

***Borrelia burgdorferi*, Signature-Tagged Mutagenesis Library Clone T06TC033 (Gene BB_0361)**

Catalog No. NR-23623

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

Bacteria Classification: *Spirochaetaceae*, *Borrelia*

Species: *Borrelia burgdorferi*

Strain: B31, clone 5A18NP1

Signature-Tagged Mutagenesis Library Clone: T06TC033

Replicon: Chromosome

Gene: BB_0361 (minD-related ATP-binding protein)

Insertion Site^{1,2}: 369704

Original Source: *Borrelia burgdorferi* (*B. burgdorferi*), clone T06TC033 was produced by signature-tagged mutagenesis (STM) of the BB_0361 gene.^{1,2}

Comments: *B. burgdorferi*, strain B31 5A18NP1 STM library clone T06TC033 lacks linear plasmids Ip5, Ip28-4 and Ip56. The plasmid profile was determined by PCR using plasmid-specific primers.²

B. burgdorferi is a Gram-negative, motile spirochete.³ 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.⁴ *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.^{3,4} 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.^{2,5} 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](https://www.ncbi.nlm.nih.gov/nuccore/AE000783)).⁶

B. burgdorferi, strain B31, clone 5A18NP1 was derived from the low-passage clone 5A18 of strain B31.⁷ Clone 5A18NP1 lacks Ip56 and Ip28-4 and the BBE02 gene (a putative restriction-modification gene on Ip25) was disrupted by homologous recombination resulting in kanamycin resistance.⁸ 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.^{1,2,9} STM is a negative selection method that identifies clones by unique DNA sequences that are incorporated into the transposable element.²

Material Provided:

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

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

Packaging/Storage:

NR-23623 was packaged aseptically in 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 micrograms per milliliter kanamycin and 40 micrograms per milliliter gentamicin

Revised Barbour-Stoenner-Kelly agar (see Appendix I) with 200 micrograms per milliliter kanamycin, 40 micrograms per milliliter gentamicin and 0.8% agar

Incubation:

Temperature: 32°C to 34°C (growth at 37°C may result in plasmid loss)¹

Atmosphere: Microaerophilic (slower growth occurs under aerobic conditions¹)

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.^{1,7}

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Borrelia burgdorferi*, Signature-Tagged Mutagenesis Library Clone T06TC033 (Gene BB_0361), NR-23623."

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. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see www.cdc.gov/biosafety/publications/bmb15/index.htm.

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References:

1. Norris, S. J., Personal Communication.
2. 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.
3. Burgdorfer, W., et al. "Lyme Disease – A Tick-Borne Spirochetosis?" *Science* 216 (1982): 1317-1319. PubMed: 7043737.
4. Johnson, R. C., et al. "*Borrelia burgdorferi* sp. nov.: Etiologic Agent of Lyme Disease." *Int. J. Syst. Bacteriol.* 34 (1984): 496-497.
5. 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.
6. Fraser, C. M., et al. "Genomic Sequence of a Lyme Disease Spirochaete, *Borrelia burgdorferi*." *Nature* 390 (1997): 580-586. PubMed: 9403685.
7. 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.
8. 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.
9. 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.

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APPENDIX I: REVISED BSK MEDIUM (ATCC® MEDIUM: 1914)

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|---|----------|
| 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 to 7.6.