

AcrS Is a Potential Repressor of *acrA* Expression in *Escherichia coli* and Its Deletion Confers Increased Kanamycin Resistance in *E. coli* BW25113

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SUPPLEMENTAL MATERIAL

Reagent	Quantity
LB Broth	85 ml
PEG-3350	10 g
DMSO	5 ml
1 M MgCl ₂	2 ml

S. TABLE 1 Recipe for 100 ml TSS (autoclave after preparation).

Strain	MIC (µg/ml) by Kanamycin Starting Concentrations			
	56 µg/ml	50 µg/ml	45 µg/ml	30 µg/ml
BW25113 (Wild-type)	28	25	22.5	15
JW3232-1 (<i>ΔacrS</i>)	28	25	22.5	30
JW0452-3 (<i>ΔacrA</i>)	14	12.5	22.5	15

S. TABLE 2 First MIC assay results - serial dilution kanamycin MIC assay using *ΔacrA*, *ΔacrS*, and BW25113.

Gene		Primer Sequences (5' to 3')	Product Size (bp)	Reference
<i>acrA</i>	Forward	TTGAAATTACGCTTCAGGAT	189	Viveiros et al. (17)
	Reverse	CTTAGCCCTAACAGGATGTG		
<i>GAPDH</i> (positive control)	Forward	GCAAACCTGACTGGTATGGCG	70	Designed by Group 2β
	Reverse	AGACGAACGGTCAGGTCAAC		

S. TABLE 3 Primer sequences used for RT-qPCR to measure *acrA* expression levels.