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SUPPORTING INFECTIOUS DISEASE RESEARCH

Antimicrobial Resistance Panel 11: *Escherichia coli* Resistance-Nodulation-Division (RND) Efflux Pumps Mutants

Catalog No. NR-55650

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

NR-55650 consists of a three-member panel of *Escherichia coli* (*E. coli*), strain BW25113 derivatives in which genes encoding Resistance-Nodulation-Division (RND) family efflux pumps were deleted. Deletions were accomplished via recombineering using DNA fragments containing a kanamycin marker *(aph)* flanked by homologous sequences of the targeted genes. This panel of gene deletions is ideal for investigations involving the roles of RND family efflux pumps and lipopolysaccharide biosynthesis as potential antibiotic targets.

NR-51923 was created by the sequential deletion of genes *acrB*, *acrD*, *acrF*, *emrB*, *emrY*, *entS*, *macB*, *mdtBC* and *mdtF* via recombineering using DNA fragments containing a kanamycin marker (*aph*) flanked by homologous sequences of the targeted genes. The kanamycin marker was subsequently removed by FLP recombinase.¹

NR-51883 was created by replacing the *acrAB* locus in NR-51923 with the kanamycin resistance cassette (Km^R).^{1,2}

NR-51862 was generated by the serial passage of NR-51883 in the presence of argyrin B, an inhibitor of acyltransferase LpxA, which is the first enzyme in the lipopolysaccharide biosynthesis pathway. The resulting substitution mutation in LpxA (Q73L) exhibits reduced susceptibility to Argyrin B.²

Table 1: Mutant Strains

Item No.	Strain	Description
NR-51923	<i>E. coli</i> NB27079- CDY0099	ΔacrB, ΔacrD, ΔacrF, ΔemrB, ΔemrY, ΔentS, ΔmacB, ΔmdtBC, ΔmdtF
NR-51883	<i>E. coli</i> , NB27079- CDY0154	ΔacrAB::Km ^R , ΔacrD, ΔacrF, ΔemrB, ΔemrY, ΔentS, ΔmacB, ΔmdtBC, ΔmdtF
NR-51862	<i>E. coli</i> , NB27079- TUP0093	ΔacrAB::Km ^R , ΔacrD, ΔacrF, ΔemrB, ΔemrY, ΔentS, ΔmacB, ΔmdtBC, ΔmdtF, LpxA(Q73L)

Material Provided:

Each panel contains one vial of each of the bacterial strains listed in Table 1. Each vial contains approximately 0.5 mL of bacterial culture in Tryptic Soy broth supplemented with 10% glycerol.

<u>Note</u>: If homogeneity is required for your intended use, please purify prior to initiating work.

Packaging/Storage:

Each isolate was packaged aseptically in cryovials. The product is provided frozen and should be stored at -60°C or colder immediately upon arrival. For long-term storage, the vapor phase of a liquid nitrogen freezer is recommended. Freeze-thaw cycles should be avoided.

Growth Conditions:

Media:

Tryptic Soy broth or equivalent Tryptic Soy agar or equivalent Incubation: Temperature: 37°C Atmosphere: Aerobic Propagation:

- 1. Keep vial frozen until ready for use, then thaw.
- 2. Transfer the entire thawed aliquot into a single tube of broth.
- 3. Use several drops of the suspension to inoculate an agar slant and/or plate.
- 4. Incubate the tube, slant and/or plate at 37°C for 1 day.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Antimicrobial Resistance Panel 11: *Escherichia coli* Resistance-Nodulation-Division (RND) Efflux Pumps Mutants, NR-55650."

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. <u>Biosafety in Microbiological and Biomedical Laboratories (BMBL)</u>. 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

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References:

- Jones, A. K., et al. "Determinants of Antibacterial Spectrum and Resistance Potential of the Elongation Factor G Inhibitor Argyrin B in Key Gram-Negative Pathogens." <u>Antimicrob. Agents and Chemother.</u> 61 (2017): e02400-16. PubMed: 28096160.
- Wooseok, H. et al. "Two Distinct Mechanisms of Inhibition of LpxA Acyltransferase Essential for Lipopolysaccharide Biosynthesis." <u>J. Am. Chem. Soc.</u> 142 (2020): 4445-4455. PubMed: 32064871.

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