Borrelia burgdorferi, Signature-Tagged Mutagenesis Library Clone T05TC398 (Gene BB_A57)

Catalog No. NR-23328

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Contributor:

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Manufacturer:

BEI Resources

Product Description:

Bacteria Classification: *Borreliaceae* (previously *Spirochaetaceae*)[1], *Borrelia*
Species: *Borrelia burgdorferi*
Strain: B31, clone 5A18NP1
Signature-Tagged Mutagenesis Library Clone: T05TC398
Replicon: Linear plasmid lp54
Gene: BB_A57 (conserved hypothetical protein)
Insertion Site[2,3]: 39014
Original Source: *Borrelia burgdorferi* (*B. burgdorferi*), clone T05TC398 was produced by signature-tagged mutagenesis (STM) of the BB_A57 gene.[2,3]
Comments: *B. burgdorferi*, strain B31 5A18NP1 STM library clone T05TC398 lacks linear plasmids lp28-4, lp38 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-23328 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 T05TC398 (Gene BB_A57), NR-23328."

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.

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References:

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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.

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