

***Salmonella enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Multiple-Gene Deletion Mutant Library, Plate MGD\_073/074\_Kan**

**Catalog No. NR-42862**

**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 multiple-gene deletion (MGD) mutant libraries contain mutants with deletions of contiguous regions of three or more genes, covering a total of 3,476 genes, between two collections of mutants differentiated by kanamycin or chloramphenicol resistance.<sup>1,2</sup> The kanamycin-resistant MGD mutant collection contains 198 mutants spanning 2,543 genes distributed among 5 96-well plates, in which each deleted region is replaced by a cassette conferring the kanamycin resistance gene. Deletions were confirmed by the depositor.<sup>1,2</sup>

Genes were targeted for deletion by primers designed to preserve the first and last 60 bases of each deleted gene.<sup>2</sup> 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.<sup>2-4</sup> Detailed information about each mutant is shown in Table 1.

**Note:** The strain designation on the plate, strain CDC 6516-60, is incorrect. The correct strain designation is strain 14028s. *S. enterica* subsp. *enterica*, strain 14028s was originally known as strain 14028. A variant of the original strain with a rough colony morphology was designated 14028r and the original smooth strain was renamed 14028s. Strain 14028 is a descendent of strain CDC 6516-60, which was isolated from pools of hearts and livers of 4-week-old chickens.<sup>5</sup> The complete genome of *S. enterica* subsp. *enterica*, strain 14028s (GenBank: [CP001363.1](#)) and plasmid (GenBank: [CP001362.1](#)) sequences are available.

Plate orientation and viability were confirmed for NR-42862.

**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-42862 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) Multiple-Gene Deletion Mutant Library, Plate MGD\_073/074\_Kan, NR-42862.”

**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](http://www.cdc.gov/biosafety/publications/bmbl5/index.htm).

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**References:**

1. McClelland, M., 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.
5. Jarvik, T., et al. "Short-Term Signatures of Evolutionary Change in the *Salmonella enterica* Serovar Typhimurium 14028 Genome." *J. Bacteriol.* 192 (2010): 560-567. PubMed: 19897643.

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**Table 1: *S. enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Multiple-Gene Deletion Mutant Library, Plate MGD\_073/074\_Kan<sup>1,2</sup>**

Well Position	Target Gene Region (Locus Tag)	Deleted Region Start	Deleted Region End
A01	STM14_3064_to_STM14_3073	2664685	2673747
A03	STM14_3125_to_STM14_3128	2737011	2740808
A05	STM14_3156_to_STM14_3151	2768207	2773426
A06	STM14_3244_to_STM14_3248	2840517	2846297
A07	STM14_3369_to_STM14_3390	2963320	2975754
A09	STM14_3436_to_STM14_3417	2995164	3010826
A11	STM14_3587_to_STM14_3572	3134961	3146049
A12	STM14_3603_to_STM14_3594	3152649	3160588
B02	STM14_3667_to_STM14_3641	3195686	3216352
B03	STM14_3748_to_STM14_3752	3282311	3286480
B05	STM14_3824_to_STM14_3816	3332278	3338176
B06	STM14_3827_to_STM14_3831	3339321	3343474
B08	STM14_3861_to_STM14_3868	3368582	3375856
B10	STM14_3899_to_STM14_3893	3399130	3407666
C03	STM14_4258_to_STM14_4250	3707714	3721903
C05	STM14_4331_to_STM14_4328	3783768	3787977
C08	STM14_4418_to_STM14_4409	3856665	3863829
C10	STM14_4443_to_STM14_4458	3889033	3907650
C11	STM14_4466_to_STM14_4470	3914172	3918728
D01	STM14_4542_to_STM14_4519	3962754	3981193
D04	STM14_4655_to_STM14_4652	4077353	4082500
D05	STM14_4685_to_STM14_4675	4100073	4113267
D07	STM14_4751_to_STM14_4758	4168455	4175090
D08	STM14_4764_to_STM14_4773	4179626	4188219
D10	STM14_4853_to_STM14_4861	4261662	4266914
E04	STM14_5105_to_STM14_5108	4481655	4485406
E05	STM14_5141_to_STM14_5136	4523003	4529137
E07	STM14_5222_to_STM14_5217	4593562	4596743
E09	STM14_5370_to_STM14_5364	4724655	4729627
E11	STM14_5446_to_STM14_5454	4802990	4812321
F02	STM14_0140_to_STM14_0131	129844	138688
G03	STM14_1057_to_STM14_1052	971367	976735
G07	STM14_1264_to_STM14_1259	1154751	1158186

Well Position	Target Gene Region (Locus Tag)	Deleted Region Start	Deleted Region End
G11	STM14_1944_to_STM14_1992	1706358	1751439
G12	STM14_2046_to_STM14_2052	1798925	1802630
H03	STM14_2424_to_STM14_2415	2087313	2093198

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

<sup>2</sup>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.