

Markerless *Escherichia coli* *rrn* Deletion Strains for Genetic Determination of Ribosomal Binding Sites

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Table S1 Strains and plasmids used in this work

Strain	Genotype	Source/Reference
MG1655	<i>ilvG rfb-50 rph-1</i>	Blattner <i>et al.</i> 1997
SQ11	$\Delta rrnE::KmR$	This work
SQ16	$\Delta rrnB::KmR$	This work
SQ20	$\Delta rrnG::KmR$	This work
SQ22	$\Delta rrnA::KmR$	This work
SQ24	$\Delta rrnD::KmR$	This work
SQ26	$\Delta rrnH::KmR$	This work
SQ34	$\Delta rrnC::KmR$	This work
SQ37	$\Delta rrnE$	This work
SQ40	$\Delta rrnEG$	This work
SQ49	$\Delta rrnGBA$	This work
SQ53	$\Delta rrnGBAD$	This work
SQ2062	$\Delta rrnGBAD(ptRNA67)$	This work
SQ2066	$\Delta rrnGBAD(pK4-16)$	This work
SQ2200	$\Delta rrnGBAD(pK4-16, ptRNA67)$	This work
SQ78	$\Delta rrnGADE$	This work
SQ2068	$\Delta rrnGADE(pK4-16)$	This work
SQ2197	$\Delta rrnGADE(pK4-16, ptRNA67)$	This work
SQ2199	$\Delta rrnGADE(ptRNA67)$	This work
SQ88	$\Delta rrnGADEH(ptRNA67)$	This work
SQ2196	$\Delta rrnGADEH(pK4-16, ptRNA67)$	This work
SQ110	$\Delta rrnGADBHC(ptRNA67)$	This work
SQ2194	$\Delta rrnGADBHC(pK4-16, ptRNA67)$	This work
SQ141	$\Delta rrnGADEHB(pKK3535, ptRNA67)$	This work
SQ2202	$\Delta rrnGADEHB(pK4-16, ptRNA67)$	This work
SQ2203	$\Delta rrnGADEHB(ptRNA67)$	This work
SQ171	$\Delta rrnGADEHBC(pKK3535, ptRNA67)$	This work
SQ2158	$\Delta rrnGADEHBC(pK4-16, ptRNA67)$	This work
Plasmid		
pKK3535	pBR322 ori, <i>rrnB</i>	Brosius <i>et al.</i> 1981
pK4-16	pSC101 ori, <i>rrnB</i>	This work
ptRNA67		Zaporjets <i>et al.</i> 2003
pKD46		Datsenko and Wanner 2000
pCP20		Cherepanov and Wackernagel 1995
pKD13		Datsenko and Wanner 2000

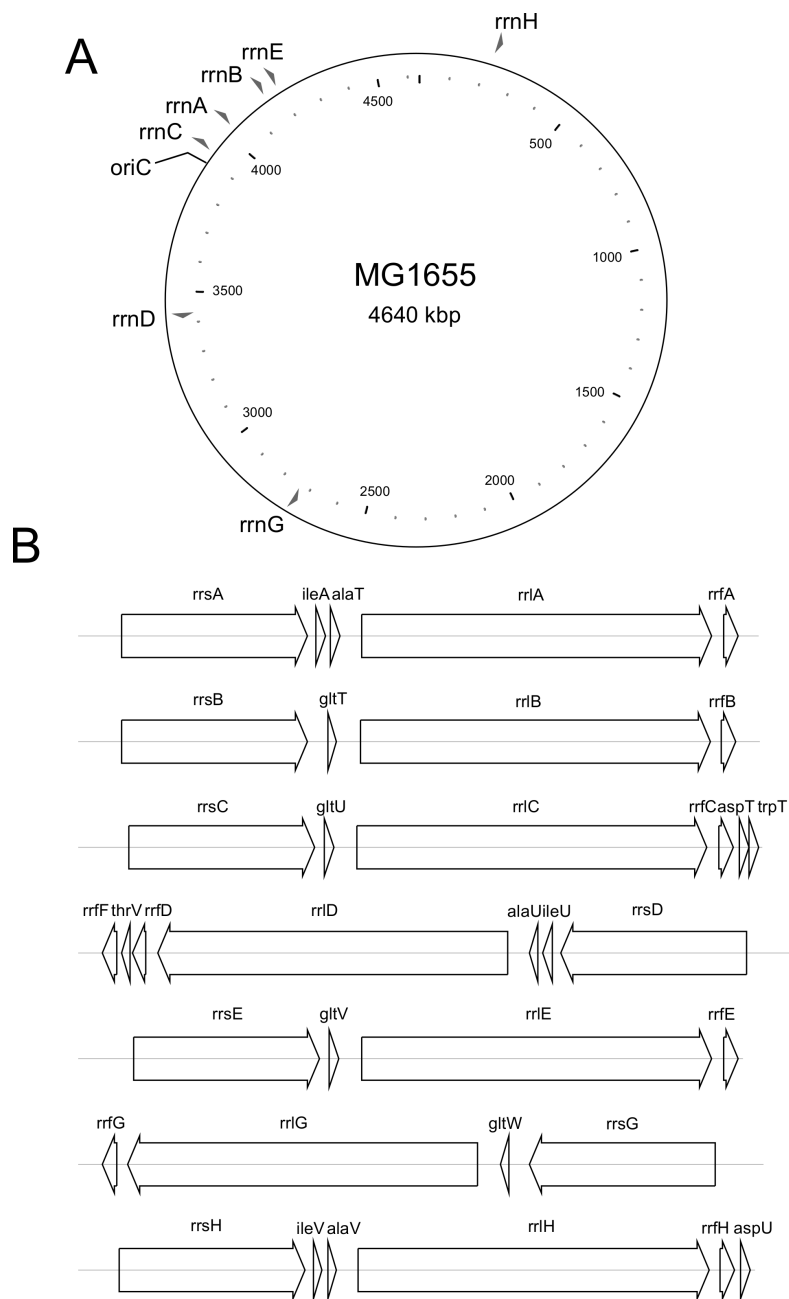


Figure S1 Extent of the deletion of each *E. coli* ribosomal RNA operon mapped with respect to Genbank version U00096.2 of the *E. coli* genomic sequence.

rrnA



4035164..4040815

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rrnB



4166298..4171959

CCAGGAGCTGAACAATTATTGCCCGTTTTACAGCGTTACGGCTTCGAAACGCTCGAAAACT
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 AAAC'TCTTCCTGTCGTCATATCTACAAGCCATCCCCCACAGATACGGTAAACTAGCCTCGT
 TTTTGCATCAGGAAAGCAGCTATGAACCACTCCTTAAAACCCTGGAACACATTTGGCATTGA
 TCATAATGCTCAGC

rrnC



3941387..3947067

AAGTTTTTCTGTGCAGCTAACTGTTGTGCGCTTAAAGGCATTACTTATCTTCCTTTTTTCTT
 TTTATTCCTCCTTAGTATGCCACCAGGAAGTGTGATTACGTGTAGGCTGGAGCTGCTTCGAA
 GTTCCTATACTTTCTAGAGAATAGGAACTTCGAACTGCAGGTTCGACGGATCCCCGGAATATC
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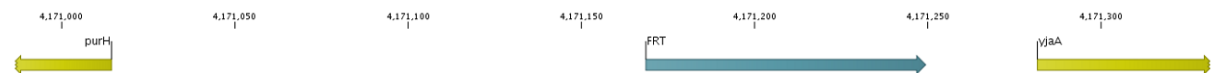
rrnD



3423217..3429164

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 AAGTTCCTATACTTTCTAGAGAATAGGAACTTCGAACTGCAGGTTCGACGGATCCCCGGAATA
 CTAAAAGACTTGCACAAGGCCAATAATGCCCCAAAGTCATTAGTAAATCATTTATTGCTGA
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 TAATGATCG

rrnE

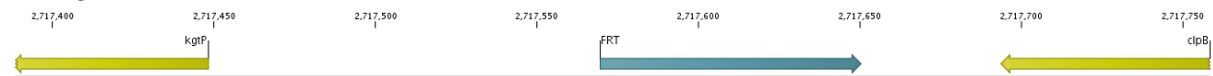


4207686..4213202

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 CCCGCATTGTAACGAAAACGTTTGCGCAACGCTCGCGAATTTTTCTCTTTCAATGGTGGTGT
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ATCCAAATTCGTCGTAATCAAATTACTGTTTCGCGA

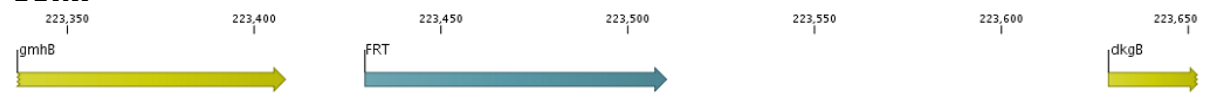
rrnG



2725867..2731558

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GGCTGGAGCTGCTTCGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGAACTGCAGGTCG
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GGACGGCGACAATCCGGTCTTCATTAACTTCCAGGCGAATCACTTTACCCGGAACCAATT

rrnH

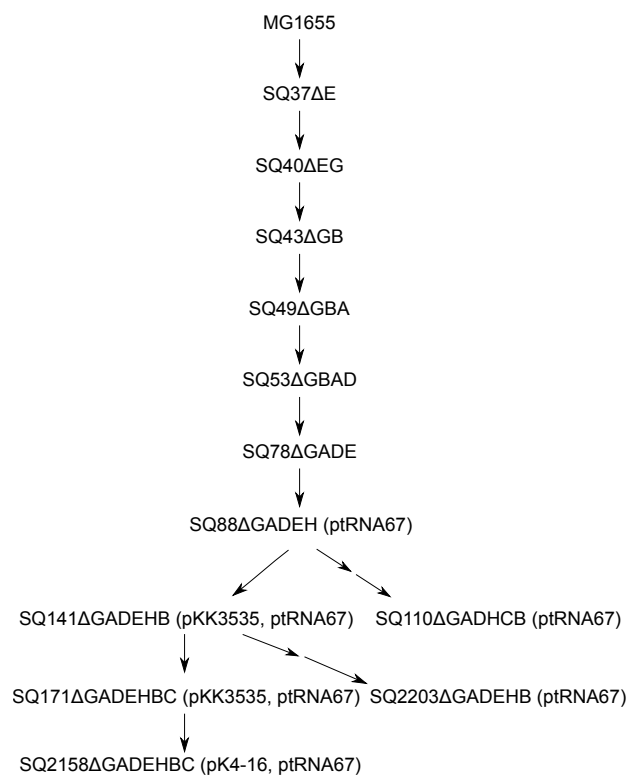


223429..229049

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ATTGCCAGAATCGAAAAATCCTCTGCATTTTACGCTCTTTTTTCTCAACAGTCTGAAGCCC
ATAATCACCTCAGTTAACGAAAATAGCATTAAAAGAGGCATATTATGGCTATCCCTGCATTT
GGTTTA

Figure S2 Schematic representation and sequence of FRT scar and regions flanking the *rrn* deletions. The FRT scar sequence is denoted as underlined text. The coordinates of the deleted regions are provided above the sequence data. Coordinates are based on the sequence of *E. coli* MG1655 (NC000913; modification date 19-Mar-2014).

A



B

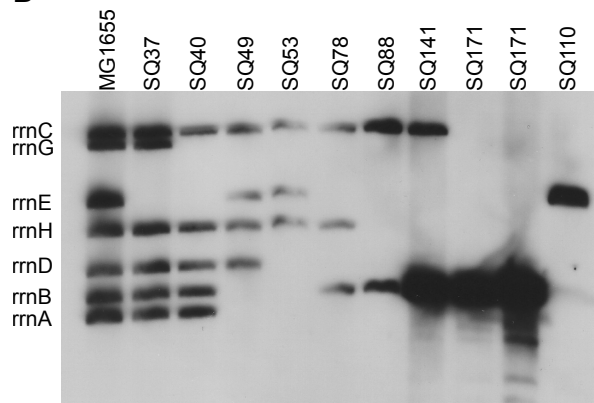


Figure S3 Knockout strategy of *rrn* operons in *E. coli* MG1655. (A) Order of ribosomal deletions to generate an *E. coli* strain with no chromosomal rRNA operons. (B) Confirmation of the *rrn* deletions by Southern blot using a 600 bp *rrs* probe.

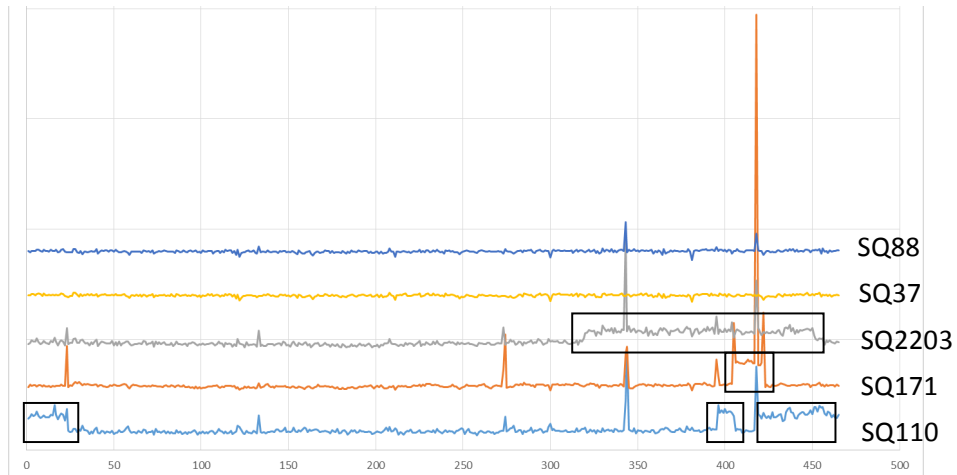


Figure S4 Relative sequence read coverage of indicated strains mapped against *E. coli* MG1655 (GenBank reference NC000913). The plot represents the average read density over 10kb windows normalized to the average genome coverage. Regions of duplication are represented by ~2x relative coverage and indicated as boxed areas on the plot. 'Spikes' in the plots represent resident plasmids, containing regions with homology to the genomic DNA, incl. tRNA and rRNA regions.

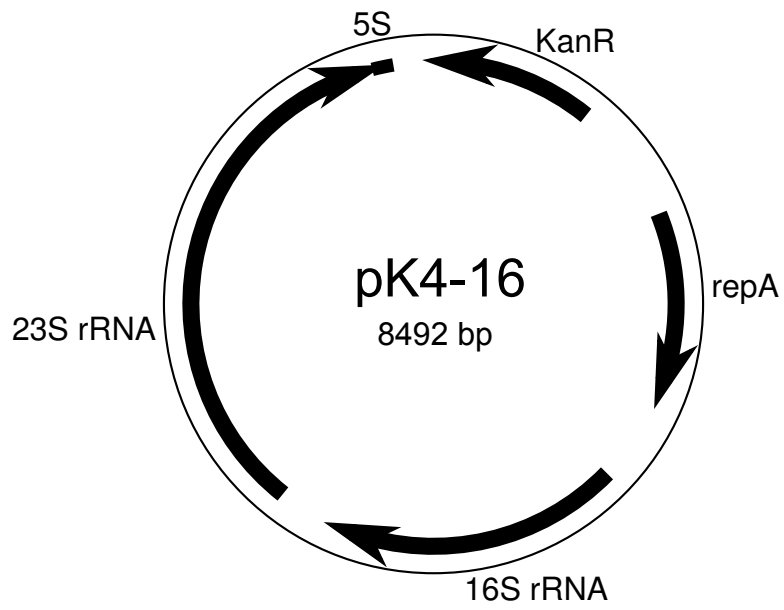


Figure S5 Schematic diagram of pK4-16 (*rrnB* plasmid with pSC101 ori)

File S1
References Supporting Information

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