Product Information Sheet for NR-29414

Salmonella enterica subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate 009/010_Cm

Catalog No. NR-29414

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 chloramphenicol-resistant mutant collection contains 3,376 mutants distributed among eleven 96-well plates. In these mutants, a single gene is replaced by a cassette conferring the chloramphenicol resistance gene, and includes 4 double mutants that contain both kanamycin and chloramphenicol cassettes. Deletions were confirmed by the depositor.[1,2] The parent strain *S. enterica* subsp. *enterica*, strain 14028s is available from BEI Resources as NR-12154.

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.

Material Provided:
Each inoculated well of the 96-well plate contains approximately 50 µL of culture in Luria Bertani (LB) broth containing 20 µg/mL chloramphenicol supplemented with 10% glycerol.

Packaging/Storage:
NR-29414 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 20 µg/mL chloramphenicol
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 009/010_Cm, NR-29414."

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. Andrews-Polymenis, H. and M. McClelland, 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.

Support Provided by NIAID

Table 1: *S. enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate 009/010_Cm[1,2]

Well Position	Deleted Region of Chromosome	Deletion Start	Deletion End	Locus Tag	14028S Gene Start	14028S Gene End	14028S Gene Strand	Description
A01	chr_14028S	17897	19936	STM14_0022	17867	19966	-	Putative exochitinase
A02	chr_14028S	95513	95743	STM14_0098	95483	95773	+	Putative secreted protein
A03	chr_14028S	236998	237777	STM14_0240	236968	237807	-	Putative outer membrane protein
A04	chr_14028S	339553	339930	STM14_0345	339523	339960	-	Putative cytoplasmic protein
A05	chr_14028S	374911	375477	STM14_0386	374881	375507	-	Putative 3-isopropylmalate isomerase
A06	chr_14028S	412236	413180	STM14_0425	412206	413210	-	Putative transcriptional regulator
A07	chr_14028S	571836	572606	STM14_0600	571806	572636	-	Putative ABC-type transport system ATPase component
A08	chr_14028S	632911	633837	STM14_0668	632881	633867	+	Putative phosphosugar isomerase
A09	chr_14028S	781964	782035	STM14_0834	781934	782065	-	Putative cytoplasmic protein
A11	chr_14028S[3]	1073599	1073991	STM14_1168	1073569	1074021	-	Lysozyme
A12	chr_14028S	1181307	1182353	STM14_1296	1181277	1182383	-	Putative dehydrogenase
B01	chr_14028S	32575	32964	STM14_0037	32545	32994	+	Putative transcriptional regulator
B02	chr_14028S	96013	96147	STM14_0099	95983	96177	-	Putative inner membrane protein
B03	chr_14028S	248685	249927	STM14_0252	248655	249957	-	
B04	chr_14028S	340816	341007	STM14_0348	340729	341037	-	Putative cytoplasmic protein
B05	chr_14028S	387855	389345	STM14_0400	387801	389375	-	Hypothetical protein
B06	chr_14028S	413386	415434	STM14_0426	413356	415464	-	Ferrioxamine receptor
B07	chr_14028S	572703	573659	STM14_0601	572673	573689	-	Putative ABC-type transport system ATPase component
B08	chr_14028S	650320	651396	STM14_0687	650290	651426	-	Ferric enterobactin transport protein FepE
B09	chr_14028S	783181	783456	STM14_0836	783151	783486	-	Putative inner membrane protein
B11	chr_14028S	1083594	1083932	STM14_1179	1083564	1083962	-	Minor tail protein
B12	chr_14028S	1190796	1191056	STM14_1312	1190766	1191086	-	Putative periplasmic protein
C04	chr_14028S	341169	341357					
C05	chr_14028S	391779	392360	STM14_0405	391749	392390	-	Putative response regulator
C06	chr_14028S	416399	416614	STM14_0428	416369	416644	-	Hypothetical protein
C07	chr_14028S	581806	582993	STM14_0610	581776	583023	-	Putative permease
C08	chr_14028S	676704	677003	STM14_0711	676674	677033	-	Molybdopterin-containing oxidoreductase iron-sulfur subunit
C09	chr_14028S	787863	788573	STM14_0841	787833	788603	-	Putative ABC transporter permease protein
C11	chr_14028S	1083989	1084258	STM14_1180	1083959	1084288	-	Minor tail protein
C12	chr_14028S	1203624	1204136	STM14_1326	1203594	1204166	+	Putative inner membrane protein
D01	chr_14028S	35369	37027	STM14_0041	35339	37057	-	Putative arylsulfatase
D02	chr_14028S	117083	117283					
D03	chr_14028S	314663	315145	STM14_0320	314633	315175	-	Putative cytoplasmic protein
D04	chr_14028S	341893	342114					
D05	chr_14028S	392431	392595	STM14_0406	392401	392625	-	Putative inner membrane protein

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Well Position	Deleted Region of Chromosome	Deletion Start	Deletion End	Locus Tag	14028S Gene Start	14028S Gene End	14028S Gene Strand	Description
D06	chr_14028S	428910	429473	STM14_0439	428880	429503	-	Putative DNA-binding transcriptional regulator
D07	chr_14028S	593758	594957	STM14_0620	593728	594987	-	Putative cytoplasmic protein
D08	chr_14028S	706303	707337	STM14_0752	706273	707367	-	Threonine-phosphate decarboxylase
D09	chr_14028S	834787	835806	STM14_0894	834757	835836	-	Putative ABC transport protein
D10	chr_14028S	1011993	1012259	STM14_1097	1011963	1012289	+	Putative cytoplasmic protein
D11	chr_14028S	1087813	1088250	STM14_1183	1087783	1088280	-	Attachment/invasion protein
D12	chr_14028S	1219233	1219928	STM14_1349	1219203	1219958	-	Flagellar basal body rod protein FlgF
E01	chr_14028S	42968	44155	STM14_0046	42932	44185	-	Putative cytoplasmic protein
E02	chr_14028S	189250	190032	STM14_0191	189220	190062	+	Putative restriction endonuclease
E04	chr_14028S	350608	351267	STM14_0360	350578	351297	+	SapA-like protein
E05	chr_14028S	399283	401511	STM14_0412	399253	401541	-	Putative cation transport ATPase
E06	chr_14028S	435438	436091	STM14_0449	435408	436121	-	Putative inner membrane protein
E08	chr_14028S	721566	722978	STM14_0766	721536	723008	-	Putative molecular chaperone
E09	chr_14028S	842190	843038	STM14_0902	842160	843068	+	Putative inner membrane protein
E10	chr_14028S	1052247	1053401	STM14_1136	1052217	1053431	-	Diaminopropionate ammonia-lyase
E11	chr_14028S	1091166	1093554	STM14_1188	1091136	1094486	-	Host specificity protein J
E12	chr_14028S	1220002	1220724	STM14_1350	1219972	1220754	-	Flagellar basal body rod protein FlgG
F01	chr_14028S	44342	45997	STM14_0047	44312	46027	-	Putative arylsulfatase
F02	chr_14028S	190935	191828	STM14_0193	190905	191858	-	2-keto-3-deoxygluconate permease
F03	chr_14028S	323135	323848	STM14_0331	323105	323878	-	Putative inner membrane protein
F04	chr_14028S	359789	360861	STM14_0370	359759	360891	-	
F05	chr_14028S	401583	401987	STM14_0413	401553	402017	-	Putative transcriptional regulator
F06	chr_14028S	438852	439904	STM14_0455	438822	439934	-	Diguanylate cyclase AdrA
F07	chr_14028S[4]	614474	614792	STM14_0648	614444	615111	+	Pseudogene
F08	chr_14028S	724815	725993	STM14_0768	724785	726023	+	Putative cytoplasmic protein
F09	chr_14028S	876669	877169	STM14_940	876639	877199	-	Putative inner membrane protein
F11	chr_14028S	1154706	1155584	STM14_1259	1154676	1155614	-	Putative periplasmic protein
F12	chr_14028S	1231439	1232122	STM14_1360	1231409	1232152	-	Putative inner membrane lipoprotein
G01	chr_14028S	66684	66863	STM14_0066	66654	66893	+	Oxaloacetate decarboxylase subunit gamma
G02	chr_14028S	191885	193096	STM14_0194	191855	193126	-	Putative inner membrane protein
G03	chr_14028S	324576	328385	STM14_0333	324546	328415	-	Putative inner membrane protein
G04	chr_14028S	372124	373296	STM14_0384	371989	373326	-	Putative permease
G05	chr_14028S	409271	409312	STM14_0421	409241	409342	-	Putative cytoplasmic protein
G06	chr_14028S	442582	443199	STM14_0461	442552	443229	-	Hypothetical protein
G07	chr_14028S	623422	624537	STM14_0662	623392	624567	+	Putative DNA repair ATPase
G08	chr_14028S	762040	762465	STM14_0816	762010	762495	+	Putative cytoplasmic protein
G09	chr_14028S	955608	956891	STM14_1035	955578	956921	-	Ascorbate-specific PTS system enzyme IIC
G10	chr_14028S	1066379	1066666	STM14_1153	1066349	1066696	-	Hypothetical protein
G11	chr_14028S	1161435	1161881	STM14_1269	1161405	1161911	-	Suppression of copper sensitivity protein
G12	chr_14028S	1339471	1339650	STM14_1492	1339441	1339680	+	Macrophage survival protein
H01	chr_14028S[5]	68667	69650	STM14_0069	68637	69680	-	Putative citrate lyase synthetase
H02	chr_14028S	193173	194069	STM14_0195	193116	194099	-	4-hydroxythreonine-4-phosphate dehydrogenase 2
H03	chr_14028S	338544	338930	STM14_0342	338514	338960	-	Putative cytoplasmic protein
H04	chr_14028S	373488	374849	STM14_0385	373458	374879	-	Isopropylmalate isomerase large subunit
H05	chr_14028S	411916	412080	STM14_0424	411886	412110	-	Putative cytoplasmic protein
H06	chr_14028S	525301	526791	STM14_0552	525271	526821	+	Hypothetical protein
H07	chr_14028S	627917	629827	STM14_0666	627887	629857	-	Outer membrane esterase
H08	chr_14028S	770814	772802	STM14_0824	770784	772832	+	Potassium-transporting ATPase subunit B
H09	chr_14028S	956981	957241	STM14_1036	956951	957271	-	Putative inner membrane protein
H10	chr_14028S	1071253	1071318	STM14_1164	1071223	1071348	-	Hypothetical protein
H11	chr_14028S	1173650	1174471	STM14_1285	1173620	1174501	+	Putative transcriptional regulator
H12	chr_14028S	1339901	1340362	STM14_1493	1339871	1340392	+	Putative envelope 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]Alternative deleted region: 2810993 – 2811385
[4]Deleted region also overlaps STM14_0647 (1.1%)
[5]Deleted region also overlaps STM14_0068 (3.1%)

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