

***Salmonella enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_166/167_Kan**

Catalog No. NR-42854

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.

Plate orientation and viability were confirmed for NR-42854.

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-42854 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_166/167_Kan, NR-42854.”

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_166/167_Kan^{1,2}

Well Position	Gene Type	Gene Start	Gene End	Target Gene (Locus Tag)	Deleted Region Start	Deleted Region End	Gene Strand	Description
A01	CDS	495278	495892	STM14_0521	495308	495860	+	Cytochrome o ubiquinol oxidase subunit III
A02	CDS	494949	495278	STM14_0520	494979	495246	+	Cytochrome o ubiquinol oxidase subunit IV
A03	CDS	494047	494937	STM14_0519	494077	494905	+	Protoheme IX farnesyltransferase
A04	CDS	486920	487510	STM14_0512	486950	487478	+	Hypothetical protein
A05	CDS	478561	480009	STM14_0503	478591	479977	-	Thiamine biosynthesis protein Thil
A07	CDS	456877	458694	STM14_0475	456907	458662	-	Maltodextrin glucosidase
A08	CDS	453951	455270	STM14_0473	453981	455238	-	Branched-chain amino acid transporter
A09	CDS	450071	451273	STM14_0468	450101	451241	+	Exonuclease subunit SbcD
A10	CDS	443622	444533	STM14_0463	443652	444501	+	Recombination associated protein
A12	CDS	434236	435330	STM14_0448	434266	435298	+	D-alanyl-alanine synthetase A
B03	CDS	535220	538582	STM14_0563	535190	538550	-	Potassium efflux protein KefA
B04	CDS	522974	523834	STM14_0548	523004	523934	+	Acyl-CoA thioesterase II
B05	CDS	521588	522874	STM14_0547	521618	522842	-	Ammonium transporter
B06	CDS	521218	521556	STM14_0546	521067	521581	-	Nitrogen regulatory protein P-II 2
B07	CDS	514925	515743	STM14_0541	514955	515711	-	Putative hydrolase
B10	CDS	502358	503656	STM14_0529	502388	503624	-	Trigger factor
C02	CDS	659758	660615	STM14_0695	659788	660583	-	2,3-dihydro-2,3-dihydroxybenzoate synthetase
C03	CDS	656949	658124	STM14_0693	656979	658092	-	Isochorismate synthase
C06	CDS	646156	650040	STM14_0686	646186	650008	-	Enterobactin synthase subunit F
C07	CDS	637722	638375	STM14_0674	637752	638343	+	Dihydropteridine reductase
C08	CDS	624893	626287	STM14_0663	624923	626255	-	Phenylalanine transporter
C10	CDS	597810	598319	STM14_0624	597840	598287	+	Phosphoribosylaminoimidazole carboxylase catalytic subunit
C11	CDS	549691	550995	STM14_0578	549676	550963	-	Inosine-guanosine kinase
C12	CDS	543904	544509	STM14_0571	543934	544477	-	Recombination protein RecR
D01	CDS	739436	741100	STM14_0792	739466	741068	+	Asparagine synthetase B
D02	CDS	734984	736408	STM14_0781	735014	736376	+	rRNA modification protein
D03	CDS	728506	729246	STM14_0772	728536	729214	+	Glutamate/aspartate transporter
D05	CDS	727107	727832	STM14_0770	727137	727800	+	Glutamate/aspartate transporter
D10	CDS	679840	680250	STM14_0717	679870	680218	+	Nucleoside diphosphate kinase regulator
E01	CDS	826795	827685	STM14_0887	826825	827653	-	Transcriptional regulator
E02	CDS	821643	822362	STM14_0881	821673	822330	-	Nucleoside/purine/pyrimidine transporter

Well Position	Gene Type	Gene Start	Gene End	Target Gene (Locus Tag)	Deleted Region Start	Deleted Region End	Gene Strand	Description
E03	CDS	820575	821618	STM14_0880	820605	821586	-	Quinolinate synthetase
E04	CDS	811610	812749	STM14_0862	811640	812717	-	Cytochrome d terminal oxidase polypeptide subunit II
E07	CDS	798872	799219	STM14_0852	798902	799187	-	Succinate dehydrogenase cytochrome b556 small membrane subunit
E08	CDS	798489	798878	STM14_0851	798519	798846	-	Succinate dehydrogenase cytochrome b556 large membrane subunit
E09	CDS	759655	760230	STM14_0814	759718	760198	-	Replication initiation regulator SeqA
E10	CDS	744628	745428	STM14_0796	744658	745396	+	Glucosamine-6-phosphate deaminase
E11	CDS	743414	744568	STM14_0795	743444	744536	+	N-acetylglucosamine-6-phosphate deacetylase
E12	CDS	741390	742142	STM14_0793	741420	742110	+	UMP phosphatase
F01	CDS	888210	890354	STM14_0953	888240	890322	-	ATP-dependent DNA helicase DinG
F02	CDS	872505	872990	STM14_0932	872535	872958	-	Molybdenum cofactor biosynthesis protein C
F03	CDS	870979	871968	STM14_0930	870976	871936	-	Molybdenum cofactor biosynthesis protein A
F04					866911	867004		
F05	CDS	864774	866795	STM14_0926	864804	866763	-	Excinuclease ABC subunit B
F06	CDS	861548	862705	STM14_0921	861578	862673	-	8-amino-7-oxononanoate synthase
F07	CDS	859135	860424	STM14_0919	859165	860494	+	Adenosylmethionine--8-amino-7-oxononanoate transaminase
F08	CDS	847480	848538	STM14_0909	847510	848506	-	Molybdate transporter ATP-binding protein
F09	CDS	846788	847477	STM14_0908	846818	847445	-	Molybdate ABC transporter permease protein
F10	CDS	843239	844714	STM14_0904	843269	844682	+	Putative molybdenum transport ATP-binding protein ModF
F11	CDS	836703	837455	STM14_0896	836733	837423	+	Phosphoglyceromutase
F12	CDS	830210	830455	STM14_0890	830240	830423	-	Oxaloacetate decarboxylase subunit gamma
G01	CDS	962095	962823	STM14_1043	962125	962791	+	Arginine transporter ATP-binding subunit
G02	CDS	960623	961339	STM14_1041	960653	961307	+	Arginine transporter permease subunit ArtQ
G03	CDS	959955	960623	STM14_1040	959985	960591	+	Arginine transporter permease subunit ArtM
G04	CDS	950637	951770	STM14_1029	950667	951738	-	Putrescine transporter ATP-binding subunit
G05	CDS	946833	947555	STM14_1025	946863	947523	-	Nitroreductase A
G06	CDS	939547	940779	STM14_1016	939577	940747	-	Multidrug translocase
G07	CDS	916763	918004	STM14_0988	916793	917972	+	Molybdopterin biosynthesis protein MoeA
G08	CDS	916014	916763	STM14_0987	916044	916731	+	Molybdopterin biosynthesis protein MoeB
G10	CDS	912399	914831	STM14_0984	912429	914799	+	Putative pyruvate formate lyase
G12	CDS	895794	896516	STM14_0962	895824	896484	+	Glutamine ABC transporter ATP-binding protein
H01	CDS	1013455	1015737	STM14_1099	1013485	1015705	+	Pyruvate formate lyase I
H02	CDS	1011047	1011844	STM14_1096	1011077	1011893	+	Pyruvate formate lyase-activating enzyme 1
H03	CDS	1003680	1006124	STM14_1089	1003710	1006092	-	Anaerobic dimethyl sulfoxide reductase subunit A
H04	CDS	993557	994525	STM14_1080	993587	994595	+	Thioredoxin reductase
H05	CDS	981027	983303	STM14_1064	981057	983271	-	ATP-dependent Clp protease ATP-binding subunit
H06	CDS	980676	980996	STM14_1063	980706	980964	-	ATP-dependent Clp protease adaptor protein ClpS
H07	CDS	980131	980352	STM14_1062	980043	980289	+	Stress response protein
H08	CDS	971292	972944	STM14_1052	971322	972912	+	Hydroxylamine reductase
H09	CDS	970312	971283	STM14_1051	970342	971251	+	HCP oxidoreductase, NADH-dependent
H10	CDS	968436	970154	STM14_1050	968466	970122	+	Pyruvate dehydrogenase
H11	CDS	967396	968397	STM14_1049	967426	968365	+	L-threonine aldolase
H12	CDS	2117454	2117798	STM14_2460	2117484	2117766	+	Lysis protein (holin)

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.