

***Mycobacterium tuberculosis* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 19**

**Catalog No. NR-19655**

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.

Plate orientation and viability were confirmed for NR-19655.

**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-19655 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 19, NR-19655.”

**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/bmbl5/index.htm](http://www.cdc.gov/biosafety/publications/bmbl5/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 19 (ZMTLI)**

Clone	Well Position	ORF Length	Locus ID	Description (Gene name)	Accession Number	Average Depth of Coverage
41329	A01	598	Rv1776c	transcriptional regulatory protein	NP_216292.1	2
41335	A02	598	Rv3209	hypothetical protein	NP_217725.1	3.548494983
41332	A03	598	Rv2043c	pyrazinamidase/nicotinamidase PNCA (PZase)	NP_216559.1	3.852842809
41330	A05	598	Rv1888c	transmembrane protein	NP_216404.1	2
41340	A06	601	Rv1205	hypothetical protein	NP_215721.1	4.364392679
41351	A07	601	Rv3920c	hypothetical protein	NP_218437.1	2
41345	A08	601	Rv2080	lipoprotein LppJ	NP_216596.1	2
41349	A09	601	Rv3567c	Oxidoreductase	NP_218084.1	1.856905158
41350	A10	601	Rv3724	cutinase	NP_216817	2
41337	A11	601	Rv0052	hypothetical protein	NP_214566.1	2
41346	A12	601	Rv2534c	elongation factor P	NP_217050.1	2
41344	B01	601	Rv1780	hypothetical protein	NP_216296.1	2
41338	B02	601	Rv0445c	RNA polymerase sigma factor SigK	NP_214959.1	2
41339	B03	601	Rv0716	50S ribosomal protein L5	NP_215230.1	2
41336	B04	601	Rv0004	hypothetical protein	NP_214518.1	2
41360	B05	604	Rv3606c	2-amino-4-hydroxy-6-hydroxymethylidihydropteridine pyrophosphokinase FolK	NP_218123.1	4.245033113
41353	B06	604	Rv1065	hypothetical protein	NP_215581.1	4.223509934
41361	B07	604	Rv3639c	hypothetical protein	NP_218156.1	2
41358	B08	604	Rv3405c	transcriptional regulatory protein	NP_217922.1	2
41356	B09	604	Rv2594c	Holliday junction resolvase	NP_217110.1	2
41357	B10	604	Rv2966c	methyltransferase (methylase)	NP_217482.1	2
41369	B11	607	Rv2405	hypothetical protein	NP_216921.1	2
41368	B12	607	Rv2116	No growth on plate	NP_216632.1	2
41364	C01	607	Rv1176c	hypothetical protein	NP_215692.1	4.439868204
41363	C02	607	Rv1031	potassium-transporting ATPase subunit C	NP_215547.1	2
41362	C03	607	Rv0067c	TetR family transcriptional regulator	NP_214581.1	2
41374	C04	610	Rv0464c	hypothetical protein	NP_214978.1	2
41373	C05	610	Rv0321	deoxycytidine triphosphate deaminase	NP_214836.1	2
41372	C06	610	Rv0114	D-alpha,beta-D-heptose-1,7-biphosphate phosphatase	NP_214628.1	2
41379	C07	613	Rv2016	hypothetical protein	NP_216532.1	2
41380	C09	613	Rv3657c	hypothetical protein	NP_218174.1	3.314845024
41377	C10	613	Rv1014c	peptidyl-tRNA hydrolase	NP_215530.1	2
41383	C11	613	Rv3832c	hypothetical protein	NP_218349.1	2
41378	C12	613	Rv1972	mce associated membrane protein	NP_216488.1	2
41384	D01	616	Rv3491	hypothetical protein	NP_218008.1	2
41393	D02	619	Rv2792c	resolvase	NP_217308.1	3.134087237

## Product Information Sheet for NR-19655

Clone	Well Position	ORF Length	Locus ID	Description (Gene name)	Accession Number	Average Depth of Coverage
41394	D03	619	Rv2875	major secreted immunogenic protein MPT70	NP_217391.1	2
41387	D04	619	Rv0274	hypothetical protein	NP_214788.1	2
41388	D05	619	Rv0921	resolvase	NP_215436.1	2
41389	D06	619	Rv1314c	hypothetical protein	NP_215830.1	3.224555735
41396	D07	619	Rv3362c	ATP/GTP-binding protein	NP_217879.1	2.337641357
41404	D08	622	Rv3773c	hypothetical protein	NP_218290.1	2.856913183
41400	D09	622	Rv2430c	PPE family protein	YP_177881.1	2
41399	D10	622	Rv1203c	hypothetical protein	NP_215719.1	2
41403	D11	622	Rv2979c	resolvase	NP_217495.1	2
41398	D12	622	Rv0196	transcriptional regulatory protein	NP_214710.1	3.135048232
41412	E01	625	Rv2613c	hypothetical protein	NP_217129.1	2
41410	E02	625	Rv2428	alkyl hydroperoxide reductase subunit C	NP_216944.1	3.5664
41413	E03	625	Rv2912c	TetR family transcriptional regulator	NP_217428.1	2
41408	E04	625	Rv1624c	hypothetical protein	NP_216140.1	4.1248
41406	E05	625	Rv1156	hypothetical protein	NP_215672.1	1.7168
41409	E06	625	Rv2134c	hypothetical protein	NP_216650.1	2
41419	E07	628	Rv2593c	Holliday junction DNA helicase RuvA	NP_217109.1	2
41418	E08	628	Rv1944c	hypothetical protein	NP_216460.1	2
41415	E09	628	Rv0113	phosphoheptose isomerase	NP_214627.1	3.109872611
41420	E10	628	Rv3233c	hypothetical protein	NP_217750.1	2
41417	E11	628	Rv1703c	catechol-o-methyltransferase	NP_216219.1	2
41416	E12	628	Rv0681	TetR family transcriptional regulator	NP_215195.1	2
41422	F01	631	Rv0089	methyltransferase/methylase	NP_214603.1	3.050713154
41424	F02	631	Rv0371c	hypothetical protein	NP_214885.1	2
41428	F03	631	Rv0992c	hypothetical protein	NP_215507.1	2
41431	F04	631	Rv1541c	lipoprotein LprI	NP_216057.1	3.846275753
41432	F05	631	Rv1910c	hypothetical protein	NP_216426.1	2
41427	F06	631	Rv0706	50S ribosomal protein L22	NP_215220.1	2
41429	F07	631	Rv1019	TetR family transcriptional regulator	NP_215535.1	2
41423	F09	631	Rv0366c	hypothetical protein	NP_214880.1	2
10111	F10	631	Rv0078A	hypothetical protein	YP_177616.1	5.199683043
9991	F11	631	Rv2306A	hypothetical protein	YP_177663.1	2
41436	F12	634	Rv2604c	glutamine amidotransferase subunit PdxT	NP_217120.1	2
41435	G01	634	Rv1233c	hypothetical protein	NP_215749.1	2
41434	G02	634	Rv0691c	transcriptional regulatory protein	NP_215205.1	3.105678233
41442	G03	637	Rv1885c	chorismate mutase	NP_216401.1	3.466248038
41445	G04	637	Rv3574	transcriptional regulatory protein TetR-family	NP_218091.1	2
41443	G05	637	Rv1986	integral membrane protein	NP_216502.1	2
41439	G06	637	Rv0789c	hypothetical protein	NP_215304.1	4.185243328
41440	G07	637	Rv1504c	hypothetical protein	NP_216020.1	2
41446	G08	637	Rv3755c	hypothetical protein	NP_218272.1	2
41437	G09	637	Rv0475	iron-regulated heparin binding hemagglutinin hbhA (adhesin)	NP_214989.1	2
41444	G10	637	Rv2949c	hypothetical protein	NP_217465.1	2
41451	G11	640	Rv2461c	ATP-dependent Clp protease proteolytic subunit	YP_177883.1	4.2625
41447	G12	640	Rv0328	TetR/AcrR family transcriptional regulator	NP_214842.1	2
41449	H01	640	Rv1401	hypothetical protein	NP_215917.1	3.090625
41452	H02	640	Rv3120	hypothetical protein	NP_217636.1	2
41454	H03	640	Rv3173c	TetR/ACRR family transcriptional regulator	NP_217689.1	3.0921875
41455	H04	640	Rv3557c	TetR family transcriptional regulator	NP_218074.1	2
41466	H05	643	Rv1167c	transcriptional regulatory protein	NP_215683.1	2
41475	H06	643	Rv3458c	30S ribosomal protein S4	NP_217975.1	2
41474	H07	643	Rv2453c	molybdopterin-guanine dinucleotide biosynthesis protein A	NP_216969.1	2

Clone	Well Position	ORF Length	Locus ID	Description (Gene name)	Accession Number	Average Depth of Coverage
41457	H08	643	Rv0133	acetyltransferase	NP_214647.1	2
41467	H09	643	Rv1412	riboflavin synthase subunit alpha	NP_215928.1	2
41464	H10	643	Rv1126c	hypothetical protein	NP_215642.1	2
41465	H11	643	Rv1163	respiratory nitrate reductase subunit delta NarJ	NP_215679.1	2