**Mycobacterium tuberculosis**, Strain CDC1551, Knockout Gateway® Clone Set, Recombinant in *Escherichia coli*, Plates 1-8

**Catalog No. NR-19438**
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:**
NR-19438 consists of Plates 1-8 (BEI Resources NR-19783 through NR-19789 and NR-20324) of the *Mycobacterium tuberculosis*, Strain CDC1551, Knockout Gateway® Clone Set. Detailed information is available on the Product Information Sheet and Certificate of Analysis for each individual plate.

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™ 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%.

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 attB substrate (attB-PCR product or a linearized attB expression clone) with an attP substrate (pDONR™ vector) to create an attL-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:**
Every inoculated well of each 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.

**Note:** 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 cannot confirm or validate any clone not identified on the plate information tables found on the Product Information Sheets for the individual plates.

**Packaging/Storage:**
NR-19438 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*, Plates 1-8, NR-19438.”

**Biosafety Level:**
1


**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 www.beiresources.org.

---

**BEI Resources**
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

© 2012 American Type Culture Collection (ATCC). All rights reserved.
While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Use Restrictions:
This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as 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:

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