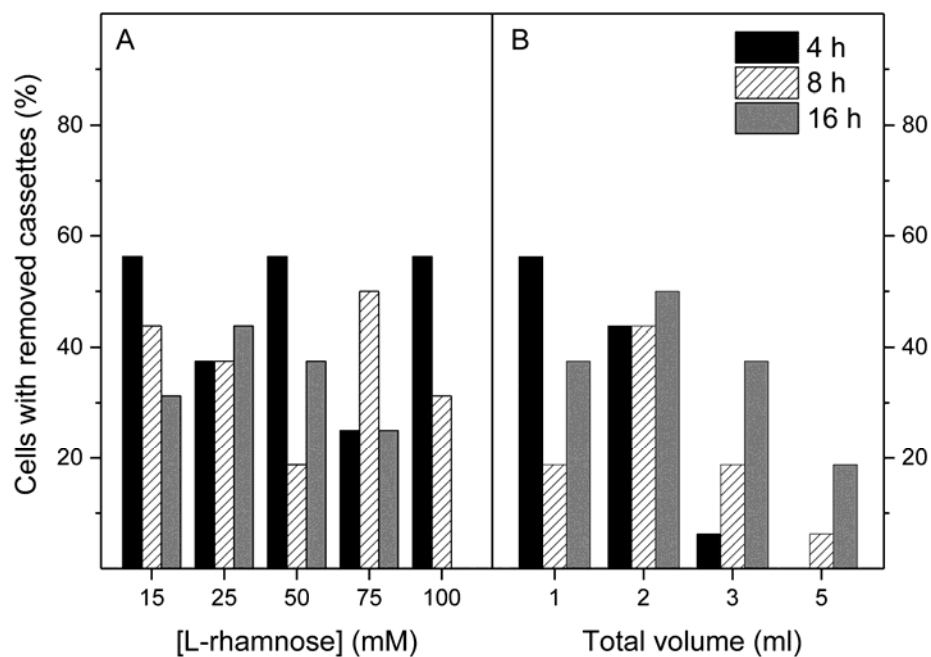


**Seven gene deletions in seven days:  
Fast generation of *Escherichia coli* strains tolerant to acetate and osmotic stress**  
Sheila I. Jensen<sup>1\*</sup>, Rebecca M. Lennen<sup>1</sup>, Markus J. Herrgård<sup>1</sup>, Alex T. Nielsen<sup>1\*</sup>



**Supplementary Figure S1.** Efficiency in percent of simultaneous excision of different integrated antibiotic cassettes in strain SIJ488 induced for 4 h (black bars), 8 h (stripes) or 16 h (grey). A) Efficiency using different concentrations of rhamnose. B) 50 µl growing cell culture inoculated into 1 ml, 2 ml, 3 ml or 5 ml LB and induced with 50 mM rhamnose (n=16).

**Supplementary Table S1.** Precursor strains and plasmids used in this study.

Strain	Genotype	Reference
XL-1 blue	recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F'proAB lacIqZΔM15 Tn10 (Tetr)]	Agilent technologies
DH5α λpir	λpir lysogen of DH5α	V. de Lorenzo <sup>2</sup>
Plasmid	Description	Reference
<b>Plasmids</b>		
pKD46	Ap <sup>R</sup> , oriR101, repA101 <sup>TS</sup> , araC-ParaB::gam-beta-exo	(Datsenko and Wanner 2000)
pEMG	Km <sup>R</sup> , oriR6K, lacZα, two I-SceI sites	(Martinez-Garcia and de Lorenzo 2011)
pSEVA 631	Gm <sup>R</sup> , oripBBR1, oriT	(Silva-Rocha et al. 2013)
pSEVA 441	sm <sup>R</sup> , oripRO1600/ColE1, oriT	(Silva-Rocha et al. 2013)
pFLP2	Ap <sup>R</sup> , ori1600, oriT, rep, cl857-FLP, sacB	(Hoang et al. 1998)
pSW-1	Ap <sup>R</sup> , oriRK2, xylS, Pm-I-sceI	(Wong and Mekalanos 2000)
pSIJ_180	pEMG ap <sup>R</sup> , frt::cm::frt	This study

<sup>2</sup>This strain was generously donated by V. de Lorenzo, Centro Nacional de Biotecnología-CSIC, Madrid, Spain

- Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci.* 97(12):6640-6645.
- Hoang TT, Karkhoff-Schweizer RR, Kutchma AJ, Schweizer HP. 1998. A broad-host-range Flp-FRT recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked *Pseudomonas aeruginosa* mutants. *Gene* 212(1):77-86.
- Martinez-Garcia E, de Lorenzo V. 2011. Engineering multiple genomic deletions in Gram-negative bacteria: analysis of the multi-resistant antibiotic profile of *Pseudomonas putida* KT2440. *Environ Microbiol* 13(10):2702-16.
- Silva-Rocha R, Martinez-Garcia E, Calles B, Chavarria M, Arce-Rodriguez A, de Las Heras A, Paez-Espino AD, Durante-Rodriguez G, Kim J, Nickel PI and others. 2013. The Standard European Vector Architecture (SEVA): a coherent platform for the analysis and deployment of complex prokaryotic phenotypes. *Nucleic Acids Res* 41(Database issue):D666-75.
- Wong SM, Mekalanos JJ. 2000. Genetic footprinting with mariner-based transposition in *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A* 97(18):10191-6.

**Supplementary Table S2: Primers used in this study**

Primers for deletions and verification	Sequence
662_rfe_EC-W-MG_fw	CTTCGTGGTTATACTTCTGCTAATAATTTTCTCTGAGAGCATGCAT TGTGTGTAGGCTGGAGCTGCTTC
974_rfe_BL21_fw	CTTCGTGGTTATACTTCTGCCAATAATTTTCTCTGAGAGCATGCAT TGTGTGTAGGCTGGAGCTGCTTC
663_rfe_rv	AGGCATTGGTTGTGTCATCACATCCTCATTTATTTGGTTAAAATTGG GGCTGCTTGCATATGAATATCCTCCTTAG
664_evga_fw	ATTACTACAGGGAGAAGGGAGATGCTTCATTGCAAAGGGAATAAT CTATGTGTAGGCTGGAGCTGCTTC
665_evga_W_rv	GAAAAAATAGGGTAAAAACTTCATGTGATTAGCCGATTTTGTTA CGTTGGCTTGCATATGAATATCCTCCTTAG
874_evga_MG-BL_rv	GAAAAATATAGGGTAAAAACTTCATGTGGTTAGCCGATTTTGTTA CGTTGCTTGCATATGAATATCCTCCTTAG
666_typA_fw	TAATCCGTGTACAATAACGCGCTATTTCTAATGCCTGAGGCAAAGT TGTGTGTAGGCTGGAGCTGCTTC
667_typA_rv	GGCAGGTTTTTATGACTAAAAACGAAATTAATCGTCTTTTCGGT GCGCGGCTTGCATATGAATATCCTCCTTAG
668_yobF_fw	TTGTATGTGATAACAGATTTTCGGGTAAACGAGGTACAGTTCTGT TTATGTGTAGGCTGGAGCTGCTTC
669_yobF_rv	CCTTTAATCTTTGCCATTTGAAAAATTCCTTAGATTGTTTTCTTCG CCCGGCTTGCATATGAATATCCTCCTTAG
670_ptsP_fw	AAAACGCATCTGCTTATCGACGTAAAAGAGGTTAAGTCACGCCAA TTATGTGTAGGCTGGAGCTGCTTC
671_pstP_rv	ACCGTTAAAAGATATGTATATGATCCGCGCTATAACCCCTCCGCGAA TCAGGCTTGCATATGAATATCCTCCTTAG
672_ackA_fw	TGGCTCCCTGACGTTTTTTTTAGCCACGTATCAATTATAGGTACTTC CATGTGTAGGCTGGAGCTGCTTC
673_ackA_rv	GCACCGCCAGCTGAGCTGGCGGTGTGAAATCAGGCAGTCAGGCGGC TCGCGCTTGCATATGAATATCCTCCTTAG
674_yciW_fw	CCGCCTTGCCTCAGGATAACGATTTTCCTTTACGACCAAGGAGCGCC CATGTGTAGGCTGGAGCTGCTTC
675_yciW_rv	TTTACGCGAATCTGTCTGACGCGGCAAGGTTAATATGTCTCACCCA ACGCGCTTGCATATGAATATCCTCCTTAG
975_rfe_seq_fw	ACTGGCAATGACCAAGACCA
976_rfe_seq_rv	CCCCAGCATATTCACCGTTG
977_typA_seq_fw	ACGATGTGAATGTTGCACCA
978_typA_seq_rv	GTCATACGTTTAATGCCACG
979_yciW_seq_fw	TCGCCGTACCGTTACTATTGA
980_yciW_seq_rv	TAATTCGGTGCACATGCATG
981_yobF_seq_fw	TAACTTCTCCCACTGGCCTG
982_yobF_seq_rv	TCAGTGGATTCGATCAGATAGCT
983_evga_seq_fw	TGCAATACAATTCTTACGCCTGT
984_evga_seq_rv	TAAGAGGCGGCGAAGTAACT
985_ptsP_seq_fw	GGTTTCTCTTGCAGCTGGTG
986_ptsP_seq_rv	GCCACGGGTCCTATTGAGAA
987_ackA_seq_fw	AGGTATCCTTTAGCAGCCTGA
988_ackA_seq_rv	CCGTCGTGGTGAAGAGTT

RL_rfe_colPCR_fwd	TTGCATATCAAATGGTAAATTTTTGACAG
RL_rfe_colPCR_rev	CGCCAGCCCCATGCCAAT
RL_evga_colPCR_fwd	CCGACTATTTATATGGTATACTTGTTCG
RL_evga_colPCR_rev	CGTAATTCCTTGTTGCTCAGACG
RL_typA_colPCR_fwd	TGACCTTTGGATAACCCTTTTTATGC
RL_typA_colPCR_rev	GACAAAGTCTCTATTGACGTAACC
RL_yobF_colPCR_fwd2	CTGATCGAGACATGTTAAAAATGGC
RL_yobF_colPCR_rev	TTCAGCCAGAGTTTTGAAGCCATTA
RL_ptsP_colPCR_fwd	GTGGTGTCAATTAACGTGATGTC
RL_ptsP_colPCR_rev	CTGTCACCACAAGTTCTTGTTATTTC
RL_ackA_colPCR_fwd	CAAAATGGCATAGACTCAAGATATTTTC
RL_ackA_colPCR_rev	CGGTAGGGATCAGCATAATAATAC
RL_yciW_colPCR_fwd	CGTTCTGCCGCCCGTTATC
RL_yciW_colPCR_rev	TTACCACCCCTTCAGCGCG
1037_evga_phos-thio_fw	T*C*GAATTATCTTAAAGGAAGCTCAG
1038_evga_phos_rv	/5PHOS/GTTGTTACTACTGATGCCACGA
1039_ackA_phos-thio_fw	T*G*CAGTGCATGATGTTAATCA
1040_ackA_phos_rv	GATCAGCATAATAATACGGGACAC
1041_yobF_phos_fw	GATTTGTGCGTCTGATCGAGA
1042_yobF_phos-thio_rv	C*A*TCAGCCGGAGTAATGAAGC
1043_ptsP_phos_fw	/5PHOS/CACTGCAGGAAAATACGCCA
1044_ptsP_phos-thio_rv	G*C*AGGTTTCAGGCGCTCC
1045_rfe_phos_fw	ATGGTAAATTTTTGCACAGGACT
1046_rfe_phos-thio_rv	C*C*ACAAGGTACGAAACAACC
1047_typA_phos_fw	ACTTGTTTGCGGAGGATGTT
1048_typA_phos-thio_rv	A*C*AGACTTATAAGGGAGATACTCGCA
1049_yciW_phos-thio_fw	C*C*GCCCGTTATCTCTGTTAT
1050_yciW_phos_rv	CTAGCAGCGGGTTGTCCT
1079_rfe50-BL21-fw	CTTCGTGGTTATACTTCTGCCA
1080_rfe50-phos-thio-rv	A*G*GCATTGGTTGTGTCATCAC
1081_typA50fw	TAATCCGTGTACAATAACGCGCT
1082_typA50-phos-thio-rv	G*G*CAGGTTTTTTATGACTAAAAACGA
1083_evga50-phos-thio-fw	A*T*TACTACAGGGAGAAGGGAGATGCT
1084_evga-MG-BL-50rv	GAAAAATATAGGGTAAAAACTTCATGTGG
1085_evga-W-50rv	GAAAAAAATAGGGTAAAAACTTCATGTGA
1086_ackA50-phos-thio-fw	T*G*GCTCCCTGACGTTTTTTTAGC
1087_ackA50rv	GCACCGCCAGCTGAGCTG
1088_yciW50-phos-thio-fw	C*C*GCCTTGCGTCAGGATAAC
1089_yciW50rv	TTTACGCGAATCTGTCTGACG
1090_yobF50fw	TTGTATGTGATAACAGATTTCCGGGT
1091_yobF-phos-thio-50rv	C*C*TTAATCTTTGCCATTTGAA
1092_ptsP50fw	AAAACGCATCTGCTTATCGACG
1093_ptsP-phos-thio-50rv	A*C*CGTTAAAAGATATGTATATGATCCGC

Primers for Plasmids	Sequence
flp_U_pkd46_rv	AACCTTATAUGCGTCTATTTATGTAGGATGAAAGG
rha_flp_U_fw	AGAAATACTAGAUGCCACAATTTGGTATATTATGT
rhamSD_AT_U_rv	ATCTAGTATTTTCUCCTCAATTTTCATTACG
RhaRS_pkd46-U_fw	ATCTGATTAAUCTTTCTGCGAATTGAGATGAC
pkd46_rha_U_rv	ATTAATCAGAUCCCTCCGTATTTAGCCAGT
pkd46_flp_U_fw	ATATAAGGDUCTTATGGCTCTGTATCTATCAGTG
795_EC_Tn7_down_U_fw	AGTCAGGUAATGGATGCCCTGCGTAAG
508_p169-rv	ACGAACGUCGGCCTCCTGTGTGAAATTG
833_p8-U_fw	ACGTTTCGUACTTTTCATACTCCCGCCATTCA
834_p8_U_rv	AGCAAGCUACTGGCCTCCTGATGTCGTC
888_p222-U_fw	ACCTGACUTATTTTCAGGAAAAGTTTCGGAGGAGATAG
889_p222-U-rv	AGCTTGCTCGTAATTATTGGGGACCCCTG
654_pSIJ180_frt-kan-U-fw	ATGCAAGCUTTCCGTACAGGTAGGCG
655_pSIJ180_frt-kan-U-rv	ATGAAGUTCCTATTCCGAAGTTCC
656_pSEVA_U_fw	ACTTCAUGGGTCCCAATAATTACGATT
657_pSEVA_gm_U_rv	ACGCGAUAGCGGAAAAGGACAACGC
754_seva_spec_frt_U_rv	AGCTTGCAUAGCGGAAAAGGACAAGGGT
648_pkd3-4-frt_EMG-U_fw	ATCTGAAUGTGTAGGCTGGAGCTGCTTC
649_pkd3-4-frt_EMG-U_rv	AGCTTGCAUATGAATATCCTCCTTAGTTCCTATTCCGA
652_pSW-amp-U_fw	AGTCCCGCUTACCAATGCTTAATCAGTGAGGCAC
653_pSW-amp-U_rv	AGCACUCTGACGCCGTTGGATACACC
411_pEMG_U_fw	ATGCAAGCUTCTAGGGATAACAGG
651_pEMG_amp_U_rv	AGCGGGACUCTGGGGTTC
650_pEMG_amp_U_fw	AGTGCUTGCGGCAGCGTG
412_pEMG_U_rv	ATTCAGAUTACCCTGTTATCCCTATACTG
887_pSW-508-fw	ACGTTTCGUTATTTTCAGGAAAAGTTTCGGAGGAGATAG
836_pSW1-U_rv	ATGCGCUCCTTCTTCGGCTACGTTGTAATCA
454_xylS_pm_U_fw	AGCGCAUACCTGCAGGCATGCAAGCT
508_p169-rv	ACGAACGUCGGCCTCCTGTGTGAAATTG
793_EC_Tn7_up_U_fw	ATCTGAAUGAGCCGTATTGAGCAGATGCT
794_EC_Tn7_up_U_rv	AAACCCGUTACTCAACCGTAACCGATTTTGC
795_EC_Tn7_down_U_fw	AGTCAGGUAATGGATGCCCTGCGTAAG
796_EC_Tn7_down_U_rv	AGCTTGCAUACCCTGGTAGTTAACTTTATTACCGGT
411_pEMG_U_fw	ATGCAAGCUTCTAGGGATAACAGG
412_pEMG_U_rv	ATTCAGAUTACCCTGTTATCCCTATACTG