May2012AJ

From Dueber Lab Wiki

--Austin.jones 11:15, 21 May 2012 (PDT)

cloning techniques

1. BglBrick/BioBrick
2. Gibson Assembly
3. Golden Gate
4. PCA
5. DNA Assembler
6. yeast genomic integrations with homing endonucleases (PMID: 21876185)

Golden Gate Assembly: plasmid each containing 1 gene of interest flanked by nucleotide cleavage sites and opposite restriction enzyme sites restriction enzymes have 4N sticky ends

75ng of each plasmid type II restriction enzymes (bsaI) ligase 97% efficiency if completely unique complementary ends at all 4 sites, 240 possible (256- 16 pallandromic) one pot one step reaction, more efficiency with less number of steps

micodes: barcodes into yeast to tell genotype of a cell upon looking under a microscope

library size: 2^3^6~200k raising one power dramatically increases the size (8^7- 2million) confers the ability to distinguish between different organelles

applications:

1. testing protein protein interactions (one with tag and other with FP): if they interact they will glow in a localized spot
2. FACS separating by fluorescence (single measure), we get information on location and combination using multiple phenotypes
3. protein shells (carboxisome) learn balance of formation of a product with multiple genes with promoter sequence/product

locations to target fluorescence: 1.mitochondria 2.PM 3.nucleus 4. golgi 5. ER 6. cortical ER 7. actin 8. bud scar 9. endosome 10. vacuole 11. peroxisome 12. microtubule 13. nucleolus 14. other

2, 8, 1, 7, 14 end of the day tomorrow find address tags in lit search choose the possibilities that can be visually differentiated

controls: dyes or antibodies to confirm that it is the organelle each take 4, compare top signaling sequences may choose membranes vs lumens of large organelles (vacuole and lumen)

nucleus: PKKKRKV

mitochondrion: http://www.pnas.org/content/96/21/11752.full.pdf 15–40 amino acid residues and is enriched in positively charged and hydroxylated (mostly serine) residues. In mitochondrial targeting peptides (mTPs), Arg, Ala and Ser are over-represented while negatively charged amino acid residues (Asp and Glu) are rare.

ATP2 deletion mutants provide evidence that the amino terminal 27 aa of the 13L subunit precursor are sufficient to direct specific sorting of the yeast protein to the mitochondria 27 aa: atggttttgc caagactata tactgctaca tcccgtgctg cttttaaagc agccaaacaa tccgctccgc ttctatccac t

(http://www.ncbi.nlm.nih.gov/gene/853585)

actin: http://jcb.rupress.org/content/107/6/2551.full.pdf a c t in-binding prot e in (designated ABPI)

plasma membrane: lipid rafts? NSF?

bud scar: http://www.science.uva.nl/research/mc/web%20protocollen/3-4-3.htm Wheat germ agglutinin (WGA) binds to N-acetyl-D-glucosamine

control dyes: http://www.science.uva.nl/research/mc/web%20protocollen/3-4-1.htm

--Austin.jones 09:12, 22 May 2012 (PDT)

Assembled oligos for PCR of PAGFP (ordered from addgene) for subsequent insertion into pWCD0436.

forward: gcatAGATCTatggtgagcaagggcgag reverse: atgcCTCGAGtcaGGATCCcttgtacagctcgtccat

Ran PCR at 65C. Product will be ~700 bp. Ran on a gel and cut out band. Digested along with vector plasmid using BglII and XhoI. Let sit at 37 for 2hrs. Ran on a gel. Bands looked good. Placed in 300 uL ADB for overnight.

other organelles: vesicle transport- between ER and golgi with tagging COPIII (inner ring of cell) and between golgi and PM (outer ring of cell) targetting clathrin spots vs solid membrane

ER- KDEL sequence

ideal properties of targeting sequences:

- small/short
- localized
- previously used to target FP

--Austin.jones 11:02, 23 May 2012 (PDT)

Continued updating the google doc with all of the possible locations for FPs. Found a UCSF study that has fused GFP with proteins localized in organelles/locations around the cell and accompanied with photos to test our ability to distinguish them apart from each other. Added these proteins to the google doc as well. Well develop primers for these already developed proteins in the UCSF study to amplify from the yeast genome.

Finished zymo cleanup. Ligated the PAGFP with backbone along with a control not including the insert in order to see how many vectors were only cut by one endonuclease in the digest and what will be background upon transformation.

Yeast Genes (http://yeastgfp.yeastgenome.org/) UCSF localized proteins fused with GFP (http://yeastgfp.yeastgenome.org/info.php)

--Austin.jones 16:55, 24 May 2012 (PDT)

Designed primers for endonuclease cloning of Venus GFP gene in pWCD0421 along with linker GS sequence. Will order them tomorrow.

1. Forward:gcatAGATCTGGATCTggttctggtagtggcagcggcagcggtagtatgtctaaaggtgaag
2. Reverse:ctagCTCGAGttaGGATCCtttgtacaattcatccatac

Attempted at designing primers for the subsequent Gibson Assembly to fuse with the organelle specific proteins. Encountered trouble in their design so will ask Will tomorrow. Miniprepped pWCD0436-PAGFP.

--Austin.jones 12:33, 25 May 2012 (PDT) Designed oligos for the Gibson assembly and ordered them. Created oligo, plasmids, and sequencing pages on the google group. Ran a test digest of the miniprep to check accuracy. Everything looks good, band was bright. Sent for sequencing to Quintara.

--Austin.jones 12:14, 29 May 2012 (PDT)

14 locations Finish vector Igem goals Automates analysis

Linking genotype to micode

1. Random assembly

Many mutations on one protein Barcode followed by cassette combination of genes Hi throughput sequencing tells combination of micode and enzyme variant

1. Predetermined

Design combinations with Nicole's explicitly then mix with different organelle genes Low combinations on many components

Ran gel of PCR product.Band looked good at 700 bp zymo purified. Digested PCR product for 1.5 hts amepWCD for 2hrs at 37C. Gel purified. Ligated for 20 mins at rt. Transformed into tg1 cells and plated on amp. Let grow overnight

Discussed mechanism to construct micode attached to yes/no gene will maintaining the link. Will do three consecutive digestions using speI/xbaI and Bamhi/bglIi scaring to reduce to only 4 endonucleases

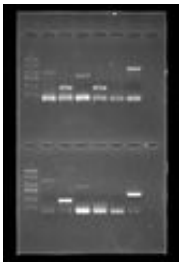
--Austin.jones 15:26, 30 May 2012 (PDT) **plan**: Pick colonies, grow up, miniprep, test digest, sequence **day**:Only 5 colonies grew up. We did not create a negative control for the ligation to test how much background there was. Picked colonies and grew up in the shaker with LB/amp for 6 hours. Miniorepped all the samples and test digests using BglII XhoI showed correct size bands. Sent two for sequnecing.

Sequencing returned for the PFGFP which looked good (mistaken deletion in sequence but chromatogram proved sequence correct.

--Austin.jones 09:52, 31 May 2012 (PDT)

Plan: PCR both the Golgi (YCR043C) and Lipid Particle (YML008C) genes out of the genome. Run Gibson assembly to fuse with the linker-Venus sequence. Transform into tg1 cells. **Day**: ran PCR both the Golgi (YCR043C) and Lipid Particle (YML008C) genes out of the genome using 65 C with 45s annealing. PCR was successful producing 400 and 1200 bp bands respectively. Zymo purified the products adding 250ul of isopropanol to the Golgi product.

LipidGolgi.jpg



Lipid (lane 3) Golgi (lane 4)

Caption2

Sequencing for 421-linker-Venus confirmed a correct sequence. Digested 421- linker Venus for gibson assembly. Let sit at 37C for 2 hours. Zymo purified. Ran the Gibson assembly using 2.5uL of both insert (Golgi and lipid particle PCR products) and vector(bglII digested pMRY001 421-linker-Venus and pHSR001 421-linker-mkate) and 15uL of 2x lab made gibson media. Heated at 50 for 1hr in thermocycler. Chemically transformed into tg1 and plated on amp.

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June2012AJ

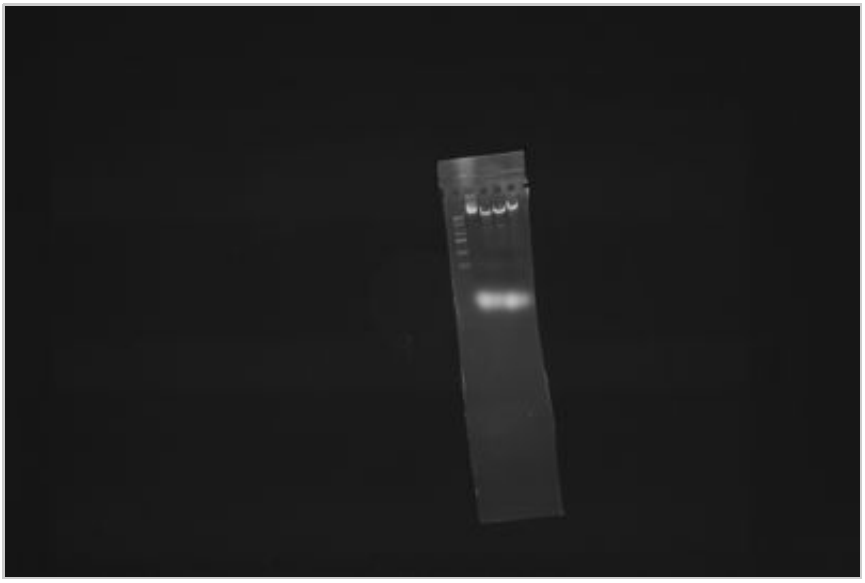
From Dueber Lab Wiki

--Austin.jones 09:59, 1 June 2012 (PDT)

plan: check plates, grow up in liquid media, miniprep, sequence.
Day: There were no colonies present for either pATJ001 or pATJ003 (both Venus) while there were only three colonies for pATJ004 and one for pATJ002 (both mKate). Most people did not have colonies, the few that did were successful in mKate but not Venus. One colony grew on the negative control, a vector that ligated to itself without the insert. Picked and grew up the 4 total colonies in LB amp for 7 hours.
To address low colony numbers: Ran electroporation transformations using pMRY-001 and pHSR-001 (2 negative controls, 2 mKate PCRs faint/bright, 2 Venus PCRs faint/light) to increase the efficiency of transformation. Grew up for 1hr in plain LB at 37. Spun down, threw out supernatant, and plated 10uL of concentrated cells onto amp plates. Placed in 37 for overnight.
To tackle colony on the negative control: Found primers in the database (AD59.omL182) to PCR the Gibson vector to eliminate sticky ends that would ligate to each other and prevent the insertion of the vector. One primer has extra bases that will be digested leaving one sticky and one blunt end in the vector. Ran orginal PCR at 65 with HF buffer but yeilded incorrect products. Reran at 55 with varying HF and GC for each reaction.

--Austin.jones 14:30, 2 June 2012 (PDT)

Plan: Test digest and sequence minipreps of pATJ002 and pATJ004.
Day: Moved plates from Friday's lectroporation transformations to the deli fridge. Ran a test digest on the four minipreps (1 sample of pATJ002 and 3 samples of pATJ004) using EcoRI and BamHI with 4uL of plasmid DNA. Let incubate at 37C for 15 minutes. Ran them on a gel expecting bands of around 6k and 2k. The 6k band looked larger than expected for all the samples but for samples 1 and 2 of pATJ004, there were faint insert bands present. Sent pATJ002 and samples 1 and 2 of pATJ004 for sequencing (lanes 2-4) Used primers O44 (reverse, binding downstream of mKate) and S16 (binds upstream of the promoter). Mixed 7uL of primer, 5uL of plasmid, and 3uL of water.



left to right: pATJ002, pATJ004a, pATJH004b, pATJ004c

--Austin.jones 10:18, 4 June 2012 (PDT)

Plan: Analyze sequencing data and redo transformations as needed.
Day Sequencing was received from Quintara. pATJ002 sequenced well on but both samples of pATJ004 showed primer dimers of the lipid particle insert. Will continue with another round of transformation, both of pATJ004 and the failed pATJ001 and pATH003 (Venus), once TG1 electrocomp cells are finished being made by the end of the day. **this week**: pATJ002 is confirmed. Need to assemble pATTj001, 003, 004. I have the PCR products of YCR043C and YML008C from the yeast genome. Need to BglII digest pMRY001 (Venus) and pHSR001 (mKate) and run GIbson assembly to recreate pATJ001, 003, and 0004. Confirm through test digests and sequencing. Send lipid 3 for sequening.

meeting: Check failed sequencing and see if possible primer dimers that were the truncated insert.
bronze- wiki, submit one part
silver: characterize function of biobrick part
gold: improve function of another biobrick part, help another iGEM team, or human practices (steer away from human practices) optimize fluorphores for yeast look at yeast parts in the regestry and check characterization (pGal promoter, homing endonucelase dsDNA breaks for high efficiency entry into the genome) special prizes: best biobrick advance, best of tracks: foundational or informational barcoding technology for specific focus or notice that registry doesnt have good parts or for targeting in yeast to improve the registry, comercial and non comerical options for this, dont rinvent something that already exists jobs: look for software, keep tabs on what is due when, familiarize yourself with yeast parts in the registry, knowing in general what is on there to build off of it standardize fluorescent microscopy, detail about how the images are taken

--Austin.jones 15:29, 5 June 2012 (PDT) **plan**: BglII digest pHSR001 and pMRY001 (2 hrs) 915-1115am. Run on gel and purify 12pm. Set up and run gibson 12-1. electroporation into Tg1 cells 115-130. recover for 1 hr in media 230. plate. **day** Not enough pHSR001 or pMRY001 to carry out the Gibson reaction. Harneet began growing more up yesterday. I will miniprep the saturated colonies later today and start the Gibson tomorrow.

Started the genome integration. Took saturated yeast cells finding an OD600 of .3 after a 100x dilution. Assumed to be .15 (or 15x concentration) so took 75 mL of cells into in 5mL of media and let them grow until reaching an OD of ~.8 for about 4 hours. Linearized the plasmid for more efficient recombination into the genome. Double

digested pATJ002 with 8 uL DNA, 8 uL of H20, 2 uL of EcoRI buffer, and 1 uL of each restriction enzyme (NotI and SacII). Let the double digest sit at 37 for 2 hours. Zymo purified and ran on a gel to check the size of the product. Saw three bands of the wrong sizes. It appears that SacI was accidentally used for the double digest instead of SacII. Reran the double digest under the same conditions with NotI and SacII. Let them digest for 45 minutes. Transformed into the yeast genome. Recovered for 35 minutes at 30C then at a 55C heat bath for 25 minutes. Plated on uracil- media.
Miniprepped both pHSR001 and pMRY001 for use tomorrow. Sent for sequencing with Z71 and O44 using 7uL of primer, 5uL of plasmid, and 3uL of H20. Also sent pATJ004 with O44 and S16.

W**plan**: Check sequencing for pHSR001 and then digest pHSR001 and pMRY001 (2 hrs) 915-1115am. Run on gel and purify 12pm. Set up and run gibson 12-1. electroporation into Tg1 cells 115-130. recover for 1 hr in media 230. plate.

day: Sequencing confirmed both pHSR001.1 and pHSR001.2. Sequencing for pATJ004.3 looked good but the primers did not overlap leaving an unconfirmed sequence. Will send for sequencing later today this time using the AF53 reverse primer.
Digested 10uL of pHSR001 and 12uL of pMRY001 with BglII for 2 hrs at 37 eluting in 10uL H20 after zymo purification (high concentration). Ran PCR from JED001 yeast genome to extract the lipid and golgi parts. Ran HF Phusion at 65C with 1 minute extension time. Ran the PCR products on a gel. Band sizes are correct (400bp for golgi and 1200bp for lipid particle).

Left: Golgi Right: Lipid Particle

Gel purified and ran two different sets of Gibson reactions.

1. 2.5 uL of BglII digested pMRY001, 2.5 uL of golgi
2. 2.5 uL of BglII digested pMRY001, 2.5 uL of lipid particle
3. 2.5 uL of BglII digested pHSR001.1, 2.5uL of lipid particle
4. 1 uL of BglII digested pMRY001, 2.5 uL of golgi
5. 1 uL of BglII digested pMRY001, 2.5 uL of lipid particle
6. 1 uL of BglII digested pMRY001, 2.5 uL of lipid particle

Mixed the vector and insert, equilibrating to 50C in the thermocycler, then adding the Gibson mastermix before allowing to run in the thermocycler for 1hr. Electroporated 0.7 uL of DNA into 30uL of electrocomp cells/rxn (purchased cells, stock cells made earlier in the week failed for everyone). Electroporation worked for only 2,3,4, and 6 (but covered pATJ001/3/4). Recovered in 500uL of LB in the 37C shaker for 30 minutes. Celia spun down cells, poured out supernatant, resuspended in remaining broth and plated 25uL on amp plates for me. Kept at 37 overnight.

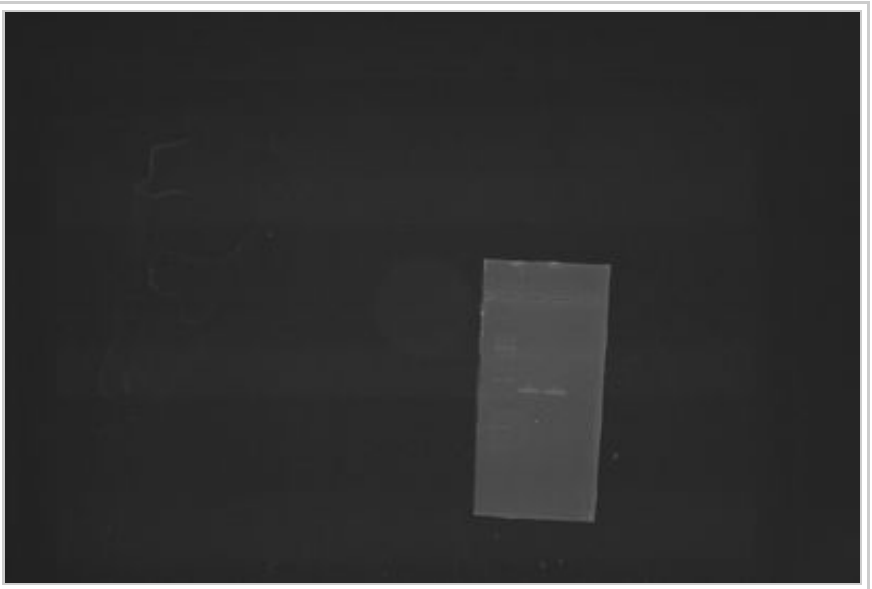
--Austin.jones 16:37, 7 June 2012 (PDT) **plan** Pick gibson assembly colonies and grow in media 9-3:30, miniprep send for sequencing 5.

day: All the plates had a good amount of colonies, although slightly smaller than normal. Picked three from each for a total of twelve and grew in 3mL of LB+amp for 6 hours. Miniprepped eluting in 50uL. Test digested using PstI and BglII for 15 minutes. Ran on a gel. Bands are difficult to distinguish; next time will digest with an enzyme whose site is in the vector. Sent 1.1a, 1.1c, 1.2a, 3a, 3c, and 3d for sequencing.

For pATJ001.1,1.2 using only S16 and for pATJ003 using S16 and Z14.

--Austin.jones 12:38, 12 June 2012 (PDT)

Plan: analyze sequencing. proceed depending on results. day: siGEM077 and 078 confirmed pATJ001. Z14 primer was mistaken for a reverse primer when it read forward on the sequence, confirming Venus and not the organelle gene. When PCRing the lipid particle gene out of the yeast genome, the wrong primers were used (70/71, not 67/68) yielding Masaki's mitochondrial gene product. However, his primers incorporated a stop codon so this construct is not viable. Will need to restart the cloning round to construct pATJ003. Next steps: PCR lipid particle gene using 67/68, gel purify, run gibson reaction with pYMR001, electroporate, grow up overnight, miniprep and sequence tomorrow.
Ran HF Phusion PCR using AU66/67 oligos at 65C for 45 sec extension time. Ran on gel and band size was correct ~1200 bp.



Lipid Particle gene

Gel purified eluting in 10uL, ran a Gibson assembly using 5uL of previously BglII digested pMRY001 and 5uL of the lipid particle gene. Let incubate for 1hr at 50C. Attempted to transform three times through electroporation but

none were successful due to arcing. Transformed 20uL of the Gibson assembly DNA with a full alliquot of cells through a KCM transformation. Let recover for 15 mins at 37 in the shaker and plated on amp.

--Austin.jones 17:58, 13 June 2012 (PDT) Plan: Check pATJ003 colonies, grow up, miniprep, test digest, sequence.

Day: Colonies looked good and were numerous. Picked four and placed in LB to grow for 6 hours. Miniprepped and ran a test digest of the four pATJ003 colones using ApaI and XhoI and NEB4 buffer. Test digest showed only one band at 9kb (size of the plasmid) without any cuts in the part.

pATJ003.1/.2/.3/.4

WIll try colony PCR tomorrow.

--Austin.jones 14:39, 14 June 2012 (PDT) Plan: Discuss with Will about the failed test digest. Transform plasmids into yeast. Day: Will recommended suspending attempting to construct pATJ003 and pick it back up later if needed. Diluted the JD003 yeast colonies grownup overnight 1:10 in YPD to achieve a 0.15 OD. Allowed to grow up 5 hours to achieve 0.8 before beginning transformations. Digested pATJ001 with SalI and NotI and pATJ004 qith NotI and PvuI (SacII stock was out) both in EcoRI buffer. Let digest for 80 minutes. Transformed into yeast cells using lithium acetate transformation. Plated pATJ001 on Leu plate and pATJ004 on Ura plate. Let sit in 30 for weekend.

--Austin.jones 10:39, 18 June 2012 (PDT) Plan: Look at transformed cells. Visualize under microscope. Day: Plated pATJ001 and pATJ004 and visualized under the microscope. Both looked nearly identical with full red glow indicative of mKate. Possible contamination from Harneet's cells? Will learn more at meeting with how to move on- whether to retransform or to drop both since pATJ003 was never successful and pATJ002 was not unique enough for identification.
everyone should be making extra venus plasmid. then in 8uL digested aliquots. nucleus brighter than most, problems with cells touching and computer detection (lower densities?) vacuole: heterogeneous, when looking at these, need to explain to an 8 year old a pattern to recognition large problem is inconsistency from cell to cell in terms of phenotype compile a list of those that are too dim collect mid log and saturated sample to visualize for each sample, in order to compare/contrast may help to narrow down by seeing pairs and taking one of each pair out three lists: 1. never 2. questionable pictures lined up: mid log, saturated, UCSF photos organize into caterogries based on shapes and pick one from each category (small dot, large dot, ring, crescent etc)

--Austin.jones 11:39, 20 June 2012 (PDT) **Plan**: Categorize organelles and look for signal sequences to replace protein chimeras **Day**: Compiled signal sequences previously found for organelles during the initial literature search in May.

- actin: MGVADLIKKFESISKEE
- peroxisome: (Will)
- nucleus: KKERLVRDKKEKKQAQKQTKLRKEKEKK/ KKRRRDFGAPANKRPRR
- vacuole: QRPL
- ER: KDEL/ KKAA/ KKFF

Phytohemmaglutin seems to be the most promising protein that is localized in the yeast vacuole (http://www.ncbi.nlm.nih.gov/pubmed/3316244) however no signal sequences seem to be known for vacuole transport or vacuolar membrane.
Found a signal sequence for cell periphery/CM exploiting GPI lipid anchors.

--Austin.jones 11:40, 20 June 2012 (PDT) **Plan**: Begin constructing mKate and Venus proteins with signal sequences to compare against current protein fused chimeras. **Day**:Narrowed down the signal sequences for the localization to organelles that are dim or diffuse with protein chimeras: vacuole, ER, and cell periphery. The vacuole's QRPL signal sequence is found in residues 24-27 and those upstream towards the N terminus are needed to target the ER where they are cleaved to expose the vacuole target sequence. Studies seem to use carboxypeptidase C (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC98912/) (http://jcb.rupress.org/content/111/2/361.full.pdf)
Plan on using KDEL for ER and CLLL and CVIM for cell periphery. Began devising cloning methods to circumvent the NotI sites in the backbone of the mKate plasmids but Will said to wait because he has devised a new method to bypass those. Began looking for direct evidence of GFP-signal sequences found in yeast before beginning the experiment.

--Austin.jones 09:39, 22 June 2012 (PDT) **Plan**: Continue finding evidence of strong fluorescence in yeast with KDEL, CLLL (Rac1), and CVIM (KRAS) signal sequences. **Day**: There is no evidence for signal sequences used to tag GFP to these proteins. Studies work with fused proteins that localize uniquely in the organelle. Dropped ER as a localization tag. Proceeded with designing primers for targeting cell periphery (CVIM and CLLL) using the Golden Gate Assembly method devised by Will.

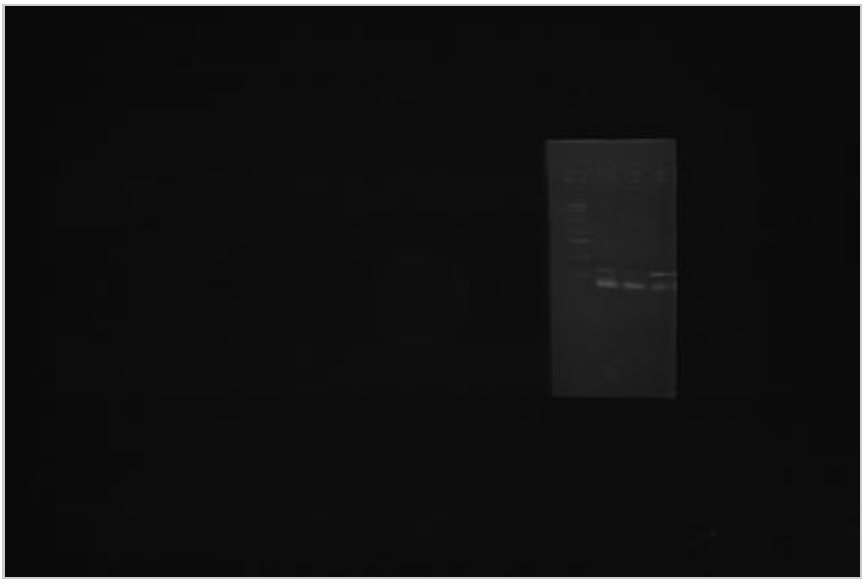
--Austin.jones 14:37, 25 June 2012 (PDT) **Plan**: meeting **Day**: Primers won't be in until tomorrow. WIll thought it would be a good idea to add a third CP tag from the Ras2 gene, adding GGCCIIC to the C terminus (CIIC being the CaaX signal). The advantage is that it well characterized to work in yeast
Monday meeting: vacuoles in different media. ER can't work with CP and nucleolus. Nucleus is bright and concentrated. Statistical determination to say that x% that it is consistent with cell type y. other directions to focus on: (1) applications (everyone for next week have one unique experiment to apply micodes to) . (2) image processing determine values of average/SD size and average/SD of intensity ---> reproducibility

applications current techniques http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2426772/ biofuels: lipid biogenesis (biodiesel): http://www.sciencedirect.com/science/article/pii/S0961953411000961 pharamcology: receptor antagonists : http://www.sciencedirect.com/science/article/pii/S1471489207001063 possible related experiement--- http://www.nature.com/nmeth/journal/v3/n5/pdf/nmeth872.pdf

--Austin.jones 16:43, 26 June 2012 (PDT) **Plan**: Run PCR when oligos arrive **Day**: PCRed CVIM, CLLL, CIIC using oligos AX35-347 (iGEM55-57). TT is ~500bp so ran extension at 20 seconds.

--Austin.jones 17:03, 27 June 2012 (PDT) **Plan**: Run on gel, xymo (1) digest (2-4), xymo ligate (20) transfrom into e coli (10) grow up overnight

Day: Ran PCR on a gel, bands look good all around 300 bp.



CVIM,CLLL,CIIS

Zymo purified and ran a Golden Gate Assembly using Bsmb1, 1.25uL of PCR product per rxn, and pWCD514. Transformed into TG1 cells using heat shock. Recovered for 1 hr (Cam) and plated.

--Austin.jones 16:05, 28 June 2012 (PDT) **Plan**: Grow up cells. Miniprep. Test digest. Sequence. **Day**: Plates looked good. Construct has an RFP dropout, picked 3 non-RFP colonies from each plate and grew up in 3mL 2YT/CAM for 6 hours. Miniprepped. Sequenced using AU45.

--Austin.jones 08:28, 29 June 2012 (PDT) **Plan**: Round 2 of GG with pATJ005-7. Transform into TG1. Plate and grow overnight. **Day**: Design of cassettes:
pATJ008: (1) Ura3_Int_5' (pWCD0526) (2) RPL18B (pWCD0530) (3) mKate (pWCD0534) (4) pATJ005 (5) Ura3_Int-3' (pWCD0560) (6)AmpR_ColE1 (pWCD0515)
pATJ009: (1) Ura3_Int_5' (pWCD0526) (2) RPL18B (pWCD0530) (3) mKate (pWCD0534) (4) pATJ006 (5) Ura3_Int-3' (pWCD0560) (6)AmpR_ColE1 (pWCD0515)
pATJ010:(1) Ura3_Int_5' (pWCD0526) (2) RPL18B (pWCD0530) (3) mKate (pWCD0534) (4) pATJ007 (5) Ura3_Int-3' (pWCD0560) (6)AmpR_ColE1 (pWCD0515)
pATJ011: (1) Leu2_Int_5' ((pWCD0524) (2) RPL18B (pWCD0530) (3) Venus (pWCD0535) (4) pATJ005 (5) Leu2_Int-3' (pWCD0559) (6)AmpR_ColE1 (pWCD0515)
pATJ012: (1) Leu2_Int_5' ((pWCD0524) (2) RPL18B (pWCD0530) (3) Venus (pWCD0535) (4) pATJ006 (5) Leu2_Int-3' (pWCD0559) (6)AmpR_ColE1 (pWCD0515)
pATJ013: (1) Leu2_Int_5' ((pWCD0524) (2) RPL18B (pWCD0530) (3) Venus (pWCD0535) (4) pATJ007 (5) Leu2_Int-3' (pWCD0559) (6)AmpR_ColE1 (pWCD0515)

Ran the GoldenGate for the 6 plasmids (using clones pATJ005.1, pATJ006.2, pATH007.2) and transformed into TG1. Plated on amp.

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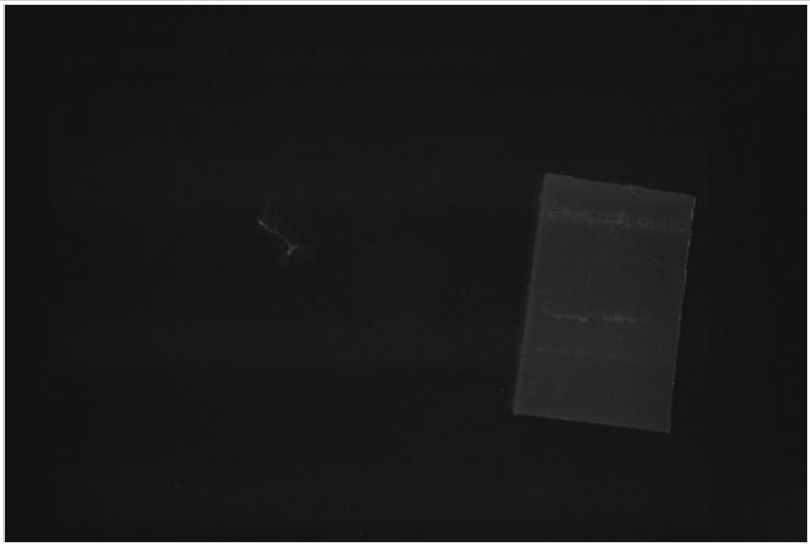
July2012AJ

From Dueber Lab Wiki

--Austin.jones 17:45, 2 July 2012 (PDT) **Plan**: Pick colonies. Grow up. Miniprep. Sequence. **Day**: Colonies grew on all plates although pATJ011 had only two. Grew colonies from each plate up for 7 hours. One of the pATJ013 did not grow. Upon miniprepping, pATH008.1, 9.1, and 10.1 showed RFP. Final plasmids miniprepped: pATH008.2, pATH009.2, pATH010.2, pATH011.1, pATH011.2, pATH012.1, pATH012.2, pATH013.1. Will test digest tomorrow.

--Austin.jones **Plan**: test digest, linearize plasmids, transform into yeast **day**: Test digested pATH008.2, pATH009.2, pATH010.2, pATH011.1, pATH011.2, pATH012.1, pATH012.2, pATH013.1. using XhoI Xba and SphI. Linearized the plasmids for integration into the yeast genome by digesting with BsmB1 for two hours.



pATJ008,9,10,11.1,11.2,12.1,12.2,13

Ran for 15 minutes and the 200 bp band must have run off. Will continue with all of them into yeast. Transformed into JD001 yeast cells. Plated pATJ008-10 on Ura- and 11-13 Leu-. Put at 30 for 48 hours.

--Austin.jones 11:02, 5 July 2012 (PDT) **Plan**:visualize and reclone if necessary **Day**:pATJ008,9,10 all grew up well (mKate strains) while pATJ 11,12,13 did not grow except for very small colonies on pATJ11.1 (letting them grow up for another day). pATJ008 and pATJ010 did not localize uniquely to the CP and the entire cell glowed red. pATJ009 looked the best. Will saturate and show mid log for tomorrow.
The failed transformations could be due to the fact that the yeast cells were not in ideal mid log growth. Back diluted and grew to 0.72 and retransformed pATJ012.1 and .2. Plated on Leu- and will take out on Saturday.

--Austin.jones 22:01, 7 July 2012 (PDT) **Plan**: Visualize pATJ0012.1 and .2 **Day**: pATJ0012.2 did not grow but pATJ0012.1 looked good. Under the microscope, the cells were small and half had distinct green CP while the other half were localized throughout the cell. Will have to optimize for max CP distinction. Possibly they are better at midlog.

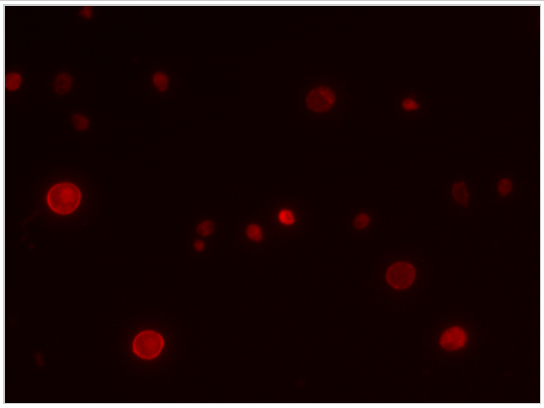
-Austin.jones 12:14, 9 July 2012 (PDT) **Plan**: Begin working with LZips **Day**: Going ahead with pATJ009 (Ras CP-mKate) and pATJ0012.1 (now pATJ0012, RasCP-Venus), will give to the cloning team (CC and RC) when ready.
meeting

- project description due on the wiki by Sunday (emphasize miCodes)- end of the day on Tuesday, ambiguous as possible: micode is a system for fluorescently labeling genotypes of yeast , don't limit to Leucine zippers, undermines the range of applications
- two independent gold medal plans by next monday
- Anderson lab Zip of known affinity, clone into cells as an assay
- be realistic with how the computer can differentiate between miCodes, better to do have proof of concept with 500 than not able to with 4000.
- cell with all organelles of all the same color (or one library size for eyeball detection and another smaller one for the computer, can theoretically mix together)

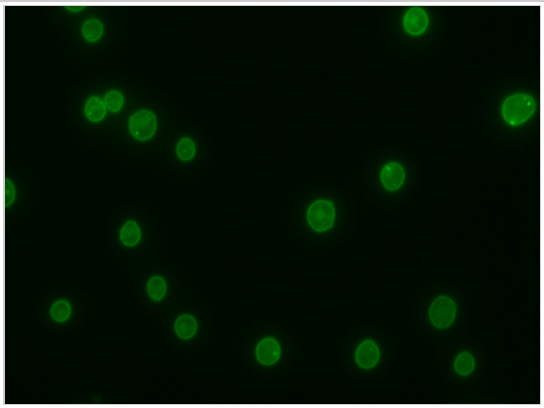
Went to the Anderson Lab to pick up the lZIPs, Zack may have them from 140L or else I can get them from Nina tomorrow.

--Austin.jones 10:48, 10 July 2012 (PDT) **Plan**: Take pictures of saturated and midlog pATJ009 and 012. Make project description. Pick up lZips.

Day: pATJ012 did not grow up. Diluted pATJ009 100uLcells:2mL SD -Ura and pATJ012 with 3.2mL SD -Leu to get 0.2 OD. Will grow up for 3.5 hours. Took pictures at full saturation.



yATJ009 saturated



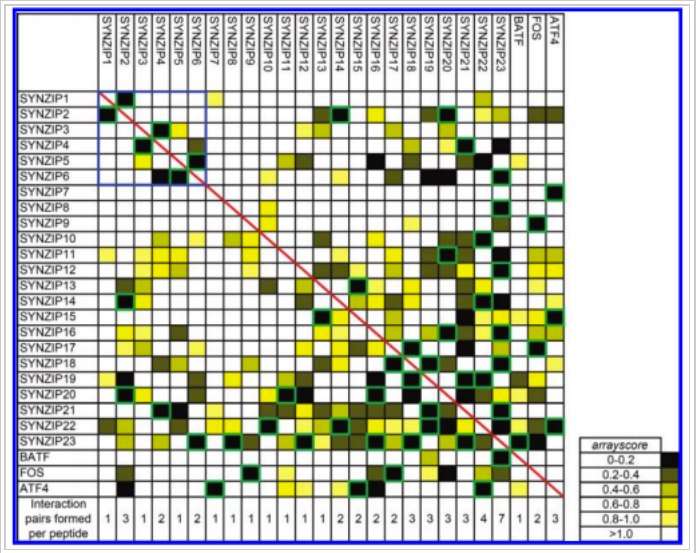
yATJ012 saturated

Checked back four hours later to visualize at midlog and both samples were at OD ~0.2. I'll start the back dilution again tomorrow.
For the leucine zipper project, the Anderson Lab lZip are homodimers and will complicate the assay. Will begin reading two Keating lab papers to find three heterodimer leucine zippers (low, medium, and high affinity) that are separated my orders of magnitude. Once synthesized, we will assemble them through GG then attach one of each coil pair to a FP and one to a localizing protein. If the organelle glows, the zipper dimerized. To measure the affinity, we can either measure the fluorescence intensity above the background or by using inducible promoters.

Project Description: miCodes (microCodes) are a system of cell barcodes that allow one to determine a yeast cell's genotype from its individual fluorescent phenotype. By localizing fluorescent proteins to distinct organelles within the cell, each yeast variant displays a unique pattern of colors. As the number of colors and target organelles raises, an exponential increase is found in the possible number of distinct barcodes. An extensive number of cells can be visualized at once by exciting the fluorescent proteins using fluorescence microscopy. Through computer or visual selection, one can sift through each combinatorial pattern of colors and targeted locations to identify a desired phenotype and elucidate the associated genotype.

--Austin.jones 14:44, 11 July 2012 (PDT) **Plan**: Find LZip sequences, order primers for assembly

Day: Read Keating's two relevant papers on known heterodimeric leucine zippers: SYNZIP (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3339576/?tool=pubmed) and coiled-coil interactome (http://pubs.acs.org/doi/abs/10.1021/ja907617a) . These are the sequences (http://pubs.acs.org/doi/suppl/10.1021/ja907617a/suppl_file/ja907617a_si_001.pdf) .



Leucine Zipper affinity levels

Chose three LZips across the levels of affinity (<.2 = high affinity, >1 = low affinity): strong= 20/2, medium=20/6, and weak= 20/13.

SYNZIP 20: STVEELLRAIQELEKRNAELKNRKEELKNLVAHLRQELAAHKYE
TCTACCGTTGAAGAATTGTTGAGAGCTATCCAAGAATTGGAAAAGAGAAACGCTGAATTGAAGAACAGAAAGGAAGAATTGAAGAACTTGGTTGCTCACTTGAGA(

SYNZIP 2: ARNAYLRKKIARLKKDNLQLERDEQNLEKIIANLRDEIARLENEVASHEQ
GCTAGAAACGCTTACTTGAGAAAGAAGATCGCTAGATTGAAGAAGGACAACTTGCAATTGGAAAGGGACGAACAAAACTTGGAAAAGATCATCGCTAACTTGAG(

SYNZIP 6: QKVAQLKNRVAYKLKENAKLENIVARLENDNANLEKDIANLEKDIANLERDVAR
CAAAAGGTTGCTCAATTGAAGAACAGAGTTGCTTACAAGTTGAAGGAAAACGCTAAGTTGGAAAACATCGTTGCTAGATTGGAAAACGACAACGCTAACTTGGAA

SYNZIP 13: QKVEELKNKIAELENRNAVKKNRVAHLKQEIAYLKDELAAHEFE
CAAAAGGTTGAAGAATTGAAGAACAAGATCGCTGAATTGGAAAACAGAAACGCTGTTAAGAAGAACAGAGTTGCTCACTTGAAGCAAGAAATCGCTTACTTGAAC

Syntheized as if was already cut by bsmbi used Davidson College Oligator (http://gcat.davidson.edu/iGem10/index.html) to assemble the oligos and added appropriate GG 3a ends as digested by BsaI (tatg to the 5' forward, g to the 3' forward, and ctacc to the 5' reverse). Removed internal restriction sites and altered so that no oligos were over 60bp. Ordered oligos.

--Austin.jones 16:04, 13 July 2012 (PDT) **Plan**: Begin gene gene synthesis by oligos. **Day**: Received primers. Phosphorylated the 5' end by mixing 20uM (diluted from 100uM) oligos independently with PNK and ligase buffer. Let sit at 37 for 1 hr. Mixed oligos together for the corresponding bZips (20, 2,6,13) added water until 200uL and then took 50uL for 6 minutes in the thermocycler to anneal.

Monday **Plan**: 12 GG reactions, transform into TG1 **Day**: Performed 6 GG reactions: each SYNZYP (20/2,6,13) with RFP and each SYNZYP (20/2,6,13) with the LP.
-Bait: zipper+filler 3b part+peroxisome targeting signal sequence
-Prey: zipper+RFP
-Using homology regions between the two corresponding LZip helices to integrate into the same region into the yeast genome. Transformed into TG1. Plated on amp.

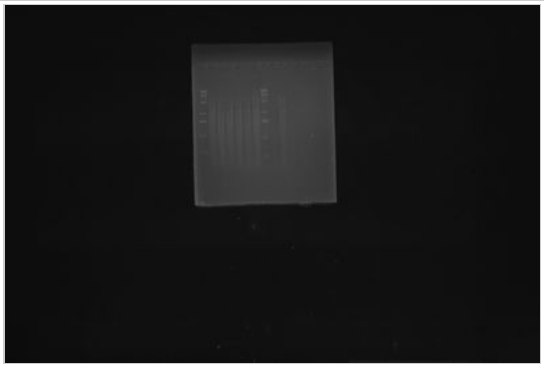
Plasmid Name	Part 1	Part 2	Part 3a	Part 3b	Part 4	Part 5	Part 6
pVY011	Ura3_Int_5'	RPL18Bp	SYNZIP20	mKate	ADH1	Con1_3'	AmpR_ColE1
	(pWCD0526)	(pWCD0530)		(pWCD0542)	(pWCD0552)	(pWCD0554)	(pWCD0515)
pVY012	Ura3_Int_5'	RPL18Bp	SYNZIP2	mKate	ADH1	Con1_3'	AmpR_ColE1
	(pWCD0526)	(pWCD0530)		(pWCD0542)	(pWCD0552)	(pWCD0554)	(pWCD0515)
pVY013	Ura3_Int_5'	RPL18Bp	SYNZIP6	mKate	ADH1	Con1_3'	AmpR_ColE1
	(pWCD0526)	(pWCD0530)		(pWCD0542)	(pWCD0552)	(pWCD0554)	(pWCD0515)
pVY014	Ura3_Int_5'	RPL18Bp	SYNZIP13	mKate	ADH1	Con1_3'	AmpR_ColE1
	(pWCD0526)	(pWCD0530)		(pWCD0542)	(pWCD0552)	(pWCD0554)	(pWCD0515)
pVY015	Con1_5'	RPL18Bp	SYNZIP20	GG3b_4x_GlySer	PTS1_ADH1	Ura3_Int_3'	AmpR_ColE1
	(pWCD0520)	(pWCD0530)		(pWCD0633)	(pWCD0553)	(pWCD0560)	(pWCD0515)
pVY016	Con1_5'	RPL18Bp	SYNZIP2	GG3b_4x_GlySer	PTS1_ADH1	Ura3_Int_3'	AmpR_ColE1
	(pWCD0526)	(pWCD0530)		(pWCD0633)	(pWCD0553)	(pWCD0560)	(pWCD0515)
pVY017	Con1_5'	RPL18Bp	SYNZIP6	GG3b_4x_GlySer	PTS1_ADH1	Ura3_Int_3'	AmpR_ColE1
	(pWCD0526)	(pWCD0530)		(pWCD0633)	(pWCD0553)	(pWCD0560)	(pWCD0515)
pVY018	Con1_5'	RPL18Bp	SYNZIP13	GG3b_4x_GlySer	PTS1_ADH1	Ura3_Int_3'	AmpR_ColE1
	(pWCD0526)	(pWCD0530)		(pWCD0633)	(pWCD0553)	(pWCD0560)	(pWCD0515)

meeting: Characterize promoters, determine strengths, take ones of different levels for our use. Use similar examples from last year. Recharacterize Dueber lab promoters. Tuesday, TDH3 promoter will be the standard (strongest) three others labeled as strong, medium, weak without empirical data will characterize those relative to TDH3. Give TDH3 to the registry in biobrick format. NLS in the database.

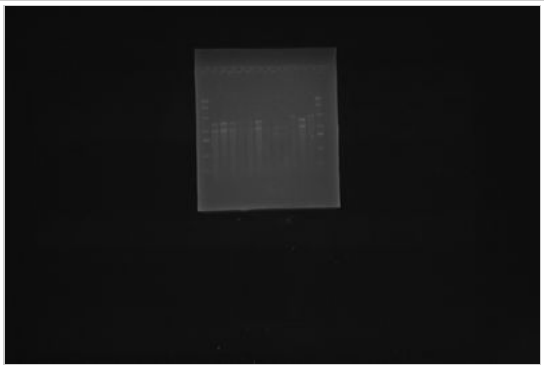
--Austin.jones 12:42, 17 July 2012 (PDT) **Plan**: pick colonies, grow up, miniprep, sequence, grow yeast **Day**: Vincent picked colonies this morning and grew them up for 6 hours. pVY13 did not grow (SYNSIP 6 prey) but all the others looked good. Redoing the GG for that pVY13. Began making bait constructs bait with PAGFP instead of linkers (pVY19-22) to serve as a control for seeing if LZa is binding to the peroxisome or not. Ran GG and transformed into TG1. Plated on Amp overnight.
Miniprepped all three clones of pVY11,12,14-18 (17 was eluted in 100uL).
Grew up pATJ009 SDS -Ura and pATJ012 in SDS -Leu overnight.

--Austin.jones 18:42, 18 July 2012 (PDT) **Plan**: Pick colonies and miniprep pVY 13,19-22. **Day**: Picked colonies from pVY13,19-22 (all about 50% RFP). Grew up for 7 hours for increased DNA concentration. Test digested 11,12, 14-18:

Plasmid	Enzymes	Expected Band Sizes
pVY011	EcoRI/XbaI	3822+1602
pVY012	(buffer 2 or 4)	3840+1602
pVY013		3852+1602
pVY014		3822+1602
pVY015	SpeI/SacII	2157+1502
pVY016	(buffer 4)	2175+1502
pVY017		2187+1502
pVY018		2157+1502

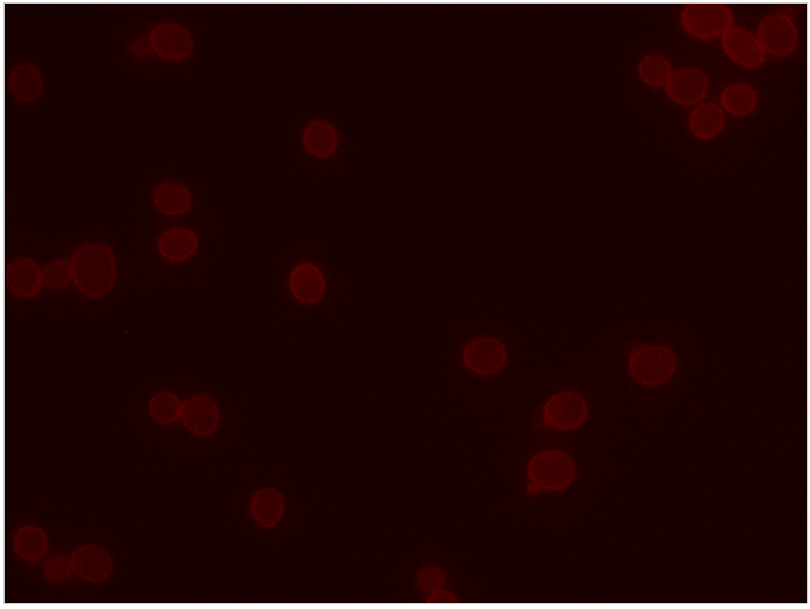


pVY11,12,14

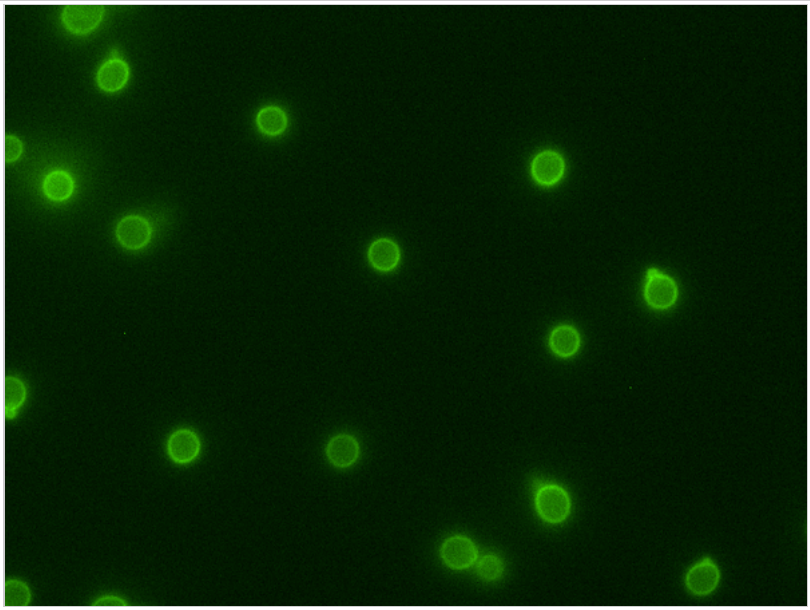


pVY15-18

Bands for 11-14 look very faint but present at the correct sizes. Vincent repicked colonies to grow up and reminiprep to improve DNA concentration. For 15-18, chose 11.3, 12.3, and 14.2 to carry on with.
Miniprepped 19-22. Will test digest them tomorrow.
Diluted pATJ009 and pATJ012 100uL cells:2500uL media and grew them up for 4 hours back to midlog. Visualizations look good.

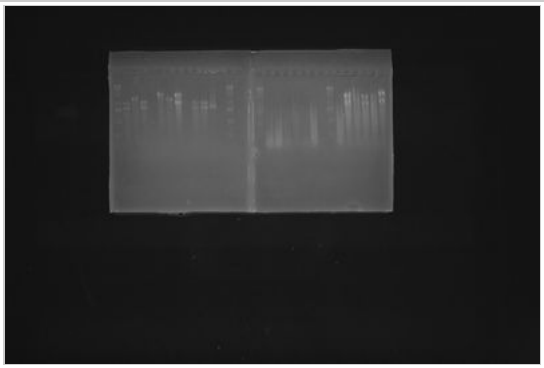


pATJ009 midlog



pATJ012 mid log

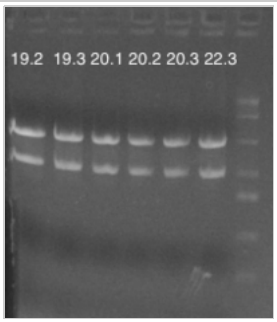
--Austin.jones 10:50, 19 July 2012 (PDT) **Plan**: test digest 13 (?), 19-22 speI sacII, miniprep 17,11,12,14 test digest, possibly bsmb1 and transform tg1 **Day**: Miniprepped the repicked clones of 17,11,12, and 14 that grew up last night. Vincent test digested those along with 19-22, 13.



21(x3), 22 (x3), 17 (x3), pVY11,12,13, 14. 19 (x3), 20 (x3)

pVY13 and pVY21 do not seem like they're working. Will try colony PCR tomorrow on multiple colonies. We are also considering that maybe the small part oligo assembly did not work well on SYNZIP 6, since pVY13 and 21 both correspond to the SYNZIP6 insert. SYNZIP 6 was the one we redesigned manually to prevent oligos over 60bp. Ended with 7 oligos.

--Austin.jones 13:40, 20 July 2012 (PDT) **Plan**: Troubleshoot pVY17,21
Day:Test digest of pVY19,20,22 using SpeI and SacII confirmed the constructs.

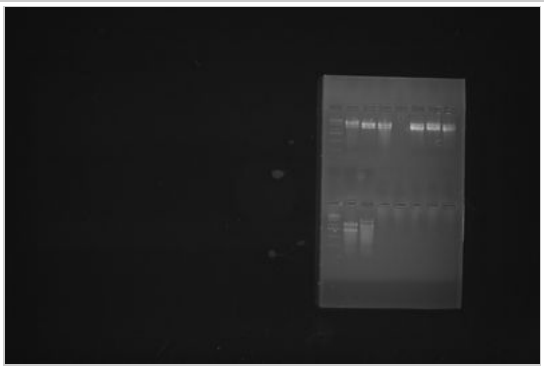


2012-07-20 TD w/ SacII/SpeI VY19,20,22

Ran colony PCR on pVY13 and 21 (10 colonies each). Used oligos that bind to the RPL18B promoter (O36) and ADH1 terminator (S16) to give a PCR product of about 1500bp. Colony PCR failed. No bands on all 18/20.

--Austin.jones16:40, 23 July 2012 (PDT) **Plan**: Redo SYNZIP 6. Go forward with SYNZIPS 20, 13, 2.

Day: Using the PNK treated oligos from the first round, Vincent annealed them in the thermocycler and digested with BsaI. I digested SYNZIPS 2, 13, 20 for both prey and bait as well as pWCD610 and 613 (vectors) using BSMBI heating them in the heat bath at 55 for 30 minutes. Ran them on a gel but showed incomplete digestion. Will rerun the digestion tomorrow for 1 hour for improved accuracy.


pVY11,12,14, 18, 19, 20, pWCD610 613

meeting:proceed with the others leaving 6 behind add SH3 domains to the construct to link with the labs bZips

--Austin.jones 13:38, 24 July 2012 (PDT) **Plan**: Redo digestion for 1 hr, redo colony PCR for pVY17 and 21. Run GG multigene assembly.
Day: Redigested pVY11,12,14, 18, 19, 20, pWCD610 613 for 1 hour at 55 using BsmBI. Vincent reran the colony PCR picking 12 each from pVY17 and 21. Used the secondary protocol taking 3uL of resuspended colony and adding to 22uL of mastermix and setting in the thermocycler. No results on the colony PCR. Retrying with HF Phusion and O36 and S16 again.
Talked to Will about problems with SYNZIP 6. Could arise with the Tm from the seventh oligo, might want to try and combining #3 and 4 for a 70+bp long one. Before continuing on we should sequence those that pass test digests to make sure that they do not have point mutations.

--Austin.jones 01:14, 26 July 2012 (PDT) **Plan**: Sequence. Order new primers. Transform 20, 2, 13.

Day: Sent all clones that appeared correct by the test digest for sequencing using Z71. Ordered a new primer for constructing ZYNSIP 6 (fused primer 3 and 4 to make a 72bp oligo). Its possible that the varied melting temperatures of the oligos before was the reason for the failed assemblies. Ran GG to assemble the 6 multigene cassettes (bait and prey for SYNZIP 2, 13, and 20. Cloned into TG1. Recovered for 30 mins and plated on Kan.

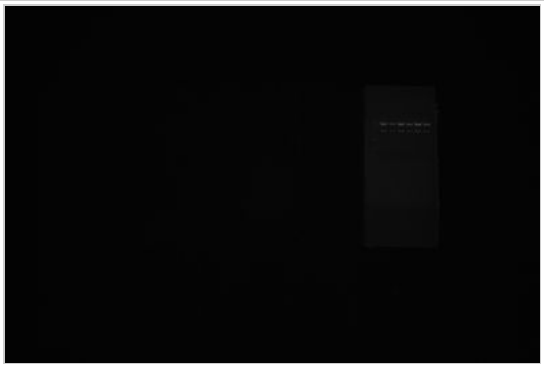
--Austin.jones 16:46, 26 July 2012 (PDT) **Plan**: Pick colonies, grow up, miniprep. Check sequencing.
SYNZIP 6 and 13 did not sequence correctly. Realized there were mixups in the oligo ordering. Will reorder for those SYNZIPS. Plates had very low efficiency (one colony for all except none for pVY025). With sequencing confirmed for SYNZIP 20 and 2, will assemble them into a multigene cassette using pVY 11/20 and 12/19 with pWCD615 backbone. Ran them in the thermocycler to digest with Bsmb1 for 2 hours. Ran on a gel to increase efficiency by isolating correct band sizes. Gel purified and will pick up GG tomorrow. Inoculated TG1 with pWCD615 to make our own stock. **Day**:

--Austin.jones 16:04, 29 July 2012 (PDT) **Plan**: Oligo should arrive. Begin reassembling SYNZIP 6. GG: 11/20, 12/19. Electroporation, recover for 30 mins, plate on Kan. p

Day: Carried forward SYNZIP 2 and 20. Made multi gene cassettes with bait and prey of 20/2 combination. pVY23 is 20 prey and 2 bait and pVY25 is 20 bait and 2 prey. Ran GG and transformed into TG1 through electroporation. Plated on Kan.

--Austin.jones 16:04, 30 July 2012 (PDT) **Plan**: Check colonies, grow up liquid cultures, miniprep, test digest

Day: Got around 80 colonies per plate. Innoculated 3 clones each of pVY023 and 025 in Kan media for 7 hours, miniprepped, and test digested using XhoI for 45 minutes. All six colonies were confirmed with expected bands at 6k and 2k. Will move forward with the yeast integration tomorrow.



pVY23.1-.3, pVY25.1-.3

--Austin.jones 17:51, 31 July 2012 (PDT) **Plan**: Transform pVY023 and 025 into yeast. **Day**: Back diluted and let yJD001 grow back to 0.8 OD. Transformed into yeast and plated on Ura-.

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August2012AJ

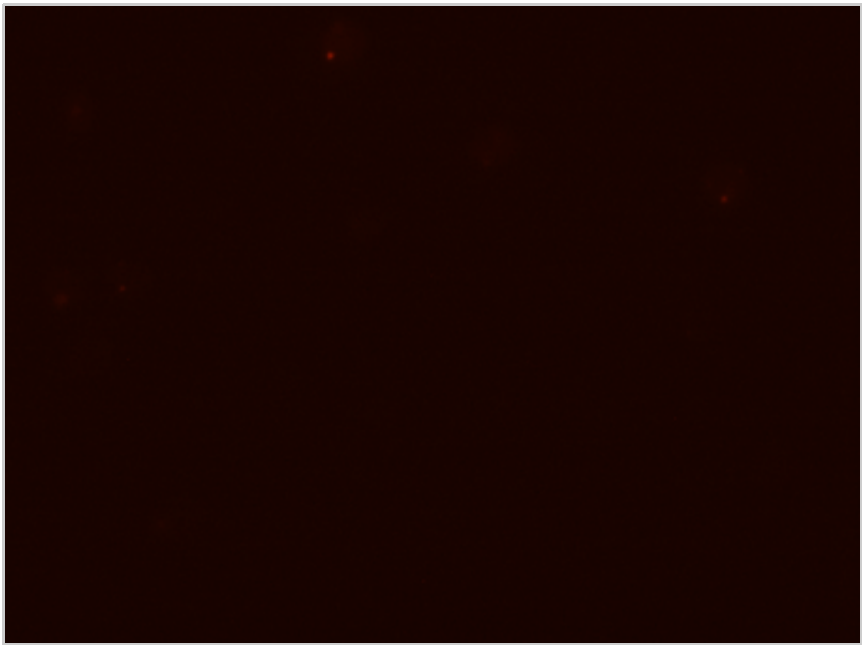
From Dueber Lab Wiki

--Austin.jones 17:25, 07 August 2012 (PDT) **day**: mini prepped overnight cultures of pVY026-028. test digest confirmed band sizes. Digested with NotI and integrated into yJD001. Out of due to the move SD ura- plates so grew up in 6uL of liquid media. Will plate on Friday upon moving back into EBB.

--Austin.jones 17:25, 10 August 2012 (PDT) **day**: pVY26 and 27 grew but pVY28 failed. Made SD Ura- plates and streaked.

--Austin.jones 17:25, 13 August 2012 (PDT) **Plan**: check colonies **Day**: pVY26 and 27 were not successful. diffuese RFP seen. Possibly hindered by the liquid media growth.

pVY26



pVY27

meeting:possible exp outpiuts p-p interactions isn't an output, its inferred from fluorescence (output) look for things that are tied to what you can measure opens up possibilities for anuthjing tied to microscope

screening only gives highest winners/loses data high throughput method to find interesting morphologies and associate them with their genotypes without association to the genotype, all you prove is that variation occurs with large #

change s to 2 have formulas animation come in last whats one way to express on or off--2 add colors-x add locations- x^y

to do pick compatible promoters and putting them all together

--Austin.jones 19:06, 14 August 2012 (PDT) **Plan**: Back dilute JD001, digest pVy26-28 with NotI, integrate into yeast, plate with SD Ura-. **day** Back diluted JD001 to OD 0.7. Digested pVY026-028 with NotI for 2 hrs. Integrated into yeast. Plated on SD Ura-.

--Austin.jones 17:20, 22 August 2012 (PDT) **Plan**: Check plates, image from plates **Day**: Images from saturation. PAGFP confirmation.

File:Atj0822a.jpg
20+2 strong, plate

File:Atj0822b.jpg
20+6 medium, plate

File:Atj0822c.jpg
20+13 weak, plate

--Austin.jones 18:07, 22 August 2012 (PDT) Imaged at midlog

File:Atj0822d.jpg
yVY026 weak, midlog

File:Atj0822e.jpg
yVY027medium, midlog

yVY028 did not image correctly

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Category: Pages with broken file links

- This page was last modified on 22 August 2012, at 18:07.
- This page has been accessed 20 times.

September2012AJ

From Dueber Lab Wiki

--Austin.jones 18:18, 4 September 2012 (PDT) Constructed: final construct LZa-RFP LZb-linker-PTS1 (+pWCD615 backbone) pVY029 pVY011 pVY016 pVY030 pVY012 pVY015 pVY031 pVY011 pVY017 pVY032 pVY013 pVY015 pVY033 pVY011 pVY018 pVY034 pVY014 pVY015

Electroporated and plated on Kan.

Heat shocked pWCD615 into TG1 because stock was running low.

--Austin.jones 15:02, 5 September 2012 (PDT) **meeting**: the advance of our project is opening the door of microscopy as a screening method that can be connected to a variety of assays. We used a direct assay to measure interaction rather than transcriptional activation of GFP (measure whole cell fluorescence.

presentation:

Intro to diversity of a wardrobe

Title- we'd lke to begin by talong about libraries Everyones done it thisn mornign ,looking at all combinations Screen an out fit for those that you like click And not like this click The idea of screening a genetic library is effectibly looking for high and low performers. There are various ways to screen genetic libraries such as click

Red libraries Titile: "what is the purpose of a screen?" The purpose is a collection of DNA and that the DNA has some kind of phenotypic output, but that is only useful if you can go back and associate the two Don't color intially, have them migrate together

No current… Replace skill with blue/white xgal Add more outputs (growth rate etc)

Many people in this room have screened libraries. Some techniqus are good while most are not very versatile. The questiion asked ourselve this summer was: how can we screen cells with a microscope and using nothing other than visual phenotypic method to link genetic identification. start off with the power of the microscope microscopy is an extemeley versitile technique. search for a variety of phenotpes in a variety of types of cells (pictures float on) weird phenotypes, tissue formation etc. howeveer looking through a library with a microsscope wold be difficult. to make it a good tech for library screens. we want to konw which cell has which version of hte gene. what is missing from micrispcy is that we cant tell the genotupe from the phenotype. next slide: there are many screening techs abvialable but they each have issues. if we could have a visual tag that is associated with a particular genotype, then we would have a screen where the output is micriscor(same tech for interesting phenotypes), its high throughput (automated tech), information loss (none, doenst screen by killing or throwing out). synbio is interested in getting trends, not only what is optimal but trends.

focus on spatial locatlziation, cant focus or assay with libraries priorties in the talk:libraries, data rentention, increasing library size, microscopy, LZa (proof of concept), spatial information

calculating library size:

grow barcodes along with pictures. cell to state. simplest way is to express with a fouresent proteins, can exprsss

more proteins with three populations, then mix different combinations of proteins, start to build up a arcode with green on.off etc 8 combinations, then this can build up. 4096, or pictures of all cells. split screen in half? write out the math. power to the power really ramps up the libary size increasing exponentially, Fit before after code.

--Austin.jones 15:46, 7 September 2012 (PDT) Transformed pVY29-34 into JD001. Plated on Ura-.

--Austin.jones 15:46, 7 September 2012 (PDT) Slides meeting with Terry

2: real microscope, include yeast, bacterial, align images, same sizes segqay is microscopy is awesome, dont give punchline (use for screens) before talking about 4: facs- whole cell flour, y2h- color, plate with different levels of activity, pulldown- binding/not binding to substrate , gated threshold strong arguments:

these are the two most comomon (others ot there) FACS and y2h: either not high throughput or you lose a lot of information when you do them, or both.

- - template

--Austin.jones 14:39, 12 September 2012 (PDT) **meeting**: bronze: targeting sequences in biobrick format
silver: data demonstrating TDH3 Venus works
clearly explain rationalization for each of the medals explaining logical argument. Show part number and registry link. gold: promoter characterization

--Austin.jones (talk) 01:50, 21 September 2012 (PDT) Oligos came in for Biobricking of PAGFP and PAmCherry. PCRd pMSY46 (PAGFP) and pMSY45 (PAmCHERRY) adding EcoRI/XbaI on the 5' end and SpeI/PtsI on the 3' end. Cut out 700bp bands on gel, purified, and digested with EcoRI and PstI for 1.5 hours. Ran on a gel, purified, and froze until Sunday. Tubes got mixed up but will verify upon photoactivation. 1 was extremely faint while 2 was normal brightness. Will ligate to pSB1C3 (iGEM backbone for registry) and transform into TG1. Need to find selective antibiotic on the backbone.

--Austin.jones (talk) 23:47, 24 September 2012 (PDT): Digest backbone with eco and pst. ligate with each of the inserts (used 4uL of 1 since band was very dim, 2uL of 2). heat shocked into into tG1. recover for 1 hr and plated on Cam-.

--Austin.jones (talk) 00:37, 26 September 2012 (PDT) Picked three colonies from each plate, grew up, and miniprepped. Test digested showed correct band sizes (2070, 700). Only use 1uL of Gel Green when making gels. Sending for sequencing tomorrow.

--Austin.jones (talk) 16:59, 27 September 2012 (PDT) Sequencing came back. pATJ14 is PAmCHERRY and pATJ15 is PAGFP. Will submit 14.1 and 15.1 to the registry.

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