Confirmation of the function of Mrub1080 as γ-glutamyl kinase (ProB) in Meiothermus ruber

Cale J. McCormick
Augustana College, Rock Island Illinois

Dr. Lori Scott
Augustana College, Rock Island Illinois

Follow this and additional works at: https://digitalcommons.augustana.edu/celebrationoflearning

Part of the Molecular Genetics Commons

Augustana Digital Commons Citation
McCormick, Cale J. and Scott, Dr. Lori. "Confirmation of the function of Mrub1080 as γ-glutamyl kinase (ProB) in Meiothermus ruber" (2016). Celebration of Learning.
https://digitalcommons.augustana.edu/celebrationoflearning/2016/posters/12
**Confirmation of the function of Mrub1080 as γ-glutamyl kinase (ProB) in Meiothermus ruber**

Cale McCormick and Dr. Lori Scott

---

**Introduction**

*Meiothermus ruber* is a gram-negative, non-motile, obligate aerobe bacterium with 3,097,457 base pairs (JGI Integrated Microbial Genomes Education Site, 2015). *M. ruber* is in the phylum Deinococcus-Thermus and was first isolated in a Russian hot spring. Organisms in the Deinococcus-Thermus phylum usually live in high temperatures and are called thermophiles, such as *M. ruber* (GENI, 2015). *M. ruber* is part of a poorly studied section of the Tree of Life and has not been routinely studied in much detail. The *M. ruber* Genome Analysis Project is of particular importance due to this reason and may help with future developments years from now (GENI, 2015). The goal of the *M. ruber* Genome Analysis Project is to develop *M. ruber* as a model organism for functional genomics research because it has an abundant amount of genomic research.

In this study, the proline biosynthesis pathway was of specific interest in hopes of expanding the *M. ruber* Genome Analysis Project. We asked the question whether *M. ruber* has a proB gene that is functionally homologous to the *E. coli* proB gene in hopes of expanding the *M. ruber* Genome Analysis Project in hopes of expanding the *M. ruber* Genome Analysis Project.

**Materials and methods**

For this experiment, lab procedures and computational analysis were used to provide evidence of orthologous genes between *E. coli* and *M. ruber*. Here was the procedure taken to identify and analyze the "weak growing" colonies from BIOL-375.

1. **Isolate plating:** *M. ruber* proB null-pl (row 1) and *M. ruber* proB null-p (row 2) Weak growing complementation assay of *E. coli* strains grown on LB-plates at 37°C for 72 hours. Growth in sector 1 of the A and B plates showed successful W.T. growth in lack of proline. Lack of growth in sector 2 of the A and B plates confirmed complementation of *E. coli* proB and proA genes on standard LB-plates. Growth in sector 4 of the A and B plates confirmed complementation with *E. coli* proB null-plasmid and proB-null (pPlasmid) plasmid. Growth in sector 4 of the A plate does "likely" ambiguous induction system. No growth in sector 5 and 6 of the A plates because *M. ruber* proB and proA null-plasmid inhibited in absence of inducers. Some growth in sector 5 and 6 of the B plasmid shows slight complementation of *M. ruber* proB null-plasmid and proB-null-plasmid. Growth in all sectors of C confirms all strains were able to grow in presence of proline.

2. **PCR:** Perform PCR to amplify the *M. ruber* proB and *M. ruber* proA gene from the plasmids from the min + E. coli proB null host. (545 bits)

**Results - Functional**

**Class complementation data**

<table>
<thead>
<tr>
<th>Samples*</th>
<th>E-value</th>
<th>Bit Score</th>
<th>% Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak proB</td>
<td>0.0</td>
<td>1494 bits</td>
<td>99%</td>
</tr>
<tr>
<td>Weak proB</td>
<td>0.0</td>
<td>1701 bits</td>
<td>99%</td>
</tr>
<tr>
<td>Weak proBA</td>
<td>0.0</td>
<td>1097 bits</td>
<td>99%</td>
</tr>
<tr>
<td>Weak proBA</td>
<td>0.0</td>
<td>1047 bits</td>
<td>99%</td>
</tr>
<tr>
<td>Wild type proB</td>
<td>0.0</td>
<td>1621 bits</td>
<td>99%</td>
</tr>
<tr>
<td>Wild type proBA</td>
<td>0.0</td>
<td>1047 bits</td>
<td>99%</td>
</tr>
<tr>
<td>Weak proBA</td>
<td>0.0</td>
<td>1919 bits</td>
<td>99%</td>
</tr>
<tr>
<td>Wild type proBA</td>
<td>0.0</td>
<td>1944 bits</td>
<td>99%</td>
</tr>
<tr>
<td>Weak proB</td>
<td>0.0</td>
<td>1684 bits</td>
<td>99%</td>
</tr>
<tr>
<td>Weak proBA</td>
<td>0.0</td>
<td>1853 bits</td>
<td>99%</td>
</tr>
</tbody>
</table>

**Figure 4:** BIOL-375 1- South proB clone insert row 1) and 2-South proB clone insert row 2) Weak growing complementation assay of *E. coli* strains grown on LB-plates at 37°C for 72 hours. Growth in sector 1 of the A and B plates showed successful W.T. growth in lack of proline. Lack of growth in sector 2 of the A and B plates confirmed complementation of *E. coli* proB and proA genes on standard LB-plates. Growth in sector 4 of the A and B plates confirmed complementation with *E. coli* proB null-plasmid and proB-null (pPlasmid) plasmid. Growth in sector 4 of the A plate does "likely" ambiguous induction system. No growth in sector 5 and 6 of the A plates because *M. ruber* proB and proA null-plasmid inhibited in absence of inducers. Some growth in sector 5 and 6 of the B plasmid shows slight complementation of *M. ruber* proB null-plasmid and proB-null-plasmid. Growth in all sectors of C confirms all strains were able to grow in presence of proline.

**Discussion**

In conclusion, this study confirmed that Mrub1080 is orthologous to *E. coli* proB. Even though the weak growing clones viewed in Figure 4 produced inconclusive results - were the few colonies that grew demonstrating complementation or where they contaminants? Mrub1080-pKt1 clones were isolated from a total of 100 colonies from BIOL-375. The proB clones were transformed into the *M. ruber* proB null host due the differences in their optional growth conditions (55°C vs 37°C, respectively). The proB and proA genes form a functional complex in *E. coli* (Smith, 1984), and an interspecies *M. ruber* proB/pKt1 proA complex might not function properly.

Two reasons for the weak complementation results could be: 1) *M. ruber* proB proteins might not function well in an *E. coli* host due to the differences in their optional growth conditions (55°C vs 37°C, respectively); and 2) proB and proA genes encode a functional complex in *E. coli* (Smith, 1984), and an interspecies *M. ruber* proB/pKt1 proA complex might not function properly. Studies in *E. coli* demonstrate that this complex is required for proB function but not required for proA function. In other studies in Dr. Scott’s lab, the *M. ruber* genes (e.g., proA) have complemented equally well to the *E. coli* genes, which demonstrates that *M. ruber* genes can be expressed in an *E. coli* host. In this study, we cloned the proB gene upstream to determine if the complementation results improved if both genes came from *M. ruber*. We still saw weak complementation. The next step would be to confirm if both the proA and proB proteins are present when the *M. ruber* proBA clones are transformed into the *E. coli* null host.

**Literature cited**


---