Mycobacterium tuberculosis, Strain CDC1551, Knockout Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 2

Catalog No. NR-19784

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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 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),* Knockout Gateway® clone set consists of 8 plates which contain 641 sequence validated knockout clones from *M. tuberculosis*, strain CDC1551. Each open reading frame was constructed with a hygromycin selectable gene replacement marker in vector pDEST-YUB, a Gateway® compatible adaptation of the cosmid cloning vector pYUB854[1] and cloned in *Escherichia coli* (*E. coli*) DH10B-T1 cells. The final construct also contains the β-lactamase gene to confer ampicillin resistance for plasmid selection in *E. coli.* The sequence was validated by full length sequencing of each 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™. A PCR product representing a functional hygromycin resistance cassette was assembled with chromosomal amplicons of approximately 600 base pairs of the regions flanking each gene targeted for replacement. The three fragments (left flank, hygromycin resistance gene, right flank) were amplified and cloned into pDONR™ entry vectors (Invitrogen™). 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™ vector) to create an *att*L-containing entry clone using the three-fragment MultiSite Gateway® Pro method. The hygromycin resistance cassette was sequence verified and experimentally verified through hygromycin resistance of DH10B-T1 *E. coli* cells. The final destination construct was confirmed by restriction digestion analysis. Please refer to the Invitrogen™ Gateway® Technology Manual for additional Gateway® product details.

Material Provided:

Each inoculated well of the 96-well plate contains approximately 60 µL of *E. coli* culture (strain DH10B-T1) in Luria Bertani (LB) broth containing 100 µg/mL ampicillin supplemented with 15% glycerol.

Packaging/Storage:

NR-19784 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 100 µg/mL ampicillin
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*, Strain CDC1551, Knockout Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 2, NR-19784."

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.

Disclaimers:

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BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
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References:

1. Bardarov, S., et. al. "Specialized Transduction: An Efficient Method for Generating Marked and Unmarked Targeted Gene Disruptions in *Mycobacterium tuberculosis*, *M. bovis* BCG and *M. smegmatis*." Microbiology 148 (2002): 3007-3017. PubMed: 12368434.

Support Provided by NIAID

Table 1: *Mycobacterium tuberculosis*, Strain CDC1551, Knockout Gateway® Clones, Plate 2 (KMTAB)

Well Position	Clone (MT Number)	Gene ID	Accession Number
A01	MT0396	923617	NP_334804.1
A02	MT0404.1	923651	NP_334813.1
A03	MT0407.1	923661	NP_334817.1
A04	MT0407	923660	NP_334816.1
A05	MT0426	923702	NP_334836.1
A06	MT0427	923706	NP_334837.1
A07	MT0432	923719	NP_334842.1
A08	MT0456	923783	NP_334865.1
A09	MT0460	923790	NP_334869.1
A10	MT0468	923799	NP_334877.1
A11	MT0484	923831	NP_334894.1
A12	MT0485	923832	N/A
B01	MT0491	923841	NP_334900.1
B02	MT0493	923845	NP_334902.1
B03	MT0500	923855	NP_334909.1
B04	MT0502	923858	NP_334911.1
B05	MT0508	923869	NP_334917.1
B06	MT0513	923882	NP_334922.1
B07	MT0514	923883	NP_334923.1
B08	MT0515	923888	NP_334924.1
B09	MT0521	923914	NP_334930.1
B10	MT0526	923965	NP_334935.1
B11	MT0558	924843	NP_334967.1
B12	MT0562	924864	NP_334970.1
C01	MT0568	924891	NP_334976.1
C02	MT0571	924899	NP_334979.1
C03	MT0572	924906	NP_334980.1
C04	MT0584	924958	NP_334993.2
C05	MT0588	924969	NP_334997.1
C06	MT0590	924978	NP_334999.1
C07	MT0592	924982	NP_335001.1
C08	MT0597	924989	NP_335006.1
C09	MT0614	925009	NP_335024.1
C10	MT0632	925029	NP_335042.1
C11	MT0639	925037	NP_335048.1

Well Position	Clone	Gene ID	Accession Number
C12	MT0645.1	925044	NP_335055.1
D01	MT0648	925049	NP_335058.1
D02	MT0653	925054	NP_335063.1
D03	MT0654	925055	NP_335064.1
D04	MT0662.1	925887	NP_335073.1
D07	MT0682	925965	NP_335093.1
D08	MT0685	925968	NP_335096.1
D09	MT0706.1	925991	NP_335118.1
D10	MT0713	925998	NP_335125.1
D11	MT0714	925999	NP_335126.1
D12	MT0717	926002	NP_335129.1
E01	MT0722	926010	NP_335137.1
E02	MT0726.1	926016	NP_335143.1
E04	MT0731	926021	NP_335148.1
E05	MT0739	926029	NP_335156.1
E06	MT0740.1	926031	NP_335158.1
E07	MT0750	926043	
E08	MT0751	926044	NP_335170.1
E09	MT0756	926049	NP_335175.1
E10	MT0768	926063	NP_335189.1
E11	MT0781	926083	NP_335208.1
E12	MT0803	926106	NP_335231.1
F01	MT0810	926113	NP_335238.1
F02	MT0811	926114	NP_335239.1
F03	MT0818	926121	NP_335246.1
F04	MT0822	926125	NP_335250.1
F05	MT0831	926134	NP_335259.1
F07	MT0833	926136	NP_335261.1
F08	MT0842	926145	NP_335270.1
F09	MT0846	926149	NP_335274.1
F10	MT0847	926150	NP_335275.1
F11	MT0849	926152	NP_335277.1
F12	MT0856	926164	NP_335284.1
G01	MT0861	926170	NP_335290.1
G02	MT0862	926171	NP_335291.1
G03	MT0873	926182	NP_335300.1
G04	MT0874	926183	NP_335301.1
G05	MT0875	926184	NP_335302.1
G07	MT0885	926194	NP_335312.1
G08	MT0893	926203	NP_335321.1
G09	MT0895	926205	NP_335323.1
G10	MT0906	926216	NP_335334.1
G11	MT0907	926217	NP_335335.1
G12	MT0910.2	926222	NP_335340.1
H01	MT0910	926220	NP_335338.1
H03	MT0936	926251	NP_335369.1
H04	MT0946	926261	NP_335379.1
H05	MT0947	926263	NP_335380.1
H06	MT0964	926302	NP_335397.1
H07	MT0972	926363	NP_335406.1
H08	MT0973	926364	NP_335407.1
H10	MT0992	926384	NP_335426.1
H11	MT1007	925190	NP_335441.2
H12	MT1022	925622	NP_335455.1