Product Information Sheet for NR-42844

Salmonella enterica subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_067/068_Kan

Catalog No. NR-42844

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 3,517 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-42844 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_067/068_Kan, NR-42844."

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_067/068_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	4392686	4393444	STM14_5003	4392716	4393413	+	Thiamine biosynthesis protein ThiF
A02	CDS	4791845	4793434	STM14_5437	4791875	4793403	+	DNA methylase M
A03	CDS	4793592	4797101	STM14_5438	4793622	4797070	+	Type I restriction enzyme EcoKI subunit R
A04	CDS	2091297	2092565	STM14_2422	2091327	2092534	+	DNA polymerase V subunit UmuC
A05	CDS	2092568	2092987	STM14_2423	2092598	2092956	+	DNA polymerase V subunit UmuD
A06	CDS	2059063	2060550	STM14_2378	2059093	2060519	+	Flagellin
A07	CDS	2935077	2935649	STM14_3339	2935107	2935618	-	DNA-invertase Hin
A08	CDS	4789880	4790212	STM14_5434	4789910	4790181	+	Endoribonuclease SymE
A10	CDS	4063526	4064746	STM14_4639	4063556	4064715	-	
A11	CDS	4755004	4756095	STM14_5391	4755034	4756064	+	Putative ABC-type sugar/spermidine/putrescine transport system ATPase component
A12	CDS	4759818	4763459	STM14_5393	4759848	4763428	+	Putative DNA repair ATPase
B01	CDS	4749510	4751594	STM14_5388	4749540	4751563	+	Putative ATP-dependent Lon protease
B02	CDS	4578830	4579162	STM14_5195	4578860	4579131	-	Putative regulatory protein
B03	CDS	4278277	4278792	STM14_4876	4278307	4278761	+	Putative C4-dicarboxylate transport system
B04	CDS	2909049	2909333	STM14_3314	2909079	2909302	-	Putative cytoplasmic protein
B06	CDS	2903475	2903690	STM14_3307	2903505	2903659	-	Putative cytoplasmic protein
B07	CDS	3952657	3953508	STM14_4510	3952687	3953477	+	Putative cytoplasmic protein
B08	CDS	4751605	4754202	STM14_5389	4751635	4754171	+	Putative cytoplasmic protein
B09	CDS	4449116	4449571	STM14_5068	4449146	4449540	+	Putative cytoplasmic protein
B10	CDS	4432921	4433376	STM14_5047	4432951	4433345	+	Putative cytoplasmic protein
B11	CDS	4441880	4442923	STM14_5059	4441910	4442892	+	Putative cytoplasmic protein
B12	CDS	3473536	3474600	STM14_3974	3473566	3474569	+	Putative cytoplasmic protein
C01	CDS	4754205	4755026	STM14_5390	4754235	4754995	+	Putative cytoplasmic protein
C02	CDS	4433373	4433978	STM14_5048	4433403	4433947	+	Putative cytoplasmic protein
C03	CDS	4763471	4764073	STM14_5394	4763501	4764042	+	Putative cytoplasmic protein
C04	CDS	794236	794373	STM14_0846	794266	794342	-	Putative cytoplasmic protein
C05	CDS	2093114	2093275	STM14_2424	2093144	2093244	+	Putative cytoplasmic protein
C06	CDS	985270	985671	STM14_1070	985300	985640	+	Putative cytoplasmic protein
C07	CDS	2614135	2615412	STM14_3011	2614165	2615381	-	Putative cytoplasmic protein
C08	CDS	4448922	4449119	STM14_5067	4448952	4449088	+	Putative cytoplasmic protein
C09	CDS	783488	784228	STM14_0837	783518	784197	-	Putative cytoplasmic protein

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Well Position	Gene Type	Gene Start	Gene End	Target Gene (Locus Tag)	Deleted Region Start	Deleted Region End	Gene Strand	Description
C10	CDS	4692204	4693019	STM14_5326	4692234	4692988	-	Putative cytoplasmic protein
C11	CDS	2911326	2912339	STM14_3318	2911356	2912308	-	Putative dehydrogenase
C12	CDS	4680739	4681749	STM14_5317	4680769	4681718	-	Putative dehydrogenase
D01	CDS	2917868	2919547	STM14_3325	2917898	2919516	-	Putative dipeptide/oligopeptide/nickel ABC-type transport system periplasmic component
D02	CDS	4693072	4693956	STM14_5327	4693102	4693925	-	Putative endonuclease
D03	CDS	4683190	4684023	STM14_5319	4683220	4683992	-	Putative endonuclease
D04	CDS	4675936	4676745	STM14_5311	4675966	4676714	+	Putative inner membrane protein
D06	CDS	630024	632753	STM14_0667	630054	632722	-	Putative inner membrane protein
D08	CDS	4162712	4163065	STM14_4741	4162775	4163034	-	Putative inner membrane protein
D09	CDS	635867	636610	STM14_0671	635897	636579	+	Putative inner membrane protein
D11	CDS	4442911	4443120	STM14_5060	4442941	4443089	+	Putative inner membrane protein
E02	CDS	4744147	4744434	STM14_5385	4744177	4744403	-	Putative integrase
E03	CDS	2602533	2603072	STM14_2997	2602563	2603041	-	Putative membrane carboxypeptidase
E04	CDS	4443120	4444073	STM14_5061	4443150	4444042	+	Putative methyl-accepting chemotaxis protein
E05	CDS	4676770	4678275	STM14_5312	4676800	4678244	+	Putative NAD-dependent aldehyde dehydrogenase
E06	CDS	4843244	4843933	STM14_5495	4843274	4843902	+	Putative outer membrane protein
E07	CDS	4838731	4839303	STM14_5491	4838761	4839272	+	Putative outer membrane protein
E08	CDS	1717819	1719021	STM14_1959	1717849	1718990	+	Putative oxidase
E09	CDS	2899686	2900279	STM14_3304	2899716	2900248	-	Putative periplasmic protein
E10	CDS	4690817	4692043	STM14_5325	4690847	4692012	-	Putative permease
E11	CDS	4441148	4441870	STM14_5058	4441178	4441839	+	Putative phage baseplate component
E12	CDS	4436356	4437471	STM14_5051	4436386	4437440	+	Putative phage baseplate protein
F01	CDS	4437462	4437821	STM14_5052	4437492	4437790	+	Putative phage baseplate protein
F02	CDS	782125	782691	STM14_0835	782155	782660	-	Putative phage integrase
F03	CDS	4433983	4435728	STM14_5049	4434013	4435697	+	Putative phage tail fiber protein H
F04	CDS	4444073	4446427	STM14_5062	4444103	4446396	+	Putative phage tail protein
F05	CDS	4435731	4436363	STM14_5050	4435761	4436332	+	Putative phage tail protein
F06	CDS	4447505	4448932	STM14_5066	4447535	4448901	+	Putative phage tail sheath protein
F07	CDS	2916104	2917645	STM14_3323	2916134	2917614	+	
F08	CDS	316755	316865	STM14_0322	316785	316834	-	Invasol SirA
F09	CDS	2909964	2910338	STM14_3316	2909994	2910307	-	Putative glucitol-specific PTS enzyme III
F10	CDS	2910335	2911315	STM14_3317	2910365	2911284	-	Putative glucitol-specific PTS enzyme III
F11	CDS	2909446	2909967	STM14_3315	2909476	2909936	-	Putative PTS system glucitol/sorbitol-specific enzyme II
F12	CDS	3998131	3998874	STM14_4564	3998161	3998843	+	Putative regulatory protein
G01	CDS	4450058	4450663	STM14_5070	4450088	4450632	+	Putative soluble lytic murein transglycosylase
G02	CDS	2914569	2915132	STM14_3321	2914599	2915101	+	Putative sugar phosphate aminotransferase
G03	CDS	2908598	2908954	STM14_3313	2908628	2908923	+	Putative transcriptional regulator
G04	CDS	635010	635864	STM14_0670	635040	635833	+	Putative PTS system mannose-specific enzyme IID
G05	CDS	636625	637095	STM14_0672	636655	637064	+	Putative PTS system mannose-specific enzyme IIAB
G06	CDS	637073	637522	STM14_0673	637103	637491	+	Putative PTS system mannose-specific enzyme IIAB
G07	CDS (LT2)[3]				4687631	4688619		
G08	CDS	4797299	4798213	STM14_5439	4797329	4798182	-	Restriction endonuclease
G10	CDS	4674147	4675583	STM14_5310	4674177	4675552	-	Sugar transporter
G11	CDS	190	255	STM14_0001	208	236	-	thr operon leader peptide
H01	CDS	340013	340288	STM14_0346	340043	340257	+	Hypothetical protein
H04	CDS	2899472	2899639	STM14_3303	2899502	2899608	-	Hypothetical protein
H07	CDS	3121988	3122278	STM14_3562	3122018	3122247	+	Hypothetical protein
H11	CDS	1315412	1315813	STM14_1466	1315442	1315782	-	Phage minor tail protein U
H12	CDS	2824132	2824290	STM14_3222	2824162	2824259	-	Hypothetical 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]Of the targeted genes, 22 CDSs and 22 sRNA were annotated in strain LT2 but not annotated in strain 14028s.