

***Mycobacterium tuberculosis* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 20**

Catalog No. NR-19656

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

For research use only. Not for use in humans.

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-19656.

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-19656 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 20, NR-19656."

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 \(BMBL\)](#), 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

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 20 (ZMTLJ)¹

Clone	Well Position	ORF Length	Locus ID	Description	Accession Number	Average Depth of Coverage
41456	A01	643	Rv0078	transcriptional regulatory protein	NP_214592.1	2
41471	A02	643	Rv1911c	lipoprotein LppC	NP_216427.1	3.833592535
41479	A03	646	Rv0525	hypothetical protein	NP_215039.1	4.27244582
41490	A04	646	Rv3609c	GTP cyclohydrolase I	NP_218126.1	2
41484	A05	646	Rv1539	lipoprotein signal peptidase	NP_216055.1	2
41481	A06	646	Rv1252c	lipoprotein LprE	NP_215768.1	2
41485	A07	646	Rv1556	regulatory protein	NP_216072.1	4.255417957
41482	A08	646	Rv1255c	transcriptional regulatory protein	NP_215771.1	2
41483	A09	646	Rv1435c	proline, glycine, valine-rich secreted protein	NP_215951.1	2
41489	A10	646	Rv3465	dTDP-4-dehydrorhamnose 3,5-epimerase RmlC	NP_217982.1	2
41487	A11	646	Rv2811	hypothetical protein	NP_217327.1	2
41480	A12	646	Rv0605	resolvase	NP_215119.1	2
41488	B01	646	Rv3066	DeoR family transcriptional regulator	NP_217582.1	2
41498	B02	649	Rv1890c	hypothetical protein	NP_216406.1	2
41495	B03	649	Rv1688	3-methyladenine DNA glycosylase	NP_216204.1	2
41501	B04	649	Rv3715c	recombination protein RecR	NP_218232.1	2
41492	B05	649	Rv0698	hypothetical protein	NP_215212.1	4.235747304
41502	B06	649	Rv3828c	resolvase	NP_218345.1	2
41500	B07	649	Rv3214	acid phosphatase	YP_177944.1	2
41499	B08	649	Rv2193	cytochrome C oxidase subunit III	NP_216709.1	2
41497	B09	649	Rv1745c	isopentenyl-diphosphate delta-isomerase	NP_216261.1	3.1201849
41510	B10	652	Rv2732c	transmembrane protein	NP_217248.1	2
41504	B11	652	Rv0238	TetR family transcriptional regulator	NP_214752.1	4.225460123
41509	B12	652	Rv1958c	hypothetical protein	NP_216474.1	2
41508	C01	652	Rv1341	putative deoxyribonucleotide triphosphate pyrophosphatase	NP_215857.1	3.685582822
41505	C02	652	Rv0316	muconolactone isomerase	NP_214830.1	2
41515	C03	652	Rv3322c	methyltransferase	YP_177958.1	2
41511	C04	652	Rv3007c	oxidoreductase	NP_217523.1	4.306748466
41518	C05	655	Rv1626	two-component system transcriptional regulator	NP_216142.1	2
41517	C06	655	Rv1498c	hypothetical protein	YP_177647.1	3.838167939
41524	C07	658	Rv2597	hypothetical protein	NP_217113.1	1.995440729
41523	C08	658	Rv2170	hypothetical protein	NP_216686.1	2
41522	C09	658	Rv1602	imidazole glycerol phosphate synthase subunit HisH	NP_216118.1	2
41519	C10	658	Rv0273c	transcriptional regulatory protein	NP_214787.1	2
41542	C11	661	Rv3588c	carbonic anhydrase	NP_218105.1	3.838124054
41539	C12	661	Rv3008	hypothetical protein	NP_217524.1	2
41527	D01	661	Rv0600c	two component sensor kinase	NP_215114.2	2
41533	D02	661	Rv2114	hypothetical protein	NP_216630.1	2
41537	D03	661	Rv2849c	cob(I)yrinic acid a,c-diamide adenosyltransferase	YP_177908.1	4.220877458
41534	D04	661	Rv2466c	hypothetical protein	NP_216982.1	2
41540	D05	661	Rv3309c	uracil phosphoribosyltransferase	NP_217826.1	4.248108926
41532	D06	661	Rv1700	hypothetical protein	NP_216216.1	2

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Clone	Well Position	ORF Length	Locus ID	Description	Accession Number	Average Depth of Coverage
41538	D07	661	Rv2926c	hypothetical protein	NP_217442.1	2
41544	D08	661	Rv3846	superoxide dismutase	NP_218363.1	2.515885023
41529	D09	661	Rv1044	hypothetical protein	NP_215560.1	2
41528	D10	661	Rv0775	hypothetical protein	NP_215289.1	2
41535	D11	661	Rv2491	hypothetical protein	NP_217007.1	2
41545	D12	664	Rv0042c	MarR family transcriptional regulator	NP_214556.1	2
41546	E01	664	Rv0329c	hypothetical protein	NP_214843.1	2
41553	E02	664	Rv2652c	phiRv2 prophage protein	NP_217168.1	2
41554	E03	664	Rv3167c	TetR family transcriptional regulator	NP_217683.1	2
41551	E04	664	Rv2065	precorrin-8X methylmutase	NP_216581.1	2
41548	E05	664	Rv1389	guanylate kinase	NP_215905.1	3.826807229
41549	E06	664	Rv1853	urease accessory protein ureD	NP_216369.1	3.832831325
41552	E07	664	Rv2306c	hypothetical protein	NP_216823.1	2
10033	E08	664	Rv3395A	hypothetical protein	NP_217912.2	2
41558	E09	667	Rv1822	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase	NP_216338.1	2
41563	E10	667	Rv3016	lipoprotein LpqA	NP_217532.1	2
41555	E12	667	Rv0421c	hypothetical protein	NP_214935.1	2
41559	F01	667	Rv2746c	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase	NP_217262.1	2
41562	F02	667	Rv2804c	hypothetical protein	NP_217320.1	4.24137931
41560	F03	667	Rv2799	hypothetical protein	NP_217315.1	2
41564	F04	667	Rv3830c	transcriptional regulatory protein TetR-family	NP_218347.1	4.217391304
41572	F05	670	Rv1601	imidazoleglycerol-phosphate dehydratase	NP_216117.1	2
41565	F06	670	Rv0264c	hypothetical protein	NP_214778.1	2
41578	F07	670	Rv3897c	hypothetical protein	NP_218414.1	2
41567	F08	670	Rv0539	dolichyl-phosphate sugar synthase	NP_215053.1	2
41577	F09	670	Rv2968c	integral membrane protein	NP_217484.1	2
41574	F10	670	Rv1733c	transmembrane protein	NP_216249.1	4.219402985
41566	F11	670	Rv0302	TetR/ACRR family transcriptional regulator	NP_214816.1	2
41570	F12	670	Rv1289	hypothetical protein	NP_215805.1	2
41569	G01	670	Rv0970	integral membrane protein	NP_215485.1	4.228358209
41571	G02	670	Rv1347c	hypothetical protein	NP_215863.1	2
41584	G03	673	Rv2421c	nicotinic acid mononucleotide adenylyltransferase	NP_216937.1	3.50371471
41583	G04	673	Rv2260	hypothetical protein	NP_216776.1	1.891530461
41590	G05	673	Rv3421c	hypothetical protein	NP_217938.1	2
41591	G06	673	Rv3641c	cell filamentation protein FIC	NP_218158.1	2
41581	G07	673	Rv1870c	hypothetical protein	NP_216386.1	2
41580	G08	673	Rv1851	urease accessory protein uref	NP_216367.1	2
41579	G09	673	Rv0195	two component transcriptional regulatory protein	NP_214709.1	-
41588	G10	673	Rv3249c	TetR family transcriptional regulator	NP_217766.1	2
41596	G12	676	Rv1377c	putative transferase	NP_215893.1	2.044378698
41593	H01	676	Rv1109c	hypothetical protein	NP_215625.1	2.221893491
41597	H02	676	Rv2295	hypothetical protein	NP_216811.1	3.125739645
41594	H03	676	Rv1219c	transcriptional regulatory protein	NP_215735.1	2
41600	H04	676	Rv3414c	RNA polymerase sigma factor SigD	NP_217931.1	2.202662722
41610	H05	679	Rv3160c	TetR family transcriptional regulator	NP_217676.1	2
41603	H06	679	Rv0398c	hypothetical protein	NP_214912.1	2
41604	H07	679	Rv0767c	hypothetical protein	NP_215281.1	4.762886598
41606	H08	679	Rv0821c	phosphate transport regulator	NP_215336.1	2
41607	H09	679	Rv0825c	hypothetical protein	NP_215340.1	2
41613	H10	679	Rv3242c	hypothetical protein	NP_217759.1	2.223858616
41611	H11	679	Rv3235	hypothetical protein	NP_217752.1	3.430044183
41608	H12	679	Rv1154c	hypothetical protein	NP_215670.1	2

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