

***Mycobacterium tuberculosis* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 12**

**Catalog No. NR-19648**

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**Contributor:**

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 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-19648.

**Material Provided:**

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

**Packaging/Storage:**

NR-19648 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 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 12, NR-19648.”

**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 12 (ZMTLB)<sup>1</sup>**

Clone	Well Position	ORF Length	Locus ID	Description	Accession Number	Average Depth of Coverage
40120	A01	286	Rv1741	hypothetical protein	NP_216257.1	2
40128	A02	289	Rv2517c	hypothetical protein	NP_217033.1	2
40126	A03	289	Rv1960c	hypothetical protein	NP_216476.1	2
40127	A04	289	Rv2076c	hypothetical protein	NP_216592.1	2
40129	A05	289	Rv3112	molybdenum cofactor biosynthesis protein D	YP_177928.1	1.626297578
10046	A06	289	Rv2094c	twin arginine translocase protein A	NP_216610.1	4.166089965
9950	A07	289	Rv0634A	hypothetical protein	YP_177629.1	1.775086505
40131	A08	292	Rv0623	hypothetical protein	NP_215137.1	2
40130	A09	292	Rv0055	30S ribosomal protein S18	YP_177688.1	2
40134	A10	292	Rv2104c	hypothetical protein	NP_216620.1	2
40139	A11	292	Rv3219	transcriptional regulatory protein WHIB-like WHIB1	NP_217735.1	2
40136	B01	292	Rv2664	hypothetical protein Rv2664	NP_217180.1	2.284246575
9952	B02	292	Rv3198A	ATP-dependent DNA helicase II UVRD2	NP_217714.1	2.657534247
10092	B03	292	Rv0590A	MCE family-like protein	YP_177627.1	2.366438356
40146	B04	295	Rv1107c	exodeoxyribonuclease VII small subunit	NP_215623.1	2
40162	B05	295	Rv3358	hypothetical protein	NP_217875.1	2
40159	B06	295	Rv2871	hypothetical protein	NP_217387.1	2.379661017
40150	B07	295	Rv1831	hypothetical protein	NP_216347.1	1.983050847
40156	B08	295	Rv2706c	hypothetical protein	NP_217222.1	2.644067797
40143	B09	295	Rv0596c	hypothetical protein	NP_215110.1	1.610169492
40157	B10	295	Rv2808	hypothetical protein	NP_217324.1	2
40161	B12	295	Rv3346c	hypothetical protein	NP_217863.1	2.613559322
10122	C01	295	Rv1638A	hypothetical protein	YP_177650.1	2.762711864
40167	C02	298	Rv2657c	phiRv2 prophage protein	NP_217173.1	2
40163	C03	298	Rv0626	hypothetical protein	NP_215141.1	2
40165	C04	298	Rv2412	30S ribosomal protein S20	NP_216928.1	2
40164	C05	298	Rv1241	hypothetical protein	NP_215757.1	2
40166	C06	298	Rv2441c	50S ribosomal protein L27	NP_216957.1	2
40173	C07	301	Rv2866	hypothetical protein	NP_217382.1	2
40169	C08	301	Rv0476	transmembrane protein	NP_214990.1	2
40170	C09	301	Rv0898c	hypothetical protein	NP_215413.1	3.485049834
40172	C10	301	Rv1839c	hypothetical protein	NP_216355.1	2
40168	C11	301	Rv0033	acyl carrier protein AcpA	NP_214547.1	2
40177	C12	304	Rv1083	hypothetical protein	NP_215599.1	3.289473684
40178	D01	304	Rv2055c	30S ribosomal protein S18	NP_216571.1	2
40174	D02	304	Rv0550c	hypothetical protein	NP_215064.1	2
40175	D03	304	Rv0569	hypothetical protein	NP_215083.1	2
40176	D04	304	Rv0616c	hypothetical protein	NP_215130.1	2
40179	D05	304	Rv2758c	hypothetical protein	NP_217274.1	1.644736842

Clone	Well Position	ORF Length	Locus ID	Description	Accession Number	Average Depth of Coverage
9946	D06	304	Rv3566A	hypothetical protein	YP_177990.1	3.161184211
40185	D07	307	Rv3260c	transcriptional regulatory protein WHIB-like WHIB2	NP_217777.1	2
40181	D08	307	Rv1519	hypothetical protein	NP_216035.1	2
40182	D09	307	Rv2312	hypothetical protein	NP_216828.1	2
40180	D10	307	Rv1247c	hypothetical protein	NP_215763.1	1.993485342
40184	D11	307	Rv2785c	30S ribosomal protein S15	NP_217301.1	2
40183	D12	307	Rv2760c	hypothetical protein	NP_217276.1	2
40186	E01	310	Rv0664	hypothetical protein	NP_215178.1	2.396774194
40192	E02	310	Rv3904c	hypothetical protein	NP_218421.1	2
40191	E03	310	Rv3769	hypothetical protein	NP_218286.1	2
40190	E04	310	Rv2662	hypothetical protein	NP_217178.1	2
40189	E05	310	Rv2422	hypothetical protein	NP_216938.1	2
40187	E06	310	Rv1580c	phiRv1 phage protein	NP_216096.1	2
40188	E07	310	Rv1993c	hypothetical protein	NP_216509.1	2
10007	E08	310	Rv3208A	hypothetical protein	YP_177943.1	-
40194	E09	313	Rv0879c	transmembrane protein	NP_215394.1	3.083067093
40195	E10	313	Rv1053c	hypothetical protein	NP_215569.1	3.562300319
40193	E11	313	Rv0424c	hypothetical protein	NP_214938.1	2
40198	E12	313	Rv3357	hypothetical protein	NP_217874.1	2
40197	F01	313	Rv2755c	type I restriction/modification system specificity determinant HsdS	YP_177904.1	2
40200	F02	316	Rv0544c	transmembrane protein	NP_215058.1	2.379746835
40202	F03	316	Rv0942	hypothetical protein	NP_215457.1	2
40203	F04	316	Rv2545	hypothetical protein	NP_217061.1	2
40201	F05	316	Rv0868c	molybdenum cofactor biosynthesis protein D	NP_215383.1	2
9985	F06	316	Rv2438A	hypothetical protein	YP_177671.1	-
9964	F07	316	Rv3197A	transcriptional regulatory protein WHIB-like WHIB7	YP_177940.1	3.155063291
40213	F08	319	Rv2865	hypothetical protein	NP_217381.1	2
40205	F09	319	Rv0705	30S ribosomal protein S19	NP_215219.1	2
40210	F10	319	Rv2632c	hypothetical protein	NP_217148.1	2
40206	F11	319	Rv0863	hypothetical protein	NP_215378.1	2
40214	F12	319	Rv3269	hypothetical protein	NP_217786.1	2
40207	G01	319	Rv1335	9.5 kDa culture filtrate antigen CFP10A	NP_215851.1	2
40209	G02	319	Rv2122c	phosphoribosyl-ATP pyrophosphatase	YP_177860.1	2.344827586
40204	G03	319	Rv0011c	putative septation inhibitor protein	NP_214525.1	2.482758621
9959	G04	319	Rv2922A	acylphosphatase	YP_177679.1	2
40222	G05	322	Rv1793	putative ESAT-6 like protein ESXN (ESAT-6 like protein 5)	YP_177838.1	2
40220	G06	322	Rv1567c	hypothetical protein	NP_216083.1	2
40221	G07	322	Rv1738	hypothetical protein	NP_216254.1	2
40219	G08	322	Rv1198	putative ESAT-6 like protein ESXL (ESAT-6 like protein 4)	NP_215714.1	2
40216	G09	322	Rv0105c	50S ribosomal protein L28	YP_177691.1	1.605590062
10056	G10	322	Rv1159A	pterin-4-alpha-carbinolamine dehydratase	YP_177641.1	4.335403727
10090	G11	325	Rv2309A	hypothetical protein	YP_177668.1	4.033846154
9954	G12	325	Rv2601A	hypothetical protein	YP_177673.1	2.593846154
10100	H01	337	Rv2077A	transmembrane protein	NP_216593.1	2.584569733
10130	H02	343	Rv3221A	putative acetyl-CoA carboxylase biotin carboxyl carrier protein subunit	NP_217737.1	3.174927114
40328	H03	349	Rv1574	phiRV1 phage related protein	NP_216090.1	2
10080	H04	349	Rv3312A	secreted protein antigen	YP_177957.1	3.277936963
40342	H05	352	Rv0741	transposase	NP_215255.1	2

Clone	Well Position	ORF Length	Locus ID	Description	Accession Number	Average Depth of Coverage
40350	H06	352	Rv3126c	hypothetical protein	NP_217642.1	2
40349	H07	352	Rv2876	transmembrane protein	NP_217392.1	2
40344	H08	352	Rv1849	urease subunit beta	NP_216365.1	2
40348	H09	352	Rv2749	hypothetical protein	NP_217265.1	2
40347	H10	352	Rv2442c	50S ribosomal protein L21	NP_216958.1	2
40346	H11	352	Rv2078	hypothetical protein	NP_216594.1	2
40343	H12	352	Rv1579c	phiRv1 phage protein	NP_216095.1	2

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