Salmonella enterica subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_057/058_Kan

Catalog No. NR-42839

For research use only. Not for human use.

Contributor:
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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-42839 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_057/058_Kan, NR-42839."

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.

Support Provided by NIAID

Table 1: *S. enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_057/058_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	893453	895675	STM14_0960	893483	895644	+	Hypothetical protein
A02	CDS	901164	902744	STM14_0972	901194	902713	+	Putative integral membrane protein
A03	CDS	904949	905869	STM14_0977	904979	905838	+	Hypothetical protein
A05	CDS	909985	911250	STM14_0981	910015	911219	+	Putative cytoplasmic protein
A07	CDS	974189	975847	STM14_1056	974219	975816	-	Hypothetical protein
A08	CDS	973088	973987	STM14_1054	973118	973956	+	Putative inner membrane protein
A09	CDS	938626	939234	STM14_1015	938656	939203	+	Undecaprenyl pyrophosphate phosphatase
A10	CDS	948612	949088	STM14_1027	948642	949057	-	Putative cytoplasmic protein
A11	CDS	953649	954122	STM14_1032	953679	954091	-	Putative inner membrane protein
A12	CDS	963053	963568	STM14_1044	963083	963537	+	Putative lipoprotein
B01	CDS	964016	964846	STM14_1046	964046	964815	-	Putative aminidase
B02	CDS	965952	967385	STM14_1048	965982	967354	+	Putative nucleoside-diphosphate-sugar epimerase
B04	CDS	1007967	1009115	STM14_1094	1007997	1009084	-	MFS family transporter
B05	CDS	1000549	1001892	STM14_1086	1000636	1001861	-	Recombination factor protein RarA
B06	CDS	1017059	1018819	STM14_1101	1017089	1018788	+	Putative cytoplasmic protein
B07	CDS	1032844	1033026	STM14_1116	1032874	1032995	-	Hypothetical protein
B08	CDS	1043379	1045220	STM14_1125	1043409	1045189	-	Hypothetical protein
B09	CDS	1034853	1035629	STM14_1120	1034883	1035598	+	Hypothetical protein
B10	CDS	1046056	1046703	STM14_1127	1046086	1046672	-	Putative metallo-beta-lactamase
B11	CDS	1106482	1107024	STM14_1202	1106512	1106993	-	Putative cytoplasmic protein
B12	CDS	1108229	1110337	STM14_1204	1108259	1110306	-	23S rRNA m(2)G2445 methyltransferase
C06	CDS	1128715	1129032	STM14_1224	1128745	1129001	+	Putative inner membrane protein
C07	CDS	1129090	1130457	STM14_1225	1129120	1130270	+	Putative SAM-dependent methyltransferase
C08	CDS	1164852	1165490	STM14_1276	1164882	1165459	-	Putative transcriptional repressor
C09	CDS	1184250	1184987	STM14_1300	1184280	1184956	-	Putative hydrolase
C10	CDS	1185011	1185565	STM14_1301	1185041	1185534	-	Putative oxidoreductase component
D03	CDS	1232654	1233238	STM14_1362	1232684	1233207	+	Maf-like protein
D06	CDS	1198725	1198952	STM14_1319	1198779	1198921	-	Hypothetical protein
D12	CDS	1243440	1244237	STM14_1375	1243470	1244206	-	Putative metallodependent hydrolase
E01	CDS	1254100	1254639	STM14_1386	1254142	1254608	-	Hypothetical protein
E02	CDS	1250770	1251795	STM14_1383	1250812	1251764	-	Beta-hexosaminidase
E04	CDS	1254714	1255349	STM14_1387	1254744	1255318	+	Putative transcriptional repressor

Well Position	Gene Type	Gene Start	Gene End	Target Gene (Locus Tag)	Deleted Region Start	Deleted Region End	Gene Strand	Description
E06	CDS	1264008	1264919	STM14_1395	1264038	1264888	-	N-acetyl-D-glucosamine kinase
E07	CDS	1915005	1916531	STM14_2181	1915029	1916500	-	Hypothetical protein
E08	CDS	1921648	1922307	STM14_2191	1921678	1922276	+	Hypothetical protein
E09	CDS	1921109	1921555	STM14_2190	1921139	1921539	+	Hypothetical protein
E10	CDS	1881988	1882797	STM14_2143	1882018	1882766	+	Putative transcriptional regulator
E11	CDS	1852693	1853340	STM14_2112	1852723	1853309	+	Hypothetical protein
E12	CDS	1892507	1893598	STM14_2157	1892537	1893567	-	Translation-associated GTPase
F01	CDS	1891252	1891530	STM14_2155	1891282	1891499	+	Hypothetical protein
F03	CDS	1879012	1879365	STM14_2139	1879042	1879334	-	Putative sulfur reduction protein
F04	CDS	1840788	1841327	STM14_2098	1840818	1841296	-	Intracellular septation protein A
F05	CDS	1839988	1840731	STM14_2097	1840018	1840700	-	Hypothetical protein
F06	CDS	1842901	1843197	STM14_2101	1842931	1843166	-	YciI-like protein
F07	CDS	1825058	1825819	STM14_2080	1825088	1825788	-	Short chain dehydrogenase
F11	CDS (LT2)[3]				1845081	1845349		
F12	CDS	1788559	1789620	STM14_2034	1788589	1789589	+	Hypothetical protein
G01	CDS	1784481	1785446	STM14_2029	1784511	1785415	+	Putative chloromuconate cycloisomerase
G03	CDS	1789617	1791020	STM14_2035	1789647	1790989	+	Putative ATPase
G04	CDS	1765254	1766201	STM14_2008	1765284	1766170	-	Universal stress protein UspE
G05	CDS	1762819	1763382	STM14_2005	1762849	1763351	+	Hypothetical protein
G06	CDS	1757286	1758221	STM14_2000	1757316	1758190	-	C32 tRNA thiolase
G07	CDS	1719804	1721459	STM14_1962	1719834	1721428	+	Glucan biosynthesis protein D
G08	CDS	1703613	1705577	STM14_1942	1703643	1705546	+	Putative collagenase
G09	CDS	1698371	1699795	STM14_1935	1698401	1699764	+	Putative regulatory protein
G10	CDS	1696811	1698235	STM14_1934	1696841	1698225	+	Gamma-aminobutyraldehyde dehydrogenase
G11	CDS	1689291	1689524	STM14_1927	1689321	1689493	+	Putative cytoplasmic protein
G12	CDS	1688837	1689286	STM14_1926	1688867	1689255	-	Putative inner membrane protein
H03	CDS	1597964	1598650	STM14_1824	1597994	1598619	+	Putative regulatory protein
H04	CDS	1570577	1570906	STM14_1792	1570655	1570875	-	Multidrug efflux system protein MdtI
H05	CDS	1564202	1565146	STM14_1785	1564232	1565115	+	Putative periplasmic protein
H07	CDS	1543746	1544324	STM14_1757	1543776	1544293	+	Electron transport complex protein RnfB
H09	CDS	1539863	1540483	STM14_1754	1539893	1540452	+	Electron transport complex protein RnfG
H10	CDS	1539168	1539860	STM14_1753	1539198	1539829	+	SoxR-reducing system protein RsxE

[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.