- Made Tet and Amp plates
- Gibson Assembly of Tet- GFP and BB- PR-DT

DpnI Reaction
- [x] 5 µL sample
- [x] 1 µL Cutsmart Buffer
- [x] 1 µL DpnI

Gibson Assembly:
- [x]
- [x] Set up following reaction on ice:
  - [x] 1µL of fragment (10µL of control)
  - [x] 10µL G.A. 2xMaster Mix
  - [x] 8µL H_2O (0µL in control)

Total volume: 20µL

- [x] Incubate in a thermo cycler for 60 minutes

Transformation of Gibson Assembly Products

- [x] Thaw a tube of NEB 5-alpha cells on ice until last ice crystals disappear. Mix gently ~~and carefully pipette~~
- [x] Add 1-5 µL containing 1pg - 100ng (we're using 2µL) of DNA to the cells mixture
  - [x] Carefully flick the tube 4-5 times to mix
- [x] Place mixture on ice for 30 minutes. Don't mix! Turn on heating block!!
- [x] Heat shock at 42°C for 30s
- [x] Place on ice for 5 minutes
- [x] Pipette 950 µL of room temp SOC into mixture
- [x] Place at 37°C for 60 minutes, shaking vigorously (250 rpm) or rotate. Meanwhile, warm selection plates to 37°C.
- [x] Mix cells thoroughly by flicking and inverting tubes
- [x] Spread 20µL and 200µL of cells onto a selection plate. Incubate overnight at 37°C. Put in at 7:00 PM

4/10/2015
Time in: 7:15am
Time out: 10:15am

Making Tetracycline Stock Solution (1000x)

- [x] Add 0.15g of Tetracycline to 10mL of 70% EtOH in a Falcon tube
- [x] Distribute to 10 1.5mL eppendorf tubes

Making 500mL LB-Tet for Plates

- [x] In a 1L flask, combine:
  - [x] 10g LB broth base
  - [x] 500 mL milliQ H_2O
  - [x] 15g Agar
- [x] Autoclave (L20 cycle)
- [x] Add 500µL of 1000x Tetracycline
- [x] Mix well
- [x] Pour plates in hood
  - Tetracycline plates are marked with a blue line

- Re-plated transformation of Gibson assembly of GFP-BB, since the plates we used last time did not have ~~exactly~~ effective tetracycline

5/2/2015
Time in: 5:40 pm
Time out: 5:50 pm

- Yesterday, Andy plated a negative control of newly made Tet plates (from 4/29). There was growth, implying that something is wrong with our Tet, since this is the third time we have made plates and had this growth.

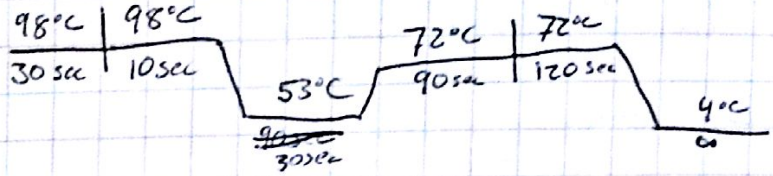
Making Terrific Broth (Better than LB)

- [ ] Make sterile solution of 0.17M KH_2PO_4 and 0.72M
  - [ ] 2.31 g KH_2PO_4
  - [ ] 12.54 g K_2HPO_4
  - [ ] 90 mL H_2O
  - [ ] After salts have dissolved, adjust to 100 mL
  - [ ] Autoclave
- [ ] To make 1L of Terrific Broth:
  - [ ] 900 mL H_2O
  - [ ] 12g Tryptone
  - [ ] 24g Yeast extract
  - [ ] 4 mL Glycerol
  - [ ] Shake until solutes have dissolved
  - [ ] Autoclave, then allow solution to cool to 260
  - [ ] Add 100 mL of sterilized 0.17M KH_2PO_4, 0.72

6/1/2015
Time in: 10:00AM
Time out: 7:00PM

PCR-ing Up pSB1T3 (Q5 HotStart High-Fidelity 2x Master Mix)

- [x] 12.5 µL Master Mix
- [x] ~~10~~ 1.25 µL primer 1 (A57)
- [x] 1.25 µL primer 2 (A58)
- [x] 0.5 µL pSB1T3 (25ng/µL)
- [x] 9.5 µL NFW



35 cycles

Started at: 12:50

Making Plates

- [x] Make LB:
  - [x] ~~10~~g Tryptone
  - [x] 10g NaCl
  - [x] ~~5~~g yeast extract
  - [x] 20g Agar (only for plates)
  - [x] Water to 1L
  - [x] Autoclave Started: 1:20PM

Got lunch at 1:30-2:30

- [x] Pour plates (Amp and Chlor)

- John and Andy are making Kan plates

Resuspending from Distribution kit:

- [ ] Top 10 Promoters
  - [ ] R0040 - 2 - 6F - Chlor
  - [ ] R0011 - 2 - 6D - Chlor
  - [ ] R0062 - 2 - 6H - Chlor
  - [ ] J23119 - 3 - 170 - Chlor
  - [ ] R0010 - 3 - 4G - Chlor
  - [ ] I13453 - 3 - 190 - Chlor
  - [ ] J23100 - 4 - 17D - Amp
  - [ ] J23101 - 4 - 17F - Amp
  - [ ] R0051 - 4 - 5L - Amp
  - [ ] I0500 - 5 - 17N - Chlor

- [ ] Backbones for PCR (J04550)
  - [ ] pSB1C3 - 4 - 4B
  - [ ] ~~pSB1T3 - 4 - 8B~~ X
  - [ ] pSB1A3 - 4 - 2H

  } 2015

  - [ ] pSB1K3 - 4 - 6B } 2014

- [ ] Functional GFP
  - [ ] I20260 - 4 - 184 - K3

To Resuspend:

- [ ] 10μL H_2O
- [ ] 15 minutes at RT

- [x] Thaw tube of NEB 5-α competent cells on ice. Mix gently
  Pipette ~~50 µL~~ 15 µL cells into transformation tube on ice
- [x] Add ~~+5 µL~~ 2 µL of resuspended distribution kit DNA
  to cell mixture. Flick 4-5 x to mix (No vortex)
- [x] Incubate 30 min on ice
- [x] Heat shock @ 42°C, 30sec
- [x] Place on ice for 5 min
- [x] Pipette 230 µL SOC (RT) into mixture
- [x] Incubate 60min @ 37°C, 250rpm. Pre-warm agar plates
- [x] Mix cells by flicking/inverting
- [x] Spread 20µL and 200µL of each transformation onto plate
- [x] Incubate overnight @ 37°C

6/12/2015
Time in: 10:00am
Time out: 5:50pm

-John and Joe started making Amp Plates

Diluting Antibiotic

10 mL of each Amp and Chlor

Chlor: 0.34g in 10mL in EtOH
Amp: 1.0g in 10mL in dH$_2$O

Aliquots of 1mL

-Added Phosphate buffer to Terrific Broth

-To inoculate, we need:

80mL Terrific Broth w/ Chlor → 80μL of Antibiotic needed
50 mL TB w/ Amp → 50μL
25 mL TB w/ Kan → 25μL

- Michael + team diluted ladder
  (Made by Michael and team)
- Ran a gel:
  - 2μL loading dye
  - 1μL PCR'd up tet backbone (from linearized)
  - 9μL H$_2$O
  - Ran with 8μL diluted ladder

Lunch: 12:50 - 1:45pm

Colony PCR (Q5 HotStart) (Caroline)

- ☑ 12.5 µL Master Mix
- ☑ 1.25 µL primer 1 (~~#~~ A57)
- ☑ 1.25 µL primer 2 (158)
- ☑ 10 µL NFW
- ☑ Tip dipped into colony (shake around)

Inoculation

- ☑ Take same tip, and dip into culture tube (Caroline)
- ☑ OR Use metal inoculation rod to inoculate colonies (Michael, Joe, Pooja, Eli)
- ☑ Put in 37°C shaker overnight

-Designed and ordered Gibson primers

-Prepped for tomorrow's MP

6/3
Time
Time

6/3/2005
Done: Ryan
Done at 8:05AM

Miniprepping

- [x] Pellet 3mL bacterial overnight culture by centrifugation at 6800 g for 3 min at RT
- [x] Resuspend pelleted bacterial cells in 250 µL Buffer P1 and transfer to a micro centrifuge tube
- [x] Add 250 µL Buffer P2 and mix by inverting the tube 4-6 times until clear. Do Not allow reaction to proceed >5 min
- [x] Add 350 µL Buffer N3 and mix immediately by inverting 4-6 times. ~~If not~~
- [x] Centrifuge for 10 min at 13,000 rpm (~17,900 g)
- [x] Apply supernatant from step 5 to the spin column by pipetting. Centrifuge for 30-60s and discard flowthrough
- [x] Wash spin col by adding 500 µL Buffer PB. Centr 30-60s and discard flowthrough
- [x] Wash spin col with 750 µL Buffer PE. Centr for 30-60 s, and discard flowthrough
- [x] Centrifuge for 1 min to remove residual wash buffer
- [x] Place the column in a clean 1.5mL tube. To elute DNA, add 50 µL Buffer EB or water to the center of the spin col. Let stand 1 min, and centrifuge for 1 min

- Panya, Joe, Michael, and John set up glycerol stocks

Nanodrop from first miniprep

Sample ID	ng/ul	A260	A280	260/280	260/230
R0040 #1	135.69	2.714	1.441	1.88	2.48
R0011 #1	101.52	2.030	1.059	1.92	2.31
R0082 #1	71.10	1.422	0.760	1.87	2.66
J23119 #1	29.18	0.584	0.316	1.84	3.25
R0010 #1	136.78	2.736	1.447	1.89	2.48
I13453 #1	15.22	0.304	0.176	1.73	2.16
J23100 #1	62.34	1.247	0.692	1.80	2.39
J23101 #1	382.16	7.643	4.111	1.86	2.33
pSB1C3 #1	284.13	5.683	3.024	1.88	2.37
pSB1A3 #1	270.43	5.409	2.897	1.87	2.51
pSB1K3 #1	102.41	2.048	1.093	1.87	2.33
I20260 #1	22.01	0.440	0.275	1.60	1.61

Lunch: 12:30 - 1:30

Safety Session: 1:30-3:10PM

- Panya and Elli ran a gel of the colony pcr's (1C3, 1K3, 1A3) [This gel contained shadow bands
- Michael and Joe are doing a second round of MPs (see Michael's)
- Panya, Elli, and I are doing a transformation
- Joe and John made gels

~~Transformation (NEB High Eff.)~~ E0044, I0500, R0051, K7500

- [x] Thaw tube of NEB 5-α cells on ice. Mix gently. Pipette 10 µL cells into transformation tube on ice
- [x] Add 2 µL of resuspended distribution kit DNA to cell mixture. Flick 4-5x to mix (No vortex)
- [x] Incubate 30 min on ice
- [x] Heat shock @ 42°C, 30sec
- [x] Place on ice for 5 min
- [x] Pipette 230 µL SOC (RT) into mixture
- [x] Incubate 60 min @ 37°C, 250 rpm. Pre-warm agar plates.
- [x] Mix cells by flicking/inverting
- [x] Spread 20 µL and 200 µL of each transformation onto a plate
- [x] Incubate overnight @ 37°C

-Andy, Taylor, and Michael set up sequencing reactions

-Panya and Elli set up a PCR of the backbones from the Minipreps

Diagnostic PCR ~~(25 samples)~~ (19 samples)

Master Mix:

- [x] ~~23.75 µL~~ ~~26.75~~ 26.2 µL A20
- [x] ~~23.75 µL~~ 26.2 µL A21
- [x] ~~237.5 µL~~ Q5 master mix 261.3 µL
- [x] ~~171 µL~~ NFW 181.1 µL

- [x] Add 24 µL of Master Mix
- [x] 1 µL Sample DNA
- [x] Thermocycle:

35 cycles

98°C 30 sec | 98°C 10 sec | 53°C 30 sec | 72°C ~~40~~ 60 sec | 72°C 120 sec | 4°C ∞

6/4/2015
Time in: 10:00 AM
Time out: 11:30 PM

- Ran gels of PCRs: (Elli, Panya, and Joe)
  - PCR of BB from MPs of J04550
  - Diagnostic PCRs of all parts:

1	- R0040	54 bp
2	- R0011	55
3	- R0062	55
4	- J23119	35
5	- R0010	200
6	- I13453	135
7	- J23100	35
8	- J23101	35
	- R0051	49 } Not being run on gels
	- I0500	1210 }
	- pSB1C3	2070
	- pSB1A3	2155
	- pSB1K3	2204
	- J04550	1069 } Not being run alone on a gel
9	- I20260	918

- Plates from transformation:
  - E0044 and R0051 did NOT grow
  - I0500 and K750000 did grow
  - Our test of untransformed cells on Chlor plates confirms that our Chlor is working

Diluting Primers: (Done by Panya and John)

A5: 17.4 nmol → 174 µL NFW
A6: 19.3 nmol → 193 µL NFW
A7: 15.5 nmol → 155 µL NFW
A8: 27.3 nmol → 273 µL NFW
} To resuspend at 100 µM

To dilute to 10 µM:
- ☑ 10 µL of resuspended 100 µM primer
- ☐ 90 µL NFW

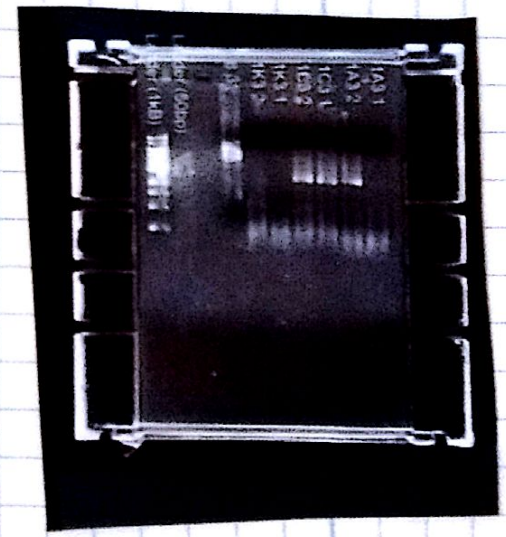
Panya, John, me

ICS+PR+DT
2 A7)

A8)

ICS+PR+DT)

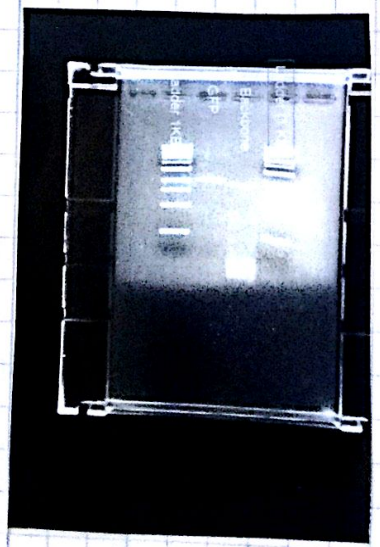
2°C
sec
4°C
∞



PCR Purification (pSB1A3 #2, pSB1C3 #1, #2) - Joe, John, and me

- [x] Add 115 µL of Buffer PB and mix
  - [x] If orange or violet, add 10 µL of 3M sodium acetate
  - [x] Mix
- [x] Place a min elute column into a provided 2mL collection tube
- [x] Apply sample to column; centrifuge for 1 min at 17,900g Discard flow through
- [x] Wash with 750 µL Buffer PE to the column and centrifuge Discard flow through.
- [x] Centrifuge to dry for 1 min
- [x] Place the column in a clean, labelled 1.5 mL tube
- [x] Add 10 µL of Buffer EB. Let stand 1 min, then centrifuge the column for 1 min.

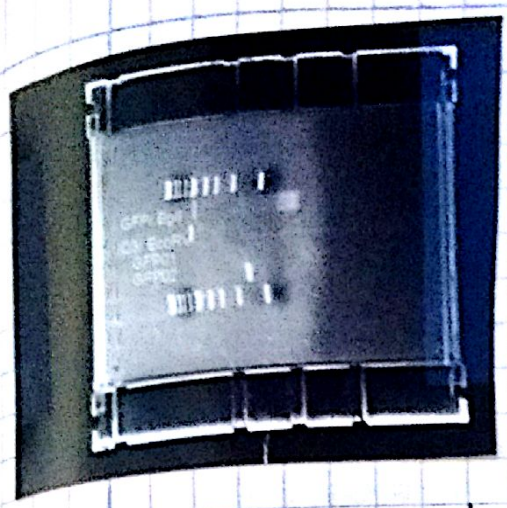
-Elli and Panya are inoculating ~~E0015~~ I0500 and K750000



-Unfortunately, the Gibson PCR of the GFP ~~produced~~ (Source: 2014 MPart ~~2~~ 1 well 26 - GFP from Amp - Part E0040 - Sequence confirmed) produced a band ~2,000 bp long, longer than the expected 720 bp. It also appeared to be very different from the amplified pSB1C3 (see right).

In order to test what's happening, we are:
John - Re-running the Gibson PCR, with both the same template GFP, and a different one (GFP-Mut3)
Joe - Running a restriction digest of the unusual GFP/1C3 PCR ~~product~~ product (BglI for GFP, EcoRV for pSB1C3)
Me - Checking that primers are what we think they are

-Elli and Panya are planning an Outreach project



- John, Elli, and Panya PCR purified:
See above gel → "GFP Amp 2 Pure PCR"
See gel to left → "IC3+PR+DT Pure PCR"

See Panya's/Elli's notebooks for nanodrop values

The results:

→ The lane labeled "GFP/BglI":
We hypothesized that, for some reason, the Gibson PCR amplified the backbone, ~~not~~ pSB1A3, instead of the GFP. If this was the case, the 1A3 would have been cut by BglI to produce fragments of size 1,226 and 929, while a GFP would not have cut at all.

The DNA did not cut, but is still way too ~~that~~ large to be GFP. Since it doesn't appear to be pSB1A3, I don't know what it could be.

→ The lane labeled "IC3/EcoRV":
This is the product of the Gibson PCR meant to amplify the backbone, pSB1C3. It should have been cut ~~to~~ by EcoRV to produce fragments 1,153 and 917 bp long.

Obviously, this was also not cut. Perhaps something went wrong with the digest?

→ This lane should have contained the Gibson PCR from GFP MP #1 (as opposed to GFP #2, which was used for the PCR on pg. 13, and resulted in the strange, large band). Obviously, there is nothing here.

→ The ~~first~~ bottom lane, "GFPO2", was the Gibson PCR product that resulted from repeating exactly the PCR on pg. 13, using GFP MP #2 again.

Mysteriously, it produced a band of the correct size. Although we don't exactly understand what went wrong last time, we ~~are~~ are going to continue by PCR-Purifying this Gibson PCR product and the PCR'd pSB1C3, and then proceeding with the Gibson reaction.

(HiFi DNA Assembly)

Gibson Assembly: GFP into pSB1C3 with promoter, RBS, and DT

GFP:
112 ng/μL
↓
0.23 pmol/μL

pSB1C3:
~26 ng/μL
↓
0.023 pmol/μL

Gibson assembly:
For 1-2 fragments, we want 0.03-~~0.06~~ 0.2 pmols of insert, and half as much vector.

We'll use 0.87 μL of GFP, and 4.35 μL of vector (pSB1C3)

Protocol:

- ☑ 0.87 μL GFP
- ☑ 4.35 μL of pSB1C3
- ☑ 10 μL HiFi Master Mix
- ☑ ~~4.78~~ 4.78 μL H_2O

Total volume: 20 μL

- ☑ Incubate 60 min at 50°C. Use 2 μL for transformation

Dinner: 8:20PM - 9:15PM

Transformation of Gibson Assembly ⟶

Transformation

- [x] Thaw cells on ice
- [x] Add 2µL of the chilled assembly product to the cells. Flick 4-5 times. Do not vortex.
- [x] Place on ice for 30 min. No mix.
- [x] Heat shock at 42°C for 30sec. No mix.
- [x] Add 950µL of RT SOC
- [x] Incubate the tube at 37°C for 60min. Shake at 250rpm.
- [x] Warm selection plates to 37°C
- [x] Spread ~~100µL~~ 20µL and 200µL of the cells onto the selection plates.
- [x] Incubate overnight at 37°C

6/5/2015
Time in: 10:00am
Time out: 5:00pm

Miniprep of Re-Transformations

- [x] Pellet 3mL of bact @ 6800g for 3 min @ RT
- [x] Resuspend pelleted cells in 250µL Buffer P1
- [x] Add 250µL Buffer P2 and invert 4-5x. <5 min
- [x] Add 350µL Buffer N3 and mix immediately by inverting 4-5x.
- [x] Centrifuge for 10 min at 13,000 rpm (~17,900g)
- [x] Apply supernatant to spin col. Centr 30-60s and discard flowthrough
- [x] Wash spin col w/ 500µL Buffer PB. Centr 60s. Discard flowthrough.
- [x] Wash spin col w/ 750µL Buffer PE. Centr 60s. Discard flowthrough.
- [x] Centr for 1 min to dry.
- [x] Elute in 50µL Buffer EB. Let stand 1 min, and centr 1 min.

K750000 MP1	169.66	3.393	1.851	1.83	1.64
K75000 MP2	130.83	2.617	1.396	1.87	1.94
I0500 MP1	95.05	1.901	0.995	1.91	2.95
I0500 MP1	165.96	3.319	1.828	1.82	1.58

Nanodrops of miniprep

Elli and Joe repeating yesterday's Gibson

Lunch: 12:50 – 1:30 PM

- We are going to use DpnI to try to eliminate the remaining template DNA (the transformation created colonies that are extremely red, indicating that ~~the~~ only the template backbone containing a functional RFP was transformed)

Restriction Digest

We are digesting:

- Gibson 1 (yesterday's Gibson assembly that produced red colonies when transformed)
- Gibson 2 (the Gibson just performed by Elli and Joe)
- GPCR'd GFP
- GPCR'd pSB1C3 + PR + DT

} Templates for both previous Gibson rxns

- Negative control: ~~template pSB1C3 + PR + RFP + DT (pSB1C3~~ just transforming the GPCR'd pSB1C3 after it's been DpnI'd with the DpnI.

- It turns out that our primers do Not match the backbone: they do not account for the scars that apparently exist in the [illegible] We (Joe) have placed the order for new, correct

- Team discussion of 2002 Elowitz paper

- Since yesterday, Michael has been diligently working on a program to automatically analyze sequence data. He has made huge amounts of progress, but is still working out bugs.

6/8/2015
I arrived at 10:30 AM
I left at 11:30 AM

- Radiation safety training: 10:00 AM - 12:00 PM
  - Planned the week
- Left for lunch/phage meeting: 1:30-3:00
  - Discussed paper
- Planned Outreach programs (high schoolers visiting tomorrow)
- Panya and Elli contacted local camps/scout troops
- Michael has almost finished his script to automate sequence analysis
- John has been working on models
- Joe has been designing gBlocks for dCas9 parts

6/9/2015
Time in: 10:00 AM
Time out: 11:00 PM

- Gibson primers have arrived! We're going to resuspend them:

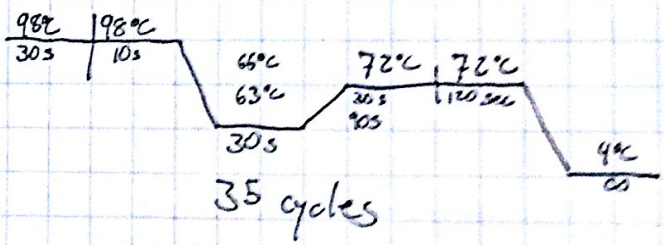
A9: GFP_fwd - 20.1 nmol → 201 µL ? 100 µM
A10: pSB1C3+PR+DT_rev - 33.6 nmol → 336 µL

To dilute to 10 µM:

- ☑ 10 µL of resuspended primer
- ☑ 90 µL NFW

Gibson PCR (Q5 HotStart) - Using new primers!

- ☑ 12.5 µL MM
- ☑ 1.25 µL (A6 or A7) (GFP / pSB1C3+PR+DT)
- ☑ 1.25 µL (A9 or A10)
- ☑ 0.5 µL DNA (GFP Amp #2 or 1C3+PR+DT → pSB1C3 #1 MP)
- ☑ 9.5 µL H_2O



35 cycles

- Panya and Elli are working on the blog
- Michael and Panya are making gels
- Elli is resuspending:

	Part	Plate	Well	Resistance
- E0022	CFP w/ LVA	2	4P	Chlor
- E0032	YFP w/ LVA	2	6B	Chlor

- ☑ Pipette 10 µL H_2O into well; pipette up and down
- ☑ Let sit for 15 min, then put in labelled tubes.

Lunch: 12:00 - 12:15

-Preparing for high schoolers

-Did activities with high schoolers (for outreach):
- Talked about lab safety
- Ran a gel
- Streaked bacteria on plates
- Extracted DNA from bananas
- Imaged a gel

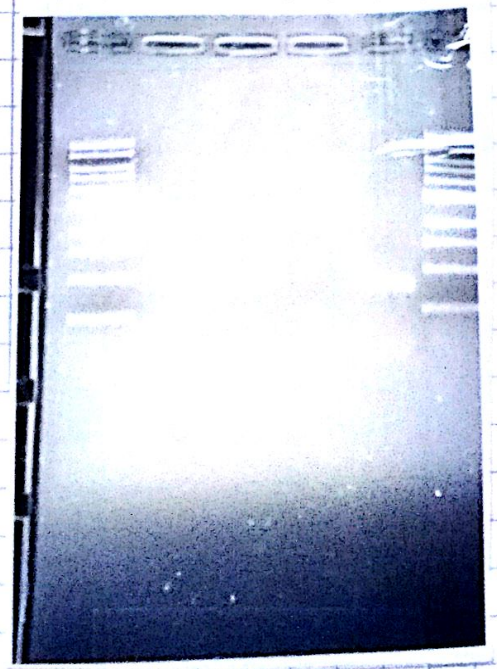
-John and Joe performed a DpnI reaction on the products of the Gibson PCR
↳ They're running the results of the PCR and the PCR+DpnI on a gel

-John and Joe are PCR purifying the DpnI'd Gibson PCR products

-I've been looking into what we need to order to use Lambda red

-Panya and Elli are working on the blog/Facebook page

pSB1C3 +DpnI | GFP +DpnI | pSB1C3 PCR Product | GFP PCR Product



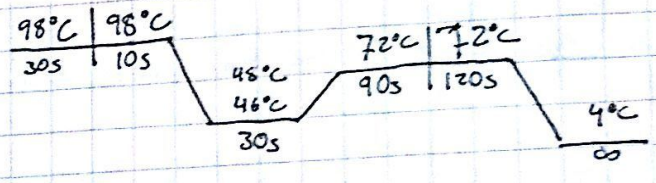
-Although it looks like the Gibson PCR of the GFP and its DpnI worked very well, it seems that the PCR of the backbone did not work.

When we checked IDT's suggested melting temp, we calculated an annealing temp of 46°C, which is very different than the 63°C recommended by NEB's melting temp calculator.

We are repeating the PCR of pSB1C3, this time with annealing temps of 46°C and 48°C.

Gibson PCR (Q5 Hotstart) - Repeat of pSB1C3

- [x] 12.5 µL MM
- [x] 1.25 µL A7
- [x] 1.25 µL A10
- [x] 0.5 µL DNA - pSB1C3 #1 MP
- [x] 9.5 µL H_2O



- Alex Kemper, who will be joining us for most of the summer, came in for a short period of time today. We discussed our project and goals for the summer.

Dinner: 6:50 PM - 8:00 PM

- PCR is finished. Before proceeding with the DpnI, we are running the PCR at on a gel.

Made figures for Phage Presentation

Done by: Joe/John (and me)

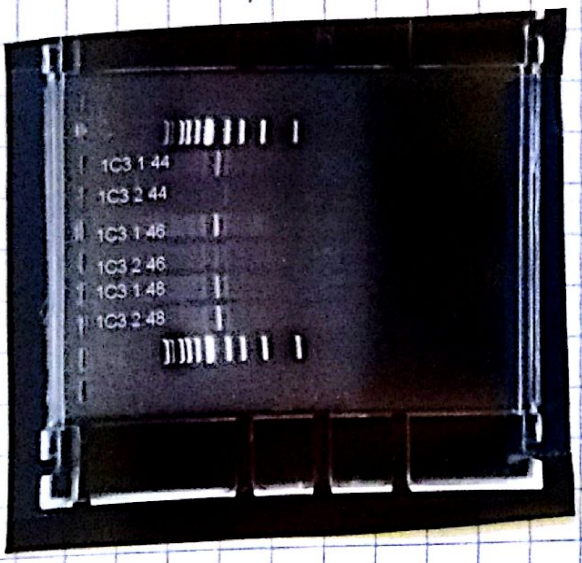
- There was no PCR product, even with the different (and lower) annealing temperatures. We're going to set up PCRs at:
  - 44°C, 46°C, 48°C,
  this time using pSB1C3 MP #1 AND #2.

Andy/Taylor

We're also going to set up a transformation of the resuspended kit parts:
- E0022 - CFP w/ LVA
- E0032 - YFP w/ LVA
- K1323002 - dCas9

- Joe has started running a gel of last night's PCR products

- Andy and I have been looking into what type of microscope has been used in past similar studies

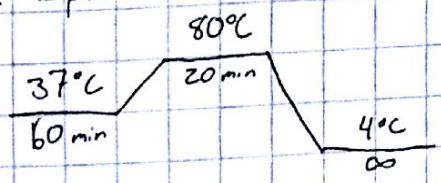


- Gel: The PCR appears to have worked for at least some of the samples, including some for which it had not worked earlier. (1C3 #1: 46 and 48)

It's not entirely clear why there are three bands? It seems that the middle band is probably the actual PCR product. Perhaps the rightmost band is the circular template (though that should be ~700 bp larger than the PCR product...)?

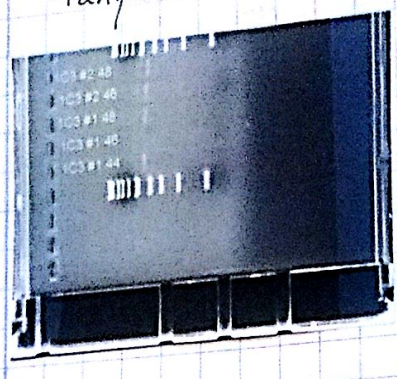
DpnI reaction - 1C3 #1 44, 1C3 #1 46, 1C3 #2 46, 1C3 #1 48, 1C3 #2 48

- ☑ 2 µL CutSmart 10x
- ☑ 5 µL PCR product
- ☑ 12 µL NFW
- ☑ 1 µL DpnI



Brian Talk: 12:30 - 2:15 PM

- Panya and Elli ran a gel of the DpnI'd PCR products:



- It appears that most of the samples worked! We will proceed with the PCR Purification.

Joe and I → PCR Purification - 1C3 #1 44, 1C3 #1 46, 1C3 #1 48, 1C3 #2 46, 1C3 #2 48

- [x] Add 95 µL of Buffer PB and mix
  - [ ] If orange or violet, add 10µL of 3M sodium acetate
  - [ ] Mix
- [x] Place a min elute column into collection tube
- [x] Apply sample to the column. Centrifuge for 1 min at 17,900g
  - [x] Discard flow through
- [x] Wash with 750 µL Buffer PE.
  - [x] Centrifuge, then discard supernatant.
- [x] Centrifuge to dry for 1 min
- [x] Place the column in a clean, labeled 1.5mL tube
- [x] Add 10µL of Buffer EB. Let stand 1 min, then centrifuge the column for 1 min.

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
1C3 #1 44	Default	6/10/2015	3:16 PM	12.09	0.242	0.134	1.81	1.61
1C3 #1 46	Default	6/10/2015	3:17 PM	25.94	0.519	0.304	1.71	1.54
1C3 #1 48 *	Default	6/10/2015	3:18 PM	23.18	0.464	0.262	1.77	1.98
1C3 #2 46	Default	6/10/2015	3:19 PM	35.07	0.701	0.437	1.61	0.92
1C3 #2 48 *	Default	6/10/2015	3:20 PM	21.80	0.436	0.226	1.93	2.02

↳ Nano drops from PCR Purification

Nanodrop from PCR Purification of yesterday's Gibson PCR + DpnI'd GFP

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
GFP AMP2 DPN1 Pure	Default	6/10/2015	3:37 PM	38.81	0.776	0.425	1.83	1.78

Gibson Assembly Using IC3 #1 45°C and IC3 #2 45°C (both w/ GFP AMP #2)

IC3 #1: 23.18 ng/μL → 0.014657 pmol/μL

IC3 #2: 21.80 ng/μL → 0.01380 pmol/μL

GFP: 38.81 ng/μL → 0.08293 pmol/μL

The formula to calculate pmol/μL: (ng/μL from nanodrop) × 1,000 / (base pairs × 650 daltons)

0.1 pmol of GFP = 1.205 μL

0.05 pmol of vector: 3.41 μL (IC3 #1)

3.62 μL (IC3 #2)

Actual Volumes

GFP: 1.205 μL

IC3: 3.41 μL of #1

3.62 μL of #2

H_2O: 5.385 μL for #1

5.175 μL for #2

Protocol:

Done by Joe and John

- ☐ 1.205 μL GFP
- ☐ 3.41 μL of IC3 #1
  - OR
  - 3.62 μL of IC3 #2
- ☐ 10 μL Hi-fi Master Mix
- ☐ 5.385 μL of H_2O for #1
  - OR
  - 5.175 μL of H_2O for #2
- ☐ Incubate in thermocycler at 50°C for 15-60 min.

Transformation of Gibson Assembly (and K1323002)

- [x] Thaw cells on ice
- [x] Add 2µL of chilled assembly product to the cells. Flick 4-5 times. Do Not vortex
- [x] Place on ice for 30 min. No Mix.
- [x] Heat shock at 42°C for 30 sec. No mix
- [x] Add 250 µL of RT SOC
- [x] Incubate the tube at 37°C for 60min. Shake at 250 rpm.
- [x] Warm selection plates to 37°C.
- [x] Spread 20µL and 200µL of the cells onto the selection plates
- [x] Incubate overnight at 37°C

6/11/2015
Time in: 10:00AM
Time out: 7:30PM

- We're going to miniprep the innoculations of CFP (E0022) w/ LVA and YFP (E0032) w/LVA
  - Panya and Michael and Joe
- Once primers arrive, we can dilute them:

A11	-pSB1C3_rev_GFPRFP_Gerald: 18.7 nmol → 187 µL	To create a G² plasmid (PR-GFP-DT)+(PR-RFP-DT) into
A12	-pSB1C3_fwd_GFPRFP_Gerald: 25.3 nmol → 253 µL	
A13	-Functional_GFP_fwd: 26.4 nmol → 264 µL	
A14	-Functional_GFP_rev: 18.5 nmol → 185 µL	
A15	-Functional_RFP_fwd: 26.5 nmol → 265 µL	
A16	-Functional_RFP_rev: 20.1 nmol → 201 µL	

More primers:

A17 - CFP_fwd: 16.4 nmol → 164 µL

A18 - CFP_rev: 21 nmol → 210 µL

A19 - DT+PR_fwd: 18.3 nmol → 183 µL

A20 - DT+PR_rev: 21.4 nmol → 214 µL

A21 - YFP_fwd: 18.8 nmol → 188 µL

A22 - YFP_rev → 19.9 nmol → 199 µL

} To create a G^2 with CFP and YFP (3 fragment Gibson)

PR + (YFP) + (DT + PR) + (RFP) + DT

pSB1C3

A23 - pSB1C3_w_PR+DT_rev: 22.2 nmol → 222 µL

A24 - YFP_fwd_into_functional_1C3: 17.7 nmol → 177 µL

A25 - YFP_rev_into_functional_1C3: 20.6 nmol → 206 µL

} To put YFP into functional unit

PR + (YFP) + DT

pSB1C3

A26 - CFP_fwd_into_functional_1C3: 17 nmol → 170 µL

A27 - CFP_rev_into_functional_1C3: 24 nmol → 240 µL

} To put CFP into functional unit:

PR + (CFP) + DT

pSB1C3

- Elli has resuspended primers and diluted primers

- To dilute 100 µM to 10 µM:

  ☑ 10 µL of stock 100 µM primer

  ☑ 90 µL sdd

- John has been doing math

- Panya has logged all of these primers into the database

Things to PCR/Gibson Assemble:

1) G² with GFP and RFP:

- Parts to combine:

  - Functional GFP
    → A13 and A14 (Colony PCR)
      ↳ Annealing temp:

  - Functional RFP
    → A15 and A16
      ↳ Annealing temp:

  - - Backbone
    → A11 and A12
      ↳ Annealing temp:

2) YFP/CFP into Functional Unit

- Parts to combine:

  - YFP/CFP
    → YFP: A24 and A25
      ↳ Annealing temp:
    → CFP: A26 and A27
      ↳ Annealing temp:

  - Backbone
    →

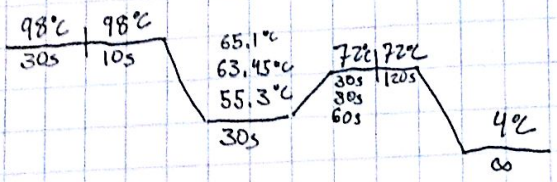
- Backbone
  →

ortunately, IDT failed to send us pSB1C3_w_PR+OT_fwd
ns that we CANNOT do Gibson #s 2 or 3

- Although we have similar primers to amplify the backbone +PR
  they do not include the scar between the insert and the RE
  which is also in the overhangs for ins
  CFP/YFP into this vector

Gibson PCR (Q5 HotStart)

	GFP	RFP (Diluted: 0.965ng/µL)	pSB1A3 (Diluted: 0.96ng/µL)
☑ 12.5 µL Master Mix			
☑ 2.5 µL Primer 1	A13	A15	A11
☑ 2.5 µL Primer 2	A14	A16	A12
☑ 0.5 µL DNA	Colony	pSB1C3 #1MP	pSB1A3 #1MP
☑ 7.0 µL sdd			



* Pro Tip from Brian: For Gibson PCR
  - Dilute MPs to use ∠1 ng of DNA
  - Only use 25 cycles
  - Double the amount of primer

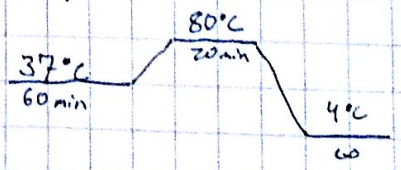
DpnI Reaction GPCR of: Funct. GFP, Funct. RFP, pSB1A3

☑ 0.5 µL DpnI

☑ 2µL CutSmart 10x

☑ 5 µL PCR product

☑ 12.5 µL H_2O

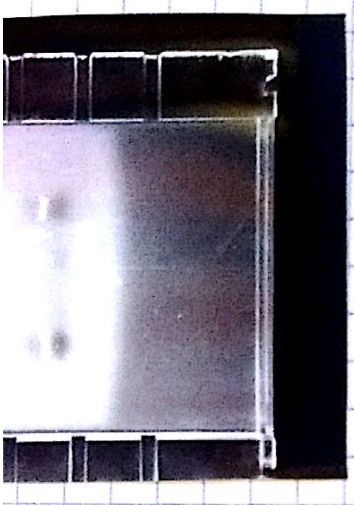


- Everyone has been doing the required CITI training

drops from earlier MP:

	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
	Default	6/11/2015	12:01 PM	19.11	0.382	0.196	1.95	1.87
	Default	6/11/2015	12:02 PM	32.06	0.641	0.354	1.81	0.80
	Default	6/11/2015	12:03 PM	51.67	1.033	0.563	1.84	1.41
	Default	6/11/2015	12:04 PM	48.37	0.967	0.504	1.92	1.89

022: CFP w/ LVA
032: YFP w/ LVA



Gel of Gibson PCRs:

- The colony PCRs of the GFP are both different in size and not the appropriate length (~1,000 bp)
- You can ~~see~~ see the primers from the the RFP PCR, but there is no sign of any amplified RFD
- There is no sign of any DNA in the lane to amplify backbone - not even any primers.

set of PCRs, we followed all of Brian's Tips. Tomorrow, we'll epeat the PCRs, one set normally, and one with Brian's advice. (Both from ead of Colony PCRs.)

ere is plenty of growth on ~~X~~ our transformations of last ibson Assembly of a Functional GFP. Most of the colonies d, even though we treated the PCR products with Dpn I, we thought, would get rid of most of the template DNA. ! There are a few colonies that appear vaguely The Gibson worked at least a little bit!

6/12/2015
Time in 10:00 AM
Time out 10:00PM

- John and Joe are miniprepping yesterday's innoculations of the functional GFP made from the Gibson Assembly

- Andy and Panya are setting up Gibson PCRs to amplify:
  - CFP
  - YFP
  - pSB1A3 + PR + DT
  } w/ overhangs to bind to the functional backbone

- Today, the primer "AZ8 pSB1C3_w-PR+DT_fwd" finally arrived!

PCRs/Gibsons to Do:

Done by: Me and John 150612

→ G² w/ Functional GFP + Functional RFP:
- A11 + A12 + pSB1A3: 67.5°C (NEB) OR 55.3°C (IDT)
- A13 + A14 + Funct. GFP: 63.0°C (NEB) OR 65.1°C (IDT)
- A15 + A16 + Funct. RFP: 63.0°C (NEB) OR 63.45°C (IDT)

Done by: Panya and Andy 150612

→ YFP into Functional Unit:
- AZ3 + AZ8 + pSB1A3: 62.5°C (NEB) OR 53.75°C (IDT)
- AZ4 + AZ5 + YFP: 63.8°C (NEB) OR 60.85°C (IDT)

→ CFP into Functional Unit:
- AZ3 + AZ8 + pSB1A3: 62.5°C (NEB) OR 53.75°C (IDT)
- AZ6 + AZ7 + CFP: 63.8°C (CFP) OR 60.95°C (IDT)

→ G² w/ Funct. CFP + Funct. YFP:
- A11 + A12 + pSB1C3/pSB1K3: 67.5°C (NEB)
-
-

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
funct. GFP 1 MP # 1	Default	6/12/2015	11:55 AM	176.53	3.531	1.914	1.84	2.31
funct. GFP 1 MP #2	Default	6/12/2015	11:56 AM	184.06	3.681	1.991	1.85	2.32
funct. GFP 2 MP # 1	Default	6/12/2015	11:57 AM	439.54	8.791	4.751	1.85	2.27
funct. GFP 2 MP # 2	Default	6/12/2015	11:58 AM	53.88	1.078	0.581	1.85	2.19

↑ Results from John's and Joe's MP of Gibson assembled Funct. GFP

R (Q5 HotStart)	(control IA3 pSD143 MP#2)	(GFP Assembled GFP MP#1)	(pJR103 #1 MP RFP)
	IA3	GFP	RFP
L MM	✓	✓	✓
	✓A11	✓A13	✓A15
	✓A12	✓A14	✓A16
Template	✓	✓	✓
H_2O	✓	✓	✓
Temp:	✓55.3°C	✓65.1°C	✓63.45°C

MM	✓	✓	✓
	✓A11	✓A13	✓A15
	✓A12	✓A14	✓A16

Diluting More 1kb Ladder:

- ☑ 100µL stock ladder
- ☑ 900µL Buffer EB (10 mM TrisCl)
- ☑ 200µL 6x loading dye

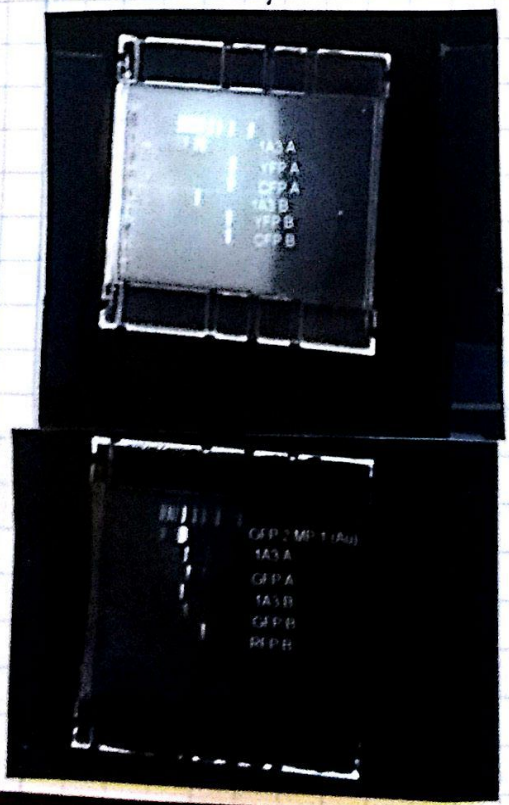
DpnI

- ☑ Making a Master Mix:
  - ☑ 6.5 µL DpnI
  - ☑ 26 µL CutSmart 10x
  - ☑ ~~77.5 µL~~ 97.5 µL H_2O

Andy

- ☑ Add:
  - ☑ 10µL Master Mix
  - ☑ 10µL PCR Products

For gels:
A: "IDT method"
B: "Brian's method"



The first gel, containing the parts to make Functional GFP and Functional YFP, appears correct.
1A3: 2518 bp (has PR+DT)
YFP: ~~762 bp~~ 762 bp
CFP: 762 bp

We used the Brian PCR products in the DpnI

The second gel does not look good.
1A3: 2155 bp (no PR+DT)
GFP: 1083 bp (w/ PR+DT)
RFP: 1069 bp (w/ PR+DT)

purifying the DpnI'd
(w/ PE2 + DT)

action YFP and Functional CFP

YFP B	CFP B
57.54 ng/μL	53.45 ng/μL
↓	↓
0.11 pmol/μL	0.11 pmol/μL

= 1 μL

= 2.5 μL

FP

Transformation:

- [x] Thaw cells on ice
- [x] Add 2μL of chilled assembly product to cells. Flick 4-5 times. Do NOT vortex.
- [x] Place on ice for 30 min. No mix.
- [x] Heat shock at 42°C for 30sec. No mix.
- [x] Add 450 μL of RT SOC
- [x] Incubate the tube at 37°C for 60 min. Shake at 250 rpm.
- [x] Warm selection plates to 37°C
- [x] Spread 20μL and 200μL of the cells onto the selection plates.
- [x] Incubate overnight at 37°C.

6/13/2015
Time in: 1:00PM
Time out: 7:15 PM

- All of our plates have growth, though we can't tell if the colonies are red yet. Andy is going to image some cells on the confocal to see if they are fluorescing the correct color (blue or yellow).

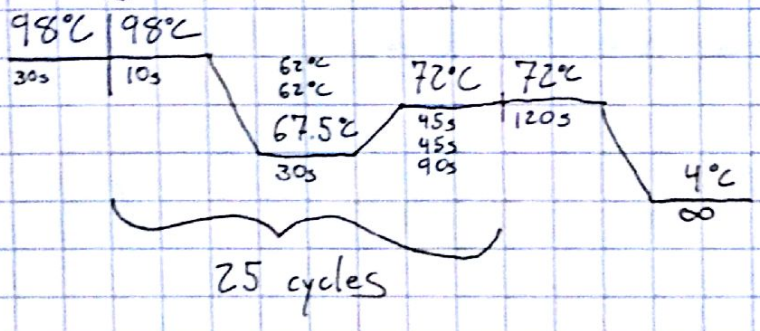
- Andy was unable to see any cells with the confocal. We're going to give them a few more hours to grow.

Lunch: 1:30-5:00 PM

- The plates have a few red colonies, but the vast majority are not red! We are doing colony GPCRs to set up to assemble a G2 with CFP and YFP, and innoculating.

Gibson PCR - Q5 Hot Start, Brian's Method

	Funct. CFP	Funct YFP	MP1, #20 pSB1K3
☑ 12.5 µL Master Mix			
☑ 2.5µL Primer 1	A13 ✓	A15	A11
☑ 2.5 µL Primer 2	A14	A16	A12
☑ 1 colony / ~~1.0~~ µL DNA	Colony	Colony	1:100 dilution, pSB1K3 #1
☑ 7.5µL / ~~6.5~~ µL sdd	7.5µL	7.5µL	~~6.5~~ µL
Annealing Temp:	62°C	62°C	67.5°C



PCR Started: 7:10 PM

-Andy will come back in a few hours to set up a DpnI reaction.

-John and Michael miniprepped yesterday's innoculations of functional CFP and YFP

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
k5100000 MP1	Default	6/14/2015	12:09 PM	311.84	6.237	3.318	1.88	2.57
k5100000 MP2	Default	6/14/2015	12:11 PM	382.31	7.646	4.158	1.84	2.06
k510012 MP1	Default	6/14/2015	12:14 PM	383.36	7.667	4.139	1.85	2.23
k510012 MP2	Default	6/14/2015	12:15 PM	337.11	6.742	3.648	1.85	2.13
func YFP for G2 MP1	Default	6/14/2015	12:18 PM	103.23	2.065	1.101	1.88	2.71
func YFP for G2 MP2	Default	6/14/2015	12:19 PM	100.04	2.001	1.080	1.85	2.27
func CFP for G2 MP1	Default	6/14/2015	12:22 PM	139.68	2.794	1.559	1.79	1.47
func CFP for G2 MP2	Default	6/14/2015	12:23 PM	111.04	2.221	1.221	1.82	2.19

K5100000: pUC18Sfi-miniTn7BB-Gm
K510012: pUC18R6KT-miniTn7BB-Gm

Name	ng/µL	260/280
fCFP QuiT Rep 1	39.76	1.83
fCFP QuiT Rep 2	44.09	1.70
fYFP QuiT Rep 1	55.93	1.71
fYFP QuiT Rep 2	49.67	1.78
IKS QuiT Rep 1	37.31	1.79
IKS QuiT Rep 2	46.99	1.68

Gibson Assembly - G² from Funch CFP and Funch YFP into pSB1K3

FCFP #1	FYFP #2	pSB1K3 #1
39.76 ng/μL	49.67 ng/μL	37.31 ng/μL
↓	↓	↓
0.054 pmol/μL	0.057 pmol/μL	0.023 pmol/μL

- 0.92 μL FCFP #1
- 0.75 μL FYFP #2
- 2.00 μL 1K3 #1

Protocol:

- [x] 0.92 μL FCFP
- [x] 0.75 μL FYFP
- [x] 2.00 μL 1K3
- [ ] 10 μL Hi Fi MasterMix
- [x] 6.63 μL H_2O
- [x] Incubate in thermocycler at 50°C for ~~45~~ 35 min

- Joe is looking into how to design chimeric RNAs for dCas9 repression
- Elli is looking up what parts we will need for the Interlab Study
- Panya is setting up sequencing reactions

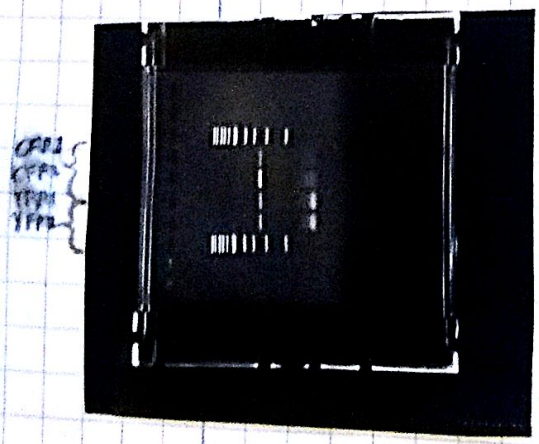
Parts to Resuspend for Interlab Study:

- I13504 ~~P1, W208, Chlor~~ P9, W218, Amp
- J23106 P1, W22A, Chlor
- J23117 P1, W22K, Chlor

} Elli and Panya

Transformation: F. CFP + F. YFP: G^2 Assembly; Resuspended from kit: I13504, J23106, J23117

- [x] Thaw cells on ice
- [x] Add 2 µL of assembly product, or 2 µL of resuspended DNA to the mixture. Flick 4-5x to mix. No vortex.
- [x] Incubate 30min on ice. Set heat block.
- [x] Heat shock @ 42°C for 30sec.
- [x] Place on ice for 5 min.
- [x] Pipette 240 µL of RT SOC into mixture
- [ ] Incubate 60 min at 37°C, 250 rpm.
  - [x] Pre-warm plates
- [x] Mix cells by flicking/inverting
- [x] Spread 20µL and 200µL of each transformation onto a plate
- [x] Incubate over night at 37°C



- We ran a gel of the PCR-Purified GPCR products. Unfortunately, it looks like the purification did NOT remove the shadow bands (probably from the primers)
- John and Elli designed primers for the Interlab study
- Elli and I worked on Gibson sheets

6/15/2015
Time in: 9:3
Time out: 8:0

- All of our plates from last night's transformations have grown!!

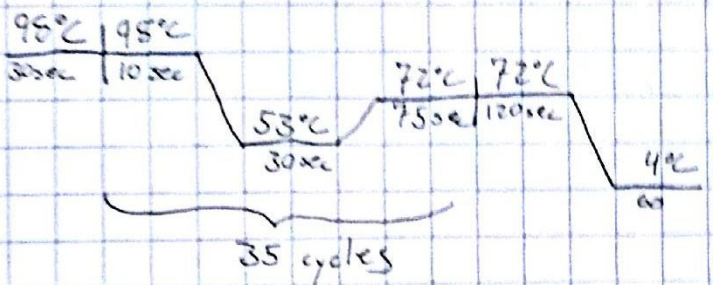
Sabs lab meeting 10:00 - 10:55 AM

- Also all of last night's innoculations have grown!

- Joe is setting up for innoculations from the transformations

Colony PCR of G² transformation

- ☑ 12.5 µL Master Mix
- ☑ ~~2.5~~ 1.25 µL A20 (VF2)
- ☑ ~~2.5~~ 1.25 µL A21 (VR)
- ☑ 1 colony
- ☑ ~~7.5~~ 9.5 µL H_2O



- Michael miniprepped yesterday's innoculations; Joe made glycerol stocks

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
K1026000 MP1	Default	6/15/2015	12:25 PM	80.03	1.601	0.836	1.92	3.59
K1026000 MP2	Default	6/15/2015	12:25 PM	129.36	2.587	1.333	1.94	2.85

- The colony PCRs that we set up are diagnostic, so we will run those on a gel when they're done. Alex Kemper who joined us today, and I also set up innoculations of the transformations of G², I13504, J23106, and J23117.

G² with F. CFP + F. YFP: 2270 bp

6/16/2015

Need to Order as IDT Ultramers:
- JZ3101_Overlap-Backbone-fwd
- JZ3106_Overlap-Backbone-fwd
- JZ3117_Overlap-Backbone-~~rev~~ fwd

Lunch: 1:30 PM - 3:00 PM

- The PCR's are done! Alex and I ran a gel:
  - Ladder
  - G^2 #1 — Run this way →
  - G^2 #2
  - ⋮
  - G^2 #8

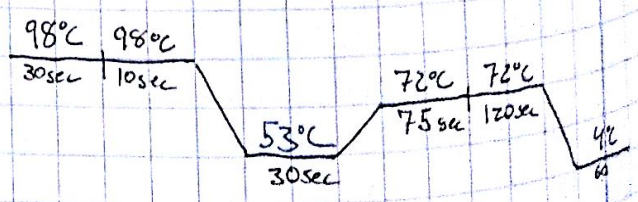
Gibson PCR - Re-PCRing F.GFP and F.RFP

Done by: Joe and Elli

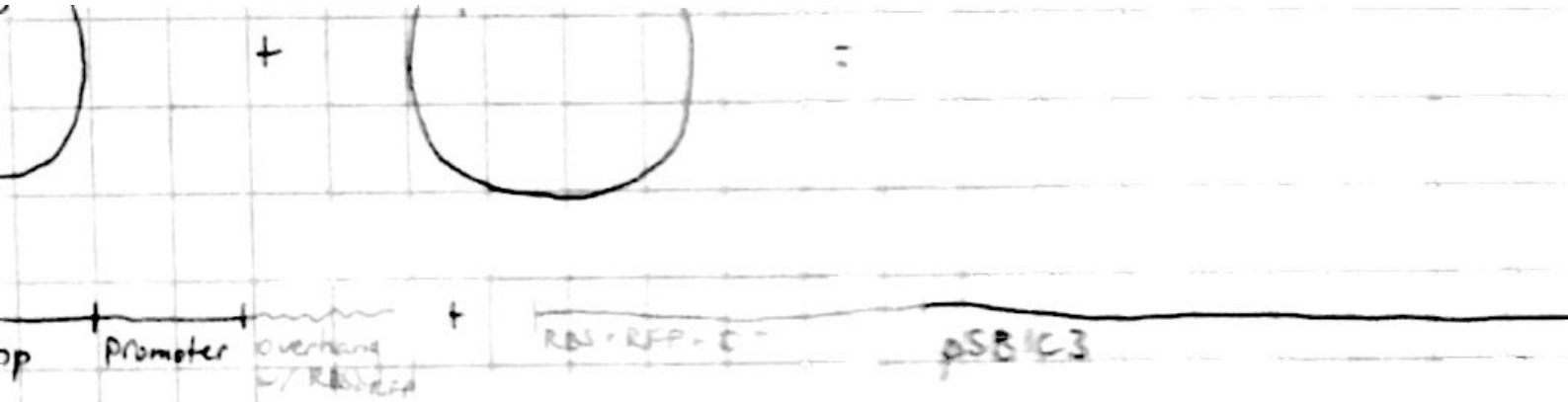
	GFP-2MP#1 Funct. GFP	pSB1K3 #1 MP Funct. RFP
☐ 12.5 µL MasterMix		
☐ 2.5 µL Primer 1	A13	A15
☐ 2.5 µL Primer 2	A14	A16
☐ ~~0.5~~ 1.0 µL DNA		
☐ 6.5 µL H_2O		
Annealing Temp:	62°C	62°C

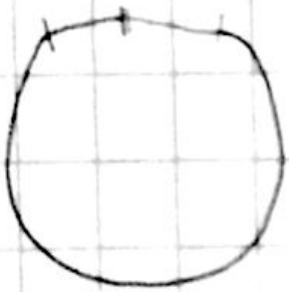
- There was no PCR product on the gel, which we ran twice. We're going to repeat the PCR/innoculation with 3 different colonies.

Colony PCRs
- ☑ 12.5 µL MasterMix
- ☑ 2.5 µL A20
- ☑ 2.5 µL A21
- ☑ 1 colony
- ☑ 7.5 µL H_2O



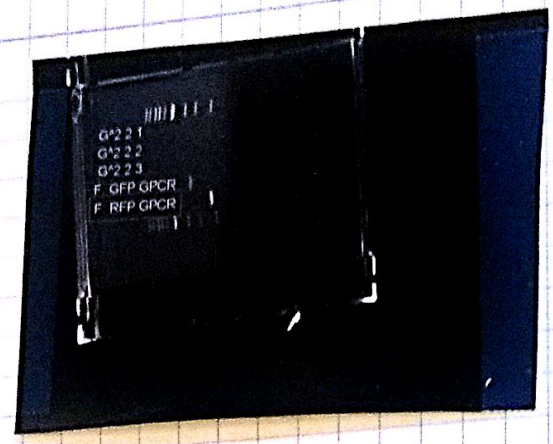
- Journal club





hould already ~~too~~ have primer #3 (see above), and ~~if~~ pri
hould only need versions for Chlor and Amp. Also primer #
be the same for RFB and for GFB

s have been ordered!



The PCR is pretty dismal. The Colony PCRs of G^2 have yielded nothing, which is pretty confusing as we should see something amplified in the PCR.

The GPCR of Funct. GFP is still not working, even after re-diluting the primers, and after (we think) PCR for CFP.

Panya { - We're going to MP one of the G^2 innoculations, just to check if perhaps the colony PCRs/primers are not amplifying correctly.

Elli { We're also going to try to use these same primers to PCR up a sample that we know works - JC11150 on pSB1K3.

Restriction Digest of the 4 G^2 MPs

- ☐ 2 µL CutSmart (10x)
- ☐ EcoRI
- ☐ PstI
- ☐
- ☐

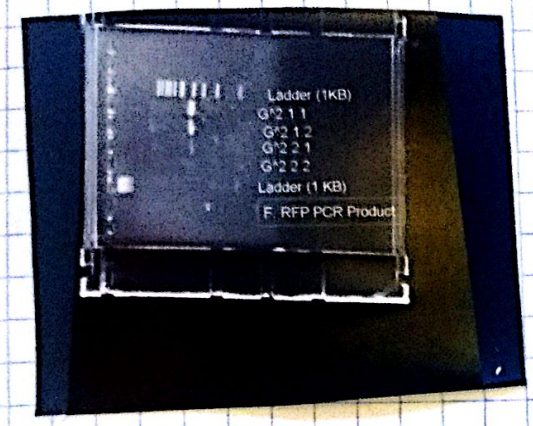
} Not done

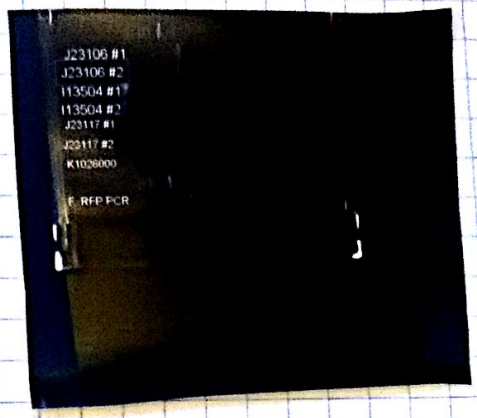
Minipreps by Panya

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
G^2 1.1	Default	6/16/2015	3:44 PM	122.64	2.453	1.340	1.83	1.83
G^2 1.2	Default	6/16/2015	3:45 PM	211.44	4.229	2.463	1.72	1.03
G^2 2.1	Default	6/16/2015	3:46 PM	40.69	0.814	0.452	1.80	1.79
G^2 2.2	Default	6/16/2015	3:46 PM	87.69	1.754	0.995	1.76	1.10
J23117 #1	Default	6/16/2015	3:47 PM	68.17	1.363	0.741	1.84	1.73
J23117 #2	Default	6/16/2015	3:48 PM	50.40	1.008	0.555	1.82	1.31
J23106 #1	Default	6/16/2015	3:48 PM	94.94	1.899	1.029	1.85	1.48
J23106 #2	Default	6/16/2015	3:49 PM	76.61	1.532	0.822	1.86	1.46
J13504 #1	Default	6/16/2015	3:50 PM	167.15	3.343	1.797	1.86	1.99
J13504 #2	Default	6/16/2015	3:51 PM	105.55	2.111	1.136	1.86	1.64
K1026000	Default	6/16/2015	3:51 PM	71.20	1.424	0.739	1.93	1.32

- Panya is re-transforming promoters from kit that didn't work

Gels





- G^2: 2268 bp + 2204 bp
- F. RFP PCR: 1069 bp

- J23106: 35 bp +
- J23117: 35 bp +
- ~~I11~~ I13504: 875 bp
- K1026000: 4311 bp

- Dinner: 7:30 PM - 9:00 PM

- iGEM team meeting

6/17/2015
- in: 10:00 AM
- out: 1:25 AM

Restriction Digest of G^2 MS

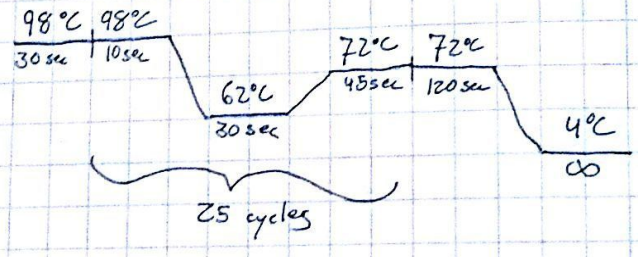
- ☑ 2 µL CutSmart (10x)
- ☑ 0.5 µL EcoRI
- ☑ Add 5 µL (1.1/1.2) or 10 µL (2.1/2.2) of minipreps
- ☑ 12.5 µL / 7.5 µL sdd

37°C, 60 min → 80°C, 20 min → 4°C, ∞

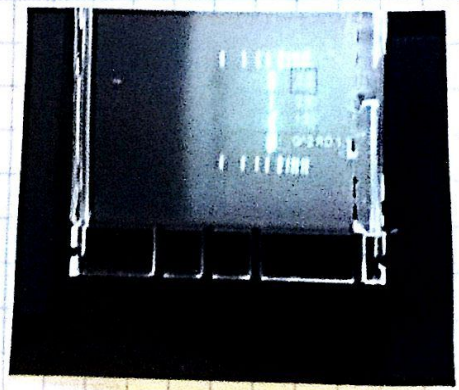
-Today, we should be receiving many primers from IDT. This should allow us to begin ~~check~~ some of the necessary PCRs.

Gibson PCR - F.RFP and F.GFP (we're going to switch the orders)

	pSB1C3 #1 MP F. RFP	F. GFP
☑ 12.5 µL Master Mix		
☑ 2.5 µL Primer 1	A13	A15
☑ 2.5 µL Primer 2	A14	A16
☑ 1.0 µL DNA		
☑ 6.5 µL H_2O		
Annealing Temp:	62°C	62°C



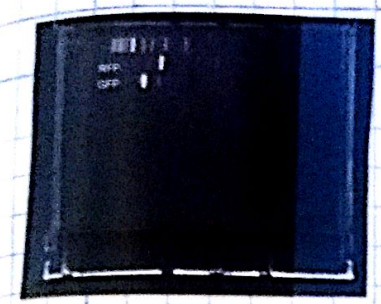
Lunch: ~~11:45~~ 12:55 - 1:15



These are the restriction digests of yesterday's MPs of our assembled G^2 with GFP and RFP. They should be ~4,000 bp (part + backbone), but are obviously closer to 2,000.

The G^2 assembly obviously did not work

The sequence data for the functional GFP that we've been using appears (for the most part) correct, EXCEPT for the DT, where the primers bind, which could explain why it's not PCR-ing correctly.



} Switched primers GAR products of F. RFP and F. GFP

The F. RFP with overhangs appears to have worked (as always). Although the F. GFP mostly is at the wrong size, there is a faint band at the correct size. If we wanted to, we could probably gel purify out this band. However, in light of the fact that the DT is wrong on the F. GFP, I'm hesitant to continue with the part.

- Also, the sequences for F. CFP and F. YFP appear to be correct, which unfortunately does not explain why the Gibson Assembly of G^2 failed.

→ Joe is going to try to remake F. GFP, this time with Brian's method.

→ Panya and Elli resuspended primers, and are going to begin the GPCR to make the parts for the Interlab Study.

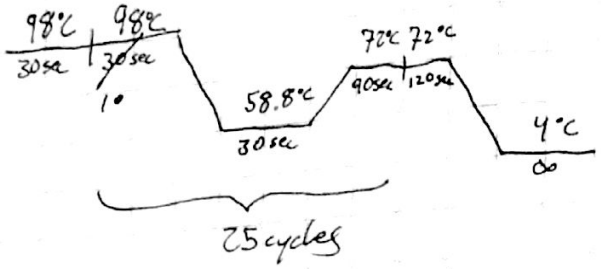
Gibson PCR - For Interlab Study I13504#1, pSB1C3 #1

	J23101		J23106		J23117	
	I13	pSB1C3	I13	pSB1C3	I13	pSB1C3
☑ 12.5 µL MM	✓	✓	✓	✓	✓	✓
☑ 2.5 µL Primer 1	A30	A29	A30	A29	A30	A29
☑ 2.5 µL Primer 2	A31	A32	A33	A34	A35	A36
☑ ~~1.0~~ µL DNA	✓	✓	✓	✓	✓	✓
☑ 6.5 µL H_2O	✓	✓	✓	✓	✓	✓
Annealing Temp	59.7	67.5	59.7	67.5	59.7	67.5
	30 sec	45 sec				

- John has been doing math all day.

Gibson PCR - Functional pSB1C3

- [x] 12.5 µL Master Mix
- [x] 2.5 µL A7
- [x] 2.5 µL A10
- [x] 1.0 µL of 1:100 diluted pSB1C3 MP #1
- [x] 6.5 µL H_2O



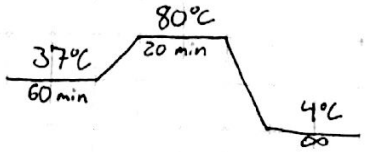
Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
1C3_for_dCas9	Default	6/17/2015	7:54 PM	24.60	0.492	0.281	1.75	1.68
1A3_for_dCas9	Default	6/17/2015	7:55 PM	19.46	0.389	0.216	1.80	1.54
GFP_AMP_2	Default	6/17/2015	7:56 PM	36.65	0.733	0.416	1.76	1.37

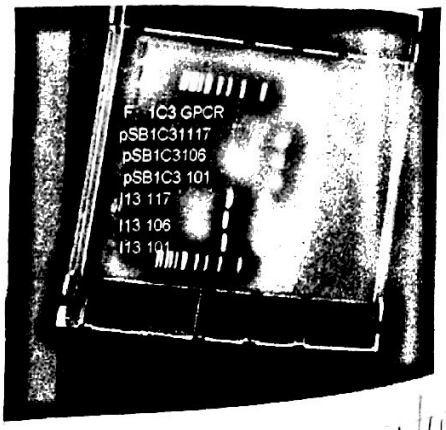
Assemblies to happen tonight

- GFP into functional pSB1C3
- dCas9 into 1C3 and 1A3
- Interlab Measurement Parts

DpnI - Functional pSB1C3, InterLab Measurement Parts

- [x] 2 µL CutSmart (10x)
- [x] 10 µL PCR product
- [x] 0.5 µL DpnI
- [x] 7.5 µL NFW





- Obviously, the gel doesn't look good. Without the backbones for the InterLab parts, and the Functional backbone for GFP, we can't proceed with those Gibson Assemblies

Andy and Michael are proceeding w/ the Gibson to put dCas9 into pSB1C3 and pSB1A3

Gibson PCR - Not Brian's method

	J23101	J23106	J23117	pSB1C3
12.5 µL Master Mix				
1.25 µL primer 1	A29	A29	A29	A29 → A17
1.25 µL primer 2	A32	A34	A36	A10
0.5 µL DNA				
9.5 µL H_2O				

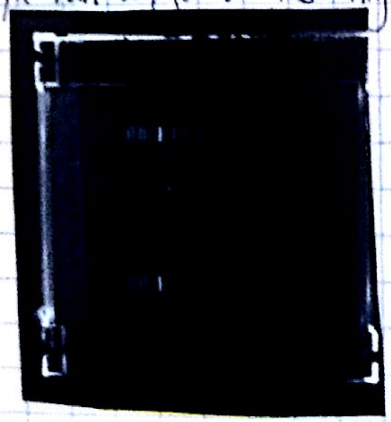
Annealing temps: 57.5°C/62.5°C for InterLab, 52°C for Funct. pSB1C3

8/8/08
Start: 10:00 AM
End: 5:30 PM

Confocal training

- Inverted scope: coverslip down
- Putting slide in place: move black things
- To switch stage (for well plates), use knob on back to loosen/move back, then unscrew 3 hex screws, then pull out, and put in new stage
- For finding sample w/ transmitted light, light must be ON for 15 min, then off for 15 min → to protect light bulbs
- Top of joystick = can switch from course to fine; control x/y location
  - Side of joystick-device = move in z direction
- Tiny Nikon display shows z location... will ALWAYS need to move up
- Kohler (sp?) illumination: to evenly distribute light
  - ~~On Move~~ Put your sample into focus, then use the "condenser" (black knobs on side) to show the ~~and~~ sides of polygon into your view more sharply
    - close field stop → black pancake knob under top white part
- Scan head controls where laser is
- On software:
  - Window "T: Pad" → Scanning needs to be in L, eyepiece = E
  - → Light turned off, and I was unable to take more notes

- Panya ran a gel of last night's backbone PCRs:



3 Functional pSB1C3 GPCR

117	103	57.5°C
106	103	57.5°C
101	103	57.5°C
117	106	62.5°C
103	117	62.5°C
103	101	62.5°C

- Most of the PCRs appear to have at least sort of worked. We're going to try the InterLab PCRs again at 60°C, since there were extra bands at 57.5°C, but the 62.5°C was very faint.

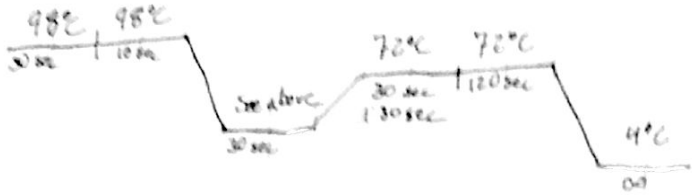
Joe and Alex set up the PCRs to put K182000 into pSB1A3

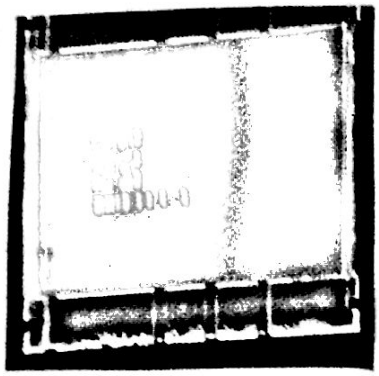
Gibson PCR

- [ ] 12.5 µL Master Mix
- [ ] 1.25 µL primer 1
- [ ] 1.25 µL primer 2
- [ ] 0.5 µL DMSO
- [ ] 9.5 µL H_2O

Annealing Temps

I13453	J23119	R0010	R0040	
A95	A41	A48	A41	A41
A96	A47	A49	A50	A51
1348 MP	2 MP	1 MP	111 MP	111 MP
61.8°C (1)	59.3° (2) (USED)	61.4°C (3) (USED)	59.3° (4) (USED)	59.3° (5) (USED)
	57°C (2)	60.95°C (3)	52.3°C (4)	51.7°C (5)



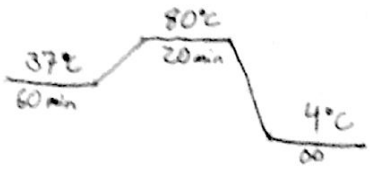


Panya repeated the PCR for the pSB1C3 for the ILM parts (InterLab Measurement parts), this time at 60°C. These clearly are the best results of the PCR, so we will be using these in the Gibsons of the ILM parts.

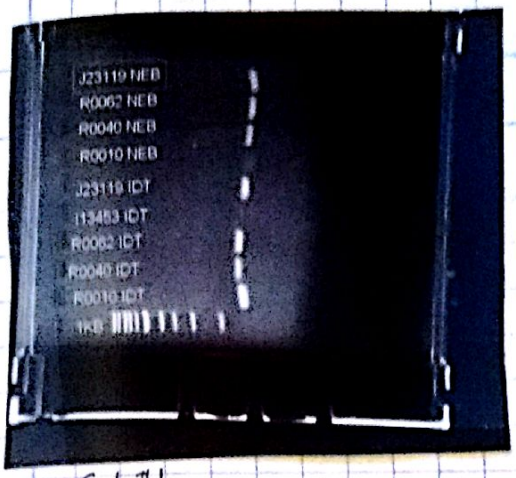
- Panya and Elli are running gels of all of today's PCR products

DpnI of Gibson PCRs

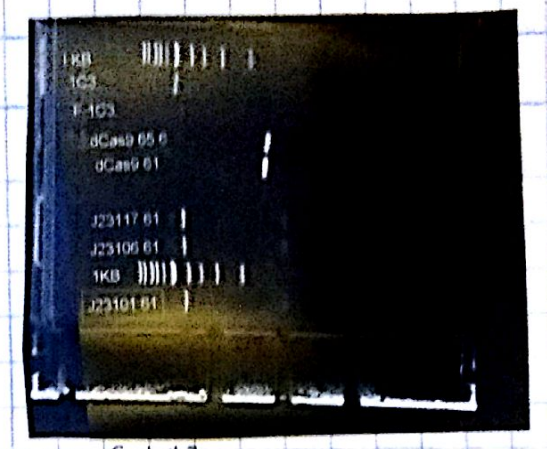
- [x] 2 µL CutSmart (10x)
- [x] 10 µL PCR product
- [ ] 0.5 µL DpnI
- [x] 7.5 µL NFW



Dinner: 7:40 PM -



Gel #1



Gel #2

On Gel #1:

- Clearly, using the "IDT method" of calculating annealing temperatures (average the Tm's of the two primers, and subtract 3) worked better than using the temperatures given by NEBuilder. We will use these (except for I13453, which didn't work at all) in the DpnI and in the Gibson.

On Gel #2:

- The dCas9 definitely did not work; it looks like perhaps the primers amplified themselves? We will look at these primers again.
- The "1C3", for the Targets of Susan (TOS) that will be assembled for the dCas9, appears perfect. It is pSB1C3 with RBS and DT (we will be inserting the promoters from Gel #1).
- The F.1C3 is a pSB1C3+PR+DT to create a functional GFP. Although it looks faint, the band still appears to be there. We will proceed.
- Parts J23117, J23106, and J23101 are ACTUALLY those promoters PCR'd onto a pSB1C3. These will be used to assemble the ILM parts, alongside the corresponding I13504 we PCR'd yesterday.

-Joe PCR-Purified the DpnI's of the PCRs that look good:

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
r0040 idt dpn1 PCR PURE	Default	6/18/2015	9:53 PM	58.13	1.163	0.712	1.63	0.96
r0062 idt dpn1 PCR PURE	Default	6/18/2015	9:54 PM	53.09	1.062	0.582	1.82	1.95
j23119 idt dpn1 PCR PURE	Default	6/18/2015	9:54 PM	86.00	1.720	1.057	1.63	1.26
F1C3 TOS DPN1 PCR PURE	Default	6/18/2015	9:55 PM	46.06	0.921	0.556	1.66	1.23
F1C3 FOR GFP DPN1 PCR PURE	Default	6/18/2015	9:56 PM	25.08	0.502	0.300	1.67	1.62
pSB1C3 106 DPN1 PCR PURE	Default	6/18/2015	9:57 PM	84.48	1.690	0.998	1.69	1.42
pSB1C3 101 DPN1 PCR PURE	Default	6/18/2015	9:57 PM	69.66	1.393	0.792	1.76	1.54
pSB1C3 117 DPN1 PCR PURE	Default	6/18/2015	9:58 PM	51.92	1.038	0.555	1.87	1.87
I13504 101 DPN1 PCR PURE	Default	6/18/2015	10:00 PM	88.47	1.769	0.963	1.84	2.17
I13504 106 DPN1 PCR PURE	Default	6/18/2015	10:00 PM	45.87	0.917	0.492	1.86	2.06
I13504 117 DPN1 PCR PURE	Default	6/18/2015	10:01 PM	51.80	1.036	0.591	1.75	1.85
GFP Amp Solo DPN1 PCR PURE	Default	6/18/2015	10:01 PM	30.62	0.612	0.319	1.92	1.66

Gibson Assembly

- [x] Basic Protocol:
  - [x] x μL insert (0.1 pmol)
  - [x] (0.5)x μL vector (0.05 pmol)
  - [x] 10 μL Hi-Fi Master Mix
  - [x] 10-x μL H_2O
    = Total volume: 20 μL
  - [x] Incubate in thermocycler for 15 min at 50°C

All Assemblies

Functional GFD (brace label for Functional GFP):

Functional GFP:
→ GFP: ~~0.03443~~ 0.06882 pmol/μL = 1.453 μL
→ F. K3: 0.01669 pmol/μL = 2.996 μL
→ 5.55 μL H_2O

InterLab Measurement Parts (brace label for J23101, J23106, J23117):

J23101:
→ I13504: 0.1573 pmol/μL = ~~[illegible]~~ 0.6357 μL
→ pSB1C3: 0.05355 pmol/μL = 0.9337 μL
→ 8.43 μL H_2O

J23106:
→ I13504: 0.08157 pmol/μL = 1.2259 μL
→ pSB1C3: 0.06494 pmol/μL = 0.7699 μL
→ 8.00 μL H_2O

J23117:
→ I13504: 0.09211 pmol/μL = ~~[illegible]~~ 1.0857 μL
→ pSB1C3: 0.03991 pmol/μL = ~~[illegible]~~ 1.2528 μL
→ 6.66 μL of H_2O

Targets of Susan (TOS) (brace label for F. J23119, F. R0062, F. R0040):

F. J23119:
→ J23119: 0.5997 pmol/μL = 1.667 μL of 1:10 dilution
→ pSB1C3: 0.02546 pmol/μL = 1.964 μL
→ 6.369 μL of H_2O

F. R0062:
→ R0062: 0.3409 pmol/μL = 2.933 μL of 1:10 dilution
→ pSB1C3: 0.02546 pmol/μL = 1.964 μL
→ 5.103 μL of H_2O

F. R0040
→ R0040: 0.3747 pmol/μL = 2.669 μL of 1:10 dilution
→ pSB1C3: 0.02546 pmol/μL = 1.964 μL
→ 5.77 μL H_2O

ons, and yesterday's (dCas9 into pSB1C3 and pSB1A3)

on ice. Aliquot into 9 transformation tubes
Product. Flick to mix, 4-5x. No vortex
e. Set heat block.
r 30sec

soc into mixture
7°C, 250 rpm

erting
uL of each transformation onto a plate.
37°C

(on the confocal)
r E coli again. While he was able to see
:ould not see the CFP. If we cannot
we can't use a CFP/YFP G^2, and will

he primers we received today, and made the

s we had for creating a KanR part did
s scar We redesigned and ordered

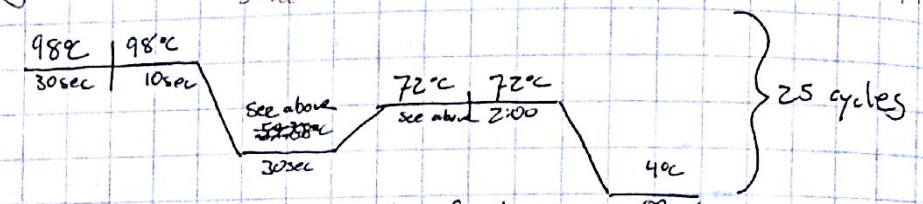
6/19/2015

Time in: 10:00AM
Time out: 11:00PM

- The new primers for making a Funct. KanR arrived! Joe is diluting, so that we can PCR.

Brian's method → Gibson PCR - pSB1K3, Funct. pSB1C3, Funct. pSB1A3, NOT funct. pSB1C3, NOT funct. pSB1A3

	pSB1K3	F. pSB1C3	F. pSB1A3	pSB1C3	pSB1A3
✓ 12.5 µL MasterMix					
✓ ~~1.25~~ 2.5 µL Primer 1	A52 ✓	A23 ✓	A23 ✓	A11 ✓	A11 ✓
✓ 1.25 µL Primer 2	A53 ✓	A28 ✓	A28 ✓	A12 ✓	A12 ✓
✓ ~~0.5~~ 1 µL DNA	pSB1K3 MP#2 ✓	pSB1C3 MP #1 ✓	pSB1A3 #2 MP ✓	pSB1C3 MP#1 ✓	pSB1C3 MP#1 ✓
✓ ~~9.5~~ 6.5 µL H_2O	✓	✓	✓	✓	✓
Annealing temp:	~~64.8~~ 59.3°C	62.5°C	62.5°C	55.3°C	55.3°C
	30 sec	1:30	1:30	1:15	1:15

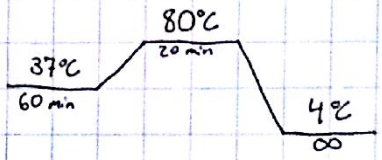


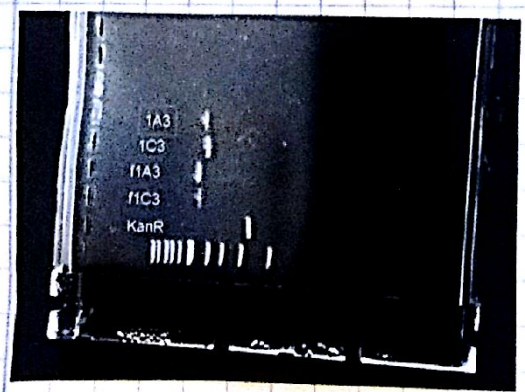
- Joe and Alex are running a gel of the PCR products

- Joe and I designed primers to change the backbones of the TOs, and to create a functional dCas9 unit.

DpnI of PCR Products:

- ✓ 2 µL CutSmart (10x)
- ✓ 10 µL PCR products
- ✓ 0.5 µL DpnI
- ✓ 7.5 µL NFW





Gel of PCR Products

KanR = 816 bp
Funct. 1C3 = ~~2419 bp~~ 2433 bp
Funct. 1A3 = 2518 bp
1C3 (for Susan) = 2070 bp
1A3 (for Susan) = 2155 bp

The gel looks great! We'll proceed with the assemblies.

PCR Purification

- [x] Add 5 volumes of Buffer PB (100µL for 20µL of DpnI rxn) and mix
- [x] Apply sample to MinElute column and centrifuge for 1 min
- [x] Discard flow through.
- [x] Add 750µL Buffer PE. Centrifuge for 1 min. Discard flowthrough.
- [x] Centrifuge 1 min to dry
- [x] Place column in final tube. Elute with 10µL of Buffer EB.
  - [x] Let the column stand for 1 min,
  - [x] Centrifuge 1 min.

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
KanR	Default	6/19/2015	5 31 PM	62 91	1 258	0 737	1 71	1.50
pSB1C3	Default	6/19/2015	5 31 PM	52 51	1 050	0 640	1 64	0 66
pSB1A3	Default	6/19/2015	5 32 PM	35 87	0 717	0 414	1 73	1 07
pSB1C3	Default	6/19/2015	5 33 PM	27 28	0 546	0 263	2 08	1 61
pSB1A3	Default	6/19/2015	5.34 PM	40 72	0 814	0.470	1 73	1 90

Gibson Assembly

	F. KanR (chlor) ~~KanR~~ ~~pSB1C3~~	F KanR (Amp)	Guide RNA ~~dCas9~~ #1		Guide RNA #2	
[x] Insert	0.8019 µL	0.8019 µL	1.885 µL	1.885 µL	1.885 µL	1.885 µL
[x] Vector	1.931 µL	2.169 µL	2.344 µL Chlor	1.635 µL Amp	2.344 µL Chlor	1.635 µL Amp
[ ] 10µL MM						
[x] 10-x µL H_2O	7.267 µL	7.0291 µL	5.771 µL	6.48 µL	5.771 µL	6.48 µL

Incubate in thermocycler for 15 min at 50°C

Transformation:

- [x] Thaw cells on ice.
- [x] Add 2µL of Assembly. Flick to mix 4-5x. No vortex.
- [x] Incubate 30 min on ice. Set heat block.
- [x] Heat shock at 42°C for 30sec.
- [x] Place on ice for 5 min.
- [x] Pipette 450 µL of RT SOC into mixture
- [x] Incubate 60min at 37°C, 250 rpm. Pre-warm plates.
- [x] Mix cells by flicking/inverting – missed
- [x] Spread 20/200µL onto a plate.
- [x] Incubate overnight at 37°C.

µCL

6/20/2015
Time in: 6:30 PM
Time out: 9:25 PM

- Inoculating last night's transformants:
  - Everything grew!! All will be inoculated. — Done by Michael
- All of last night's innoculations also grew. These will be miniprepped.
  - Done by John (help from me)

Nanodrops of MP:

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
dCas9 Amp MP 1	Default	6/20/2015	8:39 PM	95.00	1.900	1.014	1.87	0.98
dCas9 Amp MP 2	Default	6/20/2015	8:39 PM	88.15	1.763	0.958	1.84	1.85
dCas9 Chlor MP 1	Default	6/20/2015	8:41 PM	26.29 x	0.526	0.306	1.72	1.45
dCas9 Chlor MP 2	Default	6/20/2015	8:41 PM	50.90	1.018	0.549	1.85	1.42
funct. GFP MP 1	Default	6/20/2015	8:42 PM	80.27	1.605	0.837	1.92	2.28
funct. GFP MP 2	Default	6/20/2015	8:43 PM	48.69	0.974	0.510	1.91	1.78
funct. J23119 (TOS) MP 1	Default	6/20/2015	8:43 PM	116.03	2.321	1.330	1.74	1.55
funct. J23119 (TOS) MP 2	Default	6/20/2015	8:44 PM	111.72	2.234	1.174	1.90	2.28
funct. R0062 (TOS) MP 1	Default	6/20/2015	8:45 PM	100.98	2.020	1.057	1.91	2.22
funct. R0062 (TOS) MP 2	Default	6/20/2015	8:46 PM	76.61	1.532	0.811	1.89	2.30
funct. R0040 (TOS) MP 1	Default	6/20/2015	8:46 PM	11.90 x	0.238	0.149	1.60	1.91
funct. R0040 (TOS) MP 2	Default	6/20/2015	8:47 PM	16.91 x	0.338	0.142	2.37	2.28

x = Didn't sequence

- Entered MPs/Glycerols into inventory

6/21/2015
Time in: 4:40 PM
Time out: 9:00 PM

- Again, all innoculations grew!

Miniprep

- [x] Pellet bacteria at 6,800g for 3 min at RT
- [x] Resuspend pellet in 250 µL Buffer P1
- [x] Add 250 µL Buffer P2. Mix by inverting 4-6 times. (<5 min)
- [x] Add 350 µL Buffer N3. Invert 4-6x.
- [x] Centrifuge for 10 min at 13,000 rpm
- [x] Apply supernatant from step 5 to the spin col. Centr 60s; discard flowthrough
- [x] Wash by adding 500 µL Buffer PB. Centr 60s; discard flowthrough.
- [x] Wash with 750 µL Buffer PE. Centr 60s; discard flowthrough.
- [x] Centr for 1 min to dry.
- [x] Place in clean 1.5 mL tube. Elute in 50 µL Buffer EB.
  - [x] Let stand 1 min; centr 1 min

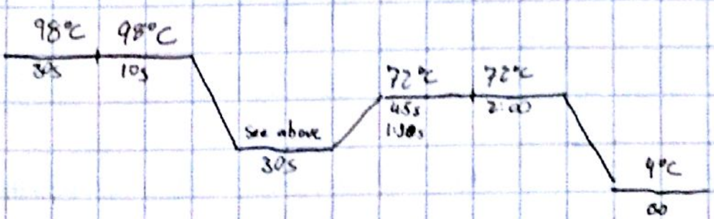
Nanodrops of MPs:

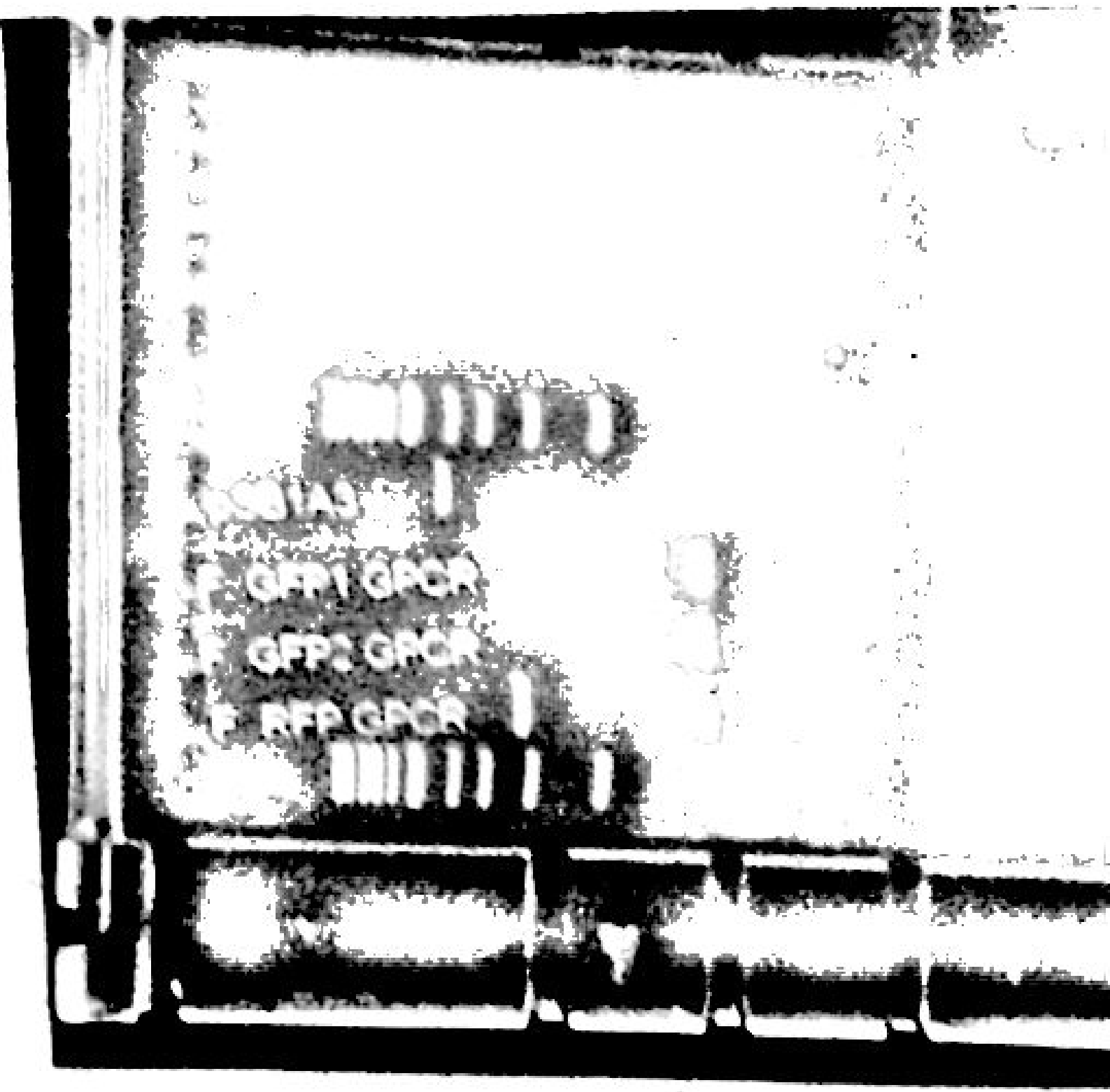
Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
funct. KanR Chlor MP 1	Default	6/21/2015	6:22 PM	73.06	1.461	0.778	1.88	2.21
funct. KanR Chlor MP 2	Default	6/21/2015	6:23 PM	43.38	0.868	0.442	1.96	2.38
funct. KanR Amp MP 1	Default	6/21/2015	6:24 PM	31.23	0.625	0.345	1.81	2.00
funct. KanR Amp MP 2	Default	6/21/2015	6:24 PM	37.22	0.744	0.370	2.01	2.33
guide RNA #1 Chlor MP 1	Default	6/21/2015	6:25 PM	38.09	0.762	0.382	2.00	1.81
guide RNA #1 Chlor MP 2	Default	6/21/2015	6:26 PM	36.53	0.731	0.384	1.90	2.03
guide RNA #1 Amp MP 1	Default	6/21/2015	6:27 PM	38.24	0.765	0.407	1.88	2.14
guide RNA #1 Amp MP 2	Default	6/21/2015	6:27 PM	35.04	0.701	0.353	1.98	2.36
guide RNA #2 Chlor MP 1	Default	6/21/2015	6:28 PM	68.44	1.369	0.722	1.90	2.23
guide RNA #2 Chlor MP 2	Default	6/21/2015	6:29 PM	39.73	0.795	0.401	1.98	2.14
guide RNA #2 Amp MP 1	Default	6/21/2015	6:29 PM	32.20	0.644	0.355	1.81	2.22
guide RNA #2 Amp MP 2	Default	6/21/2015	6:30 PM	47.88	0.958	0.504	1.90	2.26

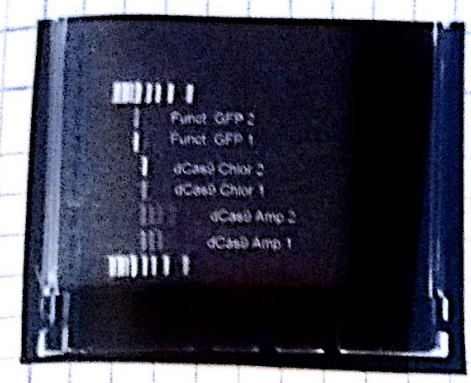
- Andy is setting up sequencing reactions of yesterday's and today's minipreps.
- John is making glycerol stocks.
- John and I are setting up PCRs for tomorrow:
  - F. GFP (2nd try) + F. RFP
  - XdCas9 for a functional unit } Primers arrive tomorrow - can't PCR length

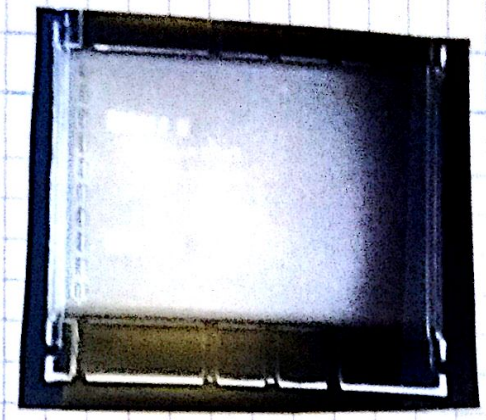
Gibson PCR

	F. GFP (try 2)	F. RFP	pSB1A3
☑ 12.5 μL MasterMix			
☑ 2.5 μL Primer 1	A13	A15	A11
☑ 2.5 μL Primer 2	A14	A16	A12
☑ 1 μL of 1:100 dilution			
☑ 6.5 μL H_2O			
Annealing temps:	63°C	63°C	67.5°C









Funct. GFP: 1083 bp
dCas9: 4113 bp
↳Cutting with PstI would create 3 bands:

The backbone is 2155bp
- 1626bp
- 1242 bp - 794 bp
- ~~2345 bp~~ 451 bp

- The F. GFP band looks to be ~3000 bp, close to the size of the Funct. unit in the backbone. It's possible that there is something incorrect in the prefix/suffix, which would prevent the enzymes from cutting, and (perhaps) primers from binding?
- The dCas9 Chlor has only one band, and it's the size of the ~~insert~~ backbone.
- It looks like the bands in dCas9 Amp (especially #2) could be correct!!

F. KanR: 1179 bp
-Although it's not easy to see on this gel, it looks like the F. KanR could be correct.

- Panya is setting up the 3A ligations to assemble
  - J23101 + I13504 (pSB1C3)
  - J23106 + I13504 (pSB1C3)
  - J23117 + I13504 (pSB1C3)

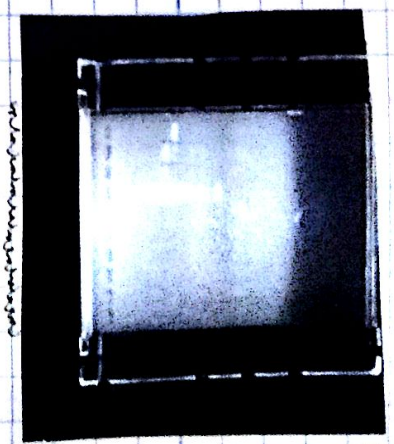
-Elli is setting up PCR to remake G^2 with F. GFP #2 + F. YFP #1 (pSB1K3#1)

-Joe is setting up a PCR of F. GFP (like we did yesterday) to create a G^2 (at 2 diff. temps)

-Joe is setting up a PCR to make a Functional GFP by putting a solo GFP into a Functional pSB1K3

~~The~~

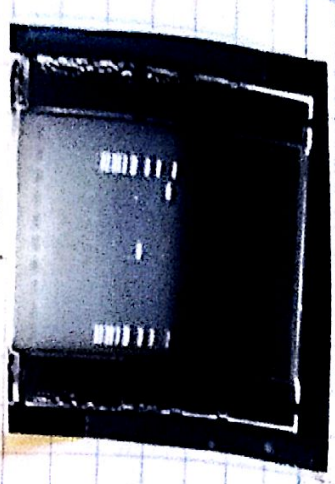
Dinner: 7:10PM - 9:00 PM



Gel:
- The functional pSB1A3 looks gorgeous!
- Using the Long Amp PCR seems to have worked! But only for the ones on Chlor.
- The regular Q5 did not appear to have worked at all.

- Lab meeting: 9:00 PM - 11:00 PM

- We accidentally reran the GPCRs from yesterday, alongside the 3A Assembly (which would not be at high enough concentration to see on a gel)

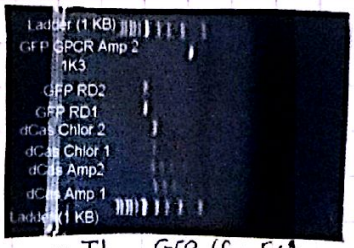


GPCR of dCas9 - Long Amp
↳ Chlor #1, Chlor #2, Amp #1, Amp #2

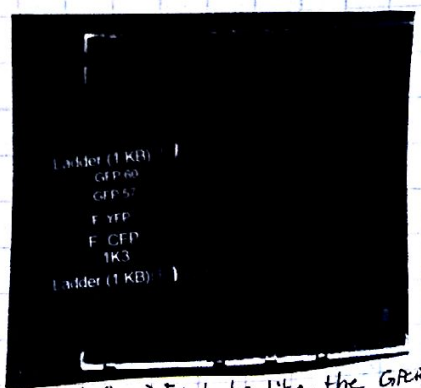
- ☑ 5 µL Buffer
- ☑ 0.75 µL dNTP
- ☑ 1 µL A54
- ☑ 1 µL A55
- ☑ 1.25 µL DNA
- ☑ 1 µL LongAmp Taq
- ☑ 15 µL NFW

- We're doing this at ~~60~~ 56°C and ~~55~~ 54°C (annealing temp)

Red: 56°C
Black: 54°C



- The GFP (for F.G) looks good! The 1K3 does not
- The RD of F.GFP does not appear to have cut.
- Again, only the dCas9 Amp 2 looks good



- Whoa? It looks like the GPCR for F.GFP into G2 could have worked!
- No F. YFP, but F. CFP and solo 1K3 look good

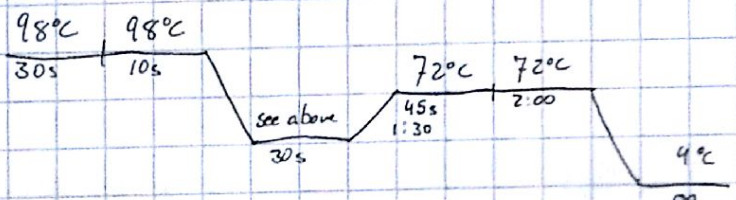
- Things to Re-PCR today:
  - Funct. pSB1K3
  - Funct. YFP

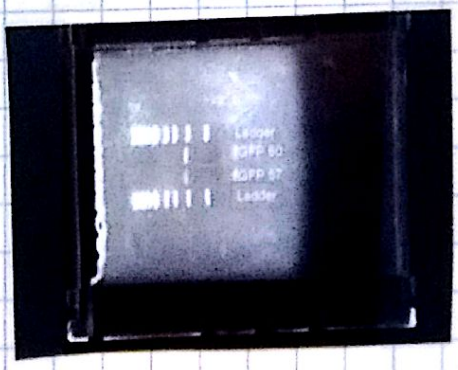
Gibson PCR

- [ ] 12.5 µL Master Mix
- [x] 2.5 µL Primer 1
- [x] 2.5 µL Primer 2
- [x] 1 µL of 1:100 dilution
- [x] 6.5 µL H_2O

Annealing temp:

	F. YFP	F. pSB1K3 (MP #2)
	A15	A7
	A16	A10
	62°C 45s	58.8°C 1:30





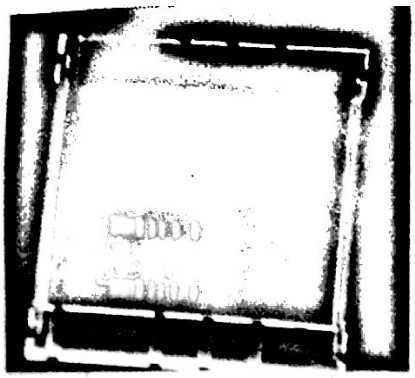
The gel confirms that the GPCR of the Funct. GFP for G2 worked! For the first time!

The "Chlor 2" dCas9 looks great, so we'll proceed with that one. We think the tube ~~was~~ labelled "Chlor 2" must be "Amp 2" as that's the only dCas9 that looks good on yesterday's gel

DpnI of ALL GPCRs

F. GFP 60°C, F. RFP, sdo 1A3 (*Unclear label on PCR tube! Could be incorrect!!), dCas9 ("C2 dCas9 54°" same as for F. GFP), F. 1K3, F. YFP (2nd try (see lab notebook page)), F. CFP, sdo 1K3, GFP solo, F. 1K3 (Same as for dCas9)

- [ ] 2 µL CutSmart (2x)
- [ ] 10 µL PCR product
- [ ] 0.5 µL DpnI
- [ ] 7.5 µL NFW



Gel of Re-PCRs
- F. YFP looks great!
- F. pSB1K3 does not look present. ~~No colonies on~~

We need to Re-PCR the F. 1K3 backbone
And they need to use different primers!

- It turns out that we need to remake F.1K3, and we'll also make F. 1A3 just in case, so that we could still create a Functional dCas9 GFP solo)

- Joe and Alex are doing those PCRs of F.1K3 and F.1A3 (for dCas9 and

- Michael is analyzing sequence data

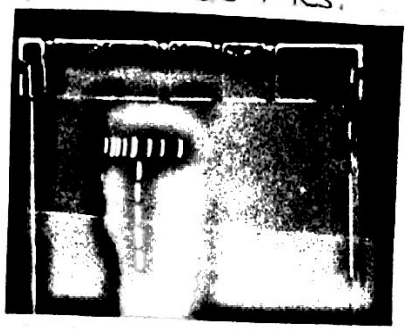
- Panya and Elli are cutting out bookmarks

- Saha Lab Meeting: 4:00 - 5:00 PM

- I'm running out the PCRs of the functional backbones on a gel. John and Joe are setting up the DpnI for these PCRs.

Ladder
F 1A3 #2 (~~56~~ 62.5°C)
F1K3 MP1 #1 (~~56~~ 62.5°C)
F1K3 MP2 #1 (~~56~~ 62.5°C)
* F1K3 MP2 #2 (56°C)
F1K3 MP1 #2 (56°C)
* F1A3 2 NS (58.8°C)

Run this way →



Everything looks great! We'll proceed with the PCRs with the asterisk

Taylor PCR purified most of the DpnIs →

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
fYFP_dpn1_pure_150623	Default	6/23/2015	6:17 PM	35.24	0.705	0.405	1.74	2.05
1A3_solo_dpn1_150623	Default	6/23/2015	6:18 PM	21.59	0.432	0.223	1.94	1.52
FGFP ~~fGFP~~_dpn1_150623	Default	6/23/2015	6:19 PM	38.80	0.776	0.419	1.85	1.91
f1K3_dpn1_150623	Default	6/23/2015	6:20 PM	8.18	0.164	0.072	2.27	1.37
1K3_dpn1_150623	Default	6/23/2015	6:21 PM	23.04	0.461	0.221	2.09	1.84
dCas9_C2_54_dpn1_150623	Default	6/23/2015	6:22 PM	14.12	0.282	0.131	2.15	1.58
fGFP_gPCR_60_dpn1_150623	Default	6/23/2015	6:24 PM	22.36	0.447	0.248	1.80	2.03
GFP_solo_dpn1_150623	Default	6/23/2015	6:25 PM	38.78	0.776	0.425	1.83	1.73
fRFP_dpn1_150623	Default	6/23/2015	6:26 PM	61.49	1.230	0.638	1.93	2.22
f1K3 MP 1 62.5 Pure	Default	6/23/2015	8:34 PM	36.14	0.723	0.428	1.69	1.88
f1K3 MP 2 62.5 Pure	Default	6/23/2015	8:35 PM	33.16	0.663	0.367	1.81	1.82
f1K3 MP 1 56 Pure	Default	6/23/2015	8:36 PM	20.11	0.402	0.226	1.78	1.95
f1K3 MP 2 56 Pure	Default	6/23/2015	8:36 PM	37.67	0.753	0.386	1.95	2.09
f1A3 #2 (Scars) Pure	Default	6/23/2015					1.82	1.82
						0.399		0.83

done by John →

sly - with Taylor and Michael (calculations with Joe + John)

ALD

µL Funct. GFP (0.06 pmol)
µL Funct. RFP (0.06 pmol)
Solo pSB1K3
µL H_2O
L HiFi Master Mix

ALD

µL Funct. CFP
µL Funct. YFP
solo pSB1K3
µL H_2O
HiFi Master Mix

Functional Unit

11 µL dCas9 (0.03 pmol)
µL Functional pSB1K3 (0.015 pmol)
µL H_2O
HiFi Master Mix

Functional Unit

µL GFP solo (0.06 pmol)
6 µL Functional pSB1A3
5 µL H_2O
L HiFi Master Mix

at 50°C for 60 min

PM - 11:15 PM

Transformation - Gibson assemblies, 3A ligations (150622)

- [x] Thaw cells on ice. Aliquot into 7 transformation tubes.
- [x] Add 2μL of Assembly. Flick 4-5x to mix. No vortex.
- [x] Incubate 30 min on ice. Set heat block.
- [x] Heat shock at 42°C for 30sec.
- [x] Place on ice for 5 min.
- [x] Pipette ~~125~~ 475 μL of RT SOC into mixture.
- [x] Incubate 60 min at 37°C, 250 rpm. Prewarm plates.
- [x] Mix cells by flicking/inverting
- [x] Spread 20μL and 200μL of each transformation onto a plate
- [x] Incubate overnight at 37°C

6/24/2015
Time in: 10:20 AM
Time out: 10:00 PM

- Joe is making Chlor and Kan plates

- Confocal training: 11:00AM - 12:00
  - Many people mount in 9:1 glycerol PBS

- Sam talk: 12:00 - 1:50PM

- Planned for presentation

- Panya and Elli innoculated yesterday's transformants, all of which grew (!)

- With Taylor and John (and Joe) began designing homologous regions for recombination

Dinner: 7:30PM - 9:00PM

6/26/2015
Time in: 10:20 AM
Time out: 5:20 PM

- Yesterday, Sam and Andy miniprepped 22 innoculations (see next page for nanodrops) and set up sequencing reactions

- Today, we'll set up diagnostic RDs to check if any of the assemblies are the correct size
  - ↳ Me, Joe, and Alex

Sample ID	ng/µL	260/280	260/230
fdCas9 #1	95.35	1.90	3.20
fdCas9 #2	81.85	1.96	2.39
fGFP #1	149.78	1.97	2.47
fGFP #2	137.24	2.00	2.46
G²GFP+RFP #1	194.98	1.94	2.25
G²GFP+RFP #2	167.32	1.94	2.45
G²GFP+RFP #3	114.79	1.91	1.71
G²GFP+RFP #4	146.13	1.96	2.67
G²GFP+RFP #5	112.84	1.95	2.25
G²GFP+RFP #6	151.19	1.86	~~2.48~~ 2.43
G²CFP+YFP #1	84.36	1.97	2.48
G²CFP+YFP #2	101.87	2.00	2.61
G²CFP+YFP #3	118.76	1.98	2.54
G²CFP+YFP #4	67.70	2.04	2.62
G²CFP+YFP #5	113.38	1.97	2.25
G2 CFP+YFP #6	103.44	2.00	2.58
J23101 #1	185.32	1.94	2.50
J23101 #2	185.43	1.92	2.37
J23106 #1	84.01	2.00	3.29
J23106 #2	204.34	1.97	2.52
J23117 #1	195.31	1.91	2.04
J23117 #2	213.01	1.91	2.39

} not correct sizes

Making gels:

- [x] 50 mL TAE
- [x] 0.5 g agarose
- [x] Microwave 90sec
- [x] Add 1 drop EtBr (in hood)

Lunch: 12:40 - 1:20 PM

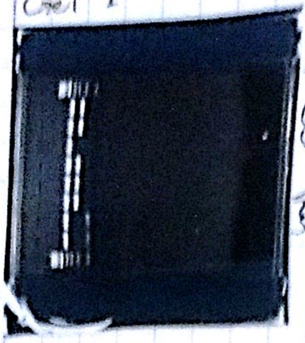
Running restriction digests on gels:

Gel 1:
↑ G² G+R #1, G²G+R#2, G²G+R #3, G²G+R #4, G²G+R#5, G²G+R #6, fdCas9 #1, fdCas9 #2

Gel 2:
↑ G²C+Y #1, G²C+Y#2, G²C+Y#3, G²C+Y #4, G²C+Y #5, G²C+Y #6, fGFP #1, fGFP#2

Gel 3:
↑ J23101 #1, J23101 #2, J23106#1, J23106#2, J23117 #1, J23117#2

Gel 1



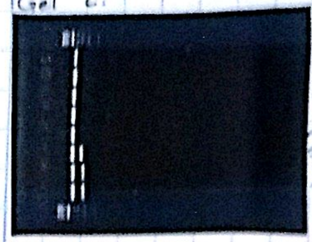
edo pSB1A3: 2155 bp

G² GFP+RFP: 2152 bp

fdCos4: 4476 bp

edo pSB1K3: 2204 bp

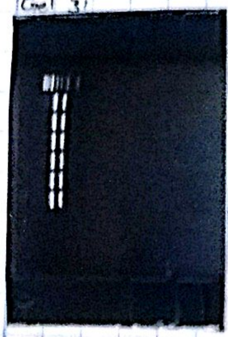
Gel 2:



G² GFP+YFP: 2270 bp

F. GFP: 1083 bp

Gel 3:



Funct. J23101: 910 bp

Funct. J23106: 910 bp

Funct. J23117: 910 bp

Obviously, the G² [illegible] at ~1000bp [illegible]. The G² with a [illegible] #2, could be correct [illegible] and [illegible] are [illegible] so we might not [illegible].

The fdCos4 [illegible] the size of a [illegible].

The GFP+YFP G² [illegible] size to the [illegible], [illegible] we can't know [illegible] worked.

The F. GFP could be [illegible]

The IMPs are all [illegible] to the expected size.

- We need sequence data before we can decide how to proceed. We'll return tomorrow.

tained sequence data and ran Michael's script on it.

of the sequences (29/~~45~~ 44) were blank - they only ined "NNNNN" All of them that did contain actual sequence rding to Michael's script) did not contain the correct parts.

going to try to re-try some of the assemblies:

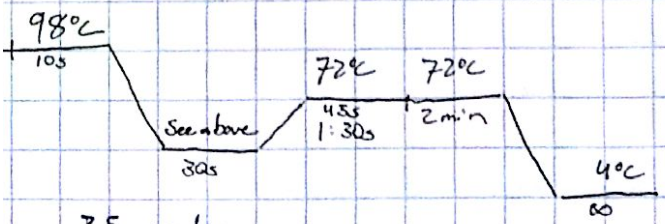
2 GFP+RFP — this time with the newly made F. GFP on np (no sequence data) as template for the GPCR

Remake TOS — using 3A assembly, and combining the promoters ith part I13504 (RBS+GFP+DT)

2 CFP+YFP- 3A assembly. Desperate times call for desperate easures.

PCR

	F. GFP (Amp)	F. RFP	pSB1K3
µL Master Mix			
µL Primer 1	A13	A16	A11
µL Primer2	A14	A15	A12
of 1:100 dilution DNA	#1 and #2 from 1506=5	~~#1~~ #1	#1
µL H_2O			
temp:	60°C	63°C	~~67°C~~ 67.5°C



25 cycles

also going to retry making F. dCas9, this time being very l with labelling

Gibson PCR - F. dCas9

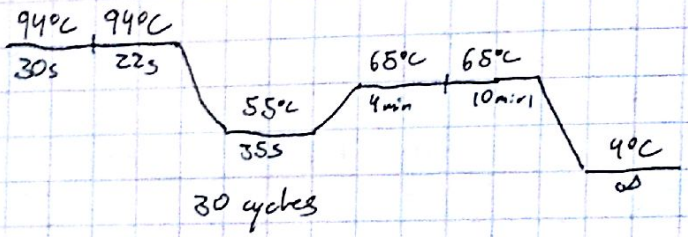
- [ ] 12.5 µL MM
- [ ] 2.5 µL Primer 1
- [ ] 2.5 µL Primer 2
- [ ] 1 µL 1:100 dilution
- [ ] 6.5 µL H_2O

} I forgot - we have to use Long Amp for dCas9

Long Amp Gibson PCR

- [x] 5 µL Buffer
- [x] 0.75 µL dNTP
- [x] 1 µL A54
- [x] 1 µL A55
- [x] 1.25 µL DNA
- [x] 1 µL Long Amp Taq
- [x] 15 µL NFW

-John is setting up the Q5 GPCR of the Funct. pSB1K3 for this assembly



John and I manually looked at all of the sequences that we DID have. Things were either:

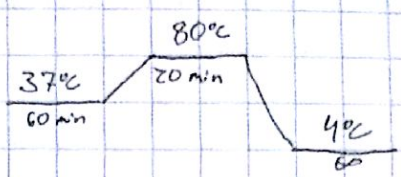
- A Functional RFP
- A REVERSED F. RFP
- Just backbone
- Unintelligible sequence

John is running a gel of today's PCR products.

G^2 GFP+RFP | Funct. dCas9

I - Solo IK3, F. GFP #1+#2, F. RFP, dCas9 Amp #2, F. IK3

- [x] 2 µL CutSmart (10x)
- [x] 10 µL PCR Products
- [x] 0.5 µL DpnI
- [x] 7.5 µL NFW



Purification

- [x] Add 100 µL Buffer PB
- [x] Place MinElute col in collection tube
- [x] Apply sample to column. Spin 1 min and discard flowthrough
- [x] Add 750 µL Buffer PE. Spin 1 min, discard flowthrough
- [x] Centrifuge to dry for 1 min
- [x] Place col in clean, labelled final tube
- [x] Elute with 10 µL of Buffer EB
  - [x] Let stand 1 min, then ~~spin~~ centr 1 min

User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
Default	6/27/2015	9:43 PM	53.85	1.077	0.554	1.94	2.84
Default	6/27/2015	9:44 PM	78.19	1.564	0.912	1.71	1.19
Default	6/27/2015	9:45 PM	83.97	1.679	0.983	1.71	1.36
Default	6/27/2015	9:45 PM	59.73	1.195	0.767	1.56	0.96
Default	6/27/2015	9:46 PM	162.39	3.248	1.738	1.87	2.25
Default	6/27/2015	9:46 PM	51.51	1.030	0.591	1.74	1.10

on Assembly

G^2	dCas9
✓0.7964 µL F.GFP#1	✓1.00 µL dCas9
✓0.5041 µL F. RFP	✓0.9867 µL F. IK3
✓0.7306 µL solo IK3	✓8.01 µL H_2O
✓7.9688 µL H_2O	
✓10 µL H.F. MM	✓10 µL H.F. MM

50°C for 1 hour

ylor and Joe are transforming the assemblies

*	G2 1 Try 2 GFP RFP	Default	6/29/2015	12:37 PM	59.08	1.182	0.636	1.86	2.19
*	G2 2 Try 2 GFP RFP	Default	6/29/2015	12:38 PM	93.36	1.867	1.030	1.81	1.58
	(2x EB Care) G2 4 Try 2 GFP RFP	Default	6/29/2015	12:38 PM	144.87	2.897	1.826	1.59	0.73
*	G2 3 Try 2 GFP RFP	Default	6/29/2015	12:40 PM	126.14	2.523	1.517	1.66	0.85
	G2 5 Try 2 GFP RFP	Default	6/29/2015	12:40 PM	131.21	2.624	1.488	1.76	1.14
*	G2 6 Try 2 GFP RFP	Default	6/29/2015	12:41 PM	105.44	2.109	1.206	1.75	1.04
	fdCas9 1 Try2	Default	6/29/2015	12:42 PM	102.89	2.058	1.089	1.89	1.77
	fdCas9 2 Try2	Default	6/29/2015	12:43 PM	68.96	1.379	0.766	1.80	1.26
*	fdCas9 3 Try2	Default	6/29/2015	12:43 PM	59.89	1.198	0.729	1.64	0.78
*	fdCas9 4 Try2	Default	6/29/2015	12:44 PM	40.02	0.800	0.471	1.70	0.81
	fdCas9 5 Try2	Default	6/29/2015	12:45 PM	49.96	0.999	0.543	1.84	1.62
	fdCas9 6 Try2	Default	6/29/2015	12:45 PM	87.93	1.759	1.007	1.75	1.20

Gibson PCR - F. CFP, F. YFP, IK3 solo

	F. CFP	F. YFP	Fdo IK3
☑ 12.5 µL MM			
☑ 2.5 µL Primer 1	A13 ✓	A15 ✓✓	A11 ✓
☑ 2.5 µL Primer 2	A14 ✓	A16 ✓✓	A12 ✓
☑ 1 µL 1:100 dilution	#2	#1-2	#1
☑ 6.5 µL NFW			
Annealing temp:	62°C 45sec	62°C 45sec	64°C 90sec

Confocal training: 12:30 - 1:30

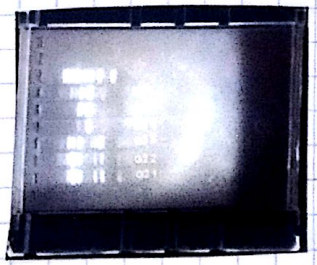
s of the RDs and the PCRs

with Amp, Chlor, and Kan resistances

2.5g

5 L

PCRs
reat!
proceed
the



RDs of G²:
Total G².4320
Bands:
~741
~334
~853
~184
~2200

nd 6 could be correct, and that they just
ough...?

re setting up sequencing reactions

Amp-Kan-Chlor plates

men

PCR Purification

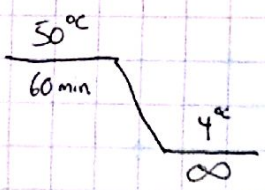
spin @ 17,900 g

- [x] Add 100 µL Buffer PB
- [x] Place MinElute col in collection tube
- [x] Apply sample to col. Spin 1 min, discard flowthrough
- [x] Add 750 µL Buffer PE. Centr 1 min, discard flow through.
- [x] Centr to dry 1 min.
- [x] Place the col in clean, labelled tube
- [x] Elute with 10µL Buffer EB.
- [x] Let stand 1 min, then centr 1 min.

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
* F. YFP 1 PCR Pure	Default	6/29/2015	6:45 PM	46.84	0.937	0.498	1.88	1.97
F. YFP 2 PCR Pure	Default	6/29/2015	6:45 PM	44.11	0.882	0.487	1.81	1.81
* F. CFP PCR Pure	Default	6/29/2015	6:46 PM	33.91	0.678	0.385	1.76	1.81
* 1K3 solo PCR Pure	Default	6/29/2015	6:47 PM	39.03	0.781	0.428	1.82	1.96

Gibson Assembly - G²: F.YFP1 + F.CFP + 1K3 solo

- [x] 1.31 µL F. CFP
- [x] 0.968 µL F. YFP #1
- [x] 1.118 µL solo pSB1K3
- [x] 6.60 µL H_2O
- [x] 10 µL H.F. Master Mix



Dinner: 7:20pm - 8:45pm

Transformation - G² (cfpYFP); F.dCas9 Try2 MP3 + gRNA #1 Amp #1 + (304450) pSB1C3 #1; F.dCas9 Try2 MP #3

- [x] Thaw cells on ice. Aliquot into transformation tubes. - 15µL
- [x] Add 2µL of Assembly / MPs (each)
- [x] Incubate 30 min on ice. Set heat block.
- [x] Heat shock at 42°C for 30 sec
- [x] Place on ice for 5 min
- [x] Pipette 290 µL of RT soc into mixture
- [ ] Incubate 60 min at 37°C, 250 rpm. Prewarm plates.
- [ ] Mix cells flicking/inverting
- [ ] Spread 20/200 µL of each transformation
- [ ] Incubate overnight at 37°C

We Are

vence confirmed

nce confirmed)
As)

yet):

+ Amp + Chlor)
!):

data for middle 1/3)

7/1/2015
Time in: 10:0
Time out: 9:0

- Joe and John are looking into how to make electrocompetent cells (to order from NEB; $174 for 6 x 0.1mL/tube).

Lunch with Sam: 12:00 - 2:00PM

- Michael says that they were able to image F. YFP and F. CFP, which is excellent! However, the G² GFP/RFP only looks like RFP.

- Yesterday, John found the primers used by Dr. Elowitz to integrate two different reporters onto two different locations of the E. coli genome. Today, we've ordered those primers so that next week, we can try to integrate those onto the genome.

- We've looked into designing primers to put F. KanR after a functional unit. However, we realized that the primers that we have for assembling G²s should work.

However! If we are to try Elowitz's method of integrating into two separate locations, we will need another antibiotic cassette. I contacted iGEM to request one, but we might need to PCR it up ourselves.

- iGEM team meeting

- Joe innoculated our F. dCas9 transformants, to start the process of making electrocompetent cells

- Michael is streaking out more F. CFP, F. YFP, and our recently assembled G²: GFP/RFP to image tomorrow.

- Panya and Elli are setting up innoculations of yesterday's G²: CFP/YFP, as well as diagnostic colony PCRs.

Dinner: 9:00 PM - 9:45 PM

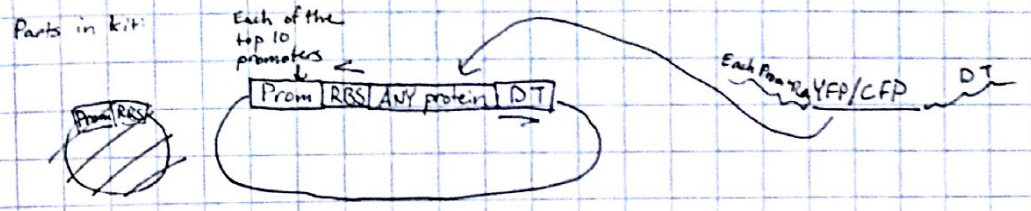
- Discussed the design of gRNAs with Joe and Elli

7/17/2015
Time in: 10:05AM
Time out: 9:00PM

- Last night, Michael discovered that some of the parts that we need or can use exist on the kit. We're double checking that.

- Panya is running last night's diagnostic PCRs on a gel (G2: CFP/YFP)

- Joe, Elli, John, and I (less so) designed/ordered primers to make Tas/Reporters to be integrated for each of the top 10 promoters by taking a ~~promoter~~ functional unit with each promoter, and creating primers for CFP and YFP to PCR into these Funct. backbones



- The colony PCRs showed that our assembly of G2: CFP/YFP was not a success. :(

- John and Joe are making electrocompetent cells

- Panya is PCR-Purifying some MPs of F dCas9, a TAS (J04450), and a gRNA to ~~et~~ transform by electroporating later (electroporation does not work well when the plasmid is eluted in a salty solution, such as Buffer EB, Tris·Cl)

- Michael resuspended and transformed parts from the kit that we need for the PCRs listed above.

- Unfortunately, IDT emailed us to say that not all of our primers will be arriving tomorrow :(

- When I left, John, Panya, Taylor, and Elli were finishing up making the electrocompetent cells. :)

7/2/2015

Time in 10:40 AM
Time out 11:00 PM

- The group last night finished making the cells. Today, we need to "arc test" the cells. Michael and Joe are looking into how to do this.

- Although some of the primers arrived, we can't begin to assemble ~~them~~ any of the Tos with the different promoters yet, because many necessary primers won't arrive till Monday.

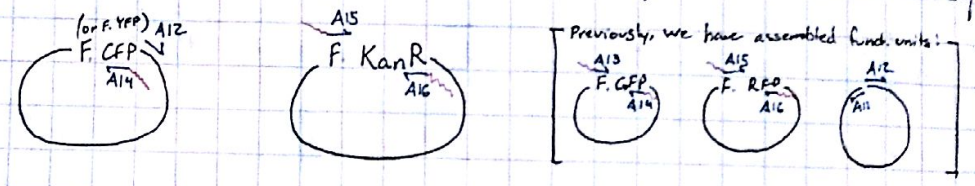
- We need to re-set up sequences from 6/29 → Michael and Joe

- Alex and I are setting up gPCRs to assemble:

  - F. CFP + F. KanR — F. CFP #1 + #2 — F. KanR (Chlor) #1 + #2
  - F. YFP + F. KanR — F. YFP #1 + #2 — F. KanR (Chlor) #1 + #2
  - G²(GFP/RFP) + F. KanR ✗ Nevermind, not doing this

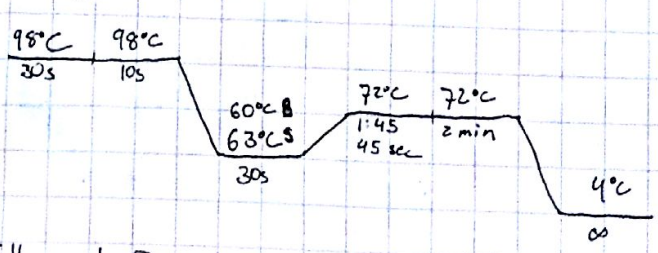
* New primer ordered to replace A14 for this assembly method on 150729

We're going to set up these up differently than we have in the past:



Gibson PCR

	From 6/15 MP F. CFP #1+#2	From 6/15 MP F. YFP #1+#2	From 6/12 MP F. KanR #1+#2 (chlor)
☑ 12.5 µL MM			
☑ 2.5 µL primer1	A12 ✓	A12 ✓	A15 ✓
☑ 2.5 µL primer2	A14 ✓	A14 ✓	A16 ✓
☑ 1 µL 1:100 dilution			
☑ 6.5 µL NFW			
Annealing temp:	60°C	60°C	63°C



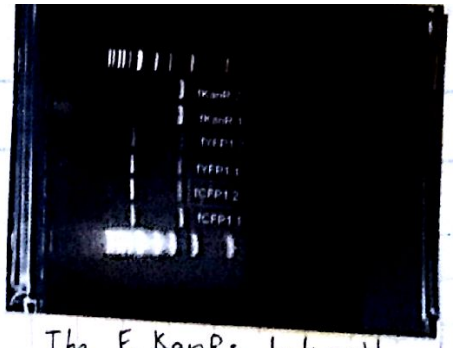
- Elli and Panya are making Kan+Amp, Kan+Chlor, and Amp+Chlor

- Joe is running the PCR products on a gel

- Michael is making an "agar pad" to image cells

DpnI of qPCR Products

- [x] 2µL CutSmart (10x)
- [x] 10µL PCR products
- [x] 7.5µL NFW
- [x] 0.5µL DpnI



The F. KanRs look good!
We're going to gel purify the F. YFP and F. CFP to get the band at ~3650 bp.

PCR Purification - Just of F. KanR DpnIs

- [x] Add 100µL Buffer PB
- [x] Place MinElute col in collection tube
- [x] Apply sample to column. Spin 1 min; discard flowthrough.
- [x] Add 750µL Buffer PE. Spin 1 min; discard flowthrough.
- [x] Centrifuge to dry for 1 min.
- [x] Place col in clean, labelled final tube.
- [x] Elute with 10µL Buffer EB. Let stand 1 min, then centr 1 min.

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
F. KanR PCR Pure #1	Default	7/2/2015	7:36 PM	65.64	1.313	0.747	1.76	2.01
F. KanR PCR Pure #2	Default	7/2/2015	7:36 PM	55.90	1.118	0.645	1.73	0.95

Gel Extraction - F. CFP #1+2, F. YFP #1+2

- [x] Excise DNA fragment from the gel with clean, sharp scalpel
- [x] Weigh the gel slice. Add 3 volumes Buffer QG to 1 volume gel (100 mg gel ~100µL) * Max amount of gel per column = 400 mg *

1.045g F. CFP1 → 1.196g → 151mg gel → 453µL QG → 604µL Total
1.039g ~~0.825g~~ F. CFP2 → 1.290g → 251mg gel → 753µL QG → 1004µL Total
1.034g ~~0.862g~~ F. YFP1 → 1.781g → 247mg gel → 741µL QG → 988µL Total
1.035g F. YFP2 → 1.323g → 288mg gel → 864µL QG → 1152µL

- [x] Incubate at 50°C for 10 min.
  - [x] Vortex every 2-3 minutes
- [x] Add 1 volume isopropanol to the sample and mix
- [x] Apply sample to QIAquick column and centr 1 min. Discard flowthrough.
- [x] To wash, add 750µL Buffer PE to QIAquick col. Centr 1 min; discard flowthrough
  - [x] Let stand 2-5 min
- [x] Place col into clean, labelled tube
- [x] Elute with 50µL Buffer EB. BUT FIRST, let sit one minute

- The concentrations from the gel extractions are very low. We're going to retry from the PCR tomorrow.

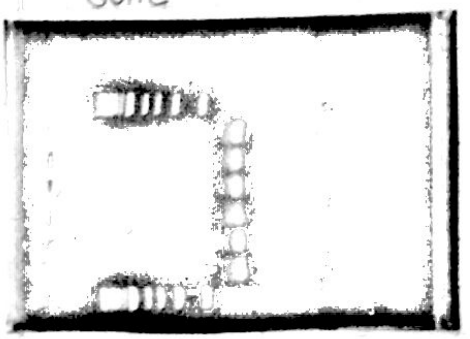
7/3/2015

Time in 9:40 AM

Time out 11:15 AM

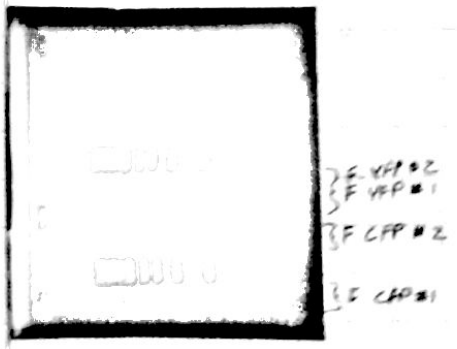
- John, Joe, and Panya set up the PCRs of the F YFP and F CFP backbone
  - They also set up PCRs to make a F. KanR to integrate, with Elowitz's primers into GalK

- John and Joe are designing gBlocks and primers for the integrati suite



The PCR of F KanR for integration yielded bands at any of the temperatures tried (6

John is going to retry the PCRs, at 60°C



The re-gPCRs of F YFP and F CFP produced the same two bands as yesterday. We're going t DpnI, and then re-gel extract

- It turns out that the ECNRZ cells that we grew up at 3 last night needed to be grown at 30°C We are going to replate at 30°C, as well as grow some (from in media at 30°C We're going to try to troubleshoot F KanR PCR today, even though we can't integrate today, s when we can integrate, it will already be optimized

Lunch 2:00-3:00pm

The F KanR did not PCR at 60°C and 57°C. Elli and Panya trying again at 43°C, 46°C, 49°C, 52°C, 55°C, and 58°C dilutions

Joe, John, and Andy executed the gel Elli and Taylor are extracting

* F YFP #1	Default	7/3/2015	7 08 PM	2 64	0 053	0 071	0 74	-0 01
F YFP #2	Default	7/3/2015	7 09 PM	1 27	0 025	0 031	0 82	-0 01
F CFP #1	Default	7/3/2015	7 10 PM	1 06	0 021	0 064	0 33	-0 00
* F CFP #2	Default	7/3/2015	7 10 PM	1 50	0 030	0 041	0 74	-0 02

Gibson Assembly:

F. CFP

1.42 ~~8.49~~ µL 1/10 F. KanR
8.66µL F. CFP
1.20µL H_2O

F. YFP

2.37µL 1/10 F. KanR
8.2µL F. YFP
1.56µL H_2O

X

} We're going to use yesterday's gel extractions

	Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
*	fYFP1 Gel Extract	Default	7/2/2015	10 22 PM	6 07	0 121	0 055	2 20	0 01
	fYFP2 Gel Extract	Default	7/2/2015	10 23 PM	3 19	0 064	0 038	1 67	0 14
	fCFP1 Gel Extract	Default	7/2/2015	10 23 PM	4 83	0 097	0 085	1 14	0 19
*	fCFP2 Gel Extract	Default	7/2/2015	10 24 PM	8 20	0 164	0 108	1 51	0 02

Gibson Assembly:

F. CFP + KanR

✓0.71 µL F. KanR
✓7.92 µL F. CFP
✓1.37µL H_2O
✓10 µl master mix

7.92 µl
-7.92 µl
7.1 µl

F. YFP + KanR

✓0.59 µL F. KanR
✓8.92µL F. YFP
✓0.49 µL H_2O
✓10 µl master mix

Chemical Transformation - Gibson Assemblies

- [ ] Thaw cells on ice Aliquot 15µL/tube
- [ ] Add 1.5/2 µL of resuspension/assembly
- [ ] Incubate 30 min on ice Set heat block
- [ ] Heat Shock at 42°C for 30sec
- [ ] Place on ice for 5 min
- [ ] Pipette 350 µL RT SOC into mixture
- [ ] Incubate 60min at 37°C, 250 rpm Pre
- [ ] Mix cells by flicking/inverting
- [ ] Spread 20/200µL onto plates
- [ ] Incubate over night at 37°C

Electroporation Transformations - From Yang Lab - TOS,

- [ ] Place electroporation cuvette, desired plasmid, ar on ice for 15 min
- [ ] Set up electroporation apparatus
  - [ ] Turn on
  - [ ] Set volts to ~~1.8 volts~~ (1.6 volts)
- [ ] Retrieve 50µL aliquot of competent cells and ~~p~~ imm
- [ ] Allow cells to thaw
- [ ] Add 0.5 - 1.0 µL of plasmid to aliquot
- [ ] Transfer cells+DNA mixture to electroporation cu
- [ ] Flick cuvette to mix, then tap on the table to drive
- [ ] Use Kimwipe to dry metal sides of cuvette
- [ ] Place cuvette in apparatus so that metal sides are metal flaps w/in the apparatus
- [ ] Attach cover of apparatus
- [ ] Have 500µL of fresh media ready to pipette
- [ ] Use 2 fingers to simultaneously press both "Pulse" bu
- [ ] Hold down buttons until beep
- [ ] Quickly disassemble apparatus + add 500µL media to the
- [ ] Pipet up+down, then transfer media+cells to fresh tu
- [ ] Place epi in shaker at 37°C for 1 hour to allow cel
- [ ] After 1 hour, plate mixture

- None of the FKanR PCRs worked (all but one had no band)

7/5/2015
Time in 8:15PM
Time out 10:30PM

- Yesterday, Joe and John inoculated things that grew from the transformation. Today, Joe came in and labelled tubes

Miniprep:

- [x] Pellet inoculations by centrifugation at 6800g for 3 min
- [x] Resuspend pelleted cells in 250μL Buffer P1
- [x] Add 250μL Buffer P2. Invert 4-6x (until clear). (< 5 min)
- [ ] Add 350μL Buffer N3 and invert 4-6x
- [x] Centr for 10min at 17,900g (13,000 rpm)
- [x] Apply supernatant from prev step to spin col by pipetting. Centr 60s; discard flowthrough.
- [ ] Wash w/ 500μL Buffer PB. Centr 60s; discard flowthrough.
- [x] Wash w/ 750μL Buffer PE. Centr 60s; discard flowthrough
- [x] Centr 60s to dry
- [x] Place col in clean tube. Elute w/ 50μL Buffer EB or water.
  - [x] Let stand one min; centr one min

- Parts from kit = eluted in water
- F. CFP + F. KanR, F. YFP + F. KanR, and retransformations of F. KanR = EB

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
I6060 #1	Default	7/5/2015	8:18 PM	77.89 [3]	1.558	0.840	1.85	1.98
I6060 #2	Default	7/5/2015	8:18 PM	67.18	1.344	0.699	1.92	2.78
J04421 #1	Default	7/5/2015	8:19 PM	67.59 [3]	1.352	0.710	1.90	3.09
J04421 #2	Default	7/5/2015	8:20 PM	78.17	1.563	0.841	1.86	1.31
I13602 #1	Default	7/5/2015	8:20 PM	232.14 [1]	4.643	2.500	1.86	2.11
I13602 #2	Default	7/5/2015	8:21 PM 27	==281.20==	5.624	3.100	1.81	1.63
KI684001 #1	Default	7/5/2015	8:21 PM	38.57	0.771	0.391	1.97	4.47
KI684001 #2	Default	7/5/2015	8:22 PM 28	==286.79== [1]	5.736	3.033	1.89	2.52
I6031 #1	Default	7/5/2015	8:22 PM	62.24 [3]	1.245	0.630	1.98	3.58
I6031 #2	Default	7/5/2015	8:23 PM	36.68	0.734	0.384	1.91	3.55
I6066 #1	Default	7/5/2015	8:24 PM	31.07 [7]	0.621	0.301	2.06	4.35
I6066 #2	Default	7/5/2015	8:24 PM	30.15	0.603	0.352	1.71	0.91
I13541 #1	Default	7/5/2015	8:25 PM	44.80	0.896	0.464	1.93	3.23
I13541 #2	Default	7/5/2015	8:25 PM	103.81 [2]	2.076	1.093	1.90	2.88
I6088 #1	Default	7/5/2015	8:26 PM	92.65 [2]	1.853	0.958	1.93	2.87
I6088 #2	Default	7/5/2015	8:27 PM	88.71	1.774	0.945	1.88	2.96
S01003 #1	Default	7/5/2015	8:27 PM	98.91 [2]	1.978	1.055	1.87	2.72
S01003 #2	Default	7/5/2015	8:28 PM	107.03	2.141	1.160	1.85	1.65

} From Kit

				ng/µL			260/280	260/230
F KanR Chlor1 #1	Default	7/5/2015	8:30 PM	108.32	2.166	1.159	1.87	2.14
F KanR Chlor1 #2	Default	7/5/2015	8:30 PM	130.02	2.600	1.410	1.84	2.19
F KanR Chlor2 #1	Default	7/5/2015	8:31 PM	98.34	1.967	1.049	1.87	1.78
F KanR Chlor2 #2	Default	7/5/2015	8:31 PM	90.25	1.805	0.967	1.87	2.28
F YFP + F KanR #1	Default	7/5/2015	8:32 PM	131.54[2]	2.631	1.400	1.88	2.21
F YFP + F KanR #1 2	Default	7/5/2015	8:32 PM	56.15[4]	1.123	0.600	1.87	2.27
F CFP + F KanR #1	Default	7/5/2015	8:34 PM	129.43[2]	2.589	1.409	1.84	1.87
F CFP + F KanR #2	Default	7/5/2015	8:34 PM	155.76[2]	3.115	1.668	1.87	2.01
								2.13

- Andy set up sequences
- I labelled tubes for and made glycerol stocks

7/6/2015

Time in: 10:45am

Time out: 12:00pm

Parts from kit		Promoter	Primers	Needs	Insert Primers
I13602	- F. CFP+LVA	R0040	A58+A59	YFP+LVA	B14+B15 - Same as B5+B6
I6031	- F. YFP (no LVA)	R0011	~~A58+A59~~ ~~B3+B4~~	CFP+LVA YFP+LVA	B3+B4
S01003	- F. CFP+LVA	R0062	~~A58+A59~~ ~~B1+B2~~	YFP+LVA	B1+B2 Same
K584001 → ~~K684001~~	- F. GFP	J23119	A58+A64	CFP+LVA YFP+LVA	B11+B12
J04421	- F. ~~G~~FP+LVA	R0010	A58+A59	YFP+LVA	DON'T PCR - ALREADY HAVE
I6060	- F. YFP+LVA	R0010	A58+A59	CFP+LVA	
I13541	- F. GFP	I13453	A58+A59	CFP+LVA YFP+LVA	B9+B10 - Same as B5+B6
I6066	- F. YFP+LVA	R0051	A58+A59	CFP+LVA	B5+B6 - Same as B14+B15
I6088	- F. YFP+LVA	I00050	A58+A59	CFP+LVA	B1+B2
Not done today		J23101 - J23106 J23117 J23100 -			

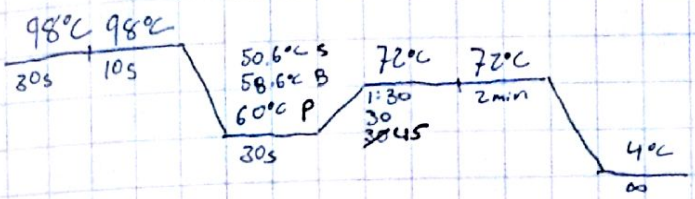
PCRs we need:

				Dilution	Primer 1	Primer 2	MM
R0040	#1	- I13602	- A58+A59 - 50.6°C	✓	A58✓	A59✓	✓
R0011	#1	- I6031	- A58+A59 - 50.6°C	✓	A58✓	A59✓	–
R0062	#1	- S01003	- A58+A59 - 50.6°C	✓	A58✓	A59✓	–
J23119	#2	- K684001	- A58+A64 - 50.2°C	✓	A58✓	A64✓	–
I13453	#2	- I13541	- A58+A59 - 50.6°C	✓	A58✓	A59✓	–
R0051	#1	- I6066	- A58+A59 - 50.6°C	✓	A58✓	A59✓	–
I00050	#1	- I6088	- A58+A59 - 50.6°C	✓	A58✓	A59✓	–
	#1	- YFP+LVA	- B5+B6 - 60°C	✓	B5✓	B6✓	–
	#1	- CFP+LVA	- B6+B6 - 60°C	✓	B5✓	B6✓	–
		- YFP+LVA	- B1+B2 - 60°C	✓	B1✓	B2✓	–
		- CFP+LVA	- B1+B2 - 60°C	✓	B1✓	B2✓	–
		- YFP+LVA	- B11+B12 - 58.6°C	✓	B11✓	B12✓	–
		- CFP+LVA	- B11+B12 - 58.6°C	✓	B11✓	B12✓	–

* THESE PRIMERS ARE NOT CORRECT Please check spreadsheet on drive

Gibson PCRs - CFP/YFP into F. backbones w/ diff promoters

- [x] 2.5 µL Primer 1
- [x] 2.5 µL Primer 2
- [x] 1 µL 1:100 dilutions
- [x] 6.5 µL Nfw
- [x] 12.5 µL M



- John has ordered the rest of the designed gBlocks + primers for integration
- Joe is setting up gPCRs/PCRs to make F. TetR, and to integrate F. CFP + F. KanR and F. YFP + F. KanR
- I re-set up four sequences for Lydia

Lunch: 2:15 PM - 4:00 PM

- Joe ran gels of the PCRs:

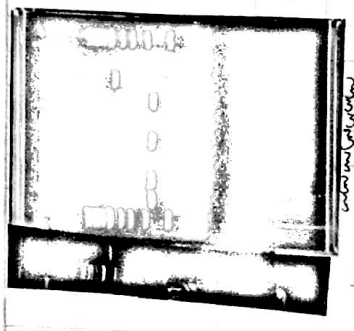
I6088: 3443 ✓
I6066: 2282 ✓
I13541: 2364bp Not present
K558001: 2269 Too large
S01003: 2288 ✓
I13602: 2288 ✓
~~I13603~~ I6031

CFP B11+B1C } Retry
YFP B11+B12 }
F. CFP + F. KanR } Elowitz integration primers failed again. We'll recheck primers
F. YFP + F. KanR }
F 143 - Retry
Solo TetR 1191 ✓

CFP B1+B2
YFP B1 + B2 ✓ Everything looks like the correct size... the CFP looks unfortunately faint
CFP B5+B6
YFP B5+B6 ✓

Dinner: 7:30 - 8:50 pm

- Elli set up DpnIs of the successful qPCRs
- Joe re-PCR'd YFP+LV4 and CFP+LV4 w/ B1+B2, B11+B12, B5; F. IA3; I13541 (Not K584001)



- Elli and Panya set up DpnIs of these PCRs that were successful
- B11 and B12 failed both times. We're going to double check these primers
- Joe and Taylor are going to try electroporating again, with: Amp (J04450) #2, gRNA #1 MP#2, F. dCas9 =

- Panya PCR Purified the DpnIs
- I inoculated in LB (out of TB).
  - J04450 in IA3 #2 (running low on MP)
  - F. dCas9 #3 + #4 (need to elute MPs in H_2O)

XOn
7/7/2015
Time in: 10:00 am
Time out: 7:25 PM

Making Tet and Tet+Amp Plates

- [ ] 5g Tryptone
- [ ] 5g NaCl
- [ ] 2.5g yeast extract
- [ ] 10g Agar
- [ ] Water to 0.5 L

- John + Panya + Michael autoclaved tubes
- Panya nanodropped last night's PCR purifications (see Panya's NB)
- John calculated volumes for the Gibsons (see John's notebook)
- Michael and Panya set up Gibsons

Gibson Assemblies:

- I6088 + CFP (B1 + B2)
- I6066 + CFP (B5 + B6)
- I13541 + CFP (B5 + B6)
- I13541 + YFP (B5 + B6)
- So1003 + YFP (B1 + B2) X Not enough concentration to proceed
- I13602 + YFP (B5 + B6)
- I6031 + CFP (B1 + B2)
- I6031 + YFP (B1 + B2)
- Tet solo + F. pSB1A3

- Michael and Panya are transforming these Gibson assemblies, as well as J04450 on Tet (from kit, we want a supply of Tet backbone), F. CFP MP#1, and F. YFP MP#1 (we've been unable to regrow these from the glycerols, and need them to image)

- John is investigating the secondary structures of the Elowitz integration primers. Every time we've tried to PCR with them, they don't amplify what we want, and instead seem to be amplifying themselves

- Elli and I made Tet and Tet + Amp plates

- I washed glassware

- Catherine's Master's Defense: 1:00 PM - 2:00 PM

- Joe and Michael miniprepped

	Concentration	260/280	260/230	
pSB1A3	123.75 ng/μL	1.76	1.16	Eluted in ~~water~~ EB
F. dCas9 3	16.47 ng/μL	1.73	1.36	Eluted in water
F. dCas9 4	26.76 ng/μL	1.87	1.83	

- Joe and Michael looked at B11 + B12 - apparently these primers amplify RBS + CFP + LVA and RBS + YFP + LVA, since K584001 has a non-standard RBS. As template, we'll need to use F YFP or F CFP.

Things we still need to assemble:

- S01003 (R0062) + YFP (B1+B2)
  ↳ Too low concentration to assemble. PCR at lower
- K584001 (J23119) + YFP/CFP (B11+B12)
  ↳ Band wrong size ↳ Need to use F. CFP/F.
- J23100 (J23100) + YFP/CFP
- J23101 (J23101) + YFP/CFP

→ Once all of these "Toss" are assembled, we
- Test Susans for all of these promoters
- Assemble the F CFP/F YFP (with each p
a functional antibiotic (F. KanR or F. Tet

- Meeting with team + Dr. Saha

- Joe and Taylor are setting up to make compet

- They are also setting up PCRs, and trying to electr
(See Joe's NB for samples, primers, temps)

7/8/2015
Time in: 10:00AM
Time out: 9:45PM

- No growth on the F Tet plate. We'll need to re
- Growth:
  - F CFP } From minipreps
  - F YFP }
  - J04450 - Tet } From kit
  - I6031 + YFP } Gibson Assemblies - only a FEW colonies
  - I6031 + CFP }

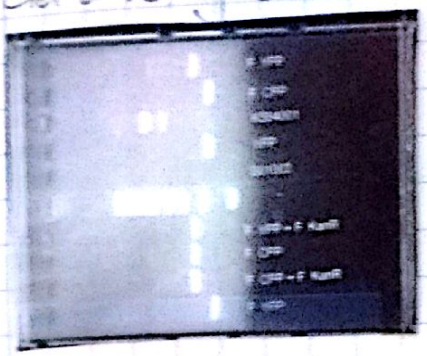
- No growth
  - I0088 + CFP
  - I6066 + CFP
  - I13602 + YFP
  - I13541 + CFP
  - ~~I6031 + YFP~~
  - I13541 + YFP
  - Funct Tet

- Panya and Alex are DpnI-ing the PCRs from last night

- Michael and John are redesigning Elowitz primers, so that they no longer bind to themselves

- Panya and Alex are re-DpnI-ing the PCRs we made on 7/6, which failed to assemble yesterday. We're going to retry these assemblies.
  ↳ ~~Michael~~ Panya is running last night's Gibson assemblies on a gel with 3x the usual amount of EtBr.

Gel of last night's PCRs:



F. YFP: 1125 ✓
F. CFP: 1125 X
K584001: 2269 ✓ (worth trying to proceed)
YFP: 762 ✓
SO1003: 2288 X
F. YFP + F. KanR: 2304 X
F. CFP: 1125 ✓
F. CFP + F. KanR: 2304 X
F. YFP: 1125 X

- We're going to retry some of the above PCRs:
  - F. CFP (B11+B12) - repeat exactly the same MP #1 from 6/14
  - K584001 - repeat w/ higher temp (A58+A64)
  - SO1003 - repeat w/ lower temp (A58+A59)
  - F. YFP + F. KanR - use #1 this time - switch primers... use A12+A14
    ↳ Check that primers (A15+A16) actually bind
  - F. CFP + F. KanR - use #2 - use A12+A14
    ↳ Check primers
  - F. YFP (A12+A14) - repeat the same. This really should work. MP #1 from 6/14

(two ways to do this)

Lunch: 1:00-2:00pm

- The plates that contain colonies that should have F. dCas9, Tes, and gRNA #1 have growth!! And they appear to NOT be red! (RFP is repressed)

	30	30	45s
	98°C, 46°C	55°C	58.6°C
	~~[illegible]~~	AE6MA69	
	#1	#2	#1

Gibson PCRs S0013, K584001, F CFP (B11+B12)

- [x] 2.5 µL Primer 1
- [x] 2.5 µL Primer 2
- [x] 1 µL of 1:50 dilution
- [x] 6.5 µL NFW
- [x] 12.5 µL MM

- Michael is washing the inoculations to image on a slide

- Panya and Elli and Alex are PCR Purifying
  - ↳ See Panya's NB and Elli's NB for nanodrops

- Tried a different imaging technique with Michael resuspending the cells in water from an inoculation pellet

- Andy, John, and Joe are imaging the (hopefully) repressed RFP from the FdCas9 transformed cells

- Taylor DpnI'd the above PCRs

- Taylor and Elli are PCR Purifying these DpnI'd PCRs

To assemble
[illegible]
S0013 + YFP (B8+B2)
K584001 + YFP (B11+B12) } YFP and CFP might be labelled "F YFP"/"F
K584001 + CFP (B11+B12) }

Everything [illegible] things not assembled with should be [illegible] [illegible] [illegible]

[illegible] [illegible]
[illegible]
[illegible]
[illegible]
[illegible]
[illegible] [illegible] Amp [illegible] plates
[illegible]

[illegible] cells [illegible] box

- Taylor and Elli are doing the Gibsons

- When we imaged the cells with Tos+dCas9+gRNA#1, there was no red fluorescence! The ones that were just Tos+dCas9 were definitely red.

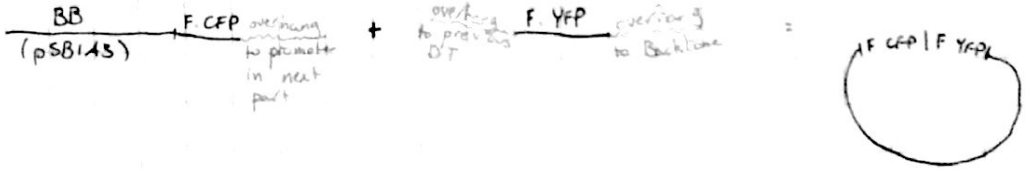
- Taylor, Elli, and Joe will transform

7/16/2015
Time in 10:00 AM
Time out 30 min

Gibson PCR - to make G²: CFP+YFP

	F. CFP + BB	F. YFP	F. YFP + BB	F. CFP
☑ 2.5 µL Primer 1	A12	A15	A12	A15
☑ 2.5 µL Primer 2	A14	A16	A14	A16
☑ 1 µL of 1:100 dilution	#1	#1	#1	#1
☑ 6.5 µL NFW				
☑ 12.5 µL MM				
Annealing temp	60°C	63°C	60°C	63°C

We're going to try (again) to remake G² CFP+YFP with the method we used to make F. CFP/YFP + F. KanR (see pg 76):



- Our cells containing Tos+dCas9+gRNA, after sitting overnight at 4°C, appear noticeably red. We're investigating potential explanations.

- John and Panya have begun making Electrocompetent cells from our cells containing ~~Tos+dCas9~~ ECNR2 cells

  ↳ Oops... they put it in a shaker at 37°C, when ECNR2 should only be grown at 30°C

DpnI:

- ☑ 2 µL CutSmart (10x)
- ☑ 10 µL PCR products
- ☑ 7.5 µL NFW
- ☑ 0.5 µL DpnI

F CFP: 1125 ✓
F YFP + BB:
F YFP: 1125 ✓
F CFP + BB

In the past, we've tried to gel purify, but it's yielded very poor results. This time we're going to just PCR Purify.

Lunch: 2:30 - 3:30

PCR Purification

- [x] Add 100µL Buffer PB
- [x] Place column into 2mL collection tube
- [x] Apply sample to column. Centr 60sec at 13,000rpm
- [x] Wash with 750µL Buffer PE. Centr 60sec.
- [x] Centr 60sec to dry
- [x] Place in clean, labelled tube
- [x] Add 30µL EB. Let sit 1 minute.
- [x] Centrifuge 60sec.

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
F YFP BB	Default	7/9/2015	4:44 PM	18.19	0.364	0.255	1.43	0.90
F CFP	Default	7/9/2015	4:44 PM	22.60	0.452	0.279	1.62	1.74
F CFP BB	Default	7/9/2015	4:45 PM	39.59	0.792	0.499	1.59	0.81
F YFP	Default	7/9/2015	4:45 PM	55.74	1.115	0.687	1.62	0.87

- Joe and John are making chemically competent cells with Tos + dCas9
- Elli ordered things for outreach
- Panya miniprepped: J04450 on Tet, I6031+YFP, I6031+CFP
- Elli resuspended the (bad) Elowitz primers, and the gBlocks on the integration suite that arrived today.
- Elli and Panya are setting up Gibson Assemblies to make G1: CFPYFP
- John, Joe, and I set up experiments to test why our dCas9 cells were not repressed (see next page)

Experiments/Tests for dCas9:

1) Everyone double check Susan sheets
2) Put half of the plates from 7/7/2015 (came out of incubator today, mostly white - ~~red~~ slightly pink) at 4°C, leave some at RT - if ones at 4°C turn red (more red, or red faster), then we know temp affects either dCas9 or gRNA... or perhaps slows down degradation of RFP
3) Take plates with cells containing the "triad" (Tos+dCas9+gRNA) that were now red out of the fridge, store at RT, and see if they become white again (as dCas9 becomes active again)
4) Take ~~either~~ a reporter protein that's under J23119 (dCas9's promoter), and store at 4°C and RT - if decreased expression at 4°C, dCas9 might be less well expressed at 4°C.
5) Retransform:
   - Tos + gRNA (assume dCas9)
   - Tos + generic thing on Chlor (assume dCas9)
   - Tos + gRNA + dCas9
   - Tos + generic chlor + dCas9

- Taylor is setting up RDs to check:
  - F. YFP + F. ~~Kan~~R
  - F. CFP + F. KanR
  - I6031 + YFP
  - I6031 + CFP

Panya's MPs:

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
I6031 YFP MP1	Default	7/9/2015	6:20 PM	387.86	7.757	4.269	1.82	1.85
I6031 YFP MP2	Default	7/9/2015	6:21 PM	231.42	4.628	2.489	1.86	2.00
I6031 CFP MP1	Default	7/9/2015	6:22 PM	387.71	7.754	4.190	1.85	2.35
I6031 CFP MP2	Default	7/9/2015	6:22 PM	379.05	7.581	4.074	1.86	2.37
J04450 tet MP1	Default	7/9/2015	6:23 PM	129.87	2.597	1.371	1.89	2.50
J04450 tet MP2	Default	7/9/2015	6:24 PM	140.59	2.812	1.502	1.87	2.32

Dinner 8:00PM - 9:50PM

- Jee is setting up glycerols

Transformation - with Taylor. G²: F. CFP + F. YFP, G³: F. YFP + F CFP

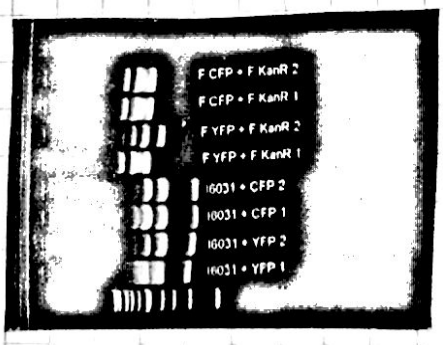
- [x] Thaw cells on ice. Aliquot 25 µL/tube.
- [x] Add 4 µL Gibson Assembly. Set heat block.
- [x] Incubate 30 min on ice
- [x] Heat shock at 42°C for 30s
- [x] Place on ice for 5 min
- [x] Pipette 450 µL SOC (RT) into mixture. Prewarm plates.
- [x] Incubate 60 min at 37°C, 250 rpm
- [x] Mix cells by flicking/inverting
- [x] Spread 20 µL/200 µL onto plates
- [x] Incubate overnight at 37°C

- Joe, Taylor, and Ell are doing chemical transformations with the new chemically competent dCas9 + TOS cells with:
  - gRNA #1 MP#2
  - DT (B0015) MP #1 from last year

Gel:

Ladder | I6031 + YFP1 | I6031 + YFP2 | I6031 + CFP1 | I6031 + CFP2 | F YFP + F KanR1 | F YFP + F KanR2 | FCFP + F KanR1 | FCFP +

Run this way ↓



The F. CFP + F. KanR and F. YFP + F. KanR have had one cut to make the plasmid linear, and should be ~4460 bp

The I6031 + CFP/YFP was cut to excise the insert, and should have two bands ~2160 and ~980.

Both things could conceivably be correct, though the F. CFP/YFP + F. KanR has strangely large bands. Also, it's impossible to tell if the insert correctly assembled, or if it's just the template DNA.

- Ell is inoculating yesterday's assemblies that grew:
  - Solo tet + F IA3, I6066+CFP, Sol003 + YFP, K584001 + CFP, I6088+CFP, K58400

Only ones w/ many colonies

- We're replating the cells from yesterday's transformations that failed.

Preliminary dCas9 results

- Yesterday, we took half of the dCas9 triad plates from 7/7/2015 and put them into the fridge, and kept half at RT
- The ones in the fridge are extremely red
- Those left at RT are very white

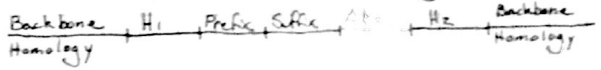
We have a few hypotheses about what's going on:

1) The gRNAs do not form the correct secondary structure at 4°C, and thus stop repressing
2) J23119, the constitutive promoter that dCas9 is under, is affected by temperature. (We're testing this with Test #4 on pg 91)
3) The RFP is degraded much more slowly at 4°C, and builds up very quickly, while the gRNA is still degraded quickly.

Joe found support in literature for both hypotheses 1 and 2

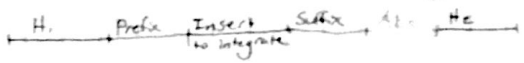
Changing gears....

We've ordered gBlocks for the integration suite that look like:

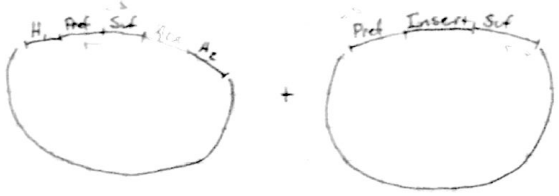


(these gBlocks will be assembled onto Chlor Backbone)

So that to integrate, you only need to put your desired insert between the prefix/suffix, and PCR up the regions of homology inward, so that we have



To do the assembly to put the insert into the gBlock integration plasmid:



So that the region of homology b/w insert and backbone is the prefix and suffix.

However, we need to test that these PCRs will actually work, since Pref/Suf tend to bind to themselves/each other

PCR to test primers

- Template = J04450 on Chlor

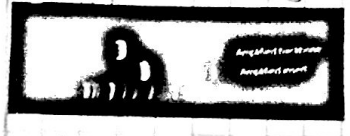
	Amplify backbone	Amplify insert
2.5 µL Primer 1	A36 (2014) - Pst rev	A26 (2014) - Pst fwd
2.5 µL Primer 2	A37 (2014) - Sal fwd	A27 (2014) - Sal rev
1 µL 1:100 dilution		
6.5 µL NFW		
12.5 µL MM		
Annealing temp	57.1°C	57.1°C

Making Chlor Plates

- 5g Tryptone
- 5g NaCl
- 2.5g Yeast extract
- 10g Agar
- Water to 0.5 L

Left for meeting 1:00pm - 2:00pm

Ran a gel of test PCRs:



Everything looks great! These primers should be able to ~~integrate~~ assemble things to integrate!

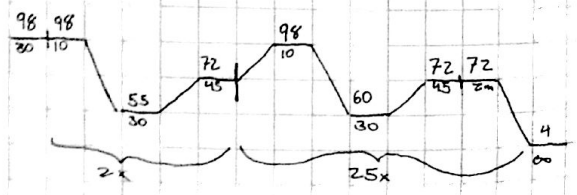
Lunch: 3:00-3:30pm

- Michael is looking at sequence data
- Joe and I inoculated ECNR2 and empty cells to make electrocompetent tomorrow
- Elli is miniprepping, John is making Glycerol stocks

Test PCRs of Elowitz integration primers

- Template:

- 2.5 µL Primer 1
- 2.5 µL Primer 2
- 1 µL 1:100 dilution
- 6.5 µL NFW
- 12.5 µL MM



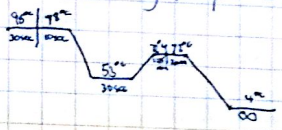
Inoculation + Diagnostic Colony PCR

✓	A1	F. YFP BB + F. CFP #1 In1	B1	F. CFP BB + F. YFP #1 In1	✓
✓	A2	" " #1 In2	B2	" " In2	✓
✓	A3	" " In3	B3	" " In3	✓
✓	A4	F. YFP BB + FCFP #2 In1	B4	F. CFP BB + F. YFP #2 In1	✓
✓	A5	" " In2	B5	" " #2 In2	✓
✓	A6	" " In3	B6	" " In3	✓

Note: #1 and #2 for each are from the same plate. Each plate has 6 inoculations/PCRs, split into two groups of three.

- Inoculated the above

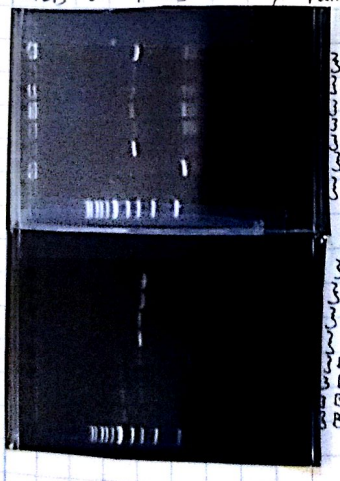
Annealing temp: 53°C



Ell:s MPs:

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
I6066 CFP MP1	Default	7/10/2015	6:12 PM	23.86	0.477	0.223	2.14	1.15
I6066 CFP MP2	Default	7/10/2015	6:13 PM	25.26	0.505	0.319	1.58	1.29
S01003 YFP MP1	Default	7/10/2015	6:14 PM	12.88	0.258	0.112	2.31	1.07
S01003 YFP MP2	Default	7/10/2015	6:15 PM	21.24	0.425	0.215	1.98	1.13
K587001 CFP MP1	Default	7/10/2015	6:17 PM	7.13	0.143	0.074	1.94	0.97
K587001 CFP MP2	Default	7/10/2015	6:18 PM	20.15	0.403	0.191	2.11	1.17
I6088 CFP MP1	Default	7/10/2015	6:19 PM	20.91	0.418	0.222	1.89	1.15
I6088 CFP MP2	Default	7/10/2015	6:20 PM	23.82	0.476	0.243	1.96	1.26
K584001 YFP MP1	Default	7/10/2015	6:21 PM	34.10	0.682	0.358	1.90	1.40

Gels of PCRs (run by Panya + Ell:)



CFP+YFP should be ~2100bp.
I'm unsure what the band at ~1700bp is.
B3-B6 could conceivably have correct bands.

Miniprep: A3-A5, B3-B6

The Elowitz integration primers worked great! On both Amp and Chlor.

7/11/2015
Time in: 1:15pm
Time out: 10:45am

- John, Joe, Taylor, Michael, and I have been making electrocompetent
  ↳ See Joe's notebook for protocol.

- Michael is miniprepping last night's inoculations of G' CFP YFP

- Re-designed all gRNA gBlocks to be under promoter R0040.
  - R0040 has no PAM sequence, and is thus not repressable
  - It is TetR protein repressable
  - None of the gRNAs for other promoters have homology to it, and thus won't bind

- We had also wanted to also have all of the gRNAs under an inducible promoter, but could not find a promoter that was both short, and inducible by something very accessable to us.

- Lunch: 3:00 pm - 4:00pm

- Continued making electrocompetent ECNR2 and blank cells

Michael miniprepped:

Sample ID		User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
FYFP bb-FCFP assembly 1	F	Default	7/11/2015	6:50 PM	132.98	2.660	1.358	1.96	2.27
FYFP bb-FCFP assembly 2 MP 1	F	Default	7/11/2015	6:51 PM	261.19	5.224	2.737	1.91	2.24
FYFP bb-FCFP assembly 2 MP 2	F	Default	7/11/2015	6:52 PM	314.03	6.281	3.308	1.90	2.26
FCFP bb-FYFP assembly 1	FR	Default	7/11/2015	6:53 PM	264.59	5.292	2.734	1.94	2.24
FCFP bb-FYFP assembly 2 MP 1	FR	Default	7/11/2015	6:54 PM	511.22	10.224	5.465	1.87	2.21
FCFP bb-FYFP assembly 2 MP 2	FR	Default	7/11/2015	6:54 PM	385.82	7.716	4.073	1.89	2.22
FCFP bb-FYFP assembly 2 MP 3	FR	Default	7/11/2015	6:55 PM	402.50	8.050	4.259	1.89	2.20

- Made glycerol stocks

- Washed so MUCH glassware

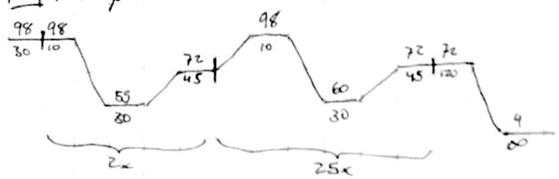
We're going to try to follow a modified version of Open Wetwares protocol for integration The basic steps are going to be:

- Make electrocompetent ECNR2 cells (done)
- PCR amplify linear fragment to integrate
- Verify PCR on a gel
- PCR purify (Harvard protocol) - Elute in H_2O!
- Add 50-200ng of PCR product (Harvard) or 5pg - 0.5μg of PCR product (Open Wetware) to cells
- Electroporate
- Incubate 60-120 min w/ moderate shaking at 37°C
- Pre warm selection plates
- Plate on selection plates, and let grow overnight

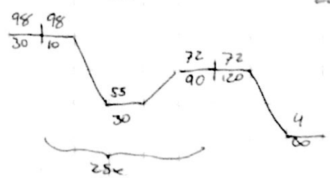
<u>Integration PCR</u> - We're going to try just using Q5 for now

- Going to try to integrate: F KanR, F CFP+KanR, ~~F YFP+F KanR~~
  150703 #1, F CFP+F Kan #1, F YFP+F Kan #1 — This doesn't do anything useful

- [x] 2.5 μL Primer 1
- [x] 2.5 μL Primer 2
- [x] 1 μL of 1:100 dilution
- [x] 6.5 μL NFW
- [ ] 12.5 μL MM



F KanR Phage lab



F CFP+F KanR Bradley lab-did not allow multiple cycles

Breakfast: 12:00 - 2:00 PM

Ran a gel of PCRs

Joe is DpnI-ing the PCRs. Although no protocol says to do this, we really don't want template plasmid.

F KanR intC	Default	7/12/2015	4:53 PM	0.99	0.020	0.013	-1.32	1.11
F KanR intC	Default	7/12/2015	4:54 PM	1.81	0.038	0.011	3.37	0.93
F KanR galK	Default	7/12/2015	4:55 PM	1.09	0.022	0.003	-7.46	0.42
F CFP galK	Default	7/12/2015	4:56 PM	1.78	0.036	0.002	16.91	0.64

- These nano drops don't look good Harvard's protocol says to use 50-200 ng, which will be very difficult however the Green Methane protocol only calls for 5pg - 0.5µg of PCR product We should be fine to proceed.

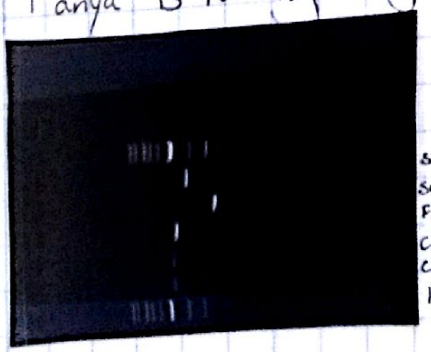
Saha Lab 6:00PM - 8:30PM

- Joe discovered that we incorrectly made our electrocompetent ECNR2 cells
  - We inoculated to remake these cells.
- Obviously, we can't continue with integration today.
- Michael and Taylor are re-setting up PCRs
  - solo TetR } To make F. TetR
  - F. IC3 }
  - solo IC3 } To put the integration gBlocks into IC3
  - int C F.CFP + F.KanR } To try to integrate tomorrow evening
  - galK F.CFP + F.KanR }

Making Electrocomp ECNR2 (for real this time) - Done by Joe, Taylor, Ell

- [ ] Take culture grown in LB at midlog, place in water bath at 42°C for 15 min to activate lambda red machinery
- [ ] Add 1-1.5 mL of mid-log cells in culture to a 1.5mL tube. Spin in centrifuge for 1 min at 15,000 rpm (max speed)
- [ ] Do EVERYTHING on ice!
- [ ] Remove liquid by decanting + pipetting - don't disturb pellet!
- [ ] Wash with 1 mL cold water, resuspend cells
  - [ ] Centr for 1 min at max speed
- [ ] Repeat wash step
- [ ] Resuspend cells with 50 µL cold water
- [ ] Add 50-200ng of insertion construct to the resuspended cells
- [ ] Place in chilled cuvette
- [ ] Set electroporation machine to "Ec01, 1.8 volts" Time constant: 4.8ms
- [ ] Dry cuvette
- [ ] Electroporate!
- [ ] Immediately place 1mL of LB in cuvette, pipette up + down to mix
- [ ] Place bacteria into 2mL of LB in a culture tube; allow them to grow for 2-3 hours at 30°C
- [ ] After 3 hours, plate culture on selection plates

- Panya is running a gel of last nights PCRs



The CFP+KanR PCRs do not look very bright. Everything is the correct size.

- Since yesterday's PCRs to integrate had very low concentrations we'll repeat them today
- Panya is setting up Dpnt of PCRs

Re-PCR - To integrate F. KanR. Done exactly as on pg 97

- [x] 2.5 µL P1
- [x] 2.5 µL P2
- [x] 1 µL of 1:100 dilution
- [x] 6.5 µL sdd
- [x] 12.5 µL MM

- Helped Panya PCR-Purify samples from last

DpnI of Re-PCRs

- [x] 2 µL of CutSmart (10x)
- [x] 10 µL PCR Product
- [x] 7.5 µL NFW
- [x] 0.5 µL DpnI

The re-A

PCR Purifications

	Sample ID	User ID	Date	Time	ng/ul	A
	solo tetR pure	Default	7/13/2015	1:30 PM	50.03	1
	f1C3 pure	Default	7/13/2015	1:31 PM	45.59	0
	solo 1C3 pure	Default	7/13/2015	1:32 PM	47.51	0
1	fCFP fKanR intC pure	Default	7/13/2015	1:34 PM	48.78	0
2	fCFP fKanR galK pure	Default	7/13/2015	1:36 PM	25.62	0

Lunch: 2:10 - 2:50

PCR Purifications

- [x] Add 100 µL Buffer PB to PCR rxn Mix.
- [x] Apply sample to spin column Centr 60s; disc
- [x] Add 750 µL Buffer PE to col Centr 60s; d
- [x] Centr 60s to dry
- [x] Place col in clean, labelled tube
- [x] Add 30 µL H_2O, let stand 1 min
- [x] Centr 60s

	Sample ID	User ID	Date	Time	ng/ul	A260
3	F KanR intC	Default	7/13/2015	3:59 PM	35.96	0.719
4	F KanR galK	Default	7/13/2015	3:59 PM	53.26	1.065

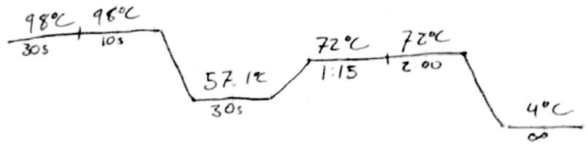
We're going to integrate with today's/last night's integra

- Panya and Elli set up Gibsons of solo TetR + F 1C3, gBlocks into 1C3

- Panya and Elli are transforming assemblies, an

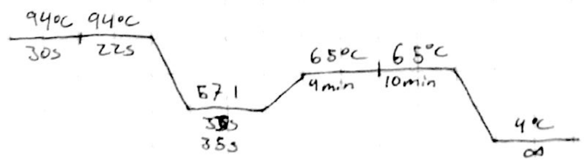
Gibson PCR - to ~~assemble~~ PUT (R0010) F dCas9 into pSB1C3

	solo pSB1C3	~~F dCas9~~ → Needs Long Amp
☑ 2.5 μL Primer 1	A36 (2014)	~~A26 (2014)~~
☑ 2.5 μL Primer 2	A37 (2014)	~~A27 (2014)~~
☑ 1 μL 1:100 dilution	#1	~~#3~~ ~~#4~~
☑ 6.5 μL NFW		
☑ 12.5 μL MM		
Annealing temp:	57.1°C	~~57.1°C~~



Long Amp of F. dCas9

- ☑ 5 μL Buffer
- ☑ 0.75 μL dNTP
- ☑ 1 μL ~~A55~~ A26 (2014)
- ☑ 1 μL A27 (2014)
- ☑ 1.25 μL DNA
- ☑ 1 μL Long Amp Taq
- ☑ 15 μL NFW



30 cycles

Lunch: 2:25 pm - 3:10 pm

- Imp primers arrived!

- Joe and Panya set up these IMP PCRs →

Imp Primers to Amplify

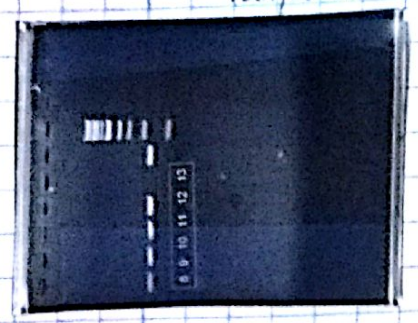
Don't have MM {
- K823005 pSB1C3 + J23101 → B23 + A37 (2014)* - 50°C → 1:30
- I13504 for J23101 → B24 + B25 - 55°C → 30s
}

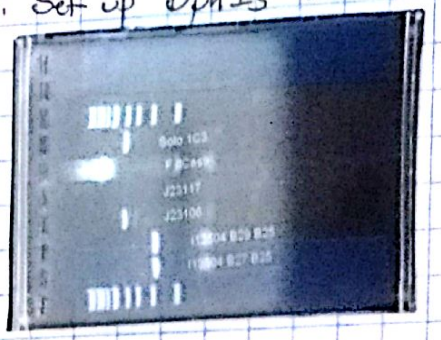
- K823008 pSB1C3 + J23106 → B26 + A37 (2014)* - 50°C → 1:30
- I13504 for J23106 → B27 + B25 - 55°C → 30s

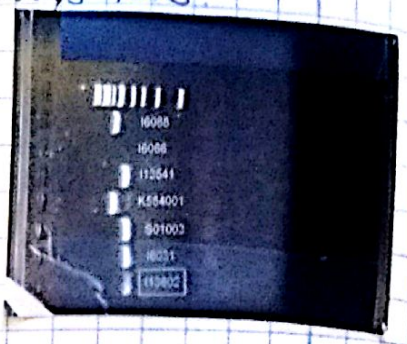
- K823013 pSB1C3 + J23117 → B28 + A37 (2014)* - 50°C → 1:30
- I13504 for J23117 → B29 + B25 - 55°C → 30s

Sequence data confirmed that we did not have any of our assembled Toss ||

- Elli and I set up PCRs to remake these Toss (see Elli's notebook)
- Panya and Elli set up DpnIs of all of today's PCRs.







Dinner
8:00-9:45PM

PCR Purifications of things that worked

- [x] Add 100µL Buffer PB
- [x] Apply to spin col, Centr 60s; discard flowthrough
- [x] Add 750µL Buffer PE. Centr 60s; discard flowthrough
- [x] Centr 60s to dry
- [x] Place col in clean labelled tube
- [ ] Add 30µL Buffer EB. Let stand 1 min
- [ ] Centr. 60s

Gibsons that WILL proceed

- F dCas9 → pSB1C3
- J23106 + I13504 (B27 + B25) — Accidentally used 2x as much backbone as insert
- I13602 + YFP (B5 + B6)
- I6031 + CFP (B1 + B2)
- S01003 + YFP (B1 + B2)
- ~~K584001 + YFP (B11 + B12)~~
- I13541 + YFP (B5 + B6)
- I6088 + CFP (B1 + B2)
- I6081 + YFP (B1 + B2)
- K584001 + F CFP (B11 + B12)
- I13541 + CFP (B5 + B6)

Gibsons that WON'T proceed

- K584001 + F YFP (B11 + B12)*
- J23117* + I13504 (B29 + B25)*
- I6066* + CFP (B5 + B6)

* Joe and Taylor are re-doing these PCRs for tomorrow

- Elli and I are doing these Gibsons. We're reducing the reaction volume (x ½) to save master mix
  ↳ See Elli's notebook for calculations (pg 64)
- Taylor + Elli are transforming the assemblies

Integration PCR

2/5/2015
Time in: 10:15am
Time out: 11:45pm

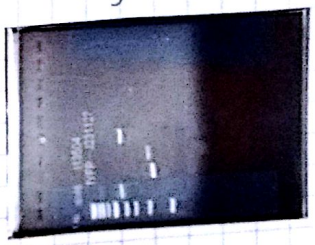
- Going to retry PCRs from pg 97 exactly the same

- [ ] 2.5μL Primer 1
- [ ] 2.5μL Primer 2
- [ ] 1μL of 1:100 dilution
- [x] 6.5μL NFW
- [ ] 12.5μL MM

} John finished doing this

- When Panya and I tried to start making electrocomp ECNR2, we found that our ODs (600nm) were surprisingly low (.28). We double checked in Forsyth (.46) and Rebscher (.60). The inconsistency is not great, but since they're close to/in midlog for ~~most~~ all three readings, we're going to put them in for ~1 hour, and then just proceed.
  Time in: 12:10

Gel of last night's PCRs:
- Everything looks great!
We'll do these assemblies, as well as re-assemble F. TetR from PCR Purifications from a few days ago.



- John DpnI'd last night's PCRs
- The integration PCRs all were not present on the gel (but ladder was). John is repeating the PCR.
- Joe is resuspending primers that arrived: primers to integrate from the integration suite.

PCR Purifications: - Started by me, finished by John

- [x] Add 100μL Buffer PB
- [x] Apply to spin col. Centr. 60s; discard flowthrough
- [x] Add 750μL Buffer PE. Centr 60s; discard flowthrough
- [x] Centr. 60s to dry
- [x] Place col in clean, labelled tube
- [J] Add 30μL Buffer EB Let Stand 1min
- [J] Centr 60 s

Lunch: 2:10 - 3:00PM

Gibsons For Today
- K584001 + F.YFP (B11 + B12)
- J23117 + I13504 (B29+B25) } From last night's PCRs
- I6066 + CFP (B5 + B6)
- solo TetR + F. pSB1C3 } From PCRs on 7/13

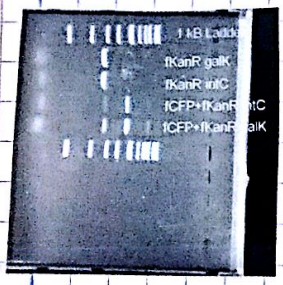
See Elli's notebook for volumes. (pg 66)

	Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
1,2	Visualized CFP+YFP 1	Default	7/15/2015	3:16 PM	40.30	0.806	0.437	1.84	2.17
3,4	Visualized CFP+YFP 2	Default	7/15/2015	3:17 PM	32.38	0.648	0.353	1.83	2.16
5,6	Visualized CFP+YFP 3	Default	7/15/2015	3:17 PM	44.91	0.898	0.491	1.83	1.81

↳ Panya miniprepped inoculations of culture that ~~was~~ appeared, on the confocal, as blue and yellow

- Elli and I did the Gibson Assemblies

- We've re-diluted our cultures of ECNR2 and put them back at 30°C (Andy says that the cells need to be constantly growing to stay at Mid Log, where their membranes are most permeable)



← Gel of PCRs to integrate

- John and Alex set up DpnI's of the integration PCRS, to make sure that template plasmid doesn't transform in, instead of PCR product

- John and I PCR purified these integration PCRs

- Joe and Taylor are making electrocompetent ECNR2 by following the Harvard iGEM 2011 protocol

- Panya and Elli are transforming the Gibsons

- Panya is inoculating yesterday's transformations.

Dinner: 9:00pm - 10:45 PM

- Plated transformations w/ Taylor

7/16/2015
Time in: 10
Time out: 2

Inoculations that Panya set up yesterday (from 7/14 assemblies):

- I13504 + J23106
- F dCas9 into pSB1C3
- I13541 + YFP
- I6031 + CFP
- Sol003 + YFP

Plates with no growth:

- I3602 + YFP
- I6088 + CFP
- I6031 + YFP
- K584001 + F. CFP
- I13541 + CFP

Plates from yesterday's assemblies with growth:

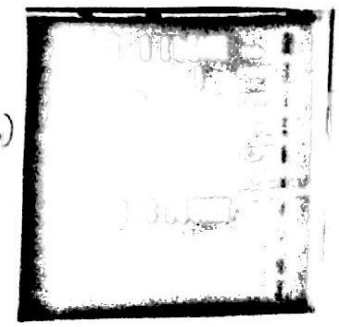
- I6066 + CFP
- J23117 + I13504
- K584001 + F. YFP
- F. TetR (ONLY one colony)

Things that grew, but failed (at the sequencing steps), from 7/6 assembly:

- I6066 + CFP
- I6088 + CFP } Re-assemble today
- K584001 + YFP
- K584001 + CFP } Re-assemble today
- Sol003 + YFP

→ Tested Michael's imaging protocol

- John and Andy PCR'd up to re-assembly the things above
- Primers arrived! John and Andy resuspended, and did the PCR with these primers to make the backbone to assemble the integrator gBlocks into pSB1C3



Lunch: 1:30 PM - 2:30 pm

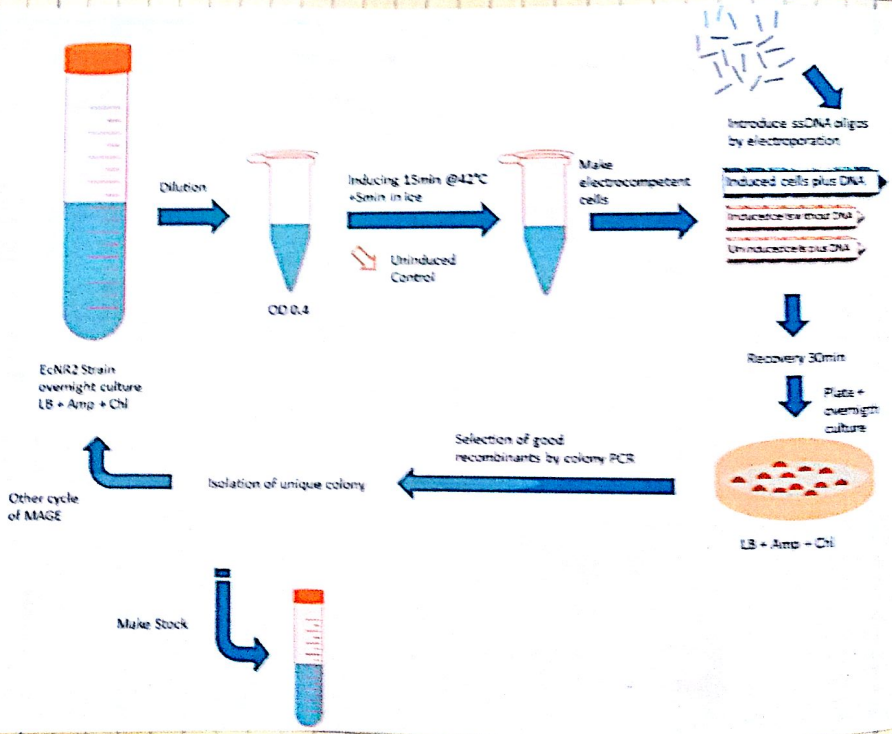
John is re-PCR-ing K584001 and IC3 (no PS)
↳ Gel looks good!
Proceed with DpnI

DpnI Gibson PCRs

- [x] 2µL CutSmart (10x)
- [x] 12.5µL PCR Products
- [x] 0.5µL DpnI

- Bill / Panya PCR purified the Gibson PCRs
- Organized plates in 4°C
- Bill and Michael Gibson assembled
- Andy and Michael transformed
- Joe, Taylor and I ~~have~~ tried integration again this time following the (largely similar to Harvard's) Ant-Bettenbourt protocol:

Andy set up integration PCRs (he ran out)



Prepare bacterial cultures

1. Inoculate EcNR2 strain from frozen glycerol stock or a single colony into 3 to 5 ml LB medium. Shake at 30° to 32°C overnight.
2. Add ~0.5 ml of the overnight culture to 35 ml of LB medium in a 250-ml (baffled) Erlenmeyer flask.
3. Place the flask in the 32°C shaking water bath and grow cells at 32°C with shaking for ~2 hr

The cells are ready when the A600 is between 0.4 and 0.6. It is important not to over-grow the cells, since stationary phase cells do not express the recombination functions well.

Induce recombination functions

1. Transfer half the culture to a 125-ml (baffled) Erlenmeyer flask and place that flask in the 42°C water bath. Shake 15 min at 220 rpm to induce. Leave the remainder of the culture at 32°C, this will be used as the uninduced control that lacks recombination activity. While the cells are inducing, fill an ice bucket with an ice-water slurry - Aliquotted into 1.5 ml tubes, put into heat block
2. Immediately after inducing for 15 min at 42°C, rapidly cool the flask in the icewater slurry with gentle swirling. Leave on ice for >5 min. Follow the same cooling protocol with the uninduced 32°C culture. While the cells are on ice, precool the centrifuge to 4°C and chill the necessary number of 35- to 50-ml plastic centrifuge tubes, labeled for induced and uninduced cells.

Make electrocompetent cells

1. Transfer both the induced and uninduced cultures to the appropriately labeled chilled 35- to 50-ml centrifuge tubes. Centrifuge 7 min at 4600g (6700 rpm in a Sorvall SA-600 rotor), 4°C. Aspirate or pour off supernatant. - 6900 rpm in table top centr
2. Add 1 ml ice-cold distilled water to the cell pellet in the bottom of each tube and gently resuspend cells with a large pipet tip (do not vortex). Add another 30 ml ice-cold distilled water to each tube, seal, and gently invert to mix, again without vortexing. Centrifuge tubes again as in last step

All subsequent resuspensions of cells should be done gently and without vortexing. Preparation of the cells for electroporation washes out any added chemical inducing agent.

3. Decant the 30-ml supernatant very carefully from the soft pellet in each tube and resuspend each cell pellet in 1 ml ice-cold distilled water.

Remove tubes from the centrifuge promptly. The pellet is very soft and care should be taken not to dislodge it, especially when processing multiple tubes.

4. Transfer resuspended cells to microcentrifuge tubes. Microcentrifuge 30 to 60 sec at maximum speed, 4°C. Carefully aspirate supernatant. In each of the tubes, resuspend the cell pellet in 200 ~~µl cold distilled water~~, which will provide enough material for ~~four or five~~ electroporations. 50µL cold sdd + PCR product to be transformed in

Electrocompetent cells can be stored at −80°C after resuspending the cell pellet in 15% (v/v) glycerol. For highest efficiency, use freshly processed cells.

Introduce DNA by electroporation

1. Chill the desired number of 0.1-cm electroporation cuvettes on ice. Turn on the electroporator and set to 1.80 kV
2. In microcentrifuge tubes on ice, 100 ng of single-stranded oligonucleotide with 50 to 100 µl of the suspension of induced or uninduced cells. Do the mixing and subsequent electroporation rapidly; do not leave the DNA-cell mixes on ice for extended periods. Be sure to include the following electroporation reactions and controls:
   1. Induced cells plus DNA.

      This is the culture that should yield the designed recombinants.

   X 2. Induced cells without DNA

      This is a control to identify contamination, determine the reversion frequency, and obtain some idea of the efficiency of the selection.

   X 3. Uninduced cells plus DNA.

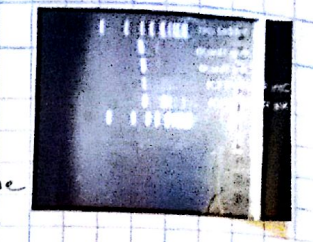
      This control tells whether there is some contaminating factor in the DNA that is contributing to the selected colonies.

3. Introduce the DNA into the cells by electroporation.

The time constant should be greater than 5 msec for optimal results. Low time constants indicate problems with the cells, the DNA, or even the

7/17/2015
Time in 11:15AM
Time out 10:50PM

Gel of last night's integration PCRs:



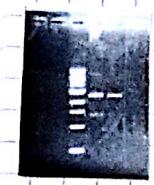
The FCFP has strong bands at the wrong size. We're re-doing these PCRs.

Elli + Joe →

Dinner: 8:15-9:30

- John is getting data of the noise from the successful plasmid assembly of G2: CFP+ YFP
- Joe is diluting the EcNR2 culture that we grew up overnight in low salt LB
- Michael is filling out UGA's collaboration form for imaging their cells
- Panya went to Swem to work on the wiki

Lunch: 2:40-3:25 PM



Re-PCRs of FCFP+F.KanR
Look good!

- A "reporter" from the school came and took pictures of us
- Made more amp (protocol on pg 7: 1g Amp → 10mL dH_2O)
- John set up DpnIs of F KanR gelK/unit and the re-PCR'd F.CFP+F.KanR
- Panya and Elli miniprepped yesterday's inoculations
  ↳ Michael made glycerols

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
K584001 + f.YFP MP1	Default	7/17/2015	4:38 PM	76.75	1.535	0.844	1.82	1.18
K584001 + f.YFP MP2	Default	7/17/2015	4:39 PM	40.94	0.819	0.553	1.48	0.66
I6066 + CFP MP1	Default	7/17/2015	4:40 PM	88.79	1.776	0.992	1.79	1.14
I6066 + CFP MP2	Default	7/17/2015	4:41 PM	111.41	2.228	1.282	1.74	1.02
J23117 + I13504 MP1	Default	7/17/2015	4:42 PM	94.89	1.898	1.116	1.70	1.02
J23117 + I13504 MP2	Default	7/17/2015	4:42 PM	204.55	4.091	2.248	1.82	1.53
fTetR MP1	Default	7/17/2015	4:43 PM	59.63	1.193	0.704	1.69	0.81

Lunch 2:40-3:20

- John DpnI'd F.CFP+F.KanR
- Someone PCP purified these integration PCRs (see next page)
- Panya inoculated yesterday's transformations

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
fKanR intC	Default	7/17/2015	6:04 PM	46.64	0.933	0.553	1.69	1.35
fKanR galK	Default	7/17/2015	6:04 PM	39.84	0.797	0.466	1.71	2.12
fCFP+fKanR intC	Default	7/17/2015	6:05 PM	14.42	0.288	0.189	1.53	1.87
fCFP+fKanR galK	Default	7/17/2015	6:06 PM	38.46	0.769	0.477	1.61	1.20

- John, Taylor, Elli and I followed the Paris-Bettencourt integration protocol (see John's notebook) to try to integrate F.CFP + F. KanR
  ↳ We have colonies on the plates from 150715's F KanR integrations, so we are not trying to integrate those

150718
Time: 9:15 AM
Time out: 6:22 PM

- Helping Elli and Panya with their Girl Scout event
- John and Andy are miniprepping yesterday's inoculations

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
galK empty Integration Suite MP 1	Default	7/18/2015	4:17 PM	114.07	2.281	1.202	1.90	2.30
galK empty Integration Suite MP 2	Default	7/18/2015	4:21 PM	48.99	0.980	0.522	1.88	2.29
I6088+CFP MP 1	Default	7/18/2015	4:22 PM	240.34	4.807	2.533	1.90	2.23
I6088+CFP MP 2	Default	7/18/2015	4:22 PM	135.33	2.707	1.482	1.83	1.43
K584001+CFP MP 1	Default	7/18/2015	4:23 PM	36.32	0.726	0.368	1.97	2.11
K584001 + CFP MP 2	Default	7/18/2015	4:24 PM	189.46	3.789	2.046	1.85	2.10
K823005 from kit MP 1	Default	7/18/2015	4:25 PM	162.66	3.253	1.709	1.90	2.29
K823005 from kit MP 2	Default	7/18/2015	4:25 PM	106.62	2.132	1.136	1.88	2.35

F ? R!

- Growth on our F.CFP + F. KanR integration plates from yesterday! The colonies (grown only on Kan, and not Kan Chlor Amp) seem a little fuzzy around the edges. That said, we inoculated 7 colonies from each intC and galK (5 Kan only, 2 Kan Chlor Amp)

7/19/2015
Time: 7 a/pm
11:50pm

- Joe and John set up PCRs to assemble:
  - F. YFP into intC integration suite
  - F. YFP into galK integration suite
  - ALL IMPs (J23101/J23106/J23117) + I13504
  - R0062 (Soloo3) + YFP

- Elli Minipepped:

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
intC suite MP1	Default	7/19/2015	8:44 PM	3.52	0.070	0.025	2.84	1.94
intC suite MP2	Default	7/19/2015	8:44 PM	12.04	0.241	0.127	1.89	1.80

- Michael was able to see our integrated F.CFP on the confocal!! Both at intC and galk.

7/20/2015
Time in: 10:05AM
-Time out: 11:20 PM

- John is running last night's PCRs on a gel
- Joe is making Amp, Chlor, TetKan, AmpChlorTetKan
- Assemblies:
  - F. YFP (R0010) into intC integrator suite
  - F. YFP (R0010) into galK integrator suite
  - J23101 + I13504 (101)
  - J23106 + I13504 (106)
  - J23117 + I13504 (117)
  - R0062 (from S01003) + YFP (B1+B2)

} Round I

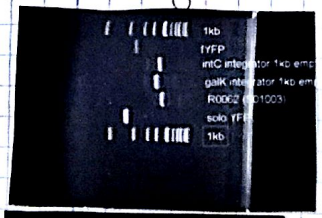
All of the gRNA gBlocks arrived!

- I0500 gRNA1 + IC3
- I13453 gRNA1 + IC3
- J23119 gRNA1 + IC3
- J23117 gRNA1 + IC3
- J23106 gRNA1 + IC3
- J23101 gRNA1 + IC3
- J23100 gRNA1 + IC3
- R0011 gRNA1 + IC3
- R0062 gRNA1 + IC3
- R0051 gRNA1 + IC3
- R0010 gRNA1 + IC3
- Scrambled gRNA1 + IC3

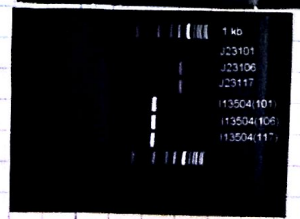
} Round II

Lunch: 12:05-1:15

- John is setting up PCRs to make solo pSB1C3 (as on pg 54) to assemble all of the gRNAs into



F. YFP: 1125 bp
intC integrator: ~3724 bp
galK integrator: ~3349 bp
R0062 (S01003): ~2688 bp
Solo YFP: 762 bp



J23101:
J23106:
J23117:
I13504(101): ~8806
I13504(106):
I13504(117).

Restriction Digest - Diagnostic of K823005, intC integrator, galK integrator

- ☑ 0.5 µL EcoRI
- ☑ 2 µL CutSmart
- ☑ 1 µg DNA
- ☑ add to 20 µL

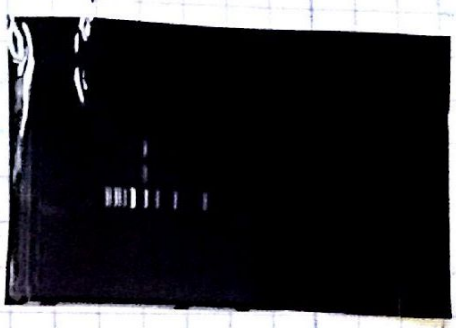
EcoRI

K823005	
#1	#2
~~6.17 µL~~ 10 µL	
11.33 µL	7.5 µL

intC integrator	
#1	#2
Concentrations too low	

galK integrator	
#1	#2
~~8.77 µL~~ 7.5 µL	
8.73 µL	0 µL

37°C for 1 hour



DpnI of pSB1C3 solo PCRs (for gRNAs)

- ☑ 17.5 µL PCR Product
- ☑ 2 µL CutSmart
- ☑ 0.5 µL DpnI

Gibsons: Round II (Reduced volume)

J23117+I13504	J23106+I13504	~~R0062~~+YFP	~~F.YFP+intC~~	F.YFP+galK
~~7.36~~ 7.326 µL I13504	~~0.66~~ 0.689 µL I13504	~~4.26~~ 2.640 µL YFP	~~3.64~~ 2.46 µL F.YFP	~~3.64~~ 2.932 µL F.YFP
1.47 µL J23117	~~4.56~~ 3.65 µL J23106	~~2.16~~ 1.44 µL R0062	~~3.51~~ 2.34 µL intC	~~1.72~~ 1.374 µL galK
~~7.15~~ 2.15 µL H_2O	~~4.58~~ 0.66 µL H_2O	~~3.58~~ .718 µL H_2O	~~2.80~~ 0.10 µL H_2O	~~4.59~~ 0.67 µL
5 µL H.F.	5 µL H.F.	5 µL H.F.	~~5 µL~~ H.F.	~~5 µL~~ H.F.

Diagnostic RD:

- K823005 ~ 2105 bp (incl. pSB1C3)
- galK on IC3 ~ 3349 bp (incl. pSB1C3)

- The K823005 (JZ3101 on IC3) is not too large, which is good!
- galK #1 is bad. galK #2 looks good. We're throwing out galK #1

Andy resuspended gBlocks

Andy set up Round II of Gibsons.

- Joe and I inoculated ~~4~~ from our cells containing RFP, GFP, CFP, and YFP for testing the plate reader
- Panya sent out sequences to Macrogen
- Joe made more low salt LB

Dinner: 6:40 - 7:30

- Team meeting
- Andy transformed the Gibson assemblies
- Taylor set up qPCRs (see Taylor's NB, pg 27)
- Elli inoculated to grow intC in IC3 from GS, things from kit to image

7/21/2015

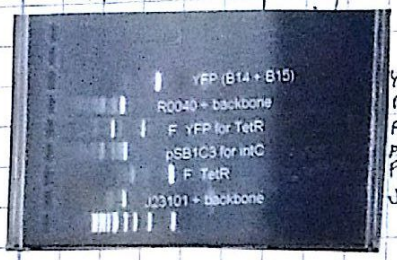
T Time in: 10:05 AM

T Time out: 11:00pm

PCRs that Taylor set up last night:

F. YFP - A12+A14 - 60°C - 1:45
F. TetR - A15+A16 - 63°C - 45sec
J23101 (IMP) - B23+A12 - 50°C - 1:30
pSB1C3 for intC - B34+B35 - 58°C - 1:30
R0040 (I13602) - 50.6°C - 1:30
YFP (E0032) - 60°C - 30sec

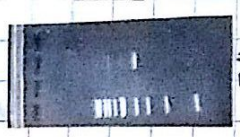
- We're running a gel of PCRs from last night:



YFP solo: 762 bp
R0040 + backbone: 2288bp
F. YFP: 1125 bp
pSB1C3 solo: 2070bp
F TetR 1554 bp
J23101 + backbone:

- I re-did the PCRs of F. YFP (63°C) for TetR, and pSB1C3 (58°C) for intC, with higher temperatures to try to get rid of ther extra bands.

- We're double checking the primers ordered on 7/1/2015, since A59 is not actually conserved



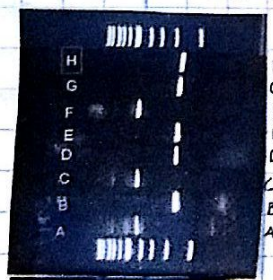
solo pSB1C3 ~ 2070 bp
F. YFP X

Dimers: 6:30-8:30

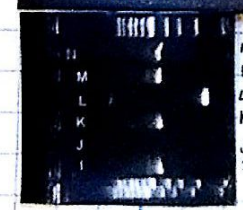
PCRs set up by Joe + Andy JJ

- I'm remaking the F YFP PCR, this time annealing at 61°C

- Set up DpnIs of PCRs from earlier that worked: F. TetR, J23101, pSB1C3 for intC

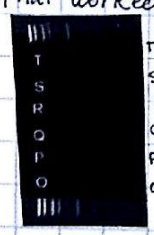


H: ~~R0040 (I13602)~~ F. YFP (B9+B10) ~770
G: F. CFP (B9+B10) ~770
F: I13543 (I13541) ~2364
E: F. YFP (B3+B4) ~770
D: F. CFP (B3+B4) ~770
C: R0011 (I14033) ~2288
B: F. YFP (B14+B15) ~770
A: R0040 (I13602) ~2288



N = J23101 (A62+A63) ~2983*
M: J23101 (A60+A61) - observed ~2983 &
L: F. YFP (B3+B3) ~770
K: J23100 (A62+A63) - standard ~2983*
J: F. CFP (B3+B3) ~770 X DID NOT WORK
I: J23100 (A60+A61) - observed ~2983*

* Band looks very small. There was a shadow band of correct size. We'll proceed w/ the assembly.

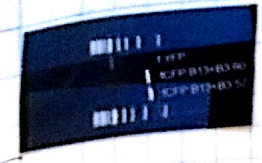


T: solo pSB1C3 (for gRNA) ~2070
S: F. YFP (B11+B12) ~770
R: F. CFP (B11+B12) ~770
Q: J23119 (pSB4001) ~2269
P: J23101 (A62+A63) - same as N ~2983 X
O: J23101 (A60+A61) - same as M ~2983 X

- Joe and Andy set up many PCRs to as... of the Functional Promoters. (See gets a bo...

- Andy set up all DpnIs. We'll PCR Purify tomorrow

- Joe re-set up PCR "J" - F. CFP B18+B3



PCRs from last night from FCFPs look good- we'll proceed with the ones from 572. F YFP does not look good.

PCR Purifications of last night's OpnIs - With Joe

- [x] Add 100µL Buffer PB to RR rxn. Mix
- [x] Apply sample to spin column. Centr. 60s; discard flowthrough
- [x] Add 750µL Buffer PE to col Centr. 60s; discard flowthrough
- [x] Centr. 60s to dry
- [x] Place col in clean, labelled tube
- [x] Add 30µL Buffer EB; let stand 1 min
- [x] Centr. 60s

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
R0040 (I13602)	Default	7/22/2015	12:32 PM	38.89	0.778	0.426	1.82	2.01
R0011 (I6031)	Default	7/22/2015	12:33 PM	72.64	1.453	0.862	1.69	1.02
I13543 (I13541)	Default	7/22/2015	12:34 PM	101.39	2.028	1.148	1.77	1.83
J23100 A60+A61 Observed Scar	Default	7/22/2015	12:35 PM	73.71	1.474	0.847	1.74	1.19
J23101 A60+A61 Observed Scar	Default	7/22/2015	12:35 PM	40.77	0.815	0.456	1.79	2.10
* J23100 A60+A61 Standard Scar	Default	7/22/2015	12:36 PM	49.60	0.992	0.561	1.77	1.62
* J23101 A60+A61 Standard Scar	Default	7/22/2015	12:37 PM	38.31	0.766	0.440	1.74	1.48
J23101 IMP	Default	7/22/2015	12:37 PM	30.21	0.604	0.352	1.72	1.81
J23119 (K584001)	Default	7/22/2015	12:39 PM	46.62	0.932	0.497	1.88	2.22
F TetR	Default	7/22/2015	12:39 PM	58.07	1.161	0.657	1.77	1.44
F CFP B3B4	Default	7/22/2015	12:41 PM	67.90	1.358	0.729	1.86	1.88
F YFP B3B4	Default	7/22/2015	12:42 PM	62.35	1.247	0.690	1.81	1.70
F YFP B14B15	Default	7/22/2015	12:42 PM	127.33	2.547	1.428	1.78	1.37
F CFP B13 B3	Default	7/22/2015	12:43 PM	25.13	0.503	0.267	1.88	2.15
F YFP B13 B3	Default	7/22/2015	12:43 PM	77.20	1.544	0.849	1.82	1.38
F CFP B9 B10	Default	7/22/2015	12:44 PM	89.28	1.786	1.049	1.70	1.13
F YFP B9 B10	Default	7/22/2015	12:44 PM	61.06	1.221	0.677	1.80	1.60
F CFP B11 B12	Default	7/22/2015	12:45 PM	71.62	1.432	0.830	1.73	1.28
F YFP B11 B12	Default	7/22/2015	12:45 PM	56.96	1.139	0.669	1.70	1.34
solo pSB1C3 for gRNA	Default	7/22/2015	12:46 PM	76.18	1.524	0.932	1.64	0.93
pSB1C3 for intC				34.4			1.75	1.86

- Panya and Elli are miniprepping
- Joe is preparing for his talk
- Andy and I Gibson Assembled (see next page)

- Lunch: 3:55PM - 4:30PM

- Michael is making slides to image

- Panya + Elli are making glycerols of their MPs

Transformation of Gibson:

- [x] Thaw cells on ice. Aliquot 10µL of cells into tubes
- [x] Add 2µL of Gibson product. Flick 4-6x.
- [x] Incubate on ice for 30 min. Set heat block.
- [x] Heat shock at 42°C for 30s.
- [x] Place on ice for 5 min.
- [x] Pipette 400µL SOC (RT) into mixture
- [x] Incubate 60 min @ 37°C, 250rpm
  - [x] Prewarm plates
- [x] Mix cells by flicking/inverting
- [ ] Spread 20/200µL of cells onto plates } Panya
- [ ] Incubate overnight at 37°C.

YFP+R0062 MP 1	Default	7/22/2015	4 51 PM	144 67	2 893	1 615	1 79	1 01
YFP+R0062 MP 2	Default	7/22/2015	4 52 PM	69 85	1 397	0 690	2 02	2 17
J23106+I13504 MP1	Default	7/22/2015	4 52 PM	68 69	1 374	0 686	2 00	1 99
J23106+I13504 MP2	Default	7/22/2015	4 53 PM	41 80	0 836	0 427	1 96	1 92
J23117+I13504 MP1	Default	7/22/2015	4 54 PM	161 16	3 223	1 721	1 87	1 32
J23117+I13504 MP2	Default	7/22/2015	4 54 PM	95 52	1 910	0 954	2 00	2 05
gRNA I13453 MP1	Default	7/22/2015	4 55 PM	45 59	0 912	0 452	2 02	1 82
gRNA J23117 MP2	Default	7/22/2015	4 56 PM	50 65	1 013	0 495	2 05	2 04
* gRNA J23119 MP1	Default	7/22/2015	4 57 PM	52 44	1 049	0 613	1 71	0 88
* gRNA J23119 MP2	Default	7/22/2015	4 57 PM	63 82	1 276	0 621	2 05	2 22
gRNA R0010 MP2	Default	7/22/2015	4 58 PM	82 38	1 648	0 924	1 78	1 03
gRNA R0062 MP1	Default	7/22/2015	4 59 PM	99 91	1 998	0 977	2 04	2 16
* gRNA R0062 MP2	Default	7/22/2015	4 59 PM	94 22	1 884	0 935	2 02	2 10
* gRNA R0011 MP2	Default	7/22/2015	5 00 PM	26 15	0 523	0 279	1 87	1 63
* gRNA J23100 MP1	Default	7/22/2015	5 01 PM	86 62	1 732	0 868	2 00	1 78
gRNA J23100 MP2	Default	7/22/2015	5 01 PM	21 02	0 420	0 206	2 04	1 45
gRNA R0051 MP1	Default	7/22/2015	5 02 PM	96 79	1 936	0 956	2 03	2 11
* gRNA J23101 MP2	Default	7/22/2015	5 03 PM	77 67	1 553	0 792	1 96	2 21
* gRNA scrambled MP2	Default	7/22/2015	5 03 PM	79 62	1 592	0 797	2 00	2 22
andy	Default	7/22/2015	5 04 PM	256 29	5 126	2 675	1 92	2 18

- Set up diagnostic RDs of the gRNAs, with the following Master Mix:
  - 7µL EcoRI
  - 7µL PstI
  - 28µL CutSmart
  - 98µL H_2O

  I added 10µL of the MP to each digest

- Taylor and I attempted double electroporations with F. dCas9 cells of:
  - R0011: I6031 + R0011 gRNA AND I6031 + Scrambled gRNA
  - J23119: K584001 + J23119 gRNA AND K584001 + scrambled gRNA
  - J23100: J23100 + J23100 gRNA AND J23100 + Scrambled gRNA
  - J23101: J23101 + J23101 gRNA AND J23101 + Scrambled gRNA

  Realized these won't work, since both TOS and gRNA are on Chlor

  The TOS is on Amp; these should be fine

  We transformed in 0.5 µL of each plasmid, into 70 µL of cells (50µL) + GYT (20µL)
  - If these fail, we should, in the future, make sure the total added DNA is <100ng AND try eluting these in NOT EB.

- Dinner: 8:10 - 10:00 PM

- We're going to set up PCRs to put the visualized TOSs onto pSB1A3

Gel

F.YFP	143	119	163
61°C	57.5	57.1	55.3°C
1145	1115	45s	78s
L-W	15	S	L-G

Amplify pSB1A3	(Again) F.YFP for TetR
A36 (1019)	A18
A37 (2081)	A11

3119 (K584001), I0500 (I0088), R0051 (I0066), I13453 (I13541)

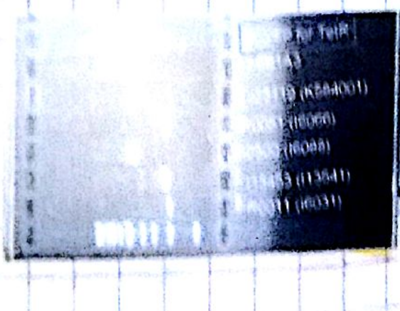
Gels of RDs:

There should be a band at ~~882~~ ~350 bp for all of the gRNAs

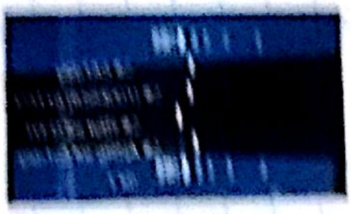
Things that definitely have the band:
- R0062 gRNA #2
- J23119 gRNA #1 + #2
- J23100 gRNA #1
- R0011 gRNA (maybe?)
- Scramble gRNA
- J23101 gRNA

7/23/2015
Time in: 10:00AM
Time out: 10:30AM

- Running a gel of last night's PCRs
  - The thermocycler that had the F.YFP for Tet R (big, old thermocycler) had numerous "temperature drifts," one that was 31°C below the set temp and one that was about 8°C above the set temp



- Panya is setting up DpnIs
- I'm re-setting up PCRs of pSB1A3 (2x) A36/A37 2.5 A57, 1:15
  K584001 F.YFP, pSRG3 for gRNAs
  57.1°C 0:45 A36/A37 (1000) | A18/A19 1:15 | 55.3°C 1:15 A44+A46



Gel of re-do PCRs
Everything looks good EXCEPT [illegible] which still has the highest band of the wrong [illegible]
[illegible]

[illegible] of Re-done PCRs

- [ ] [illegible]
- [ ] [illegible]
- [ ] [illegible]

- [illegible] is setting up colony PCRs/inoculations of the [illegible] assemblies [illegible]
  → If these PCRs look good tomorrow, we can start to assemble TUs into the integration vectors

- [illegible] and [illegible] (and [illegible] students) inoculated the rest of the element transformations of yesterday's Gibsons
  → Everything grew except for (14) and (15) both of the gRNA re-assemblies

- [illegible] PCR Purified the first set of DpnI's:

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
[illegible] Kn Amp	Default	[illegible]	2:02 PM	42.41	0.848	0.461	1.84	2.14
[illegible] Kn Amp	Default	[illegible]	2:03 PM	30.35	0.607	0.351	1.73	1.87
[illegible] Kn Amp	Default	[illegible]	2:04 PM	34.10	0.684	0.418	1.64	0.85
[illegible] Kn Amp	Default	[illegible]	2:05 PM	35.12	0.702	0.412	1.71	1.03

Lunch: 1:50pm – 3:10pm

- [illegible] PCR Purified the second set of DpnI's:

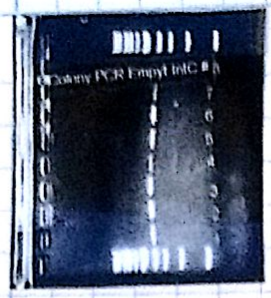
Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
[illegible] #2 Kn gRNA	Default	7/23/2018	3:36 PM	36.60	0.732	0.389	1.88	1.47
[illegible] #2 Kn TUs	Default	7/23/2018	3:37 PM	46.45	0.929	0.495	1.88	1.42
[illegible]	Default	7/23/2018	3:37 PM	52.10	1.042	0.573	1.82	1.46
[illegible] Kn TetR	Default	7/23/2018	3:38 PM	74.08	1.482	0.880	1.68	0.99

- I just looked back at yesterday's Gibsons and realized that for assemblies assembly #5 we [illegible] used a ratio of 1:5 insert of insert:backbone, when according to NEB the correct ratio would be 2:1 of insert:backbone

- [illegible] is calculating volumes for today's Gibsons: TUs → pSB1A3, [illegible], [illegible] gRNAs → pSB1C3, and remaining [illegible] → pSB1C3

- [illegible] ran a gel of [illegible] colony PCRs (see next page)

- [illegible] and I [illegible] Gibson Assemblies



As expected, all of the "intC assemblies" from yest
just look like backbone likely due to the messed
up ratios :\

Transformation of Gibsons

- [x] Thaw cells on ice. Aliquot 12µL of cells into t
- [x] Add 2µL of Gibson product. Flick 4-6x.
- [x] Incubate on ice for 30min. Set heat block.
- [x] Heat shock at 42°C for 30s.
- [x] Place on ice for 5 min.
- [x] Pipette 300µL SOC (RT) into mixture.
- [x] Incubate 60min @ 37°C, 250rpm
  - [x] Pre-warm plates
- [x] Mix cells by flicking/inverting
- [x] Spread 20/200µL of cells onto plates
- [x] Incubate overnight at 37°C

see pg 76 & John's notebook → John is setting up PCRs for tomorrow to assemble:

- (Various F. promoters + CFP) + F. KanR
- (Various F. promoters + CFP) into the Empty galK integrator

I added 2mLs of (low salt) LB to our inoculations of yesterdays assembled J23101+I13504, so that Michael can image them tomor

7/24/2015
Time in: 10:00AM
Time out: _____

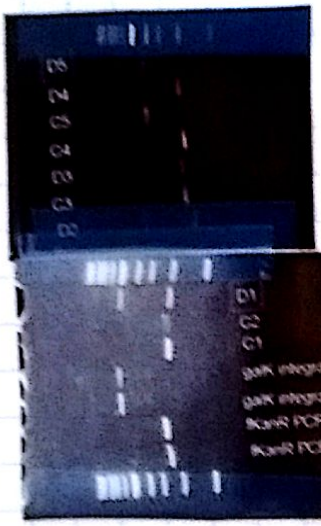
- Joe is running John's PCRs on gels:
  - D_1 - R0011+CFP for KanR Chlor
  - D_2 - R0062+CFP for KanR Chlor
  - D_3 - R0010+CFP for KanR - we already hav Amp
  - D_4 - R0040+CFP for KanR Chlor
  - D_5 - I0500+CFP for KanR Chlor

  - C_1 - R0011+CFP for galK ~~Chlor~~
  - C_2 - R0062+CFP for galK ~~Chlor~~
  - C_3 - R0010+CFP for galK - we've already integrated this here ~~Amp~~
  - C_4 - R0040+CFP for galK ~~Chlor~~
  - C_5 - I0500+CFP for ~~KanR~~ galK ~~Chlor~~

- A_1, A_2 - F. KanR (done 2x)
- B_1, B_2 - galK empty integrator (done 2x)

- I'm helping Michael make slides to image our J23106+I13504 and J23117+I13504 IMP assemblies

- I think that last night, when adding more LB to the inoculations of J23101+I13504, I added Amp instead of Chlor. Those need to be re-inoculated, with just Chlor



D5 - ~~R0040~~+CFP for KanR - 2135bp + 2070 - one band at wrong size
D4 - R0040+CFP for KanR - 979 + 2070 ✓
C5 - I0500+CFP for galK - 2135
C4 - R0040+CFP for galK - 979
D3, C3 } Ratio = NOT relevant - X
D2 - R0062+CFP for KanR - 950 + 2070 - one band at wrong size

D1 - R0011+CFP for KanR - 941 + 2070 - one band at wrong size
C2 - R0062+CFP for galK - 440 bp
C1 - R0011+CFP for galK - 941 bp
galK integrator - 3549 bp

fKanR - 1179

- Joe is PCR Purifying: galK integrator, fKanR, C1, C2, C4, ~~C5~~, D1, D2, D5 → pg 97 of JSM notebook
  - Not purifying: C5, D5, D4

- Ordered primers to assemble R0051+CFP and I0500+CFP

Gibson Assemblies:

R0011+CFP into galK
R0011+CFP ~~0.455µL~~ 0.880µL - 0.09 mol ✓
galK ~~3.78µL~~ 3.40µL - 0.095 ✓
H_2O ~~0.237µL~~ 0.707µL

R0062+CFP into galK
R0062+CFP 1.262µL ✓
galK 3.025µL ✓
H_2O 0.713µL ✓

R0040+CFP into galK
R0040+CFP ~~0.455µL~~ 0.766µL ✓
galK ~~3.78µL~~ 3.02µL ✓
H_2O ~~0.2~~ 1.204µL ✓

I0500+CFP into galK
I0500+CFP 2.13µL - 0.06 mol ✓
galK 2.264µL - 0.03 mol ✓
H_2O 0.56µL ✓

R0011+CFP + F. KanR
R0011+CFP 2.035µL - 0.05 mol ✓
~~galK~~ F. KanR 1.634µL - 0.1 mol ✓
H_2O 1.29µL ✓

R0062+CFP + F. KanR
R0062+CFP 1.923µL - 0.05 mol ✓
~~fKanR~~ F. KanR 1.634µL - 0.1 mol ✓
H_2O 1.442µL ✓

I0500+CFP + F. KanR
I0500+CFP 3.35µL - 0.04 mol ✓
~~fKanR~~ F. KanR 1.307µL - 0.05 mol ✓
H_2O 0.390µL ✓

+5µL HiFi Master Mix

- Michael and I resuspended part ~~K08109~~ K081019 (Functional luxR under promoter R0051) from the kit

Transformation - Gibson assemblies + K08109

- [x] Thaw cells on ice. Aliquot 12µL into tubes.
- [x] Add 2µL Gibson product/1µL resuspension. Flick 4-6x
- [x] Incubate 30 min on ice. Set heat block.
- [x] Heat shock at 42°C for 30sec.
- [x] Place on ice for 5 min.
- [x] Pipette 300µL SOC (RT) into mixture.
- [x] Incubate 60 min @ 37°C, 250rpm. Pre-warm plates.
- [x] Mix cells by flicking/inverting
- [x] Spread 20/200µL of cells onto plates
- [x] Incubate overnight at 37°C

- Last night's assemblies + transformations:

- I13453 (I13541) into pSB1A3 - Mostly red, some non-red
- R0051 (I6066) into pSB1A3 - Mostly red, some non-red
- R0011 (I6081) into pSB1A3 - Mostly red, some non-red
- I0500 (I6088) into pSB1A3 - No growth !!

- I0500 gRNA into pSB1C3 - All non-red colonies!
- J23106 gRNA into pSB1C3 - Mostly red, some non-red

- intC ~~toChtor~~ into pSB1C3 - Mostly red, some non-red

Joe and Taylor are inoculating these with green colonies

- Joe and Taylor are also inoculating from our electroporations from ~~yesterday~~ 150722 since yesterday's inoculations didn't grow:
  - J23101 Tos + J23101 gRNA + F. dCas9
  - J23101 Tos + Scramble gRNA + F. dCas9
  - J23100 Tos + Scramble gRNA + F. dCas9

  The plate with J23100 Tos + J23100 gRNA + F. dCas9 had only one colony, which was inoculated yesterday - it was not inoculated today !!

- They're also re-inoculating our 150722 assembly of J23101+I13504, due to my mistake described on the previous page.

Pooya + Eli miniprepped yesterday's inoculations:

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
R0040 +YFP MP1	Default	7/24/2015	6:54 PM 1	↓146.73	2.935	1.559	1.88	1.98
R0040 +YFP MP2	Default	7/24/2015	6:55 PM	173.48	3.470	1.834	1.89	2.21
R0011 +CFP MP1	Default	7/24/2015	6:56 PM 2	101.19 •	2.024	1.079	1.88	1.92
R0011 +CFP MP2	Default	7/24/2015	6:56 PM	94.63	1.893	1.010	1.87	1.78
R0011 +YFP MP1	Default	7/24/2015	6:57 PM 3	95.56 •	1.911	1.014	1.88	1.92
R0011 +YFP MP2	Default	7/24/2015	6:58 PM	86.19	1.724	0.903	1.91	1.94
I13542+ CFP MP1	Default	7/24/2015	6:59 PM 4	↓123.32	2.466	1.285	1.92	2.15
I13542+ CFP MP2 5	Default	7/24/2015	7:00 PM	60.41	1.208	0.731	1.65	1.50
I13542+ YFP MP1 6	Default	7/24/2015	7:01 PM 5	98.66 •	1.973	1.045	1.89	1.88
I13542+ YFP MP2 6	Default	7/24/2015	7:01 PM	98.96	1.979	1.039	1.91	1.97
J23119+ CFP MP1	Default	7/24/2015	7:02 PM	87.79	1.756	0.927	1.89	2.07
J23119+ CFP MP2	Default	7/24/2015	7:03 PM 6	↓126.49	2.530	1.323	1.91	2.13
J23119+ YFP MP1	Default	7/24/2015	7:04 PM	51.23	1.025	0.639	1.60	1.46
J23119 + YFP MP1	Default	7/24/2015	7:05 PM 7	117.32 •	2.346	1.319	1.78	1.90
J23119 + YFP MP2	Default	7/24/2015	7:06 PM	118.90	2.378	1.268	1.88	2.06

All the samples with [illegible] and [illegible] yellowed and [illegible]

Things to try to re-assemble:

- intC + pSB1C3 X pSB1C3 did not RR up
- Amp - J23100 + CFP
- Amp - J23100 + YFP
- Amp - J23101 + CFP
- Amp - J23101 + YFP
- ~~Chlor~~ - I0500 gRNA to pSB1C3 X Being miniprepped
- ~~Chlor~~ - J23106 gRNA to pSB1C3 X Being miniprepped
- Chlor - R0010 gRNA to pSB1C3
- Chlor - J23117 gRNA to pSB1C3
- Chlor - I13453 gRNA to pSB1C3
- J23100 gRNA to pSB1C3 X Sequence looked good!
- Chlor - R0051 gRNA to pSB1C3
- Chlor - R0011 gRNA to pSB1C3
- Amp - I0500 (I6088) into pSB1A3
- Chlor - R0062 + YFP

} See PCRs on next page

Scanned by CamScanner

PCRs we need	Primers	Primer 1	Primer 2	[illegible]	[illegible]	[illegible]
① pSB1K3 for [illegible]	[illegible] ✓	A1 ✓	A2 ✓	✓	–	[illegible]
② pSB1K3 for [illegible]	[illegible] ✓	B34 ✓	B38 ✓	✓	✓	[illegible]
③ [illegible]	[illegible] ✓	A58 ✓	B16 ✓	✓	–	[illegible]
~~④ [illegible]~~					✓	[illegible]
⑤ [illegible]	[illegible] ✓	A62 ✓	A62 ✓	✓	–	[illegible]
⑥ [illegible]	[illegible] ✓	A63 ✓	A63 ✓	✓	–	[illegible]
⑦ [illegible]	[illegible] ✓	B ✓	B2 ✓	✓	–	[illegible]
⑧ [illegible]	[illegible] ✓	B3 ✓	B3 ✓	✓	–	[illegible]
⑨ [illegible]	[illegible] ✓	B3 ✓	B3 ✓	✓	–	[illegible]
⑩ [illegible]	[illegible]	A26 ([illegible]) ✓	A26 ([illegible]) ✓	✓	✓	[illegible]
⑪ pSB1A3	[illegible] ✓	A36 ([illegible]) ✓	A37 ([illegible]) ✓	✓	✓	[illegible]

Thermocyclers

Green 57.1°C – 3

[illegible] 60.7°C – 0.30

[illegible] 59.5°C – 30

[illegible] 58°C – 13

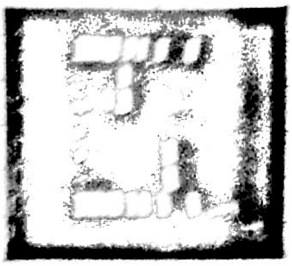
[illegible] 53.3°C – 13

7/25/2013

Time in 11:00 am

Time out 2:00 pm

- Andy came in this morning and ran gel [illegible]



- Andy re-set up PCRs ③ and ⑥

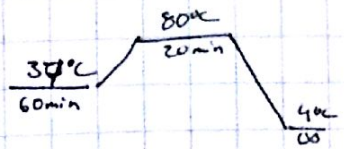
PCR ② did not work [illegible]

Repeating PCR ②

- [x] 12.5 µL 2x [illegible] Hot Start M. — [illegible]
- [x] 2.5 µL B34 — [illegible]
- [x] 2.5 µL B35
- [x] 1 µL of [illegible] dilution
- [x] 6.5 µL [illegible] H_2O

DpnIs of PCRs (except 2):

- [x] 17.5µL PCR Product
- [x] 2µL CutSmart
- [x] 0.5µL DpnI



PCR Purifications of DpnIs:

- [x] Add 100µL Buffer PB to rxn. Mix.
- [x] Apply sample to spin column. Centr. 60s; discard flowthrough.
- [x] Add 750µL Buffer PE to col. Centr. 60s; discard flowthrough.
- [x] Centr. 60s to dry
- [x] Place col in clean, labelled tube
- [x] Add 30µL Buffer EB; let stand 1 min
- [x] Centr. 60s

- The gel of the attempt to re-PCR pSB1C3 for intC had no bands. We won't try to reassemble this today. Also, we have an inoculation of a previous attempt to assemble intC into pSB1C3 to miniprep today.

Minipreps of yesterday's inoculations: - Panya is doing these

- I13453 (I13541) TOS pSB1A3 - A
- R0051 (I6066) TOS pSB1A3 - B
- R0011 ~~(I16~~ I6031) TOS pSB1A3 - C
- intC to pSB1C3 - D
- J23101 + I13504 IMP - E
- J23106 IMP MP3 - F_1
- J23117 IMP MP3 - F_2
- J23106 gRNA - G
- I0500 gRNA - H

- Michael is analyzing sequence data

- I'm calculating volumes for Gibsons (see pg 121 for list of Gibsons)

Nanodrops of PCR Purifications

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
pSB1C3 for gRNA	Default	7/25/2015	4:33 PM	34.41	0.688	0.377	1.83	1.96
R0062 (S01003) for YFP	Default	7/25/2015	4:34 PM	49.16	0.983	0.559	1.76	1.41
~~J23100 for CFP/YFP~~	Default	7/25/2015	4:35 PM	31.72	0.634	0.378	1.76	2.71
J23100 for CFP/YFP retry	Default	7/25/2015	4:36 PM	33.46	0.669	0.407	1.64	1.84
J23101 for CFP/YFP	Default	7/25/2015	4:36 PM	30.74	0.615	0.328	1.88	1.88
~~YFP (B1 + B2)~~	Default	7/25/2015	4:37 PM	35.27	0.705	0.344	2.05	2.86
YFP (B1 + B2) retry	Default	7/25/2015	4:37 PM	44.12	0.882	0.485	1.82	1.41
YFP (B13 + B3)	Default	7/25/2015	4:38 PM	37.83	0.757	0.401	1.89	1.77
CFP (B13 + B3)	Default	7/25/2015	4:38 PM	34.42	0.688	0.356	1.93	1.93
~~I0500 (I6088) for pSB1A3~~	Default	7/25/2015	4:40 PM	58.53	1.181	0.598	1.09	1.77
I0500 (I6088) for pSB1A3 retry	Default	7/25/2015	4:40 PM	57.93	1.159	0.679	1.71	1.55
pSB1A3 for TOSs	Default	7/25/2015	4:41 PM	34.21	0.684	0.383	1.79	1.60

Gibson Assemblies:

J23100 + CFP
- ✓ J23100 - 0.05 mol - 2.94 µL
- ✓ CFP(B13+B3) - 0.1 mol - 1.476 µL
- ✓ H_2O - 0.582 µL

J23100 + YFP
- ✓ J23100 - 0.05 mol - 2.94 µL
- ✓ YFP(B13+B3) - 0.1 mol - 1.54 µL
- ✓ H_2O - 0.715 µL

J23101 + CFP
- ✓ J23101 - 0.045 mol - 2.88 µL
- ✓ CFP(B13+B3) - 0.09 mol - 1.33 µL
- ✓ H_2O - 0.789 µL

J23101 + YFP
- ✓ J23101 - 0.045 mol - 2.88 µL
- ✓ YFP(B13+B3) - 0.09 mol - 1.21 µL
- ✓ H_2O - 0.909 µL

I0500 (I6088) into pSB1A3
- ✓ pSB1A3 - 0.05 mol - 2.08 µL
- ✓ I0500 (I6088) - 0.1 mol - 2.43 µL
- ✓ H_2O - 0.49 µL

R0062 + YFP
- ✓ R0062 (S01003) - 0.05 mol - 1.54 µL
- ✓ YFP(B1+B2) - 0.1 mol - 1.15 µL
- ✓ H_2O - 2.31 µL

ALL gRNAs into pSB1C3
- ✓ pSB1C3 - 0.05 mol - 1.99 µL
- ✓ gRNA gBlock - 2.16 µL
- ✓ H_2O - 0.85 µL

+5µL HiFi Master Mix

-Panya made glycerols

Transformation: Transforming assemblies + miniprep #1 of pSB1C3 (K04450) from 15a03 (running low)

- [x] Thaw cells on ice. Aliquot 12 µL into tubes
- [x] Add 2µL Gibson product/miniprep
- [x] Incubate 30 min on ice. Set heat block.
- [x] Heat shock at 42°C for 30 sec
- [x] Place on ice for 5 minutes
- [x] Pipette 300µL RT SOC into mixture.
- [x] Incubate 60 min @ 37°C, 250 rpm. Pre-warm plates.
- [x] Mix cells by flicking/inverting
- [x] Spread 20/200µL of cells onto plates.
- [ ] Incubate overnight at 37°C

I13453 (I13541) TOS 1A3 MP1	Default	7/25/2015	6:21 PM 8	↑20.93	0.4[illegible]9	0.222	1.89	1.09
I13453 (I13541) TOS 1A3 MP2	Default	7/25/2015	6:23 PM	16.78	0.3[illegible]6	0.189	1.78	0.85
R0051 (I6066) TOS 1A3 MP1	Default	7/25/2015	6:24 PM	11.50	0.2[illegible]0	0.107	2.14	1.94
R0051 (I6066) TOS 1A3 MP2	Default	7/25/2015	6:25 PM 9	↑17.90	0.3[illegible]8	0.197	1.82	0.99
R0011 (I6031) TOS 1A3 MP1	Default	7/25/2015	6:26 PM	12.81	0.2[illegible]5	0.140	1.83	0.89
R0011 (I6031) TOS 1A3 MP2	Default	7/25/2015	6:27 PM 10	↑18.44	0.3[illegible]9	0.202	1.83	1.40
intC to 1C3 MP1	Default	7/25/2015	6:28 PM 11	↑24.90	0.4[illegible]8	0.279	1.78	0.99
intC to 1C3 MP2	Default	7/25/2015	6:29 PM 47	↑9.21	0.1[illegible]4	0.091	2.03	1.68
intC to 1C3 MP3	Default	7/25/2015	6:29 PM 48	↑16.02	0.3[illegible]0	0.162	1.98	1.56
J23101 + I13504 IMP MP1	Default	7/25/2015	6:31 PM	↑15.38	0.3[illegible]8	0.160	1.92	1.46
J23101 + I13504 IMP MP2	Default	7/25/2015	6:31 PM	↑12.37	0.2[illegible]7	0.129	1.92	1.81
J23101 + I13504 IMP MP3	Default	7/25/2015	6:32 PM 12	↑22.81	0.4[illegible]6	0.274	1.67	1.00
J23106 IMP MP3	Default	7/25/2015	6:33 PM 13	↑16.29	0.3[illegible]6	0.170	1.92	1.47
J23117 IMP MP3	Default	7/25/2015	6:36 PM 14	↑12.33	0.2[illegible]7	0.109	2.27	2.16
J23106 gRNA MP1	Default	7/25/2015	6:37 PM 15	↑24.01	0.4[illegible]0	0.299	1.61	0.98
J23106 gRNA MP2	Default	7/25/2015	6:38 PM	12.93	0.2[illegible]9	0.136	1.90	2.02
I0500 gRNA MP1	Default	7/25/2015	6:38 PM 16	↑64.07	1.2[illegible]1	0.744	1.72	1.00
I0500 gRNA MP2	Default	7/25/2015	6:39 PM	45.67	0.9[illegible]8	0.491	1.86	1.68

15
4:05PM
6:55PM

- Joe came in and miniprepped this morning! ~~See~~ Nanodrops below:

7/27/2015

me in: 10:00 AM

me out: 12:15 PM.

- Elli and I miniprepped yesterday's inoculations:

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
fLuxR MP1	Default	7/27/2015	1:05 PM	74.42	1.488	0.787	1.89	2.07
fLuxR MP2	Default	7/27/2015	1:06 PM	94.20	1.884	1.067	1.77	1.05
pSB1C3 + J04450 MP1	Default	7/27/2015	1:09 PM	73.00	1.460	0.809	1.80	1.18
pSB1C3 + J04450 MP2	Default	7/27/2015	1:09 PM	3.62	0.072	0.029	2.52	1.63
R0062 + YFP MP1	Default	7/27/2015	1:10 PM	55.26	1.105	0.603	1.83	1.42
R0062 + YFP MP2	Default	7/27/2015	1:11 PM	215.39	4.308	2.294	1.88	1.66
J23101 + YFP MP1	Default	7/27/2015	1:11 PM	51.00	1.020	0.541	1.89	2.10
J23101 + YFP MP2	Default	7/27/2015	1:12 PM	68.52	1.370	0.779	1.76	0.94
J23101 + CFP MP1	Default	7/27/2015	1:13 PM	79.32	1.586	0.828	1.92	1.87
J23101 + CFP MP2	Default	7/27/2015	1:13 PM	17.64	0.353	0.194	1.82	1.91
J23100 + YFP MP1	Default	7/27/2015	1:14 PM	55.51	1.110	0.593	1.87	1.94
J23100 + YFP MP2	Default	7/27/2015	1:15 PM	52.66	1.053	0.560	1.88	2.00
J23100 + CFP MP1	Default	7/27/2015	1:15 PM	45.76	0.915	0.540	1.69	1.03
J23100 + CFP MP2	Default	7/27/2015	1:16 PM	81.04	1.621	0.926	1.75	1.04
R0011 gRNA MP1	Default	7/27/2015	1:18 PM	87.17	1.743	1.035	1.69	1.46
R0011 gRNA MP2	Default	7/27/2015	1:19 PM	17.69	0.354	0.184	1.92	1.84
R0011 gRNA MP2	Default	7/27/2015	1:19 PM	17.48	0.350	0.162	2.15	1.85
R0051 gRNA MP1	Default	7/27/2015	1:20 PM	24.12	0.482	0.280	1.72	1.57
R0051 gRNA MP2	Default	7/27/2015	1:21 PM	91.87	1.837	1.358	1.35	0.94
I13453 gRNA MP1	Default	7/27/2015	1:22 PM	46.94	0.939	0.548	1.71	1.97
I13453 gRNA MP2	Default	7/27/2015	1:23 PM	37.73	0.755	0.405	1.86	1.90
J23117 gRNA MP1	Default	7/27/2015	1:24 PM	53.75	1.075	0.559	1.92	2.02
R0010 gRNA MP1	Default	7/27/2015	1:24 PM	48.87	0.977	0.507	1.93	2.30
R0010 gRNA MP2	Default	7/27/2015	1:25 PM	53.54	1.071	0.602	1.78	1.21
I0500 (I6088) + pSB1C3 ToS MP1	Default	7/27/2015	1:27 PM	88.35	1.767	1.318	1.34	1.47
I0500 (I6088) + pSB1C3 ToS MP2	Default	7/27/2015	1:27 PM	25.47	0.509	0.286	1.78	0.97

Eluted in EB

Eluted in H_2O

- Panya sent out samples to be sequenced

- Joe and Michael (re-)imaged ~~some~~ some fluorescent proteins to make sure that we hadn't previously just been looking at autofluorescence. See MCL notebook pg 67 for a list of things imaged / settings used to image them.

Diagnostic PCRs of intC - MP #1, #2, and #3 from 150725

- [x] ~~2 µL~~ 2.5 µL A36 (2014)
- [x] 2.5 µL A37 (2014)
- [x] 1 µL of 1:100 dilution of MP
- [x] 6.5 µL H_2O
- [x] 12.5 µL MM

Annealing temp: 57.1°C Extension time: 1:45

Lunch: 3:20 - 4:20

Diagnostic RDs - Round I: gRNAs

Digesting:
- R0011 gRNA
- R0051 gRNA
- I13453 gRNA
- J23117 gRNA (only one MP)
- R0010 gRNA

Protocol

- [x] 2 µL CutSmart
- [x] 12 µL DNA (for everything)
- [x] 0.5 µL EcoRI
- [x] 0.5 µL PstI

Diagnostic PCRs - Even more!

Amplify insert (A26 + A27):

- J23101 MP #1-3
- J23106 MP #3
- J23117 MP #3

Master mix:
- 39 µL H_2O
- 15 µL A26
- 15 µL A27
- 75 µL MM

Amplify insert AND homologous regions (B32 + B33):

- R0011 + CFP into galK
- R0062 + CFP into galK
- R0040 + CFP into galK
- I0500 + CFP into galK

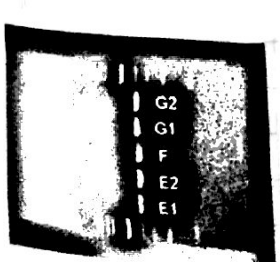
Master mix:
- 58.5 µL H_2O
- 22.5 µL B32
- 22.5 µL B33
- 112.5 µL MM

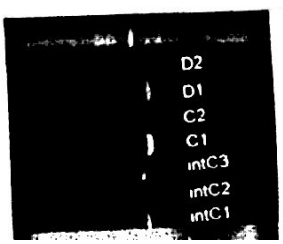
Check gRNAs (concentrations too low to RD): I0500 gRNA - A26+A27

- [x] 2.5 µL A26
- [x] 2.5 µL A27
- [x] 6.5 µL H_2O
- [x] 1 µL plasmid
- [x] 12.5 µL MM

Dinner: 7:45 - 8:45 PM

Gels of RD + intC PCR:





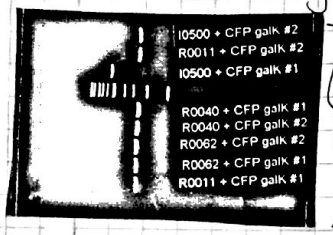
All of the RDs of gRNAs should have a band at about ~330 bp, which none of these have.
Also, the intC PCR should be abat 3920 bp. It is longer than pSB1C3 alone

Gels of PCRs

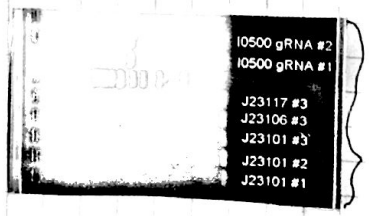
#1: J23101 #1 | J23101 #2 | J23101 #3 | J23106 #3 | J23117 #3 | Ladder | I0500 gRNA #1 | I0500 gRNA #2

#2: Ramp1 + CFP galK | Ramp1 + CFP galK #1 | Ramp1 + CFP galK #2 | Ramp2 + CFP galK #2 | Ramp2 + CFP galK | Ladder | I0500 + CFP galK #1 | I0500 + CFP galK #2 | R0040 + CFP galK #1 | R0040 + CFP galK #2

- Elli inoculated the integrated CFP (both intC and galK) to be images tomorrow from glycerols



The I0500+CFP galK #1 appears to have worked! Everything else looks bad.



There may be a faint shadow of the correct for I0500 gRNA #2 however! For some reason, it looks like backbone amplified. IMPS failed.

Elli and I set up PCRs:

- I0500 (I6088) for IA3 - A26 + A27 (2014) - 57.1°C - 1:15 } Bradley Lab
- pSB1A3 - A36 + A37 (2014) - 57.1°C - 1:15
- pSB1C3 for gRNA - A11 + A12 - 55.3°C - 1:15 } Core Lab
- R0011 + CFP + KanR TO INTEGRATE - B19 (galK) + B20 - 55°C (30 cycles) - 60°C (25 cycles) - 90s } iGEM Lab
- R0062 + CFP + KanR " " " " " "
- I0500 + CFP + KanR " " " " " "
- pSB1C3 for intC - B34 + B35 - 53°C - 1:30 } Saha Lab

Protocol:

- [x] 2.5 µL Primer 1
- [x] 2.5 µL Primer 2
- [x] 1 µL 1:100 dilution of MP
- [x] 6.5 µL H_2O
- [x] 12.5 µL Master Mix

- Elli inoculated an EcNR2 starter culture so that we can integrate tomorrow.

7/25/2015
Time in: 10:00AM
Time out: 2:15PM

- Panya is running a gel of last night's PCRs

These bands all look good!

These bands are very faint.

Redoing ultramer PCRs:
- R0011 CFP + KanR
- R0062 CFP + KanR

I realized that since we assembled I0500 into the galK integrator, we can just PCR this up.

Ultramer PCR

- [x] 2.5µL B19
- [x] 2.5µL B20
- [x] 6.5µL H_2O
- [x] 1µL 1:100 dilution (R0011+CFP+KanR MP2 / R0062+CFP+KanR MP1) } I50726
- [x] 12.5µL MM

98°C 30 | 98°C 10 | 58°C 20 | 72°C 90 | 72°C 120 | 4°C ∞

PCR to integrate I0500 from galK integrator

- [x] 2.5µL B32
- [x] 2.5µL B33
- [x] 6.5µL H_2O
- [x] 1µL 1:100 dilution (I0500+CFP into galK MP1) } I50726
- [x] 12.5µL MM

98°C 30 | 98°C 10 | 57.6°C 30 | 72°C 1:30 | 72°C 120 | 4°C ∞

DpnI - pSB1C3 for gRNA, pSB1C3 for intC, I0500 (I6088) for 1A3, pSB1A3

- [x] 17.5µL PCR Product
- [x] 2µL CutSmart
- [x] 0.5µL DpnI

37°C 60min | 80°C 20min | 4°C ∞

Lunch: 12:40 – 1:35PM

- Joe ordered gBlocks for the three IMBs (designed by Elli and me, checked by Joe and John) and for F. TetR (designed by Joe, briefly checked by me) (based on part plate)

- Joe also talked to a customer service rep at IDT, who suggested that we attempt to split the intC gBlock into three smaller gBlocks
  - Joe designed these gBlocks, which Michael and I double checked these

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
pSB1C3 for intC	Default	7/28/2015	4:44 PM	15.47	0.309	0.181	1.64	1.38
pSB1C3 for gRNA	Default	7/28/2015	4:45 PM	38.53	0.771	0.447	1.72	1.78
EB I0500 (I6088)	Default	7/28/2015	4:46 PM	28.61	0.572	0.333	1.62	1.78
pSB1A3	Default	7/28/2015	4:47 PM	85.18	1.704	1.056	1.67	1.87
H_2O R0062 + CFP + KanR	Default	7/28/2015	4:48 PM	26.45	0.529	0.298	1.78	1.73
R0011 + CFP + KanR	Default	7/28/2015	4:49 PM	29.75	0.595	0.333	1.79	1.73
I0500 + CFP from gelk integrator				16.7			1.85	1.17

- Joe is diluting last night's EcNR2 starter culture

Gibson Assemblies:

gRNAs to Assemble
- A1 - I0500 gRNA ✓
- A2 - J23106 gRNA ✓
- A3 - J23117 gRNA ✓
- A4 - R0011 gRNA ✓
- A5 - R0051 gRNA ✓
- A6 - I13453 gRNA ✓
- A7 - R0010 gRNA ✓

gRNA into pSB1C3 A
pSB1C3 - 0.033 mol - 1.17 µL ✓
gRNA - 0.1 mol - 2.165 µL ✓
H_2O - 1.665 µL ✓

intC into pSB1C3 B
pSB1C3 - 0.01 mol - 0.893 µL ✓
intC - 0.03 mol - 3.66 µL ✓
H_2O - 0.46 µL ✓

I0500 (I6088) into pSB1A3
pSB1A3 - 0.0305 mol - 2.453 µL
I0500 (I6088) - 0.003 mol - 5.217 µL
H_2O - 0 µL

- Joe, Taylor, John and I made electrocomp EcNR2, and electroporated to integrate R0062+CFP+KanR, R0011+CFP+KanR, and I0500+CFP from gBlk integrants.

- Taylor diluted primer B36 to assemble R0051 and I0500 with CFP

Transformation of Gibsons:

- [x] Thaw cells on ice. Aliquot 11 µL into tubes.
- [x] Add 2 µL Gibson PCR product
- [x] Incubate 30 min on ice. Set heat block.
- [x] Heat shock at 42°C for 30 sec.
- [x] Place on ice for 5 min.
- [x] Pipette 300 µL RT SOC into mixture
- [x] Incubate 60 min @ 37°C, 250 rpm
- [x] Mix cells by flicking/inverting
- [x] Spread 20/200 µL of cells onto plates
- [x] Incubate overnight at 37°C.

PCRs we're setting up tonight:

45s - F. RFP (J04450 on pSB1A3 #1) - A12+A14 - 60°C
45s - F. KanR (F. KanR - Chlor - MP#1) - A15+A16 - 63°C
45 30s - R0051 (J6066) (MP#1 from 150705) - A58+B36 - 50.35°C
45s - I0500 (J6068) (MP#1 from 150705) - A58+B36 - 50.35°C
30s - CFP (E0022 #1) - B5+B6 - 61.2°C

Protocol:

- [x] 6.5 µL H_2O
- [x] 2.5 µL Primer 1
- [x] 2.5 µL Primer 2
- [x] 1 µL 1:100 dilution
- [x] 12.5 µL MM

7/29/2013
Time in 10:05 AM
Time out 2:40 AM

- Joe ran a gel of last night's PCRs:

- Everything looks great! Joe set up the DpnI.

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260
fKanR for fRFP A15A16	Default	7/29/2015	1:27 PM	58.82	1.176	0.678	1.73	1
fRFP for KanR A12A14	Default	7/29/2015	1:28 PM	47.20	0.944	0.564	1.68	0
I0500 for CFP	Default	7/29/2015	1:28 PM	16.16	0.323	0.192	1.68	1
CFP B5B6	Default	7/29/2015	1:28 PM	64.86	1.297	0.731	1.77	1
R0051 for CFP	Default	7/29/2015	1:29 PM	70.99	1.420	0.809	1.75	1

Diagnostic colony PCRs of gRNAs:

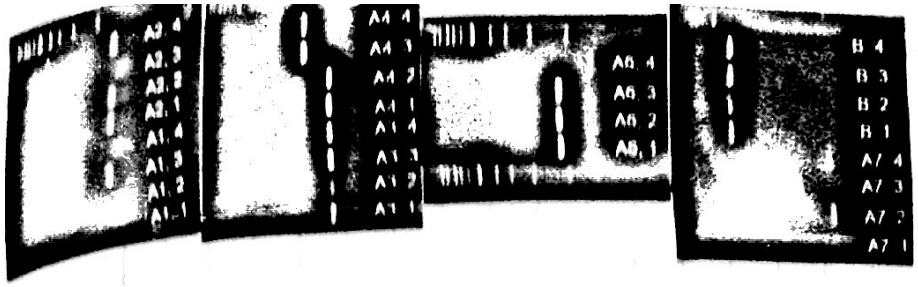
(Diagnostic colony PCRs of mtC were also done: A36+A37 [2014])

We also set up these inoculations

- [x] 5µL Master mix - ~~140~~ 120 µL
- [x] 1µL VF2 - ~~26~~ 24 µL
- [x] 1µL VR - ~~26~~ 24 µL
- [x] 3µL H_2O - ~~84~~ 72 µL

Transformation of Gibsons:

- [x] Thaw cells on ice.
- [x] Aliquot 11µL into tubes.
- [x] Add 2µL of Gibson product. Flick 4-6x to mix.
- [x] Incubate 30 min on ice. Set heat block.
- [x] Heat shock at 42°C for 30s
- [x] Place on ice for 5 min
- [x] Pipette 300µL RT SOC into the mixture
- [x] Incubate 60 min at 37°C, 250rpm
- [ ] Mix cells by flicking/inverting
- [x] Spread 20/200µL onto plates
- [x] Incubate overnight at 37°C



All gRNAs should be ~330 bp

A_1: I0500 gRNA
A_2: J23106 gRNA
A_3: J23117 gRNA
A_4: R0011 gRNA
A_6: I13453 gRNA
A_7: R0010 gRNA

It looks like we have at least one of each of the attempted six gRNA assemblies!!

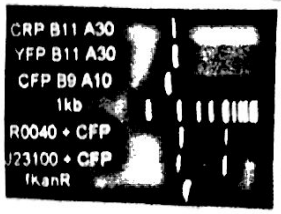
The intC assemblies look, as always, like failures.

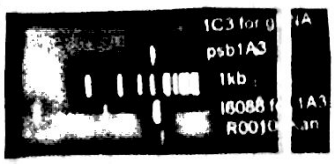
However, it's possible that we now have 11/13 gRNAs assembled! Tomorrow, we'll miniprep the inoculations that look correct.

- Michael and Taylor are setting up PCRs to do the following assemblies tomorrow:
  - J23119 gRNA into pSB1C3
  - ~~R0040 gRNA into pSB1C3~~ There is no gRNA for this!
  - J23119 + CFP (B11 + A30)
  - J23119 + YFP (B11 + A30)
  - R0040 + CFP + F. KanR
  - J23100 + CFP + F. KanR
  - I13453 + CFP (B9 + B10)
  - I6088 (I0500) into pSB1A3 (TOS)

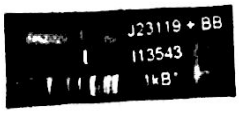
7/30/2015
Time in: 10:10AM
Time out: 10:22AM

Gels of last night's PCRs:





These PCRs had been left at 98°C. John re-did them:



DpnI of PCRs:

I0500 gRNA MP1 12	Default	7/30/2015	6:36 PM	114.77	F 2.295	1.202	1.91	2.15
J23106 gRNA MP1 13	Default	7/30/2015	6:37 PM	145.56	F 2.911	1.540	1.89	2.14
J23106 gRNA MP2 14	Default	7/30/2015	6:38 PM	114.82	F 2.296	1.271	1.81	1.94
J23117 gRNA MP1 15	Default	7/30/2015	6:38 PM	30.87	F 0.617	0.348	1.79	1.93
J23117 gRNA MP2 16	Default	7/30/2015	6:39 PM	152.32	F 3.046	1.578	1.93	1.96
R0011 gRNA MP1 17	Default	7/30/2015	6:39 PM	132.58	F 2.652	1.441	1.84	1.96
R0010 gRNA MP1 18	Default	7/30/2015	6:40 PM	170.06	F 3.401	1.798	1.89	2.15
I13453 gRNA MP1 19	Default	7/30/2015	6:41 PM	122.46	F 2.449	1.358	1.80	1.98
R0010 gRNA MP2 20	Default	7/30/2015	6:42 PM	211.38	F 4.227	2.218	1.91	2.22
R0011 gRNA MP2 21	Default	7/30/2015	6:43 PM	105.23	F 2.105	1.128	1.87	1.96

Transformation of Gibson Products

- [x] Thaw cells on ice. Aliquot 12µL into tubes.
- [x] Add 2µL Gibson product. Flick 4-6x to mix.
- [x] Incubate 30 min on ice.
- [x] Heat shock at 42°C for 30s.
- [x] Incubate on ice for 5 min.
- [x] Pipette 300µL RT SOC into the mixture
- [x] Incubate 60 min at 37°C, 250 rpm
- [x] Mix cells by flicking/inverting
- [x] Spread 20/200µL onto plates
- [x] Incubate overnight at 37°C

- Panya and Elli made glycerols of the MPs

- Many of yesterday's integration inoculations grew!! Michael is imaging these.

- Taylor and John are making electrocompetent EcNR2 to attempt to re-integrate R0010-CFP, since we can't get our glycerol to grow. (We tried twice to inoculate it in AmpChlorKan LB, and once in Kan LB, with no success. Tonight, we'll try it in just ChlorAmp.)

They also tried to electroporate/integrate from cells made on 150729, to see if stored cells remain effective.

- Panya and Elli made glycerols of the integrated R0011-CFP and R0062-CFP that Michael is imaging. These will also be stored on plates.

- Taylor and John are electroporating to test our gRNAs w/ the dCas9 cells:

A - J23100 Tos + scramble gRNA
B - J23100 Tos + J23100 gRNA
C - J23101 Tos + Scramble gRNA
D - J23101 Tos + J23101 gRNA
E - R0010 tos + scramble gRNA
F - R0010 Tos + R0010 gRNA (new!)

(Tay transformed ~1 0.5 µL of each of the two things for everything!)

Sources:
- pSB1A3-J04450 #1 150603 from kit
- J23100 #1 from kit 150603
- J23101 #1 from kit 150603
- R0010 gRNA #1 150730
- J23100 gRNA #1 150722
- J23101 gRNA #2 150722
- Scrambled gRNA #2 150724

7/31/2015
Time in 10:30am
Time out 11:30

- Last night, John, Taylor, and Andy set up PCRs to assemble:
  - J23101 + CFP
  - J23101 + YFP

PCRs from last night look good!

- Last night, Michael imaged some of the integrated CFPs
  - R0010 + CFP (I_A3, I_D3, I_E3)
  - R0062 + CFP (II_A3)

Today, he'll image and collect data on the integrate I0500+GFP (we had thought it was I0500+CFP, but sequencing disconfirmed this, showing that it had just been I6088).

Primers and gBlocks arrived!!

PCRs to put IMP gBlocks and intC gBlocks into pSB1C3:

~~IMPs~~	intC ~~IMPs~~	IMPs ~~intC~~
[✓] 2.5 µL Primer 1	B34	A11
[✓] 2.5 µL Primer 2	B35	A12
[✓] 6.5 µL H_2O		
[✓] 1 µL 1:100 dilution (J04450-pSB1C3 MP#2 150603)		
[✓] 12.5 µL MM		
Annealing temp:	53.6	55.3°C

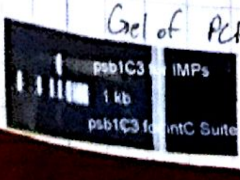
- Michael miniprepped, but yields were very low. We're going to just add more media + antibiotics and try to miniprep them again tomorrow.

Lunch 2:15-3:30

- Elli and Panya got more Outreach supplies at Radio Shack

Gel of PCRs:

pSB1C3 for IMPs	Ladder	pSB1C3 for intC



Things are correct sizes. pSB1C3 for intC looks pretty dim.

- Elli is resuspending gBlocks and primers

Scanned by CamScanner

Inoculations:
- Yesterday's dCas9 integrations - TB-AmpChlorKan
- Yesterday's EcNR2 R0010 integrations (both fresh + stored cells) - LB -
- Old R0010 integration - LB - AmpChlor (Re-typing because failed to grow in AmpChlorKan, but also not in just Kan media.)
- Adding media to cultures MP'd today (R0051+CFP and I0500+CFP)
- Test regrowing things from glycerol (J04450-Chlor) - LB - TB - Chlor
- Yesterday's assemblies:
  - I0500 (I6058) 10s into pSB1C3 - TB - Amp X Did not grow
  - E13453 + CFP - TB - Chlor 3 colonies ✓
  - J23100+CFP + KanR - TB - AmpKan X Did not grow
  - R0040+CFP + KanR - TB - ChlorKan ✓
  - J23119 + YFP - TB - Amp X Did not grow
  - J23119 + CFP - TB - Amp X Did not grow
  - J23119 gRNA into pSB1C3 - TB - Chlor ✓

Set up diagnostic colony PCRs (see below)

DpnI:

- [✓] 17.5 µL PCR Product
- [✓] 2 µL CutSmart
- [✓] 0.5 µL DpnI

-Panya PCR Purified:

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/
pSB1C3 for IMP PCR PURE	Default	7/31/2015	4:52 PM	45.60	0.912	0.541	1.6
pSB1C3 for intC integration PCR PURE	Default	7/31/2015	4:53 PM	32.56	0.651	0.399	1.6

Gibsons - other calculations are in John's notebook, pg 84. These assem
- J23101
- J23101 +

J23106 → 1C3
pSB1C3 - 0.02267 mol - 0.679 µL
J23106 - 0.068 mol - 4.313 µL
H_2O - 0

J23117 → 1C3
pSB1C3 - 0.02267 mol - 0.679 µL
J23117 - 0.068 mol - 4.313 µL
H_2O - 0

intC integrator → 1C3
pSB1C3 - 0.0157 mol - 0.6588 µL
intC 1 - 0.0314 mol - 1.459 µL
intC 2 - 0.0314 mol - 1.386 µL
intC 3 - 0.0314 mol - 1.476 µL
H_2O - 0

Transformation:

- [✓] Thaw c
- [✓] Add
- [✓] Incuba
- [✓] 42°C
- [✓] Ice fo
- [✓] 300µ
- [✓] 60 mi
- [✓] Mix c
- [✓] Spread
- [✓] Incub

22,23	R0040 + CFP + KanR MP1 FrR	Default	8/1/2015	8:57 PM	80.87	1.617	0.875	1.85	1.46
24,25	R0040 + CFP + KanR MP2 FrR	Default	8/1/2015	8:58 PM	90.66	1.813	1.135	1.60	1.09
26,27	R0040 + CFP + KanR MP3 FrR	Default	8/1/2015	8:59 PM	35.26	0.705	0.409	1.72	1.23
28,29	R0040 + CFP + KanR MP4 FrR	Default	8/1/2015	8:59 PM	122.69	2.454	1.451	1.69	0.89
30	J23119 gRNA MP1	Default	8/1/2015	9:00 PM	85.61	1.712	0.980	1.75	0.85
31	J23119 gRNA MP2	Default	8/1/2015	9:00 PM	49.75	0.995	0.554	1.79	0.89
32	J23119 gRNA MP3	Default	8/1/2015	9:01 PM	67.21	1.344	0.792	1.70	0.82
33	J23119 gRNA MP4	Default	8/1/2015	9:02 PM	105.54	2.111	1.238	1.71	0.92
34	I13453 + CFP MP1	Default	8/1/2015	9:02 PM	60.71	1.214	0.637	1.91	1.81
35	I13453 + CFP MP2	Default	8/1/2015	9:03 PM	102.42	2.048	1.113	1.84	1.56
36	I13453 + CFP MP3	Default	8/1/2015	9:04 PM	131.39	2.628	1.508	1.74	1.01
	dCas9 J23101 gRNA MP1 X	Default	8/1/2015	9:05 PM	32.22	0.644	0.391	1.65	0.84
36,37,38	I0500 + CFP MP1 FrR	Default	8/1/2015	9:05 PM	93.40	1.868	1.063	1.76	1.09
39,40	I0500 + CFP MP2 FrR	Default	8/1/2015	9:06 PM	125.07	2.501	1.406	1.78	1.09
41,42	I0500 + CFP MP3 FrR	Default	8/1/2015	9:06 PM	141.28	2.826	1.604	1.76	1.14
43	R0051 + CFP MP1	Default	8/1/2015	9:07 PM	88.43	1.769	1.034	1.71	0.99
44	R0051 + CFP MP2	Default	8/1/2015	9:08 PM	78.15	1.563	0.888	1.76	1.09
45	R0051 + CFP MP3	Default	8/1/2015	9:09 PM	40.81	0.816	0.465	1.76	0.95

- Yesterday, there were no colonies on the intC assembly plates. Repeating that assembly:

- [x] pSB1C3 from 150731 - 0.6588µL
- [x] intC1 - 1.459µL
- [x] intC2 - 1.386µL
- [x] intC3 - 1.476µL
- [x] 5µL MM

Assemblies to do tomorrow/this week:

- R0040/R0011/R0062/R0010/I13453/J23100/R0051/I0500 + YFP into intC empty integrator
- I6088 into intC empty integrator
- I13453/R0051/I0500+CFP into galK integrator
- I13453/R0051/I0500 + CFP + KanR
- R0011*/R0040*/R0051*/R0062*/I13453* + YFP into pSB1A3
- I6088 into pSB1A3
- J23106-I13504/J23117-I13504 into pSB1A3
- R0040+CFP + R0040+YFP
- R0011+CFP + R0011+YFP
- R0062+CFP + R0062+YFP
- J23100+CFP + J23100+YFP

* Can use same PCRs as the ones to put them into intC

To integrate

TOS to test gRNAs - these have to be cpt pSB1A3
- Eventually, we'll want to put a CFP under each promoter behind R0040+YFP on 1A3

Assembling G2 plasmids ~~for~~ as a backup in case integration fails

PCRs we need:	Dilutions:	Primer 1:	Primer 2:	H_2O:	MM	Temp	Time
① [illegible]	[illegible]	A[illegible] ([illegible])	A27 ([illegible])	✓	✓	57.1°C	0:30
② [illegible]	[illegible]	"	"	✓	✓	57.1°C	0:30
③ [illegible]	[illegible]	"	"	✓	✓	57.1°C	0:30
④ [illegible]	[illegible]	"	"	✓	✓	57.1°C	0:30
⑤ [illegible]	[illegible]	"	"	✓	✓	57.1°C	0:30
⑥ [illegible]	[illegible]	"	"	✓	✓	57.1°C	0:30
⑦ [illegible]	[illegible]	"	"	✓	✓	57.1°C	0:30
⑧ [illegible]	[illegible]	"	"	✓	✓	57.1°C	1:15
⑨ [illegible]	[illegible]	"	"	✓	✓	57.1°C	1:15
⑩ [illegible]	[illegible]	"	"	✓	✓	57.1°C	0:30
⑪ [illegible]	[illegible]	"	"	✓	✓	57.1°C	0:30
⑫ [illegible]	[illegible]	"	"	✓	✓	57.1°C	1:15
⑬ [illegible] K [illegible]	[illegible] ✓	A[illegible] ✓	A[illegible] ✓	✓	✓	57.1°C	1:45
⑭ pSB1K3	[illegible]	"	"	✓	✓	57.1°C	1:15
⑮ pSB1C3	[illegible]	"	"	✓	✓	57.1°C	1:13
⑯ [illegible]	[illegible]	A17	B42*			55.8°C	~~1:15~~ 1:30
⑰ [illegible]	[illegible]	"	"			55°C	~~1:15~~ 1:30
⑱ [illegible]	[illegible]	"	"			55°C	2:00
⑲ [illegible]	[illegible]	"	"			55°C	~~1:15~~ 1:30
⑳ [illegible]	[illegible]	"	"			55°C	~~1:15~~ 1:30
㉑ [illegible]	[illegible]	"	"			55°C	1:30
㉒ [illegible]	[illegible]	"	"			55°C	1:30
㉓ [illegible]	[illegible]	B38 ✓	A16 ✓	✓	✓	60°C [illegible]	0:30
㉔ [illegible]	[illegible]	B39 ✓	A16 ✓	✓	✓	60°C [illegible]	0:30
㉕ [illegible]	[illegible]	B40 ✓	A16 ✓	✓	✓	60°C [illegible]	0:30
㉖ [illegible]	[illegible]	B41 ✓	A16	✓	✓	60°C [illegible]	0:30

Transformation of re-assembled intC and R0040-GFP+KanR (MR#1-3)

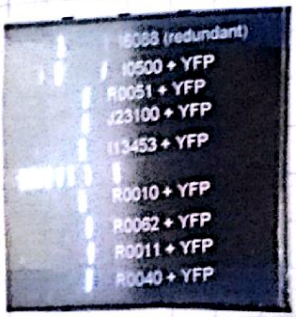
- [x] Thaw cells on ice. Aliquot 12μL into each tube.
- [x] Add 2μL Gibson product. Flick 4-6x.
- [x] Incubate 30 min on ice. Set heat block.
- [x] Heat shock at 42°C for 30s
- [x] Place on ice for 5 min.
- [x] Add 300μL RT SOC
- [x] Incubate 60 min at 37°C, 250 rpm
- [x] Mix cells by flicking/inverting
- [x] Spread 20/200μL onto plates
- [x] Incubate overnight at 37°C

-John [illegible]

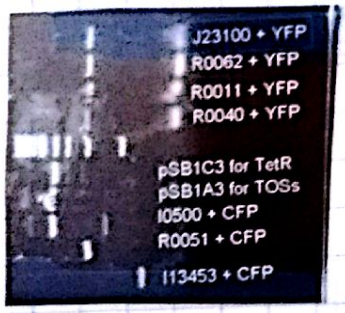
Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
J23101 + YFP MP 1 46	Default	8/2/2015	10:10 PM	90.22	1.804	0.972	1.86	2.14
J23101 + YFP MP 2 47	Default	8/2/2015	10:11 PM	52.94	1.059	0.583	1.81	2.10
J23101 + YFP MP 3 48	Default	8/2/2015	10:12 PM	95.59	1.912	1.045	1.83	2.24
J23101 + CFP MP 1 49	Default	8/2/2015	10:12 PM	50.52	1.010	0.547	1.85	2.19
J23101 + CFP MP 2 50	Default	8/2/2015	10:14 PM	56.29	1.126	0.615	1.83	1.93
J23101 + CFP MP 3 51	Default	8/2/2015	10:14 PM	57.18	1.144	0.626	1.83	2.11
J23106 gBlock MP 1 52	Default	8/2/2015	10:15 PM	98.48	1.970	1.068	1.84	2.20
J23106 gBlock MP 2 53	Default	8/2/2015	10:16 PM	106.57	2.131	1.141	1.87	2.27
J23106 gBlock MP 3 54	Default	8/2/2015	10:16 PM	88.41	1.768	0.950	1.86	2.13
J23117 gBlock MP 1 55	Default	8/2/2015	10:17 PM	52.93	1.059	0.588	1.80	2.18
J23117 gBlock MP 2 56	Default	8/2/2015	10:17 PM	77.35	1.547	0.840	1.84	2.14
J23117 gBlock MP 3 57	Default	8/2/2015	10:18 PM	40.30	0.806	0.424	1.90	2.16

-John set up Glycerols

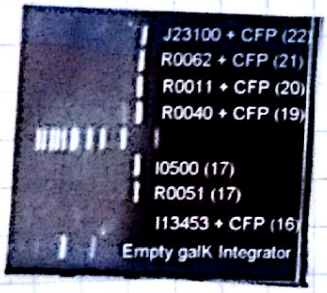
-John re-inoculated from today's MPs to image



Everything looks good!



Only I13453+CFP (10) looks bad... John is resetting these up



galK looks correct. Everything else is wrong. We'll double check these primers tomorrow

8/3/2015

Time in: 10:10 AM

Time out: 12:40 AM

DpnIs of last nights PCRs (#1-15, 23-26)

- [x] 17.5µL PCR Product
- [x] 2 µL Cutsmart
- [x] 0.5µL DpnI

- The gel of PCR (10) that John had re-set up with the three different minipreps yielded no bands, implying that the template MPs of "I13453+CFP" don't actually contain that assembly.

- I re-set up PCR (19), in order to double check the primers. A manual inspection of the primers didn't reveal any issues - they seemed to bound bind correctly.
  - The re-do of the PCR also failed, for reasons unknown

- John and I set up Gibson Assemblies (see JPM notebook, pg 88)
  - I did PCR Purifications, with nanodrops below:

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
R0040 YFP	Default	8/3/2015	2:24 PM	33.30	0.666	0.352	1.89	2.03
R0011 YFP	Default	8/3/2015	2:25 PM	31.40	0.628	0.335	1.87	1.94
R0062 YFP	Default	8/3/2015	2:26 PM	33.99	0.680	0.377	1.80	2.00
R0010 YFP	Default	8/3/2015	2:27 PM	45.21	0.904	0.508	1.78	1.22
I13453 YFP	Default	8/3/2015	2:27 PM	30.02	0.600	0.317	1.90	2.01
J23100 YFP	Default	8/3/2015	2:28 PM	41.27	0.825	0.463	1.78	1.42
R0051 YFP	Default	8/3/2015	2:29 PM	49.96	0.999	0.562	1.78	1.08
I0050 YFP	Default	8/3/2015	2:30 PM	47.68	0.954	0.530	1.80	1.79
R0051 CFP	Default	8/3/2015	2:30 PM	38.12	0.762	0.427	1.79	1.19
I0500 CFP	Default	8/3/2015	2:31 PM	47.10	0.942	0.526	1.79	1.24
Empty galK integrator	Default	8/3/2015	2:32 PM	75.15	1.503	0.892	1.68	1.00
pSB1A3 for ToS	Default	8/3/2015	2:33 PM	47.65	0.953	0.541	1.76	1.31
pSB1C3 for TetR	Default	8/3/2015	2:33 PM	37.96	0.759	0.420	1.81	1.47
R0040 YFP 2	Default	8/3/2015	2:34 PM	35.01	0.700	0.397	1.76	1.56
R0011 YFP 2	Default	8/3/2015	2:35 PM	48.73	0.975	0.572	1.70	1.20
R0062 YFP 2	Default	8/3/2015	2:36 PM	50.78	1.016	0.588	1.73	1.17
J23100 YFP 2	Default	8/3/2015	2:36 PM	46.51	0.930	0.520	1.79	1.34

- We all prepared for our presentation to Dr. Manos

  Dinner: 8:50 - 10:20 (we discussed our presentation)

- Practiced presentations

- Panya, Elli, and Taylor transformed the Gibson assemblies

- Elli is re-inoculating EcNR2 so that we can try to integrate R0040+CFP+KanR (and perhaps also RFP+KanR?)

- We discovered today that the TetR gBlock that we ordered used an incorrect sequence. Tomorrow, we'll order it again, this time using the original sequence that I had derived from the pSB1I3 backbone. We'll also try to re-PCR it up to assemble.

- Elli and Taylor are setting up PCRs to assemble:
  - F. TetR
  - I13453+CFP

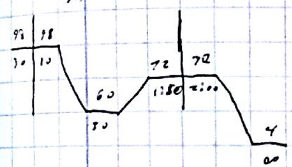
  (see Elli's notebook)

8/4/2015
Time in: 8:00 AM
Time out: 11:30 AM

-Practiced for/gave Dr. Manos talk

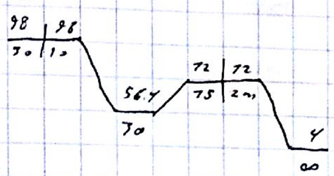
		6.5 µL	Primer 1 (2.5 µL)	Primer 2 (2.5 µL)	1 µL 1:100	12.5 µL Q5	Source
For Kate	A 1 R0051 + CFP	✓	A12 ✓	A19 ✓	✓	✓	150803 MP2
For Kate	A 2 I0500 + CFP	✓	A12 ✓	A19 ✓	✓	✓	150802 MP2
For Kate	A 3 J23100 + CFP	✓	A12 ✓	A14 ✓	✓	✓	150727 MP2
For Kate	A 4 I3453 + CFP	✓	A12 ✓	A19 ✓	✓	✓	*Sequence confirmed* 150801 MP2
For G?	A 5 R0080 + CFP	✓	A12 ✓	A14 ✓	✓	✓	150705 S13102 MP2
For G?	A 6 R0011 + CFP	✓	A12 ✓	A14 ✓	✓	✓	150715 I603??
For G?	A 7 R0062 + CFP	✓	A12 ✓	A14 ✓	✓	✓	150705 S?003 MP
	B 1 J23118	✓	A29 ✓	A64 ✓	✓	✓	150603 MP1
	C 9 FCFP	✓	A30 ✓	O11 ✓	✓	✓	150414 MP1
	C 10 FYFP	✓	A30 ✓	O11 ✓	✓	✓	150614 MP1
	D 11 FKan R	✓	A15 ✓	A16 ✓	✓	✓	150705 ? MP1

A



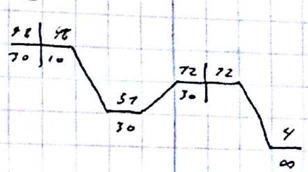
iGEM middle

B



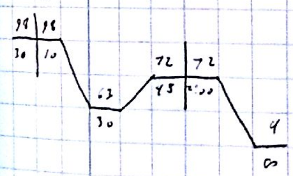
SAHA

C



~~SAHA~~ BRADLEY

D



iGEM right

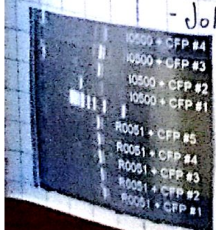
PCRs above done in the evening

-Saha lab meeting: 11:30AM - 2:30 PM

-Panya + Elli inoculated from yesterday's Gibson transformations

-John set up gels for PCRs to assemble F. TetR and I13453+CFP
-John DpnI'd and PCR-Purified, Gibson'd and transformed

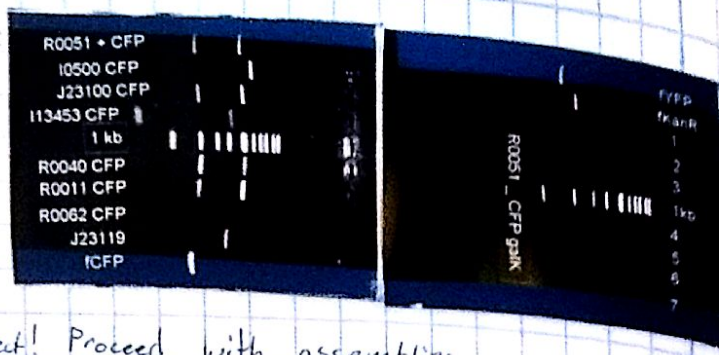
-John set up PCRs to integrate R0040+CFP and I0500+CFP (galK)
- We tried colony PCR'ing from all of the galK assemblies, but only the one worked
* only this one assembly looked good



-Taylor set up diagnostic colony PCRs of the rest of the R0051+CFP into galK colonies overnight, so tomorrow we can see if any succeeded

8/5/2013
Time in 10:05 AM
Time out 3:05 PM

Gels of yesterday's PCRs:



- R0062+CFP is not present - Redo
- I13453+CFP is not correct, but was sequence discarded last night
- None of the R0051+CFP into gelKs worked
- Everything else looks perfect! Proceed with assemblies

Today's assemblies:

- R0051+CFP + KanR
- I0500+CFP + KanR
- J23100+CFP + KanR
- R0040+CFP + ~~KanR~~ R0040+YFP
- R0011+CFP + R0011+YFP
- R0062+CFP + R0062+YFP
- J23119 + CFP
- J23119 + YFP

More assemblies:

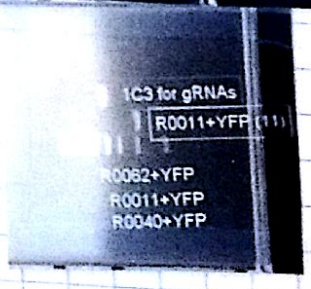
- J23101+CFP + KanR
- R0011 + YFP into pSB1A3
- R0040 gRNA into pSB1C3
- R0011 gRNA into pSB1C3
- J23119 gRNA into pSB1C3
- I0500 gRNA into pSB1C3
- I13453 gRNA into pSB1C3
- J23117 gRNA into pSB1C3
- J23106 gRNA into pSB1C3

According to sequencing data from last night, we now have 8 sets of Promoter

- R0040
- R0011
- R0062
- R0010
- J23100
- J23101
- R0051
- I0500

See pg80-81 Mel notebook

PCRs we set up:	Primer 1:	Primer 2:
For KanR → J23101+CFP	A12	A14
For G² → R0062+CFP	"	"
To make G² with KanR - THIS METHOD DOES NOT WORK. R0040+CFP+KanR	"	"
R0011+CFP+KanR	"	"
R0062+CFP+KanR	"	"
R0040+YFP	A15	A16
R0011+YFP	"	"
R0062+YFP	"	"
For TDS → R0011+YFP	A26	A27
For gRNAs → pSB1C3 for gRNAs	A11	A12



pSB1A3 + I6088 ToS MP1	Default	8/5/2015	1 17 PM	73 34	1 467	0 984	1 49	0 59
pSB1A3 + I6088 ToS MP2	Default	8/5/2015	1 18 PM	212 94	4 259	2 245	1 90	1 31
J23101 + I13504 MP1	Default	8/5/2015	1 20 PM	10 43	0 209	0 112	1 87	1 29
J23101 + I13504 MP2	Default	8/5/2015	1 21 PM	15 15	0 303	0 179	1 69	0 87
pSB1A3 + R0062 + YFP MP1	Default	8/5/2015	1 22 PM	70 17	1 403	0 892	1 57	0 66
pSB1A3 + R0062 + YFP MP2	Default	8/5/2015	1 22 PM	89 65	1 793	1 173	1 53	0 63
pSB1A3 + I13453 + YFP MP1	Default	8/5/2015	1 23 PM	7 62	0 152	0.092	1 65	1 20
pSB1A3 + I13453 + YFP MP2	Default	8/5/2015	1 24 PM	19 13	0 383	0 244	1 57	0 72
pSB1A3 + J23106 + I13504 MP1	Default	8/5/2015	1 25 PM	35 49	0 710	0 480	1 48	0 67
pSB1A3 + J23106 + I13504 MP2	Default	8/5/2015	1 27 PM	37 33	0 747	0 478	1 56	0 65
pSB1A3 + R0040 + YFP MP1	Default	8/5/2015	1 28 PM	37 13	0 743	0 490	1 51	0 64
pSB1A3 + R0040 + YFP MP2	Default	8/5/2015	1 29 PM	75 74	1 515	0 980	1 55	0 61
pSB1A3 + R0051 + YFP ToS MP1 (only 1)	Default	8/5/2015	1 30 PM	72 87	1 457	0 951	1 53	0.67

Gibsons:

R0051+CFP - 0.03 pmol - 2.528μL
F. KanR - 0.067 pmol - 2.434 μL

J23100+CFP - 0.027 pmol - 2.369μL
F. KanR - 0.066pmol - 2.398μL

I0500+CFP - 0.033 pmol - 2.377μL
F. KanR - 0.072 pmol - 2.616 μL

J23101+CFP - 0.025pmol - 8.060μL
F. KanR - 0.053 pmol - 1.926μL

J23119 - 0.033pmol - 3.437μL
CFP - 0.09 pmol - 1.54 μL

J23119 - 0.053pmol - 3.487μL
YFP - 0.09 pmol - 1.58μL } Had only ~3μL of master mix

-More reactions were calculated, but we ran out of master mix :(

PCR Purifications used in Gibsons

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230

- Taylor/Andy transformed the Gibsons

- Taylor and I electroporated to create dCas9 cells with:
  - R0051 TOS + R0051 gRNA G
  - R0051 TOS + Scramble H
  - R0062 TOS + R0062 gRNA C
  - R0062 TOS + Scramble D
  - J23100 TOS + J23100 gRNA A
  - J23100 TOS + Scramble B
  - J23101 TOS + J23101 gRNA E
  - J23101 TOS + J23110 Scramble F

- Taylor inoculated:
  - From yesterday's I13453 + CFP assembly
  - Re-transformation of J04450-pSB3K3
  - F. TetR assembly (VERY small colonies after 24+ hours of growth)
  - J23101 + I13504 onto 1C3 from G.S. to image
  - F. dCas9 from G.S. 3 (150629) - MAY or MAY NOT be sequence confirmed

8/6/2015
Time in: 10:25 AM
Time out: 11:10 PM

- ~~Yesterday~~ Worked on creating a list of PCRs and obscure protocols

- John miniprepped and made glycerols

Lunch: 12:00 - 1:40 PM

- Continued working on protocol list

Nap: 3:00 - 6:15

- John, Taylor, and I inoculated from yesterday's transformations

Dinner: 7:15 - 9:00 PM

- Worked on protocol list

8/7/2015
Time = 10:05 AM
Time out = 1:30 PM

- Selected "Track" for iGEM competition
- Met with Dr. Saha to discuss progress
- Miniprepped I0500+CFP+KanR and R0051+CFP+KanR

- [x] Pellet 3mL of bacteria
- [x] Resuspend cells in 250μL Buffer P1
- [x] Add 250μL Buffer P2. Invert 4-6x.
- [x] Add 350 μL Buffer N3. Invert 4-6x.
- [x] Centr for 10 min at 13,000 rpm
- [x] Apply supernatant to spin column. Centr 60s; discard flowthrough.
- [x] Wash w/ 500μL Buffer PB. Centr 60s; discard flowthrough
- [x] Wash w/ 750μL Buffer PE. Centr 60s; discard flowthrough
- [x] Centr 1 min to dry
- [x] Elute w/ 50μL Buffer EB

- Made glycerol stocks
- No growth in inoculations of dCas9 transformations from 150806 #
- Andy is writing/submitting our title/abstract

8/24/2015
Time = 10:45 AM
Time out = 5:30 PM

- During the weeks I'm been gone, Joe, Taylor, and John finished setting up the IMPs and taking measurements. The assembly of J23101, however, has not been sequenced, so I'm going to run a diagnostic RD.

	J23101 + I13504 #1	J23101 + I13504 #2 } from 150812 (from 150806GS1)
[x] 2μL Customark		
[x] MP:	6.51 μL	8.29 μL
[x] H_2O:	10.49 μL	8.71
[ ] 0.5μL EcoRI		
[ ] 0.5μL PstI		

- John is transforming Gibson Assemblies of F CFP-FYFP into KD (our new, low copy plasmid), and F-RFP into KD
- Joe is gel extracting the many FYFP-Backbones for 5 different promoters, to be assembled with Ptet

-It turns out that the JZ3101+I13504s from 8/12 were NOT the ones that were visualized and confirmed. Even though the MPs from 8/12 were from the 8/5 glycerols, we need to confirm the 8/5 MPs. We visualized from the 8/5 glycerols, so something must have gone wrong in the MP.

Diagnostic PCRs of JZ3101+I13504 (8/5)

- [x] 1 µL VF2
- [x] 1 µL VR
- [x] 0.5 µL MP
- [x] 2.5 µL H_2O
- [x] 5 µL Master Mix

Annealing temp: 52.6°C

8/25/2015
Time in: 2:00
Time out: 11:35am

-Last night's PCR yielded no bands. I'm going to repeat it with A26+A27 (2014)

- [x] 1 µL A26
- [x] 1.5 µL A27
- [x] 0.5 µL MP
- [x] 9.5 µL H_2O
- [x] 12.5 µL Master Mix

9:50 PM update: This PCR yielded no bands "

-Primers to amplify TetA have arrived! John resuspended those, and set up those PCRs (to assemble TetA + EYFPs under different promoters)

Only 1/4 worked

-DpnI of John's PCRs:

- [x] Entire volume of PCR -1 µL (for gel)
- [x] 2.7 µL CutSmart
- [x] 0.5 µL DpnI

-Andy PCR Purified the DpnIs

-Joe and John have been imaging the GA. samples

Gibson Assembly

① I0888 YFP - 0.025 mol - 3.44 µL
TetA - 0.075 mol - 1.52 µL

② R0051 YFP - 0.0423 mol - 2.37 µL
TetA - 0.1275 mol - 2.58 µL

③ R0040 YFP - 0.037 mol - 2.74 µL
TetA - 0.111 mol - 2.25 µL

④ R0062 YFP - 0.038 mol - 2.69 µL
TetA - 0.114 mol - 2.31 µL

⑤ R0011 YFP - 0.033 mol - 3.00 µL
TetA - 0.099 mol - 2.01 µL

Dinner/Activities fair: 6:05 PM - 9:10 PM

-John and Andy transformed the Gibson products

Just in case todays assemblies fail, we're re-setting up PCRs

(All undiluted)

YFP + BB	TetA (long)	TetA (short)
A12 + B51	B60 + B61	B59 + B61
51.6°C	57°C	57°C
1:45	0:45	0:45

Samples:

① I6088 MP1 150705 - Box 3 Well 24
② I6066 MP1 150705 - Box 3 Well 22
③ F YFP MP1 150614 - Box 1 Well 42
④ R0062 YFP MP1 150724 - Box 5 Well 26
⑤ R0011 YFP MP1 150724 - Box 4 Well 41
J04450 Tet MP1 150708 - Box 3 Well 35
J04450 Tet MP2 150708 - Box 3 Well 36

DPNI ran low #5 Purple got very little DPNI

See John's NB for full protocol

8/26/2015
Time in 12:25
Time out 11:00 PM

-Panya is MPing yesterdays inoculations of things onto low copy number plasmid

-Running gels of PCRs:

Tet1 L	Tet2 L	Tet1 3	Tet2 3	L	A3 A36 A39	C3 A34 A37	A3 A11 A12	C3 A11 A12

Gel #1

1	2	3	L	4	5

Gel #2

Tet 1 Long	Default	8/26/2015	5:56 PM	40.98	0.820	0.471	1.74	1.91
Tet 2 Long	Default	8/26/2015	5:57 PM	64.05	1.281	0.751	1.70	1.14
pSB1A3 A36+A37	Default	8/26/2015	5:58 PM	53.36	1.067	0.586	1.82	2.05
pSB1C3 A36+A37	Default	8/26/2015	5:59 PM	61.04	1.221	0.690	1.77	2.15
pSB1A3 A11+A12	Default	8/26/2015	6:00 PM	44.29	0.886	0.540	1.64	1.01

Gibson Assemble:

(I) pSB1C3 (A36 + A37) - 0.0235 pmol - 0.53 µL
J23101 + I13504 - 0.0705 pmol - 4.47 µL

(II) pSB1A3 (A36 + A37) - ~~0.626~~ 0.0235 pmol + 0.626 µL
J23101 + I13504 - 0.0705 pmol - 4.47 µL

(III) pSB1A3 (A11 + A12) - 0.0225 pmol - 0.722 µL
J23101 + I13504 - 0.0675 pmol - 4.28 µL

(IV) R0010 YFP - 0.032 pmol - 3.57 µL
TetA - 0.096 pmol - ~~0.71~~ 1.52 µL

(V) I0500 YFP - 0.031 pmol - 3.55 µL
TetA - 0.093 pmol - 1.47 µL

(VI) R0062 YFP - 0.0335 pmol - 3.39 µL
TetA - 0.1005 pmol - 1.59 µL

(VII) R0011 YFP - 0.0258 pmol - 3.64 µL
TetA - 0.084 pmol - 1.33 µL

Taylor's Gel Extraction

Drew: 7:15 - 8:15

8/27
Time
Time

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
I0500_YFP_gel_extract #1	Default	8/26/2015	7:02 PM	24.26	0.485	0.278	1.75	0.31
R0010_YFP_gel_extract #3	Default	8/26/2015	7:02 PM	18.89	0.378	0.212	1.78	0.16
R0062_YFP_gel_extract #4	Default	8/26/2015	7:03 PM	19.92	0.398	0.237	1.68	0.24
R0011_YFP_gel_extract #5	Default	8/26/2015	7:04 PM	15.49	0.310	0.164	1.89	0.42

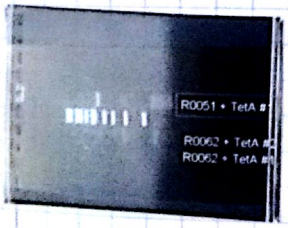
- Taylor is transforming the Gibson products
- Panya is setting up sequences of today's MPs and the J23101+I13504 from ~~Hes~~ 8/5
- I'm setting up PCRs to put F. CFP into our low copy plasmid to try to assemble G2 into this plasmid

Gibson PCR:

	Low Copy Plasmid	F. CFP
Primer 1	1 µL B54 ✓	2.5µL B52 ✓
Primer 2	1 µL B55 ✓	2.5µL B53 ✓
Miniprep	1µL 1:100 dilution ✓	1 µL Undiluted CFP
H_2O	6.5 µL ✓	9.5 µL sdd ✓
Q5	12.5 µL ✓	12.5 µL ✓
	56.5°C	63°C

The samples:

Ⓐ - I0500 CFP - 150801 MP#1 - Box 5 Well 62
Ⓑ - R0051 CFP - 150601 MP #1 - Box 6 Well 1
Ⓒ - R0010 CFP - F. CFP 150619 MP # - Box 1 Well 40
Ⓓ - R0062 CFP - 501003 150705 MP#1 - Box 3 Well 4
Ⓔ - R0011 CFP - 150724 MP#1 - Box 4 Well 39
Ⓕ - Kersch - 150816 MP#1 - Box 7 Well 8



It looks like we MIGHT have R0051 YFP + TetA!

Everything looks correct!

8/6/15
Time in: 12:00
Time out: 9:30

DpnI of last night's PCRs:

- [x] Entire volume of PCR
- [x] 2.7µL CutSmart
- [x] 0.5 µL DpnI

MinElute PCR Purification

- [x] Add 136 µl Buffer PB and mix
- [x] Apply sample to the column and centr 1 min. Discard flowthrough
- [x] Add 750 µl Buffer PE, centr 1 min. Discard flowthrough
- [x] Centr 60s to dry
- [x] Place in clean, labelled tube
- [x] Elute in 10 µl Buffer EB. Let stand 1 min.
- [x] Centrifuge for 1 min

- See John's NB for Gibson protocol (we Gibson assembled)

Class 3:15 - 5:00 pm

Transformation

- [x] Thaw cells on ice. Aliquot 10 µl into tubes
- [x] Add 2 µl Gibson product. Flick 4-5x
- [x] Incubate on ice for 30 min. Set heat block. Accidentally went 40 min
- [x] Heat shock at 42°C for 30s
- [x] Place on ice for 5 min
- [x] Pipette 300 µl SOC (RT) into mixture
- [x] Incubate 60 min at 37°C, 250 rpm. Pre-warm plates
- [x] Mix cells by flicking/inverting
- [x] Spread 20/200 µl of cells onto plates
- [x] Incubate overnight at 37°C

- We incubated yesterday's assemblies of F YFP-TetA and J23101+I13504 to K314

- Taylor miniprepped yesterday's incubations of R0051 YFP-TetA and R0062 YFP-TetA

- John set up the ultramer PCRs for these YFP-TetA to ...

8/25/2015

Time in: 10:15 am

Time out: ...

Gel of ultramer PCRs from last night. Bands should be ~2500

- Michael set up OpnT

- Class 11:00 - 2:00 pm

- Taylor PCR purified the ultramer PCRs

- John set up ultramer PCRs to integrate these ...

8/09/2015

Time in: 10:15 A...

Miniprepping

- [ ] Pellet 3mL bacterial overnight culture by centrifugation at 6800g for 3 min at RT
- [ ] Resuspend pelleted cells in 250µL Buffer P1
- [ ] Add 250µL Buffer P2, invert 4-6x
- [ ] Add 350µL Buffer N3, Invert 4-6x
- [ ] Centrifuge for 10min at 17,900g
- [ ] Apply supernatant to spin column. Centr 60s and discard flowthrough
- [ ] Wash spin col with 500µL Buffer PB. Centr 60s and discard flowthrough
- [ ] Wash with 750µL Buffer PE. Centr 60s and discard flowthrough (x2
- [ ] Centr 1 min to dry
- [ ] Place in clean 1.5mL tube
- [ ] Elute in 50µL Buffer EB. Let stand 1 min, then centr for 1min

Use set up allowed 8 days to integrate. Please

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
J23101+113504 1A3 A11+A12 #1	Default	8/28/2015	5:37 PM	184.31	3.686	1.958	1.88	2.36
J23101+113504 1A3 A11+A12 #2	Default	8/28/2015	5:38 PM	237.68	4.754	2.573	1.85	2.07
J23101+113504 1A3 A36+A37 #1	Default	8/28/2015	5:38 PM	215.85	4.317	2.320	1.86	2.31
J23101+113504 1A3 A36+A37 #2	Default	8/28/2015	5:39 PM	193.64	3.873	2.091	1.85	2.35
J0500 YFP+1gIA #1	Default	8/28/2015	5:40 PM	95.60	1.912	0.980	1.95	2.44
J0500 YFP+1gIA #1	Default	8/28/2015	5:40 PM	183.53	3.671	2.212	1.66	0.97
J0500 YFP+1gIA #2	Default	8/28/2015	5:41 PM	145.07	2.901	1.624	1.79	1.47
R0062 YFP+1gIA #1	Default	8/28/2015	5:42 PM	101.51	2.030	1.090	1.86	2.16
R0062 YFP+1gIA #2	Default	8/28/2015	5:42 PM	90.43	1.809	0.975	1.85	2.12
R0011 YFP+1gIA #1	Default	8/28/2015	5:43 PM	131.96	2.639	1.610	1.64	0.82
R0011 YFP+1gIA #2	Default	8/28/2015	5:43 PM	71.25	1.425	0.770	1.85	2.09

Dinner 6:00-7:30AM

- Set up DpnI of John's ultramer PCRs
- John re-set up the failed Ultramer PCRs
- Joe set up sequencing rxns for my MPs
- I inoculated from yesterday's transformations
- John is running a gel of the re-PCRs
- Taylor and I electroporated all gRNAs+ TDSs into dCas9 electrocomp cells (see JPM notebook pg 136 for samples)

Scanned by CamScanner

dCas9 Electroporation

To each tube of electro comp dCas9:

- [x] Add 0.5 µL gRNA (or scramble)
- [x] Add 0.5 µL TOS
- [x] Add 20 µL G/T
- [x] Electroporate (1600 - 1800 Volts)
- [x] Add 1ml SOC/LB
- [x] Incubate 1 hour
- [x] Plate

- John and Andy made electrocomp EcNR2 to integrate F. YFPs + TetA into at intC location (since none of yesterda inoculations of integrated F. CFPs + KanR grew)
  - From these, we will re-integrate the CFPs
- Andy miniprepped the assemblies onto KD (pg 39 40H NB
  - ~~Police~~ Left: 1:45 - 3:10
- Andy and I electroporated the TOSs + scramble gRNA
- ~~Joe~~ ~~Joe~~ We set up inoculations:
  - F. dCas9 (to make more electrocomp cells)
  - J23101 + I13504 assemblies from 150826(?)
  - Reinitiate of previously integrated F. CFPs
- Moved everything out of former iGEM lab
- Joe made more broths
- Taylor made more plates

inoculation did ~~not~~ grow

inoculation maybe grew

· inoculation grew

· Still no growth from the integrations, and no growth in the [illegible] electroporations from 180829

Diluting Amp

- [x] 1g Amp
- [x] 10 mL dH_2O

Saha Lab Meeting/Dinner 5:00 - 8:00 pm

- Taylor set up inoculations of EcNR2

PCR to Assemble R0010 YFP + TetA

- [x] 2.5 μL Primer 1
- [x] 2.5 μL Primer 2
- [x] 1 μL undiluted MP
- [x] 6.5 μL H_2O
- [x] 12.5 μL Master Mix

	R0010 EYFP 1500bp	TetA [illegible]
Primer 1	A12	360
Primer 2	BS1	36
	51.6°C - 1:45	57°C - [illegible]

· I set up more 023101 IMP inoculations

Ultramer PCRs to integrate CFPs

- [x] B19 2.5 µL
- [x] 2.5 µL B20
- [x] 1 µL MP
- [x] 6.5 µL H_2O
- [x] 12.5 µL MM

Annealing temp 55°C

Min. preps used

- R0011 + CFP + KanR MP2 150726
- I0500 + CFP + KanR MP1 150807 *
- R0051 + CFP + KanR MP1 150807 *
- R0010 F.CFP + F.KanR MP1 150705
- R0062 + CFP + KanR MP1 150726

* Unsure if sequencing confirmed

9/1/2015

Time in 6:00pm

Time out: 12:30

- This morning, Panya came in and ran gels of last night's PCRs
  - The PCRs to assemble R0010 + TetA failed
  - IMP colony PCRs #2 and #3 look correct, although the inoculations have not yet grown
  - The integration PCRs are all correct, except for R0051, which I will re-do

Ultramer PCR of R0051 + CFP + KanR

- [x] ~~B1~~ 2.5µL B19
- [x] 2.5 µL B20
- [x] 1 µL MP (same sample as last night)
- [x] 6.5µL H_2O
- [x] 12.5 µL MM

Dinner 6:30 - 7:30

- Before dinner, John had set up the re-inoculation of overnight EcNR2 for us to integrate

(See previous page for full protocol)

Retry of PCRs to assemble R0010 YFP + TetA

- [x] 2.5 µL Primer 1
- [x] 2.5 µL Primer 2
- [x] 1µL MP
- [x] 6.5µL H_2O
- [x] 12.5 µL qs

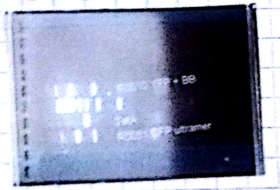
Paris-Bettencourt EcNR2:

- [x] Inoculated EcNR2 from overnight starter culture. Grow at 30°C
- [x] When OD600 is between 0.4-0.6, proceed
- [x] Place at 42°C for 15 min
- [x] Rapidly cool in icewater slurry for ≥5 min
- [x] Pre-cool the centrifuge to 4°C. Also, cool tubes and sdd
- [x] Centrifuge for 7min at 6900rpm
- [x] Pour off supernatant
- [x] Resuspend in 1mL cold sdd
- [x] Centrifuge for 7min at 6900rpm
- [x] Carefully decant
- [ ] Resuspend in 1mL H_2O
- [ ] Centrifuge 60s at max speed
- [ ] Carefully aspirate
- [ ] Resuspend in 50µL cold sdd + PCR product to be transformed
- [ ] Electroporate
- [ ] Incubate for 2hours
- [ ] Plate

9/2/2015
Time in: 3:45PM
Time out: 1:50AM

- Last night, we ended up not integrating as the EcNR2 were taking too long to grow

- The gel of yesterday's PCRs



I set up a DpnI of these PCRs
- [x] Entire vol of PCR
- [x] 2.7µL CutSmart
- [x] 0.5 µL DpnI

- Yesterday's PCR Purifications to integrate:

Sample ID	User ID	Date	Time	ng/ul (Divide all these by 12 → ~5µL)	A260	A280	260/280	260/230
I0500 CFP PCR Pur	Default	9/1/2015	4:28 PM	45.13	0.903	0.493	1.83	2.09
R0011 CFP PCR Pur	Default	9/1/2015	4:28 PM	59.90	1.198	0.669	1.79	1.92
R0062 CFP PCR Pur	Default	9/1/2015	4:29 PM	60.55	1.211	0.665	1.82	1.77
R0010 CFP PCR Pur	Default	9/1/2015	4:30 PM	68.74	1.375	0.767	1.79	1.94

- We're running the R0010 YFP + BB and R0051 CFP (to integrate) on a gel to gel extract

PCR Purification of TetA:

- [x] Add 100 (136) µL Buffer PB to rxn. Mix.
- [x] Apply sample to spin col. Centr 60s; discard flowthrough
- [x] Add 750 µL Buffer PE to col. Centr 60s; discard flowthrough
- [x] Centr 60s to dry
- [x] Place col in clean, labelled tube
- [x] Add 30 µL Buffer EB (heated); let stand 1 min
- [x] Centr 60s

Gel Extraction

- [x] Cut out DNA band from gel
- [x] Weigh slice in colorless tube
  Gel vol R0010: 61.3 µL
  R0051: 180.2 µL
- [x] Add 3 vols Buffer QG to 1 vol gel
- [x] Incubate at 50°C for 10min. Vortex the tube every 2-3 min.
  - [x] Is color yellow?
- [x] Add 1 vol isopropanol to the sample and mix
- [x] Place a QIAquick spin col in collection tube. Apply sample to col, and centr for 1 min. Discard flowthrough
- [x] Wash with 750µL Buffer PE. Centr 1min; discard flowthrough
- [x] Centr 1 min to dry
- [x] Place in clean tube, and elute in 30µL Buffer EB.

- Meeting/Dinner 7:00-8:30 PM

Taylor and John set up a Gibson of R0010 YFP + TetA

John, Taylor, and I integrated various promoters + CFP
- J23100 - Grew on plates
- R0011 - Grew
- R0062 - Grew
- R0051 - Grew, but colonies looked suspicious
- R0010 - Grew, but suspicious

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
R0051_CFP_KanR_Gel Pure	Default	9/2/2015	7:06 PM	11.48/12	0.230	0.098	2.33	0.38
R0010_YFP_BB_Gel Pure	Default	9/2/2015	7:06 PM	15.64	0.313	0.162	1.93	0.38
TetA_PCR_Pure	Default	9/2/2015	7:07 PM	75.32	1.506	0.919	1.64	1.17

- Taylor and I inoculated colonies of all 5 of yesterday's integrations in Low Salt media, if they grow, tomorrow we can Paris-Bettencourt them, and integrate their YFPs
  - Note: the R0051 CFP and R0010 CFP colonies looked very suspicious. We inoculated them, but it's possible that they won't grow

IntC Ultramer PCRs:

- [x] 2.5 µL B21
- [x] 2.5 µL B22
- [x] 1 µL 1:100 dilution of MP
- [x] 6.5 µL H_2O
- [x] 12.5 µL MM

Annealing temp: 56.5°C

The Samples:

2:30
- A - I0500 YFP + TetA #1 150825
- B - R0011 YFP + TetA #1 150826

1:30
- C - R0051 YFP + TetA #1 150822
- D - R0062 YFP + TetA #1 150827

} Note: None of these have been sequence confirmed, but they all grew in the correct resistance

- Came in to run a gel of last night's PCRs

- Class: 11:00 AM - 1:00 PM

DpnI of Ultramer PCR:

- [x] Entire vol. of PCR
- [x] 2.7 µL CutSmart
- [x] 0.5 µL DpnI

- I re-set up the PCR of ~~I0500~~ YFP + TetA with MPs 1 and 2:01 (~~#3~~ 1:45)

- All of the 150902 CFP integrations grew! Today, we can integrate the YFP + TetAs!!

- and I inoculated the integrated CFP

- Ran a gel of our re-try of I0500 YFP-TetA ultramer PCRs SH
  no success X

w/ Taka 3:00-5:00 PM

- Inoculated our assemblies of R0010 YFP-TetA

- Followed the Paris-Bettencourt protocol to electroporate and integrate
  - R0011 YFP-TetA
  - R0062 YFP-TetA
  - R0051 YFP-TetA
  - R0010 CFP-KanR
  - R0051 CFP-KanR

Ultramer PCR of R0051 CFP:

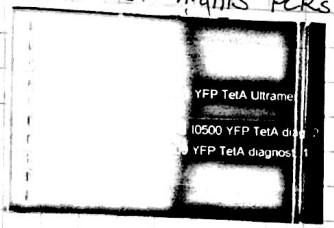
2.5 µL	B19	-56.9°C
2.5 µL	B20	-115
1 µL		
6.5 µL	H_2O	
12.5 µL	Master Mix	

Ultramer PCR of I0500 YFP-T...

Miniprep:

- [x] Pellet 3mL cells @ 6800g for 3 min at RT
- [x] Resuspend pelleted bacterial cells in 250µL Buffer P1
- [x] Add 250µL Buffer P2. Invert 4-6 times
- [x] Add 350µL Buffer N3. Invert 4-6x
- [x] Centrifuge for 10 min at 13000 rpm (~17,900g)
- [x] Apply supernatant to spin col Centr 60s, discard flowthrough
- [x] Wash with 500µL Buffer PB. Centr 60s, discard flowthrough
- [x] Wash with 750µL Buffer PE " " " "
- [x] Centr 1 min to dry
- [x] Place cols in clean, labelled tube. Elute in ~~30~~ 50µL pre-warmed Buffer EB.
  - [x] Let stand 1 min, and centr 1 min

- Gel of last night's PCRs:



- R0051 YFP + TetA looks good! Proceed to DpnI
- There are faint bands of I0500 YFP + TetA. I'll retry the integration PCRs

DpnI - Bradley Lab - Key #1

- [x] 2.7 µL CutSmart
- [x] Entire volume PCR
- [x] 0.5 µL DpnI

Ultramer PCRs:

- [x] 2.5 µL B14-21
- [x] 2.5 µL B20-22
- [x] 1 µL 1:100 dilution
- [x] 6.5 µL H_2O
- [x] 12.5 µL Master Mix

	Key	
- I0500 YFP+TetA MP1 150825	2	55°C 2:00
- I0500 YFP+TetA MP2 150825	3	
- R0010 YFP+TetA MP1 150905	4	56.5°C 1:15
- R0010 YFP+TetA MP2 150905	5	

9/8/2015

Time in: 5:30

Time out

- According to Joe, we might have a successful double integration (CFP + YFP) with R0051!
  - R0011 and R0062 do not appear to have worked clearly

- Last night, we set up inoculations to try the double integrations of:
  - R0051 (backup) } old PCRs
  - R0011
  - R0010 } New PCRs

Dinner: 6:30-7:35

Today, we'll be trying to integrate these

DpnI of R0010 ultramer PCR:

- [x] 2.7 µL CutSmart
- [x] Entire vol of PCR
- [x] 0.5 µL DpnI

Inoculations took too long to grow. We re-set them up for overnight 150909 CAG

Paris-Bettencourt Integration:

- [x] Inoculate 0.5 mL EcNR2 from overnight in 35 mL low salt LB
- [x] When OD600 is b/w 0.4-0.6, proceed.
- [x] Place at 42°C for 15 min (heat block, aliquotted into 1.5 mL tubes)
- [x] Rapidly cool in ice water slurry for ≥5 min
- [x] Pre-cool centrifuge, tubes, and sdd to 4°C
- [x] Centr for 7 min at 6900 rpm
- [x] Pour off supernatant
- [x] Resuspend in 1mL sdd
- [x] Centr 7 min at 6900rpm
- [x] Carefully decant

Protocol done 150909 CAG

- John set up the dilution of innoculations to Paris-Bettencourt

Make diluted Kan:

- [x] 0.1g Kan
- [x] 10 mL H_2O

- Re-diluted R0010 inoculation (it was growing too quickly)

- John made plates

- The dilutions of R0010, R0051, and R0062 (overshot) are growing much faster than R0011. These have been re-diluted @ ~5:40 pm

R Purification:

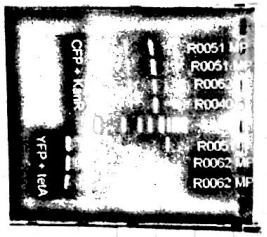
- [x] Add 136 µL Buffer PB to rxn. Mix.
- [x] Apply sample to spin col. Centr 60s, discard flowthrough
- [x] Add 750 µL Buffer PE to col. Centr 60s; discard flowthrough
- [x] Centr 60s to dry
- [x] Add 30 µL ~~Buffer EB~~ H_2O for integrations; let stand 1 min
- [x] Centr 60s

9/10/2015

Time in: 5:00pm

Time out: 2:30PM

~~All of~~ Gel of this morning's PCRs:



- John set up the inoculation for

- Dinner: 6:30-7:30

Paris Bettencourt Integration

- [x] Inoculate 0.5mL EcNR2 from overnight in 35 mL LB
- [x] When OD 600 is b/w 0.4-0.6, proceed
- [x] Place at 42°C for 15 min
- [x] Rapidly cool in ice water slurry for 5+ min
- [ ] Pre-cool centrifuge, tubes, and sdd to 4°C
- [x] Centr for 7 min at 6900 rpm (2x)
- [x] Pour off supernatant (carefully!) (2x)
- [ ] Resuspend in 1 mL cold sdd
- [ ] Centr 60s at max speed
- [ ] Carefully aspirate
- [ ] Resuspend in 50µL H_2O + PCR to integrate
- [ ] Electroporate
- [ ] Incubate for two hours
- [ ] Plate

Finished by John and Andy

Integrate
- R0
- R0
- R0
- R0
- RO

(All si

- Pokemon club: 9:00-10:10 PM

- We've now collected data for our R0051 double inte

- John set up PCRs to assemble R0040 YFP + Tet A (JPM

- John, Andy, and I set up inoculations:
  - R0010 CFP int.
  - R0011 CFP int.
  - R0051 CFP int.
  - R0062 CFP int.
  - I0500 CFP int.

  } To retry double integrations w/ YFP

  - R0010 CFP YFP double ints } To image

  - All plates we could find of dCas9 + TOS + gRNA } To

9/8/2015
Time in: 10:00AM
Time out: 10:50AM
Time in: 12:00PM
Time out: 1:30PM

Agarose gel:

- [x] 0.5g Agarose
- [x] 50mL TAE

Dilute TAE:

- [x] 40mL 50x TAE
- [x] 2L 18MΩ H_2O

Last night, we stored the successful double integrations of R0051 as glycerol stocks and plates!

Gel of PCRs to assemble R0040 YFP + TetA:

Repeat of R0040 YFP + BB PCR:

- [x] 2.5 µL A12
- [x] 2.5 µL B51
- [x] 6.5 µL H_2O
- [x] 1 µL undiluted R0040 YFP I0500S MP1 (AMP)
- [x] 12.5µL MM

Annealing temp: ① 51.6°C / ② 49.5°C
Extension time: 1:45

- Lunch: 1:20 - 2:15

Andy's PCR Purifications from yesterday:

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
r0051-yfp	Default	9/10/2015	8 39 PM	47 04[5]	0 941	0 495	1 90	2 27
r0051-cfp-1	Default	9/10/2015	8 39 PM	44 55[5]	0 891	0 495	1 80	1 58
r0051-cfp-2	Default	9/10/2015	8 40 PM	35 11[7]	0 702	0 405	1 74	1 74
r0062-yfp-1	Default	9/10/2015	8 40 PM	31 17[8]	0 623	0 366	1 70	2.33
r0062-CFP-1	Default	9/10/2015	8 41 PM	26 18[10]	0 524	0 281	1 86	1 93
I0500-YFP-1	Default	9/10/2015	8 41 PM	71 64[4]	1 433	0 813	1 76	1 74
I0500-YFP-2	Default	9/10/2015	8 42 PM	60 78[5]	1 216	0 631	1 93	2 31
R0040-CFP-1	Default	9/10/2015	8 42 PM	27 60[10]	0 552	0 301	1 83	2 08

- Taylor diluted the EcNR2 to retry the double integrations
- Left: 2:50-4:00PM
- Ran a gel of my re-attempts of R0040 YFP-BB PCR. Still no band
- The R0062 double integration plates have growth! Inoculated.

:10 - 9:25 PM

. I inoculated from yeste
to grow than double inte

ple Transformation - TOS: gRN

9/12/2015
Time in 4:00 PM
Time out: 8:40 PM

- Inoculated 35 mL of R0011 CFP integrations to try to integrate R0011 YFP today

- The PCRs of R0040 YFP + BB worked! John set up DpnIs. We'll need to gel purify the R0040 YFP + BB

Gel Purification:

- [x] Cut out DNA band from gel
- [x] Weigh slice in pre-weighed colorless tube
  Gel mass 1.2824 - 0.9946 = 0.2878 Gel volume 287.4 µL
  Tube mass 0.9946g
- [x] Add 3 volumes Buffer QG to 1 vol gel
- [x] Incubate at 50°C for 10 min. Vortex the tube every 2-3 min
  - [x] Is color yellow?
- [x] Add 1 vol isopropanol to the sample and mix
- [x] Place a QIAquick spin col in collection tube. Apply sample to col, and centr for 1 min. Discard flowthrough
- [x] Wash with 750µL Buffer PE. Centr 1 min, discard flowthrough
- [x] Centr 1 min to dry
- [x] Place in clean tube, and elute in 30µL Buffer EB

PCR Purification of TetA

- [x] Add 136µL Buffer PB to rxn Mix
- [x] Apply sample to spin col. Centr 60s, discard flowthrough
- [x] Add 750µL Buffer PE col. Centr 60s, discard flowthrough
- [x] Centr 60s to dry
- [x] Place col in clean, labelled tube
- [x] Add 30µL Buffer Heated EB; let stand 1 min
- [x] Centr 60s

Gibson of R0040 YFP + TetA
- 4.23µL R0040 YFP + BB
- 0.87µL TetA
- 5µL H2O

Dinner. 7:05 - 8:40

Transformation of Gibson

- [x] Thaw cells on ice. Aliquot 12µL of cells into tubes.
- [x] Add 2µL Gibson product. Flick 4-6x
- [x] Incubate on ice for 30 min Set heat block
- [x] Heat shock @ 42°C for 30s
- [x] Place on ice for 5 min
- [x] ~~Pipette~~ Pipette 950µL SOC
- [x] Incubate 60min @ 37°C, 250 rpm
  - [x] Pre-warm plates
- [x] Mix cells by flicking/inverting
- [x] Spread 20/200µL of cells onto plates
- [x] Incubate overnight at 37°C

Pacis-Bettencourt of R0011 cfp:

- [x] Dilute 0.5 mL overnight into 35mL LB
- [x] When the OD_{600} is b/w 0.4-0.6, proceed
- [x] Place at 42°C for 15 min
- [x] Rapidly cool in ice water slurry for 5+ minutes
- [x] Pre-cool centrifuge, tubes, and sdd at 4°C
- 2x
  - [x] Centr for 7min @ 6900 rpm
  - [x] Carefully pour off supernatant
  - [x] Resuspend in 1mL cold sdd
- [x] Centr 60s at max speed
- [x] Carefully aspirate
- [x] Resuspend in 50µL cold sdd + DNA
- [x] Electroporate
- [x] Incubate for two hours
- [x] Plate

- Inoculated many things

9/14/2015

Time in: 3:30PM - Yesterday, Andy ~~visualized~~ imaged

Time out: 2:01PM

- He also integrated

- Today, Elli is miniprepping R0040 YFP + Test

Ultramer PCR of RDDHD YEP:

- [ ] 2.5 µL B21
- [ ] 2.5 µL B22
- [x] 1 µL MP
- [ ] 6.5 µL H_2O
- [x] 12.5 µL MM