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SUPPORTING INFECTIOUS DISEASE RESEARCH

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

Catalog No. NR-19655

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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 crosscontamination 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 <u>pDONRTM221</u> 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 <u>Invitrogen</u>[™]. Recombination was facilitated through an *att*B substrate (*att*B-PCR product or a linearized *att*B expression clone) with an *att*P substrate (pDONR[™]221) to create an *att*L-containing entry clone. The entry clone contains recombinational cloning sites, *att*L1 and *att*L2 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[™] <u>Gateway[®] Technology Manual</u> for additional details.

Plate orientation and viability were confirmed for NR-19655.

Material Provided:

Each well of the 96-well plate contains approximately $60 \ \mu L$ of *E. coli* culture (strain DH10B-T1) in Luria Bertani (LB) broth containing 50 μ g/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:

<u>Media</u>: 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. <u>Biosafety in Microbiological and Biomedical Laboratories</u>. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see 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.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at <u>www.beiresources.org</u>.

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References:

- Cole, S. T., et al. "Deciphering the Biology of Mycobacterium tuberculosis from the Complete Genome Sequence." <u>Nature</u> 393 (1998): 537-544. PubMed: 9634230.
- Camus, J. C., et al. "Re-Annotation of the Genome Sequence of *Mycobacterium tuberculosis* H37Rv." <u>Microbiology</u> 148 (2002): 2967-2973. PubMed 12368430.

ATCC[®] is a trademark of the American Type Culture Collection.



Table 1: *Mycobacterium tuberculosis*, Gateway[®] Clones, Plate 19 (ZMTLI)

Position Length Number of Coverage 41329 A01 598 Rv1776c transcriptional regulatory protein NP 216252.1 2 41332 A02 598 Rv2039 hypothetical protein NP 216559.1 3.548494983 41332 A03 598 Rv1888c transmembrane protein NP 216640.1 2 41340 A06 601 Rv1205 hypothetical protein NP 215721.1 4.364392679 41345 A07 601 Rv320c hypothetical protein NP 216586.1 2 41345 A08 601 Rv320c hypothetical protein NP 218061.1 2 41345 A08 601 Rv3272 cutinase NP 218061.1 2 41346 A10 601 Rv3724 cutinase NP 2145817 2 41348 B01 601 Rv3724 cutinase NP 214566.1 2 41348 B01 R01 Rv0776 S05 ribosomal protein NP 216286.1 <t< th=""><th>Clone</th><th>Well</th><th>ORF</th><th>Locus ID</th><th>Description (Gene name)</th><th>Accession</th><th>Average Depth</th></t<>	Clone	Well	ORF	Locus ID	Description (Gene name)	Accession	Average Depth
41329 A01 598 Rv1776c transcriptional regulatory protein NP_216292.1 2 41335 A02 598 Rv2043c pyrazinamidase/nicolinamidas PNCA (PZase) NP_216559.1 3.852842809 41330 A05 598 Rv1288c transmembrane protein NP_216404.1 2 41340 A06 601 Rv1205 hypothetical protein NP_218437.1 2 41345 A08 601 Rv3206 hypothetical protein NP_218437.1 2 41349 A09 601 Rv3567c Oxidoreductase NP_216801.1 2 41340 A10 601 Rv3572 cutinase NP_216806.1 2 41343 A11 601 Rv3724 cutinase NP_217050.1 2 41344 A12 601 Rv2534c elongation factor P NP_214950.1 2 41336 B02 601 Rv0445c RNA polymerase sigma factor SigK NP_21451.1 2 41338 B03 601 <th></th> <th>Position</th> <th>Length</th> <th></th> <th></th> <th>Number</th> <th>of Coverage</th>		Position	Length			Number	of Coverage
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41340 A06 601 Rv1205 hypothetical protein NP_215721.1 4.364392679 41345 A07 601 Rv3920c hypothetical protein NP_216396.1 2 41345 A08 601 Rv2080 lipoprotein LpJ NP_216596.1 2 41345 A09 601 Rv2080 lipoprotein LpJ NP_216896.1 2 41345 A10 601 Rv3567c Oxidoreductase NP_216896.1 2 41334 A01 601 Rv052 hypothetical protein NP_216296.1 2 41346 A12 601 Rv0542 RNA polymerase sigma factor SigK NP_214959.1 2 41338 B02 601 Rv0716 50S ribosomal protein NP_214959.1 2 41336 B04 601 Rv0704 hypothetical protein NP_214958.1 2 41336 B05 604 Rv3606c hydroxymethyldihydropterfidine NP_215230.1 2 41353 B06 604	41332	A03	598	Rv2043c	pyrazinamidase/nicotinamidas PNCA (PZase)	NP_216559.1	3.852842809
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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 41372 C06 610 Rv0114 D-alpha,beta-D-heptose-1,7-biphosphate NP_214628.1 2 41379 C07 613 Rv2016 hypothetical protein NP_216532.1 2 41380 C09 613 Rv3657c hypothetical protein NP_21530.1 2 41383 C11 613 Rv3832c hypothetical protein NP_218349.1 2 41378		B09	604	Rv2594c	Holliday junction resolvase	NP_217110.1	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_214628.1 2 41372 C06 610 Rv0114 D-alpha,beta-D-heptose-1,7-biphosphate NP_214628.1 2 41379 C07 613 Rv2016 hypothetical protein NP_218530.1 2 41380 C09 613 Rv3657c hypothetical protein NP_218349.1 2 4	41357	B10	604	Rv2966c	methyltransferase (methylase)	NP_217482.1	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_218532.1 2 41380 C09 613 Rv3657c hypothetical protein NP_215530.1 2 41383 C11 613 Rv3832c hypothetical protein NP_216488.1 2 41378 C12 613 Rv1972 mce associated membrane protein NP_216488.1 2 </td <td>41369</td> <td>B11</td> <td>607</td> <td>Rv2405</td> <td></td> <td>NP_216921.1</td> <td></td>	41369	B11	607	Rv2405		NP_216921.1	
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_214581.1 2 41373 C05 610 Rv0321 deoxycytidine triphosphate deaminase NP_214836.1 2 41372 C06 610 Rv0114 D-alpha,beta-D-heptose-1,7-biphosphate NP_214628.1 2 41379 C07 613 Rv2016 hypothetical protein NP_216532.1 2 41380 C09 613 Rv3657c hypothetical protein NP_21530.1 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	41368	B12	607	Rv2116	No growth on plate	NP_216632.1	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 deaminase 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_21530.1 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 <td>41364</td> <td>C01</td> <td>607</td> <td>Rv1176c</td> <td>hypothetical protein</td> <td>NP_215692.1</td> <td>4.439868204</td>	41364	C01	607	Rv1176c	hypothetical protein	NP_215692.1	4.439868204
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 deaminase 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_21530.1 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 <td>41363</td> <td>C02</td> <td>607</td> <td>Rv1031</td> <td>potassium-transporting ATPase subunit C</td> <td>NP_215547.1</td> <td>2</td>	41363	C02	607	Rv1031	potassium-transporting ATPase subunit C	NP_215547.1	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_21530.1 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	41362	C03	607	Rv0067c	TetR family transcriptional regulator		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	41374	C04	610	Rv0464c	hypothetical protein	NP_214978.1	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	41373	C05	610	Rv0321	deoxycytidine triphosphate deaminase	NP_214836.1	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	41372	C06	610	Rv0114			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	41379	C07	613	Rv2016		NP_216532.1	2
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							
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							
41378 C12 613 Rv1972 mce associated membrane protein NP_216488.1 2 41384 D01 616 Rv3491 hypothetical protein NP_218008.1 2							
41384 D01 616 Rv3491 hypothetical protein NP_218008.1 2						NP_216488.1	
						NP_218008.1	
41393 D02 619 Rv2792c resolvase NP_217308.1 3.134087237	41393	D02	619	Rv2792c	resolvase	NP_217308.1	3.134087237

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SUPPORTING INFECTIOUS DISEASE RESEARCH

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	D10	622	Rv2979c	resolvase	NP_217495.1	2
41398	D12	622	Rv0196	transcriptional regulatory protein	NP_214710.1	3.135048232
41390	E01	625	Rv2613c	hypothetical protein	NP_217129.1	2
41412	E01 E02	625	Rv20130 Rv2428	alkyl hydroperoxide reductase subunit C	NP_216944.1	3.5664
41410	E02 E03	625				2
			Rv2912c	TetR family transcriptional regulator	NP_217428.1	
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 Lprl	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
41433	G01 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
41400	H06	643	Rv3458c	30S ribosomal protein S4	NP_217975.1	2
414/0	1100	040		molybdopterin-guanine dinucleotide biosynthesis		
41474	H07	643	Rv2453c	protein A	NP_216969.1	2

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SUPPORTING INFECTIOUS DISEASE RESEARCH

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