

Supplementary Table S1 – Mutational similarity comparison with Tenaillon *et al.*

Listed are the 9 functional units found by Tenaillon *et al.* (defined as possessing at least 5 mutations across their 114 evolved strains) that had some level of agreement with the mutations identified in this study. The remaining 17 functional units had no agreement.

Functional Unit	Gene	Number of evolved strains with mutations	
		This study (10 total strains)	Tenaillon study (114 total strains)
Extended RNA polymerase complex	<i>rpoC</i>	5	21
	<i>rho</i>	1	45
	<i>rpoB</i>	0	87
	<i>rpoD</i>	0	36
	<i>nusA</i>	0	12
	<i>rpoA</i>	0	4
Cardiolipin synthetase	<i>cls</i>	1	64
Glycogen synthesis and degradation	<i>glgP</i>	1	28
	<i>glgC</i>	0	6
	<i>glgA</i>	0	2
Leader peptide regulating isoleucine and valine biosynthesis operons	<i>ilvL</i>	2	29
General stress response regulators	<i>rpoS</i>	1	3
	<i>cspC</i>	0	13
	<i>rssB</i>	0	11
Glutamate-related genes	<i>gltP</i>	1	7
	<i>gltT</i>	1	0
	<i>gltF</i>	1	0
	<i>gltL</i>	0	3
DNA topoisomerase I	<i>topA</i>	1	8
<i>dusB-fis</i> operon	<i>dusB</i>	1	3
	<i>fis</i>	1	5
Ribosomal proteins	<i>rpsB</i>	1	0
	<i>rpsR</i>	0	5
	<i>rpsA</i>	0	3

Supplementary Table S2 – Oligos used to perform MAGE recombineering

Oligos #1-29 correspond with alleles acquired by the non-mutator ALE strains, while oligo #30 was used to determine MAGE recombineering efficiency.

#	Oligo name	Oligo sequence	Corresponding position in the reference genome (NC_000913.2)
1	ALE1_cls	TGTCGCTGCGCCATTATTCAAGCTTTGCGAGCGTgGTCAGGGGATCGCTGGGGTCAAAGGAATCAGCTA	1,306,386-1,306,307
2	ALE1_yegJ/K	TCGCTAATCAGCCGTTATATTTATTGTTTAAACTGGCGGTTGAATTGTTtTCTTCAACCGCCATGAGAAA	2,149,664-2,149,733
3	ALE1+4+5+7+9+10_pyrE/rph	GCCTTAAAAATCAGTTTGCCAGCGCCGCCTTCTGCCCGTCCCCTGCACTTCAATGATGCGCCCGTCTTCG	3,813,847-3,813,881 /3,813,963-3,813,997
4	ALE1_rpoC	ACCACGCATACCAGCAAGCTGACGAATCTGTGCCaCAGAACCACGCGCACCAGTTCGGCCATCATGTAG	4,185,607-4,185,538
5	ALE1+7_hfq	CCGACGCAGTGC GTTCAGGAACGGAgCTTGTAAAGATTGCCCTTAGCCATTCTCTCTTTCTTATATG	4,398,361-4,398,292
6	ALE1_nagA	GAGTACCTGGCAAACATCCGAATCAGGCGTTAGGGCTGAATCTGGTAAAAAAGGCACCCATAATCCGA	701,629-701,573 / 701,573-701,539
7	ALE4_mdtG/lpxL	GATGCTTCAGAATGGCATCCGaCATTACCACAGCAAATCCCCCTGATTTAGCGATAAAAAGCTCTCTGGAT	1,114,871-1,114,802
8	ALE4+5+7_pykF	TTACTACTGCGACCCAGATGCTGGATTCCATGATctAAAACCCACGCCGACTCGCGCAGAAGCCGGTGA	1,754,452-1,754,611
9	ALE4+5_ygaH/mprA	CACTGTCGTTACTATATCGGCTGAAATTAATGAGGcCATACCCAAATGGATAGTTTCGTTTACGCCATTG	2,808,747-2,808,816
10	ALE4+5+7_mlaE	TCTGCGGTTAGCGCCGAACCAGCACGCCCGGCAAAAaACAACGCGGCAACCACCGGCCAGTTCACGCA	3,336,915-3,336,984
11	ALE4_rho	GTAGGAGCTGTCTGCGGAACGGAGGAAACCAATtCATCCTGCAATATCTCCAGTACGCCATCACCAAAG	3,964,655-3,964,586
12	ALE5_yfjG	TCCAGAATCCGACTTCCGGTACAACCCGGCAAAAaTGAGGATAAGACTGAACGTCATTACTAACTGAT	2,752,626-2,752,695
13	ALE5+7+10_rpoC	CGACGCATACGATCCTaGTGGTACGCGTAACCGGTACCTGCCGGGATCAGACGACCCACGATAACGTTCT	4,187,492-4,187,418
14	ALE5_thiG	TAGTGAATACGGCGATTGCCGTCGCGGACGATCCgGTCAACATGGCGAAGGCATTTTCGCTGGCGGTAGA	4,190,054-4,189,985
15	ALE7_proQ	CTGCGCGCACAAATCAAAGACATACCCGAATTcAGCgGGACGCGGACCGCTTTTGGTGATTTCTAATAC	1,912,877-1,912,946
16	ALE7_ykfN	CCAGAAGTTGAAGTATTAACCGATCATAATGAGCgaATTTGTTCGAGCTTTATTGAACACATTGCCAACA	262,430-262,361
17	ALE7_yhjK	ATAGTGAGCACTTGCTaACCTAAGGTGATTGCGTGCCACGGTTCCTGTACACCGTTGGCAATGACAGCAA	3,682,611-3,682,680
18	ALE9_me	ACTTTTATCAGTCTGGCGaGTAGCTATCTGGTTCTGATGCCGAACAACCCGCGCGGGTGGCATTCTC	1,143,239-1,143,170
19	ALE9_yadD	TCCTGACTTACTTCTTCTCCCTGCTGAATCCCTTTCTCTTCAAACCACTGCGCCAGCGTCATCATAG	147,767-147,686
20	ALE9_barA	ATGGCCGGGCAAAAAGAGAAGCTGCTTGCGCAGGaATGAGCGATTATCTGGCGAAACCGATTGAAGAAG	2,915,332-2,915,401
21	ALE9_glgP	GCTTGTAAGCGATAGAGTGCTTAAGAGCTTCTACGaTAAGCGTGGGCGATGAATATGTAAACGGAGCATT	3,564,532-3,564,601
22	ALE10_pykF	AATGTATCCGTGCACGTAAGTCTTATCACTGCGtCCAGATGCTGGATTCCATGATCAAAAACCCACG	1,754,518-1,754,587
23	ALE10_grpE/nadK	CGTTTTCTGTTCTTTACTACTCATGAATTTCTCCGtGTTTTTTTCGATTATCTCGCTAACTTCGCTTA	2,748,707-2,748,766
24	ALE10_ygaH/mprA	TCACTGTCGTTACTATATCGGCTGAAATTAATGAGaTCATACCCAAATGGATAGTTTCGTTTACGCCATT	2,808,746-2,808,815
25	ALE10_yihT	CCACTCACACGGTCTGGTAAGCATCATTGAGCCAtTCGTCGCCCCACCGGTCGTGGCGATAAATTCGAT	4,070,228-4,070,159
26	ALE10_yiiD/E	TCCTAACAGCACCAGGATTTAAGTGAAATTAATaTTTTATCACAGACCGGTCAGTACTGCCACCAACCG	4,077,053-4,076,984
27	ALE10_ybiO	ATTTATTcAGTTTGAACGCGCATGAACACTGGatATTTGGTACTATCGGGCCGTTGACCGGCACAGTG	843,006-842,937
28	ALE4_spr	GATTGTTCTGGTTTCGTACAGCGTACATTtGatAGtAATTTGGCTTAGAACTTCGCGTTTCGACTTACG	2,268,277-2,268,346
29	ALE4_dusB	CAGGAAGACCCCTGGATCTTTCGGGAAATcTAGTgaTaaCTGGACACTGGGGAGTTGCTGCCCCCGCTGC	3,408,983-3,409,052
30	P9 malK84TAG	ATGTTTTCTGCTACTGACAGGTGGGGATAGAGCGCcTAAGACTGAAACACCATACCAACCGCGTTCTGC	