

S1 Table: List of mutations identified from Run 1 and 2 that reached $\geq 5\%$ within the population, or were identified in sequenced isolates from the end of adaptation.

Run	Mutation	Annotation	Coding Change	Locus Tag ^a	Contig	Position	Highest Freq.
1	$\Delta 12$ -bp	Ribosomal S10 protein	R53Q- $\Delta 54$ -57ATHK	1375	763	35,999	99.9%
1	$\Delta 87$ -bp	Intergenic (51-bp upstream of <i>tetM</i>)	N/A	N/A	778	138,433	90.6%
1	G \rightarrow A	Serine/threonine protein kinase PrkC, regulator of stationary phase	A196V	2283	801	27,053	16.1%
1	G \rightarrow T	N-acetylmuramoyl-L-alanine amidase family 4	E57*	2682	810	168,069	15.6%
1	$\Delta 71$ -bp	Intergenic	N/A	N/A	775	6,290	14.6%
1	C \rightarrow A	Cobalt ABC Transporter	G212V	187	730	27,780	6.3%
1	$\Delta 37$ -bp	Intergenic (98-bp upstream of <i>tetM</i>)	N/A	N/A	778	138,480	4.2%
1	$\Delta 22$ -bp	Intergenic (231-bp upstream of <i>tetM</i>)	N/A	N/A	778	138,613	0.8%
2	$\Delta 12$ -bp	Ribosomal S10 protein	R53Q- $\Delta 54$ -57ATHK	1375	763	35,999	94.7%
2	$\Delta 125$ -bp	Intergenic (36-bp upstream of <i>tetM</i>)	N/A	N/A	778	138,448	94.5%
2	T \rightarrow A	ABC Transporter 1	T278S	2766	810	260,239	23.6%
2	G \rightarrow C	ABC Transporter 2	G73A	1948	785	39,763	21.8%
2	T \rightarrow C	Type 1 restriction modification system, specificity subunit S	L187L	1696	778	18,115	14.0%
2	T \rightarrow C	Type 1 restriction modification system, specificity subunit S	L187F	1696	778	18,117	13.7%
2	C \rightarrow A	Transcriptional regulator, Cro/Ci family	S49Y	971	749	98,114	12.8%
2	G \rightarrow A	Intergenic	N/A	N/A	739	26,573	9.6%
2	G \rightarrow A	O-succinylbenzoic acid-CoA Ligase	A409A	2055	787	9,405	9.5%
2	G \rightarrow T	Cell division trigger factor	E60*	2468	809	107,415	9.5%
2	C \rightarrow G	Hypothetical Protein	G293R	842	746	25,723	8.4%
2	C \rightarrow A	Hypothetical Protein	W295L	842	746	25,716	8.1%

2	C→A	Intergenic	N/A	N/A	720	3,617	7.9%
2	Δ12-bp	Ribosomal S10 protein	Δ52-55VRAT	1375	763	35,994	7.3%
2	G→A	Type 1 restriction modification system, specificity subunit S	K195K	1696	778	18,141	6.3%
2	A→T	Zn-dependent hydrolase (Beta-lactamase superfamily)	L55F	963	749	92,039	5.7%
2	A→T	Type 1 restriction modification system, specificity subunit S	T181T	1696	778	18,099	5.4%
2	1,505 Kb insertion	Transposase IS204 insertion into Tn916	N/A	733	778	135,277	0.3%

^aLocus tags were obtained from *E. faecalis* S613 file annotated by PATRIC (<http://www.patricbrc.org>)