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SUPPORTING INFECTIOUS DISEASE RESEARCH

Mycobacterium tuberculosis Gateway[®] Clone Set, Recombinant in *Escherichia coli*, Plate 16

Catalog No. NR-19652

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 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-19652.

Material Provided:

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

Packaging/Storage:

NR-19652 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

BEI Resources www.beiresources.org 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: 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 16, NR-19652."

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 (BMBL)</u>. 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

Disclaimers:

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Product Information Sheet for NR-19652

SUPPORTING INFECTIOUS DISEASE RESEARCH

performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

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.

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Table 1: Mycobacterium tuberculosis Gateway® Clones, Plate 16 (ZMTLF)¹

Clone	Well Position	ORF Length	Locus ID	Description	Accession Number	Average Depth of Coverage
40804	A01	454	Rv2475c	hypothetical protein Rv2475c	NP 216991.1	2
40799	A02	454	Rv0841c	transmembrane protein	YP 177634.1	3.169603524
40797	A03	454	Rv0708	50S ribosomal protein L16	NP 215222.1	2
40814	A04	457	Rv2199c	integral membrane protein	NP 216715.1	2
40808	A05	457	Rv0760c	hypothetical protein Rv0760c	NP 215274.1	2
40821	A06	457	Rv2887	transcriptional regulatory protein	NP 217403.1	2
40815	A07	457	Rv2341	lipoprotein LppQ	NP 216857.1	2
40813	A08	457	Rv1982c	hypothetical protein Rv1982c	NP 216498.1	1.98249453
40807	A09	457	Rv0022c	transcriptional regulatory protein WHIB-like WHIB5	NP 214536.1	2
40820	A10	457	Rv2762c	hypothetical protein Rv2762c	NP 217278.1	2
40819	A11	457	Rv2551c	hypothetical protein Rv2551c	NP 217067.1	2
40817	A12	457	Rv2437	hypothetical protein Rv2437	NP 216953.1	1.81619256
40809	B01	457	Rv0965c	hypothetical protein Rv0965c	NP 215480.1	2
40810	B02	457	Rv1064c	lipoprotein LpgV	NP 215580.1	1.93654267
40822	B03	457	Rv3259	hypothetical protein Rv3259	NP 217776.1	2
40825	B04	457	Rv3601c	aspartate alpha-decarboxylase	NP 218118.1	2
40818	B05	457	Rv2530c	hypothetical protein Rv2530A	YP 177672.1	2
40829	B06	460	Rv0474	transcriptional regulatory protein	NP 214988.1	2.104347826
40833	B07	460	Rv1847	hypothetical protein Rv1847	NP 216363.1	2
40827	B08	460	Rv0451c	membrane protein	NP_214965.1	2
40830	B09	460	Rv0816c	thioredoxin ThiX	NP 215331.1	-
40834	B10	460	Rv1881c	lipoprotein LppE	NP 216397.1	2
40836	B11	460	Rv2513	hypothetical protein Rv2513	NP 217029.1	2
40826	B12	460	Rv0390	hypothetical protein Rv0390	NP 214904.1	2
40835	C01	460	Rv2261c	hypothetical protein Rv2261c	NP 216777.1	2
10040	C02	460	Rv2219A	hypothetical protein Rv2219A	YP 177661.1	3.906521739
40850	C03	463	Rv2620c	transmembrane protein	NP 217136.1	3.177105832
40852	C04	463	Rv3064c	integral membrane protein	NP 217580.1	2
40845	C05	463	Rv0866	molybdenum cofactor biosynthesis protein E2	NP 215381.1	2
40846	C06	463	Rv1160	7,8-dihydro-8-oxoguanine-triphosphatase	NP 215676.1	2
40849	C07	463	Rv2494	hypothetical protein Rv2494	NP 217010.1	2
40839	C09	463	Rv0061	hypothetical protein Rv0061	NP 214575.1	2
40862	C10	466	Rv0749	hypothetical protein Rv0749	NP 215263.1	2
40861	C11	466	Rv0677c	hypothetical protein Rv0677c	NP 215191.1	2
40857	C12	466	Rv0441c	hypothetical protein Rv0441c	NP 214955.1	3.096566524
40870	D01	466	Rv3320c	hypothetical protein Rv3320c	NP 217837.1	2
40867	D02	466	Rv2406c	hypothetical protein Rv2406c	NP 216922.1	3.072961373
40854	D03	466	Rv0277c	hypothetical protein Rv0277c	NP_214791.1	2.650214592
40868	D04	466	Rv2704	hypothetical protein Rv2704	NP 217220.1	2
40869	D05	466	Rv3142c	hypothetical protein Rv3142c	NP 217658.1	-
40863	D06	466	Rv1987	chitinase	NP 216503.1	2
40855	D07	466	Rv0403c	membrane protein	NP_214917.1	2

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Product Information Sheet for NR-19652

SUPPORTING INFECTIOUS DISEASE RESEARCH

Clone	Well Position	ORF Length	Locus ID	Description	Accession Number	Average Depth of Coverage
40858	D08	466	Rv0636	(3R)-hydroxyacyl-ACP dehydratase subunit HadB	NP_215150.1	2
40874	D09	469	Rv1242	hypothetical protein Rv1242	NP_215758.1	2
40881	D10	469	Rv2166c	cell division protein MraZ	NP_216682.1	2
40883	D11	469	Rv2599	hypothetical protein Rv2599	NP 217115.1	3.085287846
40877	D12	469	Rv1897c	D-tyrosyl-tRNA(Tyr) deacylase	NP_216413.1	2
40886	E01	469	Rv2645	hypothetical protein Rv2645	NP 217161.1	2.569296375
40872	E02	469	Rv0880	MarR family transcriptional regulator	NP 215395.1	2
40888	E03	469	Rv3217c	integral membrane protein	NP 217733.1	3.232409382
40878	E04	469	Rv1904	hypothetical protein Rv1904	NP 216420.1	2
40875	E05	469	Rv1546	hypothetical protein Rv1546	NP 216062.1	2
40871	E06	469	Rv0188	transmembrane protein	NP_214702.1	2
40884	E07	469	Rv2626c	hypothetical protein Rv2626c	NP 217142.1	2
40873	E08	469	Rv0997	hypothetical protein Rv0997	NP 215512.1	2
40876	E09	469	Rv1813c	hypothetical protein Rv1813c	NP 216329.1	2.33901919
40880	E10	469	Rv2011c	hypothetical protein Rv2011c	NP 216527.1	3.221748401
9956	E10	469	Rv2307B	glycine rich protein	YP 177666.1	3.752665245
40895	E12	409	Rv1081c	hypothetical protein Rv1081c	NP 215597.1	2
40895	F01	472	Rv1487	hypothetical protein Rv1081c	NP 216003.1	2
			Rv1467 Rv2185c			_
40903	F02	472		hypothetical protein Rv2185c	NP_216701.1 YP_177784.1	3.569915254
40897	F03	472	Rv1088	PE family protein		3.163135593
40901	F04	472	Rv2031c	heat shock protein hspX	NP_216547.1	2
40898	F05	472	Rv1262c	HIT-like protein	NP_215778.1	2
40906	F06	472	Rv3369	hypothetical protein Rv3369	NP_217886.1	2
40905	F07	472	Rv3317	succinate dehydrogenase hydrophobic membrane anchor subunit SdhD	NP_217834.1	2
40904	F08	472	Rv3180c	hypothetical protein Rv3180c	NP_217696.1	2
40900	F09	472	Rv1532c	hypothetical protein Rv1532c	NP_216048.1	2
40902	F10	472	Rv2103c	hypothetical protein Rv2103c	NP_216619.1	2
40894	F11	472	Rv0910	hypothetical protein Rv0910	NP_215425.1	2
40893	F12	472	Rv0771	4-carboxymuconolactone decarboxylase	NP_215285.1	2
10044	G01	472	Rv2306B	hypothetical protein Rv2306B	YP_177664.1	3.186440678
40915	G02	475	Rv3697c	hypothetical protein Rv3697c	NP_218214.1	2.932631579
40911	G03	475	Rv0661c	hypothetical protein Rv0661c	NP 215175.1	3.193684211
40914	G04	475	Rv3162c	integral membrane protein	NP 217678.1	1.593684211
40913	G05	475	Rv3103c	hypothetical protein Rv3103c	NP 217619.1	2
40907	G06	475	Rv0008c	hypothetical protein Rv0008c	NP 214522.1	2
40909	G07	475	Rv0360c	hypothetical protein Rv0360c	NP 214874.1	2
40912	G08	475	Rv1873	hypothetical protein Rv1873	NP 216389.1	2
40908	G09	475	Rv0240	hypothetical protein Rv0240	NP 214754.1	2
40918	G10	478	Rv1334	hypothetical protein Rv1334	NP_215850.1	2
40916	G10	478	Rv0723	50S ribosomal protein L15	NP 215237.1	3.487447699
40919	G12	478	Rv1615	hypothetical protein Rv1615	NP 216131.1	2
40919	H01	478	Rv2617c	hypothetical protein Rv2617c	NP 217133.1	2
40925	H01 H02	478	Rv1636	hypothetical protein Rv1636	NP_216152.1	2
				MerR family transcriptional regulator		
40927	H03	478	Rv3334		NP_217851.1	2
40926	H04	478	Rv3108	hypothetical protein Rv3108	NP_217624.1	2
40924	H05	478	Rv2602	hypothetical protein Rv2602	NP_217118.1	2
40923	H06	478	Rv2175c	putative regulatory protein	NP_216691.1	1.966527197
10005	H07	478	Rv0470A	mycolic acid synthase pcaA (cyclopropane synthase)	YP_177730.1	2
40928	H08	481	Rv0426c	hypothetical protein Rv0426c	NP_214940.1	1.98960499
40932	H09	481	Rv0854	hypothetical protein Rv0854	NP_215369.1	2.565488565
40939	H10	481	Rv2537c	3-dehydroquinate dehydratase	NP_217053.1	2
40940	H12	481	Rv2872	hypothetical protein Rv2872	NP_217388.1	2

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