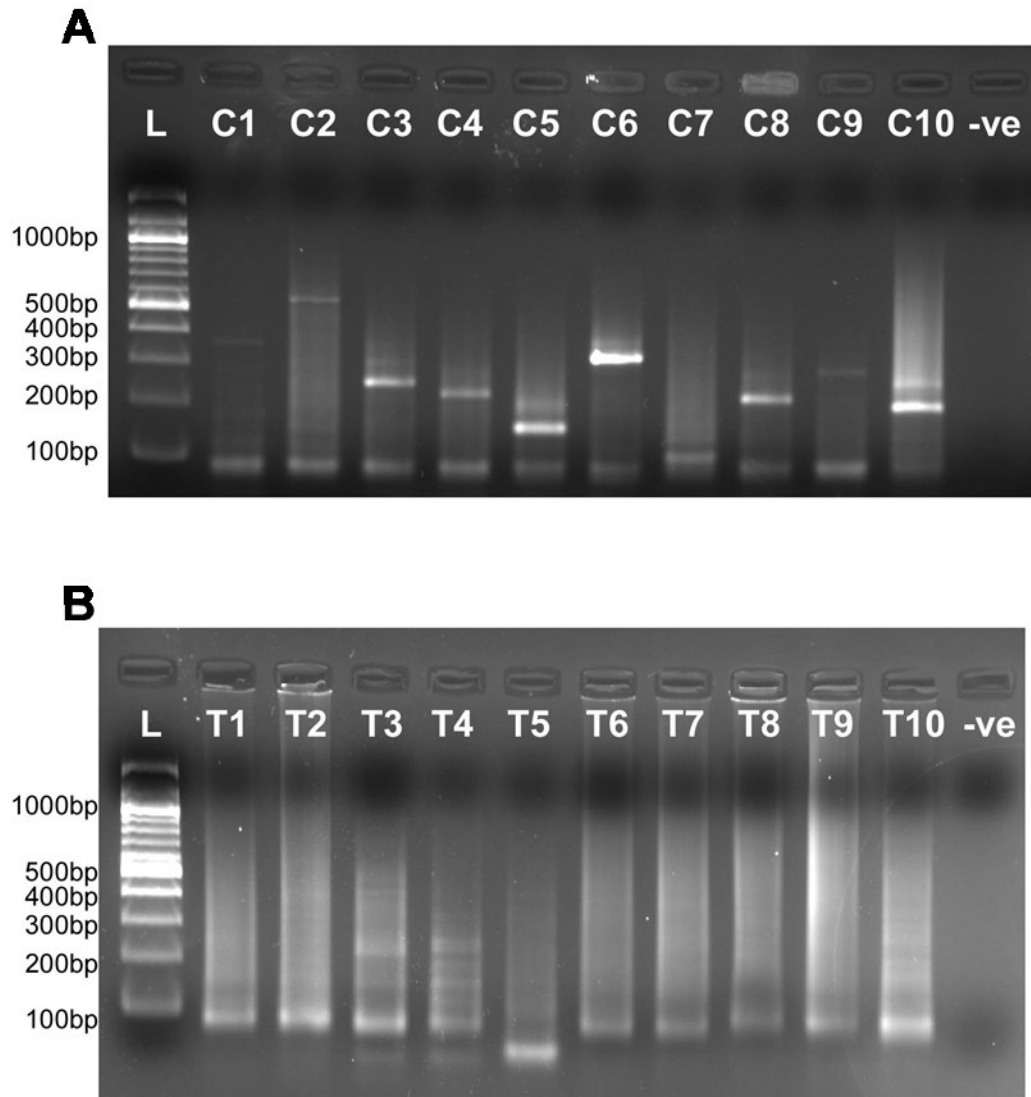
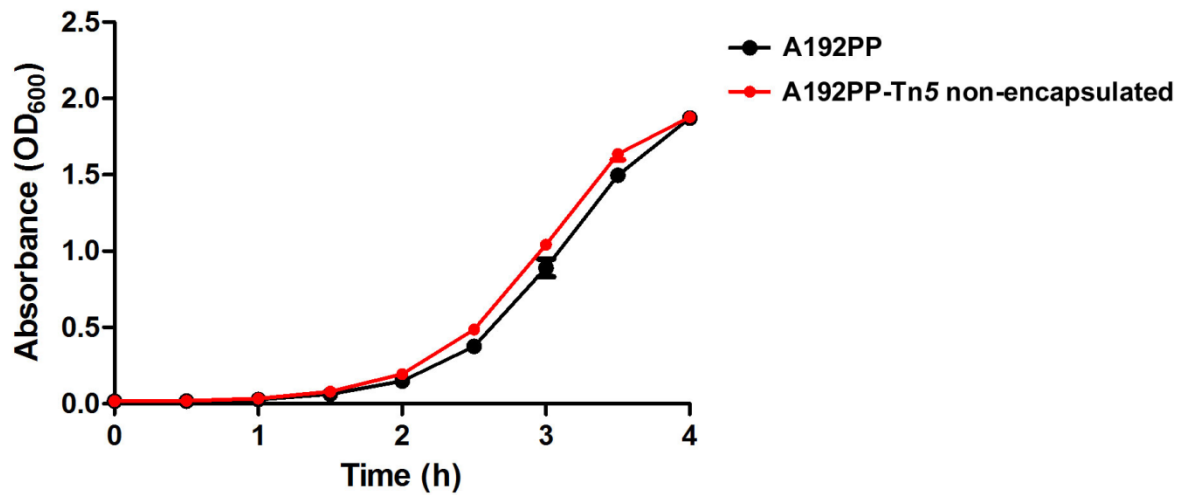
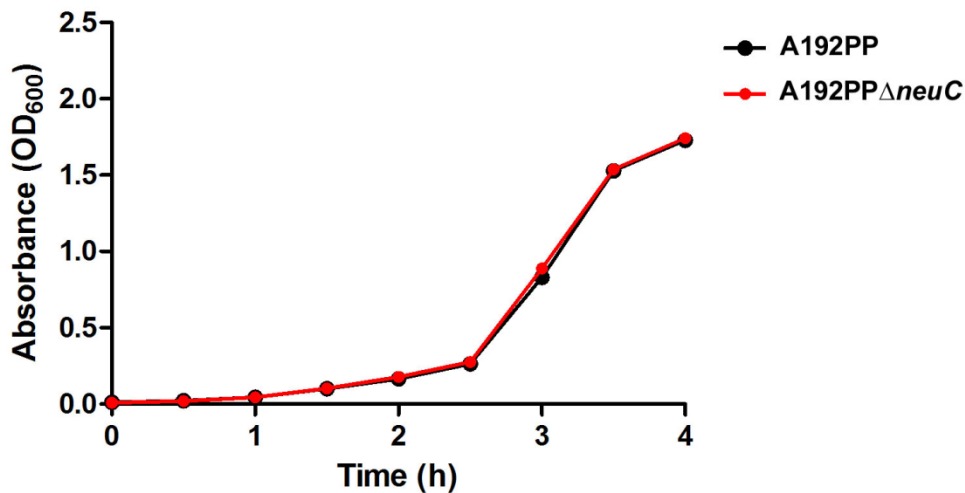


FIG S1. Linker PCR was employed to assess Tn5 insertion site diversity in: (A) ten individual adjacent colonies grown on antibiotic-supplemented Luria-Bertani agar and (B) ten individual pools of 2000-5000 colonies each.

A**B**

Strain	Mean Generation Time (min ± SD)
A192PP	24.45 ± 0.94
A192PP-Tn5 non-capsulated	23.64 ± 0.21
A192PP	30.67 ± 0.52
A192PPΔ <i>neuC</i>	30.21 ± 0.941

FIG S2. Comparison of growth kinetics of a randomly selected non-encapsulated mutant from the Tn5 TraDIS library in MH broth (A) and a non-encapsulated single gene mutant constructed using bacteriophage λ Red recombination in LB broth (B). $n=3$ in both cases. There were no significant differences in absorbance values at any time point when the log-rank [Mantel-Cox] test was applied. Student's t test was used to evaluate generation times.

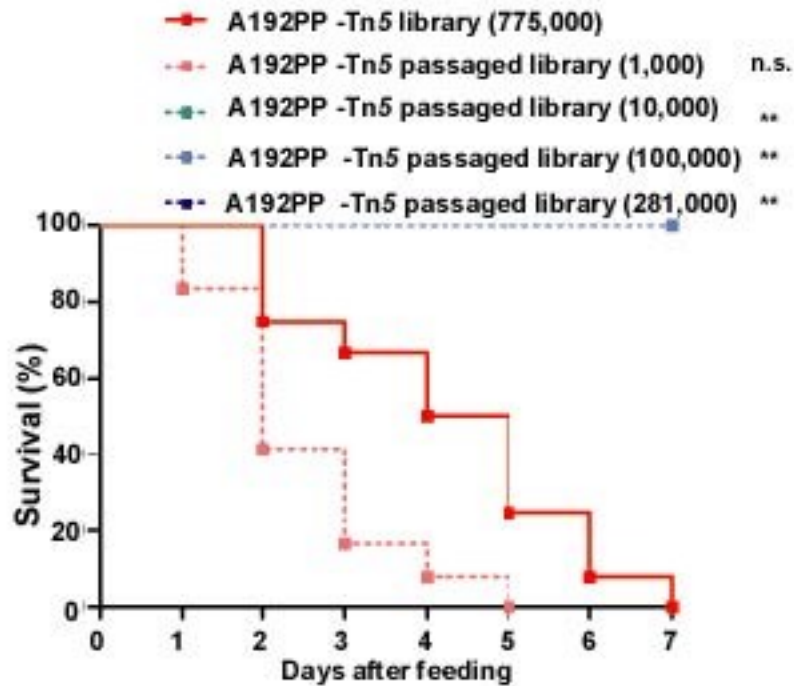


FIG S3. High-complexity cultured *E. coli* A192PP-Tn5 libraries are avirulent in neonatal rats. Survival of P2 rats colonized with *E. coli* A192PP-Tn5 libraries of differing complexities (1,000, 10,000, 100,000 or 281,000 mutants). Libraries were cultures in LB broth (8 h; 37°C) prior to initiation of colonization. Pups ($n = 12$ for each group) received $2-4 \times 10^6$ CFU by the oral route. Log-rank [Mantel-Cox] was used to compare rat survival following administration of cultured libraries with the uncultured complete library of 775,000 mutants: ns, non-significant, * $P < 0.05$, ** $P < 0.01$.

Table S4: Oligonucleotides for construction of targeted mutants

Gene	Primer	Sequence (5' to 3')
<i>lacZ</i>	<i>lacZ</i> -P1	tggatttccttacgcgaaatacgggcagacatggcctgccggttattatgtgtaggctggagctcttc
	<i>lacZ</i> -P2	tatgttggtgaaattgtgagcgaataacaatttcacacaggatacagctcatatgaatatcctccttag
<i>neuC</i>	<i>neuC</i> -P1	ctagagctgaatatggaatagttcggagacttttgacaatgctaagagaagtgtaggctggagctcttc
	<i>neuC</i> -P2	tgagaatcataacgaaagacaaaacaagcacttttttctagtcataaccatatgaatatcctccttag
<i>rfaH</i>	<i>rfaH</i> -P1	cgtaaagctttgctatccttgcgccccgattaacggataagagtcattgtgtaggctggagctcttc
	<i>rfaH</i> -P2	ctggctgccaccaggatgccaatgtcaaaactgttgggattgcttcatatgaatatcctccttag
<i>traL</i>	<i>traL</i> -P1	gtgaaatcctttcaattacaacctctgtattttccggcttcgcataaagtgtaggctggagctcttc
	<i>traL</i> -P2	cttatgataaataaaagtctcaaaattacaattacacggacatacaaaacatatgaatatcctccttag
<i>vasL</i>	<i>vasL</i> -P1	tctgcgtcatctcaaacagcaggagcggcgctactgatggcaagtaacgcgtgtaggctggagctcttc
	<i>vasL</i> -P2	aggtcacatatcccttattggtacataaatccccgatgttactgacttcatatgaatatcctccttag
<i>waaW</i>	<i>waaW</i> -P1	atagtactcatccttaattattattgtaactcagacatccatgatttttagttaggctggagctcttc
	<i>waaW</i> -P2	taaaaaattaaaaggcaagcgtaaaccaacacagtcaaaacggaaccaacatatgaatatcctccttag
<i>yaeQ</i>	<i>yaeQ</i> -P1	cgtattccgttacaatggcctcctgattcgaaaggagtttcttatggcgctgtgtaggctggagctcttc
	<i>yaeQ</i> -P2	actcgcatcagggatagcaacatgtcgggaatcacaatcatgaaggttcatatgaatatcctccttag
<i>yjiG</i>	<i>yjiG</i> -P1	gccgatgaaattcatcggcaactttgggccttttagaaatggattttgtgtaggctggagctcttc
	<i>yjiG</i> -P2	acaaatcatctctgtgtgattaatgggtattcattatattcctgcacatatgaatatcctccttag
0678	0678-P1	tagaaagtaaaattatcggacatttttatgccccacacagtcattaccgctgtaggctggagctcttc
	0678-P2	aaggcgttgatgccacacaacgcctcactgttcttttctcttttctccatatgaatatcctccttag
3010	3010-P1	tcgcaagaataatgatgaacttggcaaggatgattatgctgattaagtgtaggctggagctcttc
	3010-P2	tatctataacaaaaccatccgggtattttgtcatttttagccatcatatgaatatcctccttag

Table S5: Oligonucleotides for confirmation of targeted mutants

Gene	Primer	Sequence (5' to 3')	Fragment size	
			wildtype	Δ
<i>lacZ</i>	<i>lacZ</i> -ampF	ATGCCGGTAATAATCCACAGC	3917	1600
	<i>lacZ</i> -ampR	TGCCATGTCCGGTTTTCAA		
<i>neuC</i>	<i>neuC</i> - ampF	GACAATGCCAGGAAAAACAAG	1510	1600
	<i>neuC</i> - ampR	AAACGAAATAGCGGAGATTGT		
<i>rfaH</i>	<i>rfaH</i> - ampF	ACCACGGATGCCAATGTCA	664	1600
	<i>rfaH</i> - ampR	GTTTCATCTTTGCGATGCTGT		
<i>traL</i>	<i>traL</i> - ampF	ACACGATTCTATTGGCCCTT	873	1600
	<i>traL</i> - ampR	GTATTTTTCCGGCTTCGCAT		
<i>vasL</i>	<i>vasL</i> - ampF	TCTGCCGATCTCAGTCTGAT	1854	1600
	<i>vasL</i> - ampR	GGGCCACAGTCAAGAGGTAA		
<i>waaW</i>	<i>waaW</i> - ampF	GGGTAATCATTGCTCATCGTG	1308	1600
	<i>waaW</i> - ampR	GGTAAAAGCTGTACGGCAGA		
<i>yaeQ</i>	<i>yaeQ</i> - ampF	AACTCTGTTTCGCAAGGTGA	771	1600
	<i>yaeQ</i> - ampR	AAAACGCAGATGAATAGCCG		
0678	0678- ampF	TGTCAGGGAGTGAAGAGACAA	705	1600
	0678- ampR	AAGTGCCTCGTTTACCGTCAT		
3010	3010- ampF	TTCTGTTCTAGATGCAAGGGC	318	1600
	3010- ampR	ATGATGAACTTGGCAAAGGA		
<i>wzzE</i>	<i>wzzE</i> -ampF	AAACGCAGACTGCGTAGAAA	1195	1600
	<i>wzzE</i> -ampR	GGCGCGTACCAAATACAGTCA		

Table S6: Oligonucleotides for construction of complemented mutants

Gene	Primer	Sequence (5' to 3')
<i>neuC</i>	<i>neuC</i> -sall-F	CTAGTCGTCGACGACAATGCCAGGAAAACAAG
	<i>neuC</i> -sphi-R	GACTAGGCATGCAAACGAAATAGCGGAGATTGT
<i>rfaH</i>	<i>rfaH</i> -sall-F	CTAGTCGTCGACACCACGGATGCCAATGTCA
	<i>rfaH</i> -sphi-R	GACTAGGCATGCGTTCATCTTTGCGATGCTGT
<i>traL</i>	<i>traL</i> -sphi-F	GACTAGGCATGCACACGATTCTATTGGCCCTT
	<i>traL</i> -sall-R	CTAGTCGTCGACGTATTTTTCCGGCTTCGCAT
<i>waaW</i>	<i>waaW</i> -sall-F	CTAGTCGTCGACGGTAATCATTGCTCATCGTG
	<i>waaW</i> -sphi-R	GACTAGGCATGCGGTAAAAGCTGTACGGCAGA