Recommendation Summary

There are three major takeaways from the experiences we have collected from other teams working in non-model organisms: information gained form the literature varies widely in scope and accuracy, expertise of the sponsor lab plays a significant role in the success of a project, and many experiments take more time than initially anticipated in the planning stage.

We would recommend that future teams plan experiments that are backed by thorough literature searches, but that teams not be afraid of designing new protocols for their non-model strains. Choosing a lab whose members have prior experience in a given organism will also increase the likelihood of successful outcomes. Finally, teams beginning a research project in a non-model strain should be conservative when developing a timeline of research, and should set aside a significant amount of time (20-40% of total research time) for troubleshooting failed protocols.

Synthetic biology is only starting to realize the potential of non-model organisms. iGEM provides a fantastic opportunity to explore this potential and could further facilitate experiments in non-model organisms by establishing a "non-model consortium" within the foundation. We envision that a section of the registry and jamboree dedicated to non-model organisms that would catalyze progress in research.

Contributing Teams (2015 unless noted otherwise)

Concordia University iGEM (2014 and 2015)
Cornell University iGEM
Northeastern University iGEM
University of British Columbia iGEM
University of La Verne iGEM
Utah State University iGEM
Yale University iGEM

Statistics

- 7 teams were contacted for their experiences in non-model organisms within the United States and Canada
- Breakdown of team types:
  - 5 teams described themselves as “Undergraduate-driven with guidance from overgraduate students/professional researchers”
  - 1 team described itself as “fully undergraduate-driven”
  - 1 team described itself as “undergraduates and overgraduates contribute equally” to the project
- Role of non-model organism in project:
  - 5 teams noted that their model organism(s) is/are being used in conjunction with a model organism in research
  - 2 teams noted that their model organism(s) is/are the main focus of the project

Yale iGEM
2015

iGEM Teams in Uncharted Waters

Beginning new synthetic biology research projects in non-model organisms

Over the past few years, iGEM has increasingly centered around non-model microorganisms—organisms which are less well-characterized and have fewer resources for genetic manipulation than the model *E. coli* and *S. cerevisiae*. Despite a prevalence of non-model organisms in iGEM competition projects and the potential impact which these projects may have, few resources exist for teams hoping to initiate research in non-model strains.

The 2015 Yale University iGEM team has collaborated with several other teams working in non-model strains to design a set of considerations for future iGEM teams in order to reduce the barrier to entry into non-model organisms. We synthesized the experiences of other teams into this handout, which has been made available on our team wiki.

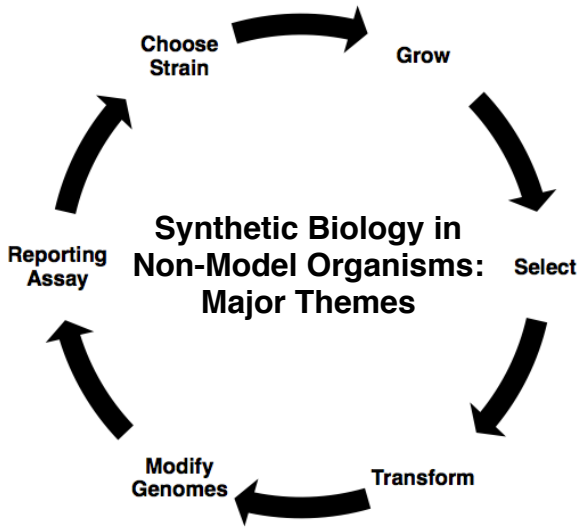

Figure 1: The 2015 Yale iGEM team identified six major themes which must be resolved before the successful implementation of a synthetic biology research project in a non-model organism. These six themes fall into three major categories: **Organism** (strain choice and growth), **Readout** (selection and reporting assays) and **Genetic Manipulation** (transformation and genome modification).

Major Themes: Challenges and Insights

Component 1: Organism

Every team who responded to the survey considered multiple non-model organisms, and several factors played a significant role in influencing teams' decisions to work with a particular non-model strain. One major factor was the expertise of the sponsoring lab; those organisms with which full-time lab members had previous experiences were more likely to be chosen. Growth time was also a major consideration, and many teams chose strains due to their fast growth rates. Many organisms were also chosen for their potential as producers of small molecules of interest, such as biofuels and therapeutics.

The success which teams had in growing their chosen strains varied widely across teams. Certain teams that chose organisms which were backed by a lot of expertise in the sponsor lab had no trouble growing their strains; other teams spent as many as four or five weeks to determine robust growth protocols. Most teams tended towards liquid culture, though many used plate growth to select for single colonies. Literature values were only somewhat useful in determining growth conditions, and many tended to report a diverse range of acceptable growth conditions.

We would recommend that teams formulating projects in non-model organisms choose strains with as much documentation in the literature as possible, as well as strains with sponsor lab expertise.

Non-Model Organisms Used by iGEM Teams (2014 and 2015)

Organism	Characteristics
Chlamydomonas reinhardtii	Algae capable of glycosylation
Chlorella kessleri	Single-cell algae
Chlorella vulgaris	Single-cell algae
Flavobacterium psychrophilum	Causes bacterial coldwater disease in fish
Gilliamella apicola	Honey- and bumblebee gut bacterium
Lactococcus lactis	Cheese bacterium
Rhizobium tropici CIAT 899	Root nodule-forming, nitrogen-fixing bacterium
Snodgrassella alvi	Honey- and bumblebee gut bacterium
Synechococcus sp. PCC 7002	Photosynthetic marine cyanobacterium capable of free fatty acid production

Major Themes: Challenges and Insights (cont.)

Component 2: Readout

Many of the most effective synthetic biology experiments lead to some readout of information. For a transformation, this may be as simple as colony growth in selective media. For more complex genetic manipulation experiments, readouts may be more elaborate.

Teams which implemented selection-based readouts had to determine the appropriate minimum inhibitory concentrations (MICs) of the antibiotics of interest in order to tease out a positive result from the background. Most iGEM teams determined MICs either through literature searches or by experimentally testing the growth of a wild-type strain at various antibiotic concentrations. Several teams used MICs suggested by the literature to guide their own resistance experiments; running this kind of experiment early on in research may be beneficial for confirming MICs, saving time, and for devising more sophisticated selection-based experiments in the future.

Fluorescent reporter proteins formed the basis for every team's readout of more complex genetic manipulation experiments. A common issue involved the autofluorescence of certain microbes; teams had to use reporters whose emission frequency would not confound with the wavelengths emitted by autofluorescent bacteria. The arsenal of reporters used thus ranged from standard GFP (green) to citrine (yellow) to tdTomato (red).

Most teams have designs for reporting assays, but few have successfully executed them. The vast majority of research time for most teams working in non-model organisms was spent determining proper growth protocols, experimentally validating MICs, and attempting DNA transformations. Teams should consider what kind of dynamic range is needed for their reporting assay, in order to better define experimental conditions.

Component 3: Genetic Manipulation

The ultimate goal of many synthetic biology projects is to implement genetic modifications in the genomes of organisms which lead to phenotypically useful traits. We identified transformations and genome modifications as two protocols associated with this goal.

Most teams sought to determine a robust transformation protocol for their non-model organism in order to express a gene of interest or to induce a pathway for eventual genome modification. The common start point for all teams was a thorough literature search, and most teams reported that little to no published information exists for their strain. Every team considered multiple possible transformation protocols, including transformation by natural competency, electroporation, chemical/heat shock, conjugation, and phage infection. Most teams then tried at least two of these methods experimentally to determine which transformation method was fastest and most effective. Other considerations which significantly affected transformation protocols were whether the organism has native restriction endonucleases, whether the organism is readily capable of homologous recombination, and whether the DNA to be transformed is linear or circular.

Fewer teams attempted genome modifications; many projects involved the expression of a gene which could remain on a plasmid rather than be incorporated into the genome. Those teams with designs for genome modification experiments did not execute them, as determining growth, transformation, and selection protocols for their organisms took much longer than expected. We recommend that teams seeking to modify genomes search the literature to see if their organism has native restriction endonucleases (a problem which could be alleviated by expressing genes in E. coli along with the associated methylase) and considering whether DNA would be more stable in a plasmid or incorporated into the organism's genome.