Robert Notebook July 2012

From Dueber Lab Wiki

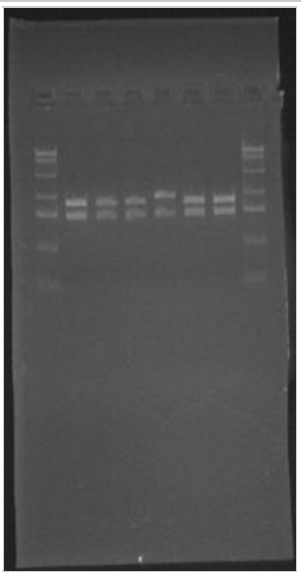
Contents

Tuesday 7/31

- 10:30am Picked three colonies each from pRC023 and pRC025 plates and seeded for minipreps. There were barely any colonies, so I'm worried about the reaction. Will re-run BsmBI tonight if that's the case.
- 4pm Imaged some of the yeast, but they all looked weird. Will send the TDH3 versions of all for sequencing.
- 5pm Miniprep.
- 6pm Test digest:

Tube	Plasmid	Enzymes	Buffer	Expected

1	pRC023A	AlwNI/NcoI	NEB3	1237+934
2	pRC023B	"	"	"
3	pRC023C	"	"	"
4	pRC025A	"	"	1216+934
5	pRC025B	"	"	"
6	pRC025C	"	"	"



All worked, but 25A is a bit iffy. Will run BsaI reaction with 23A and 25B tonight.

- 7pm Send for sequencing:

Name	Date	Construct	Clone	Primer
sIGEM238	31-Jul-12	pRC017		X45
sIGEM239	31-Jul-12	pRC017		AD41
sIGEM240	31-Jul-12	pRC028		X45
sIGEM241	31-Jul-12	pRC028		AD41
sIGEM242	31-Jul-12	pRC037		X45
sIGEM243	31-Jul-12	pRC037		AD41
sIGEM244	31-Jul-12	pRC042		X45
sIGEM245	31-Jul-12	pRC042		AD41
sIGEM246	31-Jul-12	pRC023	A	AW38
sIGEM247	31-Jul-12	pRC023	B	AW38
sIGEM248	31-Jul-12	pRC023	C	AW38
sIGEM249	31-Jul-12	pRC025	A	AW38
sIGEM250	31-Jul-12	pRC025	B	AW38
sIGEM251	31-Jul-12	pRC025	C	AW38

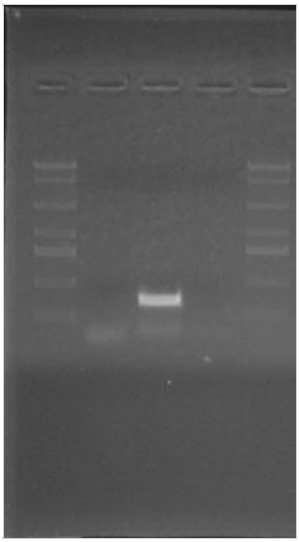
- 7pm Ran BsaI reaction to produce genomic landing pad cassette, pRC027. Plated on LB+AMP.

1. pRC022
2. pRC023
3. pRC024
4. pRC025
5. pRC026
6. pWCD0515

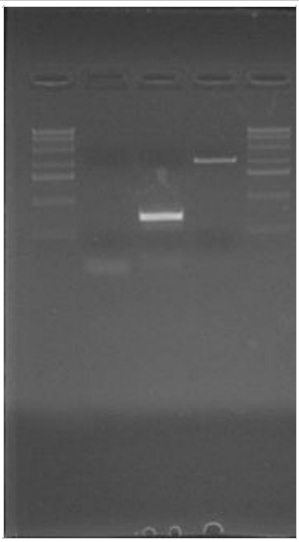
- 6pm/7pm Masaki and I ran PCR reactions to produce:

Product	Template	Primers	Temps (C)	Time	Product (bp)
pRC047	pcr pdt yJD001 genome	BA28, BA29	50, 60	30sec	654
pRC048	pcr pdt yJD001 genome	BA30, BA31	50, 60	30sec	297
pRC051	pcr pdt yJD001 genome	BA32, BA33	50, 60	30sec	1521
pRC033.1	pWCD0519	AZ52, BA06	50, 60	40sec	1678
pRC033.2	sWCD006	AZ54, AZ55	50, 60	40sec	506
pRC034.1	pWCD0558	BA07, AZ61	50, 60	40sec	1698
pRC034.2	sWCD007	BA08, BA09	50, 60	40sec	532

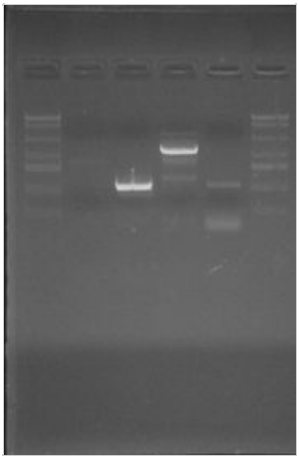
- 8pm Gel purification.
  - Forgot to add isopropanol to the pRC028 PCR pdt! Might have to redo.



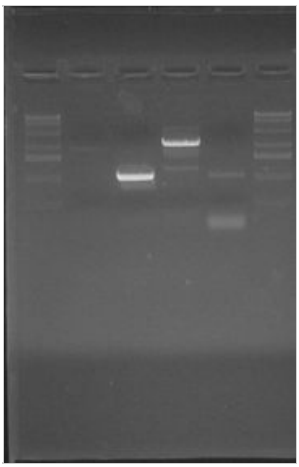
Only pRC048's 297bp product PCRed correctly at 50C. Will gel purify.



PCR done at 60C. pRC048 and pRC051 PCR products worked.



PCR 50C. All except pRC033.1 worked. pRC034.2 was dim, so will use extra.



PCR 60C. Will retry the PCR and check tomorrow.

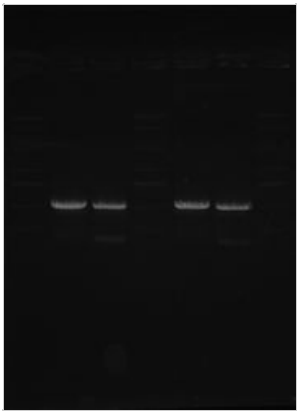
- 1am After the 1hr recovery shake, there was a clump because it didn't shake hard enough. Also, there were too many cells, so in the future, I'll do the following for electroporation:
  - Use 30ul of cells at 10x dilution.
  - Plate 100ul of the 500ul that was used for the recovery.

Monday 7/30

- 12pm Diluted yeast to 0.2OD.
- 12pm Made yeast glycerol stocks for yRC020, 21, 27, and 28.
- 4pm Image yeast plates.
- 2pm Got gBlocks! Add 20ul of ddH2O, and use 0.5ul for PCR template.
- 5pm Ran PCR reactions:

Product	Template	Primers	45	50	60	Time	Product (bp)
pRC023	pcr pdt	sWCD006	AZ42, AZ43		x	x	30sec	500
pRC025	pcr pdt	sWCD007	AZ46, AZ47		x	x	30sec	529

- 6pm Gel purified 500bp PCR products:



Lanes: 1)pRC023A @50C, 2)pRC025A @50C, 3)pRC023B @60C, 4)pRC025B @60C. Used pRC023A and pRC025A for the BsmBI reaction into pWCD0514.

- 7pm Ran BsmBI reactions. Will transform into bacteria and plate on LB+CAM.

Sunday 7/29

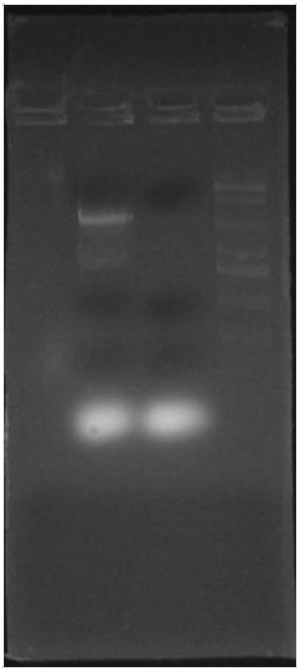
- Seeded yeast cultures into a 24-well block:

	1	2	3	4	5	6
A	yRC022	yRC023	-	-	-	yRC020
B	yRC025	yRC026 (new)	yRC026 (old)	-	-	yRC021
C	yRC030	yRC031	yRC032	yRC033	-	yRC027
D	yRC035	yRC036	yRC037	yRC038	-	yRC028

Friday 7/27

- Took yRC025 and yRC026 (7/24) out of incubator.
- Need microscope to check yeast that has already been grown.
- Waiting on homing endonuclease oligos to arrive.
- After eluting from the Zymo column, left pRC035.1 overnight in the centrifuge. Water was all evaporated in the morning. Added 10ul of ddH2O to tube. Took 1ul and ran on gel to test:

1. pRC035.1 PCR done at 45C. Will gel purify 3263bp fragment to get a spare tube.
2. pRC035.1 (from 7/25) that evaporated.
3. MW.



Lane 1 is correct size, lane 2 is faint but still there. Will use what I purified today for future Gibson reactions.

- 1pm Gel purified PCR from lane 1.
- Put in order for oligos for PCRing off the promoters (for gold medal characterization) from the genome. These I will make into type 2 parts. The pADH1 (strong) has BsmBI and BsaI sites, so I'll have to use traditional restriction enzyme cloning to insert it into the vector. I'll first clone a cassette with pSTE5 for example, then cut the vector and insert BglII/SpeI, gel purify the vector backbone fragment, then ligate the insert in.

Full Name	Sequence	Purpose	Length
BA28.iGEM156	gcatCGTCTCcTCGGTCTCcTCTAgacacgaagtgactgacaga	pSTE5 (weak) F	44
BA29.iGEM157	atgcCGTCTCaGGTCTCaCATAgatctttaaaagttgtttccgct	pSTE5 (weak) r	45
BA30.iGEM158	gcatCGTCTCcTCGGTCTCcTCTAgacagatccgccaggcgtgt	pCYC1 (medium) F	44
BA31.iGEM159	atgcCGTCTCaGGTCTCaCATAgatcttattaatttagtgtgtgtatttgtgt	pCYC1 (medium) R	53
BA32.iGEM160	gcatACTAGTttaaaacaagaagagggttga	pADH1 (strong) F	31
BA33.iGEM161	atgcAGATCTtgtatatgagatagttgattgtatgc	pADH1 (strong) R	37

Thursday 7/26

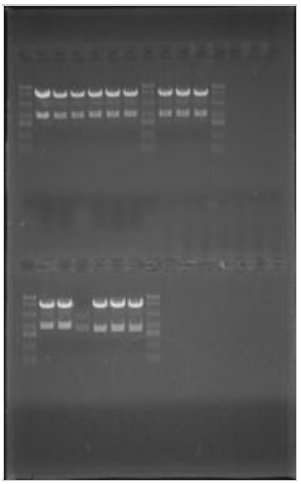
- ~~11am Dilute yRC031 to 0.2 OD.~~ Will actually dump the culture and reseed once the microscope is available again.

BsaI Plates and Yeast Integration

- 10:30am Seeded CIIC colonies into 3ml LB+AMP.
- 3:30pm Diluted yeast to 0.2OD.
- 4:30pm Miniprep.
- 5pm Test digest:

Tube	Plasmid	Enzymes	Buffer	Expected
1A	pRC037A	XhoI/XbaI	NEB 4	4903+1440
2	pRC037B	"	"	"

3	pRC037C	"	"	
4	pRC038A	"	"	4903+1460
5	pRC038B	"	"	"
6	pRC038C	"	"	"
7	pRC039A	"	"	4903+1460
8	pRC039B	"	"	"
1B	pRC039C	"	"	"
2	pRC040A	"	"	4903+1460
3	pRC040B	"	"	"
4	pRC040C	"	"	"
5	pRC041A	"	"	4903+1293
6	pRC041B	"	"	"
7	pRC041C	"	"	"



All except 40C look good. Used A plasmids for integration.

- 9pm Did yeast integrations to produce yRC030, yRC031, yRC032, yRC033, yRC034.

Homing Endonuclease

- Re-ordered some primers because we reverted back to old ConS and ConE regions after Will got those to work in multigene by using electroporation.

Full Name	Sequence	Purpose	Length
BA06.iGEM146	GAATgGAGACGtGAATgG	400bp 5'homo + KanMX R1 (replaces AZ53)	18
BA07.iGEM147	cACTGaGAGACGcACTGt	400bp 3'homo F1 (replaces AZ60)	18
BA08.iGEM148	ttttattggtagtcGGTCTCcAGTGccagactagagaatcgccg	400bp 3'homo F2 (replaces AZ62)	18
BA09.iGEM149	ctGGTCTCaCAGTgCGTCTCtCAGTgcatccgagtggcgatcac	400bp 3'homo R2 (replaces AZ63)	44

- Will need to remake pRC033.1 (pcr pdt 1) and pRC034.1 (pcr pdt 1) using the new oligos once they arrive.

Wednesday 7/25

- 1pm Miniprepped pTC005. Concentration was 238.9 ng/uL.

BsaI Plates and Yeast Integrations

- Pick colonies from restreaked plate. Run colony PCR, using AS23 as F and AS24 as R primers.

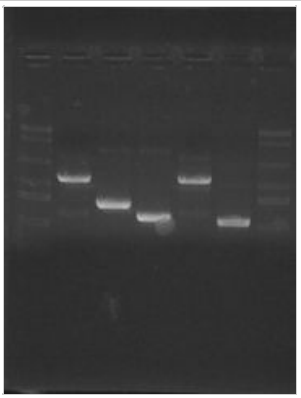
Tube	Plasmid	Condition	Band
1A	pRC042A	3x vol, electro	4829
2	pRC042B	3x vol, electro	4829
3	pRC042C	3x vol, electro	4829
4	pRC043A	3x vol, electro	4849
5	pRC043B	3x vol, electro	4849
6	pRC043C	3x vol, electro	4849
7	pRC044A	3x vol, electro	4849
8	pRC044B	3x vol, electro	4849
1B	pRC044C	3x vol, electro	4849
2	pRC045A	3x vol, electro	4849
3	pRC045B	3x vol, electro	4849
4	pRC045C	3x vol, electro	4849
5	pRC046A	3x vol, electro	4849
6	pRC046B	3x vol, electro	4849
7	pRC046C	3x vol, electro	4849
8	pRC046A	1x vol, electro	4849
1C	pRC046B	1x vol, electro	4849
2	pRC046C	1x vol, electro	4849

- 4pm Masaki redid BsaI program for CIIC plasmids, heat shocked into TGI, then plated LB+AMP. Will pick tomorrow morning.

Homing Endonuclease

- Gel purified PCR products:

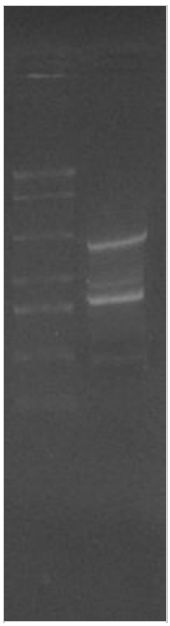
Product	Product (bp)
pRC033.1	1697
pRC033.3	850
pRC033.4	624
pRC034.1	1698
pRC035.2	559



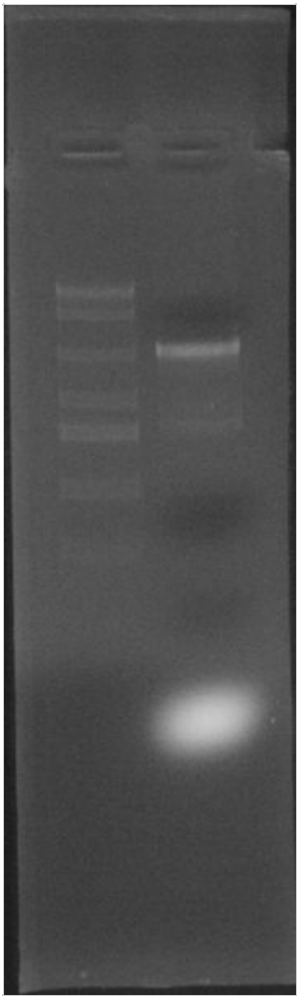
Perfect!

- Run pRC035.1 PCR reaction:

Product	Template	Primers	45	50	60	Time	Product (bp)
pRC035.1	pWCD0563	AZ64, AZ65	x	x		60sec	3263



Realized that I used pWCD0533 instead of pWCD0563 as the template. Will redo right now.



pRC035.1 redone correctly.
Cut out 3200bp band.

Tuesday 7/24

- 12pm Miniprepped pVY006, pTC005, pAJ006, pMRY030. ODs of new plasmid stocks:

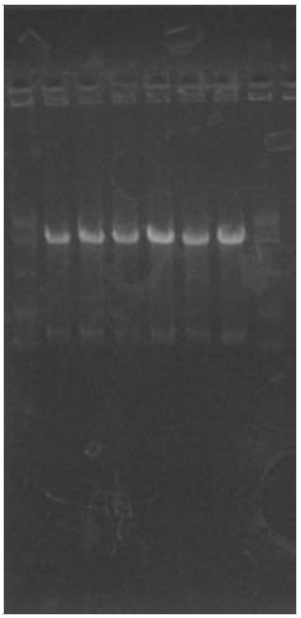
Plasmid	Description	Part	ng/ul
pVY006	ZRC1	3a	265.8
pTC005	ABP1	3a	bad curve
pAJ006	CIIC	4	136.9
pMRY030	HTA2	3a	141.6

BsaI Plates and Yeast Integrations

- 10:30am All the electroporations created lawns.
  - In future, dilute the 100ul tube of cells 10x with 10% glycerol to make 1ml total.
  - Restreaked the plate to get individual colonies.
  - Tonight, will pick colonies to colony PCR and miniprep.
- 11am Picked 3 colonies each from pRC044 and pRC046 (3x volume, heat shock). All other plates had ~5 colonies each.
- 5pm Miniprepped pRC044 and pRC046.
- 6pm Ran BsaI reaction to redo pRC037-pRC041.

- 6pm Test digest:

Tube	Plasmid	Enzymes	Buffer	Expected
1	pRC044A	BglII/XhoI	NEB 3	5609+1068+60
2	pRC044B	"	"	"
3	pRC044C	"	"	5442+1068+60
4	pRC046A	"	"	"
5	pRC046B	"	"	"
6	pRC046C	"	"	"



I think they all worked. Used 44A and 46A.

- 8pm Integrated into yeast:

Tube	Name	Description	Plasmids Used	Parental Strain	Marker	Color
1	yRC022	pRNR2-ZRC1	pRC019	yJD001	LEU	Venus
2	yRC023	pREV1-ZRC1	pRC020	"	"	"
3	yRC025	pTDH3-ABP1	pRC028	"	"	"
4	yRC026	pTEF1-ABP1	pRC029	"	"	"
5	yRC037	pRNR2-HTA2	pRC044	"	"	"
6	yRC039	pGal-HTA2	pRC046	"	"	"

Homing Endonuclease

- Sequencing confirmed everything good. Will use pRC022B, pRC024C, and pRC026A as the plasmids.
- 10pm Ran these PCR reactions (3 blocks total):

Product	Template	Primers	45	50	60	Time	Product (bp)
pRC033.1	pWCD0639	AZ52, AZ53	x	x		30sec	1697
pRC033.3	pML281	AZ56, AZ57	x	x		30sec	850
pRC033.4	pML281	AZ58, AZ59		x	x	30sec	624
pRC034.1	pWCD0650	AZ60, AZ61	x	x		30sec	1698
pRC035.2	pWCD0533	AZ66, AZ67	x	x		30sec	559

Monday 7/23

- For some reason, BsaI reactions barely worked again. Will test out conditions:
  - Maybe because part 3a concentrations are low in comparison to backbone, it doesn't get assembled correctly?
  - Maybe heat shocking the TGI will make them prefer smaller plasmids?
- Summary concentrations of all the plasmids used in the cassette BsaI assemblies. Concentration of part 3a's is low, so might miniprep more.

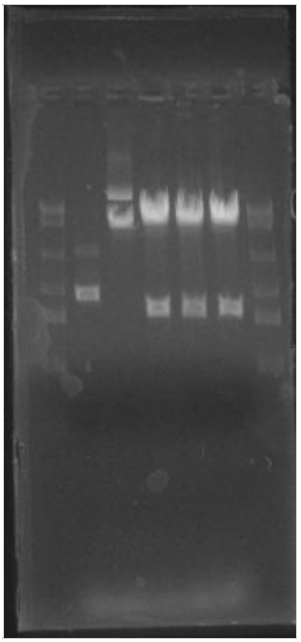
Plasmid	Description	Part	ng/ul
pVY006	ZRC1 (VM)	3a	117.8
pTC005	ABP1 (Actin)	3a	176.4
pAJ006	CIIC (CP)	4	119.0
pMRY030	HTA2 (Nucleus)	3a	85.7
pWCD0524	Leu 5' Int	1	308.9
pWCD0543	Venus	3b	268.4
pWCD0552	ADH1	4	213.9
pWCD0559	Leu 3' Int	5	232.0
pWCD0515	AmpR ColE1	6	174.4
pWCD0528	TDH3	2	302.4
pWCD0529	TEF1	2	282.2
pWCD0531	RNR2	2	266.2
pWCD0532	REV1	2	271.5
pWCD0533	pGal	2	156.2

- 9pm Electroporated to transform pCC020 and pCC021.

Yeast integrations

- 10:30am Picked colonies for miniprep.
- 4pm Diluted yeast to 0.2 OD.
- 5pm Miniprep. Test digest. pRC044 was red.

Tube	Plasmid	Enzymes	Buffer	Expected
1	pRC042A	BglII/XhoI	NEB 3	5589+1068+60
2	pRC042B	"	"	"
3	pRC043A	"	"	5609+1068+60
4	pRC043B	"	"	"
5	pRC045	"	"	"



Integrated with 42B (iffy), 43A, and 45.

- 6pm Linearize with BsmBI.
- 8pm Do yeast integration.

BsaI Reaction Re-Do

- 12pm Retrying BsaI reaction with triple part 3a volume:

Ingredient	Volume (ul)
ddH2O	3.5
T4 Ligase buffer	1
T4 Ligase	0.5
BsaI	0.5
pWCD0524	0.5
pWCD0543	0.5
pWCD0552	0.5
pWCD0559	0.5
pWCD0515	0.5
pMRY030	1.5
total	9.5
part 2	0.5

Tube	Product
1	pRC042
2	pRC043
3	pRC044
4	pRC045
5	pRC046
6	pRC042 (old conc, as control)

- 4pm With these BsaI products:

    - Take 1ul and do electroporation.
    - With remaining 9ul, do normal heat shock.
- In summary, will have 4 conditions. In heat shock, we think TGI selectively take up smaller (wrong) plasmids.

1. Normal BsaI recipe, heat shock (what I've been doing).
2. Normal BsaI recipe, electroporation.
3. Equal conc. triple part 3a volume, heat shock.
4. Equal conc. triple part 3a volume, electroporation.

Yeast Imaging

- 11am Diluted yeast into 5ml of SD-LEU to ~0.2OD. Will image after meeting.
- 4:30pm Take out yeast. Put on slides using these conditions:

1. 1. Normal spin-down prep.
   2. Take 4ul straight from culture.
   3. Add EDTA.
   4. Fixing to the slide.
   5. Passing through filter or screen.

Robert.c 22:49, 24 July 2012 (PDT)

Sunday 7/22

- 4pm Took out pRC022, 024, and 026 LB+CAM plate restreaks.
- 4pm Ran these BsaI reactions, transformed into TGI, plated on LB+CAM.

Plasmid Name	Part 1	Part 2	Part 3a	Part 3b	Part 4	Part 5	Part 6
pRC042	Leu2_Int_5'	TDH3p	HTA2 (Nucleus)	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1
pRC043	Leu2_Int_5'	TEF1p	HTA2 (Nucleus)	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1
pRC044	Leu2_Int_5'	RNR2p	HTA2 (Nucleus)	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1
pRC045	Leu2_Int_5'	REV1p	HTA2 (Nucleus)	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1
pRC046	Leu2_Int_5'	Gal1p	HTA2 (Nucleus)	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1

- 4pm Took out the yeast plates that grew enough.
- 4pm Seeded the yeast that grew.

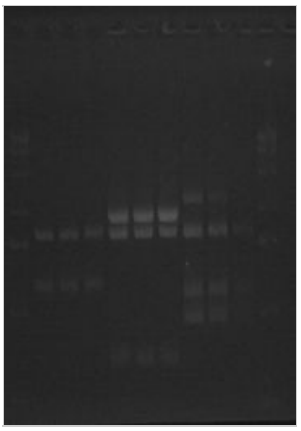
Robert.c 22:49, 24 July 2012 (PDT)

Saturday 7/21

- 10am Took out BsmBI plates.
- 10am Seeded BsmBI reactions (pRC024, pRC026, pRC022) into 3ml of LB+Cam for miniprep.
- 5pm Miniprepped plasmids.
- 5pm Test digested:

Tube	Plasmid	Enzymes	Buffer	Expected
1A	pRC022A	SacI/AlwNI	NEB 4	1089+637
2	pRC022B	"	"	"
3	pRC022C	"	"	"
4	pRC024A	"	"	1326+1089+312

5	pRC024B	"	"	
6	pRC024C	"	"	
7	pRC026A	"	"	1089+600+467
8	pRC026B	"	"	
lone	pRC026C	"	"	



Everything looks like it worked.

- 7pm Sent for sequencing. Think I missed it, and they'll pick up on Monday morning :-(

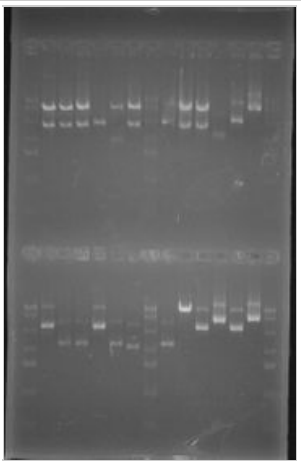
Name	Date	Construct	Clone	Primer
sIGEM212	12-Jul-12	pRC014	1	AA05
sIGEM213	21-Jul-12	pRC022	A	AW38
sIGEM214	21-Jul-12	pRC022	B	AW38
sIGEM215	21-Jul-12	pRC022	C	AW38
sIGEM216	21-Jul-12	pRC024	A	AW38
sIGEM217	21-Jul-12	pRC024	A	AW39
sIGEM218	21-Jul-12	pRC024	B	AW38
sIGEM219	21-Jul-12	pRC024	B	AW39
sIGEM220	21-Jul-12	pRC024	C	AW38
sIGEM221	21-Jul-12	pRC024	C	AW39
sIGEM222	21-Jul-12	pRC026	A	AW38
sIGEM223	21-Jul-12	pRC026	B	AW38
sIGEM224	21-Jul-12	pRC026	C	AW38

Robert.c 19:18, 21 July 2012 (PDT)

Friday 7/20

- 10am Picked colonies from BsaI reactions for miniprep. Put 2.5ml per well in 24-well block.
- 10am Transformed BsmBI reactions (pRC024, pRC026) onto LB+Cam plates.
- 12pm Ran PNK Treat on AZ40 and AZ41 to produce pRC022 "PCR product." Tube labeled RC22.
- 12pm Minipreped pWCD0610. Concentration 157.9 ng/uL.
- 3pm Insert pRC022 PCR product into pWCD0514 with BsmBI short program.
- 3pm Measured pWCD0514 I've been using at 48.5 ng/uL, which is very low. Picking colony from Will's plate, restreaking for myself, and seeding in 5ml of LB+Cam to miniprep tomorrow.
- 4pm Seed yeast to 30ml of 0.2 OD.
- 5pm Transform BsmBI reaction product into TGI, plate on LB+Cam.
- 5pm Miniprep BsaI reactions from block.
- 6pm Test digests:

Tube	Plasmid	Enzymes	Buffer	Expected
1A	pRC028A	BglII/XhoI	NEB 3	5589+2470+38
2	pRC028B	"	"	"
3	pRC028C	"	"	"
4	pRC029A	"	"	5609+2470+38
5	pRC029B	"	"	"
6	pRC029C	"	"	"
7	pRC030A	"	"	5609+2470+38
8	pRC030B	"	"	"
1B	pRC030C	"	"	"
2	pRC037A	"	"	5589+754
3	pRC037B	"	"	"
4	pRC037C	"	"	"
5	pRC038A	"	"	5609+754
6	pRC038B	"	"	"
7	pRC038C	"	"	"
8	pRC039A	"	"	5609+754
1C	pRC039B	"	"	"
2	pRC039C	"	"	"
3	pRC040A	"	"	5609+754
4	pRC040B	"	"	"
5	pRC040C	"	"	"
6	pRC041A	"	"	5442+754
7	pRC041B	"	"	"
8	pRC041C	"	"	"



Picked pRC028A, pRC029C, pRC030B, pRC037C, pRC038A, pRC039A, pRC040C, pRC041A; pRC037A, pRC038B, pRC039B, and pRC040A.

- Don't know what happened for for the CIIC constructs, so picked one of each "type" of product I saw: 8000+4000bp and 5000+2000bp. Don't have any clue how those are formed, so will redo the BsaI reaction Sunday probably.

Robert.c 19:18, 21 July 2012 (PDT)

Thursday 7/19

- 9:30am Picked colonies from pRC018, pRC031, pRC032. Other reactions failed.
- 11am Took ODs. Put new pWCD0560 into iGEM -20 GG box.

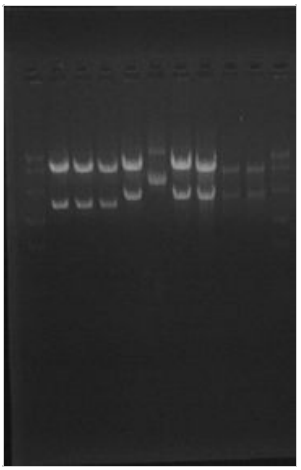
Plasmid	Description	Part	ng/ul
pWCD0560	Ura3_Int-3'	1	202.1
pVY006	ZRC1 (VM)	3a	117.8
pTC005	ABP1 (Actin)	3a	176.4
pAJ006	CIIC	4	119.0

- Ran more BsaI reactions:

PCR Tube	Plasmid Name	Part 1	Part 2	Part 3a	Part 3b	Part 4	Part 5	Part 6
1	pRC028	Leu2_Int_5'	TDH3p	ABP1_Actin	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1
2	pRC029	Leu2_Int_5'	TEF1p	ABP1_Actin	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1
3	pRC030	Leu2_Int_5'	RNR2p	ABP1_Actin	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1
4	pRC037	Leu2_Int_5'	TDH3p	Venus		CIIC	Leu2_Int-3'	AmpR_ColE1
5	pRC038	Leu2_Int_5'	TEF1p	Venus		CIIC	Leu2_Int-3'	AmpR_ColE1
6	pRC039	Leu2_Int_5'	RNR2p	Venus		CIIC	Leu2_Int-3'	AmpR_ColE1
7	pRC040	Leu2_Int_5'	REV1p	Venus		CIIC	Leu2_Int-3'	AmpR_ColE1
8	pRC041	Leu2_Int_5'	Gal1p	Venus		CIIC	Leu2_Int-3'	AmpR_ColE1

- 2pm Seeded 10ml of yJD001 to 0.2 OD.
- 3pm Miniprep cultures. Test digest:

Tube #	Plasmid	Enzymes	Buffer	Expected
1	pRC018A	BglII/XhoI	NEB 3	5609+2058
2	pRC018B	"	"	"
3	pRC018C	"	"	"
4	pRC031A	"	"	5609+2470+38
5	pRC031B	"	"	"
6	pRC031C	"	"	"
7	pRC032A	"	"	5442+2470+38
8	pRC032B	"	"	"
lone	pRC032C	"	"	"

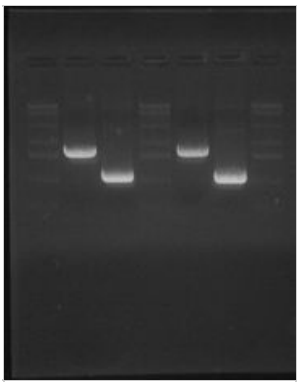


Chose 18A, 31A, 32A to linearize.

- 7pm Did yeast integration to produce

- 7pm Transformed BsaI reactions into TG1. Plated on LB+Amp.
- 6pm Ran these Phusion PCR reactions (30sec anneal):

Tube	Product	Parts Included
1	pRC024 1107bp PCR pdt (55C)	AZ44, AZ45, pWCD0526
2	pRC026 536bp PCR pdt (55C)	AZ48, AZ49, pWCD0559
3	pRC024 1107bp PCR pdt (62C)	AZ44, AZ45, pWCD0526
4	pRC026 536bp PCR pdt (62C)	AZ48, AZ49, pWCD0559



All are good! Cut them out and elute separately, just to have a backup gel purification.

- 9pm Ran BsmBI protocol with PCR products 3 and 4 (1 and 2 labels rubbed off slightly, so there's uncertainty which one is which) and pWCD0514 for bright band. Tubes are labeled RC24 and RC26.
- 9pm Seeded pWCD0610 in 5ml of LB+Kan.

Robert.c 11:27, 20 July 2012 (PDT)

Wednesday 7/18

- 11am None of the BsaI reactions worked (plates completely red). Redid reactions.
- 5pm Plated on LB+Amp. Pick 3 colonies each tomorrow and seed for minipreps.
- 5pm Reseeded pWCD0560 into 5ml of LB+Cam. Miniprep tomorrow.

Robert.c 19:14, 19 July 2012 (PDT)

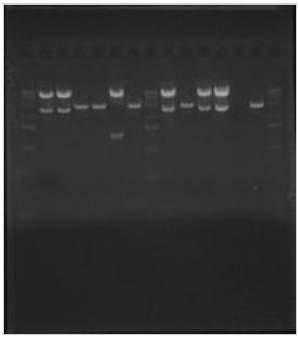
Tuesday 7/17

- 12:30pm Picked 3 colonies from each. pRC018 had pretty much only red colonies.
- 1pm Did BsaI reactions for pTC005, ABP1 (Actin) and redid pRC018.

Tube	Plasmid Name	Part 1	Part 2	Part 3a	Part 3b	Part 4	Part 5	Part 6
1	pRC018	Leu2_Int_5'	TEF1p	ZRC1 (VM)	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1
2	pRC028	Leu2_Int_5'	TDH3p	ABP1_Actin	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1
3	pRC029	Leu2_Int_5'	TEF1p	ABP1_Actin	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1
4	pRC030	Leu2_Int_5'	RNR2p	ABP1_Actin	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1
5	pRC031	Leu2_Int_5'	REV1p	ABP1_Actin	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1
6	pRC032	Leu2_Int_5'	Gal1p	ABP1_Actin	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1

- 5pm Transformed BsaI reactions into TG1, plated on Amp.
- 5pm Diluted yeast to 0.2 OD.
- 7pm Did minipreps.
- 8pm Test digested:

Tube #	Plasmid	Enzymes	Buffer	Expected
1	pRC017A	BglII/XhoI	NEB 3	5589+2058
2	pRC017B	"	"	"
3	pRC017C	"	"	"
4	pRC019A	"	"	5609+2058
6	pRC019B	"	"	"
6	pRC019C	"	"	"
7	pRC020A	"	"	5609+2058
8	pRC020B	"	"	"
1	pRC020C	"	"	"
2	pRC021A	"	"	5442+2058
3	pRC021B	"	"	"
4	pRC021C	"	"	"



pRC017A+B, pRC019B, pRC020A+C, and pRC021A worked.

- 11pm Yeast integration:

Strain Name	Description	Parental Strain	Plasmids Used	Marker
yRC021	pTEF1-ZRC1(VM)-Venus	yJD001	pRC018	LEU
yRC022	pRNR2-ZRC1(VM)-Venus	yJD001	pRC019	LEU
yRC023	pREV1-ZRC1(VM)-Venus	yJD001	pRC020	LEU
yRC024	pGal-ZRC1(VM)-Venus	yJD001	pRC021	LEU

- 11pm Poured more SD-Leu plates for these integrations.

Robert.c 11:26, 19 July 2012 (PDT)

Monday 7/16

- 2pm Meeting:
  - Decided that Nucleolus: NOP56, NP: NIC96, and VM: ZRC1 are the best.
  - Won't do promoter library stuff with Nucleolus and NP.
- 5pm Start BsaI for reactions:

Plasmid Name	Part 1	Part 2	Part 3a	Part 3b	Part 4	Part 5	Part 6
pRC017	Leu2_Int_5'	TDH3p	ZRC1 (VM)	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1

pRC018	Leu2_Int_5'	TEF1p	ZRC1 (VM)	Venus	ADH1	Leu2_Int-3' AmpR_ColE1
pRC019	Leu2_Int_5'	RNR2p	ZRC1 (VM)	Venus	ADH1	Leu2_Int-3' AmpR_ColE1
pRC020	Leu2_Int_5'	REV1p	ZRC1 (VM)	Venus	ADH1	Leu2_Int-3' AmpR_ColE1
pRC021	Leu2_Int_5'	Gal1p	ZRC1 (VM)	Venus	ADH1	Leu2_Int-3' AmpR_ColE1

- Mastermix:

Plasmid	Description
pWCD0515	AmpR/ColEI
pWCD0524	Leu 5' Int
pWCD0543	Venus
pWCD0552	ADH1
pWCD0559	Leu 3' Int
pVY006	ZRC1 (VM)

- 5pm Grew up more pWCD0560 for miniprepping tomorrow.
- 11pm Plated. Will pick at ~9am tomorrow.
- 11pm Transformed and plated pWCD0610.

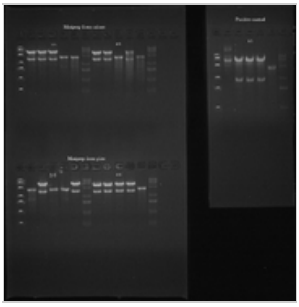
Robert.c 11:26, 19 July 2012 (PDT)

Sunday 7/15

- 1pm Miniprep cultures.
- 2pm Test digest:

Tube #	Plasmid	Enzymes	Buffer	Expected
A1	pRC013A old	BglII/XhoI	NEB 3	5609+2178
A2	pRC013B old	"	"	"
A3	pRC013C old	"	"	"
A4	pRC013D old	"	"	"
A5	pRC013E old	"	"	"
A6	pRC014A old	"	"	4897+2160
A7	pRC014B old	"	"	"
A8	pRC014C old	"	"	"
B1	pRC014D old	"	"	"
B2	pRC014E old	"	"	"
B3	pRC013A new	"	"	5609+2178
B4	pRC013B new	"	"	"
B5	pRC013C new	"	"	"
B6	pRC013D new	"	"	"
B7	pRC013E new	"	"	"
B8	pRC014A new	"	"	4897+2160
C1	pRC014B new	"	"	"
C2	pRC014C new	"	"	"
C3	pRC014D new	"	"	"
C4	pRC014E new	"	"	"
C5	control A	"	"	5609+2244

C6	control B	"	"	"
C7	control C	"	"	"
C8	control D	"	"	"
lone	control E	"	"	"



It seems that picking from culture and from plate makes no difference, and that my minipreps are equivalent to Will's.

- 3pm Pick Vincent's yeast plates.

Robert.c 17:32, 16 July 2012 (PDT)

Saturday 7/14

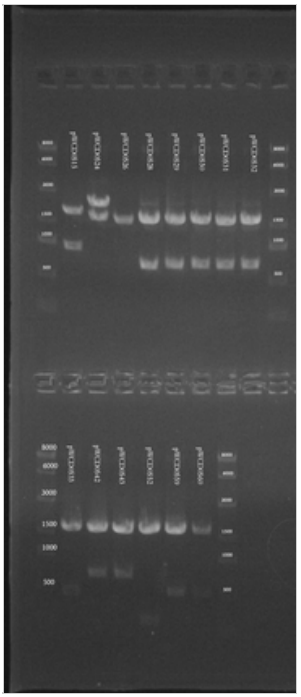
- Pick colonies.

Friday 7/13

- 10am Miniprepped pWCD plasmids.
- 11am Ran BsaI test digest on new plate minipreps.
- Summary of all the plasmids taken from plate. pWCD0560 will be redone because OD graph looked bad.

Tube #	Plasmid	Description	Part	OD (ngl/uL)	Enzymes	Buffer	Expected
1	pWCD0515 (old)	AmpR/ColEI	6	174.4	BsaI	NEB 3	1851+901
2	pWCD0524	Leu 5' Int	1	308.9	"	"	2314+1650
3	pWCD0526 (old)	Ura 5' Int5	1	143.6	"	"	1650+1612
4	pWCD0528	TDH3	2	302.4	"	"	1650+703
5	pWCC0529	TEF1	2	282.2	"	"	1650+723
6	pWCD0530	RPL18B	2	274.7	"	"	1650+723
7	pWCD0531	RNR2	2	266.2	"	"	1650+723
8	pWCD0532	REV1	2	271.5	"	"	1650+723
1	pWCD0533	pGal	2	156.2	"	"	1650+492
2	pWCD0542	mKate	3b	282.8	"	"	1650+712
3	pWCD0543	Venus	3b	268.4	"	"	1650+730
4	pWCD0552	ADH1	4	213.9	"	"	1650+253
5	pWCD0559	Leu 3' Int	5	232	"	"	1650+520

6	pWCD0560	Ura 3' Int	5	149.0 *	"	"	1650+510



All were correct.

- 11am Ran BsaI reaction comparing minipreps from culture and minipreps from plate.
- 3pm Looked at yRC016 and yRC017 again:
  - Second colonies from both had no color when imaged.
  - Could be due to bad folding.
- 4pm Transformed BsaI reaction into TG1 and plated on LB+Amp. Tomorrow, will pick 5 per plate, grow up, then run test digest.

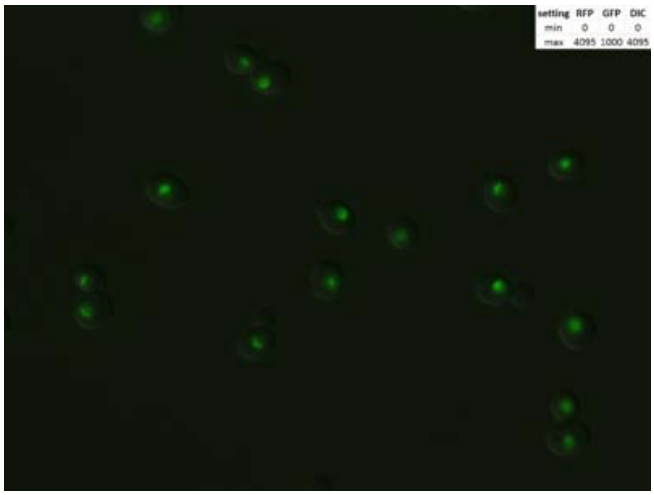
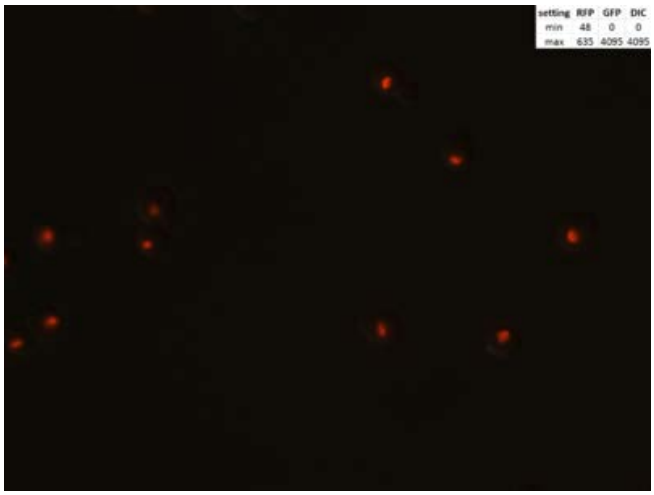
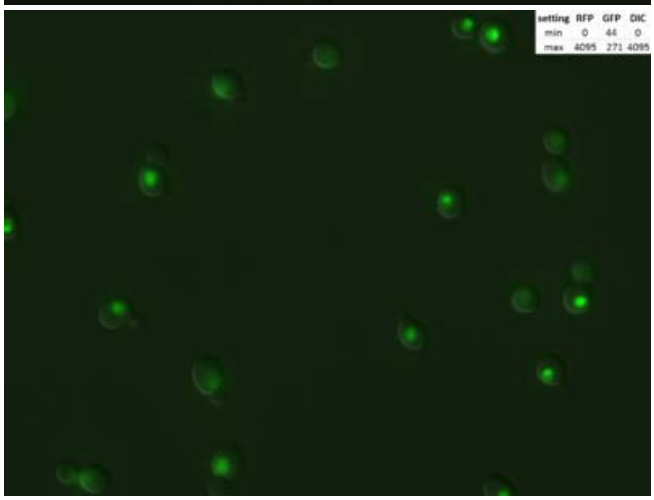
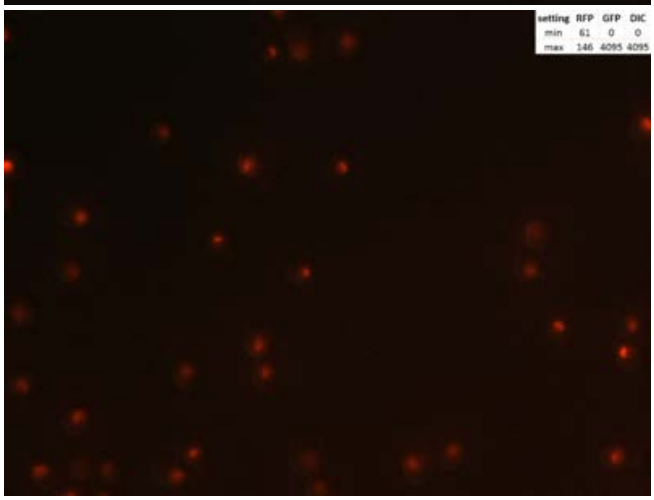
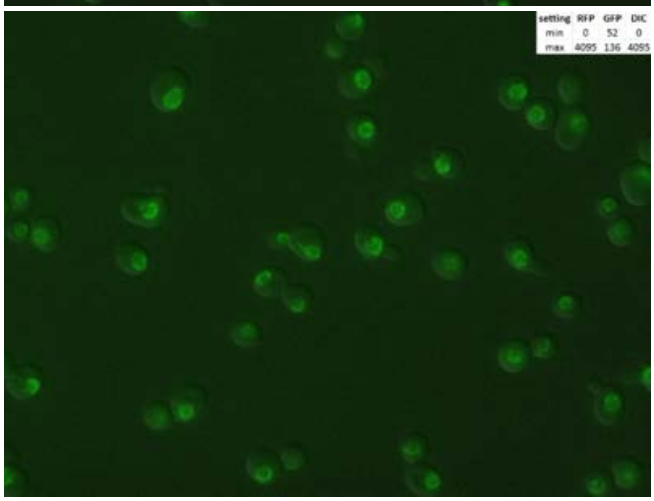
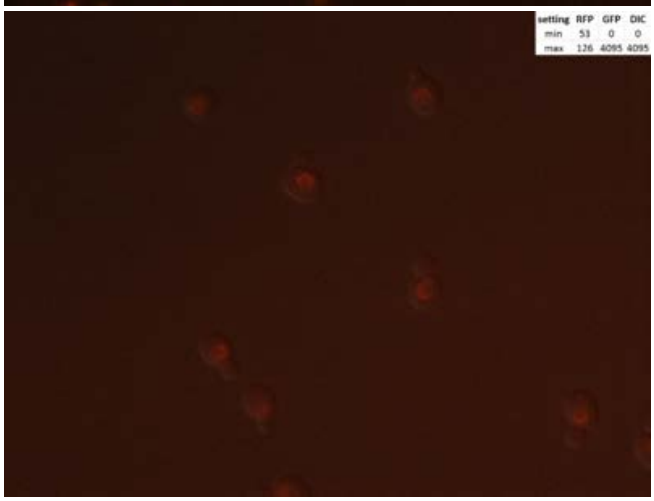
Robert.c 21:43, 13 July 2012 (PDT)

Thursday 7/12

- 10am Diluted yRC.
- 11am Miniprepped the pWCD I seeded. Will test digest with the rest tomorrow

Plasmid	Description	Part	OD
pWCD0524	Leu 5' Int	1	308.9
pWCD0528	TDH3	2	302.4
pWCD0529	TEF1	2	282.2
pWCD0530	RPL18B	2	274.7
pWCD0531	RNR2	2	266.2
pWCD0532	REV1	2	271.5
pWCD0533	pGal	2	156.2
pWCD0542	mKate	3b	282.8
pWCD0543	Venus	3b	268.4

- 5pm Seeded other yRC cultures for glycerol stock. Froze saturated yRC cultures from today.

strain/condition	Venus	mKate
NOP56 Nucleolus		
BFR2 Nucleolus		
NIC96 NP		

- 6pm Because neither DBP5 yeast worked, I sent parent plasmids (pRC013, pRC014) for sequencing:

Name	Date	Construct	Clone	Primer
sIGEM209	12-Jul-12	pRC013	1	S16
sIGEM210	12-Jul-12	pRC013	1	AD41
sIGEM211	12-Jul-12	pRC014	1	S16
sIGEM212	12-Jul-12	pRC014	1	AA05

Robert.c 18:57, 12 July 2012 (PDT)

Wednesday 7/11

- 12pm Picked colonies from pWCD plates.
- 12pm Transformed pWCD0552, pWCD0559, and pWCD0560 onto LB+CAM plates.
- 7pm Picked colonies from my yeast plates to look at midlog tomorrow. Left Leu plates in longer, but put Ura in fridge.

Plate	Marker	Parents	Description
yRC012	Leu	pRC009 in yJD001	NOP56 nuco -Venus
yRC016	Leu	pRC013 in yJD001	DBP5 np -Venus
yRC017	Ura	pRC014 in yJD001	DBP5 np -mKate
yRC019	Ura	pRC016 in yJD001	NIC96 np -mKate

Robert.c 18:34, 11 July 2012 (PDT)

Tuesday 7/10

- 11am Diluted yRC018, to be visualized while at midlog.
- 4pm Made more SD-Leu and SD-Leu-Ura media that was contaminated.
- 4pm Transformed into bacteria and plated:

Tube #	Plasmid	Description	Part
1	pWCD0524	Leu 5' Int	1
2	pWCD0528	TDH3	2
3	pWCC0529	TEF1	2
4	pWCD0530	RPL18B	2
5	pWCD0531	RNR2	2
6	pWCD0532	REV1	2
7	pWCD0533	pGal	2
8	pWCD0542	mKate	3b
9 (lone)	pWCD0543	Venus	3b

- Need to do pWCD0552, 0559, 0560 tomorrow after I make more LB+Cam plates.

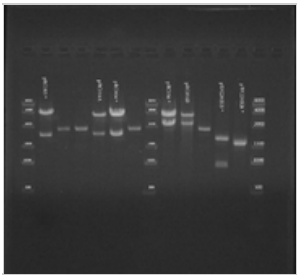
Robert.c 11:58, 11 July 2012 (PDT)

Monday 7/9

- 9:30am Picked 3 colonies per BsaI transformed plate. Plates only had 5-10% red colonies.
- 9:30am Picked colony from pWCD0526 transformation plate and seeded in 5ml LB+CAM.
- 10am Miniprepped pWCD0515. OD was 174.4 ng/uL. Will test digest later with the rest.
- 11:30am Seeded yRC014(100ul in 3ml) to look at under scope at 3:30pm.
- 2pm Seeded yJD001 for yeast (500ul in 20ml) integration at 6pm.
- 6pm Took OD of pWCD0526: 143.6 ng/uL
- 6pm Test digested:

Tube #	Plasmid	Description	Enzymes	Buffer	Expected
1	pRC013A	DBP5-Venus	BglII/XhoI	NEB 3	5609+2178
2	pRC013B	"	"	"	"

3	pRC013C	"	"	"	"
4	pRC014A	DBP5-mKate	"	"	4897+2160
5	pRC014B	"	"	"	"
6	pRC014C	"	"	"	"
7	pRC016A	NIC96-mKate	"	"	4897+3231
8	pRC016B	"	"	"	"
1	pRC016C	"	"	"	"
2	pWCD0515 (morning)	AmpR/ColE1	BsaI	"	1871+901
3	pWCD0526 (now)	Ura 5' Int	"	"	1650+1612



The *'d plasmids have a blue tough tag added and were placed into the box. The pWCD plasmids replaced the old ones.

- 7:30pm Linearized with BsmBI (30min at 55C):

Tube #	Plasmid	Description	Enzymes	Buffer
1	pRC009 (from Will)	NOP56-Venus	BsmBI	NEB 3
2	pRC013A	DBP5-Venus	"	"
3	pRC014B	DBP5-mKate	"	"
4	pRC016A	NIC96-mKate	"	"

- 8:30pm Did Zymo cleanup.
- 9pm Did yeast integration
- 9pm Transform Vincent's BsaI into TGI, plated on LB+Amp.

Robert.c 22:54, 9 July 2012 (PDT)

Sunday 7/8

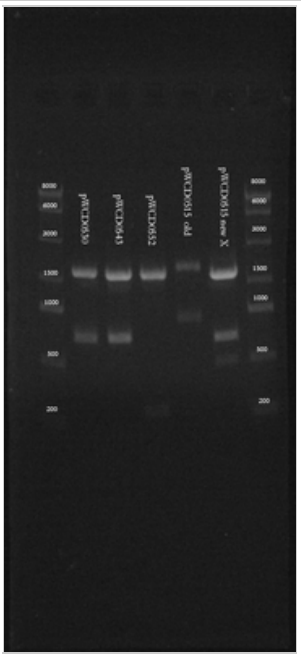
- 4pm Miniprep pWCD plasmids. New pWCD0515 is wrong because it was not red and grew in LB+CAM. Instead, used old pWCD0515.
- 5pm Ran BsaI program.
- Summary OD table:

Plasmid	Description	Part	OD
pWCD0524	Leu 5' Int	1	129.6
pWCD0526	Ura 5' Int	1	81.4
pWCD0530(new)	RPL18B	2	160.0
pWCD0542	mKate	3b	129.6

pWCD0543(new)	Venus	3b	173.2
pWCD0552(new)	ADH1	4	127.0
pWCD0559	Leu 3' Int	5	184.4
pWCD0560	Ura 3' Int	5	116.1
pWCD0515(old)	AmpR/ColE1	6	63.8
-			
pRC005	NOP56 nuco	3a	113.3
pRC006	BFR2 nuco	3a	130.4
pRC007	DBP5 np	3a	151.7
pRC008	NIC96 np	3a	194.6

- 5pm Test digested newly-miniprepped DNA:

Tube #	Plasmid	Description	Enzymes	Buffer	Expected
1	pWCD0530	RPL18B	BsaI	NEB 3	1650+723
2	pWCD0543	Venus	"	"	1650+730
3	pWCD0552	ADH1	"	"	1650+253
4	pWCD0515 old	AmpR/ColE1	"	"	1871+901
5	pWCD0515 new	AmpR/ColE1	"	"	1871+901



The new pWCD0515 is bad. Good thing I didn't use it.

- 6pm Picked pWCD0515 from Will's plate, seeded in 5ml of LB-Amp.
- 6pm Picked Vincent's plates, 3 colonies per. Seeded in 3ml of LB-Amp.
- 6pm Transformed pWCD0526 (81.3 OD, slightly low) into TG1, plated on LB-Amp. Tomorrow, will pick and seed in 5ml of LB-Amp.
- 9pm Transformed BsaI result into TG1, plated on LB-Amp. Did not do pRC009 because Will got it to work.
- 9pm Will gave me pRC009 that he tried for himself. BglII/XhoI test digest:

Correctly cut. Will use this in future.

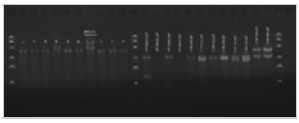
Saturday 7/7

- 1pm Miniprepped cultures seeded yesterday
- 2pm Took OD of pWCD plasmid miniprepped:

```
pWCD0542 new - 129.6 ng/uL
pWCD0560 new - 116.1 ng/uL
```

- 2pm Did several test digests:

Tube #	Plasmid	Description	Enzymes	Buffer	Expected
1A	pRC009C	NOP56-Venus	BglII / XhoI	NEB 3	5609+2244
2A	pRC009D	"	"	"	"
3A	pRC013C	DBP5-Venus	"	"	5609+2178
4A	pRC013D	"	"	"	"
5A	pRC014C	DBP5-mKate	"	"	4897+2160
6A	pRC014D	"	"	"	"
7A	pRC015C	NIC96-Venus	"	"	5609+3249
8A	pRC015D	"	"	"	"
1B	pRC016C	NIC96-mKate	"	"	4897+3231
2B	pRC016D	"	"	"	"
3B	-	-	-	-	
4B	-	-	-	-	
5B	pWCD0542 new	mKate part3b	BsaI	NEB 3	1650+712
6B	pWCD0542 old	"	"	"	1650+712
7B	pWCD0560 new	Ura 3' Int part5	"	"	1650+510
8B	pWCD0560 old	"	"	"	1650+510
1C	pRC005 tube1	NOP56 part3a	"	"	1650+1526
2C	pRC005 tube2	"	"	"	1650+1526
3C	pRC006 tube1	BFR2 part3a	"	"	1650+1616
4C	pRC006 tube2	"	"	"	1650+1616
5C	pRC007 tube1	DBP5 part3a	"	"	1650+1460
6C	pRC007 tube2	"	"	"	1650+1460
7C	pRC008 tube1	NIC96	"	"	2531+1650
8C	pRC008 tube2	"	"	"	2531+1650



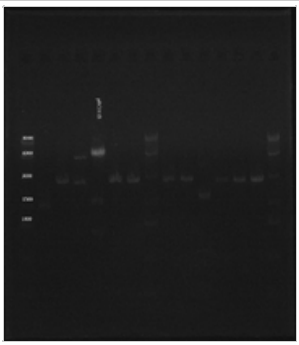
Only the labeled one, pRC015C, is correct and will be integrated. New pWCD plasmids are good. pRC005-009 are good.

- 3pm Transformed and put into 5ml culture more pWCD0530, pWCD0543, pWCD0552, and pWCD0515 to increase concentration.
- 4pm Linearized pRC015C with BsmBI (NEB 3) and Zymo cleanup elute in 20ul.
- 5pm Integrated pRC015 into yJD001 to produce yRC018 (Leu-RPL18B-NIC96 np-Venus).

Friday 7/6

- 12pm Picked 2 colonies per plate and seeded in 3ml of broth.
- 6pm Miniprepped plasmid
- 7pm Test digested plasmids:

PCR Tube	Plasmid	Enzymes	Buffer	Expected
1	pRC009A	BglII / XhoI	NEB 3	5609+2244
2	pRC009B	"	"	"
3	pRC011A	"	"	5609+1521+813
4	pRC011B	"	"	"
5	pRC013A	"	"	5609+2178
6	pRC013B	"	"	"
7	pRC014A	"	"	4897+2160
8	pRC014B	"	"	"
1	pRC015A	"	"	5609+3249
2	pRC015B	"	"	"
3	pRC016A	"	"	4897+3231
4	pRC016B	"	"	"



Only 11B worked it seems.

- Plan for redoing constructs:

1. Because those bands were dim in the test digest, will miniprep up more pWCD0560 and pwCD0524 overnight.
2. Will pick two more colonies, miniprep them up, and test digest per the plates that did not work: pRC009, 013, 014, 015, 016.

3. Will redo BsaI cassette assembly after seeing results tomorrow afternoon and talking to Will.

- 8pm Linearized plasmids with BsmBI (NEB 3) 30min at 55C.
- 9pm Integrated into yeast.

Robert.c 22:47, 6 July 2012 (PDT)

Thursday 7/5

- 9am Diluted yeast (yRC and yJD strains) to 0.2OD.
- 9am Miniprepped pRC014C+D and pRC016C+D.
- 12pm Test digested all of pWCD with BsaI in NEB3 to troubleshoot.

PCR	Plasmid	Description	Part	Expected Bands
1	pWCD0524	Leu 5' Int	1	2314+1650
2	pWCD0530	RPL18B	2	1650+723
3	pWCD0543	Venus	3b	1650+730
4	pWCD0552	ADH1	4	1650+253
5	pWCD0559	Leu 3' Int	5	1650+520
6	pWCD0515	AmpR/ColE1	6	1871+901
7	pWCD0526	Ura 5' Int	1	1650+1612
8	pWCD0542	mKate	3b	1650+712
1	pWCD0560	Ura 3' Int	5	1650+510
2	pWCD0528	TDH3	2	1650+703
3	pWCD0529	TEF1	2	1650+723
4	pWCD0531	RNR2	2	1650+723
5	pWCD0532	REV1	2	1650+723
6	pWCD0533	pGal	2	1650+492
7	pWCD0533 + control		2	1650+492

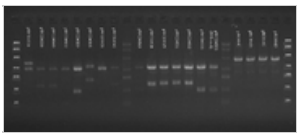
- Also did nanodrop OD measurements earlier. DNA concentration of iGEM stock plasmids is very low.

Plasmid	DNA Concentration (ng/uL)
pWCD0524	113.4
pWCD0530	46.9
pWCD0543	55.1
pWCD0559	184.4
pWCD0515	63.8
pWCD0526	81.4
pWCD0542	37.0
pWCD0552	42.1
pWCD0560	34.4
pWCD0533 stock	132.6
pRC005	113.3
pRC006	130.4
pRC007	151.7
pRC008	194.6

- 12pm Test digested pRC014C+D and pRC016C+D:

Plasmid	Enzymes	Buffer	Expected
pRC014C	BglII/ XhoI	NEB 3	4897+2160
pRC014D	"	"	4897+2160
pRC016C	"	"	4897+3231
pRC016D	"	"	4897+3231

- Ran all the test digests together:



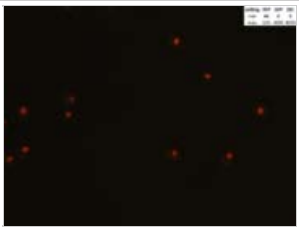
Test digests documented by the tables above. All the pWCD parts are correct. Mine are all wrong.

- 2pm Based on test digest gel, will:

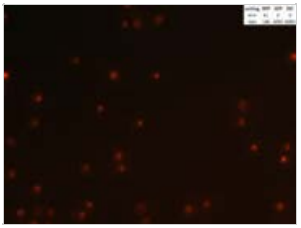
1. Integrate pRC014C+D and pRC016C+D into yeast. X will not do
2. Pick other colonies from cassette plates pRC009, pRC011, pRC013, pRC015 and grow up. X will not do
3. Redo BsaI cassette construction:

PCR	Cassette Constructed
1	pRC005-V
2	pRC006-V
3	pRC007-V
4	pRC008-V
5	-
6	pRC007-mK
7	pRC008-mK
8	-

- 3pm Imaged yRC yeast at mid-log. yRC012, yRC014, yRC016, yRC018 did not have color again, so will redo those.



yRC013: NOP56, Nucleolus-mKate



yRC015: BFR2, Nucleolus-mKate

- 9pm Transformed and plated all of my and VY's BsaI reactions and CC's BsmBI reaction.

Robert.c 22:48, 5 July 2012 (PDT)

Wednesday 7/4

- 2pm Checked on yeast plates:

Strain	Description	State
yRC012	NOP56-V	Clone B grew
yRC013	NOP56-mK	Grew, in fridge. Image confirmed.
yRC014	BFR2-V	Clone B grew
yRC015	BFR2-mK	Clone B grew, in fridge. Image confirmed.
yRC016	DBP5-V	Clone B grew
yRC017	DBP5-mK	No growth
yRC018	NIC96-V	Clone B grew
yRC019	NIC96-mK	No growth

- 3pm Miniprepped pRC005-008.
- 3pm Picked new colonies from the pRC014 and prC016 cassette plates and seeded in LB-Amp.
- 3pm Picked colonies from plates with growth and seeded in SD-Leu/Ura to look at midlog growth for tomorrow.

Robert.c 20:24, 4 July 2012 (PDT)

Tuesday 7/3

- yRC013 and yRC015B (both NP-mKate) grew! Took images. Others I'll wait another day then redo.
- Transformed pRC005-008 into bacteria.

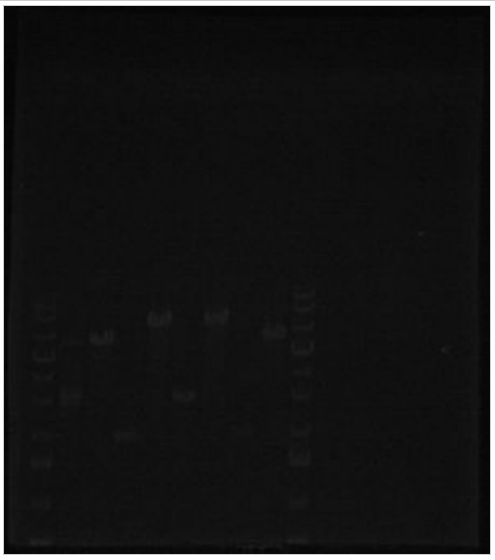
Robert.c 16:39, 3 July 2012 (PDT)

Monday 7/2

- 4am Picked colonies from pRC009, pRC011, pRC013, pRC015. Seeded for miniprep.
- 12pm Colonies from morning were all red.
- 1pm Picked more colonies from pRC009, pRC011, pRC013, pRC015.
- 1:30pm Moving meeting
- 3pm iGEM meeting
- 5pm Diluted yJD001 to 0.2 OD.

- 7pm Miniprepped cultures seeded at 1pm.
- 7pm Test digested:

Tube	Plasmid	Enzymes and Buffer	Expected Bands
1	pRC009A	PvuI/XhoI NEB3	5806+2047
2	pRC009B	PvuI/XhoI NEB3	5806+2047
3	pRC011A	PvuI/XhoI NEB3	5896+2047
4	pRC011B	PvuI/XhoI NEB3	5896+2047
5	pRC013A	PvuI/XhoI NEB3	5740+2047
6	pRC013B	PvuI/XhoI NEB3	5740+2047
7	pRC015A	PvuI/XhoI NEB3	6811+2047
8	pRC015B	PvuI/XhoI NEB3	6811+2047



Test digest of pRC009-15 odd. Strange cutting patterns; we'll see in a few days.

- 7pm Digested above plasmids with BsmBI (NEB3) to linearize. Double volume, 55C for 30min.
- 9pm ~~Zymo purified digestion.~~ (forgot to purify, which may affect results)
- 9pm Did yeast integrations with pRC009, pRC011, pRC013, pRC015.

Robert.c 22:18, 2 July 2012 (PDT)

Sunday 7/1

- 1pm Diluted yeast to 0.2OD
- 1pm Digested (double volume) the following plasmids with SacII (NEB 4) to linearize:

```
pRC010
pRC012A
pRC012B
pRC014A
pRC014B
pRC016A
pRC016B
```

- 2pm Reseeded WCD plasmids because shaker stopped so culture was clumpy and not saturated.
- 5pm Did yeast integration. Produced these plates:

Strain Name	Description	Parental Strain	Plasmids Used	Marker
yRC013	RPL18B-NOP56(Nuco)-mKate	yJD001	pRC010	URA3
yRC015A	RPL18B-BFR2(Nuco)-mKate	yJD001	pRC012A	URA3
yRC015B	RPL18B-BFR2(Nuco)-mKate	yJD001	pRC012B	URA3
yRC017A	RPL18B-DBP5(NP)-mKate	yJD001	pRC014A	URA3
yRC017B	RPL18B-DBP5(NP)-mKate	yJD001	pRC014B	URA3
yRC019A	RPL18B-NIC96(NP)-mKate	yJD001	pRC016A	URA3
yRC019B	RPL18B-NIC96(NP)-mKate	yJD001	pRC016B	URA3

- 6pm Transformed pRC009, pRC011, pRC013, and pRC015 into bacteria. LB+Amp plates.

Robert.c 18:38, 1 July 2012 (PDT)

Robert Notebook August 2012

From Dueber Lab Wiki

Contents

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.

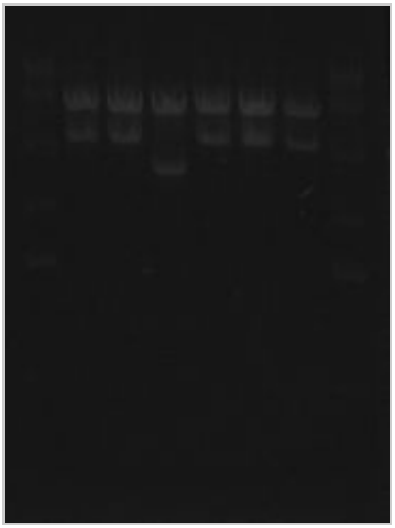
Thursday 8/30

- 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

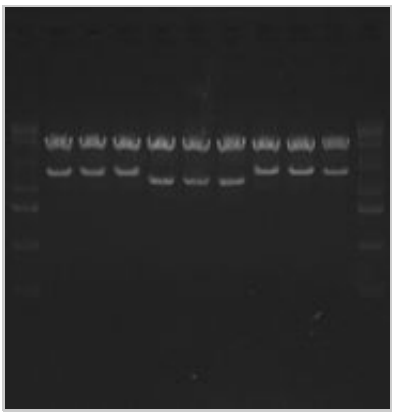
- 9pm Yeast transformations.
  - pRC036 into yRC040 to make yRC053, plate on the 5418-YPD plates.
  - pRC057-60 into yJD001 to make yRC049-52, plate in SD-LEU.
  - Used 1ul of plasmid, resuspended yeast into 1,000ul of ddH2O, and plated 100ul.

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.

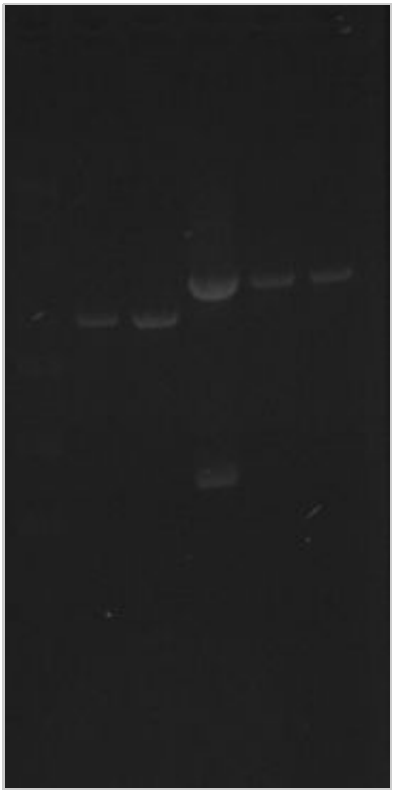
Monday 8/27

- 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



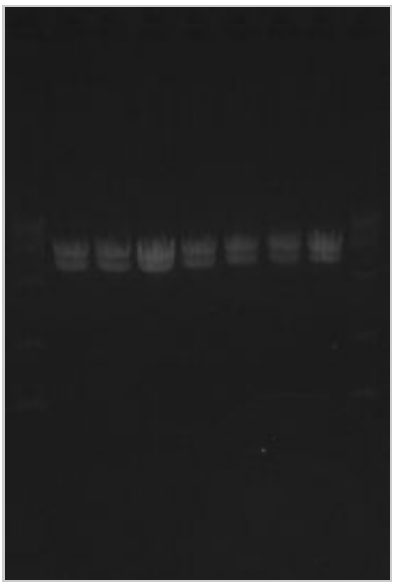
All good. I think I've proved that pRC027 is correctly integrated into yJD001 to produce yRC040.

- 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

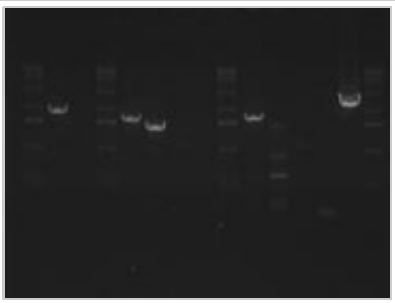
- 10pm Figure out yRC040 genome issues by trying out another primer set.

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.

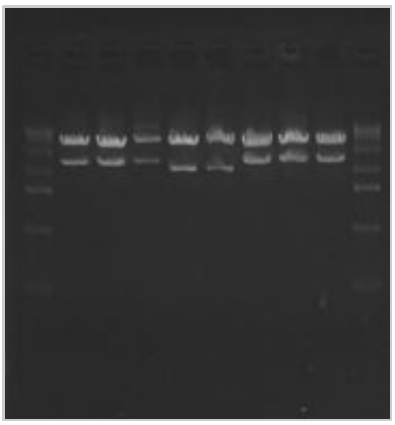
- Colony PCR or just pick colonies from pRC036 plates.
- 5pm Miniprep pRC055, test digest, sequence.

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

Thursday 8/23

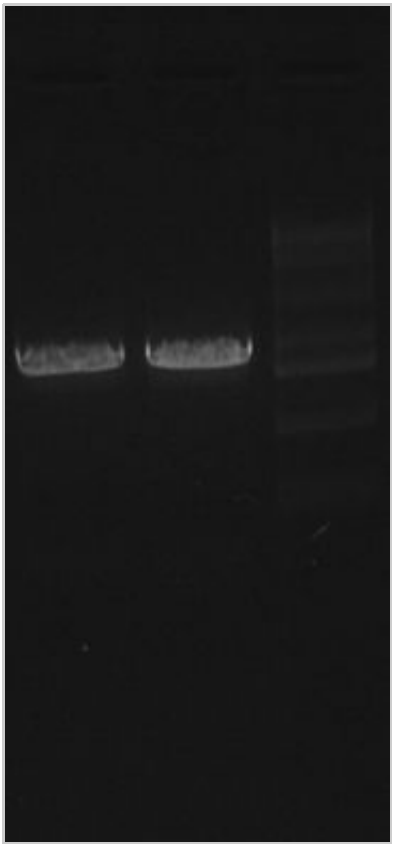
- 4pm Checked on newly-electroporated pRC053, pRC054, and pRC056 plates for a difference from before. More colonies, so if the ones from today don't work, will pick ~3 colonies each.
- Miniprep pRC053, pRC054, pRC056. Test digest:

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.

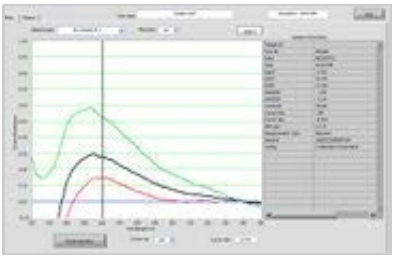


As expected. Combined lanes, eluted into 10ul of ddH2O. Nanodropped 78.6 ng/ul.

- 7pm Sent for sequencing:

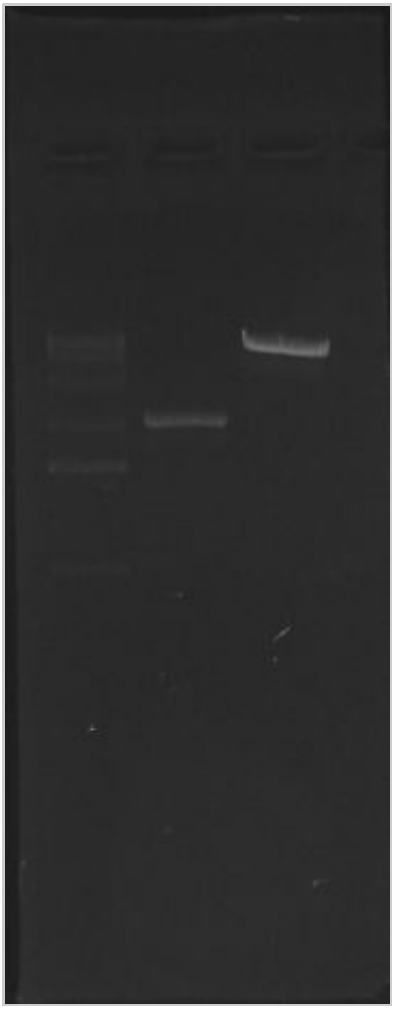
Name	Date	Construct	Clone	Primer
sIGEM344	23-Aug-12	pRC035	B	PL03_A06
sIGEM345	23-Aug-12	pRC053	A	V19
sIGEM346	23-Aug-12	pRC053	A	AU71
sIGEM347	23-Aug-12	pRC053	B	V19
sIGEM348	23-Aug-12	pRC053	B	AU71
sIGEM349	23-Aug-12	pRC054	A	V19
sIGEM350	23-Aug-12	pRC054	A	AU71
sIGEM351	23-Aug-12	pRC054	B	V19
sIGEM352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

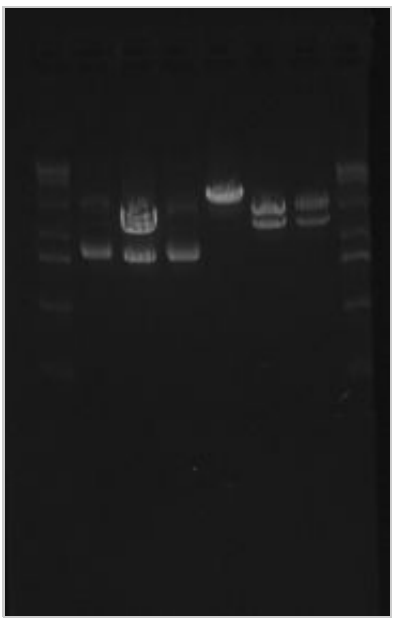
2	Insertion region	yRC040B	AS23, AS24	"	"	2611
3	Insertion region	pRC027@	AS23, AS24	"	"	2611

@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.

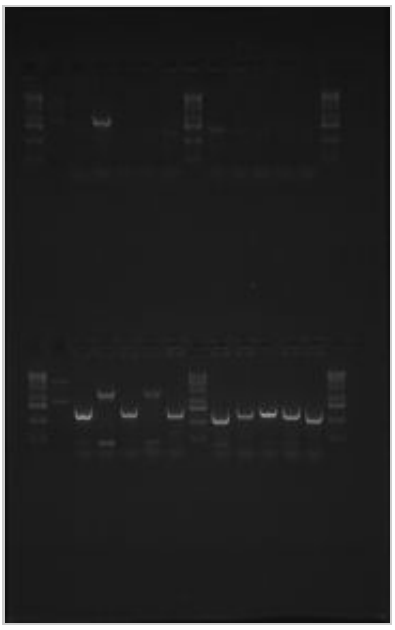
Product	Template	Primers	Temps (C)	Time	Pdt (bp)
pRC034 +C	pRC034 stock	BA08, AH17	Taq	2min30sec	1594
pRC033 cPCR	pRC033 bead plate	AZ42, AZ59	"	"	1876
pRC035 cPCR	pRC035 bead plate	AR62, AW39	"	"	2434

- 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



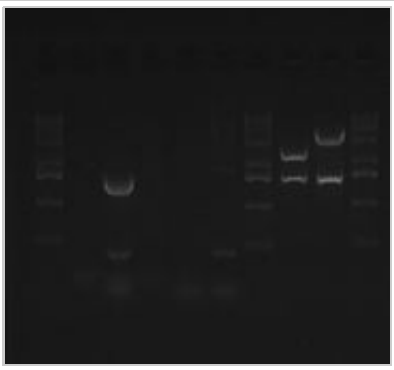
Tomorrow, miniprep up pRC033A,B,F; pRC035A,B,D; test digest then sequence.

- 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

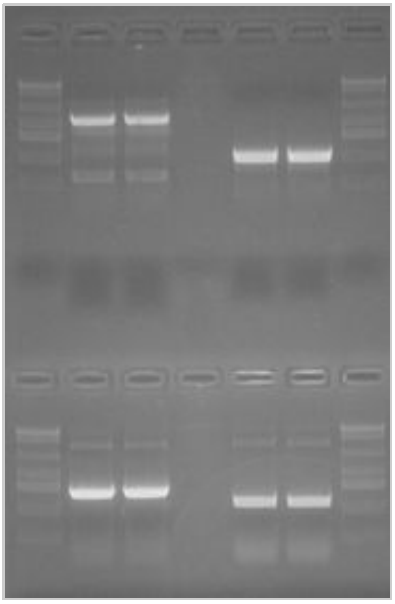
- 1pm Seeded yeast cultures, 100ul into 3ml SD-LEU.
- Will image at mid-log.
- GLycerol stocked some of the correct yeast cultures.
- 1pm Will need to hold off on yRC040 colony PCR until later because gel room is unavailable.
    - Want to choose primers to cover begnning and end of insertion homologies.
- 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.

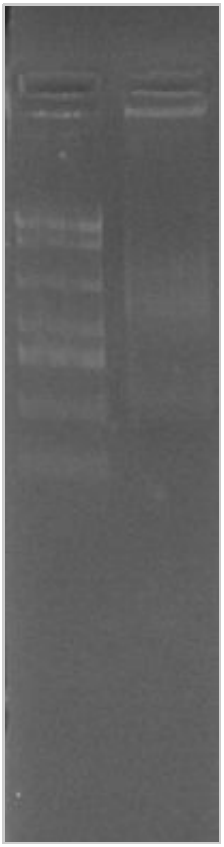
Promoter Characterization

- 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

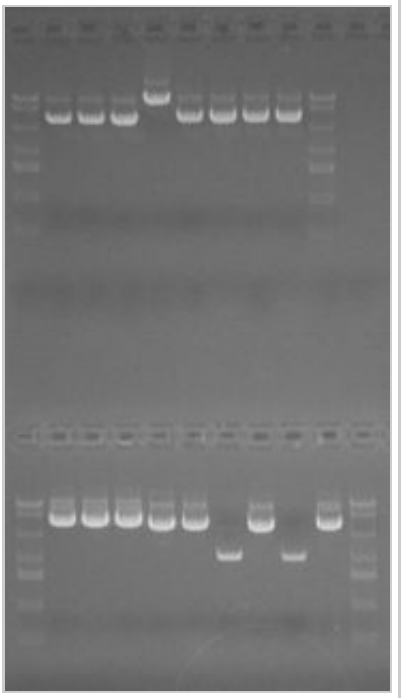
- 1am Transformed 1ul into EPI300 with electroporation. Plated 150ul of recovery solution onto LB+CAM.

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	"	"	"

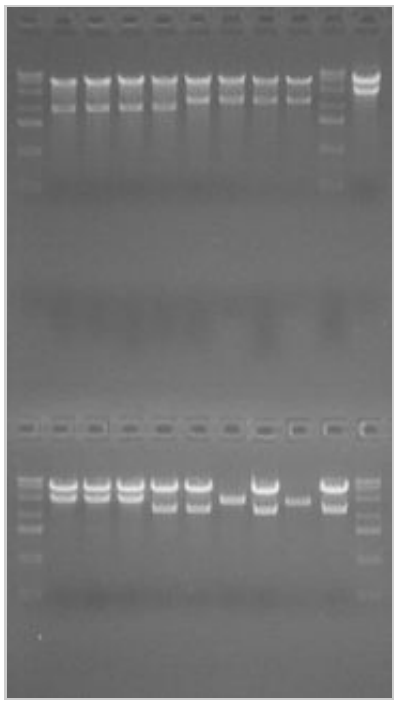


Something weird...Will retry digest with XbaI/XhoI and +C.

- 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

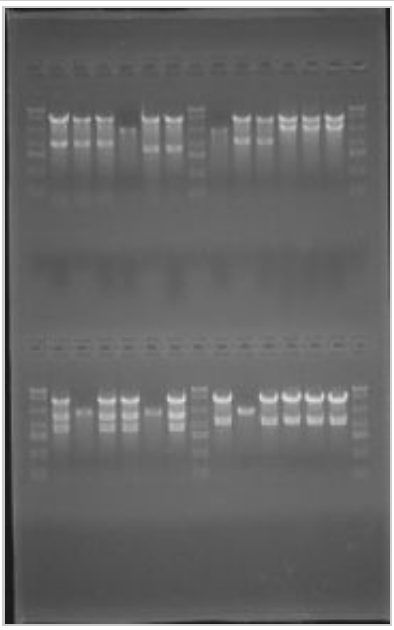
- 9pm Checked sequencing.
  - pRC033 is missing parts 1 and 2.
  - pRC034 is correct. Labeled tube in freezer with blue tag.

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	"	"	"

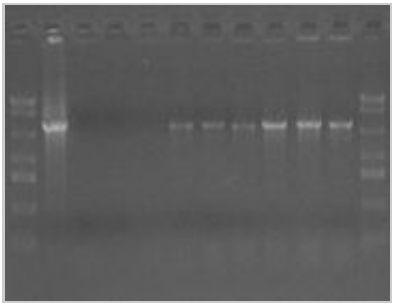
Making pADH1 for Promoter Characterization

- ~~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	"	"

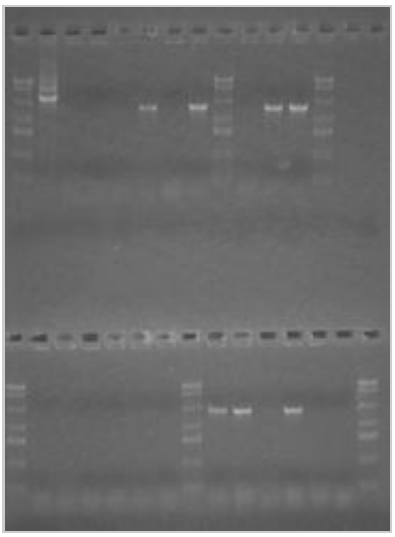


The water colony method is far better. Will do colony PCR this way in the future.

- 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.

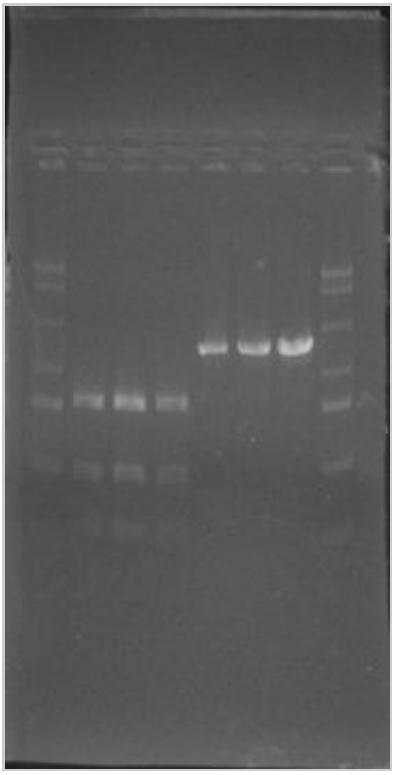
Promoter Characterization

- 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:

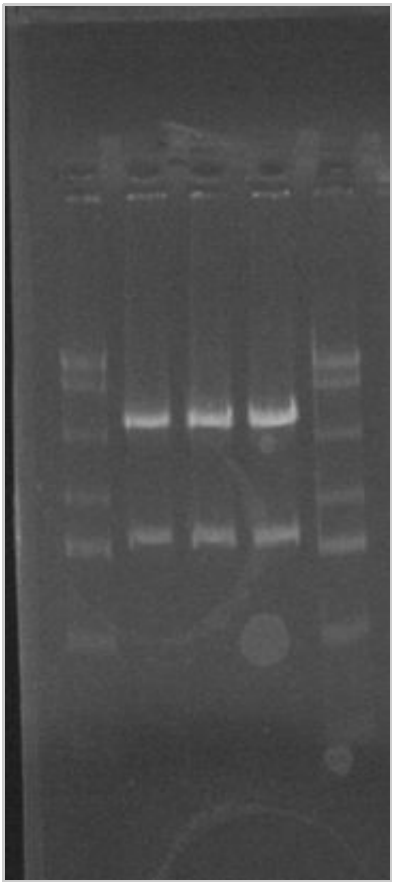
Name	Date	Construct	Clone	Primer
sIGEM274	2-Aug-12	pVY006		AW38
sIGEM275	2-Aug-12	pVY006		AW39
sIGEM276	2-Aug-12	pTC005		AW38
sIGEM277	2-Aug-12	pTC005		AW39
sIGEM278	2-Aug-12	pATJ006		AW38
sIGEM279	2-Aug-12	pMRY030		AW38
sIGEM280	2-Aug-12	pMRY030		AW39
sIGEM281	2-Aug-12	pRC027	A	G66
sIGEM282	2-Aug-12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

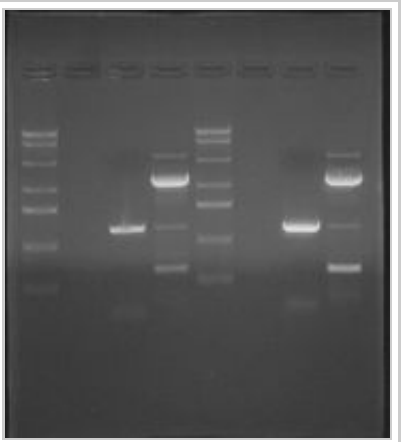
Plasmid Name	Part 1	Part 2	Part 3	Part 4	Part 5	Part 6	'
pRC049	Leu2_Int_5'	pSTE5 (weak)	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1	
pRC050	Leu2_Int_5'	pCYC1 (medium)	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1	
pRC052	Leu2_Int_5'	TDH3p	Venus	ADH1	Leu2_Int-3'	AmpR_ColE1	

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.