iGEM Advisor's Meeting
5/25/12

PPT Presentation

Biobrick with PUF binding site

- Grow on Xgal to test blue/white colonies rather than quantifying LacZ
- Need a plasmid without Lac I already. Easiest way is to get the strain from the people who made that biobrick already. A different option is to do a Lac I knockout.
- Rao: Faster and easier to just do GFP expression.
    - A blue/white screen is just a yes or no. It's not a quantitative assay.
    - Can do LacZ and GFP in parallel. That's a stronger experiment.

Potential Plan

- Assemble biobrick
- Then introduce PUF binding site and mutated PUF binding site. Insert before RBS of Lac I, after RBS of Lac I,
- Assemble last biobrick of PUF+PIN
- The biobrick that we intend to make is made of smaller biobricks that we will hopefully process all in parallel.

- Different assembly methods?
- Rao: forget fancy PCR things, including Gibson assembly.
    - Need a simple control to test the PUF. GFP is a 2 week control experiment to test PUF levels.
    - The modular Lac I/Z system is worth pursuing, but it should be done in parallel with the GFP experiment.
- The terminators are small, they are going to be hard to cut out of a gel. How will we tackle small segment assembly?
    - 3A assembly is made so that we don't have to do gel purification. Therefore we will be fine on the promoters, RBS, and terminators. The hard part is the PUF sites
    - A possibility is to biobrick PUF by itself so that we can simply order primers and such for it.
    - Other option: ignore the existing biobrick BBa_Q04121 and simply put the RBS and PUF binding site on the forward primer for the reporter so it's all together to begin with. The reverse primer would have both the terminators.
        - Constraint – RBS must be no more than 10 base pairs away from the start ATG. The PUF binding site will be better suited to before the RBS
        - Also, we can expand the biobrick with the addition of each primer, by adding aroud 40 bp everytime

Biobricks

- PUF is too small to add on its own. Would need to put PUF with the RBS or something.
- Ideal biobrick: RBS, PUF, and Lac I reporter all together
- It's too small to biobrick the PUF binding site
    - Really makes a problem with creating a PUF toolkit

Final Plan

- PUF binding site with Lac Z
- Mutated PUF binding site with Lac Z
- PUF binding site with Lac I
- Mutated PUF binding site with Lac I
- PUF binding site with YFP
- Mutated PUF binding site with YFP
- PUF+PIN
- Mutated PUF + PIN
- The RBS and PUF binding sites will be in the promoter for the varying gene. That simplifies putting that tiny sequence into E. Coli

Remaining questions

- How extensively should things be characterized?
    - Worry about it when we get there.
- Should we link PUF to another protein domain on top of the endonuclease?
    - Don't worry about it, we need to basics first.
    - We have 3 strains of PUF: Wild type PUF (just PUF), PUF with endonuclease, PUF with mutated endonuclease

PHAT project

- Korean lab is making stocks of stuff and preparing to ship them early next week. Will hopefully get them on Wednesday. We will also receive a plasmid map.
    - Need to get them a FedEx account number because normal mail will take weeks
- First step is to biobrick the wild type BM3 (cytochrome P50), and 2 other mutants (one with the highest activity level and the other with the highest longevity)
- Second step, redo their assay to make sure that biobrick formatting doesn't affect the enzyme's activity
    - How do we measure piceatannol? We don't have that capability to run the mass spec.
    - Look into measuring via engineering or some way other than machinery. Look at reduction reactions. Send an email to the Korean professor to see if they used any other methods.
- Next step, order powder resveratrol. Mix it in the media and see if piceatannol would form.

    - Contact Matthew Koffas because he published about producing piceatannol in E. Coli. Tell him that despite patenting issues, we just want any strain for a proof of concept idea.
- Still looking to play around with the chemical drawing software. It would be a nice theoretical presentation at iGEM.

Announcements

- Isiah: Will start using a virtual lab notebook that everyone should write when they use something up. There will be a piece of paper on the bench, but digital is better.
- Daily Team meeting at 5 in the Union basement.
- Advisor's meeting every Friday from 3-4 PM.