

***Salmonella enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate 011/012_Kan**

Catalog No. NR-29404

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-29404 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 011/012_Kan, NR-29404.”

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/bmbI5/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 011/012_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
A01	chr_14028S	1341493	1341610	STM14_1498	1341463	1341640	-	
A03	chr_14028S	1440196	1441776	STM14_1640	1440166	1441806	+	Short chain acyl-CoA synthetase
A04	chr_14028S	1560873	1561171	STM14_1778	1560843	1561201	-	
A05	chr_14028S	1618035	1618862	STM14_1850	1618005	1618892	+	Putative dehydrogenase protein
A07	chr_14028S	1691102	1694023	STM14_1929	1691072	1694053	-	Putative virulence protein
A08	chr_14028S	1756622	1756900	STM14_1998	1756592	1756930	+	Putative membrane transporter of cations
A09	chr_14028S	1837652	1838470	STM14_2094	1837622	1838500	-	Putative catalase
A10	chr_14028S	1970942	1971796	STM14_2256	1970912	1971826	-	Integral membrane protein
A11	chr_14028S	2037567	2038064	STM14_2348	2037537	2038094	+	Putative intracellular protease/amidase
A12	chr_14028S	2144388	2144489					
B01	chr_14028S	1343547	1343662	STM14_1504	1343517	1343692	-	
B02	chr_14028S	1359626	1359715	STM14_1530	1359596	1359745	+	Putative cytoplasmic protein
B03	chr_14028S	1446709	1447800	STM14_1647	1446679	1447830	+	Putative acyl-CoA dehydrogenase
B04	chr_14028S	1579679	1580266	STM14_1803	1579649	1580296	+	Putative ABC transporter permease component
B05	chr_14028S	1620695	1621243	STM14_1854	1620665	1621273	+	Putative hydrogenase maturation protease
B07	chr_14028S	1694080	1696164	STM14_1930	1694050	1696194	-	Putative virulence protein
B09	chr_14028S	1852026	1852280	STM14_2111	1851996	1852310	-	Putative inner membrane protein
B10	chr_14028S	1973524	1973646	STM14_2262	1973494	1973676	+	
B11	chr_14028S	2050650	2050745	STM14_2366	2050620	2050775	-	Putative inner membrane protein
B12	chr_14028S	2144538	2144804	STM14_2496	2144508	2144834	+	Putative cytoplasmic protein
C01	chr_14028S	1344050	1344334	STM14_1505	1344020	1344364	+	Lysozyme inhibitor
C02	chr_14028S	1359858	1360343	STM14_1531	1359828	1360373	-	Chorismate mutase
C03	chr_14028S	1450426	1451232	STM14_1650	1450396	1451262	+	Quinate/shikimate dehydrogenase
C04	chr_14028S	1580336	1581178	STM14_1804	1580306	1581208	+	Putative ABC transporter periplasmic component
C05	chr_14028S ³	1621309	1621992	STM14_1855	1621279	1622022	+	Putative Ni/Fe hydrogenase 1 b-type cytochrome subunit
C07	chr_14028S	1696269	1696472	STM14_1931	1696239	1696502	+	Putative inner membrane protein
C09	chr_14028S	1864445	1865914	STM14_2127	1864415	1865944	+	TPR repeat-containing protein
C10	chr_14028S ⁴	1329656	1329796	STM14_1480	1329626	1329826	+	PagJ
C11	chr_14028S	2057809	2058954	STM14_2377	2057779	2058984	+	Lysine-N-methylase
C12	chr_14028S	2190257	2192473	STM14_2555	2190227	2192503	+	Thiosulfate reductase precursor
D01	chr_14028S	1345097	1345309	STM14_1508	1345067	1345339	-	Putative cytoplasmic protein
D02	chr_14028S	1375232	1376368	STM14_1561	1375202	1376398	-	Arylsulfatase regulator
D03	chr_14028S	1451359	1452534	STM14_1651	1451329	1452564	+	Putative transport protein
D04	chr_14028S	1593481	1594440	STM14_1819	1593451	1594470	-	Putative dehydrogenase
D05	chr_14028S	1628327	1629553	STM14_1862	1628297	1629583	+	Putative transport protein
D06	chr_14028S	1644752	1645894	STM14_1880	1644722	1645924	-	Putative aminotransferase

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	1700990	1701160	STM14_1937	1700960	1701190	+	Putative inner membrane protein
D08	chr_14028S	1775652	1776701					
D09	chr_14028S	1920285	1920398	STM14_2186	1920255	1920428	+	Putative cytoplasmic protein
D10	chr_14028S ^{5,6}	1328870	1329498	STM14_1479	1328944	1329528	-	Phage tail assembly-like protein
D12	chr_14028S	2212513	2213883	STM14_2576	2212483	2213913	+	Undecaprenol-phosphate galactosephosphotransferase/O-antigen transferase
E01	chr_14028S	1346327	1347310	STM14_1511	1346297	1347340	-	Putative cytoplasmic protein
E03	chr_14028S	1476361	1479363	STM14_1677	1476331	1479393	+	Tetrathionate reductase complex subunit A
E04	chr_14028S	1602384	1602851	STM14_1830	1602354	1602881	+	Competence damage-inducible protein A
E05	chr_14028S	1631416	1632597	STM14_1864	1631386	1632627	+	Putative multidrug efflux protein
E06	chr_14028S	1646067	1648082	STM14_1881	1646037	1648112	+	Putative glycosyl hydrolase
E07	chr_14028S	1701253	1701591	STM14_1938	1701223	1701621	+	Hypothetical protein
E08	chr_14028S	1776975	1777151	STM14_2020	1776945	1777199	+	Putative outer membrane lipoprotein
E09	chr_14028S	1936313	1937854	STM14_2209	1936181	1937884	-	Putative diguanylate cyclase/phosphodiesterase
E10	chr_14028S	2015953	2017764	STM14_2324	2015923	2017794	-	Putative penicillin-binding protein
E11	chr_14028S	2066937	2067263	STM14_2387	2066907	2067293	-	Putative 50S ribosomal protein
E12	chr_14028S	2214015	2215388	STM14_2577	2213985	2215418	+	Phosphomannomutase
F01	chr_14028S	1352532	1353269	STM14_1518	1352502	1353299	-	Putative ABC-type transport system ATPase component
F02	chr_14028S	1418226	1418480	STM14_1613	1418196	1418510	-	Putative inner membrane protein
F03	chr_14028S	1479416	1480378	STM14_1678	1479386	1480408	+	Tetrathionate reductase complex subunit C
F04	chr_14028S ⁷	1615554	1615820	STM14_1847	1615524	1615850	-	Putative inner membrane protein
F05	chr_14028S	1632647	1634095	STM14_1865	1632617	1634125	+	Hypothetical protein
F06	chr_14028S	1663831	1664595	STM14_1896	1663744	1664625	-	Hypothetical protein
F07	chr_14028S	1717365	1717742	STM14_1958	1717335	1717772	-	Cryptic aminoglycoside resistance protein
F08	chr_14028S	1791193	1791447	STM14_2036	1791163	1791477	+	Thiosulfate:cyanide sulfurtransferase (rhodanese)
F10	chr_14028S	2019646	2021664	STM14_2327	2019616	2021694	+	Flagellar biosynthesis protein FlhA
F11	chr_14028S	2081178	2082830	STM14_2408	2081148	2082860	+	Putative inner membrane protein
G01	chr_14028S	1353323	1353853	STM14_1519	1353293	1353883	-	Putative ABC-type transport system ATPase component
G02	chr_14028S	1419564	1419710	STM14_1615	1419534	1419740	-	Hypothetical protein
G03	chr_14028S	1520127	1520921	STM14_1730	1520097	1520951	+	Putative cell wall-associated hydrolase
G04	chr_14028S	1616422	1617480	STM14_1848	1616392	1617510	-	Putative outer membrane protein
G05	chr_14028S	1634920	1635900	STM14_1868	1634851	1635930	-	Putative S-adenosylmethionine/tRNA-ribosyltransferase-isomerase
G06	chr_14028S	1666412	1666732	STM14_1899	1666322	1666762	-	Putative cytoplasmic protein
G07	chr_14028S	1732647	1732931	STM14_1975	1732617	1732961	+	Putative inner membrane protein
G09	chr_14028S	1967231	1967440	STM14_2251	1967201	1967470	-	Putative cytoplasmic protein
G10	chr_14028S	2021717	2022808	STM14_2328	2021687	2022838	+	Flagellar biosynthesis protein FlhB
H02	chr_14028S	1420093	1421256	STM14_1616	1420063	1421286	+	O-antigen polymerase
H03	chr_14028S ⁸	1559683	1560756	STM14_1777	1559653	1560786	+	Outer membrane protein N precursor
H04	chr_14028S	1617681	1617962	STM14_1849	1617651	1617992	+	Putative hydrogenase
H05	chr_14028S	1636572	1636796	STM14_1870	1636542	1636826	+	Putative cytoplasmic protein
H06	chr_14028S	1682226	1682387	STM14_1917	1682196	1682417	+	Putative outer membrane lipoprotein
H07	chr_14028S	1735338	1736009	STM14_1979	1735308	1736039	-	Putative ABC-type polar amino acid transport system ATPase component
H08	chr_14028S	1807261	1809183	STM14_2058	1807231	1809213	-	RNase II stability modulator
H09	chr_14028S ⁹	1969270	1969578					
H10	chr_14028S	2034175	2034231	STM14_2343	2034145	2034261	-	Hypothetical protein
H11	chr_14028S	2140253	2141923	STM14_2492	2140223	2141953	-	Putative periplasmic protein

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.

³Deleted region also overlaps STM14_1856 (1.1%)

⁴Alternative deleted regions: 1974614 - 1974754, 2784599 - 2784739

⁵Alternative deleted region: 1974911 - 1975732

⁶Deleted region also overlaps STM14_1478 (3.2%)

⁷Deleted region also overlaps STM14_1846 (22.5%)

⁸Deleted region also overlaps STM14_1776 (2.6%)

⁹Alternative deleted region: 1969270 - 1979297