# IGEM Vincent Notebook - May-July 2012

From Dueber Lab Wiki

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## Vinyeh923 - 31 July 2012 (PDT)

-Grow up pVY13 and 21 (4 clones each), miniprep and test digest with BsmBI
-Expect bands of sizes 3400+2030 for pVY13, and bands of sizes 2500+1871 for pVY21
-no positives from test digest

-oligos came in at around 4
-Try 1:5 dilution of oligos in pnk phosphorylation step, run annealing program (to make synzip parts 6 and 13), and BsaI digest program (to make pVY13,14 and 21,22)
-Plate on LB Amp

## Vinyeh923 - 27 July 2012 (PDT)

-Plate pVY013 and pVY021 on LB Amp
-no colonies grew

## Vinyeh923 - 26 July 2012 (PDT)

-SYNZIP 6 and 13 did not sequence correctly. Noticed an error in the oligos ordered for SYNZIP 13, will reorder

| Full Name | Sequence | Purpose | Length |
|---|---|---|---|
| BA15.iGEM150 | tatgCAAAAGGTTGAAGAATTGAAGAACAAG | SYNZIP 13 F1 | 31 |

| | | |
|---|---|---|
| BA16.iGEM151 | ATCGCTGAATTGGAAAACAGAAACGCTGTTAAGAAGAACAGAGTTG | SYNZIP 13 F2 46 |
| BA17.iGEM152 | CTCACTTGAAGCAAGAAATCGCTTACTTGAAGGACGAATTGGCTGCTCACGAATTTGAAg | SYNZIP 13 F3 60 |
| BA18.iGEM153 | GTTTCTGTTTTCCAATTCAGCGATCTTGTTCTTCAATTCTTCAACCTTTTG | SYNZIP 13 R3 51 |
| BA19.iGEM154 | GCGATTTCTTGCTTCAAGTGAGCAACTCTGTTCTTCTTAACAGC | SYNZIP 13 R2 44 |
| BA20.iGEM155 | ctaccTTCAAATTCGTGAGCAGCCAATTCGTCCTTCAAGTAA | SYNZIP 13 R1 42 |

-To make SYNZIP 6 part: use AY72/73/76/77/78/and BA05
-Phosphorylate oligos, run annealing program, transform to create RFP prey and PAGFP bait constructs (pVY013 and pVY021)

## Vinyeh923 - 25 July 2012 (PDT)

-Send in pVY011, 012, 014, 019, 020, 021, 022 for sequencing
-Transform multi-gene cassettes using electroporation and plate on LB Km<br.
-Reorder oligos for SYNZIP 6 (basically combine 3f and 4f into one long oligo >60bp).

## Vinyeh923 - 24 July 2012 (PDT)

-Retry colony pcr with phusion instead of taq, use primers s16 and o36
-Digest pVY11,12,14,19,20,22, and WCD615 with BsmBI
-Gel purify and start BsmBI digest/ligation program to create multigene constructs

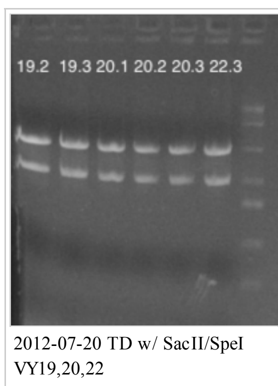
| Plasmid | SYNZIP prey | SYNZIP bait | Pairings |
|---|---|---|---|
| pVY023 | 20 | 2 | pVY011+pVY020 |
| pVY024 | 20 | 13 | pVY011+pVY022 |
| pVY025 | 2 | 20 | pVY012+pVY019 |
| pVY026 | 13 | 20 | pVY014+pVY019 |

## Vinyeh923 - 23 July 2012 (PDT)

-Redo pVY13 and 21 transformations
Tomorrow:
-Digest w/ BsmBI and gel purify parts pVY11,12,14,19,20,22 as well as backbone vector pWCD615

## Vinyeh923 - 20 July 2012 (PDT)

-Redid test digests (SpeI/SacII) on pVY19,20,22 to confirm



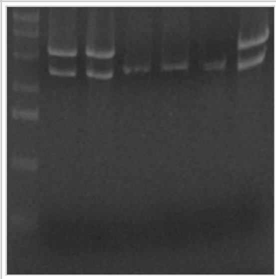
2012-07-20 TD w/ SacII/SpeI VY19,20,22

-All of these constructs give bands as expected
-Run colony PCR on pVY13 and 21 (about 10 colonies each). Picked oligos that bind to the RPL18B promoter and ADH1 terminator to give a PCR product of about 1000-1500bp

## Vinyeh923 - 19 July 2012 (PDT)

-Test digest pVY19-22 as well as regrown pVY11-14,17

| Plasmid | Enzymes | Band Sizes |
|---|---|---|
| pVY019 | SpeI/SacII | 2847+1502 |
| pVY020 | buffer 4 | 2865+1502 |
| pVY021 | | 2877+1502 |
| pVY022 | | 2847+1502 |



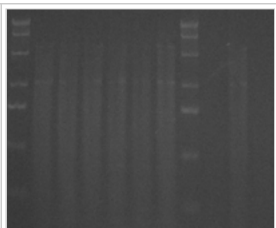
2012-06-19 TD w/ BsmBI VY11-14 (13.1-3)

2012-07-19 TD w/ SpeI/SacII VY19-22

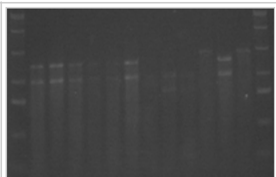
-pVY13 and pVY21 do not seem like they're working. Austin and I will try colony PCR tomorrow on multiple colonies. We are also considering that maybe the small part oligo assembly did not work well on SYNZIP 6, since pVY13 and 21 both correspond to the SYNZIP6 insert.

## Vinyeh923 - 18 July 2012 (PDT)

-Pick colonies from pVY13,pVY19-22. Miniprep and test digest later
-Test digest pVY11-18 according to table on 17 July



20-12-07-18 VY11,12,14 TD (Eco/Xba clones#1-3)



2012-07-18 VY14-18 TD (SpeI/SacII clones#1-3)

-Chose pVY11.3, 12.3, 14.2, 15.2, 16.3, 18.2
-Re-pick pVY11.3,12.3, and 14.2 and culture overnight to get higher concentrations from miniprep
-Pick 3 more colonies from pVY17 plate since none were positive

## Vinyeh923 - 17 July 2012 (PDT)

-pVY11-18 grew well with the exception of pVY13 (corresponds to SYNZIP6 prey)
-Redo the BsaI digest/ligation for pVY13
-Create bait constructs with PAGFP instead of linkers (pVY19-22): this would give us a control to see whether LZa is binding to the peroxisome or not
-Transform all of these constructs into TG1, LB Amp

-Tomorrow: Test Digest these constructs

| 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

# Vinyeh923 - 16 July 2012 (PDT)

-Create constructs for zipper assay
-Bait: zipper+filler 3b part+peroxisome targeting signal sequence
-Prey: zipper+RFP
-Want to combine bait and prey model into a multi-gene cassette so only one integration will be needed

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

-Transform into TG1, plate on LB Amp

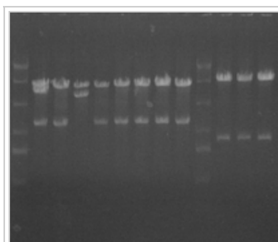
# Vinyeh923 - 13 July 2012 (PDT)

-PNK-treat individual oligos (5x diluted)
-Set up oligo-annealing reaction according to protocol

# Vinyeh923 - 11 July 2012 (PDT)

-Miniprep colonies, test digest

| Plasmid | Enzymes | Buffer | Expected Bands |
|---|---|---|---|
| pVY007 | PstI | 3 | 4945+1816 |
| pVY010 | BamHI | 3 | 5712+1225 |



2012-07-11 VY test digest
pVy7(PstI, clones#1-8)
pVY10(BamHI clones #1,2,4)

We see the expected bands from pVY7 clones 4-8 and all of the clones for pVY10
-Use pVY7 clone 4 and pVY10 clone 2 for linearization (BsmBI, 55C for 30min) and transformation

-Ordered oligos for PCA of SYNZIP parts for preliminary micode assay
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

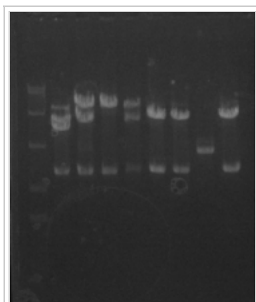
Syntheized as if was already cut by bsmbi used Davidson College Oligator (http://gcat.davidson.edu/iGem10/index.html) to assemble the oligos and added appropriate GG 3a ends as digested by BsaI (tatg to the 5' forward, g to the 3' forward, and ctacc to the 5' reverse). Removed internal restriction sites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

| Full Name | Sequence | Purpose | Length |
|---|---|---|---|
| AY54.iGEM094 | tatgTCTACCGTTGAAGAATTGTTGAG | SYNZIP 20 F1 | 27 |
| AY55.iGEM095 | AGCTATCCAAGAATTGGAAAAGAGAAACGCTGAATTGAAGAACAGAAAGG | SYNZIP 20 F2 | 50 |
| AY56.iGEM096 | AAGAATTGAAGAACTTGGTTGCTCACTTGAGACAAGAATTGGCTGCTCACAAGTACGAAg | SYNZIP 20 F3 | 60 |
| AY57.iGEM097 | CTCTTTTCCAATTCTTGGATAGCTCTCAACAATTCTTCAACGGTAGA | SYNZIP 20 R3 | 47 |
| AY58.iGEM098 | GCAACCAAGTTCTTCAATTCTTCCTTTCTGTTCTTCAATTCAGCGTTT | SYNZIP 20 R2 | 48 |
| AY59.iGEM099 | ctaccTTCGTACTTGTGAGCAGCCAATTCTTGTCTCAAGTGA | SYNZIP 20 R1 | 42 |
| AY60.iGEM100 | tatgGCTAGAAACGCTTACTTGAGAAAGAAGATCG | SYNZIP 2 F1 | 35 |
| AY61.iGEM101 | CTAGATTGAAGAAGGACAACTTGCAATTGGAAAGGGACGAACAAAACTTGGAAAAGATCA | SYNZIP 2 F2 | 60 |
| AY62.iGEM102 | TCGCTAACTTGAGGGACGAAATCGCTAGATTGGAAAACGAAGTTGCTTCTCACGAACAAg | SYNZIP 2 F3 | 60 |
| AY63.iGEM103 | CAATTGCAAGTTGTCCTTCTTCAATCTAGCGATCTTCTTTCTCAAGTAAGCGTTTCTAGC | SYNZIP 2 R3 | 60 |
| AY64.iGEM104 | CTAGCGATTTCGTCCCTCAAGTTAGCGATGATCTTTTCCAAGTTTTGTTCGTCCCTTTC | SYNZIP 2 R2 | 59 |
| AY65.iGEM105 | ctaccTTGTTCGTGAGAAGCAACTTCGTTTTCCAAT | SYNZIP 2 R1 | 36 |
| AY66.iGEM106 | tatgCAAAAGGTTGCTCAATTGAAGAACAGAG | SYNZIP 6 F1 | 32 |
| AY67.iGEM107 | CTAGATTGAAGAAGGACAACTTGCAATTGGAAAGGGACGAACAAAACTTGGAAAAGATCA | SYNZIP 6 F2 | 60 |
| AY68.iGEM108 | TCGCTAACTTGAGGGACGAAATCGCTAGATTGGAAAACGAAGTTGCTTCTCACGAACAAg | SYNZIP 6 F3 | 60 |
| AY69.iGEM109 | CAATTGCAAGTTGTCCTTCTTCAATCTAGCGATCTTCTTTCTCAAGTAAGCGTTTCTAGC | SYNZIP 6 R3 | 60 |
| AY70.iGEM110 | CTAGCGATTTCGTCCCTCAAGTTAGCGATGATCTTTTCCAAGTTTTGTTCGTCCCTTTC | SYNZIP 6 R2 | 59 |
| AY71.iGEM111 | ctaccTTGTTCGTGAGAAGCAACTTCGTTTTCCAAT | SYNZIP 6 R1 | 36 |
| AY72.iGEM112 | tatgCAAAAGGTTGCTCAATTGAAGAACAGAG | SYNZIP 13 F1 | 32 |
| AY73.iGEM113 | TTGCTTACAAGTTGAAGGAAAACGCTAAGTTGGAAAACATCGTTGCTAGATTGGAAAAC | SYNZIP 13 F2 | 59 |
| AY74.iGEM114 | GACAACGCTAACTTGGAAAAGGACATCGCTAACTTGGAAAAGGACATCGg | SYNZIP 13 F3 | 50 |
| AY75.iGEM115 | CTAACTTGGAAAGAGATGTTGCTAGA | SYNZIP 13 F4 | 26 |
| AY76.iGEM116 | CTTAGCGTTTTCCTTCAACTTGTAAGCAACTCTGTTCTTCAATTGAGCAACCTTTTG | SYNZIP 13 R3 | 57 |
| AY77.iGEM117 | GTCCTTTTCCAAGTTAGCGTTGTCGTTTTCCAATCTAGCAACGATGTTTTCCAA | SYNZIP 13 R2 | 54 |
| AY78.iGEM118 | ctaccTCTAGCAACATCTCTTTCCAAGTTAGCGATGTCCTTTTCCAAGTTAGCGAT | SYNZIP 13 R1 | 56 |

## Vinyeh923 - 10 July 2012 (PDT)

-Pick colonies off plate from fresh transformation of pVY7 and 10 constructed from BsaI ligation/digest
-Miniprep after cultures reach saturation, test digest with BsmBI (30 min at 55C)

| Plasmid | Enzymes | Buffer | Expected Bands |
|---|---|---|---|
| pVY007 | BsmBI | 3 | 4883+1879 |
| pVY010 | BsmBI | 3 | 5059+1879 |



2012-07-10 VY test digest pvy7 and 10 clones #1-4

-Linearize with BsmBI (30 min at 55C)
-Plate on SD plates

-Not sure of pVY007 was correct according to digest b/c of smear. Pick more colonies and test digest tomorrow

## Vinyeh923 - 9 July 2012 (PDT)

-Picked 3 colonies for each of pVY007 and pVY010, grow until saturation
-Miniprep colonies and test digest using:

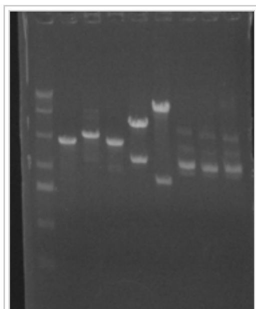
| Plasmid | Enzymes | Buffer | Expected Bands |
|---|---|---|---|

| | | | |
|---|---|---|---|
| pVY007 | PstI | 3 | 4945+1816 |
| pVY010 | BamHI | 3 | 5712+1225 |

-Test digest did not show positive results
-Will said the problem might be with the plasmids iGEM miniprepped. I'll try to redo the BsaI reaction to create pVY7 and 10 using Will's plasmid stock.
-Seeded yJD001 in 5mL of YPD for yeast transformation tomorrow
-Tomorrow: pick colonies, test digest, linearize, yeast integration

# Vinyeh923 - 6 July 2012 (PDT)

| Plasmid | Enzymes | Buffer | Expected Bands |
|---|---|---|---|
| pVY007 | PstI | 3 | 4945+1816 |
| pVY008 | PstI | 3 | 4243+1788 |
| pVY009 | BamHI | 3 | 6424+1243 |
| pVY010 | BamHI | 3 | 5712+1225 |

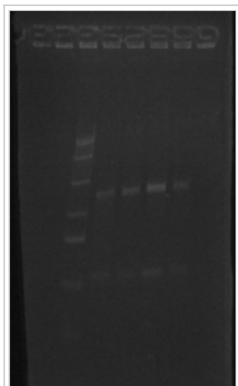


2012-07-06 Test digest pVY7-8 w PstI, pVY9-10 w BamHI (clones #2 and 3 of each)

-We see the expected band sizes for pVY8 clone 3 and pVY9 clone 2
-Linearize with SacII
-Transform into yJD001

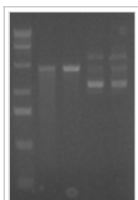
# Vinyeh923 - 5 July 2012 (PDT)

-PvuI/XhoI test digest of constructs from 7/3/12:



Test digest of pVY7-10 using PvuI and XhoI

-It seems like none of these plasmids are of the correct size... should repick colonies and miniprep tomorrow
-Set up another test digest using PstI for SNA4 plasmids and BamHI for ZRC1 plasmids (should cut within the gene to create two bands)



Test digest of pVY7/8 using PstI, pVY9/10 using BamHI

-It seems that the colony I picked for pVY7 and 8 did not have a correct insert. pVY9 and 10 seem to be impure as well

Tomorrow:
-Run test digests on repicked colonies, verify whether or not correct construct and sizes are present
-If constructs are correct, set up linearization digest and backdilute 5mL of yJD001 in 50mL
-Zymo purify linearization digests, transform into yeast

## Vinyeh923 - 3 July 2012 (PDT)

-Pick colonies, grow cultures for miniprep
-Inoculate yeast culture for transformation, prepare cells and stop at last LiOAc wash+pellet step
-Test digest

| Plasmid | Enzymes | Buffer | Expected Bands |
|---|---|---|---|
| pVY007 | PvuI/XhoI | 3 | 4714+2047 |
| pVY008 | PvuI/XhoI | 3 | 3994+2037 |
| pVY009 | PvuI/XhoI | 3 | 5620+2047 |
| pVY010 | PvuI/XhoI | 3 | 4900+2037 |

-Digest using EcoRI, 37C for 2 hours
-Zymo purify DNA
-Yeast Transformation w/ LiOAc, plate

| Strain Name | Description | Parental Strain | Method | Plasmids Used | Marker |
|---|---|---|---|---|---|
| yVY013 | LEU5'-RPL18B-SNA4(VM)-Venus-ADH1-LEU3' | yJD001 | Integration | pVY007 | LEU |
| yVY014 | URA5'-RPL18B-SNA4(VM)-mKate-ADH1-URA5' | yJD001 | Integration | pVY008 | URA |
| yVY015 | LEU5'-RPL18B-ZRC1(VM)-Venus-ADH1-LEU3' | yJD001 | Integration | pVY009 | LEU |
| yVY016 | URA5'-RPL18B-ZRC1(VM)-mKate-ADH1-URA5' | yJD001 | Integration | pVY010 | URA |

## Vinyeh923 - 2 July 2012 (PDT)

-Set up round 2 of cloning (see table from last day)
-Transform BsaI digest/ligation reactions into chemically competent TG1 cells (plate on LB Amp)

## Vinyeh923 - 29 June 2012 (PDT)

-Sequencing for pVY006 (ZRC1, clone 1) came back positive
-Picked 2 colonies from SNA4 and ZRC1 to miniprep
-Sent in SNA4 (pVY005) for sequencing
-Set up round 2 of cloning

| Plasmid Name | Part 1 | Part 2 | Part 3a | Part 3b | Part 4 | Part 5 | Part 6 |
|---|---|---|---|---|---|---|---|
| pVY007 | Leu2_Int_5' (pWCD0524) | RPL18B (pWCD0530) | SNA4 (pVY005) | Venus (pWCD0543) | ADH1 (pWCD0552) | Leu2_Int-3' (pWCD0559) | AmpR_ColE1 (pWCD0515) |
| pVY008 | Ura3_Int_5' (pWCD0526) | RPL18B (pWCD0530) | SNA4 (pVY005) | mKate (pWCD0542) | ADH1 (pWCD0552) | Ura3_Int-3' (pWCD0560) | AmpR_ColE1 (pWCD0515) |
| pVY009 | Leu2_Int_5' (pWCD0524) | RPL18B (pWCD0530) | ZRC1 (pVY006) | Venus (pWCD0543) | ADH1 (pWCD0552) | Leu2_Int-3' (pWCD0559) | AmpR_ColE1 (pWCD0515) |
| pVY010 | Ura3_Int_5' (pWCD0526) | RPL18B (pWCD0530) | ZRC1 (pVY006) | mKate (pWCD0542) | ADH1 (pWCD0552) | Ura3_Int-3' (pWCD0560) | AmpR_ColE1 (pWCD0515) |

## Vinyeh923 - 28 June 2012 (PDT)

2012-06-28 SNA4 and ZRC1 part 2 pcr product EXPAND

-Colonies for pWCD514-ZRC1 golden gate cloning grew on LB CM, picked two colonies to miniprep
-Started golden gate assembly on pWCD514+SNA4 and ZRC1 pcr products

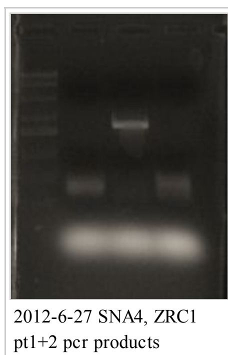
-Transform both constructs into TG1 in LB CM

## Vinyeh923 - 27 June 2012 (PDT)

-Ran PCR to make pcr products SNA4, ZRC1 part 1, and ZRC1 part 2 using yeast genomic DNA

| pcr part | forward primer | reverse primer | expected band size (bp) |
|---|---|---|---|
| SNA4 | AX19.iGEM039 | AX20.iGEM040 | ~460 |
| ZRC1 part 1 | AX21.iGEM041 | AX49.iGEM069 | ~1200 |
| ZRC1 part 2 | AX48.iGEM068 | AX22.iGEM042 | ~150 |

-Expected band sizes shown in table above



2012-6-27 SNA4, ZRC1 pt1+2 pcr products

ZRC1 Part 2 is faint in the gel, and it seems that the SNA4 pcr product is of the incorrect size

## Vinyeh923 - 22 June 2012 (PDT)

-Designed primers for GG assembly of 2 possible VM targeting proteins

| Full Name | Sequence | Purpose |
|---|---|---|
| AX19.iGEM039 | GCATCGTCTCCTCGGTCTCCTATGtgttgctactgtgtttgt | VM SNA4/YDL123W: forward primer |
| AX20.iGEM040 | ATGCCGTCTCAGGTCTCACTACCtggcacataaggaggagg | VM SNA4/YDL123W: reverse primer |
| AX21.iGEM041 | GCATCGTCTCCTCGGTCTCCTATGatcaccggtaaagaattg | VM ZRC1/YMR243C: external forward primer |
| AX22.iGEM042 | ATGCCGTCTCAGGTCTCACTACCcaggcaattggaagtatt | VM ZRC1/YMR243C: external reverse primer |
| AX48.iGEM068 | gcatCGTCTCcCtTCTCAAGAAGCCTTTGACAGCCATGG | VM ZRC1/YMR243C: internal forward primer |
| AX49.iGEM069 | atgcCGTCTCagaAgAAGATGGTGAACCACCTGC | VM ZRC1/YMR243C: internal reverse primer |

## Vinyeh923 - 20 June 2012 (PDT)

-Digested 3 yeast transformations worth of pVY004
-Integrate Venus plasmids for Vacuole, Nuclear Periphery, Nucleolus, Nucleus, and Mitochondria into yVY003 mKate Vacuolar Membrane strain

| Strain Name | Description | Parental Strain | Method | Plasmids Used | Marker |
|---|---|---|---|---|---|
| yVY008 | (RPL18B-VMA4-mKate) + (RPL18B-Mito-Venus) | yVY003 | Integration | pMRY018 | URA3, LEU |
| yVY009 | (RPL18B-VMA4-mKate) + (RPL18B-Nucleus-Venus) | yVY003 | Integration | pMRY027 | URA3, LEU |
| yVY010 | (RPL18B-VMA4-mKate) + (RPL18B-Vacuole-Venus) | yVY003 | Integration | pCLC003 | URA3, LEU |
| yVY011 | (RPL18B-VMA4-mKate) + (RPL18B-NucPeriph-Venus) | yVY003 | Integration | pRC001 | URA3, LEU |
| yVY012 | (RPL18B-VMA4-mKate) + (RPL18B-Nucleolus-Venus) | yVY003 | Integration | pRC003 | URA3, LEU |

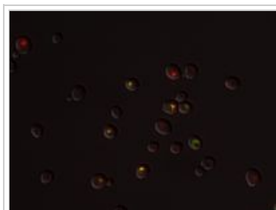
## Vinyeh923 - 19 June 2012 (PDT)

-Backdiluted saturated cultures of yVY003-004 to 0.2 OD600. View microscopy of both midlog and saturated cultures after they reach OD600 of 0.8 (~4 hrs)

## Vinyeh923 - 18 June 2012 (PDT)

-Pick cotransformed strain colonies and view under microscopy

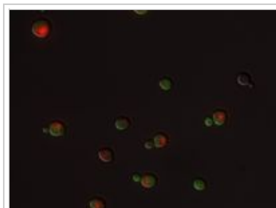
| Venus Insert | mKate VM strain |
|---|---|
| VM | |


yVY005 (mkate and venus vm)

Actin

yVY006 (mkate vm + venus actin)

ER


yVY007 (mkate vm + venus ER)

-Also picked same colonies to grow to saturation
-Tomorrow: prepare slides for microscopy after backdiluted 3mL culture grows to mid-log phase, restreak plates for yVY005-007

## Vinyeh923 - 15 June 2012 (PDT)

-Miniprep pVY004
-Pour SD-URA-LEU plates

## Vinyeh923 - 14 June 2012 (PDT)

-Restreak yeast strains
-Try a colocalization experiment, transform Venus parts into mKate counterpart strains

- Transform Venus Actin, ER, and VM parts into VM mKate strain yVY003 using integration

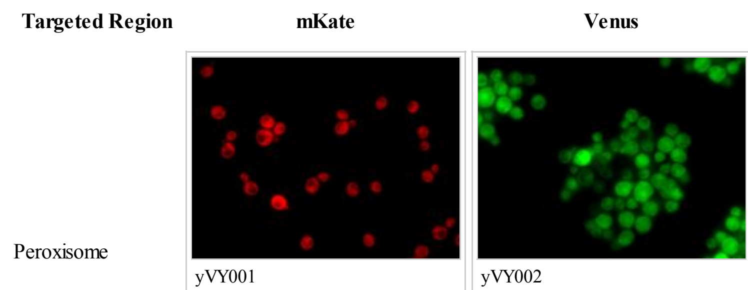
-Transform pVY004 into TG1 and plate onto LB Amp
-Grow overnights of pVY004 for miniprep tomorrow

## Vinyeh923 - 13 June 2012 (PDT)

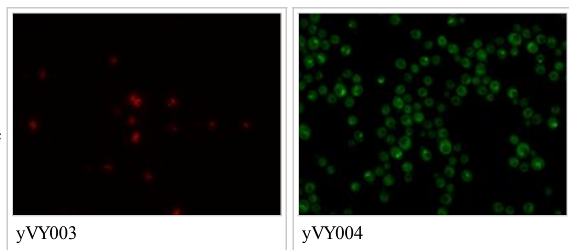
-Prepared slides of cells in mid-log growth phase - variability seemed to improve. However, yVY001-002 (encoding peroxisome-targeting proteins) do not seem to be viable because of diffusiveness and effect on cell health (seems to be toxic). yVY003 is clearly defined while yVY004 is more diffuse when visualizing the vacuolar membrane.

| Targeted Region | mKate | Venus |
|---|---|---|

Peroxisome


yVY001 yVY002

Vacuolar Membrane



yVY003 yVY004

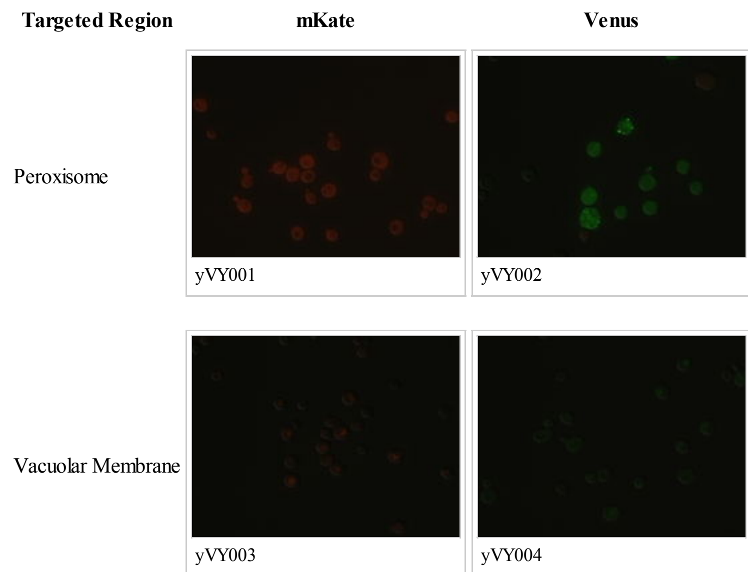
## Vinyeh923 - 12 June 2012 (PDT)

-Backdiluted saturated cultures of strains yVY001-004
-Prepared slides for microscopy
-Prepare cell stock of strains yVY001-004 using 500uL of culture and 500uL 50% glycerol

## Vinyeh923 - 11 June 2012 (PDT)

-Sequencing confirmed
-Yeast plates grew well
-Prepare slides for microscopy (4uL of PBS and cell pellet)

| Targeted Region | mKate | Venus |
|---|---|---|
| Peroxisome | yVY001 | yVY002 |
| Vacuolar Membrane | yVY003 | yVY004 |



-Need to restreak plates

## Vinyeh923 - 8 June 2012 (PDT)

-Test digest of pVY004 using AatII and BglII for 2 hours
-Send in samples for sequencing

| Sequence ID | Construct | Clone | Primer |
|---|---|---|---|
| sIGEM046 | pVY001 | 3 | S16 |
| sIGEM047 | pVY001 | 3 | AF47 |
| sIGEM048 | pVY002 | 1 | S16 |
| sIGEM049 | pVY002 | 1 | AA30 |
| sIGEM050 | pVY003 | 1 | S16 |
| sIGEM051 | pVY003 | 1 | AF47 |
| sIGEM052 | pVY004 | 1 | S16 |
| sIGEM053 | pVY004 | 1 | AA30 |
| sIGEM054 | pVY004 | 2 | S16 |
| sIGEM055 | pVY004 | 2 | AA30 |
| sIGEM056 | pVY004 | 3 | S16 |
| sIGEM057 | pVY004 | 3 | AA30 |

-Linearize confirmed plasmids using SacII and NotI (double the volume of each digest to a final volume of 20uL), zymo cleanup to 10uL final volume
-Yeast transformation and plate

## Vinyeh923 - 7 June 2012 (PDT)

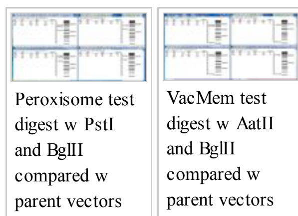
-Plates pVY001-004 (Peroxisome-mKate, Peroxisome-Venus, VacuolarMembrane-mKate, VacuolarMembrane-Venus in that order) had good ratios compared to negative controls for each vector
-Pick 3 colonies per plate, grow in 3mL LB Amp, miniprep when saturated

-Test digest Peroxisome vectors with BglII and PstI (NEBuffer 3)

- We expect Peroxisome-mKate to have three bands (~6.1kb, ~1.3kb, and 0.5kb), and the mKate parent vector to have two bands (~6.1kb, ~1.1kb)
- We expect Peroxisome-Venus to have three bands (~6.2kb, ~1.3kb, and 0.5kb), and the Venus parent vector to have two bands (~6.2kb, ~1.2kb)

-Test digest VacuolarMembrane vectors with AatII and BglII (50% and 100% activity in NEBuffer 3)

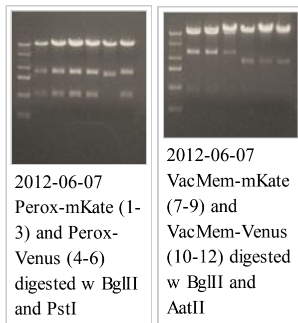
- We expect VacuolarMembrane-mKate to have three bands (~5.6kb, ~1.9kb, ~0.4kb), and the mKate parent vector to have two bands (~5.4kb, ~1.9kb)
- We expect VacuolarMembrane-Venus to have three bands(~6.4kb, ~1.3kb, ~0.4kb), and the Venus parent vector to have two bands (~6.1kb, ~1.3kb)



Peroxisome test digest w PstI and BglII compared w parent vectors

VacMem test digest w AatII and BglII compared w parent vectors

Test digest results



2012-06-07 Perox-mKate (1-3) and Perox-Venus (4-6) digested w BglII and PstI

2012-06-07 VacMem-mKate (7-9) and VacMem-Venus (10-12) digested w BglII and AatII

We see expected bands for each test digest for pVY001-003. Should re-run test digest for pVY004 for longer to make sure there are three bands.

## Vinyeh923 - 6 June 2012 (PDT)

-Cells did not grow in the plates. We figure the comp cell stock was not good (cell density not high enough)
-Use commercial stock and re-transform the Gibson assembly products, plate onto LB Amp

## Vinyeh923 - 5 June 2012 (PDT)

-Start electrocomp cell prep

- Backdilute cultures 50x to an OD600 of about 0.1 in two 25mL flasks
- Check OD600 readings until about 0.6
- Keep on ice, perform 10% glycerol washes
- Aliquot into cell stock

-Perform transformations of Gibson assemblies, plate onto LB Amp

## Vinyeh923 - 4 June 2012 (PDT)

-Check vector PCR's on gel

2012-06-03 HSR/MRY HF (1,2) and GC (3,4)

The expected bands are not seen. PCR does not seem to work for these vectors.

-Alternatively, we will digest the vectors with BglII and use them for Gibson assembly after gel purification
-Start Gibson assembly, use 15uL of mix and 5uL of total DNA (2.5uL PCR Product, 2.5uL backbone)
-4 total reactions (VacMem-mKate, VacMem-Venus, Perox-mKate, Perox-Venus)
-will prep electrocomp TG1 cells tomorrow, transform w/ electroshock

## Vinyeh923 - 1 June 2012 (PDT)

-No colonies grew on the VacMem-Venus and VacMem-mKate plates from Gibson assembly

-Troubleshooting:

- check if we have primers to run PCR on vectors (without having to digest for BglII)
- try electroporation, for two pcr products(one faint, one thick band) for each vector and negative controls for each vector [6 total transformations]

Set up PCR reactions for Venus and m-Kate vectors to create linearized plasmids for Gibson assembly

- Venus: AU60 F, AD59 R
- m-Kate: AU62 F, AD59 R

-These PCRs will create linear plasmids with two homology regions corresponding to the organelle PCR products for Gibson assembly
-Need to digest backbone PCR product with BglII to remove junk DNA at forward end

## Vinyeh923 - 31 May 2012 (PDT)

-Sequencing results were what we expected, although there is a point mutation (that encodes for the same amino acid)

-Set up PCR for organelle-targeting genes in yeast genome. Primers AV07/08 (peroxisome) and AU78/79 (vacuolar membrane)
-Annealing temp was set to 65C

2012-05-31 peroxisome and vacuolar membrane (far right)

We see the expected ~700bp pcr product for the vacuolar membrane targeting orf(far right), but the ~700bp pcr product for the peroxisome targeting orf is not seen (second to the right)
. -Retry pcr (peroxisome w/ primers AV07/08) at 55C annealing temp

2012-05-31 peroxisome (far right)

We see the expected ~700bp pcr product for the peroxisome targeting orf

-Purified pcr product that worked (vacuolar membrane vm) for Gibson assembly
-Purified BglII-cut Venus and m-Kate plasmids for Gibson assembly
-Perform Gibson assembly using **vm pcr product** and each plasmid
-Transform into TG1 E.Coli, plate on LB Amp

-Need to perform Gibson assembly on peroxisome targeting orf pcr product

## Vinyeh923 - 30 May 2012 (PDT)

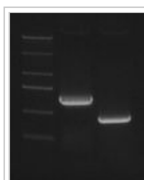
-We see ~20 colonies on the negative control plate, ~200 colonies on the ligation plate. Pick two colonies to grow in LB+Amp, then miniprep. -Test digest with BamHI and SpeI

2012-05-30 pWCD421-Venus BglXho and pWCD480-mKate SpeBam

We see the expected ~5700 and ~1400 bp fragments
-Sent in for sequencing with forward primer Z71 and reverse primer O44

## Vinyeh923 - 29 May 2012 (PDT)

-Check individual SOEing parts on gel


2012-05-29 pWCD480 AU62-65, AU64-63 soeing pcr products

We see the expected ~440 bp from A, and ~760 bp from B.

-Purify A and B together and use as template for final SOEing step (external primers AU62,AU63)

2012-05-29 pWCD480 final soeing product AU62-63

We see the expected ~1.1kb product

-Purify SOEing product and digest with BglII and PstI
-Digest backbone vector pWCD0480 with BglII and PstI

2012-05-29 pWCD480 final soeing product (left) and vector (right) digest w BglII+PstI

We see the expected ~1.1kb product and ~6kb vector backbone

-Purify backbone and SOEing PCR product, ligate with a negative control.
-Transform into TG1 chemically competent cells, skip rescue step b/c we're using AmpR selection

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# IGEM Vincent Notebook - Aug-Oct 2012

From Dueber Lab Wiki

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## Vinyeh923 - 1 Oct 2012 (PDT)

-SH3 peptide and domain (Crk) data:

| Binding Affinity | Peptide Sequence | Trans Kd, uM | Sequence |
|---|---|---|---|
| stng | PPPALPPKRRR | 0.1 | cctccacctgcattaccgccaaagcgtcgtcgt |
| mdhi | PPPVPPRR | `5-20 | ccaccgcctgttccacctcgtaga |
| mdlo | PPAIPPRQPT | 127 | ccgcctgcaattccaccgcgtcaacctact |
| wk | GPPVPPRQST | 663 | ggtccacctgttcctccacgtcaatctact |
| Crk domain | SH3 Domain | | gcagagtatgtgcgggccctgtttgactttaatgggaatgatgaagaagaccttccctttaagaaaggagacatcctgagaatccg ggataagcctgaagagcagtggtggaatgcagaggacagcgaaggaaagagggggatgattcctgtcccttacgtggagaagtat |

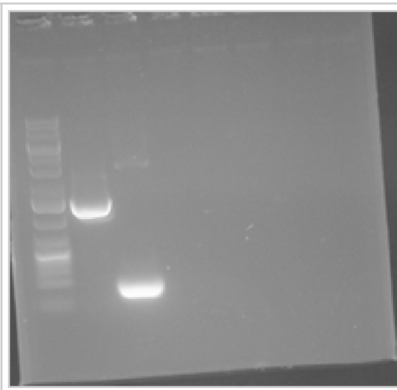
-Oligo data

| Oligo Name | Sequence | Purpose |
|---|---|---|
| BD08.iGEM197 | GCATCGTCTCCTCGGTCTCCGTAGCatcaccggtaaagaattg | ZRC1_3b F |
| BD09.iGEM198 | ATGCCGTCTCAGGTCTCAGGATCCcaggcaattggaagtatt | ZRC1_3b R |
| BD10.iGEM199 | GCATCGTCTCCTCGGTCTCCTATGgcagagtatgtgcgggcc | CrK SH3_3a F |
| BD11.iGEM200 | ATGCCGTCTCAGGTCTCACTACCatacttctccacgtaagg | CrK SH3_3a R |
| | | SH3 pep |

| | | |
|---|---|---|
| BD12.iGEM201 | GCATCGTCTCCTCGGTCTCCTATGCC | stng F1 |
| BD13.iGEM202 | TCCACCTGCATTACCGCCAAAGCGTCGTCGTGGTAGTGAGACCTGAGACGGCAT | SH3 pep stng F2 |
| BD14.iGEM203 | CGACGCTTTGGCGGTAATGCAGGTGGAGGCATAGGAGACCGAGGAGACGATGC | SH3 pep stng R1 |
| BD15.iGEM204 | ATGCCGTCTCAGGTCTCACTACCACGA | SH3 pep stng R2 |
| BD16.iGEM205 | GCATCGTCTCCTCGGTCTCCTATG | SH3 pep mdhi F1 |
| BD17.iGEM206 | CCACCGCCTGTTCCACCTCGTAGAGGTAGTGAGACCTGAGACGGCAT | SH3 pep mdhi F2 |
| BD18.iGEM207 | CTACGAGGTGGAACAGGCGGTGGCATAGGAGACCGAGGAGACGATGC | SH3 pep mdhi R1 |
| BD19.iGEM208 | ATGCCGTCTCAGGTCTCACTACCT | SH3 pep mdhi R2 |
| BD20.iGEM209 | GCATCGTCTCCTCGGTCTCCTATGC | SH3 pep mdlo F1 |
| BD21.iGEM210 | CGCCTGCAATTCCACCGCGTCAACCTACTGGTAGTGAGACCTGAGACGGCAT | SH3 pep mdlo F2 |
| BD22.iGEM211 | GGTTGACGCGGTGGAATTGCAGGCGGCATAGGAGACCGAGGAGACGATGC | SH3 pep mdlo R1 |
| BD23.iGEM212 | ATGCCGTCTCAGGTCTCACTACCAGTA | SH3 pep mdlo R2 & SH3 pep wk R2 |
| BD24.iGEM213 | GCATCGTCTCCTCGGTCTCCTATGG | SH3 pep wk F1 |
| BD25.iGEM214 | GTCCACCTGTTCCTCCACGTCAATCTACTGGTAGTGAGACCTGAGACGGCAT | SH3 pep wk F2 |
| BD26.iGEM215 | GATTGACGTGGAGGAACAGGTGGACCCATAGGAGACCGAGGAGACGATGC | SH3 pep wk R1 |

-BD08 & BD09: PCR to make ZRC1 (VM targeting protein) 3b part
-BD10 & BD11: PCR to make Crk SH3 domain 3a part
-PNK treat the other oligos (1:5 dilution)

-Gel Purify PCR products (expect ~200bp for SH3 domain, ~1400 for ZRC1)



2012-10-01 PCR Zrc1 and crk sh3

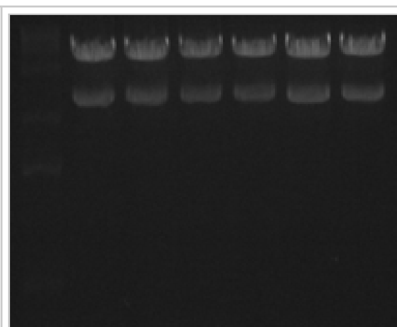
-BsmBI digest using pWCD514 as a backbone for 3a/3b parts
-Plated pVY37-40 on Cam and left in the 37C incubator
-pVY35-36 are at the ligation/digest stage. need to plate tomorrow

# Vinyeh923 - 19 Sept 2012 (PDT)

-Start an experiment using SH3 domains and measure binding affinity. Will target bait constructs to two different organelles: one set will have bait constructs targeted to the peroxisome (much like our original LZ experiment), and the other set will be targeted to VM or Nuclear Periphery. We are concerned that the peroxisome importing system may create an artificial threshold for binding such that only strong interactions will be imported well. Will test to see if there is a greater dynamic range and sensitivity if we target the bait constructs to the outer membranes of organelles instead.
-Need to ask for SH3 sequences and binding data

# Vinyeh923 - 6 Sept 2012 (PDT)

-Test digest pVY29-34 with XbaI (expect bands at 5000 and 2000bp)



2012-09-06 TD VY29-34 using XbaI

-Linearize with NotI
-Plate on SD Ura (6 total, two of each interaction)

**Proof of concept experiment for LZassay program**
-Transform linearized pMRY34 (gfp targeted nucleus) to yVY17 (strong interaction) to get a basic micode
-Will mix with yVY20 (weak interaction), and see if we can distinguish between them using Harneet's program. Basic micode will be there so we can determine actual identities and program accuracy

# Vinyeh923 - 5 Sept 2012 (PDT)

-Created LZa-RFP+LZb-linker-PTS1 constructs w/ BsmBI ligation digest and pWCD615 backbone
-Plated these constructs (pVY29-34) on Kan

# Vinyeh923 - 1 Sept 2012 (PDT)

-pVY16,17 sequenced correctly. pVY15,18 had random inserts and omits within the SYNZIP parts. will try more colonies later to see if it actually worked.

# Vinyeh923 - 31 Aug 2012 (PDT)

-Picked 3 colonies each off plates yesterday
-Today, miniprepped cultures, digest with XhoI/EcoRI in EcoRI Buffer
-Expect bands of sizes ~2600+1100bp

# Vinyeh923 - 29 Aug 2012 (PDT)

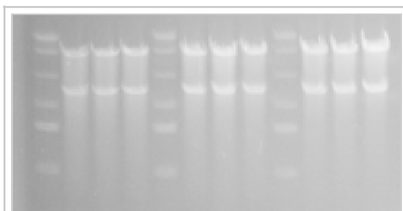
-Create LZb-GlySer-PTS1 constructs (pVY15-18)

| Plasmid Name | Part 1 | Part 2 | Part 3a | Part 3b | Part 4 | Part 5 | Part 6 |
|---|---|---|---|---|---|---|---|
| pVY015 | Con1_5' (pWCD0520) | RPL18Bp (pWCD0530) | SYNZIP20 | GG3b_4x_GlySer (pWCD0633) | PTS1_ADH1 (pWCD0553) | Ura3_Int_3' (pWCD0560) | AmpR_ColE1 (pWCD0515) |
| pVY016 | Con1_5' (pWCD0520) | RPL18Bp (pWCD0530) | SYNZIP2 | GG3b_4x_GlySer (pWCD0633) | PTS1_ADH1 (pWCD0553) | Ura3_Int_3' (pWCD0560) | AmpR_ColE1 (pWCD0515) |
| pVY017 | Con1_5' (pWCD0520) | RPL18Bp (pWCD0530) | SYNZIP6 | GG3b_4x_GlySer (pWCD0633) | PTS1_ADH1 (pWCD0553) | Ura3_Int_3' (pWCD0560) | AmpR_ColE1 (pWCD0515) |
| pVY018 | Con1_5' (pWCD0520) | RPL18Bp (pWCD0530) | SYNZIP13 | GG3b_4x_GlySer (pWCD0633) | PTS1_ADH1 (pWCD0553) | Ura3_Int_3' (pWCD0560) | AmpR_ColE1 (pWCD0515) |

-Run BsaI ligation digestion reaction, electroshock and plate on LB Amp

# Vinyeh923 - 07 Aug 2012 (PDT)

-miniprep pVY26,27,28
-test digest with XhoI, expect bands at 5700 and 2000bp



2012-08-07 TD XhoI VY26 27 28 clones #1-3

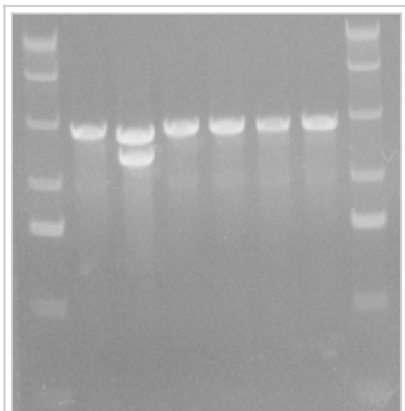
Looks like the bands are the right size for every clone. Choose clone 3 to integrate into yeast.
-digest with NotI to linearize

-transform into yJD001

# Vinyeh923 - 03 Aug 2012 (PDT)

-Sequencing for pVY13, 14, and 21 were positive

-Miniprep pVY22 colonies, test digest w/ BsmBI (expect bands at 2500 and 1900 bp)

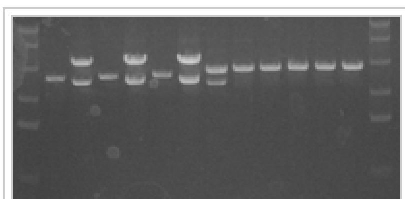


2012-08-03 VY TD 22 (clones 4-9)

-Clone 5 looks to be the right band sizes
-Send in for sequencing (use Z71 as sequencing primer)
-Create construct for leucine zipper assay using pVY22 and pVY11

| Plasmid | Cassette 1 | Cassette 2 | Backbone |
|---|---|---|---|
| pVY024 | pVY011 (SYNZIP 20 prey) | pVY022 (SYNZIP 13 bait) | pWCD615 |

# Vinyeh923 - 02 Aug 2012 (PDT)



2012-08-02 VY TD 13,14,21,22 (clones 1-3)

-pVY13 (clone 2), 14 (clone 1+3) , 21 (clone 1) appear to have correct band sizes after digesting with BsmBI
-Send for sequencing
-Create constructs for leucine zipper assay

| Plasmid | Cassette 1 | Cassette 2 | Backbone |
|---|---|---|---|
| pVY026 | pVY014 (SYNZIP 13 prey) | pVY019 (SYNZIP 20 bait) | pWCD615 |
| pVY027 | pVY011 (SYNZIP 20 prey) | pVY021 (SYNZIP 6 bait) | pWCD615 |
| pVY028 | pVY013 (SYNZIP 6 prey) | pVY019 (SYNZIP 20 bait) | pWCD615 |

-Plate on LB Kan

-Pick more colonies of pVY22 for overnight, miniprep and test digest tomorrow

## Vinyeh923 - 01 Aug 2012 (PDT)

-Electrocompetent cells grow at a slower rate than TG1's... will pick colonies (3-4 per plate) and let them grow overnight
-Tomorrow: miniprep DNA and test digest, then send for sequencing

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