Salmonella enterica subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate 005/006_Cm

Catalog No. NR-29412

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]

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.

Plate orientation and viability were confirmed for NR-29412.

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-29412 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 1 day.

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 005/006_Cm, NR-29412."

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 005/006_Cm[1,2]

Well Position	Deleted Region of Chromosome or Plasmid	Deletion Start	Deletion End	Locus Tag	14028S Gene Start	14028S Gene End	14028S Gene Strand	Description
A01	plasmid_14028S	7933	8085	STM14_5538	7903	8115	+	Plasmid-encoded fimbriae; regulatory
A02	plasmid_14028S	77654	77887	STM14_5616	77624	77917	-	Conjugative transfer: fimbrial synthesis
A03	chr_14028S[3]	30024	30482	STM14_0033	29994	30512	-	Fimbrial subunit
A05	chr_14028S	342936	343376	STM14_0352	342894	343406	-	Putative outer membrane protein
A06	chr_14028S	387031	387271	STM14_0399	387001	387501	-	Putative periplasmic protein
A07	chr_14028S	604854	605336	STM14_0635	604824	605366	-	Fimbrin
A09	chr_14028S	908343	909002	STM14_979	908136	909032	-	Putative inner membrane protein
A10	chr_14028S	1053706	1055082	STM14_1138	1053676	1055112	-	Putative transcriptional regulator
A11	chr_14028S	1186217	1186990	STM14_1303	1186187	1187020	+	Putative curli operon transcriptional regulator
A12	chr_14028S	1351726	1352478	STM14_1517	1351696	1352508	-	Putative ABC transporter protein
B01	plasmid_14028S	8577	9392	STM14_5539	8547	9422	+	Putative outer membrane protein
B02	chr_14028S	15044	15931	STM14_0016	15014	15961	-	Putative transcriptional regulator
B03	chr_14028S[4]	30508	31179	STM14_0034	30478	31209	-	Fimbrial chaperone
B04	chr_14028S	231391	231891	STM14_0234	231361	231921	-	Putative fimbrial subunit
B05	chr_14028S	349325	350212	STM14_0358	349295	350242	-	Transcriptional regulator
B06	chr_14028S	389505	390188	STM14_0401	389475	390218	-	Putative response regulator
B07	chr_14028S	605472	605945	STM14_0636	605442	605975	-	Fimbrial protein
B08	chr_14028S	641532	642176	STM14_0681	641502	642206	+	Phosphopantetheinyltransferase component of enterobactin synthase multienzyme complex
B10	chr_14028S[5]	1072324	1072776	STM14_1166	1072294	1072980	+	Bacteriophage virulence determinant
B11	chr_14028S	1187520	1187855	STM14_1305	1187490	1187885	+	Curli assembly protein CsgE
B12	chr_14028S	1368291	1369724	STM14_1553	1368261	1369754	+	Putative methyl-accepting chemotaxis protein
C01	plasmid_14028S	9705	10202	STM14_5540	9675	10232	+	Putative outer membrane protein
C02	chr_14028S	17073	17456	STM14_0019	17043	17486	+	Hypothetical protein
C03	chr_14028S	31304	32089	STM14_0035	31274	32119	-	Putative thiol-disulfide isomerase
C04	chr_14028S	232037	234634	STM14_0235	232007	234664	-	Putative fimbrial outer membrane usher
C05	chr_14028S	365243	365584	STM14_0375	365213	365614	-	DNA-binding transcriptional regulator Crl
C06	chr_14028S	390221	390649	STM14_0402	390191	390679	-	Putative inner membrane protein
C07	chr_14028S	606049	606681	STM14_0637	606019	606711	-	Periplasmic chaperone
C08	chr_14028S	673875	674375	STM14_0709	673845	674405	-	Putative anaerobic dehydrogenase component
C09	chr_14028S[6]	927696	928490	STM14_1002	927666	928520	-	Putative electron transfer protein beta subunit
C10	chr_14028S	1088427	1088900	STM14_1184	1088397	1088930	+	Superoxide dismutase precursor
C11	chr_14028S	1187920	1188510	STM14_1306	1187890	1188540	+	DNA-binding transcriptional regulator CsgD
C12	chr_14028S[7]	1390224	1390439	STM14_1578	1390194	1390469	-	Putative periplasmic protein
D01	plasmid_14028S	13598	14056	STM14_5543	13568	14086	+	Plasmid-encoded fimbriae; major fimbrial subunit
D02	chr_14028S	23365	24009	STM14_0025	23335	24039	-	Putative cytoplasmic protein
D03	chr_14028S	33394	34338	STM14_0038	33364	34368	-	Putative transcriptional regulator
D04	chr_14028S	234712	235404	STM14_0236	234682	235434	-	Putative periplasmic fimbrial chaperone
D05	chr_14028S	379251	379589	STM14_0391	379221	379619	-	Putative outer membrane protein

Well Position	Deleted Region of Chromosome or Plasmid	Deletion Start	Deletion End	Locus Tag	14028S Gene Start	14028S Gene End	14028S Gene Strand	Description
D06	chr_14028S	490979	492430	STM14_0517	490949	492460	-	Putative periplasmic protein
D07	chr_14028S	606772	609324	STM14_0638	606742	609354	-	Outer membrane usher protein precursor
D08	chr_14028S	696559	697452	STM14_0739	696529	697482	+	Putative DNA-binding transcriptional regulator
D09	chr_14028S	928561	929448	STM14_1003	928531	929478	-	Putative electron transfer protein alpha subunit
D10	chr_14028S	1100657	1100884	STM14_1196	1100627	1100926	+	Hypothetical protein
D11	chr_14028S	1189822	1190217	STM14_1310	1189792	1190247	-	Cryptic curlin major subunit
D12	chr_14028S	1397259	1398167	STM14_1586	1397229	1398197	-	Succinylglutamate desuccinylase
E01	plasmid_14028S	14391	14633	STM14_5544	14361	14663	+	Plasmid-encoded fimbriae; regulation
E02	chr_14028S	25142	25768	STM14_0029	24971	25798	-	Fimbrial chaperone
E03	chr_14028S	38803	39504	STM14_0043	38773	39534	+	Putative outer membrane/exported protein
E04	chr_14028S	235483	235935	STM14_0237	235453	235965	-	Putative minor fimbrial subunit
E06	chr_14028S	492712	493935	STM14_0518	492472	493965	-	TPR repeat-containing protein
E07	chr_14028S	610416	610874	STM14_0640	610386	610904	-	Putative fimbrial protein
E08	chr_14028S	825290	825775	STM14_0885	825260	825805	+	Fumarate hydratase
E09	chr_14028S	929802	930905	STM14_1005	929772	930935	-	Putative acyl-CoA dehydrogenase
E10	chr_14028S	1100941	1101567	STM14_1197	1100911	1101597	+	Hypothetical protein
E11	chr_14028S	1190339	1190605	STM14_1311	1190309	1190635	-	Putative autoagglutination protein
E12	chr_14028S	1418537	1419331	STM14_1614	1418507	1419361	-	Putative DNA/RNA non-specific endonuclease
F01	plasmid_14028S	31449	32282	STM14_5564	31419	32312	+	Regulation of spv operon, lysR family
F02	chr_14028S	25833	28394	STM14_0030	25803	28424	-	Fimbrial usher
F04	chr_14028S	235992	236408	STM14_0238	235962	236438	-	Putative minor fimbrial subunit
F05	chr_14028S[8]	380420	381685	STM14_0393	380390	381715	+	Putative fimbrial usher
F06	chr_14028S	557688	558455	STM14_0585	557658	558485	+	Putative periplasmic protein
F07	chr_14028S	612216	612878	STM14_0642	612186	612908	+	Putative regulatory protein
F08	chr_14028S	825850	826635	STM14_0886	825820	826665	+	Fumarate hydratase
F09	chr_14028S	931414	933039	STM14_1007	931384	933069	-	Putative dehydrogenase
F10	chr_14028S	1174816	1176252	STM14_1287	1174786	1176282	+	Putative sodium/glucose cotransporter
F12	chr_14028S	1445743	1446573	STM14_1646	1445713	1446603	-	Putative transcriptional regulator
G01	plasmid_14028S	45044	45958	STM14_5576	45011	45988	-	Plasmid partition protein B
G02	chr_14028S	28455	29402	STM14_0031	28425	29432	-	Fimbrial subunit
G04	chr_14028S	236468	236938	STM14_0239	236438	236968	-	Putative minor fimbrial subunit
G05	chr_14028S	381750	384257	STM14_0394	381720	384287	+	Putative fimbrial usher
G06	chr_14028S	579985	580701	STM14_0608	579955	580731	-	Hydroxypyruvate isomerase
G07	chr_14028S	612957	613208	STM14_0643	612927	613238	+	Hypothetical protein
G08	chr_14028S	875376	875969	STM14_938	875292	875999	-	Putative integral membrane protein
G09	chr_14028S	933105	933935	STM14_1008	933075	933965	+	Putative transcriptional regulator
G11	chr_14028S	1342256	1342753	STM14_1501	1342226	1342783	-	Virulence membrane protein PAGC precursor
G12	chr_14028S	1449623	1450321	STM14_1649	1449593	1450351	+	3-dehydroquinate dehydratase
H01	plasmid_14028S	62640	62942	STM14_5597	62610	62972	-	Conjugative transfer: fimbrial subunit
H02	chr_14028S	29463	29948	STM14_0032	29433	29978	-	Fimbrial subunit
H03	chr_14028S	208387	209010	STM14_0208	208357	209040	+	Putative fimbrial chaperone
H05	chr_14028S	385124	385600	STM14_0396	385094	385630	+	Putative fimbrial major subunit
H07	chr_14028S	613430	613966	STM14_0644	613400	613996	+	Putative fimbrial protein
H08	chr_14028S	876059	876601	STM14_939	876023	876631	-	Putative inner membrane protein
H10	chr_14028S	1180016	1181236	STM14_1295	1179986	1181266	-	Putative sialic acid transporter
H11	chr_14028S	1348132	1348386	STM14_1513	1348102	1348416	+	Putative outer membrane lipoprotein
H12	chr_14028S	1452958	1454109	STM14_1654	1452928	1454139	+	Putative transport 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]Deleted region also overlaps STM14_0034 (0.7%)
[4]Deleted region also overlaps STM14_0033 (1.0%)
[5]Alternative deleted region: 2812208 - 2812660
[6]Deleted region also overlaps STM14_1001 (10.3%)
[7]Deleted region also overlaps STM14_1579 (2.6%)
[8]Deleted region also overlaps STM14_0392 (0.7%)

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