Borrelia burgdorferi, Signature-Tagged Mutagenesis Library Clone T06TC322 (Gene IR_BB_M26-BB_M27)

Catalog No. NR-23841

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Manufacturer:
BEI Resources

Product Description:
Bacteria Classification: Borrelliaceae (previously Spirochaetaceae), Borrelia
Species: Borrelia burgdorferi
Strain: B31, clone 5A18NP1
Signature-Tagged Mutagenesis Library Clone: T06TC322
Replicon: Circular plasmid cp32-6
Gene: IR_BB_M26-BB_M27 (intergenic region)
Insertion Site: 16488

Original Source: Borrelia burgdorferi (B. burgdorferi), clone T06TC322 was produced by signature-tagged mutagenesis (STM) of the intergenic region between the BB_M26 and BB_M27 genes. 3, 4

Comments: B. burgdorferi, strain B31 5A18NP1 STM library clone T06TC322 lacks linear plasmids Ip28-4 and Ip56. The plasmid profile was determined by PCR using plasmid specific primers. 3

B. burgdorferi 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 dammin) 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 Ip56 and Ip28-4 and the BBE02 gene (a putative restriction-modification gene on Ip25) 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 broth 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-23841 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 loss2)
Atmosphere: Microaerophilic (slower growth occurs under aerobic conditions2)

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°C 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 T06TC322 (Gene IR_BB_M26-BB_M27), NR-23841.”

Biosecurity Level: 2

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References:

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APPENDIX I: REVISED BARBOUR-STOENNER-KELLY MEDIUM (ATCC® MEDIUM 1914)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
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<tbody>
<tr>
<td>HEPES</td>
<td>5.64 g</td>
</tr>
<tr>
<td>Neopeptone</td>
<td>4.7 g</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>0.7 g</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.64 g</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>2.0 g</td>
</tr>
<tr>
<td>TC-Yeastolate</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Sodium pyruvate</td>
<td>0.75 g</td>
</tr>
<tr>
<td>N-acetylglucosamine</td>
<td>0.37 g</td>
</tr>
<tr>
<td>Bovine serum albumin, fraction V</td>
<td>47.0 g</td>
</tr>
<tr>
<td>CMRL 1066, 10X (w/o Glutamine or NaHCO₃)</td>
<td>100.0 mL</td>
</tr>
<tr>
<td>Rabbit serum (heat inactivated)</td>
<td>60.0 mL</td>
</tr>
<tr>
<td>Distilled water</td>
<td>840 mL</td>
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</tbody>
</table>

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.