

***Salmonella enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate 013/014_Kan**

Catalog No. NR-29405

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-29405 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 013/014_Kan, NR-29405."

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.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at www.beiresources.org.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI

Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Use Restrictions:

This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

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.

ATCC® is a trademark of the American Type Culture Collection.



Table 1: *S. enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate 013/014_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	2219201	2220142	STM14_2581	2219171	2220172	+	Abequosyltransferase
A02	chr_14028S	2227252	2228070	STM14_2589	2227222	2228100	+	dTDP-glucose pyrophosphorylase
A03	chr_14028S	2332526	2332711	STM14_2692	2332496	2332741	-	Putative inner membrane protein
A04	chr_14028S	2462901	2463098	STM14_2842	2462871	2463128	+	Polymyxin resistance protein B
A05	chr_14028S	2522847	2524184	STM14_2907	2522817	2524214	+	Putative diaminopimelate decarboxylase
A06	chr_14028S ³	2657738	2658031	STM14_3055	2657708	2658061	+	Putative glycerate kinase
A07	chr_14028S	2741893	2742471	STM14_3130	2741863	2742501	+	Hypothetical protein
A08	chr_14028S	2813630	2814133	STM14_3201	2813600	2814163	+	Antirepressor-like protein
A09	chr_14028S	2925730	2926266					
A10	chr_14028S	2984819	2986129	STM14_3401	2984789	2986159	+	Putative glycoporin
A11	chr_14028S	3294663	3295037	STM14_3766	3294633	3295067	+	Putative lactoylglutathione lyase
A12	chr_14028S	3457410	3457880	STM14_3960	3457380	3457910	-	Putative cytoplasmic protein
B02	chr_14028S	2228178	2229017	STM14_2590	2228148	2229047	+	dTDP-4-dehydrorhamnose reductase
B04	chr_14028S	2491475	2491567	STM14_2872	2491445	2491597	+	Putative cytoplasmic protein
B05	chr_14028S	2537533	2537847	STM14_2922	2537503	2537877	+	Putative cytoplasmic protein
B06	chr_14028S	2670167	2672320	STM14_3068	2670137	2672350	+	Putative diguanylate cyclase
B07	chr_14028S	2756991	2757695	STM14_3142	2756961	2757725	+	Hypothetical protein
B08	chr_14028S	2814466	2815083	STM14_3203	2814436	2815113	+	Antiterminator-like protein
B09	chr_14028S	2926707	2927124	STM14_3331	2926750	2927154	+	Putative transposase
B10	chr_14028S	3113590	3114327	STM14_3555	3113560	3114357	+	Putative metal-dependent hydrolase
B11	chr_14028S	3303160	3303657	STM14_3776	3303130	3303687	+	Putative cytoplasmic protein
B12	chr_14028S	3531907	3532545	STM14_4042	3531877	3532608	+	Putative inner membrane protein
C03	chr_14028S	2361219	2361281	STM14_2729	2361189	2361311	+	Putative inner membrane protein
C04	chr_14028S	2491707	2492585	STM14_2873	2491677	2492615	+	NADH dehydrogenase transcriptional repressor
C05	chr_14028S	2538080	2538268	STM14_2923	2538050	2538298	-	Putative regulatory protein
C06	chr_14028S ⁴	2672685	2672864	STM14_3070	2672655	2672915	+	Putative inner membrane protein
C07	chr_14028S	2766790	2768091	STM14_3150	2766760	2768121	+	Putative phosphotransferase system IIB component
C08	chr_14028S	2839130	2839648	STM14_3242	2839091	2839678	-	Neutral amino-acid efflux protein
C09	chr_14028S	2928050	2928628	STM14_3333	2928020	2928658	+	Putative cytoplasmic protein
C10	chr_14028S	3157743	3158570	STM14_3601	3157686	3158600	-	Putative integral membrane protein
C11	chr_14028S	3308875	3309195	STM14_3784	3308845	3309225	+	Putative cytoplasmic protein
C12	chr_14028S	3536793	3537632	STM14_4047	3536763	3537662	+	Tartrate dehydratase subunit alpha
D02	chr_14028S	2245331	2245825	STM14_2605	2245301	2245855	+	Putative colanic acid biosynthesis acetyltransferase WcaF
D03	chr_14028S	2383075	2383305	STM14_2757	2383045	2383335	-	Virulence protein
D04	chr_14028S	2505677	2506447	STM14_2886	2505647	2506477	+	Putative transketolase
D06	chr_14028S	2673540	2673794	STM14_3073	2673495	2673824	-	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
D07	chr_14028S	2768162	2768995	STM14_3151	2768132	2769025	+	N-acetylmuramic acid-6-phosphate etherase
D08	chr_14028S	2849644	2850039	STM14_3253	2849614	2850069	+	Putative cytoplasmic protein
D09	chr_14028S	2928685	2930607	STM14_3334	2928655	2930637	+	Putative DNA/RNA helicase
D10	chr_14028S	3215127	3215318					
D11	chr_14028S	3324355	3325347	STM14_3806	3324325	3325377	+	Putative periplasmic ferrichrome-binding protein
D12	chr_14028S	3571540	3571644	STM14_4085	3571510	3571674	-	Putative periplasmic protein
E02	chr_14028S	2246660	2247814	STM14_2607	2246630	2247844	+	Putative colanic acid biosynthesis protein
E03	chr_14028S	2386947	2388128	STM14_2761	2386917	2388158	+	Hypothetical protein
E04	chr_14028S	2506504	2507835	STM14_2887	2506474	2507871	+	Ascorbate-specific PTS system enzyme IIC
E05	chr_14028S	2578140	2580269	STM14_2964	2578110	2580299	+	Hypothetical protein
E06	chr_14028S	2685985	2693231	STM14_3082	2685955	2693261	+	
E07	chr_14028S	2770997	2772133	STM14_3154	2770967	2772163	+	Putative permease
E08	chr_14028S	2867443	2868603	STM14_3275	2867413	2868633	-	Hypothetical protein
E09	chr_14028S	2946560	2946811	STM14_3349	2946329	2946862	-	
E10	chr_14028S	3229957	3230790	STM14_3685	3229927	3230820	+	Putative outer membrane protein
E11	chr_14028S	3335329	3335784	STM14_3820	3335299	3335814	+	Putative ATP-dependent RNA helicase-like protein
E12	chr_14028S	3573957	3574559	STM14_4087	3573927	3574589	+	DNA-binding transcriptional regulator EnvR
F02	chr_14028S	2284161	2284517	STM14_2637	2284131	2284547	+	Putative cytoplasmic protein
F03	chr_14028S	2396597	2396740	STM14_2772	2396453	2396770	-	Virulence protein
F04	chr_14028S	2508269	2508652	STM14_2889	2508239	2508682	+	Putative phosphotransferase system enzyme II A component
F06	chr_14028S	2713067	2713816	STM14_3101	2713037	2713846	+	Putative dimethylsulfoxide reductase
F07	chr_14028S	2782918	2783400	STM14_3166	2782885	2783430	-	Transposase-like protein
F09	chr_14028S	2948638	2949507	STM14_3352	2948608	2949537	-	Virulence protein
F10	chr_14028S	3260983	3261396	STM14_3723	3260953	3261426	+	Putative mannitol dehydrogenase
F12	chr_14028S	3649857	3649967	STM14_4191	3649827	3649997	-	Putative outer membrane lipoprotein
G02	chr_14028S	2319585	2320286	STM14_2678	2319555	2320316	+	Acetoin dehydrogenase
G03	chr_14028S	2447623	2448366	STM14_2826	2447593	2448396	+	Putative aldolase
G04	chr_14028S	2520230	2521273	STM14_2905	2520200	2521303	+	Putative cytoplasmic protein
G05	chr_14028S	2601392	2602051	STM14_2995	2601362	2602081	-	Glutamine amidotransferase
G06	chr_14028S	2725577	2726185	STM14_3108	2725547	2726215	-	Putative cytoplasmic protein
G07	chr_14028S	2786285	2788597	STM14_3170	2786255	2788627	+	Tail fiber-like protein
G08	chr_14028S	2906328	2907551	STM14_3310	2906298	2907581	-	Putative ATPase
G09	chr_14028S	2949860	2950696	STM14_3354	2949830	2950726	-	Putative transcriptional activator
G10	chr_14028S	3263535	3264947	STM14_3726	3263505	3264977	+	Putative mannitol dehydrogenase
G11	chr_14028S	3374600	3375211	STM14_3867	3374570	3375241	-	Putative disulfide bond formation protein
G12	chr_14028S	3693621	3693836	STM14_4235	3693591	3693866	+	Putative cytoplasmic protein
H03	chr_14028S	2451016	2451738	STM14_2829	2450986	2451768	+	Putative transcriptional regulator
H04	chr_14028S	2521335	2522696	STM14_2906	2521305	2522726	+	Putative amino acid transporter
H05	chr_14028S	2613707	2613976	STM14_3010	2613677	2614006	-	Putative inner membrane protein
H06	chr_14028S	2736966	2737784	STM14_3125	2736936	2737814	-	Putative hydrolase
H07	chr_14028S ⁵	1317350	1320385	STM14_1470	1317320	1320415	-	Phage tail component H-like protein
H08	chr_14028S	2912664	2913806	STM14_3319	2912634	2913836	-	Putative hexulose 6 phosphate synthase
H09	chr_14028S	2955844	2956218	STM14_3362	2955814	2956248	-	Tricarboxylic transport
H10	chr_14028S	3266249	3266938	STM14_3727	3266219	3266968	+	Putative regulatory protein
H11	chr_14028S	3456532	3457191	STM14_3959	3456502	3457221	+	Putative inner membrane protein
H12	chr_14028S	3704014	3704499	STM14_4247	3703984	3704529	+	Hypothetical 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_3056 (48.2%)

⁴Deleted region also overlaps STM14_3071 (53.1%)

⁵Alternative deleted regions: 2794745 - 2797780