Product Information Sheet for NR-29410

Salmonella enterica subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate 001/002_Cm

Catalog No. NR-29410

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-29410 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 001/002_Cm, NR-29410."

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. 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 001/002_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
A07	chr_14028S	48402	50381	STM14_0050	48372	50411	+	Putative glycosyl hydrolase
A09	chr_14028S	347689	348450	STM14_0356	347659	348480	-	Putative xylanase/chitin deacetylase
A10	chr_14028S	2277429	2278253	STM14_2631	2277399	2278283	-	Putative cytoplasmic protein
A12	chr_14028S	3731328	3732029	STM14_4273	3731298	3732059	-	Putative cytoplasmic protein
B07	chr_14028S	50481	51794	STM14_0051	50451	51824	+	Putative sodium galactoside symporter
B08	chr_14028S	319394	319819	STM14_0327	319364	319849	-	Putative cytoplasmic protein
B09	chr_14028S	466031	466336	STM14_0484	466001	466366	-	Hypothetical protein
B10	chr_14028S	2278313	2278969	STM14_2632	2278283	2278999	-	Putative inner membrane protein
B11	chr_14028S	3215653	3216399	STM14_3667	3215623	3216429	-	Putative inner membrane protein
B12	chr_14028S	3732143	3733054	STM14_4274	3732113	3733084	-	Putative inner membrane protein
C07	chr_14028S	58976	60118	STM14_0060	58946	60148	-	Putative nitrite reductase
C08	chr_14028S[3]	318206	320422	STM14_0328	319916	320452	-	Putative outer membrane lipoprotein
C09	chr_14028S	466422	467054	STM14_0485	466392	467084	-	Putative regulatory protein
C10	chr_14028S	2279040	2281091	STM14_2633	2279010	2281121	-	Putative inner membrane protein
C11	chr_14028S	3438763	3439650	STM14_3936	3438733	3439680	+	Putative sugar kinase
C12	chr_14028S	3733111	3734085	STM14_4275	3733081	3734115	-	Putative phosphotriesterase
D07	chr_14028S	305395	306390	STM14_0313	305365	306420	+	Putative cytoplasmic protein
D08	chr_14028S	320486	321769	STM14_0329	320456	321799	-	Putative cytoplasmic protein
D09	chr_14028S	617161	618027	STM14_0651	617131	618057	+	Putative glycosyltransferase
D10	chr_14028S	2378409	2378969	STM14_2751	2378379	2378999	+	Putative inner membrane protein
D11	chr_14028S	3439721	3440494	STM14_3937	3439691	3440524	+	AGA operon transcriptional repressor
D12	chr_14028S	3852395	3852802	STM14_4404	3852365	3852832	+	Putative acetyltransferase
E08	chr_14028S	321826	323070	STM14_0330	321796	323100	-	Hypothetical protein
E09	chr_14028S	712521	712751	STM14_0757	712491	712781	-	Putative hydrolase
E10	chr_14028S	2473267	2474988	STM14_2853	2473237	2475018	+	Hypothetical protein
E11	chr_14028S	3440888	3441682	STM14_3939	3440858	3441712	-	Tagatose-bisphosphate aldolase
E12	chr_14028S	3894950	3899275	STM14_4450	3894920	3899305	-	Putative inner membrane protein
F07	chr_14028S	309352	309786	STM14_0316	309322	309816	+	Putative cytoplasmic protein
F08	chr_14028S	343544	344197	STM14_0353	343514	344227	-	Putative fimbrial assembly chaperone
F09	chr_14028S	714050	714943	STM14_0759	714020	714973	-	2-keto-3-deoxygluconate permease
F10	chr_14028S	2539450	2540046	STM14_2926	2539420	2540076	+	Putative inner membrane protein
F12	chr_14028S	4080850	4082547	STM14_4655	4080820	4082577	-	Putative dipeptide/oligopeptide/nickel ABC-type transport system periplasmic component
G08	chr_14028S	344281	346731	STM14_0354	344251	346761	-	Putative fimbrial usher
G09	chr_14028S	715071	716939	STM14_0760	715041	716969	-	Putative sigma-54 dependent transcriptional regulator
G12	chr_14028S	4164658	4164987	STM14_4745	4164628	4165017	+	Putative inner membrane protein
H07	chr_14028S	310654	311496	STM14_0318	310624	311526	+	Putative cytoplasmic protein
H08	chr_14028S	346813	347223	STM14_0355	346783	347253	-	Putative fimbrial subunit

Well Position	Deleted Region of Chromosome	Deletion Start	Deletion End	Locus Tag	14028S Gene Start	14028S Gene End	14028S Gene Strand	Description
H09	chr_14028S	1640412	1641818	STM14_1877	1640382	1641848	+	Putative coiled-coil protein
H10	chr_14028S	2874894	2875439	STM14_3285	2874864	2875469	-	Putative cytoplasmic protein
H11	chr_14028S	3643059	3648893	STM14_4188	3643029	3648923	-	Putative surface-exposed virulence protein
H12	chr_14028S	4253263	4253631	STM14_4844	4253233	4253661	+	Putative cytoplasmic 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: 319991 - 320422