

***Salmonella enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_156/157_Kan**

Catalog No. NR-42849

For research use only. Not for human use.

Contributor:

Michael McClelland, Professor, Scientific Director, Vaccine Research Institute of San Diego, San Diego, California, USA

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 11 96-well plates, in which 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}

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.

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.⁵ The complete genome of *S. enterica* subsp. *enterica*, strain 14028s (GenBank: [CP001363.1](#)) and plasmid (GenBank: [CP001362.1](#)) sequences are available.

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-42849 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 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 SGD_156/157_Kan, NR-42849."

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:

- McClelland, M., Personal Communication.

- 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.
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Table 1: *S. enterica* subsp. *enterica*, Strain 14028s (Serovar Typhimurium) Single-Gene Deletion Mutant Library, Plate SGD_156/157_Kan^{1,2}

Well Position	Gene Type	Gene Start	Gene End	Target Gene (Locus Tag)	Deleted Region Start	Deleted Region End	Gene Strand	Description
A01	CDS	4420750	4421574	STM14_5034	4420780	4421543	+	Transcriptional repressor IclR
A03	CDS	4415578	4417179	STM14_5029	4415605	4417148	-	Malate synthase
A04	CDS	4414380	4415309	STM14_5028	4414410	4415278	-	Homoserine O-succinyltransferase
A05	CDS	4404742	4406031	STM14_5016	4404772	4406000	+	Phosphoribosylamine--glycine ligase
A06	CDS	4403420	4404745	STM14_5015	4403450	4404714	-	Transcriptional regulatory protein ZraR
A07	CDS	4400290	4400562	STM14_5011	4400320	4400531	-	Transcriptional regulator HU subunit alpha
A10	CDS	4393437	4394072	STM14_5004	4393467	4394041	+	Thiamine-phosphate pyrophosphorylase
A11	CDS	4390264	4390452	STM14_4999	4390204	4390271	-	Putative cytoplasmic protein
B01	CDS	4357565	4358965	STM14_4960	4357595	4358934	+	Soluble pyridine nucleotide transhydrogenase
B02	CDS	4355004	4356380	STM14_4958	4355034	4356349	-	Argininosuccinate lyase
B03	CDS	4354113	4354886	STM14_4957	4354143	4354855	-	Acetylglutamate kinase
B04	CDS	4351858	4353009	STM14_4955	4351888	4352978	+	Acetylornithine deacetylase
B05	CDS	4348838	4351489	STM14_4954	4348868	4351458	+	Phosphoenolpyruvate carboxylase
B06	CDS	4337403	4338065	STM14_4940	4337433	4338034	+	Fructose-6-phosphate aldolase
B07	CDS	4336288	4337391	STM14_4939	4336318	4337360	+	Glycerol dehydrogenase
B08	CDS	4333168	4335348	STM14_4936	4333198	4335317	-	Hydroperoxidase
B09	CDS	4332113	4333003	STM14_4935	4332143	4332972	-	5,10-methylenetetrahydrofolate reductase
B10	CDS	4323157	4324458	STM14_4928	4323187	4324427	-	Cystathionine gamma-synthase
B12	CDS	4314076	4314606	STM14_4920	4314106	4314575	+	ATP-dependent protease peptidase subunit
C01	CDS	4312735	4314066	STM14_4919	4312765	4314035	+	ATP-dependent protease ATP-binding subunit
C02	CDS	4311739	4312668	STM14_4918	4311769	4312637	+	1,4-dihydroxy-2-naphthoate octaprenyltransferase
C03	CDS	4311161	4311646	STM14_4917	4311191	4311615	+	Ribonuclease activity regulator protein RraA
C05	CDS	4306805	4307905	STM14_4911	4306835	4307784	+	Fructose 1,6-bisphosphatase II
C06	CDS	4305962	4306708	STM14_4910	4305992	4306677	+	Ferredoxin-NADP reductase
C07	CDS	4303850	4304617	STM14_4906	4303880	4304586	+	Triosephosphate isomerase
C09	CDS	4274483	4275517	STM14_4871	4274513	4275486	+	Rhamnose-proton symporter
C10	CDS	4273638	4274486	STM14_4870	4273668	4274455	-	Transcriptional activator RhaR
C11	CDS	4272649	4273485	STM14_4869	4272679	4273454	-	Transcriptional activator RhaS
C12	CDS	4270892	4272361	STM14_4866	4270922	4272330	+	Rhamnulokinase
D01	CDS	4269636	4270895	STM14_4865	4269666	4270864	+	L-rhamnose isomerase
D02	CDS	4268666	4269493	STM14_4864	4268696	4269462	+	Rhamnulose-1-phosphate aldolase
D03	CDS	4261587	4262423	STM14_4853	4261617	4262392	-	Formate dehydrogenase accessory protein
D04	CDS	4257427	4258329	STM14_4851	4257457	4258298	+	Formate dehydrogenase-O beta subunit
D06	CDS	4255869	4256798	STM14_4849	4255899	4256767	+	Formate dehydrogenase accessory protein FdhE

Product Information Sheet for NR-42849

Well Position	Gene Type	Gene Start	Gene End	Target Gene (Locus Tag)	Deleted Region Start	Deleted Region End	Gene Strand	Description
D09	CDS	4226441	4227850	STM14_4817	4226471	4227819	+	Nitrogen regulation protein NR(I)
D10	CDS	4224755	4226128	STM14_4815	4224701	4226097	-	Coproporphyrinogen III oxidase
D12	CDS	4205380	4206711	STM14_4790	4205410	4206680	-	Proline dipeptidase
E01	CDS	4196615	4197103	STM14_4783	4196645	4197144	+	Transcriptional activator RfaH
E02	CDS	4188555	4189316	STM14_4774	4188585	4189285	-	Uridine phosphorylase
E03	CDS	4187484	4188296	STM14_4773	4187514	4188265	+	Putative diene lactone hydrolase
E04	CDS	4183453	4185717	STM14_4769	4183483	4185686	-	5-methyltetrahydropteroyltriglutamate—homocysteine methyltransferase
E05	CDS	4182251	4183204	STM14_4767	4182281	4183173	+	MetE/methH regulator
E06	CDS	4179455	4180567	STM14_4764	4179581	4180536	-	Lysophospholipase L2
E07	CDS	4178820	4179440	STM14_4763	4178850	4179409	+	Homoserine/homoserine lactone efflux protein
E08	CDS	4178160	4178780	STM14_4762	4178190	4178749	-	Threonine efflux system
E09	CDS	4173754	4174638	STM14_4757	4173784	4174607	+	Chloramphenicol resistance
E10	CDS	4171866	4172816	STM14_4754	4171896	4172785	-	Magnesium/nickel/cobalt transporter CorA
E11	CDS	4160058	4162604	STM14_4739	4160088	4162573	-	Adenylate cyclase
E12	CDS	4156812	4157981	STM14_4735	4156842	4157950	+	Putative uroporphyrinogen III C-methyltransferase
F01	CDS	4155613	4156812	STM14_4734	4155643	4156781	+	Putative protoheme IX biogenesis protein
F02	CDS	4144108	4145370	STM14_4719	4144138	4145339	-	UDP-N-acetyl-D-mannosamine dehydrogenase
F03	CDS	4141879	4142925	STM14_4717	4141909	4142894	-	Lipopolysaccharide biosynthesis protein WzzE
F04	CDS	4138527	4138856	STM14_4713	4138557	4138825	-	Thioredoxin
F05	CDS	4132635	4132916	STM14_4707	4132665	4132885	+	Peptidyl-prolyl cis-trans isomerase C
F09	CDS	4125678	4127528	STM14_4701	4125708	4127497	-	Dihydroxy-acid dehydratase
F11	CDS	4124310	4124570	STM14_4699	4124337	4124539	-	Acetolactate synthase 2 regulatory subunit
F12	CDS	4109304	4110233	STM14_4682	4109334	4110202	-	Ribokinase
G01	CDS	4105779	4107284	STM14_4679	4105764	4107253	-	D-ribose transporter ATP binding protein
G02	CDS	4103287	4105155	STM14_4677	4103317	4105124	-	Potassium transport protein Kup
G03	CDS	4099001	4099993	STM14_4674	4099031	4099962	-	Asparagine synthetase AsnA
G04	CDS	4097859	4098302	STM14_4672	4097889	4098271	+	Flavodoxin
G05	CDS	4095591	4097480	STM14_4671	4095621	4097449	+	tRNA uridine 5-carboxymethylaminomethyl modification enzyme GidA
G06	CDS	4094865	4095494	STM14_4670	4094895	4095463	+	Glucose-inhibited division protein B
G07	CDS	4093869	4094249	STM14_4668	4093899	4094218	+	F0F1 ATP synthase subunit I
G09	CDS	4092759	4092998	STM14_4666	4092789	4092967	+	F0F1 ATP synthase subunit C
G10	CDS	4092229	4092699	STM14_4665	4092259	4092668	+	F0F1 ATP synthase subunit B
G12	CDS	4090127	4091668	STM14_4663	4090157	4091637	+	F0F1 ATP synthase subunit alpha
H01	CDS	4089213	4090076	STM14_4662	4089243	4090045	+	F0F1 ATP synthase subunit gamma
H03	CDS	4087364	4087783	STM14_4660	4087394	4087752	+	F0F1 ATP synthase subunit epsilon
H04	CDS	4076183	4077142	STM14_4651	4076213	4077111	+	Phosphate transporter permease subunit
H05	CDS	4075293	4076183	STM14_4650	4075323	4076152	+	Phosphate transporter permease subunit
H06	CDS	4074433	4075206	STM14_4649	4074463	4075175	+	Phosphate transporter subunit
H07					4045502	4046058		
H08					4012055	4012108		
H09					4012166	4012194		
H10	CDS	4009609	4011297	STM14_4577	4009639	4011266	+	Acetolactate synthase catalytic subunit
H11	CDS	4001406	4002734	STM14_4569	4001436	4002703	+	Regulatory protein UhpC
H12					3966604	3966716	+	

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.