Salmonella enterica subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate 025/026_Kan

Catalog No. NR-29409

For research use only. Not for human use.

Contributor:
Helene Andrews-Polymenis, 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]

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.

Plate orientation and viability were confirmed for NR-29409.

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-29409 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 025/026_Kan, NR-29409."

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.

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Table 1: *S. enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate 025/026_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[3]	4617335	4617394					
A03	chr_14028S[3]	4771073	4771252					
A04	chr_14028S	4743796	4743918					
A05	chr_14028S[3]	4734978	4735175					
A07	chr_14028S	3136539	3136870					
A09	chr_14028S	2080073	2080128					
A10	chr_14028S[3]	3155153	3155290					
A11	chr_14028S[3]	4400118	4400228					
A12	chr_14028S[3]	3065172	3065240					
B02	chr_14028S[3]	2987107	2987154					
B04	chr_14028S	2418376	2418528					
B05	chr_14028S[3]	4356477	4356549					
B06	chr_14028S	1928394	1928489	STM14_2200	1928329	1928535	-	Hypothetical protein
B07	chr_14028S	1454819	1454915					
B09	chr_14028S	129286	129512	STM14_0130	129166	129492	+	Hypothetical protein
B10	chr_14028S	903103	903181	STM14_974	903009	903137	+	Conserved hypothetical protein
B11	chr_14028S	943615	943711	STM14_1020	943024	943644	-	Putative regulatory protein
C01	chr_14028S[3]	1739629	1739704					
C02	chr_14028S	1979467	1979590	STM14_2277	1979524	1979643	+	Putative cytoplasmic protein
C03	chr_14028S	2282644	2282798	STM14_2635	2282708	2282821	+	Hypothetical protein
C04	chr_14028S[3]	2726444	2726769					
C05	chr_14028S[3]	3190141	3190243					
C06	chr_14028S[3]	3242063	3242193					
C07	chr_14028S	3380592	3380732	STM14_3875	3380454	3380651	-	Hypothetical protein
C09	chr_14028S[4]	4222626	4222748	STM14_4810	4222650	4222715	-	Spot 42 RNA
C10	chr_14028S[3]	506088	506142					
C11	chr_14028S[3]	1233257	1233391					
C12	chr_14028S[3]	3189942	3190041					
D01	chr_14028S	3410330	3410500	STM14_3904	3410298	3410435	-	Hypothetical protein
D02	chr_14028S[3]	3504277	3504327					
D03	chr_14028S	4155360	4155532	STM14_4733	4155257	4155382	-	Hypothetical protein
D04	chr_14028S	4518484	4518553	STM14_5129	4518503	4518631	+	Hypothetical protein
D05	chr_14028S	127454	127548	STM14_0128	127427	127546	+	Putative lipoprotein
D06	chr_14028S[3]	556676	556768					
D07	chr_14028S[3]	729269	729396					
D08	chr_14028S	2759226	2759432	STM14_3144	2759420	2759548	-	Hypothetical protein
D10	chr_14028S	4828403	4828646	STM14_5479	4828502	4828636	-	Hypothetical protein
D12	chr_14028S	4555771	4555845	STM14_5170	4554739	4555815	+	DNA-binding transcriptional regulator MelR
E01	chr_14028S	529101	529415	STM14_0558	529071	529445	+	Hypothetical protein

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Well Position	Deleted Region of Chromosome	Deletion Start	Deletion End	Locus Tag	14028S Gene Start	14028S Gene End	14028S Gene Strand	Description
E02	chr_14028S	4475061	4475213	STM14_5097	4475031	4475243	-	Putative stress-response protein
E04	chr_14028S[5]	1948354	1948437	STM14_2226	1948324	1948467	+	Hypothetical protein
E05	chr_14028S	4216464	4216673	STM14_4804	4216434	4216703	-	Putative cytoplasmic protein
E06	chr_14028S	1922387	1922620	STM14_2192	1922357	1922689	+	Putative cytoplasmic protein
E07	chr_14028S	1837126	1837572	STM14_2093	1837096	1837602	-	Putative cytoplasmic protein
E09	chr_14028S	3225571	3225777	STM14_3679	3225541	3225807	+	Hypothetical protein
E10	chr_14028S	4428462	4428941	STM14_5039	4428432	4428971	-	Putative cytoplasmic protein
E11	chr_14028S	3611811	3611945	STM14_4147	3611781	3611975	+	Bacterioferritin-associated ferredoxin
E12	chr_14028S	1570258	1570560	STM14_1791	1570228	1570590	-	Multidrug efflux system protein MdtJ
F01	chr_14028S	3430756	3431634	STM14_3927	3430726	3431664	+	DNA-binding transcriptional activator TdcA
F02	chr_14028S	1942711	1943121	STM14_2215	1942681	1943139	-	Hypothetical protein
F04	chr_14028S	3270489	3270728	STM14_3732	3270459	3270758	+	Putative inner membrane protein
F05	chr_14028S	302092	302787	STM14_0306	302062	302817	+	Hydroxyacylglutathione hydrolase
F06	chr_14028S	2303596	2303994	STM14_2659	2303566	2304024	-	Putative lipoprotein
F07	chr_14028S	2090660	2090812	STM14_2420	2090630	2090842	+	Putative cold-shock protein
F08	chr_14028S	539659	539766	STM14_0565	539629	539796	+	Hypothetical protein
F09	chr_14028S	4222677	4222706					
F10	chr_14028S	527076	527276	STM14_0553	527046	527306	-	50S ribosomal protein L31 type B
F11	chr_14028S	4717885	4717965	STM14_5355	4717965	4718453	+	Putative arginine repressor
F12	chr_14028S	527342	527422	STM14_0554	527312	527452	-	50S ribosomal protein L36
G01	chr_14028S	1979554	1979613	STM14_2277	1979524	1979643	+	Putative cytoplasmic protein
G02	chr_14028S	4373464	4373577	STM14_4977	4373434	4373607	+	Putative cytoplasmic protein
G03	chr_14028S	2565379	2565561	STM14_2951	2565349	2565591	+	Putative inner membrane protein
G04	chr_14028S	3228006	3228257	STM14_3683	3227976	3228287	+	Hypothetical protein
G05	chr_14028S	3339017	3339094	STM14_3826	3338987	3339136	+	Putative inner membrane protein
G06	chr_14028S	4869045	4869125	STM14_5520	4869015	4869155	-	Putative inner membrane protein
G07	chr_14028S	3611262	3611678	STM14_4146	3611232	3611708	+	Bacterioferritin, iron storage and detoxification protein
G08	chr_14028S	3270063	3270134	STM14_3730	3270033	3270164	+	Putative inner membrane protein
G09	chr_14028S	4373336	4373380	STM14_4976	4373306	4373410	+	Pseudogene
G10	chr_14028S	2078862	2079425	STM14_2403	2078832	2079455	-	Colanic acid capsular biosynthesis activation protein A
G11	chr_14028S	4627316	4627660	STM14_5252	4627286	4627690	-	Putative cytoplasmic protein
G12	chr_14028S	3543485	3543697	STM14_4057	3543455	3543727	+	Putative cytoplasmic protein
H02	chr_14028S	693441	693590	STM14_0732	693411	693620	-	Cold shock protein CspE
H03	chr_14028S	280781	280981	STM14_0279	280751	281011	+	Rho-binding antiterminator
H04	chr_14028S	528855	529013	STM14_0557	528825	529043	+	Hemolysin expression-modulating protein
H05	chr_14028S[6]	797724	798050	STM14_0850	797694	798080	-	Putative inner membrane protein
H06	chr_14028S	3849581	3849811	STM14_4398	3849551	3849841	-	Putative transcriptional regulator
H08	chr_14028S	1924353	1924559	STM14_2195	1924323	1924589	-	Cell division topological specificity factor MinE
H09	chr_14028S	2965965	2966063	STM14_3373	2965935	2966093	+	Putative transport protein
H10	chr_14028S	3031234	3031929	STM14_3463	3031204	3031959	+	Transcriptional regulator
H11	chr_14028S	4199838	4200491	STM14_4787	4199808	4201631	-	Arylsulfotransferase
H12	chr_14028S	4573742	4574557	STM14_5188	4573712	4574587	+	Putative DNA-binding protein

[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]sRNA was targeted
[4]Deleted region also overlaps STM14_4811 (28.5%)
[5]Deleted region also overlaps STM14_3731 (6.8%)
[6]Deleted region also overlaps STM14_0849 (0.9%)

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