

8/10/18 iGEM Meeting

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# Updates

- Did another Gibson Assembly of pADS088 backbone and LuxR
- Successfully transformed iGEM Comp. Cells with this assembled plasmid
- Ordered new iPCR primers to isolate the lead promoter (PbrAP) and pLux
- Did our first liquid culture and miniprep
- Sent DNA from transformed cells to sequencing to verify the Gibson Assembly
- Got sponsored by BioBasic
- Ordered pipette tips, Phusion PCR mix, Gibson master mix, scale on the way

# Chromium Promoter and pADS088 backbone

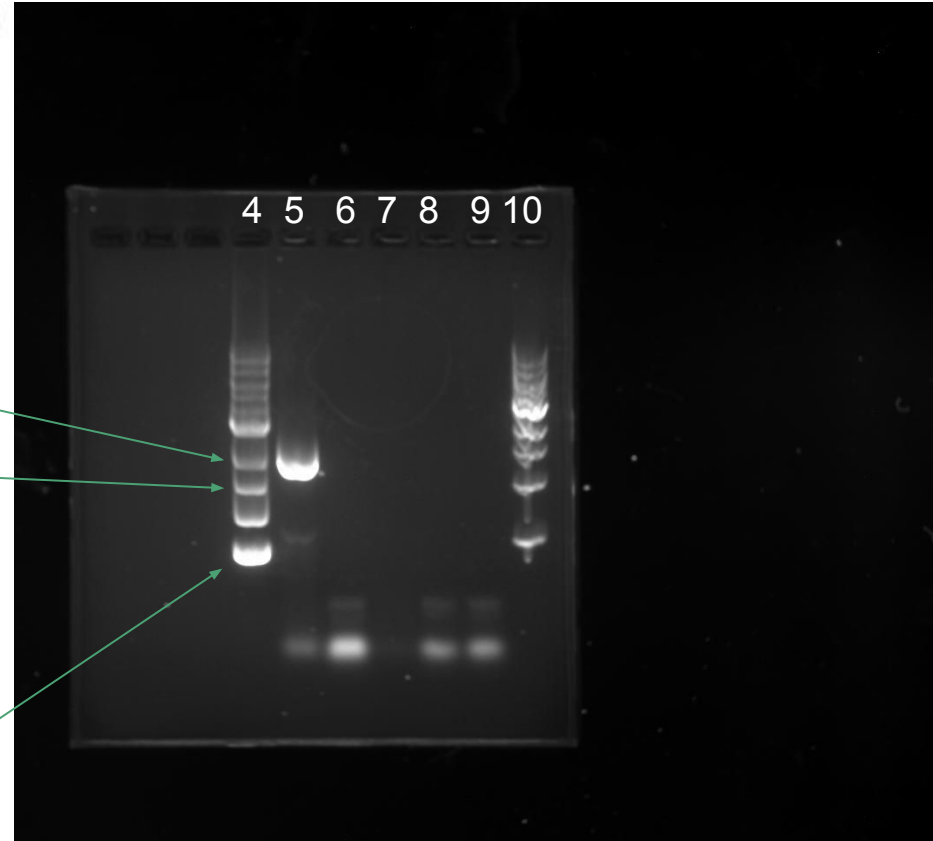
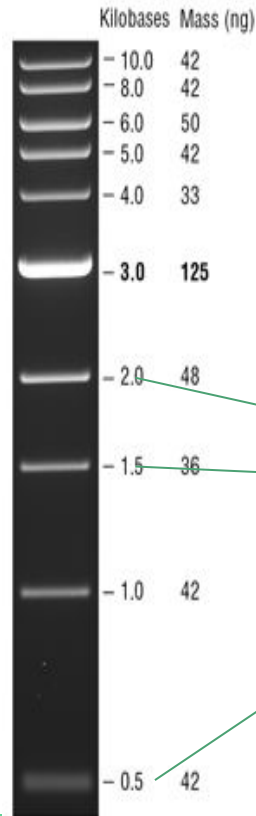
Lane 4, 10: 1 kb ladder

Lane 5: pADS088 backbone

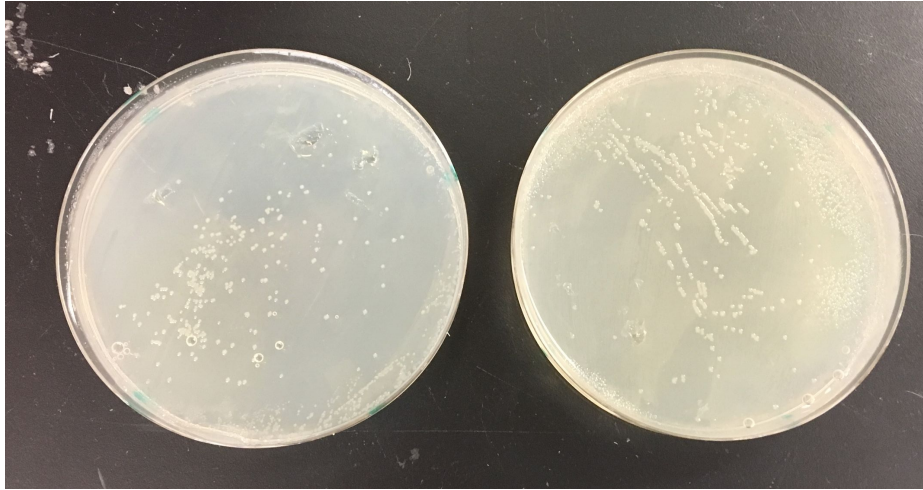
- 1700 bp

Lane 6-9: Chromium promoter

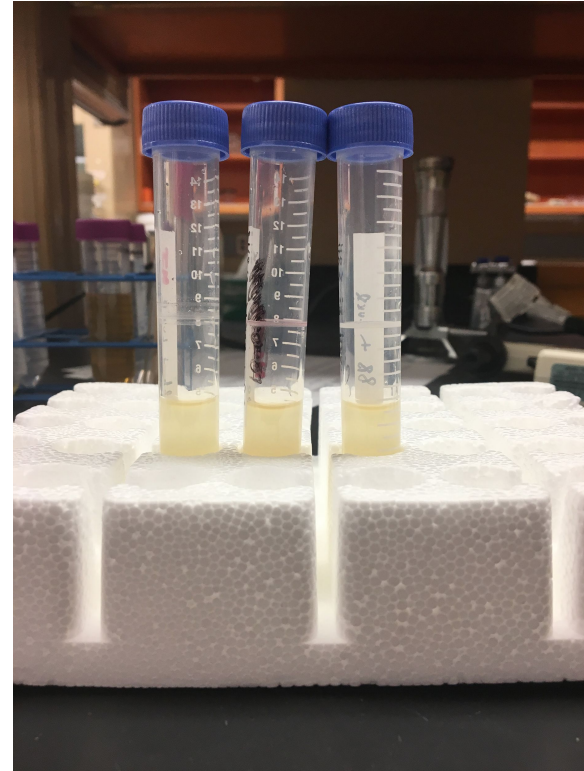
- 160 bp
- Bottom row = primers
- Top row = primer dimers / the promoter
- Next step: Use 500 bp ladder?
  - Suggestions?



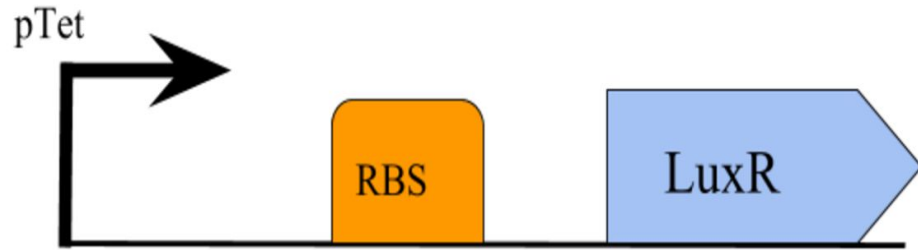
# Assembled pADS088 backbone and LuxR gene



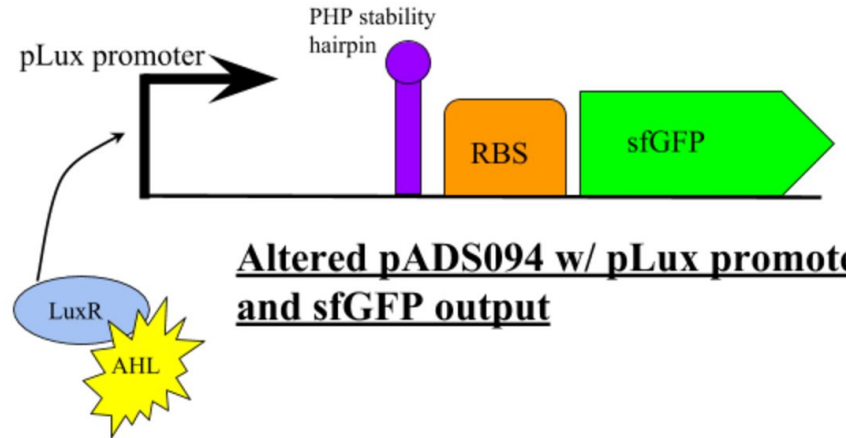
- Transformation protocol:
  - 40  $\mu$ l cells, 10  $\mu$ l assembled Gibson DNA, 50  $\mu$ l SOC, plated 80  $\mu$ l
- Liquid culture, Mini-prepped, sent to sequencing yesterday



(sent into sequencing)



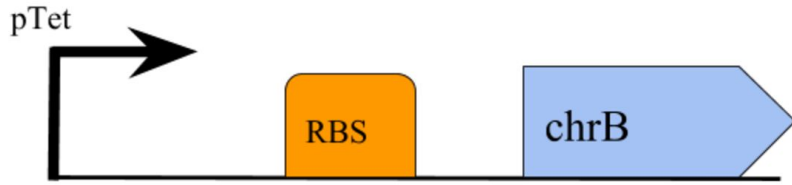
**Altered pADS088 w/ pTet promoter, produces LuxR**



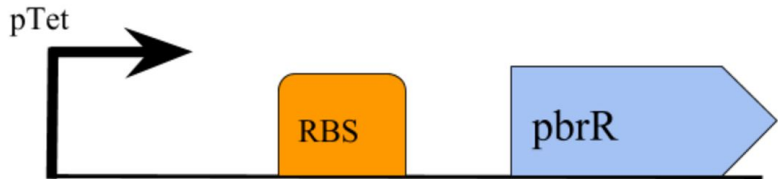
**Altered pADS094 w/ pLux promoter and sfGFP output**

# Next Week:

- iPCR Primers, new iGEM DNA will arrive Monday
- Finally complete InterLab
- Run an iPCR to achieve pLux promoter in pADS094 backbone
  - If successful, we will now (hopefully) have both plasmids to complete Step 2 (pLux/LuxR system in 88/94 plasmids). See if this two-plasmid system will produce sfGFP in vitro.
- Run an iPCR to achieve Lead promoter (PbrAP) in pADS094 backbone
- Gibson assemble/Use iPCR to achieve:
  - Chromium promoter (ChrP) in pADS094 backbone
  - Chromium repressor (ChrR) in pADS088 backbone (GFP output)
  - Lead repressor (PbrR) in pADS088 backbone (GFP output)

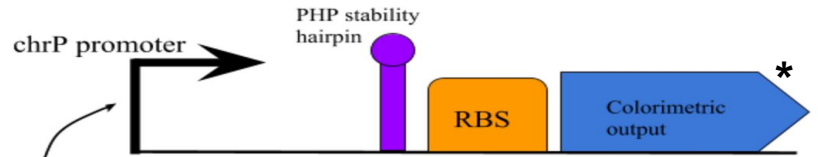


**Altered pADS088 w/ pTet promoter:  
produces chromium repressor chrB**

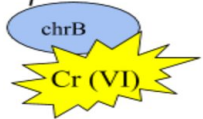


**Altered pADS088 w/ pTet promoter:  
produces lead repressor pbrR**

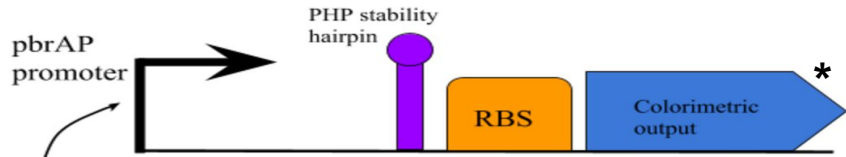
&



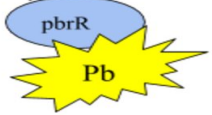
**Altered pADS094 w/ chrP promoter  
and colorimetric output**



&

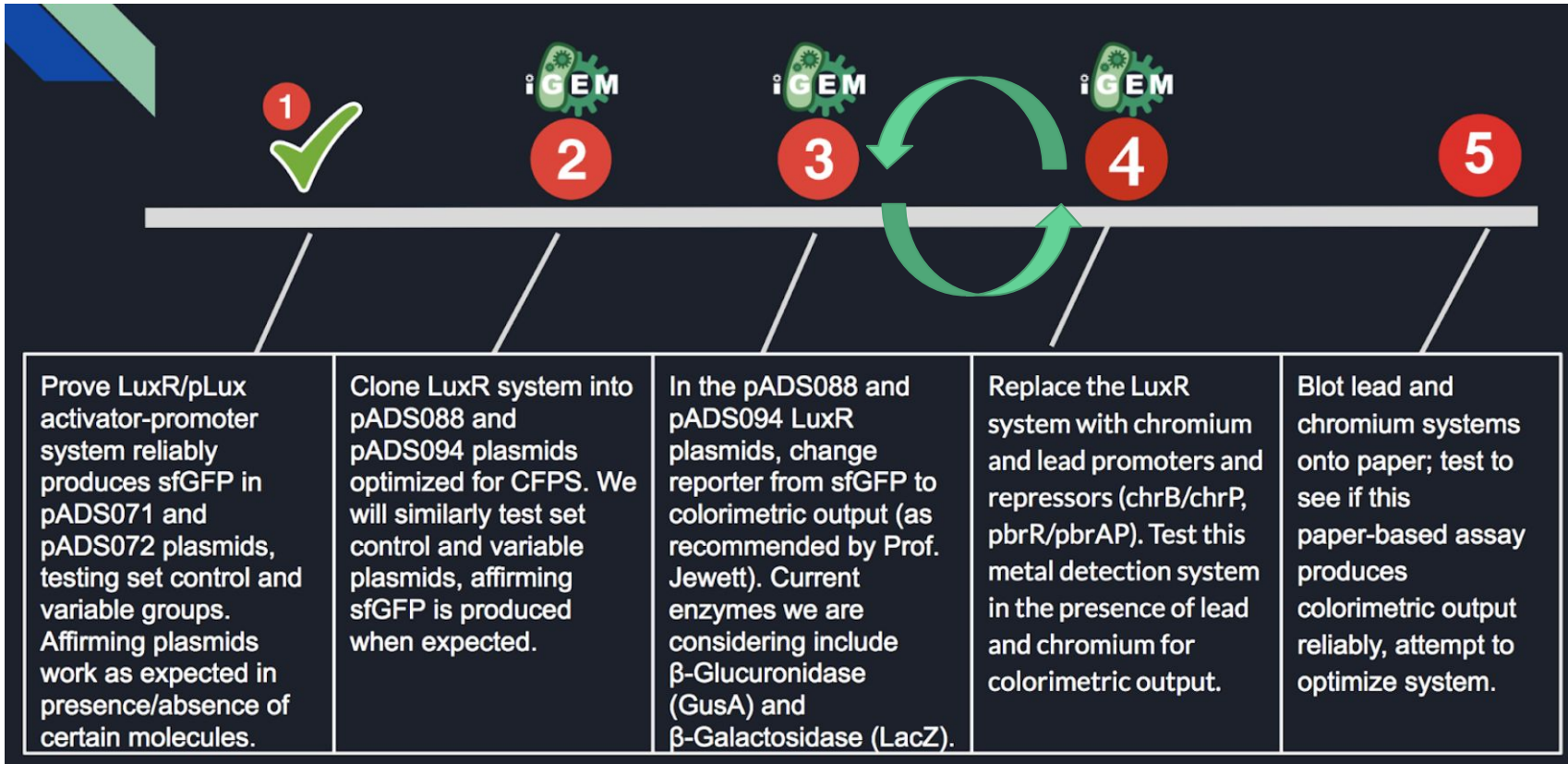


**Altered pADS094 w/ pbrAP  
promoter and colorimetric output**



\* Will not be replacing the sfGFP output with colorimetric output just yet

# Next Steps Cont.





# Budget

Northwestern

## Summary Budget Status Report For Fiscal Year 2018 As of Accounting Period 11 - July 2018 (Closed)

**Fiscal Year/Budget Period:** Previous Period  
**Accounting Period:** Previous Period  
**Fund:** All  
**Current Department:** 4732000 -  
 ChBE-Chemical  
 & Biol Eng Dept  
**Project Status:** Active Projects  
 Only  
**Project:** 30040117 - J

**Department Manager:** Young, Jennifer Ann  
**Project Manager:** Leonard, Joshua N

**Fund :** 320 Unrestricted Gifts

**Department :** 4732000 ChBE-Chemical & Biol Eng Dept

**Project :** 30040117 J Leonard Research Gift [\(Detail Drill\)](#)

Account	Current Period Summary			Fiscal Year to Date			Current Budget(Un)	Favorable Balance
	Pre-Enc/Enc	Actual	Total	Pre-Enc/Enc	Actual	Total		
Revenue 40301 Private Gifts -Unrestricted	0.00	0.00	0.00	0.00	3,180.54	3,180.54	0.00	3,180.54
49999 Budget Carryforward Revenue*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
88802 Non-Mandatory Transfer from	0.00	0.00	0.00	0.00	4,000.00	4,000.00	0.00	4,000.00
<b>Revenue Total</b>	<b>\$0.00</b>	<b>\$0.00</b>	<b>\$0.00</b>	<b>\$0.00</b>	<b>\$7,180.54</b>	<b>\$7,180.54</b>	<b>\$0.00</b>	<b>\$7,180.54</b>
Expense 73010 Paper And Office Supplies	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
73475 Lab Supplies and Hardware	0.00	0.00	0.00	0.00	213.35	213.35	0.00	(213.35)
75130 External-Printing Services	0.00	0.00	0.00	0.00	301.44	301.44	0.00	(301.44)
75340 Laboratory Services	0.00	0.00	0.00	0.00	161.00	161.00	0.00	(161.00)
75520 Common Carrier	0.00	0.00	0.00	0.00	12.02	12.02	0.00	(12.02)
76730 Professional Development	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
76765 Domestic Travel-Transportation	0.00	0.00	0.00	0.00	549.62	549.62	0.00	(549.62)
76766 Domestic Travel-Hotel/Lodging	0.00	0.00	0.00	0.00	453.33	453.33	0.00	(453.33)
76777 Domestic Travel-Mtg& Conf Fees	0.00	0.00	0.00	0.00	5,390.00	5,390.00	0.00	(5,390.00)
79999 Budget Carry Forward*	0.00	0.00	0.00	0.00	0.00	0.00	8,617.19	8,617.19
<b>Non-Personnel Total</b>	<b>\$0.00</b>	<b>\$0.00</b>	<b>\$0.00</b>	<b>\$0.00</b>	<b>\$7,080.76</b>	<b>\$7,080.76</b>	<b>\$8,617.19</b>	<b>\$1,536.43</b>
<b>Expense Total</b>	<b>\$0.00</b>	<b>\$0.00</b>	<b>\$0.00</b>	<b>\$0.00</b>	<b>\$7,080.76</b>	<b>\$7,080.76</b>	<b>\$8,617.19</b>	<b>\$1,536.43</b>
<b>Net Position: Revenue less Expense</b>				<b>\$0.00</b>	<b>\$99.78</b>	<b>\$99.78</b>	<b>(\$8,617.19)</b>	<b>\$8,716.97</b>
<b>Net Position Excluding Benefits</b>				<b>\$0.00</b>	<b>\$99.78</b>	<b>\$99.78</b>	<b>(\$8,617.19)</b>	<b>\$8,716.97</b>
<b>Expense Total Excluding Carry Forward</b>				<b>\$0.00</b>	<b>\$7,080.76</b>	<b>\$7,080.76</b>	<b>\$0.00</b>	<b>(\$7,080.76)</b>
<b>Net Position Excluding Carry Forward</b>				<b>\$0.00</b>	<b>\$99.78</b>	<b>\$99.78</b>	<b>\$0.00</b>	<b>\$99.78</b>

\* Budgetary Only Account  
 Northwestern  
 INFORMATION TECHNOLOGY