

***Salmonella enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate 025/026_Cm**

Catalog No. NR-29420

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-29420 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 025/026_Cm, NR-29420."

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/bmb15/index.htm.

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References:

1. Andrews-Polymeris, 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 025/026_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 |
|---------------|------------------------------|----------------|--------------|------------|-------------------|-----------------|--------------------|----------------------------------|
| A02 | chr_14028S ³ | 4617335 | 4617394 | | | | | |
| A03 | chr_14028S ³ | 4771073 | 4771252 | | | | | |
| A07 | chr_14028S | 3136539 | 3136870 | | | | | |
| A09 | chr_14028S | 2080073 | 2080128 | | | | | |
| A11 | chr_14028S ³ | 4400118 | 4400228 | | | | | |
| A12 | chr_14028S ³ | 3065172 | 3065240 | | | | | |
| B01 | chr_14028S ⁴ | 4011747 | 4011840 | STM14_4582 | 4011777 | 4011950 | - | Hypothetical protein |
| B02 | chr_14028S ³ | 2987107 | 2987154 | | | | | |
| B05 | chr_14028S ³ | 4356477 | 4356549 | | | | | |
| B07 | chr_14028S | 1454819 | 1454915 | | | | | |
| B09 | chr_14028S | 129286 | 129512 | STM14_0130 | 129166 | 129492 | + | Hypothetical protein |
| B11 | chr_14028S | 943615 | 943711 | STM14_1020 | 943024 | 943644 | - | Putative regulatory protein |
| C01 | chr_14028S ³ | 1739629 | 1739704 | | | | | |
| C02 | chr_14028S | 1979467 | 1979590 | STM14_2277 | 1979524 | 1979643 | + | Putative cytoplasmic protein |
| C03 | chr_14028S | 2282644 | 2282798 | STM14_2635 | 2282708 | 2282821 | + | Hypothetical protein |
| C04 | chr_14028S ³ | 2726444 | 2726769 | | | | | |
| C05 | chr_14028S ³ | 3190141 | 3190243 | | | | | |
| C06 | chr_14028S ³ | 3242063 | 3242193 | | | | | |
| C07 | chr_14028S | 3380592 | 3380732 | STM14_3875 | 3380454 | 3380651 | - | Hypothetical protein |
| C08 | chr_14028S ³ | 3729136 | 3729184 | | | | | |
| C09 | chr_14028S ⁵ | 4222626 | 4222748 | STM14_4810 | 4222650 | 4222715 | - | Spot 42 RNA |
| C11 | chr_14028S ³ | 1233257 | 1233391 | | | | | |
| D01 | chr_14028S | 3410330 | 3410500 | STM14_3904 | 3410298 | 3410435 | - | Hypothetical protein |
| D02 | chr_14028S ³ | 3504277 | 3504327 | | | | | |
| D03 | chr_14028S | 4155360 | 4155532 | STM14_4733 | 4155257 | 4155382 | - | Hypothetical protein |
| D04 | chr_14028S | 4518484 | 4518553 | STM14_5129 | 4518503 | 4518631 | + | Hypothetical protein |
| D05 | chr_14028S | 127454 | 127548 | STM14_0128 | 127427 | 127546 | + | Putative lipoprotein |
| D06 | chr_14028S ³ | 556676 | 556768 | | | | | |
| D07 | chr_14028S ³ | 729269 | 729396 | | | | | |
| D08 | chr_14028S | 2759226 | 2759432 | STM14_3144 | 2759420 | 2759548 | - | Hypothetical protein |
| D10 | chr_14028S | 4828403 | 4828646 | STM14_5479 | 4828502 | 4828636 | - | Hypothetical protein |
| E01 | chr_14028S | 529101 | 529415 | STM14_0558 | 529071 | 529445 | + | Hypothetical protein |
| E02 | chr_14028S | 4475061 | 4475213 | STM14_5097 | 4475031 | 4475243 | - | Putative stress-response protein |
| E04 | chr_14028S | 1948354 | 1948437 | STM14_2226 | 1948324 | 1948467 | + | Hypothetical protein |
| E05 | chr_14028S | 4216464 | 4216673 | STM14_4804 | 4216434 | 4216703 | - | Putative cytoplasmic protein |
| E06 | chr_14028S | 1922387 | 1922620 | STM14_2192 | 1922357 | 1922689 | + | Putative cytoplasmic protein |
| E07 | chr_14028S | 1837126 | 1837572 | STM14_2093 | 1837096 | 1837602 | - | Putative cytoplasmic protein |
| E08 | chr_14028S ⁶ | 3595526 | 3595735 | STM14_4114 | 3595496 | 3595765 | + | Putative cytoplasmic protein |

| Well Position | Deleted Region of Chromosome | Deletion Start | Deletion End | Locus Tag | 14028S Gene Start | 14028S Gene End | 14028S Gene Strand | Description |
|---------------|------------------------------|----------------|--------------|------------|-------------------|-----------------|--------------------|---|
| E09 | chr_14028S | 3225571 | 3225777 | STM14_3679 | 3225541 | 3225807 | + | Hypothetical protein |
| E10 | chr_14028S | 4428462 | 4428941 | STM14_5039 | 4428432 | 4428971 | - | Putative cytoplasmic protein |
| E11 | chr_14028S | 3611811 | 3611945 | STM14_4147 | 3611781 | 3611975 | + | Bacterioferritin-associated ferredoxin |
| E12 | chr_14028S | 1570258 | 1570560 | STM14_1791 | 1570228 | 1570590 | - | Multidrug efflux system protein MdtJ |
| F02 | chr_14028S | 1942711 | 1943121 | STM14_2215 | 1942681 | 1943139 | - | Hypothetical protein |
| F03 | chr_14028S | 2209540 | 2210646 | STM14_2574 | 2209510 | 2210676 | + | UDP-glucose/GDP-mannose dehydrogenase |
| F04 | chr_14028S ⁷ | 3270489 | 3270728 | STM14_3732 | 3270459 | 3270758 | + | Putative inner membrane protein |
| F05 | chr_14028S | 302092 | 302787 | STM14_0306 | 302062 | 302817 | + | Hydroxyacylglutathione hydrolase |
| F06 | chr_14028S | 2303596 | 2303994 | STM14_2659 | 2303566 | 2304024 | - | Putative lipoprotein |
| F07 | chr_14028S | 2090660 | 2090812 | STM14_2420 | 2090630 | 2090842 | + | Putative cold-shock protein |
| F08 | chr_14028S | 539659 | 539766 | STM14_0565 | 539629 | 539796 | + | Hypothetical protein |
| F09 | chr_14028S | 4222677 | 4222706 | | | | | |
| F10 | chr_14028S | 527076 | 527276 | STM14_0553 | 527046 | 527306 | - | 50S ribosomal protein L31 type B |
| F11 | chr_14028S | 4717885 | 4717965 | STM14_5355 | 4717965 | 4718453 | + | Putative arginine repressor |
| G01 | chr_14028S | 1979554 | 1979613 | STM14_2277 | 1979524 | 1979643 | + | Putative cytoplasmic protein |
| G03 | chr_14028S | 2565379 | 2565561 | STM14_2951 | 2565349 | 2565591 | + | Putative inner membrane protein |
| G04 | chr_14028S | 3228006 | 3228257 | STM14_3683 | 3227976 | 3228287 | + | Hypothetical protein |
| G05 | chr_14028S | 3339017 | 3339094 | STM14_3826 | 3338987 | 3339136 | + | Putative inner membrane protein |
| G07 | chr_14028S | 3611262 | 3611678 | STM14_4146 | 3611232 | 3611708 | + | Bacterioferritin, iron storage and detoxification protein |
| G08 | chr_14028S | 3270063 | 3270134 | STM14_3730 | 3270033 | 3270164 | + | Putative inner membrane protein |
| G09 | chr_14028S | 4373336 | 4373380 | STM14_4976 | 4373306 | 4373410 | + | Pseudogene |
| G10 | chr_14028S | 2078862 | 2079425 | STM14_2403 | 2078832 | 2079455 | - | Colanic acid capsular biosynthesis activation protein A |
| G11 | chr_14028S | 4627316 | 4627660 | STM14_5252 | 4627286 | 4627690 | - | Putative cytoplasmic protein |
| G12 | chr_14028S | 3543485 | 3543697 | STM14_4057 | 3543455 | 3543727 | + | Putative cytoplasmic protein |
| H01 | chr_14028S | 1947204 | 1947287 | STM14_2221 | 1947174 | 1947317 | + | Putative cytoplasmic protein |
| H02 | chr_14028S | 693441 | 693590 | STM14_0732 | 693411 | 693620 | - | Cold shock protein CspE |
| H03 | chr_14028S | 280781 | 280981 | STM14_0279 | 280751 | 281011 | + | Rho-binding antiterminator |
| H04 | chr_14028S | 528855 | 529013 | STM14_0557 | 528825 | 529043 | + | Hemolysin expression-modulating protein |
| H05 | chr_14028S ⁸ | 797724 | 798050 | STM14_0850 | 797694 | 798080 | - | Putative inner membrane protein |
| H06 | chr_14028S | 3849581 | 3849811 | STM14_4398 | 3849551 | 3849841 | - | Putative transcriptional regulator |
| H07 | chr_14028S | 1204459 | 1204512 | STM14_1328 | 1204429 | 1204542 | + | Putative inner membrane protein |
| H09 | chr_14028S | 2965965 | 2966063 | STM14_3373 | 2965935 | 2966093 | + | Putative transport protein |
| H10 | chr_14028S | 3031234 | 3031929 | STM14_3463 | 3031204 | 3031959 | + | Transcriptional regulator |

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.

³sRNA targeted

⁴Deleted region also overlaps STM14_4581 (14.1%)

⁵Deleted region also overlaps STM14_4811 (28.5%)

⁶Deleted region also overlaps STM14_4113 (8.5%)

⁷Deleted region also overlaps STM14_3731 (6.8%)

⁸Deleted region also overlaps STM14_0849 (0.9%)