Salmonella enterica subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate 019/020_Kan

Catalog No. NR-29408

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 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.[1,2] 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.[2] 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.[2-4] 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-29408 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 019/020_Kan, NR-29408."

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. Andrews-Polymenis, 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.

Support Provided by NIAID

Table 1: *S. enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate 019/020_Kan[1,2]

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	350389	350544	STM14_0359	350359	350574	-	Putative cytoplasmic protein
A03	chr_14028S	755880	756746	STM14_0807	755850	756776	+	Putative transcriptional regulator
A04	chr_14028S[3]	1358196	1358984	STM14_1527	1358166	1359014	-	Putative transcriptional regulator
A05	chr_14028S	1486842	1489544	STM14_1687	1486812	1489574	+	Sensor kinase
A06	chr_14028S	1642137	1643084	STM14_1878	1642107	1643114	-	Putative transcriptional regulator
A07	chr_14028S	1767896	1768438	STM14_2011	1767866	1768468	+	Putative transcriptional regulator
A08	chr_14028S	2051093	2051755	STM14_2368	2051063	2051785	+	DNA-binding transcriptional activator SdiA
A10	chr_14028S	2954855	2955772	STM14_3361	2954825	2955802	-	Tricarboxylic transport
B01	chr_14028S	94873	95229	STM14_0096	94843	95259	-	Putative secreted protein
B02	chr_14028S	351658	352005	STM14_0362	351628	352035	+	VirG-like protein
B03	chr_14028S	756921	757313	STM14_0808	756891	757343	+	Ferric uptake regulator
B04	chr_14028S	1362188	1362769	STM14_1537	1362158	1362799	-	Putative nitric oxide reductase
B05	chr_14028S[4]	1519419	1519940	STM14_1729	1519389	1519970	+	Superoxide dismutase
B06	chr_14028S	1648199	1650667	STM14_1882	1648169	1650697	+	Putative glycosyl hydrolase
B07	chr_14028S	1768542	1769168	STM14_2012	1768512	1769198	-	Putative cytoplasmic protein
B09	chr_14028S	2699174	2700073	STM14_3084	2699144	2700103	+	Putative outer membrane protein
B10	chr_14028S	2956289	2957743	STM14_3363	2956259	2957773	-	Tricarboxylic transport
B11	chr_14028S	3332605	3334188	STM14_3817	3332575	3334218	+	Putative methyl-accepting chemotaxis protein
B12	chr_14028S	3942156	3942998	STM14_4499	3942126	3943028	+	Putative transcriptional regulator
C01	chr_14028S	116219	116779	STM14_0118	116189	116941	+	Putative secreted protein
C02	chr_14028S	406022	408934	STM14_0418	405989	408964	-	DNA restriction enzyme
C03	chr_14028S	1021023	1022246	STM14_1106	1020993	1022276	-	3-phosphoshikimate 1-carboxyvinyltransferase
C04	chr_14028S	1471746	1473029	STM14_1673	1471716	1473059	+	Putative amino acid permease
C05	chr_14028S	1526056	1526517	STM14_1738	1526026	1526547	-	Superoxide dismutase
C06	chr_14028S	1668421	1668939	STM14_1901	1668391	1668969	-	Putative transcriptional regulator
C07	chr_14028S	1771207	1773129	STM14_2016	1771177	1773159	+	Invasin-like protein
C08	chr_14028S	2189664	2190182	STM14_2554	2189634	2190212	+	Thiosulfate reductase electron transport protein
C09	chr_14028S	2700191	2702323	STM14_3085	2700161	2702353	+	Intimin-like protein
C10	chr_14028S	2986535	2986990	STM14_3402	2986505	2987020	+	S-ribosylhomocysteinase
C11	chr_14028S	3357598	3358404	STM14_3847	3357568	3358434	+	Putative regulatory protein
C12	chr_14028S[5]	3943132	3943953	STM14_4500	3943102	3943983	-	Putative Zn-dependent hydrolase
D01	chr_14028S	274917	276620	STM14_0275	274887	276650	-	Putative endochitinase
D02	chr_14028S[6]	440928	441323	STM14_0458	440898	441353	-	Hypothetical protein
D03	chr_14028S	1138793	1138843	STM14_1239	1138763	1138873	+	Putative cytoplasmic protein
D04	chr_14028S	1473111	1473944	STM14_1674	1473081	1473974	+	Putative proline iminopeptidase
D05	chr_14028S	1529886	1530266	STM14_1742	1529862	1530296	-	Transcriptional regulator SlyA
D06	chr_14028S	1686104	1686709	STM14_1921	1686074	1686739	+	Putative DNA-binding transcriptional regulator

Product Information Sheet for NR-29408

Well Position	Deleted Region of Chromosome	Deletion Start	Deletion End	Locus Tag	14028S Gene Start	14028S Gene End	14028S Gene Strand	Description
D07	chr_14028S	1841979	1842647	STM14_2100	1841949	1842800	+	Transport protein TonB
D08	chr_14028S	2431121	2431756	STM14_2809	2430902	2431786	-	Putative regulatory protein
D09	chr_14028S	2738026	2739009	STM14_3126	2737996	2739039	-	Anaerobic sulfide reductase
D10	chr_14028S	3032374	3033201	STM14_3465	3032344	3033231	+	Invasion regulatory protein
D11	chr_14028S	3399085	3400590	STM14_3893	3399055	3400620	+	Putative methyl-accepting chemotaxis protein
D12	chr_14028S	4040174	4042849	STM14_4618	4040144	4042879	-	Hybrid sensory histidine kinase TorS
E01	chr_14028S	329670	330131	STM14_0336	329640	330161	-	Putative cytoplasmic protein
E02	chr_14028S	452277	453512	STM14_0471	452247	453542	-	Phosphate regulon sensor protein
E03	chr_14028S[7]	1272843	1274828	STM14_1406	1273020	1273478	-	Transposase
E04	chr_14028S	1480439	1481131	STM14_1679	1480409	1481161	+	Tetrathionate reductase complex subunit B
E05	chr_14028S	1530374	1530781	STM14_1743	1530344	1530811	+	Putative outer membrane lipoprotein
E06	chr_14028S	1689778	1691037	STM14_1928	1689748	1691067	-	Putative virulence protein
E08	chr_14028S	2472225	2473166	STM14_2852	2472195	2473196	-	Putative chemotaxis signal transduction protein
E09	chr_14028S	2739073	2739831	STM14_3127	2739043	2739861	-	Anaerobic sulfite reductase subunit B
E10	chr_14028S	3038103	3038972	STM14_3474	3038073	3039002	-	Invasion protein regulatory protein
E11	chr_14028S	3668402	3670051	STM14_4212	3668372	3670081	+	Putative inner membrane protein
E12	chr_14028S[8]	4280153	4280713	STM14_4878	4280123	4280743	-	Superoxide dismutase
F01	chr_14028S	330588	332717	STM14_0338	330558	332747	-	Putative cytoplasmic protein
F02	chr_14028S	638870	639391	STM14_0676	638840	639421	+	Putative regulatory protein
F03	chr_14028S	1276130	1277533	STM14_1408	1276100	1277563	+	Sensor protein PhoQ
F04	chr_14028S[9]	1481355	1483073	STM14_1680	1481325	1483103	-	Sensory histidine kinase
F05	chr_14028S	1545541	1545696	STM14_1760	1545511	1545741	+	oriC-binding nucleoid-associated protein
F06	chr_14028S	1694080	1696164	STM14_1930	1694050	1696194	-	Putative virulence protein
F07	chr_14028S	2030632	2031501	STM14_2337	2030602	2031531	+	Flagellar motor protein MotB
F08	chr_14028S	2504731	2505624	STM14_2885	2504701	2505654	+	Putative transketolase
F09	chr_14028S	2739902	2740855	STM14_3128	2739872	2740885	-	Anaerobic sulfide reductase
F10	chr_14028S	3040123	3041724	STM14_3475	3040093	3041754	-	Invasion protein regulator
F11	chr_14028S	3672185	3673477	STM14_4216	3672155	3673507	+	Osmolarity sensor protein
F12	chr_14028S	4476185	4477384	STM14_5099	4476104	4477414	-	Conjugative transfer protein
G01	chr_14028S	332801	333187	STM14_0339	332771	333217	-	Putative cytoplasmic protein
G02	chr_14028S	692681	693193	STM14_0731	692651	693223	-	Palmitoyl transferase for Lipid A
G03	chr_14028S	1277593	1278207	STM14_1409	1277563	1278237	+	DNA-binding transcriptional regulator PhoP
G04	chr_14028S	1483108	1483668	STM14_1681	1483060	1483698	-	Response regulator
G05	chr_14028S	1626477	1627208	STM14_1860	1626447	1627238	-	Putative regulatory protein
G06	chr_14028S	1761284	1762378	STM14_2003	1761254	1762408	+	Putative methyl-accepting chemotaxis protein
G07	chr_14028S	2031558	2032328	STM14_2338	2031528	2032415	+	Flagellar motor protein MotA
G08	chr_14028S	2508965	2509924	STM14_2891	2508935	2509954	-	Putative transcriptional regulator
G09	chr_14028S	2933495	2934955	STM14_3338	2933465	2934985	+	Flagellin
G10	chr_14028S	3085761	3086693	STM14_3526	3085731	3086723	+	RNA polymerase sigma factor RpoS
G11	chr_14028S	3673534	3674193	STM14_4217	3673504	3674223	+	Osmolarity response regulator
G12	chr_14028S	4681876	4683159	STM14_5318	4681846	4683189	-	Lysosomal glucosyl ceramidase-like protein
H02	chr_14028S	754379	755722	STM14_0806	754349	755752	+	Tricarballylate dehydrogenase
H03	chr_14028S	1353995	1355068	STM14_1520	1353965	1355098	+	Putative inner membrane protein
H04	chr_14028S	1486173	1486751	STM14_1686	1486143	1486781	+	Transcriptional activator
H05	chr_14028S	1634200	1634628	STM14_1866	1634170	1634658	-	Putative transcriptional regulator
H07	chr_14028S	2049682	2050278	STM14_2365	2049652	2050308	+	Response regulator
H08	chr_14028S	2557637	2558515	STM14_2946	2557607	2558545	+	Outer membrane protease
H09	chr_14028S	2954026	2954640	STM14_3360	2953996	2954670	+	Regulatory protein
H10	chr_14028S	3126359	3129055	STM14_3566	3126329	3129085	-	Hybrid sensory histidine kinase BarA
H11	chr_14028S	3730365	3731252	STM14_4272	3730068	3731282	-	Putative transcriptional regulator

[1]All information in this table was provided by the depositor at the time of deposition.
[2]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.
[3]Deleted region also overlaps STM14_1528 (3.6%)
[4]Deleted region also overlaps STM14_1728 (12.1%)
[5]Deleted region also overlaps STM14_4501 (14.2%)
[6]Deleted region also overlaps STM14_0457 (9.3%)
[7]Deleted region also overlaps TM14_1405: (3.1%)
[8]Alternative deleted regions: 4280153 – 4304453
[9]Deleted region also overlaps STM14_1681 (2.2%)

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