iGEM Meeting 8/9/17

- Wiki Freeze → Nov. 1

* work w/ other team

* improve function of previous part

- Project Description - characterizing chromoprotein under varying conditions with a goal in making data / research available to underfunded labs

My work

- RFP
- background research / wiki
- Human practices
- Maker Faire

*$350 by Sept 1st

- Human Practices

- PDF → design, construction manual, video
- send in video, skype or present real genetic research to genetic classes

BFP Redo #3

- must remake IPTG LB
- check cells if grown → plated from old transformation

Calculations

1mM: (1M)(V) = (.001M)(250 mL)

.25 mL 1M IPTG stock

250 µL in 250 mL LB

10µM: (1M)(V) = (10 × 10⁻⁶)(250mL)

.0025mL 1M IPTG stock

2.5 µL in 250 mL LB

100nM (1M)(V) = (100 × 10⁻⁶)(250 mL)

.025 mL

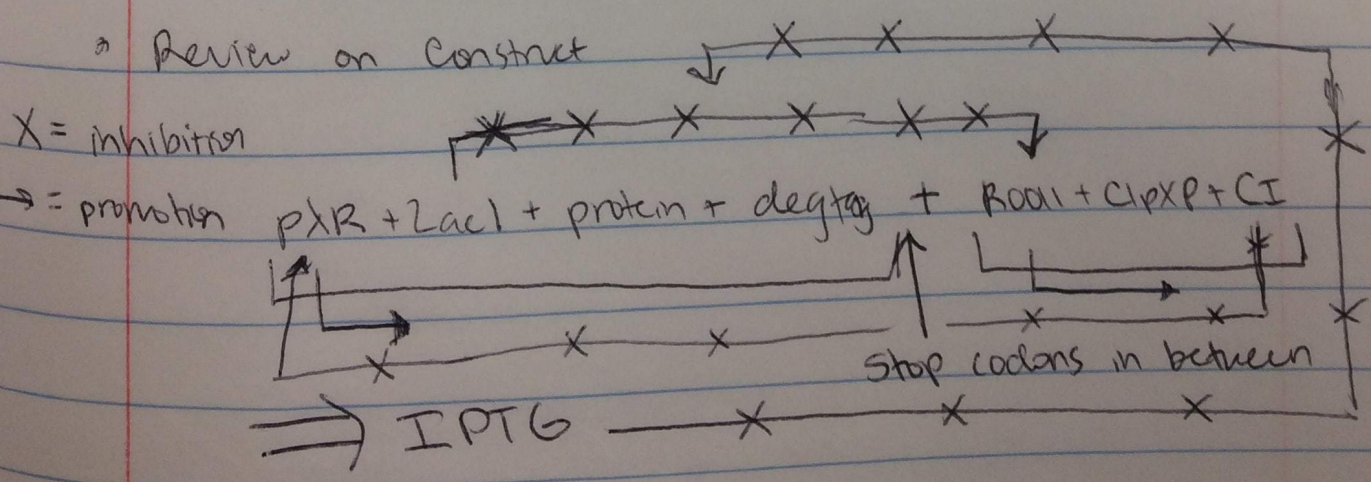
25 µL 1M IPTG Stock

* take from 1M Stock for all dilutions to reduce batch effect

8/16/17

- made LB

* Review on Construct



- Wet Lab Update
  - weak plac ?? ⇒ replacement for ROII
    - will be repressed by Lac molecules
  - Tspanpk + degtag ⇒ miniprepped by Thurs 8/17
    ⇒ digested by Friday 8/18
  - ROII

iGEM 9/6/17 Wed. Morning

- Transf of ~~PXR RFP~~ PXR LacI R.gl RFP
  - Kan resistant
  - 1 ng/µl
  - used 5 µl of plasmid rather than 2µl as protocol says

* 2 tubes of plasmid ① 1 ng/µl RFP plasmid in Aks
② diluted RFP plasmid in Carb backbone

- transformation done in purple tube
- may have left DH10 Beta Cells out for too long, but should be ok * NEXT TIME → have Beta cells out after everything else is at

10/2/17

- IPTG Stock at X-Gal: 1000x concentration

1:1000

want to make 1 mM concentration

$$\frac{1}{1000} = \frac{x}{(1 \times 10^{-3})}$$

1000

10/4/17

* IPTG / XGAL conc.? → 1M in 1mL b/c working conc (1x) = 1:1

Plan: • retransform constructs on kan + carb plates

• -induce IPTG (10mL) in 5mL of 9/16 kan

parallel RFP (10mL), 9/20 carb pTR ——— RFP,

- kan cells (w/o IPTG)
- carb cells (w/o IPTG)

• ~~make dilutions of IPTG in dH2O → freeze~~ then dry

1mM: (1M)(x) = (1x10^-3)(1mL)

* Question → do I make concentration of IPTG based on LB? → YES

• 0 10 100 1

0 10 100 1

0 10 100 1

-0 -10 -100 -1

0 10 100 1

25mL

* make stocks (used immediately) of 40mL stocks of IPTG (concentrated LB (to accompensate)

Calculations:

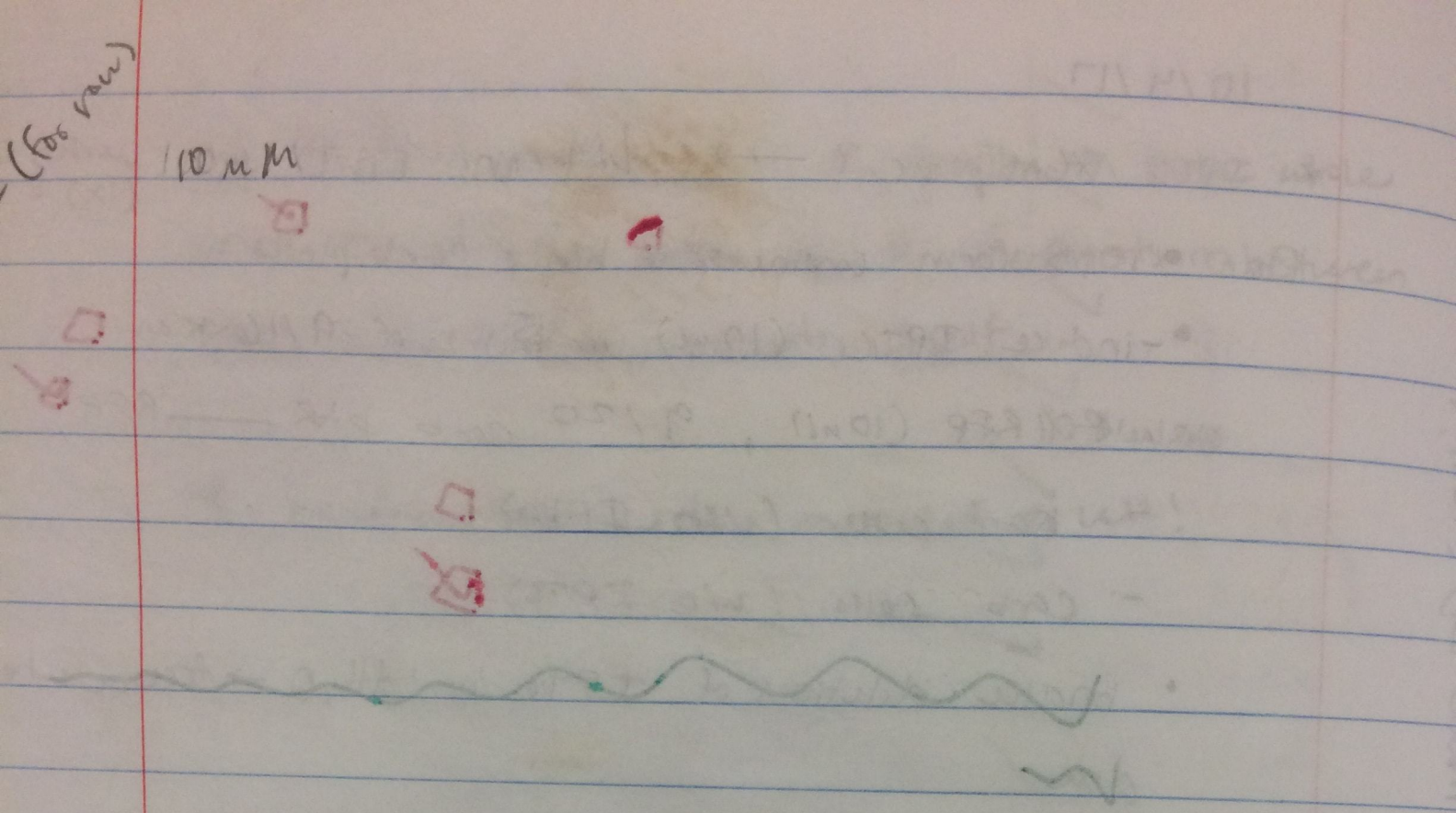
1mM: (1M)(x) = (1x10^-3)(40mL)

x = 0.04mL = 40 uL 5mL → 5uL

5mL → .5uL

(for raw)

10 mM



10/4/14 Carb Transf.

pXRLacI ROII RFP in amp/carb → taken from stock and put in our tube in room - freezes (red 1 mL tube)

transf: pXRLacI ROII RFP in carb → 10 uL in DH10 Beta

pUC 19 → 5 uL

plates:
- carb ☑
- cam (- control) ☑
- plain ~~LB~~ DH10 ☑
- plain pUC 19 (+ control) ☑

10/5/17

- transformation of 10/5 → successful; all plates correct
- liquid cultures (x2) (carb transf + IPTG / no IPTG) → NOT SUCCESFUL today
  - made liquid cultures of new, 10/4 transf + add IPTG to 1 culture
  - re-added IPTG to 1 LC of 10/4
  - placed at back windowsill

* IPTG may be heat sensitive ?

10/6/17

* FLUORESCENCE → IPTG heat sensitive so grow on windowsill
* plan for monday → trials (2 to see if it works)
  - amp LB ☑
  - transformation on carb ☑

* Thoughts → Change conc. of IPTG to greater

2mM $(1M)(V_1) = (2M)(5mL)$

$(1M)(V_1) = (2mM)(5mL)$

$= 10uL$

200uM $(1M)(V_1) = (200 \times 10^{-6})(5mL)$

$= 1uL$

20uM $(1M)(V_1) = (20 \times 10^{-6})(5mL)$

$.0001 = .1uL$

$(1M)(V_1) = (.1M)(1mL)$

$.1mL \quad .1 \quad 100uL$

238.3g | mol

5g | mol | 238.3g

238.3g | mol | 5g

5g → 283.3g/mol

238.3 ,238

283.3g | 1mol | 1L = 0.8383 g/mL for 1M IPTG
mol | 1L | 1000mL

0.5666g/mL for 1M

10/18/17

- made IPTG stocks

  .238 g / 1 ml → 2x's (one is better than other b/c I used less water for 1 mL microcentrifuge tube)

  → stored in iGEM Sophomores "no touch" box in freezer

  → stored powder in fridge

- 1mM IPTG (5mL) + 9/18 PLRRFP Kan streak singles
- 100uM IPTG (5mL) + 9/18 PLRRFP Kan streak singles

  → window sill

* To do:
  - retransform on Kan ☑
  - full trial from 40 mL LB stocks
  - RFP write up
  - miniprep cells for sequencing ☐

10/20/17

- 15 mL LB → 1mM IPTG, 100 μM IPTG

1mM: 15uL + 15mL LB

100μM: 1.5uL + 15mL LB

6 types: 1mM, 100μM → 5uL construct

* 1mM, 100μM from plate 1mM, 100μM → 10uL construct

- make eq. stocks of cells (70uL water + cells)

* vortex cells?

10/27/17

- replace TB w/ PXK ?
- Take TspE out
  - Bsu36I — EcoR1