Robert Notebook October 2012

From Dueber Lab Wiki

Contents

Yeast Expected Fluorescences

New Assignments

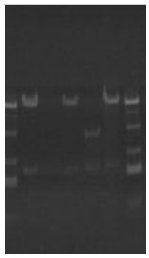
LZ-mKate	Red	Green	Blue	'	LZ-PTS1	Red	Green	Blue
1A	off	Nuc on	Nuc on		1A	off	CP on	CP on
1B	off	Nuc on	VM on		1B	off	CP on	Actin on
11A	off	Nuc on	off		11A	off	CP on	off
11B	off	VM on	Nuc on		11B	off	Actin on	CP on
14A	off	VM on	VM on		14A	off	Actin on	Actin on
14B	off	VM on	off		14B	off	Actin on	off
16A	off	off	Nuc on		16A	off	off	CP on
16B	off	off	VM on		16B	off	off	Actin on

Old Assignments

LZ-mKate	Red	Green	Cyan	'	LZ-PTS1	Red	Green	Cyan
1A	VM on	VM PA	VM on		1B	off	off	Actin on
11B	Nuc on	VM on	off		11A	Actin on	Actin on	CP on
14A	Nuc on	Nuc PA	VM on		14B	Actin on	CP PA	off
16A	VM on	off	off		16B	CP on	off	Actin on

Thursday 10/25

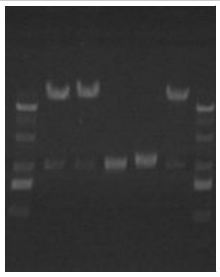
- 2am Miniprep from ~~130, 131,~~(were red) 132 cultures. Test digested:



132AC look correct. Will use 132C because it looks like more DNA.

- 5am Picked from yRC123, 124, 127. Seeded in block for 2nd integration later and for mid-log imaging.
- I'm having trouble with yJD001 not growing up. I seeded a culture at 6pm last night, and at 5am, it's still not growing.
  - I made more YPD and seeded a 0.2OD culture there. We'll see if it overtakes the old one.
  - Others in the lab have been having this issue.
- 8am Picked 1 clone from pRC130 and 3 clones from 131 plates.
- 8:30am Integrated pRC121, 122, 126, 128 (Leu); 129, 132-136 (Ura).
  - The Leu strains will be used for the sequential integrations later.
  - I accidentally plated 132 on the plate either labeled 126 or 128. I will know later. Will redo the 132 integration later today.
  - The Ura strains are to verify that that half-code is correct.
- 12pm Picked 4 colonies from pRC125 plate.
- 2pm Reran reaction for pRC130 by accident. Should have done 131 instead.
- 4pm Miniprep and test digest pRC130 and 131.

Lane	Contents	Bands
1	MW	MW
2	pos control 201-L	1766+10kb
3	130A	-
4	130B	1766+10kb
5	130C	1766+10kb
6	131	1766+10kb



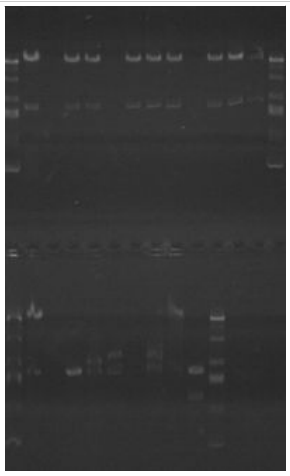
130A and 131 look good.

- 5pm ~~Depending on test digest results, re-transform 131 (old BsmBI rxn) with stock undiluted E-TGI.~~They were good, so no need to
- 5pm Integrated 130, 131, and re-integrated 132 (because of mix-up earlier today).
- 10pm Miniprep and test digest pRC125.

Wednesday 10/24

- 1am Electroporated and plated 130, 131 (new rxns), and 132 (old rxn). This time did a 5x dilution of E-TGI.
- ~~2am Miniprep 2x each of 125, 126, 130, 131. I doubt these will work, but there might be a chance.~~ Won't bother. When I tried with 121, 122, 128 heat shocked, 9/9 were wrong.
- 4am Inoculated culture with 3 clones each off the 125, 126 E-TGI plates.
- 12pm Inoculated culture with 130, 131 (new BsmBI reaction into ETGI) and 132 (old BsmBI reaction into ETGI). I don't think that the 130, 131 will work because there were ~98% red colonies, so I will rerun the BsmBI reaction for those.
- 12pm Miniprep and test digest all the 121, 122, 128 EPI300 clones and the 125, 126 E-TGI clones.

Lane	Contents	Bands	' Lane	Contents 2	Bands 2
1	MW	MW	1	MW	MW
2	pos control 201-L	1766+10kb	2	pos control 201-L	1766+10kb
3	-	-	3	-	-
4	121A (new BsmBI)	1766+10kb	4	125A (re-transformation of old BsmBI)	1766+10kb
5	121B	1766+10kb	5	125B	1766+10kb
6	-	-	6	125C	1766+10kb
7	122A (new BsmBI)	1766+10kb	7	-	-
8	122B	1766+10kb	8	126A (re-transformation of old BsmBI)	1766+10kb
9	122C	1766+10kb	9	126B	1766+10kb
10	-	-	10	126C	1766+10kb
11	128A (new BsmBI)	1766+10kb			
12	128B	1766+10kb			
13	128C	1766+10kb			

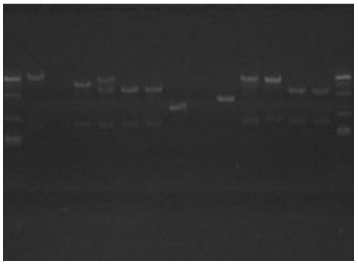


121A, 122A, 128A all worked. None of 125 or 126 worked, so will rerun those reactions and plate those.

- 4pm Reran BsmBI reaction for 125 and 126 because the test digests did not work out, so I think my original reaction failed. Will use 0.5ul of each part and 1.0ul of the backbone.
- 5pm Miniprepped and test digested 125, 126 that came from heat shock of old BsmBI reaction. Doubt these will work, but it's worth a try.

Lane	Contents	Bands
1	MW	MW
2	pos control 201-L	1766+10kb
3	-	-
4	125A	1766+10kb
5	125B	1766+10kb
6	125C	-
7	125D	1766+10kb
8	125E	1766+10kb
9	-	-
10	126A	1766+10kb
11	126B	1766+10kb
12	126C	1766+10kb

13	126D	1766+10kb
14	126E	1766+10kb



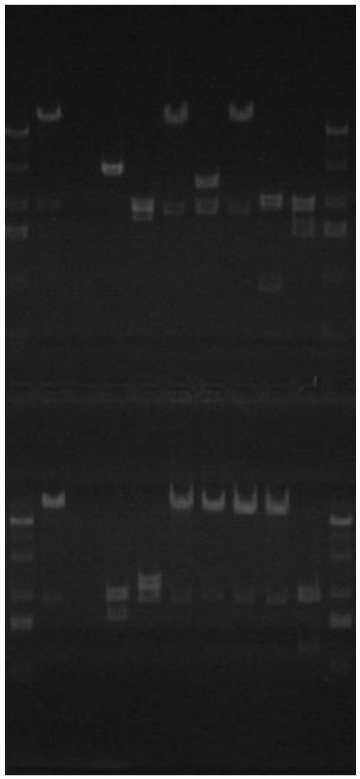
Only 126B worked. The second band for the + control (pRC221) did not show up, but I'm not too concerned because it showed up correctly before. This time, the NotI might just be too dilute.

- 5pm Electroporated 130, 131 with old BsmBI reaction. I won't need these if the 130, 131 that grow up later (that were electroporated with E-TGI with new BsmBI reaction) work. However, I don't trust them because there were ~98% red.
- 10:30pm Electroporated and plated new BsmBI reaction of pRC125.
- 11pm Seed yRC123, 124, 127 for midlog imaging.

Tuesday 10/23

- 12:30am Test digested:

Lane	Contents	Bands	' Lane	Contents 2	Bands 2
1	MW	MW	1	MW	MW
2	pos control 201-L		2	pos control 201-L	1766+10kb
3	123A	1766+10kb	3	126A	1766+10kb
4	123B	1766+10kb	4	126B	1766+10kb
5	123C	1766+10kb	5	127A	1766+10kb
6	124A	1766+10kb	6	127B	1766+10kb
7	124B	1766+10kb	7	129A	1766+10kb
8	125A	1766+10kb	8	129B	1766+10kb
9	125B	1766+10kb	9	129C	1766+10kb

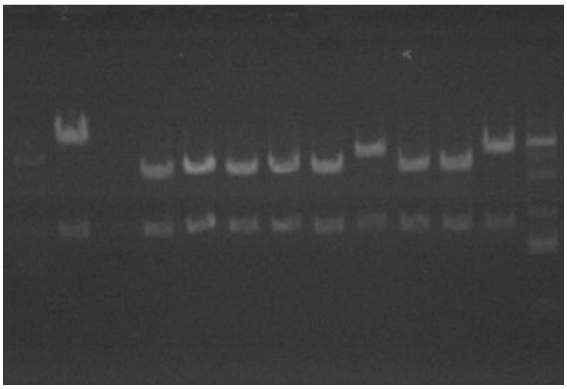


123C, 124B, 127A, 129A worked, so will integrate the first 3.

- 2am Picked 5 colonies per pRC121, 122, 128 TGI plate.
- 2am Ran colony PCR to screen through these colonies. Depending on this screen, will decide whether to transform the new BsmbI reaction.
- 2am Heat shocked 5.0ul of pRC125, 126. Will pick at 3:30pm later today.
- 3:30am Linearized and integrated pRC123, 124, and 127 into yJD001. Plated on SD-Leu.
- 12pm Miniprep and test digested the pRC121, 122, 128 clones.
    - Colony PCrs showed no bands because the DNA got "caught" in the well (I could see bright band).
    - Initially prepped 3/5 colonies I seeded this morning.

What I mean by it getting "caught"

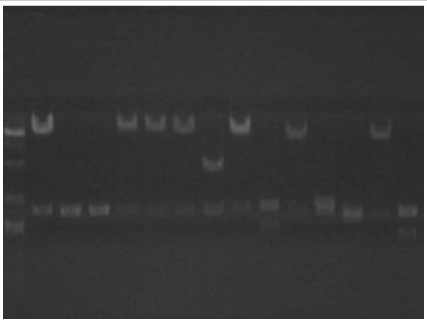
Lane	Contents	Bands
1	MW	MW
2	pos control 201-L	1766+10kb
3	-	-
4	121A	1766+10kb
5	121B	1766+10kb
6	121C	1766+10kb
7	122A	1766+10kb
8	122B	1766+10kb
9	122C	1766+10kb
10	128A	1766+10kb
11	128B	1766+10kb
12	128C	1766+10kb



I think all of them are bad.

- 12pm Suspended pRC132-136 in A1. Will miniprep at 3:30 because these won't be needed until the next round of integrations so aren't as urgent.
- 1pm Seeded yJD001 for integrations later today.
- 4pm Seeded 5 clones each of 125, 126, 130, 131 TGI that came from the original BsmBI reaction.
- 4pm Seeded 121, 122, 128 EPI300 that came from the rerun reaction.
- 4pm Electroporated pRC125, 126 with the original reaction. Used 1.0ul of DNA and 30ul of 5x diluted E-TGI (instead of 10x before). 10% chance I might have switched the tubes. We'll know after the yeast grow.
- 5pm Harneet miniprepped 132-136 for me.
- 7pm Test digested the miniprepped 132-136:

Lane	Contents	Bands
1	MW	MW
2	pos control 129	1766+10kb
3	132A	1766+10kb
4	132B	1766+10kb
5	133A	1766+10kb
6	133B	1766+10kb
7	134A	1766+10kb
8	134B	1766+10kb
9	134C	1766+10kb
10	135A	1766+10kb
11	135B	1766+10kb
12	135C	1766+10kb
13	136A	1766+10kb
14	136B	1766+10kb
15	136C	1766+10kb



133AB, 134AC, 135B, 136B worked. All except 132 are represented, so I will re-transform.

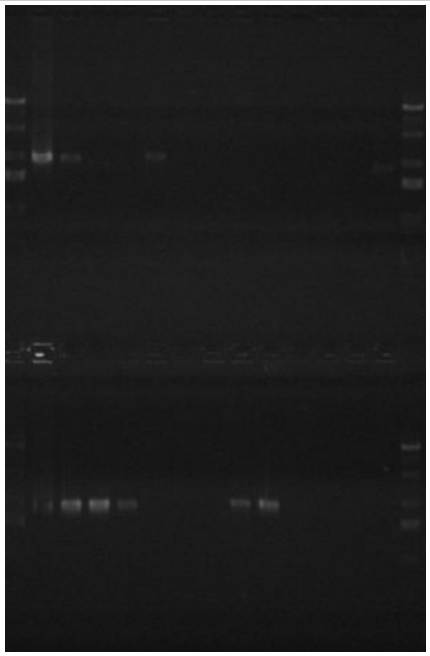
Monday 10/22

- 1am Because I don't want to be delayed at all, I electroporated all 16 plasmids.

    - 121-129 were E-TGI.
    - 130-136 were EPI300 because I ran out of E-TGI. Shouldn't be a problem because I'll be integrating then in the 2nd round anyways.
- 12pm Pick from plates pRC123-127, 129. The other 3 E-TGI ones had no colonies.
- 1pm Heat shocked 5.0ul of 121, 122, 128 into TGI because I either got no colonies at all (121, 122) or no white (128).
- 1:30pm In parallel, redid the assemblies, this time just adding 0.5ul of each part and 1.0ul of the digested backbone. Later, will both heat shock and electroporate.
- 4pm Sent several PAmCherry and PAGFP cassettes for sequencing.
    - pRC070 with AN59 F, AU72 F, O44 R.
    - pRC096 with S16 F, O44 R.
    - pRC075 with AN59 F, AU72 F, O44 R.
    - pRC092 with S16 F, O44 R.
- 9pm Picked colonies from 132-136.
- 9pm Heat shocked 5.0ul of 130, 131 into TGI.
- 10pm Redid assemblies for 130, 131, this time adding 0.5ul of each part and 1.0ul of the digested backbone.
- 10pm Miniprep.

Sunday 10/21

- I keep getting contaminated plates, so in the future, I will try using water+P/s in the last couple washes next time and adding it to my plates when I pour them.
- 1am Checked yeast that was growing. Seeded new 5ml culture for integration later today.
- 1am Ran test colony PCR with new primers:

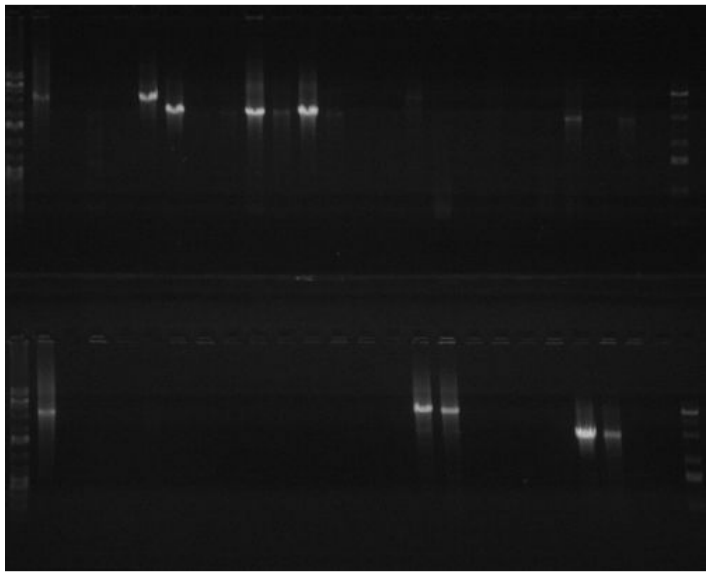


Some bands did not show up because the GG concentration was too high and precipitated the DNA in the well I think. Next time, will use the dilution that Jonas made.

- 1pm Seeded my yeast strains for integration later today. I'm guessing they'll be ready at 3pm.
- 2pm Linearized the Ura integration vectors with NotI. Did each alone, and will combine all four of them later post-elution.
- 5pm Integration.
    - Grew up 10ml of culture, reduced down to 75ul in last 100uM LiOAc step.
    - Combined 25ul of each Leu strain together to get a mix.
    - Used the normal amount of cells (117ul).
    - Plated 5 different SD-L-U plates, 4 for each 1x1 and 1 for the 4x4.
- 7:30pm Redid MiCode assignments to leave out PA proteins. This will cut down the combinations to 3*3*3*3 (on1/on2/off), which means we can still do a 8x8.
    - Chose the 4 pairings that showed strong interactions and their reverse (1A-1B, 1B-1A)x4 = 8.
    - Prey = pRC121-128: 1A, 1B, 11A, 11B, 14A, 14B, 16A, 16B.
    - Bait = pRC129-136: 1A, 1B, 11A, 11B, 14A, 14B, 16A, 16B.

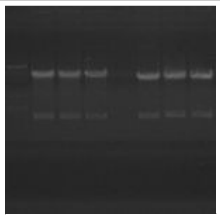
Saturday 10/20

- 1am Ran colony PCrs. Most of them failed because I expect ~66% correct for the plasmid MGs.



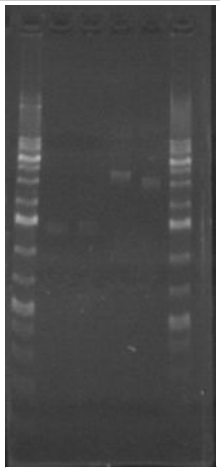
Positive control worked, but most colonies did not.

- 3am Gel purified more pRC116 and pRC117 because I will be using 1.0ul per reaction.

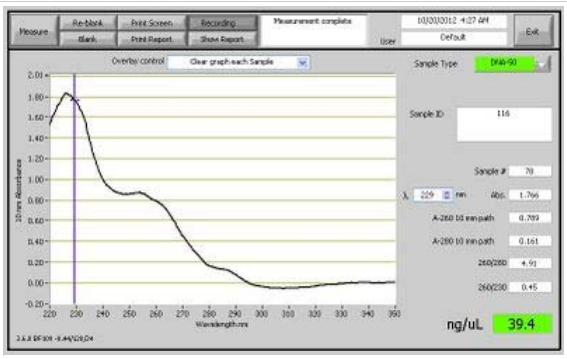


All as expected

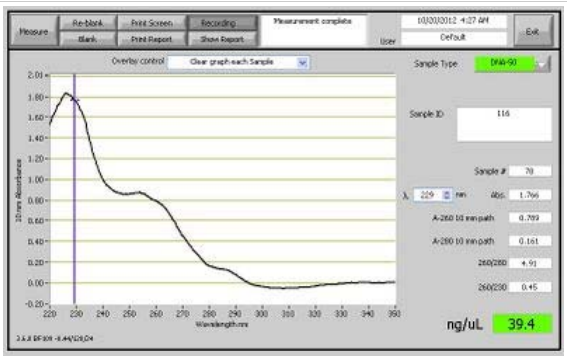
- 4am Ran gel of and nanodropped purified gel elution:



Same dilution I do all my DNA test digests at (1ul of DNA, load 5ul in well)

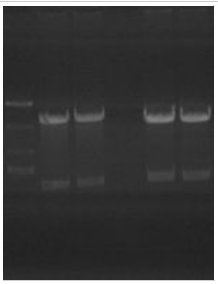


pRC116 gel pure= 39.4ng/uL



pRC117 gel pure = 40.7ng/uL

- 6am Purifed more because I only had ~36ul each.


All as expected

- 6am Tested titration of backbone. Will heat shock (with Will's new cells) to find optimal conditions -- decent number of colonies with fewest red.

Tube	Description
1	201-L, 0.,5ul backbone
2	201-L, 0.75ul backbone
3	201-L, 1.0ul backbone (planner's request)
4	242-U, 0.5ul backbone
5	242-U, 0.75 backbone
6	242-U, 1.0ul backbone (planner's request)

- 6am Picked 3 colonies each off integrant Leu plates so I can quickly do integrations after confirming them on a scope.
- 12pm Check on Leu integration plates.
- 2pm Heat shocked the test backbone titration. Will hold off on the 80 BsmBI reactions until I see these plates and count colonies.
- 7pm Rerun colony PCR off of integration heat shock test plates to characterize % correct assemblies better.
  - Forward will be same for both bait half-code, prey half-code, on mTurquoise, and off EmCFP-containing parts: BB18.
  - Reverse for left half: AU40 for left half (Con4 rev). Expect 1057bp band.
  - Reverse for right half: AJ23 for right half (Con3 rev). Expect 1126bp band.
  - Picking 3 colonies per plate, 24 total.

- Using pRC201-L as positive control again.

Friday 10/19

- 2pm Pick from pre-digested library assembly plates.
- 2pm Colony PCR and pick from integration heat shock plates.
  - For pRC116/Leu halves, use AJ24 For and AS24 Rev, which cover Con4 into Leu3'homo. Expect 2561bp.
  - For pRC117/Ura halves, use AR62 For and V24 Rev, which covers from end of Ura5'homo into Con2. Expect 1762bp.
- 2pm Test a colony PCR screen of the predigested plasmid MG heat shock plates in the fridge.

4x4 Testing Summary

Plasmid Approach MG round

- Need to pre-digest otherwise red colonies
- Heat shock gives ~66% correct product if colony is white. 3ul of DNA and plating everything gives me good results.
- Electroporation has given me 100% correct product if colony is white. 1ul of DNA and plating everything gives me good results.
- I don't fully trust all white colonies through heat shock, but I do for electroporations.
- Need to pre-digest the backbone, which I have stock of.
- Test colony PCRs.
- **When I scale up:**
  - Pre-digest backbone, which I have done already.
  - Do first-round heat shock (3ul DNA and plate all cells), colony PCR 3 clones while growing up in a 24well block (8x3clones ea).
  - If none good, then electroporate (1ul DNA and plate all cells).

Full MiCode/Library Assembly round

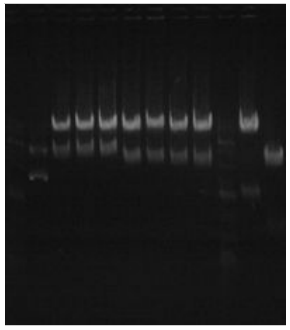
- Lots of colonies, but >95% red if I don't predigest backbones. Red % seems to be the same between heat shock and electroporation.
- I'm getting almost no (3-5) colonies if I predigest, use 3ul of DNA, and heat shock or electroporation (similar-looking plates). However, I think the colonies are correctly assembled.
- I fully trust any white colonies produced in the BsaI library assembly to be correct.
- Not sure if pre-digesting the backbone even helps.
- **Find a way to do this efficiently.**
  - Nanodrop gel purified backbones.
  - Rerun with 1.0ul of pre-digested backbone instead of 0.5ul.
  - Try predigesting only 517, which has RFP.
  - Ligase buffer?
  - Nanodrop to equalize molar amounts
  - Heat shock with more DNA and plate all cells.
  - Electroporate with undiluted tube of cells.

Integration Approach

- Heat shock then plating all the cells gives 10-20 colonies, which is not super great.
- Almost all (90%) electroporation white colonies were correct.
- I almost fully trust all the white colonies if I electroporate.
- Test colony PCRs.
- **When I scale up:**
  - Pre-digest backbone, which I have done already.
  - Do first-round heat shock (3ul DNA and plate all cells), colony PCR 3 clones while growing up in a 24well block (8x3clones ea).
  - If none good, then electroporate (1ul DNA and plate all cells).

Thursday 10/18

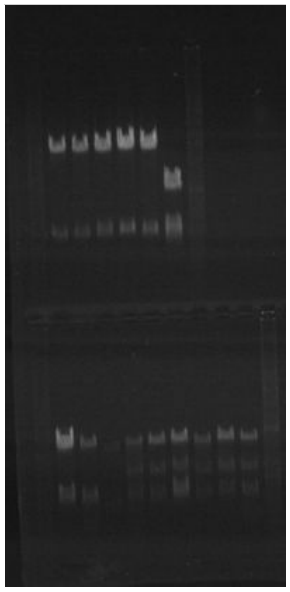
- 1am Miniprepped the colonies that Austin seeded (E-TGI integrations).
  - Test digested with BsaI, expecting 4558+10kb for Leu half, and 3846+10kb for Ura half.
  - Used pRC202 for pos (will have similar banding for the larger chunk) pRC117 for neg.
  - If these work, will integrate Leu halves into yJD001.



All worked expect for 201! For this gel, I added no GG while pouring gel, diluted GG 10x with loading buffer, then added the typical amount I add (2.5ul/10ul digest). I did a weird dilution of the ladder, which made me end up adding less ladder. For the next gel, I will do a 1:1 dilution ladder:GG and add 1-2ul to the well.

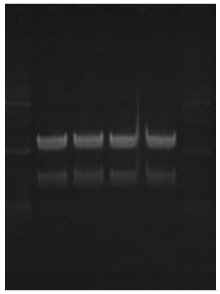
- 4am Miniprep and test digest E-TGI library cloning un-predigested (3 clones each of 1Ax1A, 1Ax1B, 1Bx1B in a block)

Tube	Plasmid	Clone	Enzymes	Buffer	Bands
1	201-L	B	NotI	3	14439+1766
2	201-L	C	NotI	3	14439+1766
3	222 + control		NotI	3	10kb+1766
4	227 + control		NotI	3	10kb+1766
5	231 + control		NotI	3	10kb+1766
6	116 - control		NotI		3629+1766
7	1Ax1A	A	EcoRI/PstI	3	14793+3429+3123+2297+1233+1233
8	1Ax1A	B	EcoRI/PstI	3	"
1	1Ax1A	C	EcoRI/PstI	3	"
2	1Ax1B	A	EcoRI/PstI	3	18500+3429+1270+1233
3	1Ax1B	B	EcoRI/PstI	3	"
4	1Ax1B	C	EcoRI/PstI	3	"
5	1Bx1B	A	EcoRI/PstI	3	12014+4987+3429+1233
6	1Bx1B	B	EcoRI/PstI	3	"
7	1Bx1B	C	EcoRI/PstI	3	"



Confirmed that all of the integration MGs should work. I think that 1Ax1A, 1Ax1B, 1Bx1B are correct, so will transform 1ul of clone A, C, and B respectively.

- 4am Check on plates for pre-digested library assembly for plasmid 4x4.
  - Only the TGI plate had colonies. Very few for the E-TGI plates.
  - I got very few colonies...3-5 per plate. However, they were almost all white except for the 4x4 plate, which had 3/15 red.
  - The number I pick will depend on the test digest above.
- 4am Miniprepped pRC70, 115, 116, 117.
- 7am Transformed 1Ax1A, 1Ax1B, 1Bx1B (not pre-digested), plate on URA. Should be ready Saturday morning.
- 7am Integrated pRC201, 222, 227, 231, plate on LEU. Should be ready Saturday morning.
- 1pm Nanodropped pWCD517 and pWCD563 gel purifications.
  - pWCD517 = -2.2 ng/ul!
  - pWCD563 = 64.2 ng/ul.
  - Will only gel purify 517 in future because 563 doesn't have RFP and has two bands that are too similar in size to purify.
- 2pm For integration MGs, heat shocked 3ul of DNA and plated all cells.
- 4pm Redigested pWCD517.

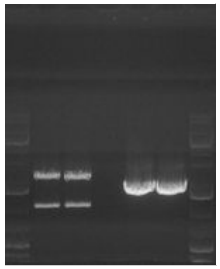


I loaded with the LD+GG, so will do that in the future. Also, I used 5ul of my ladder cocktail: 9ul ladder, 1ul GG (diluted), 10ul dH2O.

- 5pm Gel purified out larger band and nanodrop. 22.1 ng/ul.
- 6pm Ran new BsaI reaction for 4x4, 1Ax1A, 1Ax1B, 1Bx1B.
  - Used 1.0ul of pWCD517 gel purified, 0.5ul of pWCD563, and 1.0ul of each half.
- 11pm Plate with TGI and E-TGI.

Wednesday 10/17

- 1:30am Plated E-TGI electroporation the BsaI library assemblies because heat shocks were primarily red.
  - Streaked 10ul out and used beads for remaining 190ul.
- 4am Checked BsaI library assembly plates, and had tons of red. Some non-red though, so will pick later.
- 4am Digested pWCD517 and 563 to see if predigesting backbone will help.
  - pWCD517 expect 1870+901bp, purify larger band.
  - pWCD563 expect 1650+1586bp, could not separate the two, so just purified it together.
  - Ran BsaI assembly reaction with predigested backbones, 0.5ul of each, and 1ul of half-MiCodes.



Couldn't separate out bands of pWCD0563.

- 11am Picked colonies from MMG, integration parents pRC116 and 117, and cassette pRC70 and 115 plates.
- 11am Heat shock and electroporated BsaI library reactions that were pre-digested.
- 12pm Austin picked from the E-TGI integration plates.
- 4pm Picked 3 colonies each off of 1Ax1A, 1Ax1B, 1Bx1B E-TGI not pre-digested plates.
- 5pm Scraped 4x4 plate and miniprepped. I will get a lot less DNA because I accidentally overloaded the reagents with too many cells.
- 9pm Transformed the 4x4 miniprep into yJD001. Used 1ul of DNA.

Meeting Notes

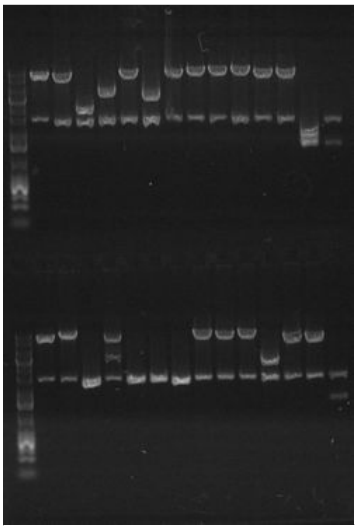
- For 4x4 do full characterization.
  - Show error bars in graphs.
  - Heat map.
- For 40x40:
  - Show picture.
  - Do nuclei check.
- Slide 1.
  - 4x4, sample image micode, sample image zipper.
- Slide 2.
  - Characterized N cells
  - Graph with SDs.
- Slide 3.
  - Heat map.
- Slide 4
  - 40x4x0, sample image micode, sample image zipper.

Tuesday 10/16

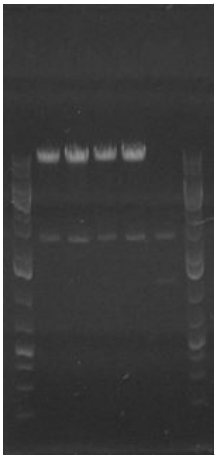
- 6am Miniprep.
- 8am Test digest, comparing to + (old 221, 262) and - (61, 62) controls.

Lane	Contents	Bands	Lane	Contents 2	Bands 2
1	MW	MW	1	MW	MW
2	pos control	1766+10kb	2	pos control	1766+10kb
3	201A	1766+10kb	3	241A	1766+10kb
4	201B	1766+10kb	4	241B	1766+10kb
5	201C	1766+10kb	5	241C	1766+10kb

6	202A	1766+10kb	6	242A	1766+10kb
7	202B	1766+10kb	7	242B	1766+10kb
8	202C	1766+10kb	8	242C	1766+10kb
9	203A	1766+10kb	9	243A	1766+10kb
10	203B	1766+10kb	10	243B	1766+10kb
11	203C	1766+10kb	11	243C	1766+10kb
12	204A	1766+10kb	12	244A	1766+10kb
13	204B	1766+10kb	13	244B	1766+10kb
14	204C	1766+10kb	14	244C	1766+10kb
15	neg control	1766+937	15	neg control	1766+937



IT WORKED...somewhat; not all of them assembled correctly. Will use 201A, 202A, 203A, 204A, 241A, 243A, 244B in the next round of assembly. Am going to miniprep the elect-TGI clones of 242 to see if there is correct product in the mix but it was disfavored.

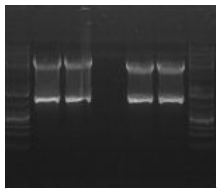


Miniprepped pRC242A-C elec-TGI, which worked perfectly. So for the rest of the assemblies, will first do heat shocks, then electroporate any that did not work.

- 9:30am Ran BsaI reaction to produce:

Tube	Description
1	4x4 library
2	LZ1A-LZ1A
3	LZ1A-LZ1B
4	LZ1B-LZ1B

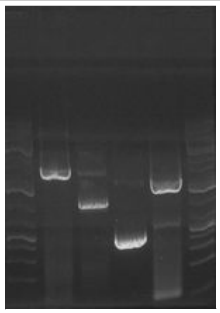
- 2pm Heat shocked the full MiCodes.
  - 3ul DNA, 50ul cell cocktail.
  - After shock, added 100ul LB and plated 50ul to dilute cells (got lawns last times).
- ~~2pm Digested backbone. Used 8ul of DNA for each (20ul total digestion mix). will load 10ul per well and elute in 10ul TAE. This way, I have ~30ul total (combined with yesterday), of which I will use 0.5ul per MG assembly reaction.~~Not going to do the large 80 right now. Also, unsure if I digested with BsaI or BsmBI, so will redo everything.
- 4pm Digested pRC116, 117 backbones with BsmBI. Will try to do a 4x4 integration library in parallel to see which looks better.
  - Expect 901+4594bp for pRC116, and 901+3882bp for pRC117.



Cut out larger bands, which seemed correct.

- 4pm Heat shocked transformed my 116 and 117 stocks and plated. 0.5ul plasmid, streaked out 5ul.
- 4pm Because I don't trust my pRC116, re-PCRed 116 parts, WCD phusion 55C with 35 cycles instead of 30:

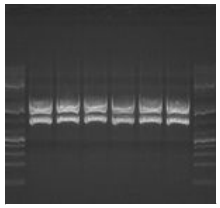
Description	Primers	Tm	Result
pWCD524, Leu2_Int_5'	BC38	51°	2320bp
	BC39	55°	
pRC61, mV1	BC40	52°	976bp
	BC41	54°	
pWCD559, Leu2_Int_3'	BC42	52°	516bp
	BC43	53°	
pRC61, mV1	BC44	51°	1766bp
	BC45	50°	



All look correct. I will gel purify, eluting into 10ul, run Gibson and electroporate in case tonight reaction doesn't work.

Part #	Part desc.	Ci (uM) enter	Vi (uL)
1	pRC116.1	31.6	1.184039113
2	pRC116.2	17.3	2.162753524
3	pRC116.3	77.9	0.480303414
4	pRC116.4	31.9	1.172903949
Total			5

- 6pm Re-gel purified pRC061 and pRC062 with BsmBI because I think I did it with BsaI by accident.
  - Did 3x volume so will have 30ul total later.
  - Expect 1802+901bp for both. Want the 1802 chunk.



All as expected.

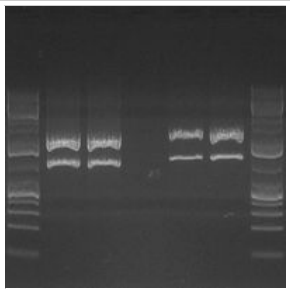
- 6:30pm Did BsmBI reaction to create the following with the pRC116 and pRC117 pre-digested backbones.

Tube	Description
1	1A-mKate, pRC201-L
2	11B-mKate, pRC222-L
3	14A-mKate, pRC227-L
4	16a-mKate, pRC231-L
5	1B-PAGFP-PTS1, pRC242-U
6	11A-PAGFP-PTS1, pRC261-U
7	14B-PAGFP-PTS1, pRC268-U
8	16B-PAGFP-PTS1, pRC272-U

- 12am Plated BsmBI integrations. Streaked out 10ul of TGI, and beaded out all E-TGI.

Monday 10/15

- 12am Redo BsmBI reaction for 4 prey (201-204) and 4 bait (241-244) using pre-digested, gel purified backbone. Want the 1802bp backbone.
  - Used 0.5ul of the backbone and 1ul of all other parts.

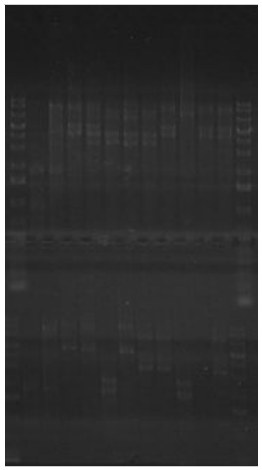


Cut out larger bands, gel purified in separate volumes so I would have 20ul of each total.

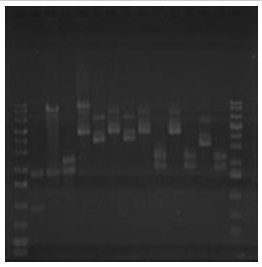
- 5am Electroporate and heat shock simultaneously.
  - For electroporation, use 30ul of freshly diluted TGI electrocomp, 1ul of DNA; plate all.
  - For heat shock, use 5ul of DNA, 50ul of our TGI cocktail; plate all.
  - Rescued both in LB ~70min.
- 7am Plated both electroporations and heat shocks.
- 8pm Picked from plates. Picked 3 clones each from both TGI and elecTGI. Will only prep TGI at first.

Tuesday 10/9

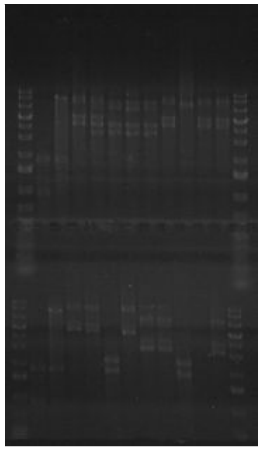
- 12am Test digested all plasmids I've miniprepped already.
  - The 18 TGI: pRC201, 209, 218 219, 224, 229, 231, 236, 238, 239, 257, 261, 262, 264, 275, 276, 277, 278, 280.
  - The 11 EPI300: pRC206, 207, 208, 226, 230, 235, 237, 240, 244, 247, 268.
  - + control = the MGs that I used before (pRC221 and 262).
  - - control = backbones pRC061, 62.



1st lane is - control, 2nd lane is +, then same order as before.



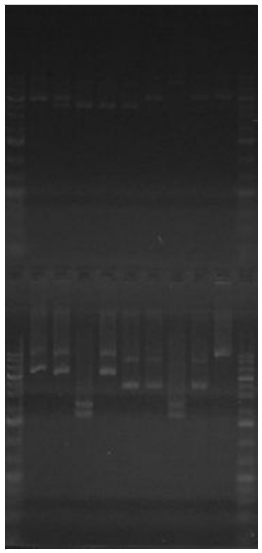
1st lane - control, 2nd lane + control, then the order for the EPI300 listed above.



Stained gel 1 for longer for clearer bands. I don't expect the MMG I did yesterday to work, and am going to be amazed if it works.

Monday 10/8

- 12am Rerun test digest, this time with only post-staining so GG doesn't affect banding.

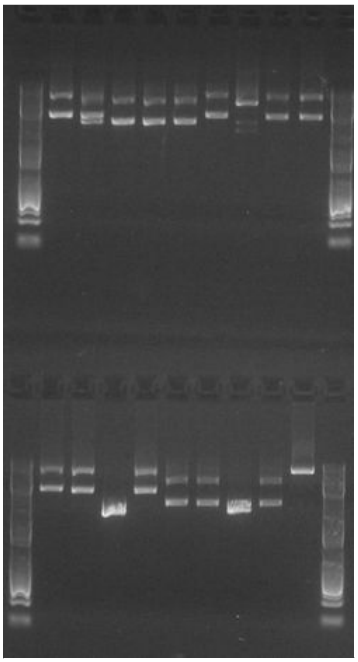


Looks the same as before. Strange.

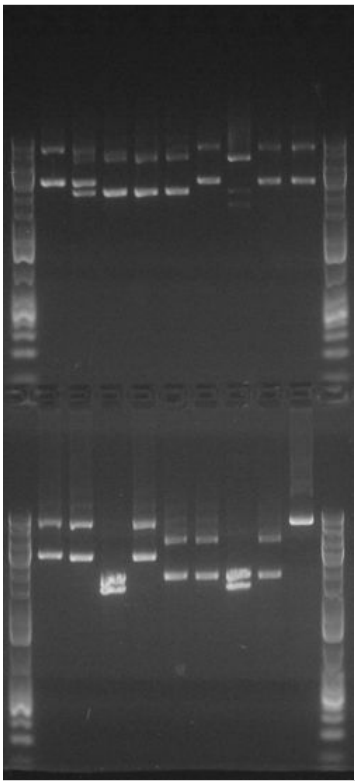
- 5am Test growth of all the bacteria I miniprepped and others that look pink-white.
  - Ones I miniprepped already: pRC201, 209, 218 219, 224, 229, 231, 236, 238, 239, 257, 261, 262, 264, 275, 276, 277, 278, 280
  - Ones that look kind of white: pRC202, 203, 211, 212, 213, 214, 223, 228, 240, 243, 248, 249, 250, 252, 253, 254, 258, 259, 273.
  - Will let grow for ~24hrs to allow redness to manifest itself.
- 6am Miniprepped.
- 7am Run test assembly with 201, 218, 224, 229; 257, 261, 264, 275 and electroporate.
  - Mixed together 1ul of each side together (4ul total per side), then added 1ul of that to the reaction mix.
  - Used 0.5ul each of the backbones, pWCD517 (AmpR ColE1) and pWCD563 (Ura Cen6).
- 7pm Transformed MMG into yeast.
  - Added 2ml of dH2O, scraped plate.
  - Streaked out 10ul for future.
  - Added 1ul of miniprep to smallish pellet (Vinay followed an older protocol and diluted yeast).
  - Plated all the yeast.

Sunday 10/7

- 1am Put yeast into glycerol stock.
- 2am Miniprepped only a few of them because the rest grew up red: pRC201, 209, ~~218~~ 219, 224, 229, 231, 236, 238, 239, 257, 261, 262, 264, 275, 276, 277, 278, 280. This makes 9 each, so I can run a test BsaI library combination if I wanted to tomorrow.
- 3am Test digested with BsaI. Expect 1766 + ~10,000bp. Backbone would be 1766+937bp.



I think some of them worked, but will put gel into bath for hour to see if the ladder is correct. This means that my reaction worked, just that the rest need to be electroporated. Will check on the elctroporated plates in the afternoon to see if I have more white colonies because those will likely be correct.

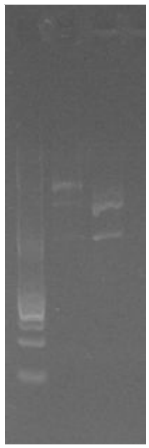


Ran a bit longer then put into GG bath for hour.

- 4pm Picked from electroporations: pRC206, 207, 208, 226, 230, 235, 237, 240, 244, 247, 268.
  - pRC245, 246, 266 did not have a single white colony.
  - In future, do a 2x dilution of the 10x frozen, and plate all.

Saturday 10/6

- 12:00am Plated all the MG cassettes. At noon, pick. At midnight, miniprep.
- 12:30am Miniprepped more pRC061 and pRC062. Test digested with BsmBI. Expect 1802+901 for both.



I think both are good. pRC061 wasn't cut as well since I only digested for 15min. Labeled with tough tag and put in my working box.

- 205, 207, 208, 226, 230, 235, 237, 240, 244, 245, 246, 247, 266, 268 did not have a single red colony. Electroporated and plated at 5pm.
  - For 205-240, I used the 10x dilution, recovered in 500ul, and plated 100ul.
  - For 244-268, I diluted the 10x to a total of 50x dilution, recovered in 100ul, and plated all 100ul.
  - Ran a negative control with just EPI300 and no DNA to see if the cuvettes are clean.
- ~~I might want to remake pRC061 and 62 because the RFP promoter might have been truncated.~~Checked sequencing, and it is ok. Still very strange that RFP doesn't show up as bright.
  - Will thinks something is funny with the media.
  - Negative control media (just media in culture tube) did not grow.

Friday 10/5

- 10:30am Seeded pRC061 and 62 for miniprep.
- 10:30am Seeded yRC045-52 for glycerol stocks.
- Dilutions needed, standardized to 0.02 pmol/ul. Will add:
  - 1.0ul of backbone post-dilution. I modified dilution calculation to make it 1/2 as concentrated.
  - 0.5ul of LZ pre-dilution because I don't want to do 80 dilutions
  - 1ul of all the MiCode components post-dilution.

In excel: File:MG Cloning.xlsx

Plasmid (pRC…)	Description	Length	Times Used	Conc (ng/ul)	Molar Conc. (pmol/ul)	Dilution factor	Total Volume Needed	Plasmid to add	Water to add
61	mV1	2703	40	73.5	0.041200013	0.242718367	42	10.2	31.8
62	mV2	2703	40	83.4	0.046749403	0.213906475	42	9.0	33.0
70	PAmCherry Nucleus on	4273	15	207.7	0.073647781	0.271562831	17	4.6	12.4
74	Venus Nucleus on	4280	9	200.2	0.070872274	0.282197802	11	3.1	7.9

75	PAGFP Nucleus on	4283	7	275.6	0.097496091	0.20513643	9	1.8	7.2
112	mTurquoise2 Nucleus on	4282	13	183	0.064753089	0.308865574	15	4.6	10.4
98	PAmCherry VM on	3888	11	138.7	0.054051316	0.370018745	13	4.8	8.2
100	Venus VM on	5210	9	180.2	0.052405049	0.381642619	11	4.2	6.8
101	PAGFP VM on	5213	7	1399	0.406617489	0.049186276	9	0.4	8.6
113	mTurquoise2 VM on	5212	14	234.5	0.06817019	0.293383369	16	4.7	11.3
71	PAmCherry Nucleus off	4280	14	185.6	0.065703767	0.304396552	16	4.9	11.1
76	Venus Nucleus off	2596	8	151.8	0.088597843	0.22573913	10	2.3	7.7
81	EmCFP Nucleus off	4286	13	151.4	0.053521684	0.373680317	15	5.6	9.4
96	PAmCherry CP on	3888	13	164.7	0.064183502	0.311606557	15	4.7	10.3
91	Venus CP on	3892	9	197.2	0.076769753	0.26051927	11	2.9	8.1
92	PAGFP CP on	3895	9	180.5	0.070214339	0.284842105	11	3.1	7.9
115	mTurquoise2 CP on	3896	13	265.9	0.103408313	0.193408048	15	2.9	12.1
110	PAmCherry Actin on	5655	15	153.4	0.041100662	0.486610169	17	8.3	8.7
107	Venus Actin on	5659	9	229.1	0.061339673	0.326053252	11	3.6	7.4
108	PAGFP Actin on	5662	5	196.1	0.052476371	0.381123916	7	2.7	4.3
114	mTurquoise2 Actin on	5663	15	240.7	0.064399959	0.310559202	17	5.3	11.7
97	PAmCherry CP off	3895	12	194.5	0.075660326	0.264339332	14	3.7	10.3
93	Venus CP off	3899	8	126.5	0.049157904	0.406852174	10	4.1	5.9
87	EmCFP CP off	3900	12	108.3	0.042074592	0.47534626	14	6.7	7.3
Zipper	Any zipper part	6746	-1	241.8	0.054308277	0.36826799	1	0.4	0.6
pWCD802	LZ1B-mKate	6746	-1	239	0.053679397	0.372582427	1	0.4	0.6
pWCD901	LZ1A-PAGFP	5579	-1	154.1	0.041850663	0.477889682	1	0.5	0.5
pWCD902	LZ1B-PAGFP	5579	-1	135.1	0.03669062	0.545098446	1	0.5	0.5

- 6pm Ran BsmBI reaction. pWCD0839 and 0939 did not work (toxic), so those wells are blank. pRC253 is made wrong (added different zipper in).

Thursday 10/4

- Will do the MG assemblies with equimolar volumes except for the backbones pRC061 and pRC062, which I will add 1/2 of what the calculation tells me to.
- Nanodropped all plasmids to calculate
- 8pm Transformed and plated more pRC061 and pRC062 because I might run out tomorrow.

Wednesday 10/3

- 4pm Picked many colonies of yRCmmg to look for recombination. 9/10 were the same according to Celia.

Tuesday 10/2

- 8pm Miniprepped and test digested:

Tube	Plasmid	Clone	Enzymes	Buffer	Bands
1	pRC116	G	NotI	3	3729+1766
2	pRC221, condition 3	A	NotI	3	12576+1766
3	pRC221, condition 3	B	NotI	3	12576+1767
4	pRC221, condition 3	C	NotI	3	12576+1768

- Send pRC116G for sequencing. Would rather use this than pRC116C, which gave mixed reads.

Monday 10/1

- 3:30am Heat shocked 5ul of BsmBI reaction mix from yesterday. Plated all.
- 6pm Masaki checked on the plates. Some of them gave very good white/red ratio, which I will document here when I have the time.

Robert Notebook September 2012

From Dueber Lab Wiki

Contents

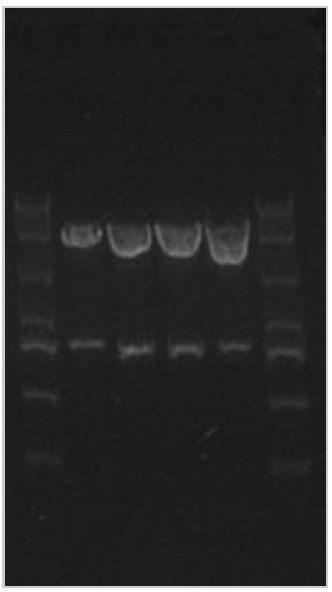
Sunday 9/30

- 9pm Rann BsmBI to make pRC221:

Tube	Plasmid	Description	Backbone Used
1	pRC221	0.50ul of everything	pRC116C
2	pRC221	Equimolar everything	"
3	pRC221	0.25ul of just pRC116C	"
4	pRC221	0.25ul of everything	"
5	pRC221	0.50ul of everything	pRC116G
6	pRC221	Equimolar everything	"
7	pRC221	0.25ul of just pRC116G	"
8	pRC221	0.25ul of everything	"

- 9pm Was thinking that BsmBI was getting overloaded by the DNA in the reaction, so experiment to test BsmBI cutting efficiency. Expect 4594+901bp=5495total.

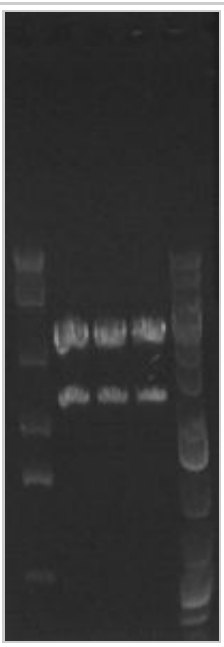
Tube	Plasmid	Description
1	1ul of pRC116G	Test BsmBI reaction
2	2ul of pRC116G	Test BsmBI reaction
3	3ul of pRC116G	Test BsmBI reaction
4	4ul of pRC116G	Test BsmBI reaction



I think BsmBI cutting isn't the issue here.

Saturday 9/29

- 4am Reran BsmBI reaction to produce pRC221, this time using only 0.25ul of pRC116 backbone. Hopefully will get different result.
- 10pm Did pRC116C, F, G minipreps and test digested with NotI again. Expect 3729+1766.



Seems correct.

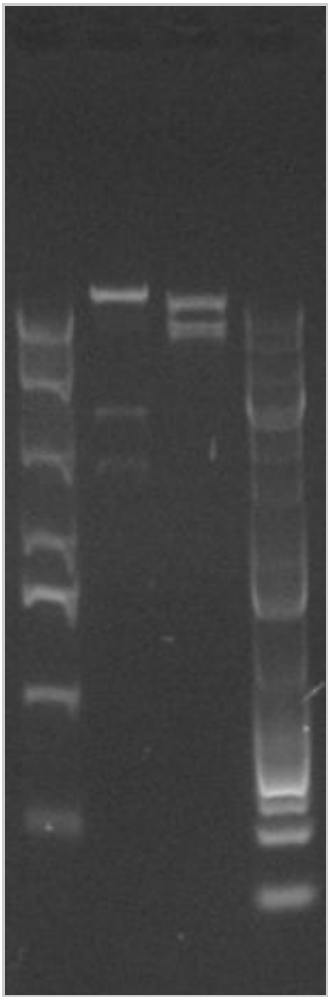
Troubleshoot bad assemblies

- Test digest ALL the cassettes I've made again.
- Gel purify.
- Try running reaction with less DNA. Maybe BsaI is too heavily loaded with DNA to cut.
- Pick more pRC117 colonies.

Friday 9/28

- Worked on wiki.
- 11pm Re-test digested pRCmmg to get smaller fragments.

Tube	Plasmid	Enzymes	Buffer	Bands
1	pRCmmg	EcoRI/SalI/Acc651	3	13619+6487+1856
2	pRCmmg	AatII/ClaI/EcoRI	4	8985+7097+5880

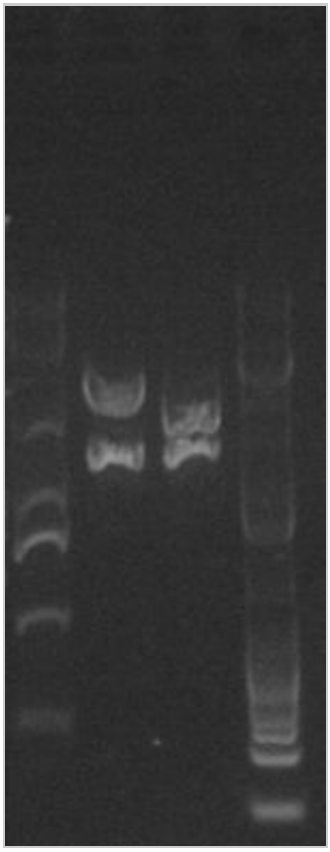


Hmm...getting much different banding than expected.

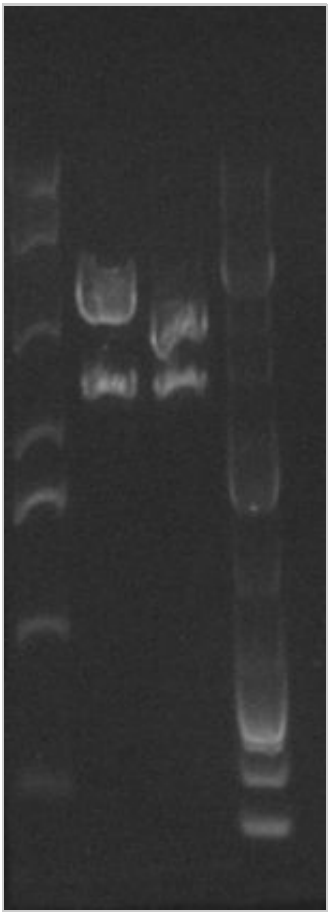
Thursday 9/27

- 4am Now that I figured out how to get good gels, test digested pRC116 and 117 again:

Tube	Plasmid Clone	Enzymes	Buffer	Bands
1	pRC116	NotI	3	3729+1766
2	pRC117	NotI	3	3017+1766



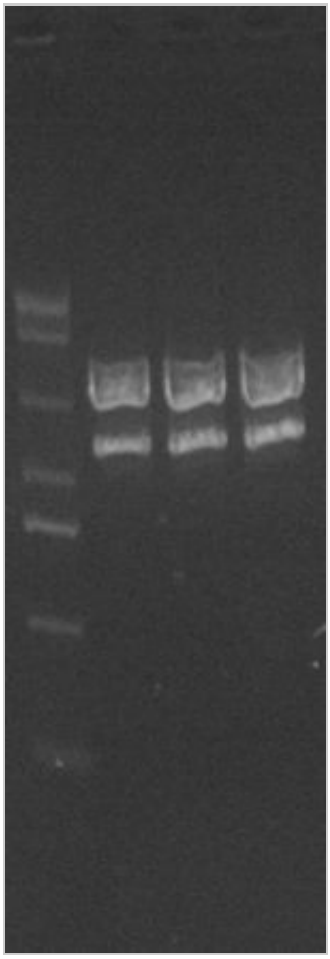
I think both are correct?



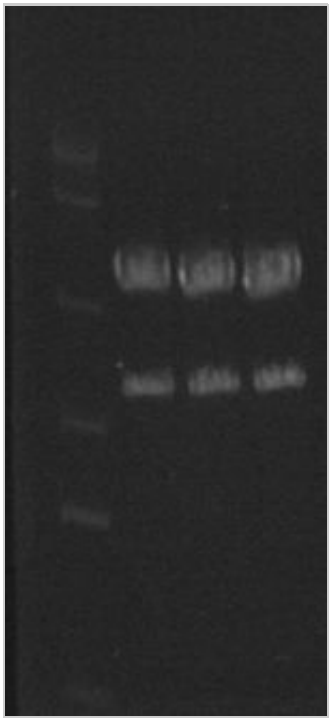
Ran it for a bit longer, and I think pRC116 is missing a small chunk. 117 looks perfect though, so I don't know why the MGs aren't assembling correctly.

- 1pm Miniprepped pRC116C-E. Test digested.

Tube	Plasmid Clone	Enzymes	Buffer	Bands
1	pRC116 C	NotI	3	3729+1766
2	pRC116 D	NotI	3	3729+1766
3	pRC116 E	NotI	3	3729+1766



I think they're good?



Ran longer.

- Since they look identical, I sent 116C for sequencing.
- 8pm Redid Gibson assembly for pRC116, electroporated, then plated.

Part #	Part desc.	Ci (uM) enter	Vi (uL)
1	pRC116.1	33.4	1.838022257
2	pRC116.2	39.4	1.55812039
3	pRC116.3	78.5	0.782037495
4	pRC116.4	74.7	0.821819858
Total			5

- 11pm Test integrated pRC262A and B (A should not work). Plated on -URA.

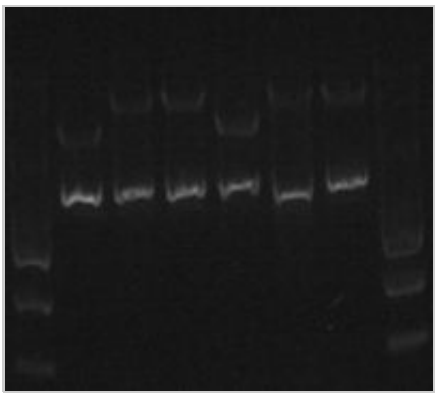
Wednesday 9/26

- I think LB+KAN and YPD contaminations that grow up once we put them into the shaker have been giving

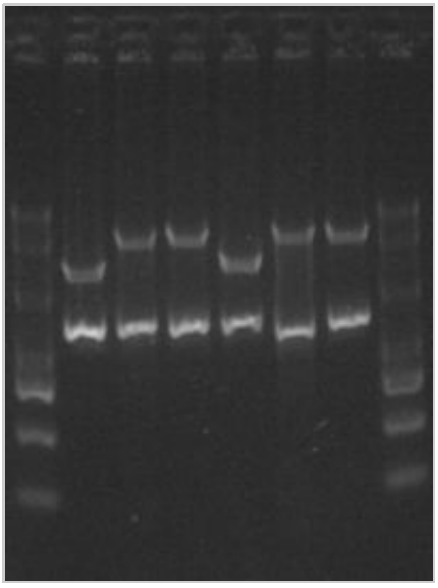
me trouble. Got new stocks today.
- We're having recurring issues with the 3000, 6000, and 8000bp ladder bands. I'm trying a post-stain to see if it helps anything.

- 3am Redid yeast transformation with MMG to hopefully not have the same issue with contamination.
- 2pm Seeded more 3 colonies of pRC116. Hopefully will get one that is correct.
- 10pm Miniprepped pRC262 retries. Hopefully they work:

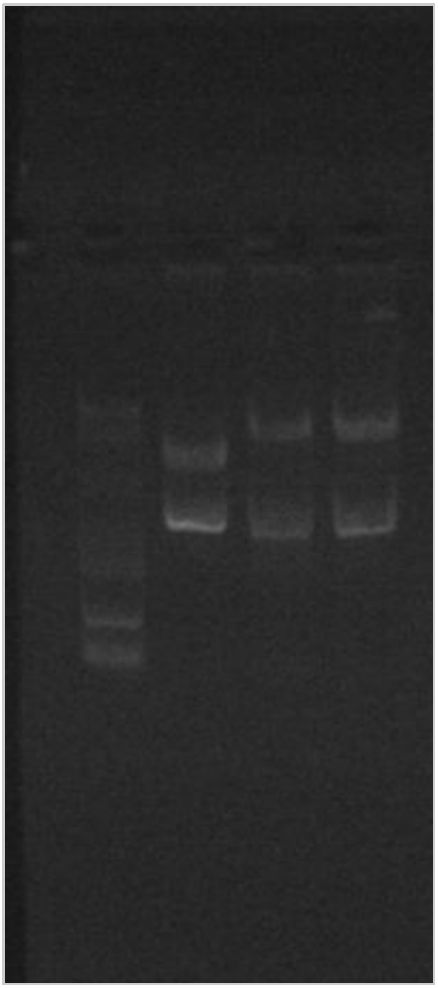
Tube	Plasmid	Clone	Enzymes	Buffer	Bands
1	pRC262-Ura retry	A	AatII/XmaI	4	7890+3617+1864+1705
2	pRC262-Ura retry	B	AatII/XmaI	4	7890+3617+1864+1705
3	pRC262-Ura retry	C	AatII/XmaI	4	7890+3617+1864+1705
4	pRC262-Ura retry equimolar	A	AatII/XmaI	4	7890+3617+1864+1705
5	pRC262-Ura retry equimolar	B	AatII/XmaI	4	7890+3617+1864+1705
6	pRC262-Ura retry equimolar	C	AatII/XmaI	4	7890+3617+1864+1705



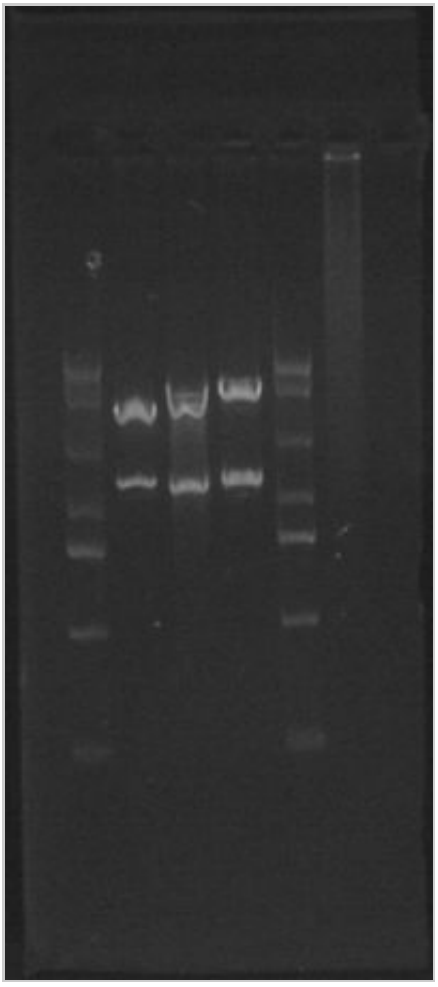
No idea which is correct because the ladder is nonexistent. Guessing that the larger ones are correct.



And now post-stained. This is a surefire fix, but I'd like to not have to do this all the time, so I'm testing other conditions



Here, I added 2uL of buffer w/GG to 10ul of ladder, and loaded 5ul in the well. I used the same gel as before.



Here, I used 5ul of diluted GG (in brown tube) into 20ml of gel (1:4000x dilution). Will do this in future

- 10pm MMG assembly worked! Saw fluorescence in yeast. Fallback if the mV1-Leu and mV2-Ura don't work is just stick with what I had before.

Tuesday 9/25

- 2am Redo BsmBI reaction normally as before and with these changes:

Plasmid	Length	Times Used	Conc (ng/ul)	Molar Conc. (pmol/ul)	Dilution factor	Total Volume Needed	Plasmid to add
pWCD821	6746	1	268.6	0.060327557	0.414404319	1.1	0.5
pRC116	5495	1	262.3	0.072324703	0.345663363	1.1	0.4
pRC070	4273	1	186.1	0.065988696	0.378852767	1.1	0.4
pRC074	4280	1	220.9	0.078200227	0.319692168	1.1	0.4

pRC113	5212	1	182.4	0.053024489	0.471480263	1.1	0.5
pWCD922	5579	1	181	0.049156197	0.508582873	1	0.5
pRC117	4783	1	226.2	0.071655294	0.348892573	1	0.3
pRC110	5655	1	184.1	0.049326153	0.506830527	1	0.5
pRC107	5659	1	239.8	0.064204512	0.389380734	1	0.4
pRC115	5212	1	229	0.066571316	0.375537118	1	0.4

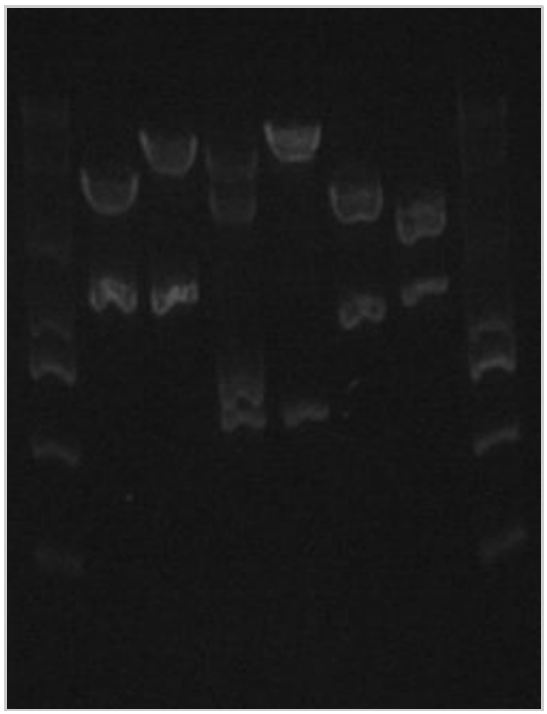
Tube	Plasmid	Description
1	pRC221	retry
2	pRC221	equimolar
3	pRC262	retry
4	pRC262	equimolar

- 7:30am Plated both TGI and EPI300.
- 7pm Picked colonies from TGI plates.

Monday 9/24

- 10:30am Picked two colonies each out of MG TGI plates.
  - 262 had very high % red, so something might be off. Will see if white colonies are even correct, then we can tell if the pRC117 was made correctly.
- 6pm Sequenced pRC116 with S23, AU45, and AW39.
- 6pm Re-sequenced pRC117 with AW39.
- 8pm Test digested:

Tube	Plasmid	Clone	Enzymes	Buffer	Bands
1	pRC221-Leu	A	NotI/SacI	2	8698+3878+1766
2	pRC221-Leu	B	NotI/SacI	2	8698+3878+1766
3	pRC262-Ura	A	NotI/ClaI	3	13310+1043+723
4	pRC262-Ura	B	NotI/ClaI	3	13310+1043+723
5	pRC116		ScaI/PvuI	3	4080+1415
6	pRC117		ScaI/PvuI	3	3268+1515

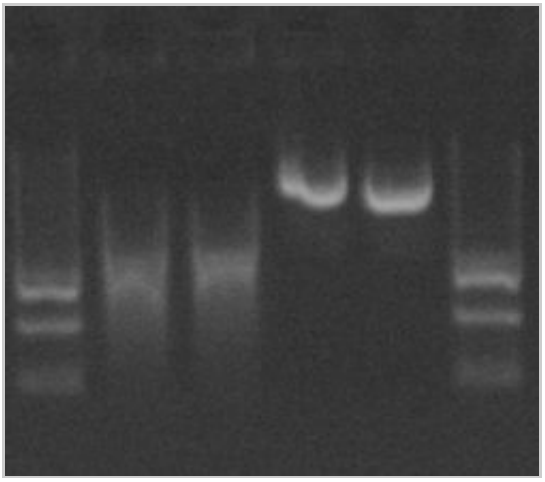


221 and 262 look all off. Not even sure what happened. 116 and 117 look correct at least, thankfully.

- 11pm Checked on EPI300 plates. All colonies on 221 were red. Most colonies on 262 were red. Not sure what the problem was, will redo BsmBI assembly.
- MG to-dos.
  - Monday morning, pick from heat shock plate.
  - Monday afternoon, miniprep TGI. Test digest.
  - Monday late night, integrate left side into yeast. Also try MMG BsaI assembly.
  - Tuesday early morning, transform MMG into yeast.

Sunday 9/23

- 3pm Miniprepped and test digested pRC116A. B was white. Used PvuI/ScaI like yesterday. Also ran alongside pRC117A re-digested just to verify.



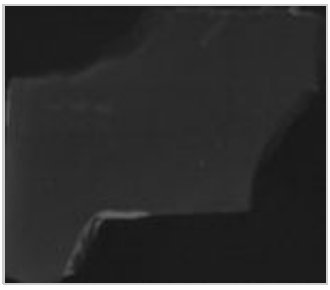
116, 117 digested. 116, 117 undigested. Something weird with the gel and ladder. I think it's correct. Will sequence tomorrow.

- 4:30pm Started BsmBI reaction to produce pRC221 and 262 with the new mV1-Leu and mV2-Ura backbones.
  - For electroporations, used 1ul of DNA, 30ul of 10x diluted refrozen EPI300, and plated all. Plated at 10:30pm.
  - For heat shock, used 3ul of DNA, 50ul of flash frozen TGI, and plated all. Plated at 10:30pm.

Saturady 9/22

- Miniprep and sequence pRC117.

Tube	Plasmid Clone	Description	Enzymes	Buffer	Bands
1	pRC117 A	AmpR_ColE1 type 6b	PvuI/ScaI	NEB 3	3268+1515
2	pRC117 B	Ura3_Cen6 type 6a	PvuI/ScaI	NEB 3	3268+1515



Gel fell apart in my hand. Looks like 117A is correct.

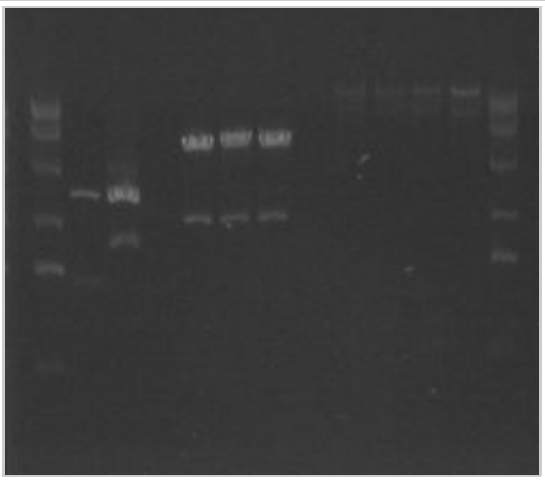
Friday 9/21

- 1am Run Gibson reaction, using 1.5ul of each PCR pdt.
- 3am Electroporate and plate.
  - pRC116: Sparked, so used 200/500ul.
  - pRC117: TC 3.6, so used 100/500ul.
- 5pm Transformed pRCmmgD into yJD001. Used 1.0ul DNA, plated half of transformation mix.
- 5pm Picked 2 colonies of pRC117.
- 6:30pm Redid electroporation for pRC116 and plated.
- Start thinking about VM tag.

Thursday 9/20

- 12pm Miniprepped pWCD563, pRC114A-C, and pRCmmgA-D. Test digest with pWCD517 later.

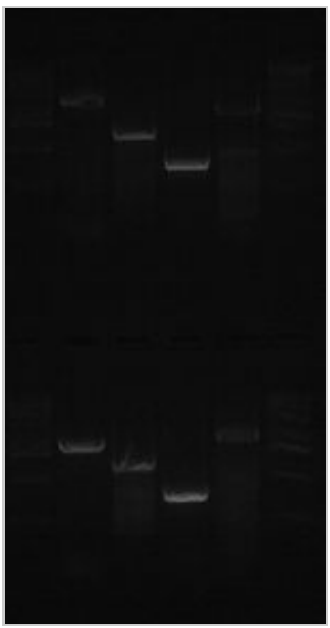
Tube	Plasmid	Clone	Description	Enzymes	Buffer	Bands
1	pWCD0517		AmpR_ColE1 type 6b	AlwNI/NcoI	NEB 4	1911+860
2	pWCD0563		Ura3_Cen6 type 6a	ApaI/SacI	NEB 4	2059+1177
3	pRC114	A	Cas6 mTurquoise2 Actin on	BglII/PvuI	NEB 3	4227+1436
4	pRC114	B	Cas6 mTurquoise2 Actin on	"	"	"
5	pRC114	C	Cas6 mTurquoise2 Actin on	"	"	"
6	pRCmmg	A	MMG test	SacI/EcoRI	NEB 4	15564+6398
7	pRCmmg	B	MMG test	"	"	"
8	pRCmmg	C	MMG test	"	"	"
9	pRCmmg	D	MMG test	"	"	"



All look correct. Sent pRC114B and pRCmmgD for sequencing.

- 11pm Ran gel:

Description	Primers	Tm	Result
pWCD524, Leu2_Int_5'	BC38	51°	2320bp
	BC39	55°	
pRC61, mV1	BC40	52°	976bp
	BC41	54°	
pWCD559, Leu2_Int_3'	BC42	52°	516bp
	BC43	53°	
pRC61, mV1	BC44	51°	1766bp
	BC45	50°	
pWCD526, Ura3_Int_5'	BC46	50°	1618bp
	BC47	51°	
pRC62, mV2	BC48	51°	971bp
	BC49	57°	
pWCD560, Ura3_Int_3'	BC50	60°	506bp
	BC51	52°	
pRC62, mV2	BC44	51°	1792bp



All look correct.

Meeting Notes

- Harneet should mention yeast per slide on his slide.
- Thomas MiCode examples unclear. Images not impressive. Show both original and false colored/thresholded
- Our image analysis needs to be more dramatic and self-confident.

- We should say what scope we used.
- Label results.
- Masaki should talk about how common orthognonality is.

Wednesday 9/19

- 1am Electroporated and plated all of pRC MMG. TC 4.0, used a bit over 1.0ul.
- 1pm Miniprep pWCD0517. Seeded pWCD0563 for miniprep later.
- 1pm Seeded pRC114 for miniprep later.

Meeting Notes

- We might want to do LZ-VM instead of perox because perox might be too stringent.
- Austin will be testing out a handful of LZ-VM.
- Vincent will do SH3 domain.

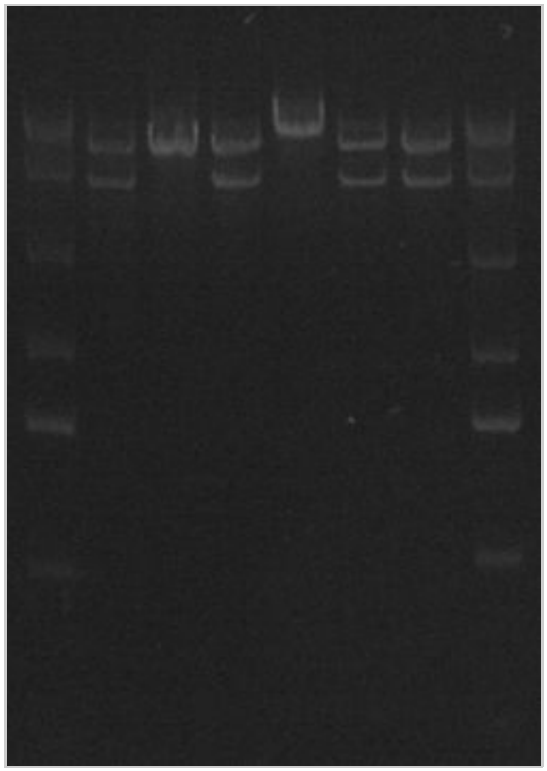
Tuesday 9/18

- 2am Redid miniprep and test digests from yesterday.

I think all except pRC114 are correct. Will send 261D for sequencing tomorrow.

- 3am Ran pRC114 cassette reaction again. Electroporate later today.
- 12pm Spun down pRC221, but did not begin miniprep yet. Do minipreps and test digest pRC221.
- 12:40pm Electroporated and plated 100ul of pRC114.
- 6pm Ran BsaI reaction for RC MMG.
- 6pm Seeded pWCD517 for miniprep.

- 6pm Miniprepped pRC221 tests A-F. Test digested XmaI/SacI (NEB4). Expect 6945+4602bp.



I think there is a 2/3 success rate for TGI. For some reason, it seems like this assembly was very efficient. I believe it was because I used the frozen stock T4 ligase buffer.

Monday 9/17

- Renamed the plasmids to match Will's LZs.
  - pRC220--> pRC221.
  - pRC261--> pRC262.
- 2pm Miniprepped pWCD520-522, pRC114E-H, pRC261. Test digest all to confirm.

Tube	Plasmid	Clone	Description	Enzymes	Buffer	Bands
1	pWCD0520		Con1_5'	AlwNI/SacI	NEB 4	1089+749
2	pWCD0521		Con2_5'		"	
3	pWCD0522		Con3_5'		"	
4	pRC114	E	Cas6 mTurquoise2 Actin on	BglII/EcoRI	NEB 3	4779+884
5	pRC114	F	Cas6 mTurquoise2 Actin on	"	"	"
6	pRC114	G	Cas6 mTurquoise2 Actin on	"	"	"

7	pRC114	H	Cas6 mTurquoise2 Actin on	"	"	"	
8	pRC261	D	LZ11B-PTS1	PvuI/SalI	NEB 3	6267+4272	
9	pRC261	E	LZ11B-PTS1	"	"	"	
10	pRC261	F	LZ11B-PTS1	"	"	"	

- 5pm Sent pRC112, 113, 115 for seqencing.
- 5pm Added more LB+Antibiotic to media. Minipreps failed because I thought A4 had ethanol added already.

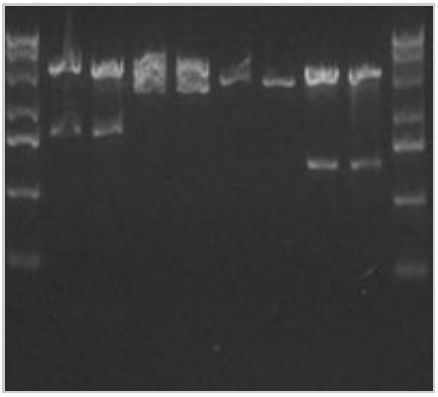
Sequence confirm pRC112-115, 262.

- If pRC262 is correct, assemble with pRC221.
- Pick more pRC221 from TGI to check.
- Build PAmCherry and PAGFP for registry.
- Build new mV1/2 backbones that include LEU/URA and homology regions.

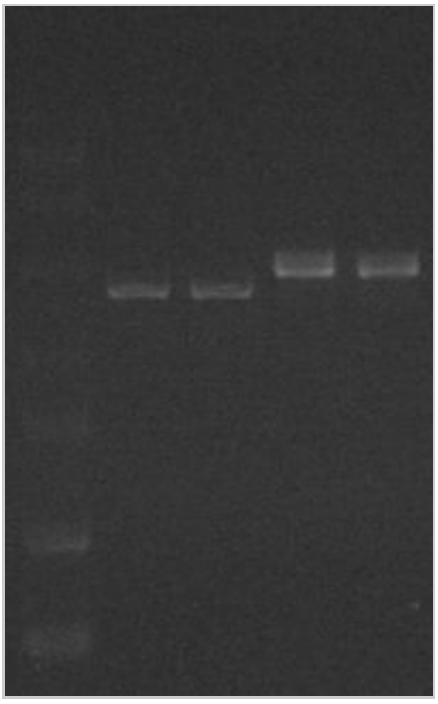
Sunday 9/16

- 3pm Seeded pWCD520-522 and pRC261A-C for miniprep tomorrow.
- 3:30pm Miniprepped and test digested pRC112-115.

Tube	Plasmid	Clone	Description	Enzymes	Buffer	Bands
1	pRC112	A	Cas3 mTurquoise2 Nucleus on	BglII/XhoI	NEB 3	3152+10710+60
2	pRC112	B	Cas3 mTurquoise2 Nucleus on	"	"	"
3	pRC113	A	Cas3 mTurquoise2 VM on	"	"	3151+2061
4	pRC113	B	Cas3 mTurquoise2 VM on	"	"	"
5	pRC114	A	Cas6 mTurquoise2 Actin on	"	"	3152+2473+38
6	pRC114	B	Cas6 mTurquoise2 Actin on	"	"	"
7	pRC115	A	Cas6 mTurquoise2 CP on	"	"	3152+744
8	pRC115	B	Cas6 mTurquoise2 CP on	"	"	"



Miniprepped pRC114C,D. Still looks wrong, so will pick 4 more colonies.



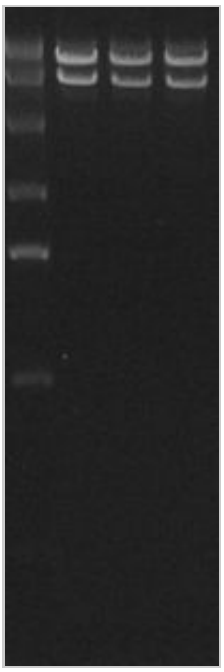
Reran 114s. Are all wrong it seems.

- 8pm Counted colonies on plates.

Strain	White	Red	Success
TGI flash frozen	13	21	38%
TGI slow frozen	18	15	55%
EPI300 10x fresh	172	52	77%

Saturday 9/15

- 6am Heat shocked pMRY8-81 and pRC112, 113, 115 and plated on AMP. Electroporated pRC114 and 261, plated on AMP and KAN. For 261, used 10x dilution and plated all.
- 3pm Miniprepped pRC220A-C. Test digested with XmaI/SacI (NEB4). Expect 6945+4602bp.



These all look correct. The smaller band is a bit iffy, but that could be because it's picking up all the GelGreen and slowing down. Will send pRC220A for sequencing.

- 7pm Picked pRC112-115, seeded for miniprep. Two colonies each, except for 114, which I picked 4 of in case they are actually red.

Friday 9/14

- 2:30pm Picked 3 colonies from pRC220 plate.
- 11pm Transform remaining MG reaction. Use 1ul of DNA and plate all cells.

1. TGI.
2. TGI no N2.
3. EPI300 stock again. This time plated 100ul/500ul recovery.
4. EPI300 10x fresh.
5. EPI300 10x refrozen.

- 10pm Ran BsmBI reaction to make MG pRC261, which has 11B that should interact with the 11A I made already.
- 10pm Ran BsaI reactions to make mTurquoise on cassettes:

Plasmid Name	Part 1	Part 2	Part 3	Part 3a	Part 3b	Part 4	Part 5	Part 6
pRC112	Con3_5'	RNR2p		HTA2 (Nucleus)	mTurquoise2	ADH1	Con4_3'	AmpR_ColE1
pRC113	Con3_5'	RPL18Bp		ZRC1 (VM)	mTurquoise2	ADH1	Con4_3'	AmpR_ColE1
pRC114	Con2_5'	RPL18Bp		ABP1 (Actin)	mTurquoise2	ADH1	Con3_3'	AmpR_ColE1
pRC115	Con2_5'	RPL18Bp	mTurquoise2			CIIC (CP)	Con3_3'	AmpR_ColE1

Thursday 9/13

- Electroporation plate grew. Restreaked at noon.
- Pour more KAN plates later.

Wednesday 9/12

- ~~Pick colonies from electroporation plates.~~ All colonies on all plates were red :-(
- With just pRC220, redo with the following protocol changes:
  - Nanodrop and standardize part concentrations.
  - Use Ligase buffer aliquots.
  - Use undiluted EPI300.
  - TC 3.6
  - Rescue in 250ul, plate all.
  - Plated at 12:30am.

Plasmid	Length	Times Used	Conc (ng/ul)	Molar Conc. (pmol/ul)	Dilution factor	Total Volume Needed	Plasmid to add	Water to add

pWCD821	2771	1	280.7	0.153483591	0.162883862	1	0.2	0.8
pRC103	5209	1	162.9	0.047383026	0.527615101	1	0.5	0.5
pRC74	4280	1	207.8	0.07356273	0.339846006	1	0.3	0.7
pRC70	4273	1	206.4	0.073186818	0.34159157	1	0.3	0.7
pRC61	2703	1	91.2	0.051121649	0.489029605	1	0.5	0.5

Tuesday 9/11

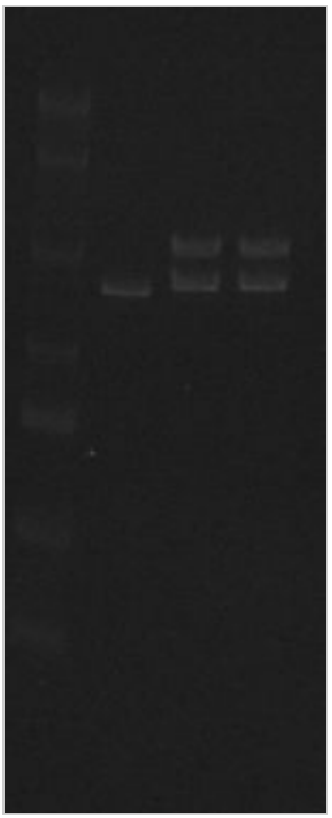
- 11:30am Seeded TGI for ~~transformations later.~~ We decided to just electroporate.
- 6pm Sent pRC105 and pRC086 for sequencing.
- Ran multigene cassettes, do 4 bait, 4 prey.
  - Electroporate all, but save the rest of the reaction in case. Test TGI, non-Liq N2 TGI, and fresh TGI.
  - Used 0.80ul of 2, 7 because they kept shorting.
  - Having lots of trouble with 8, so used 1ul into 50ul of EPI300. Even after I did that, it still sparked. Zach said they still worked after that, so will continue.
  - Used pRC261 for heat shock. Added 3ul of DNA into the 230ul of TGI-KCM cocktail, plated 100ul.
  - 2am Plated electroporations. 3am plated heat shocks.

Tube	Plasmid	Description
1	pRC220	LZ 11A mKate
2	pRC221	LZ 11B mKate
3	pRC228	LZ 15A mKate
4	pRC229	LZ 15B mKate
5	pRC260	LZ 11A PTS1
6	pRC261	LZ 11B PTS1
7	pRC268	LZ 15A PTS1
8	pRC269	LZ 15B PTS1

Monday 9/10

- 11am Picked colonies from electroporated pRC105 plate.
- Send pRC061, 62, 64, 106, 108 for sequencing.
- 9:30pm Miniprepped and test digested pRC105.
  - Tried 55C AE elute.

- Tried 1ul digestion. BglII/XhoI (NEB3), expecting 3152+2470+38bp.
- Put 5ul into well.



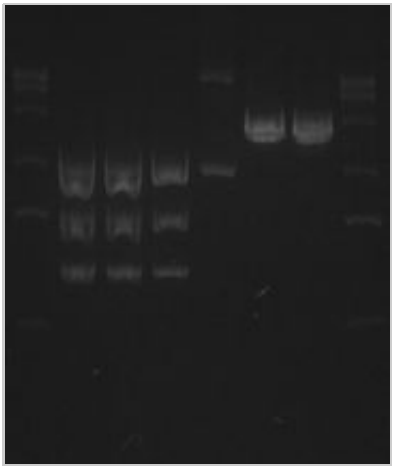
Clones B and C look correct. Will send B for sequencing. Gel probably looked dim because it was older, so will continue doing 1ul digestions and pipetting 5ul per well.

Sunday 9/9

- 3am Picked 3 more clones each of bad plasmids.
- 3:30am Ran new BsaI cassette reaction to make pRC105.
- 7pm Miniprep and test digested:

Tube	Plasmid	Clone	Description	Enzymes	Buffer	Bands
1	pRC064	B	Cas1 PAmCherry VM on	AlwNI/ScaI	NEB 3	1689+1048+710
2	pRC064	C	Cas1 PAmCherry VM off	"	"	1689+1048+710

3	pRC064 D	Cas2 Venus VM on	"	"	1689+1048+710
4	pRC105 B	Cas2 PAGFP VM on	BglII/XhoI	"	3152+2470+38
5	pRC105 C	Cas2 Venus VM off	"	"	3152+2470+38
6	pRC105 D	Cas3 EmCFP VM on	"	"	3152+2470+38



For each plasmid, ran 10, 8, then 6ul of test digest to test GelGreen sensitivity. In future, will use 5ul of test digest. pRC064D is good. None of the pRC105s look good.

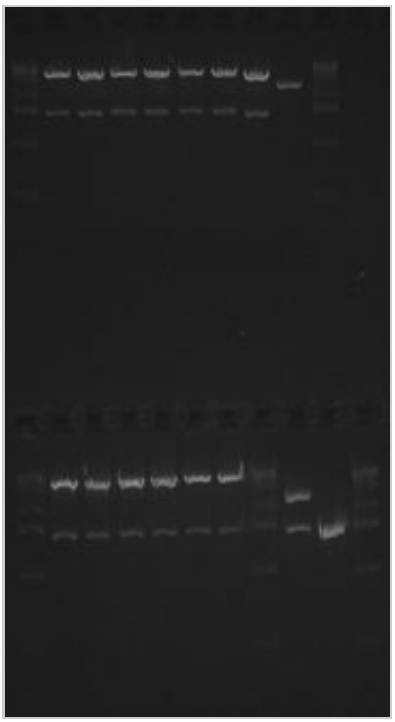
- 10pm Electroporated and plated pRC105. TC 4.4, plated 100ul.

Saturday 9/8

- Miniprep new cassettes and pWCD0552 and 515.

Tube	Plasmid	Description	Enzymes	Buffer	Bands
1A	pRC098	Cas1 PAmCherry VM on	BglII/XhoI	NEB 3	3151+2052
2	pRC099	Cas1 PAmCherry VM off	"	"	3151+2059
3	pRC100	Cas2 Venus VM on	"	"	3152+2058
4	pRC101	Cas2 PAGFP VM on	"	"	3152+2061
5	pRC102	Cas2 Venus VM off	"	"	3152+2065
6	pRC103	Cas3 EmCFP VM on	"	"	3151+2058
7	pRC104	Cas3 EmCFP VM off	"	"	3151+2065
8	pRC105	Cas6 EmCFP Actin on	"	"	3152+2470+38
1B	pRC106	Cas6 EmCFP Actin off	"	"	3152+2470+45

2	pRC107	Cas7 Venus Actin on	"	"	3151+2470+38
3	pRC108	Cas7 PAGFP Actin on	"	"	3151+2473+38
4	pRC109	Cas7 Venus Actin off	"	"	3151+2470+45
5	pRC110	Cas8 PAmCherry Actin on	"	"	3153+2464+38
6	pRC111	Cas8 PAmCherry Actin off	"	"	3153+2464+45
7	pWCD0515	AmpR_ColE1	AlwNI/ScaI		1911+861
8	pWCD0552	ADH1	"		969+934



105 looks iffy.

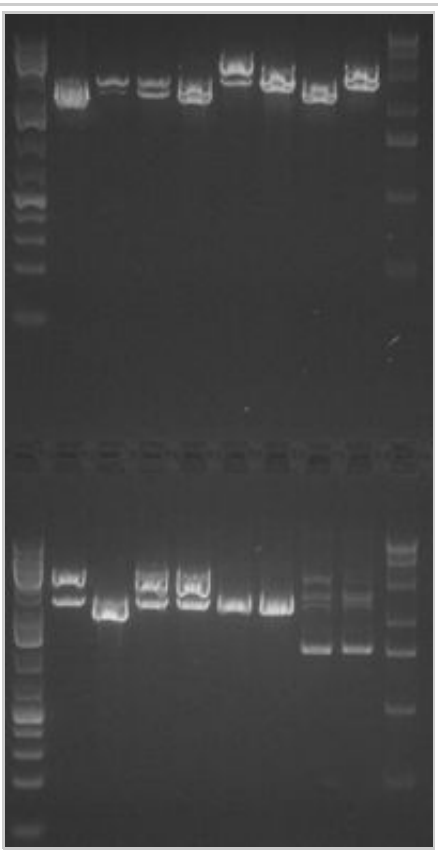
- See what to do with strangely-digested plasmids.
- 7pm Did Do TECAN readings.
- 7pm Sent sequencing out:

Name	Date	Construct Clone	Primer
sIGEM479	8-Sep-12	pRC098	AW39
sIGEM480	8-Sep-12	pRC099	AW39
sIGEM481	8-Sep-12	pRC100	AW39
sIGEM482	8-Sep-12	pRC101	AW39
sIGEM483	8-Sep-12	pRC102	AW39
sIGEM484	8-Sep-12	pRC103	AW39
sIGEM485	8-Sep-12	pRC104	AW39
sIGEM486	8-Sep-12	pRC105	AW39
sIGEM487	8-Sep-12	pRC106	AW39

sIGEM488	8-Sep-12	pRC107	AW39
sIGEM489	8-Sep-12	pRC108	AW39
sIGEM490	8-Sep-12	pRC109	AW39
sIGEM491	8-Sep-12	pRC110	AW39
sIGEM492	8-Sep-12	pRC111	AW39

Friday 9/7

- 12am Seeded off cassettes and part plasmids for miniprep later today.
- 1am Miniprep and test digest "on" cassettes (hopefully without cross-contamination this time). Use same enzymes as yesterday.

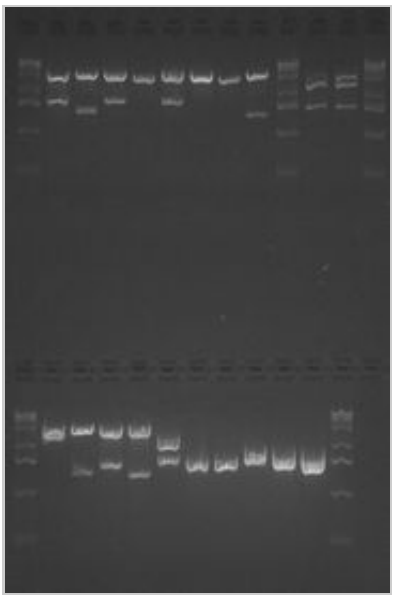


Not sure what happened to the last two...

- 2am Seeded yeast into YPD for re-TECANing later today.
- 4am Transform BsaI cassette reactions into TGI. Streaked out 20ul on LB+AMP.
- 4:30pm Miniprepped and began to test digest plasmids:

Tube	Plasmid	Description	Enzymes	Buffer	Bands

1A	pRC071 Cas1 PAmCherry Nucleus off	BglII/XhoI	NEB 3	3151+1062+67
2	pRC073 Cas1 PAmCherry Actin off	"	"	3151+2464+45
3	pRC076 Cas2 Venus Nucleus off	"	"	3152+1068+67
4	pRC079 Cas2 Venus Actin off	"	"	3152+2470+45
5	pRC081 Cas3 EmCFP Nucleus off	"	"	3151+1068+67
6	pRC083 Cas3 EmCFP Actin off	"	"	3151+2470+45
7	pRC085 Cas6 EmCFP VM off	"	"	3152+2065
8	pRC087 Cas6 EmCFP CP off	"	"	3152+748
1B	pRC090 Cas7 Venus VM off	"	"	3151+2065
2	pRC093 Cas7 Venus CP off	"	"	3151+748
3	pRC095 Cas8 PAmCherry VM off	"	"	3153+2059
4	pRC097 Cas8 PAmCherry CP off	"	"	3153+742
5	pRC063 ZRC1 dead	AlwNI/ScaI	"	1949+1048
6	pRC064 ABP1 dead	"	"	1689+1048+710
7	pRC065 HTA2 dead	"	"	1048+1019
8	pRC066 PAmCherry dead	"	"	1332+1048
1C	pRC067 Venus dead	"	"	1338+1048
2	pRC069 EmCFP dead	"	"	1338+1048
3	pRC061 mV1	AlwNI/NcoI	"	1829+874
4	pRC062 mV2	AlwNI/NcoI	"	1829+874



pRC073 and 95's smaller band looks funny, as does 64's bands. Otherwise everything else looks correct.

- 7pm Sent sequencing.

Name	Construct	Clone	Primer
sIGEM439	pRC070	new	G66
sIGEM440	pRC070	new	AW39
sIGEM441	pRC072	new	AW39
sIGEM442	pRC074	new	AW39
sIGEM443	pRC075	new	AW39
sIGEM444	pRC077	new	AW39
sIGEM445	pRC077	new	AW39
sIGEM446	pRC078	new	AW39
sIGEM447	pRC080	new	G66
sIGEM448	pRC080	new	AW39
sIGEM449	pRC082	new	AW39
sIGEM450	pRC084	new	AW39
sIGEM451	pRC086	new	AW39
sIGEM452	pRC088	new	AW39
sIGEM453	pRC089	new	AW39
sIGEM454	pRC091	new	AW39
sIGEM455	pRC092	new	G66
sIGEM456	pRC092	new	AW39
sIGEM457	pRC071		AW39
sIGEM458	pRC073		AW39
sIGEM459	pRC076		AW39
sIGEM460	pRC079		AW39
sIGEM461	pRC081		AW39
sIGEM462	pRC083		AW39
sIGEM463	pRC085		G66
sIGEM464	pRC085		AW39
sIGEM465	pRC087		AW39
sIGEM466	pRC087		AW39
sIGEM467	pRC090		AW39
sIGEM468	pRC093		AW39
sIGEM469	pRC095		AW39
sIGEM470	pRC097		AW39
sIGEM471	pRC063		AW38
sIGEM472	pRC064		AW38
sIGEM473	pRC065		AW38

sIGEM474	pRC066	AW38
sIGEM475	pRC067	AW38
sIGEM476	pRC069	AW38
sIGEM477	pRC061	AW39
sIGEM478	pRC062	AW39

- Take TECAN readings tomorrow.

Thursday 9/6

- 12am Ran cassette and part assembly reactions in parallel:
  - Cassette:

Tube	Result	Description
1	pRC071	Cas1 PAmCherry Nucleus off
2	pRC073	Cas1 PAmCherry Actin off
3	pRC076	Cas2 Venus Nucleus off
4	pRC079	Cas2 Venus Actin off
5	pRC081	Cas3 EmCFP Nucleus off
6	pRC083	Cas3 EmCFP Actin off
7	pRC085	Cas6 EmCFP VM off
8	pRC087	Cas6 EmCFP CP off
1B	pRC090	Cas7 Venus VM off
2	pRC093	Cas7 Venus CP off
3	pRC095	Cas8 PAmCherry VM off
4	pRC097	Cas8 PAmCherry CP off

- Part. Used 0.5 uL of PCR pdt for all.

Tube	Result	Description	Type
1	pRC063	ZRC1 dead	3a
2	pRC064	ABP1 dead	3a
3	pRC065	HTA2 dead	3a
4	pRC066	PAmCherry dead	3
5	pRC067	Venus dead	3
6	pRC069	EmCFP dead	3

- 2am Electroporated pRC061 and pRC062 Gibson rxns, plated LB+KAN. Should look red in morning.
- 12pm Heat shocked cassettes and parts.
  - Plate cassettes on AMP. Streak out 20ul.
  - Plate parts on CAM. Spread all 60ul with beads.
- 1pm Repicked on cassettes because there was probably cross-contamination.
- 11pm Ran new BsaI cassette reactions because we want Nucleus+VM together, and CP+Actin together. That way, we will never get Nucleus and VM in the same channel because those two can be tough to distinguish.

Tube	Result	Description
1	pRC098	Cas1 PAmCherry VM on
2	pRC099	Cas1 PAmCherry VM off
3	pRC0100	Cas2 Venus VM on
4	pRC0101	Cas2 PAGFP VM on
5	pRC0102	Cas2 Venus VM off
6	pRC0103	Cas3 EmCFP VM on
7	pRC0104	Cas3 EmCFP VM off
1B	pRC0105	Cas6 EmCFP Actin on
2	pRC0106	Cas6 EmCFP Actin off
3	pRC0107	Cas7 Venus Actin on
4	pRC0108	Cas7 PAGFP Actin on
5	pRC0109	Cas7 Venus Actin off
6	pRC0110	Cas8 PAmCherry Actin on
7	pRC0111	Cas8 PAmCherry Actin off

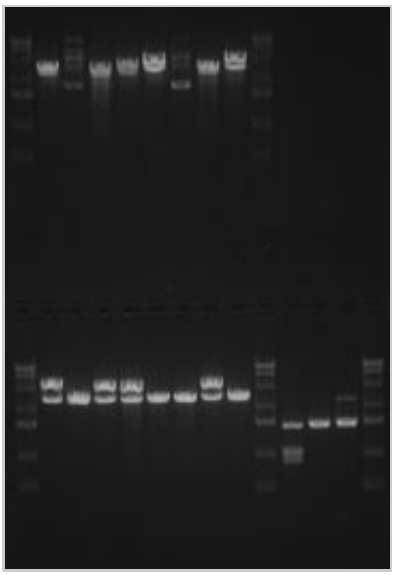
- 11pm Transformed and plated more pWCD0552 and 515 to make more.

Wednesday 9/5

- 2pm Miniprepped on cassettes and pWCD plasmids. Test digest.

Tube	Plasmid	Description	Enzymes	Buffer	Bands
1A	pRC070	PAmCherry nucleus on	EcoRI/PstI	NEB 3	2417+1856
2	pRC072	PAmCherry actin on	EcoRI/BamHI	"	3377+2276

3	pRC074	Venus nucleus on	EcoRI/PstI	"	2424+1856
4	pRC075	PAGFP nucleus on	EcoRI/PstI	"	2427+1856
5	pRC077	Venus actin on	EcoRI/BamHI	"	3383+2277
6	pRC078	PAGFP actin on	EcoRI/BamHI	"	3386+2277
7	pRC080	EmCFP nucleus on	EcoRI/PstI	"	2423+1856
8	pRC082	EmCFP actin on	EcoRI/BamHI	"	3383+2276
1B	pRC084	EmCFP VM on	EcoRI/PstI	"	3354+1856
2	pRC086	EmCFP CP on	"	"	2037+1856
3	pRC088	Venus VM on	"	"	3353+1856
4	pRC089	PAGFP VM on	"	"	3356+1856
5	pRC091	Venus CP on	"	"	2036+1856
6	pRC092	PAGFP CP on	"	"	2039+1856
7	pRC094	PAmCherry VM on	"	"	3349+1856
8	pRC096	PAmCherry CP on	"	"	2032+1856
1C	pWCD0519	ConS	AlwNI/NcoI	"	934+498+404
2	pWCD0555	Con2_3'	"	"	934+904
3	pWCD0558	ConE	"	"	934+906



Top row looks sketchy. Bottom row looks ok. pWCD558 I think didn't cut completely.

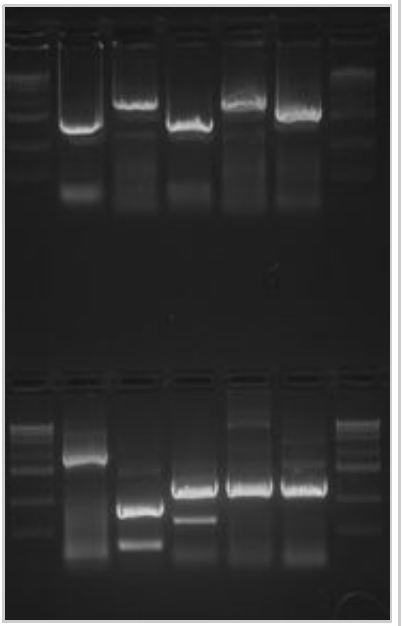
Name	Construct	Clone	Primer
sIGEM407	pRC070		G66
sIGEM408	pRC070		AW39
sIGEM409	pRC072		G66
sIGEM410	pRC072		AW39

sIGEM411	pRC074	G66
sIGEM412	pRC074	AW39
sIGEM413	pRC075	G66
sIGEM414	pRC075	AW39
sIGEM415	pRC077	G66
sIGEM416	pRC077	AW39
sIGEM417	pRC078	G66
sIGEM418	pRC078	AW39
sIGEM419	pRC080	G66
sIGEM420	pRC080	AW39
sIGEM421	pRC082	G66
sIGEM422	pRC082	AW39
sIGEM423	pRC084	G66
sIGEM424	pRC084	AW39
sIGEM425	pRC086	G66
sIGEM426	pRC086	AW39
sIGEM427	pRC088	G66
sIGEM428	pRC088	AW39
sIGEM429	pRC089	G66
sIGEM430	pRC089	AW39
sIGEM431	pRC091	G66
sIGEM432	pRC091	AW39
sIGEM433	pRC092	G66
sIGEM434	pRC092	AW39
sIGEM435	pRC094	G66
sIGEM436	pRC094	AW39
sIGEM437	pRC096	G66
sIGEM438	pRC096	AW39

- 7pm Once oligos arrive:
  - Make mV1 and mV2 parts through PCR and Gibson assembly.
  - Make off PCR products. After the gel purification, make in parallel the part plasmid (BsmBI rxn) and the cassette (BsaI rxn).

Tube	Product	Template	Primers	Temps (C)	Time	Pdt (bp) '
1	pRC061 backbone	pWCD0608	BB31, BB32	45, 55	40sec	948
2	pRC061 insert	pWCD0608	BB33, BB34	"	"	1812

3	pRC062 backbone	pWCD0608	BB35, BB36	"	"	948
4	pRC062 insert	pWCD0608	BB37, BB38	"	"	1812
5	pRC063	pVY006	BB39, AX22	"	"	1378
6	pRC064	pTC005	BB40, AX58	"	"	1827
7	pRC065	pMRY030	BB41, AX24	"	"	448
8	pRC066	pWCD0536	BB42, PL03_E05	"	"	760
1B	pRC067	pWCD0535	BB43, PL03_E04	"	"	766
2	pRC069	pWCD0537	BB45, AW20	"	"	766



All look good.

- 11:30 Ran Gibson reaction to make pRC061 and pRC062.
  - Used 2.5ul of each pRC061 PCR product.
  - While doing gel purification for pRC062, accidentally combined both PCR products, so just eluted in 10ul of AE and used 5ul in the reaction.
  - Will electroporate.

Tuesday 9/4

- 1pm Picked colonies, seeded in 24-well block.

Monday 9/3

- 11pm Transformed and plated pWCD0519, 555, and 558 to miniprep up more. Plated 5ul of transformation cocktail.
- 11pm Ran BsaI reaction and heat shock transformed into TGI. Streaked out 10ul.

Tube	Result	Description
1A	pRC070	PAmCherry nucleus on
2	pRC072	PAmCherry actin on
3	pRC074	Venus nucleus on
4	pRC075	PAGFP nucleus on
5	pRC077	Venus actin on
6	pRC078	PAGFP actin on
7	pRC080	EmCFP nucleus on
8	pRC082	EmCFP actin on
1B	pRC084	EmCFP VM on
2	pRC086	EmCFP CP on
3	pRC088	Venus VM on
4	pRC089	PAGFP VM on
5	pRC091	Venus CP on
6	pRC092	PAGFP CP on
7	pRC094	PAmCherry VM on
8	pRC096	PAmCherry CP on

Sunday 9/2

- Check GG plasmid stocks and seed more if necessary.
- 120 minipreps!

Saturday 9/1

- 4pm Ran TECAN measurements.
- 5pm Order oligos.