

***Salmonella enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate 007/008_Kan**

Catalog No. NR-29402

For research use only. Not for human use.

Contributor:

Helene Andrews-Polymeris, Associate Professor, Department of Microbial Pathogenesis and Immunology, College of Medicine, Texas A&M Health Science Center, Bryan, Texas, USA and Michael McClelland, Professor, Scientific Director, Vaccine Research Institute of San Diego, San Diego, California, USA

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-29402 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 007/008_Kan, NR-29402."

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 007/008_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	1454310	1454609	STM14_1655	1454280	1454639	+	Putative cytoplasmic protein
A02	chr_14028S	1730158	1730814	STM14_1971	1730128	1730844	+	Putative inner membrane protein
A03	chr_14028S	2002539	2003374	STM14_2307	2002455	2003404	-	
A04	chr_14028S	2290123	2291028	STM14_2645	2290093	2291058	-	Putative sugar kinase
A05	chr_14028S	2385596	2385979	STM14_2759	2385566	2386009	+	Putative cytoplasmic protein
A06	chr_14028S	2772309	2773175	STM14_3155	2772279	2773205	-	Putative transcriptional regulator
A07	chr_14028S	3026636	3027493	STM14_3458	3026606	3027523	-	Putative periplasmic binding protein
A08	chr_14028S	3311428	3312244	STM14_3788	3311398	3312274	+	
A10	chr_14028S	3997670	3998083	STM14_4563	3997640	3998113	+	Phosphotransferase system mannitol/fructose-specific IIA component
A11	chr_14028S	4439562	4440431	STM14_5055	4439532	4440461	+	Putative phage glycosyltransferase
A12	chr_14028S	4798786	4799028	STM14_5442	4798756	4799058	-	Putative cytoplasmic protein
B01	chr_14028S	1474063	1474740	STM14_1675	1474033	1474770	+	Putative cytoplasmic protein
B02	chr_14028S	1733898	1734599	STM14_1977	1733868	1734629	-	Putative periplasmic binding protein
B03	chr_14028S	2004061	2004600	STM14_2308	2004031	2004630	-	Hypothetical protein
B04	chr_14028S	2294415	2295362	STM14_2652	2294385	2295392	+	Putative outer membrane lipoprotein
B05	chr_14028S	2386417	2386884	STM14_2760	2386387	2386914	+	Putative phage tail fiber assembly protein
B06	chr_14028S	2785464	2786228	STM14_3169	2785434	2786258	+	Phage tail assembly-like protein
B08	chr_14028S	3318900	3319898	STM14_3799	3318870	3319928	+	Putative methyl-accepting chemotaxis protein
B09	chr_14028S	3840387	3841025	STM14_4386	3840357	3841055	+	Long polar fimbrial chaperone precursor
B10	chr_14028S	4005966	4007222	STM14_4573	4005936	4007252	+	Putative L-fucose permease
B11	chr_14028S	4575420	4575653	STM14_5191	4575390	4575683	-	Hypothetical protein
B12	chr_14028S ³	4819666	4820280	STM14_5464	4819687	4820310	-	DNA-binding transcriptional activator BglJ
C01	chr_14028S	1474812	1475978	STM14_1676	1474782	1476008	+	Putative regulatory protein
C02	chr_14028S	1736066	1736686	STM14_1980	1736036	1736716	-	Putative ABC-type transport system membrane component
C03	chr_14028S	2044024	2044290	STM14_2357	2043994	2044320	+	Putative glucose-6-phosphate dehydrogenase
C04	chr_14028S	2295438	2297867	STM14_2653	2295408	2297897	+	Putative outer membrane protein
C05	chr_14028S	2389407	2390678	STM14_2765	2389377	2390708	-	Hypothetical protein
C06	chr_14028S	2832610	2833200	STM14_3233	2832580	2833230	+	Anti-RNA polymerase sigma factor SigE
C07	chr_14028S	3176243	3176590	STM14_3613	3176213	3176620	+	Hypothetical protein
C08	chr_14028S	3414143	3414466	STM14_3909	3414113	3414496	-	Hypothetical protein
C09	chr_14028S	3841170	3841646	STM14_4387	3841140	3841676	+	Long polar fimbrial protein A precursor
C10	chr_14028S	4172895	4173215	STM14_4755	4172865	4173245	+	Putative inner membrane protein
C11	chr_14028S	4593517	4594044	STM14_5217	4593487	4594074	+	Putative regulatory protein
C12	chr_14028S	4841752	4842399	STM14_5493	4841722	4842429	+	Putative periplasmic chaperone protein
D01	chr_14028S	1484134	1485033	STM14_1683	1484104	1485063	+	Putative inner membrane protein
D02	chr_14028S	1736883	1738373	STM14_1981	1736853	1738403	-	Putative inner membrane protein

Well Position	Deleted Region of Chromosome	Deletion Start	Deletion End	Locus Tag	14028S Gene Start	14028S Gene End	14028S Gene Strand	Description
D03	chr_14028S	2062662	2062970	STM14_2382	2062632	2063000	-	Flagellar biosynthesis protein FlIT
D04	chr_14028S	2297941	2298564	STM14_2654	2297911	2298594	+	Putative periplasmic chaperone protein
D06	chr_14028S	2907656	2908414	STM14_3311	2907626	2908444	-	Putative cytoplasmic protein
D07	chr_14028S	3207608	3208291	STM14_3656	3207578	3208321	+	Putative fimbrial chaperone
D08	chr_14028S	3441789	3442607	STM14_3940	3441723	3442637	-	Putative fructose-1-phosphate kinase
D09	chr_14028S	3892270	3892800	STM14_4445	3892240	3892830	-	Mannitol repressor protein
D10	chr_14028S	4276166	4276789	STM14_4874	4276136	4276819	-	Putative outer membrane protein
D11	chr_14028S	4689752	4690714	STM14_5324	4689722	4690744	-	Myo-inositol 2-dehydrogenase
D12	chr_14028S	4842537	4843166	STM14_5494	4842507	4843196	+	Putative outer membrane protein
E01	chr_14028S ⁴	1622003	1623745	STM14_1856	1621973	1623775	+	Putative hydrogenase-1 large subunit
E02	chr_14028S	1766779	1767750	STM14_2010	1766749	1767780	-	Putative integral membrane protein
E03	chr_14028S	2138502	2140136	STM14_2491	2137998	2140166	-	TPR repeat-containing protein
E04	chr_14028S	2298680	2299150	STM14_2655	2298650	2299180	+	Putative fimbrial-like protein
E05	chr_14028S	2428340	2429482	STM14_2807	2428310	2429512	+	Putative dehydratase
E06	chr_14028S	2913940	2914515	STM14_3320	2913910	2914545	+	Putative hexulose 6 phosphate synthase
E07	chr_14028S ⁵	3208392	3210821	STM14_3657	3208362	3210851	+	Putative outer membrane usher protein
E08	chr_14028S	3522045	3522878	STM14_4030	3522015	3522908	+	N-acetylneuraminatase lyase
E10	chr_14028S	4292635	4293297	STM14_4892	4292605	4293327	-	Putative regulatory protein
E11	chr_14028S	4715375	4715761	STM14_5350	4715345	4715791	+	Putative transposase
E12	chr_14028S	4860674	4861699	STM14_5512	4860644	4861729	+	Putative major fimbrial subunit
F01	chr_14028S	1629790	1631286	STM14_1863	1629760	1631316	+	PhoPQ-regulated protein
F03	chr_14028S	2161265	2162344	STM14_2520	2161235	2162374	+	Cobalt-precorrin-6A synthase
F04	chr_14028S	2325215	2325856	STM14_2684	2325185	2325886	+	Putative glutathione S-transferase
F05	chr_14028S	2462529	2462846	STM14_2841	2462499	2462876	-	Hypothetical protein
F06	chr_14028S	2937838	2941431	STM14_3345	2937808	2941461	-	Putative ABC transporter protein
F07	chr_14028S	3211001	3211501	STM14_3659	3210971	3211681	+	Putative fimbrial-like protein
F08	chr_14028S	3539204	3539809	STM14_4049	3539174	3539839	+	Putative regulatory protein
F09	chr_14028S	3986271	3989009	STM14_4550	3986241	3989039	+	Putative transcriptional regulator
F11	chr_14028S	4728547	4729674	STM14_5370	4728517	4729704	-	Putative inner membrane protein
F12	chr_14028S	4861800	4862297	STM14_5513	4861770	4862327	+	Putative fimbrial subunit
G01	chr_14028S	1653565	1653834	STM14_1885	1653535	1653864	+	Acid-resistance protein
G02	chr_14028S	1774403	1775201	STM14_2018	1774373	1775231	-	
G03	chr_14028S	2249063	2249491	STM14_2609	2249033	2249521	+	Putative colanic acid biosynthesis acetyltransferase WcaB
G04	chr_14028S	2345849	2346676	STM14_2711	2345819	2346706	-	Putative phosphoserine phosphatase
G05	chr_14028S	2599136	2599942	STM14_2993	2599106	2599972	+	Pyridoxal kinase
G06	chr_14028S	2943854	2945974	STM14_3348	2943824	2946004	+	Outer membrane receptor FepA
G07	chr_14028S	3296458	3296925	STM14_3768	3296428	3296955	+	Putative monoamine oxidase
G08	chr_14028S	3788260	3788919	STM14_4332	3788230	3788949	+	Putative regulatory protein
G09	chr_14028S	3992516	3992815	STM14_4556	3992486	3992845	-	Putative cytoplasmic protein
G10	chr_14028S	4301766	4302581	STM14_4903	4301736	4302611	-	Aldolase
G11	chr_14028S	4744826	4748281	STM14_5386	4744796	4748311	-	Putative DNA helicase
G12	chr_14028S	4862375	4864852	STM14_5514	4862345	4864882	+	Putative fimbrial usher protein
H01	chr_14028S	1719063	1719569	STM14_1960	1719033	1719608	+	Putative periplasmic protein
H02	chr_14028S	1972157	1972711	STM14_2258	1972127	1972741	-	Putative inner membrane protein
H03	chr_14028S	2250483	2252582	STM14_2611	2250453	2252612	+	Tyrosine kinase
H04	chr_14028S	2383713	2385482	STM14_2758	2383683	2385512	-	O-antigen acetylase
H05	chr_14028S	2600085	2601317	STM14_2994	2600055	2601347	-	Putative regulatory protein
H06	chr_14028S	2965416	2965805	STM14_3371	2965386	2965835	+	Hypothetical protein
H07	chr_14028S	3297943	3298761	STM14_3770	3297913	3298791	-	Putative transcriptional regulator
H08	chr_14028S	3836216	3836683	STM14_4383	3836186	3836713	+	Long polar fimbrial minor protein
H09	chr_14028S	3993552	3994352	STM14_4559	3993522	3994382	+	Putative fructose-1,6-bisphosphate aldolase
H10	chr_14028S	4438015	4439502	STM14_5054	4437985	4439532	+	Putative inner membrane protein
H11	chr_14028S	4790469	4791818	STM14_5436	4790439	4791848	+	Type I restriction enzyme specificity protein
H12	chr_14028S	4864958	4865581	STM14_5515	4864928	4865611	+	Putative fimbrial chaperone

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_5463 (1.4%)

⁴Deleted region also overlaps STM14_1855 (2.7%)

⁵Deleted region also overlaps STM14_3658 (65.0%)