September 2012 Notes- Masaki

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9/20/12

post synberc talk notes

- Talk about automated microscopy: give time scales etc
- Have real numbers for answers
  - Don't say "were working on it" => say what we're doing
- **Slow down!**

- State what microscope we are using at some point in the presentation
- "low res but we have other options etc"

- Flow cytometer possibility?

- Data on other organelles, or expression optimization
  - Finding robust targeting sequences and compatible loads

- Focus on imaging (make pretty)

FRET

Suggest a few things after the End w/ take home message

Meeting:

Application with LZ could be done with FRET pairs in E Coli But it would require a lot of cotransformations

Yes you can do it in various ways but

Unknown kinase functions (in human cells?) Nice things about setup, could be used in other organisms

Setup- getting there but not there yet

Questions to answer How many cells in a slide view

Make sure it is dramatic changes for cartoons

Harneet: Language + slides

Austin: Slides ok, but slow way down

Masaki: A bit slower

Thomas: needs most work Micode examples were unclear False coloring? Or explaining thresholding Need to label results slides Add data for other organelles

Registry entry of localization parts digitally -pictures that show targetting -updated promoter

Tendencies: To get hung up or to fly past things

Fly past- take a deep breathe and slow down

Hung up- rediscuss so we can figure out how to verbalize

9/14/12

slides

- Need to state presence of 40 by 40 library
- 40 zippers to test the interaction with the other 40
- Make it for a specific example

- Every time something changes (it is a message) don’t confuse audience

Terry's changes:

-
  - Find out where pics came from (citation)
  - Add to corner
  - Change title
  - Microscopy provides really interesting information

9/12/12

Make micodes for P.O.C. leucine zippers green cp/blue nucleus blue cp/green nucleus

Add photoactivatibles to the registry

slides

states, channels, organelles states

- on, off, on if

9/11/12

72, 67 have addition pstI sites and need to be digested with a different enzyme All will be digested with EcoRI and SacII

Need to redo digestion and yeast growths

9/7/12

Slide review -need to make background and template

9/6/12

Innoculated cells from plates of zach's new parts to be miniprepped tomorrow

- new parts consist of new fluorescent proteins
  - mTurqoise, sYFP, amilCP, iLOV

Miniprepped extra parts for gg MG assembly Miniprepped pMRY055-pMRY062

Test digested with ecoRI, PstI

9/5/12

Made test cassettes for new cfp,yfp and others Ran out of 614 so used 515 instead (no notI site)

group meeting

Me- micode Assay

- Write up brief instructions
- Making an assortment of micode (at least 12)

Format the testing so that it is repeatable Phenotypic assay of high and low Kd doesn't come out of the assay but it is within ____ range

9/2/12

Zipper part test digests

- Making mastermix with eco and pstI
  - 10 ul total,
  - 25% the normal enzyme concentration
  - .125 ul per rxn

August 2012 Notes- Masaki

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8/30/12

Transform experimental promoter cassettes (pMRY55-62)

Innoculate other parts to be imaged (yMRY20-25)

slide review

Clothing analogy

- Don't know why other outfits didn't make it,

Thresholding

- Don't know if you've explored all true combinations
- Downside of other methods: Not sure if entire library was represented

- Need a switch to genetic libraries
  - Creating a large library of mutants and screen for desired activity

- Good screen should link genotypes to an observable phenotype
    - Pictures- gfp cell of different brightness
    - Or drawing of some living and dying
    - Double helices => to arrow => to observable phenotypes
    - High throughput
    - End with preserves information about all genotypes

- Most have threshold that causes them to lose information

Information loss

- Take off microscopy
    - Facs- under all throughput
    - Pull down- fails to bind
    - Rearrange so pulldown is underneath chromatography

Use pictures

- Beta galactose screen, blue pigment

Barcode phenotype associated with fluorseece

Walkthrough boxes one by one

8/27/12

- Made multi fp nucleus cassettes to assemble
- Used wrong promoter RC048 pCYC1
- Should use RNR2p (low strength) for nucleus, will go through with one set to see if works
- Need to reBSAI using correct promoter strengths

8/6/12

slide review

- Condense
    - Too much text
    - Terry's advice page
    - Make sure text is bring home message or words to remmeber
- Growth selections?
- Start at how do you screen librarys?
    - Cant if looking under microscope etc.
- Break down further
    - What you are detecting /sorting? Flow chart?

    - FACs - fluorescence intensity
    - Two hybrid- only to p-p interations
- Give story line and how they use it experimentally
- Micode 1, micode 2
- Plain pictures of yeast cells- undistiguishable
- Then click to show micodes

- See a cell but cant identify genotype
- Spend more time with story

- Make sure slides have transitions into the successive slide

- Sell libraries
- Keep things active "working with micodes"

8/2/12

- Bands for 47A, C 48 A,B were strong
- BsmbI reaction w. 47 A and 48 A

8/1/12

safety page

- EHS.BERKELEY.EDU
- Look up if questionable
- Edta cannot go down sink

schedule

- In email?
- Tomorrow-grad students going to ebb~2 pm to pick out lab benches
- Friday-packing day
    - 10 am group stuff
    - Before 10-own benches
- Monday will pick up liquids
- Tuesday afternoon - all should be in place
- Tuesday/Wednesday unpacking

wetlab

- New promoter assembly
- BSMBI for first 2 (Ste5, CYC1)
- ADH1 has bsmbI and BsaI sites so we need to assemble once the other cassettes are made. w/ xba and BglII

July 2012 Notes- Masaki

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7/30/12

- Celia transformed multi gene cassettes on Sunday
- Transformed well, lots of colonies present on plates
- Grew up with other PA single diffused fps

To do for next week

- Draft slides
  - What we are doing and why
  - No leucine zippers, just micodes
  - Demonstrate why micodes are better and how to use them
  - Run a workflow
  - How does a user use a micode
  - Show math
  - ~ 6 slides
  - If use diagram need to stay consistent (all graphics)
  - Make diagrams in illustrator
  - Use animations/ create narrative

    - Sell it to them

- Practice talk in mid september
- Making six slides per week
- Sketch design for shirt design

- Look at Nucleus tags

7/25/12

- Ran bsaI cassette assembly for cell periphery promoter library on igem side thermocyclers
- heat shock, and grow in Amp (no recovery needed)

- Heat shocked ecoli to grow more plasmid for parts 535, 610, 615 - need to recover in cam

- Electroporate pMRY 051-054: recover in LB and grow on LBKan plates

7/24/12

- Realized I used a 6b part instead of a 6 part for the multigene cassettes (could account for lack of ecoli growth on plates)
- Test digesting ecoli plasmid and reassembling using bsmbi

7/18/12

- checked plates: all grew red meaning only single cut plasmid religated.
- tried rerunning bsmBI and using electroporation w/ a negative control of just pWCD610

7/17/12

- ran bsmbI digestion
- gel purified

[image|image] ligated and transformed into TG1 using heat shock

7/16/12

- Integrated 34-46
- Ran bsmbI for multigene integrations need to gel purify and ligation and transformation into ecoli then yeast.

Meeting Notes

- Wanted to be able to use parts (promoters) for our project
- Use gg to put them all in the same plasmid as a standardized method (same copy number etc)
- Or make new library using some promoter and using mutagenesis
- TDH3 promoter as standard (make a registry insert)
  - Use strong medium and weak
  - Characterize strength relative to those three

7/10/12

- pMRY 036, 38 test digested correctly but did not grow yeast
- pMRY037, 040, 041 test digested correctly and did integrate correctly

7/08/12

Still no growth.

7/05/12

Yeast transformations did not grow. Maybe due to too high of a rediluted OD before cell wash step. was ~1, should be 0.8

Next dilution grew to 0.73 and reintegrated.

June 2012 Notes- Masaki

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6/29/12

Sent for sequencing, plates looked good. Grew up colonies for miniprep: YDL066W_mito, YBR010W_nuc, YBL003C_nuc

6/28/12

Robert transformed YCL017C_mit

picked colony that was not red under the green LED. Grew up for 6 hours

Transformed YDL066W_mito, YBR010W_nuc, YBL003C_nuc

6/27/12

All pcrs worked except YCL017C. Used wrong primers. Redid PCR. PCR redo lengths (1,2,3) 756, 245, 588

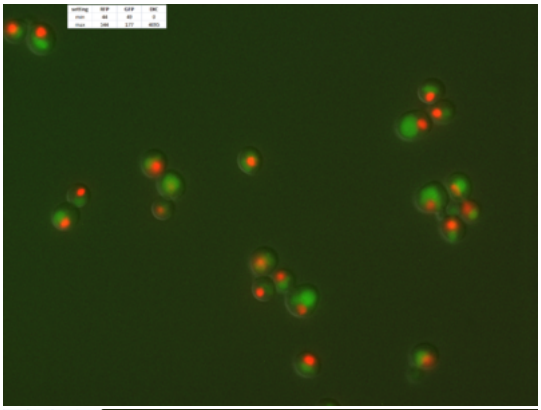
Started digestion and ligations of other genes YDL066W_mito, YBR010W_nuc, YBL003C_nuc(wcdBsmBI). I will finish transformations this evening.

6/25/12

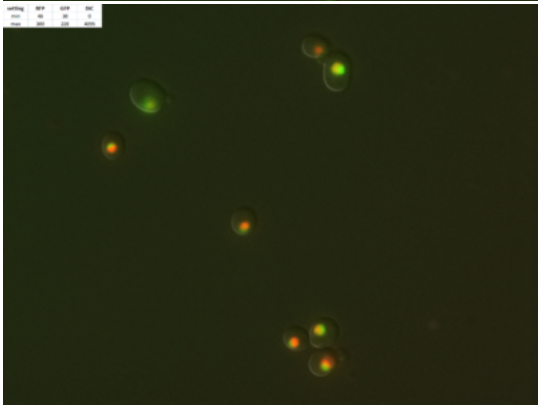
Cotransformation Microscopy

strain	Venus target	mKate target	midlog

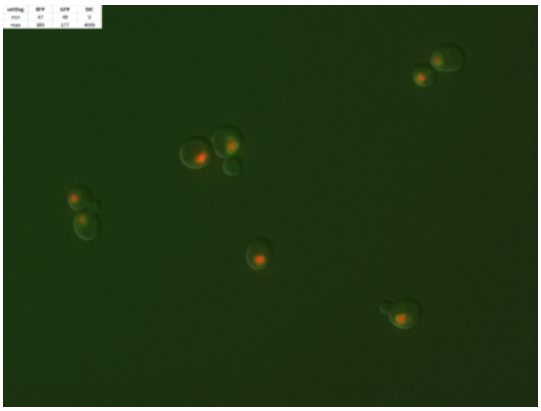
yMRY005 Vacuole Nucleus



yMRY006 Nucleolus Nucleus



yMRY007 Nuclear Periphery Nucleus



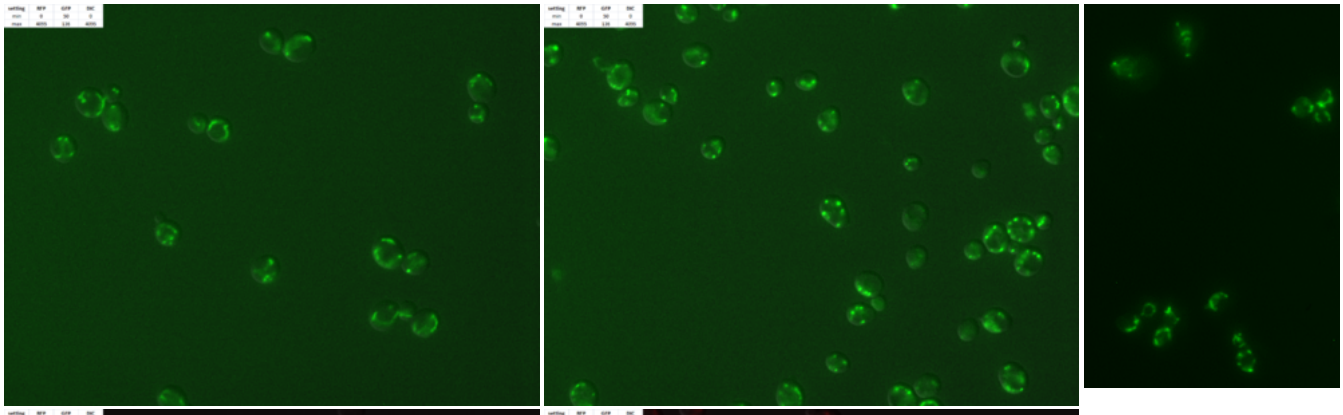
yMRY008 Mito Actin 400px

6/22/12

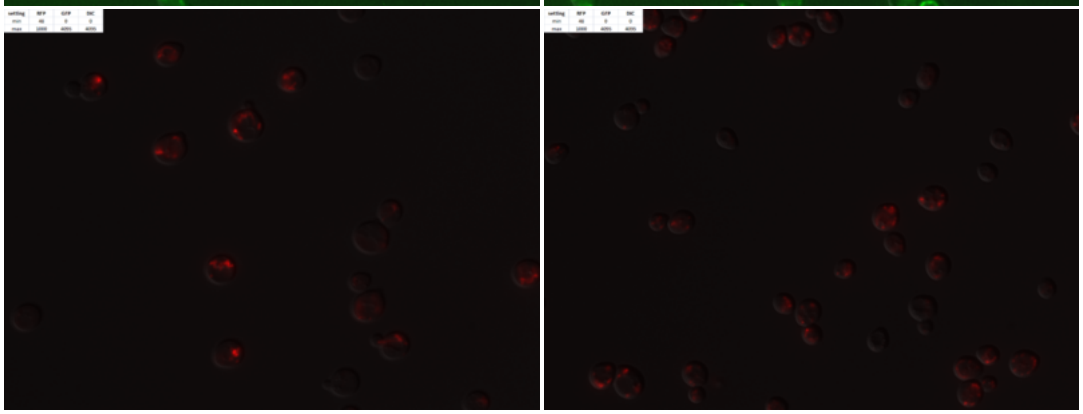
Microscopy

strain/condition	midlog	saturated	ygd image

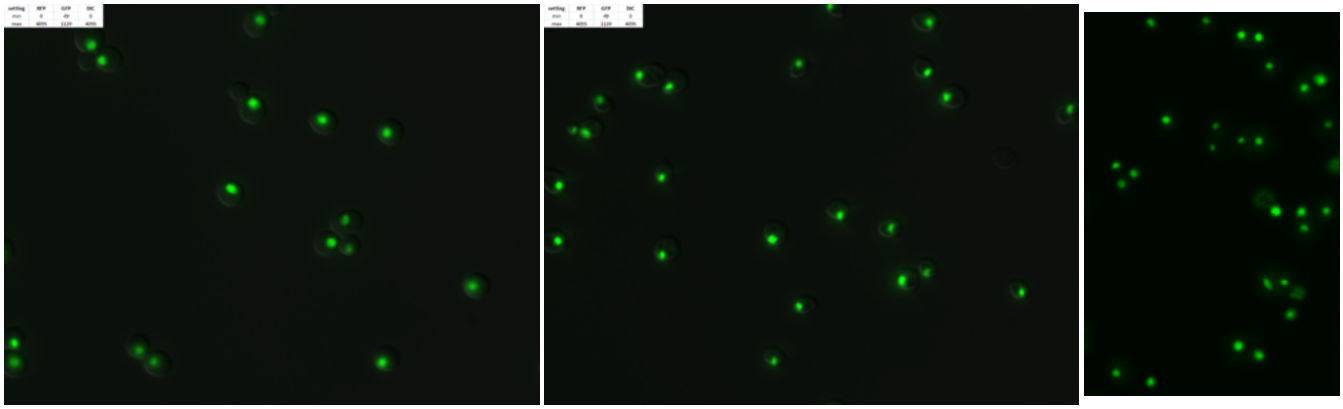
yMRY001
Mito-Venus



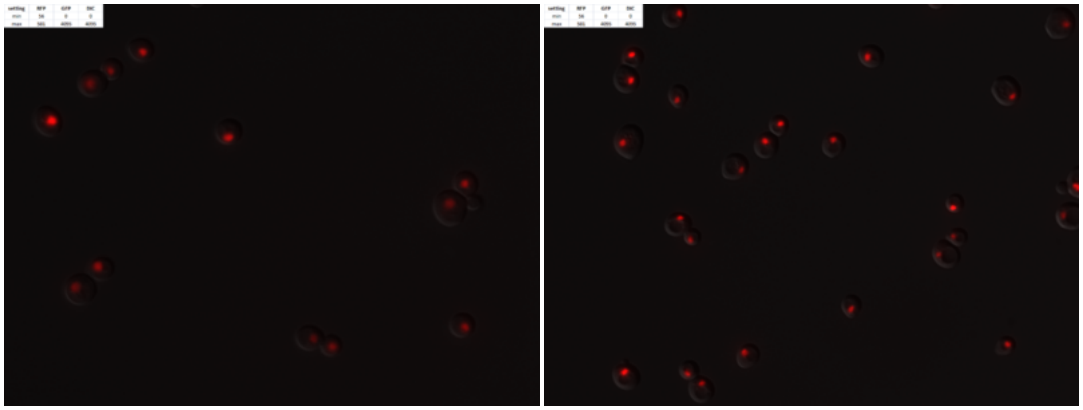
yMRY002
Mito-mKate



yMRY003
Nucleus-Venus



yMRY004
Nucleus-mKate



6/20/12

Double digests

Digest plasmids w/ NotI and PvuI, buffer 3

1. pMRY018-mito Venus (colony 1 from plate)
2. pMRY022-mito mKate (colony 1 from plate)
3. pMRY027- nucleus Venus (colony 2 from plate)
4. pMRY029- nucleus mKate (colony 2 from plate)

2 hours digesting

Transformed venus nucleolus, nuclear periphery and vacuole inserts into nucleus mkate strain (yMRY004)

6/19/12

Yeast and ecoli grew up overnight

Replated ecoli and miniprepped for more plasmid dna

Want an OD600 of 0.2 for yeast cultures to grow up to midlog phase and visualize using microscopy
Will also visualize saturated cultures

Undiluted OD600s:

yMRY001	9.25
yMRY002	4.52
yMRY003	9.48
yMRY004	8.37

6/18/12

Microscopy

Microscopy from a plated culture

1. Drop 4uL of PBS on a glass slide.
2. Dip the pipette into the colony.
3. Pipette the PBS up and down until it becomes cloudy.
4. Carefully drop the cover slip on top of the liquid

Microscopy from a liquid culture

1. Grow yeast in selective media up to 0.6-0.8 OD
2. Take 100uL of that broth and spin it down 8000rpm for 1min.
3. Drop 4ul of PBS on a glass slide.
4. Touch the tip to the pellet, then pipette the PBS up and down until it becomes cloudy.
5. Carefully place cover slip on top of droplet.

All four strains imaged well

strain/cond	setting	RFP	GFP	DIC
yMRY001p	min	0	60	0
	max	4095	163	4095
yMRY002p	min	0	71	0
	max	4095	200	4095
yMRY003p	min	65	0	0
	max	170	4095	4095
yMRY004p	min	45	0	0
	max	155	4095	4095

strain/condition	plate
yMRY001 Mito-Venus	400px
yMRY002 Mito-mKate	400px
yMRY003 Nucleus-Venus	400px
yMRY004 Nucleus-mKate	400px

6/15/12

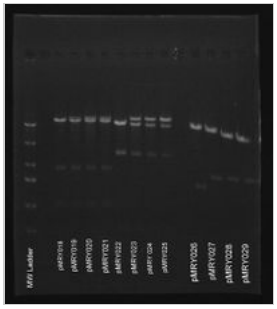
Digested plasmids for 2 hours w/ NotI and PvuI, buffer 3 to insert into yeast

1. pMRY018-mito Venus (colony 1 from plate)
2. pMRY022-mito mKate (colony 1 from plate)
3. pMRY027- nucleus Venus (colony 2 from plate)
4. pMRY029- nucleus mKate (colony 2 from plate)

6/14/12

Gibson product- test digest results:

Mito-venus: all good
Mito-mkate: all good
Nuc-venus: none?
Nuc-mkate:none?



Test digest

Sequence all four nuc, 1 from each for mito
Grow up another set for tomorrow just incase

Sequencing results

pMRY018-mito Venus	confirmed forward and reverse
pMRY022-mito mKate	confirmed forward and reverse
pMRY026-nucleus Venus	no insert
pMRY027- nucleus Venus	confirmed
pMRY028- nucleus mKate	bad read
pMRY029- nucleus mKate	confirmed

6/13/12

redid gibson

1. 2.5 insert
2. 2.5 backbone
3. 5 ul master mix (store bought)

zymoclean up and chemical transformation

6/12/12

Restarted PCR using new primers **without stop codon**
Mito:

```
AU70.iGEM0011 agaagaaaacaaaaaacaaaAGATCTatgttgaaatcaactgc F
AW11.iGEM0037 ccgctgccactaccagaaccAGATCCatgacctgaccatttgatg R
```

Nucleus:

```
AU72.iGEM0013 agaagaaaacaaaaaacaaaAGATCTatgtccggtggtaaaggtg F
AW12.iGEM0038 ccgctgccactaccagaaccAGATCCcagttcttgagaagctttggc R
```

6/11/2012

Looked at sequencing

Both gibson reactions worked but realized there is a stop codon in the reverse primer

Microscopy

100x dse objective microscope

Peroxisome pts 1

Gfp in cytosol control

Think in terms of pixels and pixel length

Microscopy applications

1. Mammalian applications

   Tissue growth
   Monitor how cells develop in a multicellular environment

1. Need a proof of concept -emulating a usable product

   Be user oriented
   When reading papers- ask if could you do this using the micodes

1. Monitoring a population
2. Quantization of population

   Compared to PCR which is unable to do

- Next steps:

1. Expression level testing
2. Combining of organelles/tags

By next week:
Finish targeting and determine which organelles are worth targeting

6/5/2012

Made new stock of electrocompetant cells
Electroporated Gibson product and plated

Celia and Austin had working plasmids from the chemical transformation

- performed yeast transformations

6/6/2012

Transformations did not work
Determined it was due to the competent cells

Used store bought cells - retransformed using 30uL electrocompetent cells

Plates labeled MRY120606:

1-Mito-Venus
2-Mito-mKate
3-Nucleus-Venus
4-Nucleus-mKate

6/7/2012

Cells grew on plates
Positive control worked
Negative control had ~1/3 the colonies seen on experimental plates
Inoculated 3 colonies from each plate in 3 mL of LB Kan

6/4/2012

Ran out of Gibson product

- made more

Did celia's minipreps pCLC003 (1-3), pCLC004 (4-6)

Meeting w/ Dueber:

Look at existing yeast parts in the registry

Homing endonuclease
Optimizing fluorophores for yeast

Download imageJ
Research commercial and non commercial options for imaging software

Need people for administrative tasks
-software

Create strict protocols for imaging

May 2012 Notes- Masaki

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5/31/2012

Checking for restriction sites in protein sequences

BglII, BamHI, XbaI, SpeI

Nucleus:
YBR010W: none
YBL003C: 1 BglII site
Mitochondria:
YDL006W: 1BglII, 1 BamHI, 1 XbaI
YCL017C: 2 BglII

Will remove if necessary in the future.

PCR to obtain protein sequences from the genome
Primers used: AU70, AU 71 for - mitochondria
AU 72, AU73 for YBL003C-nucleus

Performed gibson
Added 2.5 insert, 2.5 backbone and 5 of MM
Incubated for 1 hour

Chemically transformed 10 uL of gibson product into 50 ul cells+KCM

Plated
no colonies grew as of 6/1/2012, determined chemical transformation efficiency was too low. Will try electroporation next

5/30/12

Didn't grow neg control, need to do test digest
5 colonies grew on plate

- Grew up all 5
- Miniprepped

Testing all 5 with test digest of BglII/ XhoI

Backbone:insert=6609:765
Backbone:unwanted vector=6609:2727

Sequencing

- O44(18bp) gttttaaaacctaag r
- Z71 TCACGCCCAAGAAATCAGGC f

Should be:
AGATCTggatctggttctggtagtggcagcggcagcggtagtatgtctaaaggtgaagaattattcactggtgttgtcccaattttggttgaattagatggtgatgttaatggtc

Primers arrived- AU 70, 71, 72, 73: diluted to 100mM

</tab>

5/29/12

2 methods for utilizing MiCodes: linking genotype to MiCode

- Random assembly
  - Best used for single mutagenesis, for linker proteins, enzymes etc
  - Inserted gene-barcode-micode expression cassette
  - High throughput sequencing to determine which genes correlate to which barcodes (each barcode is already assigned to a micode)
  - Downside:
    - Possibility of same micode going to multiple barcodes
- Predetermined assembly
  - For single enzyme mutagenesis (very tedious)
  - For generating combination of gene and different strength promoters (less tedious)

A component is defined as a genetic part that can be isolated

Backbone-[Barcode]-- xbaI--bglII--[gene (w/or w/out start codon)]--backbone
Insert: speI-- [Barcode]-- xbaI--bglII--[gene (w/or w/out start codon)]-BamHI---

Xba/Spe scar

Bgl/Bam scar

5/25/2012

Gene extraction from chromosome using gibson

Forward: promoter homology (20 bp)-AGATCT-gene homology (annealing)

AGAAGAAAACAAAAAACAAA-AGATCT- gene homology

Reverse: gene end homology (annealing/**remove stop codon!**)-GGATCT-linker homology (20 bps) [rev compliment]

Gene homology (end)- GGATCT- ggttctggtagtggcagcgg

Ordering reverse: ccgctgccactaccagaacc-AGATCC- revcomp gene homology

- Gibson overhangs should be 26 or higher
- **Don't forget to remove stop codon at the end of the gene homology region**

Ordered oligos

AU70.iGEM0011	agaagaaaacaaaaaacaaaAGATCTatgttgaaatcaactgc	Mitochondria YCL017C: forward primer for gibson assembly pWCD0421-Venus and pWCD0480-mKate
AU71.iGEM0012	ccgctgccactaccagaaccAGATCCatgacctgaccatttgatg	Mitochondria YCL017C: reverse primer for gibson assembly pWCD0421-Venus and pWCD0480-mKate
AU72.iGEM0013	agaagaaaacaaaaaacaaaAGATCTatgtccggtggtaaaggtg	Nucleus YBL003C: forward primer for gibson assembly pWCD0421-Venus and pWCD0480-mKate
AU73.iGEM0014	ccgctgccactaccagaaccAGATCCcagttcttgagaagctttggc	Nucleus YBL003C: reverse primer for gibson assembly pWCD0421-Venus and pWCD0480-mKate
AV09.iGEM0031	agaagaaaacaaaaaacaaaAGATCTatgagcacgggagatttttttaac	Endosome YPR173C: forward primer
AV10.iGEM0032	ccgctgccactaccagaaccAGATCCgttaccttcttgaccaaaatc	Endosome YPR173C: Reverse primer
AV11.iGEM0033	agaagaaaacaaaaaacaaaAGATCTatggctttggaacctattg	Actin YCR088W: forward primer
AV12.iGEM0034	ccgctgccactaccagaaccAGATCCgttgcccaaagacacataattgc	Actin YCR088W: Reverse primer

5/24/2012

Organelle Targeting

Mitochondria

IDP1 / YDL066W

http://www.yeastgenome.org/cgi-bin/locus.fpl?locus=YDL066W

Mitochondrial NADP-specific isocitrate dehydrogenase:

> catalyzes the oxidation of isocitrate to alpha-ketoglutarate; not required for mitochondrial respiration and may function to divert alpha-ketoglutarate to biosynthetic processes (1, 2)

- IDP1/YDL066W on chromosome IV from coordinates 334835 to 336121.

ATGAGTATGTTATCTAGAAGATTATTTTCCACCTCTCGCCTTGCTGCTTTCAGTAAGATT
AAGGTCAAACAACCCGTTGTCGAGTTGGACGGTGATGAAATGACCCGTATCATTTGGGAT
AAGATCAAGAAGAAATTGATTCTACCCTACTTGGACGTAGATTTGAAGTACTACGACTTA
TCTGTCGAATCTCGTGACGCCACCTCCGACAAGATTACTCAGGATGCTGCTGAGGCGATC
AAGAAGTATGGTGTTGGTATCAAATGTGCCACCATCACTCCTGATGAAGCTCGTGTGAAG
GAATTCAACCTGCACAAGATGTGGAAATCTCCTAATGGTACCATCAGAAACATTCTCGGC
GGTACAGTGTTCAGAGAGCCCATTGTGATTCCTAGAATTCCTAGACTGGTCCCACGTTGG
GAAAAACCAATCATTATTGGAAGACACGCCCACGGTGATCAATATAAAGCTACGGACACA
CTGATCCCAGGCCCAGGATCTTTGGAACTGGTCTACAAGCCATCCGACCCTACGACTGCT
CAACCACAAACTTTGAAAGTGTATGACTACAAGGGCAGTGGTGTGGCCATGGCCATGTAC
AATACTGACGAATCCATCGAAGGGTTTGCTCATTCGTCTTTCAAGCTGGCCATTGACAAA
AAGCTAAATCTTTTCTTGTCAACCAAGAACACTATTTTGAAGAAATATGACGGTCGGTTC
AAAGACATTTTCCAAGAAGTTTATGAAGCTCAATATAAATCCAAATTCGAACAACTAGGG
ATCCACTATGAACACCGTTTAATTGATGATATGGTCGCTCAAATGATAAAATCTAAAGGT
GGCTTTATCATGGCGCTAAAGAACTATGACGGTGATGTCCAATCTGACATCGTCGCTCAA
GGATTTGGCTCCTTAGGTTTGATGACTTCTATCTTAGTTACACCAGACGGTAAAACTTTC
GAAAGTGAAGCTGCTCATGGTACCGTGACAAGACATTATAGAAAGTACCAAAAGGGTGAA
GAAACTTCTACAAACTCCATTGCATCCATTTTCGCGTGGTCGAGAGGTCTATTGAAGAGA
GGTGAATTGGACAATACTCCTGCTTTGTGTAAATTTGCCAATATTTTGGAATCCGCCACT
TTGAACACAGTTCAGCAAGACGGTATCATGACGAAGGACTTGGCTTTGGCTTGCGGTAAC
AACGAAAGATCTGCTTATGTTACCACAGAAGAATTTTTGGATGCCGTTGAAAAAAGACTA
CAAAAAGAAATCAAGTCGATCGAGTAA

Amino Acid Sequence

1 MSMLSRRLFS TSRLAAFSKI KVKQPVVELD GDEMTRIIWD KIKKKLILPY

51 LDVDLKYYDL SVESRDATSD KITQDAAEAI KKYGVGIKCA TITPDEARVK

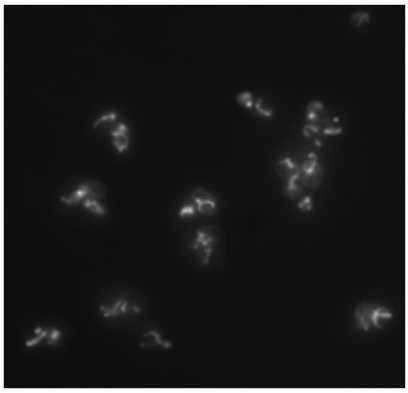
101 EFNLHKMWKS PNGTIRNILG GTVFREPIVI PRIPRLVPRW EKPIIIGRHA 151 HGDQYKATDT LIPGPGSLEL VYKPSDPTTA QPQTLKVYDY KGSGVAMAMY

201 NTDESIEGFA HSSFKLAIDK KLNLFLSTKN TILKKYDGRF KDIFQEVYEA

251 QYKSKFEQLG IHYEHRLIDD MVAQMIKSKG GFIMALKNYD GDVQSDIVAQ 301 GFGSLGLMTS ILVTPDGKTF ESEAAHGTVT RHYRKYQKGE ETSTNSIASI

351 FAWSRGLLKR GELDNTPALC KFANILESAT LNTVQQDGIM TKDLALACGN

401 NERSAYVTTE EFLDAVEKRL QKEIKSIE*



YDL066W with gfp c-terminus tag

http://www.yeastgenome.org/cgi-bin/protein/proteinPage.pl?dbid=S000002224

NFS1/YCL017C

http://www.yeastgenome.org/cgi-bin/locus.fpl?locus=YCL017C

Cysteine desulfurase:

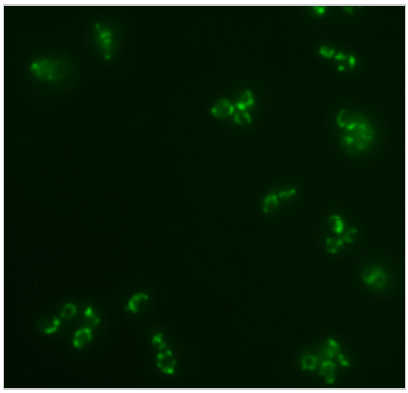
> involved in iron-sulfur cluster (Fe/S) biogenesis and in thio-modification of mitochondrial and cytoplasmic tRNAs; essential protein located predominantly in mitochondria (1, 2, 3)

NFS1/YCL017C on chromosome III from coordinates 94270 to 92777.

The sequence you have requested is oriented with respect to the Crick (bottom) strand. Thus, the chromosomal coordinate of the beginning of the gene or sequence is larger than that of the end. The sequence shown here is the 5'-3' direction of the Crick strand and is the reverse complement of the Watson (top) strand.

ATGTTGAAATCAACTGCTACAAGATCGATAACAAGATTATCTCAAGTTTACAACGTTCCA
GCGGCCACATATAGGGCTTGTTTGGTAAGCAGGAGATTCTATTCCCCTCCTGCAGCAGGC
GTGAAGTTAGACGACAACTTCTCTCTGGAAACGCATACCGATATTCAGGCTGCTGCAAAG
GCACAGGCTAGTGCCCGTGCGAGTGCATCCGGTACCACCCCAGATGCTGTAGTAGCTTCT
GGTAGCACTGCAATGAGCCATGCTTATCAAGAAAACACAGGTTTTGGTACTCGTCCCATA
TATCTTGACATGCAAGCCACTACACCAACAGACCCTAGGGTTTTGGATACGATGTTGAAG
TTTTATACGGGACTTTATGGTAATCCTCATTCCAACACTCACTCTTACGGTTGGGAAACA
AATACTGCTGTGGAAAATGCTAGAGCTCACGTAGCAAAGATGATCAATGCCGACCCCAAG
GAAATAATATTCACTTCGGGAGCGACCGAATCTAATAATATGGTTCTTAAGGGTGTCCCA
AGATTTTATAAGAAGACTAAGAAACACATCATCACCACTAGAACGGAACACAAGTGTGTC
TTGGAAGCCGCACGGGCCATGATGAAGGAGGGATTTGAAGTCACTTTCCTAAATGTGGAC
GATCAAGGTCTTATCGATTTGAAGGAATTGGAAGATGCCATTAGACCAGATACCTGTCTC
GTCTCTGTGATGGCTGTCAATAATGAAATCGGTGTCATTCAACCTATTAAAGAAATTGGT
GCAATTTGTAGAAAGAATAAGATCTACTTTCATACTGACGCCGCACAAGCCTATGGTAAG
ATTCACATTGATGTCAATGAAATGAACATTGATTTACTATCAATTTCTTCTCACAAGATT
TACGGTCCAAAGGGAATAGGTGCCATCTATGTAAGAAGGAGACCAAGAGTTAGATTAGAA
CCTTTACTATCCGGTGGTGGCCAAGAGAGAGGATTGAGATCTGGTACTTTGGCCCCCCCA
TTGGTAGCGGGATTTGGTGAAGCTGCGAGATTGATGAAGAAAGAATTTGACAACGACCAA
GCTCACATCAAAAGACTATCCGATAAATTAGTCAAAGGTCTATTATCCGCTGAACATACC

ACGTTGAACGGATCTCCAGATCATCGTTATCCAGGGTGTGTTAACGTTTCTTTCGCCTAC
GTGGAAGGAGAATCTTTATTGATGGCACTAAGGGATATCGCATTATCCTCGGGTTCAGCC
TGTACATCTGCTTCCCTAGAACCTTCTTATGTTTTACATGCGCTGGGTAAGGATGATGCA
TTAGCCCATTCTTCCATCAGATTTGGTATTGGTAGATTTAGTACTGAAGAGGAGGTCGAC
TACGTCGTTAAGGCCGTTTCTGACAGAGTAAAATTCTTGAGGGAACTTTCACCATTATGG
GAAATGGTTCAAGAAGGTATTGACTTAAACTCCATCAAATGGTCAGGTCATTGA



YCL017C with gfp c-terminus tag

Nucleus

HTA2/YBL003C

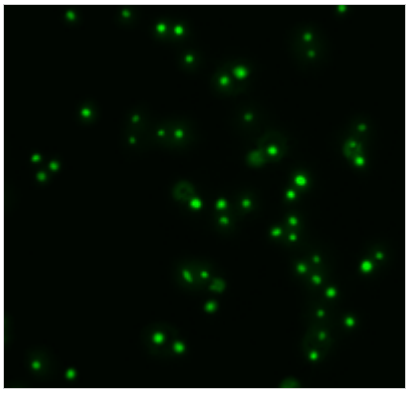
http://www.yeastgenome.org/cgi-bin/locus.fpl?locus=YBL003C

Histone H2A:

> core histone protein required for chromatin assembly and chromosome function; one of two nearly identical (see also HTA1) subtypes; DNA damage-dependent phosphorylation by Mec1p facilitates DNA repair; acetylated by Nat4p (1, 2, 3, 4, 5 and see Summary Paragraph)

The sequence you have requested is oriented with respect to the Crick (bottom) strand. Thus, the chromosomal coordinate of the beginning of the gene or sequence is larger than that of the end. The sequence shown here is the 5'-3' direction of the Crick strand and is the reverse complement of the Watson (top) strand.

Chr 2
ATGTCCGGTGGTAAAGGTGGTAAAGCTGGTTCAGCTGCTAAAGCTTCTCAATCTAGATCT
GCTAAAGCTGGTTTAACATTCCCAGTTGGTAGAGTGCACAGATTGCTAAGAAGAGGTAAC
TACGCCCAGAGAATTGGTTCTGGTGCTCCAGTCTATCTAACTGCTGTCTTAGAATATTTG
GCTGCTGAAATTTTAGAATTGGCTGGTAATGCTGCTAGAGATAACAAAAAAACCAGAATT
ATTCCAAGACATTTACAATTGGCCATCAGAAATGATGATGAATTGAACAAGCTATTGGGT
AATGTTACCATCGCCCAAGGTGGTGTTTTGCCAAACATTCACCAAAACTTGTTGCCAAAG
AAGTCTGCCAAGACTGCCAAAGCTTCTCAAGAACTGTAA
http://www.yeastgenome.org/cgi-bin/getSeq?query=YBL003C&seqtype=ORF%20Genomic%20DNA&format=fasta



YBL003C with gfp c-terminus tag

http://yeastgfp.yeastgenome.org/displayLocImage.php?loc=16754
500 msec

HHT1/YBR010W
http://www.yeastgenome.org/cgi-bin/locus.fpl?locus=YBR010W

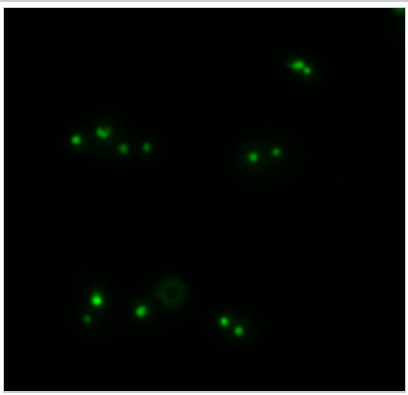
Histone H3:

> core histone protein required for chromatin assembly, part of heterochromatin-mediated telomeric and HM silencing; one of two identical histone H3 proteins (see HHT2); regulated by acetylation, methylation, and phosphorylation (2, 3, 4, 5, 6, 7, 8, 9 and see Summary Paragraph)

HHT1/YBR010W on chromosome II from coordinates 256331 to 256741.

http://www.yeastgenome.org/cgi-bin/getSeq?query=YBR010W&seqtype=ORF%20Genomic%20DNA&format=fasta

ATGGCCAGAACAAAGCAAACAGCAAGAAAGTCCACTGGTGGTAAGGCCCCAAGAAAGCAA
TTAGCTTCTAAGGCTGCCAGAAAATCCGCCCCATCTACCGGTGGTGTTAAGAAGCCTCAC
AGATATAAGCCAGGTACTGTTGCTTTGAGAGAAATCAGAAGATTCCAAAAATCTACTGAA
CTGTTGATCAGAAAGTTGCCTTTCCAAAGATTGGTCAGAGAAATCGCTCAAGATTTCAAG
ACCGACTTGAGATTTCAATCTTCTGCCATCGGTGCCTTGCAAGAATCTGTCGAAGCCTAC
TTAGTCTCTTTATTTGAAGATACCAACTTGGCTGCCATTCACGCCAAGCGTGTCACTATC
CAAAAGAAGGATATCAAGTTGGCTAGAAGATTAAGAGGTGAAAGATCATAG



YBR010W with gfp c-terminus

tag

200 msec
http://yeastgfp.yeastgenome.org/displayLocImage.php?loc=11276

5/21/2012

iGEM registry: www.2012.igem.org

Regionals: **October 13-14th** , Stanford
World champ jamboree: **November 2-5**, MIT

Cloning technologies
○ BglBrick/BioBrick Cloning
○ Gibson Assembly

Gibson, Daniel G et al. "Enzymatic assembly of DNA molecules up to several hundred kilobases." Nature methods 6.5 (2009): 343-345.
One step
Good for making combinations of multiple genes
Primers can get expensive

○ Golden Gate Cloning

Advantage
9 genes + template in one step
BsaI RE, type 2s RE, reach over enzyme- recognizes a site and cuts somewhere else
GGTCTCN|NNNN...
CCAGAGNNNNN|

○ Polymerase cycling Assembly

synthesizing genes from oligos

○ DNA Assembler

Assembles multiple gene fragments at the same time in a yeast cell
Cassette construct design
5'hom-promoter-gene-terminator-3'hom
Moderate efficiency with larger pathways
High eff with short pathways
Ura selection (for plasmid transformations; uracil producing gene)
Advantages
One step cloning
Can assemble large pieces of DNA (9-19 kb)
Doesn’t require exogenous recombinase

Versatile in vivo using plasmid or chromosomal construction

○ Yeast Genomic Integrations w/ homing endonucleases

PMID: 21876185
Reiterative recombination
Goal: to assemble genes together
High efficiency
Homing endonucleases
Recognizes long sequences ~40 bases
Will be unique in genome
Uses recombination to insert genes
2 endonucleases, 2 markers

Codon optimization

To normalize gc content, you can recode for same amino acids but different gc content

MiCodes:Microscopic Barcodes

Genotypes by phenotype using fluorescent proteins
Big questions
How hard is it to distinguish individual organelles
Applications
Orthogonal protein-protein interactions pairs
Finding protein-protein interaction pairs that don't crosstalk
Things to look into
Targeting tag specificity
Fluorescent protein production/lifespan
Current competing process:
FACS- Fluorescence activated cell sorting
Run cells through capillary tube
Laser reads fluorescence one at a time
Sorting Gates based on reading
Does not give info on localization
Need to find reason why miCodes are better
TBDetermined
Directions of applications
Which organelles we can use
Current list
1. Mitochondria
2. Plasma membrane
3. Nucleus
4. Golgi apparatus
5. ER

6. Cortical ER
7. Actin
8. Bud scar -Crater-like ring of chitinous scar tissue located on the surface of the mother cell. It is formed after the newly emerged daughter cell separates thereby marking the site of cytokinesis and septation. The number of bud scars that accumulate on the surface of a cell is a useful determinant of replicative age.
9. Endosomes
10. Vacuole

    Mmr

    Membrane

    Cortisol

1. Peroxyzome
2. Microtubules
3. Nucleolus
4. Other targets

Target fluorescent protein to all locations in yeast and determining what can be differentiated

Use two fluorophores to target each organelle

Cross examine in 12x12 matrix

1 green, 1 red, 1 b&w to determine if we can distinguish organelles

Antibodies?-subcellular specificity

Potential for nucleus

Dyes for confirmation

Need to run a literature search for organelle targeting

Protein shells

Carboxysomes

Balancing protein distribution/ expression level

Can use varying strength promoters

Match promoter levels with miCodes to easily determine what balance of gene expression is required