Borrelia burgdorferi, Signature-Tagged Mutagenesis Library Clone T08TC701 (Gene BB_0002)

Catalog No. NR-25134

For research use only. Not for use in humans.

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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: T08TC701
Replicon: Chromosome
Gene: BB_0002 (glycosyl hydrolase family 3 N domain protein)
Insertion Site[1,2]: 1277
Original Source: *Borrelia burgdorferi (B. burgdorferi)*, clone T08TC701 was produced by signature-tagged mutagenesis (STM) of the BB_0002 gene.[1,2]
Comments: *B. burgdorferi*, strain B31 5A18NP1 STM library clone T08TC701 lacks linear plasmids lp28-4 and lp56. 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).[6]

B. burgdorferi, strain B31, clone 5A18NP1 was derived from the low-passage clone 5A18 of strain B31.[7] 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.[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-25134 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 conditions[1])
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 T08TC701 (Gene BB_0002), NR-25134."

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/bmbl5/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)

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.

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