

***Salmonella enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_033/034_Kan**

Catalog No. NR-42827

For research use only. Not for human use.

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 3517 mutants distributed among 11 96-well plates, in which 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 pCLF3 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.

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-42827 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 SGD_033/034_Kan, NR-42827."

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. 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.

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Table 1: *S. enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_033/034_Kan^{1,2}

Well Position	Gene Type	Gene Start	Gene End	Target Gene (Locus Tag)	Deleted Region Start	Deleted Region End	Gene Strand	Description
A01	CDS	3298839	3300578	STM14_3771	3298869	3300547	+	Putative acetyl-CoA/acetoacetyl-CoA transferase beta subunit
A02	CDS	1447844	1449439	STM14_1648	1447874	1449366	+	4-hydroxyphenylacetate catabolism
A03	CDS	1149319	1150170	STM14_1252	1149349	1150139	-	Putative glycosyl transferase
A04	CDS	2232994	2234214	STM14_2594	2233024	2234183	+	Putative transposase
A05	CDS	2927301	2927567	STM14_3332	2927331	2927536	+	Catabolic arginine decarboxylase
A06	CDS	4552239	4554509	STM14_5169	4552269	4554478	+	Putative PTS system enzyme IIB component
A07	CDS	1712890	1713171	STM14_1952	1712920	1713140	-	Putative inner membrane protein
A08	CDS	3378034	3378654	STM14_3873	3378064	3378623	-	Hypothetical protein
A09	CDS	4815106	4815567	STM14_5457	4815136	4815536	+	Putative nucleoside-diphosphate-sugar pyrophosphorylase
A10	CDS	1710042	1711022	STM14_1948	1710072	1710991	-	Putative cytoplasmic protein
A11	CDS	2017848	2018987	STM14_2325	2017878	2018956	+	Zinc resistance protein
A12	CDS	4401308	4401763	STM14_5013	4401338	4401732	+	Putative 3-polyphenyl-4-hydroxybenzoate decarboxylase
B01	CDS	3084012	3085439	STM14_3524	3084042	3085408	-	Putative DNA-damage-inducible protein
B02	CDS	3693869	3694129	STM14_4236	3693899	3694098	+	Nitrate reductase 2 beta subunit
B03	CDS	1674401	1675945	STM14_1906	1674431	1675914	-	Putative transposase
B04	CDS	3102091	3102651	STM14_3546	3102121	3102620	+	Putative DNA-binding transcriptional regulator
B05	CDS	4777899	4778807	STM14_5418	4777929	4778776	+	Menaquinone-specific isochorismate synthase
B06	CDS	2468935	2470230	STM14_2848	2468965	2470199	+	Putative oxidoreductase
B07	CDS	2573772	2574770	STM14_2960	2573802	2574739	-	Adenosylcobinamide kinase/adenosylcobinamide-phosphate guanylyltransferase
B08	CDS	2150691	2151236	STM14_2506	2150721	2151205	+	2-methylisocitrate lyase
B09	CDS	418670	419557	STM14_0431	418700	419526	-	Putative cellulase protein
B11	CDS	1711775	1712893	STM14_1951	1711805	1712862	-	2,3-diketo-L-gulonate reductase
B12	CDS	3869330	3870328	STM14_4425	3869360	3870297	-	Putative cytoplasmic protein
C01	CDS	3305373	3305747	STM14_3780	3305403	3305716	+	Transcriptional regulator NanR
C02	CDS	3523043	3523834	STM14_4031	3523073	3523803	+	
C03	CDS	3146681	3147997	STM14_3589	3146711	3147966	-	Sensor kinase
C04	CDS	619284	619613	STM14_0655	619314	619582	+	Putative permease
C05	CDS	436290	437501	STM14_0450	436320	437470	-	Putative cytoplasmic protein
C06	CDS	2828879	2829118	STM14_3226	2828909	2829087	-	Phosphonoacetaldehyde hydrolase
C07	CDS	486011	486823	STM14_0511	486041	486792	-	Putative amino-acid transporter
C08	CDS	1009333	1010754	STM14_1095	1009363	1010723	-	Pyrimidine (deoxy)nucleoside triphosphate pyrophosphohydrolase
C09	CDS	1390429	1390845	STM14_1579	1390459	1390814	+	Putative cytoplasmic protein
C10	CDS	2971389	2971820	STM14_3385	2971419	2971789	-	Putative transposase

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Product Information Sheet for NR-42827

Well Position	Gene Type	Gene Start	Gene End	Target Gene (Locus Tag)	Deleted Region Start	Deleted Region End	Gene Strand	Description
C11	CDS	341440	341637	STM14_0350	341470	341606	-	Ribonucleoside transporter
C12	CDS	3991077	3992270	STM14_4555	3991107	3992239	+	Putative periplasmic protein
D01	CDS	3283463	3284470	STM14_3749	3283493	3284439	+	DNA-binding transcriptional repressor UxuR
D02	CDS	4773885	4774658	STM14_5411	4773915	4774627	-	Pydrogenase 2 protein HybA
D03	CDS	3329906	3330892	STM14_3813	3329936	3330861	+	
D04	CDS	370609	371314	STM14_0382	370639	371283	-	Putative entericidin A precursor
D05	CDS	4593026	4593202	STM14_5215	4593056	4593171	-	Putative cytoplasmic protein
D06	CDS	986705	987565	STM14_1073	986735	987534	+	Putative ATP-binding protein
D07	CDS	3027520	3028341	STM14_3459	3027550	3028310	-	Putative sugar transporter
D08	CDS	2326951	2328309	STM14_2686	2326981	2328278	+	Peptidoglycan hydrolase
D10	CDS	1222616	1223566	STM14_1353	1222646	1223535	-	Phosphoglycerate transport system sensor protein
D11	CDS	2560050	2562056	STM14_2948	2560080	2562025	+	Xanthosine phosphorylase
D12	CDS	2586410	2587333	STM14_2977	2586440	2587212	+	Sigma N
E01	CDS	3698566	3700149	STM14_4241	3698596	3700118	-	Vitamin B12 biosynthetic protein
E02	CDS	2159311	2160084	STM14_2517	2159341	2160053	+	Hypothetical protein
E03	CDS	4346894	4348627	STM14_4952	4346924	4348596	+	Putative outer membrane lipoprotein
E04	CDS	392683	393042	STM14_0407	392713	393011	-	Methyl-accepting chemotaxis protein III
E05	CDS	1725856	1727481	STM14_1966	1725886	1727450	+	Hypothetical protein
E06	CDS	3378673	3380352	STM14_3874	3378703	3380321	-	Dihydropyrimidine dehydrogenase
E07	CDS	2334181	2335416	STM14_2694	2334211	2335385	-	Putative regulatory protein
E08	CDS	639710	640051	STM14_0678	639740	640020	-	Glyoxylate carboligase
E09	CDS	578161	579942	STM14_0607	578191	579911	-	Putative FlgK/FlgL export chaperone
E10	CDS	1214782	1215204	STM14_1341	1214812	1215173	+	GPH family transport protein
E11	CDS	4240821	4242203	STM14_4831	4240851	4242172	+	Citrate lyase subunit gamma
F01	CDS	686769	687065	STM14_0724	686799	687034	+	Ferric-rhodotorulic acid outer membrane transporter
F02	CDS	1246050	1248224	STM14_1378	1246080	1248193	+	Putative transcriptional repressor
F03	CDS	2307921	2308652	STM14_2664	2307951	2308621	-	Nitrate reductase 2 alpha subunit
F04	CDS	1670664	1674404	STM14_1905	1670694	1674373	-	Putative cell envelope opacity-associated protein A
F05	CDS	4645907	4646542	STM14_5280	4645937	4646511	+	Putative outer membrane lipoprotein
F06	CDS	94319	94549	STM14_0095	94349	94518	-	Putative fructose-like phosphotransferase EIIb subunit 2
F07	CDS	4341980	4342300	STM14_4945	4342010	4342269	-	Putative ABC-type cobalt transport system ATP-binding component
F08	CDS	3254832	3255488	STM14_3716	3254862	3255457	-	Reactivating factor for ethanolamine ammonia lyase
F09	CDS	2620176	2621579	STM14_3017	2620206	2621548	+	Nitrate reductase 2 delta subunit
F10	CDS	1675945	1676640	STM14_1907	1675975	1676609	-	Putative ABC-type cobalt transport system ATP-binding component
F11	CDS	3254161	3254838	STM14_3715	3254191	3254807	-	Putative permease
F12	CDS	2436737	2438023	STM14_2815	2436767	2437896	+	Vitamin B12 biosynthetic protein
G01	CDS	2155965	2156759	STM14_2513	2155995	2156728	+	Putative periplasmic protein
G03	CDS	3659648	3660427	STM14_4204	3659678	3660396	+	Biofilm formation regulatory protein BssR
G04	CDS	926137	926520	STM14_0998	926167	926489	-	Putative ethanolamine utilization protein
G05	CDS	2628908	2629597	STM14_3026	2628938	2629566	+	DNA-binding transcriptional activator AlIS
G06	CDS	575544	576470	STM14_0604	575574	576439	+	Putative sugar-specific PTS enzyme II
G07	CDS	1715447	1715884	STM14_1955	1715477	1715853	-	Putative transcriptional repressor of sgc operon
G08	CDS	1716544	1717335	STM14_1957	1716574	1717304	-	Putative ferredoxin
G09	CDS	92456	92743	STM14_0093	92486	92712	-	Putative regulatory protein
G10	CDS	1777310	1777900	STM14_2021	1777340	1777869	+	Precorin-3B C17-methyltransferase
G11	CDS	2157550	2158275	STM14_2515	2157580	2158244	+	Citrate lyase synthetase
G12	CDS	687062	688138	STM14_0725	687092	688107	+	Histidine ammonia-lyase
H01	CDS	856996	858516	STM14_0917	857026	858485	-	Peptide chain release factor-like protein
H02	CDS	360888	361502	STM14_0371	360918	361471	-	Putative ferredoxin
H04	CDS	1441896	1442189	STM14_1641	1441926	1442158	+	Putative cytoplasmic protein
H05	CDS	3980329	3981270	STM14_4542	3980359	3981239	+	Putative aldo/keto reductase
H07	CDS	1778782	1779651	STM14_2023	1778812	1779587	+	DNA gyrase inhibitor
H08	CDS	2186957	2187424	STM14_2551	2186987	2187393	+	Putative PTS permease
H10	CDS	4808204	4808665	STM14_5450	4808234	4808634	-	Transcriptional activator FlhD
H11	CDS	2033121	2033471	STM14_2341	2033151	2033440	+	Putative acetyl-CoA/acetoacetyl-CoA transferase beta subunit

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.