

***Salmonella enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate 001/002\_Kan**

**Catalog No. NR-29399**

**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.<sup>1,2</sup> 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.<sup>1,2</sup> The parent strain *S. enterica* subsp. *enterica*, strain 14028s is available from BEI Resources as NR-12154.

Genes were targeted for deletion by primers designed to preserve the first and last 30 bases of each deleted gene.<sup>2</sup> Gene replacement followed a modified Lambda-Red technique, with an added T7 RNA polymerase promoter positioned in plasmid [pCLF4](#) to generate a gene-specific transcript from the *Salmonella* genome directly downstream of each mutant.<sup>2-4</sup> Detailed information about each mutant is shown in Table 1.

**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-29399 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 001/002\_Kan, NR-29399."

**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](http://www.cdc.gov/biosafety/publications/bmb15/index.htm).

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**References:**

1. Andrews-Polymeris, H. and M. McClelland, 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.

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**Table 1: *S. enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate 001/002\_Kan<sup>1,2</sup>**

Well Position	Deleted Region of Chromosome	Deletion Start	Deletion End	Locus Tag	14028S Gene Start	14028S Gene End	14028S Gene Strand	Description
A01	chr_14028S	117558	117917	STM14_0119	117528	117947	-	Putative cytoplasmic protein
A02	chr_14028S	610980	611552	STM14_0641	610950	611582	+	Transcriptional regulator FimZ
A03	chr_14028S	1134824	1135639	STM14_1233	1134794	1135669	+	secreted effector protein
A04	chr_14028S	1734666	1735253	STM14_1978	1734636	1735283	-	Putative ABC transporter permease component
A05	chr_14028S	2780583	2781992	STM14_3164	2780529	2782022	-	Leucine-rich repeat-containing protein
A06	chr_14028S	3295108	3296373	STM14_3767	3295078	3296403	+	Putative acetyl-CoA hydrolase
A07	chr_14028S	48402	50381	STM14_0050	48372	50411	+	Putative glycosyl hydrolase
A08	chr_14028S	311924	314503	STM14_0319	311894	314533	-	Putative chaperone ATPase
A09	chr_14028S	347689	348450	STM14_0356	347659	348480	-	Putative xylanase/chitin deacetylase
A10	chr_14028S	2277429	2278253	STM14_2631	2277399	2278283	-	Putative cytoplasmic protein
A12	chr_14028S	3731328	3732029	STM14_4273	3731298	3732059	-	Putative cytoplasmic protein
B01	chr_14028S	315229	316662	STM14_0321	315199	316692	-	Putative cytoplasmic protein
B02	chr_14028S	633964	634947	STM14_0669	633934	634977	+	Putative inner membrane protein
B03	chr_14028S	1136605	1138230	STM14_1237	1136575	1138260	+	Secreted effector protein
B04	chr_14028S	1800876	1802189	STM14_2050	1800846	1802219	+	Putative inner membrane protein
B05	chr_14028S	2921735	2923549	STM14_3328	2921705	2923579	-	Putative inner membrane protein
B07	chr_14028S	50481	51794	STM14_0051	50451	51824	+	Putative sodium galactoside symporter
B08	chr_14028S	319394	319819	STM14_0327	319364	319849	-	Putative cytoplasmic protein
B09	chr_14028S	466031	466336	STM14_0484	466001	466366	-	Hypothetical protein
B10	chr_14028S	2278313	2278969	STM14_2632	2278283	2278999	-	Putative inner membrane protein
B11	chr_14028S <sup>3</sup>	3215653	3216399	STM14_3667	3215623	3216429	-	Putative inner membrane protein
B12	chr_14028S	3732143	3733054	STM14_4274	3732113	3733084	-	Putative inner membrane protein
C01	chr_14028S	318398	318823	STM14_0325	318368	318853	-	Putative cytoplasmic protein
C02	chr_14028S	784544	785635	STM14_0838	784514	785665	-	Putative UDP-galactopyranose mutase
C03	chr_14028S	1177835	1178935	STM14_1293	1177805	1178965	-	Putative inner membrane protein
C04	chr_14028S	1836277	1836399	STM14_2091	1836247	1836429	-	Putative cytoplasmic protein
C05	chr_14028S	2923869	2925092	STM14_3329	2923839	2925122	+	Putative inner membrane protein
C06	chr_14028S	3571855	3573894	STM14_4086	3571825	3573924	-	Putative signal transduction protein
C07	chr_14028S	58976	60118	STM14_0060	58946	60148	-	Putative nitrite reductase
C08	chr_14028S <sup>4,5</sup>	318206	320422	STM14_0328	319916	320452	-	Putative outer membrane lipoprotein
C09	chr_14028S	466422	467054	STM14_0485	466392	467084	-	Putative regulatory protein
C10	chr_14028S	2279040	2281091	STM14_2633	2279010	2281121	-	Putative inner membrane protein
C11	chr_14028S	3438763	3439650	STM14_3936	3438733	3439680	+	Putative sugar kinase
C12	chr_14028S	3733111	3734085	STM14_4275	3733081	3734115	-	Putative phosphotriesterase
D01	chr_14028S	337810	338490	STM14_0341	337687	338520	-	Putative RHS-like protein
D02	chr_14028S	789441	791159	STM14_0843	789411	791189	-	Putative glycosyltransferase
D03	chr_14028S	1336088	1337152	STM14_1486	1336058	1337182	-	Putative cytoplasmic protein
D04	chr_14028S	1836549	1836992	STM14_2092	1836519	1837022	-	Putative cytoplasmic protein

Well Position	Deleted Region of Chromosome	Deletion Start	Deletion End	Locus Tag	14028S Gene Start	14028S Gene End	14028S Gene Strand	Description
D05	chr_14028S	3005336	3006715	STM14_3431	3005306	3006745	-	Anaerobic nitric oxide reductase flavorubredoxin
D06	chr_14028S	3793199	3793741	STM14_4338	3793169	3793771	+	Putative transcriptional regulator
D07	chr_14028S	305395	306390	STM14_0313	305365	306420	+	Putative cytoplasmic protein
D08	chr_14028S	320486	321769	STM14_0329	320456	321799	-	Putative cytoplasmic protein
D09	chr_14028S	617161	618027	STM14_0651	617131	618057	+	Putative glycosyltransferase
D10	chr_14028S	2378409	2378969	STM14_2751	2378379	2378999	+	Putative inner membrane protein
D11	chr_14028S	3439721	3440494	STM14_3937	3439691	3440524	+	AGA operon transcriptional repressor
D12	chr_14028S	3852395	3852802	STM14_4404	3852365	3852832	+	Putative acetyltransferase
E01	chr_14028S	386294	386998	STM14_0398	386264	387028	-	Putative inner membrane protein
E02	chr_14028S	826864	827655	STM14_0887	826795	827685	-	Transcriptional regulator
E03	chr_14028S	1338194	1338922	STM14_1490	1338164	1338952	+	Putative envelope lipoprotein
E04	chr_14028S	1893787	1894992	STM14_2159	1893757	1895022	+	Putative cytoplasmic protein
E05	chr_14028S	3048124	3049293	STM14_3483	3048094	3049323	+	Translocation machinery component
E06	chr_14028S	3871929	3872342	STM14_4428	3871899	3872372	-	2,3-diketo-L-gulonate TRAP transporter small permease protein YiaM
E08	chr_14028S	321826	323070	STM14_0330	321796	323100	-	Hypothetical protein
E09	chr_14028S	712521	712751	STM14_0757	712491	712781	-	Putative hydrolase
E10	chr_14028S	2473267	2474988	STM14_2853	2473237	2475018	+	Hypothetical protein
E11	chr_14028S	3440888	3441682	STM14_3939	3440858	3441712	-	Tagatose-bisphosphate aldolase
E12	chr_14028S	3894950	3899275	STM14_4450	3894920	3899305	-	Putative inner membrane protein
F01	chr_14028S	391111	391575	STM14_0404	391081	391605	-	Putative outer membrane protein
F02	chr_14028S	828994	830130	STM14_0889	828913	830160	-	Putative cation transporter
F03	chr_14028S	1497063	1497419	STM14_1698	1497033	1497449	-	Secreted effector protein
F04	chr_14028S <sup>6</sup>	2260113	2263043	STM14_2620	2260083	2263073	-	Putative diguanylate cyclase/phosphodiesterase
F05	chr_14028S	3069038	3069634	STM14_3509	3069008	3069664	-	Serine/threonine-specific protein phosphatase 2
F06	chr_14028S	4492058	4493386	STM14_5118	4492028	4493416	-	Putative methyl-accepting chemotaxis protein
F07	chr_14028S	309352	309786	STM14_0316	309322	309816	+	Putative cytoplasmic protein
F08	chr_14028S	343544	344197	STM14_0353	343514	344227	-	Putative fimbrial assembly chaparone
F09	chr_14028S	714050	714943	STM14_0759	714020	714973	-	2-keto-3-deoxygluconate permease
F10	chr_14028S	2539450	2540046	STM14_2926	2539420	2540076	+	Putative inner membrane protein
F12	chr_14028S	4080850	4082547	STM14_4655	4080820	4082577	-	Putative dipeptide/oligopeptide/nickel ABC-type transport system periplasmic component
G01	chr_14028S <sup>7</sup>	583129	583323	STM14_0611	583099	583353	-	Putative cytoplasmic protein
G02	chr_14028S <sup>8</sup>	927263	927718	STM14_1001	927233	927748	-	Putative cytoplasmic protein
G03	chr_14028S	1679511	1680083	STM14_1912	1679481	1680113	+	Putative cytoplasmic protein
G04	chr_14028S	2395503	2396234	STM14_2771	2395473	2396264	+	Putative tail fiber protein of phage
G05	chr_14028S	3206535	3207551	STM14_3655	3206505	3207581	+	Putative outer membrane protein
G07	chr_14028S	309843	310607	STM14_0317	309813	310637	+	Putative cytoplasmic protein
G08	chr_14028S	344281	346731	STM14_0354	344251	346761	-	Putative fimbrial usher
G09	chr_14028S	715071	716939	STM14_0760	715041	716969	-	Putative sigma-54 dependent transcriptional regulator
G10	chr_14028S	2770058	2770915	STM14_3153	2770028	2770945	+	2-dehydropanoate 2-reductase
G12	chr_14028S	4164658	4164987	STM14_4745	4164628	4165017	+	Putative inner membrane protein
H01	chr_14028S <sup>9</sup>	583290	584543	STM14_0612	583260	584573	-	Allantoin permease
H02	chr_14028S	1101898	1102029	STM14_1198	1101868	1102059	+	MsgA-like protein
H03	chr_14028S	1729628	1729969	STM14_1970	1729598	1729999	-	Putative dipicolinate reductase
H05	chr_14028S	3212304	3212876	STM14_3660	3212274	3212906	+	Hypothetical protein
H07	chr_14028S	310654	311496	STM14_0318	310624	311526	+	Putative cytoplasmic protein
H08	chr_14028S	346813	347223	STM14_0355	346783	347253	-	Putative fimbrial subunit
H09	chr_14028S	1640412	1641818	STM14_1877	1640382	1641848	+	Putative coiled-coil protein
H10	chr_14028S	2874894	2875439	STM14_3285	2874864	2875469	-	Putative cytoplasmic protein
H12	chr_14028S	4253263	4253631	STM14_4844	4253233	4253661	+	Putative cytoplasmic protein

<sup>1</sup>All information in this table was provided the depositor at the time of deposition.

<sup>2</sup>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.

<sup>3</sup>Deleted region also overlaps STM14\_3666 (7.3%)

<sup>4</sup>Alternative deleted region: 319991 - 320422

<sup>5</sup>Deleted region also overlaps STM14\_0325 - STM14\_0327 (100%)

<sup>6</sup>Deleted region also overlaps STM14\_2621 (0.3%)

<sup>7</sup>Deleted region also overlaps STM14\_0612 (4.9%)

<sup>8</sup>Deleted region also overlaps STM14\_1002 (6.2%)

<sup>9</sup>Deleted region also overlaps STM14\_0611 (25.1%)