

***Salmonella enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_037/038_Kan**

Catalog No. NR-42829

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 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 [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-42829.

Material Provided:

Each inoculated well 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-42829 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:

LB broth or agar containing 60 µg/mL kanamycin

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_037/038_Kan, NR-42829."

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.

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Table 1: *S. enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_037/038_Kan^{1,2}

Well Position	Gene Type	Gene Start	Gene End	Target Gene (Locus Tag)	Deleted Region Start	Deleted Region End	Gene Strand	Description
A03	CDS	3099630	3100280	STM14_3543	3099660	3100249	+	Putative cytoplasmic protein
A06	CDS	2615612	2616664	STM14_3012	2615642	2616633	+	Transcriptional regulator EutR
B03	CDS	4344662	4345540	STM14_4947	4344692	4345509	-	Pyruvate formate lyase II activase
B04	CDS	1899172	1899768	STM14_2164	1899202	1899737	-	Hydrogenase 1 maturation protease
B05	CDS	1746016	1746465	STM14_1987	1746046	1746434	+	Putative inner membrane protein
B08	CDS	3867710	3868183	STM14_4422	3867740	3868152	+	Putative oxidoreductase
B09	CDS	3177171	3177641	STM14_3615	3177201	3177610	+	Hypothetical protein
B11	CDS	2459625	2460524	STM14_2838	2459655	2460493	-	Putative cytoplasmic protein
C03	CDS	1882801	1883190	STM14_2144	1882831	1883159	+	Putative transcriptional regulator
C08	CDS	3536146	3536763	STM14_4046	3536176	3536732	+	L(+)-tartrate dehydratase subunit beta
D02	CDS	3736028	3736444	STM14_4278	3736058	3736413	-	Hypothetical protein
D03	CDS (LT2) ³				467879	468144		
D04	CDS	171573	172523	STM14_0177	171603	172492	+	Putative cytoplasmic protein
D05	CDS	480053	480850	STM14_0504	480083	480819	+	2-aminoethylphosphonate transporter
D06	CDS	3100262	3101008	STM14_3544	3100292	3100977	+	Putative cytoplasmic protein
D08	CDS	3520409	3521899	STM14_4029	3520439	3521868	+	Putative sialic acid transporter
D09	CDS	3148029	3149804	STM14_3590	3148059	3149773	-	L-fucose isomerase
D10	CDS	2177773	2179167	STM14_2540	2177803	2179136	-	CoA-dependent propionaldehyde dehydrogenase
D11	CDS	2617219	2617878	STM14_3014			+	Putative carboxysome structural protein
D12	CDS	4726178	4726990	STM14_5366	4726208	4726959	-	Hydroxylase
E01	CDS	2179179	2180291	STM14_2541	2179209	2180260	-	Propanol dehydrogenase
E04	CDS	3307225	3308709	STM14_3782	3307255	3308678	-	Putative NAD-dependent aldehyde dehydrogenase
E07	CDS	2562053	2563363	STM14_2949	2562083	2563215	+	Phosphoglycerate transport regulatory protein precursor
E11	CDS	2398255	2398902	STM14_2775	2398285	2398871	-	Transcriptional regulator NarP
F02	CDS	3416751	3417737	STM14_3915	3416781	3417706	-	Putative glutathione S-transferase
F03	CDS	91173	92459	STM14_0092	91203	92428	-	Putative oxidoreductase FixC
F05	CDS (LT2) ³				3270328	3270431		
F06	CDS	4642598	4642873	STM14_5271	4642628	4642842	+	Putative outer membrane protein
F08	CDS	2618803	2620164	STM14_3016	2618833	2620133	+	Ethanolamine ammonia-lyase heavy chain

Product Information Sheet for NR-42829

Well Position	Gene Type	Gene Start	Gene End	Target Gene (Locus Tag)	Deleted Region Start	Deleted Region End	Gene Strand	Description
F10	CDS	1715896	1716528	STM14_1956	1715926	1716497	-	Ribulose-phosphate 3-epimerase
F11	CDS	4340213	4340539	STM14_4942	4340243	4340508	-	
G03	CDS	4631196	4632359	STM14_5258	4631226	4632328	-	Putative glutathionylspermidine synthase
G04	CDS	602509	603054	STM14_0630	602539	603023	+	Putative membrane-bound metal-dependent hydrolase
G06	CDS	3879591	3881546	STM14_4436	3879621	3881515	-	Putative cytoplasmic protein
G09	CDS	4685247	4687178	STM14_5321	4686264	4687153	+	Putative carbohydrate kinase
G12	CDS	2969966	2971300	STM14_3384	2969996	2971269	+	Putative regulatory protein
H02	CDS	3640506	3641315	STM14_4186	3640536	3641284	-	Nitrite transporter NirC
H03	CDS	2080354	2081169	STM14_2407	2080384	2081138	-	Mannosyl-3-phosphoglycerate phosphatase
H04	CDS	4330362	4331918	STM14_4933	4330392	4331887	-	Putative 5'-nucleotidase/2',3'-cyclic phosphodiesterase
H05	CDS	4697485	4697787	STM14_5333	4697515	4697756	-	Putative cytoplasmic protein
H06	CDS	762560	763879	STM14_0817	762590	763848	+	Putrescine transporter
H08	CDS	2969044	2969394	STM14_3381	2969074	2969363	+	Hypothetical protein
H10	CDS	1099932	1100558	STM14_1195	1099962	1100527	+	Hypothetical protein
H11	CDS	2936612	2937727	STM14_3344	2936642	2937696	-	Putative glycosyl transferase
H12	CDS	4401933	4403414	STM14_5014	4402047	4403383	-	Sensor protein ZraS

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.