

***Salmonella enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_065/066_Kan**

Catalog No. NR-42843

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. 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.

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-42843 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 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_065/066_Kan, NR-42843."

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/bmb15/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_065/066_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	4611198	4612745	STM14_5237	4611228	4612714	-	Hypothetical protein
A03	CDS	4589814	4590185	STM14_5209	4589850	4590154	-	Putative outer membrane lipoprotein
A04	CDS	4591372	4592400	STM14_5213	4591402	4592369	+	Putative aminomutase
A05	CDS	4600129	4601631	STM14_5226	4600159	4601600	-	Putative amino-acid transport protein
A06	CDS	4602064	4605390	STM14_5228	4602094	4605335	+	Hypothetical protein
A07	CDS	4610060	4611199	STM14_5236	4610090	4611183	+	Putative FeS protein
A08	CDS	4626423	4627154	STM14_5251	4626453	4627123	-	23S rRNA (guanosine-2'-O-)-methyltransferase
A09	CDS	4634111	4634431	STM14_5260	4634141	4634400	+	Putative inner membrane protein
A10	CDS	4635776	4636531	STM14_5263	4635806	4636500	+	Transcriptional repressor UlaR
A11	CDS	4636636	4637700	STM14_5264	4636666	4637669	+	Putative L-ascorbate 6-phosphate lactonase
A12	CDS	4694012	4694563	STM14_5328	4694042	4694532	+	Hypothetical protein
B01	CDS	4725750	4726166	STM14_5365	4725780	4726135	-	Hypothetical protein
B02	CDS	4715866	4716252	STM14_5351	4715896	4716221	+	Putative translation initiation inhibitor
B04	CDS	4727819	4728322	STM14_5369	4727849	4728291	+	Putative acetyltransferase
B06	CDS	4783517	4784788	STM14_5424	4783547	4784757	+	Putative inner membrane protein
B07	CDS	4784875	4786116	STM14_5425	4784905	4786085	+	Putative transport protein
B08	CDS	4800100	4800303	STM14_5444	4800130	4800272	+	Putative cytoplasmic protein
B09	CDS	4800398	4802548	STM14_5445	4800428	4802517	+	Putative carbon starvation protein
B10	CDS	4825952	4826629	STM14_5477	4825979	4826598	-	Nucleotidase
B11	CDS	4849992	4851659	STM14_5502	4850022	4851607	+	Putative ABC transporter ATP-binding protein
B12	CDS	4829743	4830816	STM14_5482	4829773	4830785	-	Putative phosphoesterase
C01	CDS	527508	527978	STM14_0555	527538	527947	+	Putative inner membrane protein
C03	CDS	922656	923576	STM14_0993	922686	923545	-	Putative ABC transporter periplasmic binding protein
C08	CDS	1282422	1283087	STM14_1415	1282452	1283056	+	Putative ribosomal large subunit pseudouridine synthase
C09	CDS	1756108	1756542	STM14_1997	1756138	1756511	-	Putative universal stress protein
C12	CDS	1591284	1591625	STM14_1815	1591314	1591594	+	Hypothetical protein
D01	CDS	1576425	1577120	STM14_1800	1576455	1577089	-	Putative dithiobiotin synthetase
D02	CDS	1574052	1574951	STM14_1798	1574082	1574920	-	Putative transcriptional regulator
D03	CDS	1572679	1573932	STM14_1797	1572709	1573901	+	Putative transport protein
D04	CDS	1467394	1467810	STM14_1668	1467424	1467779	-	Cysteine desulfuration protein SufE
D05	CDS	1413133	1413669	STM14_1607	1413163	1413638	-	Putative regulatory protein

Well Position	Gene Type	Gene Start	Gene End	Target Gene (Locus Tag)	Deleted Region Start	Deleted Region End	Gene Strand	Description
D06	CDS	1412316	1412984	STM14_1606	1412346	1412953	+	2-deoxyglucose-6-phosphatase
D07	CDS	1929920	1931830	STM14_2203	1929950	1931799	+	Putative DNA helicase
D08	CDS	1937906	1939465	STM14_2210	1937936	1939434	+	Putative inner membrane protein
D09	CDS	2318191	2318778	STM14_2675	2318221	2318747	+	Putative transport protein
D10	CDS	2318910	2319506	STM14_2677	2318940	2319475	-	Hypothetical protein
D11	CDS	2322277	2323215	STM14_2681	2322307	2323184	+	tRNA-dihydrouridine synthase C
D12	CDS	2329475	2329873	STM14_2688	2329505	2329842	-	Hypothetical protein
E02	CDS	3203592	3203864	STM14_3651	3203622	3203833	+	Putative cytoplasmic protein
E03	CDS	2412315	2413958	STM14_2793	2412345	2413927	+	Multidrug transporter membrane component/ATP-binding component
E04	CDS	2418861	2421530	STM14_2800	2418891	2421499	-	Phosphotransfer intermediate protein in two-component regulatory system with RcsBC
E05	CDS	2988803	2989228	STM14_3404	2988833	2989197	+	Putative inner membrane protein
E07	CDS	3135354	3136136	STM14_3573	3135384	3136105	+	tRNA pseudouridine synthase C
E08	CDS	3137691	3138539	STM14_3577	3137721	3138508	-	7-cyano-7-deazaguanine reductase
E09	CDS	3199467	3200645	STM14_3646	3199497	3200614	+	Acetyl-CoA acetyltransferase
E10	CDS	3227153	3227812	STM14_3682	3227183	3227781	+	Putative hemolysin
E11	CDS	3293586	3294293	STM14_3764	3293616	3294262	-	Putative inner membrane protein
E12	CDS	3277224	3277787	STM14_3740	3277254	3277756	-	Hypothetical protein
F02	CDS	3334525	3335019	STM14_3818	3334555	3334988	+	Hypothetical protein
F03	CDS	3341296	3342213	STM14_3829	3341326	3342182	+	Putative transcriptional regulator
F04	CDS	3342388	3343551	STM14_3831	3342418	3343520	-	Putative alcohol dehydrogenase
F05	CDS	3343657	3344484	STM14_3832	3343687	3344453	-	2,5-diketo-D-gluconate reductase A
F06	CDS	3364134	3364715	STM14_3855	3364164	3364684	+	Esterase YqiA
F07	CDS	3365567	3365989	STM14_3857	3365597	3365958	+	Hypothetical protein
F08	CDS	3365986	3366618	STM14_3858	3366016	3366587	+	ADP-ribose pyrophosphatase NudF
F10	CDS	3415359	3415757	STM14_3912	3415389	3415726	-	Putative inner membrane protein
F11	CDS	3416198	3416683	STM14_3914	3416228	3416652	-	Hypothetical protein
F12	CDS	3445584	3446447	STM14_3945	3445614	3446416	+	Putative methyltransferase
G01	CDS	3448511	3448906	STM14_3947	3448541	3448875	-	Hypothetical protein
G02	CDS	3448928	3449518	STM14_3948	3448958	3449487	-	DnaA initiator-associating protein DiaA
G03	CDS	3449528	3450103	STM14_3949	3449558	3450072	-	Hypothetical protein
G04	CDS	3450170	3450838	STM14_3950	3450200	3450774	+	Putative nucleoside-diphosphate-sugar epimerase
G05	CDS	3490104	3490397	STM14_3995	3490134	3490366	+	Putative transcriptional regulator
G06	CDS	3490842	3491477	STM14_3998	3490872	3491446	+	Putative transport protein
G07	CDS	3491496	3492047	STM14_3999	3491526	3492016	+	Putative transport protein
G08	CDS	3492052	3492834	STM14_4000	3492082	3492803	+	Putative transport protein
G09	CDS	3492842	3493654	STM14_4001	3492872	3493623	+	Putative ABC transporter ATP-binding protein YrbF
G10	CDS	3493867	3494844	STM14_4002	3493897	3494813	-	Putative calcium/sodium:proton antiporter
G11	CDS	3494858	3495844	STM14_4003	3494888	3495813	-	D-arabinose 5-phosphate isomerase
G12	CDS	3586340	3586894	STM14_4102	3586370	3586863	-	Putative ferripyochelin-binding protein
H02	CDS	3588524	3589066	STM14_4106	3588554	3589035	+	Putative DNA topoisomerase
H03	CDS	3663196	3663759	STM14_4206	3663226	3663761	+	ADP-ribose diphosphatase NudE
H05	CDS	3666279	3666947	STM14_4209	3666309	3666916	-	Putative hydrolase
H06	CDS	3666958	3667359	STM14_4210	3666988	3667328	-	Ribosome-associated heat shock protein Hsp15
H08	CDS	4649199	4649861	STM14_5283	4649229	4649830	+	Cell morphogenesis/cell wall metabolism regulator
H09	CDS	4649964	4650929	STM14_5284	4649994	4650898	+	Putative cationic amino acid transporter
H10	CDS	4651010	4651858	STM14_5285	4651040	4651827	+	Putative reductase
H11	CDS	4651946	4652332	STM14_5286	4651976	4652301	-	Putative transcriptional regulator
H12	CDS	4655331	4655888	STM14_5290	4655361	4655857	+	Putative transcriptional regulator

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.