Borrelia burgdorferi, Signature-Tagged Mutagenesis Library Clone T05TC025 (Gene BB_0629)

Catalog No. NR-23032

For research use only. Not for human use.

Contributor:

Steven J. Norris, Ph.D., Professor and Vice Chair for Research, Department of Pathology and Laboratory Medicine, University of Texas Health Science Center at Houston Medical School, Houston, Texas, USA

Manufacturer:

BEI Resources

Product Description:

Bacteria Classification: *Borreliaceae* (previously *Spirochaetaceae*)[1], *Borrelia*
Species: *Borrelia burgdorferi*
Strain: B31, clone 5A18NP1
Signature-Tagged Mutagenesis Library Clone: T05TC025
Replicon: Chromosome
Gene: BB_0629 [phosphotransferase system (PTS), fructose-specific IIABC component]
Insertion Site[2,3]: 611156
Original Source: *Borrelia burgdorferi* (*B. burgdorferi*), clone T05TC025 was produced by signature-tagged mutagenesis (STM) of the BB_0629 gene.[2,3]
Comments: *B. burgdorferi*, strain B31 5A18NP1 STM library clone T05TC025 lacks linear plasmids lp28-4 and lp56. The plasmid profile was determined by PCR using plasmid specific primers.[3]

B. burgdorferi is a Gram-negative, motile spirochete.[4] It is a zoonotic, vector-borne pathogen transmitted by ticks and the etiologic agent of Lyme disease, now the most common tick-transmitted disease in the United States.[5] *B. burgdorferi* is predominant in North America, but also exists in Europe.

B. burgdorferi, strain B31 was originally isolated in 1981 from adult ticks (*Ixodes dammini*) collected from lower vegetation on Shelter Island, New York, USA.[4,5] Strain B31 is composed of a 910 kilobase (kb) linear chromosome, 9 circular plasmids (cp) and 12 linear plasmids (lp). Plasmids range in size from 5 kb to 56 kb and total 610 kb.[3,6] Continuous passage of *B. burgdorferi* is known to cause spontaneous loss of plasmids. The complete genome of *B. burgdorferi*, strain B31 has been sequenced (GenBank: AE000783).[7]

B. burgdorferi, strain B31, clone 5A18NP1 was derived from the low-passage clone 5A18 of strain B31.[8] Clone 5A18NP1 lacks lp56 and lp28-4 and the BBE02 gene (a putative restriction-modification gene on lp25) was disrupted by homologous recombination resulting in kanamycin resistance.[9] Inactivation of BBE02 results in increased transformation efficiency and therefore, clone 5A18NP1, was used to create the STM library through the *mariner*-based transposition suicide *Himar1* delivery vector, pMarGent, containing *aacC1* which confers gentamicin resistance.[2,3,10] STM is a negative selection method that identifies clones by unique DNA sequences that are incorporated into the transposable element.[3]

Material Provided:

Each vial contains approximately 0.5 mL of bacterial culture in Revised Barbour-Stoenner-Kelly medium supplemented with 200 µg/mL kanamycin, 40 µg/mL gentamicin and 15% glycerol.

Note: If homogeneity is required for your intended use, please purify prior to initiating work.

Packaging/Storage:

NR-23032 was packaged aseptically, in screw-capped plastic cryovials. 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:
Revised Barbour-Stoenner-Kelly broth (see Appendix I) with 200 µg/mL kanamycin and 40 µg/mL gentamicin
Revised Barbour-Stoenner-Kelly agar (see Appendix I) with 200 µg/mL kanamycin, 40 µg/mL gentamicin and 0.8% agar
Incubation:
Temperature: 32°C to 34°C (growth at 37°C may result in plasmid loss)[2]
Atmosphere: Microaerophilic (slower growth occurs under aerobic conditions[2])
Propagation:

1. Keep vial in dry ice during inoculations.
2. Inoculate new cultures from scraping of frozen stock into a single tube of Revised Barbour-Stoenner-Kelly Medium.
3. Incubate the tube at 32 to 34°C for 2 to 14 days. Do not shake culture during growth.

Note: Subculturing should be minimized to avoid plasmid loss.[2,8]

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Borrelia burgdorferi*, Signature-Tagged Mutagenesis Library Clone T05TC025 (Gene BB_0629), NR-23032."

Biosafety Level: 2

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:

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.

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:

1. Gupta, R. S., S. Mahmood, and M. Adeolu. "A Phylogenomic and Molecular Signature Based Approach for Characterization of the Phylum *Spirochaetes* and Its Major Clades: Proposal for a Taxonomic Revision of the Phylum." Front. Microbiol. 4 (2013): 217. Erratum in: Front. Microbiol. 4 (2013): 322. PubMed: 23908650.
2. Norris, S. J., Personal Communication.
3. Lin, T., et al. "Analysis of an Ordered, Comprehensive STM Mutant Library in Infectious *Borrelia burgdorferi*: Insights into the Genes Required for Mouse Infectivity." PLoS One 7 (2012): e47532. PubMed: 23133514.
4. Burgdorfer, W., et al. "Lyme Disease - A Tick-Borne Spirochetosis?" Science 216 (1982): 1317-1319. PubMed: 7043737.
5. Johnson, R. C., et al. "*Borrelia burgdorferi* sp. nov.: Etiologic Agent of Lyme Disease." Int. J. Syst. Bacteriol. 34 (1984): 496-497.
6. Casjens, S., et al. "A Bacterial Genome in Flux: The Twelve Linear and Nine Circular Extrachromosomal DNAs in an Infectious Isolate of the Lyme Disease Spirochete *Borrelia burgdorferi*." Mol. Microbiol. 35 (2000): 490-516. PubMed: 10672174.
7. Fraser, C. M., et al. "Genomic Sequence of a Lyme Disease Spirochaete, *Borrelia burgdorferi*." Nature 390 (1997): 580-586. PubMed: 9403685.
8. Purser, J. E. and S. J. Norris. "Correlation between Plasmid Content and Infectivity in *Borrelia burgdorferi*." Proc. Natl. Acad. Sci. USA 97 (2000): 13865-13870. PubMed: 11106398.
9. Kawabata, H., S. J. Norris and H. Watanabe. "BBE02 Disruption Mutants of *Borrelia burgdorferi* B31 Have a Highly Transformable, Infectious Phenotype." Infect. Immun. 72 (2004): 7147-7154. PubMed: 15557639.
10. Botkin, D. J., et al. "Identification of Potential Virulence Determinants by *Himar1* Transposition of Infectious *Borrelia burgdorferi* B31." Infect. Immun. 74 (2006): 6690-6699. PubMed: 17015459.

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

Support Provided by NIAID

Appendix I: Revised BSK Medium (ATCC® Medium: 1914)

HEPES	5.64 g
Neopeptone	4.7 g
Sodium citrate	0.7 g
Glucose	5.64 g
$NaHCO_3$	2.0 g
TC-Yeastolate	2.0 g
Sodium pyruvate	0.75 g
N-acetylglucosamine	0.37 g
Bovine serum albumin, fraction V	47.0 g
CMRL 1066, 10X (w/o Glutamine or $NaHCO_3$)	100.0 mL
Rabbit serum (heat inactivated)	60.0 mL
Distilled water	840 mL

For agar, add 0.8% agarose.

Dissolve ingredients up to and including bovine serum albumin one at a time in distilled water. Adjust to pH 7.5 with NaOH and filter-sterilize. Aseptically add CMRL 1066 and rabbit serum. Mix well and aseptically dispense into appropriate vessel. Final pH of complete medium should be 7.5 - 7.6.

BEI Resources
www.beiresources.org

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