Robert Notebook June 2012

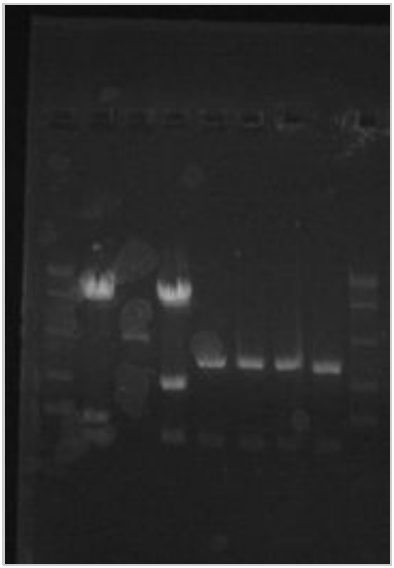
From Dueber Lab Wiki

Contents

Saturday 6/30

- 7am Pick colonies with a RFP screen (want white colonies).
  - Grew up in 3ml LB+Amp.
  - pRC013 did not have any white colonies
  - Will probably redo all the -Venus ones because I used the wrong Leu2_Int_3' part (I should have used pWCD0559).
- Miniprepped more pWCD0514 (ran out of our aliquot) - Parts backbone with RFP-CamR-ColE1
- Only these grew. Did test digests:

Tube	Plasmid	Enzymes and Buffer	Expected Bands
1	pRC010	PvuI/XhoI NEB3	5086+2037
2	pRC012A	PvuI/XhoI NEB3	5176+2037
3	pRC012B	"	"
4	pRC014A	PvuI/XhoI NEB3	5020+2037
5	pRC014B	"	"
6	pRC016A	PvuI/XhoI NEB3	6091+2037
7	pRC016A	"	"



Test digest of pRC010, pRC012A, pRC012B, pRC014A, pRC014B, pRC016A, pRC016B

- Seeded yJD001 for yeast integration
- Transformed and seeded into 5ml for minipreps tomorrow:

Plasmid	Description	Part Type	Reason
pWCD0559	Leu2_Int_3'	5	I used the wrong plasmid (pWCD0529 or something) for Leu2_Int_3'
pWCD0515	AmpR_ColE1	6	Didn't grow because I thought if followed the CamR part format. Will do in LB+Amp
pWCD0528	TDH3	2	Promoter library
pWCD0529	TEF1	2	"
pWCD0531	RNR2	2	"
pWCD0532	REV1	2	"

pWCD0533	pGal	2	"

- Redid all the Venus BsaI reactions. Will transform tomorrow.

Friday 6/29

- Miniprepped these plasmids for today's GG colonings:

Plasmid	Description	Part Type
pWCD0526	Ura3_Int_5'	1
pWCD0524	Leu2_Int_5'	1
pWCD0530	RPL18B	2
pWCD0542	mKate	3b
pWCD0543	Venus	3b
pWCD0552	ADH1	4
pWCD0560	Ura3_Int_3'	5
pWCD0529	Leu2_Int_3'	5
pWCD0515	AmpR_ColE1	6

- Did GG cloning:

PCR Tube	Plasmid Name	Part 1	Part 2	Part 3	Part 3a	Part 3b	Part 4	Part 5	Part 6a	Part 6b	Part 6
1	pRC009	Leu2_Int_5'	RPL18B		NOP56 (nucleolus)	Venus	ADH1	Leu2_Int-3'			AmpR_ColE1
2	pRC010	Ura3_Int_5'	RPL18B		NOP56 (nucleolus)	mKate	ADH1	Ura3_Int-3'			AmpR_ColE1
3	pRC011	Leu2_Int_5'	RPL18B		BFR2 (nucleolus)	Venus	ADH1	Leu2_Int-3'			AmpR_ColE1
4	pRC012	Ura3_Int_5'	RPL18B		BFR2 (nucleolus)	mKate	ADH1	Ura3_Int-3'			AmpR_ColE1
5	pRC013	Leu2_Int_5'	RPL18B		DBP5 (NP)	Venus	ADH1	Leu2_Int-3'			AmpR_ColE1
6	pRC014	Ura3_Int_5'	RPL18B		DBP5 (NP)	mKate	ADH1	Ura3_Int-3'			AmpR_ColE1
								Leu2_Int-			

7	pRC015	Leu2_Int_5'	RPL18B	NIC96 (NP)	Venus	ADH1	3'	AmpR_ColE1
8	pRC016	Ura3_Int_5'	RPL18B	NIC96 (NP)	mKate	ADH1	Ura3_Int-3'	AmpR_ColE1

- Vincent transformed then plated on LB+Amp (no need for rescue)

Robert.c 00:34, 1 July 2012 (PDT)

Thursday 6/28

- Miniprepped these plasmids:

Plasmid Name	Description	Coli Origin	Antibiotic Resistance
pRC005	NOP56(Nuco)	ColE1	Cam
pRC006	BFR2(Nuco)	ColE1	Cam
pRC007	DBP5(NP)	ColE1	Cam
pRC008	NIC96(NP)	ColE1	Cam

- Sent them for sequencing:

Sequence ID	Date	Construct	Clone	Primer
siGEM142	28-Jun-12	pRC005	1	AW38
siGEM143	28-Jun-12	pRC005	1	AW39
siGEM144	28-Jun-12	pRC005	2	AW38
siGEM145	28-Jun-12	pRC005	2	AW39
siGEM146	28-Jun-12	pRC006	1	AW38
siGEM147	28-Jun-12	pRC006	1	AW39
siGEM148	28-Jun-12	pRC006	2	AW38
siGEM149	28-Jun-12	pRC006	2	AW39
siGEM150	28-Jun-12	pRC007	1	AW38
siGEM151	28-Jun-12	pRC007	1	AW39
siGEM152	28-Jun-12	pRC007	2	AW38
siGEM153	28-Jun-12	pRC007	2	AW39
siGEM154	28-Jun-12	pRC008	1	AW38
siGEM155	28-Jun-12	pRC008	1	AW39

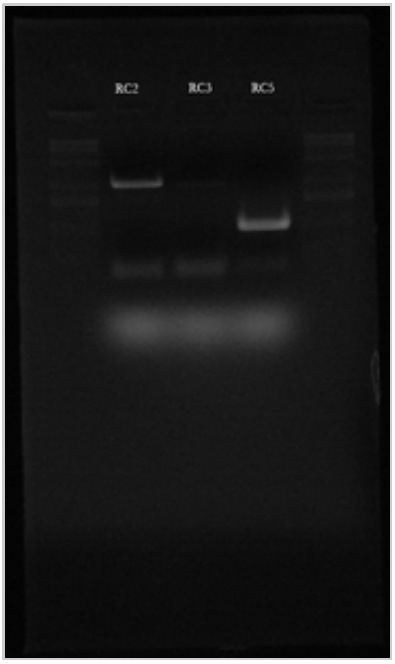
siGEM156	28-Jun-12 pRC008	2	AW38
siGEM157	28-Jun-12 pRC008	2	AW39

Robert.c 00:34, 1 July 2012 (PDT)

Wednesday 6/27

- Finished PCR cleanups.

First PCR run, with all primers. Ran overnight. 60degrees anneal for 30sec, and 50sec for just longer piece.



Reran RC2, RC3, and RC5. 50 degrees anneal for 30sec.

- Started wcdbsmbi program to ligate the PCR products into pWCD514. Added various amounts of insert:

```
RC1 0.5ul of zymo cleanu
RC2 0.5ul
RC3 2ul
RC4 0.5ul front piece + 0.5ul back piece
```

- 1hr rescue. After, spun down 14.8k rpm 2min, took off 75ul of supernatatnt, and plated cells resuspended in ~75ul of residual. Beads to cover plate.
- Have these plates:

```
RC1 Nucleolus NOP56
RC2 Nucleolus BFR2
RC3 NP DBP5
RC4 NP NIC96
```

After heat shock, did 30min rescue in 100ul of sterile LB. Then spun down 14.8k rpm 2min (like Electroporation Protocol), poured off supernatant, resuspended in residual.

Plan for next few days:

- Today 9pm: Transform wcdbsmbi into TG1 (requires 1hr rescue)
- Thur 9am: Pick plate, seed culture
- Thur 4pm: Miniprep, send for sequencing
- Thurs 5pm: GG singlepot reaction, transform into TG1
- Sat 9am: Take out plates

Robert.c 20:48, 28 June 2012 (PDT)

Tuesday 6/26

- Got oligos:

```
AX38 - Nucleolus NOP56 F primer
AX39 - Nucleolus NOP56 R primer
AX40 - Nucleolus BFR2 F primer
AX41 - Nucleolus BFR2 R primer
AX42 - NP DBP5 F primer
AX43 - NP DBP5 R pirmer
AX44 - NP NIC96 external F primer
AX45 - NP NIC96 internal F primer
AX46 - NP NIC96 internal R primer
AX47 - NP NIC96 external R primer
```

- Ran these PCR reactions:

```
1. Nucleolus NOP56 (AX38,39) 1520bp
2. Nucleolus BFR2 (AX40,41) 1600bp
3. NP DBP5 (AX42,43) 1450
4. NP NIC96 front piece (AX44,46) 2500bp
5. NP NIC96 back piece (AX45,47) 470bp


All pieces are 1.5-1.6kbp, so used 35sec annealing for all 5 reactions.
```

Robert.c 17:43, 27 June 2012 (PDT)

Monday 6/25

- Took images of colocalization experiments at mid-log.
- Need to find an application of our project by next meeting.

Robert.c 12:21, 26 June 2012 (PDT)

Friday 6/22

- Miniprepped
- Discussed upcoming stuff to do:

```
Wetlab:
*Signal sequences, new proteins
*Promoter library
*Colocalizations (by Mon)
Imaging
*Standard contrast
*Matlab/ImageJ
```

Go on with these organelles:

1. Cell periphery CC
2. Nucleus MRY
3. Vacuole CC
4. Nucleolus RC
5. Actin TC
6. Mitochondria MRY
7. NP RC
8. VM VY

- With each organelle, we need to find ~4 ways for GFP/RFP to colocalize to the organelle. So we need to find a new protein, and order F and R primers for it, in addition to new F and R primers to our old proteins.
- I chose a new protein for NP because it doesn't show well: DBP5 (NP).
- For new proteins, I chose the ones that were used as markers, NOP56 (nucleolus) and NIC96 (NP).
- Ones I chose to use:
  - NIC96 (NP): Linker nucleoporin component of the nuclear pore complex.
  - DBP5 (NP): Cytoplasmic ATP-dependent RNA helicase of the DEAD-box family.
  - BFR2 (nucleolus): Essential protein that is a component of 90S preribosomes.
  - NOP56 (nucleolus): Essential evolutionarily-conserved nucleolar protein component...

Robert.c 23:13, 22 June 2012 (PDT)

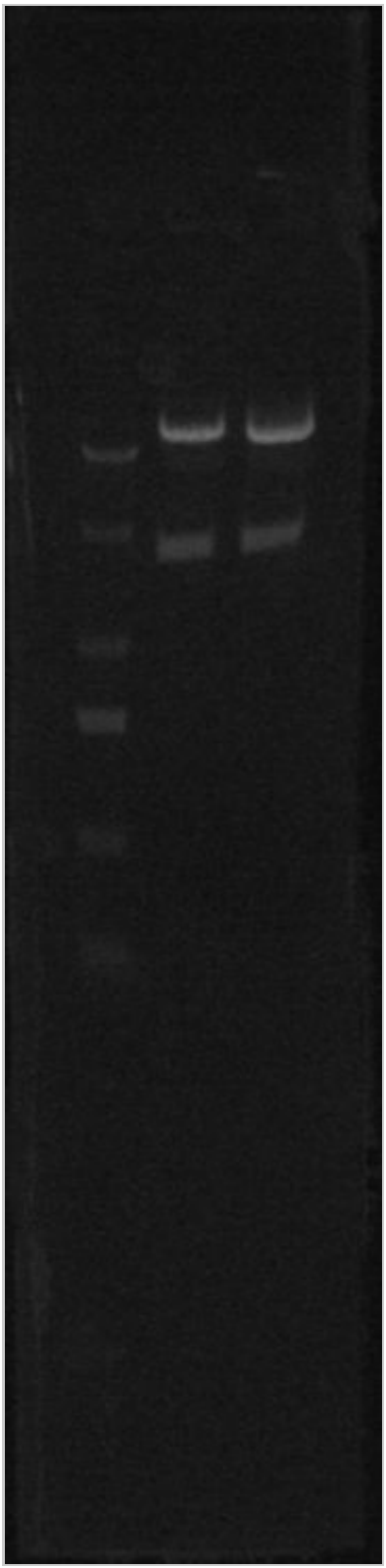
Thursday 6/21

- Meeting with other team:
  - Clostridia butanol pathway.
  - 2-component system.
  - System that can sense changes in O2 and light.
  - Transformation via conjugation.
  - Light and O2 activate another promoter that causes butanol production.

- Digested more Venus plasmid:

```
pRC001 (NP): NotI/NsiI, NEB 3. 7143+2409.
pRC003 (Nucleolus): NotI/SacII, EcoRI buffer. 6590+2386.
```

Do x14 digestion volume total, aliquot it into 6 tubes. With remaining, run test gel.



pRC001 (NP-V) digested with NotI/NsiI and pRC003 (Nuco-V) digested with NotI/SacII. Gel shows that digestion went as expected.

Wednesday 6/20

- Did these cotransformations (https://docs.google.com/spreadsheet/ccc?key=0ApdDUHORhUhAdDlzcTJRQkJwTTB1ZkdDU3hrMVlDUlE#gid=0) .

yRC005	[RPL18B-BFR2(Nucleolus)-Venus] + [RPL18B-NUP84(NP)-mKate]	yRC002 '	pRC003	URA3, LEU
yRC006	[RPL18B-HTA2(Nucleus)-Venus] + [RPL18B-NUP84(NP)-mKate]	yRC002	pMRY027	URA3, LEU
yRC007	[RPL18B-SEC63(ER)-Venus] + [RPL18B-NUP84(NP)-mKate]	yRC002	pHSR002	URA3, LEU
yRC008	[RPL18B-VMA4(VM)-Venus] + [RPL18B-NUP84(NP)-mKate]	yRC002	pVY004	URA3, LEU
yRC009	[RPL18B-NUP84(NP)-Venus] + [RPL18B-BFR2(Nucleolus)-mKate]	yRC004	pRC001	URA3, LEU
yRC010	[RPL18B-SNA3(Vacuole)-Venus] + [RPL18B-BFR2(Nucleolus)-mKate]	yRC004	pCC003	URA3, LEU
yRC011	[RPL18B-HTA2(Nucleus)-Venus] + [RPL18B-BFR2(Nucleolus)-mKate]	yRC004	pMRY027	URA3, LEU

- Made more 50% PEG for integrations.
- Tomorrow, need to do more Not/Sac-equivalent digestions.
- Tomorrow, TC and MRY will do their integrations.
- Tomorrow, will have meeting with other team.

Robert.c 17:28, 20 June 2012 (PDT)

Tuesday 6/19

- 10am Diluted yRC001-004 to 0.2OD. Will be ready for imaging in 4hrs
- 11am Miniprepped pRC001 and pRC003. Combined all in same tube
- 11am Test digested some of the pRC001 and pRC003:

```
NotI, NsiI, NEB 3 for both
pRC001, or NP-V: 7143+2409
pRC003, or Nucl-V: 5539+2409+1028
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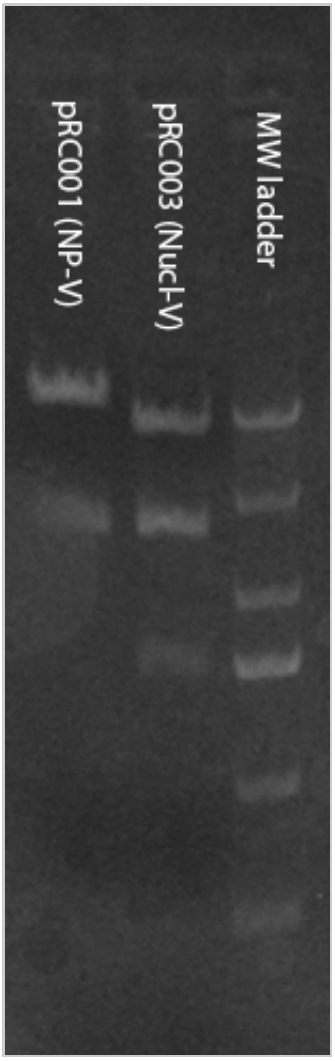


NotI/NsiI digest of pRC001 and pRC003. Everything as expected. Bright bands, so decent DNA concentration.

- 2pm Put the mid-log and saturated yeast on slides and imaged.
- Tomorrow, we're going to do the following cotransformations (https://docs.google.com/spreadsheet/ccc?key=0ApdDUHORhUhAdDlzcTJRQkJwTTB1ZkdDU3hrMVlDUlE#gid=0) , with Venus + mKate and vice versa.
- Grew up more NP-mKate and Nucleolus-mKate, to be seeded into YPD tomorrow.

Robert.c 19:10, 19 June 2012 (PDT)

Monday 6/18

- 10am Looked at Nucl-mK under scope. Both looked ok.
- 11am Seeded Nucl-mK clone 2 into 3ml of SD-Ura, in order to get the 0.2-->0.8OD microscopy and for freezing down
- 11am Digested NP-V with NotI/NsiI and Nucl-V with NotI/PvuI for yeast integrations. Did x13 digestion volume (6 transformations + 1 extra volume to run on gel as a test immediately after digestion). Will Zymo purify the digestion and save for today's and tomorrow's integrations.
- 12pm Diluted Actin-mK (yTC004) and VM-mK (yVY003) to 0.2OD in YPD. Will grow to 0.8OD later today and do genomic integration.
- 1pm Seeded NP-V and Nucl-V TG1 in LB-Amp for minipreps tomorrow morning.
- 3pm Seeded NP-mK and Nucl-mK in SD-Ura from colony to get saturated in order to do yeast integrations tomorrow.
- 4pm Meeting notes:
  - Image processing - Harneet, Thomas
  - Image acquisition - HArneet, Celia
  - Cotransformations - Everyone
  - Better targeting tags/Expression optimization - Masaki, Austin
  - Experiments to use MiCodes
    - Cloning strategy - Masaki
    - Making libraries of ??? - Vincent
  - Yeast parts in registry (promoters?) - Celia
  - Optimizing transformation efficiency - Robert
  - Add CFP to FP targeting constructs - Harneet, Robert
  - Building MiCode libraries - Austin
- 5pm Diluted NP-V and Nucl-V TG1 to get more miniprep broth for tomorrow.
- 7pm Streaked new pure colony plates, using half a plate: NP-V/NP-mK and Nucl-V/Nucl-mK TG1.

Robert.c 00:42, 19 June 2012 (PDT)

Sunday 6/17

- Came in to check on my yeast transformation plates, both Nucl-mKate. Clone #1 did not seem to work, while clone #2 did.
- Seeded some yJD001 from plate in case Masaki or I need it tomorrow.
- Seeded the following yeast mKate cultures to do more co-transformations tomorrow:

```
Actin yTC004
Vac. membrane yVY003
ER yHSR002
```

- Tomorrow, will maybe restreak the plates in order to get two types of cells on one plate (to cut down the number of plates in the fridge)

Robert.c 17:03, 17 June 2012 (PDT)

Friday 6/15

- Seeded yJD001 to 0.2OD for yeast integration later today.
- Seeded NP-Venus and NP-mKate to 0.2OD to put on slides later today.
- Did double digest of Nucl-mKate clones 1 and 2 in double volume with NotI/PvuI (since SacII is out) in NEB 3.
- Yeast LiOAc transformation of Nucl.-mKate clones 1 and 2.
  - 20ul digestion reaction.
  - Zymo cleanup into 10ul ddH2O.
  - After heat shock, spin down, pour off supernatant, resuspend in the 75ul residual in bottom of Eppendorf, and plate.
  - Check plates on Monday.
- Miniprepped more of NP-Venus, NP-mKate, Nucl-Venus plasmid for future use.
- Poured SD-Ura-Leu plates and made SD-Ura, SD-Leu, and SD-Ura-Leu media.
- Took out yeast NP-Venus and put on slide. Looked at NP-mKate later in the day.
  - NP-Venus look as expected. Will use this recent plate from now on. Put tape label on older plate.
  - NP-mKate looked as expected, but was dim.

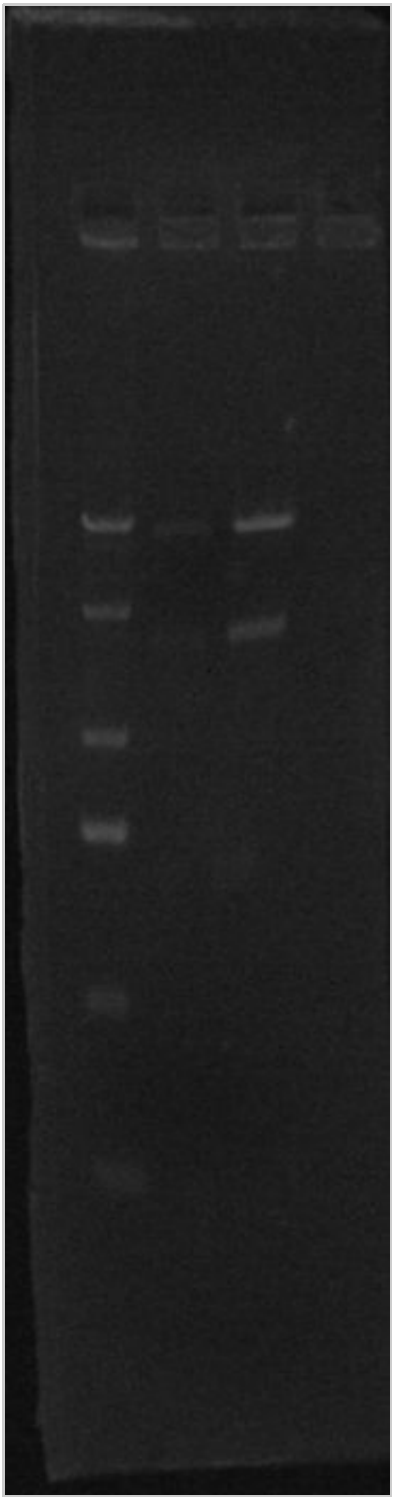
Tomorrow, check on:

- TG1 mono-colony plates in 37C incubator.
- HSR, VY, TC yeast transformation plates.

Robert.c 19:14, 15 June 2012 (PDT)

Thursday 6/14

- 10am Masaki picked two colonies from TG1 transformed with Nucleolus-mKate Gibson product. He only picked 2 because negative control had 7 colonies and mine had 71. (7/71)^2 = 1%
- 3pm Miniprepped Nucleolus-mKate from culture.
- 3pm Did test digestion BglII/PvuI (NEB 3) on miniprep product and ran on gel. Expected 5622+2402+813 if correct, 6331+1832+813 if Nucleolus-Venus, and 4827+2402 if original pHSR001 vector (no insert).



Nucleolus-mKate clones 1 and 2 test digested with BglII/PvuI. No 813bp portion, but it could just be hard to see. Still sending for sequencing. I think it is correct because the 5622 and 2402 portions seem right.

- 6pm Sent Nucleolus-mKate plasmids for sequencing with S16 and O44 (sIGEM114-117)
- 6pm Picked what was left of NP-Venus clone 2, NP-mKate clone 2, Nucl-Venus clone 1 and seeded in 3ml of LB+amp broth for miniprepping and streaking on new plates tomorrow.
- 6pm Started yJD001 seed culture in YPD broth for tomorrow's transformation.
- 7pm Checked on NP-Venus (yRC001) and NP-mKate (yRC002) yeast plates. Colonies were really small Picked largest colony for various things:
    - Grew both cultures in 3ml of SD-Leu to dilute to 0.2 OD and grow up to 0.8 then image tomorrow.
    - Seeded 3ml SD-xxx culture.
    - Will streak on half of Leu plate. On other half, streaked the colony from Nucl.-Venus. These half-plates represent the single colony that we used for microscopy.
    - Will use that culture to seed 0.2OD culture to grow up to 0.8OD.
    - Will freeze down some of the culture later. 500ul saturated culture + 500ul of 50% glycerol.
    - Put back into 30C incubator so colonies grow larger.

Robert.c 18:39, 14 June 2012 (PDT)

Wednesday 6/13

- Miniprepped pHSR001, pMRY001, and Nucleolus-mKate clones #1-3.
- Seeded plate with Nucleolus-mKate clones with the miniprep broth (did a 1:10 dilution before adding ~3ul of broth).
- Digested pHSR001 and pMRY001 with BglII.
- Zymo purified digestion. Eluted in 10ul.
- Did Gibson with 2.5ul of Nucleolus insert, 2.5ul of pHSR001 vector, and 15ul of Mike's Gibson mix (20ul total)
- Once finished, did a standard heat shock transformation:

1. Used 10ul of Gibson product (saved 10ul in case) and 50ul of TGI-KCM cocktail.
2. After heat shock, did 30min rescue in 100ul of sterile LB.
3. Then spun down 14.8k rpm 2min (like Electroporation Protocol (http://dueber.berkeley.edu/wiki/Template:IGEM_Electroporation_Protocol)), poured off supernatant, resuspended in residual.
4. Plated all of the broth and did a dilution streak.

- Checked sequences (sIGEM096-099). Turns out "Nucleolus-mKate" was actually Nucleolus-Venus. Good thing I redid starting from Gibson and will have colonies to pick and miniprep tomorrow.
- Diluted yCC004 to ~0.2 OD600. Let grow to ~4hrs (didn't care about OD then), then spun down and put on slide.
- Will took fluorescence microscopypicture.

- The following organelles I picked and reseeded for GFP/RFP colocalization experiments in a couple days. I only did the mKate versions because we will insert in the Venus. Dipped in marked colony from original plate and seeded in 3ml of SD-URA.

```
Actin yTC004
Endosome yTC002
Peroxisome yVY001
Vac. membrane yVY003
ER yHSR002
Cortical ER yHSR004
```

- Check tomorrow morning.

- Timeline of upcoming days for the Nucleolus-mKate plate:
  - Thurs morning check plates. Pick colonies (last time we did 3) depending on negative control.
  - Thurs morning grow up culture. Do miniprep Thurs afternoon.
  - Thurs afternoon after eluting DNA, do test digest and send for sequencing.
  - Thurs night, start yJD001 culture for Fri transformations.
  - Fri morning do back-dilution of yJD001 culture.
  - Fri noon do digest with NotI/SacII: 6451+2382.
  - Fri noon do gel purification. We want the 6451 band.
  - Fri afternoon do yeast genome integration with LiOAc
  - Fri afternoon plate yeast on SD-Ura plates.
  - Mon morning check yeast plate.
  - Mon morn take yeast colony and put on slide to image right before meeting.

Robert.c 17:24, 13 June 2012 (PDT)

Tuesday 6/12

Today, I:

- Diluted yeast to 0.15 OD in ~50ml of YPD total. We waited 4hrs, then begun the transformation.
- Begin digesting the mKate vectors asap (started at 10:15am). I'll do these digestions and, depending on the gels, transformations:

```
NP-Venus  NotI/PvuI clone 2
NP-mKate NotI/NsiI clone 2
Nucleolus-mKate NotI/SacII clone 1
Nucleolus-mKate NotI/SacII clone 2
Nucleolus-mKate NotI/SacII clone 3
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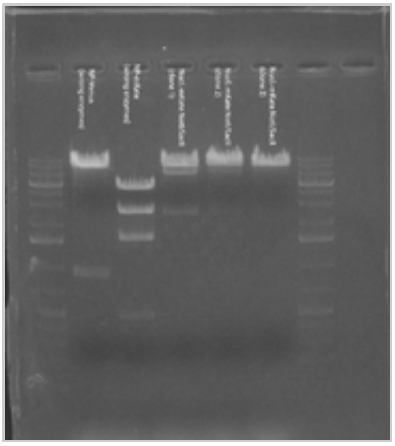

First round of digestion. I accidentally switched the enzymes for NP-Venus and NP-mKate.

- Made a mistake....

```
NP-Venus should be NotI/NsiI (NEB 3) digest: 7143+2409.
NP-mKate should be NotI/PvuI (NEB 3) digest: 8521+892.
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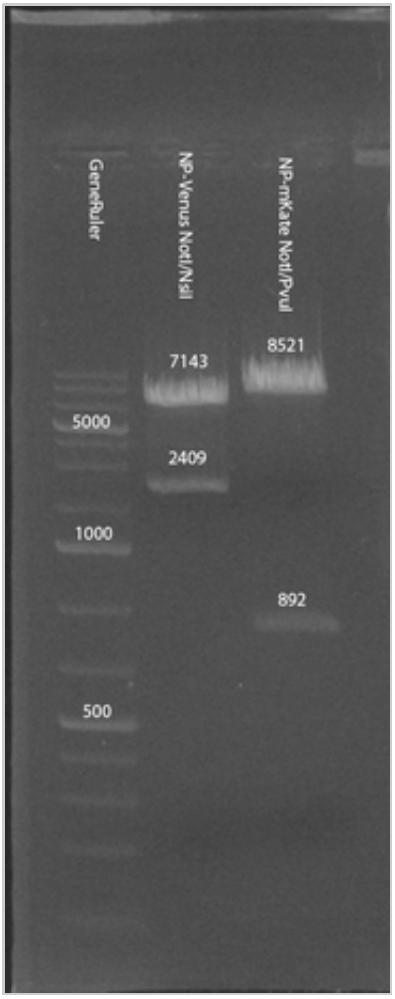


Redid NP-Venus and NP-mKate with correct enzymes. Turned out as expected

Standardizing the cell growth phase:

1. Grow cells that we microscoped yesterday to saturation overnight.
2. Dilute and grow up cells starting from 0.2OD.
3. Grow for 4hrs at 30C.
4. Immediately put on slides and image.

- I'm running into problems with the Nucleolus-mKate, so I'm going to do several things in parallel in the next few days:

1. Send for sequencing with another reverse primer, O44.
2. Rerun the Gibson reaction. First, I need to make more pHSR001 and pMRY001. Picked colonies from plates in deli fridge and started growing some for miniprep tomorrow.
3. Miniprep more DNA from the original colonies because I'm using so much for troubleshooting. There wasn't very much bacteria from the original colony left on the plate, so I just scraped the surface where the colony used to be. Hopefully, I will have growth. If not, I'll either pick from the plate I have already (those colonies

haven't been sequenced yet, so I would waste a lot of troubleshotting effort) or do another electroporation (lots of work).

4. Digests of the vectors seem to be correct, so I tried to transform all 3 clones today into yeast. Wednesday, I'll see how they turned out.

Robert.c 18:50, 12 June 2012 (PDT)

Monday 6/11

Today:

- Checked sequencing results. We may have messed up one of the R primers.
- Something is weird with the nucleolus-mKate R sequencing. Sending out all 3 clone-miniprep Gibson results with just Z72 R to sequence the tail end of the insert, including part of the homology and hopefully mKate. I don't need to do the F reactions because those turned out correctly already. They are sIGEM088-090.
- We got some colonies! URA plates had more growth.
- Put 4ul of PBS on slide, tap pipette to colony, then

```
pipette up and down in the PBS to suspend cells. Add cover slip on top. The 4ul of fluid should com
```

- We need to grow up the specific colony we picked in selective media, and then freeze down in glycerol. I've seeded the colony in 3ml in selective media overnight. Because only two of my plates grew, I only grew up NP-Venus and Nucl.-Venus in Leu-selective media.
- Later, we want to grow up all the successful cells up to a standard concentration. Use the same Leu-selective growth for this.

Tomorrow:

- Dilute the cells to 0.15OD (do as early as possible because it will take time to grow up to 0.8 OD)
- Check sequencing. Choose which clone for Nucleolus-mKate to use based on this.
- Freeze down some of the yeast in glycerol.
- Dilute some of the yeast and grow up. Image later that day.
- Digest the NP-mKate and Nucleolus-mKate Gibson miniprep product with NotI and SacII. Just do this with each of the 3 clones of the miniprep product. Call the resulting yeasts yRC002.1, yRC002.2, etc.

```
NP-Venus NotI/PvuI (NEB 3) digest: 7143+2409.
NP-mKate NotI/NsiI (NEB 3) digest: 8521+892.
Nucleolus-mKate NotI/SacII (EcoRI) digest: 6451+2386.
```

- Note: Use PvuI and NsiI instead of SacII because SacII exists inside the protein.

- Note: PvuI for the NP-mKate is not ideal because it is so downstream of the URA3 homology. However, because it is efficient for others, it should be ok.
- Instead of Zymo cleanup, I'll do a gel purification just to see if the digest was the issue.
- Do the yeast LiOAc transformation later with the 10ul from the gel purification.
- Plate the yeast cells.

Meeting Discussion:

- We might want to mess with the promoters so that the expression levels across all fluorophors across all organelles is relatively similar.
- We like...

```
Nucleolus
Nuclear periphery
Vacuole
Vacuolar membrane
Cell periphery
Mitohondria
```

- We'll want to keep growth phase consistent for the future.
- Later on, the use for our idea:
  - Mutation needs to be integrated near our fluorescent cassettes/barcodes.
  - It's a good way to tell cells apart without having to sequence them always.
  - Good in population studies, when there are various competing cultures on a single plate.
  - We can see what fell out and what grew more, for example with antibiotic growth.
- We need to show a proof-of-concept later and a narrative that works for this.
- A good option would be for mutagenizing mammalian cell
- The narrative can go both ways...problem <--> solution.
- This idea (in comparison to FACS) is good at spatial organization
- Next steps:
  - After we finish all organelles, we want to cut down our list of organelles to make things simpler and to cut out ones that are hard to tell apart
  - We want to maybe switch a few to tags to eliminate stress on cells.
  - We want to characterize cell growth rate and standardization. For controls:

```
Unmodified cells
Cell with cytoplasmic GFP
```

- - Next, we want equal brightness, so we want to clone in a library of promoters.
  - By next week, we want to see what half of the chart is worth pursuing and why.

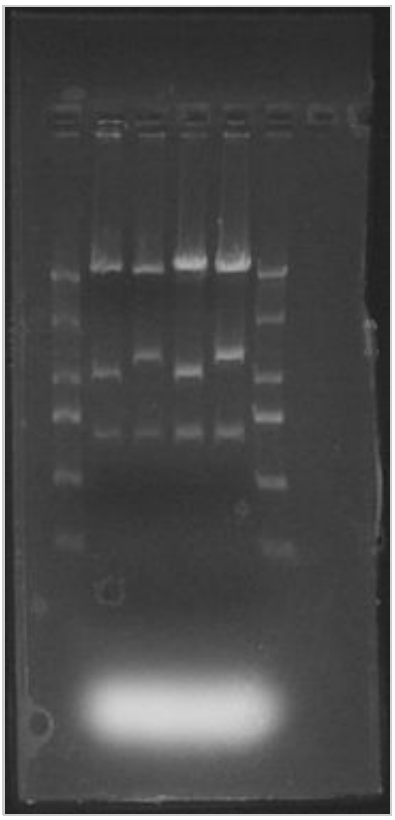
Robert.c 18:06, 11 June 2012 (PDT)

Friday 6/8

Today:

- I'm rerunning a test digest. In this one, I'll take clones 1 and 2 of Nucleolus for just venus and digest them with the enzymes for venus and mKate. Details here:

```
Lane 1: Ladder
Lane 2: "Nucleolus-Venus clone 1" digested with BglII/XhoI.
 Expected 6609+1554+813
Lane 3: "Nucleolus-Venus clone 1" digested with BglII/PvuI
 Expected 6331+1832+813
Lane 4: "Nucleolus-mKate clone 2" digested with BglII/XhoI
 Expected 4460+1738++1536+813+290
Lane 5: "Nucleolus-mKate clone 2" digested with BglII/PvuI
 Expected 5622+2402+813
Lane 6: Ladder
```



Second test digest, done according to the lanes shown above. Lanes 2 and 4 are the same, as are 3 and 5.

- Results of this digest are that I think clone #1 is correct, but clone #2 is switched. Went back and relabeled with small white dot in freezer box.
- We're still going to go ahead with the yeast genome transformations.
- I digested my plasmids with NotI and SacII. Each digestion was 20ul and there were 4 total digestion tubes. I digested the following clones:

```
Nucleolus-Venus: Clone 1 (sent for sequencing and just test digested)
Nucleolus-mKate: Clone 2 (clone 3 was sent for sequencing, clone 2 was just tested by test digest s
NP-Venus: Clone 2 (sent for sequencing)
NP-mKate: Clone 2 (sent for sequencing)
```

- After 2 hours of digestion, I did a Zymo (small surface-area column) cleanup and eluted into 10ul of water.
- Harneet and Vincent prepared some yeast cells with LiOAc. Those will keep for ~2 hours.
- I'll take 50ul of the cells suspended in LiOAc + the 10ul of my Zymo cleanup elution.
- I screwed up and added 117 of ssDNA instead of the 117 of cells+mastermix. So, I made a new cell+mastermix without ssDNA volume and added 3x the amount necessary for each transformation. So each tube has...

```
10ul of digested plasmid that was Zymo purified
117ul ssDNA
240ul 50% PEG
36ul 1M LiOAc
50ul ddH2O
```

- With this, I'll go through the same process, but will have a larger pellet. The 10ul of DNA should hopefully be plenty for the transformation.
- After the heat shock and pellet, I resuspended in 300ul of water and only applied 100ul to the plates.
- I sent Nucleolus clone 3 out for sequencing. F reaction using S16 is sIGEM066. R reaction using AF47 is SIGEM067.

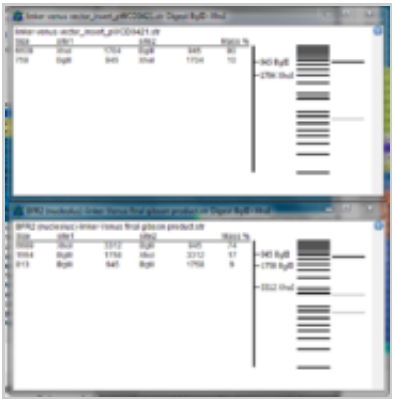
Robert.c 18:30, 8 June 2012 (PDT)

Thursday 6/7

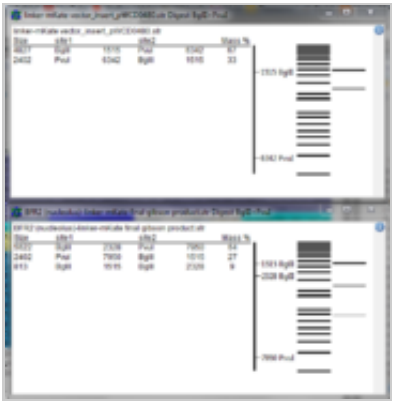
Today's agenda:

- Grow 3ml of culture up for minipreps. We picked 3 colonies per plate. Plates are kept in deli fridge.
- Checked that the insert didn't have NotI or SacII in it. Neither NUP84 (nuclear periphery) nor BFR2 (nucleolus) had those sites, so we're good for the genomic insertion step later.
- I need to do the miniprep and run test digests on the vectors.

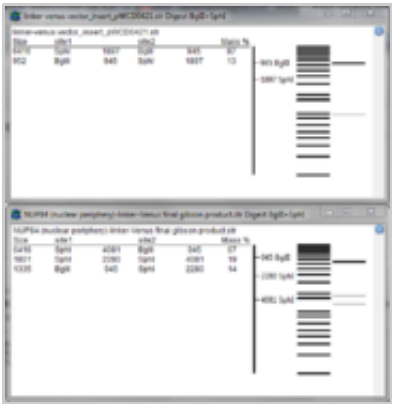
```
BFR2-Venus: BglII/XhoI (NEB3), 6609+759 parent, 6609+1554+813 expected.
BFR2-mKate: BglII/PvuI (NEB3), x4827+2402 parent, 5622+2402+813 expected.
NUP84-Venus: BglII/SphI (NEB2), 6416+952 parent, 6416+1801+1335 expected.
NUP84-mKate: BglII/SphI, 6295+934 parent, 6295+1783+1335 expected.
```



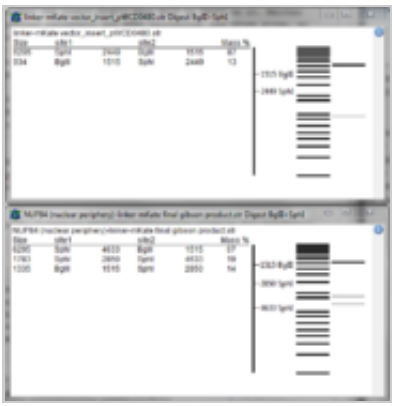
Test digest of linker-Venus parent vector and BFR2-linker-Venus gibson product with BglII/XhoI.



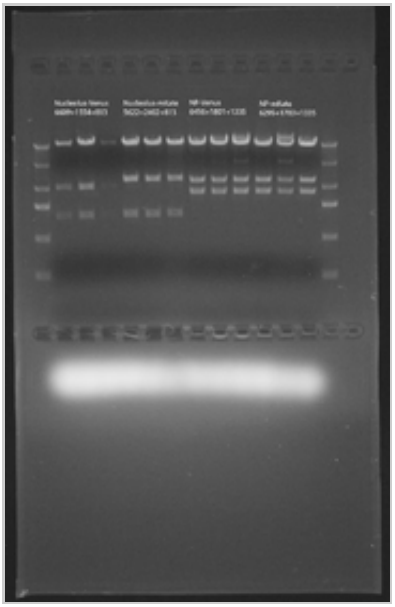
Test digest of linker-mKate parent vector and BFR2-linker-mKate gibson product with BglII/PvuI.



Test digest of linker-Venus parent vector and NUP84-linker-Venus gibson product with BglII/SphI.



Test digest of linker-mKate parent vector and NUP84-linker-mKate gibson product with BglII/SphI.



Results of test digest. An issue that may have arisen is that Nucleolus Venus and mKate were switched. Clones were in right order, but they might have been swapped. Will run another test digest tomorrow to fix.

- I might've switched Nucleolus Venus and mKate. The results of this swap would be:

```
"Nucleolus-Venus" cut by BglII/XhoI
  expected (if actually Venus): 6609+1554+813
  wrong (if mKate): 4460+1738+1536+813+290
"Nucleolus-mKate" cut by BglII/PvuI
  expected (if actually mKate): 5622+2402+813
  wrong (if Venus): 6331+1832+813
```

- Afterwards, I need to send the miniprepped plasmids for sequencing. I used S16 forward primer and AA30 reverse for venus AF47 for mkate.
- Summary of sequencing:

```
NUP84-Venus (nuclear periphery): Clone 2, pRC001 vector, sIGEM058+059 sequences
NUP84-mKate: Clone 2, pRC002 vector, sIGEM060+061 sequences
BFR2-Venus (nucleolus): Clone 1, pRC003 vector, sIGEM062+063 sequences
BFR2-mKATE: Clone 3, pRC004 vector, sIGEM064+065 sequences
```

Robert.c 21:29, 7 June 2012 (PDT)

Wednesday 6/6

Today's agenda:

- Transformations did not work. It was likely the TG1's problem because the + control even did not work.
- We're going to use the commercial TG1 cells and follow protocol (http://dueber.berkeley.edu/wiki/Template:IGEM_Electroporation_Protocol) exactly.
- I'll check on plates tomorrow.

Robert.c 10:04, 7 June 2012 (PDT)

Tuesday 6/5

Today's agenda:

- Back-dilute (we did 50-fold dilution of the culture from last night) and allow to grow back up to 0.6-0.8 OD600. When at that concentration, it should be mid-log growth. We then chilled on ice, then washed 4x with 10% glycerol in water. We then resuspended in 1ul of the 10% glycerol.
- With the electro-compotent cells, we did electoporation to transform in our Gibson product. Instead of transforming with 30ul of starting bacteria solution, we used 50ul. We still used 1ul of DNA. After the rescue, we resuspended in what was left after pouring off the broth and plated all of it. We then plated them on LB-amp and grew them overnight.
- I transformed a pWCD437 vector into our TG1 to act as a cell positive control.
- We watched Celia and Austin do their genome transformations.

Tomorrow, I will be out earlier. I will need to:

- Check on plates. If they didn't work and the + control also didn't work, then it's the cells.
- If they did work, I will pick a colony or two, miniprep it up, and separate out the plasmid from the miniprep broth later in the afternoon.
- Then, I will do a test digestion and send it for sequencing.

Robert.c 18:41, 5 June 2012 (PDT)

Monday 6/4

Today's agenda:

- Grow up two 25-ml cultures of TGI, which will be turned into electropore-competent cells later today by glycerol washes. We want to halt the TGI growth during the logarithmic phase.
- Do electropore transformations with the TGIs.
- Make more LB agar with ampicillin plates.
- Do Gibson assembly with the redone PCR inserts from last Tuesday. Need to do this tomorrow instead.

Meeting Discussion:

- For gold, we should improve or characterize a part.
- We should go through the parts registry and see what we could maybe incorporate into our design.
- Maybe include PGAL inducible promoter? Maybe homing endonucleases?
- We need to be very clear and standardized about how we collect our fluorescent images.

Robert.c 17:15, 4 June 2012 (PDT)

Friday 6/1

Transformations were unsuccessful. Plates did not grow up for most people. Austin, Celia, and Thomas got a few (1-3) colonies. Plan of action:

- Linearize vectors for Gibson using PCR primers. We want to choose a F primer after the BglII site, and R primer before. They'll copy in opposite directions and make a linear strand. We ended up choosing:

```
Venus: AU60 F, AD59 R
mKate: AU62 F, AD59 R
```

- After running the PCR we need to digest with BglII because one of the primers contains a BglII site. Digesting with BglII will make one end of the linearized vector have a blunt end, the other have a sticky end. They shouldn't come back together.
- Electroporation to increase transformation efficiency. For this, we need competent cells.

Robert.c 15:01, 4 June 2012 (PDT) (forgot to timestamp on Fri)

Retrieved from "https://dueberlab.com/w/index.php?title=Robert_Notebook_June_2012&oldid=10709"

- This page was last modified on 1 July 2012, at 18:42.
- This page has been accessed 256 times.

Robert Notebook May 2012

From Dueber Lab Wiki

Contents

Thursday 5/31

Today, we:

- PCRed up the Gibson inserts using YJD001 (yeast genome template) and the two primers we ordered per organelle. We followed these instructions: http://dueber.berkeley.edu/wiki/Template:IGEM_Phusion_PCR. My primers were as follows:

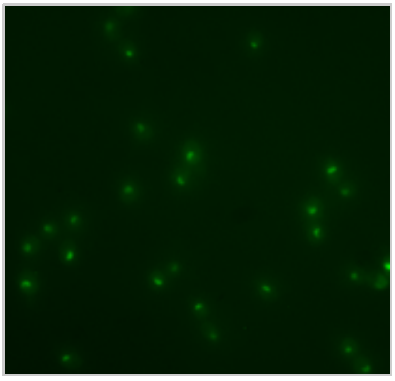
```
Nucleolus BFR2: AV03, AV04


Nuclear periphery NUP84: AV05, AV06
```

PCR tubes were labeled from the top with my initials and from the sides with BFR2 and NUP84 (in that order). Tube numbers are 1 and 2.

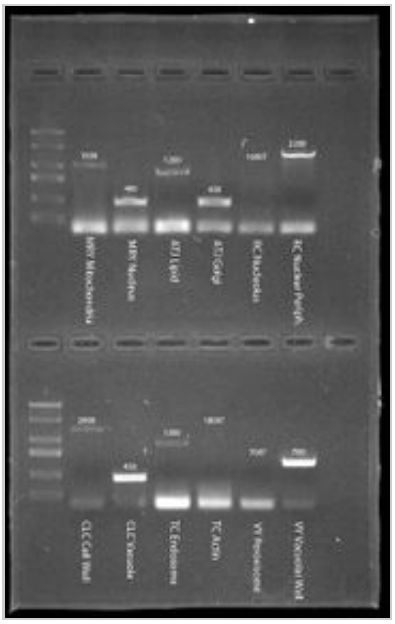
- Realized that I didn't check the protein coding region for BglII, BamHI, SpeI, XbaI sites! My BFR2 (nucleolus) has an internal BglII coding region. So, I need to choose a different protein.
- For the Nucleuolus, I chose RRP9, Protein involved in pre-rRNA processing, associated with U3 snRNP, http://www.yeastgenome.org/cgi-bin/locus.fpl?locus=YPR137W

```
F primer (AV13.iGEM0035): agaagaaaacaaaaaacaaaAGATCTatgtcagatgttacccaacag (47bp)
R primer (AV14.iGEM0036): ccgctgccactaccagaaccAGATCCaaagcctgtttggtc (41bp)
```



RRP9 colocalized with GFP onto the nucleolus of yeast cells

- We won't be ordering though, since all we want is a proof-of-concept GFP-tagging. If it becomes an issue later, we can just remove the internal restriction sites.
- With this PCR product, we will be inserting it into a vector that has been cut by BglII at once site and chewed back by exonuclease to reveal homologous regions.



Organelle PCR products to be inserted into via Gibson assembly

- My Nucleolus PCR did not work, so I redid at 55 degrees.
- We digested the pWCD480-mKate and pWCD0504-Venus with BglII in preparation for the Gibson assembly.
- We followed the Gibson protocol here: http://dueber.berkeley.edu/wiki/Template:IGEM_Gibson_Assembly, using 2.5ul of PCRed insert, 2.5ul of digested vector, and 5ul of the master mix. Master mix ran out before I added, so I used Mike's solution and added 15ul of his mix.
- The PCR rerun gel is shown below:

Organelle PCR reruns that failed to work the first time

- We transformed 50ul of TGI-KCM bacteria mix with 5ul of our Gibson product, saving the rest just in case. Plates are growing in 37 incubator.

Notes on how to choose homology region (for the future in PCR reactions):

- Should end (at 3') with a G or C (GC clamp).
- 50-65% GC content.
- Overall base composition should be balanced; no missing bases
- Ideally should be random base distribution, with no large sections of repeats.
- Sequence can be modified to be 18-25bp.
- Ideally Tm should be under 40 degrees.
- Sources: http://openwetware.org/wiki/Arking:JCAOligoTutorial1b and http://www.nfstc.org/pdi/Subject04/pdi_s04_m01_02_f.htm

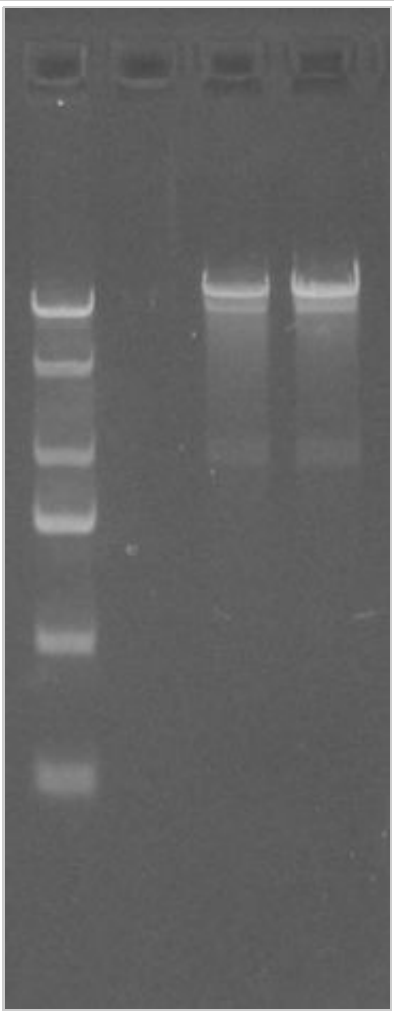
Robert.c 19:56, 31 May 2012 (PDT)

Wednesday 5/30

Today, we:

- Checked on the plates. Product plate had ~200 colonies. Negative control had ~20 colonies. We picked two colonies and grew them up in LB+amp media.

- Miniprepped the resulting growth.
- Checked the pWCD0504-PAGFP sequencing result from Friday. Because we used a reverse primer, to compare the sequence, we had to reverse complement it first. There were a few single nucleotide errors, but those were found to be due to algorithm error after checking the graphs in Finch TV>
- Test digested the plasmid to check that it was transformed correctly and that we got the correct plasmid. Spe/Bam 5800 and 1400bp should be the results from our desired product, while 5800 and 3400bp should be the results from an incorrect plasmid.



pWCD480-mKate SpeI/BamHI test digest

- Worked, so we'll send both tubes for one sequence each, one forward (Z71) and one reverse (O44) to cover more area with the reads. In total, we'll be sending 2 tubes. The other group doing pWCD421-Venus is doing the same. Our sequences are cataloged in our system as sIGEM004 and sIGEM005.

Robert.c 18:31, 30 May 2012 (PDT)

Tuesday 5/29

Will's question: How do we get the barcode and the miCode cassette to associate one-to-one in the final plasmid?

- Associate each organelle with a residue?
- How do we link the barcodes with the cassettes without doing it manually?
- The idea is to make the cassette out of individual components that have a tag and coding region. When assembling the components together, the tags condense into the barcode and the coding regions condense into the cassette.

```
An example for the nucleus:


GFP: ---[G tag]-|-|-(GFP coding)---


RFP: --|-[R tag]---(RFP coding)-|--


When you cut at the sites shown: | you can insert all of RFP (tag + coding) into GFP to get:
---[G]-[R]---(RFP)-(GFP)---


By alternating the enzymes used, you can sequentially add more sequences into the middle.
```

- We decided on using two pairs of enzymes that form a scar together: BglII/BamHI and SpeI/XbaI

```
BglII  A|GATCT
       TCTAG|A
BamHI  G|GATCC
       CCTAG|G
```

```
SpeI   A|CTAGT
       TGATC|A
XbaI   T|CTAGA
       AGATC|T
```

Today we:

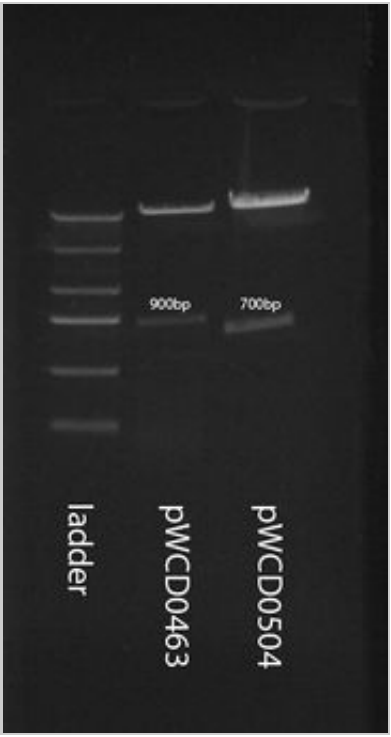
- Finished the SOEing reaction (PCR phase two)
- Gel purified the result
- Digested the SOEing result and a vector (pWCD0480) with BglII and PstI
- Ligated the cut insert and vector together. Also made a negative control with just the vector ligated back onto itself.
- Transformed TGI with the plasmids (product and negative control) and grew them on separate plates overnight.

Robert.c 10:17, 30 May 2012 (PDT)

Friday 5/25

Test digestion run of vector and vector+PAGFP plasmids:

- Left lane = ladder
- Middle lane = just vector (463)
- Right lane = vector with PAGFP ligated (504)
- Digested with BglII/XhoI. Just vector should have 6000 and 900bp bands, while vector+PAGFP should have 6000 and 700bp bands.



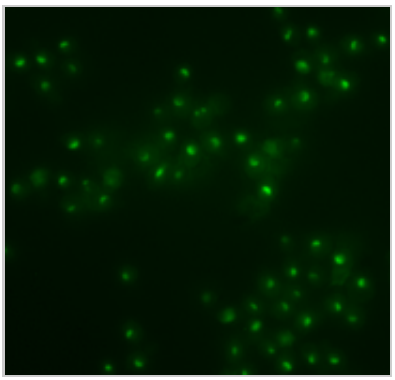
PAGFP BglII/XhoI test digest

Send for sequencing:

- 5ul plasmid
- 10ul primer volume (O44) at 0.66uM. To get this, we take the 100uM stock primer, dilute it 100x (990ul water + 10ul primer), and add 10ul of that 100x dilution to the final sequencing reaction.

GFP-linked Proteins
Nucleolus: BFR2, Essential protein that is a component of 90S preribosomes, http://www.yeastgenome.org/cgi-bin/locus.fpl?locus=YDR299W

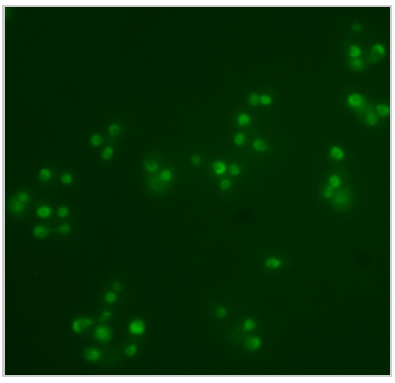
```
F primer: agaagaaaacaaaaaacaaaAGATCTatggaaaaatcactagcgga (46bp)
R primer: ccgctgccactaccagaaccAGATCCaccaaagatttggatatcat (46bp)
```



BFR2 colocalized with GFP onto the nucleolus of yeast cells

Nuclear periphery: NUP84, Subunit of the nuclear pore complex, http://www.yeastgenome.org/cgi-bin/locus.fpl?dbid=S000002274

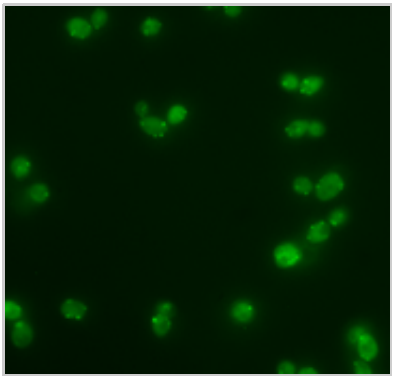
```
F primer: agaagaaaacaaaaaacaaaAGATCTatggaattatcccctactta (46bp)
R primer: ccgctgccactaccagaaccAGATCCgttcgaaagggttgctgtgc (46bp)
```



NUP84 colocalized with GFP onto the nuclear peripheryof yeast cells

Peroxisome: PNC1, Nicotinamidase that converts nicotinamide to nicotinic acid as part of the NAD(+) salvage pathway, http://www.yeastgenome.org/cgi-bin/locus.fpl?locus=YGL037C

```
F primer: agaagaaaacaaaaaacaaaAGATCTatgaagactttaattgttgt (46bp)
R primer: ccgctgccactaccagaaccAGATCCtttatccacgacattgatgt (46bp)
```



PNC1 colocalized with GFP onto the peroxisome of yeast cells

mKate Vector Creation
We got the 4 different oligos today to make the mKate vector. We put the external forward + internal reverse, and internal forward + external reverse in their own tubes to get the first round of PCR amplification. Next, we'll combine the products with external forward + external reverse to do a sewing reaction.

Robert.c 17:06, 25 May 2012 (PDT)

Thursday 5/24

Thurs 5/24 to-dos:

- Miniprep the culture seeded from the transformation plate from yesterday (4pm)
- Keys (1pm)
- Protein lookup
- Oligo ordering

Yeast GFP Colocalization Database Search

Methodology

1. Go to http://yeastgfp.yeastgenome.org/ 'Advanced Search'
2. Under 'Display Options', uncheck the 1st 'Show graphical scoring...' and check the 3rd 'Show ORF Summary Table'
3. Under 'Search Criteria' select organelle of interest
4. Submit
5. You'll have a list of ORFs that localize to the organelle. Search for the ones that have a check mark only in the column for your organelle of interest and have no modifying letters (H, M, B).

6. Check the ones that have a reasonable TAP Abundance since that indicates number of molecules/cell
7. At the very bottom of the table, go 'Display Localizations for these ORFs'
8. Click on the cartoon yeast cell diagrams on the right to see the color images taken. If the images show that the protein seems to localize correctly, click on the ORF name.
9. This takes you to the http://www.yeastgenome.org/ page for your protein, which gives you all the information about it.
10. While on the 'Summary' tab, scroll down to the 'Sequence Information' section and click on the 'Get Sequence' button to get the sequence coding for the protein.

Nucleolus:

- BFR2, Essential protein that is a component of 90S preribosomes, http://www.yeastgenome.org/cgi-bin/locus.fpl?locus=YDR299W
- RRP9, Protein involved in pre-rRNA processing, http://www.yeastgenome.org/cgi-bin/locus.fpl?locus=YPR137W
- NAN1, U3 snoRNP protein, component of the small (ribosomal) subunit (SSU) processosome containing U3 snoRNA, http://www.yeastgenome.org/cgi-bin/locus.fpl?locus=YPL126W

Nuclear Periphery:

- PML39, Protein required for nuclear retention of unspliced pre-mRNAs along with Mlp1p and Pml1p; anchored to nuclear pore complex via Mlp1p and Mlp2p,

http://www.yeastgenome.org/cgi-bin/locus.fpl?locus=YML107C

- NUP84, Subunit of the nuclear pore complex, http://www.yeastgenome.org/cgi-bin/locus.fpl?dbid=S000002274
- NUP188, Subunit of the nuclear pore complex, http://www.yeastgenome.org/cgi-bin/locus.fpl?locus=YML103C

Peroxisome:

- GPD1, NAD-dependent glycerol-3-phosphate dehydrogenase, http://www.yeastgenome.org/cgi-bin/locus.fpl?locus=YDL022W
- PEX25, Peripheral peroxisomal membrane peroxin required for the regulation of peroxisome size and maintenance, http://www.yeastgenome.org/cgi-bin/locus.fpl?locus=YPL112C
- PEX5, Peroxisomal membrane signal receptor for the C-terminal tripeptide signal sequence (PTS1) of peroxisomal matrix proteins, http://www.yeastgenome.org/cgi-bin/locus.fpl?locus=YDR244W
- CTA1, Catalase A, breaks down hydrogen peroxide in the peroxisomal matrix formed by acyl-CoA oxidase (Pox1p) during fatty acid beta-oxidation, http://www.yeastgenome.org/cgi-bin/locus.fpl?locus=YDR256C (use this because it is located within peroxisome)

Robert.c 14:30, 24 May 2012 (PDT)

Plasmid Design

- Celia, Harneet, Robert
- Plasmid pWCD0480, which has mKate (RFP) in it that we want. We will want to modify the plasmid into a template, which we will insert our FP-linked proteins.
- To do this, we will make the active site of the vector into this: --(promoter)--(BglII)--(linker)--(GFP/RFP)--(BamHI)--(STOP codon)--(XhoI)--
- Plasmid is currently like this: --(promoter)--(BglII)--(pot1)--(mKate)--(DOD)--(BamHI)--(STOP)--(Xho)--(terminator)--(PstI)
- Plasmid we currently have has more than 1 XhoI site, so we need to use an alternate restriction site, PstI
- We will do a splice to cut out DOD, which we don't want. For this, we'll use the (BamHI)--(STOP)--(Xho) area as a homologous overlap region.
- We'll run 3 PCR reactions:

1. One to PCR out mKATE and add BglII and a linker region. F primer inserts the BglII and linker and overlaps with mKate. R primer overlaps with both mKate and (BamHI)--(STOP)--(Xho).
2. Two to PCR out the (terminator)--PstI region. F primer overlaps with both (BamHI)--(STOP)--(Xho) and terminator. R primer overlaps with PstI and extra junk on the end.
3. Glue those two together to have a final sequence of (BglII)--(linker)--(mKate)--(BamHI)--(STOP)--(XhoI)--(terminator)--(PstI)

- We'll need 4 primers total, because in the 3rd PCR, we'll use some of the same primers.
- When we insert the gene, the result will be: (A)GATCT--gene--G(GATCT)--linker--GFP with the parenthesis being parts of the BglII site that we clone into.
- Going to order the plasmids tomorrow.

Robert.c 17:56, 24 May 2012 (PDT)

Wednesday 5/23 Developments

- We found a really good database with GFP-localized proteins, so we will use that primarily
- Want to order oligos tomorrow, but we need to know the regions that will eventually flank the gene first.
- Layout of plasmid: (promoter)--(Gene)--(GFP/RFP)...
- Today, we added more to the Google Doc of targeting
- We ligated the test vector and insert together, and transformed bacteria with them.
- Tomorrow, we will see the colonies and compare to the negative control (just vector).
- Tomorrow, we need to do a miniprep on the growth we started on Tuesday.

Robert.c 15:37, 23 May 2012 (PDT)

Targeting Research

(4) Golgi targeting:

- Going from ER to endosomes: cis-Golgi network --> Golgi stack (cis, medial, trans cisternae) --> trans-Golgi networks (http://www.springerlink.com/content/y0l390681p73585w/)
- pEYFP-Golgi plasmid for mammalian cells (http://www.ebiotrade.com/buyf/productsf/clontech/632358.htm)
- A-sensitive trans-Golgi network-localised protein p230 (http://jcs.biologists.org/content/112/11/1645.short)

(5) ER targeting:

- Calreticulin;KDEL retrieval sequence (Bos taurus) targets to ER lumen (http://www.amalgaam.co.jp/products/coral_hue/organelle.html)
- KDEL at C-terminus (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC207004/)
- KKAA localized to ER permanently, while KKFF have access to post-ER compartments (http://www.jbc.org/content/274/21/15080.short)
- H2N-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln- from Wikipedia

(10) Vacuole targeting:

- "Our studies indicate that the tetrapeptide QRPL found very near the NH2-terminus of proCPY functions as a vacuolar targeting signal." (http://jcb.rupress.org/content/111/2/361.full.pdf)
- 24-QRPL-27 in Pro-region of CPY allow interaction with Vps10p (http://jcb.rupress.org/content/111/2/361.abstract)
- VPS10 codes for vacuole sorting of CPY (http://www.cell.com/abstract/0092-8674%2894%2990219-4)

(11) Microtubule targeting:

-

Meeting thoughts:

- We want to look into both the specific tag that localizes a protein and proteins that always localize that we can piggyback off of.
- Want to add stuff to the Google Docs so we can add together.

Progress:

1. Mitochondria - Leads, couple potential localized proteins
2. PM - How it looks depends on the proteins used. Spotted ones localize at lipid rafts.
3. Nucleus - 7residue N-terminus. Nucleus is well-defined. Celia found protein that stays in nucleus that we could piggyback GFP onto.
4. Golgi - A couple of proteins localized
5. ER - Lots of short 4-residue targets

6. Lipid rafts?
7. Chromosome?

Properties of targeting sequences that we like:

- Shorter sequences are better for cloning ease.
- Stay localized.
- Previously used to target fluorescent protein.
-

Robert.c 15:37, 23 May 2012 (PDT)

Project Discussion

Looks like we're going to do miCodes.
Organelles:

1. Mitochondria
2. PM
3. Nucleus
4. Golgi
5. ER
6. Cortical ER
7. Actin
8. Bud scar
9. Endosome
10. Vacuole
11. Microtubule
12. Nucleolus

Thoughts:

- We'll want to use two colors at a time. That way, you can convert the colors into a black-white image to see if you can tell them apart.
- First things to look up are address tags.
- Controls: antibodies, dyes that target specific organelles
- Each person take 4 organelles, find 3 ways to target. No need for peroxisome.
- I am working on: (4) Golgi, (5) ER, (10) Vacuole, (11) Microtubule.
- Celia also has #4 and 10
- Harneet also has #5 and 11.

- Look up any way to get a fluorescent protein to target to the organelle.

Robert.c 14:54, 21 May 2012 (PDT)

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