

***Borrelia burgdorferi*, Signature-Tagged Mutagenesis Library Clone T04TC322 (Gene BB\_A60)**

**Catalog No. NR-22864**

**For research use only. Not for human use.**

**Contributor:**

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**Manufacturer:**

BEI Resources

**Product Description:**

Bacteria Classification: *Borreliaceae* (previously *Spirochaetaceae*)<sup>1</sup>, *Borrelia*

Species: *Borrelia burgdorferi*

Strain: B31, clone 5A18NP1

Signature-Tagged Mutagenesis Library Clone: T04TC322

Replicon: Linear plasmid lp54

Gene: BB\_A60 (surface lipoprotein P27)

Insertion Site<sup>2,3</sup>: 40456

Original Source: *Borrelia burgdorferi* (*B. burgdorferi*), clone T04TC322 was produced by signature-tagged mutagenesis (STM) of the BB\_A60 gene.<sup>2,3</sup>

Comments: *B. burgdorferi*, strain B31 5A18NP1 STM library clone T04TC322 lacks linear plasmids lp28-4 and lp56 and circular plasmid cp9. The plasmid profile was determined by PCR using plasmid specific primers.<sup>3</sup>

*B. burgdorferi* is a Gram-negative, motile spirochete.<sup>4</sup> 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.<sup>5</sup> *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.<sup>4,5</sup> 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.<sup>3,6</sup> 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)).<sup>7</sup>

*B. burgdorferi*, strain B31, clone 5A18NP1 was derived from the low-passage clone 5A18 of strain B31.<sup>8</sup> 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.<sup>9</sup> 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.<sup>2,3,10</sup> STM is a negative selection method that identifies clones by unique DNA sequences that are incorporated into the transposable element.<sup>3</sup>

**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-22864 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)<sup>2</sup>

Atmosphere: Microaerophilic (slower growth occurs under aerobic conditions<sup>2</sup>)

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.<sup>2,8</sup>

**Citation:**

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Borrelia burgdorferi*, Signature-Tagged Mutagenesis Library Clone T04TC322 (Gene BB\_A60), NR-22864."

**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](http://www.cdc.gov/biosafety/publications/bmbl5/index.htm).

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**References:**

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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 <sub>3</sub> .....	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 <sub>3</sub> ).....	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.