

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

Catalog No. NR-29401

For research use only. Not for use in humans.

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 3,517 mutants distributed among eleven 96-well plates. In these mutants, 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} 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 pCLF4 to generate a gene-specific transcript from the *Salmonella* genome directly downstream of each mutant.^{2,3,4} 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 60 µg/mL kanamycin supplemented with 10% glycerol.

Packaging/Storage:

NR-29401 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 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_Kan, NR-29401."

Biosafety Level: 1

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. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; 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.

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Table 1: *S. enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate 005/006_Kan^{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
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
B09	chr 14028S	918231	919112	STM14_990	918201	919142	-	L-asparaginase
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
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

Well Position	Deleted Region of Chromosome or Plasmid	Deletion Start	Deletion End	Locus Tag	14028S Gene Start	14028S Gene End	14028S Gene Strand	Description
C12	chr 14028S ⁶	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
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
E05	chr 14028S ⁷	379696	380394	STM14_0392	379666	380424	+	Putative fimbrial chaperone
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
F03	chr 14028S	90212	91093	STM14_0091	90182	91123	-	Putative electron transfer flavoprotein FixB
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
G10	chr 14028S	1179041	1179673	STM14_1294	1179011	1179703	-	Putative outer membrane protein
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
H04	chr 14028S	324111	324482	STM14_0332	324081	324512	-	Putative Shiga-like toxin A subunit
H05	chr 14028S	385124	385600	STM14_0396	385094	385630	+	Putative fimbrial major subunit
H06	chr 14028S	580862	581680	STM14_0609	580832	581710	-	Tartronic semialdehyde reductase
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
H09	chr 14028S	934260	934625	STM14_1009	934230	934655	+	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

¹All information in this table was provided by the depositor at the time of deposition.

²Construction 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.

³Deleted region also overlaps STM14_0034 (0.7%)

⁴Deleted region also overlaps STM14_0033 (1.0%)

⁵Alternative deleted region: 2812208 – 2812660

⁶Deleted region also overlaps STM14_1579 (2.6%)

⁷Deleted region also overlaps STM14_0393 (0.4%)

⁸Deleted region also overlaps STM14_0392 (0.7%)