Robert Notebook October 2012

From Dueber Lab Wiki

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

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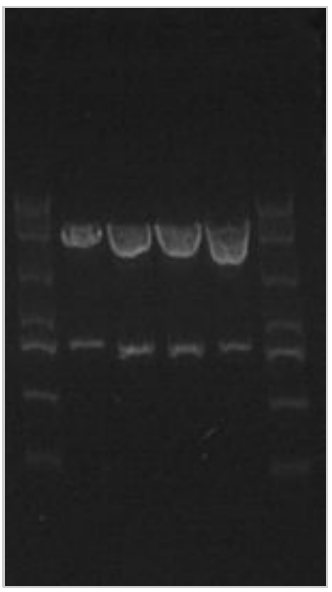
Sunday 9/30

- 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.

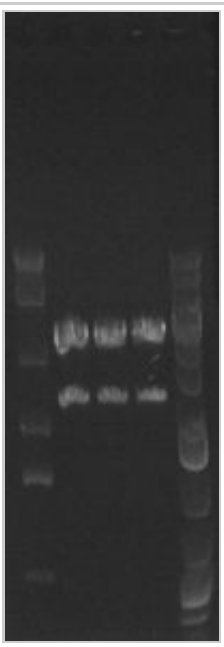
Tube	Plasmid	Description
1	1ul of pRC116G	Test BsmBI reaction
2	2ul of pRC116G	Test BsmBI reaction
3	3ul of pRC116G	Test BsmBI reaction
4	4ul of pRC116G	Test BsmBI reaction



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.

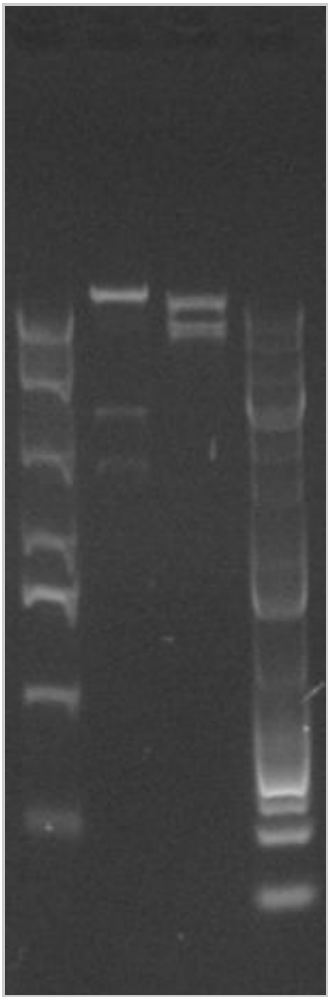
Troubleshoot bad assemblies

- 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

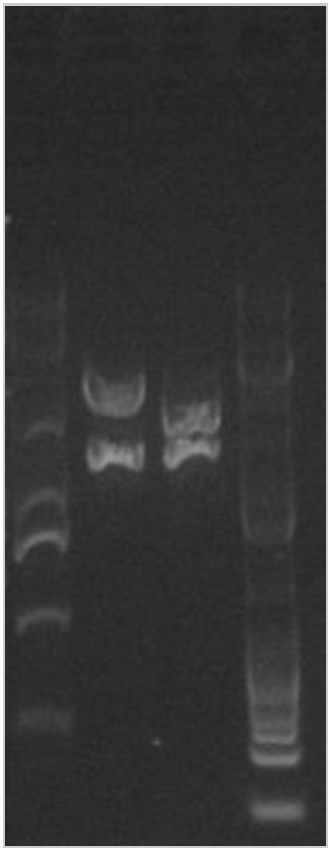


Hmm...getting much different banding than expected.

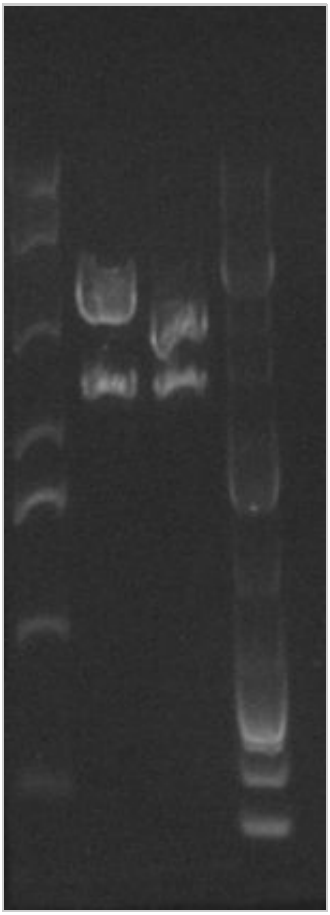
Thursday 9/27

- 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



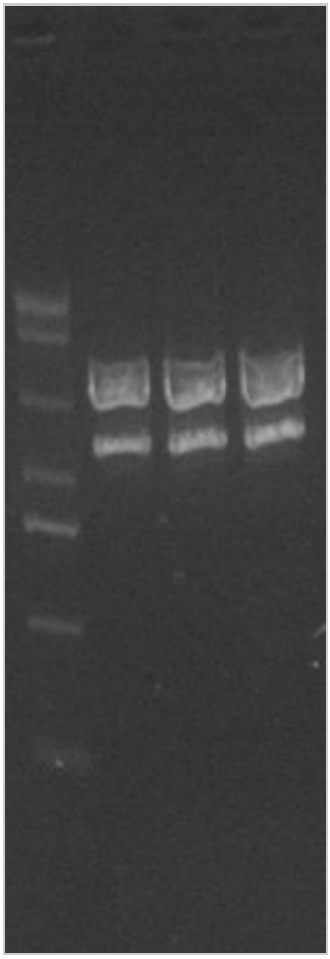
I think both are correct?



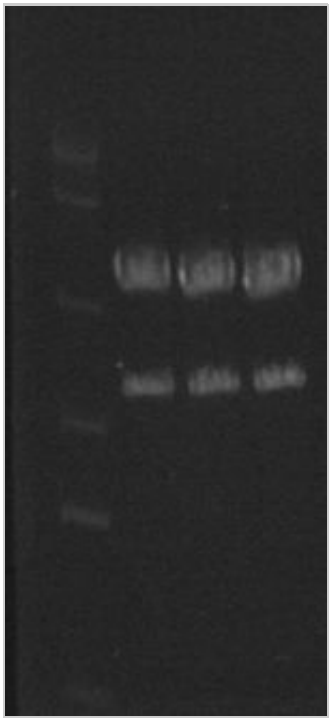
Ran it for a bit longer, and I think pRC116 is missing a small chunk. 117 looks perfect though, so I don't know why the MGs aren't assembling correctly.

- 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.

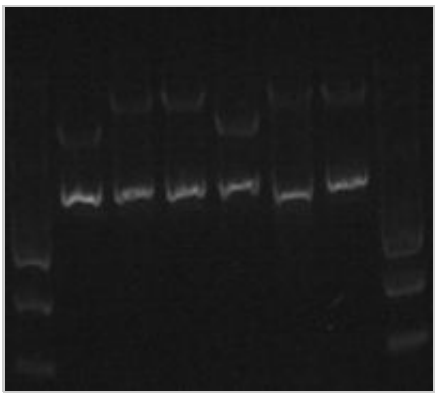
Wednesday 9/26

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

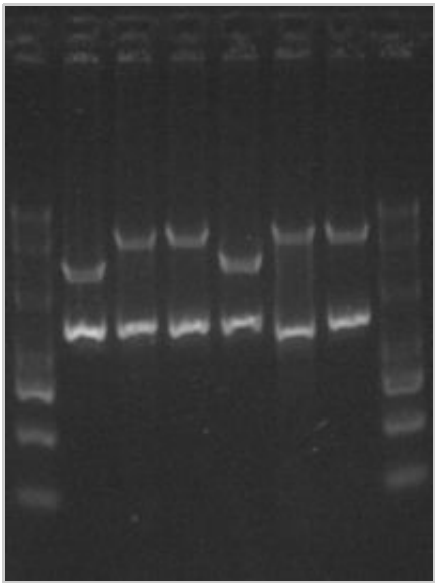
me trouble. Got new stocks today.
- We're having recurring issues with the 3000, 6000, and 8000bp ladder bands. I'm trying a post-stain to see if it helps anything.

- 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:

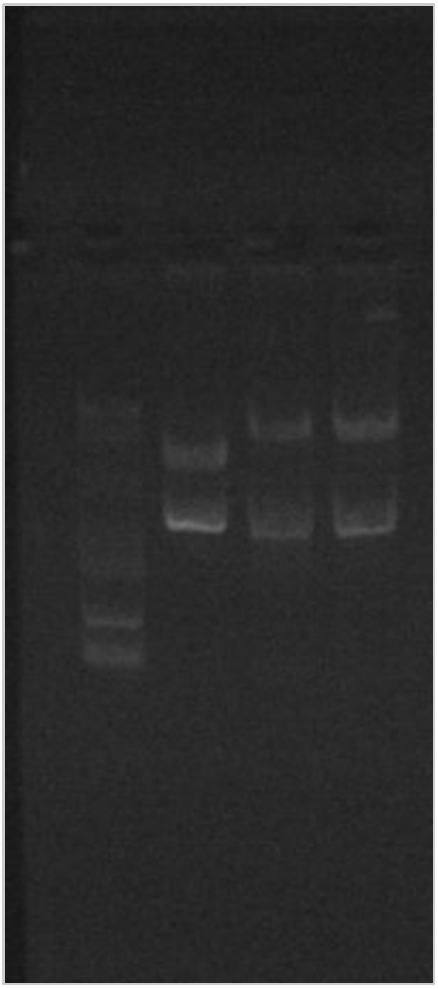
Tube	Plasmid	Clone	Enzymes	Buffer	Bands
1	pRC262-Ura retry	A	AatII/XmaI	4	7890+3617+1864+1705
2	pRC262-Ura retry	B	AatII/XmaI	4	7890+3617+1864+1705
3	pRC262-Ura retry	C	AatII/XmaI	4	7890+3617+1864+1705
4	pRC262-Ura retry equimolar	A	AatII/XmaI	4	7890+3617+1864+1705
5	pRC262-Ura retry equimolar	B	AatII/XmaI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



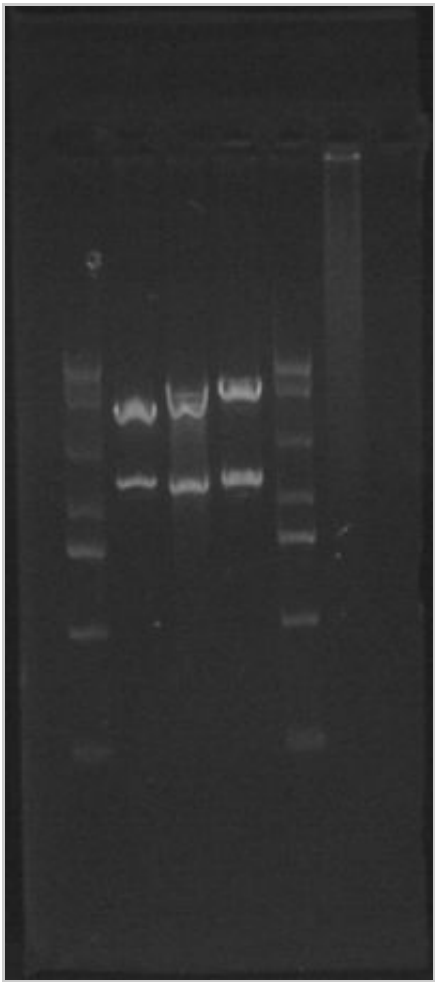
No idea which is correct because the ladder is nonexistent. Guessing that the larger ones are correct.



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

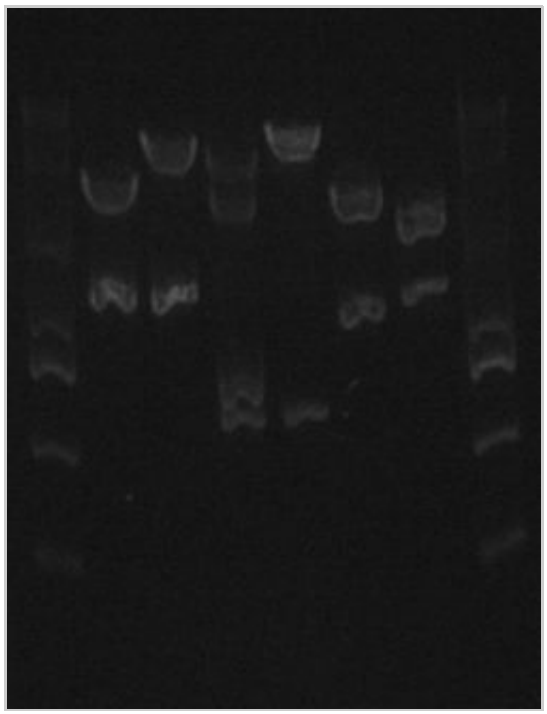
Tube	Plasmid	Description
1	pRC221	retry
2	pRC221	equimolar
3	pRC262	retry
4	pRC262	equimolar

- 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

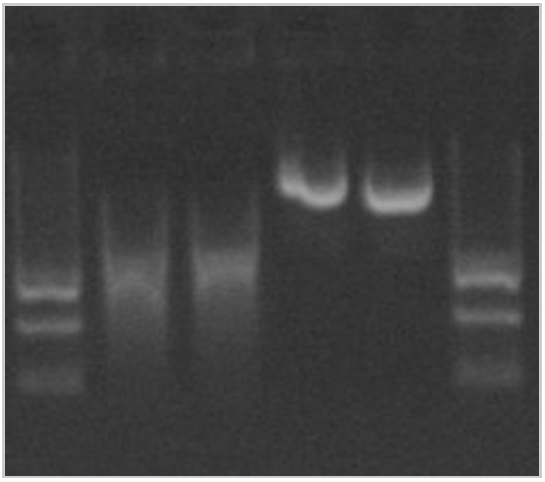


221 and 262 look all off. Not even sure what happened. 116 and 117 look correct at least, thankfully.

- 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.



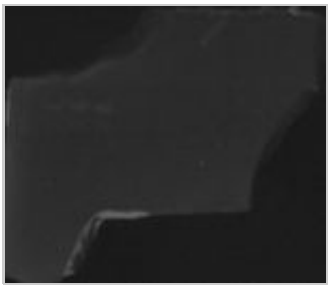
116, 117 digested. 116, 117 undigested. Something weird with the gel and ladder. I think it's correct. Will sequence tomorrow.

- 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.

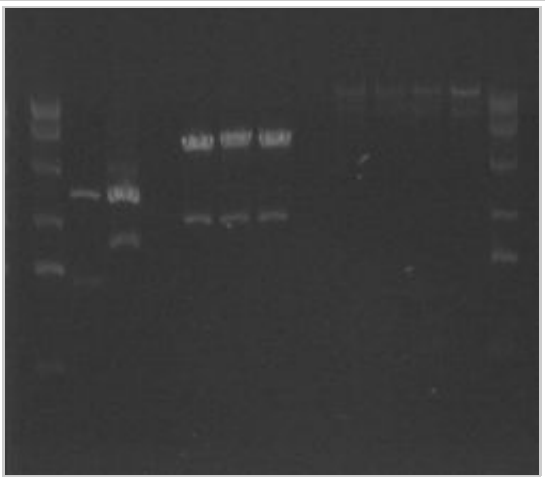
Friday 9/21

- 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.

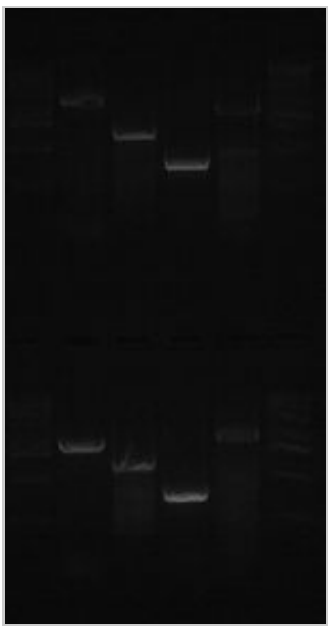
Tube	Plasmid	Clone	Description	Enzymes	Buffer	Bands
1	pWCD0517		AmpR_ColE1 type 6b	AlwNI/NcoI	NEB 4	1911+860
2	pWCD0563		Ura3_Cen6 type 6a	ApaI/SacI	NEB 4	2059+1177
3	pRC114	A	Cas6 mTurquoise2 Actin on	BglII/PvuI	NEB 3	4227+1436
4	pRC114	B	Cas6 mTurquoise2 Actin on	"	"	"
5	pRC114	C	Cas6 mTurquoise2 Actin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

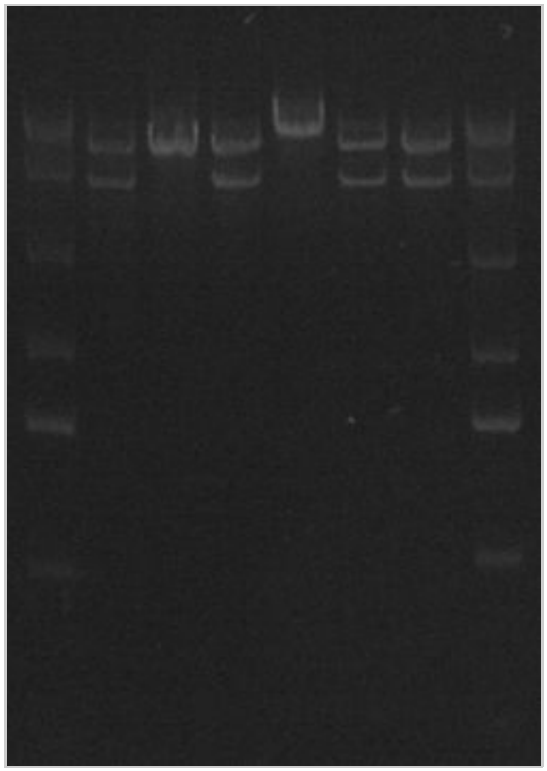
Tuesday 9/18

- 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.

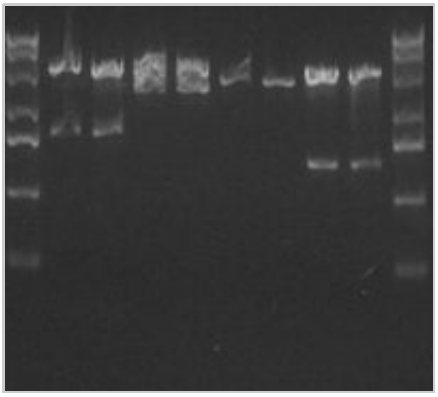
Sequence confirm pRC112-115, 262.

- 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.

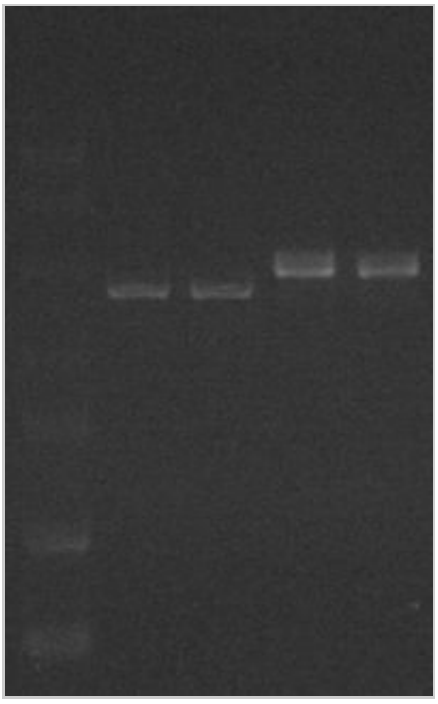
Sunday 9/16

- 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.



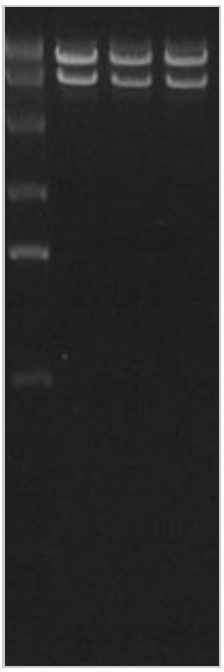
Reran 114s. Are all wrong it seems.

- 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

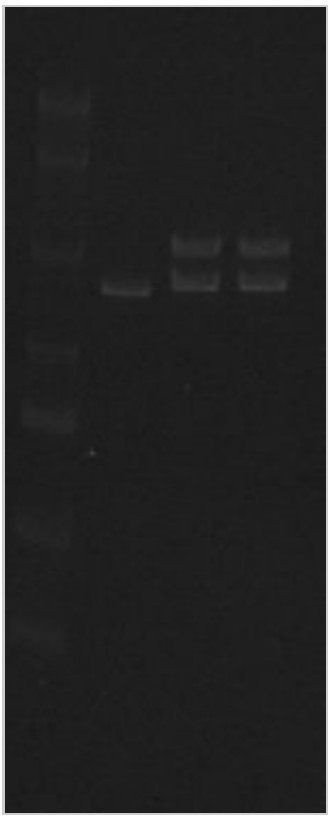
- 11:30am Seeded TGI for ~~transformations later.~~ We decided to just electroporate.
- 6pm Sent pRC105 and pRC086 for sequencing.
- Ran multigene cassettes, do 4 bait, 4 prey.
  - Electroporate all, but save the rest of the reaction in case. Test TGI, non-Liq N2 TGI, and fresh TGI.
  - Used 0.80ul of 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.



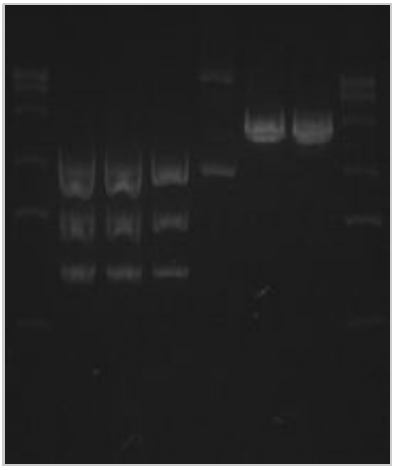
Clones B and C look correct. Will send B for sequencing. Gel probably looked dim because it was older, so will continue doing 1ul digestions and pipetting 5ul per well.

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.

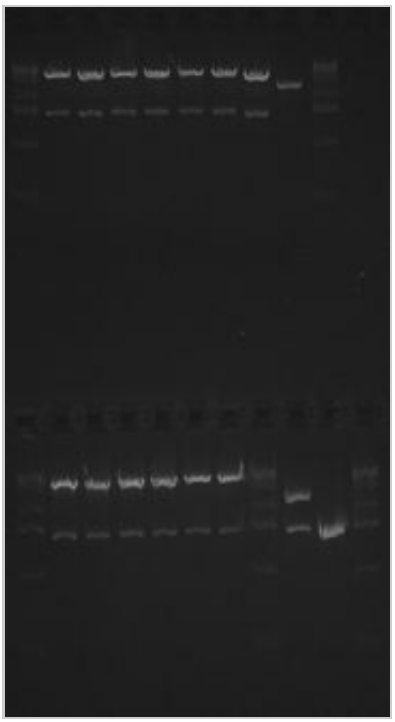
- 10pm Electroporated and plated pRC105. TC 4.4, plated 100ul.

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.

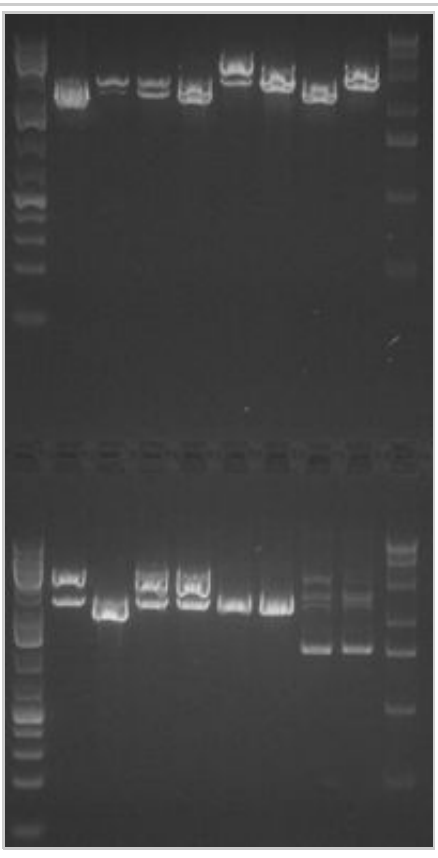
- See what to do with strangely-digested plasmids.
- 7pm Did Do TECAN readings.
- 7pm Sent sequencing out:

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.

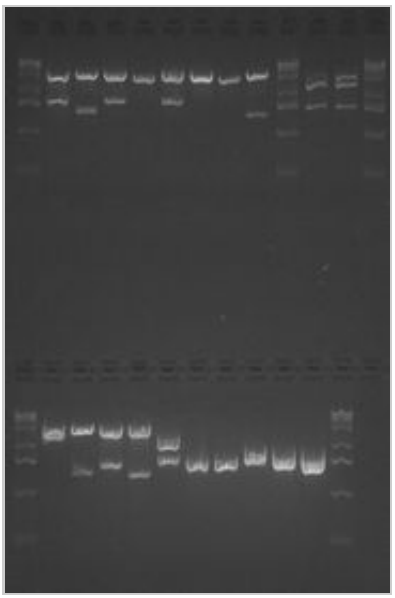


Not sure what happened to the last two...

- 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

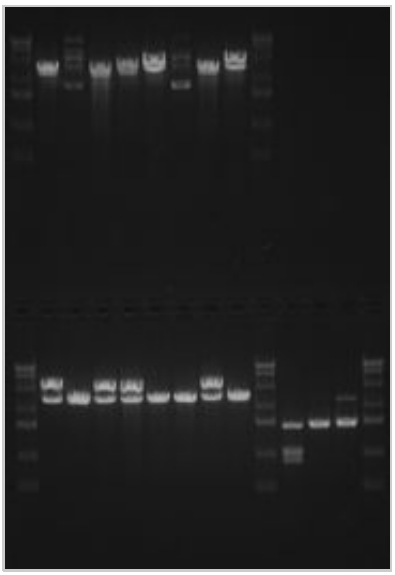
- 11pm Transformed and plated more pWCD0552 and 515 to make more.

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.

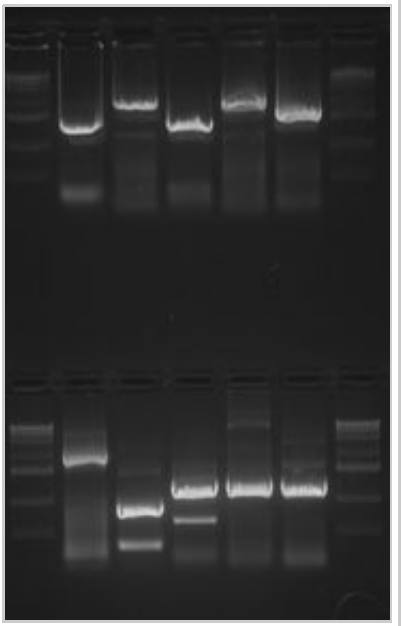
Name	Construct	Clone	Primer
sIGEM407	pRC070		G66
sIGEM408	pRC070		AW39
sIGEM409	pRC072		G66
sIGEM410	pRC072		AW39

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.

Robert Notebook August 2012

From Dueber Lab Wiki

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Friday 8/31

- Take out yeast plates.
  - 9pm Seeded the promoter characterization strains on a plate for TECAN. 8x500ul for each yeast strain. yRC045-52.
  - Seed homing endonuclease stuff.

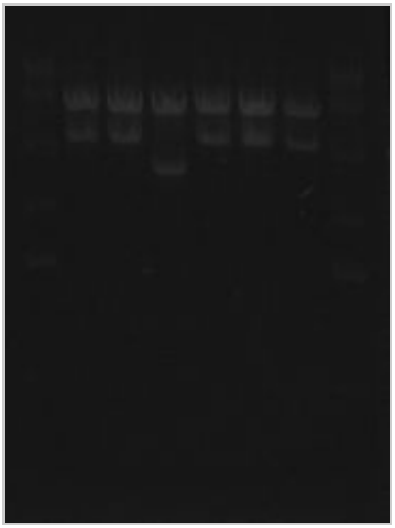
Thursday 8/30

- Send pRC059 for sequencing with BA52 and Q68.
- Take out yeast restreak plate.

Wednesday 8/29

- 12:30pm Seeded pRC059 for miniprep. Picked 6 colonies.
- 2pm Seeded yJD001 and yRC040 to 0.2OD for transformations.
- 6pm Miniprepped pRC059 and test digest.

Tube	Plasmid Clone	Enzymes	Buffer	Bands
1	pRC059 A	EcoRI/PstI	NEB 3	4597+2809
2	pRC059 B	"	"	
3	pRC059 C	"	"	"
4	pRC059 D	"	"	"
5	pRC059 E	"	"	"
6	pRC059 F	"	"	"



Will try out both A and C. Too bad I didn't send C for sequencing.

- 7pm Sent for sequencing:

Name	Date	Construct	Clone	Primer
sIGEM386	29-Aug-12	pRC059	A	V19
sIGEM387	29-Aug-12	pRC059	A	PL03_D11
sIGEM388	29-Aug-12	pRC059	A	AU71
sIGEM389	29-Aug-12	pRC059	B	V19
sIGEM390	29-Aug-12	pRC059	B	PL03_D11
sIGEM391	29-Aug-12	pRC059	B	AU71

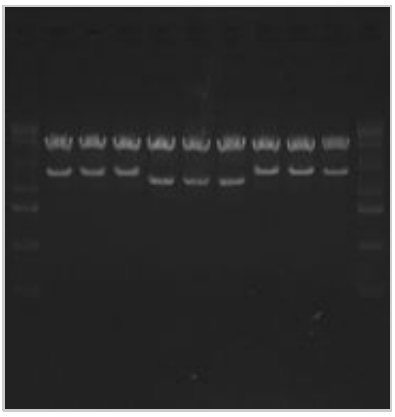
- 9pm Yeast transformations.
  - pRC036 into yRC040 to make yRC053, plate on the 5418-YPD plates.
  - pRC057-60 into yJD001 to make yRC049-52, plate in SD-LEU.
  - Used 1ul of plasmid, resuspended yeast into 1,000ul of ddH2O, and plated 100ul.

Tuesday 8/28

- 4pm Miniprepped and test digested pRC057, pRC058, pRC060.

Tube	Plasmid	Clone	Enzymes	Buffer	Bands

1	pRC057 A	EcoRI/PstI	NEB 3	4597+1909
2	pRC057 B	"	"	"
3	pRC057 C	"	"	"
4	pRC058 A	"	"	4597+1552
5	pRC058 B	"	"	"
6	pRC058 C	"	"	"
7	pRC060 A	"	"	4597+1988
8	pRC060 B	"	"	"
lone	pRC060 C	"	"	"



All look good. Will send only A for sequencing.

- 7pm Sent for sequencing:

Name	Date	Construct	Clone	Primer
sIGEM377	28-Aug-12	pRC057	A	V19
sIGEM378	28-Aug-12	pRC057	A	PL03_D11
sIGEM379	28-Aug-12	pRC057	A	AU71
sIGEM380	28-Aug-12	pRC058	A	V19
sIGEM381	28-Aug-12	pRC058	A	PL03_D11
sIGEM382	28-Aug-12	pRC058	A	AU71
sIGEM383	28-Aug-12	pRC058	A	V19
sIGEM384	28-Aug-12	pRC060	A	V19
sIGEM385	28-Aug-12	pRC060	A	PL03_D11

- 7pm Restreaked yRC045-48 because they are too dense. For the mKate transformations, use 1ul of plasmid and plate only half the cells.
- 8pm Double digested parent, pRC057A with BglII/XbaI, result should 5899bp. Already did a purification of the insert. Will ligate together, heat shock transformation into TGI, then plate on AMP.

Looks correct.

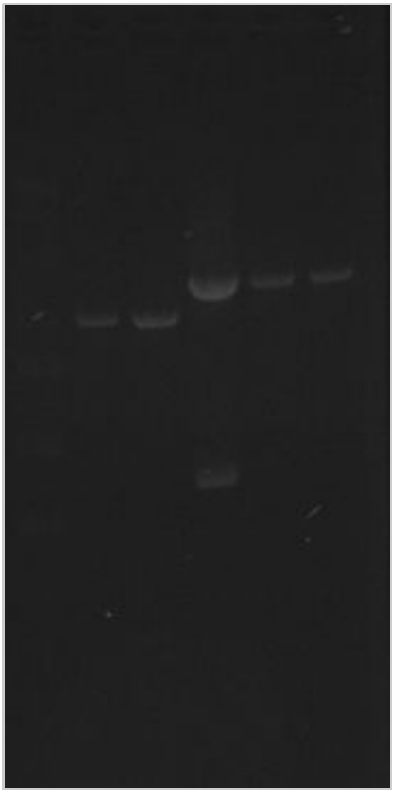
Monday 8/27

- 4pm Picked 3 colonies per electroporated plates for miniprep tomorrow.
- ~~Maybe transform pRC036 into yRC040.~~ Wait til Will gets back.

Sunday 8/26

- 4pm Ran PCR reactions on gel.

Lane	Contents	Template	Bands
1	MW	-	MW
2	Genome test (pADH1 pcr pdt)	yJD001 HSR	1521
3	Genome test (pADH1 pcr pdt)	yJD001 RC	1521
4	Insertion region pos control	pRC027	2052
5	Insertion region	yRC040A	2052
6	Insertion region	yRC040A	2052



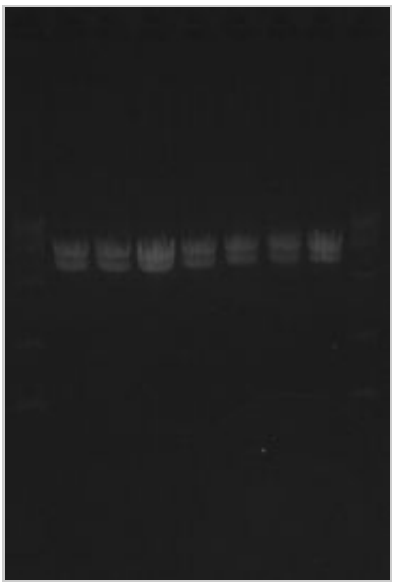
All good. I think I've proved that pRC027 is correctly integrated into yJD001 to produce yRC040.

- 5pm Electroporated cassette reactions.
  - pRC057 tc 4.8, pRC058 tc 4.6, pRC060 tc 4.6.
- 6pm Both pRC036C,D sequenced correctly, so labeled with tough tag and miniprepped more.

Saturday 8/25

- 12pm Miniprepped 36C-J. A and B were red. Accidentally combined E and F.

Tube	Plasmid Clone	Enzymes	Buffer	Bands
1	pRC036 C	Acc651/XbaI	NEB 3	5196+3750
2	pRC036 D	"	"	"
3	pRC036 E/F	"	"	"
4	pRC036 G	"	"	"
5	pRC036 H	"	"	"
6	pRC036 I	"	"	"
7	pRC036 J	"	"	"



Bands are close, but they seem right. I'll send clones C and D for sequencing and toss the rest.

- 1pm Diluted yJD001 to transform into later today.
- 5pm Send pRC036 for sequencing:

Name	Date	Construct	Clone	Primer
sIGEM361	25-Aug-12	pRC036	C	G66
sIGEM362	25-Aug-12	pRC036	C	AZ58
sIGEM363	25-Aug-12	pRC036	C	AN57
sIGEM364	25-Aug-12	pRC036	C	O35
sIGEM365	25-Aug-12	pRC036	C	PL03_H05

sIGEM366	25-Aug-12	pRC036	C	AR62
sIGEM367	25-Aug-12	pRC036	C	AM50
sIGEM368	25-Aug-12	pRC036	C	AW39
sIGEM369	25-Aug-12	pRC036	D	G66
sIGEM370	25-Aug-12	pRC036	D	AZ58
sIGEM371	25-Aug-12	pRC036	D	AN57
sIGEM372	25-Aug-12	pRC036	D	O35
sIGEM373	25-Aug-12	pRC036	D	PL03_H05
sIGEM374	25-Aug-12	pRC036	D	AR62
sIGEM375	25-Aug-12	pRC036	D	AM50
sIGEM376	25-Aug-12	pRC036	D	AW39

- 9pm Transformed pRC053-56 into yeast. Used 3ul of the miniprepped plasmid. Plated on SD-LEU.
- 9pm Made cassettes for registry promoters linked to mKate:

Plasmid Name	Part 1	Part 2	Part 3	Part 4	Part 5	Part 6a	Part 6b
pRC057	ConS	pSTE5 (weak)	mKate	ADH1	ConE	Leu2_Cen6	AmpR_ColE1
pRC058	ConS	pCYC1 (medium)	mKate	ADH1	ConE	Leu2_Cen6	AmpR_ColE1
pRC060	ConS	TDH3p	mKate	ADH1	ConE	Leu2_Cen6	AmpR_ColE1

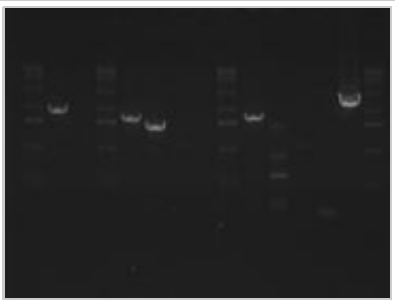
- 10pm Figure out yRC040 genome issues by trying out another primer set.

Tube	Product	Template	Primers	Temps (C)	Time	Pdt (bp)
1	Genome test (pADH1 pcr pdt)	yJD001 HSR	BA52, BA33	Taq	2min15sec	1521
2	Genome test (pADH1 pcr pdt)	yJD001 RC (today)	"	"	"	1521
3	Insertion region pos control	pRC027	AZ42, AZ47	"	"	2052
4	Insertion region	yRC040A	"	"	"	2052
5	Insertion region	yRC040A	"	"	"	2052

Friday 8/24

- 10:30am Picked pRC055 colonies, seeded in LB+AMP
- 4pm Ran gel for PCRs from yesterday.

Lane	Contents	Template	Bands
1	MW	-	MW
2	Genome test (pADH1 pcr pdt)	yJD001 HSR	1521
3	Genome test (pADH1 pcr pdt)	yJD001 RC	1521
4	MW	-	MW
5	5'Leu homo neighborhood	yRC040A	1242
6	3'Leu homo neighborhood	yRC040A	1003
7	Outwards neg control	yRC040A	0
8	Insertion region	yRC040A	2611
9	MW	-	MW
10	5'Leu homo neighborhood	yRC040B	1242
11	3'Leu homo neighborhood	yRC040B	1003
12	Outwards neg control	yRC040B	0
13	Insertion region	yRC040B	2611
14	Insertion region	pRC027	2611



My yJD001 genome prep didn't work. yRC040A Leu homo neighborhoods seem correct. The outwards negative controls have faint wrong bands. The pRC027 positive control is correct, but the homologous yRC PCRs don't show.

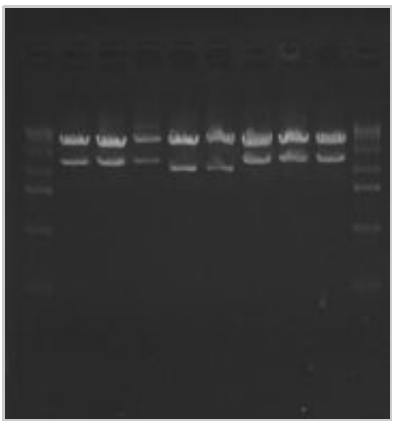
- Colony PCR or just pick colonies from pRC036 plates.
- 5pm Miniprep pRC055, test digest, sequence.

Tube	Plasmid Clone	Enzymes	Buffer	Bands
1	pRC055 A	EcoRI/PstI	NEB 3	4597+2827
2	pRC055 B	"	"	"
3	pRC055 C	"	"	"

Thursday 8/23

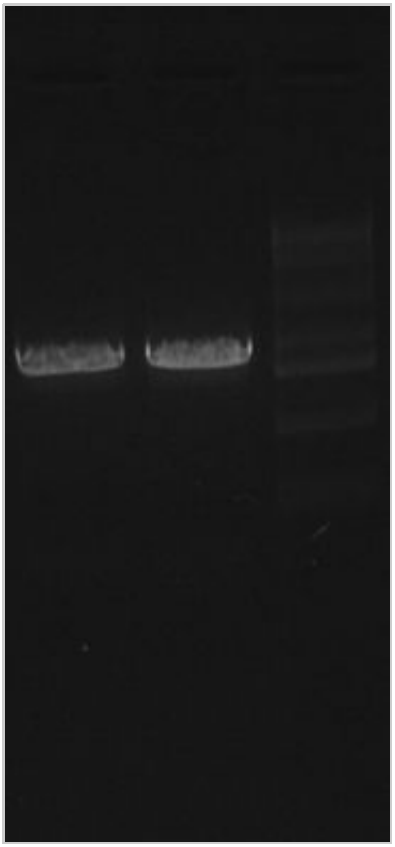
- 4pm Checked on newly-electroporated pRC053, pRC054, and pRC056 plates for a difference from before. More colonies, so if the ones from today don't work, will pick ~3 colonies each.
- Miniprep pRC053, pRC054, pRC056. Test digest:

Tube	Plasmid Clone	Enzymes	Buffer	Bands
1	pRC053 A	EcoRI/PstI	NEB 3	4597+1927
2	pRC053 B	"	"	"
3	pRC053 C	"	"	"
4	pRC054 A	"	"	4597+1570
5	pRC054 B	"	"	"
6	pRC056 A	"	"	4597+2006
7	pRC056 B	"	"	"
8	pRC056 C	"	"	"



All worked it seems.

- 5pm Gel purified ADH1 fragment (pRC051/55 fragment). Expect 1521bp.

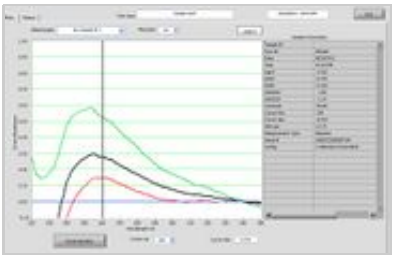


As expected. Combined lanes, eluted into 10ul of ddH2O. Nanodropped 78.6 ng/ul.

- 7pm Sent for sequencing:

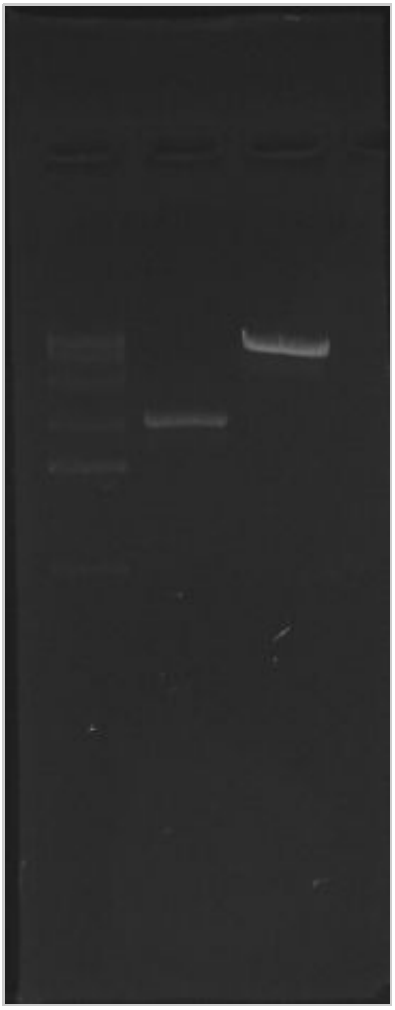
Name	Date	Construct	Clone	Primer
sIGEM344	23-Aug-12	pRC035	B	PL03_A06
sIGEM345	23-Aug-12	pRC053	A	V19
sIGEM346	23-Aug-12	pRC053	A	AU71
sIGEM347	23-Aug-12	pRC053	B	V19
sIGEM348	23-Aug-12	pRC053	B	AU71
sIGEM349	23-Aug-12	pRC054	A	V19
sIGEM350	23-Aug-12	pRC054	A	AU71
sIGEM351	23-Aug-12	pRC054	B	V19
sIGEM352	23-Aug-12	pRC054	B	AU71
sIGEM353	23-Aug-12	pRC056	A	V19
sIGEM354	23-Aug-12	pRC056	A	AU71
sIGEM355	23-Aug-12	pRC056	B	V19
sIGEM356	23-Aug-12	pRC056	B	AU71

- 9pm Electroporated and plated pRC036 reactions. Plated 100ul of rescue broth onto LB+AMP.
  - pRC036A. Should not grow because 33A is wrong.
  - pRC036B, used 1.0ul of cassette reaction. Time constant of 4.6.
  - pRC036B, used 1.5ul. Time constant of 4.0.
- 9pm Genomic purification of yJD001, yRC040A, yRC040B.
  - Added 3x10^7 cells by taking OD.
  - Nanodropped final products.



yJD001=13.8ng/ul,
yRC040A=7.4ng/ul,
yRC040B=26.3ng/ul.

- 9pm Digest both pRC035A (parent) and pADH1 (insert) with XbaI/BglII:



Insert on left, parent on right. Both seem right, so will go ahead and do ligation.

- 10pm Ligated, heat shocked, then plated on LB+AMP.
- 11pm Ran these PCR reactions:

Tube	Product	Template	Primers	Temps (C)	Time	Pdt (bp)
1A	Genome test (pADH1 pcr pdt)	yJD001 HSR	BA52, BA33	Taq	2min30sec	1521
2	Genome test (pADH1 pcr pdt)	yJD001 RC	BA52, BA33	"	"	1521
3	5'Leu homo neighborhood	yRC040A	AK07, AZ55	"	"	1242
4	3'Leu homo neighborhood	yRC040A	AZ46, AK08	"	"	1003
5	Outwards neg control	yRC040A	AZ55, AZ46	"	"	0
6	Insertion region	yRC040A	AS23, AS24	"	"	2611
7	5'Leu homo neighborhood	yRC040B	AK07, AZ55	"	"	1242
8	3'Leu homo neighborhood	yRC040B	AZ46, AK08	"	"	1003
1B	Outwards neg control	yRC040B	AZ55, AZ46	"	"	0

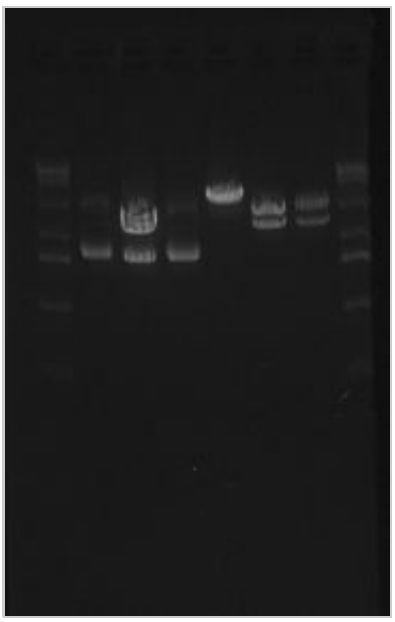
2	Insertion region	yRC040B	AS23, AS24	"	"	2611
3	Insertion region	pRC027@	AS23, AS24	"	"	2611

@Accidentally added pRC047 instead, added pRC027 on top immediately after it started. Should be ok since AS23 and AS24 don't bind to pRC047.

Wednesday 8/22

- 2pm Meeting
- Troubleshoot yRC040 colony PCR.
  - ~~Redo with larger pellet of yeast.~~
  - Genomic purification. Seed 2nd colony and yJD001.
- Give pWCD0561 and pWCD0517 to Will.
- 11am Miniprepped pRC033 and pRC035.
- 1pm Test digested.

Tube	Plasmid Clone	Enzymes	Buffer	Bands
1	pRC033 A	NdeI/HindIII	NEB 2	2507+1047
2	pRC033 B	"	"	"
3	pRC033 F	"	"	"
4	pRC035 A	"	"	2869+1822
5	pRC035 B	"	"	"
6	pRC035 D	"	"	"



pRC033B looks weird, A and F heavier bands seem off. pRC035B,D are correct. Will send both for sequencing.

- 6pm Sent for sequencing. Sent 35A instead of 35D by accident.

Name	Date	Construct	Clone	Primer
sIGEM332	22-Aug-12	pRC033	A	AW38
sIGEM333	22-Aug-12	pRC033	A	AW39
sIGEM334	22-Aug-12	pRC033	B	AW38
sIGEM335	22-Aug-12	pRC033	B	AW39
sIGEM336	22-Aug-12	pRC035	B	AW38
sIGEM337	22-Aug-12	pRC035	B	AZ45
sIGEM338	22-Aug-12	pRC035	B	AZ46
sIGEM339	22-Aug-12	pRC035	B	AW39
sIGEM340	22-Aug-12	pRC035	D	AW38
sIGEM341	22-Aug-12	pRC035	D	AZ45
sIGEM342	22-Aug-12	pRC035	D	AZ46
sIGEM343	22-Aug-12	pRC035	D	AW39

- 7pm Seeded pRC053, pRC054, and pRC056 cassettes in LB+AMP.
  - Will redo BsaI reaction tonight.
  - Will redo electroporation.
- 8pm Ran these cassette assemblies:

Tube	Plasmid	Description
1	pRC036A	Insertion vector using pRC033A and 35B.
2	pRC036B	Insertion vector using pRC033B and 35B.
3	pRC053	pSTE5-Venus-Cen6
4	pRC054	pCYC1-Venus-Cen6
5	pRC056	pTDH3-Venus-Cen6

- 8pm Seeded yDJ001, yRC040A, and yRC040B for genomic extraction tomorrow.
- 9pm Redid electroporation of pRC053, pRC054, pRC056. Time constants were 4.2-4.6, like last time, so I think that there might not be too much DNA in the transformation. Plated 200ul of the rescue broth.

Tuesday 8/21

- 12pm Seeded pWCD0561 (CAM) and pWCD0517 (AMP) for miniprep.
- 9pm Miniprepped, test digested. pWCD0517 was 126.1 ng/ul, pWCD0561 was 424.4 ng/ul. Results shown below.

Tube	Plasmid	Clone	Enzymes	Buffer	Expected
1	pWCD0517	A	AlwNI/NcoI	NEB 3	1911+860
2	pWCD0561	C	"	"	3470+934

Homing Endonuclease

- 3pm Pick colonies from pRC033 and pRC035 electrocomp plates to screen. 10 colonies each.

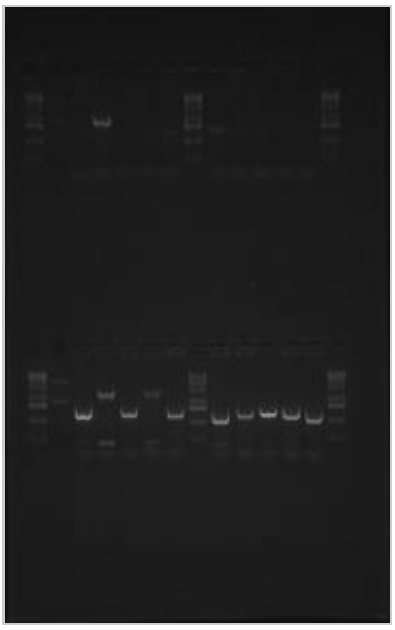
Product	Template	Primers	Temps (C)	Time	Pdt (bp)
pRC034 +C	pRC034 stock	BA08, AH17	Taq	2min30sec	1594
pRC033 cPCR	pRC033 bead plate	AZ42, AZ59	"	"	1876
pRC035 cPCR	pRC035 bead plate	AR62, AW39	"	"	2434

- 6:30pm Ran gel.

Lane	Contents	Bands
1	MW	MW
2	34 +c	1594
3	33A	1876
4	33B	1876
5	33C	1876
6	33D	1876
7	33E	1876
8	MW	MW
9	33F	1876
10	33G	1876
11	33H	1876
12	33I	1876
13	33J	1876
14	MW	MW

Lane	Contents 2	Bands 2
1	MW	MW
2	34 +c	1594
3	34A	2434
4	34B	2434

5	34C	2434
6	34D	2434
7	34E	2434
8	MW	MW
9	34F	2434
10	34G	2434
11	34H	2434
12	34I	2434
13	34J	2434
14	MW	MW



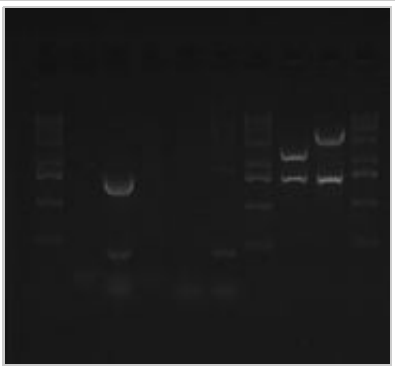
Tomorrow, miniprep up pRC033A,B,F; pRC035A,B,D; test digest then sequence.

- 7pm Colony PCR using yRC040. 60ul of saturated culture. 50ul of 20mM NaOH. 10ul of supernatant per PCR.

Product	Template	Primers	Temps (C)	Time	Pdt (bp)
5'Leu homo neighborhood	yRC040	AK07, AZ55	Taq	2min45sec	1242
3'Leu homo neighborhood	yRC040	AZ46, AK08	"	"	1003
Outwards neg control	yRC040	AZ55, AZ46	"	"	0
Insertion region	yRC040	AS23, AK08	"	"	2637
Insertion region	pRC027	AS23, AK08	"	"	2637

- 11pm Ran gel containing both test digest (above) and cPCR.

Lane	Contents	Bands
1	MW	MW
2	5'Leu homo neighborhood	1242
3	3'Leu homo neighborhood	1003
4	Outwards neg control	0
5	Insertion region (yRC040)	2637
6	Insertion region (pRC027)	2637
7	MW	MW
8	pWCD0517 tDigest	1911+860
9	pWCD0561 tDigest	3470+934



pWCD plasmids are correct. All of the yeast looks strange. Even the positive control lane 6 looks off. Will troubleshoot tomorrow.

- Hold off on gel purifying PCR product.

Promoter Characterization

- Hold off on gel purifiying PCR product.
- 4pm Electroporated the cassettes. Plated 100 of the 500ul of recovery onto AMP plates. Tomorrow, pick colonies to miniprep.

Monday 8/20

- 7pm Did yeast integration for Celia.

Promoter Library

- Look at yRC025 and yRC035 under scope to see if new plates are correct.
- Make yeast glycerol stocks for yRC023, yRC036, yRC038 (old plate).
- Toss yRC038 new plate.
- Figured that high expression of ABP1 and HTA2 kill cells. Will not try to use TDH3 or TEF1 for those.
- Done with Promoter Library for now.

Promoter Characterization

- Celia tried re-integrating pCYC1 (pRC050), but saw no fluorescence again.
  - F Sequencing shows that promoter matches perfectly
  - Will resend R sequencing.
- 3pm Ran PCR to make ADH1 (pRC051) insert. See below for specifics.
- 7pm Ran BsaI reaction to produce Cen6 plasmids:

Plasmid Name	Part 1	Part 2	Part 3	Part 4	Part 5	Part 6a	Part 6b
pRC053	ConS	pSTE5 (weak)	Venus	ADH1	ConE	Leu2_Cen6	AmpR_ColE1
pRC054	ConS	pCYC1 (medium)	Venus	ADH1	ConE	Leu2_Cen6	AmpR_ColE1
pRC056	ConS	TDH3p	Venus	ADH1	ConE	Leu2_Cen6	AmpR_ColE1

- 7pm Transformed pWCD0561 (CAM) and pWCD0517 (AMP) into TGI because Will's stock is almost out.
  - Streaked 5ul onto plates.

Homing Endonuclease

- 1pm Redid pRC033 Gibson rxn.
  - Nanodrop PCR pdts needed to run it to look for relative concentrations.
  - Use 1x stock EPI300 instead of 10x dilution.
  - Will need to pick more colonies.

Part #	Part desc.	Ci (uM) enter	Vi (uL)
1	pRC033.1	87.1	1.640782021
2	pRC033.2	153.3	0.932238187

3	pRC033.3	219.3	0.651674027
4	pRC033.4	80.5	1.775305765
Total			5

- 3pm Ran pRC035 Gibson rxn.
- 4pm Ran these PCR:

Product	Template	Primers	Temps (C)	Time	Pdt (bp)
pRC033.4	pML281	AZ58, AZ59	50	30sec	624
pRC035.1	pWCD0563	AZ64, AZ65	50	60sec	3263
pRC035.2	pWCD0533	AZ66, AZ67	50	30sec	559
pRC051 pcr pdt	yJD001	BA33, BA52	55	40sec	1521

- 7pm Electroporated and plated pRC033 and pRC035 gibson product.
  - Used 30ul of EPI300 from stock (not 10x dilution)
  - Did both a 5ul+streak and a 50ul+bead shake out of the 500ul recovery LB.
- 7pm Seeded yRC040 for PCR tomorrow.

8/18-8/25 Break

https://docs.google.com/spreadsheet/ccc?key=0ApdDUHORhUhAdDdsNHlXVmVremZCY2lFQkw1VXhXTHc

Promoter Library

- Someone re-integrate pRC029 (pTEF1-ABP1) to make yRC026.
- All plasmids are in the iGEM GG Cloning box, and are arranged in columns by organelle. Top of the organelle is the part 3a or 4 that tags to the organelle. Closest to the top is TDH3, and closest to the bottom is pGal (which I made but we're not using).

Promoter Characterization

- Someone re-integrate pRC050 (pCYC1-Venus) to make yRC042.
- All promoter characterization parts are in my WB1.
- My plasmids pages are all updated.

Tuesday 8/7

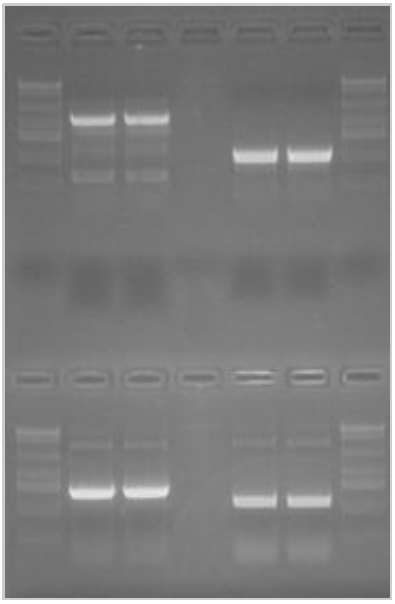
- 1pm Seeded yeast cultures, 100ul into 3ml SD-LEU.
- Will image at mid-log.
- GLycerol stocked some of the correct yeast cultures.
- 1pm Will need to hold off on yRC040 colony PCR until later because gel room is unavailable.
    - Want to choose primers to cover begnning and end of insertion homologies.
- pRC033 did not grow, so will OD the PCR pdts before running another reaction.

Monday 8/6

Homing Endonuclease

- Colony PCR yRC040, using pRC027 as +C.
- Seed yRC040 for glycerol stocks.
- Only 5 colonies on entire pRC033 plate, so will re-PCR all the parts.

1	2	3	4	5	6	7
MW	pRC033.1	pRC033.1	-	pRC033.2	pRC033.2	MW
MW	1678	1678	-	506	506	MW
MW	pRC033.3	pRC033.3	-	pRC033.4	pRC033.4	MW
MW	850	850	-	624	624	MW



All bands as expected. Gel purified both lanes into one Zymo column and eluted into 10ul.

- 8pm Ran Gibson reaction, using 1.25ul of each product.
- 10:30pm Did electroporation, plated 100ul on LB+CAM.

Promoter Library

- ~~Miniprep pWCD0515 and pWCD0559.~~ No need.
- 5pm Sent pRC020A, 39A, 40B, 42A, 43A, 44A, 45A for sequencing. Use X45 (end of 5' LEU) as F and AZ21 (end of ADH1) as R primers.
- 5pm Re-sent pRC049A, pRC050B, pRC052B, pRC019A, pRC028A, pRC029A, pRC037A, pRC038A with AZ21 as R primer.

Name	Date	Construct	Clone	Primer
sIGEM309	5-Aug-12	pRC017 (+C)	A	AZ21
sIGEM310	5-Aug-12	pRC049	A	AZ21
sIGEM311	5-Aug-12	pRC050	B	AZ21
sIGEM312	5-Aug-12	pRC052	B	AZ21
sIGEM313	5-Aug-12	pRC019	A	AZ21
sIGEM314	5-Aug-12	pRC028	A	AZ21
sIGEM315	5-Aug-12	pRC029	A	AZ21
sIGEM316	5-Aug-12	pRC037	A	AZ21
sIGEM317	5-Aug-12	pRC038	A	AZ21
sIGEM318	5-Aug-12	pRC020	A	X45

sIGEM319	5-Aug-12	pRC020	A AZ21
sIGEM320	5-Aug-12	pRC039	A X45
sIGEM321	5-Aug-12	pRC039	A AZ21
sIGEM322	5-Aug-12	pRC040	B X45
sIGEM323	5-Aug-12	pRC040	B AZ21
sIGEM324	5-Aug-12	pRC042	A X45
sIGEM325	5-Aug-12	pRC042	A AZ21
sIGEM326	5-Aug-12	pRC043	A X45
sIGEM327	5-Aug-12	pRC043	A AZ21
sIGEM328	5-Aug-12	pRC044	A X45
sIGEM329	5-Aug-12	pRC044	A AZ21
sIGEM330	5-Aug-12	pRC045	A X45
sIGEM331	5-Aug-12	pRC045	A AZ21

- 6pm Checked yeast on microscope.
  - Turns out I was using Fiji wrong.
  - From past week, yRC032, 33, 36, 37 were right when I thought they were wrong.
  - From yesterday, yRC022, 30, 31, 32, 33, 36 are correct.
  - Need to reimage yRC023 and 38.
  - Seeded all the correct colonies for midlog imaging.

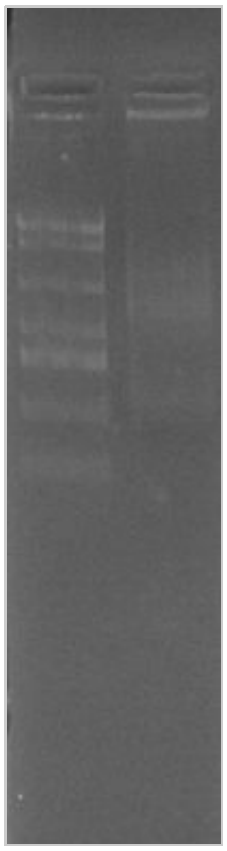
Promoter Characterization

- 6pm Checked yeast on microscope.
  - yRC041 and 44 are correct. Seeded for midlog and glycerol.
  - Pick another colony from yRC042 tomorrow.

Sunday 8/5

Homing Endonuclease

- 8pm Took 20ul of Gibson reaction product, added 1ul of DpnI, incubated 45min in 37. Ran all on gel.



Will rerun the Gibson reaction.

- 10pm Rerun Gibson reactions for pRC033. Use 1.25ul of each part. Use different 33.1 and 33.2 parts (parts A).

Part #	Part desc.	Volume
1	pRC033.1A	1.25
2	pRC033.2A	1.25
3	pRC033.3	1.25
4	pRC033.4	1.25
	Total	5

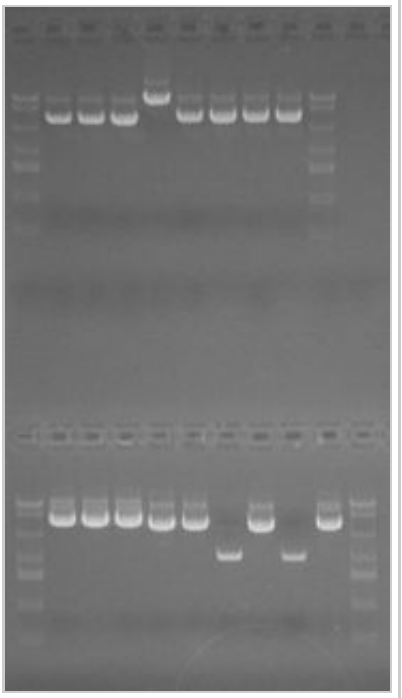
- 1am Transformed 1ul into EPI300 with electroporation. Plated 150ul of recovery solution onto LB+CAM.

Promoter Array

- 6pm Miniprepped cultures from yesterday.
- 7pm Test digested:

Tube	Plasmid	Clone	Enzymes	Buffer	Expected
1	pRC039	A	XbaI/SpeI	NEB 4	4672+1691

2	pRC039 C	"	"	"
3	pRC040 B	"	"	"
4	pRC040 C	"	"	"
5	pRC044 A	"	"	4672+2065
6	pRC044 B	"	"	"
7	pRC045 A	"	"	"
8	pRC045 D	"	"	"
1	pRC020 A	"	"	4672+2995
2	pRC020 B	"	"	"
3	pRC020 C	"	"	"
4	pRC042 A	"	"	4672+2045
5	pRC042 B	"	"	"
6	pRC042 C	"	"	"
7	pRC043 A	"	"	4672+2065
8	pRC043 B	"	"	"
lone	pRC043 C	"	"	"

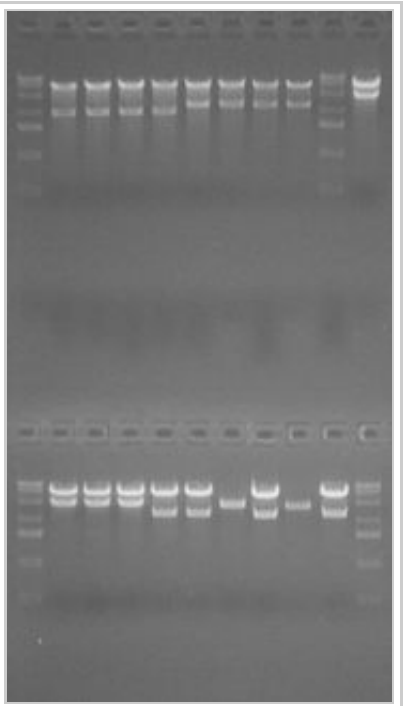


Something weird...Will retry digest with XbaI/XhoI and +C.

- 9pm Retried test digest.

Tube	Plasmid	Clone	Enzymes	Buffer	Expected
1A	pRC039	A	XbaI/XhoI	NEB 4	4903+1460

2	pRC039	C	"	"	"
3	pRC040	B	"	"	"
4	pRC040	C	"	"	"
5	pRC044	A	"	"	4903+1834
6	pRC044	B	"	"	"
7	pRC045	A	"	"	"
8	pRC045	D	"	"	"
lone	pRC017 (+C)	-	"	"	4903+2744
1B	pRC020	A	"	"	4903+2764
2	pRC020	B	"	"	"
3	pRC020	C	"	"	"
4	pRC042	A	"	"	4903+1814
5	pRC042	B	"	"	"
6	pRC042	C	"	"	"
7	pRC043	A	"	"	4903+1834
8	pRC043	B	"	"	"
lone	pRC043	C	"	"	"



Something was wrong with SpeI. Will go ahead with integrations.

#	Name	Description	Plasmids Used	Parental Strain	Marker	Color
1	yRC023	pREV1-ZRC1	pRC020A	yJD001	LEU	Venus
2	yRC032	pRNR2-CIIC	pRC039A	"	"	"

3	yRC033	pREV1-CIIC	pRC040B	"	"	"
4	yRC035	pTDH3-HTA2	pRC042A	"	"	"
5	yRC036	pTEF1-HTA2	pRC043A	"	"	"
6	yRC037	pRNR2-HTA2	pRC044A	"	"	"
7	yRC038	pREV1-HTA2	pRC045A	"	"	"

- Use X45 (end of 5' LEU) as F and AZ21 (end of ADH1) as R primers for cassette sequencing in the future.
- ~~7pm Seeded pWCD0515 and pWCD0559 into 3ml broth.~~ Decided against making more...almost out of agarose.
- Might want to send our stocks of pWCD plasmids for sequencing.

Saturday 8/4

Homing Endonuclease

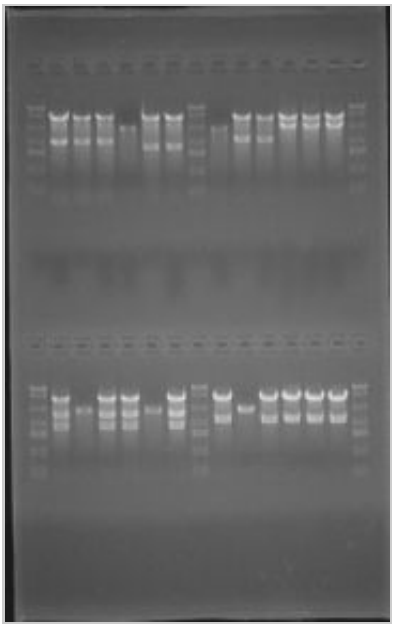
- 9pm Checked sequencing.
  - pRC033 is missing parts 1 and 2.
  - pRC034 is correct. Labeled tube in freezer with blue tag.

BsaI Reactions from Yesterday

- 12pm Seeded yJD001 to 0.2 for yeast integrations.
- 1pm Miniprepped BsaI reactions for Promoter Array and Promoter Characterization block.
- 4pm Test digested.

Tube	Plasmid	Clone	Enzymes	Buffer	Expected	'
1A	pRC049	A	SpeI/XbaI	NEB 4	4672+1469+98	
2	pRC049	B	"	"	"	
3	pRC049	C	"	"	"	
4	pRC050	A	"	"	4672+1210	
5	pRC050	B	"	"	"	
6	pRC050	C	"	"	"	
7	pRC052	A	"	"	4672+1646	
8	pRC052	B	"	"	"	
1B	pRC052	C	"	"	"	

2	pRC019 A	"	"	4672+2995
3	pRC019 B	"	"	"
4	pRC019 C	"	"	"
5	pRC028 A	"	"	4672+2123+1302
6	pRC028 B	"	"	"
7	pRC028 C	"	"	"
8	pRC029 A	"	"	4672+2143+1302
1C	pRC029 B	"	"	"
2	pRC029 C	"	"	"
3	pRC037 A	"	"	4672+1671
4	pRC037 B	"	"	"
5	pRC037 C	"	"	"
6	pRC038 A	"	"	4672+1691
7	pRC038 B	"	"	"
8	pRC038 C	"	"	"



Use 49A, 50B, 52B, 19A, 28A, 29A, 37A, 38A.

- 5pm Sent for sequencing.

Name	Date	Construct	Clone	Primer
sIGEM293	4-Aug-12	pRC049	A	X45
sIGEM294	4-Aug-12	pRC049	A	AW39
sIGEM295	4-Aug-12	pRC050	B	X45

sIGEM296	4-Aug-12	pRC050	B	AW39
sIGEM297	4-Aug-12	pRC052	B	X45
sIGEM298	4-Aug-12	pRC052	B	AW39
sIGEM299	4-Aug-12	pRC019	A	X45
sIGEM300	4-Aug-12	pRC019	A	AW39
sIGEM301	4-Aug-12	pRC028	A	X45
sIGEM302	4-Aug-12	pRC028	A	AW39
sIGEM303	4-Aug-12	pRC029	A	X45
sIGEM304	4-Aug-12	pRC029	A	AW39
sIGEM305	4-Aug-12	pRC037	A	X45
sIGEM306	4-Aug-12	pRC037	A	AW39
sIGEM307	4-Aug-12	pRC038	A	X45
sIGEM308	4-Aug-12	pRC038	A	AW39

- 7pm Did yeast integrations. Might have swapped pRC028 and pRC029:

#	Name	Description	Plasmids Used	Parental Strain	Marker	Color
1	yRC041	pSTE5-Venus	pRC049A	yJD001	LEU	Venus
2	yRC042	pCYC1-Venus	pRC050B	"	"	"
3	yRC044	TDH3p-Venus	pRC052B	"	"	"
4	yRC022	pRNR2-ZRC1	pRC019A	"	"	"
5	yRC025	pTDH3-ABP1	pRC028A	"	"	"
6	yRC026	pTEF1-ABP1	pRC029A	"	"	"
7	yRC030	pTDH3-CIIC	pRC037A	"	"	"
8	yRC031	pTEF1-CIIC	pRC038A	"	"	"

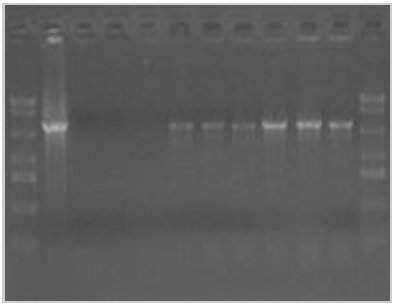
Making pADH1 for Promoter Characterization

- ~~Made pRC051 with traditional cloning.~~
  - ~~Used pRC049B as the parent vector.~~
  - ~~Double digest both pRC049B and PCR pdt with SpeI/BglII (NEB 2), total volume 20ul so I will have brighter band.~~
  - Realized that I made the front end of the promoter wrong. It should have a XbaI site (GG method) instead of a SpeI site (Bglbrick). Ordered new F primer to correct the issue, BA52.

Colony PCR

- 12pm In parallel, tried various colony PCR methods.
  - Used X45 F and AS24 R primers.
  - If successful, run colony PCR on next set of BsaI plates.

Tube	Plasmid	Condition	Band
1	pRC017	PC from miniprep	3563bp
2	pRC019A	From culture	3583bp
3	pRC019B	"	"
4	pRC019C	"	"
5	pRC019D	Direct from colony	"
6	pRC019E	"	"
7	pRC019F	"	"
8	pRC019G	Colony into water	"
1	pRC019H	"	"
2	pRC019I	"	"

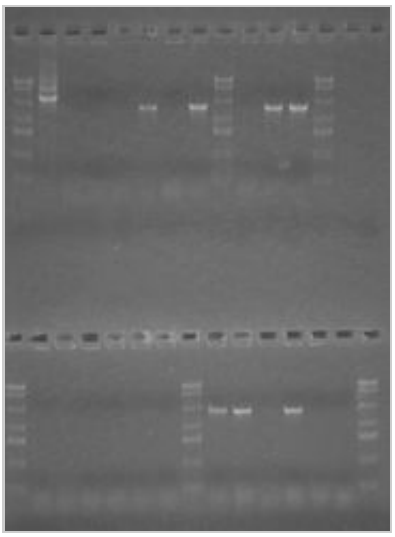


The water colony method is far better. Will do colony PCR this way in the future.

- 6pm Ran colony PCR on the BsaI plates from yesterday:

Tube	Plasmid	Clone	Band
1A	pRC017 (+)	-	3563
2	pRC020	A	3583
3	pRC020	B	"
4	pRC020	C	"
5	pRC039	A	2279
6	pRC039	B	"
7	pRC039	C	"
8	pRC040	A	2279
1B	pRC040	B	"
2	pRC040	C	"

3	pRC042	A	2633
4	pRC042	B	"
5	pRC042	C	"
6	pRC043	A	2653
7	pRC043	B	"
8	pRC043	C	"
1C	pRC044	A	2653
2	pRC044	B	"
3	pRC044	C	"
4	pRC045	A	2653
5	pRC045	B	"
6	pRC045	C	"



39AC, 40BC, 44AB, and 45A worked. Will pick a colony D for 45. Will seed all of 20, 42, 43 for miniprep. Hopefully those will work when miniprepped.

Friday 8/3

Promoter Array

- 3am Electroporated BsaI reaction, plated on LB+AMP.
- 2am Seeded colonies that worked that were ~~imaged correctly~~ for yeast glycerol stocks.
- 4pm Realized that the above "imaged correctly" colonies were actually wrong. Will run BsaI reaction for

these. Ran pRC042 again because it didn't grow after electroporation.

Tube	Plasmid	Description	Yeast Product
1	pRC020	pREV1-ZRC1	yRC023
2	pRC039	pRNR2-CIIC	yRC032
3	pRC040	pREV1-CIIC	yRC033
4	pRC043	pTEF1-HTA2	yRC036
5	pRC044	pRNR2-HTA2	yRC037
6	pRC045	pREV1-HTA2	yRC038
lone	pRC042	pTDH3-HTA2	yRC035

- 6pm Picked colonies from BsaI plate. Seeded in 3ml of LB+AMP on block.
  - This time, after miniprepping the cassette, send for sequencing.
- 11pm Electroporated BsaI reactions and plated on LB+AMP.
  - pRC039's time const. was a bit high, so might not have been much DNA.
  - Recovery for 45min.
  - Tomorrow, pick for miniprep.

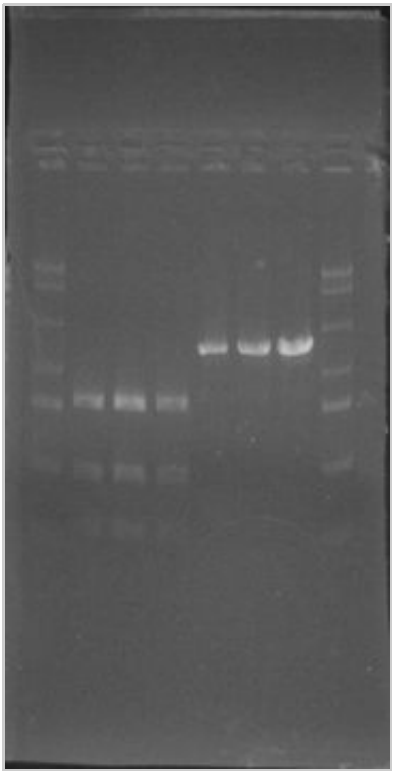
Promoter Characterization

- 3am Plated Promoter Array re-do and Promoter Characterization BsaI reactions.
  - Might have ran electroporation on pRC050 twice by accident...25% chance I did it wrong.
  - Plated 100ul of reaction product onto LB+AMP.
- 6pm Picked colonies from BsaI plate. Seeded for minipreps on same block as the BsaI reactions.

Homing Endonuclease

- Miniprep pRC033 and pRC034.
- Test digest:

Tube	Plasmid	Enzymes	Buffer	Expected
1	pRC033A	AlwNI/SacI	NEB 4	2465+1089
2	pRC033B	"	"	"
3	pRC033C	"	"	"
4	pRC034A	AlwNI/BglII	NEB 3	1421+739
5	pRC034B	"	"	"
6	pRC034C	"	"	"



Not sure what the issue is, so will send pRC033B and pRC034C for sequencing. Kept 33A and 34B as backup.

- 6pm Sent for sequencing:

Name	Date	Construct	Clone	Primer
sIGEM289	3-Aug-12	pVY022	5	Z71
sIGEM290	3-Aug-12	pRC033	B	AW38
sIGEM291	3-Aug-12	pRC033	B	AW39
sIGEM292	3-Aug-12	pRC034	C	AW38

Thursday 8/2

Promoter Array

- I think yRC037 sequenced incorrectly.
- 2pm Checked yeast:

Yeast	Description	Imaging Result	Redo?
yRC022	pRNR2-ZRC1(VM)	Nothing	x

23	pREV1-ZRC1(VM)	x	
25	pTDH3-ABP1(Actin)	Nothing	x
26 new	pTEF1-ABP1(Actin)	Not localized correctly	x
26 old	pTEF1-ABP1(Actin)	Nothing	x
30	pTDH3-CIIC(CP)	Not localized correctly	x
31	pTEF1-CIIC(CP)	Not localized correctly	x
32	pRNR2-CIIC(CP)	x	
33	pREV1-CIIC(CP)	x	
35	pTDH3-HTA2(Nucleus)	Nothing	x
36	pTEF1-HTA2(Nucleus)	x	
37	pRNR2-HTA2(Nucleus)	x	
38	pREV1-HTA2(Nucleus)	?	

- 7pm Ran another BsaI reaction:

Tube	Plasmid	Description	Yeast Product
1	pRC019	pRNR2-ZRC1	yRC022
2	pRC028	pTDH3-ABP1	yRC025
3	pRC029	pTEF1-ABP1	yRC026
4	pRC037	pTDH3-CIIC	yRC030
5	pRC038	pTEF1-CIIC	yRC031
6	pRC042	pTDH3-HTA2	yRC035

- 7pm Sent for sequencing:

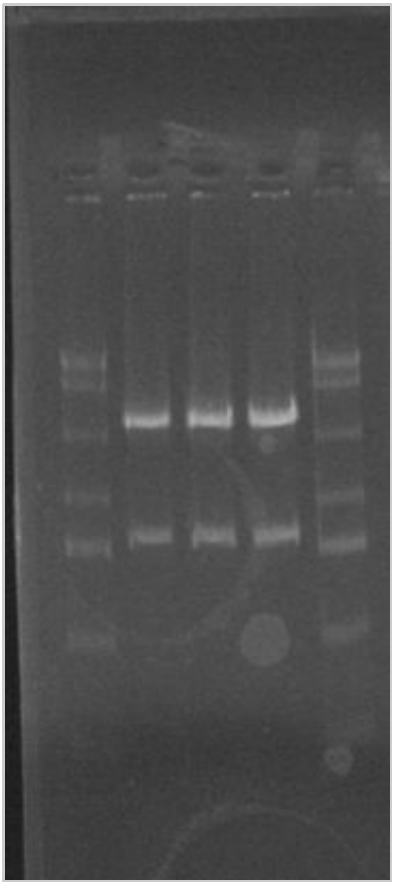
Name	Date	Construct	Clone	Primer
sIGEM274	2-Aug-12	pVY006		AW38
sIGEM275	2-Aug-12	pVY006		AW39
sIGEM276	2-Aug-12	pTC005		AW38
sIGEM277	2-Aug-12	pTC005		AW39
sIGEM278	2-Aug-12	pATJ006		AW38
sIGEM279	2-Aug-12	pMRY030		AW38
sIGEM280	2-Aug-12	pMRY030		AW39
sIGEM281	2-Aug-12	pRC027	A	G66
sIGEM282	2-Aug-12	pRC027	A	AW39
sIGEM283	2-Aug-12	pRC027	B	G66

sIGEM284	2-Aug-12	pRC027	B	AW39
sIGEM285	2-Aug-12	pRC027	C	G66
sIGEM286	2-Aug-12	pRC027	C	AW39
sIGEM285	2-Aug-12	pRC047	A	AW38
sIGEM286	2-Aug-12	pRC047	B	AW38
sIGEM287	2-Aug-12	pRC048	A	AW38
sIGEM288	2-Aug-12	pRC048	B	AW38

Homing Endonuclease

- 10:30am Minprepped pRC027.
- 11am Dilute yeast to 0.2OD.
- 4pm Test digest:

Tube	Plasmid	Enzymes	Buffer	Expected
1	pRC027A	BglII/BamHI	NEB3	3410+1068
2	pRC027B	"	"	"
3	pRC027C	"	"	"



All worked. Will use just clone A for integration. Will toss clone C because only one extra is ok.

- 6pm Sent pRC027 for sequencing. Use G66 as F and AW39 as R primers. See above.
- 6pm Yeast integrate miniprepped pRC027.
  - Linearized with EcoRI and PstI (NEB 3) for 1hr.
  - Plated on SD-URA.

Promoter Characterization

- 10:30am Picked colonies from pRC047 and pRC048 BsmBI plates. Seeded in LB+CAM for miniprep.
- 5pm Miniprep pRC047 and pRC048.
- 6pm Test digest pRC047 and pRC048:

Tube	Plasmid	Enzymes	Buffer	Expected
1	pRC047A	AlwNI/NcoI	NEB3	1340+934
2	pRC047B	"	"	"
3	pRC047C	"	"	"

4	pRC048A	"	"	983+934
5	pRC048B	"	"	"
6	pRC048C	"	"	"

- 6pm Masaki found clones A and B were correct for both pRC047 and pRC048.
- 7pm Sent pRC047, and pRC048 for sequencing. Use AW38 as F primer. See above.
- 7pm Ran BsaI reaction to produce:

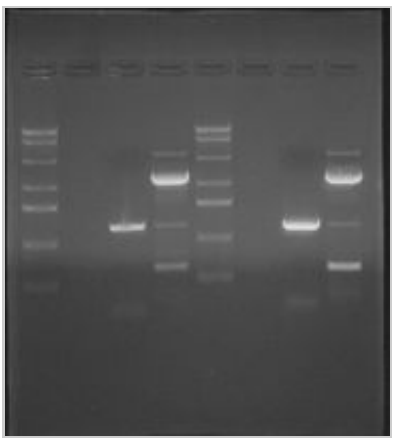
Plasmid Name	Part 1	Part 2	Part 3	Part 4	Part 5	Part 6	'
pRC049	Leu2_Int_5'	pSTE5 (weak)	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1	
pRC050	Leu2_Int_5'	pCYC1 (medium)	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1	
pRC052	Leu2_Int_5'	TDH3p	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1	

Wednesday 8/1

- 11am Checked sequencing results. All were right. Need to rerun the pTDH3-CIIC one because I didn't catch the terminator region.
- 12pm Ran PCR to retry parts for homing endonuclease and promoter characterization.

Product	Template	Primers	Temps (C)	Time	Product (bp)	'
pRC047 pcr pdt	yJD001 genome	BA28, BA29	45, 55	40sec	654	
pRC033.1	pWCD0519	AZ52, BA06	45, 55	40sec	1678	

- 3pm Gel purification:



All worked! Will use pRC033.1B for Gibson.

- 5pm Masaki ran BsmBI reactions to produce pRC047 (pSTE5 type 2 part) and pRC048 (pCYC1 type 2 part).

- 5pm Sent for sequencing:

Name	Date	Construct	Clone	Primer
sIGEM245 (rerun)	1-Aug-12	pRC042		AD41
sIGEM246 (rerun)	1-Aug-12	pRC023	A	AW38
sIGEM247 (rerun)	1-Aug-12	pRC023	B	AW38
sIGEM248 (rerun)	1-Aug-12	pRC023	C	AW38
sIGEM249 (rerun)	1-Aug-12	pRC025	A	AW38
sIGEM250 (rerun)	1-Aug-12	pRC025	B	AW38
sIGEM251 (rerun)	1-Aug-12	pRC025	C	AW38
sIGEM252	1-Aug-12	pRC037		AS24
sIGEM253	1-Aug-12	pCLC020		Z72
sIGEM254	1-Aug-12	pCLC020		AV11
sIGEM255	1-Aug-12	pCLC020		AW21
sIGEM256	1-Aug-12	pCLC020		AU72
sIGEM257	1-Aug-12	pCLC021		AX21
sIGEM258	1-Aug-12	pCLC021		AV11

- 5pm Ran Gibson reaction to produce pRC033 and pRC034. Used Harneet's excel sheet for calculating Gibson.

Part #	Part desc.	Ci (uM) enter	Vi (uL)
1	pRC033.1B	58.6	1.540528978
2	pRC033.2B	71.5	1.262587386
3	pRC033.3	95.2	0.948266787
4	pRC033.4	72.3	1.248616848
Total			5

Part #	Part desc.	Ci (uM) enter	Vi (uL)
1	pRC034.1B	53.3	0.324561404
2	pRC034.2B	3.7	4.675438596
Total			5

- 8pm Transformed Gibson products into EPI300, plated on LB+CAM.
  - Used 30ul of 10x dilution + 1ul of Gibson product.
  - Plated only 100ul of the 500ul rescue solution.
  - Saved the other 19ul of Gibson product in case I want to do a DpnI digest.
- 8pm Picked colonies from BsaI reaction plate, seeded in LB+AMP for minipreps tomorrow.
- 10pm Transformed pRC047, pRC048, and negative control reactions into TGI, plated on LB+CAM.
