Robert Notebook July 2012

From Dueber Lab Wiki

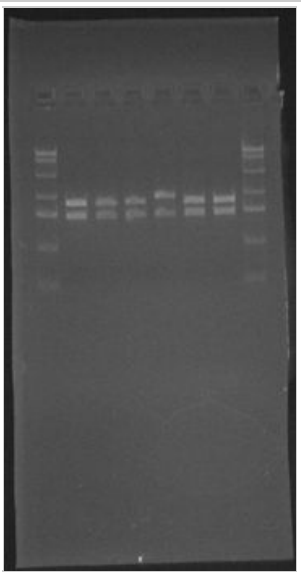
Contents

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	pRC023 + Ab	NheI + NotI	NEB3	1237 + 934

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

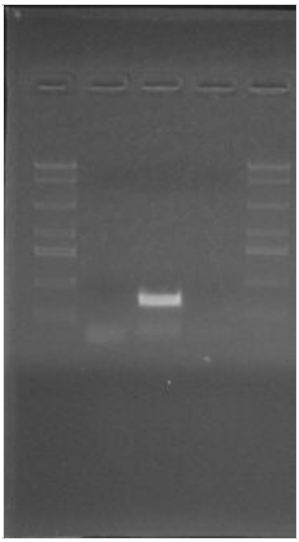
- 7pm Ran BsaI reaction to produce genomic landing pad cassette, pRC027. Plated on LB+AMP.

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

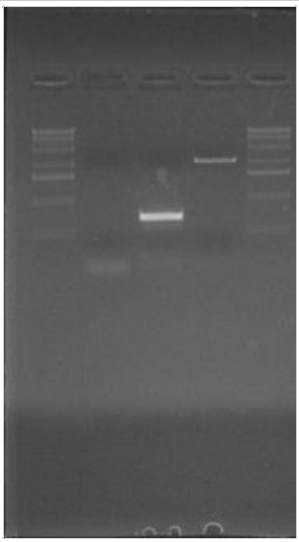
- 6pm/7pm Masaki and I ran PCR reactions to produce:

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

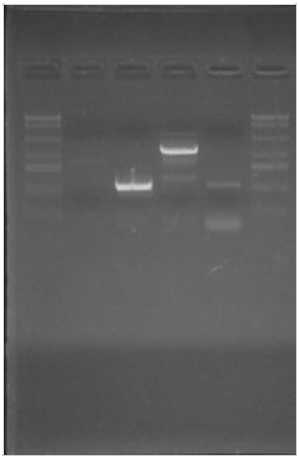
- 8pm Gel purification.
  - Forgot to add isopropanol to the pRC028 PCR pdt! Might have to redo.



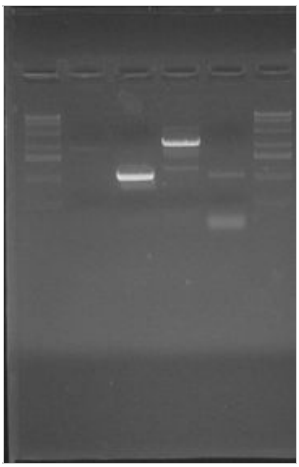
Only pRC048's 297bp product PCRed correctly at 50C. Will gel purify.



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.

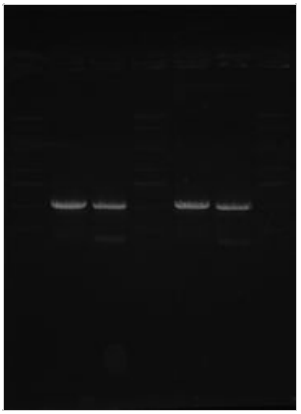
- 1am After the 1hr recovery shake, there was a clump because it didn't shake hard enough. Also, there were too many cells, so in the future, I'll do the following for electroporation:
  - Use 30ul of cells at 10x dilution.
  - Plate 100ul of the 500ul that was used for the recovery.

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

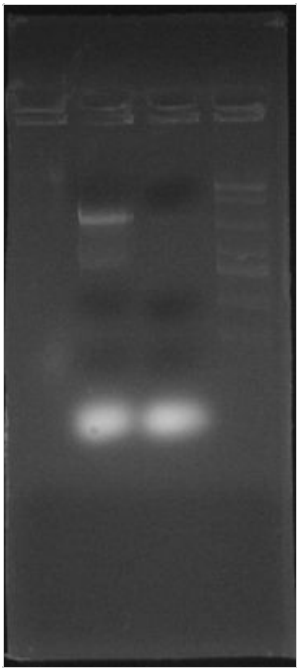
- Seeded yeast cultures into a 24-well block:

	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

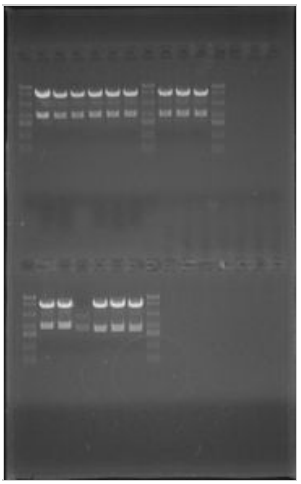
- ~~11am Dilute yRC031 to 0.2 OD.~~ Will actually dump the culture and reseed once the microscope is available again.

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.

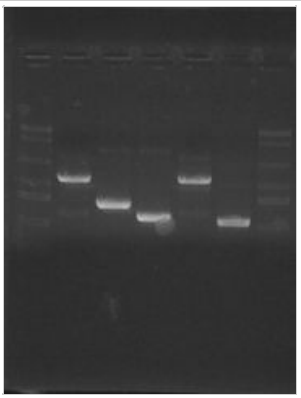
Tube	Plasmid	Condition	Band
1A	pRC042A	3x vol, electro	4829
2	pRC042B	3x vol, electro	4829
3	pRC042C	3x vol, electro	4829
4	pRC043A	3x vol, electro	4849
5	pRC043B	3x vol, electro	4849
6	pRC043C	3x vol, electro	4849
7	pRC044A	3x vol, electro	4849
8	pRC044B	3x vol, electro	4849
1B	pRC044C	3x vol, electro	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:

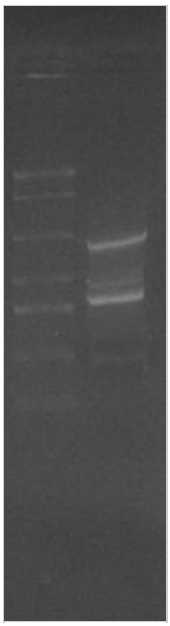
Product	Product (bp)
pRC033.1	1697
pRC033.3	850
pRC033.4	624
pRC034.1	1698
pRC035.2	559



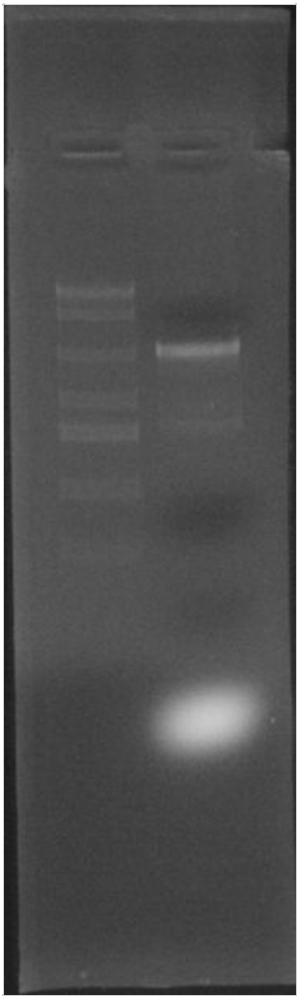
Perfect!

- 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:

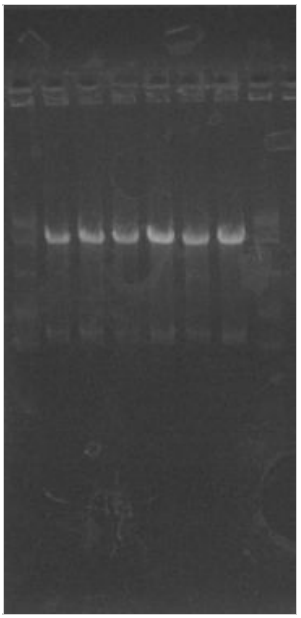
Plasmid	Description	Part	ng/ul
pVY006	ZRC1	3a	265.8
pTC005	ABP1	3a	bad curve
pAJ006	CIIC	4	136.9
pMRY030	HTA2	3a	141.6

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.

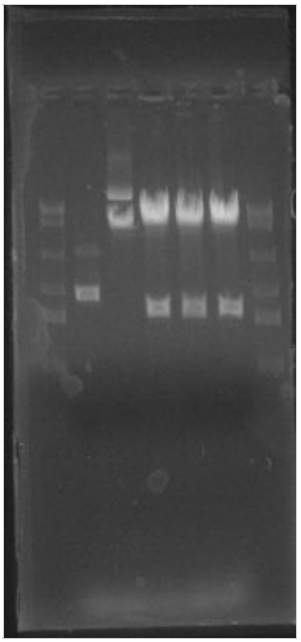
Plasmid	Description	Part	ng/ul
pVY006	ZRC1 (VM)	3a	117.8
pTC005	ABP1 (Actin)	3a	176.4
pAJ006	CIIC (CP)	4	119.0
pMRY030	HTA2 (Nucleus)	3a	85.7
pWCD0524	Leu 5' Int	1	308.9
pWCD0543	Venus	3b	268.4
pWCD0552	ADH1	4	213.9
pWCD0559	Leu 3' Int	5	232.0
pWCD0515	AmpR ColE1	6	174.4
pWCD0528	TDH3	2	302.4
pWCD0529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

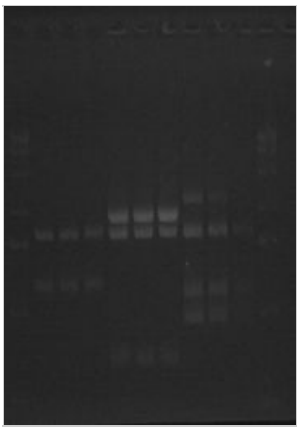
Robert.c 22:49, 24 July 2012 (PDT)

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 :-(

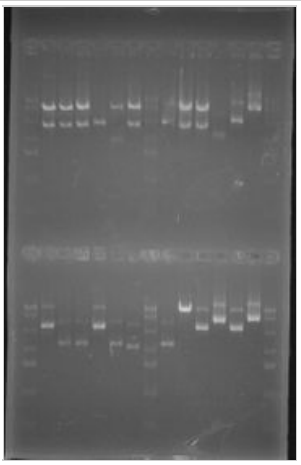
Name	Date	Construct	Clone	Primer
sIGEM212	12-Jul-12	pRC014	1	AA05
sIGEM213	21-Jul-12	pRC022	A	AW38
sIGEM214	21-Jul-12	pRC022	B	AW38
sIGEM215	21-Jul-12	pRC022	C	AW38
sIGEM216	21-Jul-12	pRC024	A	AW38
sIGEM217	21-Jul-12	pRC024	A	AW39
sIGEM218	21-Jul-12	pRC024	B	AW38
sIGEM219	21-Jul-12	pRC024	B	AW39
sIGEM220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

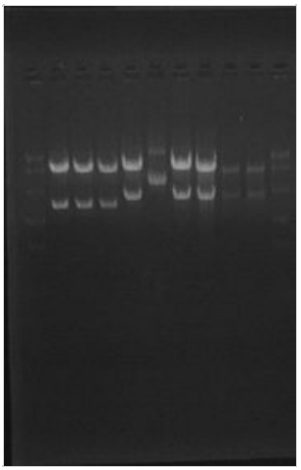
Plasmid	Description	Part	ng/ul
pWCD0560	Ura3_Int-3'	1	202.1
pVY006	ZRC1 (VM)	3a	117.8
pTC005	ABP1 (Actin)	3a	176.4
pAJ006	CIIC	4	119.0

- 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	"	"	"

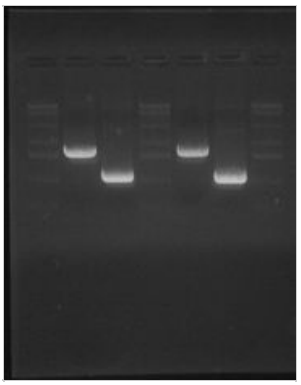


Chose 18A, 31A, 32A to linearize.

- 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)

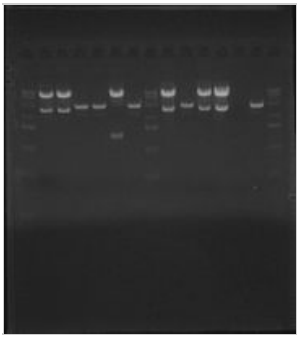
Tuesday 7/17

- 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.

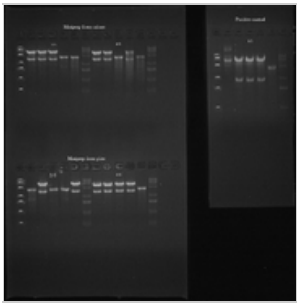
Robert.c 11:26, 19 July 2012 (PDT)

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

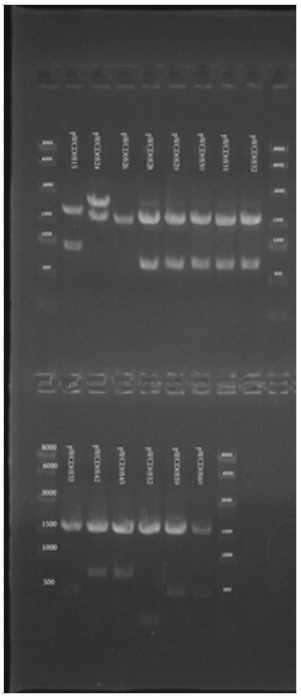
- Pick colonies.

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.

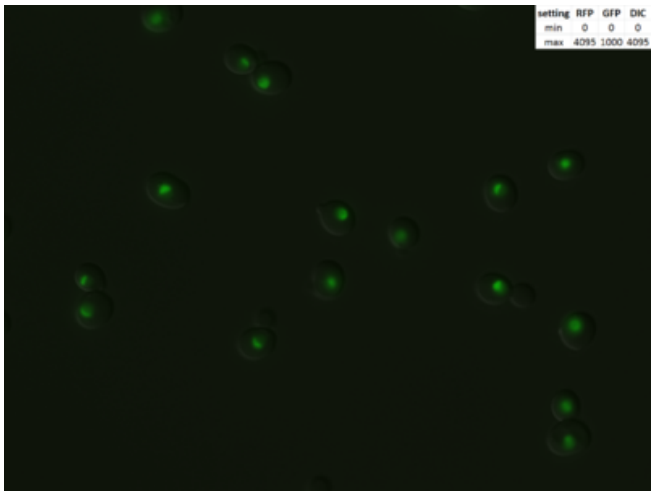
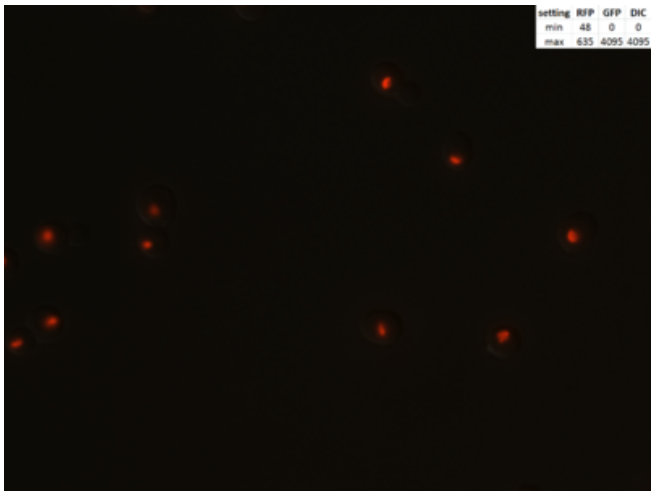
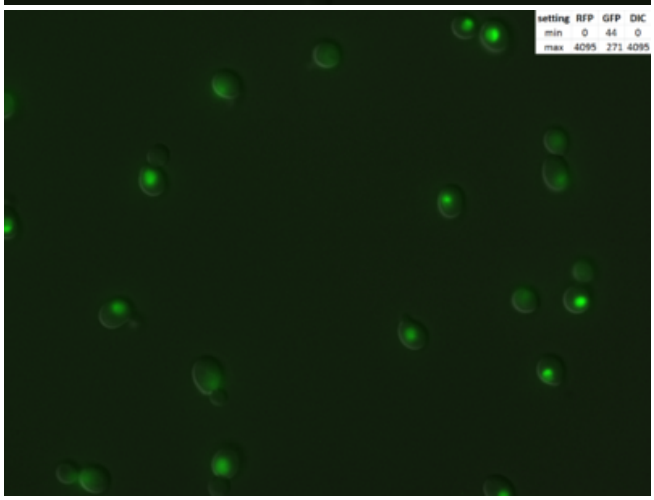
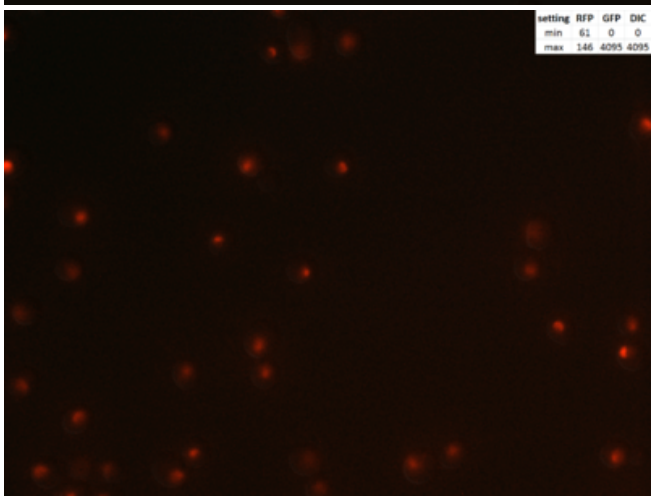
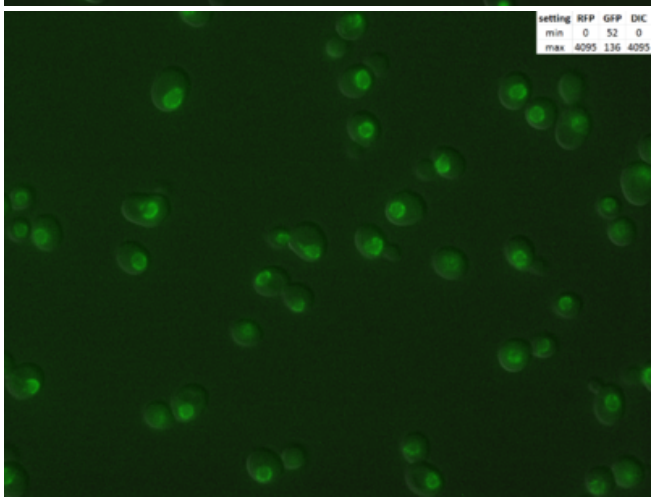
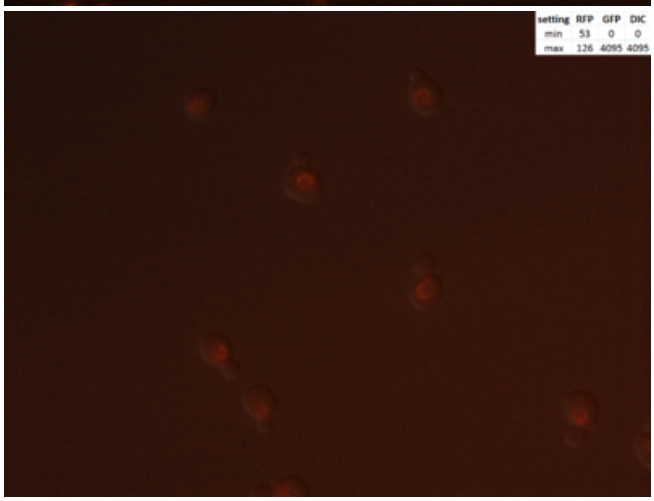
Robert.c 21:43, 13 July 2012 (PDT)

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.

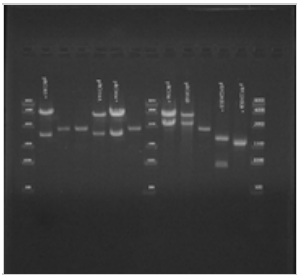
Robert.c 11:58, 11 July 2012 (PDT)

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

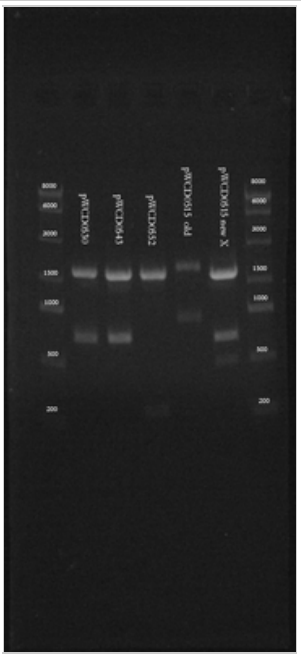
- 4pm Miniprep pWCD plasmids. New pWCD0515 is wrong because it was not red and grew in LB+CAM. Instead, used old pWCD0515.
- 5pm Ran BsaI program.
- Summary OD table:

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:

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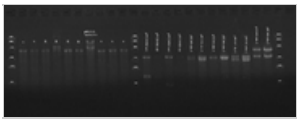
pWCD0542 new - 129.6 ng/uL
pWCD0560 new - 116.1 ng/uL
```

- 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



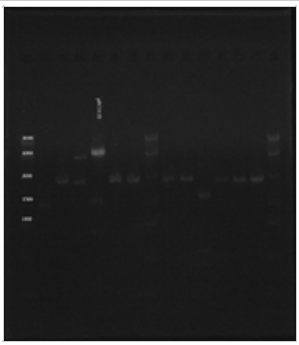
Only the labeled one, pRC015C, is correct and will be integrated. New pWCD plasmids are good. pRC005-009 are good.

- 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

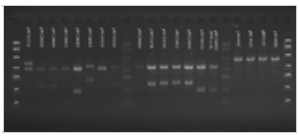
- Also did nanodrop OD measurements earlier. DNA concentration of iGEM stock plasmids is very low.

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:



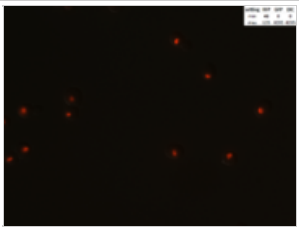
Test digests documented by the tables above. All the pWCD parts are correct. Mine are all wrong.

- 2pm Based on test digest gel, will:

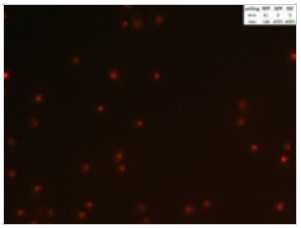
1. Integrate pRC014C+D and pRC016C+D into yeast. X will not do
2. Pick other colonies from cassette plates pRC009, pRC011, pRC013, pRC015 and grow up. X will not do
3. Redo BsaI cassette construction:

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.

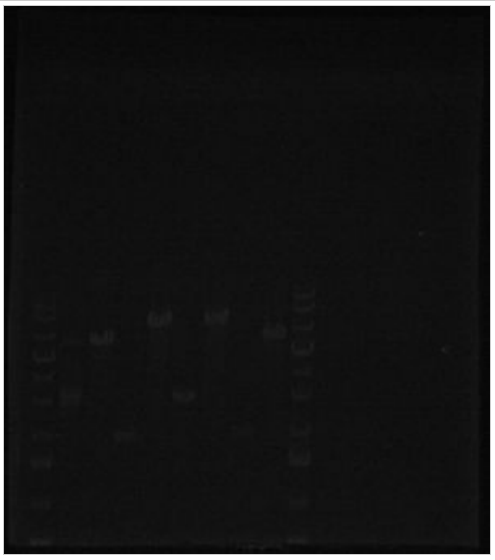
Robert.c 16:39, 3 July 2012 (PDT)

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)
