Introduction

*Methanococcus maripaludis* is a model organism for Archaea, which affords researchers the beneficial qualities such as (1) producing methane used as biogas and (2) manufacturing isoprenoids as precursors for high-value biochemicals. However, there are few genetic tools available to metabolically engineer Archaea. Our goal is to develop some useful tools for synthetic biology of Archaea. Building on our past *M. maripaludis* projects, which created and characterized an mCherry reporter system and a recombinant mutant making geraniol, our team is now working to (1) create, characterize and model a ribosome-binding site (RBS) library using the mCherry reporter system and (2) model geraniol production of the recombinant *M. maripaludis* using flux balance analyses. Additionally, our team has initiated an Archael InterLab Study to further characterize the reproducibility of our mCherry reporter system.

### Methodology

**Archaeal Ribosome Binding Site (RBS) Library Development:**

Random oligonucleotides were cloned into the RBS upstream of the gene encoding mCherry in a methanococal expression vector. Clones were picked and the oligonucleotide identified by PCR and sequencing. Fluorescence was then determined to evaluate mCherry expression (Figure 1). In addition, the measurement reproducibility was determined through the Archael InterLab Study.

**Characterization of an Archaeal RBS Library**

A continuing project for our iGEM Team, developing a library of varying translational efficiency for Archaea, is a tool missing in Synthetic Biology. Previously we characterized a native and two theoretical sequences (BBA-K1383000-2). This year we created a new part: BBA-K1635000 (Figure 2). Figure 3 shows the representative mutants from our library that have met standards of cultivation, screening, and sequencing.

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**Archaeal InterLab Study**

Inspired by the iGEM HC. coli Interlab Measurement Study, we extended this type study into the realm of Archaea. We used mCherry extracts from our developed *M. maripaludis* transformants (Figure 4L). Our goal was to determine if our mCherry quantification protocol was reproducible among many different labs. Collected data from participating teams (Figure 4R) and data analysis are shown in Tables 1 and 2.

### Metabolic Modeling

Using the metabolic model for *M. maripaludis* that our 2014 team amended by adding geraniol metabolites and reactions, we discovered a significance in the CO2/NH4 ratio for the production of geraniol via BBA-K1383000 (Figure 6).

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**RBS Library:** Created a library, characterized 13 mutants and submitted one new part, BBA-K1635000, to the iGEM registry. InterLab Study: Measured fluorescence of previous part BBA-K1383000 and 9 other mutants to demonstrate high reliability of our data, and reproducibility of our measurement protocols.

**UTR Modeling:** UTR designer is not an effective translation efficiency predictor for *M. maripaludis*. Metabolic Modeling: Modeled previous part BBA-K1383000 for higher production of isoprenoid compounds such as geraniol.

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### Conclusions

#### Limiting Nitrogen: No change in geraniol production or specific growth rate (Table 3):

<table>
<thead>
<tr>
<th>C/N</th>
<th>CO2 uptake rate (mmol g DW-1 h-1)</th>
<th>NH4+ uptake rate (mmol g DW-1 h-1)</th>
<th>Specific growth rate (h-1)</th>
<th>Geraniol production rate (mmol g DW-1 h-1)</th>
</tr>
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<tbody>
<tr>
<td>1.5</td>
<td>10.60</td>
<td>0.67</td>
<td>0.81</td>
<td>0.93</td>
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<td>2</td>
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<td>0.51</td>
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</tbody>
</table>

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**Figure 1:** The steps through which our translational library development occurs. From Library: anamorphic transformation; PCR and sequencing of mutant, mCherry extraction and maturation, and collection of fluorescent data.

**Figure 2:** Transcription unit including promoter (P_hum), ribosome binding site (RBS), and mCherry reporter gene for BBA-K1635000. The fourth base in the RBS (designated in red) has a G to T transversion.

**Table 3:** Correlation matrix of mCherry fluorescence data across 7 participating iGEM teams after removing individual outlier data (see figure for the ESD table).

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**Table 4:** Increasing C/N and maintaining NH4+, and observing change in geraniol production (with some specific growth rate increase). As increasing the amount of CO2, the specific growth rate increased, but not the geraniol production.