Use of \( rpsL \) as a Counterselectable Marker in *Borrelia burgdorferi*

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We have demonstrated that \( rpsL \), encoding the S12 protein of the small ribosomal subunit, can be used as a counterselectable marker in *Borrelia burgdorferi*, the causative agent of Lyme disease. Mutations in \( rpsL \) confer streptomycin resistance. Streptomycin susceptibility is dominant in an \( rpsL \) merodiploid, and streptomycin selects for the loss of wild-type \( rpsL \) carried in trans. This is the first description of a counterselectable marker in *B. burgdorferi*.

The approach for genetically manipulating *Borrelia burgdorferi*, a spirochete that causes Lyme borreliosis (2, 6, 23), has slowly matured over the past 15 years (17, 18). A number of selectable markers conferring antibiotic resistance have been developed and used to disrupt genes and maintain plasmids, including gyrB (Cou1) (21), aphI (Kan1) (5), ermC (Erm1) (22), aadA (SptStr) (9), and aacC1 (Gen1) (8), which have enabled effective, if not efficient, molecular genetic tools. However, to date, no counterselectable marker has been available to select for the loss of a particular DNA sequence (16). We now demonstrate that susceptibility to streptomycin is dominant in a merodiploid carrying a wild-type \( rpsL \) allele and a streptomycin-resistant \( rpsL \) allele and that \( rpsL \) can function as a counterselectable marker in *B. burgdorferi*. This now provides a genetic tool, previously unavailable, for studying the biology of *B. burgdorferi* and the pathogenesis of Lyme disease.

In *Escherichia coli*, the \( rpsL \) gene encodes the S12 ribosomal protein of the 30S subunit. Streptomycin exerts its antimicrobial activity by binding to 16S rRNA, near the binding interface of S12, to inhibit protein synthesis by increasing translational errors through the recruitment of incorrect tRNAs (11, 12). Mutations in \( rpsL \) confer resistance in numerous bacteria, including *E. coli* (4, 10, 14) and the spirochete *Leptospira biflexa* (15). Streptomycin inhibits the growth of streptomycin-resistant \( rpsL \) mutants when wild-type (streptomycin-sensitive) \( rpsL \) is expressed in trans, indicating that the antibiotic susceptibility phenotype is dominant (13). Furthermore, growing merodiploid \( rpsL \) strains in the presence of streptomycin can select for the loss of wild-type \( rpsL \), demonstrating the utility of this gene as a counterselectable marker (16).

We recently isolated *B. burgdorferi* mutants that were 10-fold more resistant to streptomycin than the parental strain, B31-A (7). One isolate contained a mutation in \( rpsL \) encoding the single amino acid substitution K88E in S12, the same residue that was found to be mutated in streptomycin-resistant *L. biflexa* (15) and *E. coli* (10). We constructed an \( rpsL \) merodiploid strain to test the feasibility of streptomycin susceptibility as a counterselectable marker in *B. burgdorferi*.

**Construction of a counterselectable marker.** \( rpsL \) was amplified from *B. burgdorferi* strain B31-A3 genomic DNA by PCR using the primers pLSU133F/SgrAI (5'-CGCCGGTACTGGAACACTGGTATGGGTC-3') and rpsL375R/SgrAI (5'-CAATTGTTACCGAGCTTCAAGGAAA-3') and KanR488R (5'-TCACTCGCATCAACCAAACC-3') (Fig. 1A). Low-passage B31-A was transformed with pBSpSrL, and the streptomycin-resistant strain DCSmR4 (7) was transformed with either pBSpSrL or the empty vector pBSV2, essentially as described previously (19). Cultures of transformants were diluted with Barbour-Stoenner-Kelly (BSK) II medium (1) containing kanamycin (200 \( \mu \)g/ml) in 96-well plates (25) to select for those containing pBSV2 or pBSpSrL harboring the kanamycin resistance gene aphI. Positive wells were screened for the presence of the plasmids by PCR using the primers PfBG5-MfeI (5'-CAATTGTTACCGAGCTTCAAGGAAA-3') and KanR488R (5'-TCACTCGCATCAACCAAACC-3') to detect aphI. Clones were chosen from 96-well plates that had fewer than 10 positive wells because the probability that a well was inoculated with a single cell is greater than 0.94 (J. M. Graham and D. S. Samuels, unpublished data).

**Phenotype of \( rpsL \) merodiploid.** We first tested the hypothesis that streptomycin susceptibility is dominant in an \( rpsL \) merodiploid. Each strain was initially grown in liquid BSK II medium containing only kanamycin to maintain pBSV2 or pBSpSrL. *Borrelia* spirochetes were then counted using a Petroff-Hausser counting chamber, and 1,000 spirochetes were plated in semisolid BSK medium (19) containing (i) no antibiotics, (ii) kanamycin (200 \( \mu \)g/ml), (iii) streptomycin (50 \( \mu \)g/ml), or (iv) kanamycin plus streptomycin. Plates were incubated at 37°C and 5% CO\(_2\) for 2 weeks before enumeration. The merodiploid strain DCSmR/pBSpSrL did not form colonies in the presence of both kanamycin and streptomycin, suggesting that streptomycin susceptibility is dominant (Table 1). A few DCSmR/pBSpSrL colonies formed in the presence of streptomycin alone (Table 1). Similar results were confirmed using a second DCSmR/pBSpSrL clone (data not shown). The frequency of loss of pBSpSrL (0.04%) is much lower than we...
have observed with another counterselectable marker system using fluoroquinolones and parC (~3%) (18); the higher rate of plasmid loss in the parC mutants may be due to a partitioning defect. However, the pBSV2 backbone vector is stable, with 100% retention after ~90 generations without selection (24).

Counterselection with rpsL. Streptomycin should select for the loss of the rpsL allele, conferring streptomycin susceptibility, in a merodiploid (DCSmR/pBSpsL). Thus, rpsL can function as a counterselectable marker and should be a useful molecular tool for genetic experiments that require the loss of a DNA sequence. We are currently isolating rpsL mutations in low-passage infectious strains, and we are attempting to apply the counterselectable marker system for use in the animal model of Lyme borreliosis.

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REFERENCES

TABLE 1. Antibiotic selection for the loss of pBSpsL.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>% of colonies compared to no selection, in the presence of:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Kanamycin</td>
</tr>
<tr>
<td>B31-A</td>
<td>pBSpsL</td>
<td>101 (±5.1)</td>
</tr>
<tr>
<td>DCSmR4</td>
<td>pBSV2</td>
<td>105 (±9.2)</td>
</tr>
<tr>
<td>DCSmR4</td>
<td>pBSpsL</td>
<td>106 (±20.0)</td>
</tr>
</tbody>
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* Values are means ± standard errors of the means for four independent experiments.
adA confers streptomycin resistance in 