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SUPPORTING INFECTIOUS DISEASE RESEARCH

Mycobacterium tuberculosis Gateway[®] Clone Set, Recombinant in *Escherichia coli*, Plate 7

Catalog No. NR-19643

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 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>pDONR™221</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[™] Gateway[®] Technology Manual for additional details.

Plate orientation and viability were confirmed for NR-19643.

Material Provided:

Each inoculated well of the 96-well plate contains approximately $60 \ \mu$ L of culture in Luria Bertani (LB) broth containing 50 μ g/mL kanamycin supplemented with 15% glycerol.

Packaging/Storage:

NR-19643 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 μ g/mL kanamycin

Incubation:

Temperature: 37°C Atmosphere: Aerobic

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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 7, NR-19643."

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 7 (ZMTDG)¹

Clone	Well Position	ORF Length	Locus ID	Description (Gene name)	Accession Number	Average Depth of Coverage
74787	A01	1534	Rv2004c	hypothetical protein	NP_216520.1	3.322685789
74777	A02	1540	Rv3490	alpha,alpha-trehalose-phosphate synthase	NP_218007.1	3.507142857
74918	A03	1549	Rv0336	13E12 repeat family protein	NP_214850.1	2.276307295
74957	A04	1555	Rv0895	hypothetical protein	NP_215410.1	3.427652733
74903	A05	1558	Rv0147	aldehyde dehydrogenase	NP_214661.1	3.486521181
74866	A06	1561	Rv3062	ATP-dependent DNA ligase	NP_217578.1	3.624599616
74906	A07	1561	Rv3561	acyl-CoA synthetase	NP_218078.1	2.864189622
75055	A08	1564	Rv0592	MCE-family protein MCE2D	NP_215106.1	4.098465473
74893	A09	1567	Rv3698	hypothetical protein	NP_218215.1	5.124441608
74971	A10	1567	Rv3887c	transmembrane protein	NP_218404.1	4.257817486
74921	A11	1573	Rv0234c	succinic semialdehyde dehydrogenase	NP_216247.2	4.27972028
74810	A12	1576	Rv2209	integral membrane protein	NP_216725.1	3.556472081
74897	B01	1576	Rv2733c	hypothetical protein	NP_217249.1	3.277284264
74697	B02	1582	Rv2414c	hypothetical protein	NP_216930.1	5.06573957
75046	B03	1585	Rv0171	MCE-family protein MCE1C	NP_214685.1	2.998107256
75041	B04	1585	Rv2213	leucyl aminopeptidase	NP_216729.1	5.203154574
74833	B05	1585	Rv2329c	nitrite extrusion protein 1 NarK1	NP_216845.1	3.013249211
74994	B06	1585	Rv3509c	hypothetical protein	NP_218026.1	3.176656151
75037	B07	1588	Rv2174	integral membrane protein	NP_216690.1	4.08186398
74737	B08	1594	Rv0453	PPE family protein	YP_177727.1	2.640526976
74938	B09	1597	Rv3776	hypothetical protein	NP_218293.1	3.046963056
74758	B10	1600	Rv2223c	exported protease	NP_216739.1	3.515
75058	B11	1603	Rv1345	long-chain-fatty-acid	NP_215861.1	3.670617592
74769	B12	1612	Rv2154c	FtsW-like protein FtsW	NP_216670.1	4.265508685
74849	C01	1612	Rv3049c	monooxygenase	NP_217565.1	2.905086849
74713	C02	1621	Rv3211	ATP-dependent RNA helicase RhIE	NP_217727.1	3.312152992
74927	C03	1624	Rv2672	secreted protease	NP_217188.1	3.253694581
74935	C04	1627	Rv2502c	acetyl-/propionyl-CoA carboxylase subunit beta	NP_217018.1	3.525507068
74981	C05	1627	Rv3737	transmembrane protein	NP_218254.1	4.163491088
74855	C06	1636	Rv0806c	UDP-glucose-4-epimerase CpsY	NP_215321.1	3.647310513
74989	C07	1642	Rv3308	phosphomannomutase	NP_217825.1	3.950669915
75069	C08	1645	Rv0897c	oxidoreductase	NP_215412.1	4.145896657
74838	C09	1648	Rv3379c	1-deoxy-D-xylulose-5-phosphate synthase	NP_217896.1	3.52973301
74843	C10	1651	Rv3885c	hypothetical protein	NP_218402.1	4.235614779
75035	C11	1654	Rv1186c	hypothetical protein	NP_215702.1	3.477629988
74953	C12	1657	Rv3811	hypothetical protein	YP_178018.1	3.02051901
74883	D01	1666	Rv0672	acyl-CoA dehydrogenase FADE8	NP_215186.1	3.662665066
74998	D02	1672	Rv1013	acyl-CoA synthetase	NP_215529.1	3.003588517

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Clone	Well Position	ORF Length	Locus ID	Description (Gene name)	Accession Number	Average Depth of Coverage
75005	D03	1684	Rv1217c	tetronasin-transport integral membrane protein ABC transporter	NP_215733.1	3.866983373
75074	D04	1687	Rv2800	hydrolase	NP_217316.1	3.462359218
75065	D05	1690	Rv2100	hypothetical protein	NP_216616.1	3.956213018
74789	D06	1696	Rv0782	oligopeptidase B	NP 215295.2	4.264150943
74914	D07	1699	Rv0946c	glucose-6-phosphate isomerase	NP 215461.1	3.380812243
74782	D08	1702	Rv0166	acvl-CoA synthetase	NP 214680.1	3.456521739
75449	D09	1708	Rv0256c	PPE family protein	YP 177704.1	4.266978923
75125	D10	1729	Rv1754c	hypothetical protein	NP 216270.1	4.053209948
75323	D11	1741	Rv3245c	two component sensory transduction histidine kinase MTRB	NP_217762.1	3.998276852
75289	D12	1753	Rv1550	fatty-acid-CoA ligase	NP_216066.1	4.19965773
75182	E01	1768	Rv0226c	transmembrane protein	NP_214740.1	3.182126697
75393	E02	1774	Rv1185c	acyl-CoA synthetase	NP 215701.1	4.117249154
75317	E03	1786	Rv3533c	PPE family protein	YP 177985.1	3.555431131
75110	E04	1789	Rv2565	hypothetical protein	NP_217081.1	3.474007826
75142	E05	1792	Rv3590c	PE-PGRS family protein	YP 177993.1	1.938616071
75129	E06	1807	Rv2088	transmembrane serine/threonine-protein kinase J	NP_216604.1	4.122302158
75453	E07	1813	Rv1280c	periplasmic oligopeptide-binding lipoprotein OppA	NP 215796.1	4.079426365
75275	E08	1813	Rv3635	transmembrane protein	NP 218152.1	3,237727523
75262	E09	1828	Rv2572c	aspartyl-tRNA synthetase	NP 217088.1	2,791575492
75410	E10	1843	Rv0126	trehalose synthase TRES	NP 214640 1	2 295713511
75265	F11	1846	Rv1297	transcription termination factor Rho	NP 215813.1	2 908992416
75145	E12	1867	Rv2115c	ATPase	NP 216631.1	4 086234601
75330	F01	1873	Rv0244c	acyl-CoA dehydrogenase FADE5	NP 214758 1	2 849973305
75097	F02	1876	Rv1281c	oligopeptide-transport ATP-binding protein ABC transporter OppD	NP_215797.1	4.065565032
75351	F03	1885	Rv1492	methylmalonyl-CoA mutase small subunit	NP_216008.1	4.187798408
75083	F04	1885	Rv2356c	PPE family protein	YP_177872.1	3.975066313
75339	F05	1894	Rv1768	PE-PGRS family protein	YP_177832.1	2.343189018
75191	F06	1897	Rv3884c	CBXX/CFQX family protein	NP_218401.1	4.112282551
75375	F07	1900	Rv1925	acyl-CoA synthetase	NP_216441.1	3.796315789
75345	F08	1903	Rv1551	glycerol-3-phosphate acyltransferase	NP_216067.1	3.280084078
75137	F09	1933	Rv0282	hypothetical protein	NP_214796.1	3.829280911
75238	F10	1933	Rv1272c	drugs-transport transmembrane ATP-binding protein ABC transporter	NP_215788.1	3.167615106
75206	F11	1951	Rv3801c	acyl-CoA synthetase	NP_218318.1	3.150691953
75253	F12	1951	Rv3808c	bifunctional UDP-galactofuranosyl transferase GLFT	NP_218325.1	3.897488467
75333	G01	1954	Rv2682c	1-deoxy-D-xylulose-5-phosphate synthase	YP_177898.1	3.217502559
75305	G02	1972	Rv3710	2-isopropylmalate synthase	NP_218227.2	3.303245436
75113	G03	1978	Rv0248c	succinate dehydrogenase flavoprotein subunit	NP_214762.1	3.364509606
75219	G04	1978	Rv1420	excinuclease ABC subunit C	NP_215936.1	4.003538928
75367	G05	1981	Rv2299c	heat shock protein 90	NP_216815.1	3.467945482
75193	G06	1993	Rv3667	acetyl-CoA synthetase	NP_218184.1	3.538384345
75327	G07	2008	Rv2079	hypothetical protein	NP_216595.1	3.840139442
75170	G08	2020	Rv3743c	cation transporter P-type ATPase CtpJ	NP_218260.1	2.593069307
75159	G09	2023	Rv0102	integral membrane protein	NP_214616.1	3.739495798
75163	G10	2032	Rv0931c	transmembrane serine/threonine-protein kinase D PKND (protein kinase D) (STPK D)	NP_215446.1	3.758366142
75117	G11	2038	Rv3779	transmembrane protein alanine and leucine rich	NP 218296.1	4.407752699
75443	G12	2041	Rv2395	integral membrane protein	NP 216911.1	4.24399804
75242	H01	2059	Rv0457c	peptidase	NP 214971.1	3.639630889
75437	H02	2059	Rv1084	hypothetical protein	NP_215600.1	4.066537154

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75249	H03	2071	Rv1836c	hypothetical protein	NP_216352.1	4.142926123
75593	H04	2101	Rv1877	integral membrane protein	NP_216393.1	4.360780581
75691	H05	2131	Rv2326c	transmembrane ATP-binding protein ABC transorter	NP_216842.1	3.916001877
75765	H06	2182	Rv0120c	elongation factor G	NP_214634.1	4.250229148
75579	H07	2203	Rv1564c	maltooligosyltrehalose synthase TreX	YP_177821.1	3.860190649
75622	H08	2212	Rv1781c	4-alpha-glucanotransferase MalQ	NP_216297.1	3.775768535
75697	H09	2266	Rv2984	polyphosphate kinase	NP_217500.1	7.053398058
75799	H10	2317	Rv0938	ATP-dependent DNA ligase	NP_215453.1	3.981009927
75702	H11	2350	Rv0969	metal cation transporter P-type ATPase CtpV	NP_215484.1	3.098297872
75843	H12	2353	Rv0949	ATP-dependent DNA helicase II UVRD1	YP_177772.1	3.552911177

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

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