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SUPPORTING INFECTIOUS DISEASE RESEARCH

Salmonella enterica subsp. enterica, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_043/044_Kan

Catalog No. NR-42832

For research use only. Not for human use.

Contributor:

Michael McClelland, Professor, Scientific Director, Vaccine Research Institute of San Diego, San Diego, California, USA

Manufacturer:

BEI Resources

Product Description:

Production in the 96-well format has increased risk of crosscontamination between adjacent wells. Individual clones should be purified (e.g. single colony isolation and purification using good microbiological practices) and sequence-verified prior to use. BEI Resources does not confirm or validate individual mutants provided by the contributor.

The Salmonella enterica (S. enterica) subsp. enterica, strain 14028s (serovar Typhimurium) targeted single-gene deletion (SGD) mutant library contains a total of 3,773 individual genes deleted simultaneously across two collections of mutants differentiated by kanamycin or chloramphenicol resistance.^{1,2} The kanamycin-resistant mutant collection contains 3517 mutants distributed among 11 96-well plates, in which a single gene is replaced by a cassette conferring the kanamycin resistance gene, and includes 9 double mutants that contain both kanamycin and chloramphenicol cassettes. Deletions were confirmed by the depositor.^{1,2}

Genes were targeted for deletion by primers designed to preserve the first and last 30 bases of each deleted gene.² Gene replacement followed a modified Lambda-Red technique, with an added T7 RNA polymerase promoter positioned in plasmid <u>pCLF3</u> to generate a gene-specific transcript from the *Salmonella* genome directly downstream of each mutant.²⁻⁴ Detailed information about each mutant is shown in Table 1.

Note: The strain designation on the plate, strain CDC 6516-60, is incorrect. The correct strain designation is strain 14028s. *S. enterica* subsp. *enterica*, strain 14028s was originally known as strain 14028. A variant of the original strain with a rough colony morphology was designated 14028r and the original smooth strain was renamed 14028s. Strain 14028 is a descendent of strain CDC 6516-60 which was isolated from pools of hearts and livers of 4week-old chickens.⁵ The complete genome of *S. enterica* subsp. *enterica*, strain 14028s (GenBank: <u>CP001363.1</u>) and plasmid (GenBank: <u>CP001362.1</u>) sequences are available.

Material Provided:

Each inoculated well of the 96-well plate contains approximately 50 μL of culture in Luria Bertani (LB) broth containing 60 $\mu g/mL$ kanamycin supplemented with 10% glycerol.

Packaging/Storage:

NR-42832 was packaged aseptically in a 96-well plate. The product is provided frozen and should be stored at -80°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:

<u>Media</u>: LB broth or agar containing 60 µg/mL kanamycin <u>Incubation</u>: Temperature: 37°C Atmosphere: Aerobic <u>Propagation</u>: 1. Scrape top of frozen well with a pipette tip :

- 1. Scrape top of frozen well with a pipette tip and streak onto agar plate.
- 2. Incubate the plates at 37°C for 24 hours.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Salmonella enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_043/044_Kan, NR-42832."

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</u> <u>Microbiological and Biomedical Laboratories</u>. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

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References:

1. McClelland, M., Personal Communication.

- Porwollik, S., et al. "Defined Single-Gene and Multi-Gene Deletion Mutant Collections in *Salmonella enterica* sv Typhimurium." <u>PLoS One</u> 9 (2014): e99820. PubMed: 25007190.
- Santiviago, C. A., et al. "Analysis of Pools of Targeted Salmonella Deletion Mutants Identifies Novel Genes Affecting Fitness during Competitive Infection in Mice." <u>PLoS Pathog.</u> 5 (2009): e1000477. PubMed: 19578432.
- Datsenko, K. A. and B. L. Wanner. "One-step Inactivation of Chromosomal Genes in *Escherichia coli* K-13 Using PCR Products." <u>Proc. Natl. Acad. Sci. USA</u> 97 (2000): 6640-6645. PubMed: 10829079.
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Table 1: S. enterica subsp. enterica, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_043/044_Kan^{1,2}

Well Position	Gene Type	Gene Start	Gene End	Target Gene (Locus Tag)	Deleted Region Start	Deleted Region End	Gene Strand	Description
A02	CDS	1614130	1615077	STM14_1845	1614160	1615046	-	Putative inner membrane protein
A03	CDS	81260	81655	STM14_0081	81290	81624	-	DNA-binding transcriptional activator CaiF
A04	CDS	1361381	1361743	STM14_1534	1361411	1361712	-	Putative cytoplasmic protein
A05	CDS	954165	955295	STM14_1033	954195	955264	-	23S rRNA methyluridine methyltransferase
A06	CDS	4531194	4531760	STM14_5144	4531137	4531729	-	Cytochrome c-type protein NrfB
A07	CDS	3305762	3307063	STM14_3781	3305792	3307032	+	Putative oxidoreductase
A08	CDS	3315674	3317146	STM14_3796	3315704	3317115	-	Putative D-mannonate oxidoreductase
A09	CDS	1367693	1368211	STM14_1552	1367723	1368180	+	Putative cytoplasmic protein
A10	CDS	2028058	2028561	STM14_2335	2028088	2028530	+	Purine-binding chemotaxis protein
A11	CDS	4524322	4525011	STM14_5138	4524352	4524980	-	Putative inner membrane protein
A12	CDS	1147851	1149317	STM14_1251	1147881	1149286	-	4-hydroxyphenylacetate catabolism
B01	CDS	446934	450074	STM14_0467	446964	450043	+	Exonuclease subunit SbcC
B02	CDS	855309	856994	STM14_0916	855339	856963	-	
B03	CDS	1896632	1898425	STM14_2162	1896662	1898394	-	Hydrogenase 1 large subunit
B04	CDS	3327038	3328741	STM14_3811	3327068	3328710	+	Hydrogenase 2 large subunit
B06	CDS	2240053	2241489	STM14_2600	2240083	2241464	+	Mannose-1-phosphate guanylyltransferase
B06	CDS	4427535	4428224	STM14_5037	4427565	4428193	+	Peptidase E
B07	CDS	402582	403835	STM14_0416	402612	403804	-	Putative inner membrane protein
B08	CDS	458766	459368	STM14_0476	458796	459337	+	Putative thiol-alkyl hydroperoxide reductase
B09	CDS	2602140	2602520	STM14_2996	2602170	2602489	-	Hypothetical protein
B10	CDS	4298615	4299658	STM14_4900	4298645	4299627	-	Putative sugar transport protein
B11	CDS	1208513	1209160	STM14_1334	1208543	1209081	+	Glutaredoxin 2
B12	CDS	2160636	2161241	STM14_2519	2160666	2161210	+	Cobalt-precorrin-6Y C(5)-methyltransferase
C01	CDS	2410707	2411198	STM14_2790	2410737	2411167	+	Ferredoxin-type protein
C02	CDS	751868	753172	STM14_0804	751898	753141	+	Citrate-proton symporter
C03	CDS	3658261	3658662	STM14_4201	3658291	3658631	+	Putative inner membrane protein
C04	CDS	1723236	1724579	STM14_1964	1723266	1724548	+	Putative cytoplasmic protein
C05	CDS	682902	683798	STM14_0720	682932	683767	+	Triphosphoribosyl-dephospho-CoA synthase
C06	CDS	1899758		STM14_2165	1899788	1900131	-	Hydrogenase-1 operon protein HyaE
C08	CDS	2149951	2150694	STM14_2505	2149981	2150663	+	Cobalamin synthase
C09	CDS	2583946	2584830	STM14_2974	2583976	2584799	+	DNA-binding transcriptional activator XapR
C10	CDS	3537695	3538963	STM14_4048	3537725	3538932	+	Putative cation transporter
C11	CDS	621022	622347	STM14_0658	621052	622316	-	Pyridine nucleotide-disulfide oxidoreductase

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Product Information Sheet for NR-42832

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H01 CDS 165840 167042 STM14_0170 165870 167011 + Type IV pilin biogenesis protein H02 CDS 1220809 1221507 STM14_1351 1220839 1221476 - Flagellar basal body L-ring protein H03 CDS 3421604 3423043 STM14_3921 3421634 3422904 + Putative transport protein H04 CDS 1121444 1122049 STM14_1217 1121474 1122018 - Putative DNA transformation protein H05 CDS 3252887 3253465 STM14_3713 3252917 3253434 - Putative inner membrane protein H06 CDS 371424 371747 STM14_0383 371478 371716 - Putative cytoplasmic protein H07 CDS 4249339 4250142 STM14_4839 4249369 4250111 - Putative glycerol-3-phosphate regulon repressor H08 CDS 3149905 3151323 STM14_3591 3149935 3151292 - L-fuculokinase </th <th>Well Position</th> <th>Gene Type</th> <th>Gene Start</th> <th>Gene End</th> <th>Target Gene (Locus Tag)</th> <th>Deleted Region Start</th> <th>Deleted Region End</th> <th>Gene Strand</th> <th>Description</th>	Well Position	Gene Type	Gene Start	Gene End	Target Gene (Locus Tag)	Deleted Region Start	Deleted Region End	Gene Strand	Description
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¹All information in this table was provided by the depositor at the time of deposition.

²Construction of each listed mutant has been confirmed either by PCR or by an array indicating a functional T7 promoter in the correct location and orientation. Mutants that did not produce such a signal on the array, or did not yield the expected mutant product during PCR, are not listed.
³Of the targeted genes, 22 CDSs and 22 sRNA were annotated in strain LT2 but not annotated in strain 14028s.

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