

***Salmonella enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate 009/010\_Kan**

**Catalog No. NR-29403**

**For research use only. Not for use in humans.**

**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 3,517 mutants distributed among eleven 96-well plates. In these mutants, 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>

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>2,3,4</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-29403 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 009/010\_Kan, NR-29403."

**Biosafety Level: 1**

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 (BMBL). Current Edition. Washington, DC: U.S. Government Printing Office.

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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-12 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 009/010\_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
A02	chr_14028S	95513	95743	STM14_0098	95483	95773	+	Putative secreted protein
A03	chr_14028S	236998	237777	STM14_0240	236968	237807	-	Putative outer membrane protein
A04	chr_14028S	339553	339930	STM14_0345	339523	339960	-	Putative cytoplasmic protein
A05	chr_14028S	374911	375477	STM14_0386	374881	375507	-	Putative 3-isopropylmalate isomerase
A07	chr_14028S	571836	572606	STM14_0600	571806	572636	-	Putative ABC-type transport system ATPase component
A08	chr_14028S	632911	633837	STM14_0668	632881	633867	+	Putative phosphosugar isomerase
A09	chr_14028S	781964	782035	STM14_0834	781934	782065	-	Putative cytoplasmic protein
A10	chr_14028S <sup>3</sup>	1273050	1273448	STM14_1406	1273020	1273478	-	Transposase
A11	chr_14028S <sup>4</sup>	1073599	1073991	STM14_1168	1073569	1074021	-	Lysozyme
A12	chr_14028S	1181307	1182353	STM14_1296	1181277	1182383	-	Putative dehydrogenase
B01	chr_14028S	32575	32964	STM14_0037	32545	32994	+	Putative transcriptional regulator
B02	chr_14028S	96013	96147	STM14_0099	95983	96177	-	Putative inner membrane protein
B03	chr_14028S	248685	249927	STM14_0252	248655	249957	-	
B04	chr_14028S	340816	341007	STM14_0348	340729	341037	-	Putative cytoplasmic protein
B05	chr_14028S	387855	389345	STM14_0400	387801	389375	-	Hypothetical protein
B06	chr_14028S	413386	415434	STM14_0426	413356	415464	-	Ferrioxamine receptor
B07	chr_14028S	572703	573659	STM14_0601	572673	573689	-	Putative ABC-type transport system ATPase component
B08	chr_14028S	650320	651396	STM14_0687	650290	651426	-	Ferric enterobactin transport protein FepE
B09	chr_14028S	783181	783456	STM14_0836	783151	783486	-	Putative inner membrane protein
B11	chr_14028S	1083594	1083932	STM14_1179	1083564	1083962	-	Minor tail protein
B12	chr_14028S	1190796	1191056	STM14_1312	1190766	1191086	-	Putative periplasmic protein
C01	chr_14028S	34406	34786	STM14_0039	34376	34816	+	Putative transcriptional regulator
C02	chr_14028S	96701	98530	STM14_0100	96671	98560	-	Putative sulfatase
C03	chr_14028S	307453	309276	STM14_0315	307423	309306	+	Putative cytoplasmic protein
C04	chr_14028S	341169	341357					
C05	chr_14028S	391779	392360	STM14_0405	391749	392390	-	Putative response regulator
C06	chr_14028S	416399	416614	STM14_0428	416369	416644	-	Hypothetical protein
C07	chr_14028S	581806	582993	STM14_0610	581776	583023	-	Putative permease
C08	chr_14028S	676704	677003	STM14_0711	676674	677033	-	Molybdopterin-containing oxidoreductase iron-sulfur subunit
C09	chr_14028S	787863	788573	STM14_0841	787833	788603	-	Putative ABC transporter permease protein
C11	chr_14028S	1083989	1084258	STM14_1180	1083959	1084288	-	Minor tail protein
C12	chr_14028S	1203624	1204136	STM14_1326	1203594	1204166	+	Putative inner membrane protein
D01	chr_14028S	35369	37027	STM14_0041	35339	37057	-	Putative arylsulfatase
D02	chr_14028S	117083	117283					
D03	chr_14028S	314663	315145	STM14_0320	314633	315175	-	Putative cytoplasmic protein
D04	chr_14028S	341893	342114					

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	392431	392595	STM14_0406	392401	392625	-	Putative inner membrane protein
D06	chr_14028S	428910	429473	STM14_0439	428880	429503	-	Putative DNA-binding transcriptional regulator
D07	chr_14028S	593758	594957	STM14_0620	593728	594987	-	Putative cytoplasmic protein
D08	chr_14028S	706303	707337	STM14_0752	706273	707367	-	Threonine-phosphate decarboxylase
D09	chr_14028S	834787	835806	STM14_0894	834757	835836	-	Putative ABC transport protein
D10	chr_14028S	1011993	1012259	STM14_1097	1011963	1012289	+	Putative cytoplasmic protein
D11	chr_14028S	1087813	1088250	STM14_1183	1087783	1088280	-	Attachment/invasion protein
D12	chr_14028S	1219233	1219928	STM14_1349	1219203	1219958	-	Flagellar basal body rod protein FlgF
E02	chr_14028S	189250	190032	STM14_0191	189220	190062	+	Putative restriction endonuclease
E03	chr_14028S	317073	317294	STM14_0323	316878	317324	-	Putative cytoplasmic protein
E04	chr_14028S	350608	351267	STM14_0360	350578	351297	+	SapA-like protein
E05	chr_14028S	399283	401511	STM14_0412	399253	401541	-	Putative cation transport ATPase
E06	chr_14028S	435438	436091	STM14_0449	435408	436121	-	Putative inner membrane protein
E07	chr_14028S <sup>5</sup>	614317	614445	STM14_0647	614287	614475	+	Integrase
E08	chr_14028S	721566	722978	STM14_0766	721536	723008	-	Putative molecular chaperone
E09	chr_14028S	842190	843038	STM14_0902	842160	843068	+	Putative inner membrane protein
E10	chr_14028S	1052247	1053401	STM14_1136	1052217	1053431	-	Diaminopropionate ammonia-lyase
E11	chr_14028S	1091166	1093554	STM14_1188	1091136	1094486	-	Host specificity protein J
F01	chr_14028S	44342	45997	STM14_0047	44312	46027	-	Putative arylsulfatase
F02	chr_14028S	190935	191828	STM14_0193	190905	191858	-	2-keto-3-deoxygluconate permease
F03	chr_14028S	323135	323848	STM14_0331	323105	323878	-	Putative inner membrane protein
F04	chr_14028S	359789	360861	STM14_0370	359759	360891	-	
F05	chr_14028S	401583	401987	STM14_0413	401553	402017	-	Putative transcriptional regulator
F06	chr_14028S	438852	439904	STM14_0455	438822	439934	-	Diguanylate cyclase AdrA
F07	chr_14028S <sup>6</sup>	614474	614792	STM14_0648	614444	615111	+	
F08	chr_14028S	724815	725993	STM14_0768	724785	726023	+	Putative cytoplasmic protein
F09	chr_14028S	876669	877169	STM14_0940	876639	877199	-	Putative inner membrane protein
F10	chr_14028S	1056617	1057849	STM14_1140	1056587	1057879	+	Integrase
F11	chr_14028S	1154706	1155584	STM14_1259	1154676	1155614	-	Putative periplasmic protein
G01	chr_14028S	66684	66863	STM14_0066	66654	66893	+	Oxaloacetate decarboxylase subunit gamma
G02	chr_14028S	191885	193096	STM14_0194	191855	193126	-	Putative inner membrane protein
G03	chr_14028S	324576	328385	STM14_0333	324546	328415	-	Putative inner membrane protein
G04	chr_14028S	372124	373296	STM14_0384	371989	373326	-	Putative permease
G05	chr_14028S	409271	409312	STM14_0421	409241	409342	-	Putative cytoplasmic protein
G06	chr_14028S	442582	443199	STM14_0461	442552	443229	-	Hypothetical protein
G07	chr_14028S	623422	624537	STM14_0662	623392	624567	+	Putative DNA repair ATPase
G08	chr_14028S	762040	762465	STM14_0816	762010	762495	+	Putative cytoplasmic protein
G09	chr_14028S	955608	956891	STM14_1035	955578	956921	-	Ascorbate-specific PTS system enzyme IIC
G10	chr_14028S	1066379	1066666	STM14_1153	1066349	1066696	-	Hypothetical protein
G11	chr_14028S	1161435	1161881	STM14_1269	1161405	1161911	-	Suppression of copper sensitivity protein
G12	chr_14028S	1339471	1339650	STM14_1492	1339441	1339680	+	Macrophage survival protein
H02	chr_14028S	193173	194069	STM14_0195	193116	194099	-	4-hydroxythreonine-4-phosphate dehydrogenase 2
H03	chr_14028S	338544	338930	STM14_0342	338514	338960	-	Putative cytoplasmic protein
H04	chr_14028S	373488	374849	STM14_0385	373458	374879	-	Isopropylmalate isomerase large subunit
H05	chr_14028S	411916	412080	STM14_0424	411886	412110	-	Putative cytoplasmic protein
H06	chr_14028S	525301	526791	STM14_0552	525271	526821	+	Hypothetical protein
H07	chr_14028S	627917	629827	STM14_0666	627887	629857	-	Outer membrane esterase
H08	chr_14028S	770814	772802	STM14_0824	770784	772832	+	Potassium-transporting ATPase subunit B
H09	chr_14028S	956981	957241	STM14_1036	956951	957271	-	Putative inner membrane protein
H10	chr_14028S	1071253	1071318	STM14_1164	1071223	1071348	-	Hypothetical protein
H11	chr_14028S	1173650	1174471	STM14_1285	1173620	1174501	+	Putative transcriptional regulator
H12	chr_14028S	1339901	1340362	STM14_1493	1339871	1340392	+	Putative envelope protein

<sup>1</sup>All information in this table was provided by 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>Alternative deleted regions: 1786329 - 1786727, 1926005 - 1926403, 2057170 - 2057568, 2630913 - 2631311, 3477946 - 3478344, 3649085 - 3649483, 80529 - 80927, 874654 - 875052, 983525 - 983923

<sup>4</sup>Alternative deleted regions: 2810993 - 2811385

<sup>5</sup>Deleted region also overlaps STM14\_0646 (15.6%) and STM14\_0648 (0.3%)

<sup>6</sup>Deleted region also overlaps STM14\_0647 (1.1%)