

***Mycobacterium tuberculosis* Gateway®  
Clone Set, Recombinant in *Escherichia coli*, Plate 40****Catalog No. NR-19676**

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

**Manufacturer:**

BEI Resources

**Product Description:**

Clone plates are replicated using a BioMek® FX robot. 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 only confirms the clone plate orientation and viability of randomly picked clones. BEI Resources does not confirm or validate individual clone identities 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.

Plate orientation and viability were confirmed for NR-19676.

**Material Provided:**

Each inoculated 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-19676 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 40, NR-19676."

**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).

**Disclaimers:**

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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 40 (ZMTMD)<sup>1</sup>**

Clone	Well Position	ORF Length	Locus ID	Description	Accession Number	Average Depth of Coverage
11825	A01	133	MT3223	hypothetical protein MT3223	NP_337749.1	-
11965	A02	133	MT2326	hypothetical protein MT2326	NP_336794.1	-
11925	A03	136	MT3156	hypothetical protein MT3156	NP_337678.1	-
11991	A05	142	MT0603	hypothetical protein MT0603	NP_335012.1	-
11916	A06	145	MT3536	hypothetical protein MT3536	NP_338062.1	2
11821	A07	145	MT0506	hypothetical protein MT0506	NP_334915.1	-
11951	A08	145	MT3174.1	hypothetical protein MT3174.1	NP_337697.1	-
11843	A10	145	MT0294	hypothetical protein MT0294	NP_334704.1	-
11873	A11	145	MT3436	hypothetical protein MT3436	NP_337965.1	-
11909	A12	148	MT2424	hypothetical protein MT2424	NP_336904.1	-
11927	B01	148	MT3847	hypothetical protein MT3847	NP_338397.1	-
11969	B02	151	MT0416	hypothetical protein MT0416	NP_334826.1	-
11869	B03	151	MT2417	hypothetical protein MT2417	NP_336896.1	-
11826	B04	154	MT0540	hypothetical protein MT0540	NP_334949.1	2
11819	B05	154	MT2375	hypothetical protein MT2375	NP_336854.1	-
11816	B06	154	MT2895	hypothetical protein MT2895	NP_337406.1	2
11853	B07	154	MT2083	hypothetical protein MT2083	NP_336548.1	-
12003	B08	154	MT3846	hypothetical protein MT3846	NP_338396.1	-
11905	B09	157	MT1771.1	hypothetical protein MT1771.1	NP_336230.1	-
11976	B10	157	MT0868	hypothetical protein MT0868	NP_335297.1	1.955414013
11985	B11	160	MT2092	hypothetical protein MT2092	NP_336557.1	-
11814	B12	160	MT2115	hypothetical protein MT2115	NP_336580.1	2
11829	C01	160	MT3290.2	hypothetical protein MT3290.2	NP_337819.1	-
11899	C02	160	MT3767.3	hypothetical protein MT3767.3	NP_338320.1	-
11903	C03	160	MT3580.2	hypothetical protein MT3580.2	NP_338125.1	-
11839	C04	163	MT0116.1	hypothetical protein MT0116.1	NP_334525.1	-
11955	C05	163	MT2369	hypothetical protein MT2369	NP_336845.1	-
11913	C06	166	MT3628	hypothetical protein MT3628	NP_338175.1	-
11963	C07	166	MT2168	hypothetical protein MT2168	NP_336637.1	-
11837	C08	166	MT3994	hypothetical protein MT3994	NP_338548.1	-
11961	C09	166	MT3491.1	hypothetical protein MT3491.1	NP_338013.1	-
11941	C10	166	MT2510	hypothetical protein MT2510	NP_336993.1	-
11895	C11	169	MT2325	hypothetical protein MT2325	NP_336793.1	-
11915	C12	169	MT0740.1	hypothetical protein MT0740.1	NP_335158.1	-
11901	D01	169	MT3876.1	hypothetical protein MT3876.1	NP_338427.1	-
12001	D02	169	MT1578.1	hypothetical protein MT1578.1	NP_336031.1	-
11953	D03	172	MT3362	hypothetical protein MT3362	NP_337889.1	-

Clone	Well Position	ORF Length	Locus ID	Description	Accession Number	Average Depth of Coverage
11882	D04	172	MT0573.1	hypothetical protein MT0573.1	NP_334982.1	1.947674419
11823	D05	175	MT0773.1	hypothetical protein MT0773.1	NP_335200.1	-
11849	D06	175	MT1070	hypothetical protein MT1070	NP_335507.1	-
11939	D07	175	MT0492	hypothetical protein MT0492	NP_334901.1	-
11855	D08	178	MT3972.1	hypothetical protein MT3972.1	NP_338526.1	-
11920	D09	178	MT0991	hypothetical protein MT0991	NP_335425.1	2
11993	D10	178	MT1098	hypothetical protein MT1098	NP_335540.1	-
11929	D11	178	MT1556.1	hypothetical protein MT1556.1	NP_336009.1	2
11889	D12	181	MT2960	hypothetical protein MT2960	NP_337472.1	-
11831	E01	181	MT0031	hypothetical protein MT0031	NP_334440.1	-
11983	E02	184	MT1414.1	hypothetical protein MT1414	NP_335863.1	-
11865	E03	184	MT1321	hypothetical protein MT1321	NP_335768.1	-
11897	E04	184	MT3919	hypothetical protein MT3919	NP_338471.1	2
11871	E05	184	MT0946	hypothetical protein MT0946	NP_335379.1	-
11919	E06	184	MT0910.4	hypothetical protein MT0910.4	NP_335342.1	-
11959	E07	187	MT0910.3	hypothetical protein MT0910.3	NP_335341.1	-
11890	E08	187	MT1122.1	hypothetical protein MT1122.1	NP_335566.1	1.679144385
11944	E09	187	MT2138.1	hypothetical protein MT2138.1	NP_336605.1	2.85026738
11986	E10	187	MT3953	hypothetical protein MT3953	NP_338506.1	1.743315508
11863	E11	187	MT1356	hypothetical protein MT1356	NP_335804.1	-
11846	E12	190	MT1813	hypothetical protein MT1813	NP_336270.1	1.994736842
11877	F01	190	MT1077	hypothetical protein MT1077	NP_335515.1	-
12079	F02	190	MT0431	hypothetical protein MT0431	NP_334841.1	2
12058	F03	190	MT2554	hypothetical protein MT2554	NP_337042.1	2
12026	F04	190	MT1288	hypothetical protein MT1288	NP_335732.1	2
12117	F05	193	MT0576	hypothetical protein MT0576	NP_334985.1	2
12032	F06	193	MT1178	hypothetical protein MT1178	NP_335623.1	2
12067	F07	193	MT0910.1	hypothetical protein MT0910.1	NP_335339.1	-
12094	F08	196	MT1497.2	hypothetical protein MT1497.2	NP_335947.1	1.964285714
12185	F09	199	MT0196	hypothetical protein MT0196	NP_334602.1	-
12167	F10	199	MT3780	hypothetical protein MT3780	NP_338333.1	2
12129	F11	199	MT0069	hypothetical protein MT0069	NP_334479.1	-
12187	F12	199	MT3275.1	hypothetical protein MT3275.1	NP_337802.1	2
12031	G01	199	MT0553	hypothetical protein MT0553	NP_334962.1	-
12011	G02	202	MT2467	hypothetical protein MT2467	NP_336948.1	2
12072	G03	202	MT1746.1	hypothetical protein MT1746.1	NP_336202.1	2
12015	G04	202	MT2370.2	hypothetical protein MT2370.2	NP_336848.1	-
12119	G05	205	MT1585.1	hypothetical protein MT1585.1	NP_336038.1	2
12155	G06	205	MT0600	hypothetical protein MT0600	NP_335009.1	2
12056	G07	205	MT2370	hypothetical protein MT2370	NP_336846.1	2
12052	G08	205	MT0663	50S ribosomal protein L33	NP_335074.1	2
12170	G09	208	MT0853	hypothetical protein MT0853	NP_335280.1	2
12077	G10	208	MT3671.2	hypothetical protein MT3671.2	NP_338216.1	-
12115	G11	208	MT2396	hypothetical protein MT2396	NP_336874.1	-
12121	G12	208	MT3412	hypothetical protein MT3412	NP_337941.1	-
12087	H01	208	MT3014	hypothetical protein MT3014	NP_337527.1	-
12163	H02	211	MT0638.1	hypothetical protein MT0638.1	NP_335047.1	-
12180	H03	211	MT0290	hypothetical protein MT0290	NP_334696.1	2
12152	H04	211	MT1266	hypothetical protein MT1266	NP_335710.1	2
12005	H05	214	MT1054.1	hypothetical protein MT1054.1	NP_335492.1	-
12195	H06	214	MT3103	hypothetical protein MT3103	NP_337618.1	-
12183	H07	217	MT3959	hypothetical protein MT3959	NP_338512.1	-

Clone	Well Position	ORF Length	Locus ID	Description	Accession Number	Average Depth of Coverage
12176	H08	217	MT3268	hypothetical protein MT3268	NP_337793.1	2
12149	H09	220	MT3149.1	hypothetical protein MT3149.1	NP_337669.1	-
12061	H10	220	MT2365.2	hypothetical protein MT2365.2	NP_336840.1	-
12045	H11	220	MT1121	hypothetical protein MT1121	NP_335564.1	2
12125	H12	223	MT2588	hypothetical protein MT2588	NP_337079.1	2

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