Product Information Sheet for NR-42859

Salmonella enterica subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_BLUES

Catalog No. NR-42859

For research use only. Not for human use.

Contributor:
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Manufacturer:
BEI Resources

Product Description:
Production in the 96-well format has increased risk of cross-contamination 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 3,517 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. The chloramphenicol-resistant mutant collection contains 3,376 mutants distributed among eleven 96-well plates. In these mutants, a single gene is replaced by a cassette conferring the chloramphenicol resistance gene, and includes 4 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 pCLF3 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 4-week-old chickens.[5] The complete genome of *S. enterica* subsp. *enterica*, strain 14028s (GenBank: CP001363.1) and plasmid (GenBank: CP001362.1) sequences are available.

Plate orientation and viability were confirmed for NR-42859.

Material Provided:
Each inoculated well of rows A, B,C and D of the 96-well plate contains approximately 50 µL of culture in Luria Bertani (LB) broth containing 60 µg/mL kanamycin supplemented with 10% glycerol. Each inoculated well of rows E, F and H of the 96-well plate contains approximately 50 µL of culture in Luria Bertani (LB) broth containing 60 µg/mL kanamycin supplemented with 10% glycerol.

Packaging/Storage:
NR-42859 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:
Media:
Note: NR-42859 contains both chloramphenicol-resistant and kanamycin-resistant mutants. Please refer to Table 1 for the appropriate antibiotic to use for each mutant in plate SGD_BLUES.
LB broth or agar containing 60 µg/mL kanamycin
LB broth or agar containing 20 µg/mL chloramphenicol
Incubation:
Temperature: 37°C
Atmosphere: Aerobic
Propagation:
1. Scrape top of frozen well with a pipette tip and streak onto agar plate.
2. Incubate the plates at 37°C for 1 day.

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_BLUES, NR-42859."

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

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References:

1. McClelland, M., Personal Communication.
2. Porwollik, S., et al. "Defined Single-Gene and Multi-Gene Deletion Mutant Collections in *Salmonella enterica* sv Typhimurium." PLoS One 9 (2014): e99820. PubMed: 25007190.
3. Santiviago, C. A., et al. "Analysis of Pools of Targeted *Salmonella* Deletion Mutants Identifies Novel Genes Affecting Fitness during Competitive Infection in Mice." PLoS Pathog. 5 (2009): e1000477. PubMed: 19578432.
4. Datsenko, K. A. and B. L. Wanner. "One-Step Inactivation of Chromosomal Genes in *Escherichia coli* K-13 Using PCR Products." Proc. Natl. Acad. Sci. USA 97 (2000): 6640-6645. PubMed: 10829079.
5. Jarvik, T., et al. "Short-Term Signatures of Evolutionary Change in the *Salmonella enterica* Serovar Typhimurium 14028 Genome." J. Bacteriol. 192 (2010): 560-567. PubMed: 19897643.

Support Provided by NIAID

Table 1: *S. enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_BLUES[1,2]

Well Position	Antibiotic-Resistance[3]	Gene Type	Gene Start	Gene End	Target Gene (Locus Tag)	Deleted Region Start	Deleted Region End	Gene Strand	Description
A02	KAN	CDS	2833262	2833837	STM14_3234	2833292	2833805	+	RNA polymerase sigma factor RpoE
A04	KAN	CDS	2001478	2002089	STM14_2304	2001508	2002057	+	Holliday junction DNA helicase motor protein
A06	KAN	CDS	3223564	3224460	STM14_3676	3223594	3224428	+	Site-specific tyrosine recombinase XerD
A08	KAN	CDS	3462431	3464566	STM14_3964	3462461	3464564	+	Polynucleotide phosphorylase/polyadenylase
B01	KAN	CDS	4825102	4825539	STM14_5475	4825132	4825507	-	DNA polymerase III subunit psi
B02	KAN	CDS	4621926	4623224	STM14_5248	4621956	4623192	-	Adenylosuccinate synthetase
B05	KAN	CDS	2596138	2596395	STM14_2989	2596168	2596363	-	Phosphohistidinoprotein-hexose phosphotransferase component of PTS system (Hpr)
B10	KAN	CDS	478109	478351	STM14_0502	478139	478319	+	Exodeoxyribonuclease VII small subunit
C01	KAN	CDS	250848	253520	STM14_0254	250878	253488	+	PII uridylyl-transferase
C04	KAN	CDS	13595	14734	STM14_0014	13625	14702	-	Chaperone protein DnaJ
D01	KAN	CDS	3172521	3175892	STM14_3611	3172551	3175860	+	Exonuclease V subunit gamma
D05	KAN	CDS	4324461	4326893	STM14_4929	4324491	4326861	-	Bifunctional aspartate kinase II/homoserine dehydrogenase II
E01	CM	CDS	2001478	2002089	STM14_2304	2001508	2002057	+	Holliday junction DNA helicase motor protein
E02	CM	CDS	1064144	1064518	STM14_1150	1064174	1064486	-	Regulatory protein
E03	CM	CDS	2833262	2833837	STM14_3234	2833292	2833805	+	RNA polymerase sigma factor RpoE
E04	CM	CDS	2995089	2996150	STM14_3417	2995119	2996118	+	Recombinase A
E05	CM	CDS	3223564	3224460	STM14_3676	3223594	3224428	+	Site-specific tyrosine recombinase XerD
F01	CM	CDS	3462431	3464566	STM14_3964	3462461	3464564	+	Polynucleotide phosphorylase/polyadenylase
F02	CM	CDS	4825484	4825930	STM14_5476	4825457	4825898	-	Ribosomal-protein-alanine N-acetyltransferase
H05	CM	CDS	967396	968397	STM14_1049	967426	968365	+	L-threonine aldolase
H05	CM	CDS	439949	440758	STM14_0456	439979	440726	+	Pyrroline-5-carboxylate reductase
H06	CM	CDS	434236	435330	STM14_0448	434266	435298	+	D-alanyl-alanine synthetase A
H06	CM	CDS	2117454	2117798	STM14_2460	2117484	2117766	+	Lysis protein (holin)
H08	CM	CDS	759655	760230	STM14_0814	759718	760198	-	Replication initiation regulator SeqA
H08	CM	CDS	690887	692272	STM14_0729	690917	692240	+	C4-dicarboxylate transporter DcuC

[1]All information in this table was provided by the depositor at the time of deposition.
[2]Construction 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.
[3]Antibiotic-resistance: KAN = kanamycin-resistant mutant; CM = chloramphenicol-resistant mutant

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