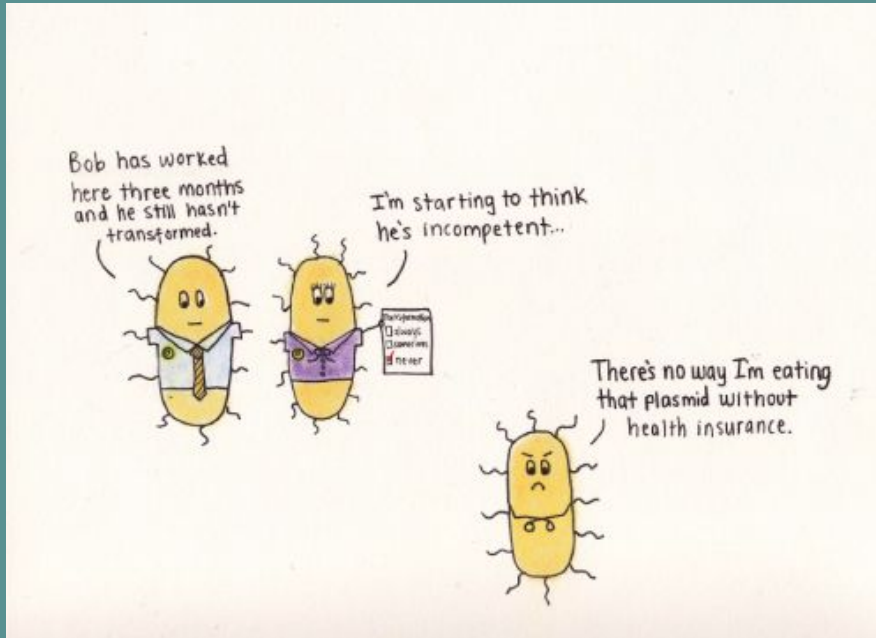


7/27 iGEM Meeting





Updates

- Received \$3530.86 funding from the ChemE Department covering individual registration, flights, and hotel costs for three ChemE majors
 - Individual Registration: \$695 per person
 - Flights: ~\$250 round trip per person
 - Hotel: \$695.86 for two nights
- We got some free stuff
 - 10 Gibson Assembly Master Mix Rxns
- We ran our first gel!
- Completed Interlab calibrations and submitted that data
 - We received an extension on the Interlab cell measurement data
 - The data must be sent in by the end of August



Updates Cont.

- We have transformed twice (Monday and Tuesday) with no success





Interlab



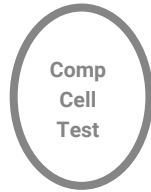
Used iGEM's protocol
All our own reagents
*used 10x less CAM

Growth on less than $\frac{1}{2}$
plates
No fluorescence



Same Protocol
*got LB-Agar from Jack
*added only 50 uL of SOC
*plated 100 uL (duplicate)

No growth at all



Followed iGEM protocol
*used iGEM DNA

No initial growth, but after
48 hours, red colonies
formed showing the
transformation worked
but was delayed
(low level of competency)



Same Protocol
*used Adam's comp cells
*used fresh SOC from
Bon

No initial growth, but after
48 hours we had growth
on $\frac{1}{4}$ of the plates



Same Protocol
*used 2 CAM plates (Bon)
*pipetted 3uL of DNA
*recovered in 900 uL SOC

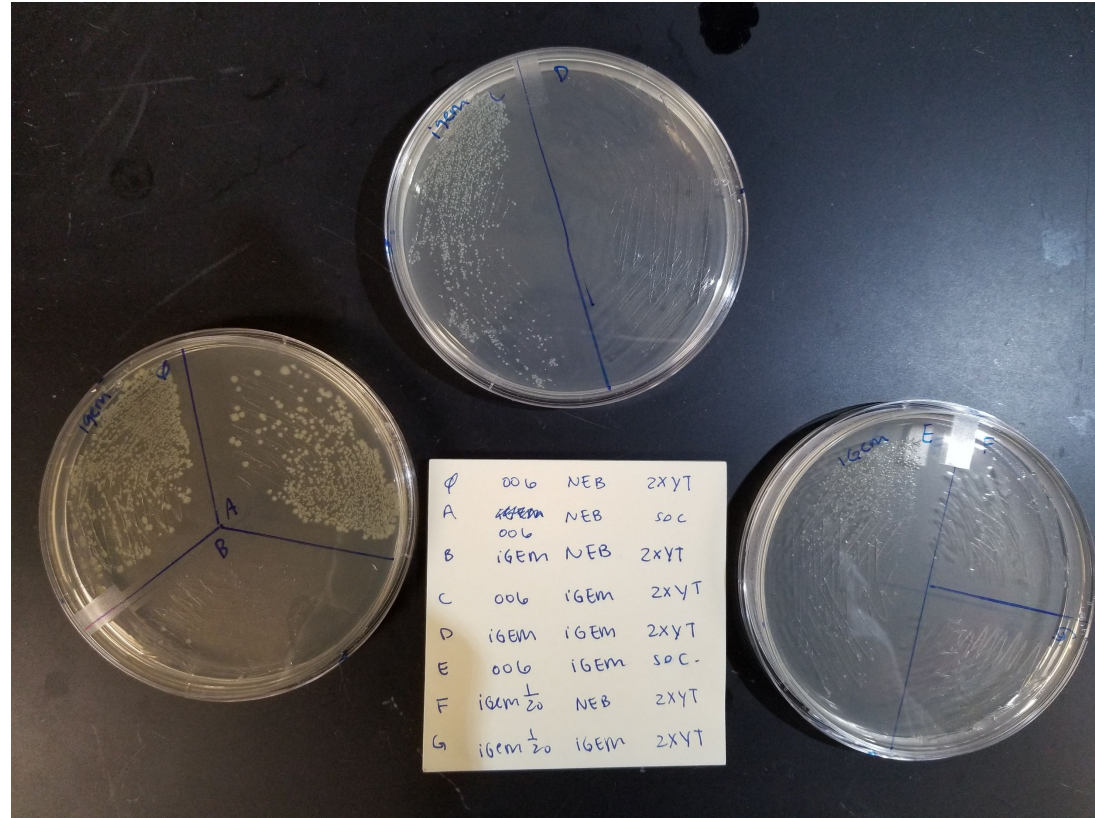
Growth in non-CAM plates,
but no fluorescence

Interlab (Continued)

Question: What is the reason for our failed transformations?

Lulu ran a transformation with our pos. control plasmid DNA. The following plates were the result.

Indicates that our comp. Cells are only slightly competent and don't efficiently take up DNA. Our DNA also seemed to not have the concentration Needed (See B).



Gel Results



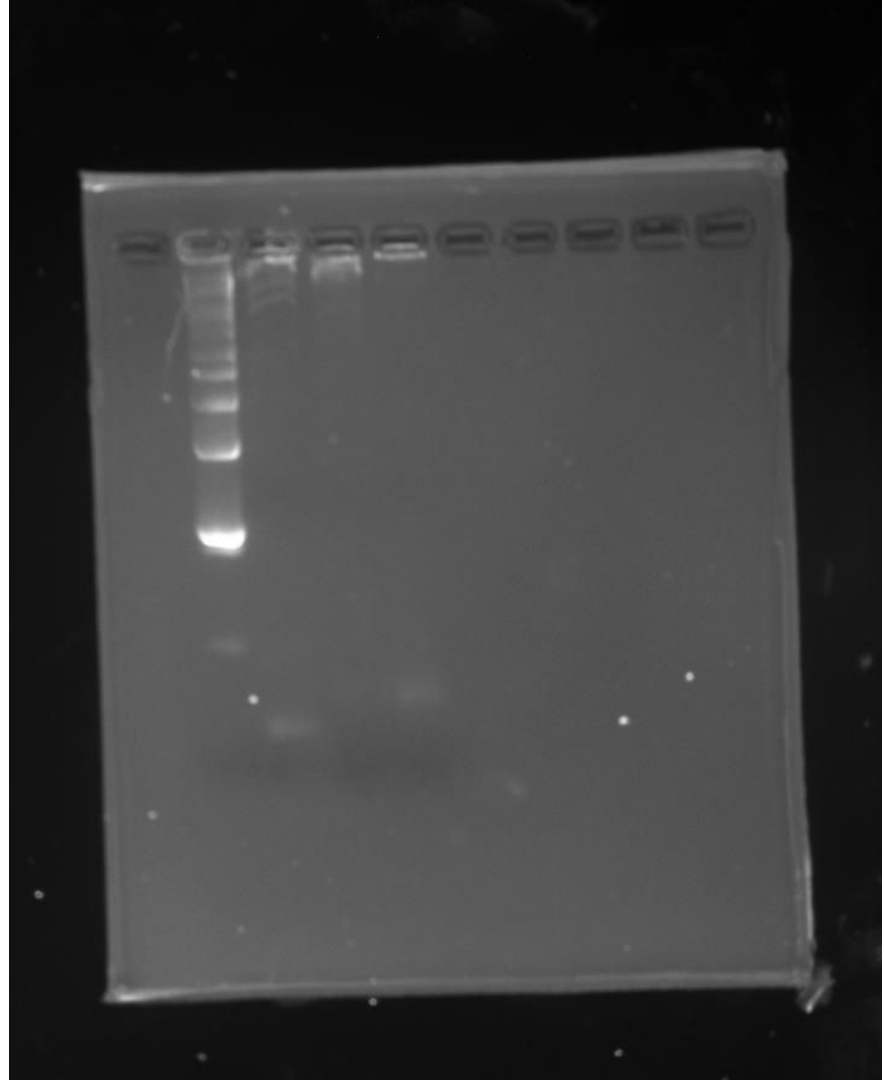
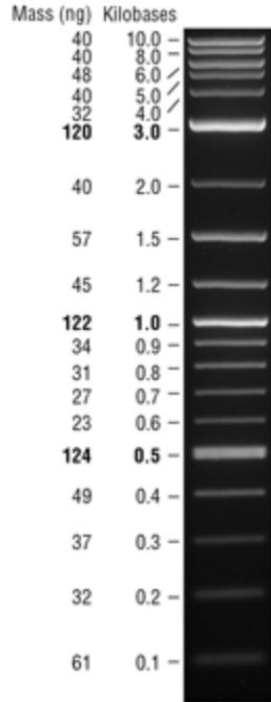
Question: Was our PCR successful?

Lane 2: 1 kb Ladder

Lane 3: pADS088 backbone

Lane 4: LuxR Gene

Lane 5: Adam's Positive Control



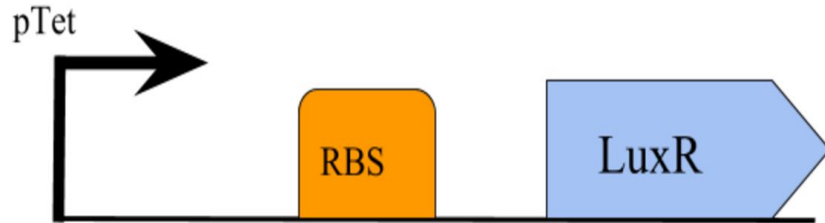


Next Steps

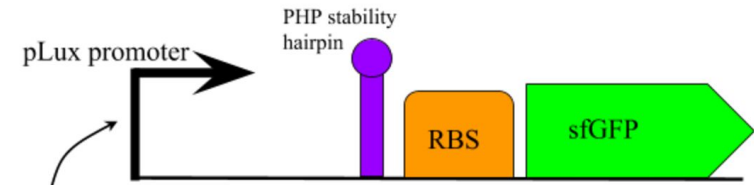
- Interlab
 - Extended Deadline: August 24th
 - We want to talk to Walter about making comp. cells
 - Collab with UChicago???
 - We're planning on using the backup DNA included in the iGEM kit
 - Shedd collaboration still a possibility (haven't gotten back to us)
 - Backup plan for Human Practices is creating a study

Next Steps Cont.

- We have run PCRs that amplified both pADS088 backbone and LuxR gene (plan to use Gibson Assembly to clone LuxR gene into this backbone, shown below).
 - Gels inconclusive?
- Have designed iPCR primers to achieve pADS094 plasmid backbone with pLux promoter (shown below).
- Rest of summer: Lots and lots of PCR and Gibson Assemblies



Altered pADS088 w/ pTet promoter, produces LuxR



Altered pADS094 w/ pLux promoter and sfGFP output



Next Steps Cont.



Prove LuxR/pLux activator-promoter system reliably produces sfGFP in pADS071 and pADS072 plasmids, testing set control and variable groups. Affirming plasmids work as expected in presence/absence of certain molecules.

Clone LuxR system into pADS088 and pADS094 plasmids optimized for CFPS. We will similarly test set control and variable plasmids, affirming sfGFP is produced when expected.

In the pADS088 and pADS094 LuxR plasmids, change reporter from sfGFP to colorimetric output (as recommended by Prof. Jewett). Current enzymes we are considering include β -Glucuronidase (GusA) and β -Galactosidase (LacZ).

Replace the LuxR system with chromium and lead promoters and repressors (chrB/chrP, pbrR/pbrAP). Test this metal detection system in the presence of lead and chromium for colorimetric output.

Blot lead and chromium systems onto paper; test to see if this paper-based assay produces colorimetric output reliably, attempt to optimize system.



Questions, Comments, Concerns?

- AbbVie contact?
- Best person to help with making comp cells?
 - Walter?
- Transformations