8/3/18 iGEM Meeting

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Updates

- PCR of LuxR gene and pADS088 backbone was successful
- We attempted Gibson Assembly of pADS088 backbone and LuxR gene
  - The transformation we did to check if it was successful failed
- Control transformation using pADS071 plasmid was successful
- Interlab transformations still aren't working despite using 5 uL of DNA as opposed to 1 and following the exact same protocol as our control transformation
- We emailed iGEM asking for more DNA
- We received $300 funding from the ESAM department

Gel Results of LuxR

Lane 1, 7, 10: 1 kb ladder

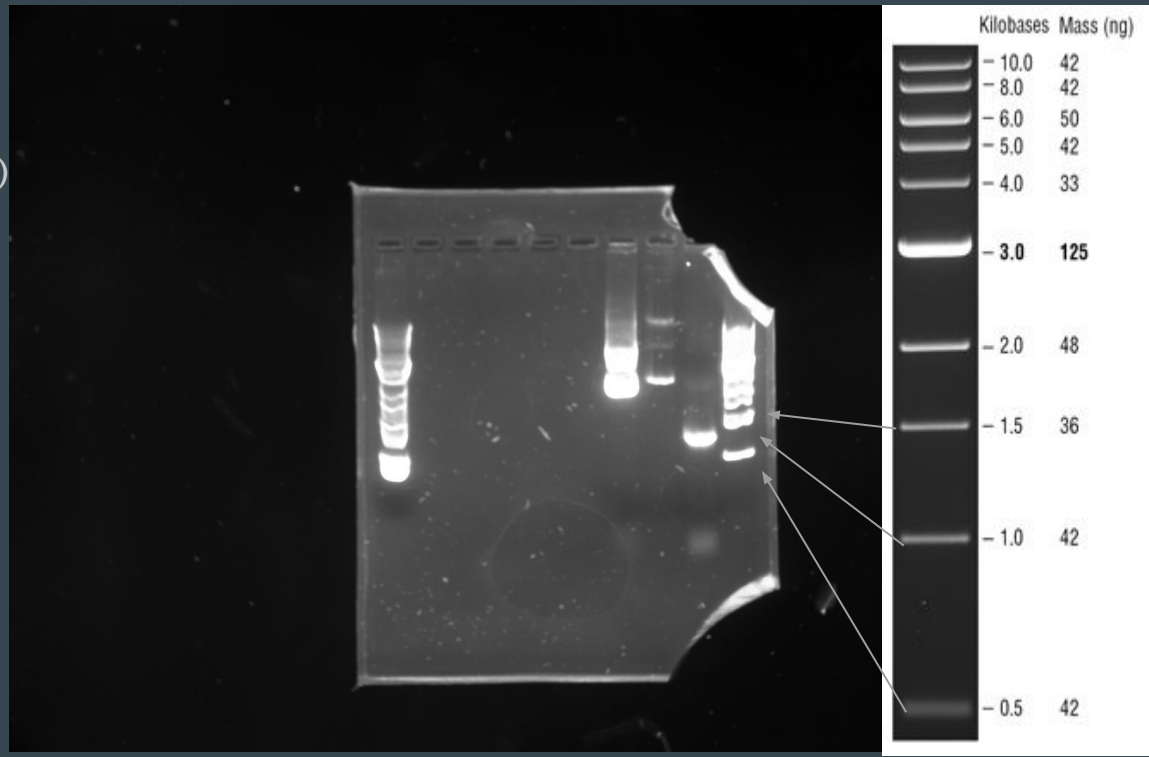
Lane 8: pADS071 (~3350 bp) (inconclusive?)

Lane 9: LuxR (~750 bp) w/ 3 lines

Top line: remainder of pADS072

Middle line: LuxR (~750 bp)

Bottom line: Primer (~40 bp)



Gel Results of pADS088

Lane 1, 2, 4, 10: 1 kb Ladders

Lane 6, 8: pADS088 (~2350 bp)

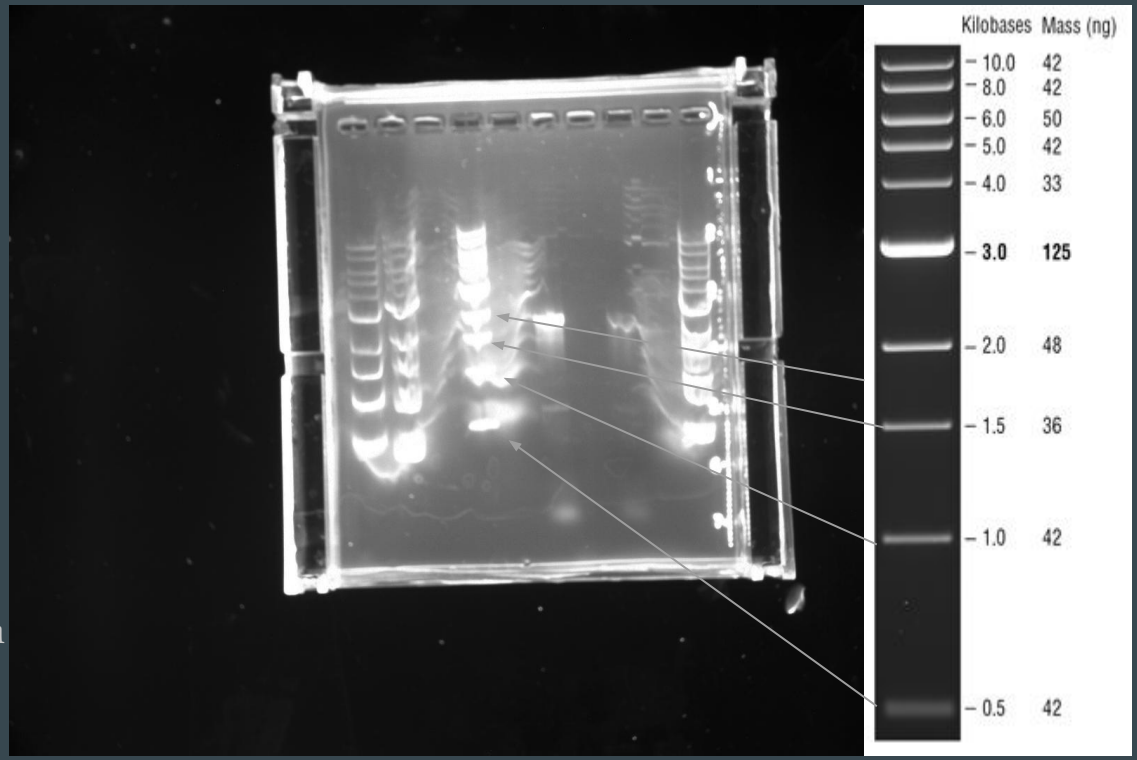
Three Lines Visible for Lanes 6,8

Line 1: Backbone (~1750 bp)

Line 2: Remainder of Plasmid: (~600 bp)

Line 3: Primer (~40 bp)

Performed Gel extraction on Line 1 to obtain pADS088 backbone.



Gel Results for Chromium and Lead Repressors

Addition of 20 bp homology on both ends

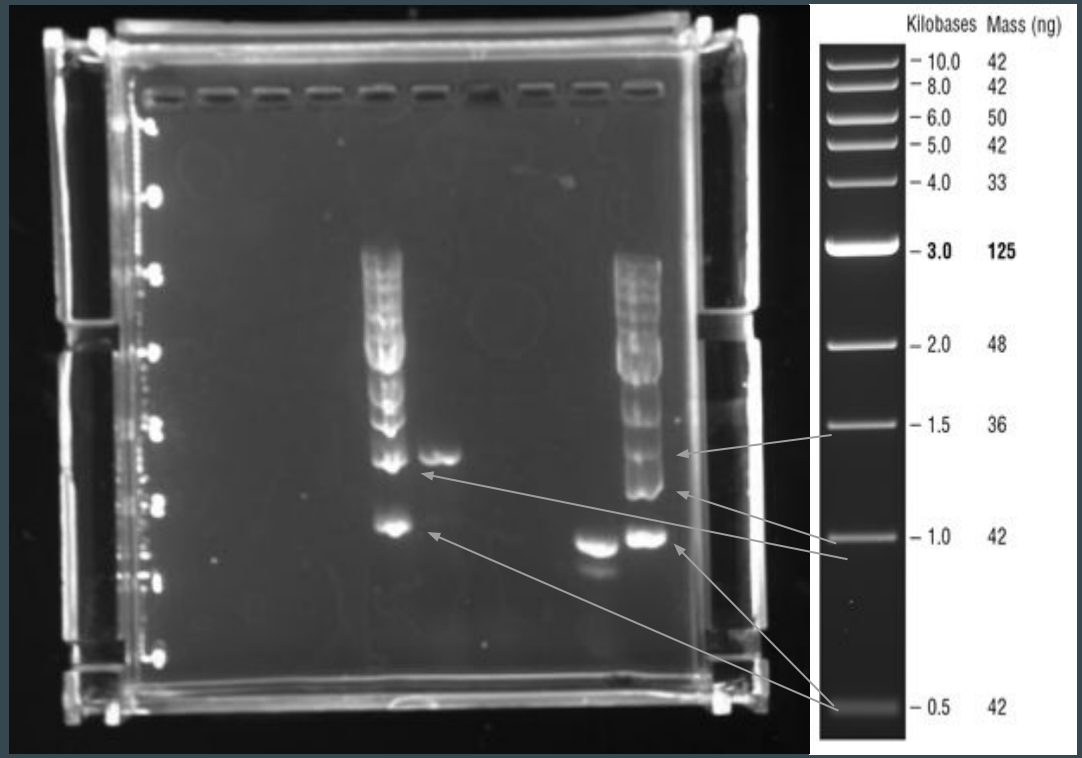
for Gibson Assembly

Lane 5 & 10: 1 Kb Ladder

Lane 6 & 7: Chromium Repressor (~ 979 bp)

Lane 8 & 9: Lead Repressor (~ 479 bp)

~BEST GEL YET~



Gibson Assembly Results

- Attempted Gibson Assembly of pADS088 backbone and LuxR gene
- Followed NEB Gibson Assembly protocol
- Transformed iGEM comp cells w/ Gibson Assembly product
  - Gibson Assembly product should be kanamycin resistant
  - 40 uL cells, 10 uL Gibson Product, 50 uL SOC
  - Obtained kanamycin plate from Lucks Lab
  - There was no growth
- Possible reasons
  - Gibson Assembly was unsuccessful
  - Transformation failed (water bath?)

Transformations!

- Did a transformation using pADS071 as opposed to iGEM DNA
  - Added 1 uL of 34 ng/uL DNA (on accident)
  - Used both iGEM comp cells and comp cells from Adam
  - Both had a lot of growth
- Next, we did another transformation following the same protocol but with iGEM DNA
  - We used 1 uL of iGEM DNA - it should be noted that the mass of DNA provided to us is unknown as iGEM never released that information - but their transformation protocol calls for 1 uL
  - Used iGEM comp cells only, as well as CAM plates from the same batch that was used in control transformation
  - Only had growth on positive and negative control plates

Transformations Cont.

- Transformed again using iGEM DNA the following day
  - Used 5 uL of DNA as opposed to 1 uL
  - Used plates from the same batch again
  - Only transformed plasmids 1-6; we didn't transform the positive and negative control again
  - No growth!
- Possible reasons
  - Our water bath is unreliable; it's set to 42 °C yet we checked the temperature w/ 3 thermometers and found it to be at 38 °C, 39 °C, and 45 °C
  - Our comp cells have a low transformation efficiency (via comp cell test that we performed 2 weeks ago)
  - We have no idea what the DNA concentration is that iGEM sent us, so we may be transforming w/ a very small amount of DNA

Human Practices Update

- Original plan: work with the Shedd or the Evanston Water Treatment Center
  - Shedd showed interest then hasn't responded
  - "Evanston Chemist" hasn't answered our two emails
- Potential plan: create a survey and use the results to impact our project
  - iGEM has standards for the surveys to meet medal requirements
  - Purdue Team advised that we get our survey IRB approved (what they did last year)

Silver

To qualify for a silver medal, teams must demonstrate how they have identified and investigated one or more Human Practices issue in the context of their project. Demonstrate to the judges that your have thought carefully and creatively about whether your work is safe, responsible, and good for the world. You could accomplish this through engaging with your local, national and/or international communities, or through developing new creative approaches (see **exemplary projects**). **Please note that conducting a survey will not necessarily fulfill this requirement.** See "How to conduct a valid and legitimate survey".

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Gold

To qualify for a gold medal, teams must complete two of the four requirements listed on the **official medal criteria page**. To qualify for gold using Human Practices work, teams must expand on their silver medal activities by demonstrating how the investigation of their HP issues has been integrated into the design and/or execution of their project. Just talking about your project with people outside your lab DOES NOT meet this requirement. Show us that your conversations with people outside the lab have influenced your project. **We want to see how your iGEM project (lab design, parts selection/development, overall application, etc.) has evolved based on your Human Practices work.** Think of the design/build/test/learn cycle of engineering.

Next Steps

- Continue Interlab transformations
  - Maybe make new comp cells? Obtain comp cells from another lab?
  - Attempt the transformations in a lab w/ equipment that we know works
  - Use new DNA sent to us from iGEM
  - Possibly share cells w/ UChi team if we can't get our transformations to work
- Check Gibson Assembly product
  - Sequencing?
- Start PCRing lead/chromium repressors and promoters
- Complete a survey for Human Practices in case the Shedd and Evanston Water Department don't come through
- Continue working on Wiki