Product Information Sheet for NR-42828

Salmonella enterica subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_035/036_Kan

Catalog No. NR-42828

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 CDC6516-60, 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-42828 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_035/036_Kan, NR-42828."

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.

Disclaimers:
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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_035/036_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	2263041	2264012	STM14_2621	2263071	2263879	+	3-methyl-adenine DNA glycosylase II
A02	CDS	1902638	1903774	STM14_2168	1902668	1903743	-	Putative cytochrome oxidase subunit II
A04	CDS	2171874	2172395	STM14_2531	2171904	2172364	-	Propanediol dehydratase small subunit
A05	CDS	1680626	1682119	STM14_1916	1680656	1682088	-	L-asparagine transport protein
A06	CDS	3102648	3104204	STM14_3547	3102678	3104173	+	Putative cytoplasmic protein
A07	CDS	4698598	4699242	STM14_5335	4698628	4699211	-	Putative inner membrane protein
A08	CDS	4738626	4739945	STM14_5378	4738656	4739914	+	L-idonate transport protein
A09	CDS	2325902	2326939	STM14_2685	2325932	2326908	+	Putative 1,2-dioxygenase
A10	CDS	2174227	2174577	STM14_2533	2174257	2174546	-	Propanediol dehydratase reactivation protein
A11	CDS	1665150	1666238	STM14_1898	1665180	1666207	-	Putative outer membrane porin precursor
A12	CDS	4740007	4740771	STM14_5379	4740037	4740740	+	Gluconate 5-dehydrogenase
B01	CDS	4034503	4037055	STM14_4614	4034533	4037024	+	Trimethylamine N-oxide reductase subunit
B02	CDS	1986648	1986938	STM14_2287	1986678	1986907	+	DNA damage-inducible protein YebG
B04	CDS	2624949	2626352	STM14_3021	2624979	2626321	+	Putative aldehyde oxidoreductase
B05	CDS	4265683	4266342	STM14_4860	4265713	4266311	+	Putative branched-chain amino acid permease
B07	CDS	2237124	2238518	STM14_2597	2237154	2238487	+	Putative UDP-glucose lipid carrier transferase
B08	CDS	2979675	2980859	STM14_3395	2979705	2980828	-	Putative inner membrane protein
B10	CDS	224443	226632	STM14_0228	224473	226601	-	Ferrichrome outer membrane transporter
B11	CDS	1152380	1153756	STM14_1257	1152410	1153725	-	4-hydroxyphenylacetate catabolism
B12	CDS	1883187	1884020	STM14_2145	1883217	1883989	+	N5-glutamine S-adenosyl-L-methionine-dependent methyltransferase
C01	CDS	433419	433727	STM14_0445	433449	433696	+	Putative inner membrane protein
C03	CDS (LT2)[3]				935032	935651		
C04	CDS	184559	186173	STM14_0189	184589	186142	+	
C05	CDS	2249524	2250366	STM14_2610	2249554	2250335	+	Glycosyl transferase
C06	CDS	3779865	3781136	STM14_4324	3779895	3781105	+	Putative phosphatase
C07	CDS	986008	986685	STM14_1072	986038	986654	+	SlsA
C08	CDS	4015641	4016564	STM14_4588	4015671	4016533	+	DNA-binding transcriptional regulator DsdC
C09	CDS	1658872	1659528	STM14_1892	1658902	1659497	+	Formate dehydrogenase-N subunit gamma
C10	CDS	3328738	3329916	STM14_3812	3328768	3329885	+	Putative hydrogenase 2 b cytochrome subunit
C11	CDS	4215268	4215783	STM14_4802	4215298	4215752	+	Molybdopterin-guanine dinucleotide biosynthesis protein B

Well Position	Gene Type	Gene Start	Gene End	Target Gene (Locus Tag)	Deleted Region Start	Deleted Region End	Gene Strand	Description
C12	CDS	1904220	1905545	STM14_2170	1904250	1905514	-	Putative glutamic dehyrogenase-like protein
D01	CDS	1592226	1593440	STM14_1818	1592256	1593409	-	Putative dehydratase
D02	CDS	2332946	2334187	STM14_2693	2332976	2334156	-	Putative oxidoreductase
D03	CDS	70880	72400	STM14_0072	70910	72369	-	Putative citrate lyase alpha chain/citrate-ACP transferase
D04	CDS	4742602	4743621	STM14_5383	4742632	4743590	+	Putative alcohol dehydrogenase
D05	CDS	2171185	2171859	STM14_2530	2171215	2171828	-	Propanediol dehydratase medium subunit
D06	CDS	3420268	3421578	STM14_3920	3420298	3421547	+	Putative inner membrane protein
D07	CDS	3191472	3192494	STM14_3637	3191502	3192463	-	Putative transcriptional regulator
D08	CDS	4037045	4038229	STM14_4615	4037075	4038198	+	Trimethylamine N-oxide reductase cytochrome c-like subunit
D09	CDS	3783693	3784736	STM14_4328	3783723	3784705	+	Putative L-asparaginase
D10	CDS	3634805	3634972	STM14_4178	3634835	3634941	+	Hypothetical protein
D11	CDS	2151233	2152753	STM14_2507	2151263	2152722	+	Cobyric acid synthase
D12	CDS	3515734	3516987	STM14_4024	3515764	3516956	-	Cytosine permease
E02	CDS	4696774	4697112	STM14_5331	4696804	4697081	-	Putative cytoplasmic protein
E03	CDS	1779925	1780830	STM14_2025	1779955	1780799	+	Putative transcriptional regulator
E04	CDS	89396	90166	STM14_0090	89426	90135	-	Putative electron transfer flavoprotein FixA
E05	CDS	1216410	1216826	STM14_1345	1216440	1216795	-	Flagellar basal body rod protein FlgB
E06	CDS	1740447	1741247	STM14_1984	1740477	1741216	+	Putative inner membrane protein
E07	CDS	1677423	1678316	STM14_1909	1677453	1678285	-	Hypothetical protein
E08	CDS	3082061	3082825	STM14_3521	3082127	3082794	-	Putative regulatory protein
E09	CDS	422381	424267	STM14_0434	422411	424236	-	Propionyl-CoA synthetase
E10	CDS	1022419	1023180	STM14_1107	1022449	1023149	-	Putative Zn-dependent protease
E11	CDS	69710	70003	STM14_0070	69740	69972	-	Citrate lyase subunit gamma
E12	CDS	548567	549676	STM14_0577	548597	549507	+	Acetyl esterase
F01	CDS	1780949	1781878	STM14_2026	1780979	1781847	-	Putative 2'-hydroxyisoflavone reductase
F03	CDS	1026487	1028757	STM14_1112	1026523	1028726	-	Hypothetical protein
F04	CDS	4831619	4832482	STM14_5484	4831649	4832451	+	Pyruvate formate lyase-activating enzyme
F05	CDS	3225147	3225560	STM14_3678	3225177	3225529	+	Putative inner membrane protein
F06	CDS	3514514	3515617	STM14_4022	3514544	3515586	-	Putative cytoplasmic protein
F07	CDS	2449767	2450984	STM14_2828	2449797	2450953	+	Putative galactonate dehydratase
F08	CDS	87398	88915	STM14_0088	87428	88884	+	L-carnitine/gamma-butyrobetaine antiporter
F09	CDS	299797	300567	STM14_0304	299827	300536	-	Putative methyltransferase
F10	CDS	3856590	3856892	STM14_4409	3856620	3856861	+	Putative outer membrane lipoprotein
F11	CDS	2763950	2765494	STM14_3147	2763980	2765463	-	Putative transglycosylase
G01	CDS	2896630	2897793	STM14_3300	2896660	2897762	-	HlyD family secretion protein
G02	CDS	2158275	2159330	STM14_2516	2158305	2159299	+	Cobalamin biosynthesis protein CbiG
G03	CDS	420889	422340	STM14_0433	420919	422309	-	2-methylcitrate dehydratase
G04	CDS	2174596	2174871	STM14_2534	2174626	2174840	-	Polyhedral body protein
G05	CDS	2503132	2504652	STM14_2884	2503162	2504621	-	Hypothetical protein
G06	CDS	1907957	1908691	STM14_2174	1907987	1908660	-	Putative inner membrane protein
G07	CDS	4016790	4018127	STM14_4590	4016820	4018096	-	Permease DsdX
G09	CDS	2714465	2716870	STM14_3103	2714495	2716839	+	Putative anaerobic dimethylsulfoxide reductase
G10	CDS	833679	834377	STM14_0893	833709	834346	-	Putative cytoplasmic protein
G11	CDS	4032288	4033688	STM14_4612	4032318	4033657	+	Putative cytochrome c peroxidase
G12	CDS	3385254	3386555	STM14_3878	3385284	3386524	+	Putative cytoplasmic protein
H01	CDS	3104216	3106879	STM14_3548	3104246	3106848	+	Putative helicase
H02	CDS	1739800	1740330	STM14_1983	1739830	1740299	+	Cytochrome b561
H03	CDS	1947966	1948253	STM14_2225	1947996	1948222	+	Hypothetical protein
H04	CDS	2019224	2019616	STM14_2326	2019254	2019585	+	Flagellar protein
H05	CDS	484901	486004	STM14_0510	484931	485973	-	2-aminoethylphosphonate--pyruvate transaminase
H06	CDS	1414679	1414969	STM14_1609	1414709	1414938	+	Putative cytoplasmic protein
H07	CDS	4342399	4344696	STM14_4946	4342429	4344665	-	Putative formate acetyltransferase 2
H07	CDS	4345542	4345895	STM14_4948	4345572	4345864	-	Putative fructose-like phosphotransferase EIIB subunit 3
H11	CDS	1215594	1216253	STM14_1343	1215624	1216222	+	Flagellar basal body P-ring biosynthesis protein FlgA

[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.