

***Mycobacterium tuberculosis* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 22**

**Catalog No. NR-19658**

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**For research use only. Not for human use.**

**Contributor:**

Pathogen Functional Genomics Resource Center at the J. Craig Venter Institute

**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 *Mycobacterium tuberculosis* (*M. tuberculosis*), Gateway® clone set consists of 42 plates which contain 3724 sequence validated clones (3294 *M. tuberculosis*, strain H37Rv clones supplemented with 430 unique open reading frames (ORF) from *M. tuberculosis*, strain CDC1551) cloned in *Escherichia coli* (*E. coli*) DH10B-T1 cells. Each ORF was recombined in vector pDONR™221 with an ATG start codon and no stop codon. The sequence was validated by full length sequencing of each entry clone with greater than 1X coverage and a mutation rate of less than 0.2%. Detailed information about each clone is shown in Table 1.

Information related to the use of Gateway® Clones can be obtained from [Invitrogen™](#). Recombination was facilitated through an *attB* substrate (*attB*-PCR product or a linearized *attB* expression clone) with an *attP* substrate (pDONR™221) to create an *attL*-containing entry clone. The entry clone contains recombinational cloning sites, *attL1* and *attL2* to facilitate gene transfer into a destination vector, M13 forward and reverse priming sites for sequencing and a kanamycin resistance gene for selection. Please refer to the [Invitrogen™ Gateway® Technology Manual](#) for additional details.

**Material Provided:**

Each well of the 96-well plate contains approximately 60 µL of *E. coli* culture (strain DH10B-T1) in Luria Bertani (LB) broth containing 50 µg/mL kanamycin supplemented with 15% glycerol.

**Packaging/Storage:**

NR-19658 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 50 µg/mL kanamycin

Incubation:

Temperature: *E. coli*, strain DH10B-T1 clones should be grown at 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 18 to 24 hours.

**Citation:**

Acknowledgment for publications should read “The following reagent was obtained through BEI Resources, NIAID, NIH: *Mycobacterium tuberculosis* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 22, NR-19658.”

**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](#). 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see [www.cdc.gov/biosafety/publications/bmb15/index.htm](http://www.cdc.gov/biosafety/publications/bmb15/index.htm).

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**References:**

1. Cole, S. T., et al. "Deciphering the Biology of *Mycobacterium tuberculosis* from the Complete Genome Sequence." *Nature* 393 (1998): 537-544. PubMed: 9634230.
2. Camus, J. C., et al. "Re-Annotation of the Genome Sequence of *Mycobacterium tuberculosis* H37Rv." *Microbiology* 148 (2002): 2967-2973. PubMed 12368430.

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**Table 1: *Mycobacterium tuberculosis*, Gateway® Clones, Plate 22 (ZMTLL)**

Clone	Well Position	ORF Length	Locus ID	Description (Gene name)	Accession Number	Average Depth of Coverage
41796	A01	718	Rv1686c	integral membrane protein ABC transporter	NP_216202.1	4.229805014
41793	A02	718	Rv1321	hypothetical protein Rv1321	NP_215837.1	2
41788	A03	718	Rv0813c	hypothetical protein Rv0813c	NP_215328.1	2.823119777
41787	A04	718	Rv0324	ArsR family transcriptional regulator	NP_214838.1	4.181058496
41800	A05	718	Rv3674c	endonuclease III	NP_218191.2	4.181058496
41792	A06	718	Rv1265	hypothetical protein Rv1265	NP_215781.1	4.157381616
41808	A07	721	Rv2794c	hypothetical protein Rv2794c	NP_217310.1	2.056865465
41810	A08	721	Rv3036c	hypothetical protein Rv3036c	NP_217552.1	2.685159501
41801	A09	721	Rv0364	transmembrane protein	NP_214878.1	2
41806	A10	721	Rv2691	TRK system potassium uptake protein CEOB	YP_177900.1	2
41803	A11	721	Rv1158c	hypothetical protein Rv1158c	NP_215674.1	3.198335645
41811	A12	721	Rv3673c	membrane-anchored thioredoxin-like protein	NP_218190.1	4.056865465
41809	B01	721	Rv2976c	uracil-DNA glycosylase	NP_217492.1	2
41802	B02	721	Rv0491	two component sensory transduction protein RegX3	NP_215005.1	1.993065187
41821	B03	724	Rv3208	TetR family transcriptional regulator	NP_217724.1	1.990331492
41820	B04	724	Rv2788	transcriptional repressor SIRR	NP_217304.1	1.991712707
41822	B05	724	Rv3246c	two component sensory transduction transcriptional regulatory protein MTRA	NP_217763.1	1.991712707
41816	B06	724	Rv1418	lipoprotein LprH	NP_215934.1	1.991712707
41819	B07	724	Rv2716	hypothetical protein Rv2716	NP_217232.1	1.995856354
41812	B08	724	Rv0583c	lipoprotein LpqN	NP_215097.1	1.993093923
41815	B09	724	Rv1035c	transposase	NP_215551.1	3.832872928
41817	B10	724	Rv1874	hypothetical protein Rv1874	NP_216390.1	3.821823204
41818	B11	724	Rv1980c	immunogenic protein MPT64 (antigen MPT64/MP64)	NP_216496.1	2
41813	B12	724	Rv0784	hypothetical protein Rv0784	NP_215298.1	3.827348066
41814	C01	724	Rv1024	hypothetical protein Rv1024	NP_215540.1	1.990331492
41824	C02	727	Rv0232	TetR/ACRR family transcriptional regulator	NP_214746.1	1.991746905
41823	C03	727	Rv0200	transmembrane protein	NP_214714.1	4.127922971
41833	C04	727	Rv3766	hypothetical protein Rv3766	NP_218283.1	1.993122421
41832	C05	727	Rv3757c	osmoprotectant (glycine betaine/carnitine/choline/L-proline) transport integral membrane protein ABC transporter PROW	NP_218274.1	1.994497937
41831	C06	727	Rv3481c	integral membrane protein	NP_217998.1	2
41828	C07	727	Rv3102c	putative cell division ATP-binding protein FTSE (septation component-transport ATP-binding protein ABC transporter)	NP_217618.1	1.993122421

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Clone	Well Position	ORF Length	Locus ID	Description (Gene name)	Accession Number	Average Depth of Coverage
41826	C08	727	Rv1104	hypothetical protein Rv1104	NP_215620.1	1.980742779
41827	C09	727	Rv2335	serine acetyltransferase CysE	NP_216851.1	2
41846	C10	730	Rv2217	lipoyltransferase	NP_216733.1	4.212328767
41848	C11	730	Rv2536	transmembrane protein	NP_217052.1	2
41849	C12	730	Rv2711	IRON-dependent repressor and activator IDER	NP_217227.1	4.195890411
41847	D01	730	Rv2286c	hypothetical protein Rv2286c	NP_216802.1	2
41850	D02	730	Rv2906c	tRNA (guanine-N(1)-)-methyltransferase	NP_217422.1	4.209589041
41845	D03	730	Rv2203	hypothetical protein Rv2203	NP_216719.1	2.221917808
41839	D04	730	Rv0981	two component response transcriptional regulatory protein MprA	NP_215496.1	1.989041096
41836	D05	730	Rv0966c	hypothetical protein Rv0966c	NP_215481.2	4.205479452
41843	D06	730	Rv1712	cytidylate kinase	NP_216228.1	2.201369863
41857	D07	733	Rv0653c	TetR family transcriptional regulator	NP_215167.1	4.233287858
41863	D08	733	Rv3210c	hypothetical protein Rv3210c	NP_217726.1	4.218281037
41853	D09	733	Rv0518	hypothetical protein Rv0518	NP_215032.1	2
41865	D10	733	Rv3582c	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase	NP_218099.1	2
41861	D11	733	Rv2676c	hypothetical protein Rv2676c	NP_217192.1	1.989085948
41854	D12	733	Rv0618	galactose-1-phosphate uridylyltransferase galTa	YP_177741.1	4.203274216
41862	E01	733	Rv2728c	hypothetical protein Rv2728c	NP_217244.1	4.241473397
41867	E03	733	Rv3713	cohyric acid synthase CobQ2	NP_218230.1	2.556616644
41856	E04	733	Rv0632c	hypothetical protein Rv0633c	NP_215147.1	3.181446112
41858	E05	733	Rv1507c	hypothetical protein Rv1507A	YP_177648.1	2.233287858
41877	E06	736	Rv3837c	phosphoglycerate mutase	NP_218354.1	3.502717391
41872	E07	736	Rv1115	hypothetical protein Rv1115	NP_215631.1	1.991847826
41873	E08	736	Rv1268c	hypothetical protein Rv1268c	NP_215784.1	1.991847826
41876	E09	736	Rv3668c	protease	NP_218185.1	2
41875	E10	736	Rv3426	PPE family protein	YP_177972.1	1.993206522
41874	E11	736	Rv1408	ribulose-phosphate 3-epimerase	NP_215924.1	2
41888	E12	739	Rv3699	hypothetical protein Rv3699	NP_218216.1	2.219215156
41885	F01	739	Rv2989	transcriptional regulatory protein	NP_217505.1	3.427604871
41884	F02	739	Rv2945c	lipoprotein LppX	NP_217461.1	2
41878	F03	739	Rv1100	hypothetical protein Rv1100	NP_215616.1	4.220568336
41890	F04	739	Rv3702c	hypothetical protein Rv3702c	NP_218219.1	1.700947226
41893	F05	742	Rv1676	hypothetical protein Rv1676	NP_216192.1	1.991913747
41892	F06	742	Rv0558	ubiquinone/menaquinone biosynthesis methyltransferase	YP_177738.1	1.991913747
41895	F07	742	Rv1816	transcriptional regulatory protein	NP_216332.1	2
41891	F08	742	Rv0472c	TetR family transcriptional regulator	NP_214986.1	2
41894	F09	742	Rv1811	Mg <sup>2+</sup> transport P-type ATPase C	NP_216327.1	1.993261456
41897	F10	742	Rv3404c	hypothetical protein Rv3404c	NP_217921.1	2
41896	F11	742	Rv3386	transposase	NP_217903.1	1.989218329
41902	F12	745	Rv0641	50S ribosomal protein L1	NP_215155.1	1.987919463
41901	G01	745	Rv0351	GRPE protein (HSP-70 cofactor)	NP_214865.1	1.991946309
41900	G02	745	Rv0071	maturase	NP_214585.1	1.991946309
41905	G03	745	Rv2695	hypothetical protein Rv2695	NP_217211.1	4.253691275
41903	G04	745	Rv1610	hypothetical protein Rv1610	NP_216126.1	4.177181208
41907	G05	745	Rv3174	short chain dehydrogenase	NP_217690.1	1.991946309
41918	G06	748	Rv2821c	hypothetical protein Rv2821c	NP_217337.1	1.994652406
41910	G07	748	Rv0781	oligopeptidase B	NP_215296.2	2.113636364
41908	G08	748	Rv0452	transcriptional regulatory protein	NP_214966.1	1.993315508
41912	G09	748	Rv1411c	lipoprotein LprG	NP_215927.1	3.252673797
41914	G10	748	Rv1725c	hypothetical protein Rv1725c	NP_216241.1	2
41917	G11	748	Rv2558	hypothetical protein Rv2558	NP_217074.1	4.20855615

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Clone	Well Position	ORF Length	Locus ID	Description (Gene name)	Accession Number	Average Depth of Coverage
41919	G12	748	Rv3147	NADH dehydrogenase subunit C	NP_217663.1	4.219251337
41920	H01	748	Rv3390	lipoprotein LpqD	NP_217907.1	1.99197861
41911	H02	748	Rv0903c	two component response transcriptional regulatory protein PRRA	NP_215418.1	1.99197861
41913	H03	748	Rv1593c	hypothetical protein Rv1593c	NP_216109.1	1.99197861
41930	H04	751	Rv3528c	hypothetical protein Rv3528c	NP_218045.1	1.986684421
41923	H05	751	Rv0634c	Glyoxalase II GlxII	NP_215148.1	4.215712383
41926	H06	751	Rv2687c	Integral membrane protein ABC transporter	NP_217203.1	4.215712383
41931	H07	751	Rv3576	lipoprotein LppH	YP_177991.1	1.994673768
41929	H08	751	Rv3434c	transmembrane protein	NP_217951.1	2.427430093
41925	H09	751	Rv2054	hypothetical protein Rv2054	NP_216570.1	2
41924	H10	751	Rv1151c	NAD-dependent deacetylase	NP_215667.1	1.99334221
41928	H11	751	Rv2972c	hypothetical protein Rv2972c	NP_217488.1	2
41932	H12	751	Rv3821	integral membrane protein	NP_218338.1	2