

## ***Mycobacterium tuberculosis* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 39**

### **Catalog No. NR-19675**

This reagent is the tangible property of the U.S. Government.

### **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 inoculated well of the 96-well plate contains approximately 60 µL of culture in Luria Bertani (LB) broth containing 50 µg/mL kanamycin supplemented with 15% glycerol.

#### **Packaging/Storage:**

NR-19675 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: 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: *Mycobacterium tuberculosis* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 39, NR-19675."

#### **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 <http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>.

#### **Disclaimers:**

You are authorized to use this product for research use only. It is not intended for human use.

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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 39 (ZMTMC)<sup>1</sup>**

Clone	Well Position	ORF Length	Locus ID	Description (Gene name)	Accession Number	Average Depth of Coverage
11444	A01	2296	Rv0103c	cation-transporter P-type ATPase B	NP_214617.1	7.807926829
11440	A02	2296	Rv2783c	polynucleotide phosphorylase/polyadenylase	NP_217299.1	6.198606272
11358	A03	2302	Rv0803	phosphoribosylformylglycinamide synthase II	NP_215318.1	3.953084275
11324	A04	2335	Rv1563c	maltooligosyltrehalose synthase TreY	YP_177820.1	7.291648822
11464	A05	2353	Rv1992c	metal cation transporter P-type ATPase G CtpG	NP_216508.1	7.819379516
11454	A06	2368	Rv3729	transferase	NP_218246.1	8.083192568
11468	A07	2377	Rv2900c	formate dehydrogenase H	NP_217416.1	7.596129575
11527	A08	2437	Rv0373c	carbon monoxide dehydrogenase large subunit	NP_214887.1	6.924907673
11570	A09	2446	Rv3909	hypothetical protein Rv3909	NP_218426.1	8.07686018
11518	A10	2467	Rv2823c	hypothetical protein Rv2823c	NP_217339.1	7.892987434
11530	A11	2554	Rv0006	DNA gyrase subunit A	NP_214520.1	7.652701644
11596	A12	2557	Rv1937	oxygenase	NP_216453.1	7.380524052
11664	B01	2584	Rv0384c	endopeptidase ATP binding protein	NP_214898.1	8.087074303
11504	B03	2635	Rv1747	transmembrane ATP-binding protein ABC transporter	NP_216263.1	8.182922201
11660	B04	2662	Rv0058	replicative DNA helicase	NP_214572.1	8.223891811
11644	B05	2692	Rv2567	hypothetical protein Rv2567	NP_217083.1	7.604383358
11478	B07	2842	Rv3646c	DNA topoisomerase I	NP_218163.1	7.754750176
11622	B08	2863	Rv1832	glycine dehydrogenase	NP_216348.1	7.1742927
11563	B09	2869	Rv1475c	aconitate hydratase	NP_215991.1	6.112582781
11492	B10	2887	Rv3240c	preprotein translocase subunit SecA	YP_177950.1	8.116383789
11551	B11	2932	Rv0676c	transmembrane transport protein MmpL5	NP_215190.1	7.303888131
11511	B12	2938	Rv0202c	transmembrane transport protein MmpL11	NP_214716.1	7.515997277
11601	C01	2947	Rv0041	leucyl-tRNA synthetase	NP_214555.1	7.543603665
11612	C02	3016	Rv3193c	hypothetical protein Rv3193c	NP_217709.1	7.952254642
11631	C03	3022	Rv2221c	glutamate-ammonia-ligase adenyllyltransferase GLNE (glutamine-synthetase adenyllyltransferase)	NP_216737.1	7.176704169
11508	C04	3091	Rv1664	polyketide synthase pks9	NP_216180.1	7.915237787
11580	C05	3163	Rv1536	isoleucyl-tRNA synthetase	NP_216052.1	8.117609864
11566	C06	3235	Rv3728	hypothetical protein Rv3728	NP_218245.1	7.74064915
11797	C07	3556	Rv0667	DNA-directed RNA polymerase subunit beta	NP_215181.1	7.754780652

<sup>1</sup>All information in this table was provided by J. Craig Venter Institute at the time of deposition.