Product Information Sheet for NR-42830

Salmonella enterica subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_039/040_Kan

Catalog No. NR-42830

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-42830 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_039/040_Kan, NR-42830."

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_039/040_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	1221519	1222616	STM14_1352	1221549	1222585	-	Flagellar basal body P-ring protein
A02	CDS	1150703	1151506	STM14_1255	1150733	1151475	-	4-hydroxyphenylacetate catabolism
A03	CDS	1142797	1143207	STM14_1243	1142827	1143176	-	Hypothetical protein
A04	CDS	1217246	1217944	STM14_1347	1217276	1217913	-	Flagellar basal body rod modification protein
A05	CDS	1766256	1766642	STM14_2009	1766286	1766479	+	Putative inner membrane protein
A06	CDS	4018145	4019467	STM14_4591	4018175	4019436	-	D-serine dehydratase
A07	CDS	2153574	2154251	STM14_2509	2153604	2154220	+	Vitamin B12 biosynthetic protein
A08	CDS	595812	596705	STM14_0622	595842	596674	-	Carbamate kinase
A10	CDS	2028582	2030597	STM14_2336	2028612	2030560	+	Chemotaxis protein CheA
A11	CDS	3326544	3327038	STM14_3810	3326574	3327007	+	Hydrogenase 2 maturation endopeptidase
A12	CDS	2247819	2249036	STM14_2608	2247849	2249005	+	Glycosyl transferase
B02	CDS	4737564	4738562	STM14_5377	4737594	4738531	+	L-idonate regulator
B03	CDS	4215780	4216364	STM14_4803	4215810	4216276	+	Molybdopterin-guanine dinucleotide biosynthesis protein A
B04	CDS	2637473	2638516	STM14_3037	2637503	2638485	+	Putative periplasmic protein
B05	CDS	4786595	4787110	STM14_5428	4786625	4787079	-	Putative inner membrane protein
B06	CDS	441538	442083	STM14_0459	441568	442052	-	Shikimate kinase II
B07	CDS	4297086	4298621	STM14_4899	4297116	4298590	-	Putative ABC-type aldose transport system ATPase component
B08	CDS	2406413	2407282	STM14_2786	2406443	2407251	+	Quinol dehydrogenase membrane component
B09	CDS	4629446	4630129	STM14_5255	4629476	4630098	-	Putative cytoplasmic protein
B10	CDS	2163017	2163976	STM14_2522	2163047	2163945	+	CbiB
B11	CDS	4265360	4265683	STM14_4859	4265390	4265652	+	Putative inner membrane protein
B12	CDS (LT2)[3]				1973098	1973162		
C01	CDS	2598870	2599109	STM14_2992	2598900	2599078	+	Putative cytoplasmic protein
C02	CDS	2585099	2586355	STM14_2976	2585129	2586324	+	Xanthosine permease
C03	CDS	3851427	3851912	STM14_4401	3851457	3851881	+	Putative acetyltransferase
C04	CDS	577258	578076	STM14_0606	577288	578045	-	DNA-binding transcriptional repressor AllR
C05	CDS	2713839	2714468	STM14_3102	2713869	2714437	+	Putative anaerobic dimethylsulfoxide reductase
C06	CDS	1216830	1217234	STM14_1346	1216860	1217203	-	Flagellar basal body rod protein FlgC
C07	CDS	3741634	3742002	STM14_4284	3741664	3741971	+	Death-on-curing protein
C08	CDS	832259	833560	STM14_0892	832289	833529	-	Oxaloacetate decarboxylase beta chain

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C09	CDS	1127421	1128083	STM14_1222	1127451	1128052	+	Hypothetical protein
C10	CDS	3758540	3760183	STM14_4305	3758570	3760152	-	Methyl-accepting transmembrane citrate/phenol chemoreceptor
C11	CDS	3878512	3879327	STM14_4435	3878542	3879296	+	Putative regulatory protein
C12	CDS	4248403	4249305	STM14_4838	4248439	4249274	-	Putative sugar kinase
D01	CDS	296593	297507	STM14_0301	296623	297476	+	Putative transcriptional regulator
D02	CDS	4531757	4532428	STM14_5145	4531787	4532397	-	Putative formate-dependent nitrite reductase
D03	CDS	4039024	4040064	STM14_4617	4039054	4040033	+	Periplasmic sensory protein associated with the TorRS two-component regulatory system
D04	CDS	2175986	2176477	STM14_2537	2176016	2176446	-	Propanediol utilization protein
D05	CDS	2243188	2244153	STM14_2603	2243218	2244122	+	GDP-fucose synthetase
D06	CDS	2180288	2181643	STM14_2542	2180318	2181612	-	Polyhedral body protein
D07	CDS	2969544	2969882	STM14_3383	2969574	2969851	-	Hypothetical protein
D08	CDS	1986227	1986580	STM14_2286	1986257	1986549	+	Hypothetical protein
D09	CDS	1165487	1165882	STM14_1277	1165517	1165851	+	Putative periplasmic protein
D10	CDS	1150180	1150560	STM14_1253	1150210	1150529	-	4-hydroxyphenylacetate catabolism
D12	CDS	2032540	2033118	STM14_2340	2032570	2033093	+	Transcriptional activator FlhC
E01	CDS	2643176	2645014	STM14_3042	2643344	2644983	-	Nitrate/nitrite sensor protein NarQ
E02	CDS	4802915	4804576	STM14_5446	4802945	4804545	-	Methyl-accepting chemotaxis protein I
E04	CDS	3944848	3946533	STM14_4503	3944878	3946502	+	NAD-dependent DNA ligase LigB
E05	CDS	3766656	3769397	STM14_4314	3766686	3769315	+	Putative ABC-type multidrug transport system ATPase component
E06	CDS	4702290	4704203	STM14_5339	4702359	4704172	-	Phosphotransferase system mannitol/fructose-specific IIA component
E07	CDS	2581269	2581661	STM14_2968	2581299	2581630	-	Putative negative regulator
E08	CDS	2156762	2157553	STM14_2514	2156792	2157522	+	Cobalt-precorrin-6x reductase
E09	CDS	2175357	2175989	STM14_2536	2175387	2175958	-	Propanediol utilization protein
E10	CDS	3723857	3725197	STM14_4264	3723590	3723909	+	Low affinity gluconate transporter
E10	CDS	3723857	3725197	STM14_4264	3723590	3723909	+	Low affinity gluconate transporter
E11	CDS	3079685	3080947	STM14_3519	3079715	3080916	+	Putative tRNA synthase
E12	CDS	2454620	2455225	STM14_2833	2454650	2455194	+	Aluminum-inducible protein
F02	CDS	2241492	2242715	STM14_2601	2241522	2242684	+	Glycosyl transferase
F03	CDS	4294190	4295782	STM14_4896	4294220	4295751	+	Autoinducer-2 (AI-2) kinase
F04	CDS	4340886	4341965	STM14_4944	4340916	4341934	-	Putative fructose-like permease EIIC subunit 2
F05	CDS	1584396	1586831	STM14_1809	1584426	1586800	+	Putative dimethyl sulphoxide reductase
F06	CDS	1900159	1901007	STM14_2166	1900189	1900976	-	Putative hydrogenase-1 protein
F07	CDS	410859	411869	STM14_0423	410889	411838	-	Cytochrome BD2 subunit II
F08	CDS	3029189	3030037	STM14_3461	3029219	3030006	-	Putative permease
F09	CDS	3401008	3402528	STM14_3894	3401038	3402497	+	Aerotaxis sensor receptor
F10	CDS	4046948	4047637	STM14_4623	4046978	4047606	+	Galactonate operon transcriptional repressor
F11	CDS	4780111	4780572	STM14_5420	4780141	4780541	+	Hypothetical protein
F12	CDS	2402468	2402947	STM14_2779	2402498	2402916	+	Cytochrome c-type biogenesis protein CcmE
G01	CDS	4699301	4700434	STM14_5336	4699331	4700403	-	Dihydroorotase
G02	CDS	4859237	4860586	STM14_5511	4859267	4860555	-	Hypothetical protein
G03	CDS	480853	481713	STM14_0505	480883	481682	+	2-aminoethylphosphonate transporter
G04	CDS	1655468	1655611	STM14_1888	1655498	1655580	-	30S ribosomal subunit S22
G05	CDS	2438089	2438967	STM14_2816	2438119	2438936	-	Putative transcriptional regulator
G06	CDS	2172405	2174237	STM14_2532	2172435	2174206	-	Propanediol dehydratase reactivation protein
G07	CDS	2025140	2026006	STM14_2333	2025170	2025975	+	Chemotaxis methyltransferase CheR
G08	CDS	943024	943644	STM14_1020	943054	943613	-	Putative regulatory protein
G09	CDS	2182200	2182550	STM14_2544	2182230	2182519	-	Polyhedral body protein
G10	CDS	3151805	3152515	STM14_3593	3151835	3152484	-	DNA-binding transcriptional activator FucR
G11	CDS	940833	941648	STM14_1017	940863	941617	+	Putative hydrolase
G12	CDS	1215209	1215502	STM14_1342	1215239	1215471	+	Anti-sigma28 factor FlgM
H01	CDS	416780	418405	STM14_0429	416810	418374	+	prp operon regulator
H02	CDS	3539826	3540455	STM14_4050	3539856	3540379	+	Putative regulatory protein
H03	CDS	2838089	2838988	STM14_3241	2838119	2838957	+	Putative transcriptional regulator
H04	CDS	524049	524618	STM14_0549	524079	524587	-	Hypothetical protein
H05	CDS	723030	724709	STM14_0767	723060	724678	+	Putative heatshock protein
H06	CDS	515792	516847	STM14_0542	515822	516816	+	Putative cysteine synthase/cystathionine beta-synthase

Well Position	Gene Type	Gene Start	Gene End	Target Gene (Locus Tag)	Deleted Region Start	Deleted Region End	Gene Strand	Description
H07	CDS	3176617	3177180	STM14_3614	3176647	3177101	+	Hypothetical protein
H08	CDS	1051439	1051900	STM14_1134	1051469	1051869	+	Putative leucine response regulator
H09	CDS	3874654	3876150	STM14_4431	3874684	3876119	-	L-xylulose kinase
H10	CDS	1321447	1322052	STM14_1473	1321630	1322168	+	Hypothetical protein
H11	CDS	425858	428794	STM14_0438	425888	428763	-	Flagellar protein
H12	CDS	1144070	1145632	STM14_1247	1144100	1145601	+	4-hydroxyphenylacetate catabolism

[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.