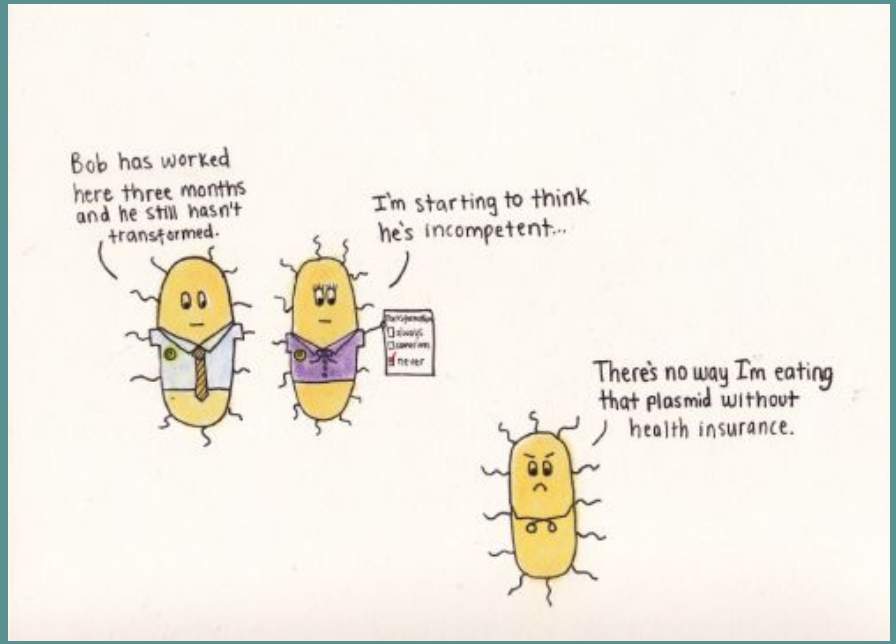
7/27 iGEM Meeting



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

Trial 1

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

Growth on less than ½ plates
No fluorescence

Trial 2

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

No growth at all

Comp Cell Test

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)

Trial 3

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

No initial growth, but after 48 hours we had growth on ¼ of the plates

Trial 4

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

Growth in non-CAM plates, but no fluorescence

http://parts.igem.org/Help:Protocols/Transformation

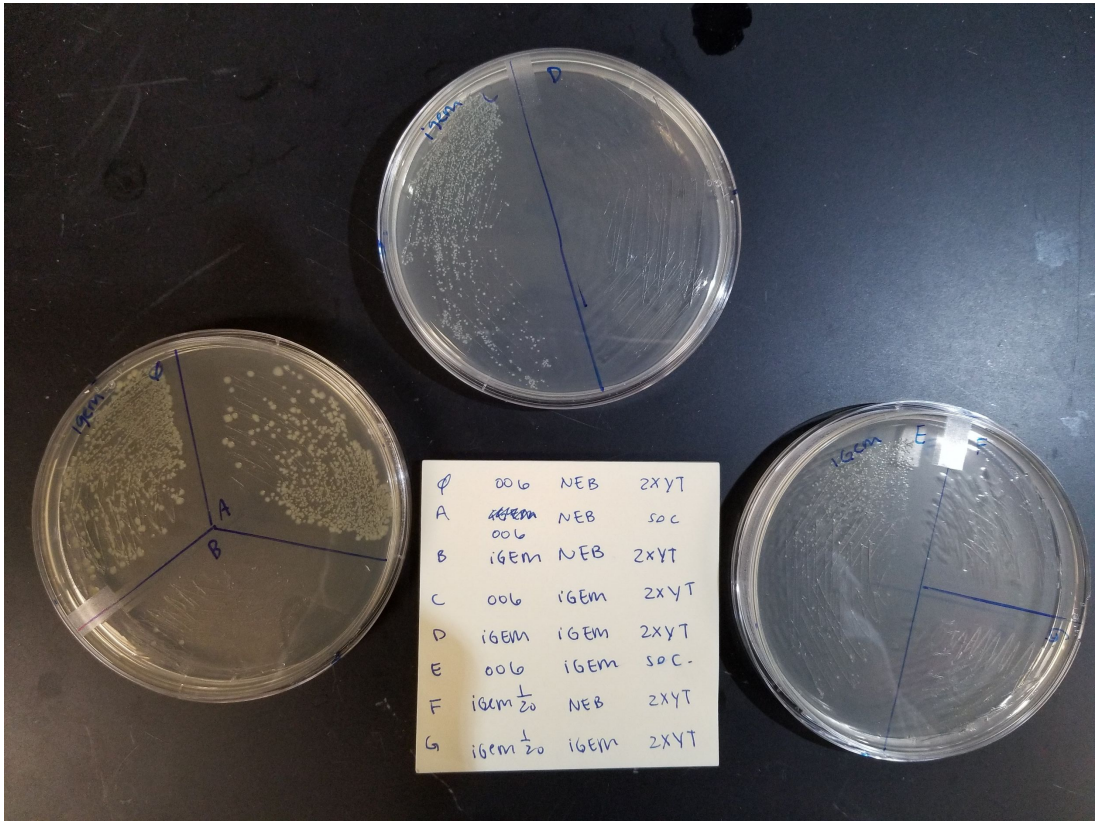
http://parts.igem.org/Help:2017_Competent_Cell_Test_Kit

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

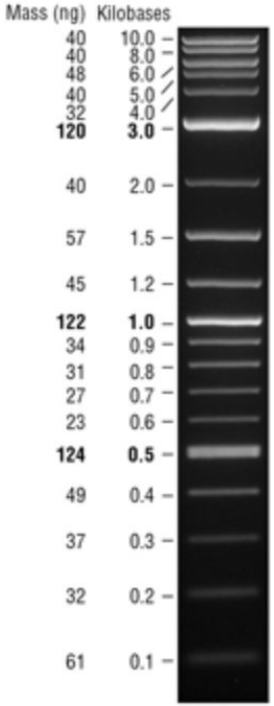
Question: Was our PCR successful?

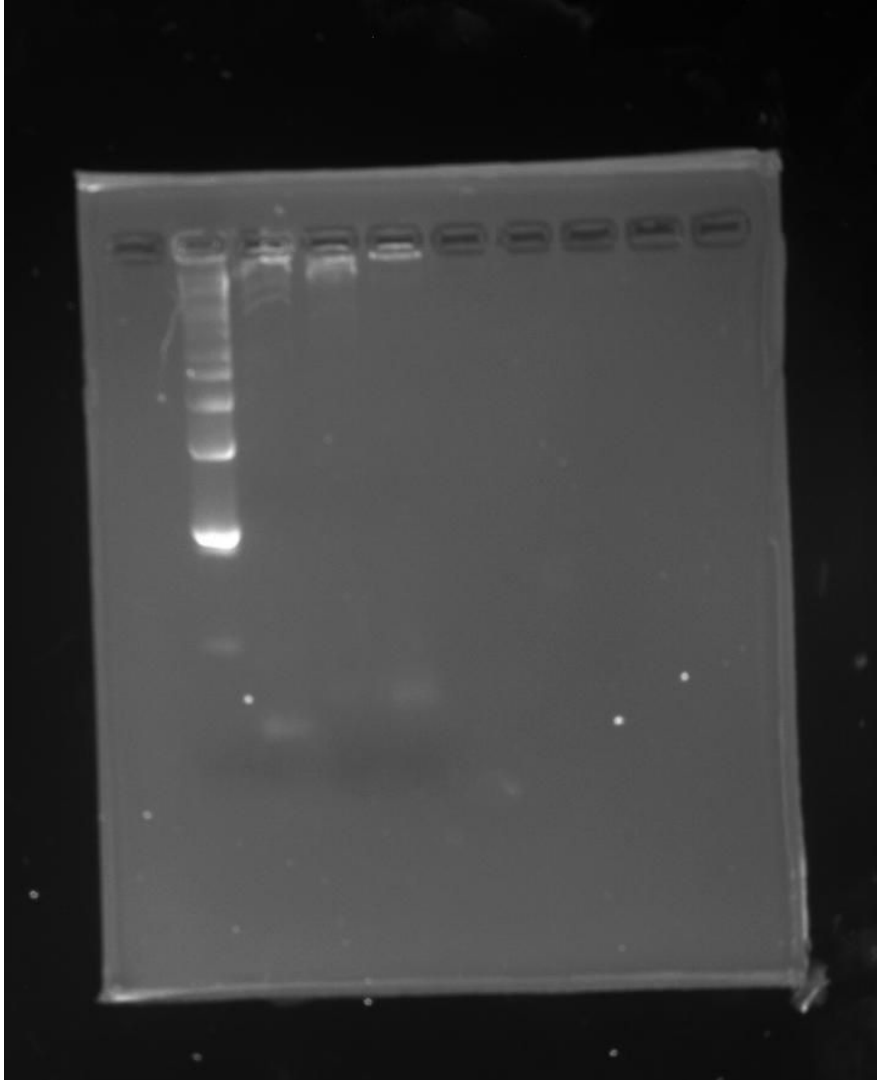
Lane 2: 1 kb Ladder

Lane 3: pADS088 backbone

Lane 4: LuxR Gene

Lane 5: Adam’s Positive Control



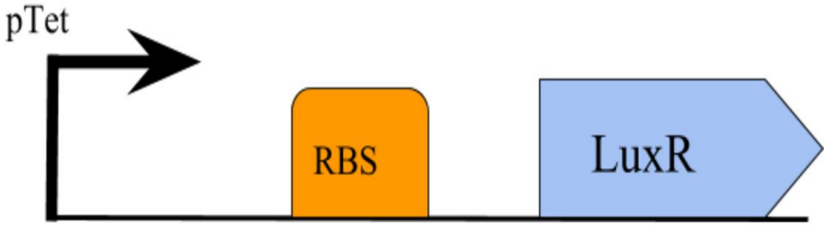


Next Steps

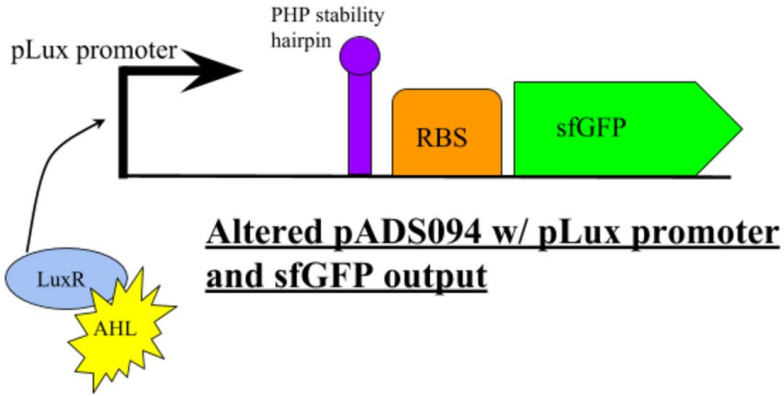
- Interlab
  - Extended Deadline: August 24th
  - We want to talk to Walter about making comp. cells
  - Collab with UChicago???
  - We're planning on using the backup DNA included in the iGEM kit
  - Shedd collaboration still a possibility (haven't gotten back to us)
    - Backup plan for Human Practices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

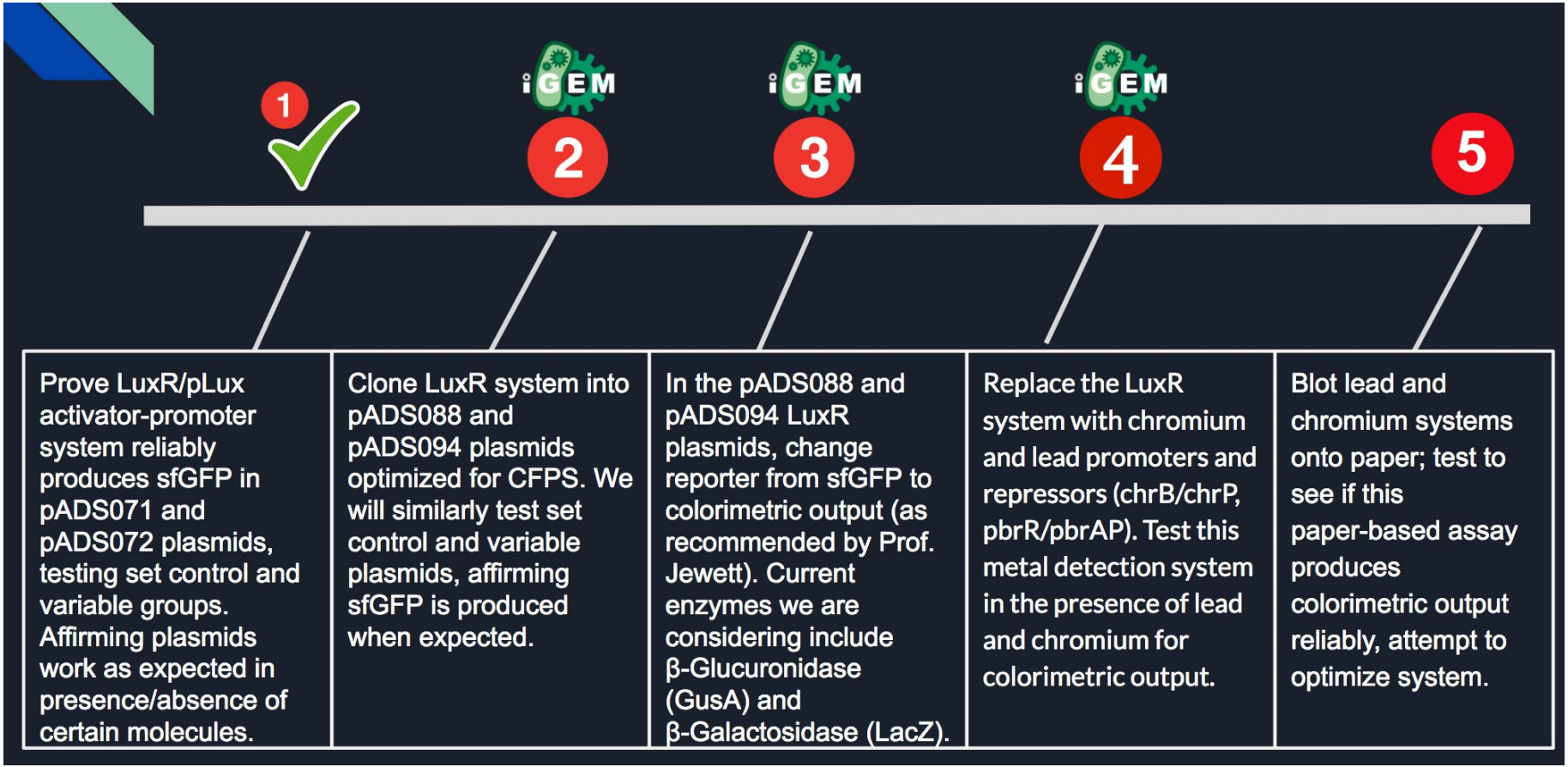


Altered pADS088 w/ pTet promoter, produces LuxR



Altered pADS094 w/ pLux promoter and sfGFP output

Next Steps Cont.



Questions, Comments, Concerns?

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