Salmonella enterica subsp. enterica, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_029/030_Kan

Catalog No. NR-42825

For research use only. Not for use in humans.

Contributor:
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.[2,3,4] Detailed information about each mutant is shown in Table 1.

Note: The strain designation on the plate, strain CDC 6516-60, is incorrect. The correct strain designation is strain 14028s. *S. enterica* subsp. *enterica*, strain 14028s was originally known as strain 14028. A variant of the original strain with a rough colony morphology was designated 14028r and the original smooth strain was renamed 14028s. Strain 14028 is a descendent of strain CDC 6516-60 which was isolated from pools of hearts and livers of 4-week-old chickens.[5] The complete genome of *S. enterica* subsp. *enterica*, strain 14028s (GenBank: CP001363.1) and plasmid (GenBank: CP001362.1) sequences are available.

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-42825 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 SGD_029/030_Kan, NR-42825."

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 (BMBL). 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

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. McClelland, M., 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.
5. Jarvik, T., et al. "Short-Term Signatures of Evolutionary Change in the *Salmonella enterica* Serovar Typhimurium 14028 Genome." J. Bacteriol. 192 (2010): 560-567. PubMed: 19897643.

Support Provided by NIAID

BEI Resources is committed to ensuring digital accessibility for people with disabilities. This Product Sheet contains complex tables and may not be fully accessible. Please let us know if you encounter accessibility barriers and a fully accessible document will be provided:
E-mail: Contact@BEIResources.org. We try to respond to feedback within 24 hours.

Table 1: *S. enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_029/030_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	CDS	3708970	3710685	STM14_4251	3709000	3710654	-	Putative dihydroxyacid dehydratase
A02	CDS	2693427	2699024	STM14_3083	2693457	2698993	+	Putative outer membrane protein
A03	CDS	602079	602516	STM14_0629	602109	602485	+	Putative inner membrane protein
A04	CDS	3419991	3420155	STM14_3919	3420021	3420124	-	Putative cytoplasmic protein
A05	CDS	618839	619084	STM14_0654	618869	619053	+	
A06	CDS	2942847	2943782	STM14_3347	2942877	2943751	-	Putative hydrolase
A07	CDS	1071484	1071933	STM14_1165	1071514	1071902	+	Hypothetical protein
A08	CDS	573682	574341	STM14_0602	573763	574310	-	Putative ABC transporter permease component
A09	CDS	4390264	4390452	STM14_4999	4390294	4390421	-	Putative cytoplasmic protein
A10	CDS	2946329	2946862	STM14_3349	2946359	2946612	-	
A11	CDS	2637115	2637345	STM14_3035	2637145	2637314	+	Putative cytoplasmic protein
A12	CDS	3625666	3625901	STM14_4165	3625696	3625870	+	
B01	CDS	3214295	3214693	STM14_3663	3214325	3214662	+	Plasmid maintenance protein
B02	CDS	712798	713970	STM14_0758	712828	713939	-	Putative hydrolase
B04	CDS	2430902	2431786	STM14_2809	2431121	2431755	-	Putative regulatory protein
B05	CDS	41723	42913	STM14_0045	41753	42882	-	Putative arylsulfatase regulator
B06	CDS	1606827	1607042	STM14_1836	1606857	1607011	+	Hypothetical protein
B07	CDS	3122250	3122525	STM14_3563	3122280	3122575	+	Putative transcriptional regulator
B08	CDS	712491	712781	STM14_0757	712470	712750	-	Putative hydrolase
B10	CDS	4165005	4165527	STM14_4746	4165035	4165496	+	
B11	CDS	4665998	4667161	STM14_5300	4666028	4667130	+	Putative metallo-dependent hydrolase
B12	CDS	1962968	1963132	STM14_2242	1962998	1963101	+	Putative inner membrane protein
C01	CDS	4788756	4788899	STM14_5431	4788786	4788868	+	Putative cytoplasmic protein
C02	CDS	4428296	4428397	STM14_5038	4428326	4428366	-	Putative cytoplasmic protein
C03	CDS	2277081	2277399	STM14_2630	2277111	2277368	-	
C04	CDS	1985474	1986133	STM14_2285	1985504	1986102	+	Putative inner membrane protein
C05	CDS	1158909	1160795	STM14_1267	1158939	1160764	-	Suppression of copper sensitivity protein
C07	CDS	2361739	2362142	STM14_2732	2361769	2362111	-	
C08	CDS	2538467	2538835	STM14_2924	2538497	2538804	-	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
C09	CDS	1920620	1920757	STM14_2188	1920650	1920726	+	Putative cytoplasmic protein
C11	CDS	946559	946849	STM14_1024	946589	946818	-	Hypothetical protein
C12	CDS	4293425	4293589	STM14_4893	4293455	4293558	-	Putative periplasmic protein
D01	CDS	3658652	3659125	STM14_4202	3658682	3659064	+	Putative inner membrane protein
D02	CDS	1615133	1615675	STM14_1846	1615163	1615644	+	Putative outer membrane protein
D04	CDS	3786168	3787013	STM14_4330	3786198	3786982	+	Putative sugar kinase
D05	CDS	4664793	4666001	STM14_5299	4664823	4665970	+	Putative permease
D06	CDS	397750	398955	STM14_0410	397780	398945	+	Putative cation efflux pump
D07	CDS	3983349	3984206	STM14_4545	3983379	3984178	+	Putative phosphotransferase system enzyme II
D08	CDS	3784795	3786114	STM14_4329	3784825	3786083	+	Anaerobic C4-dicarboxylate transporter
D10	CDS	4244329	4245180	STM14_4833	4244359	4245155	+	Putative aldose-1-epimerase
D11	CDS	2382514	2382984	STM14_2756	2382544	2382986	+	DNA polymerase V subunit
D12	CDS	3525350	3526102	STM14_4035	3525380	3526071	+	Putative cytoplasmic protein
E01	CDS	4541394	4542302	STM14_5156	4541424	4542271	+	Putative dioxygenase
E02	CDS	1089020	1089715	STM14_1185	1089050	1089684	-	Minor tail protein
E03	CDS	1627297	1628322	STM14_1861	1627327	1628291	+	Putative zinc-binding dehydrogenase
E04	CDS	3261465	3262472	STM14_3724	3261495	3262441	+	Putative malate/L-lactate dehydrogenase
E05	CDS	4255532	4255822	STM14_4848	4255562	4255791	-	Putative regulatory protein
E06	CDS	4050325	4051518	STM14_4626	4050355	4051487	-	Putative mandelate racemase
E07	CDS	4798482	4798769	STM14_5441	4798512	4798738	-	Putative inner membrane protein
E08	CDS	1625310	1626401	STM14_1859	1625340	1626370	-	Putative hydrolase
E09	CDS	3212953	3213489	STM14_3661	3212983	3213458	+	Ail/OmpX-like protein
E11	CDS	4237589	4238437	STM14_4828	4237619	4238406	+	Putative cytoplasmic protein
E12	CDS	375522	376400	STM14_0387	375552	376369	-	Putative fumarylacetoacetate hydrolase
F01	CDS	2388219	2388737	STM14_2762	2388249	2388706	+	Hypothetical protein
F02	CDS	378258	378530	STM14_0390	378288	378499	+	Putative cytoplasmic protein
F03	CDS	2881497	2892971	STM14_3297	2881527	2892940	-	
F05	CDS	37103	38674	STM14_0042	37133	38643	+	Putative 5'-nucleotidase
F06	CDS	3704539	3706098	STM14_4248	3704569	3706067	+	Putative periplasmic phosphate-binding protein
F07	CDS (LT2)[3]				1978461	1979296		
F08	CDS	4236551	4237576	STM14_4827	4236581	4237128	+	Putative periplasmic protein
F09	CDS	4302994	4303758	STM14_4905	4303024	4303727	-	Ribulose-phosphate 3-epimerase
F11	CDS	3300647	3301831	STM14_3772	3300677	3301800	+	Putative arylsulfatase regulator
F12	CDS	4079907	4080725	STM14_4654	4079937	4080694	+	Shikimate 5-dehydrogenase
G01	CDS	40131	41624	STM14_0044	40161	41593	-	Putative arylsulfatase
G02	CDS	1074726	1075259	STM14_1170	1074756	1075228	-	Hypothetical protein
G03	CDS	2391120	2392109	STM14_2767	2391150	2392042	+	Putative cytoplasmic protein
G04	CDS	72929	73837	STM14_0074	72959	73806	-	Triphosphoribosyl-dephospho-CoA synthase
G05	CDS	394586	397753	STM14_0409	394616	397722	+	Putative cation efflux system protein
G06	CDS	601258	602082	STM14_0628	601288	602051	+	Putative outer membrane protein
G07	CDS	2388751	2389080	STM14_2763	2388781	2389049	+	Putative inner membrane protein
G08	CDS	1977760	1978413	STM14_2274	1977949	1978382	-	RecE-like protein
G09	CDS	677033	677800	STM14_0712	677063	677769	-	Putative hydrogenase protein
G10	CDS	2135043	2135361	STM14_2487	2135073	2135330	-	
G11	CDS	3351367	3352674	STM14_3843	3351397	3352643	-	Putative transporter
G12	CDS	4234440	4235681	STM14_4825	4234470	4235650	+	Coproporphyrinogen III oxidase
H01	CDS	4233565	4234443	STM14_4824	4233595	4234412	+	Putative inner membrane protein
H02	CDS	719398	720831	STM14_0764	719428	720800	-	Putative molecular chaperone
H04	CDS	3981506	3982249	STM14_4543	3981536	3982218	+	Putative cytoplasmic protein
H05	CDS	717574	718569	STM14_0762	717604	718538	+	TPR repeat-containing protein
H06	CDS	1090360	1091064	STM14_1187	1090390	1091033	-	Tail assembly protein
H07	CDS	3984206	3984955	STM14_4546	3984236	3984924	+	Putative phosphotransferase system enzyme IIC
H08	CDS	1073337	1073585	STM14_1167	1073286	1073554	-	Hypothetical protein
H09	CDS	4575680	4576171	STM14_5192	4575710	4576140	-	Putative acetyltransferase
H10	CDS	2673844	2674200	STM14_3074	2673874	2674115	+	Putative transposase
H11	CDS	4051633	4052529	STM14_4627	4051663	4052498	-	Putative transcriptional regulator
H12	CDS	3259783	3260598	STM14_3721	3259813	3260705	+	Putative hydrolase/acyltransferase

[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]Of the targeted genes, 22 CDSs and 22 sRNA were annotated in strain LT2 but not annotated in strain 14028s.

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898