11/14/13

Dissecting Fitness Effects and Genetic Interactions of Evolved Mutations

Now that I am set up at Lehigh, I plan to do a lot of backcrossing experiments. Previously I've used the strategy of backcrossing the evolved to ancestor and genotyping/phenotyping individual segregants. Here I want to try bulk segregant methods. The idea is to select for a pool of MATa segregants and propagate this pool forward in time. The question becomes how to best look at the ratio of evolved to ancestral alleles.

My plan is to do this bulk segregant method on 12 populations and pool them ~~into one lane~~ and do whole-genome sequencing. I would be looking at probably 50-100 mutations scattered throughout the genome. It seems easier (certainly quicker, and possibly cheaper) to do it this way rather than PCRing up each mutation.

Each evolved SNP would start at 1/24 of the population — this may be a bit low — with 200x coverage ~~it would be~~ there would be ~10 reads calling the mutation. Over time, though, beneficial mutations would increase in frequency as:

$$\ln\left(\frac{E_t}{A_t}\right) = \ln\left(\frac{E_0}{A_0}\right) + st$$

There are several things to do before I can really move ahead with this. The first is to construct a good MATα strain for the backcrossing experiment.

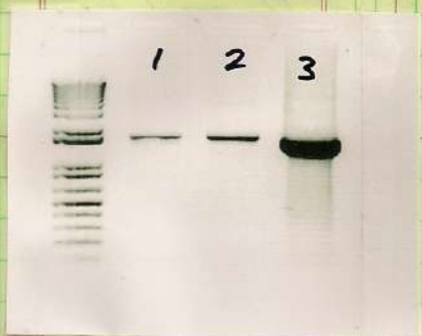
11/14/13

INTEGRATING URA3 NEAR MATα IN yGIL646

I attempted to do this before but without success (see Notebook Evolution 8, p 133).

A few days ago Remi did a PCR of the URA3MX cassette off of pAG60 using primers oGIL446 & 447.

I then did a 500 λ PCR (spread out over 8 tubes). I pooled these PCRs and eluted in 30 λ EB



LANE
1 Remi's PCR
2 My PCR
3 Pooled/Concentrated PCR

I transformed 10 λ of the PCR using standard protocol
- Diluted 1:100 λ into duplicate 10 mL YPD ~~et~~ tubes (~9am)
- Transformation started ~2pm
- I resuspended in only 20 λ LiOAc Mix — I did duplicate 10 mL tubes, rather than split 1 culture
- Heat shock 30' (rather than 15')
- Plated onto SD+his+trp

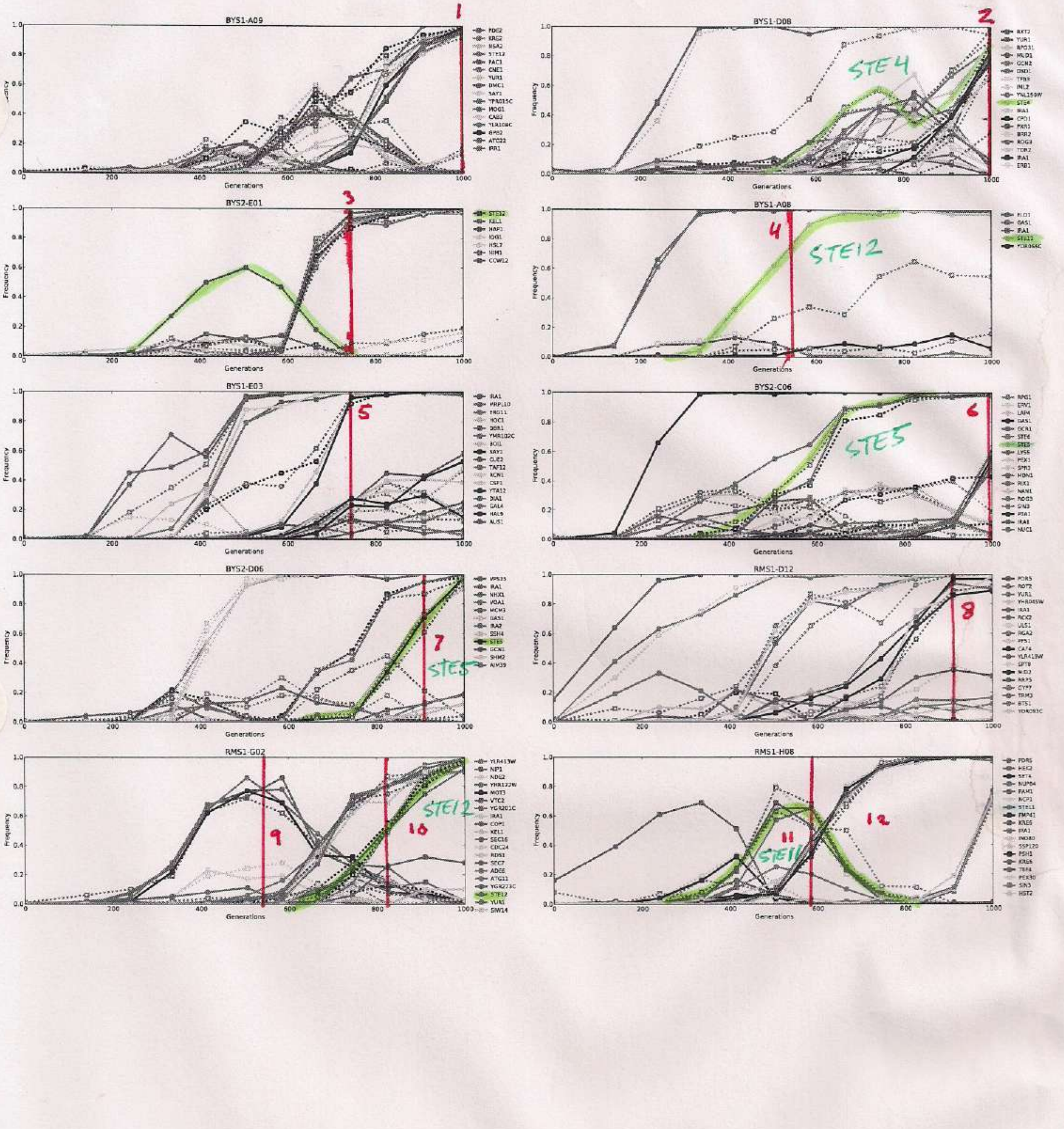
11/17/13

I see a bunch of colonies (background high in some places)

For each of the two plates I will
- pick two colonies → inoculate SD+his+trp
- Take two streaks → " " " "
- pick two colonies → restreak on SD+his+trp

11/18/13

It look like stuff is growing on all of the plates & tubes (aside from maybe one of the clones) so I am hopeful that I have succeeded in integrating URA3 near MATα → the next thing to do is to mate and show that it segregates w/ mating type


BYS1-A09
1
BYS1-D08
2
STE4
BYS2-E01
3
BYS1-A08
4
STE12
BYS1-E03
5
BYS2-C06
6
STE5
BYS2-D06
7
STE5
RMS1-D12
8
RMS1-G02
9
10
STE12
RMS1-H08
11
STE11
12
Generations
Frequency

	Initials	Date
Prepared By		
Approved By		

11/18/13

STREAKING CULTURES FOR BULK-SEGREGANT FITNESS ASSAYS

On Friday (11/15) I streaked from the freezer the following populations. These were selected using the following criteria:

- Only small cultures (therefore everything can be done on a 24-h cycle)
- Mix of RM/BY
- Inclusion of steriles
- Avoid FDS
- Avoid repeated identical mutations (ie IRA1)
- Try to capture lots of mutations
- Try to get a few cases where multiple clones/cohort
- Try to get a few matched samples

Population

1. BYS1-A09-1000
2. BYS1-D08-1000
3. BYS2-E01-745
4. BYS1-A08-545
5. BYS1-E03 - 745 ←
6. BYS2-C06-1000
7. BYS2-D06-910
8. RMS1-D12-910
9. RMS1-G02-545
10. RMS1-G02-825

11/12 RMS1-H08-585 → 11 sterile (stell) → 12

11/18/13
BYS1-E03 colonies are smaller than all the others on YPD plates. Clearly more fit in liq, but maybe not on agar.

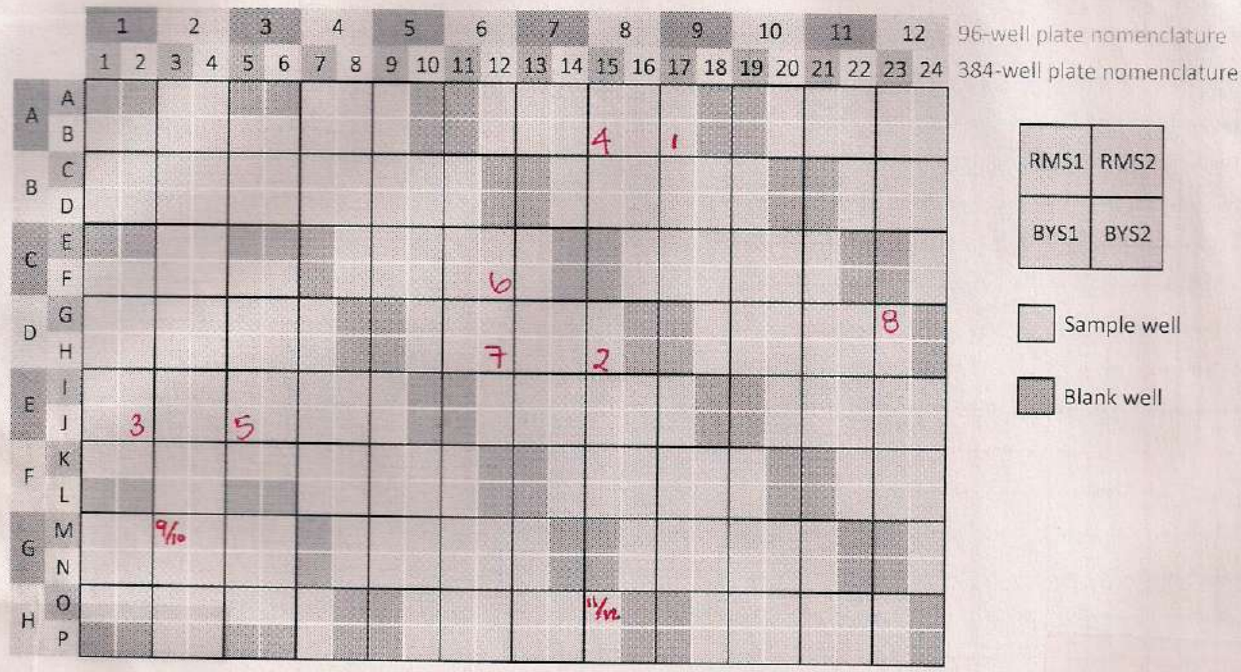
All streaked onto YPD on friday, put in fridge yesterday

Remi picked singles and set up the following plates:

I added 4 λ of each of the 7 clones of (2, 3, 4, 6, 7, 8, 9, 10, 11/12) to YPD + αF

We did a 1:1000 serial transfer of both plates.

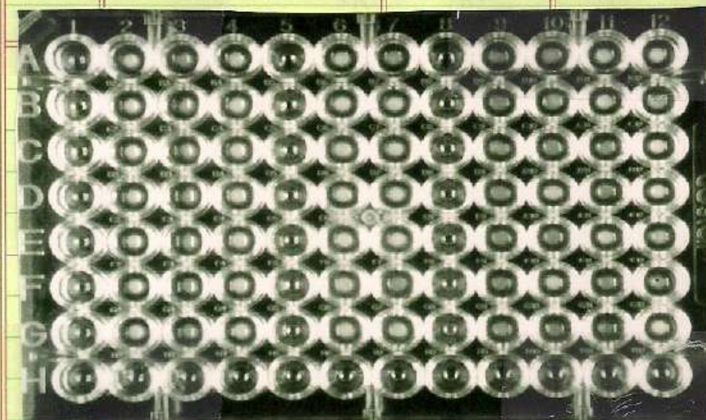
Small population size cultures



	Initials	Date
Prepared By		
Approved By		

11/19/13

Phenotyping picked clones



It is not so easy to see in the plate picture above, but it was obvious which ~~ones~~ clones grew on YPD+αF (steriles) and which did not. I checked column 12 A&B under the scope, just to be sure. The #s agree w/ expectations given the freq. of steriles of each sample point (p. 3).

2	BYS1-D08-1000	All Sterile
3	BYS2-E01-745	None sterile
4	BYS1-A08-545	All Sterile
6	BYS2-C06-1000	All Sterile
7	BYS2-D06-910	A&F Not sterile BCDEG Sterile
9	RMS1-G02-545	None Sterile
10	" " -825	ABCEG# Not Sterile DF Sterile
11	RMS1-H08-585	CDFG# NS ABE Sterile
12		BEF# Not sterile ACDG Sterile

11/20/13

FITNESS ASSAYS ON EVOLVED CLONES

Yesterday at 3:30 Remi & I started the fitness assays on the picked evolved clones.

We mixed the freshly grown Exp 3 Ref plates (501:501) and diluted 1:1000 into 3 plates (using the same serial middle plate).

We will wash & reuse the tips during the expt.

We put the gen. 0 sample into $\frac{1}{4}$ of a 384 well plate.

Today I ran FACS on the Gen 0 samples.

Yesterday we also froze down the Exp. plate

11/26/13

Dilutions (started @ 3:30)

11/21/13

NOTES ON FACS: FACS is going great so far. Some issues with air bubbles, perhaps I will reduce mixing volume/speed or go without mixing entirely. I had similar issues at Princeton and I wonder if I could have fixed this there by adjusting params. Now I am doing:

1 μL/s counting
10 μL volume
10 μL mixing volume
100 μL/s mixing speed

I will try these tomorrow and see.

Dilutions done ~ 4pm (~3:50)

11/22/13

Dilutions ~3:30 (on incubator ~ 3:50)

11/25/13

FITNESS ASSAYS ON EVOLVED CLONES

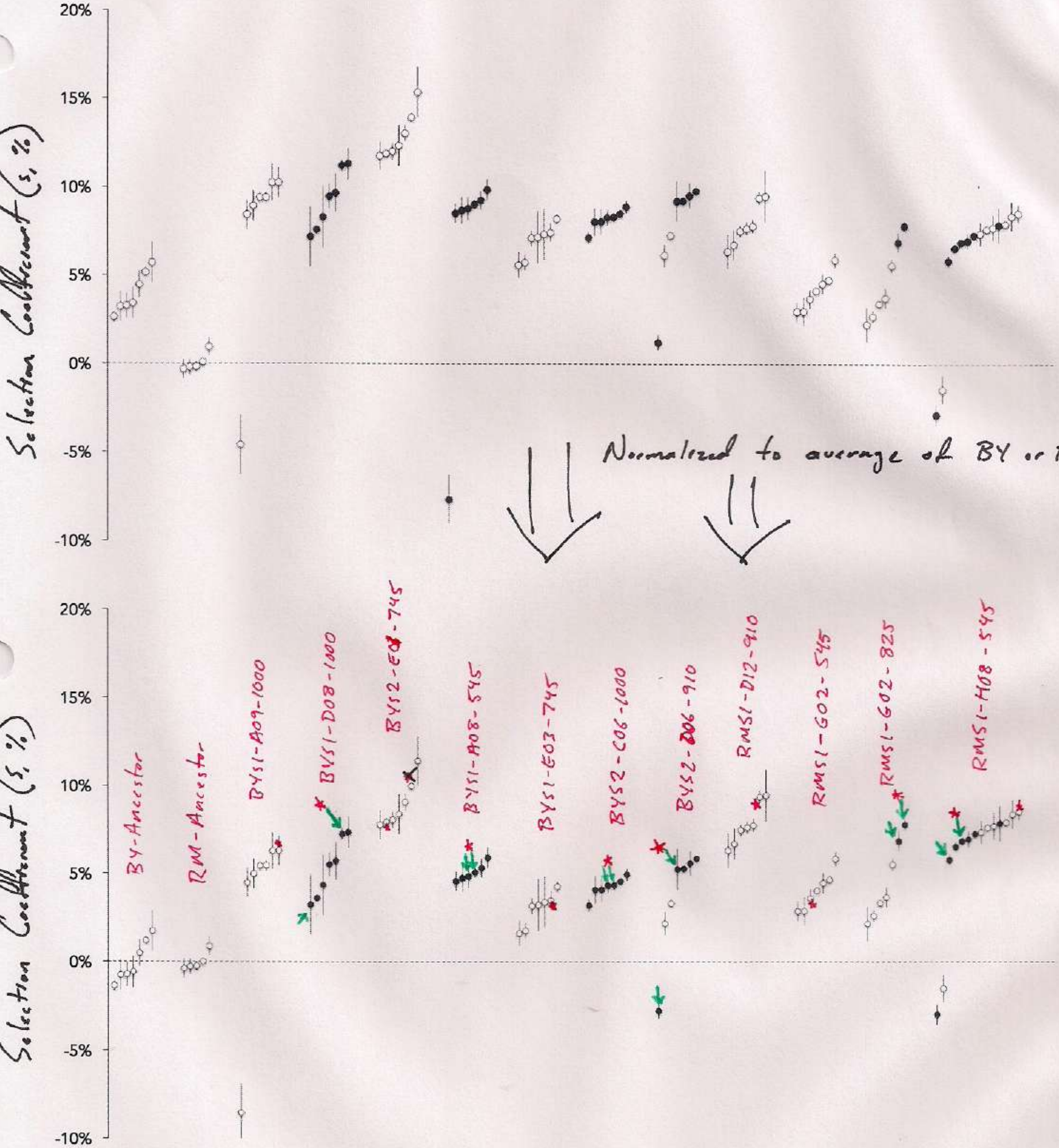
Sample	Replicate		Average Nonsterile	Average Sterile	SteDev
BY-Anc	Clone 1	1	-1.4%		0.3%
BY-Anc	Clone 7	2	-0.8%		0.9%
BY-Anc	Clone 6	3	-0.7%		0.7%
BY-Anc	Clone 4	4	-0.6%		0.9%
BY-Anc	Clone 3	5	0.5%		0.8%
BY-Anc	Clone 2	6	1.2%		0.2%
BY-Anc	Clone 5	7	1.7%		1.1%
RM-Anc	Clone 1	7	-0.4%		0.6%
RM-Anc	Clone 5	8	-0.3%		0.4%
RM-Anc	Clone 2	9	-0.2%		0.3%
RM-Anc	Clone 3	10	0.0%		0.1%
RM-Anc	Clone 4	11	0.9%		0.5%
BYS1-A09-1000	Clone 2	11	-8.5%		1.7%
BYS1-A09-1000	Clone 1	12	4.5%		0.8%
BYS1-A09-1000	Clone 7	13	5.0%		0.8%
BYS1-A09-1000	Clone 4	14	5.4%		0.3%
BYS1-A09-1000	Clone 5	15	5.5%		0.3%
BYS1-A09-1000	Clone 3	16	6.3%		1.0%
BYS1-A09-1000	Clone 6	17	6.3% ★		0.8%
BYS1-D08-1000	Clone 2	17		3.2% ←	1.7%
BYS1-D08-1000	Clone 5	18		3.6%	0.2%
BYS1-D08-1000	Clone 4	19		4.3%	1.7%
BYS1-D08-1000	Clone 7	20		5.5%	0.7%
BYS1-D08-1000	Clone 6	21		5.7%	1.1%
BYS1-D08-1000	Clone 1	22		7.2% ← ★	0.3%
BYS1-D08-1000	Clone 3	23		7.4%	0.9%
BYS2-E01-665	Clone 4	23	7.8%		0.8%
BYS2-E01-665	Clone 1	24	7.9% ★		0.2%
BYS2-E01-665	Clone 6	25	8.1%		0.5%
BYS2-E01-665	Clone 2	26	8.4%		1.1%
BYS2-E01-665	Clone 5	27	9.1%		0.5%
BYS2-E01-665	Clone 3	28	10.0%		0.3%
BYS2-E01-665	Clone 7	29	11.4%		1.4%
BYS1-A08-545	Clone 3	29		-11.7%	1.4%
BYS1-A08-545	Clone 6	30		4.6%	0.5%
BYS1-A08-545	Clone 7	31		4.8%	0.8%
BYS1-A08-545	Clone 2	32		4.8% ←	0.7%
BYS1-A08-545	Clone 1	33		5.1% ← ★	0.3%
BYS1-A08-545	Clone 5	34		5.3%	0.5%
BYS1-A08-545	Clone 4	35		5.9%	0.6%
BYS1-E03-745	Clone 7	35	1.6%		0.7%
BYS1-E03-745	Clone 5	36	1.8%		0.4%
BYS1-E03-745	Clone 1	37	3.2%		0.5%
BYS1-E03-745	Clone 6	38 .	3.2%		1.5%
BYS1-E03-745	Clone 4	39	3.4%		1.4%
BYS1-E03-745	Clone 3	40	3.5% ★		0.5%
BYS1-E03-745	Clone 2	41	4.3%		0.3%
BYS2-C06-1000	Clone 6	41		3.2%	0.3%
BYS2-C06-1000	Clone 4	42		4.1%	0.8%
BYS2-C06-1000	Clone 3	43		4.1%	0.7%
BYS2-C06-1000	Clone 1	44		4.3% ←	0.4%
BYS2-C06-1000	Clone 2	45		4.4% ← ★	0.2%
BYS2-C06-1000	Clone 5	46		4.6%	0.1%
BYS2-C06-1000	Clone 7	47		4.9%	0.3%
BYS2-D06-~~665~~ 910	Clone 3	47		-2.8% ←	0.4%
BYS2-D06-~~665~~ 910	Clone 6	48	2.2%		0.6%
BYS2-D06-~~665~~ 910	Clone 1	49	3.3%		0.2%
BYS2-D06-~~665~~ 910	Clone 2	50		5.3% ← ★	1.1%
BYS2-D06-~~665~~ 910	Clone 7	51		5.3%	0.2%
BYS2-D06-~~665~~ 910	Clone 4	52		5.6%	0.7%
BYS2-D06-~~665~~ 910	Clone 5	53		5.9%	0.2%
RMS1-D12-910	Clone 5	53	6.3%		1.0%
RMS1-D12-910	Clone 7	54	6.7%		0.9%
RMS1-D12-910	Clone 6	55	7.5%		0.3%
RMS1-D12-910	Clone 3	56	7.6%		0.4%
RMS1-D12-910	Clone 2	57	7.7%		0.4%
RMS1-D12-910	Clone 4	58	9.4% ★		0.4%
RMS1-D12-910	Clone 1	59	9.5%		1.5%
RMS1-G02-545	Clone 2	59	2.9%		0.6%
RMS1-G02-545	Clone 6	60	2.9%		0.8%
RMS1-G02-545	Clone 1	61	3.6% ★		0.5%
RMS1-G02-545	Clone 3	62	4.1%		0.0%
RMS1-G02-545	Clone 5	63	4.5%		0.6%
RMS1-G02-545	Clone 7	64	4.7%		0.2%
RMS1-G02-545	Clone 4	65	5.9%		0.4%
RMS1-G02-825	Clone 2	65	2.2%		1.0%
RMS1-G02-825	Clone 1	66	2.6%		0.3%
RMS1-G02-825	Clone 7	67	3.4%		0.1%
RMS1-G02-825	Clone 5	68	3.7%		0.6%
RMS1-G02-825	Clone 3	69	5.5%		0.4%
RMS1-G02-825	Clone 4	70		6.8% ←	0.6%
RMS1-G02-825	Clone 6	71		7.8% ← ★	0.3%
RMS1-H08-585	Clone 02	71		-2.9%	0.6%
RMS1-H08-585	Clone 07	72	-1.5%		0.8%
RMS1-H08-585	Clone 03	73		5.8% ←	0.3%
RMS1-H08-585	Clone 08	74		6.5%	0.3%
RMS1-H08-585	Clone 01	75		6.9% ← ★	0.2%
RMS1-H08-585	Clone 06	76		6.9%	0.4%
RMS1-H08-585	Clone 09	77		7.3%	0.2%
RMS1-H08-585	Clone 13	78	7.4%		0.7%
RMS1-H08-585	Clone 11	79	7.6%		0.3%
RMS1-H08-585	Clone 05	80	7.7%		0.7%
RMS1-H08-585	Clone 14	81		7.8%	1.0%
RMS1-H08-585	Clone 04	82	7.9%		0.2%
RMS1-H08-585	Clone 10	83	8.4%		0.8%
RMS1-H08-585	Clone 12	84	8.5% ★		0.5%

- The green arrows (←) indicate which sterile clones were transformed.
  - ↳ Unfortunately some of them have low fitness
- Red star (★) indicates the ~~[illegible]~~ strains I plan to backcross

12A
11D
11B
12D
11A
12C
11E
11G
11F
11C
12G
12B
12E
12F

11/25/13

FITNESS ASSAYS ON EVOLVED CLONES



○ Nonsterile

● Sterile

11/25/13

NOTES ON FITNESS ASSAYS

Overall the data look great. There were some issues with the cytometer — but this was just first-time use stuff. (leaking, switching waste, etc) but it is good now. I changed the params again:

1 µl/s counting
20 µl volume
20 µl mixing volume
200 µl/s mixing speed

- It looks like the BY-reference I picked had a significant fitness defect. There are two observations that substantiate this: both BY-Anc and BYS1-A08-545 were higher than expected. After normalizing to the mean of the ancestor they look as expected.

- BYS2-E01-745 had the highest fitness advantage, but ~~at~~ only one sweep (that does not contain any known drivers)

- BYS1-E03-745 showed strange pellet morphology — I will need to look more carefully to see if this is sliming FDS.

- It is good to see that the nonsterlies are more fit than the sterlies in RMS1-H08-545, since they outcompeted the sterlies during the expt.

- It is good to see that the sterlies in RMS1-G02-825 are more ~~fit~~ fit than both the nonsterlies from the same timepoint and also the nonsterlies from generation 545

→ However I expected the nonsterlies from gen 825 to be more fit than the nonsterlies from 545 → not clear if this is true.

11/20/13

	Initials	Date
Prepared By		
Approved By		

PREPARING STRAINS FOR BACKCROSSING

Yesterday Rami picked colonies off of the -ura restreak plates (page 2). Today I will freeze down these putative URA3 transformants and I will plate them to Canavanine plates. ⟹ yGIL675 → 678

Also today I will streak plasmids from the MoBY collect which contain STE4, STE5, STE11, & STE12.

11/21/13

MINIPREPS ON MoBY PLASMIDS

As it turns out the MoBY plasmid's are not Amp^R, they are Kan^R. So I inoculated 2.5 ml of LB+Kan w/ ~~4~~ 2 λ of bact stock (last night ~5pm).

This morning I prepped 1 ml culture

LANE
1 pMoBY - STE4
2 " 5
3 " 11
4 " 12

→ this is consistent w/ previous obs. (see notebook Evolution 8, p 160) in that ~~??~~ pMoBY-STE5 ran as high MW and pMoBY-STE12 as lower MW

Tonight I will pick strains to transform.

11/22/13

I ~~pick~~ started o/n cultures of the following (pulled from plate in fridge):

② BYS1-D08-1000 A & B
④ BYS1-A08-545 A & B
⑥ BYS2-C06-1000 A & B
⑦ BYS2-D06-910 B & C
⑩ RMS1-G02-825 D & F
⑪ RMS1-H08-545 A & B

11/23/13

- Dilute o/n cultures 1:100 ~10am
- Start transformations ~ ~~#~~ 2:30 pm

11/25/13

Transformations look good — hundreds of putative transformants per plate. This afternoon I will pick 3 putative transformants each and inoculate into 5ml SD+his+trp. Tomorrow I will check αF^s

→ I inoculated 5 ml SD+his+trp w/ 4 putative transformants each from 2A, 4A, 6B, 7B, 10F, & 11A (see p. 9 & 11, red stars)

11/25/13

· On 11/23 I restreaked ~~on~~ putative can1 mutants of yGIL675→678 onto YPD

· Today I picked one single colony and inoculated 5 ml YPD.

11/27/13

· Yesterday I spotted the picked putative can1 mutants to SD+his+trp+Can.

· Not much growth o/n (t.s. 8:30am) and the growth does not look like a lawn on the patch, rather looks like papillation → not sure what that means.

· No growth, though, of the CAN1 strain BY51-00-L000-2A

· Also, yGIL675-can1, ~~[illegible]~~ yGIL676-can1, ~~[illegible]~~ seem to be growing best, yGIL676-can1 a little less, and yGIL678-can1 not much at all on the Can plate. I will leave the plate @ 30 over break.

→ Freezedown yGIL675-can1 ⟶ yGIL679
yGIL676-can1 ⟶ yGIL680

~~NOTE: These cultures grew on SD+his+trp. I will spin down and resuspend in YPD for freezedown, but should be recognized on [illegible]~~

11/27/13

SCREENING FOR COMPLEMENTATION OF STERILE MUTATIONS

- Last evening I took 4λ of each of the SD+his+trp cultures containing the putative plasmid transformants and inoculated 800λ of YPD+αF
- This morning I am screening for those that respond to αF – given that the culture grew o/n and given the rate of plasmid loss, there are lots of budding cells under the scope, but I am just looking for any shmoos in the mix.

~~PUTATIVE~~
PUTATIVE
TRANSFORMANT

2A	1	Shmoos	Freeze down as → yGIL681
	2	Shmoos	→ yGIL682
	3	Shmoos	
	4	Shmoos	
4A	1	Shmoos	→ yGIL683
	2	Shmoos – but not many	
	3	Shmoos	→ yGIL684
	4	Shmoos	
6B	1	Shmoos	→ yGIL685
	2	Shmoos	→ yGIL686
	3	Shmoos	
	4	Shmoos	
7B	1	Shmoos	→ yGIL687
	2	Shmoos	→ yGIL688
	3	Shmoos	
	4	Shmoos	
10F	1	Shmoos	→ yGIL689
	2	No shmoos	
	3	Shmoos	→ yGIL690
	4	Shmoos	
11A	1	No shmoos	
	2	Maybe a couple shmoos	→ yGIL691
	3	Shmoos	→ yGIL692
	4	No shmoos	

NOTE: These cultures were frozen down from SD+his+trp cultures. Should be restreaked on -ura medium to maintain plasmid.

12/6/13

Generating Diploids for Bulk Segregant fitness Assays

· Streak the MATα strains w/ genotype: MATα::URA3, can1

- yGIL679
- yGIL680

Streak the steriles (complemented by URA plasmid) onto SD+his+trp:

yGIL681
" 683
" 685
" 687
" 689
" 692

Streak non-sterile evolved clones onto YPD from 96-well plate in freezer:

STRAIN	CLONE	WELL
BYS1-A09-1000	6	F1
BYS2-E01-665	1	A3
BYG1-E03-745	3	C5
RMS1-D12-910	4	D8
RMS1-G02-545	1	A9
RMS1-H08-585	12	F12

12/9/13

My plan was to do crosses w/ all of these, but I am debating now. I wonder if I should make an RM version of the Bulk-segregant ancestor. → I suppose that is a good thing to do — it will also allow me to test how well the bulk-segregant selection works. I will put all the plates above in my fridge for now.

Streak: yGIL429 (RM-Ancestor)
yGIL679 (Bulk-segregant MATα strain)
yGIL680 (")

12/12/13

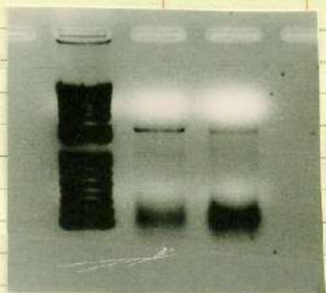
· Two days ago I crossed yGIL429 to both yGIL679 + 680 by manipulating spores → for 3 matings each.

· Yesterday I picked these putative diploid colonies and restreaked to YPD.

12/12/13

SEQUENCING Canl MUTANT ALLELES FROM yGIL679 & yGIL680

~~Yesterday I~~ Two days ago I did genome preps on yGIL679 and yGIL680 and PCR'd the canl locus using primers oGIL280 & oGIL281



2 kb product → PCR cleanup → elute 30 λ EB

→ submit for sequencing w/ outside primers

NOTE: I may have mixed up the PCRs

12/17/13

Sequencing is not great → I can't really tell what the canl mutations are → doesn't really matter.

12/17/13

PREPARING STRAINS FOR BACKCROSSING — CONTINUED

I succeeded in constructing ~~an~~ a BY strain for backcrossing which ~~is~~ has URA3 integrated adjacent to MATα and is a can1 mutant. There are two can1 mutants (yGIL679 & yGIL680).

I will need to make an RM version of this strain.

I crossed yGIL429 (Ancestor-RM) to both yGIL679 & 680 by micromanipulating to arrange matings

↳ picked 3 putative diploid colonies and restreaked to singles (A → C)

↳ picked ~~3~~ 2 singles each (A1 → C2) & mating type test.

12/18/13

All twelve clones from the two crosses (yGIL429 × 679 & yGIL429 × 680) did not mate w/ either MATa or MATα testers. Control strains (yGIL519 & yGIL679) mated expected.

I froze down the following

Cross of	yGIL429	& yGIL679	Clone A1	→	yGIL693
"	"	"	" B1	→	" 4
"	"	yGIL680	" A1	→	" 5
"	"	"	" B1	→	" 6

NOTE: I made note on the original mating plate, that the "A mating" of yGIL429 & yGIL680 made a slightly smaller colony → though they didn't look any smaller when I restreaked. Could have been that it just arrested longer & took longer to mate and restart growth.

I want to test a few sporulation protocols on the diploids yGIL693 & yGIL696.
(1) My crude protocol of saturating growth in YPD for 3 days then putting in 2% KAc + His/Trp
(2) The Murray lab protocol: o/N in YPD; 6h in YP KAc; 2% KAc + His/Trp
(3) The Dunham Lab protocol: o/N in YPD; into SPO++ (~~[illegible]~~ 1.5% KAc, 0.25% Yeast Extract, 0.25% Glucose)

12/19/13

The Murray lab & Dunham lab protocols are similar, so I will just do Dunham lab protocol

· Yesterday I started o/n cultures of yGIL693 & 696 in 10 mL YPD
· Today I spun down and resuspended in 20 mL SPO++ media (1.5% KAc, 0.25% YE, 0.25% Glucose, +H+T)
↳ on RT rollerdrum.

12/23/13 The direct-to-KAc sporulations were poor, but the SPO++ look great.
I will leave them on the roller drum until tomorrow, then put them in the fridge.

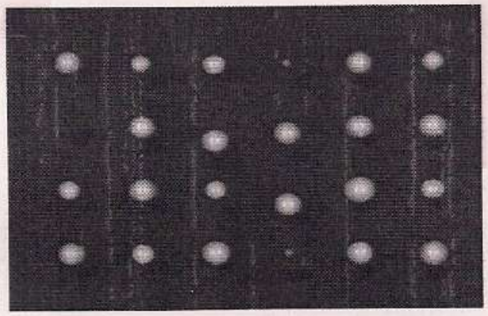
12/31/13

DISSECTING TETRADS

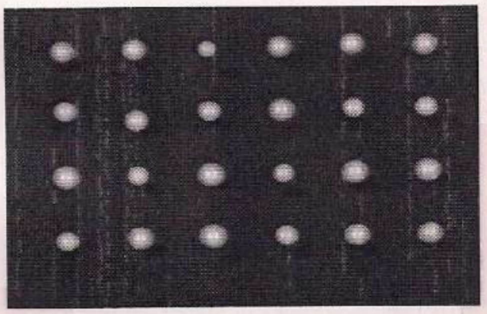
Yesterday I dissected six tetrads each of yGIL693 & yGIL696.

1/1/14

yGIL693



yGIL696



Some noticeable differences in colony size — kind of looks 2:2.

In these crosses ~~three~~ four things are segregating: Mating type, URA3, ~~and can1.~~ can1, and GPA1

↳ although Mating type and URA3 are linked!

Replica plate each to YPD, 5FOA, Can, a-tester, & α-tester

12/31/13

BULK SEGREGANT TEST

Today I am going to take the sporulated cultures of yGIL6933 696 (p.21) and try the Bulk Segregant random-spore protocol that Ian Ehrenreich used.

My starting material is 20ml of sporulated culture

Ian's Protocol	My Modified Protocol
· 100 ml YPD culture	10 mL YPD
· Resus in 200 ml SPO++	20 mL SPO++
· Spin 50ml	~~Resuspend in 200λ~~ Spin 10mL
· Resuspend 1ml H_2O	Resuspend in 200λ (results in ~500λ volume)
· Add 300λ β-glucoronidase	Add 10λ Zymolyase (1000x)
— 30° for 1h —	— 30° for 1h —
· Add 50λ glass beads	
— Vortex 2 min —	
— 30° for 1h —	
— Vortex 2 min —	
· Add 19 ml H_2O	
· Plate 100λ per plate	

I looked under the scope after the 1h incubation and I did not see any tetrads still intact. I didn't think the extra hour incubation was necessary.

I added 50λ glass beads and vortexed for 2 min.

After this I noticed that there were v. large cell aggregates as well as cells sticking to the plastic — presumably these are sticky spores. I then added the cells (~300λ) to 5ml H_2O in a falcon tube. I noticed that the cells were pelleting a lot. I decided to spin the falcon tubes. (300RPM - slowest speed for 2 min)

I separated the supernatant 3ml into 3 tubes. I discarded the rest.

I also washed the cells that were sticking to the wall twice w/ ~~4~~ 1ml H_2O. Then I tried to remove the cells w/ 2ml PBSTween.

I plated 100λ of the supernatant & PBST prep onto SD+his,trp,ura, SD+htu+can, SD+htu+5FOA, & SD+htu+Can+5FOA.

NOTES:

- So basically I had good digestion w/ 10λ Zymolyase. I could get away w/ less and perhaps I should try 5λ next time.
- I essentially did the spore enrichment that Sebastian & Megan have done. Ian, though, didn't do an enrichment. In the future I should probably bring up to 5ml in PBST, not H_2O, and I should use glass tubes not plastic.
- It would be useful to sonicate the samples, but unfortunately I don't have one.
- I wonder if glass beads are necessary — I would think the cells would stick to the glass beads — the clumping didn't happen until after vortexing.
- Even in the finished preps, I noticed some cells that seem to be spores stuck together.
- Overall good, but I feel it could be improved. We will see what grows up.

1/3/[redacted]14

NOTES ON TETRAD DISSECTIONS AND BULK-SEGREGANT TEST

Well, I took two steps back. First I'll start w/ the results of the tetrad dissections of yGIL693 & yGIL696.

Parent	Spore	+MATa	+MATalpha	5FOA	Can	Big Size
yGIL693	1A	x				x
	[redacted]					
	1C		x	x	x	
	1D	x				x
	2A		x	x	x	
	2B	x				x
	2C	x			x	x
	2D		x	x		
	3A		x		x	
	3B	x		x		x
	3C		x	x	x	
	3D	x				x
	4A		x	x		
	4B	x		x	x	x
	4C	x				x
	4D		x		x	
	5A	x		x	x	x
	5B	x			x	x
	5C					x
	5D		x			x
	6A		x			
	6B	x			x	x
	6C		x	x	x	
	6D	x		x		x
yGIL696	1A	x				x
	1B		x	x	x	
	1C	x			x	x
	1D		x	x		
	2A	x		x	x	x
	2B		x			
	2C		x			
	2D	x		x	x	x
	3A		x	x		
	3B		x	x		
	3C	x			x	x
	3D	x			x	x
	4A	x				x
	4B	x			x	x
	4C		x	x		
	4D		x	x	x	
	5A	x			x	x
	5B		x			
	5C	x		x		x
	5D		x	x	x	
	6A	x				x
	6B		x			
	6C	x		x		x
	6D		x	x		

URA3 is NOT linked to MATα

So the strains are wrong. But where is URA3. It could be at the URA3 locus - I doubt this is the case since it is the C. albicans URA3. It could have gone to GPA1 where there is a NatMX cassette.

↳ I could test both of these possibilities

- Check to see if MATa, URA3 has the yEVenus by Flow
- [redacted] Check to see if yGIL679 & 680 are ~~NatMX~~ CloNAT R.

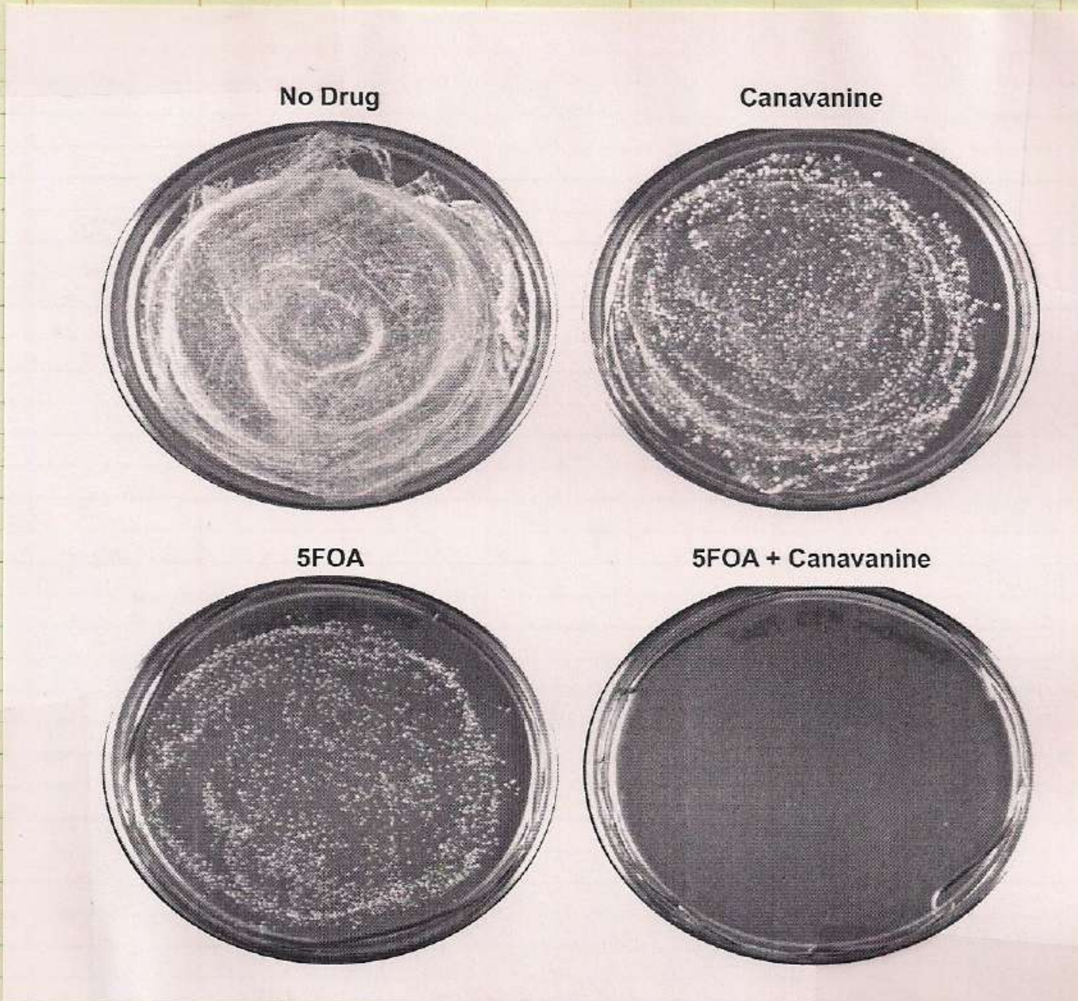
When I made these strains I froze down 4 putative CaURA3 transformants yGIL675 → 678 (p16).

↳ I will patch these onto YPD then replica plate to YPD + Nat.

NOTE: I still have the original transformation plates — I could rescreen more. They are pretty old though (see p. 7)

1/3/14

NOTES ON BULK-SEGREGANT, RANDOM-SPORE TEST



This plate is from the y61L693 spundulis PBST prep (see p. 23)

↳ The other 3 sets look similar.

The second issue I had today concerned the plating efficiency on double drug (canavanine + 5FOA). In theory there should be the same # of colonies on 5FOA & Canavanine — that seems to be the case, but there should also be about half that # on the double drug. It seems like there are none on the double drug. There are a few things that come to mind:

(1) They could be growing more slowly — indeed, there seem to be some small colonies coming up.

(2) It could be that all the colonies on 5FOA & Canavanine are LOH diploids

(3) The could be protein perdurance issues ~~although I think~~

I will do two things here — I will replica plate the "No Drug" to 5FOA+Can → this will test the perdurance issue.

Also, I will pick a bunch of colonies off the 5FOA plate and mating-type test to see if they are haploids.

1/7/14

FOLLOW-UP ON DOUBLE-DRUG SELECTION

After waiting a few extra days I noticed a number of colonies growing up on the double drug plates.

NOTES:

As I mentioned on p. 25 there still far fewer than half the # of colonies on the double drug compared to either single drug

→ I suspect that all of these are haploids. I had picked & mating-type tested 48 colonies from the "yGIL693 + 5FOA, PBST" (see p. 25). Of these 9 were obviously MATa, it looks like 10 MATα (although it is hard to tell because the diploids often produce colonies - mate - with MATα tester), which means ~29 diploids

I will pick another 48 colonies off the yGIL693-double drug-supernatant. and test mating type.

→ 1/10/14

Of the 48, 45 are clearly MATα, 2 clearly MATa, and 1 perhaps diploid.

→ I seems that the MATαs were mostly the MATas — perhaps by adding a little αF to the plates I could select for only MATα.

1/10/14

SCREENING yGIL677 & yGIL678 FOR LINKAGE ~~___~~ OF URA3 & MATα

- I crossed yGIL677 & yGIL678 to yGIL521 (MRM-Anc-Ref) by micromanipulating vegetative cells → I set up 4 ~~___~~ pairings each.
- Yesterday I picked these colonies (labeled yGIL521×677 A-D & yGIL521×678 A-D) and inoculated 5mL YPD.
- Today I spun down 250μl and resuspended in 2mL SPO++
  → on RT rollerdrum @ 5:30pm.

1/20/14

- Friday (1/17/14) I dissected tetrads of the 521×678 and 521×677.
- Tried to get 5 full tetrads per cross but only got 4 full ones on the 521×678 and 4 attempts on the 521×677 (none completely successful).
- Replica plated both to -Ura, +Mat a, and +Mat α each.
- Restreaked yGIL643 (mat α ancestor) from freezer.

1/22/14

- Replica plating results were very unclear. I definitely pressed too hard onto the velvet so all of the tetrads were very smooshed making it too hard to phenotype
  - Will re-do these dissections.
- Restreaked 519×697 C and 519×698 A-C to singles on YPD (These are the 4 diploids of the 12 colonies picked).
- Streaked the Mat a reference (519) and three Mat α references (697-700) from freezer.

1/28/14

- Picked 2 colonies of yGIL643 to sporulate and plate on SD w/ Canavanine to select for can1 mutants.

1/31/14

- Redid tetrad dissections → Hopefully got 2 sets of 6 tetrads for the 521x678 (I did 521x678 twice by accident, then ran out of time to get any for 521x677 → will do 521x677 soon)

- Streaked 2 can1Δ mutant colonies from each yGIL643 +SD + canavenine plates to YPD.

2/4/14

- Tetrad dissectections were not as successful as hoped → No full tetrads

2/7/14

- We will abandon dissecting tetrads for now.
- A couple days ago we made YNB + glutamate plates for future use to select for Mat α, can1, ura3, and GPA1
- Yesterday we froze down the can1 mutantants
  - yGIL643 A1 → 705
  - yGIL643 B1 → 706
- Also struck out yGIL 429 (RM ancestor) and yGIL432 (BY ancestor) from freezer and started cultures
- Today, we crossed yGIL ~~643A1~~ 705 X yGIL429 and ~~yGIL432~~ yGIL ~~643A1~~ 705 X yGIL432 to be sporulated.

2/10/14

- ~~Fri~~ Saturday (2/8/14) I created a αF gradient of 0 uM, 1 uM, and 10 uM ~~αF~~ spread with 429x705 to test for αF effect on Mat a arrest.

- Today, ~~replicated~~ replica plated each

Of the 429x705's on 0, 1, & 10 μM αF onto SD, -Ura, and +5FOA

- SD selects for everything
- -Ura selects for Mat α
- +5FOA selects for Mat a

2/14/14

-429x705 replica plates showed $\boxed{1 \alpha F}$ is most likely enough suppression to ~~get~~ select for Mat a

- Saw a few colonies on the 10 αF + 5FOA, however those are probably just mutants.

- Added ~~3λ αF,~~ drugs to 4 YNB glutamate plates (2 done by me & 2 done by DLang (·))

• 10,000x αF	3λ	percipitated quickly w/ poor distribution → we will see what happens.
• 100x 5FOA	300λ	
• 1,000x Can	30λ	
• 1,000x Nat	30λ	

- Bulk Segregant Protocol
  - 5mL spot
  - spin down 5min (1800rpm)
  - resuspend pellet in 200λ H_2O
  - Add 5λ 1000x Zymolygase
  - 1hr in 30°C roller drum.
  - Add ~~E~~ 50λ glass beads & 50λ 10% Trition → vortex 2min
  - ~~Brought up to 5mL with H_2O al~~
  - 30min back in the 30°
  - vortex 2min
  - Brought up to 5mL with H_2O for sonicator at power 4 for 4-5 seconds
  - 100λ to YNB glutamate + 5FOA + can + Nat + 1αF + htu plates
  - 30°C to grow up.

* made sure to check spore seperation under scope after each appropriate step.

2/19/14

- Pickedand resuspended the 10αF, 1αF, & 0αF mutants on Monday (2/17) in 96 well plate
- Pinned them to +Mat a & +Mat α yesterday (2/18)
- replica plated to SD today

2/21/14

- Mated yGIL683, yGIL685, yGIL687, yGIL692, RMS1-H08-585, RMS1-G02-545, yGIL429, and yGIL 432 to yGIL705.
  * 4 each.
- Then wedecided ~~to try~~ to stop mating directly for the day and ~~to~~ try plating 50/50 mixes of each of the 14 strains with yGIL705 to YPD to grow up and replica plate to -Ura, +Nat to select for mated.
  * yGIL681 + yGIL689 didn't grow in suspension
  ∴ we picked 2 colonies from each into the 50/50 mix
  * plated yGIL429 (top) & yGIL705 (bottom) alone as control when replicaplating

2/22/14

Replica plated the crosses between evolved clones & yGIL705 to SD glutamate + his + trp + Nat

2/19/14

STREAK STRAINS FOR BACKCROSSING

I streaked the following strains to YPD

- yGIL429 (RM Anc.)
- yGIL432 (BY Anc.)

- yGIL705 (Bulk-segregant MATα Anc.)

- yGIL681 BYS1-D08-1000 w/ pMoBY-STE4
- 683 BYS1-A08-545 — STE12
- 685 BYS2-C06-1000 — STE5
- 687 BYS2-D06-910 — STE5
- 689 RMS1-G02-825 — STE12
- 692 RMS1-H08-585 — STE11

see p.17

- BYS1-A09-1000 Clone 6 (Well F1)
- BYS2-E01-665 Clone 1 (Well A3)
- BYS1-E03-745 Clone 3 (Well C5)
- RMS1-D12-910 Clone 4 (Well D8)
- RMS1-G02-545 Clone 1 (Well A9)
- RMS1-H08-585 Clone 12 (Well F12)

These were taken from the 96-well plate that was frozen down on 11/18/13 (p. 5). The specific clones are the ones indicated by a star (*) on pages 9 & 11.

All strains in incubator @ 5:30

2/22/14

Remy crossed all the evolved & Ancestral strains to yGIL705 (see p.30)

Labelled as follows:

1	yGIL705 x yGIL681	8	yGIL705 x yGIL432
2	" " YGIL683	9	" " ~~YGIL~~ RMS1-G02-545
3	" " YGIL685	10	" " RMS1-H08-595
4	" " YGIL687	11	" " BYS2-E01-665
5	" " YGIL689	12	" " BYS1-E03-745
6	" " YGIL692	13	" " RMS1-D12-910
7	" " YGIL429	14	" " BYS1-A09-1000

2/22/14

NOTES ON BULK SEGREGANT APPROACH

There is some growth on the SD glutamate+his&trp + 5FOA+Can+Nat+αF plates from 2/14 (p. 29), but the growth is very slow and non-uniform — we overdosed the drugs on the plate & we see more growth around the edges where concentrations are lower. It has now been over a week — too long.

There are a few options:
(1) Decrease drug conc. → for instance ClonNat is effective at 12.5 μg/ml. (according to Gilbury)
(2) Use NH_4 rather than glutamate (Nat works @ 100 μg/mL on ~~glutamate~~ NH_4)
(3) Get rid of the Nat issue by moving GPA1::Nat into the 705 bkg.
↳ not having Nat in the MATα strain does allow selection for mating on -ura+Nat, as we are trying now.
(4) Use CSM-arg rather than Minimal (+his+trp
(5) Any combination of 1-4 above.

2/24/14

- Picked colonies from the yGIL429x705 and yGIL432x705 on SD+his/trp glutamate+5FOA+Can+Nat+αF and suspended in 100λ YPD in a 96well plate

A1 – A6	A7 – A9	A10 – A12
Labelled specific colonies	429x705	432x705

mix of all 4 — Dr Lang's plates

REMY	CROSS	~~Dr. Lang~~
A2-A5 B1 F3-F4	429	~~C1-C6~~ ~~B2-B6~~ ~~D1~~ ~~F5-F6~~
B2-B6 C1-C6 D1 F5-F6	432	

Dr Lang	Cross
G1-G6 H2-H5	429
E1-E6 D2-D6 F1-F2	432

- Streaked all diploids from 2/21/14 ~~to YPD~~ that were replica plated on 2/22/14 to -ura + Nat (all were proven diploid). to YPD
- Picked all diploids from needle manipulation on 2/21/14 to grow up in 5mL YPD → in incubator.

2/25/14

- 96 well plate from yesterday shows no growth in wells B1 (Remy, yGIL429 x yGIL705) OR C11 (Dr Lang, yGIL432 x yGIL705)
- Replicaplated the 96-well plate from yesterday to SD → check for diploids
- ~~Pinned~~ Pinned 5x of each of the suspended micromanipulated diploids to -ura + Nat to check for diploids.

2/26/14

- From micromanipulated diploids plated to -ura + Nat yesterday, Most grew up (AKA are intact diploid), but the following did NOT: 2C, 3A-3B, 4A-4B, 6A, 7B, 7C, 8C, & 10C-10D.

2/27/14

- Picked 2 colonies from each of the restreaked plates from the replica plating on **2/22/14** and resuspended in 5mL YPD to grow up in incubator

(see pg 30 2/21/14)
- Neg Control (yGIL429 + yGIL705 from SD-ura+Nat) did not grow up on YPD BUT yGIL429 did
- ~~Pos Control (yGIL429 x yGIL705 from SD-ura+Nat) grew up on YPD → 7A & 7B~~

2/28/14

I ~~pelleted~~ spun 1mL of each of the 28 cultures from (2/27). Resuspended in 2 mL SPO++ medium

↳ · I did not do the neg. control which regrew on YPD.
· The cultures were a little dense – prob. doesn't matter.
· Tubes labeled by number (see p. 31) using "A" & "B" for replicates.
· RT rollerdrum @ 4:45 pm.

3/3/14

All 28 cultures had tetrads.

I am setting up the following 96-well plate to freezedown the diploids:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	1A	2A	3A	4A	5A	6A	9A	10A	11A	12A	13A	14A
C												
D												
E	1B	2B	3B	4B	5B	6B	9B	10B	11B	12B	13B	14B
F												
G												
H	7A			7B			8A			8B		

⇐ #s correspond to p. 31

3/10/14

- poured YNB + CSM – arg + 5FOA + can + ClonNat + αF plates
- started overnight cultures of all 1A – 14B (5λ in 5mL YPD)
- made 200 mL SPO++ media: 200 mL H_2O, 3g KAc, 0.5g yeast extract, 0.5g glucose, 400λ His, and 400 µL Trp. → filter sterilized

3/11/14

- froze down all 14 diploids (A) in duplicate in 250λ glycerol + 1mL culture:

YGIL705 x YGIL429 ——— 707
YGIL705 x YGIL432 ——— 708
YGIL705 x YGIL681 ——— 709
YGIL705 x YGIL683 ——— 710
YGIL705 x YGIL685 ——— 711
YGIL705 x YGIL687 ——— 712
YGIL705 x YGIL689 ——— 713
YGIL705 x YGIL692 ——— 714
YGIL705 x RMS1-G02-545 ——— 715
YGIL705 x RMS1-H08-585 ——— 716
YGIL705 x BYS2-E01-665 ——— 717
YGIL705 x BYS1-E03-745 ——— 718
YGIL705 x RMS1-D12-910 ——— 719
YGIL705 x BYS1-A09-1000 ——— 720

- Started Sporulation of all 14 diploids using last 3mL of each after freeze down: 3mL culture, 5 min spin down, resuspend in 10mL SPO++ media, on room temp. rollerdrum.

3/14/14

- Conducted the following bulk segregant protocol on the sporulated yGIL708, yGIL709, yGIL715, and yGIL716:
    - 10mL culture
    - spin down 5min @ 1800 rpm
    - Resuspend in 200λ H_2O
    - Add 5λ 1000x zymolygase
    - Incubate 30° roller drum for 1hr
    - Add 50λ Glass beads + 50λ 10% Triton
    - Vortex 2mins
    - Incubate 30° rollerdrum for 1hr (checked scope @ 30min)
    - Vortex 2mins
    - Bring up to 5mL with H_2O
    - Sonicate 4 seconds @ power 4
- plated 100λ and 1mL to drug plates made on 3/10/14 and that had sat out to dry overnight, parafilmed and put in 30° incubator to hopefully grow up in ~3-4 days.
- Restreaked yGIL701 from freezer to YPD

3/17/14

- The 1mL & 100λ bulk segregant drug plates grew up nicely
- Yesterday Dr. Lang plated 10λ of each to drug plates to grow up as there was so much growth on the 1mL & 100λ (see pictures on pg 36-37)
- Picked 90 single colonies from the yGIL708 (100λ) plate and suspended them in 100λ YPD in a 96-well plate labelled as "BSI" = Bulk Segregant Individuals

YGIL 708 (100λ)
BSI
000 1000

- Bottom 6 wells left w/ 100λ YPD awaiting controls for mating type testing.

~~Plate is in -80°~~

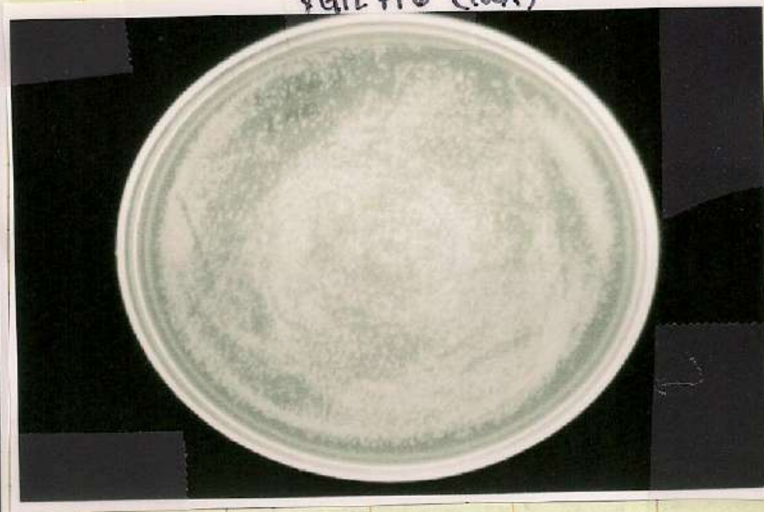
- In incubator for colonies to grow up
- Performed Bulk Segregant Pools (BSP) protocol on the YGIL 708 (100λ) +drug plate:
    - Add 2 mL YPD to plate → * Needs more like 3mL for 2mL yield
    - Use glass spreader to make uniform
    - Transfer BSP to tube
    - Add 500 λ 75% Glycerol
    - 1.25 mL in cargo tube → freeze down
    - Remaining distributed evenly through 1 column of 96 well plate

YGIL 709 (100λ)

YGIL 708 (100λ)

YGIL 715 (100λ)

YGIL 716 (100λ)



3/18/14

- Performed BSP protocol on yGIL709 (100λ) & yGIL715 (100λ)
  → freeze down in cargo tube & plate
- Made the following BSI plates for YGIL709 & YGIL715

yGIL709 (100λ) BSI

H XX 67

A x 6 7 x x 12 x
B YGIL715 (100λ) BSI
yGIL715 (10λ) BSI
H x x x x

Both in incubator

- BSP Plate in freezer :

A B C
YGIL709 (100λ)
1 2 3

1A = yGIL709 (100λ) BSP
2B = yGIL709 (100λ) BSP
3C = YGIL715 (100λ) BSP

3/21/14

- Pinned all 4 plates (708, 709, 715, 716) to +Nat, +can, + 5FOA, + Mat a, and + Mat α (in that order) with a pin switch between Mat a & Mat α

* On the yGIL708 A1-A6 → H1-H6 set, I by accidently put the pin back in the wells after Mat a pinning
→ possible contamination

- I also repinned the full plates to new ones w/ 125λ YPD to grow up fresh for freeze down tomorrow

3/22/14

Added glycerol (50 λ of 60%) to the o/n plates in YPD & froze down
↳ on plate 716 there was growth in well A7 but not C9

3/23/14

- I replica plated the mating test plates to SD.
- Started o/n cultures of the other diploid cultures (707-720) other than the four already done
  ↳ see p. 34
  ↳ I used the cultures from the fridge from 2/27 (5 λ into 5 ml YPD)

3/24/14

- replica plating from yesterday shows no growth on Matα ☺
- on Mata { * 709 shows irregular growth → they lost the plasmid and are ~½ STE (~30 out of ~90) * 708 had missing growth, but could be due to improper pinning and/or replicating }
- Pinned plates from 3/21/14 show full growth of all BSI^s on +5FOA, +can, +Nat
  * some contaminant growth on +Nat plates
- Began sporulation of cultures from 3/23/14
- Noticed some small colonies forming on the leftover YGIL708 & 709 (10λ) plates from 3/17/14 → possible sterile mutations? Will check for sure soon

3/28/14

- Performed Bulk segregant protocol (pg. 35) on yGIL707, 710, 711, 712, 713, 714, 717, 718, 719, and 720
- plated 100λ & 10λ of each to bulk segregant media

4/1/14

- 100λ plates ranged from sparse (yGIL713) to practically a lawn (yGIL714). (see pictures, pg 39-41)
    - The plates w/ less colonies were those with the plasmid (yGIL709-714)
- replated from leftovers from 3/28/14 that had bee in water in the fridge
    - 200λ: 707, 710, 711, 712, 713
    - 100λ: 714, 717, 718, 719, 720
- 10λ plates had little growth

yGIL707 (100λ)

yGIL710 (100λ)

YGIL 711 (100λ)

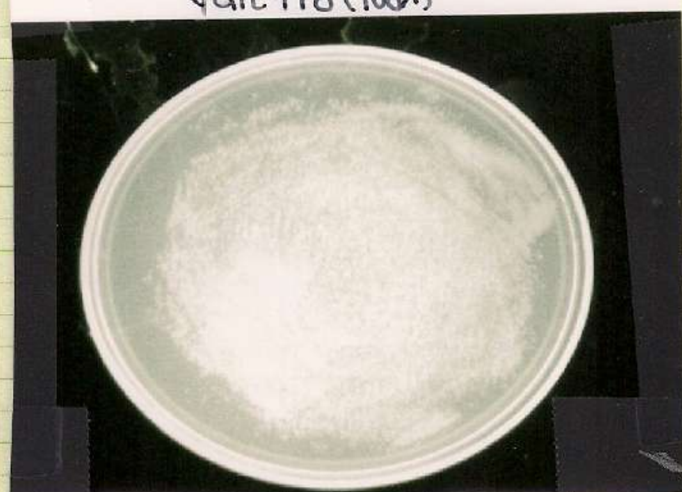
YGIL 712 (100λ)

YGIL 713 (100λ)

YGIL 714 (100λ)

YGIL 717 (100λ)

YGIL 718 (100λ)

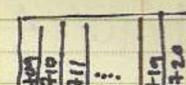


YGIL719 (100λ)

YGIL720 (100λ)

4/3/14

- Performed bulk segregant ~~pro~~ pools protocol (pg 36) on all ~~plates from~~ 100λ plates from 4/1/14
  - Redistributed pools in freezedown plate



- Due to poor yields on 10λ plates, we ~~plated~~ streaked 5λ of the BSPs to YPD to grow up for BSI's
  - Streaked to YPD after glycerol addition by accident
- 200λ/100λ 're-do' plates from 4/1/14 looked even worse than originals → possibly due to ~~a~~ overnight in water

4/8/14

~~Friday (3/4/14) I picked singles from~~

- My plates from 4/3/14 were ~~a~~ too populated to pick singles (due to 2 streak through per dilution on plate) so Dr. Lang restreaked them again on Saturday (4/5/14)
- Monday (4/7/14) I picked singles from 707 – 717 (from 4/5/14) to grow up and check for mating type + can + 5FOA by pinning
- Today I picked the final 3 plates (718, 719, 720) and will check for same as above tomorrow
- Yesterday we began the pool propagation to generation 10 and made 3 copies of the original BSP plate from 4/3/14

- Today we continued BSP propagation to gen. 20 and pooled gen. 10, ~~spstrt~~ spun down @ 1800rpm for 5 min and poured out supernatant to store pellet in -20° freezer.
- I pinned all BSI's (picked on 4/7/14) to +Mat a, +Mat α, and A1-A6 to +Can and +5FOA.
- As I went to store all BSI's in -80°c, I dropped the plates and ruined all samples ~~(xxx)~~ (except 707)
- Froze down all 3 copies of original pools

4/10/14

- Yesterday ~~and today~~ I replica plated all pinned plates from 4/8/14 to SD, +can SD
- I finished pinning the last 3 plates today (718, 719, and 720) but could only test for +can for 718 and 720 since we didn't have enough +can plates
- Pinned +can and +5FOA of all plates from 4/8/14 (A1-A6) show complete growth, but were slow to grow.
- The replica plates are looking really ~~r~~ weird, ~~mostly~~ most likely due to the wetness of the plates when they were pinned

4/11/14

- No growth on +Mat α plates from 4/9/14 except on 711:

~~7##~~ + Mat a A1-A6

711 + Mat a A7-A12

711 +Mat α A1-A6

711 +Mat α A7-A12

4/18/14

- The BSP propagation is complete with the last pool preparation today.
- Wells H06 and G07 have wierd morphologies similar to those of column 10 (YGIL718) → may have developed new mutation and effect decison of which generations that will be sequenced, but will still do genomic preps for all generations next week.
- Continuing the re-do's of the BSI's with 710, 712, and 713 ready to be frozen down today ~~and 714~~.
- Will redo mating type testing on 711 BSI's (after they grow up tonight) due to ~~mat~~ growth on the Mat α plates after replica plating.
- ~~We had restreaked 711 again, due to over population, to pick for BSI's today.~~
- BSI re do's for 714 and 717 used restreaked plates while 711 used original plate.

4/24/14

- All BSI's are picked and in −80°c
- 2nd mating type test of 711 confirms Mat α, However the ~~#~~ 711 BSI's that yielded the wierd mating type results were the ones used in the BSP experiments so we will see how those results turn out.

- performed genomic preps (day 1) on BSPs 010 - 110. (See page 45)

4/25/14

- performed genomicpreps (day 2) on BSPs 010-110 (see pg. 45)

Qubit Data:

BSP	Qubit DNA concentration	Final concentration
010	0.632 ug/mL	63.2
020	0.617	61.7
030	0.715	71.5
040	0.728	72.8
050	0.718	71.8
060	0.636	63.6
070	0.666	66.6
080	0.713	71.3
090	0.777	77.7
100	0.695	69.5
110	0.660	66.0

5/22/14

- Struck out yGIL646 (BY Anc), yGIL697 (BY Ref), and BYSI-A03-545 Ref (11/18 plate A4 - see pg 9) from freezer for BSI fitness assay of 710 BSIs

5/27/14

- Began 710 BSI competition on 5/24/14 (saturday) w/ cultures going into incubator @ 1pm.

A	EA	B
	710 BSIs	
E	EA	A

A: A07, A01, H07, H12 } A = Anc Ref

E: A06, A12, H01, H06 } E = Evo Ref

Quick yeast genomic preparation (04/24/14)

This protocol starts with frozen cell pellets from 5 ml of culture.

Resuspend cells in 300 μl of Lysis buffer.

Add ~0.3 g Glass beads

Add 300 μl of phenol:chloroform:isoamyl alcohol

Vortex for 10 min.

Spin 5 min at top speed.

Remove aqueous layer (~250 μl) and transfer to a new tube.

Add 5 μl RNase (100 mg/ml). Incubate 37°C for at least 1 hour.

Add 300 μL of phenol:chloroform:isoamyl alcohol. Vortex for 15 seconds.

Transfer contents to a phase-lock gel (light) tube.

Spin 5 min at top speed.

Dump aqueous layer into a tube with 500 μl of ethanol. Precipitate DNA in –20° C freezer for at least 30 min (overnight).

Day 1

Pellet DNA by spinning for 10 minutes at top speed. Decant or aspirate ethanol.

Add 1 ml of 70% ethanol, vortex, and spin for 2 min at top speed. Decant or aspirate ethanol.

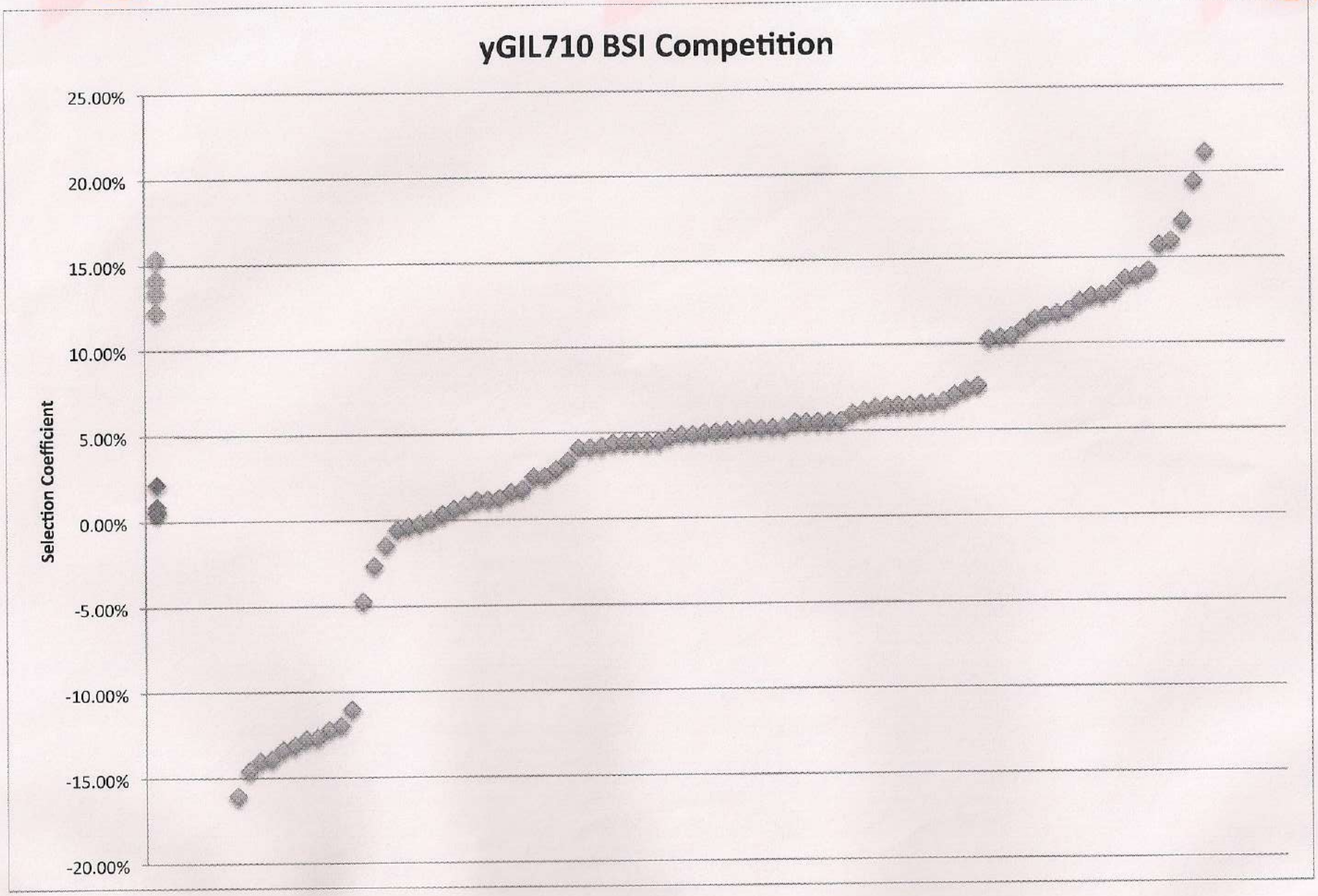
Dry DNA pellet in speed-vac for ~10 min. Resuspend pellet in 100 ul of ddH_20.

Check and record DNA concentration using Qubit fluorometer

Day 2

Lysis Buffer
2% Triton X-100
1% SDS
100 mM NaCl
10 mM Tris-Cl, pH 8.0
1 mM EDTA

To make 50 ml of Lysis Buffer
40.9 ml H_2O
5 ml of 20% Triton X-100
2.5 ml of 20% SDS
1 ml of 5 M NaCl
500 ul of 1 M Tris-Cl, pH 8.0
100 ul of 0.5 M EDTA



yGIL710 BSI Competition
Selection Coefficient
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
-5.00%
-10.00%
-15.00%
-20.00%

6/2/14

- 710 BSI competition (pg 46) results were unexpected w/ ~4 groups of segregants (ü) over a very wide range (-15% → 20% → ¨ ??)
- Prompted mating type testing of yGIL709-712 on +Mata
- I also ~~set up~~ began 1:32 overnights of 709-712 inorder to perform competitions of all 4 at once tomorrow.
- Ancestor and Evolved references in same spots as 710 original (see bottom of pg. 44 67), but yGIL709 has the following setup:

A | E
H | EE

~~and yGIL712 had an extra mess up well at E07~~

* yGIL712 has a blank well at E07

- Note: all evolved references used in past 710 BSI competition and those starting today are Mat a

6/3/14

- Began competitions of yGIL709-712 w/ Gen 10 into incubator @ 1:50pm. & Gen 0 FACS ready for tomorrow.
- Replica plated mating type test plates from yesterday
- Evolved references were inoculated into respective wells (4 uL per well) from corresponding wells in the frozen down plate from 11/18
  - 709 ref = A02 (BYS1-D09-1000)
  - 710 ref = A04 (BYS1-A08-545) → picked colony from plate (5/22) pg 44)
  - 711 ref = B06 (BYS2-C06-1000)
  - 712 ref = B07 (BYS2-D06-910)

6/4/14

- Results from mating type testing show 710 STEs are distributed throughout the fitness's recorded.

- yGIL 709 BSI + Mat a mating type test results: (following are Mat α)

A: 01, 04, 05, 06, 08, 09 B: 09 C: 04, 06, 09
D: 07, 08, 11, 12 E: 04, 07, 09, 12 F: 04,
G: 03, 05, 08 H: 04

- yGIL 711 BSI' Mat α's:

A: 02, 09, 10, 11 B: 01, 02, 08, 10 C: 01, 03, 04, 08, 11
D: 03, 04, 07, 09 E: 01, 03, 05, 06, 07, 09, 11 F: 01, 04
G: 01, 04, 05, 11, 12 H: 02, 08, 09, 10

- yGIL 712 BSI' Mat α's

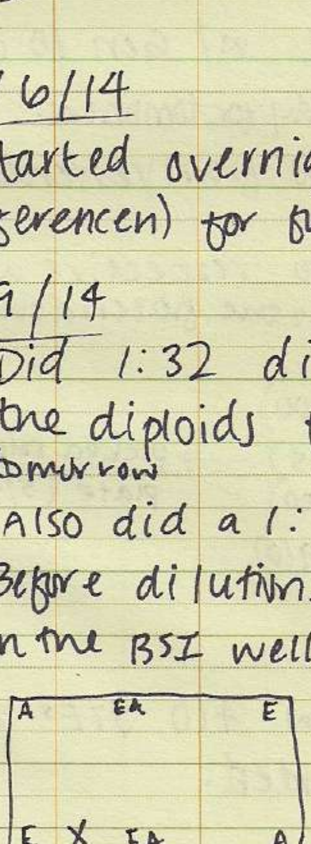
A: 02, 11 B: 06, 11 C: 04, 06, 12
D: 11 E: 09 F: 04, 06, 07, 08, 10
G: — H: 05, 10

6/6/14

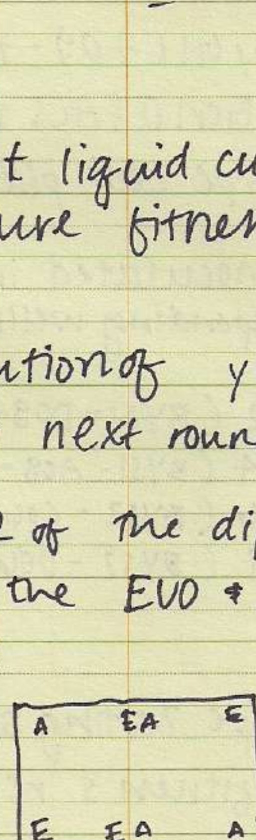
- Started overnight liquid culture of yGIL701 (The Diploid referencen) for future fitness assay.

6/9/14

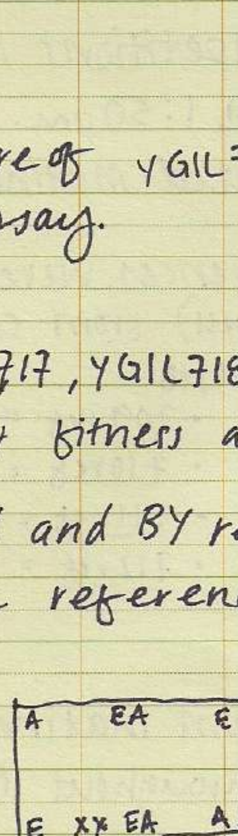
- Did 1:32 dilution of yGIL717, yGIL718, yGIL 720 BSI's, and the diploids for next round of fitness assays starting tomorrow
- Also did a 1:32 of the diploid and BY references.
- Before dilutions the EVO + Anc references were placed in the BSI wells:



yGIL 717



yGIL 718



yGIL 720

- The X's in ~~previous~~ drawings indicate wells that were contaminated before the initial freeze down and transfer tips for those wells will be removed for the competion.
  - YGIL717: X = H03 • ~~YGIL718 X =~~
  - YGIL720: X = H03, H04
- Diploid plate setup on pg. 34
- Evolved ref wells from plate "1/13 found on pg 31:
  - YGIL717: BYS2-E01-665 → A03
  - YGIL718: BYS2-E03-745 → C05
  - YGIL720: BYS1-A09-1000 → F01

6/10/14

- Began competitions of YGIL717, YGIL718, YGIL720, and Diploids w/ Gen10 into incubator @ 2:10 PM.
- Froze down plate of YGIL701 (Diploid reference) w/ 45 μL of 75% Glycerol.

6/19/14

- Fitness assays of 717, 718, & 720 BSI's and the Diploids were completed yesterday
- Data looks good; however I used the ~~wron evolved ancestor~~ Mat α BY reference and the evolved reference were Mat a so they mated leading to weird/unexpected data.
- Due to unexpected BSI fitness assay results with very large spreads (example: YGIL710 BSI pg 46) we decided we want to go back to the original diploids and perform fitness assays on with respect to mating type since the original data was completed with Mat a and our BSI's are Mat α → possible source of weird BSI fitness assay data.
- Chose YGIL710 and YGIL720 for initial fitness assay on mating type

- ~~7e~~ 2 days ago (6/16/14) I struck out the yGIL710 and yGIL720 diploids from wells C02 and C12, respectively, from the freezer.
- Yesterday I picked a single colony from each plate and suspended in 5 mL of YPD
- Today I began sporulation of 710 & 720 following protocol from pg. 35 w/ 5 mL culture into 10 mL spo++

6/23/14

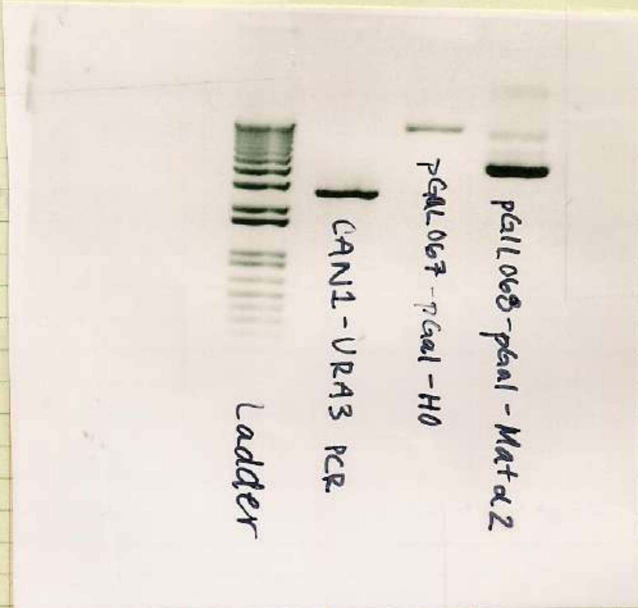
- Sporulation of yGIL 710 + 720 look good in YPD, but a bit worse in YPGal
  ↳ began sporulation of yGIL 710 & 720 after growing up in YPGal on 6/19/14
- Will perform Bulk segregant protocol (pg. 35) on the sporulated yGIL 710 & 720 (YPD) on to Bulk segregant media from 3/31/14.
  * Gave 100λ of each to Kevin for dissecting tetrads before doing protocol.
- plated 100λ, 50λ, and 10λ of 710 & 720 each to BSP media
- Struck out yGIL 705 & yGIL 706 from freezer

6/25/14

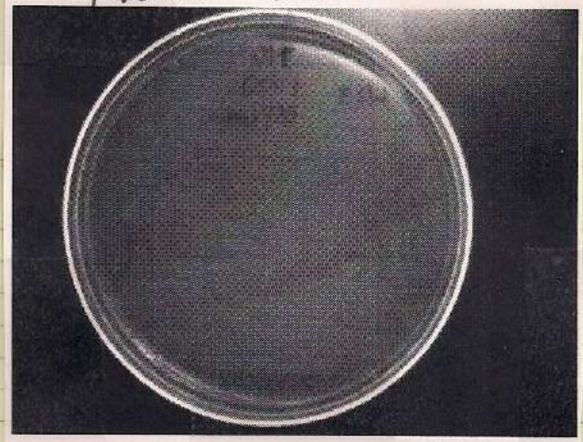
- Created pools of the 100λ plates of 710 & 720 w/ 2 mL of YPD and struck out to YPD to grow up singles

6/26/14

- Dr. Lang did successful PCR of CAN1-URA3, pGIL067-pGal-HO, and pGIL068-pGal-Matα2 (pg. 51)
- Then I performed transformation of yGIL705 with CAN1-URA3. (followed Yeast Transformation (12/18/08) protocol).
- Chose 100λ plates to create pools, however in future BSP experiments we should use 50λ plates for picking individuals (pg. 51)



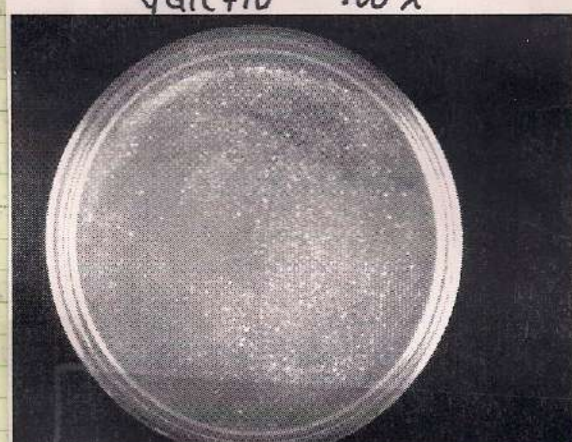
yGIL710 10λ



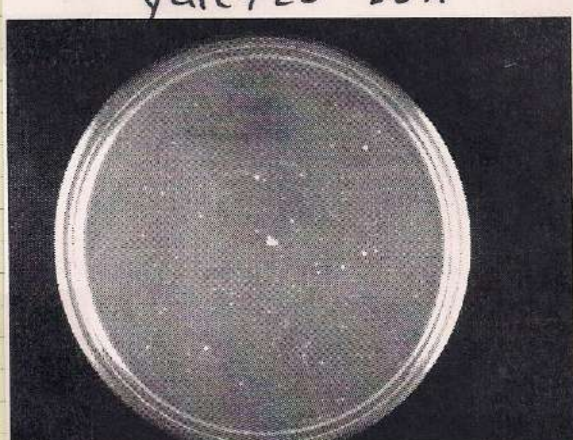
yGIL710 50λ

yGIL720 10λ

yGIL710 100λ



yGIL720 50λ



yGIL720 100λ

6/30/14

- +5FOA plates of the control and transformed yGIL705 show expected results with about double the amount of CFUs on the transformed yGIL705 + 5FOA.

- The mating type testing of yGIL710 & yGIL720 BSPs showed an error in the original pinning as the same exact pattern was found for both 710 & 720 → will re-pin and ~~test~~. replica plate tomorrow to SD.
- Picked 12 single colonies from the transformed yGIL705 on +5FOA and ~~plate~~ streaked out each to YPD

7/2/14

- Yesterday I replica plated the mating type tests of yGIL710 and 720 BSPs:
    - yGIL710 BSPs: → No growth on +Mat α
    → No growth on +Mat a for wells A03, A05, A09, A10, B02-B05, B09, B09, B11, B12, C01, C03, C04, C06, C07, C09-C11, D04, D08, D10-D12, E01, E03, E05, E06, E09, E12, F01, F02, F05-F08, F10, G01, G04, G11, H04, H05, H09, H11.
    - yGIL720 BSPs: → No growth on +Mat α
    → No growth on +Mat a in well B12
- Yesterday I picked singles and started overnight cultures in 5mL YPD ~~of a~~ from each of the 12 YPD plates of the transformed yGIL705 from the +5FOA (6/30/14).
- Started overnight cultures in 5mL YPD of yGIL720 BSP well B12 and yGIL710 BSPs wells A05, B02, B11, C06, D04, E09, & G01
  ↳ Will sporulate to test if sterile or diploid
- Struckout the 12 evolved Mat a clones used in original BSA. (pg 31) onto YPD
  ↳ from 11/18/13 frozen down plate & corresponding tubes

- Spotted 5λ of each of the 12 transformed yGIL705's to +Can and +5FOA

7/3/14

- Began sporulations (1mL culture in 2mL Spo++) of ~~the~~ the 8 BSP wells found to have no growth on +Mat a that I started overnight cultures of yesterday along w/ a diploid reference.

7/7/14

- Sporulation showed all 710s were sterile while the 720 BSP well B12 was diploid → ~1% diploids were getting through the BSP protocol

- Started overnights of the 12 evolved Mat a clones that I struckout on 7/2/14 (pg 51.) ↳ 6 with plasmid in -ura, 6 w/out plasmid in YPD

- Greg picked 96 more of the transformed 705 and found 4 that successfully integarted the plasmid and struck them out to YPD labelled yGIL705 ura3Δ::CAN1 A-D → I started overnight cultures of all 4

7/9/14

- Froze down the transformed yGIL705:
  - yGIL705 A ——— yGIL 737
  - yGIL705 B ——— yGIL 738

- Crossed each of the 12 evolved Mat a clones with yGIL737 in 4 replicate.

- Greg spotted yGIL705A-D to +Can and +5FOA to confirm transformation.

7/10/14

- Struck all 4 of each of the 12 crosses to yGIL737 to YPD (A+B) (C+D) * only 3 replicate of yGIL737 X to 692, 639, 695, and BY52-E01-H5

7/16/14

- started overnight cultures of single colonies ~~of~~ from all plates struck out on 7/10/14 and began sporulating them Saturday 7/12/14
- Found 6 diploids (confirmed on scope): yGIL737 X yGIL681 C, X yGIL ~~697~~ 687 D, X BYS1-A09-1000 B, X BYS2-E01-665 A & C, and X RMS1-D12-910 B
- Transformed all 6 with the HO plasmid yesterday following the revised Yeast Transformation (12/18/08) protocol (pg. 55)
- Spotted all 6 to SD-ura and +5FOA to make sure X yGIL681 C and X yGIL687 D lost their sterile plasmids and found no growth on SD-ura & full growth on +5FOA :)
- Yesterday I restruck the rest of the crosses that are not confirmed diploids to YPD inorder to pick different singles and sporulate and hopefully find diploids.
- Will freezedown the 6 confirmed diploids today.
- Yesterday confirmed diploid of yGIL737 X RMS1-G02-545 D

yGIL737 X yGIL681 C	yGIL739
yGIL737 X yGIL697 D	yGIL740
yGIL737 X BYS1-A09-1000 B	yGIL741
yGIL737 X BYS2-E01-665 A	yGIL742
yGIL737 X BYS2-E01-665 C	yGIL743
yGIL737 X RMS1-D12-910 B	yGIL744
yGIL737 X RMS1-G02-545 D	yGIL745

- Yesterday I crossed yGIL737 to yGIL429 and yGIL432 in quadruple replicate (labelled A-D) and today I struck out all 8 crosses to YPD.

* yGIL429 and yGIL432 are the BY & RM ancestors and I had started overnight cultures of them from the freezer on Monday (7/14)

Yeast Transformation (12/18/08)

Grow overnight culture

In the morning, dilute culture 1:~~100~~ 50 (100 ul into 10 ml YPD)

Grow for ~6 hours until mid-log (~2×10^7 cells/ml)

Transfer culture to a 15 ml falcon tube.

Spin for 5 minutes at 2000 rpm in Beckman Coulter centrifuge (~850 x g). Decant media.

Resuspend cells in 1 ml H_2O. Transfer to a microfuge tube and spin 1 min at top speed.

Aspirate H_2O and repeat water wash 3 times with 1 ml H_2O.

Wash twice with 1 ml LiOAc Mix.

Resuspend cells in ~~70~~ 20 ul LiOAc Mix. Add 10 ul of carrier DNA (boiled salmon sperm DNA). This should yield a volume of ~~~100~~ 50 ul; ~~separate equally into two tubes of ~50 each.~~

Add ~~10~~ 1 ul of PCR or plasmid to cells. Vortex briefly.

Add 360 ul (6-fold volume) of PEG Mix and incubate 30° C for 30 min. *Alternative: If strain is temperature sensitive, incubate at room temperature.*

Add 47 ul of DMSO (final concentration ~10%).

Heat shock for 15 min at 42° C. *Alternative: If strain is temperature sensitive, heat shock at 37° C.*

Sediment cells for 3 minutes at 2000 rpm in microcentrifuge.

Resuspend cells with 200 ul of ~~YPD~~ ~~-ura~~ → YPD and plate onto ~~YPD~~ -ura. *Alternative: For some selections, such as nutrient markers, cells can be plated directly onto selective media. For many selections such as dominant drug markers and 5FOA, cells need to grow vegetatively for several generations before transformants show the selectable phenotype. An alternative to the replica plating method I use is to hold the cells in 1 ml of YPD for >1.5 hours then plate directly onto the selective media.*

Incubate plates overnight.

Replica plate lawn onto selective media.

LiOAc Mix
100 mM Lithium Acetate
10 mM Tris
1 mM EDTA

To make 50 ml of LiOAc Mix
44.4 ml H_2O
5 ml of 1 M LiOAc (Lithium Acetate)
500 ul 1 M Tris-Cl, pH 7.5
100 ul 0.5 M EDTA

PEG Mix
40% Poly Ethylene Glycol
100 mM Lithium Acetate
10 mM Tris
1 mM EDTA

To make 50 ml of PEG Mix
44.4 ml of 45% PEG (Poly Ethylene Glycol)
5 ml of 1 M LiOAc (Lithium Acetate)
500 ul 1 M Tris-Cl, pH 7.5
100 ul 0.5 M EDTA

7/17/14

- Began sporulation of 1mL culture in 2mL SPO++ for each of the replicates of the 4 ~~remaining~~ crosses we did not find diploids of in the first round of sporulation.
  * These are different singles from the restreaked plates (7/15)
  * 2 of the populations I restreaked on 7/15 were labelled incorrectly and I will start overnights of ~~most~~ 4 new singles from each in order to sporulate tomorrow.
- Picked 2 singles from each of the confirmed diploids transformed w/ HO (YGIL739 - YGIL744) and resuspended them in 5mL SD-ura to watch for growth and confirm transformation.
- Picked singles from each of the YGIL737 x YGIL429 + x YGIL432 A-D plates to start overnights and sporulate tomorrow.

7/19/14

- Began Gal induction of the one diploid that successfully transformed HO and grew up in -ura (YGIL744 A):
  - YPRaf: 0.5mL culture, 2mL YPRaf (2%) w/ one wash in H_2O before inoculating → plate to YPD at 3hr and 6hr
  - YPG: 0.5mL culture, 1mL H_2O wash, 2mL YPG → plate at 1hr and 3hr and 6hr.

7/20/14

- Began Sporulation of YGIL737 x YGIL689, x YGIL692, x YGIL429, and x YGIL432
- Found that none of the crosses that were sporulated on 7/17/14 are diploid: YGIL737 x YGIL683, x YGIL685, x BYS1-E03-745, and x RMS1-H08-545 → may need to recross.
- Began Gal induction on the remaing 11 diploids (A + B of YGIL739-744)
  * YPG - 1hr + 3hr, YPRaf - 3hr + 6hr

7/22/14

Yesterday (7/21/14)
- Suspended 16 ~~duplicates of~~ single colonies from each of the Gal induction plates of 744A (YPG - 1hr, 3hr, & 6hr and YPRaf 3hr & 6hr) in 1% αF and checked for shmoos after 3 hours
  → Found NO shmoos for all 16 of YPG 1hr & YPRaf (all)
  → Found shmoos on 15 of the 16 from YPG 3hr and all 16 of YPG 6hr.
- Spotted the original 15 colonies from YPG 3hr (744A) to +5FOA and SD-ura and resuspended them in YPD

- Today, found full growth on SD-ura for all 15 colonies of 744A confirmed a/a → they havent lost the plasmid
  * Chose colony 10 & colony 15 to pick from the +5FOA plate to get a/a w/out plasmid and suspended in YPD for overnight & freeze down tomorrow.
- Suspended 8 single colonies from each of the 11 Gal inductions done on 7/20 (only YPG 3hr) in 1% αF to check for shmoos and confirm a/a.
- Crossed yGIL737 to yGIL683 and will cross yGIL737 to yGIL685 as soon as it grows up fully.
- Current standing of all 12 desired crosses & Ancestors: ~~yGIL737 X~~ ⊛

~~① yGIL681 = BYS1-D08-1000~~ * added 10/20/14

	yGIL737 X ____	~~Diploid?~~ Crossed?	Sporulated	Diploid?	+ HO	a/a?	a/a No plasmid
1	681/BYS1-D08-1000	✓	✓	yGIL739	✓	755	
2	687/BYS2-D06-910	✓	✓	yGIL740	✓	756	
3	BYS1-A09-1000	✓	✓	yGIL741	✓	762	
4	BYS2-E01-665	✓	✓	yGIL742 yGIL743	✓ ✓	 ~~757~~	
5	RMS1-D12-910	✓	✓	yGIL744	✓	753 754	
	~~RMS1-D12-910~~						

	YGIL737X ______	crossed?	sporulated?	Diploid?	+HO	a/a?	a/a + NO plasmid?
6	RMS1-G02-545	✓	✓	YGIL745	X	X 791	X
7	693/BYS1-A08-545	✓	X	X	X	X 795	X
8	695/BYS2-C06-100	X	X	771 X	X	790 X	X
9	689/RMS1-G02-825	✓	✓	793 794 X	X	X	X
10	692/RMS1-H08-585	✓	✓	X	X	808 X	X
11	RMS1-H09-595	✓	X	X	X	797 X	X
12	BYS1-E03-745	✓	X	X	X	806 X	X
Anc	429	✓	✓	YGIL~~746~~ 749 YGIL~~747~~ 750	X	821 X	X
Anc	432	✓	✓	YGIL~~748~~ 751 YGIL~~749~~ 752	X	793 X 794	X

- Started overnights to freeze down tomorrow:
- ~~Froze down~~ YGIL7~~3~~7X YGIL~~4~~29 B ——— YGIL~~746~~ 749
  YGIL737X YGIL429 D ——— YGIL~~747~~ 750
  YGIL737X YGIL432 C ——— YGIL~~748~~ 751
  YGIL737X YGIL432 D ——— YGIL~~749~~ 752

- Individuals in 1% αF from 7/20 Gal induction confirmed only 1/16 for 739, 740, and 743 were a/a
  * Confirmed a/a colonies were spotted to +5FOA and SD -ura, and were ~~re~~suspended in 5mL YPD
  * Will go back to YPG 3hr plates for YGIL741 and YGIL742 to try and find a/a w/ 1% αF tomorrow

- Streaked 5x of YGIL741 A + B that have been inducting since 7/20 to YPD

7/23/14

- Froze down YGIL744 A Mat a/a w/out HO ——— ~~YGIL~~
  colony 10 ——— YGIL753
  colony 15 ——— YGIL754

- Started overnights of YGIL739 B3, YGIL740 B3, and YGIL743A8 (the confirmed a/a) that grew on +5FOA to freeze down tomorrow:

  YGIL739 B3 a/a (NO HO) ——— YGIL755
  YGIL740 B3 a/a (NO HO) ——— YGIL756
  YGIL743 A8 a/a (NO HO) ——— YGIL757

- Started overnights of yGIL737 x yGIL689 A (1-3), B (1-3), C(1-3) and yGIL737 x yGIL 692 A (1-3), B (1-3), and C(1-3) to sporulate tomorrow.

7/24/14

- Sporulated all viable duplicates of each cross to yGIL737 (699, 692, BYS1-E03-745, and RMS1-H08-585).

- Froze down yGIL 755 - yGIL757

7/27/14 → SCREENING FOR MATa/MATa VERSION OF yGIL741

· On Thursday (7/24) I started o/N cultures from tubes of yGIL741 + HO.
↳ two tubes from independent transformants "A" + "B".
· Inoculated 5ml SD+his+trp (-ura)

· On Friday (7/25) I spun 1 ml culture and resuspended in 2 ml YPGal
↳ cultures on rollerdrum (30°) @ noon.
· Induce HO for 3h then streak to singles on YPD

· Yesterday I screened 12 colonies each of the 3 day induction plates (see p. 58). → No shmoos after 3h in YPD+αF.

· Today I checked 12 colonies each from ~~the~~ Friday's 3h gal induction
↳ After 3h in YPD+αF all but one of the 24 are a/α
↳ Pick four each A1, A2, B1, B2 → patch to -ura, & 5FOA, and start 5ml YPD culture.
A3, A5 B3, B4

CROSSING yGIL685 & yGIL737

· On Friday (7/25) I started o/N cultures of yGIL685 & 737.
· Yesterday (7/26) I set up 10 crosses of these strains by micromanipulating single cells.
· Today I restreaked these 10 on YPD
↳ Note cross #10 - the colony on the original cross plate looks small.

SPORULATION

· On Friday (7/25) I put xBYS1-E03-745 A1, A2, D1, & D2 (which were not grown up on Thursday) into SPO++

· For the cross between BYS1-A08-545 (yGIL683) & 737 I picked 2 singles each (8 total A1-D2) and started o/N cultures. - Friday (7/25).
· Yesterday (7/26) I put these 8 into SPO++

7/29/14

- Yesterday I screened the 30 sporulations I started on on 7/24/14 and found following confirmed diploids: yGIL737 x RMS1-H08-585 A1, A2, C1, and D2, and yGIL737 x BYS1-E03-745 C1 and C2
- Yesterday I started overnights from the +5FOA plate of yGIL741 confirmed a/a to freeze down today:

yGIL741 A2 (a/a, NO plasmid) —— yGIL762
yGIL741 B3 (a/a, NO plasmid) —— yGIL763

- Started overnights of confirmed diploids to freeze down tomorrow:

yGIL737 x RMS1-H08-585 A1 —— yGIL764
yGIL737 x RMS1-H08-585 D2 —— yGIL765
yGIL737 x BYS1-E03-745 C1 —— yGIL766
yGIL737 x BYS1-E03-745 C2 —— yGIL767

7/31/14

- Yesterday I began sporulating 2 singles from crosses #1-9 of yGIL737 x yGIL685 done on 7/26 by Greg. (9A wasn't grown up enough so there are only 17)
- Yesterday I picked from a +5FOA plate & grew up overnight the following confirmed diploids that were crossed by Greg on 7/25 and am freezing them down now:

(yGIL683)
yGIL737 x BYS1-A08-545 C1 —— yGIL768
yGIL737 x BYS1-A08-545 C2 —— yGIL769
yGIL737 x BYS1-A08-545 D1 —— yGIL770

8/1/14

Screen for diploids the cross between yGIL685 & 737. (see p. 60).

↳ Of the 17, only 8A & 8B have tetrads.
· Start o/n cultures to freezedown 8A & 8B as yGIL771 & 772, respectively.
→ NOTE: This means that only 1 of 10 arrayed matings produced diploids.

Streak Strains

I struck out the following strains:

↓

To be transformed with pGAL-HO:

Isogenic	Strain	Alias	Transformation
yGIL750	yGIL749	RM Anc	HO
yGIL752	yGIL751	BY Anc	HO
yGIL765	yGIL764	RMS1-H08-585	HO
yGIL767	yGIL766	BYS1-E03-745	HO
yGIL772	yGIL771	BYS2-C06-1000	HO
yGIL769-770	yGIL768	BYS1-A08-545	HO
	yGIL745	RMS!-G02-545	HO

To be transformed with pGAL-MATalpha2:

Isogenic	Strain	Alias	Transformation
	yGIL755	BYSI-D08-1000	MATalpha2
	yGIL756	BYS2-D06-910	MATalpha2
yGIL763	yGIL762	BYS1-A09-1000	MATalpha2
	yGIL757	BYS2-E01-665	MATalpha2
yGIL754	yGIL753	RMS1-D12-910	MATalpha2

To be crossed:

	yGIL689	RMS1-G02-825
	yGIL692	RMS1-H08-585 (ste)
	yGIL737	MATalpha Bulk Segregant Ancestor

· Except yGIL771, which I'll streak out tomorrow

· Strains for crosses that contain plasmid (689 & 692) were struck onto SD+his+trp to select for plasmid. (URA3 marker)

8/2/14

· Freezedown 8A → yGIL771
8B → yGIL772

· Streak yGIL771 to singles on YPD.

8/3/14

START O/N CULTURES FOR TRANSFORMATION / CROSSES

- I started o/n cultures of the 12 strains to be transformed (p.61) by picking single colony → 5 mL YPD.
- O/N cultures of the strains to be crossed → yGIL737 into YPD → yGIL689, 692 into SD+hist+trp

8/4/14 TRANSFORMATION W/ pGAL-HO

- Dilute o/n cultures 1:100 in 10 mL YPD ~ 9:45am.
- Katie transformed the seven confirmed diploids (yGIL 749, 751, 764, 766, 771, 768, 3745) with pGIL067 (Gal-inducible HO) → 1 μl plasmid (see p. 55)
  → transformation started ~3:30; ended ~6:30
- I realized that I did not have pGIL068 (Gal-inducible MATα2) out.
  - inoculate 2.5 mL LB+amp with strain from freezer
- Leave the 5 to be transformed w/ MATα2 on rollerdrum

8/5/14

TRANSFORMATION W/ pGAL-MATα2

- Dilute the 5 o/n cultures 1:50 in YPD (200 λ into 10 mL) → 30° rollerdrum ~8:50am
- Miniprep the plasmid pGIL068 (GAL-MATα2) in duplicate → elute in 30 μL EB.

- transformed yGIL 755, 756, 762, 757, 753 w/ pGal-MATα2 w/ 1 ul plasmid
- inoculated O/N cultures of yGIL 737 & 692

8/6/14

- streaked colonies resulting from yGIL689 x 737 (8/4) A-K on YPD
- Picked 2 colonies from each pGal-HO transformation (8/4) & inoculated in 5 ml -URA
  - plates all had ≈ 5-10 lg. colonies which were avoided when picking.

8/7/14

- Picked 2 colonies from each ~~pGal-HO~~ pGal-MATα transformation ~~(8/4)~~ 8/5 & inoculated in 5 ml -ura.
  - plates all had more uniform colony size than pGal-HO transformation.

8/8/14

yGIL 737 x 689 A-K

- singles picked from B, C, F, G, H, I & put into 5 ml YPD.
- plates for A, D, J, K were too crowded to select singles so these were restreaked on YPD.
  - E grew up slower than the rest so this was excluded.

yGIL 737 x 692 A-G

- A-G were all streaked onto YPD

pGal-HO transformation (8/4)

- 745, 771, 764, 768 - only 1 tube grew up. A 3rd colony was picked & put into 5 ml SD.

8/9/14

yGIL 737 x 689

- ADJK plates still @ 30°C
- B, C, F, G, H, I (2 each) cultures we sporulated in spo ++ (onto RT rollerdrum @ 12:30)

pGal-HO transformation → Induction

- 745, 71, 64, 68 induced in YPG for 3 hrs. ~~[illegible]~~ (10:09 - 1:20) ~~[illegible]~~ ~~[illegible]~~ & streaked onto YPD.

pGal-MATα transformant induction

- 755, 756, 762, 757, 753 + α2 transformants were assigned strain #'s and frozen down.

New strain #'s

yGIL753 → 773, 774

yGIL755 → 775, 776

yGIL756 → 777, 778

yGIL757 → 779, 780

yGIL762 → 781, 782

- pGal-MATα was induced in YPG for ≈3 hrs. (~~[illegible]~~) 9:45 - 12:45
- inductions transferred to spo++ & put on rollerdrum @ ~~[illegible]~~ 1:20 pm

8/10/14

· Yesterday I started o/n cultures of two colonies from the restreak the seven crosses between yGIL737 & 692 (labeled A1, A2, B1, B2, ..., G1, G2), as well as the restreaks of the 737 & 689 cross (A1, A2, D1, D2, J1, J2, K1, K2).

· Today I spun 1ml of the o/n and resuspended in 2ml SPO++ → RT rollerdrum.

· All YPD o/n are in fridge.

8/20/14

yGIL 737 × 689

confirmed diploids: A1, A2, F1, G1, G2, I1, I2

yGIL 737 × 692

confirmed diploids: G1 & G2

pGal-MATα2 - was sporulated on 8/9. Tetrads present in all cultures but efficiency was very poor.

8/21/14

- All strains transformed w/ pGal-HO (8/4) & streaked onto YPD (749, 51, 64, 66, 71, 68, 45) (2 replicates each except for 745)
  - singles (2 each) were picked & transferred into 100 ul 1xαF YPD.
  - plate into 30°C at 11:00 am. - 2:30 pm.

pGal-HO transformant strains	a/a ?
yGIL 749A1	NO
749A2	NO
749B1	NO
749B2	NO
764A1	yes
764A2	yes
764C1	NO
764C2	yes
751A1	NO
751A2	NO
751B1	NO
751B2	NO
766A1	NO
766A2	NO
766B1	NO
766B2	NO
	NO

strain	a/a ?
yGIL 768A1	NO
768A2	NO
768B1	NO
768B2	NO
771A1	yes
771A2	yes
771B1	NO
771B2	NO
745B1	NO
745B2	NO

yGIL 764 → A1/A2
yGIL 771 → A1/A2 spotted on -ura & 5FOA to check for plasmid loss.

8/25/14

- started O/N cultures: (1:5)

737 x 689 A1, F1 } both confirmed
737 x 692 G1, G2 } diploid on 8/20

- yGIL 764 & 771 grew up on 5FOA (a/a confirmed)

764 A1
" A2 → picked off 5FOA & into 5 ml YPD
771 A1
" A2

8/26/14

a/α
737x689 A1 → yGIL 783
737 x 689 F1 → yGIL 784
737x692 G1 → yGIL 785
737x692 G2 → yGIL 786

a/a
yGIL 764 A1 → yGIL 787
764 A2 → yGIL 788
771 A1 → yGIL 789
771 A2 → yGIL 790

- frozen down

8/29/14

- Singles picked from +HO Gal induced plates with no a/a confirmation were found to have lost the plasmid due to no growth in -ura.

- Yesterday started overnights from the least diluted portion of the yGIL 745 B, 749 A & B, 751 A & B, 766 A & B, and 769 A & B in -ura to try and find some that had not lost the plasmid inorder to redo the induction.

- Performed the following Gal inductions ~~on~~ based on amount of growth in -ura

• Low Growth: 5mL culture spindown → 1mL H_2O wash → 2mL YPG → plate @ 3hr

* done w/ 749A, 751A, 766A, and 769 A&B

• Normal Growth: (745B, 749B, 751B, 766B) 0.5mL culture spindown → 1mL H_2O wash → 2mL YPG → plate at 3hr.

8/30/14

The nine gal-HO induction strains from yesterday stayed @ 30 overnight. I struck for singles on YPD (labeled "gal-HO 20h induction") ~ 10am.

9/2/14

- Began the following methods for inducing MATα2: (773 + 775)

* on RT @ 4:20PM	A	O/N -ura → 3hr YPG → SPO++ (Glucose)
* on RT @ 7:20PM	B	O/N -ura → 6hr YPG → SPO++ (Glucose)
* on RT @ 7:20PM	C	O/N -ura → 6hr YPG → SPO++ (Galactose)
* on room temp @ 1PM	E	O/N -ura → SPO++ (Galactose)

- Checked for a/a of 3 singles each from the 3hr and 20hr Gal inductions of 745B, 749A+B, 751A+B, 766A+B, and 768A+B → in incubator @ 2:15PM.

* Confirmed a/a for 745B clones 1+2, 751B clones 1+2, and 768A clone 1

↳ spotted to -ura and +5FOA to freezedown without HO

9/3/14

- Started overnights from the confirmed a/a +5FOA plates to freeze tomorrow:

yGIL745B	clone 1	yGIL791
yGIL745B	clone 2	yGIL792
yGIL751B	clone 1	yGIL793
yGIL751B	clone 2	yGIL794
yGIL768A	clone 1	yGIL795

in incubator @ 1:15pm

- Picked 12 more clones from 749 A+B and 766 A+B 3hr Gal induced plates and suspended in 1% αF to check for confirmed a/a: none confirmed :(
- started cultures of yGIL $\underbrace{783, 785}_{\text{HO transform to be done}}$, $\underbrace{787, \text{and } 789}_{\text{Matα2 transform to be done.}}$
- started overnights of yGIL749 and 766 to re-do the HO transformations due to no confirmed a/a.

To be transfomed with pGAL-HO:			
Isogenic	**Strain**	**Alias**	**Transformation**
yGIL784	yGIL783	RMS1-G02-825	pGAL-HO
yGIL786	yGIL785	RMS1-H08-585	pGAL-HO
yGIL750	yGIL749	yGIL429 (RM Anc.)	pGAL-HO
yGIL767	yGIL766	BYS1-E03-745	pGAL-HO

To be transfomed with pGAL-MATalpha2:			
Isogenic	**Strain**	**Alias**	**Transformation**
yGIL788	yGIL787	RMS1-H08-585	pGAL-MATalpha2
yGIL790	yGIL789	BYS2-C06-1000	pGAL-MATalpha2
yGIL792	yGIL791	RMS1-G02-545	pGAL-MATalpha2
yGIL794	yGIL793	yGIL432 (BY Anc.)	pGAL-MATalpha2
	yGIL795	BYS1-A08-545	pGAL-MATalpha2

Done		
Isogenic	**Strain**	**Alias**
yGIL774	yGIL773	RMS1-D12-910
yGIL776	yGIL775	BYS1-D08-1000
yGIL778	yGIL777	BYS2-D06-910
yGIL780	yGIL779	BYS2-E01-665
yGIL782	yGIL781	BYS1-A09-1000

9/5/14

- Checked sporulations ABCE of 773 and 775. Greg estimated 775 E had ~1% efficiency (2/170) and 773E had < 1%. (1/190) while neither of us found any on A B or E.

9/8/14

- HO transformations showed moderate efficency for 785, 749, and 766 and created a lawn on 783.
- MATα2 transformations all showed high efficiency.
- Restreaked 2 colonies each of 787, 789, 792, 793, and 795 +MAT α2 to YPD.
- Resuspended 2 colonies each of 785, 749, an 766 in -ura and a sample from the 783 lawn into -ura.

*NOTE: yGIL791 and yGIL794 did not grow up in YPD when Greg diluted them back before transformation w/ +MATα2.
↳ just grew slow!

- checked sporulations again → relatively the same.

9/9/14:

- Started O/N of 773 and 775 in -ura (Glu) + -ura (GAL) last night to perform following sporulation methods today:

D) O/N -ura(GAL) —*→ SPO++ (GLU) "D1"
　　　　　　　　—*→ SPO++ (GAL) "D2"

E) O/N -ura(GLU) —*→ SPO++(GAL) "F1"

F) O/N -ura(GLU) —*→ -ura(GAL)3hr —*→ SPO++(GLU)
　　　　　　　　　　　　　　　　　—*→ SPO++(GAL) "F2"

G) O/N -ura(GLU) —*→ -ura(GAL) 6hr —*→ SPO++ (GLU) "G1"
　　　　　　　　　　　　　　　　　—*→ SPO++(GAL) "G2"

* 1mL H_2O wash

- I messed up the F1 & F2 procedure by transferring the 773 into the 775 (oops!) so we restarted a 3hr -ura(GAL) that went into the incubator at 1:30pm that will now be a good version of F1 and F2 BUT now G1 and G2 will be completed on the "MIX" and the pure 775

- All steps going into SPO++ used 0.5mL culture → 1mL H_2O wash → 1mL SPO++ EXCEPT for procedure D1 and D2 due to low growth O/N in -ura (GAL) ∴ used 1mL culture → ~~1mL H_2O wash~~ → 1mL SPO++

- All (F) & (D) procedures had no H_2O wash before SPO++ due to small pellet.

9/10/14

- Greg and I performed Gal inductions on 749, 766, and 795 A & B (each) + H_2O from 9/8/14 (they grew slow)

  * 0.5mL culture → 1mL H_2O wash → 2mL YPG → Plate @ 3hr

- Resuspended 2 colonies from each A & B plates of 787, 789, 792, 793, and 795 + MATα2 in -ura for freeze down tomorrow.

9/12/14

- Freeze down the following:

yGIL787 A1 + Mat α2	yGIL796
yGIL787 B1 + MAT α2	yGIL797
yGIL789 A1 + MAT α2	yGIL798
yGIL789 B1 + MAT α2	yGIL799
yGIL792 A1 + MAT α2	yGIL800
yGIL792 B1 + MAT α2	yGIL801
yGIL793 A1 + MAT α2	yGIL802
yGIL793 B1 + MAT α2	yGIL803

yGIL795 A1 +MATα2 ———————— yGIL804
yGIL795 B1 +MATα2 ———————— yGIL805

- checked for a/a in the ^(4hr) Gal induced (3hr) 749, 766, and 795 A+B for each ∴ None in 749 or 785 ∴, but 9 confirmed a/a for 766 ☺

~~Started O/N of 766 A clones A and B confirmed a/a~~

- Checked sporulations and found nothing ∴ Not even in the re-do of method Ⓔ → possibly due to spending 1st day of sporulation in microfuge tubes = bad oxygenation

- Spotted 766 A confirmed a/a colonies A and B to -ura & +5FOA

9/15/14

- ~~DF~~ yGIL766 A ^(MATa/a) clones Ⓐ + Ⓑ did not grow on -ura ∴ picked from +5FOA and started O/N to freeze down tomorrow.

9/16/14

- froze down yGIL766 A Ⓐ a/a ———— yGIL806
yGIL766 A Ⓑ a/a ———— yGIL807

- Checked 30 more colonies from 795 A+B and 19 colonies from 749 A+B in ~~that~~ αF and found colony 6 from 785B confirmed a/a → patched to -ura + +5FOA and started O/N in YPD for freeze down.

9/17/14

- Performed following sporulation methods on yGIL775:

Ⓓ -URA (GAL) O/N (.5mL) $\xrightarrow{spin}$ 1mL H_2O wash → SPO++ (Glu) ①
→ SPO++ (GAL) ②

Ⓔ -Ura O/N → .5mL spin → 1mL H_2O wash → SPO++ (Gal)

Ⓕ -ura O/N → -ura (GAL) $\xrightarrow{3hr}$ 1mL spin → 1mL H_2O wash → SPO++(Glu) ①
→ SPO++(Gal) ②

Ⓖ -ura O/N ⟶ -ura(GAL) $\xrightarrow{6hr}$ 1mL spin → SPO++(Glu) ①
→ SPO++(Gal) ②

* H_2O washes not done except for on 2nd round of Ⓔ done w/ cells in exponential phase done in duplicate (1 w/ water wash & 1 without).

- Greg did transfer from -ura O/N → -ura (Gal) for Ⓕ & Ⓖ of 100x culture into 2mL -ura (Gal).

- picked the 795 B ⑥ confirmed a/a from +5FOA and started O/N in YPD

9/19/14

- froze down yGIL795B ⑥ a/a ⟶ yGIL808

9/22/14

- Checked sporulations from 9/17/14 and found best results in Ⓔ exponential w/ & w/out H_2O wash. (w/ ≈ 1/100) (w/out ≈ 4/254). All other methods had comparable and lower yeilds than that observed in all Ⓔ methods.

- Started O/N's of 773 ~~##~~ and 775 in -ura and -ura(Raf) to do following sporulations tomorrow

Ⓗ -ura (O/N) —①→ SPO++ (Glu)
② → Exp → SPO++ (Glu)

Ⓘ -ura(Raf) —①→ SPO++ (Glu)
② → Exp → SPO++ (Glu)
③ → SPO++(Gal)
④ → Exp → SPO++(Gal)

NO H_2O washes

- Struck out yGIL749, 783 (Need HO transform) and 806 & 808 (Need a2 transform) to YPD from freezer.

9/23/14

- Started H1 & H2 Sporulation methods for 773 & 775 due to low growth in SD-ura (Raf).

9/24/14

- SD Raf cultures of 773 & 775 from 9/22/14 were not supplemented with His & Trp ∴ didn't grow ∴ → started new ones w/ His & Trp. (10x each in 5mL of SD (900λ SD : 100λ Raf)).
- Started O/N's of 749, 783, 806, and 808 in YPD.

9/25/14

- O/N cultures of yGIL773 & 775 in Raffinose grew well
  ↳ 1:50 dilution (100λ into 5mL) done at 9:45 (in duplicate)

22.5 mL	1.1x SD (no sugar)
2.5 mL	20% Raffinose
50 λ	Histidine (500x)
50 λ	Tryptophan (500x)

- @ ~~8~~ 2:45 I spun 1mL of the original O/N culture in triplicate for both 773 & 775.
  ↳ · no water wash
  · Resuspend the three replicates in
    - SPO++ GLUCOSE (I1)
    - SPO++ GALACTOSE (I3)
    - 2% KAc + His & Trp (labeled I5).
- @ ~~8~~ 2:45 the 1:50 dilutions from this morning are too dilute to do anything.

@ 5:45 I spun 5mL of the exponentially growing cultures and resuspended in 2mL of either SPO++ & GLUCOSE (I2) or SPO++ GALACTOSE (I4).

↳ They still didn't seem very saturated (small pellets) → maybe I didn't supplement correctly.

Also, I started new O/N cultures of 749, 783, 806, & 808 in 5 mL YPD.

9/26/14

- I checked the H1 + H2 sporulations from 9/23 and found NO tetrads ¨ → back on RT roller drum.
- transformed yGIL749 + 783 w/ HO and 806 + 809 with α2 ⟶ plated to -ura in incubator (protocal on pg. 55, no separation).

9/29/14

~~- Started O/N of transformed 749 (HO), 806 (α2), + 809 (α2) (2 colonies each) in -ura~~

- The transformed 783 created a lawn ⟶ we did this before on 9/8/14 and realized its because 783 came from a sterile w/ the ura (pg. 68)
- Started O/N of 2 colonies from yGIL749 + HO in -ura
- Streaked 2 colonies each of yGIL806 + MATα2 and yGIL809 + Matα2 to YPD
- ~~Strea~~ Restreaked from freezer yGIL753 (a/a, NO α2 of 773) and yGIL755 (a/a, NO α2 of 775) from freezer

9/30/14

- Started O/N in YPD of yGIL784 (yGIL783 w/out sterile plasmid) for HO transformation tomorrow.
- Picked singles from the yGIL753 + 757 YPD plates into 5mL YPD for transformation w/ new Matα plasmid (pGIL069) that I prepped yesterday (protocal on pg. 74).
- yGIL749 ① + ② + HO are growing very slow, but will be ready for Gal induction tomorrow.
- Started O/N of 773 + 775 in -ura (Gal) for sporulate tomorrow.

QIAGEN MINI PREP PROTOCOL

Spin down 1 mL of O/N bacterial culture

Resuspend pellet in 250 µL Buffer P1

Add 250 µL Buffer P2 and mix thoroughly (4-6 inversions→clear solution)

  **NO more than 5 mins

Add 350 µL Buffer N3 and mix immediately (inversions)

Centrifuge for 10 mins at 13,000 rpm

Pipette supernatant into QIAGEN spin column

Centrifuge for 30 sec at 13,000 rpm and discard flow-through

Wash with 500 µL Buffer PB

Centrifuge for 30 sec at 13,000 rpm and discard flow-through

Wash with 750 µL Buffer PE

Centrifuge for 30 sec at 13,000 rpm and discard flow-through

Transfer spin column to collection tube

Centrifuge for 1 min at 13,000 rpm to remove residual wash buffer

Place column in sterile microfuge tube and add 50 µL Buffer EB to the center of the spin column and let it stand for 1 min

Centrifuge for 1 min at 13,000 rpm

Label and store in -20°C freezer

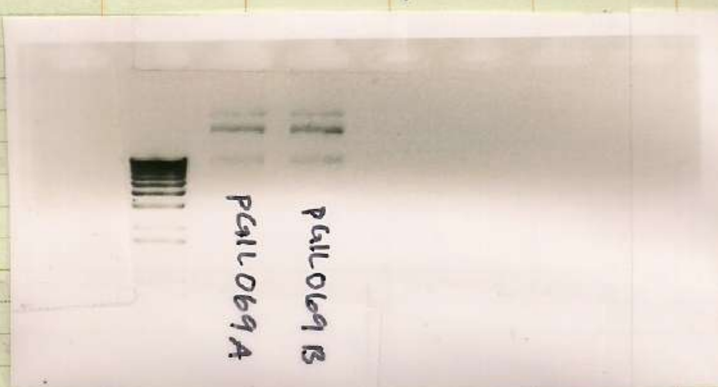
10/1/14

- Started O/N^s in YPD of 806 & 808 + α2 (① + ② each) to be frozen down tomorrow:

yGIL 806 + Mat α2 ①	———	yGIL ~~809~~ 811
yGIL 806 + Mat α2 ②	———	yGIL ~~810~~ 812
yGIL 808 + Mat α2 ①	———	yGIL ~~811~~ 813
yGIL 808 + Mat α2 ②	———	yGIL ~~812~~ 814

- Transformed yGIL753* and yGIL757 w/ pGIL069 (Mat α2) and yGIL784 with pGIL067 (+HO)

- Gal inductions started at 9:15AM (by Greg) of yGIL749 + HO (①+②) were plated @ 12:40pm and then again at 3:30pm after being left at RT.



10/2/14

ANOTHER ATTEMPT TO GET GAL-INDUCED MATα2 STRAINS TO SPORULATE

- O/N cultures of yGIL773 & yGIL775 in SC-ura + Gal were started on 9/30 (p. 73)
  → this morning there were dense but still not saturated (773 more dilute than 775).
- Attempt to induce sporulation by growing exponentially in YPD → during this time the plasmid may be lost & the MATα2 repressed; the conc. of α2 will dilute out each generation → therefore it is worth trying different times in YPD.
- Set up three time points: → in 5mL YPD

DILUTION	TIME DILUTED into YPD	TIME IN SPO++	HOURS IN YPD	
1:50	9 am	2:30pm	5.5 h	← spin 5mL, resusp. 2mL SPO++ (Glucose)
1:100	9 am	5:30 pm	8.5 h	← — " —
1:10	2:30 pm	5:30 pm	3 h	← — " —

10/3 Dilute o/n of yGIL811 & 813 (containing pGIL069) into YPD (1:100 & 1:50) at 9 am

DILUTION	TIME DILUTED	TIME IN SPO++	HOURS IN YPD
1:50	9 am	3:15 pm	6.25 h
1:100	9 am	5 pm	8 h

10/6/14

The 806(1), 806(2), 808(1), & 808(2) o/N cultures in YPD have been sitting on my bench for several days (p. 75)

↳ I spun 1ml each, washed once in 1ml H_2O, then resuspended in 2.5ml of newly made 2% KAc + His & Trp → on RT rollerdrum ~4:30pm

10/9/14

- yGIL784 also still has the STE plasmid → struck out 783 & 784 to YPD from freezer to patch to +5FOA tomorrow so they lose the plasmid and we can transform them with HO.

- Greg was using yGIL 811 & 813 which have the +Mat α2, NOT +Mat α (pGIL069)

- The yGIL753* and yGIL757 transformed w/ Mat α (pGIL069) on 10/1/14 (pg. 75) were still in the incubator

  ↳ I picked 4 colonies each → 2 in -ura / 2 in YPD } o/N

  to re-do sporulations tomorrow and freeze down.

10/10/14

~~- froze down yGIL753* + MAT α (pGIL069) → yGIL815, yGIL816~~

~~yGIL757 + MAT α (pGIL069) → yGIL817, yGIL819~~

~~- froze down yGIL753* (1) + Mat α (pGIL069)~~

- froze down:

  yGIL753* + MAT α (pGIL069) → 1 yGIL817, 2 yGIL818

  yGIL757 + MAT α (pGIL069) → 1 yGIL819, 2 yGIL820

- This morning Greg diluted the 817-820 in -ura 1:50 and 1:100 at 10:45 AM for me to do the following sporulation methods on all 4 strains.

* O/N SD -ura → 1:50 into YPD → 3hr → Spin down 5mL → 2mL SPO++
  → 1:100 into YPD → 6hr → Spin down 5mL → 2mL SPO++

* O/N YPD → 1mL spindown → 2mL SPO++.

10/11/14

- patched 793 & 794 to +5FOA so they lose the STE plasmid and we can freezedown tomorrow.
- resuspended the 3 confirmed a/a colonies of 749 that were patched to +5FOA in YPD to freeze down tomorrow.

10/13/14

- froze down the 749 a/a's from 10/11

YGIL749 a/a 3hr colony 5	YGIL821
YGL 749 a/a 3hr colony 7	YGIL822
YGIL749 a/a 6hr colony 5	YGIL823

- started O/N in YPD of 793 & 794 NO STE to freezedown tomorrow

YGIL793 No STE	YGIL ~~824~~ 826
YGIL794 No STE	YGIL ~~825~~ 827

- checked sporulation of 817-820 started on 10/10/14 and they look really Good!! ☺☺
  * 1:50 → 3hr YPD looks best (~120/199 = 64%)
  * 1:100 → 6hr YPD looked roughly the same (~34/64 = 56%)
  * YPD O/N looked almost the same as the 1:50
  * No difference between $\underbrace{\text{817/818}}_{753^*}$ and $\underbrace{\text{819/820}}_{757}$

10/15/14

- froze down YGIL 826 & 827
- started overnights of Mat a/a (NO mat α2) to transform with pGIL069 (Mat α) on Friday:

Mat a/a	YGIL756	BYS2-D06-910
	YGIL762	BYS1-A09-1000
	YGIL 791	RMS1-G02-545
	YGIL 795	BYS1-A08-545
	YGIL808	RMS1-H08-585 (STE)
	YGIL787	RMS1-H08-585
	YGIL 806	BYS1-E03-745

10/20/14

- Saturday (10/18/14) I transformed the 7 Mat a/a's on previous page w/ pGIL069 (Mat α)
    - ↳ very high efficiency for 791 & 795 → restreak to -ura
    - ↳ all others - pick 2 colonies each to resuspend in ~~-ura~~ and freeze down tomorrow.
- 7 out of 12 Mat a/a's now transformed w/ Mat α (pGIL069).

Still need:

Mat a/α:	Mat a/a	
yGIL739	yGIL755	BY51 - D08-1000
yGIL771	yGIL790	BY52 - C06 -1000
yGIL783	DON'T HAVE	RMS1-G02-825
↳ NON sterile mat needs HO = yGIL826 & 827		
yGIL749	yGIL821	RM Anc.
yGIL751	yGIL793	BY Anc

* See table on next page

10/21/14

- Big plates came in today, figured out 500mL ~ 6 big plates
- Made 500mL of Bulk segregant Media:

start: 500mL SD agar
+ 0.4g CSM -arg
+ 0.5g 5FOA
} Microwave → + 500x ClonNat (100 mg/mL)
+ 500x Can. (60mg/mL)
plate

↳ used ~3.1mL of 100mg/mL because we ran out of 5FOA → should not matter too much since the drugs are in excess & only need it to kick out plasmid

* 500mL = 6 big + 5 little plates

	yGIL737 X ???	MAT a/alpha		Switch to MAT a/a	pGIL069 (MATalpha)?
1	BYS1-D08-1000	yGIL681	yGIL739	yGIL755	X
2	BYS2-D06-910	yGIL687	yGIL740	yGIL756	✓ 828, 829
3	BYS1-A09-1000		yGIL741	yGIL762; yGIL763	✓ 830, 831
4	BYS2-E01-665		yGIL742; yGIL743	yGIL757	✓ yGIL819; yGIL820
5	RMS1-D12-910		yGIL744	yGIL753; yGIL754	✓ yGIL817; yGIL818
6	RMS1-G02-545		yGIL745	yGIL791; yGIL792	✓ yGIL838, 839
7	BYS1-A08-545	yGIL683	yGIL768; yGIL769; yGIL770	yGIL795	✓ yGIL840, 841
8	BYS2-C06-1000	yGIL685	yGIL771; yGIL772	yGIL789; yGIL790	X
9	RMS1-G02-825	yGIL689	yGIL783; yGIL784; **yGIL826; yGIL827**	X	X
10	RMS1-H08-585 (STE)	yGIL692	yGIL785; GIL786	yGIL808	✓ 836, 837
11	RMS1-H08-585		yGIL764; yGIL765	yGIL787; yGIL788	✓ 832, 833
12	BYS1-E03-745		yGIL766; yGIL767	yGIL806; yGIL807	✓ 834, 835
13	RM Anc	yGIL429	yGIL749; yGIL750	yGIL821; yGIL822; yGIL823	X
14	BY Anc	yGIL432	yGIL751; yGIL752	yGIL793; yGIL794	X

10/22/14

- froze down 2 of each of the 5 Mat a/a's transformed with pGIL069 from 10/20 in -ura that took 2 days to grow up.

yGIL756 + α — yGIL828, yGIL829
yGIL762 + α — yGIL830 X, yGIL831
yGIL787 + α — yGIL832, yGIL833
yGIL806 + α — yGIL834, yGIL835
yGIL808 + α — yGIL836, yGIL ~~839~~ 837

- picked 2 colonies each from the restreaked plates (791 + 795) into 5mL -ura to freeze when saturated

- Did a pilot of this round of Bulk Segregant approach on 817 + 819 that were sporulated on 10/10/14

Bulk Segregant Protocol (pg 35)

- 2mL culture
- Spindown 1min at 15000
- Resuspend in 200λ H_2O (good H_2O)
- Add 5λ Zymolyase (~~10mg/mL~~)
- Incubate 30° 1hr
- Add 50λ glass beads
- Add 50λ 10% Triton
- vortex 2mins
- Incubate 30° 30min - CHECK
- ~~Sonicate 4sec @ power 4~~
- ~~CHECK~~

protocol continued...

~~plated 500λ~~ - bring up to 5 mL w/ MilliQ H_2O
- sonicate @ power 4, 4 seconds
- check on scope

plated: - 500λ each to big plates of stock on BSA media
- 100λ each of stock, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} dilutions onto the 5 little BSA media plates

10/24/14

- Started overnights of YGIL755, 790, 794, and 822 to be transformed w/ pGIL069 tomorrow and YGIL826 to be transformed w/ HO (pGIL067) tomorrow.

10/25/14

- froze down the following in -ura:

yGIL791 + pGIL069 — yGIL838, yGIL839

yGIL795 + pGIL069 — yGIL840, yGIL841

- Katie diluted yGIL755, 790, 794, 822, and 826 1:50 into 10 mL YPD for me this morning to perform ~~[illegible]~~ transformations w/ pGIL069 (α) & 067 (HO)

10/27/14

- started O/N in -ura of 2 colonies each from 755, 790, 822, and 826 transformed w/ α and HO
- yGIL794 (BY Anc) had exceptional efficiency and needed to be restreaked to -ura before being able to pick single colonies
- ~~started~~ streaked 817 an 819 to -ura from freezer to start sporulating after O/N in YPD.

10/29/14

- Spun down 1 mL of yGIL826 + HO ① + ② and resuspended in 2mL YPGal @ 3pm → plated 10x at 6pm.

- froze down the following strains from yesterday in -ura:

yGIL755 + pGIL069 —— yGIL847, yGIL848

yGIL790 + pGIL069 —— yGIL849, yGIL850

yGIL822 + pGIL069 (RM anc) —— yGIL851 X, yGIL852 X

- Started O/N of yGIL851 and 852 ~~in 10m~~ (10x in 10mL) to begin sporulation tomorrow (in at 3:30pm).

10/29/14

- I picked 2 colonies from the ~~first~~ restreak plate on -ura of yGIL794 + pGIL069 to freeze down tomorrow (in 5mL -ura)

yGIL794 + pGIL069 —— yGIL853 X, yGIL854 X

- Started O/N in 10 mL YPD of $\underbrace{\text{yGIL 817, 819, 853, 854}}_{\text{1 colony from each - wrap plate}}$ to sporulate tomorrow (Greg).

* yGIL 817 & 819 from 10/27

- Began sporulation in 20mL SPO++ from spin down of all 10mL of yGIL851 and 852 → SPO++ from 9/15 (orange tape)

11/3/14

- Greg froze down yGIL 853 & 854 on Saturday (11/1/14)

~~- Greg checked the~~

~~- Friday 10/31/14 I did gal inductions of yGIL826 + HO ① and ② and plated at 3hrs.~~ Greg

- on 10/31/14 I picked 24 colonies from yGIL826 + HO ① and 18 colonies from yGIL826 + HO ② and suspended in αF
  ↳ greg found none of the ① and 12 of the ② were confirmed a/a mating type. → Patched to +5FOA
- Today I resuspended 2 of the confirmed a/a colonies of yGIL826 + HO ② that were patched to +5FOA in 5mL YPD to freeze down tommorrow.
  ↳ yGIL826 a/a ⑤ —— yGIL857
  yGIL826 a/a ⑮ —— yGIL858
- Struck out yGIL $\boxed{818, 820}$, $\boxed{830, 834}$, $\boxed{847, 850, 851, 852, 853, \text{and } 854}$ from freezer to -ura $\boxed{849}$, and $\boxed{836}$ ROUND 2 ROUND 1 ROUND 3
- Started O/N in 10mL YPD of 817 + 819 from 10/27 -ura plate to sporulate in 20mL spo++ tomorrow.

11/4/14

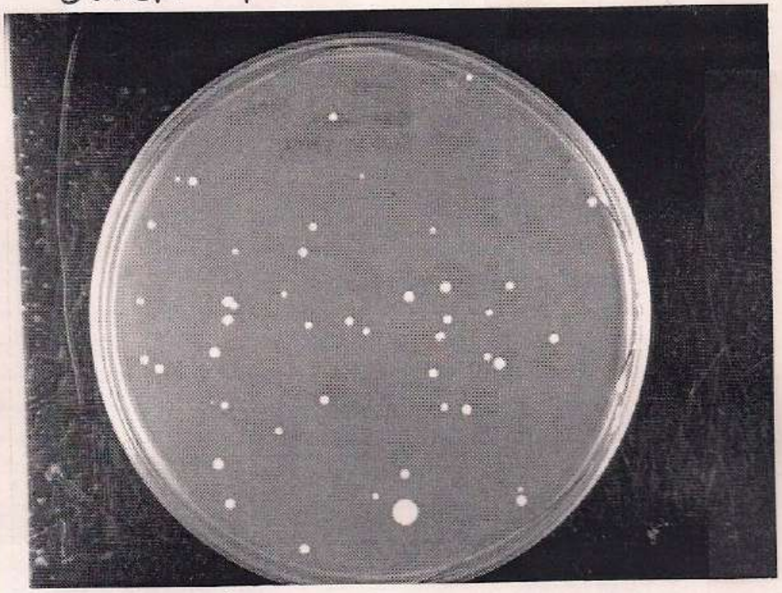
- froze down yGIL857 + 858
- Began Sporulation of 917 + 919 w/ spo++ from 10/27
  * Need to make more spo++ for 12 20mL sporulations this week

11/5/14

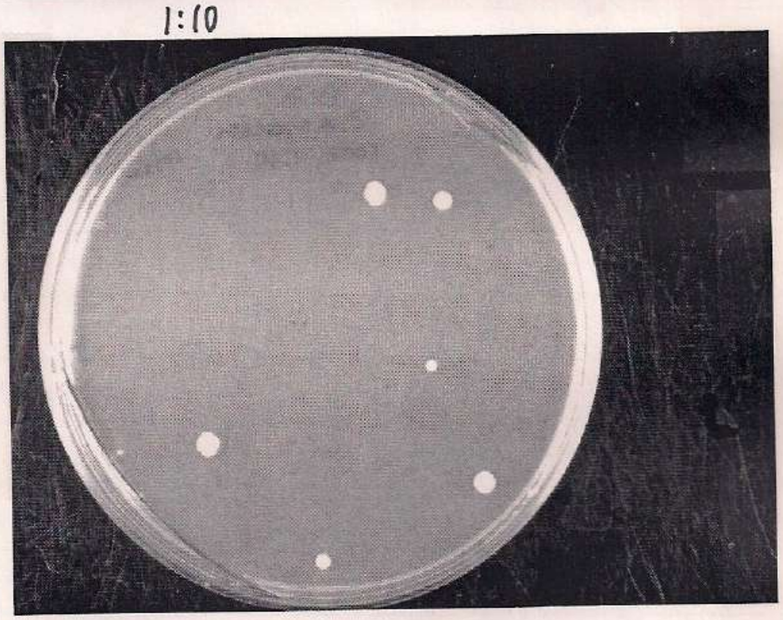
- My YPD liq was contaminated → maybe why 851 + 852 aren't sporulating?
  * restarted 5mL YPD O/N of 857 + 858 from +5FOA patches to redo freeze down tomorrow
- 12 populations chosen to sporulate to check each round of my 3 transformation sets are still really small on -ura plate
  ↳ Greg will start 10mL YPD O/N tomorrow so I can sporulate on Friday

BSP plates

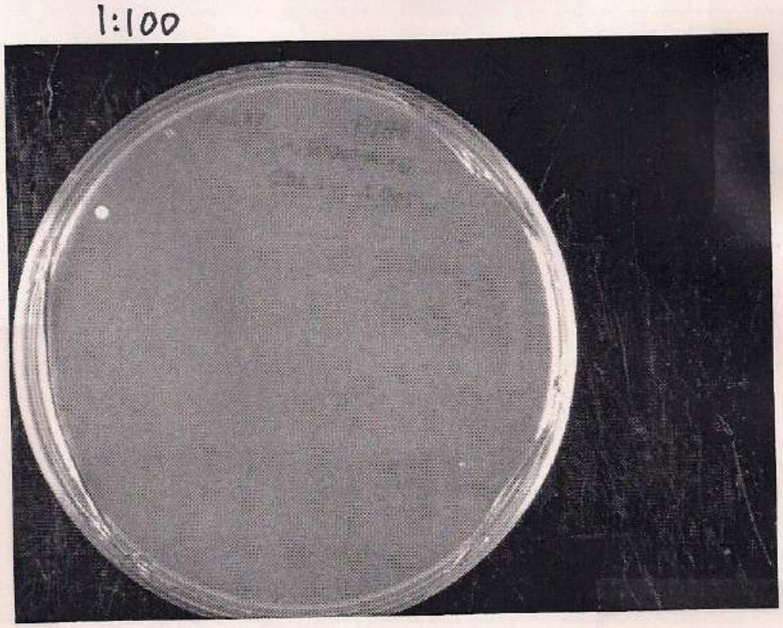
STOCK YGIL 817 (100λ) little plates



1:10



1:100



and no growth on 1:1,000 & 1:10,000

plated: 10/22/14
pictured: 10/29/14

11/7/14
(817 & 819 from 11/4)
Bulk Segregant Protocol

- 20 mL culture → spin
- 200λ H_2O resuspend
- 5λ 1000x zymolyase

→ ended up being 750λ all together

- Incubate @ 30° for 1hr
- 50λ glass beads
- 50λ 10% Triton
- vortex 2 mins
- Incubate @ 30° for 40 min (CHECK)
- Bring up to 5mL w/ H_2O
- sonicate @ power 4 for 4 seconds
- Plate
  - ↳ 500λ on big plate
- YPD hold: 500λ into 5mL YPD for 1.5hrs → plate 500λ to big plates

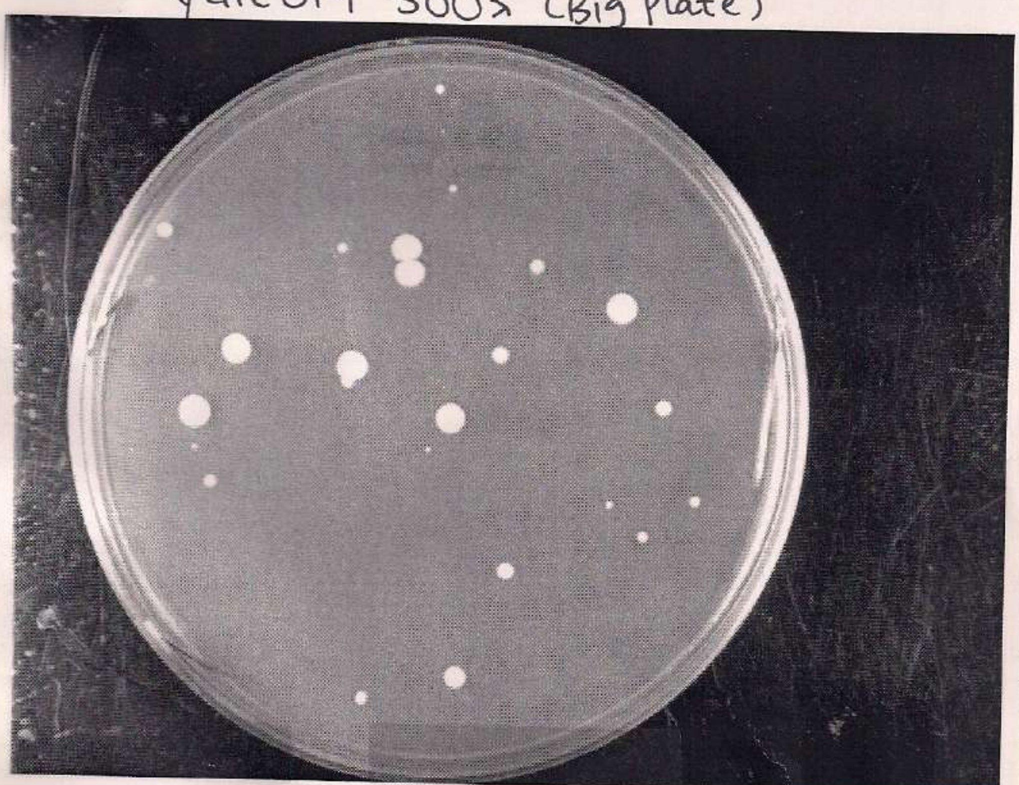
→ Also plated 100λ of 10^{-6} & 10^{-5} diluted samples to YPD plates to replica plate to BSP media when grown.

* NOTE: I did not add clonat + CAN to agar before pouring →

BSP Plates

yGIL 817 500x (Big Plate)

yGIL 819 500x (Big Plate)



the BSP media little plates ∴ concentrations may be off after re-melting & adding ~~also~~
↳ will plate to little BSP plates tomorrow (817 & 819 in fridge till then).

- Today I also sporulated all 12 cultures grown in YPD tomorrow O/N (1mL culture into 2mL SPO++)

11/10/14

- Checked the sporulations from 11/7: 7 out of 12 had tetrads one from round 2 (yGIL830) did not, and four from round 3 did not (yGIL851-854).
  * confusing that there are ones that work and don't work from rounds 2 & 3
- Bulk segregant plates from the YPD hold look much better :)
- Replica plated both dilution factors of 817 & 819 that were plated to YPD after the bulk seg. protocol. to the BSA media made on 11/7 that has full amount of 5FOA BUT concentrations may be a little off.

11/11/14

- a/a's + pGIL069 that sporulate: yGIL817-820, 834, 836, 847, 849, and 850
- a/a's + pGIL069 that DON'T sporulate: yGIL830, yGIL851-854
  ↳ must Re-do transformations ∴ struck to YPD from freezer
- a/a's + pGIL069 to CHECK for sporulation: 828, 829, 831-833, 835, 837, 838-841, 848, and 849
  ↳ Struck 828-838 from freezer to -ura (didn't have enough plates to do all 13)
  * YPD o/n on Thurs → sporulate Friday

11/12/14

- Struck out 839, 840, 841, 848, + 849 from freezer to -ura to check for sporulation (YPD o/n on Friday → sporulate on Saturday)
- Transformations to be done on Friday: yGIL762 (original of 830) yGIL794 (original of 853/854) and yGIL822 (original of 851/852) AND yGIL857 (yGIL826 a/a - the one that never got pGIL069).
  ↳ struck to YPD today. (other 3 done yesterday)
- Replica plating to BSP media results:

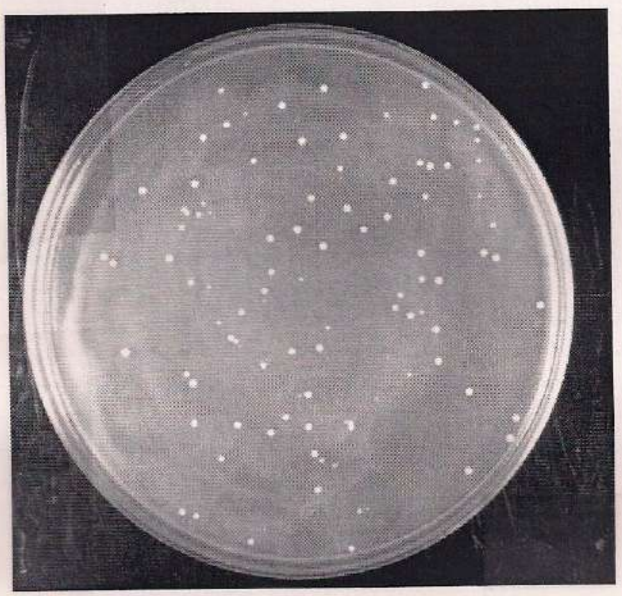
NO 4° O/N YPD → BSP
- 817: 5/46 @ 10^{-6}; ~22/250 @ 10^{-5}
- 819: 4/42 @ 10^{-6}; ~17/200 @ 10^{-5}

accurate ⟶ estimation

4° O/N
YPD → BSP { 817: 3/50 @ 10^{-6}; ~35/200 @ 10^{-5}; ~70/300 @ 10^{-4}
819: 5/49 @ 10^{-6}; ~31/200 @ 10^{-5}; ~30/300 @ 10^{-4}

yGIL817 Bulk Segregants

No YPD hold



1.5hr YPD hold

# of colonies on ~1/4 of YPD HOLD plate: 1,698
of 'Big' colonies on entire YPD HOLD plate: ~24

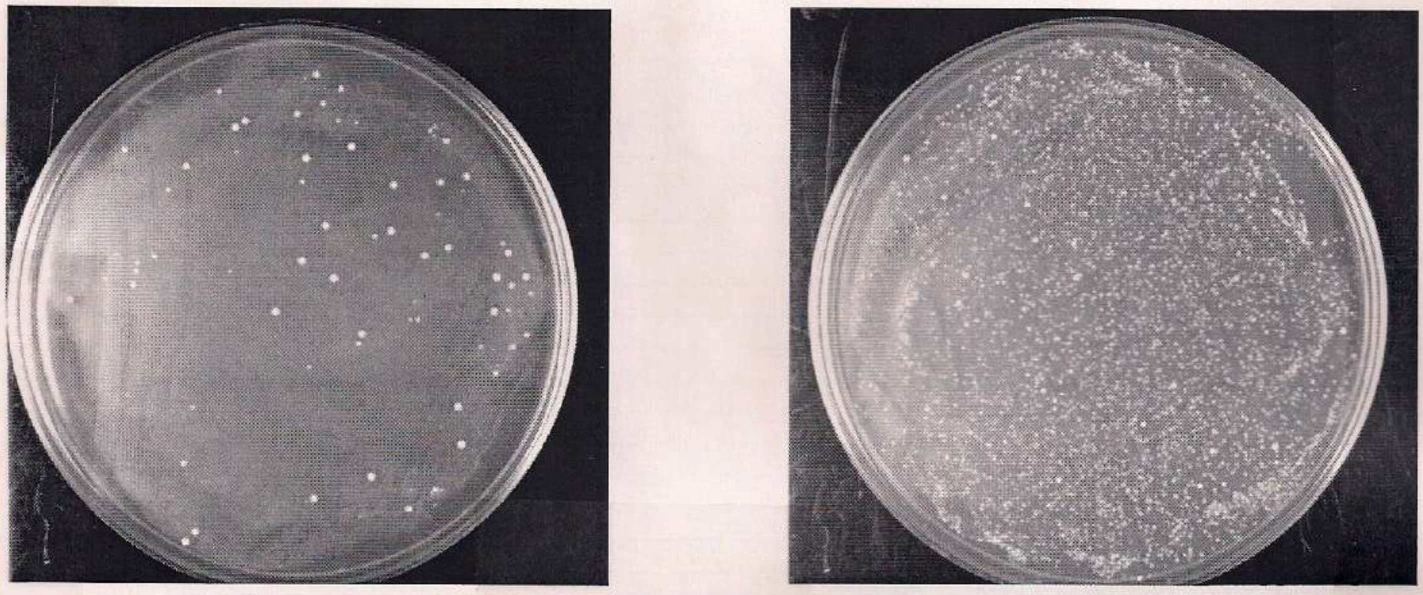
- Greg started picking colonies from 817 & 819 YPD HOLD so we can start making sure the segregants are Mat a haploid w/ DNA staining content.

↳ I struckout YGIL429 & 432 (RM & BY Anc's), YGIL354 and 355 (a & α testers) and YGIL757 & 753* (819 & 817 aka no pal1069) from the freezer to YPD.

* 817 & 819 BS's plated on 11/7 & pictured on 11/12

yGIL819 Bulk Segregants

No YPD hold — 1.5hr YPD hold



# of colonies on ~1/4 YPD HOLD plate: 983
of "Big" colonies on entier YPD HOLD plate: ~30

11/~~13~~ 14/14

- Struck out the Mat a Evolved versions of 817 (RMS1-D12-960) and 819 (BYS2-E01-665) from 11/18/13 plate in freezer (pg. 18)
- Began YPD O/N of yGIL 839, 840, 841, 848, and 849 to begin sporulation tomorrow
- Transformed yGIL 762, 794, 822, and 857 w/ pGIL069. (Mat α)
- Greg picked 2 plates each of colonies from 817 & 819 BS's
  - ↳ made copies of all plates in duplicate
  - ↳ Mating Type Testing CONTROL WELLS — yGIL646 — α; yGIL432 — a

817 plate ① —— NONE
817 plate ② —— ROW F : 02 & 06 — a
                                   04 & 08 — α
819 plate ① —— ROW D : 02 & 06 — a
                                   04 & 08 — α
819 plate ② —— ROW E : " "
                                   " "

- Began sporulations of yGIL 828, 829, 831, 832, 833, 835, 837 and 839

11/15/14

- Began sporulation of 839, 840, 841, 843, and 849
- Started 5mL YPD O/N of yGIL 429, 432, and the a/a versions of 817 & 819 (753* & 757) to add to the BSR plates ~~for~~ as controls.
- picked 2 more plates each from 817 & 819 big BS plates

11/16/14

- Replica plated the mating type testing from 11/14 to SD
- Added controls to all copy 1 plates of 817/819 BSI ① + ② for DNA content:

~~817 ①② F02,06 = 432 (a); F04,08 = 753* (a/a)~~
~~817 ③~~

432(a): ROW __ well 02 ~~08~~ 06

753* OR 757 (a,a): ROW __ well 04,08

- Started 5mL YPD O/N of 10 pGIL069 transformants each of yGIL 762, 794, 822, and 857 to check for sporulation quality before freezedown
- started YPD O/N of Mat a Evolved strains of 817/819 to add to the copy plates for Fitness assays

11/17/14

- sporulation results of yGIL __ + pGIL069:

✓ 753* — 817, 818
✓ 757 — 819, 820
✓ 756 — 828, 829
✓ 762 — 830 X, 831 ✓
✓ 797 — 832, 833
✓ 806 — 834, 835
✓ 808 — 836, 837
X 791 — 838 x, 839 x

X 795 — 840 x, 841 x
X 794 — 853 x, 854 x
✓ 755 — 847, 848
✓ 790 — 849, 850
X 822 — 851 x, 852 x

TBD: <u>857</u>

* 794 & 822 already re-transformed (ready for sporulation check.)
* 791 & 795 <u>Need</u> re-transformation

- out of the 40 YPD O/N started yesterday of pGIL069 transformants, I started sporulations of 30 of them: 10 each of yGIL 794, 822, and 857 + pGIL069 after realizing we already have a good sporulator of yGIL 762 + pGIL069 (yGIL831) that was checked on the scope today.

- Added the following controls to plates ① * ② of 817 (RM) and 819 (BY) COPY 2 for future fitness assays:

Strain	Plate	Row	Well	Control
817	①/②	G/F	02,06	YGIL 429 (RM Anc)
817	①/②	G/F	04,08	Mat a Evo RM11-D12-910
819	①/②	D/E	02,06	YGIL 432 (BY anc)
819	①/②	D/E	04,08	Mat a Evo BYS2-E01-665

↳ added 50λ 60% Glycerol * froze down.

11/19/14

- made COPY 1 (DNA content) and COPY 2 (Fitness Assay) of 817 * 819 plates 3 * 4 (mating type testing) originals picked on 11/13/14
- added controls to original plates and diluted controls ~~into~~ into new copy plates and into incubator

WELLS	MTT	DNA Content	Fitness assay
02, 06	YGIL432 (a)	YGIL432 (a)	817: YGIL 429 (RM) 819: YGIL 432 (BY)
04, 08	YGIL 646 (α)	817: YGIL753* (a/a) 819: YGIL757 (a/a)	Mat a Evo 817: RM11-D12-910 819: BYS2-E01-665

11/19/14

- Checked sporulations from 11/17/14:
  yGIL857, yGIL822, yGIL794 ⇒ 10/10 good sporulation.

- started -ura O/N of 2 good sporulators each from the original -ura transformant plate to be frozen down as: (GS = good sporulator)

yGIL794 + pGIL069 GS ── yGIL859, yGIL860

yGIL822 + pGIL069 GS ── yGIL861, yGIL862

yGIL857 + pGIL069 GS ── yGIL863, yGIL864

11/21/14

- froze down the COPY2 (fitness assay) plates 3 & 4 of 817 & 819 bulk segregants and gave COPY 1 (DNA content) to Greg to wherever break
- struck out yGIL 753, 757 (YPD), 817, 819 (-ura)
- transformed yGIL791 & 795 with pGIL069.

11/23/14

- Started YPD O/N of 3 pGIL069 transformants each of 791 & 795 to sporulate tomorrow & freeze down GS"
- Started YPD O/N of 817, 819, 831, & 862 and their a/a No pGIL069 versions 753, 757, 762, & 822 to sporulate tomorrow to confirm a/a behavior

12/19/14

SUMMARY OF MATING TYPE & OTHER PHENOTYPES FROM BULK SEGREGANTS

Strain	Plate	Well	+MATalpha	+MATa	Notes	+MATa (1st round)
817	Plate 1	A01	+			
817	Plate 1	A02	+			
817	Plate 1	A03	+			
817	Plate 1	A04	+		Large	
817	Plate 1	A05		+	Large	+
817	Plate 1	A06	+			
817	Plate 1	A07	+			
817	Plate 1	A08	+			
817	Plate 1	A09				
817	Plate 1	A10	+			
817	Plate 1	A11	+			
817	Plate 1	A12	+			
817	Plate 1	B01	+			
817	Plate 1	B02	+			
817	Plate 1	B03	+			
817	Plate 1	B04	+			
817	Plate 1	B05	+			
817	Plate 1	B06				
817	Plate 1	B07		+	Large	+
817	Plate 1	B08	+			
817	Plate 1	B09	+			
817	Plate 1	B10	+			
817	Plate 1	B11	+			
817	Plate 1	B12	+			
817	Plate 1	C01	+			
817	Plate 1	C02		+	Large	+
817	Plate 1	C03	+			
817	Plate 1	C04	+			
817	Plate 1	C05	+			
817	Plate 1	C06		+		+
817	Plate 1	C07	+			
817	Plate 1	C08	+			
817	Plate 1	C09	+			
817	Plate 1	C10	+			
817	Plate 1	C11	+			
817	Plate 1	C12	+			
817	Plate 1	D01	+			
817	Plate 1	D02	+			
817	Plate 1	D03	+			
817	Plate 1	D04	+			
817	Plate 1	D05	+			
817	Plate 1	D06	+			
817	Plate 1	D07	+			
817	Plate 1	D08		+	Large	+
817	Plate 1	D09		+		
817	Plate 1	D10	+			
817	Plate 1	D11	+			
817	Plate 1	D12	+			
817	Plate 1	E01	+			
817	Plate 1	E02				
817	Plate 1	E03	+			
817	Plate 1	E04	+			
817	Plate 1	E05	+			
817	Plate 1	E06	+			
817	Plate 1	E07	+			
817	Plate 1	E08		+		+
817	Plate 1	E09	+			
817	Plate 1	E10	+			
817	Plate 1	E11	+			
817	Plate 1	E12	+			
817	Plate 1	F01	+			
817	Plate 1	F02	+			
817	Plate 1	F03	+			
817	Plate 1	F04	+			
817	Plate 1	F05	+			
817	Plate 1	F06	+			
817	Plate 1	F07	+			
817	Plate 1	F08	+			
817	Plate 1	F09	+			
817	Plate 1	F10	+			
817	Plate 1	F11	+			
817	Plate 1	F12	+			
817	Plate 1	G01	+			
817	Plate 1	G02	+		Control	
817	Plate 1	G03	+			
817	Plate 1	G04		+	Control	
817	Plate 1	G05	+			
817	Plate 1	G06	+		Control	
817	Plate 1	G07	+			
817	Plate 1	G08		+	Control	
817	Plate 1	G09	+			

Strain	Plate	Well	+MATalpha	+MATa	Notes	+MATa (1st round)
819	Plate 1	A01	+			
819	Plate 1	A02	+			
819	Plate 1	A03				
819	Plate 1	A04	+			
819	Plate 1	A05				
819	Plate 1	A06	+			
819	Plate 1	A07		+	Large	+
819	Plate 1	A08	+			
819	Plate 1	A09	+			
819	Plate 1	A10				
819	Plate 1	A11				
819	Plate 1	A12				
819	Plate 1	B01				
819	Plate 1	B02				
819	Plate 1	B03	+			
819	Plate 1	B04	+			
819	Plate 1	B05	+			
819	Plate 1	B06				
819	Plate 1	B07	+			
819	Plate 1	B08	+			
819	Plate 1	B09				
819	Plate 1	B10	+			
819	Plate 1	B11	+			
819	Plate 1	B12	+			
819	Plate 1	C01	+			
819	Plate 1	C02				
819	Plate 1	C03	+			
819	Plate 1	C04				
819	Plate 1	C05				
819	Plate 1	C06	+			
819	Plate 1	C07				
819	Plate 1	C08	+			
819	Plate 1	C09				
819	Plate 1	C10	+			
819	Plate 1	C11				
819	Plate 1	C12	+			
819	Plate 1	D01	+			
819	Plate 1	D02	+		Control	
819	Plate 1	D03				
819	Plate 1	D04		+	Control	+
819	Plate 1	D05	+			
819	Plate 1	D06	+		Control	
819	Plate 1	D07				
819	Plate 1	D08		+	Control	+
819	Plate 1	D09	+			
819	Plate 1	D10	+			
819	Plate 1	D11				
819	Plate 1	D12	+			
819	Plate 1	E01	+			
819	Plate 1	E02	+		Large	
819	Plate 1	E03	+			
819	Plate 1	E04				
819	Plate 1	E05				
819	Plate 1	E06				
819	Plate 1	E07				
819	Plate 1	E08				
819	Plate 1	E09	+			
819	Plate 1	E10	+			
819	Plate 1	E11				
819	Plate 1	E12				
819	Plate 1	F01				
819	Plate 1	F02				
819	Plate 1	F03			Large	
819	Plate 1	F04	+			
819	Plate 1	F05				
819	Plate 1	F06				
819	Plate 1	F07				
819	Plate 1	F08	+			
819	Plate 1	F09				
819	Plate 1	F10				
819	Plate 1	F11				
819	Plate 1	F12	+			
819	Plate 1	G01				
819	Plate 1	G02		+		+
819	Plate 1	G03				
819	Plate 1	G04	+			
819	Plate 1	G05				
819	Plate 1	G06				
819	Plate 1	G07				
819	Plate 1	G08	+			
819	Plate 1	G09	+			

Strain	Plate	Well	+MATalpha	+MATa	Notes	+MATa (1st round)
817	Plate 1	G10	+			
817	Plate 1	G11	+			
817	Plate 1	G12	+			
817	Plate 1	H01	+			
817	Plate 1	H02	+			
817	Plate 1	H03	+			
817	Plate 1	H04	+			
817	Plate 1	H05	+			
817	Plate 1	H06	+			
817	Plate 1	H07	+			
817	Plate 1	H08	+			
817	Plate 1	H09	+			
817	Plate 1	H10	+			
817	Plate 1	H11	+			
817	Plate 1	H12	+			
817	Plate 2	A01	+			
817	Plate 2	A02	+			
817	Plate 2	A03	+			
817	Plate 2	A04	+			
817	Plate 2	A05	+			
817	Plate 2	A06	+			
817	Plate 2	A07	+			
817	Plate 2	A08	+			
817	Plate 2	A09	+			
817	Plate 2	A10	+			
817	Plate 2	A11	+			
817	Plate 2	A12	+			
817	Plate 2	B01	+			
817	Plate 2	B02	+			
817	Plate 2	B03	+			
817	Plate 2	B04	+			
817	Plate 2	B05	+			
817	Plate 2	B06	+			
817	Plate 2	B07		+	Large	+
817	Plate 2	B08	+			
817	Plate 2	B09	+			
817	Plate 2	B10	+			
817	Plate 2	B11	+			
817	Plate 2	B12	+			
817	Plate 2	C01	+			
817	Plate 2	C02	+			
817	Plate 2	C03	+			
817	Plate 2	C04	+			
817	Plate 2	C05	+			
817	Plate 2	C06	+			
817	Plate 2	C07	+			
817	Plate 2	C08	+			
817	Plate 2	C09		+	Large	+
817	Plate 2	C10	+			
817	Plate 2	C11	+			
817	Plate 2	C12	+			
817	Plate 2	D01	+			
817	Plate 2	D02	+			
817	Plate 2	D03	+			
817	Plate 2	D04	+			
817	Plate 2	D05	+			
817	Plate 2	D06	+			
817	Plate 2	D07	+			
817	Plate 2	D08	+			
817	Plate 2	D09	+			
817	Plate 2	D10	+			
817	Plate 2	D11	+			
817	Plate 2	D12	+			
817	Plate 2	E01	+			
817	Plate 2	E02	+			
817	Plate 2	E03	+			
817	Plate 2	E04	+			
817	Plate 2	E05	+			
817	Plate 2	E06	+			
817	Plate 2	E07	+			
817	Plate 2	E08	+			
817	Plate 2	E09	+			
817	Plate 2	E10	+			
817	Plate 2	E11	+			
817	Plate 2	E12	+			
817	Plate 2	F01	+			
817	Plate 2	F02	+		Control	
817	Plate 2	F03	+			
817	Plate 2	F04		+	Control	+
817	Plate 2	F05	+			
817	Plate 2	F06	+		Control	

Strain	Plate	Well	+MATalpha	+MATa	Notes	+MATa (1st round)
819	Plate 1	G10				
819	Plate 1	G11				
819	Plate 1	G12	+			
819	Plate 1	H01	+			
819	Plate 1	H02	+			
819	Plate 1	H03	+			
819	Plate 1	H04	+			
819	Plate 1	H05				
819	Plate 1	H06	+			
819	Plate 1	H07				
819	Plate 1	H08				
819	Plate 1	H09				
819	Plate 1	H10	+			
819	Plate 1	H11				
819	Plate 1	H12	+			
819	Plate 2	A01				
819	Plate 2	A02			Large	
819	Plate 2	A03				
819	Plate 2	A04	+			
819	Plate 2	A05				
819	Plate 2	A06			Large	
819	Plate 2	A07	+			
819	Plate 2	A08	+			
819	Plate 2	A09				
819	Plate 2	A10				
819	Plate 2	A11				
819	Plate 2	A12				
819	Plate 2	B01	+			
819	Plate 2	B02				
819	Plate 2	B03				
819	Plate 2	B04	+			
819	Plate 2	B05	+			
819	Plate 2	B06				
819	Plate 2	B07	+			
819	Plate 2	B08			Large	
819	Plate 2	B09		+	Large	+
819	Plate 2	B10	+			
819	Plate 2	B11				
819	Plate 2	B12	+			
819	Plate 2	C01	+			
819	Plate 2	C02	+			
819	Plate 2	C03	+			
819	Plate 2	C04		+		
819	Plate 2	C05	+			
819	Plate 2	C06	+			
819	Plate 2	C07	+			
819	Plate 2	C08				
819	Plate 2	C09				
819	Plate 2	C10				
819	Plate 2	C11	+			
819	Plate 2	C12				
819	Plate 2	D01	+			
819	Plate 2	D02				
819	Plate 2	D03				
819	Plate 2	D04				
819	Plate 2	D05	+			
819	Plate 2	D06	+			
819	Plate 2	D07	+			
819	Plate 2	D08				
819	Plate 2	D09				
819	Plate 2	D10	+			
819	Plate 2	D11	+			
819	Plate 2	D12	+			
819	Plate 2	E01	+			
819	Plate 2	E02	+		Control	
819	Plate 2	E03	+			
819	Plate 2	E04	+		Control	+
819	Plate 2	E05	+			
819	Plate 2	E06	+		Control	
819	Plate 2	E07				
819	Plate 2	E08		+	Control	+
819	Plate 2	E09	+			
819	Plate 2	E10	+			
819	Plate 2	E11	+		Large	
819	Plate 2	E12				
819	Plate 2	F01				
819	Plate 2	F02	+			
819	Plate 2	F03	+			
819	Plate 2	F04	+			
819	Plate 2	F05	+			
819	Plate 2	F06				

Strain	Plate	Well	+MATalpha	+MATa	Notes	+MATa (1st round)
817	Plate 2	F07	+			
817	Plate 2	F08	+	+	Control	+
817	Plate 2	F09	+			
817	Plate 2	F10	+			
817	Plate 2	F11	+			
817	Plate 2	F12	+			
817	Plate 2	G01	+			
817	Plate 2	G02	+			
817	Plate 2	G03	+			
817	Plate 2	G04	+			
817	Plate 2	G05	+			
817	Plate 2	G06	+			
817	Plate 2	G07	+			
817	Plate 2	G08	+			
817	Plate 2	G09	+			
817	Plate 2	G10	+			
817	Plate 2	G11	+			
817	Plate 2	G12	+			
817	Plate 2	H01	+			
817	Plate 2	H02	+			
817	Plate 2	H03	+			
817	Plate 2	H04	+			
817	Plate 2	H05	+			
817	Plate 2	H06		+	Large	+
817	Plate 2	H07	+			
817	Plate 2	H08	+			
817	Plate 2	H09	+			
817	Plate 2	H10	+			
817	Plate 2	H11	+			
817	Plate 2	H12	+			
817	Plate 3	A01	+		Large	
817	Plate 3	A02	+		Control	
817	Plate 3	A03				
817	Plate 3	A04		+	Control	
817	Plate 3	A05	+			
817	Plate 3	A06	+		Control	
817	Plate 3	A07				
817	Plate 3	A08		+	Control	
817	Plate 3	A09	+			
817	Plate 3	A10	+			
817	Plate 3	A11	+			
817	Plate 3	A12	+			
817	Plate 3	B01				
817	Plate 3	B02	+			
817	Plate 3	B03	+			
817	Plate 3	B04				
817	Plate 3	B05	+			
817	Plate 3	B06	+			
817	Plate 3	B07	+			
817	Plate 3	B08	+			
817	Plate 3	B09	+			
817	Plate 3	B10	+			
817	Plate 3	B11	+			
817	Plate 3	B12	+			
817	Plate 3	C01	+			
817	Plate 3	C02	+			
817	Plate 3	C03	+			
817	Plate 3	C04				
817	Plate 3	C05	+			
817	Plate 3	C06				
817	Plate 3	C07			Blank	
817	Plate 3	C08	+			
817	Plate 3	C09	+		Large	
817	Plate 3	C10	+			
817	Plate 3	C11	+			
817	Plate 3	C12	+			
817	Plate 3	D01	+			
817	Plate 3	D02	+			
817	Plate 3	D03	+			
817	Plate 3	D04	+			
817	Plate 3	D05	+			
817	Plate 3	D06	+			
817	Plate 3	D07	+			
817	Plate 3	D08	+			
817	Plate 3	D09	+			
817	Plate 3	D10	+			
817	Plate 3	D11			Blank	
817	Plate 3	D12	+			
817	Plate 3	E01	+			
817	Plate 3	E02	+			
817	Plate 3	E03				

Strain	Plate	Well	+MATalpha	+MATa	Notes	+MATa (1st round)
819	Plate 2	F07				
819	Plate 2	F08	+		Large	
819	Plate 2	F09				
819	Plate 2	F10	+			
819	Plate 2	F11	+			
819	Plate 2	F12				
819	Plate 2	G01				
819	Plate 2	G02				
819	Plate 2	G03	+			
819	Plate 2	G04				
819	Plate 2	G05	+			
819	Plate 2	G06	+			
819	Plate 2	G07				
819	Plate 2	G08				
819	Plate 2	G09	+		Large	
819	Plate 2	G10	+			
819	Plate 2	G11	+			
819	Plate 2	G12				
819	Plate 2	H01				
819	Plate 2	H02	+			
819	Plate 2	H03				
819	Plate 2	H04				
819	Plate 2	H05	+			
819	Plate 2	H06	+			
819	Plate 2	H07	+			
819	Plate 2	H08				
819	Plate 2	H09	+			
819	Plate 2	H10				
819	Plate 2	H11	+			
819	Plate 2	H12	+			
819	Plate 3	A01				
819	Plate 3	A02				
819	Plate 3	A03	+			
819	Plate 3	A04				
819	Plate 3	A05				
819	Plate 3	A06				
819	Plate 3	A07	+			
819	Plate 3	A08				
819	Plate 3	A09	+			
819	Plate 3	A10				
819	Plate 3	A11				
819	Plate 3	A12	+			
819	Plate 3	B01	+			
819	Plate 3	B02	+			
819	Plate 3	B03	+			
819	Plate 3	B04	+			
819	Plate 3	B05				
819	Plate 3	B06				
819	Plate 3	B07				
819	Plate 3	B08	+			
819	Plate 3	B09	+			
819	Plate 3	B10				
819	Plate 3	B11				
819	Plate 3	B12				
819	Plate 3	C01				
819	Plate 3	C02	+		Control	
819	Plate 3	C03				
819	Plate 3	C04			Control	
819	Plate 3	C05	+			
819	Plate 3	C06	+		Control	
819	Plate 3	C07	+			
819	Plate 3	C08		+	Control	
819	Plate 3	C09				
819	Plate 3	C10			Large	
819	Plate 3	C11				
819	Plate 3	C12	+			
819	Plate 3	D01				
819	Plate 3	D02	+			
819	Plate 3	D03	+			
819	Plate 3	D04	+			
819	Plate 3	D05				
819	Plate 3	D06	+		Large	
819	Plate 3	D07				
819	Plate 3	D08				
819	Plate 3	D09				
819	Plate 3	D10	+			
819	Plate 3	D11				
819	Plate 3	D12				
819	Plate 3	E01				
819	Plate 3	E02	+			
819	Plate 3	E03				

Strain	Plate	Well	+MATalpha	+MATa	Notes	+MATa (1st round)
817	Plate 3	E04	+			
817	Plate 3	E05	+			
817	Plate 3	E06	+			
817	Plate 3	E07	+			
817	Plate 3	E08	+			
817	Plate 3	E09	+			
817	Plate 3	E10	+			
817	Plate 3	E11	+			
817	Plate 3	E12	+			
817	Plate 3	F01				
817	Plate 3	F02	+			
817	Plate 3	F03	+			
817	Plate 3	F04	+			
817	Plate 3	F05	+			
817	Plate 3	F06				
817	Plate 3	F07	+			
817	Plate 3	F08	+			
817	Plate 3	F09	+			
817	Plate 3	F10	+			
817	Plate 3	F11	+			
817	Plate 3	F12				
817	Plate 3	G01		+	Large	
817	Plate 3	G02	+			
817	Plate 3	G03	+			
817	Plate 3	G04	+			
817	Plate 3	G05	+			
817	Plate 3	G06	+			
817	Plate 3	G07	+			
817	Plate 3	G08	+			
817	Plate 3	G09	+			
817	Plate 3	G10	+			
817	Plate 3	G11	+			
817	Plate 3	G12	+			
817	Plate 3	H01	+			
817	Plate 3	H02	+			
817	Plate 3	H03	+			
817	Plate 3	H04	+			
817	Plate 3	H05	+			
817	Plate 3	H06	+			
817	Plate 3	H07	+			
817	Plate 3	H08	+			
817	Plate 3	H09	+			
817	Plate 3	H10	+			
817	Plate 3	H11	+			
817	Plate 3	H12	+			
817	Plate 4	A01	+			
817	Plate 4	A02				
817	Plate 4	A03				
817	Plate 4	A04	+			
817	Plate 4	A05	+			
817	Plate 4	A06	+			
817	Plate 4	A07	+			
817	Plate 4	A08	+			
817	Plate 4	A09	+			
817	Plate 4	A10	+			
817	Plate 4	A11	+			
817	Plate 4	A12	+			
817	Plate 4	B01				
817	Plate 4	B02	+		Control	
817	Plate 4	B03	+			
817	Plate 4	B04		+	Control	
817	Plate 4	B05	+			
817	Plate 4	B06	+		Control	
817	Plate 4	B07	+			
817	Plate 4	B08		+	Control	
817	Plate 4	B09	+			
817	Plate 4	B10	+			
817	Plate 4	B11	+			
817	Plate 4	B12	+			
817	Plate 4	C01	+			
817	Plate 4	C02	+			
817	Plate 4	C03				
817	Plate 4	C04				
817	Plate 4	C05	+			
817	Plate 4	C06	+			
817	Plate 4	C07	+			
817	Plate 4	C08	+			
817	Plate 4	C09				
817	Plate 4	C10	+			
817	Plate 4	C11	+			
817	Plate 4	C12	+			

Strain	Plate	Well	+MATalpha	+MATa	Notes	+MATa (1st round)
819	Plate 3	E04		+		
819	Plate 3	E05	+			
819	Plate 3	E06	+			
819	Plate 3	E07	+			
819	Plate 3	E08				
819	Plate 3	E09				
819	Plate 3	E10				
819	Plate 3	E11				
819	Plate 3	E12	+			
819	Plate 3	F01				
819	Plate 3	F02				
819	Plate 3	F03				
819	Plate 3	F04	+			
819	Plate 3	F05				
819	Plate 3	F06				
819	Plate 3	F07	+		Large	
819	Plate 3	F08				
819	Plate 3	F09	+			
819	Plate 3	F10	+			
819	Plate 3	F11	+			
819	Plate 3	F12				
819	Plate 3	G01				
819	Plate 3	G02				
819	Plate 3	G03	+			
819	Plate 3	G04				
819	Plate 3	G05				
819	Plate 3	G06				
819	Plate 3	G07	+			
819	Plate 3	G08	+			
819	Plate 3	G09				
819	Plate 3	G10	+			
819	Plate 3	G11	+			
819	Plate 3	G12	+			
819	Plate 3	H01	+			
819	Plate 3	H02	+			
819	Plate 3	H03	+			
819	Plate 3	H04	+			
819	Plate 3	H05	+			
819	Plate 3	H06	+			
819	Plate 3	H07	+			
819	Plate 3	H08	+			
819	Plate 3	H09				
819	Plate 3	H10	+			
819	Plate 3	H11				
819	Plate 3	H12	+		Large	
819	Plate 4	A01	+			
819	Plate 4	A02	+			
819	Plate 4	A03	+			
819	Plate 4	A04	+			
819	Plate 4	A05	+			
819	Plate 4	A06	+			
819	Plate 4	A07	+			
819	Plate 4	A08	+			
819	Plate 4	A09	+			
819	Plate 4	A10	+			
819	Plate 4	A11	+			
819	Plate 4	A12	+			
819	Plate 4	B01	+			
819	Plate 4	B02	+			
819	Plate 4	B03	+			
819	Plate 4	B04	+			
819	Plate 4	B05	+			
819	Plate 4	B06	+			
819	Plate 4	B07	+			
819	Plate 4	B08	+			
819	Plate 4	B09	+			
819	Plate 4	B10	+			
819	Plate 4	B11	+			
819	Plate 4	B12	+			
819	Plate 4	C01	+			
819	Plate 4	C02	+			
819	Plate 4	C03	+			
819	Plate 4	C04	+			
819	Plate 4	C05	+			
819	Plate 4	C06	+			
819	Plate 4	C07	+			
819	Plate 4	C08	+			
819	Plate 4	C09	+			
819	Plate 4	C10	+			
819	Plate 4	C11	+			
819	Plate 4	C12	+			

Strain	Plate	Well	+MATalpha	+MATa	Notes	+MATa (1st round)
817	Plate 4	D01	+			
817	Plate 4	D02	+			
817	Plate 4	D03	+			
817	Plate 4	D04	+			
817	Plate 4	D05	+			
817	Plate 4	D06	+			
817	Plate 4	D07	+			
817	Plate 4	D08				
817	Plate 4	D09	+			
817	Plate 4	D10	+			
817	Plate 4	D11	+			
817	Plate 4	D12	+			
817	Plate 4	E01	+			
817	Plate 4	E02	+			
817	Plate 4	E03	+			
817	Plate 4	E04	+			
817	Plate 4	E05				
817	Plate 4	E06	+			
817	Plate 4	E07	+			
817	Plate 4	E08	+			
817	Plate 4	E09	+			
817	Plate 4	E10	+			
817	Plate 4	E11	+			
817	Plate 4	E12	+			
817	Plate 4	F01	+			
817	Plate 4	F02	+			
817	Plate 4	F03	+		Large	
817	Plate 4	F04				
817	Plate 4	F05	+		Large	
817	Plate 4	F06	+			
817	Plate 4	F07	+			
817	Plate 4	F08	+			
817	Plate 4	F09	+			
817	Plate 4	F10	+			
817	Plate 4	F11	+			
817	Plate 4	F12	+			
817	Plate 4	G01	+			
817	Plate 4	G02	+			
817	Plate 4	G03	+			
817	Plate 4	G04	+			
817	Plate 4	G05	+			
817	Plate 4	G06	+		Large	
817	Plate 4	G07	+			
817	Plate 4	G08	+			
817	Plate 4	G09	+			
817	Plate 4	G10	+			
817	Plate 4	G11	+			
817	Plate 4	G12	+			
817	Plate 4	H01				
817	Plate 4	H02	+			
817	Plate 4	H03				
817	Plate 4	H04	+			
817	Plate 4	H05	+			
817	Plate 4	H06	+			
817	Plate 4	H07	+			
817	Plate 4	H08	+		Large	
817	Plate 4	H09	+		Large	
817	Plate 4	H10	+			
817	Plate 4	H11	+			
817	Plate 4	H12	+			

Strain	Plate	Well	+MATalpha	+MATa	Notes	+MATa (1st round)
819	Plate 4	D01	+			
819	Plate 4	D02	+			
819	Plate 4	D03	+			
819	Plate 4	D04	+			
819	Plate 4	D05	+			
819	Plate 4	D06	+			
819	Plate 4	D07	+			
819	Plate 4	D08	+			
819	Plate 4	D09	+			
819	Plate 4	D10	+			
819	Plate 4	D11	+			
819	Plate 4	D12	+			
819	Plate 4	E01	+			
819	Plate 4	E02	+			
819	Plate 4	E03	+			
819	Plate 4	E04	+			
819	Plate 4	E05	+			
819	Plate 4	E06	+			
819	Plate 4	E07	+			
819	Plate 4	E08	+			
819	Plate 4	E09	+		Large	
819	Plate 4	E10	+		Large	
819	Plate 4	E11	+			
819	Plate 4	E12	+			
819	Plate 4	F01	+			
819	Plate 4	F02	+		Large	
819	Plate 4	F03	+			
819	Plate 4	F04	+			
819	Plate 4	F05	+			
819	Plate 4	F06	+			
819	Plate 4	F07	+			
819	Plate 4	F08	+			
819	Plate 4	F09	+			
819	Plate 4	F10	+			
819	Plate 4	F11	+			
819	Plate 4	F12	+			
819	Plate 4	G01	+			
819	Plate 4	G02	+			
819	Plate 4	G03	+			
819	Plate 4	G04	+			
819	Plate 4	G05	+			
819	Plate 4	G06	+			
819	Plate 4	G07	+			
819	Plate 4	G08	+			
819	Plate 4	G09	+			
819	Plate 4	G10	+			
819	Plate 4	G11	+			
819	Plate 4	G12	+			
819	Plate 4	H01	+			
819	Plate 4	H02	+		Control	
819	Plate 4	H03	+			
819	Plate 4	H04	+		Control	
819	Plate 4	H05	+			
819	Plate 4	H06	+		Control	
819	Plate 4	H07	+			
819	Plate 4	H08	+		Control	
819	Plate 4	H09	+			
819	Plate 4	H10	+			
819	Plate 4	H11	+			
819	Plate 4	H12	+			

12/1/14

- checked sporulations from 11/24 and found all a/a + α (817, 819, 831, 862) to have normal amount of tetrads. Also found that the "a/a"s 753 & 757 had no tetrads but a lot of shmoo-looking cells and large vacuoles. The "a/a"s 762 and 822 had very few, but unexpected tetrads.

12/2/14

- dissected tetrads of 817 & 819 to mating type test and confirm a/a or a/?

12/3/14

- froze down

yGIL791 + pGIL069 GS → yGIL865, yGIL866

yGIL795 + pGIL069 GS → yGIL867, yGIL868

12/5/14

- did 10 tetrad dissections of yGIL831
- patched all tetrads of yGIL 817, 819, & 862 to YPD and replica plated to +Nat, +CAN, +α, +a

* patched plates went into incubator to grow a little more

* Dan will replica plate +α/+a to SD for me tomorrow

12/19/14

SUMMARY OF TETRAD DISSECTIONS

yGIL817									
		Mate as				+CAN			+Nat
	Notes	MATa	MATalpha	Non-mater	alpha Mutants	Sensitive	Mutants	Resistant	
1A		X				X			
1B		X			X			X	X
1C									
1D		X			X		X		X
2A		X				X			X
2B		X					X		
2C		X			X	X			
2D									
3A	small	X				X			
3B		X				X			X
3C		X			X	X			
3D		X						X	X
4A		X			X			X	
4B		X				X			
4C		X					X		X
4D		X					X		X
5A									
5B									
5C		X						X	X
5D		X			X	X			
6A		X			X		X		
6B		X				X			X
6C		X				X			X
6D		X						X	
7A		X			X			X	
7B		X			X	X			
7C		X				X			X
7D	on edge	X				X			X
8A		X					X		X
8B		X			X		X		
8C		X				X			
8D		X						X	X

↑

- These spores mostly mate as MATa, but a few colonies appeared within the patch (Remy patched before replica plating), suggesting that mutations appeared allowing them to mate as MATα.
- I replica plated the original YPD plate to -ura. The ones that produced MATα mutants also showed growth on -ura suggesting that they retained the plasmid.

12/19/14

yGIL819									
		Mate as				+CAN			+Nat
	Notes	MATa	MATalpha	Non-mater	alpha Mutants	Sensitive	Mutants	Resistant	
1A									
1B		X				X			
1C				X			X		
1D		X						X	X
2A				X		X			
2B		X			X			X	
2C		X					X		X
2D				X		X			X
3A		X			X	X			X
3B				X		X			
3C		X			X			X	X
3D				X			X		
4A		X				X			
4B		X					X		X
4C				X			X		X
4D				X			X		
5A				X				X	X
5B		X				X			X
5C				X			X		
5D		X				X			
6A		X				X			X
6B				X		X			
6C		X			X		X		
6D				X				X	X
7A									
7B		X				X			X
7C				X		X			
7D				X				X	
8A		X			X			X	X
8B				X		X			X
8C		X					X		
8D				X		X			
9A		X			X		X		X
9B		X			X			X	
9C									
9D									
10A				X		X			
10B		X				X			X
10C				X		X			
10D		X						X	X
11A	small	X				X			
11B	big	X					X		X
11C	small			X				X	X
11D	big			X		X			
12A				X		X			X
12B		X				X			
12C		X						X	
12D									
13A									
13B	big			X		X			
13C									
13D	small			X				X	X
14A		X				X			X
14B				X				X	
14C				X		X			X
14D		X						X	

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12/19/14

yGIL862									
		Mate as				+CAN			+Nat
	Notes	MATa	MATalpha	Non-mater	alpha Mutants	Sensitive	Mutants	Resistant	
1A		X				X			
1B		X			X	X			X
1C		X					X		X
1D		X					X		
2A		X			X			X	
2B		X			X	X			
2C		X				X			X
2D		X						X	X
3A		X				X			X
3B		X			X			X	
3C		X				X			
3D									
4A		X				X			
4B		X				X			X
4C		X				X			X
4D		X					X		
5A		X				X			X
5B		X			X	X			
5C		X			X		X		
5D		X					X		X
6A		X				X			
6B		X					X		X
6C		X			X	X			X
6D		X			X	X			
7A		X				X			
7B		X				X			X
7C									
7D		X						X	X
8A		X			X			X	X
8B		X			X	X			X
8C		X				X			
8D		X					X		

12/19/14

SUMMARY OF STRAINS

#	yGIL737 X ???		MAT a/α	MAT a/a		w/ pGIL069	
				Name	Sporulate?	BS?	GS?
1	BYS1-D08-1000	yGIL681	yGIL739	yGIL755	TBD	None	yGIL847-848
2	BYS2-D06-910	yGIL687	yGIL740	yGIL756	TBD	None	yGIL828-829
3	BYS1-A09-1000		yGIL741	yGIL762-763	Some tetrads, but very low efficiency	yGIL830	yGIL831
4	BYS2-E01-665		yGIL742-743	yGIL757	No tetrads but weird vacuoles and shmoos	None	yGIL819-820
5	RMS1-D12-910		yGIL744	yGIL753-754	No tetrads but weird vacuoles and shmoos	None	yGIL817-818
6	RMS1-G02-545		yGIL745	yGIL791-792	TBD	yGIL838-839	yGIL865-866
7	BYS1-A08-545	yGIL683	yGIL768-770	yGIL795	TBD	yGIL840-841	yGIL867-868
8	BYS2-C06-1000	yGIL685	yGIL771-772	yGIL789-790	TBD	None	yGIL849-850
9	RMS1-G02-825	yGIL689	yGIL783-784 (STE) yGIL826-827	yGIL857-858	TBD	None	yGIL863-864
10	RMS1-H08-585 (STE)	yGIL692	yGIL785-786	yGIL808	TBD	None	yGIL836-837
11	RMS1-H08-585		yGIL764-765	yGIL787-788	TBD	None	yGIL832-833
12	BYS1-E03-745		yGIL766-767	yGIL806-807	TBD	None	yGIL834-835
13	RM Anc	yGIL429	yGIL749-750	yGIL821-823	Some tetrads, but very low efficiency	yGIL851-852	yGIL861-862
14	BY Anc	yGIL432	y GIL751-752	yGIL793-794	TBD	yGIL853-854	yGIL859-860

12/19/14

GENOTYPING THE MAT LOCUS

- From Remy's dissections* (p. 97) I picked two 4-spore tetrads from gGIL817 (4 & 6, p99) and gGIL819 (8 & 14, p101) as well as 4 clones from the bulk segregant prep that mated as MATα (gGIL817 B7, D8, E8 & 819 E2) ← p. 91-95

↳ made gDNA preps and genotyped the MAT locus using primers:

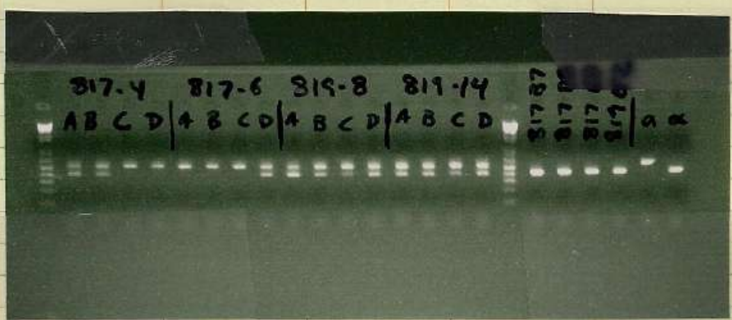
128064846 IDT G.LANG 180615302 04-Dec-2014 **MAT-external-R** 5'-AGT CAC ATC AAG ATC GTT TAT GG-3' Tm=52.8 °C MW=7,062.6 5.4 OD_{260}= 23.4 nm= 0.17mg	128064847 IDT G.LANG 180615303 04-Dec-2014 **MATalpha-internal-F** 5'-GCA CGG AAT ATG GGA CTA CTT CG-3' Tm=57.4 °C MW=7,088.6 5.5 OD_{260}= 24.4 nm= 0.17mg	128064848 IDT G.LANG 180615304 04-Dec-2014 **MATa-internal-F** 5'-ACT CCA CTT CAA GTA AGA GTT TG-3' Tm=52.6 °C MW=7,022.6 6.1 OD_{260}= 27.1 nm= 0.19mg

→ 54° annealing temp.

From: http://yeaki.org/index.php?title=Yeast_Mating_Type_PCR_Assay

- 5'-AGTCACATCAAGATCGTTTATGG-3'
  - This primer corresponds to a sequence at the right end of and directed towards the *MAT* locus.
- 5'-GCACGGAATATGGGACTACTTCG-3'
  - This primer corresponds to a sequence within the α-specific DNA located at *Matα* and *HMLα*.
- 5'-ACTCCACTTCAAGTAAGAGTTTG-3'
  - This primer corresponds to a sequence within the a-specific DNA located at *Mat***a** and *HML***a**.

MATα 404 bp; MAT**a** 544 bp; diploid strains will give both bands



To 1 μl of gDNA, add:

40.5845245	H2O
5	10 x Buffer
0.4	dNTPs
1.06837607	Primer R
1.02459016	Primer a
0.92250923	Primer alpha
1	Taq

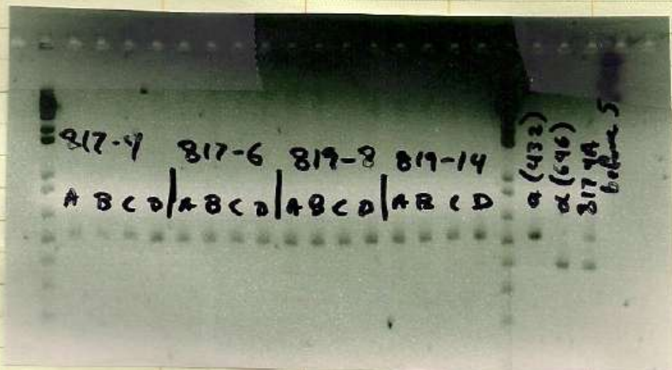
NOTES ON MAT GENOTYPING

- All four spores have MATa → so the diploids are MATa/a.
- Some haploids show a MATα product too.
  ↳ this is consistent w/ the ones that produce MATα and can grow on -ura suggesting that they retain the plasmid
- Interestingly the clones from the Bulk segregant protocol that mate as α have lost the MATa band
  ↳ this suggests that the MATαs that come through do so by gene converting off the plasmid after meiosis.

12/20/14

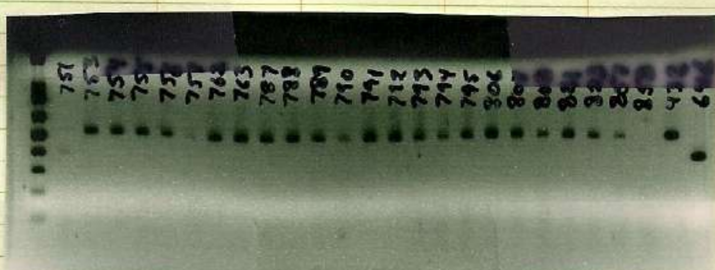
MAT LOCI GENOTYPING CONT.

To demonstrate that the MATα band comes from the plasmid, I replica plated to 5FOA and re-genotyped.



After plating to 5FOA, the MATα band is gone suggesting that the MATα gene was coming from the plasmid.

Since the MAT PCR is working well, I went to the freezer and genotyped all of the putative MATa/a strains (see p. 105).



- Looks good. The a/α control (751, 1st lane) is light.
- 751 is a little light too
- 858 didn't ~~really~~ work.
- Otherwise all look like true MATa/a

12/20/14

NOTES ON BULK SEGREGANT STRAINS & PLASMID

Some things are becoming clear:

- The strains really are MATa/a (p. 108)
- The 5% or so ~~spo~~ segregants that mate as MATα arise via gene conversion w/ the MATα locus on the plasmid (p. 107)

→ The problem therefore lies w/ the plasmid, not the strains. I will need to fix the plasmid.

→ pGIL069 originally came from the Branch lab (plasmid: B1311) → see p.

The plasmid contains a large >4kb fragment w/ both MATα genes and some neighboring ORFs → lots of homology

My plan is to amplify just MATα2 and reclone it into the HindIII site on YCp50.

CONSTRUCTION OF MATα2 PLASMID

Amplify MATα2 w/ flanking HindIII sites from pGIL069 using the following primers

128477208 IDT
G.LANG
181178787 17-Dec-2014
MATalpha2-HindIII-Forwar
5'-TAC AAA AAGCTT TCG TCT TGT TCA AGA AG
Tm=60.1 °C MW=10,504.9
8.2 OD_{260}= 24.3 nm= 0.25mg

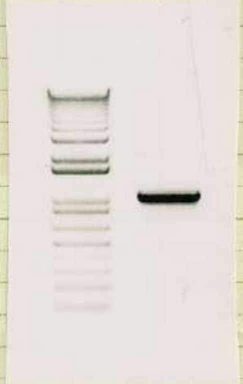
128477209 IDT
G.LANG
181178788 17-Dec-2014
MATalpha2-HindIII-Revers
5'-CGA AGT AAGCTT ATT GTGAAT GTC GTC TA
Tm=57.3 °C MW=10,510.9
9.0 OD_{260}= 26.4 nm= 0.28mg

84.33 H2O
10.00 10 x Buffer
0.80 dNTPs
1.23 Primer Forw
1.14 Primer Rever
1.00 pGIL069
1.50 HiFi

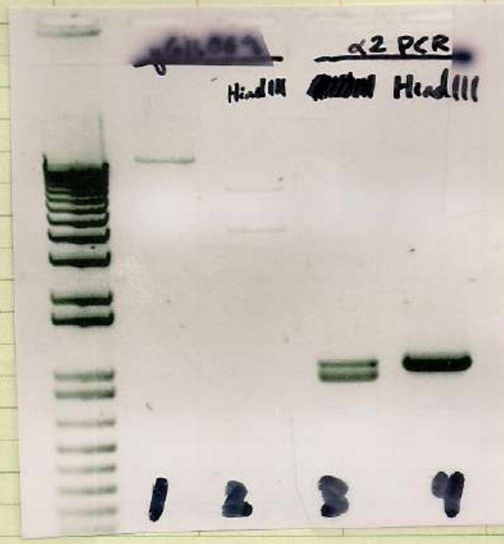
Annealing temp: 50°
Extension time: 1 min.

- I did not realize the plasmid was so dilute
- Don't know what is w/ the double band of PCR product
  → should only be 10-15 bp diff.

Digest 17.3 λ of PCR product & pGIL069 w/ HindIII
2h @ 37°



→ elute in 30 λ H_2O



12/22/14

PLASMID CONSTRUCTION

Ok I'm a little confused. On p. 109 the last two lanes are the PCR product before & after HindIII digestion, though the tubes may be mislabeled. The last lane looks like the uncut PCR and lane 3 looks like partially cut PCR.

↳ I suspect that I did not add enzyme to the digestion, rather I added enzyme to the eluted PCR product and in effect did a RT digestion.

I mixed lanes 3 & 4 and reran the PCR purification → elute in 30 λ H_2O and redigest 17.3 λ w/ HindIII (~noon)

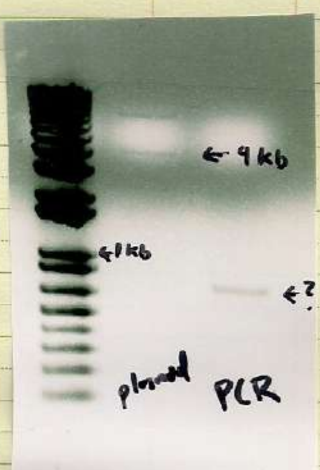
VECTOR
- I heat inactivated HindIII (80°C for 20 min)
- Add ~~0.5~~ 0.5 λ CIP, incubate @ 37°C → 12:20

- Heat kill @ 80° for 20 min (3 - 3:20)
- PCR cleanup on vector & PCR digest

Nanodrop (~3:20)

PLASMID: 5 ng/µL, 5.3 ng/µL → ~5 ng/µL

PCR: 91.3 ng/µL, 92.8 ng/µL → ~90 ng/µL



The ~~plasmid~~ PCR seems to be running closer to 700 bp – it should be 1 kb. (compare to p. 109).

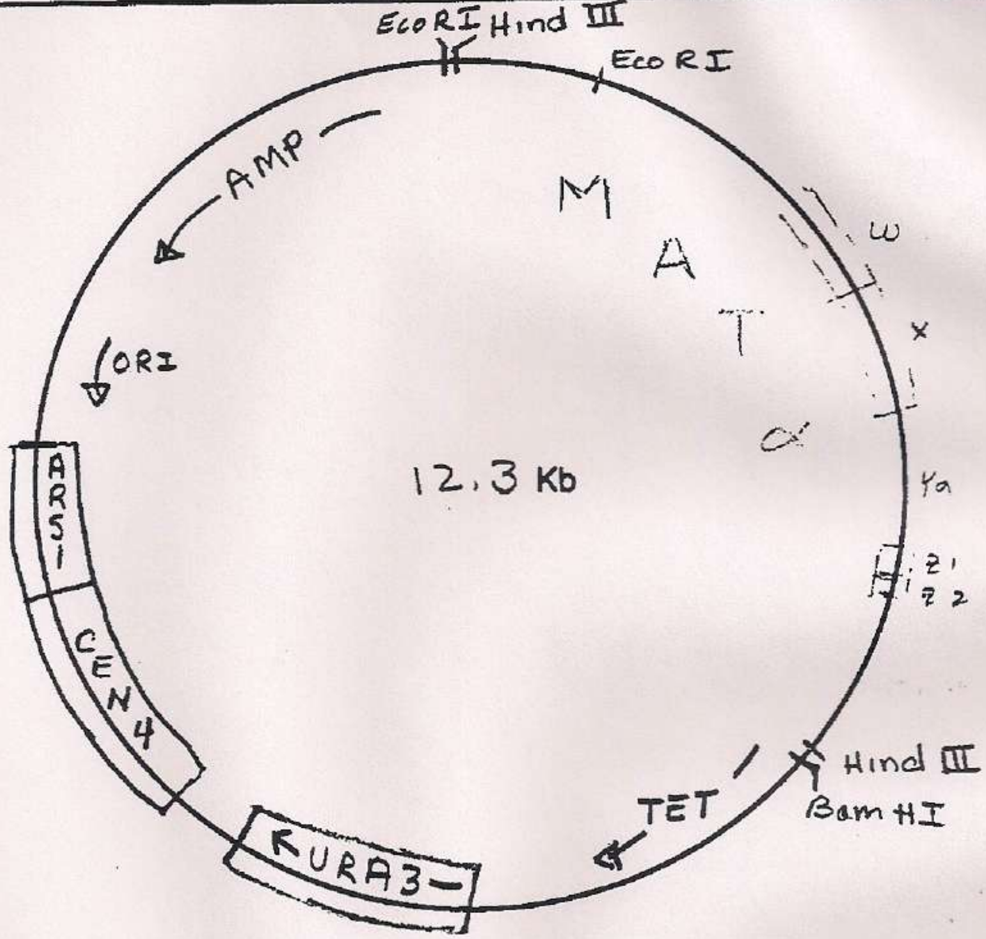
The plasmid looks good.

Perhaps there is an internal ~~Hd~~ HindIII site that is not in my sequence.

BROACH LAB PLASMIDS

Department of Biology, Princeton University, Princeton NJ 08544 -1014
Phone#- 609-452-5987

Yeast genes	Bacterial genes and/or other DNA	Vector	Plasmid name
Matα URA3 CEN4, ARS1	AMP, TET	YCp 50	pKSW26
		Host strain	**Strain #**
		MC1066	B1311



Comments and Notes: The Matα HindIII fragment was cloned from LCIII 3a 2α6 (B1296) into YCp50.

Source: Karen Weiler

Enterer: KSW **Date:** 12/20/89

>YCp50

Hind III

```
gaattctcatgtttgacagcttatcatcgatAAGCTTCTTGCAGTAGTATTGAACAGTGTATATCTTCATATTGCTTTTGTT
GTAAATATTGCGCAAAAGCGACTATCTTTGTGATTTGGTTGAACTTTTGCATATTCACGTTATTACCATCTTTAGTGAATGTA
TCGTTTCCGTCTCTTATGAAGGTAATGTCCCTGATAAGCAGTGATGTAAAAGGTACACAAGGAAGCTGACTGTGGAAAATATG
TTTAATTGTTCTTCTATAAACGTTGTAGTTGTTGTTTGGATGTACGACGACTTTTAGGCGCTGAAAAAGGTGGTCTGATTTAA
CATCTATCGGGAGAGATAGTCTTTCTATTGAATGATTTTGCAATGATGTAATGATTGAAGCAAGTGAGTTTAAGTTTCGTAAG
TATAGACAAGACAGTGCAACTTGTAACCAGTAGGAAATGGTGTGCGTTTGCTGCAAAGTTGTCTCAAGAACGTAGGACGATAA
CTGGTTGGAAAGCGTAAACACGGAGTCAATTGTTGTATCGTTGTGTTTGAAATGTCTTGTGAATTCTATTGTTTCAATATCCA
AGTAAAGAGAAGATTCTAAGAGAGTCAATGTCTTTGCAAGTGACCATGGAGATACGTTTAGAGCGAAGGCTTCGACTTCTAGA
AGTTCACCCTGTTTCCATTGGAAAAGTAAATCACTGAGGTCAGTTGCACCGCACAATTCATCATTTGCGTTCGTTTTGTCTAT
CTCGTATTGGAGAAATAAGTTTTCGTAGTCCAGGGGCGGTTTATTTTCTTCGATTTCCTTATCAGTTAGACCAATGTAATGAA   overlap
TCTTCAATTGCCTCGTTCTTTTTTTGTTTATAAACAGCTGAACTATGTCTGCAAACAGTTCTTGGTATTCATAATATTCAGCC   of MATα2
AAGTGACTGTACCAAAAATTGAGGATATCTTTTAATGTTGTCTCACTATCTTGCCAATAAGACTCTACCCAGATTTGTATTAG   & BUD5
ACGAGGGACGGAGTGATTTTTGTGTTTGTTTTTATTAATTGTGGGATAGGATAGTAGCAACTCTTGGAGGAGAGCATTGTCAG
TTGTCCAGTCTCTGAAGTTAAGTAGTAAGTTTGCGGAGTCAAAGGGGGATGGCTTTTGCCATTTGTGAGAGTTGTGCGGCAGC
ATCTTATTCAAATAGAGCTGTATTCTGAAGACCTCTTGTAGAACATCATCCATACTAAAAAGTAAATCGTCCTGTCCCATTAC
GAGCTGTATTAGTGCTGTGACCCTCTGTATATTTACGTTGCCATGAAGAAGGTAATGGGCGATATTTTGATACAATTCCTGAG
TTGCATGTTGGATTGAGTTTACGAAGGGTCGCCAGACGGCCAGAAACCTCCAGGCGGAGTTAACAACTAGTAATACGGCATCC
ATGTTTGCATCAGCGCCGAGCCTATACCAGTCACTGAGTAGACGTTTTCTTGCTCTTTTTATGTCCTGACTTCTTTTGACGAG
GGGGCATTCTCTAGAGACACAGGCAGTTGCTTCCAGCAACTGCCGTACGGCCGTTCTATGCTGTCGAGGATTTTTTTTGGGA
CGATATTGTCATTATAGGGCAGTGTGTGACTTATGAATTGTTGTAGAAGGACGTCTGTGATGTTGGAGATATGTATTTGTTA   Forward
ACTCTTCTTGAGACGATTTGGCCCTGGATAGCGAAGCGTGCGGTTACAAATAGGTCGTCTTGTTCAAGAAGGTAGGCGAGGAC   Primer
ATTATCTATCAGTACAAACATCTTAGTAGTGTCTGAGGAGAGGGTTGATTGTTTATGTATTTTTGCGAAATATATATATATAT   Binding Site
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CATCCGCCGATTTATTTTTCTGTGTAAGTTGATAATTACTTCTATCGTTTTCTATGCTGCGCATTTCTTTGAGTAATACAGTA   ← MATα2
ATGGTAGTAGTGAGTTGAGATGTTGTTTGCAACAACTTCTTCTCCTCATCACTAATCTTACGGTTTTTGTTGGCCCTAGATAA
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CATCATATGAAACAACTTAAACTCTTAACTACTTCTTTTAACCTTCACTTTTTATGAAATGTATCAACCATATATAATAACTT   ← Reverse
AATAGACGACATTCACAATATGTTTACTTCGAAGCCTGCTTTCAAAATTAAGAACAAAGCATCCAAATCATACAGAAACACAG   Primer
CGGTTTCAAAAAAGCTGAAAGAAAAACGTCTAGCTGAGCATGTGAGGCCAAGCTGCTTCAATATTATTCGACCACTCAAGAAA   Binding Site
GATATCCAGATTCCTGTTCCTTCCTCTCGATTTTTAAATAAAATCCAAATTCACAGGATAGCGTCTGGAAGTCAAAATACTCA
GTTTCGACAGTTCAATAAGACATCTATAAAATCTTCAAAGAAATATTTAAACTCATTTATGGCTTTTAGAGCATATTACTCAC   ← MATα1
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                 Hind III
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```

12/23/14

LIGATION OF MATα2 INTO YCp50 & TRANSFORMATION

Even though the purified plasmid & PCR look less than ideal (p. 110) I will move ahead and do the ligation & transformation.

	conc (ng/μl)	size (bp)	conc nM	μl for ligation
PLASMID	5	12325	0.66	10
PCR	90	1042	140	2

→ molar ratio: ~40:1 insert:vector

LIGATION

- 5 λ H_2O
- 2 λ 10× T4 ligase buffer
- 10 λ plasmid cut w/ HindIII
- 2 λ MATα2 PCR cut w/ HindIII (or H_2O for control)
- 1 λ T4 DNA ligase

20 λ

- ligate for 20 min @ RT; heat kill ligase @ 65°C for 10 min, ~~or~~ put on ice.
- ~~Heat shock~~
- add 2 λ ligation
- incubate on ice for 30 min
- heat shock 30s @ 45° (I meant to do 42)
- add 1 mL LB
- incubate 30 min @ 37°
- spin down.
- resuspend in ~~100~~ 150 λ LB
- plate on LB Amp.

12/24/14

- I'm not sure it worked. 51 colonies on the transformation & 38 on the empty vector control.
- Tomorrow I will pick 20 colonies and inoculate 4 mL of LB Amp.
- Parafilm plates and put in the fridge.

12/26/14

MINIPREP PUTATIVE PLASMIDS

· I miniprepped the 20 ~~plasmid~~ bacterial colonies from yesterday (labeled A→T) → elute 30λ EB

· Digest 5λ of each w/ HindIII

80.3 λ H_2O
22 λ 10x Buffer E
2.2 λ BSA
5.5 λ HindIII
110 λ → 5λ + 5λ miniprep

EXPECTED INSERT: ~ 1 kb

CORRECT PLASMIDS: A, B, C, G, H, I, J, K, M, N, O, P, R, T

EMPTY VECTOR: D, E, F, Q, S

I don't know what L is? → contaminant?

pGIL069 is the original (~4kb) insert.

→ I don't know from this digest the orientation (though it shouldn't matter). I will freeze down 4 (A, B, C, & G) as pGIL071-074.

12/29/14

TESTING PLASMIDS IN YEAST

· On Friday (12/26) I struck out yGIL793 from the freezer → this is the BY ancestor crossed to the bulk segregant MATa strain and then converted to MAT a/α.

→ In retrospect this may not have been the best ~~choice~~ choice since the pGIL069 transformants of this strain sporulated poorly (p. 105). But I did confirm that ~~they are MATa/α (p.~~ yGIL793 is MATa/α (p. 108).

· Last night I started o/n cultures in YPD of yGIL793.

· This morning I diluted 1:50 (200λ into 10mL YPD) → 9:20am

I would like to transform today and start sporulations on Thursday. As a control for sporulation I struck yGIL859 to -ura (this is a pGIL069 containing strain, though one derived from ~~yGIL~~ yGIL794, not 793).

Start transformations @ 1:45pm (same protocol as p. 55)

12/31/14

Thousands of colonies on -ura plates.

→ I picked two each and inoculated 5mL YPD. I also inoculated 5mL of the positive control (yGIL859) and the negative control yGIL794 → 10am.

1/1/2015

Spin down 1mL of the saturated cultures from yesterday.

Resuspend in 2mL SPO++ → on RT rollerdrum @ 11am.

1/12/15

The new plasmids work. No tetrads in negative control (yGIL793). Lots of tetrads in the positive control (yGIL859) and the 8 yGIL793s transformed with plasmids pGIL071-074. → All four plasmids are good.

1/20/15 Sporulation Efficiency of pGIL071-074

pos. control (yGIL859): 107/~150 = ~71%

pGIL071	A: 133/~175 = ~76%	
	B: 96/~125 = ~77%	
pGIL072	A: 100/~125 = ~80%	
	B: 131/~175 = ~75%	
pGIL073	A: 116/~150 = ~77%	
	B: 89/~120 = ~74%	
pGIL074	A: 89/~120 = ~74%	
	B: 62/~80 = ~77%	

1/20/15 Tetrad Dissections of yGIL~~739~~ 793 + pGIL071 & yGIL859

Protocol: 48.5λ H_2O

1.5 λ Zymolyase (150 mg/mL)

5 λ 10^8 Culture

10 mins @ 37°c + 150 λ cold H_2O → put on ice.

- Got 9 tetrads each
- Struck out the remaining 13 Mat a/a strains (all but 793) → pg. 105 to YPD from the freezer to be transformed with pGIL071
- Started ~~Y~~ SD-ura 5mL O/N of 793+pGIL071 A to freeze down tomorrow.

1/21/15

- Froze down yGIL793 + pGIL071 — yGIL871, yGIL872
- Made Bulk segregant media (pg. 78) (7 big, 2 little plates) to perform the Bulk seg. protocol on the sporulated 739 + pGIL071 (now 'yGIL871') tomorrow.

1/22/15

- We forgot that the B.S. protocol calls for 20mL culture, so I struck yGIL871 to -ura from the freezer so we can re-do the sporulation
- I patched the the tetrads from 1/20/15 to YPD to mating type test tomorrow
  - ↳ started YPD O/N of yGIL354 (a) + 355 (α) from freezer
- I transformed 12 of the 13 Mat a/a strains with pGIL071 (protocol on pg. 55) and plated to -ura
  - 1:50 dilution @ 9:50 AM, began at 2PM

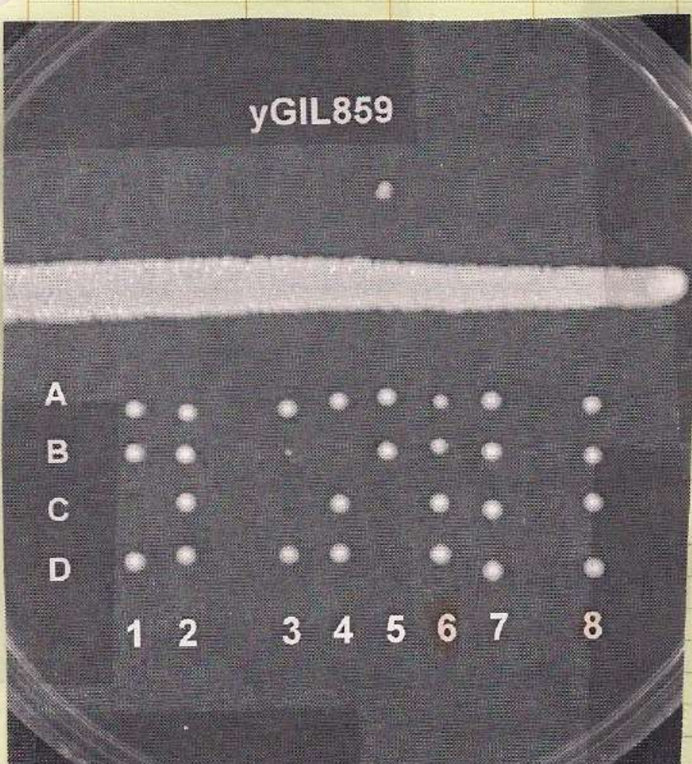
- we did not transform yGIL 787 because the colonies that grew were very small and the YPD O/N culture was barely saturated
    - This could mean something is wrong w/ yGIL 787 ∴ I poured YPD plates so we can streak 787 & 788 (its duplicate) and see if they both grow small.

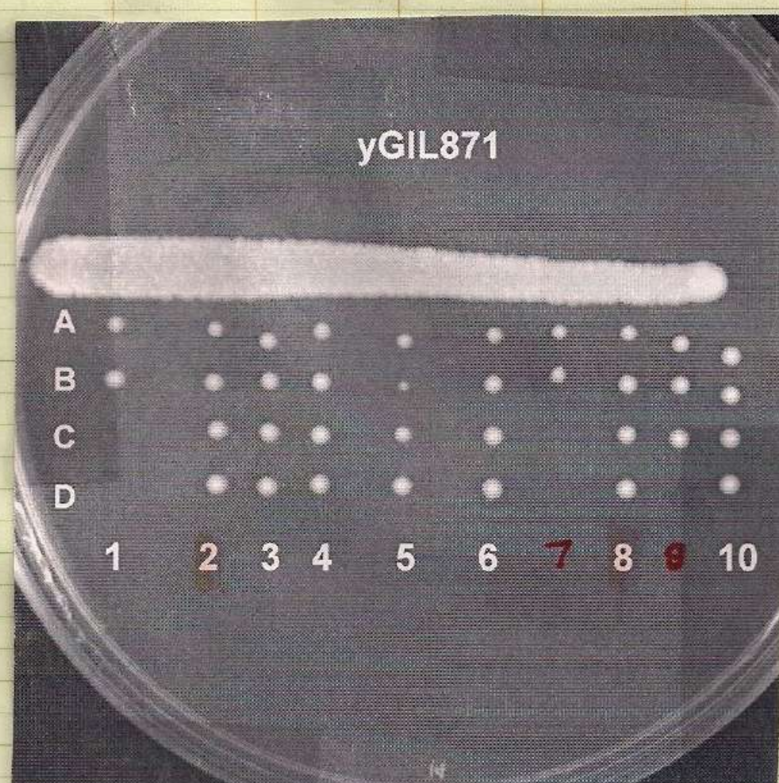
1/23/15

- Replica plated the patched yGIL 871 & 859 tetrads to -ura, +5FOA, +Mat a, +Mat α in that order.

- Struck out yGIL 787 & 788 from the freezer.

1/24/15 Tetrad Replicaplating Results





* Tetrads 7 & 9 from yGIL871 were mess ups, could have >1 tetrad growing in any that grew.

yGIL859							
		Mate as				-URA	+5FOA
	Notes	MATa	MATalpha	Non-mater	alpha Mutants		
1A		X					X
1B		X					X
1C							
1D		X			X 1	X	X
2A		X					X
2B		X					X
2C		X				X	X
2D		X			X 4	X	X
3A		X				X	X
3B	small	X				X	X
3C							
3D		X			X 1		X
4A		X			X 2	X	X
4B							
4C		X			X 1		X
4D		X			X 2	X	X
5A		X			X 1	X	X
5B		X					X
5C							
5D							
6A		X					X
6B		X			X 1	X	X
6C		X			X 2	X	X
6D		X					X
7A		X				X	X
7B		X					X
7C		X				X	X
7D		X			X 1		X
8A		X			X 6	X	X
8B		X					X
8C		X			X 1	X	X
8D		X			X 1		X

Total
α Mutants: 24

yGIL871

	Notes	Mate as				-URA	+5FOA
		MATa	MATalpha	Non-mater	alpha Mutants		
1A		X			0		X
1B		X			0	X	X
1C							
1D							
2A		X			0	X	X
2B		X			0	X	X
2C		X			1		X
2D		X			1		X
3A		X			0	X	X
3B		X			0		X
3C		X			0		X
3D		X			0	X	X
4A		X			1	X	X
4B		X			0	X	X
4C		X			0		X
4D		X			0		X
5A		X			0	X	X
5B	small	X			0	X	X
5C		X			0		X
5D		X			0		X
6A		X			0		X
6B		X			0	X	X
6C		X			0	X	X
6D		X			0		X
7A		X			0	X	X
7B		X			1	X	X
7C							
7D							
8A		X			1		X
8B		X			0	X	X
8C		X			0	X	X
8D		X			0		X
9A		X			0	X	X
9B		X			0	X	X
9C		X			0		X
9D							
10A		X			0	X	X
10B		X			0	X	X
10C		X			0		X
10D		X			1		X

Total
α Mutants: 6

1/24/15 pGIL071 Transformants

- I started 5mL SD-ura O/N of 2 random colonies each from the 12 strains transformed w/ pGIL071 on 1/22/15
- I started a 10mL YPD O/N of yGIL871 to begin 20mL Sporulation tomorrow.
- yGIL787 & 788 both look very small → something may be wrong with them?

1/26/15

- Froze down the 12 pGIL071 Transformants in duplicate:

yGIL753 + pGIL071	yGIL876 yGIL877
yGIL755 + pGIL071	yGIL878 yGIL879
yGIL756 + " "	yGIL880 yGIL881
yGIL757 + " "	yGIL882 yGIL883
yGIL762 + " "	yGIL884 yGIL885
yGIL789 + " "	yGIL886 yGIL887
yGIL791 + " "	yGIL888 yGIL889
yGIL795 + " "	yGIL890 yGIL891
yGIL806 + " "	yGIL892 yGIL893
yGIL808 + " "	yGIL894 yGIL895
yGIL821 + " "	yGIL896 yGIL897

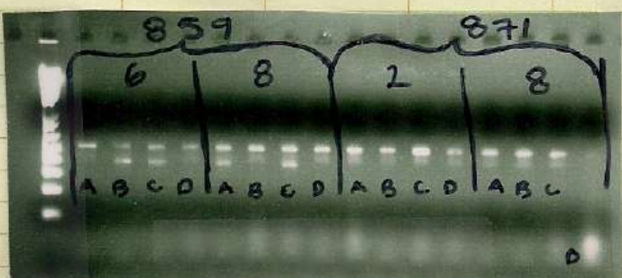
yGIL857 + pGIL071 —— yGIL898
yGIL899

- Observed Mat α mutants on both 859 & 871 ∴ kept in incubater to make sure which do/do not create mutants.
- Began 20mL sporulation of yGIL871 (No H_2O wash after 10mL YPD O/N & spindown).

1/27/15 Genotyping the MAT locus

- Chose tetrads 6 & 8 from yGIL859 & 2 & 8 from yGIL871 to PCR at the MAT locus (did not do YPD O/N)
- did Genomic preps & followed PCR set up on pg. 107 with the following thermal cycler settings: 5' 94° initial denature 30 x (1' 94°, 1' 54°, 1' 72°) and 10' ~~72~~ 72° final extension

1/29/15



859: 6B, 6C } retained the plasmid AND showed growth on -URA (expected ! :))

8A, 8C } same as 6B & C

BUT 8D showed α Mutants w/ no evidence of retaining the plasmid.

871 2: None appeared to retain the plasmid by the PCR results while C & D allowed for α mutants w/ out growth on -Ura, and A & B did the opposite.

8: only 8A allowed for α mutants & PCR results show none retained the plasmid, while 8B & C grew on -ura.

1/29/15 Bulk Segregant Protocol (yGIL871)

- Began 20 mL Sporulation on 1/26/15 (~57/75 ≈ 76%)
- 20mL culture spin down
- resuspend in 200λ H_2O (ends up @~750λ)
- Add 5λ 1000x Zymolyase
- incubate @ 30° for ~~30 min~~ 1 hr → Check: still pretty stuck together
- Add 50λ glass beads
  50λ 10% Triton X-100
- Vortex for 2 mins → Check: comparable to above.
- Incubate at 30° for 40 min → Check: Needs 2min Vortex

~~- Bring up to 5mL w/ H_2O + sonicate at power 4 for 4sec.~~
~~Check:~~

- Needed another 40 min 30° incubation after 2nd 2min vortex
- Bring up to 5mL w/ 4.25mL H_2O + sonicate at power 4 for 4 seconds → Check: still tetrads, but much better → move forward
- Take 500λ of sonicated culture into 5mL YPD for 1.5 hr hold. (in at 1:10 PM) @ 30° RD
- Spun down all 5mL, but realized that's not what we did last time so I vortexed and plated 500λ to Big BS media plate

2/4/15 Bulk Segregant Results

- Only 2 colonies have grown → even if we plated 100% of the 5mL YPD hol we would have only gotten 20 ∴
  1) something wrong with the media?
  2) YPD hold wasn't long enough?
  3) Tetrads were not broken up enough?
- plated the rest of the yGIL871 YPD hold (now 6 days) just to see what happens (to BSP media)
- ~~Started~~ Streaked yGIL871 to -ura from the freezer to re-do entire protocol.
- Started YPD O/N of YGIL789 to transform with pGIL071 tomorrow

2/6/15

- Yesterday I transformed YGIL788 w/ pGIL071
- The 6-day YPD hold BS. plated on 2/4/15 showed a full lawn of growth
  ↳ ∴ maybe the YPD hold was the only issue? → will test next round
  ↳ will scrape some cells and plate to YPD to check singles for Mat a by αF
- Started YPD O/N of yGIL871 to start 20mL sporulation tomorrow

2/7/15

- Started 20mL sporulation of yGIL871
- picked 2 transformants of yGIL788 + pGIL071 for -ura O/N to freeze

2/9/15

- froze down yGIL 798 + pGIL071 → yGIL902, yGIL903
- Resuspended 48 singles from the 6-day YPD Hold yGIL871 BS. in YPD to mating type test tomorrow

* Started YPD O/N of 354(a) + 355 (α)

2/9/15

- Checked sporulation of yGIL871 : 47/~120 = ~40%

2/10/15 BULK Segregant Protocol (Pilot #2)

- sporulated 20mL yGIL871 (started on 2/7/15) efficiency: ~30/65 = ~46%
- 20mL culture spindown
- resuspend in 200λ H_2O (endsup as ~ 550λ ~~550λ~~)
- add 5λ 1000x Zymolyase
- Incubate at 30° for 1hr → Check: Still a good amount of tetrads
- Add 50λ Glass beads & 50λ 10% triton then Vortex 2min → less tetrads, but still very present.
- Incubate at 30° for 40min → Check: a bit less tetrads.
- 2min Vortex → Check: much better (~1-2% tetrads present).
- Bring up to 5mL w/ H_2O (about 4.5mL H_2O) and sonicate at power 4 for 4 seconds → Check: still tetrads, but very little. good to move on.
- Started 4 ~~3~~ YPD holds of 500λ into 5mL YPD (in at 1:30pm ~~1:25pm~~)
  1. 1.5hrs YPD hold (3pm) → all 5mL resuspended in 500λ YPD → plate
  2. 3hrs YPD hold (4:30pm) → " "
  3. 4.5hrs YPD hold (6pm) → " "

2/11/15

- Yesterday I pinned the 48 singles from the restreaked 6-Day YPD B.S. plate (2/9/15) to +Mata and +Mata to mating type test
- Today I replicaplated to SD

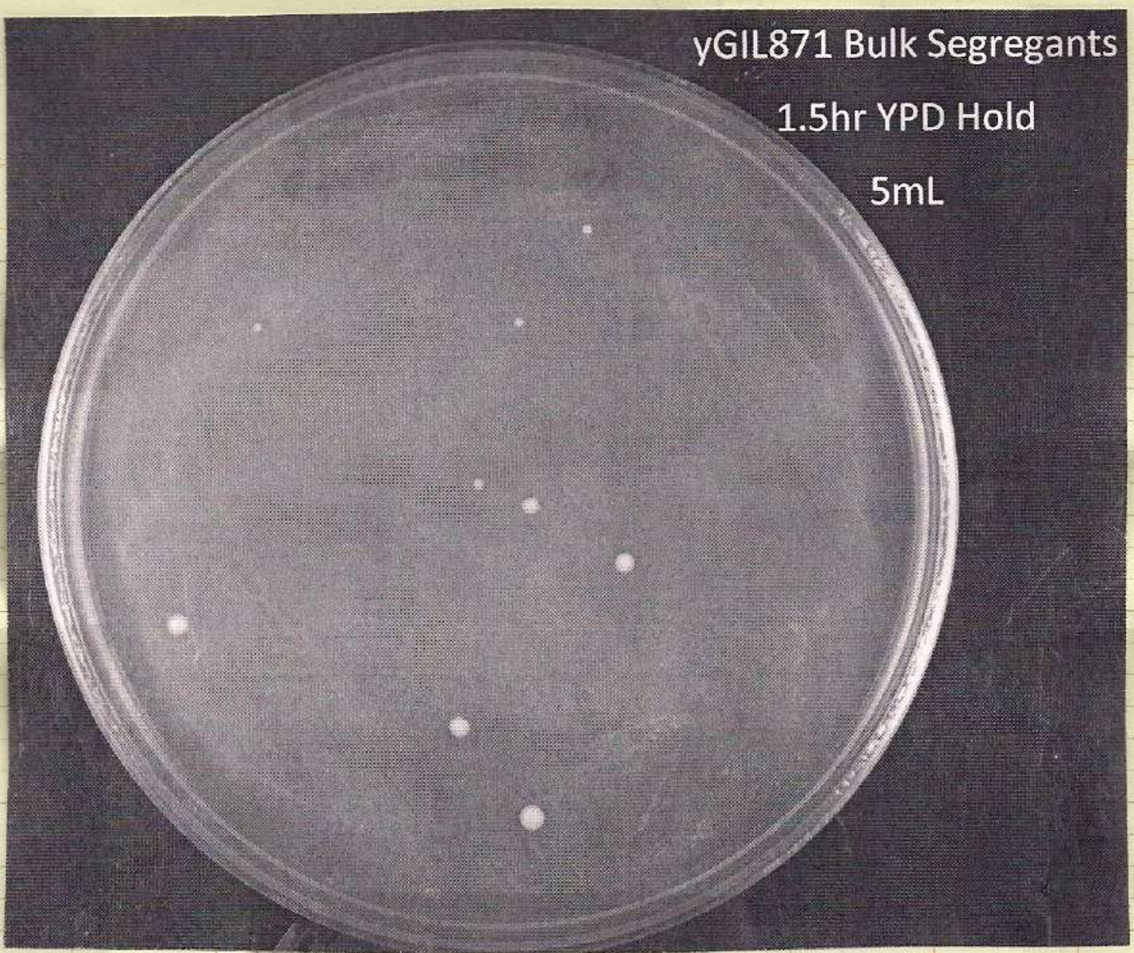
2/12/15

- All singles mated as Mata :) (<2% persist/convert to α)

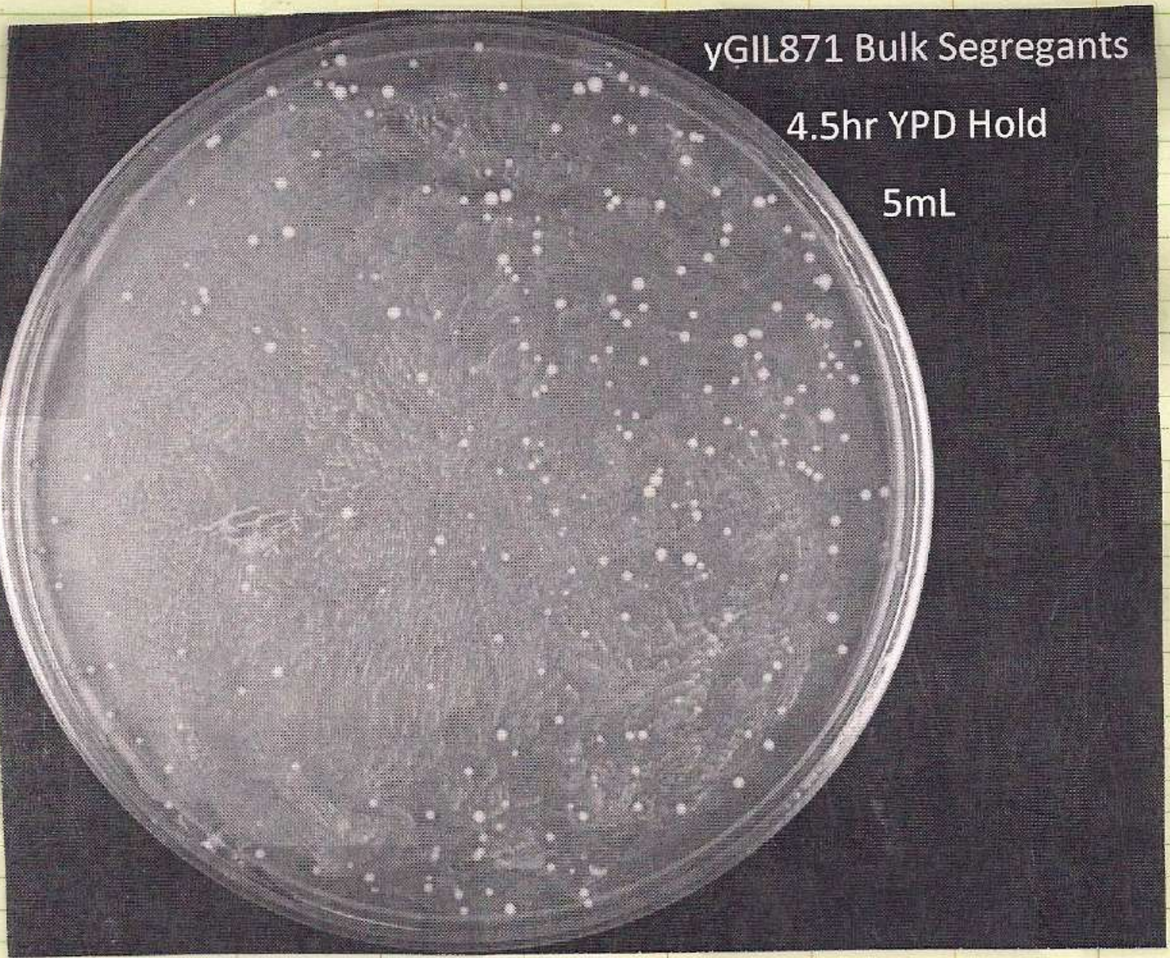
2/14/15

- Began sporulation of yGIL871 (20mL).

Plated: 2/10/15
Photographed: 2/16/15



9 colonies



303 colonies

Plated: 2/13/15
Photographed: 2/16/15

#of colonies on 1/8 of the plate: ~1,477

#of colonies on whole plate: ~11,816

2/16/15

- picked all 9 singles from 871 BS. 1.5hr YPD Hold and 87 singles from the 4.5hr Hold plates to be mating type tested when the testers grow up on 2/19

2/17/15

- Started 2 additional 20mL yGIL871 sporulations.
- Made a Bulk Segregant Pool of the 3day Hold plate.
    - Add 5mL YPD + spread
    - suck up as much as possible → ~2.5mL
    - Add another 5mL + re-spread
    - suck up as much as possible → ~3mL

    * End volume was ~5.5mL → next time add 5 and then 3.5 to try and get an even 5mL yeild.

128

- I struck 2λ of the BSP to YPD to mating type test.
- Froze down the following:

yGIL871 3-day Hold BSP — 001, 002

2/20/15
~~2/19/15~~

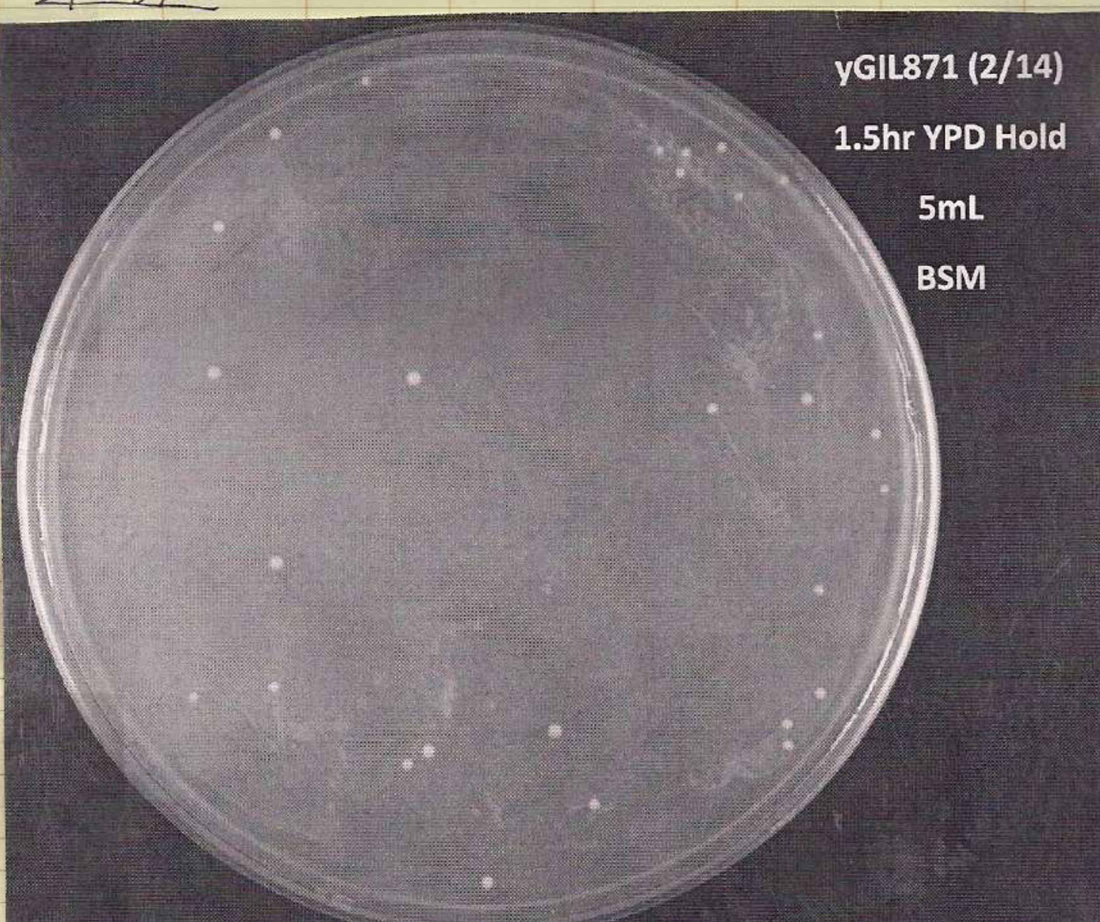
Bulk Segregant Protocol (pg. 126)

- 1st incubation check: many tetrads
- 1st vortex check: significantly less tetrads, but still a lot
- 2nd incubation & vortex check: very few tetrads (greg)
- Sonicate check: comparable to above.
- YPD Holds (4 each) went in at 3:50 pm.

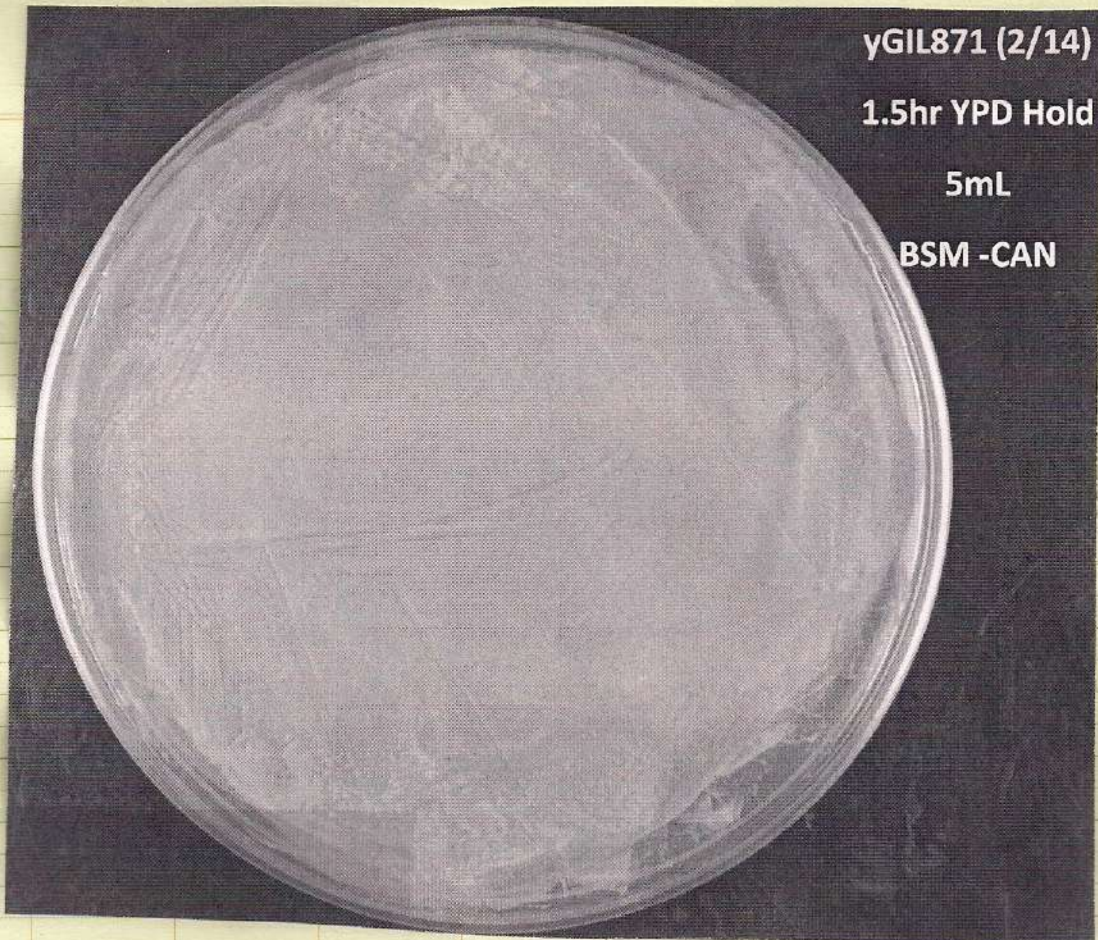
2/21/15

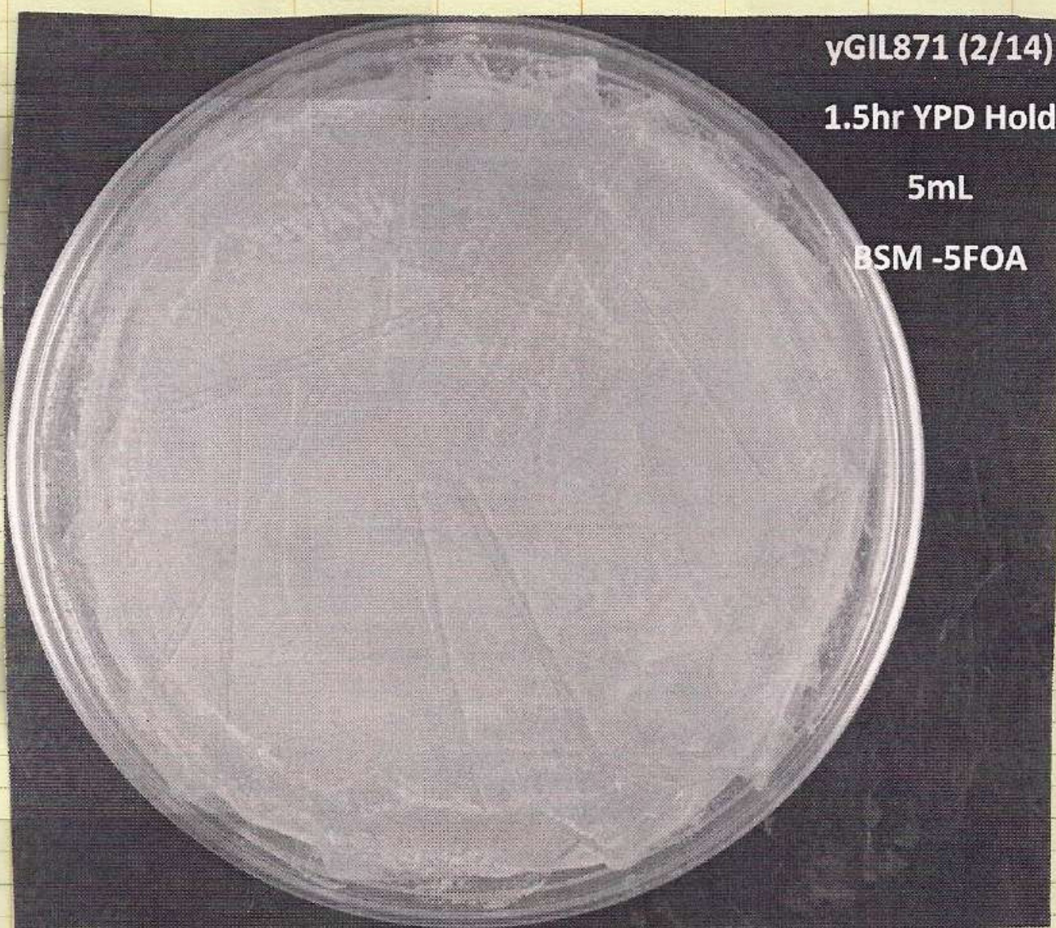
- plated the 24hr YPD hold to BSM, -CAN, & -5FOA (500λ)

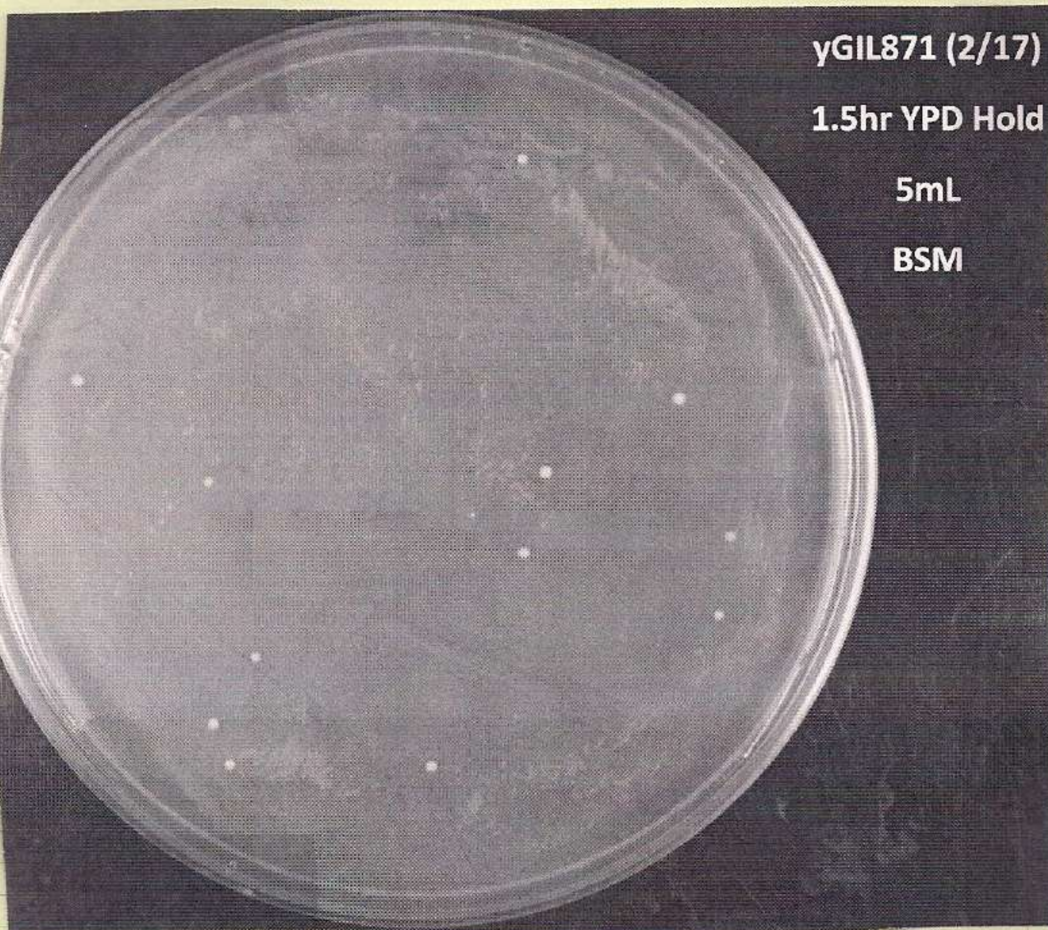
2/23/15

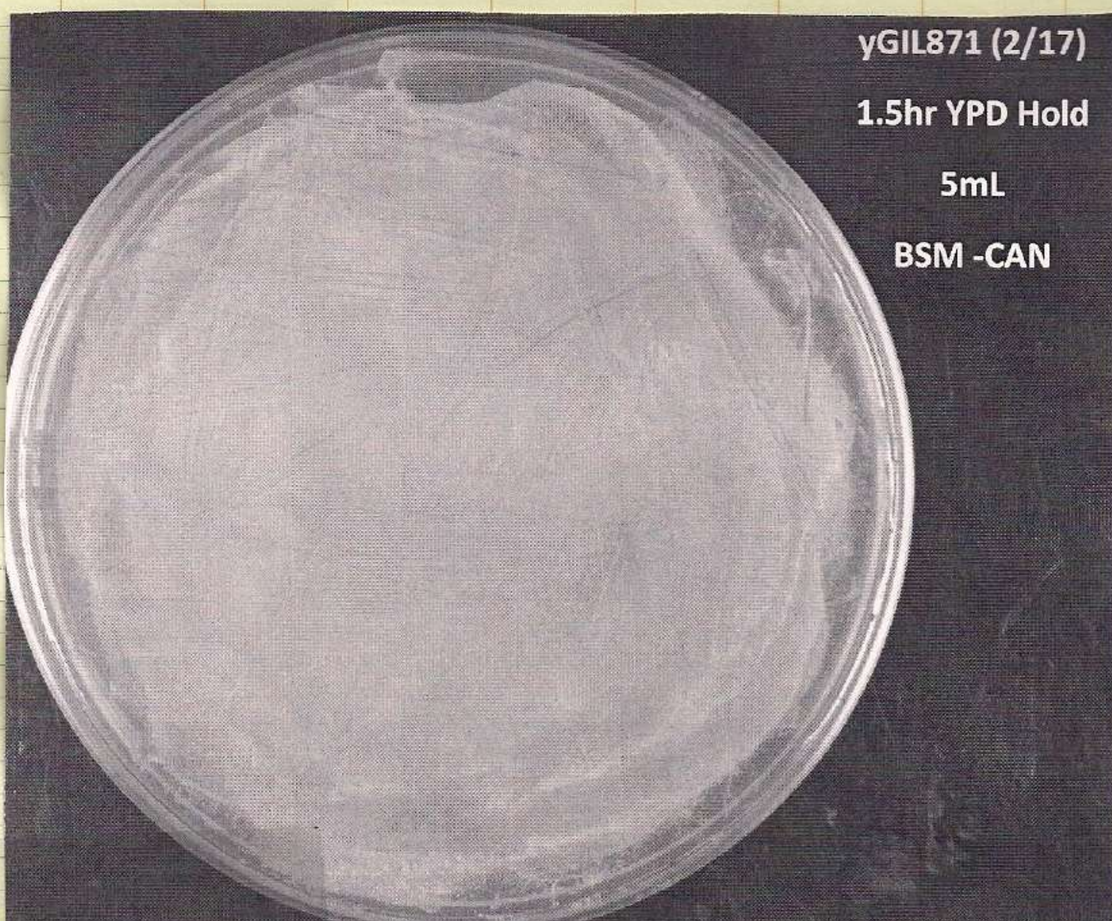


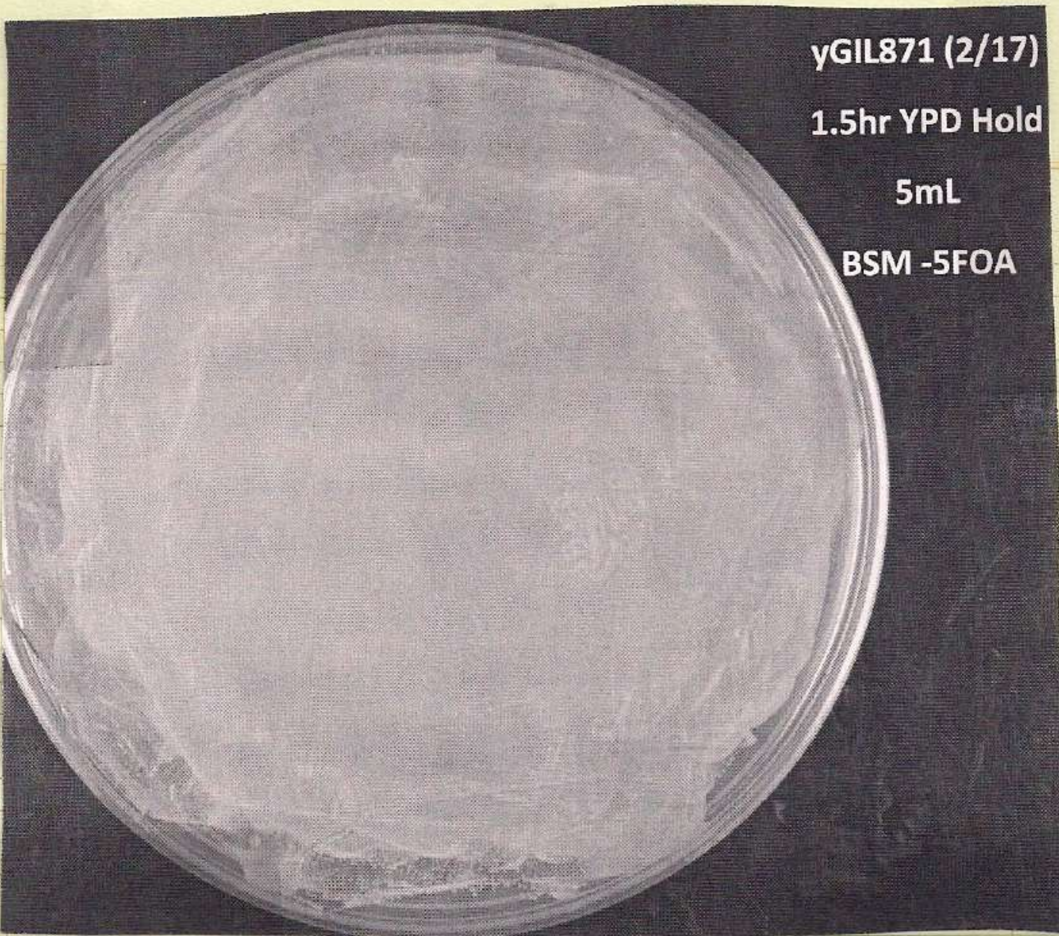
plated: 2/20/15
photographed: 2/23/15


yGIL871 (2/14)
1.5hr YPD Hold
5mL
BSM -CAN

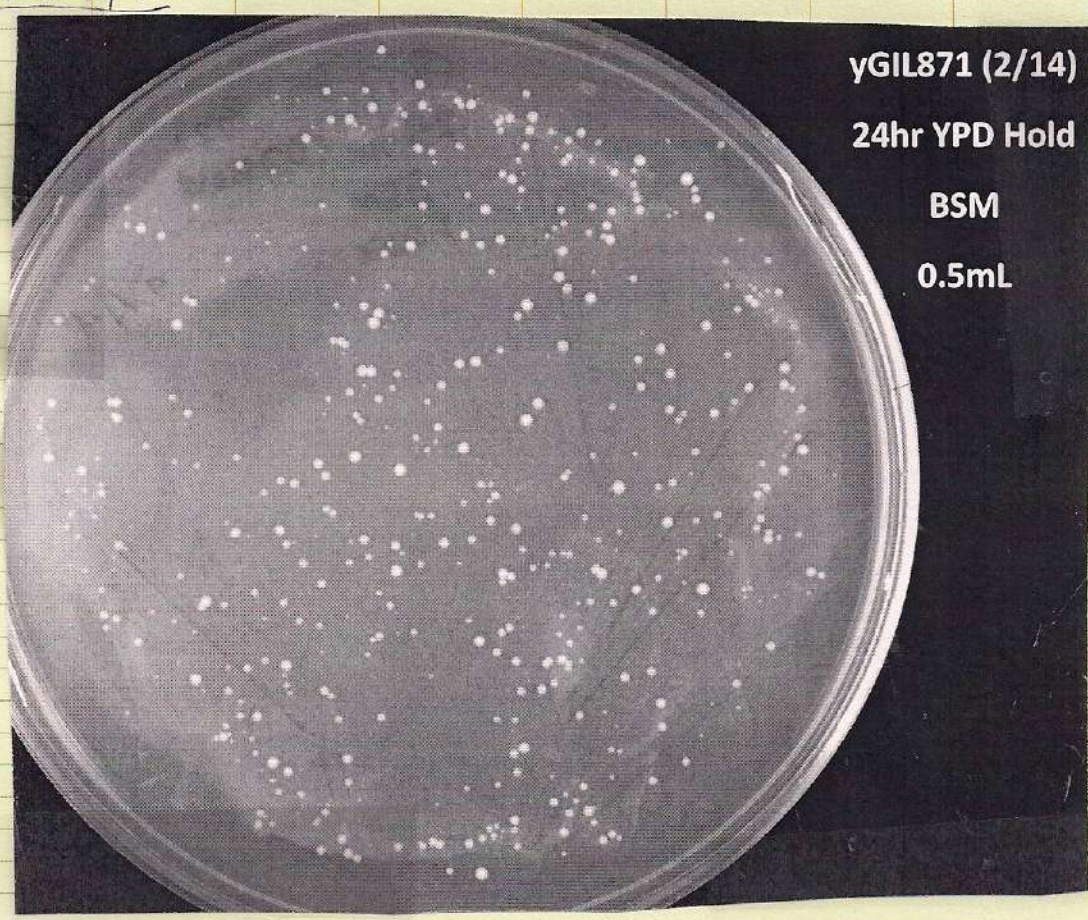

yGIL871 (2/14)
1.5hr YPD Hold
5mL
BSM -5FOA


yGIL871 (2/17)
1.5hr YPD Hold
5mL
BSM


yGIL871 (2/17)
1.5hr YPD Hold
5mL
BSM -CAN

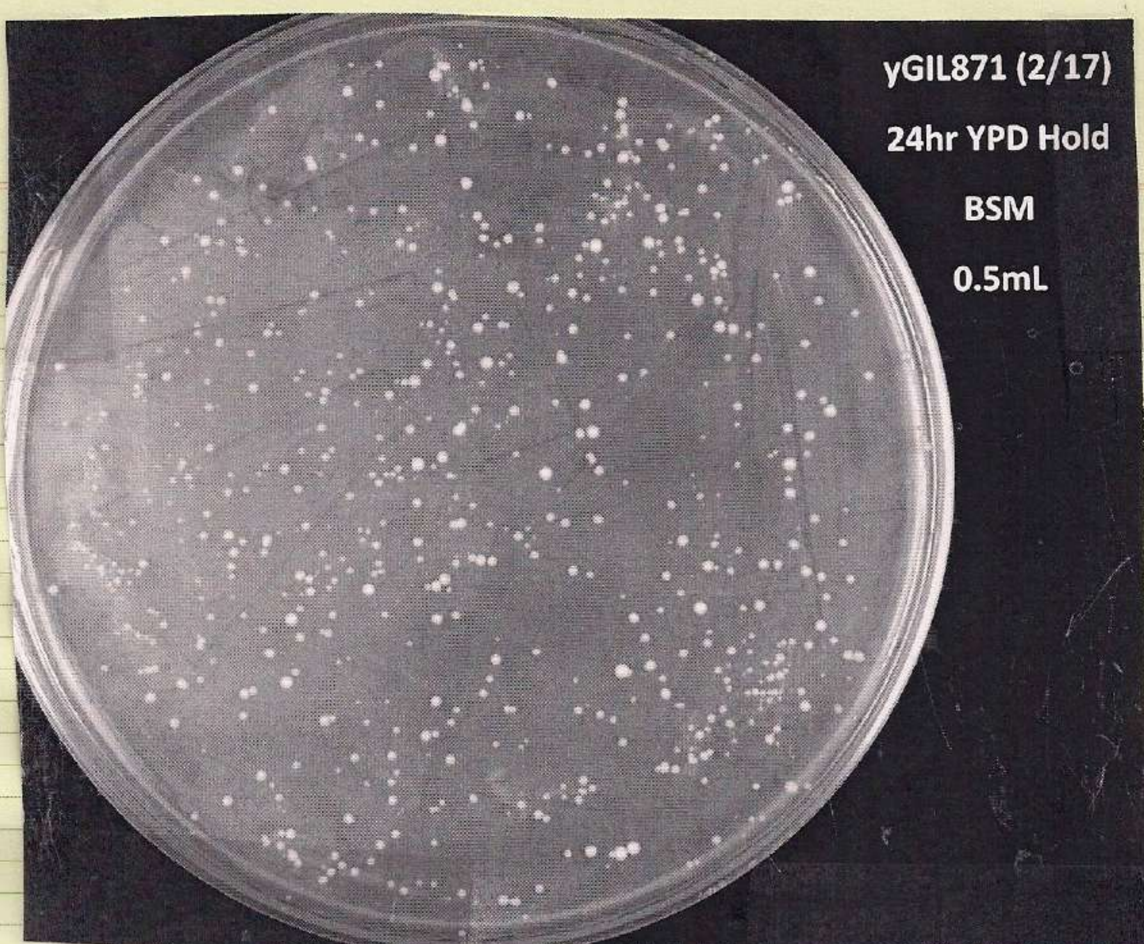


2/24/15



plated: 2/21/15
photo'd: 2/24/15

colonies
= 573



Colonies

= 328

2/24/15 (Final) BULK Segregant Protocol

- 20mL sporulation (98/~125 = ~78.4%) spindown
  ↳ Began on 2/17/15
- Resuspend in 200λ H_2O → Final volume = ~650λ
- Add 5λ Zymolyase
- Incubate at 30° for 1 hr ⇒ Check: practically no change from
- Add 50λ Glass Beads and 50λ 10% Triton
- Vortex 2 mins ⇒ Check: less than before, but still many tetrads.
- Incubate @ 30° for 40 min. ⇒ Check: way less tetrads :)
- vortex 2min ⇒ Check: ready for sonicate & YPD Hold.

~~- Sonicate at power 4 for~~

- Bring up to 5mL w/ H_2O (~4.4mL)
- Sonicate at power 4 for 4 seconds.

m

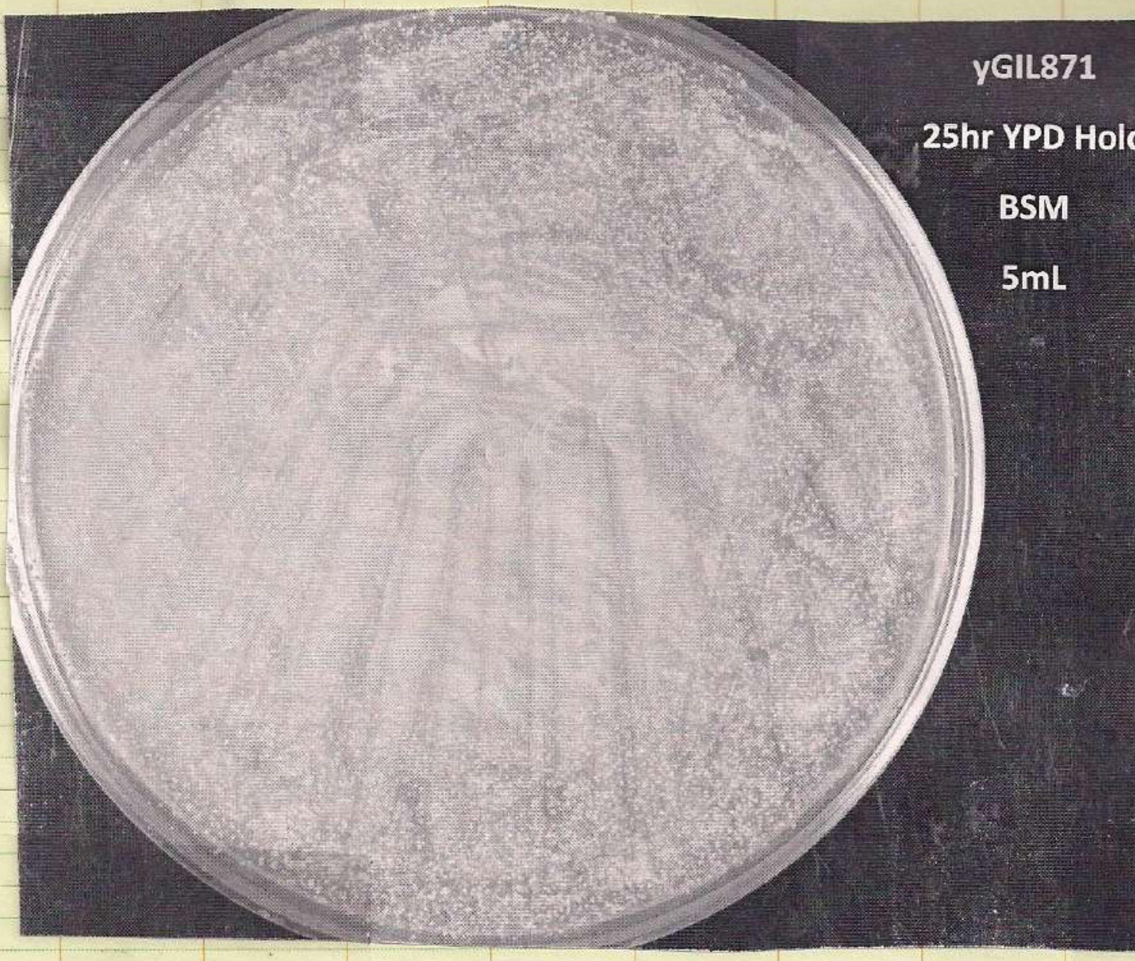
- 500x into 5mL YPD (started 4 of them) in @ 12:15pm.

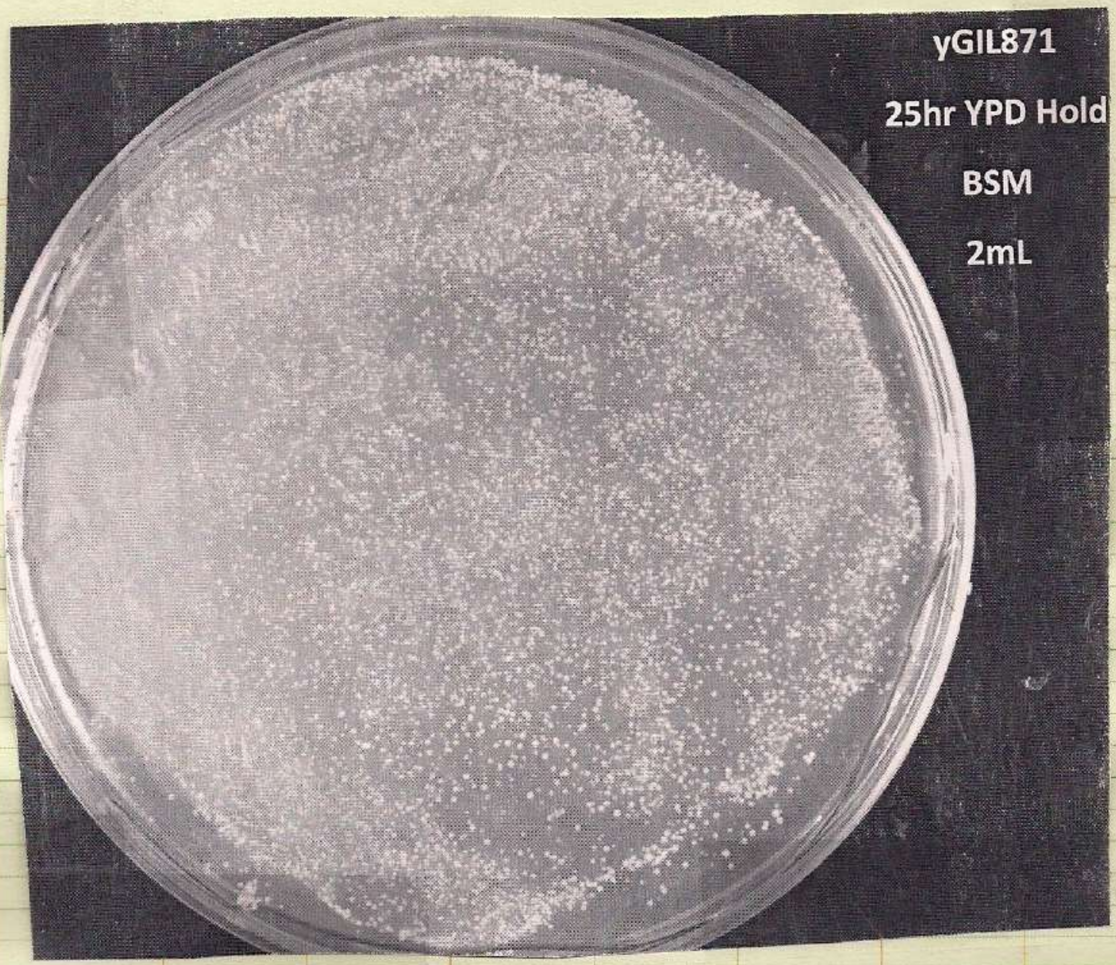
plated: 2/25/15

Photographed: 2/28/15

colonies ≈ 7,544


yGIL871
22hr YPD Hold
BSM
5mL


yGIL871
25hr YPD Hold
BSM
5mL

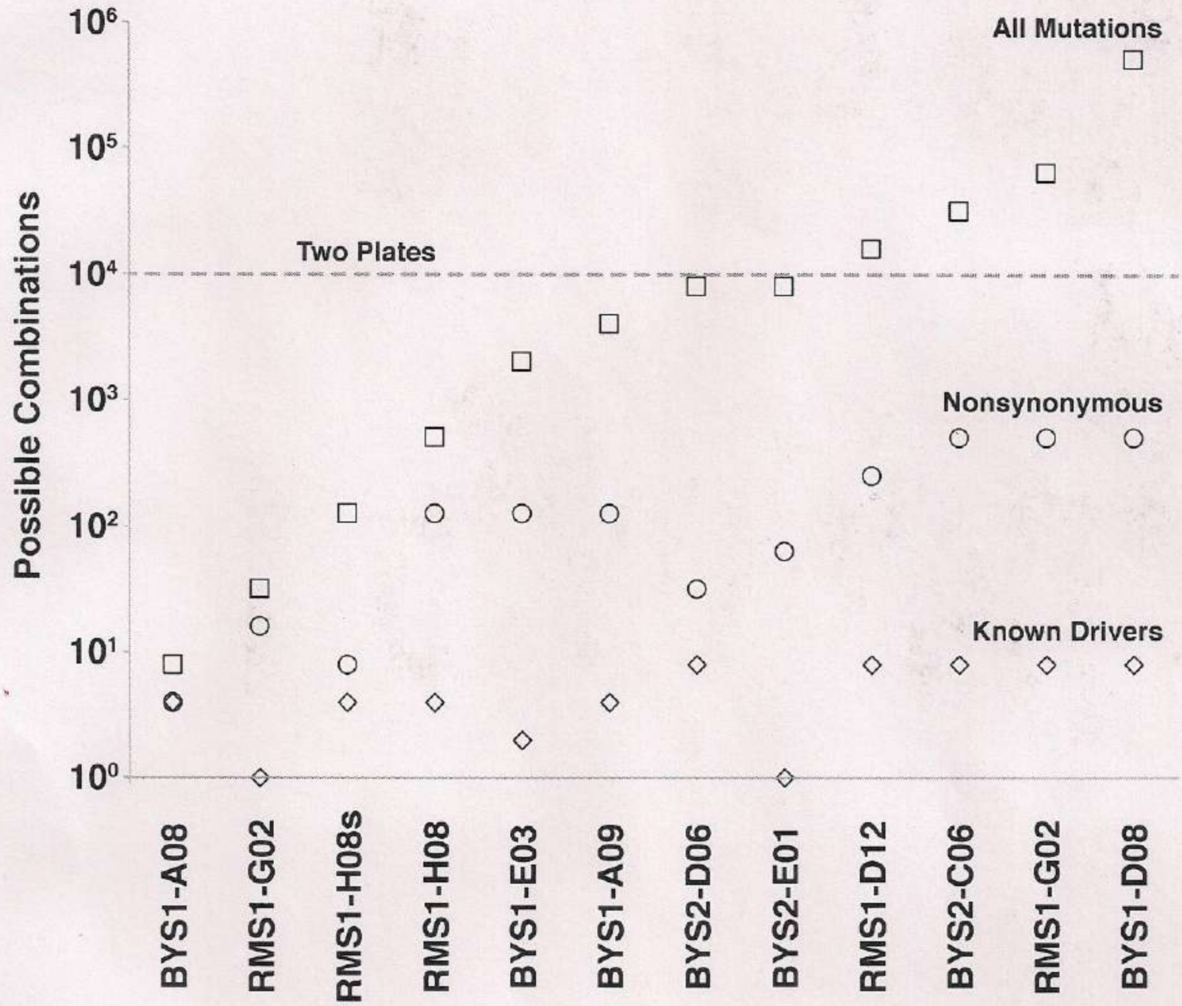


#colonies

~9,112

#colonies

~5,904



		BYS1-A08	RMS1-G02	RMS1-H08s	RMS1-H08	BYS1-E03	BYS1-A09	BYS2-D06	BYS2-E01	RMS1-D12	BYS2-C06	RMS1-G02	BYS1-D08
Number of Mutations	All Mutations	3	5	7	9	11	12	13	13	14	15	16	19
	Nonsynonymous	2	4	3	7	7	7	5	6	8	9	9	9
	Known Drivers	2	0	2	2	1	2	3	0	3	3	3	3
Pool Representation	All Mutations	1250	313	78	20	5	2	1	1	0.6	0.3	0.2	0.02
	Nonsynonymous	2500	625	1250	78	78	78	312	156	39	20	20	20
	Known Drivers	2500	10000	2500	5000	2500	2500	1250	10000	1250	1250	1250	1250

2/28/15

- picked 2 96 well plates of singles from the 25hr YPD hold, 1mL plate
- started YPD O/N of 354 (a) and 355 (α) to mating type test the singles

3/2/15 strain update

yGIL737 X ???			MAT a/α	MAT a/a	w/ pGIL071	
1	BYS1-D08-1000	yGIL681	yGIL739	yGIL755	yGIL878-879	
2	BYS2-D06-910	yGIL687	yGIL740	yGIL756	yGIL880-881	1
3	BYS1-A09-1000		yGIL741	yGIL762-763	yGIL884-885	
4	BYS2-E01-665		yGIL742-743	yGIL757	yGIL882-883	
5	RMS1-D12-910		yGIL744	yGIL753-754	yGIL876-877	
6	RMS1-G02-545		yGIL745	yGIL791-792	yGIL888-889	
7	BYS1-A08-545	yGIL683	yGIL768-770	yGIL795	yGIL890-891	2
8	BYS2-C06-1000	yGIL685	yGIL771-772	yGIL789-790	yGIL886-887	
9	RMS1-G02-825	yGIL689	yGIL783-784 (STE) yGIL826-827	yGIL857-858	yGIL898-899	
10	RMS1-H08-585 (STE)	yGIL692	yGIL785-786	yGIL808	yGIL894-895	3
11	RMS1-H08-585		yGIL764-765	yGIL787-788	yGIL902-903	
12	BYS1-E03-745		yGIL766-767	yGIL806-807	yGIL892-893	
13	RM Anc	yGIL429	yGIL749-750	yGIL821-823	yGIL896-897	
14	BY Anc	yGIL432	yGIL751-752	yGIL793-794	yGIL871-872	

Bulk Segregant Pool 003 (yGIL871)

- Add 5mL YPD ~~to~~ 25hr hold, 2mL plate (pretty cracked)
- suck up as much as possible (~2.2mL)
- Added ~2.5mL YPD → end volume: ~4mL.
- Added 1mL 75% glycerol → froze down 1mL in tube and the rest distributed into ~~a~~ 1 row of the BSP 96well plate

yGIL871 BSP ———— 003

3/3/15 BSI Mating Type Results

- all 192 singles mated as Mat a :) < 1/192 = 0.5% chance
- Froze down both BSI plates.

3/21/15

- On saturday 3/14/15 & sunday 3/15/15 I had Katie streak-out the first & second 4 strains to be used for the BSA (1 & 2)
  * Told her to streak to YPD by accident
- I started sporulations of 1 on Tuesday 3/17/15 and saw on Friday 3/20/15 that 3 out of 4 (880, 882, 884) were able to sporulate well while 878 didnot.
- I restarted sporulations of 879 and all of 2 on Friday 3/20
- Today I will run the BSA Protocol on 880, 882, 884 (1) as well as 876, 886, & 888 (2) → YPD hold in at 1:45pm
  * The 2 strains started sporulations on wednesday 3/18/15 ∴ had 1 less day of sporulation, but have reached ~75% efficiency.
  * 890 from 2 also sporulated well, but ~~tt~~ I will not use it today to make it even.
- Today I began sporulating 3 (which was properly struck to -ura)

3/24/15

*pulled @ 1:30pm → entire process: ~3hr
plating: ~2.5hr

- Sunday (3/22/15) I plated all 4 plates each of the 1st BSA run on 880, 882, 884, 876, 886, and 888 (plates were cloudy)
- Today I am running the 2nd BSA protocol round on 879, 890, 892, 894, 896, 898, and 902 that all had ~75% S.E.
  * 879 & 890 began sporulating on 3/20 while the rest began on 3/21

* in @ 2:15pm

BULK SEGREGANT PROTOCOL

Grow 10mL YPD overnight

Spin down and resuspend in 20mL SPO++ until ~75% sporulation efficiency is reached

Spin down and resuspend in 200λ H_2O (Final volume ~500-750λ)

Add 5λ zymolyase (1,000x, 150 mg/mL)

** Note tetrad prevalence after each of the following steps**

Incubate at 30°C for 1hr

Add 50λ glass beads and 50λ 10% Triton

Vortex for 2mins

Incubate at 30°C for 40mins

Vortex for 2mins

Bring up to 5mL with H_2O (~4-4.5mL H_2O needed)

Sonicate at power 4 for 4secs

Start two 5mL YPD holds (500λ sample into 5mL YPD)

After 24hrs plate 4 BSM plates: two 2mL (BSP's) and two 1mL (BSI's)

Grow for ~3 days and pick two 96well plates of singles from the 1mL plates and pool the 2mL plates:

> Spread 5mL YPD (collected volume ~2-3mL)
> Spread 2.5mL YPD (additional collected volume ~1-2mL)
> End volume ~4mL
> Add 1mL 75% Glycerol
> Freeze down in single row of 96well BSP plate and 1mL in cryo tube

BULK SEGREGANT MEDIA (BSM)

500mL SD agar
Add 0.4g CSM –arg and 0.5g 5FOA before autoclaving
Add 500λ ClonNat (100mg/mL) and 500λ CAN (60mg/mL) to melted agar

YIELD: ~7-8 Big plates

3/25/15

- pulled the YPD Hold @ 1:15pm (23 hr hold). and plated all 4 plates for each.
- put the fully grown plate from 3/22/15 into the fridge (parafilmed) to ~~pick~~ begin pick & pool tomorrow.

3/26/15

- picked 1 96-well plate of singles per 1mL plate of the first round (876, 880, 882, 884, 886, & 888)

3/27/15

- Made the BSP's of 876, 880, 882, 884, 886, & 888 from both 2mL plates each:
  - Add 3mL YPD to first plate → yield ~2mL
  - Add 2mL YPD to first plate & get all possible → final ~5mL
  - Take 3mL of the pool from first plate and add to second plate → yield 1mL (left in tube) + ~2mL
  - Add 2mL YPD to second plate → get ~1mL
  - Add 1mL YPD final to get anything left → Final ~5mL

- Froze down pools:

yGIL876 BSP	004
yGIL880 BSP	005
yGIL882 BSP	006
yGIL884 BSP	007
yGIL886 BSP	008
yGIL888 BSP	009

- Froze pools in BSP plate. (red dot means they were frozen down today - 3/27/15)

MM

3/28-29/15

- 3/28/15 I pulled the second round of plates and put them in the fridge
↳ (878, 890, 892, 894, 896, 898, + 902)

- I picked one 96-well plate each of BSI's from the 1mL plates

3/30/15

- I froze down all 26 BSI plates (2 per strain for all 13)

- Before freezing down I pinned to +5FOA, +CAN, + -ura and mating type tested:

• +5FOA growth on all plates tested (48 BSI'): 876 1, 880 1, 884 1, 888 1, and 892 1

• +CAN growth on all plates tested (48 BSI'): 876 1 + 2, 878 1, 880 1, 882 1 + 2, 884 1, 886 1, 888 1, 890 1, 892 1, 894 1 + 2, and 896 1

• -ura No growth on all plates tested (48 BSI'): 876 1, 878 1, 880 1, 882 1, 884 1, 886 1, 888 1, 890 1, 892 1, 894 1, and 896 1

3/31/15 • +Mat α growth AND +Mat a NO growth on all plates tested (48 BSI'): 876 1 + 2, 878 1, 880 1, 882 1 + 2, 884 1, 886 1, 888 1, 890 1, 892 1, 894 1 + 2, and 896 1

* While mating type testing 896 1 (→ BY ANC.) 1A-6H I double dipped the pins after +Mat α → should be okay, but it is marked on the lid just in case

3/31/15

- Froze down / made last seven pools:

yGIL878 BSP ——— 010

yGIL890 BSP ——— 011

yGIL892 BSP ——— 012

yGIL894 BSP —— 013

yGIL896 BSP —— 014

* yGIL898 BSP —— 015

yGIL902 BSP —— 016

* Accidently touched the second 2mL plate ∴ finished the pool w/ the two 1mL plates I picked from and put the touched 2mL plate back in fridge w/ note and marked this strain w/ a green dot on the BSP plate.

BSP ①:

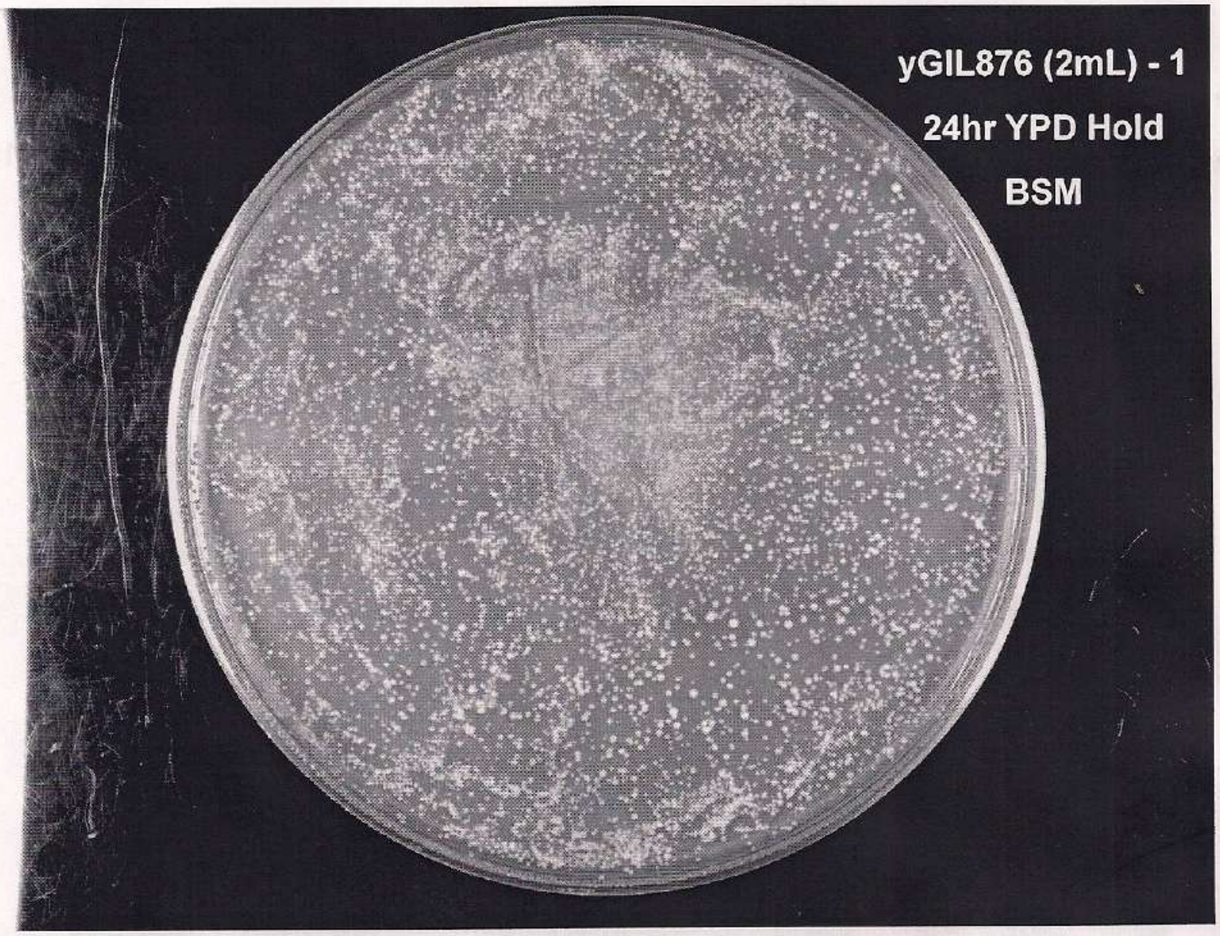
871 (BY Anc)	003
896 (RM Anc)	014
878 (BYS1-D08-1000)	010
880 (BYS2-D06-910)	005
884 (BYS1-A09-1000)	007
882 (BYS2-E01-665)	006
~~887~~ 876 (RMS1-D12-910)	004

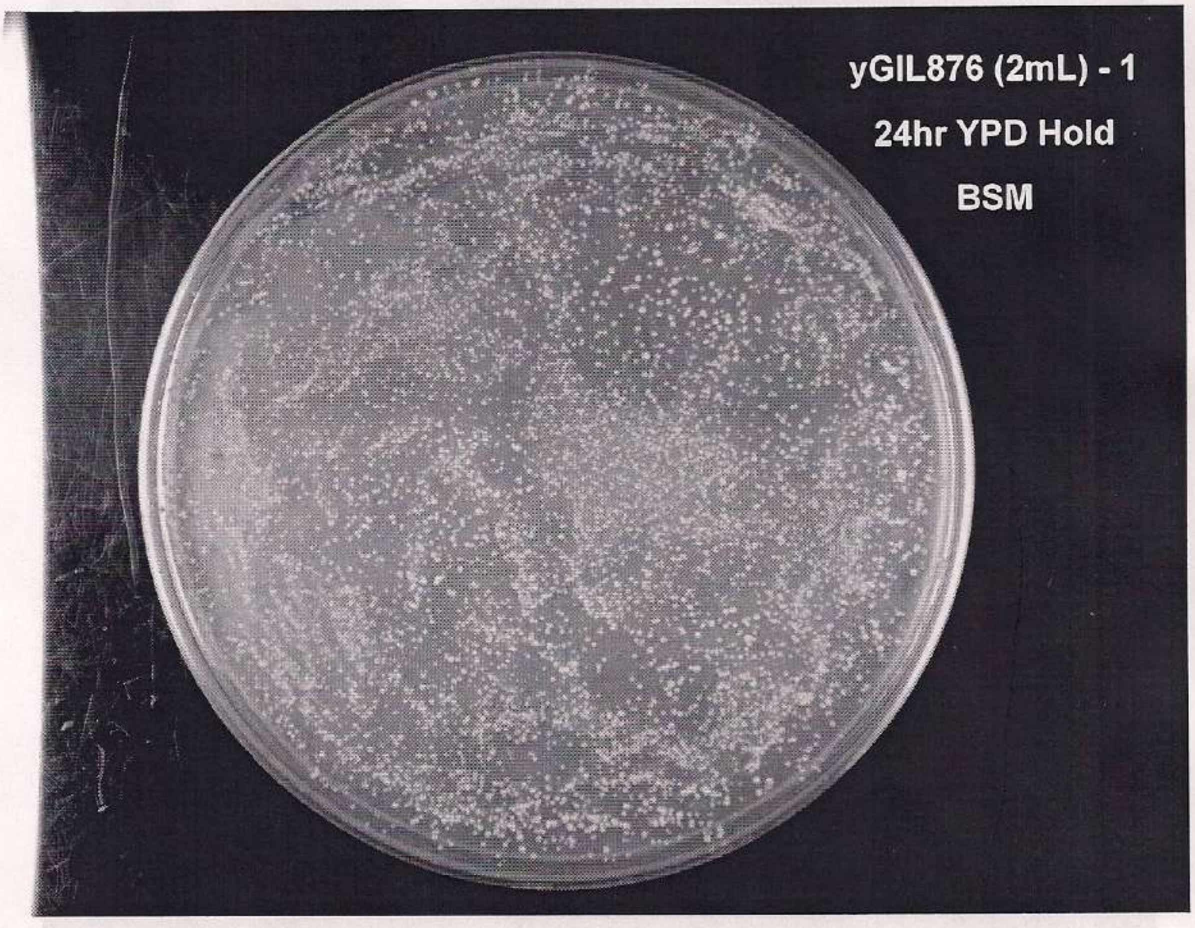
BSP ②:

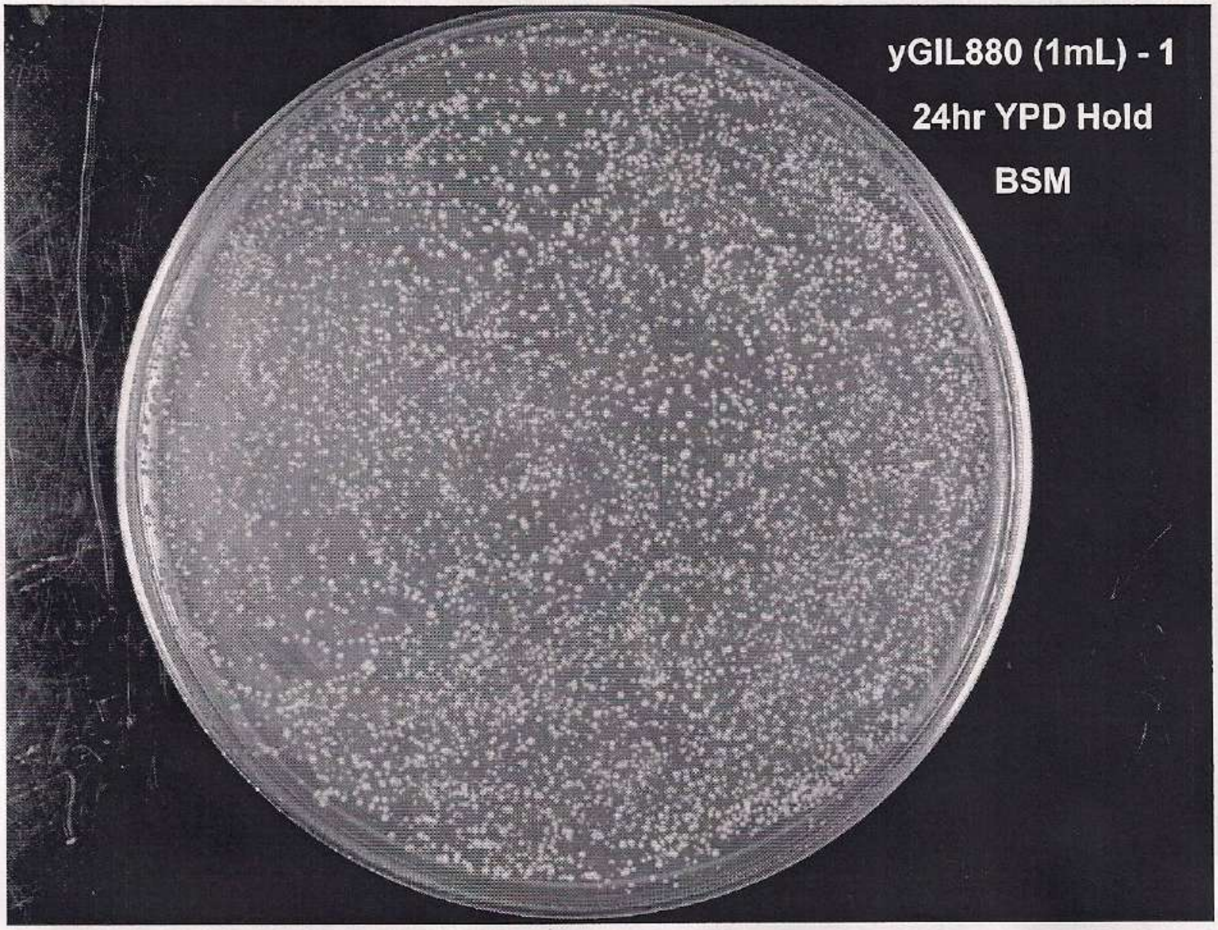
888 (RMS1-G02-545)	009	
890 (BYS1-A08-545)	011	
886 (BYS2-C06-1000)	008	
898 (RMS1-G02-825)	015	*
894 (RMS1-H09-585 (STE))	013	
902 (RMS1-H09-585)	016	
892 (BYS1-E03-745)	012	

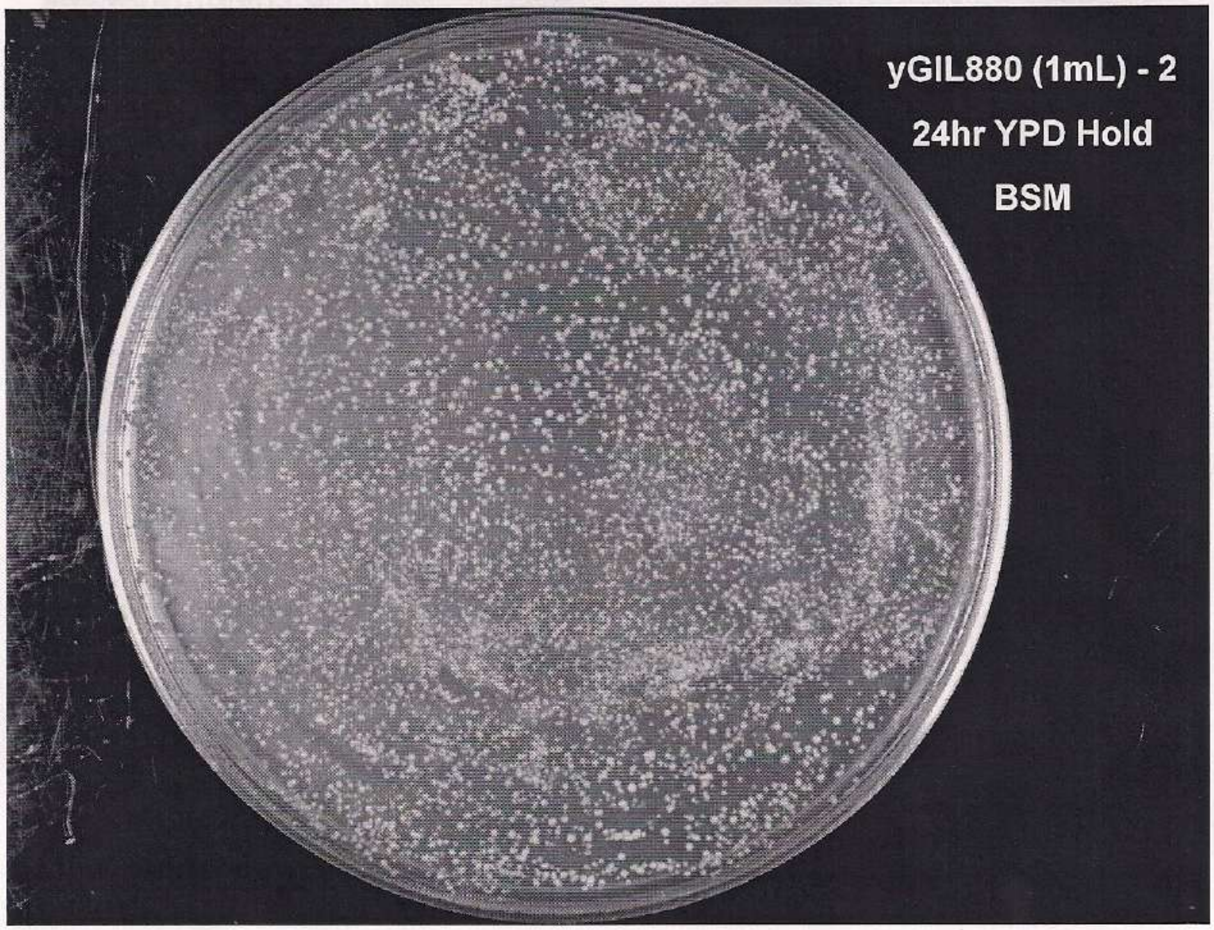
Bulk Segregant Protocol Round 1:

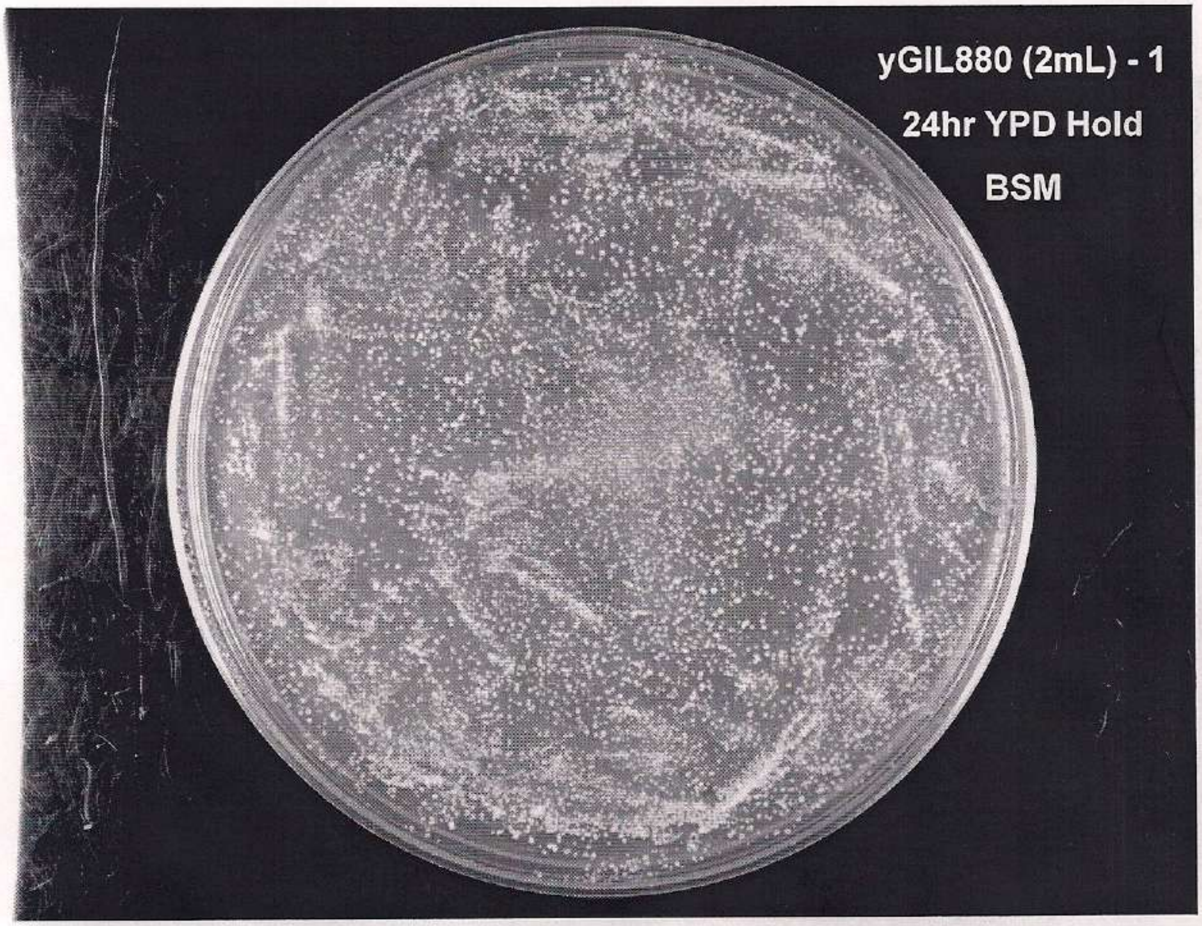
Conducted: 3/22/15 Plated: 3/22/15 Photographed: 3/25/15

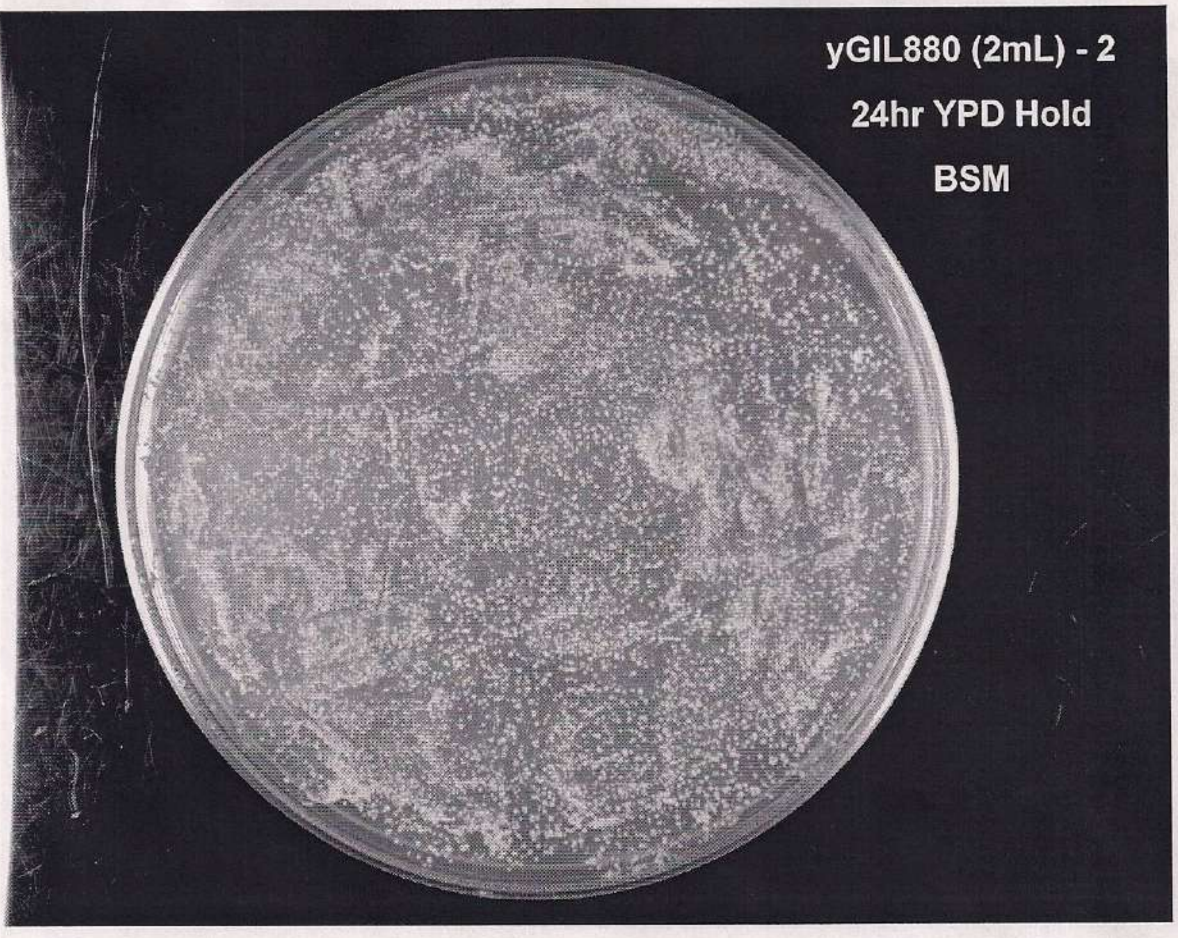

yGIL876 (2mL) - 1
24hr YPD Hold
BSM

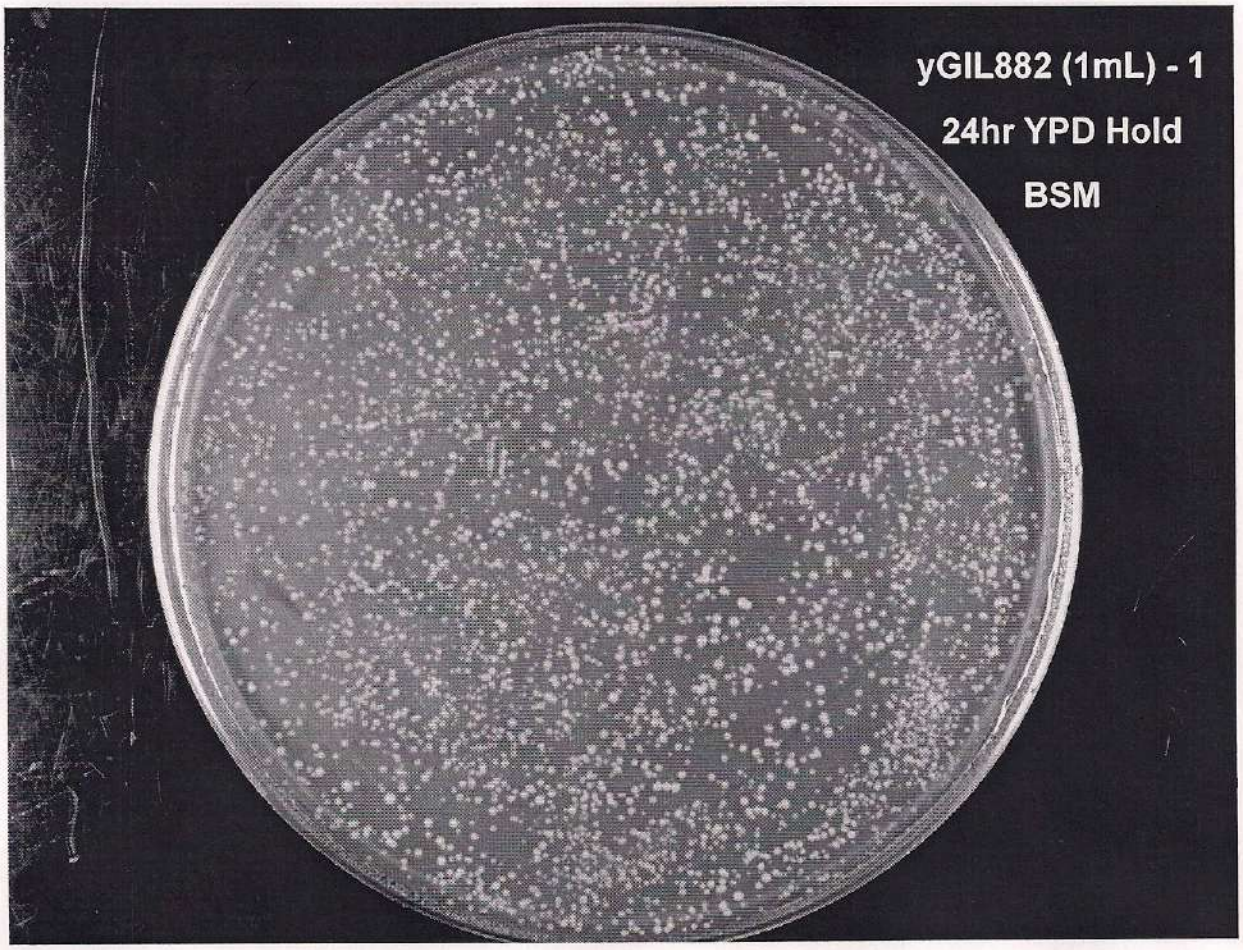

yGIL876 (2mL) - 1
24hr YPD Hold
BSM

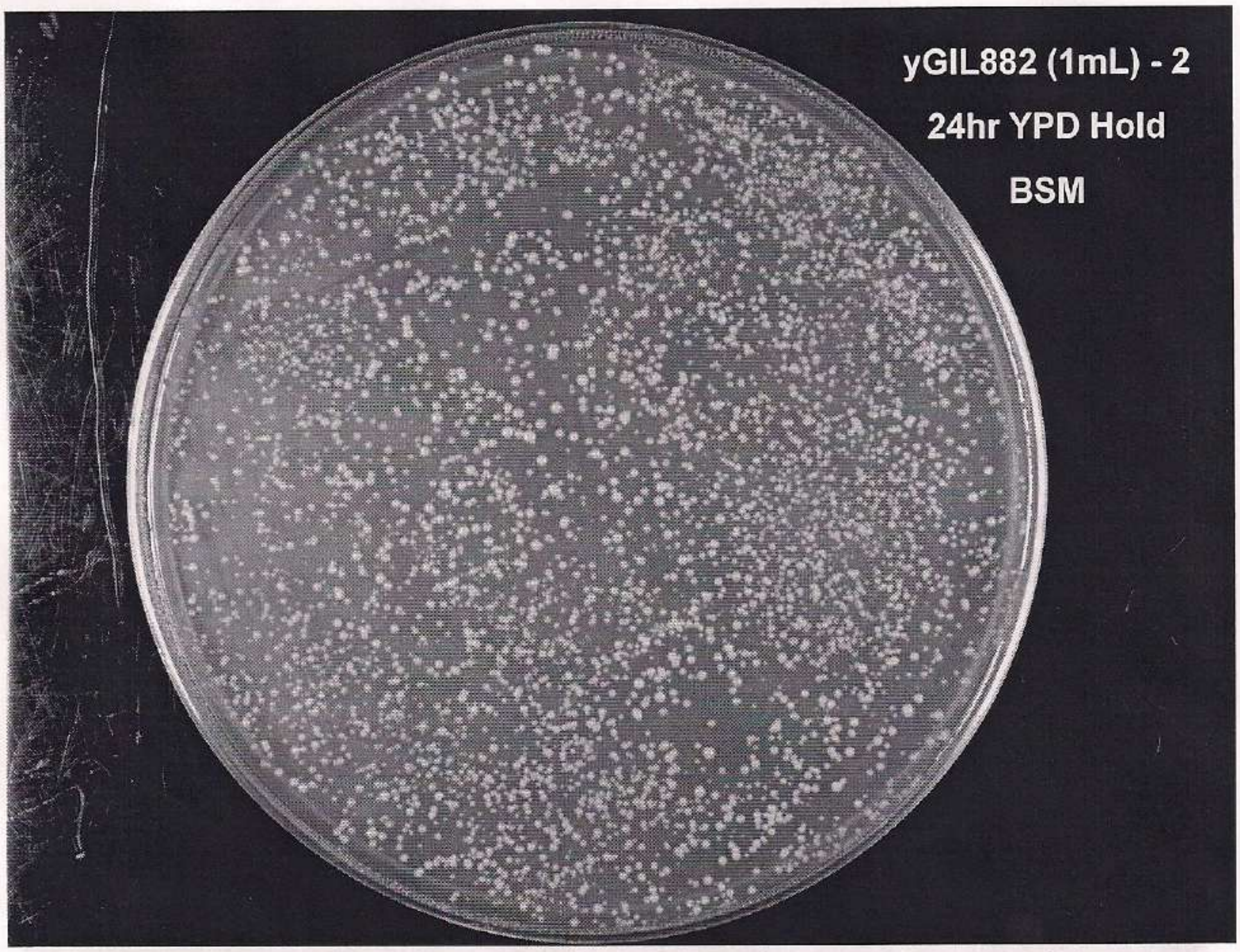

yGIL880 (1mL) - 1
24hr YPD Hold
BSM

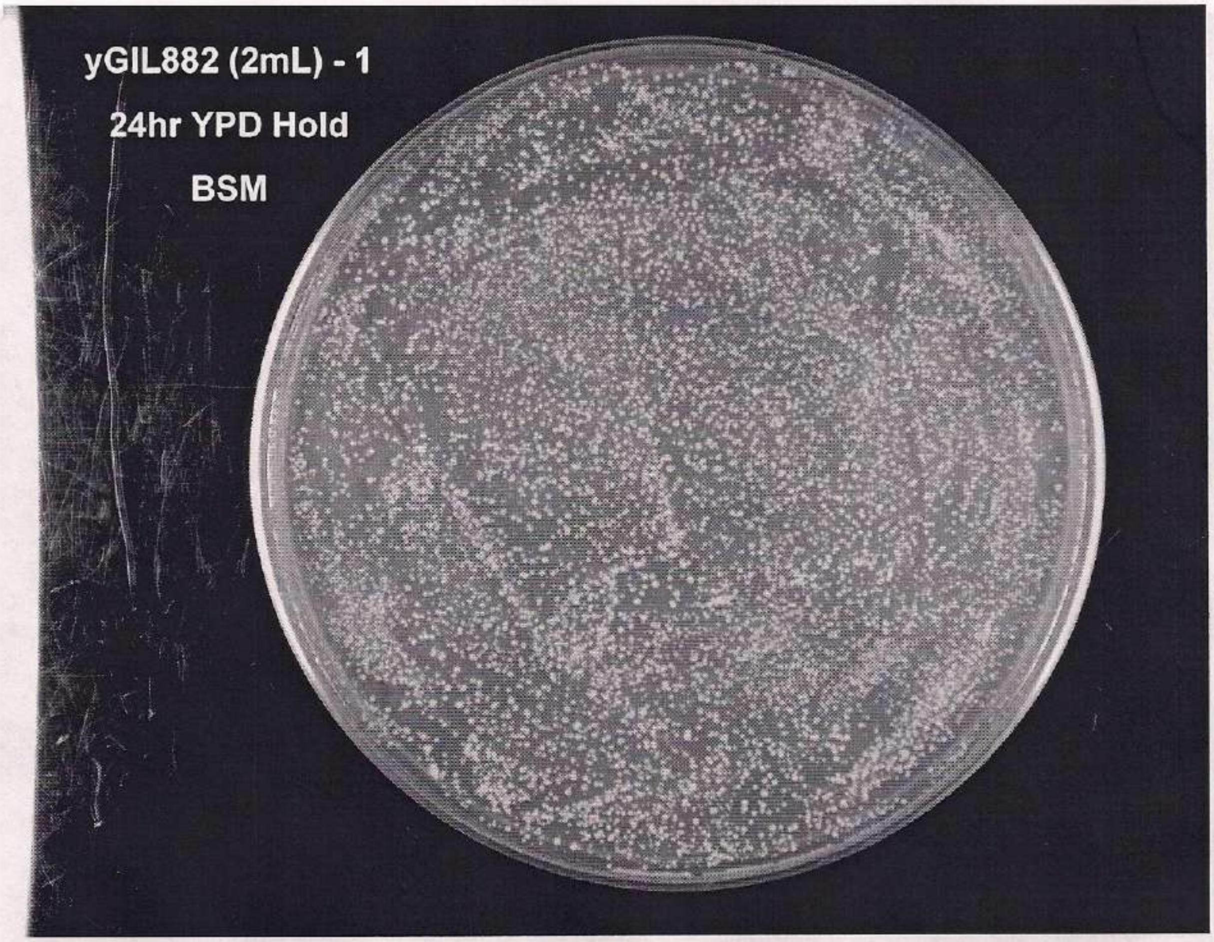

yGIL880 (1mL) - 2
24hr YPD Hold
BSM

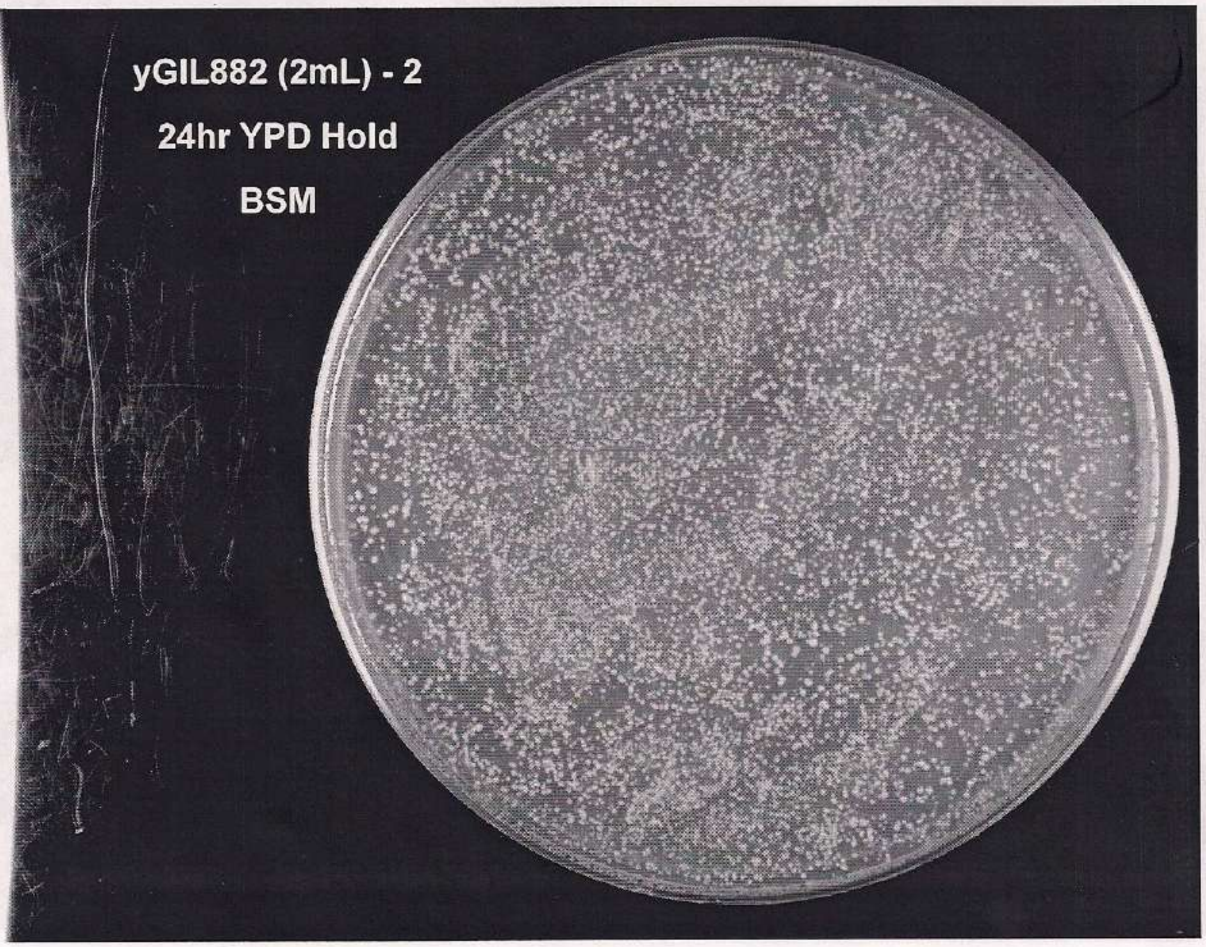

yGIL880 (2mL) - 1
24hr YPD Hold
BSM

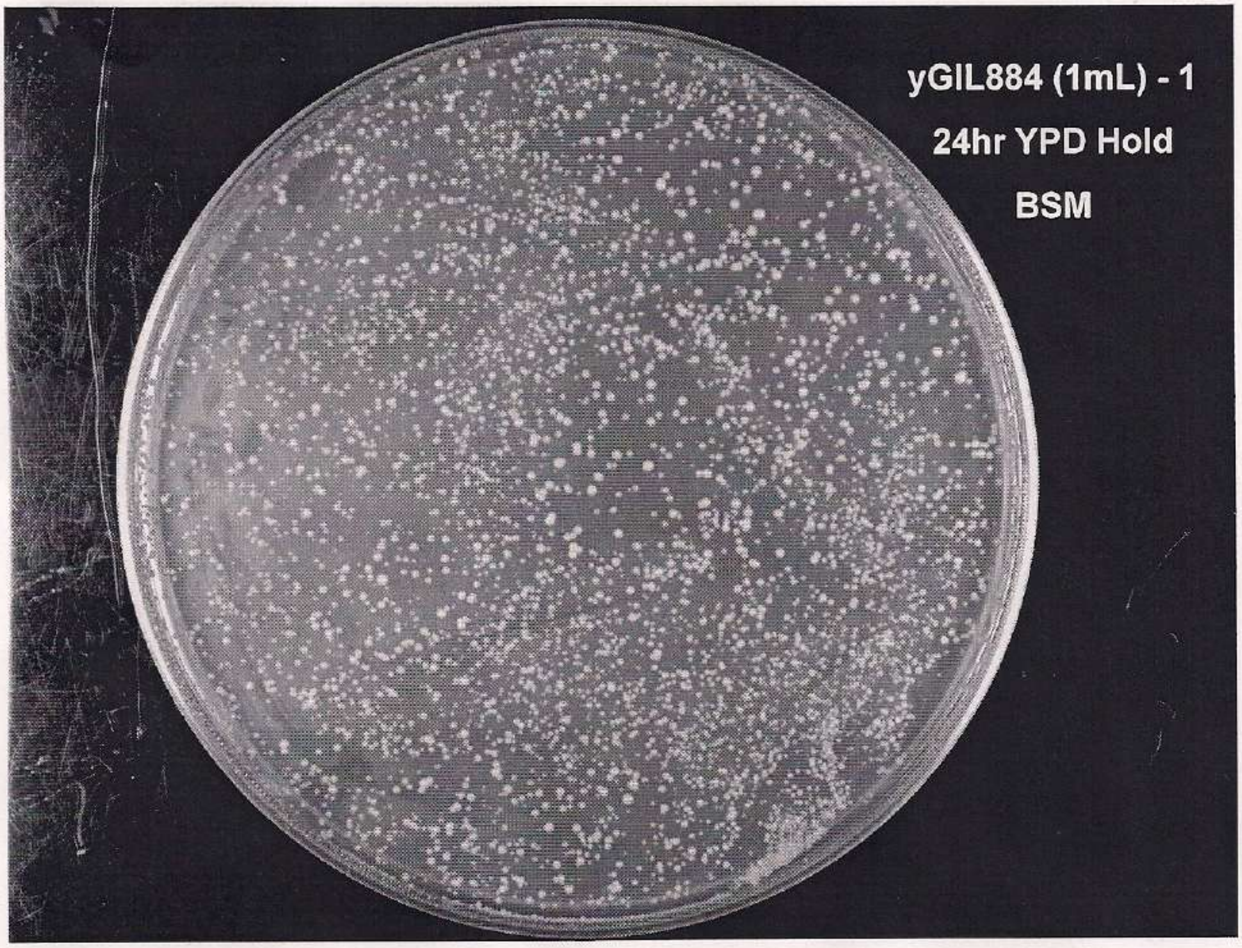

yGIL880 (2mL) - 2
24hr YPD Hold
BSM

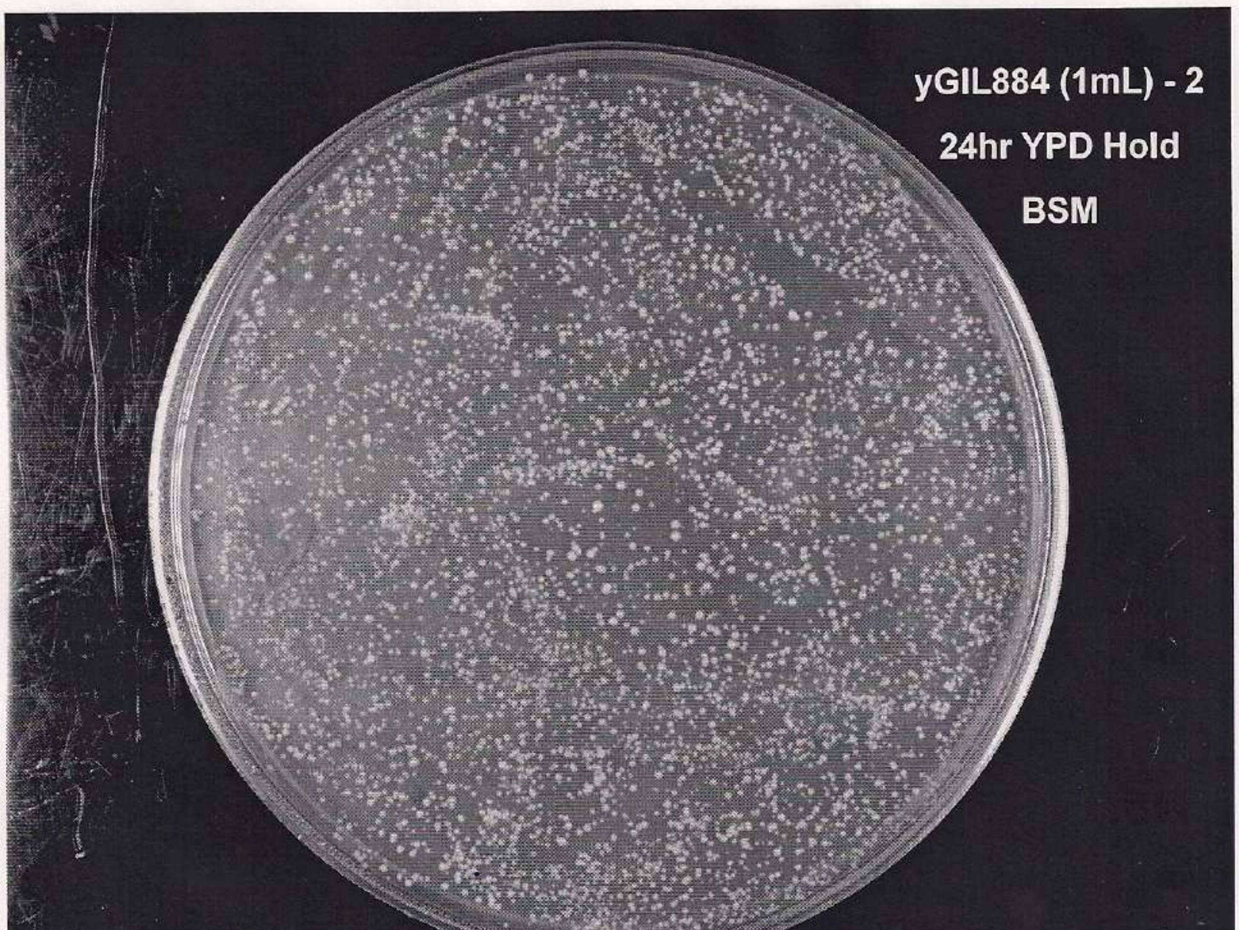

yGIL882 (1mL) - 1
24hr YPD Hold
BSM

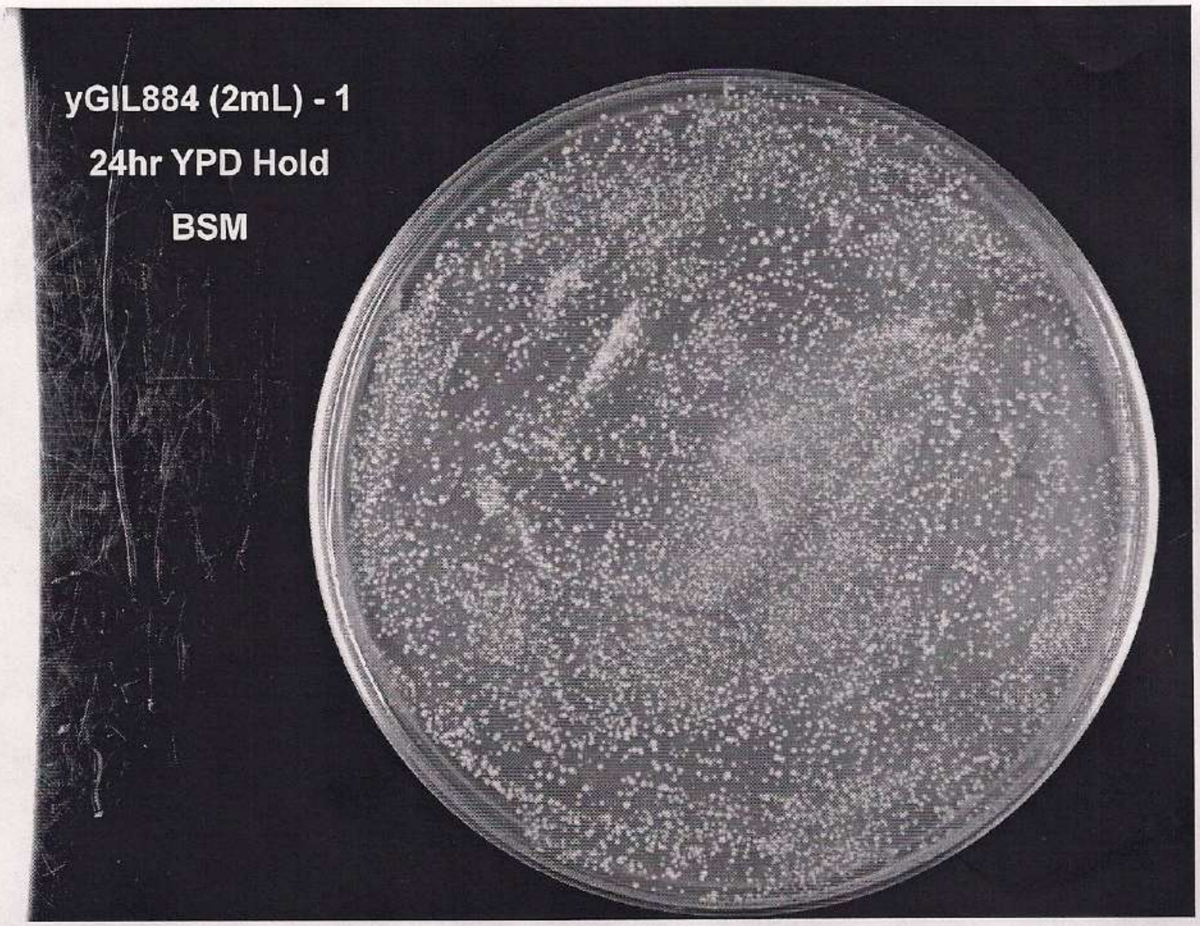

yGIL882 (1mL) - 2
24hr YPD Hold
BSM

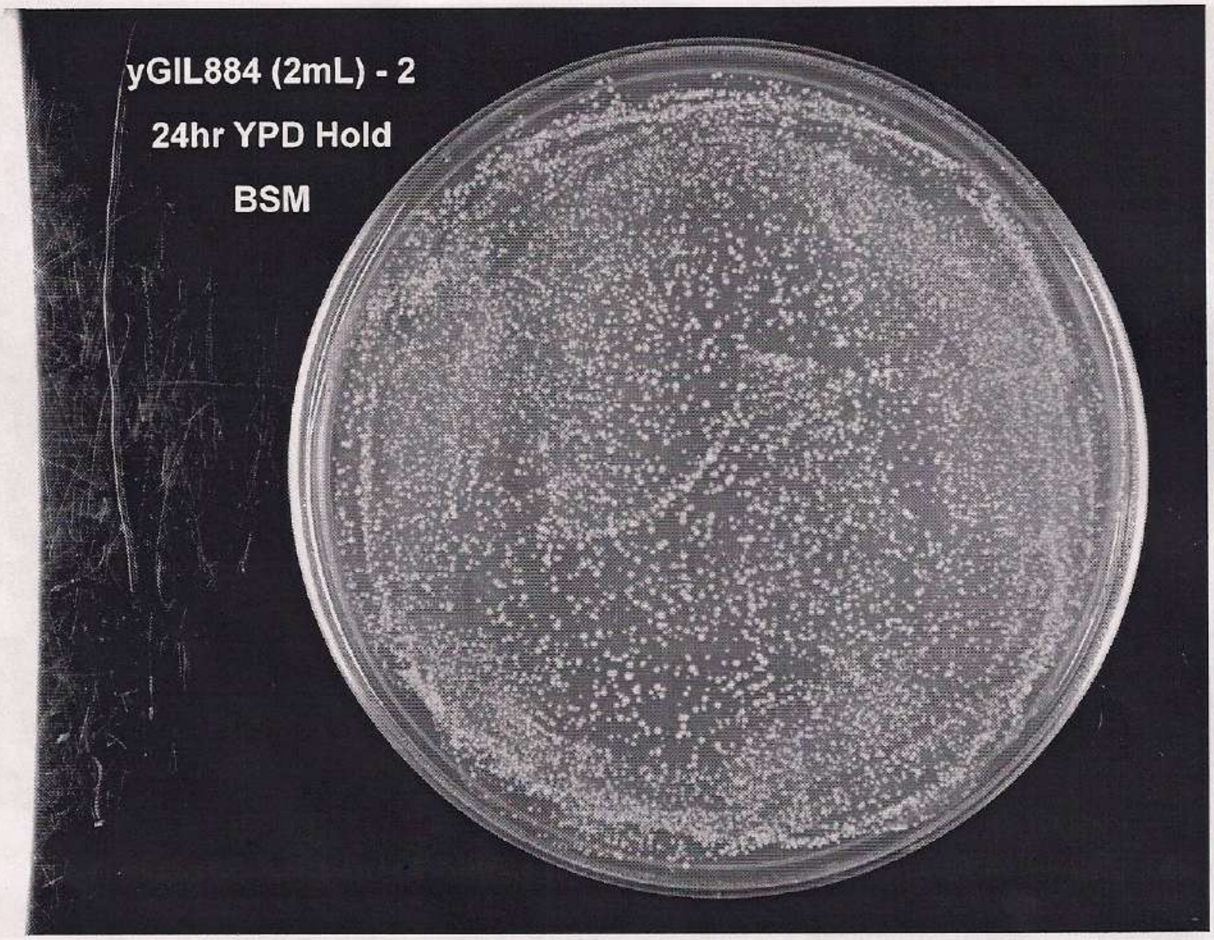

yGIL882 (2mL) - 1
24hr YPD Hold
BSM

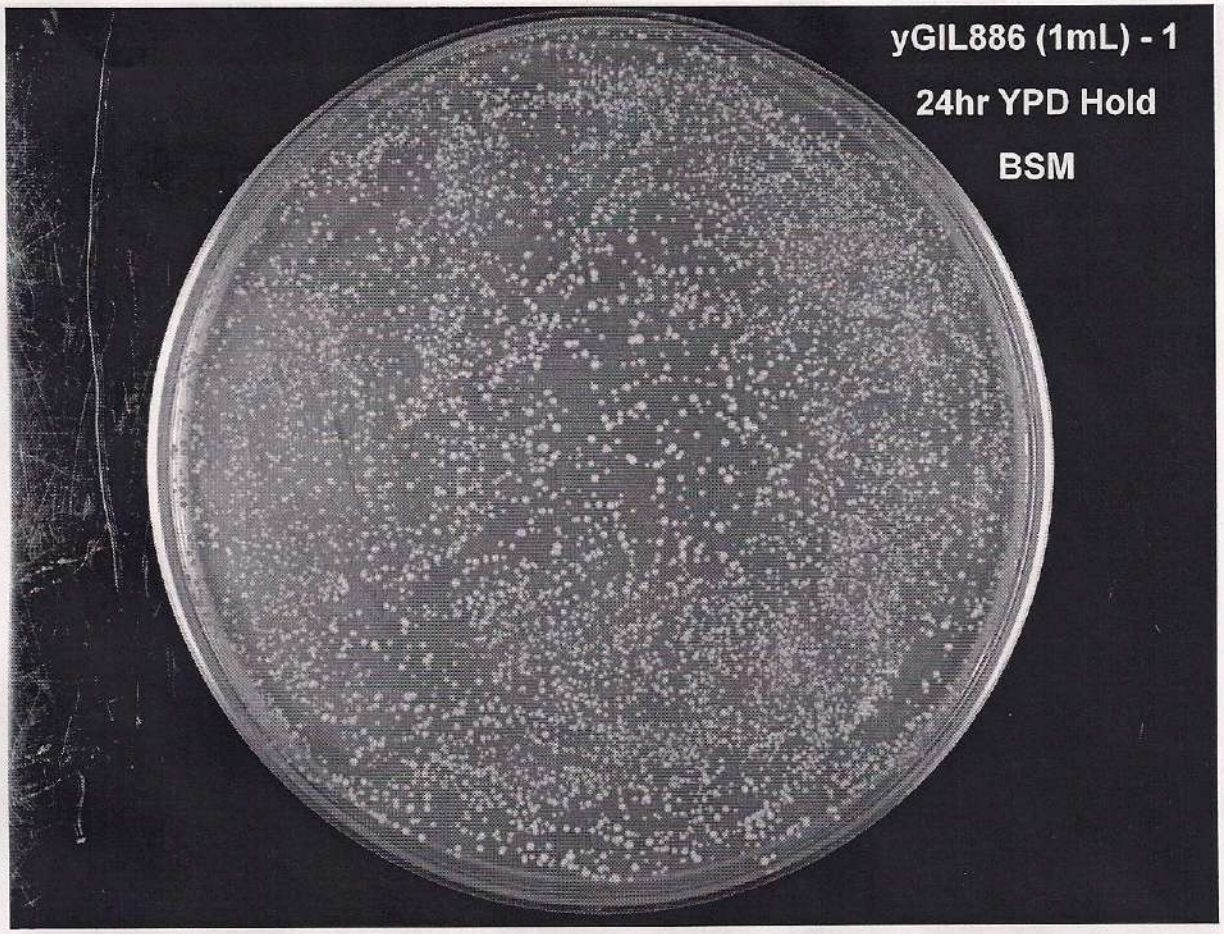

yGIL882 (2mL) - 2
24hr YPD Hold
BSM


yGIL884 (1mL) - 1
24hr YPD Hold
BSM

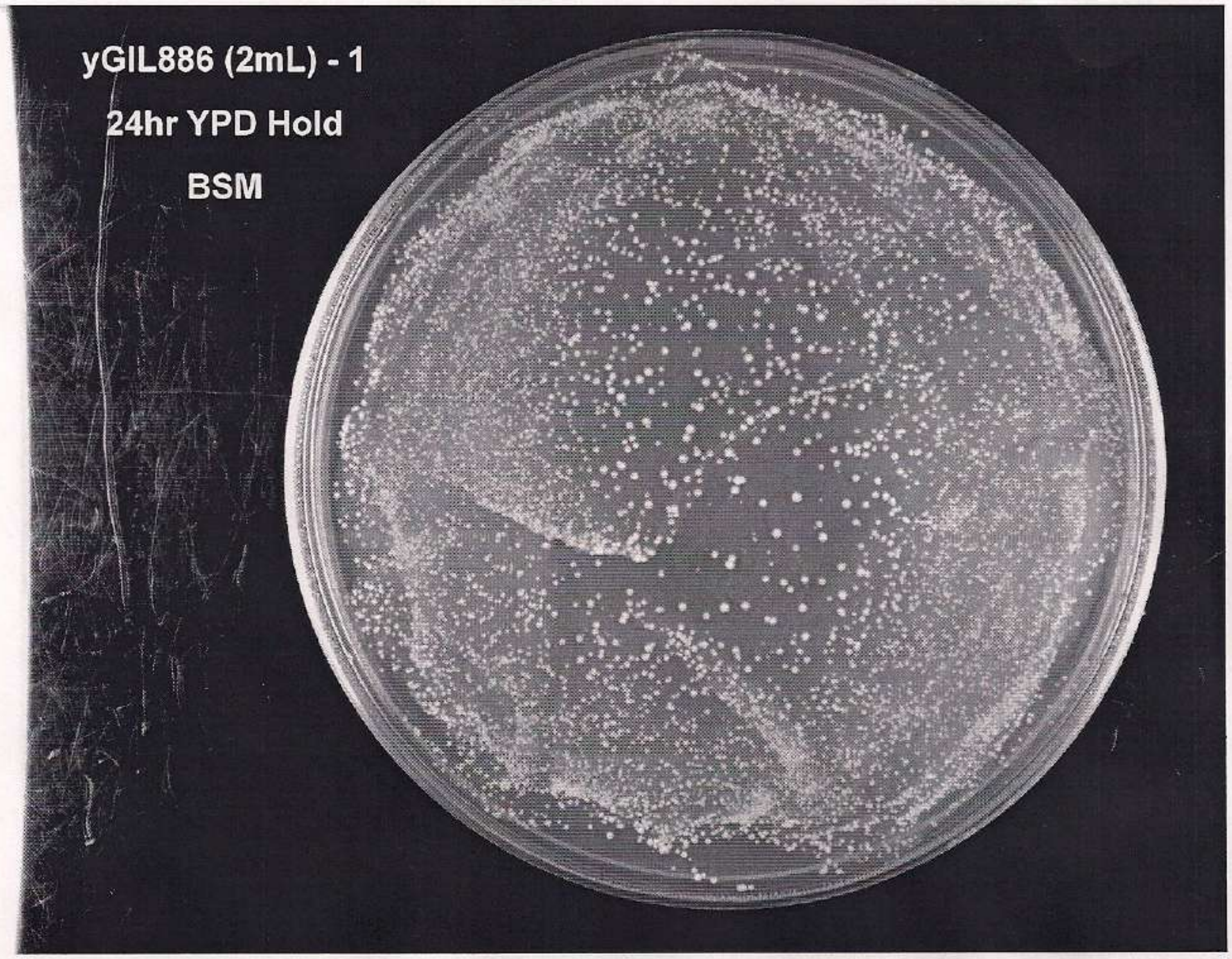

yGIL884 (1mL) - 2
24hr YPD Hold
BSM

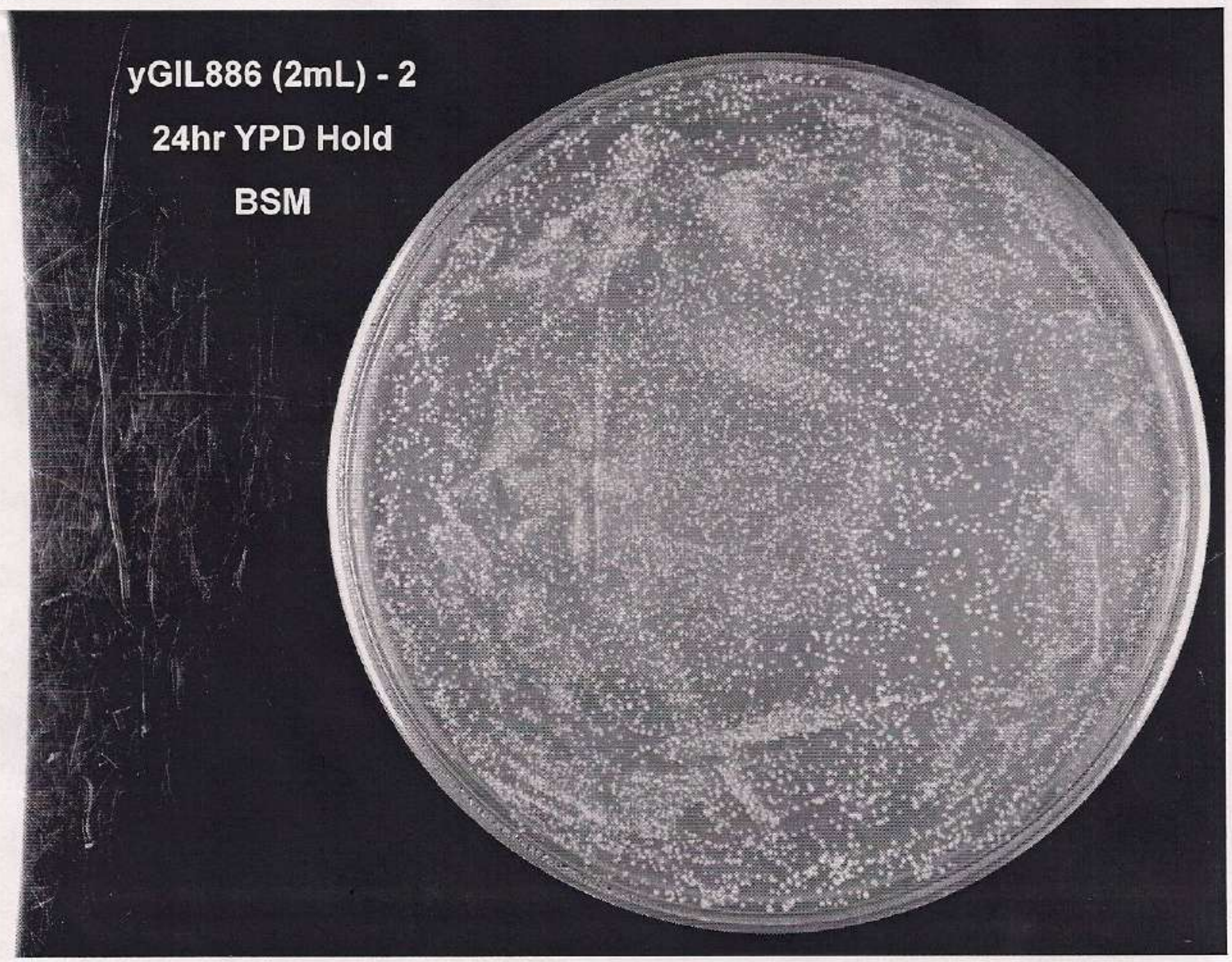

yGIL884 (2mL) - 1
24hr YPD Hold
BSM

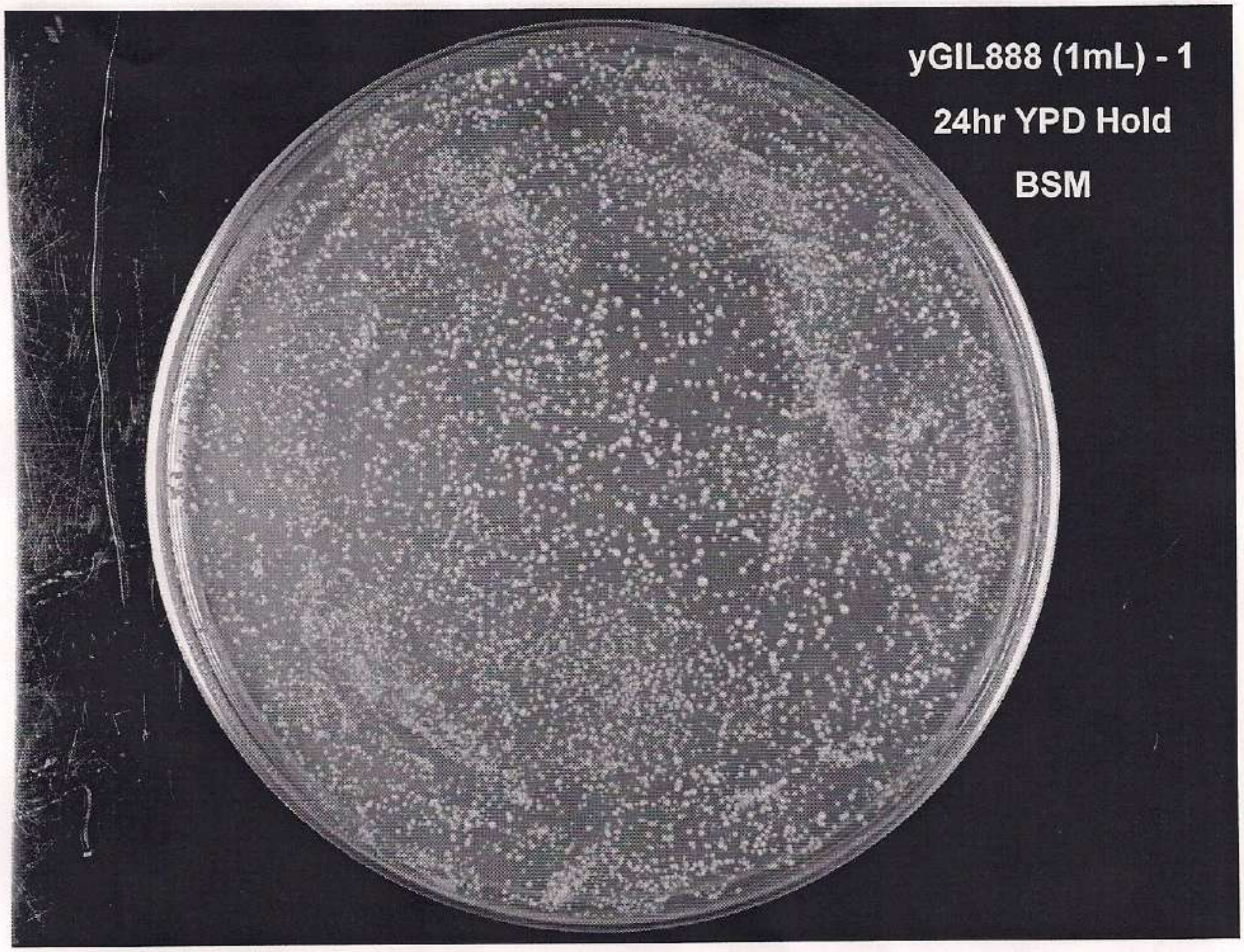

yGIL884 (2mL) - 2
24hr YPD Hold
BSM

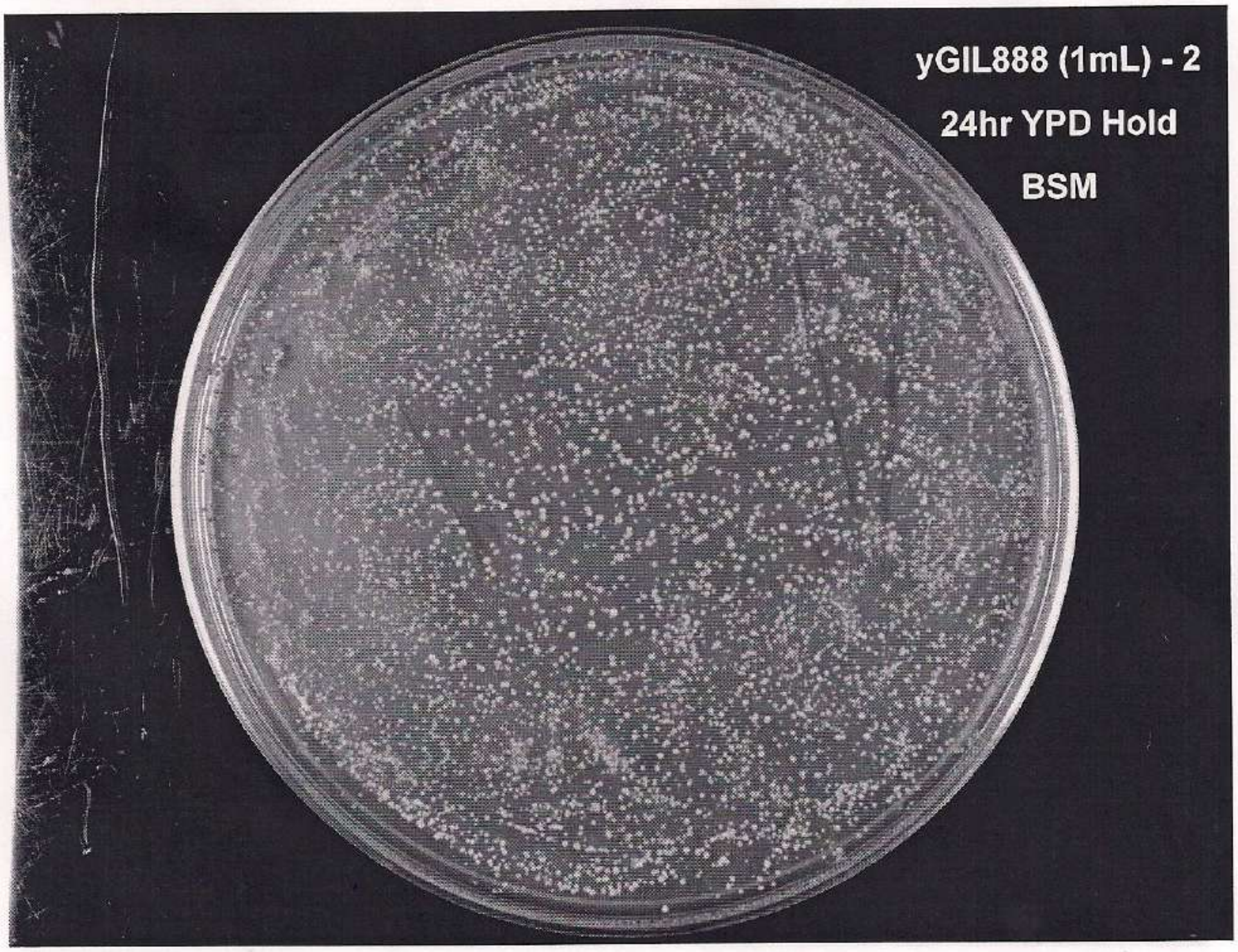

yGIL886 (1mL) - 1
24hr YPD Hold
BSM

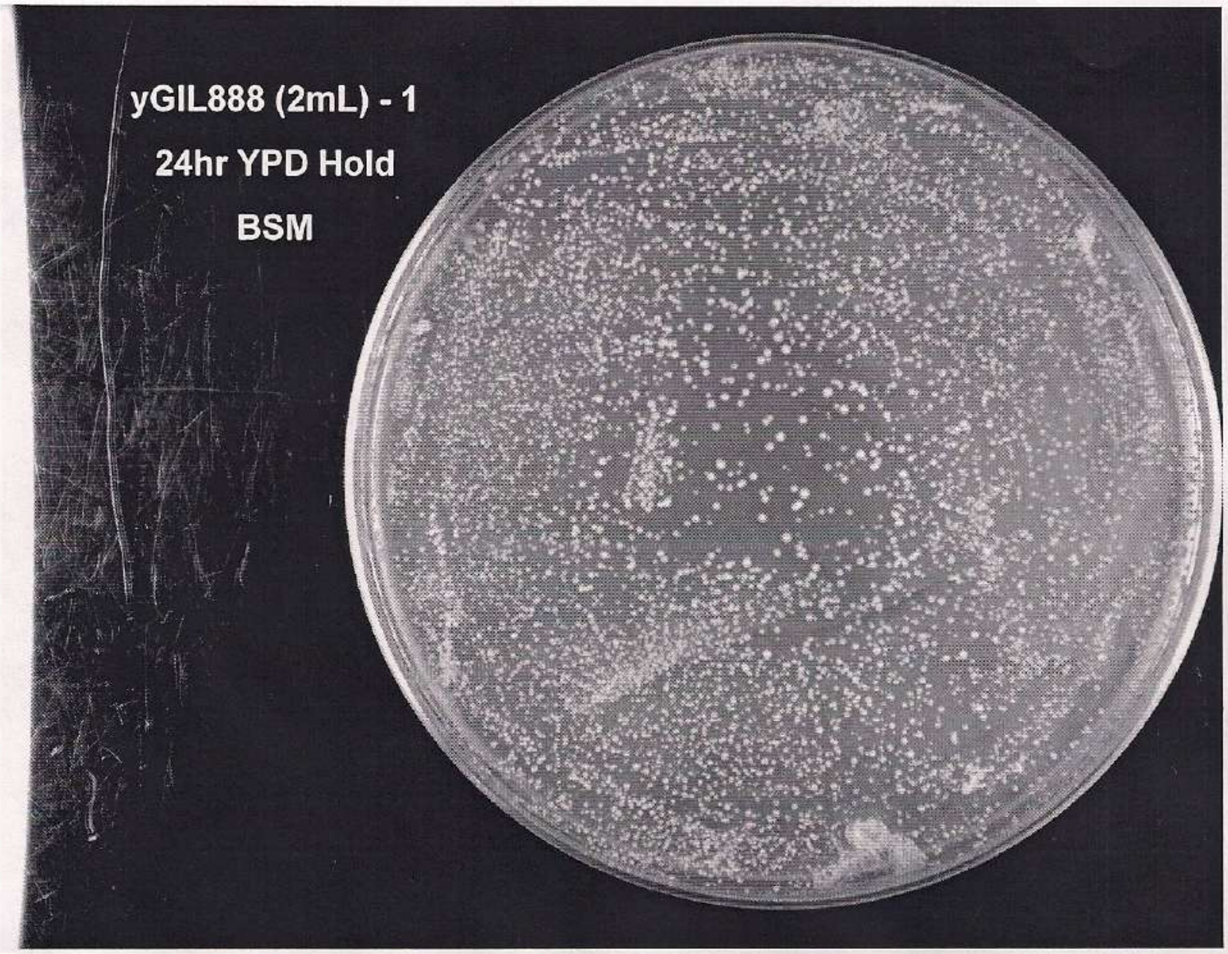

yGIL886 (1mL) - 2
24hr YPD Hold
BSM

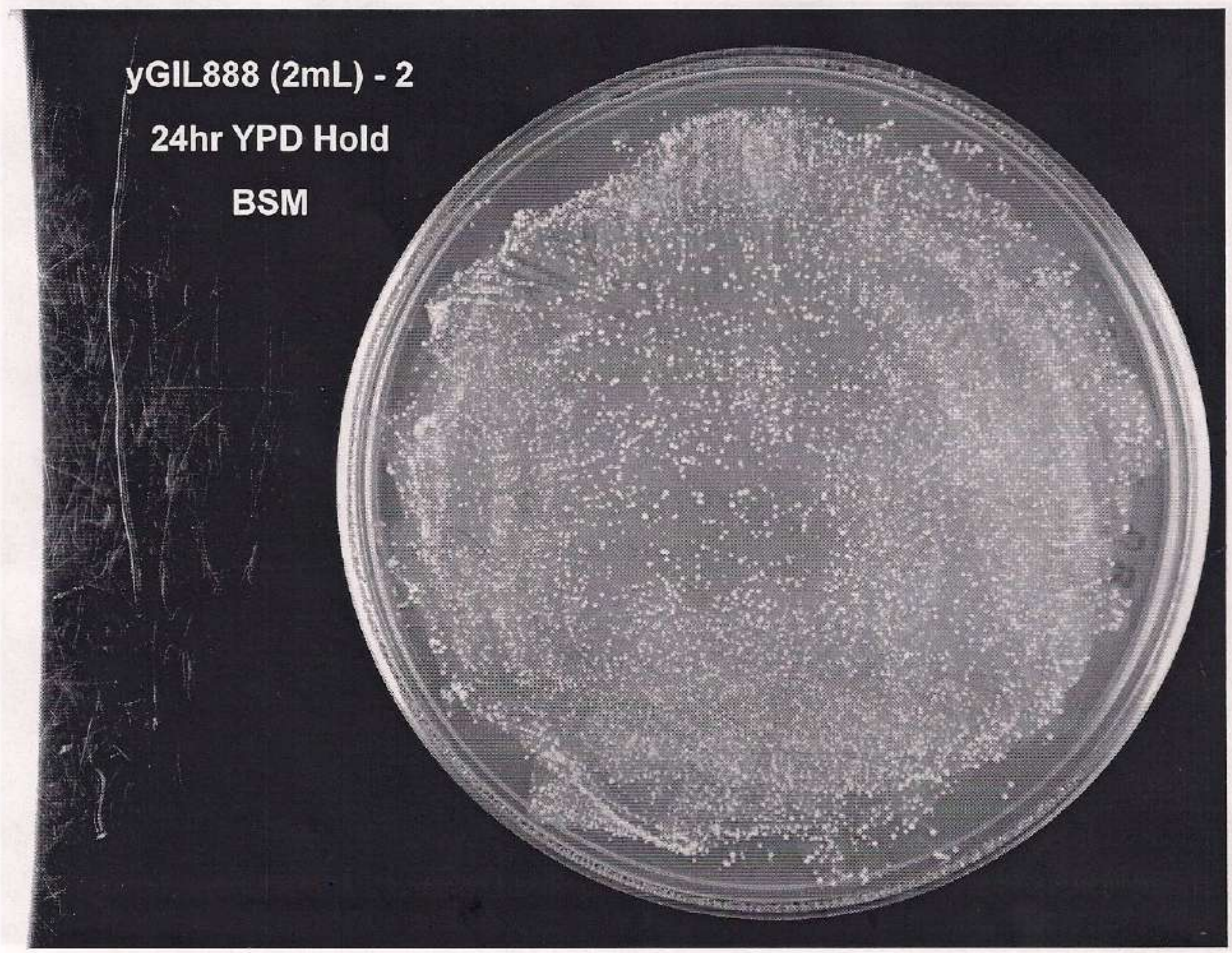

yGIL886 (2mL) - 1
24hr YPD Hold
BSM


yGIL886 (2mL) - 2
24hr YPD Hold
BSM


yGIL888 (1mL) - 1
24hr YPD Hold
BSM

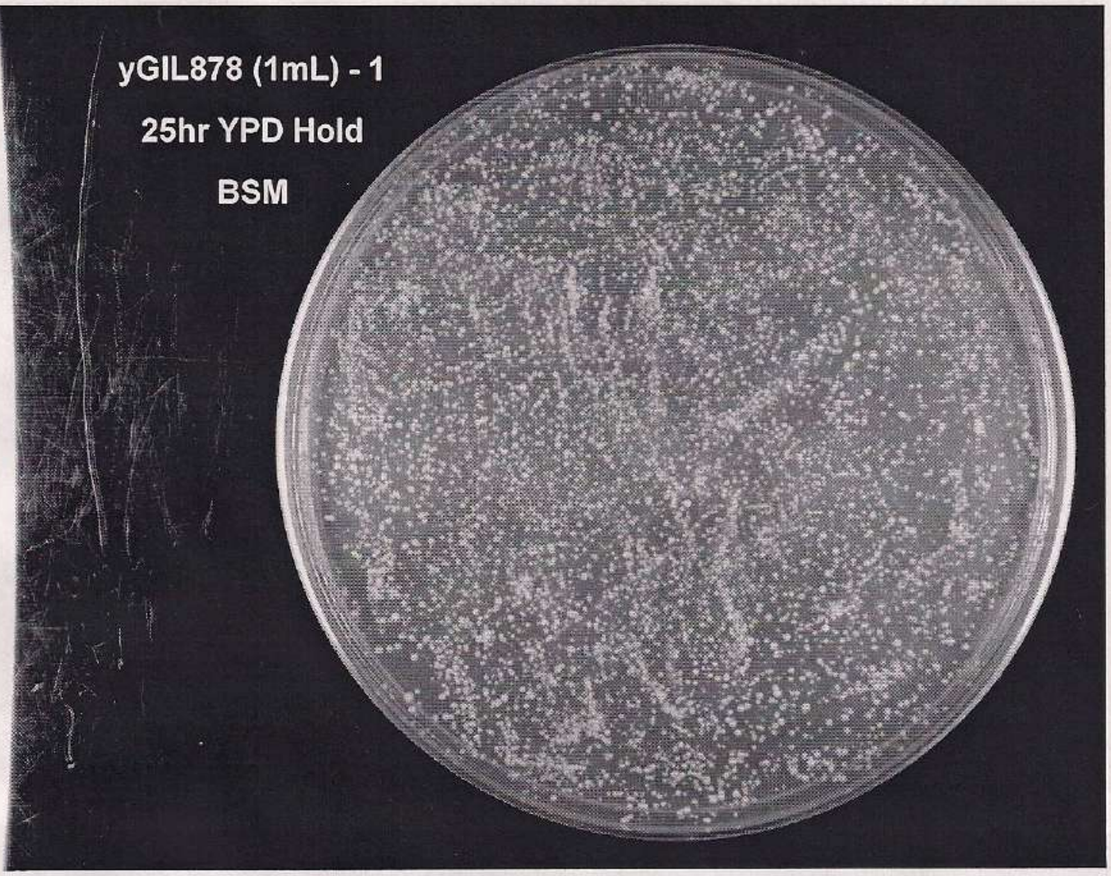

yGIL888 (1mL) - 2
24hr YPD Hold
BSM

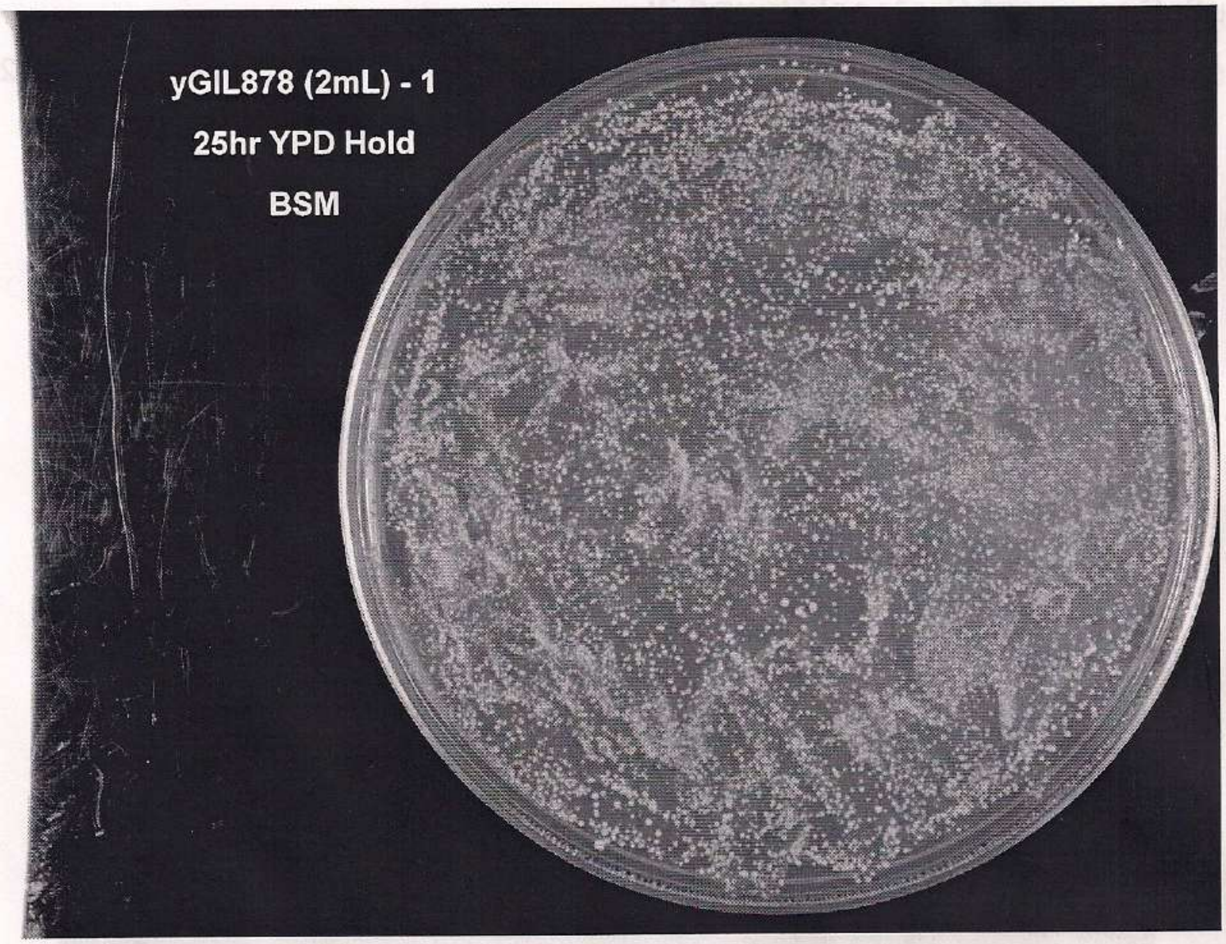

yGIL888 (2mL) - 1
24hr YPD Hold
BSM

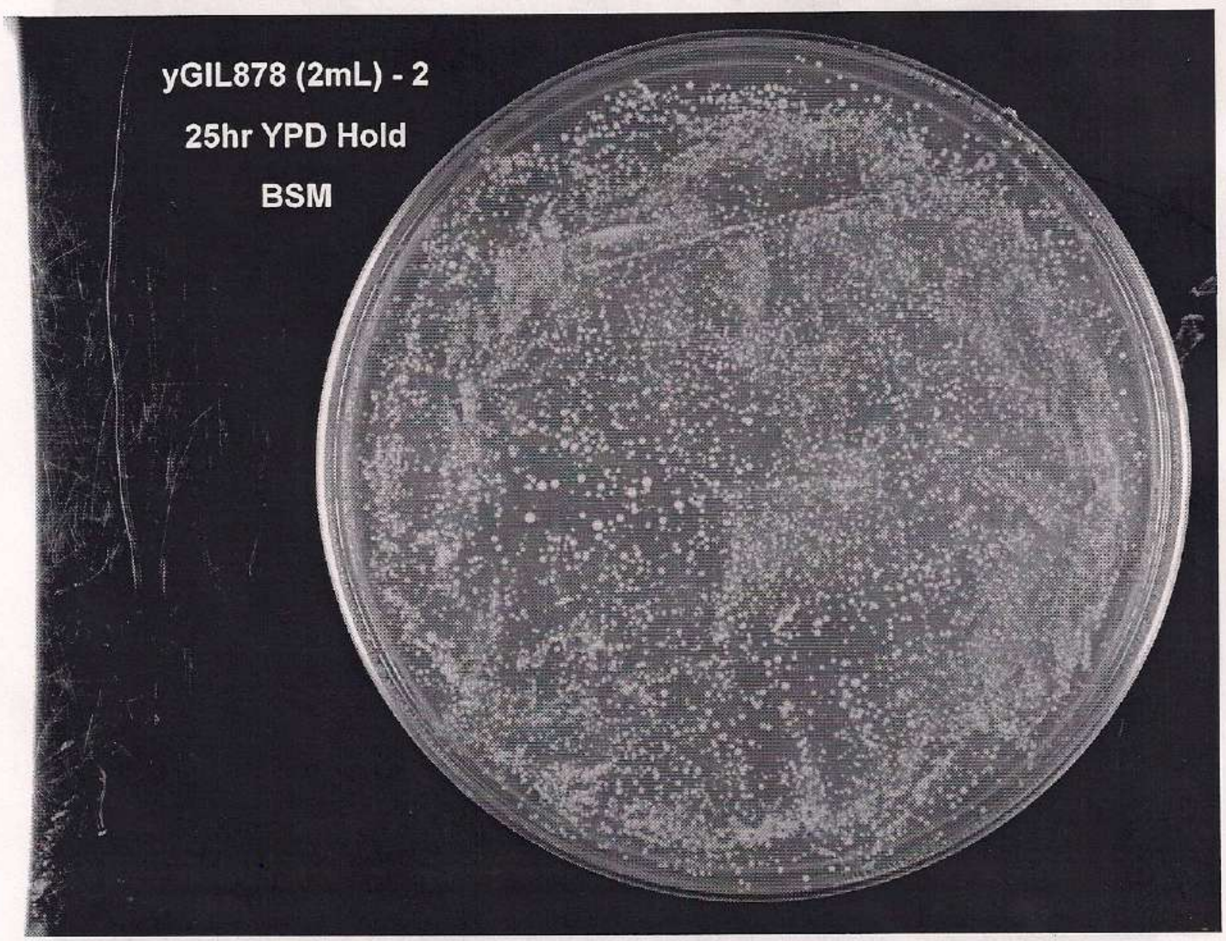

yGIL888 (2mL) - 2
24hr YPD Hold
BSM

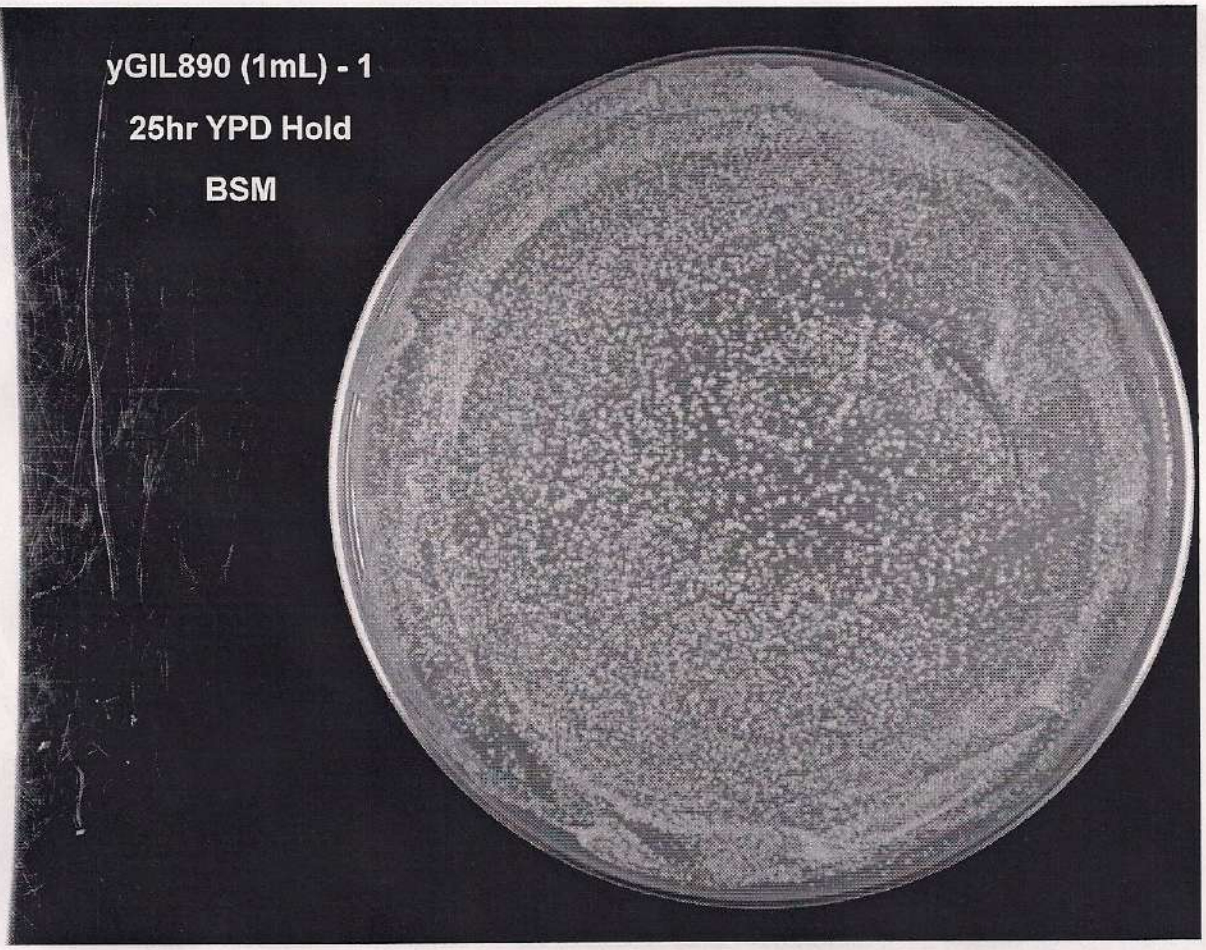
Bulk Segregant Protocol Round 2:

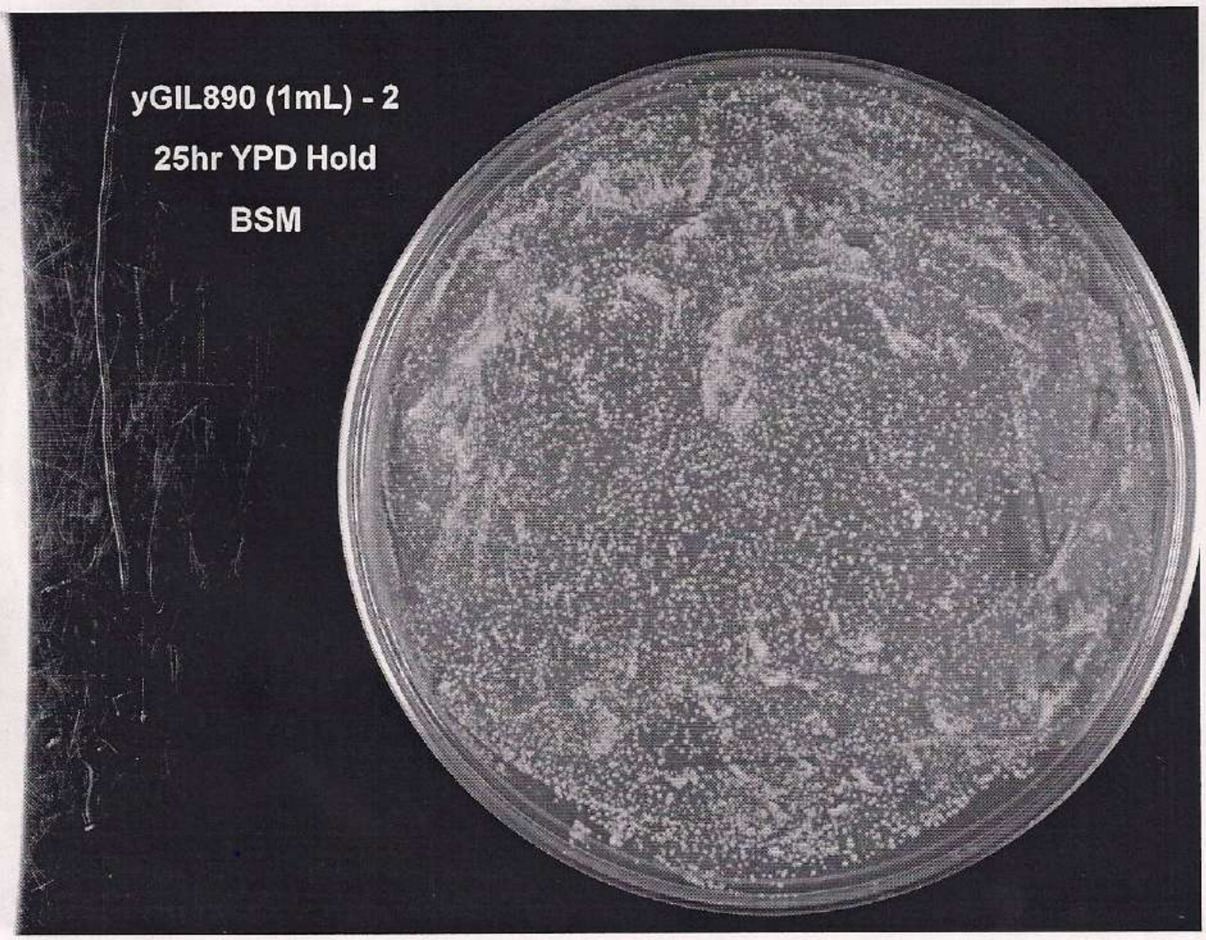
Conducted: 3/24/15 Plated: 3/25/15 Photographed: 3/28/15

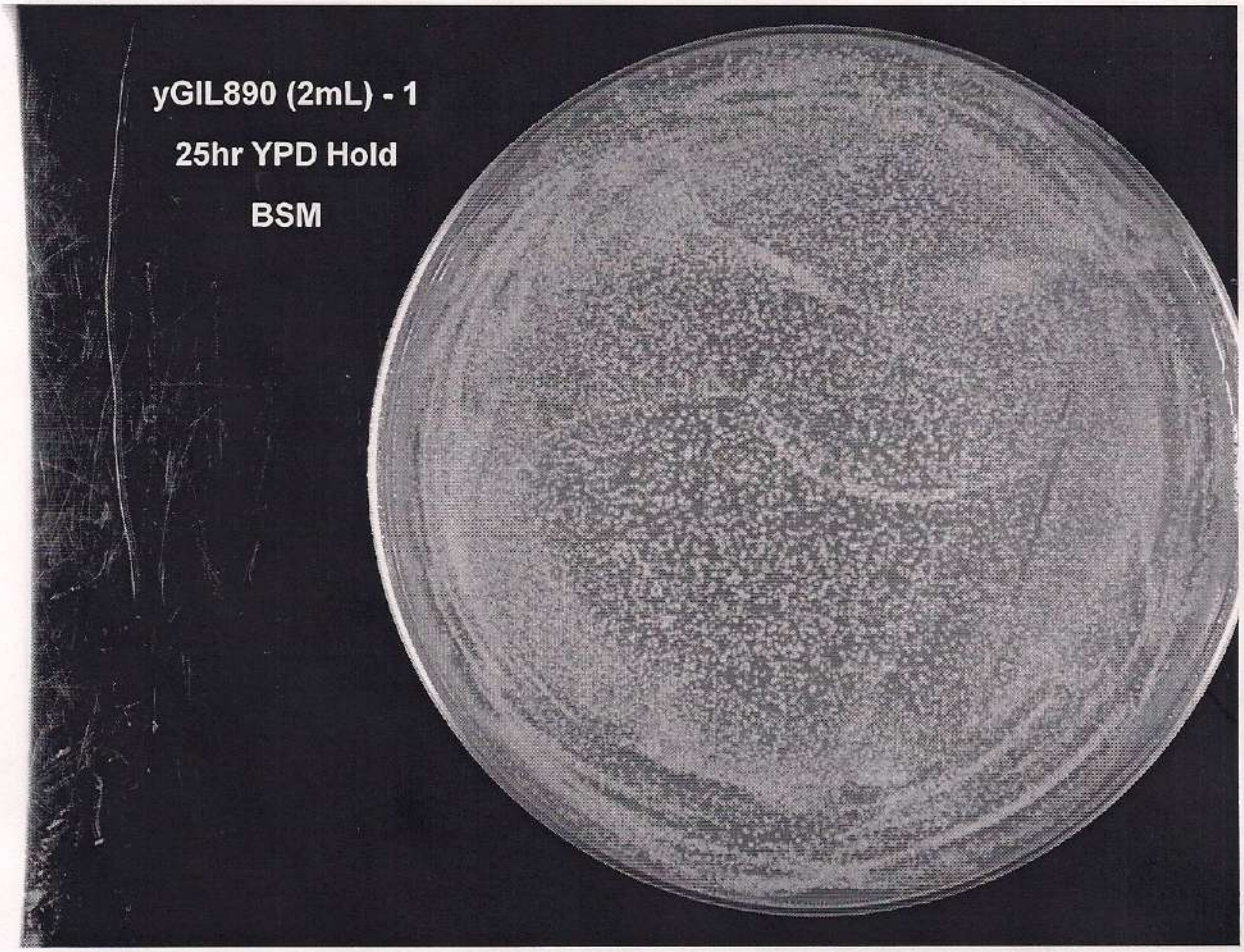


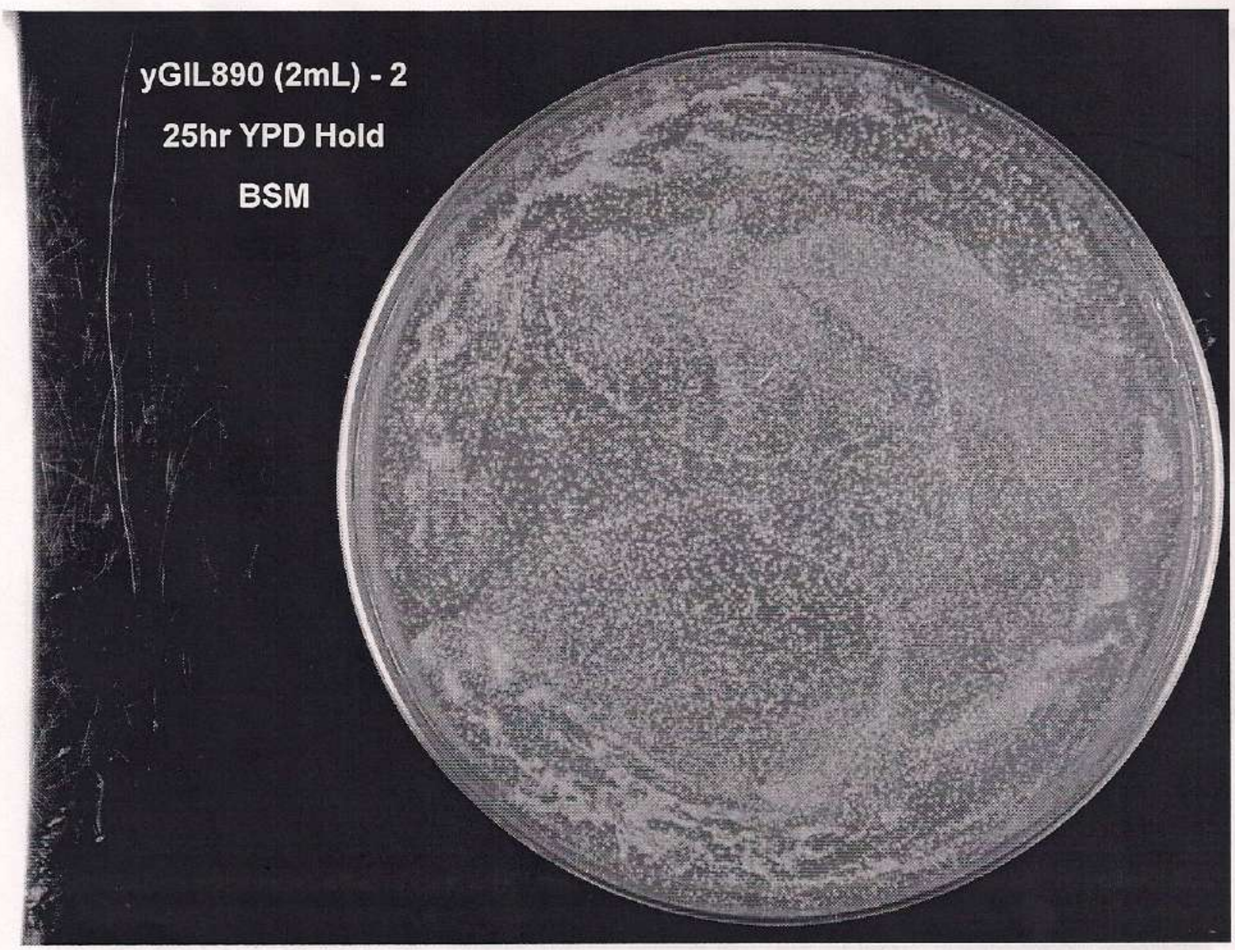

yGIL878 (2mL) - 1
25hr YPD Hold
BSM

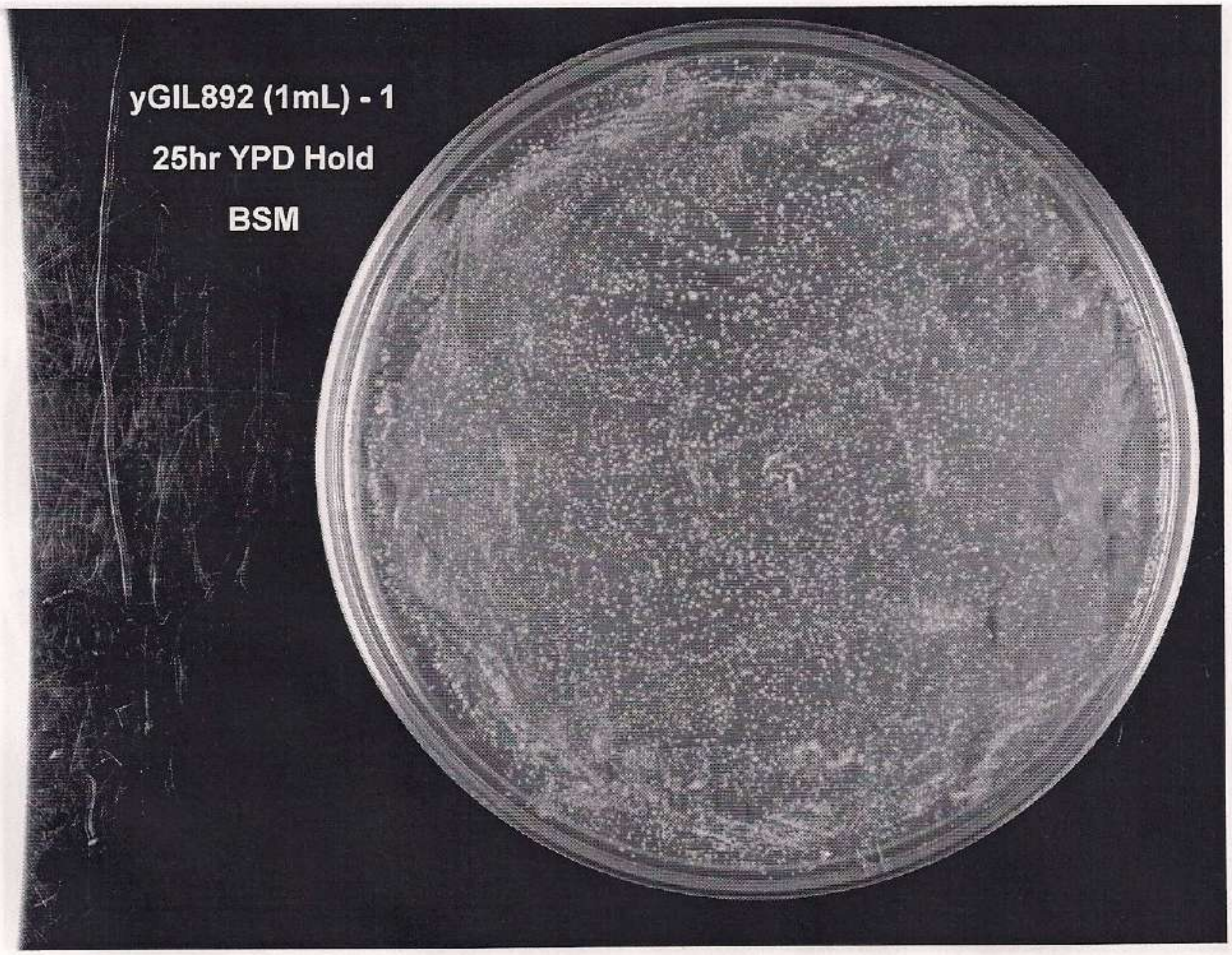

yGIL878 (2mL) - 2
25hr YPD Hold
BSM

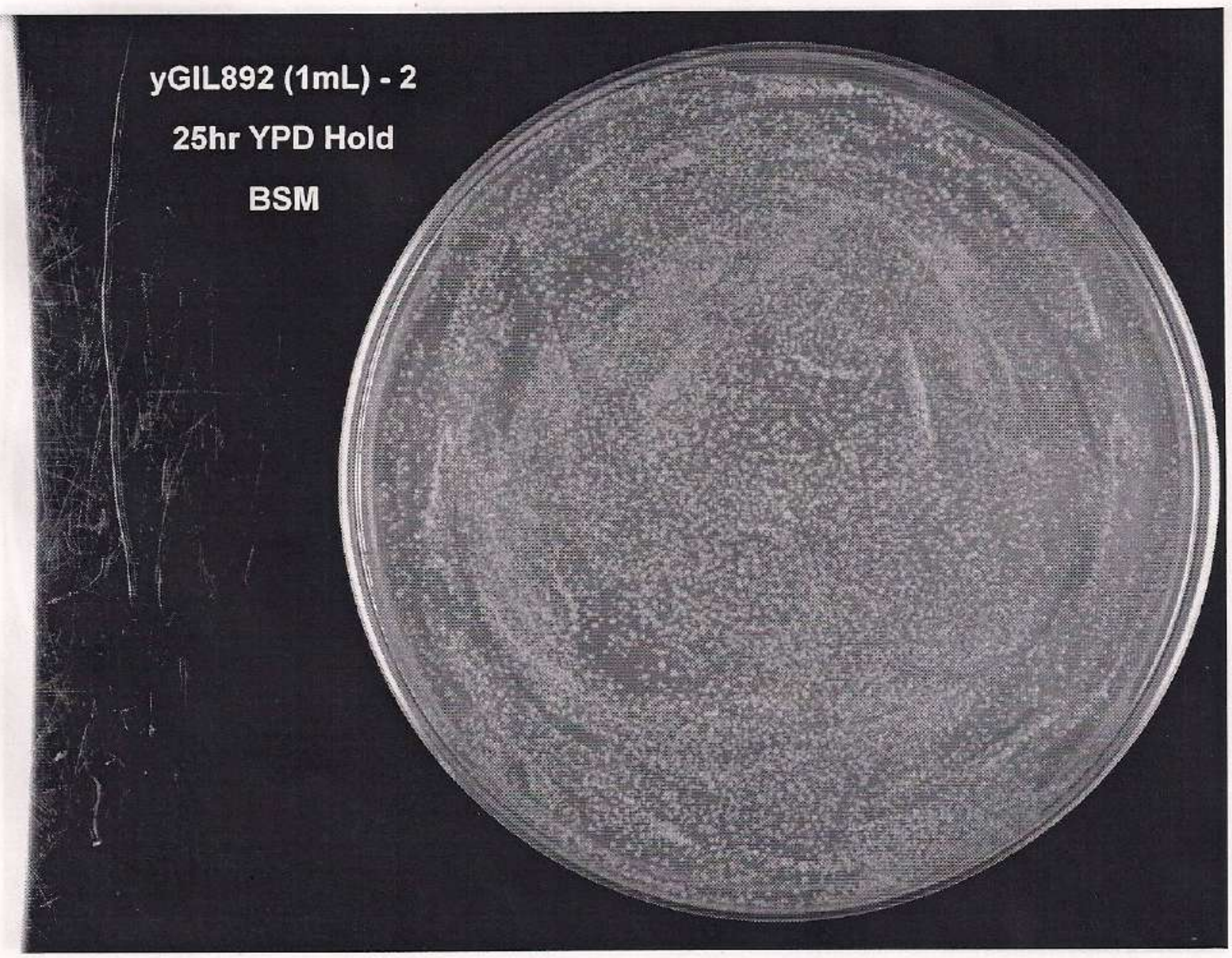

yGIL890 (1mL) - 1
25hr YPD Hold
BSM

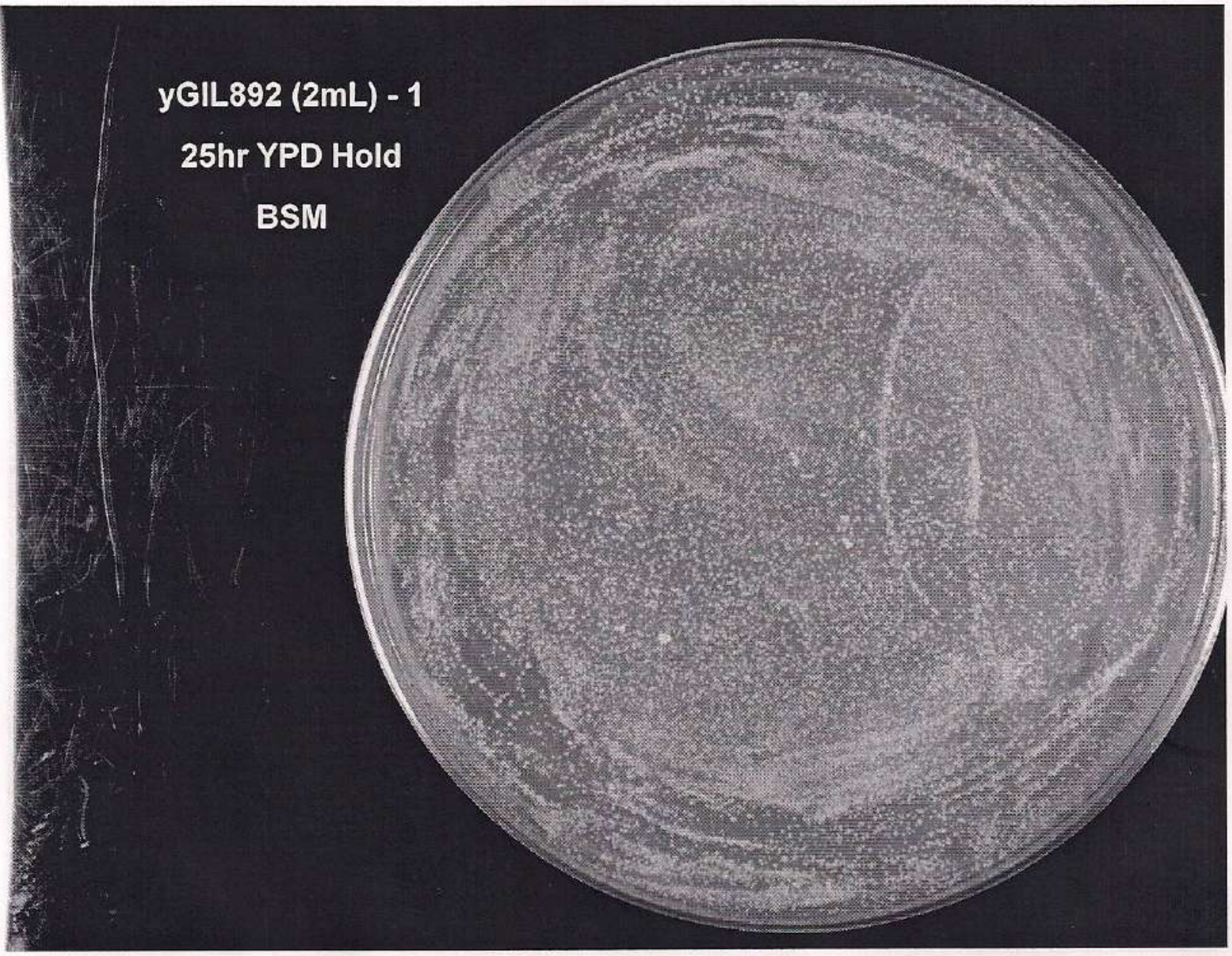

yGIL890 (1mL) - 2
25hr YPD Hold
BSM

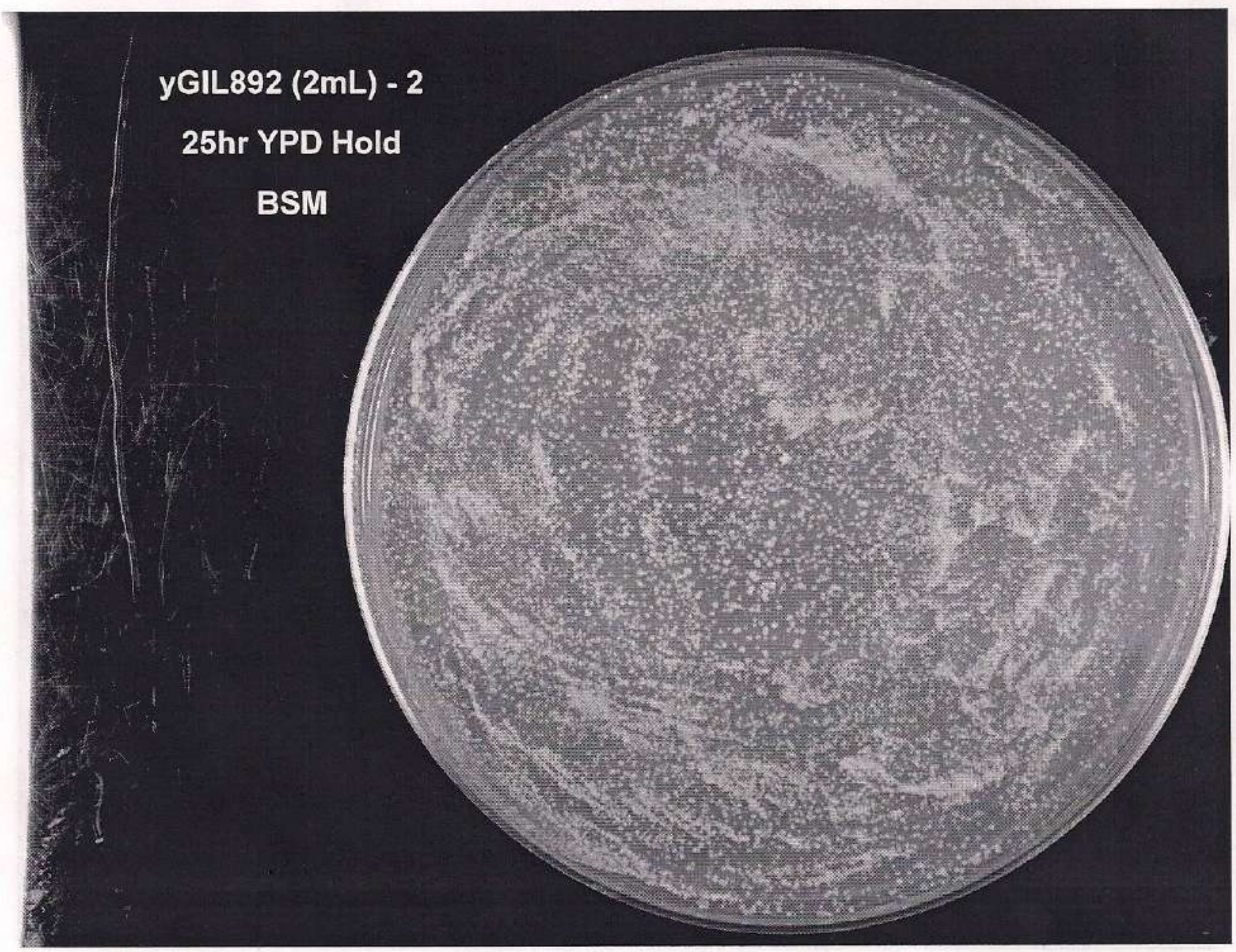

yGIL890 (2mL) - 1
25hr YPD Hold
BSM

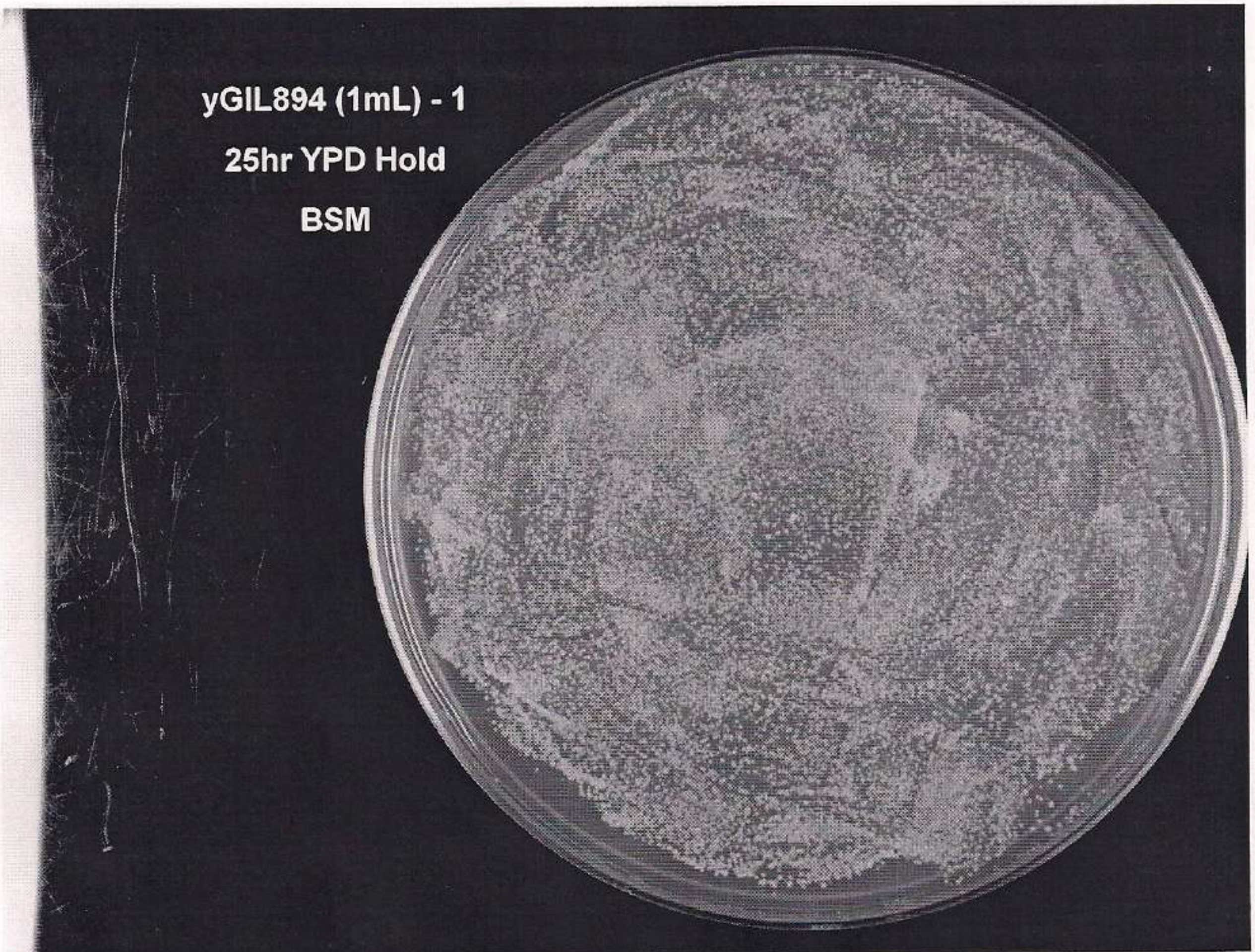

yGIL890 (2mL) - 2
25hr YPD Hold
BSM

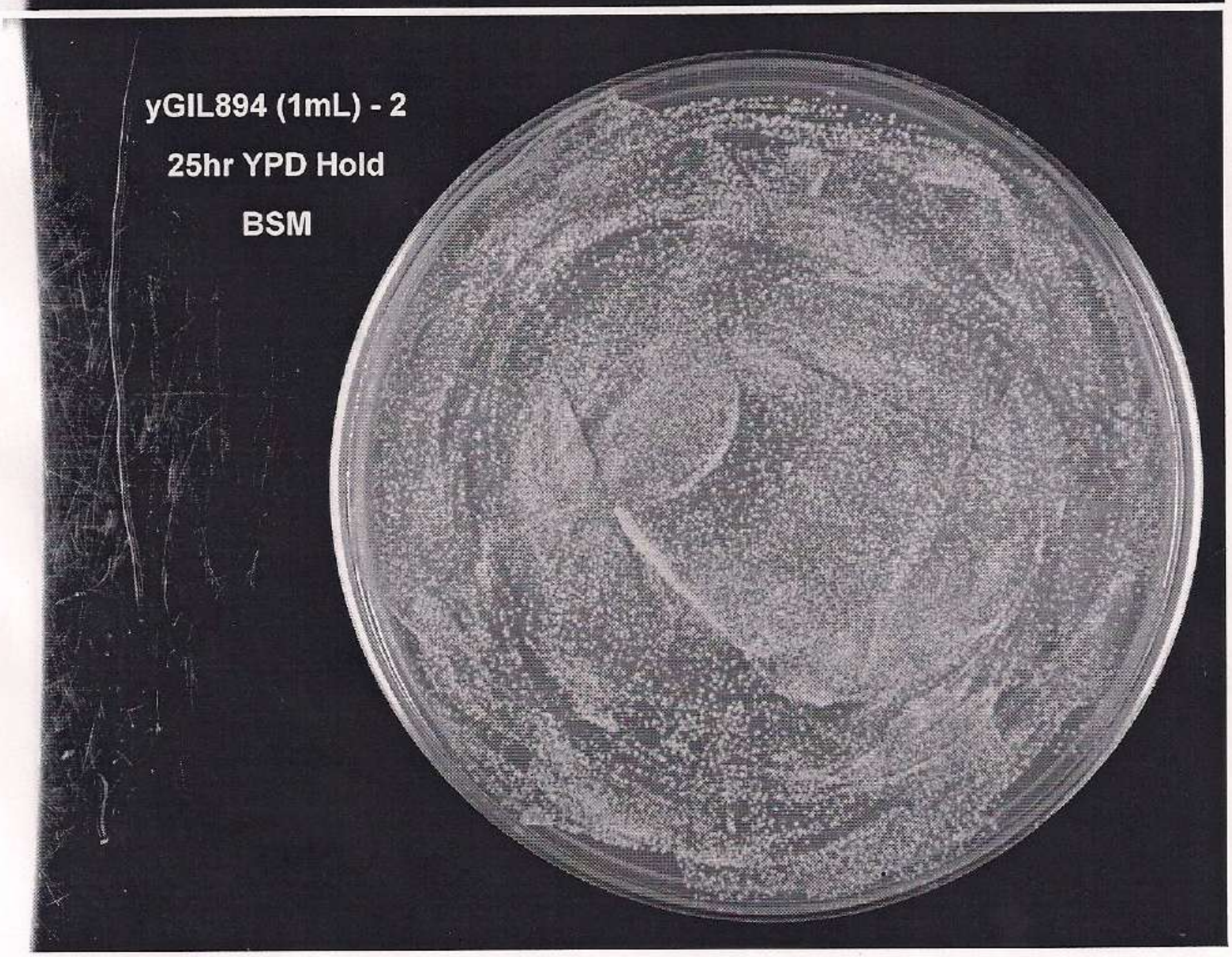

yGIL892 (1mL) - 1
25hr YPD Hold
BSM


yGIL892 (1mL) - 2
25hr YPD Hold
BSM

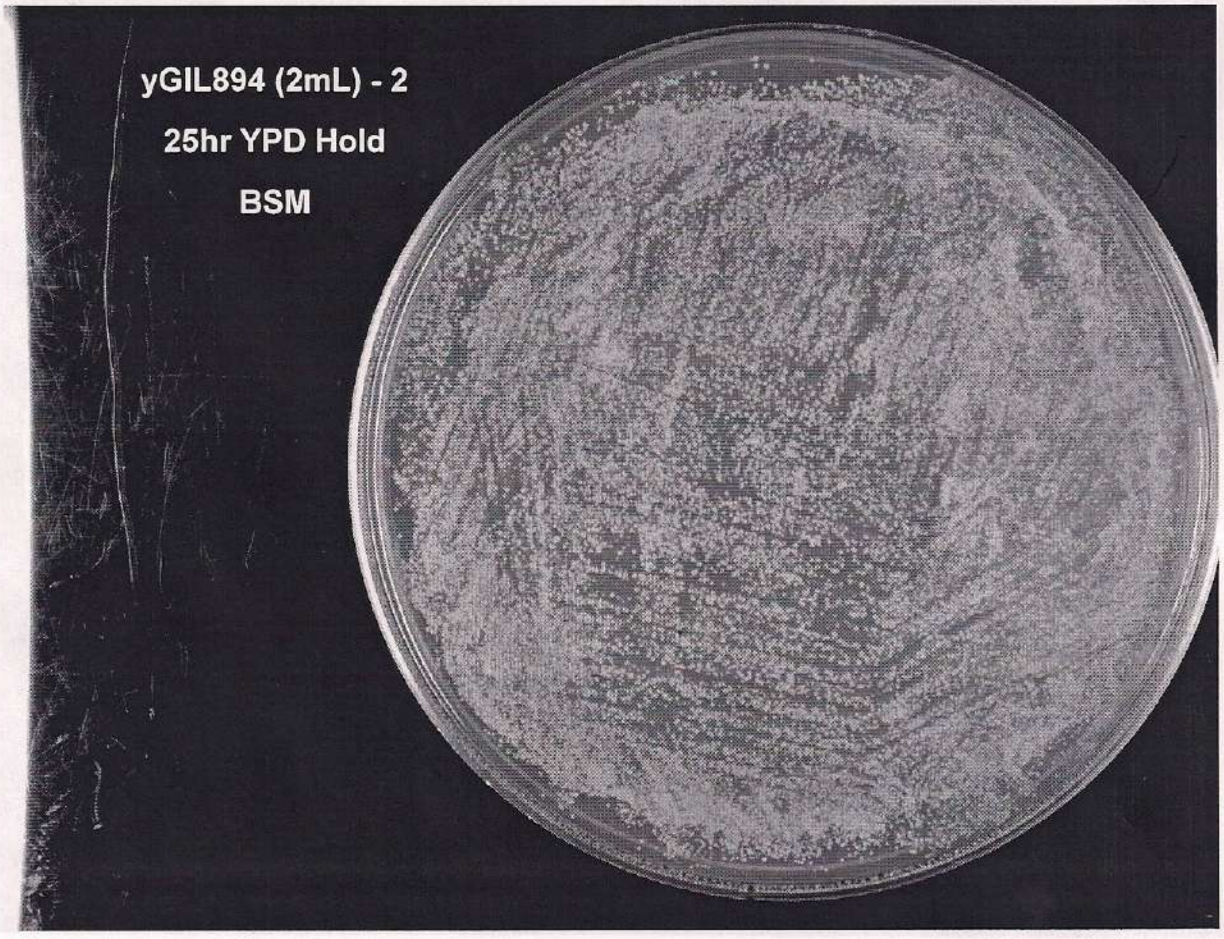

yGIL892 (2mL) - 1
25hr YPD Hold
BSM


yGIL892 (2mL) - 2
25hr YPD Hold
BSM

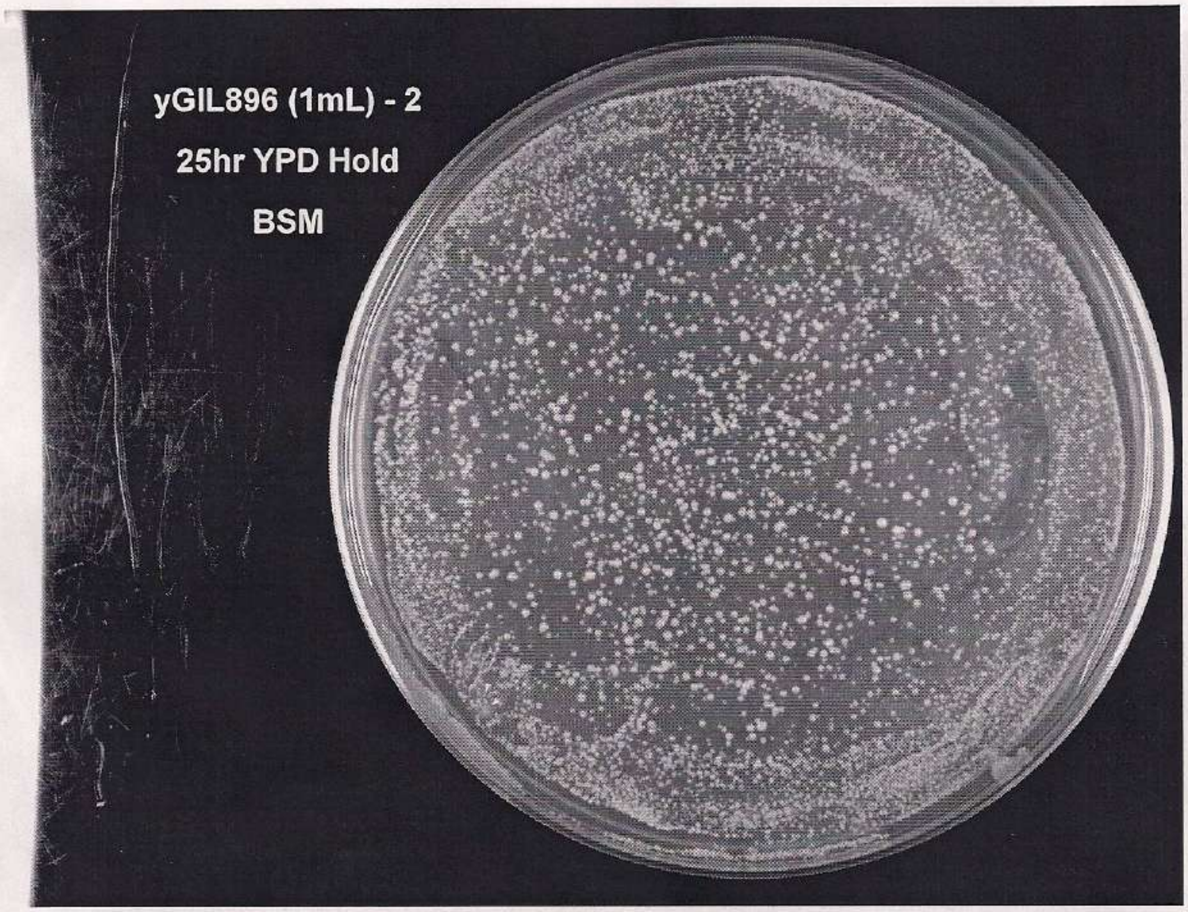

yGIL894 (1mL) - 1
25hr YPD Hold
BSM

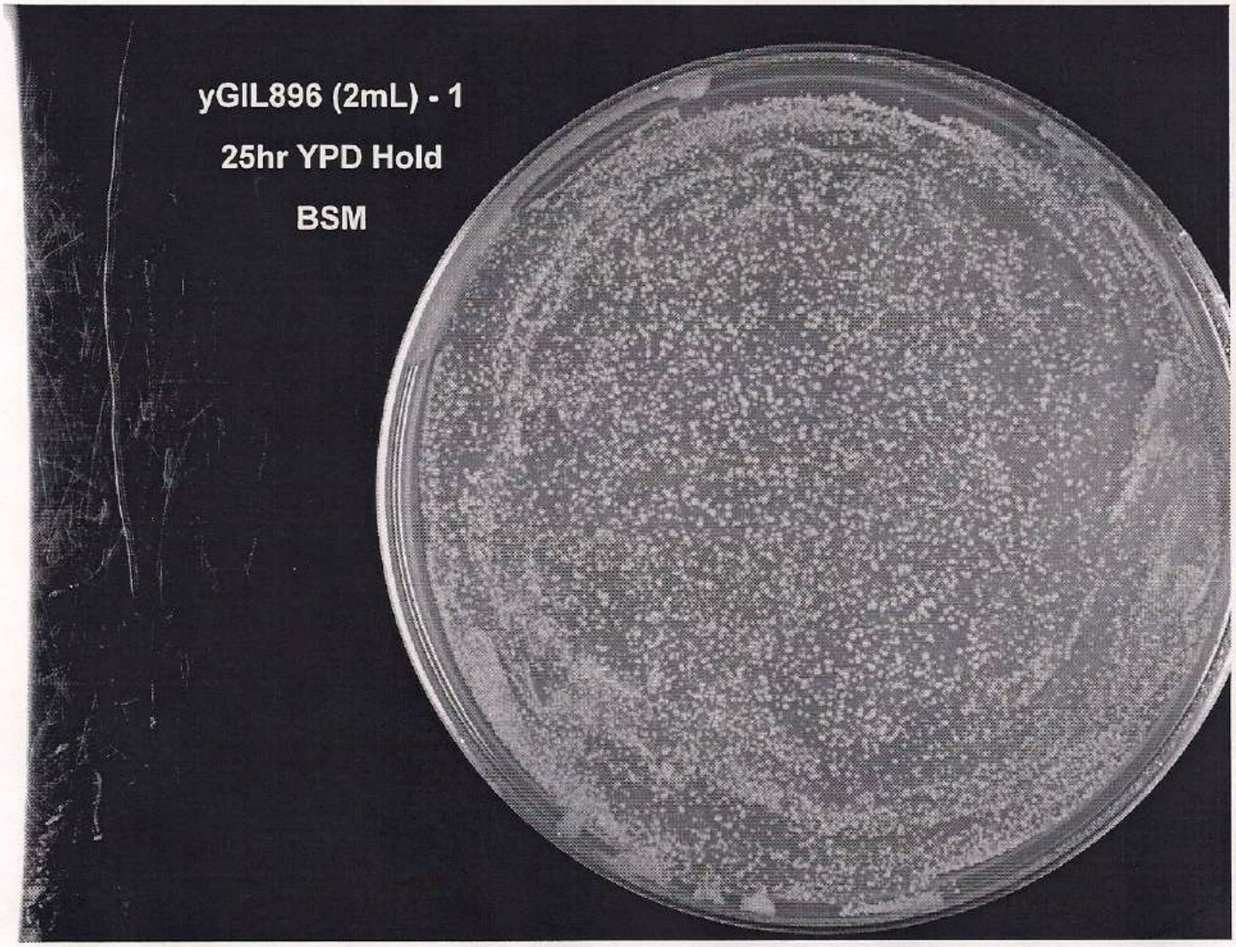

yGIL894 (1mL) - 2
25hr YPD Hold
BSM

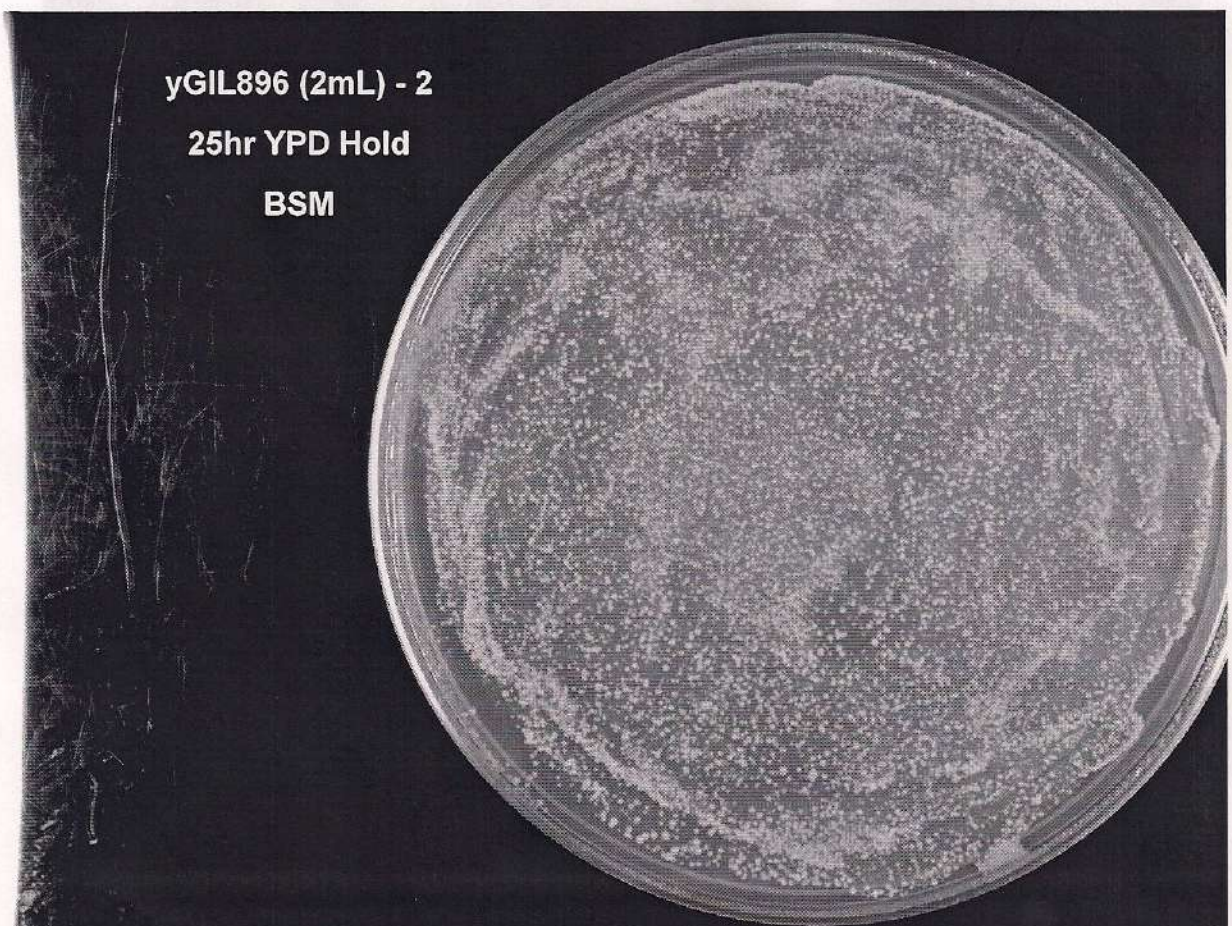

yGIL894 (2mL) - 1
25hr YPD Hold
BSM

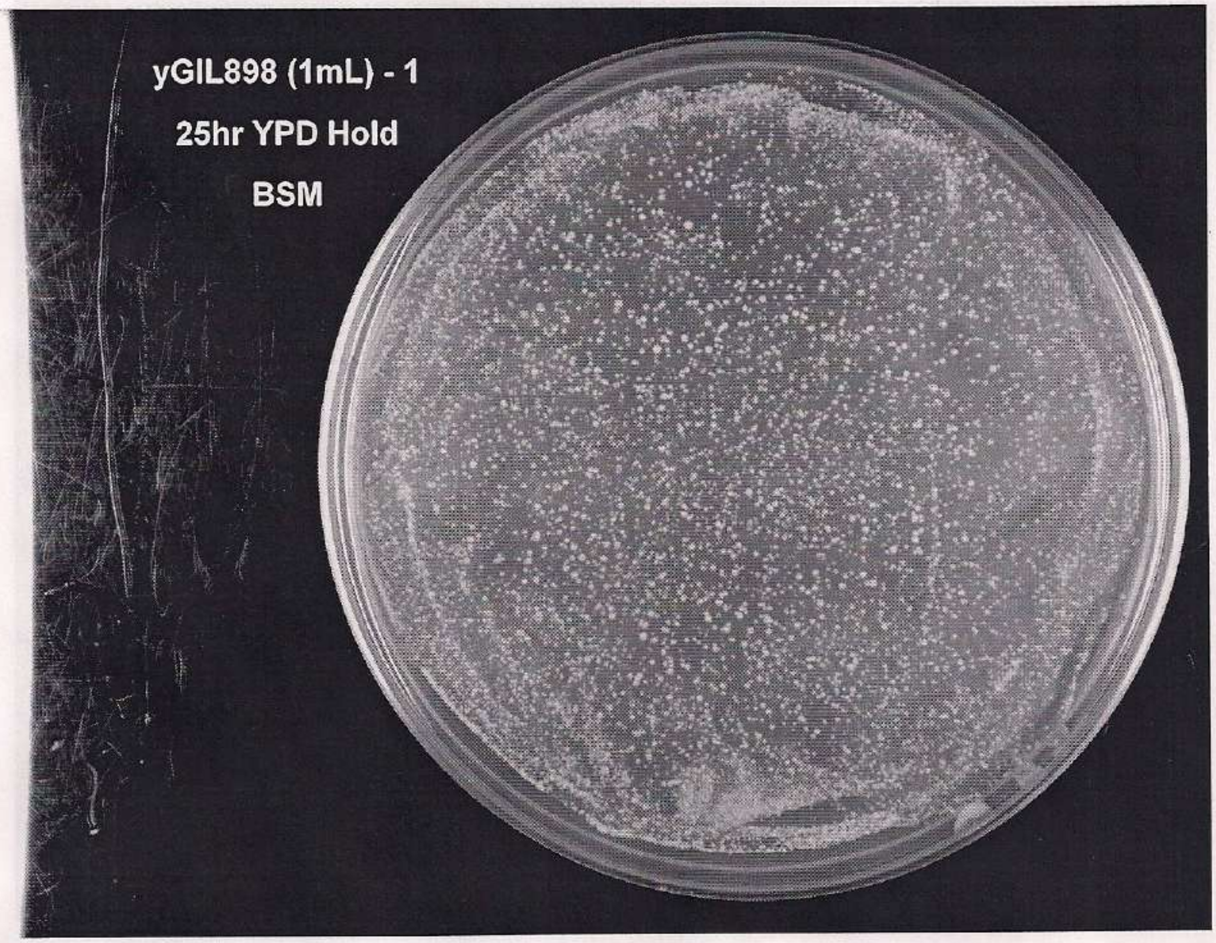

yGIL894 (2mL) - 2
25hr YPD Hold
BSM

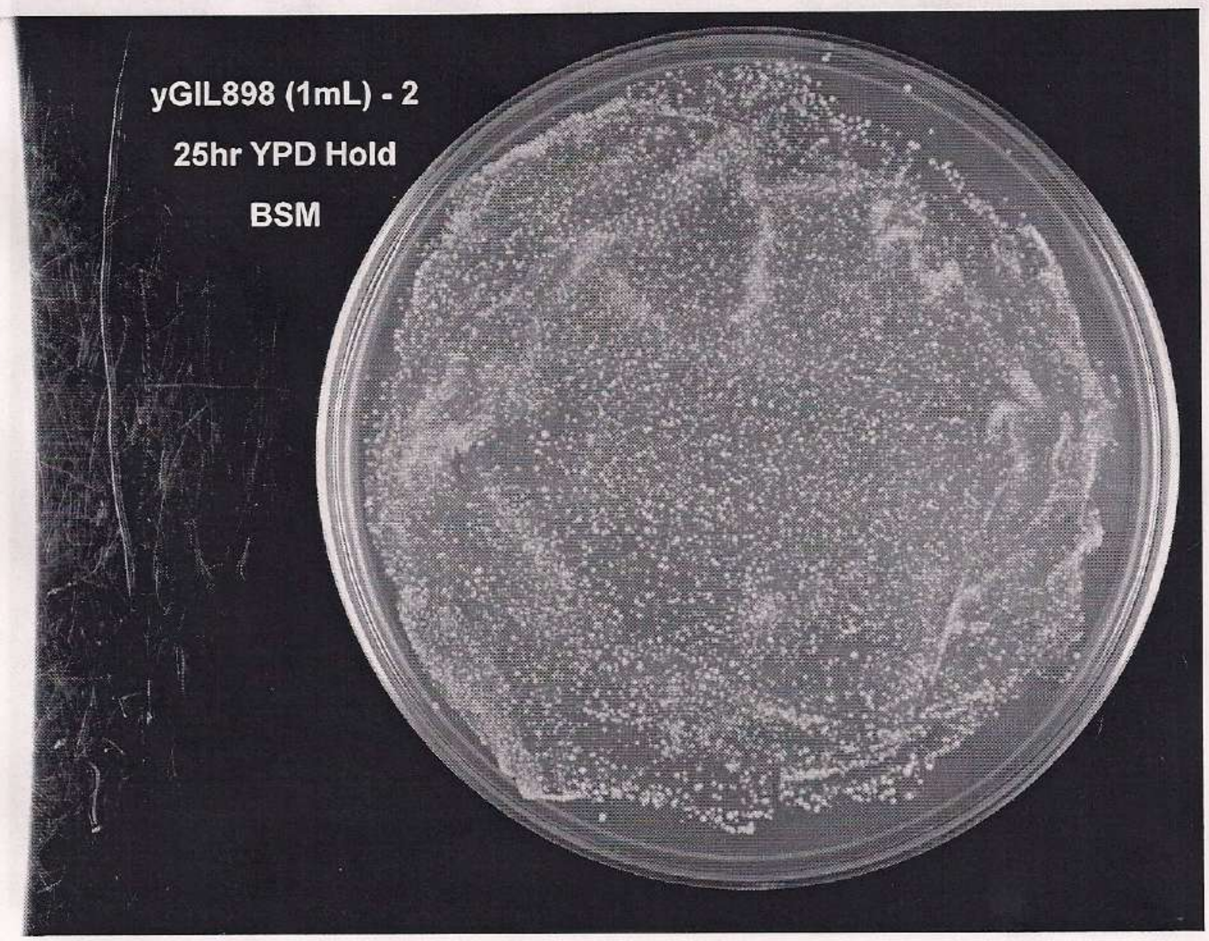

yGIL896 (1mL) - 1
25hr YPD Hold
BSM

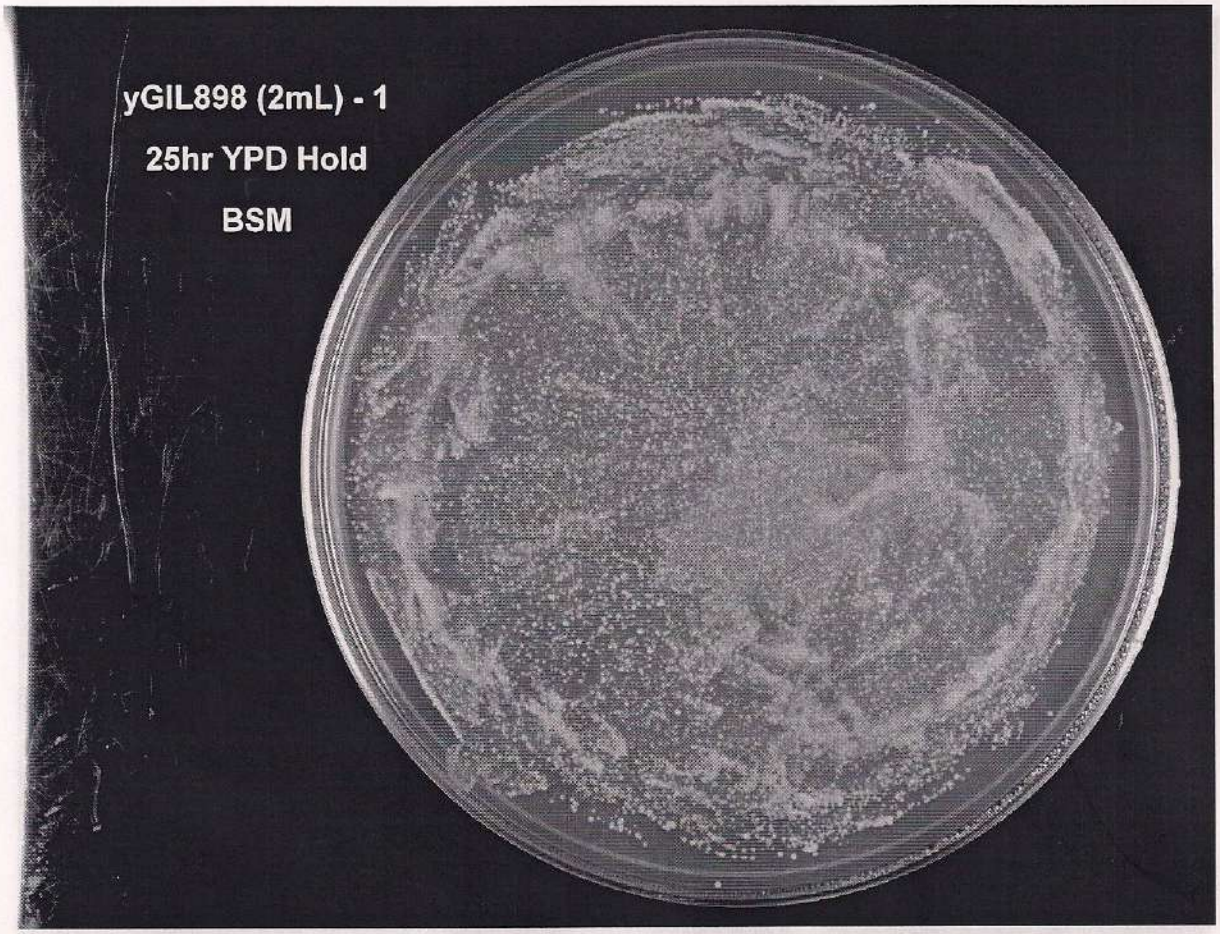

yGIL896 (1mL) - 2
25hr YPD Hold
BSM

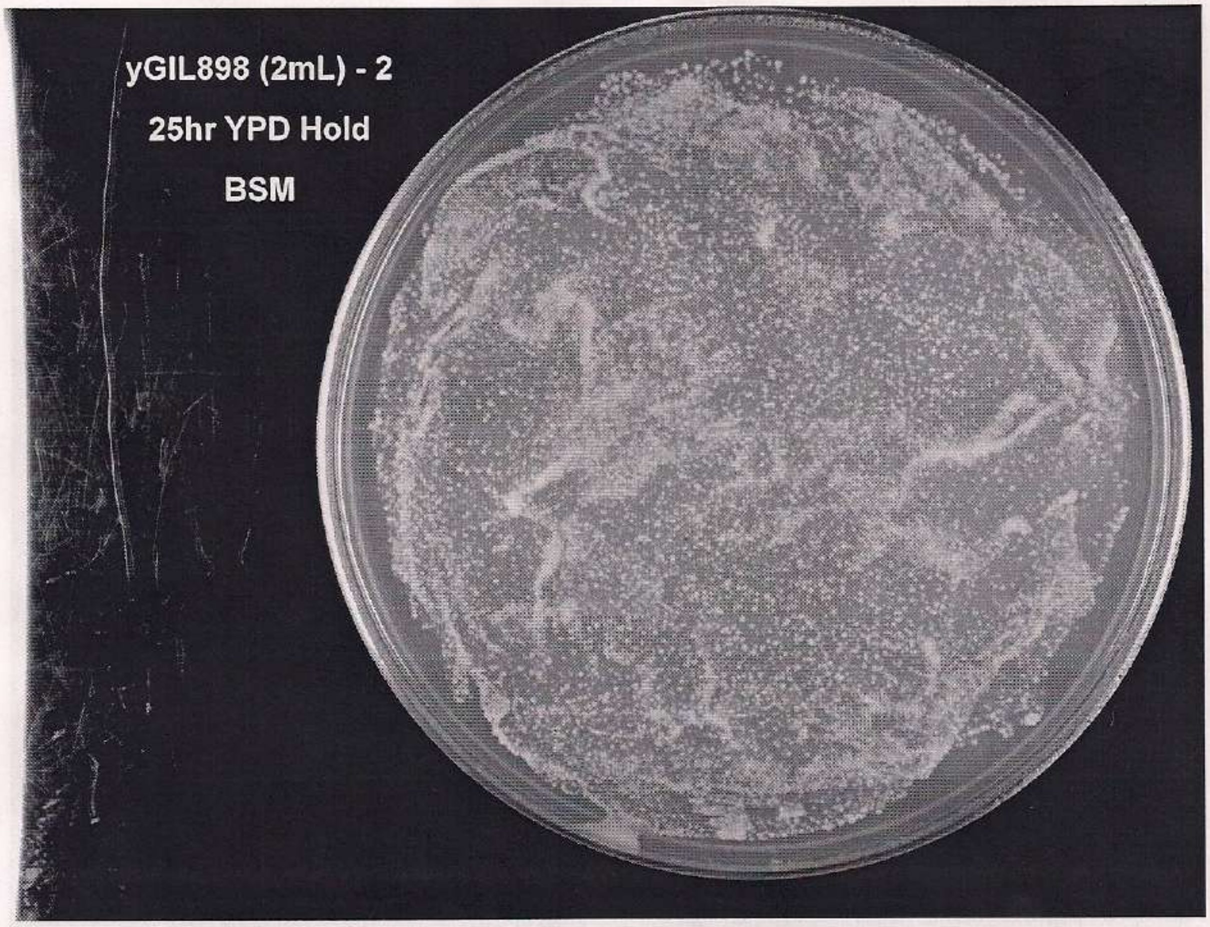

yGIL896 (2mL) - 1
25hr YPD Hold
BSM

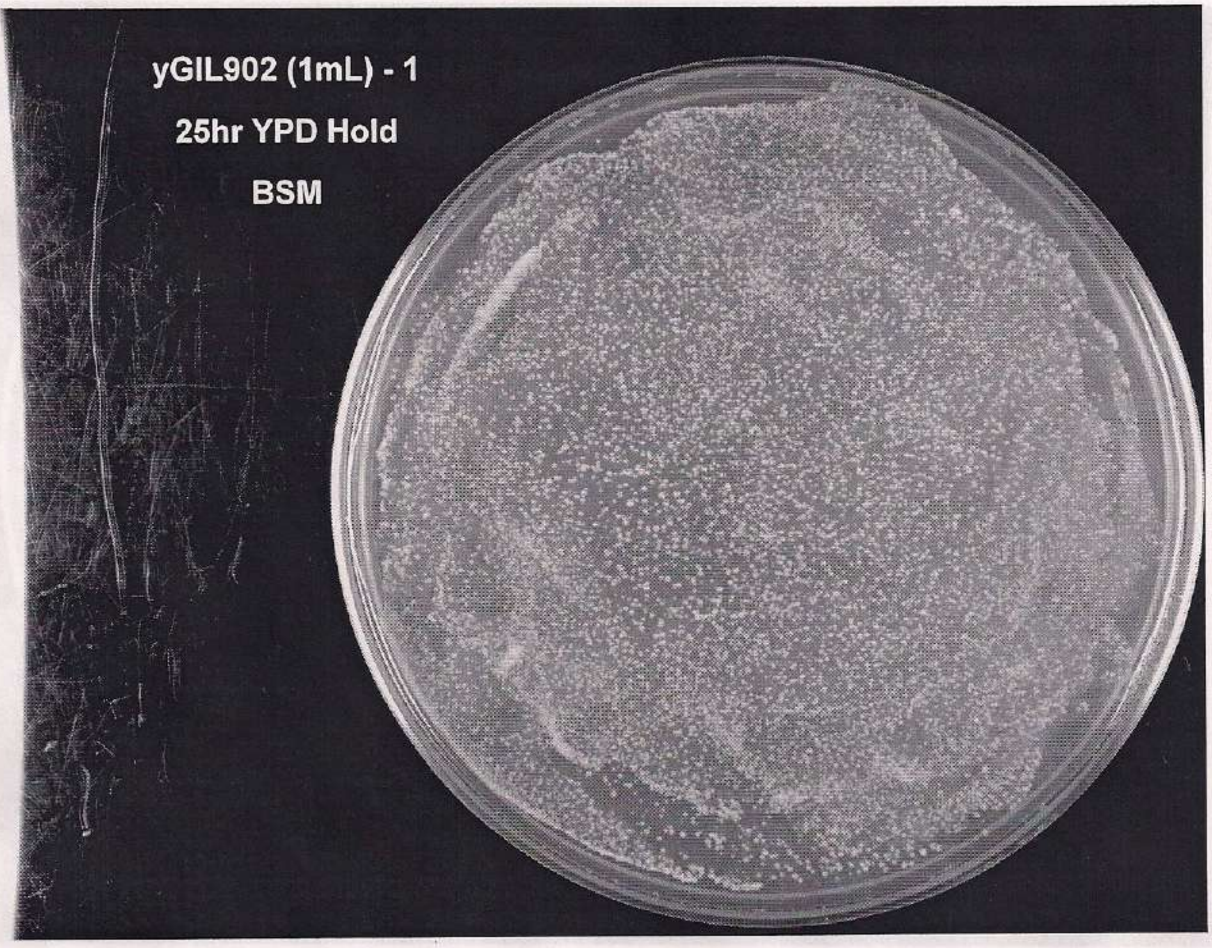

yGIL896 (2mL) - 2
25hr YPD Hold
BSM

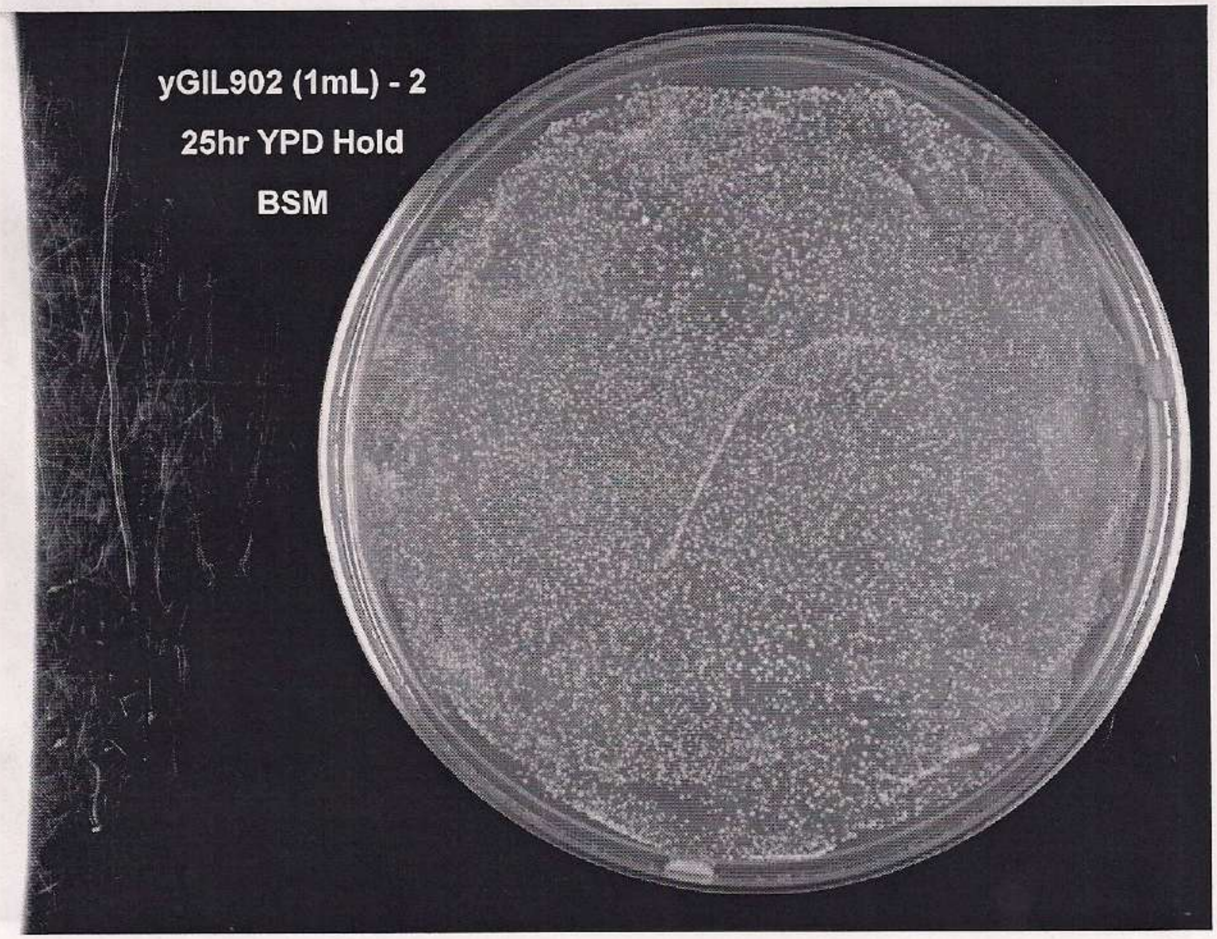

yGIL898 (1mL) - 1
25hr YPD Hold
BSM

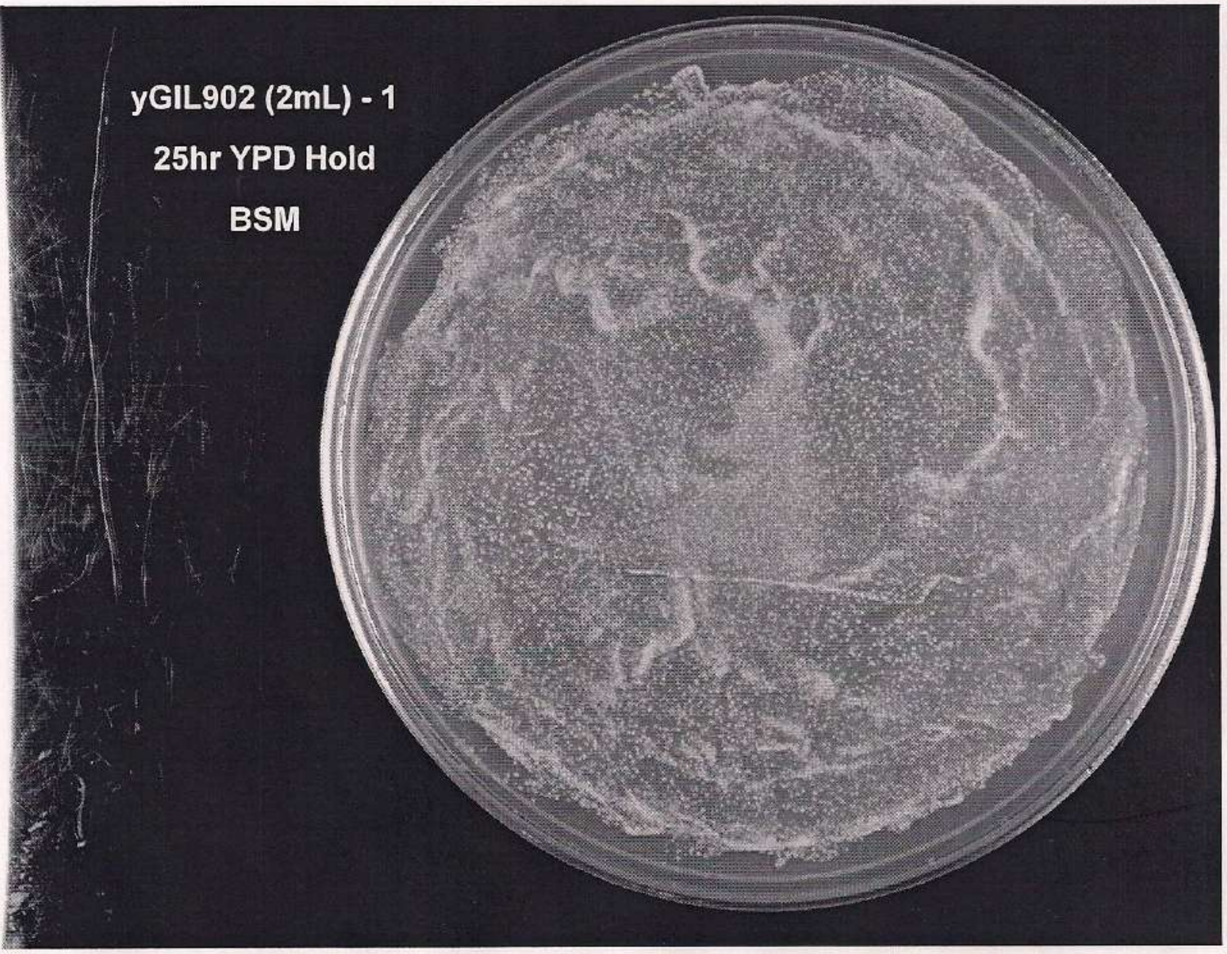

yGIL898 (1mL) - 2
25hr YPD Hold
BSM

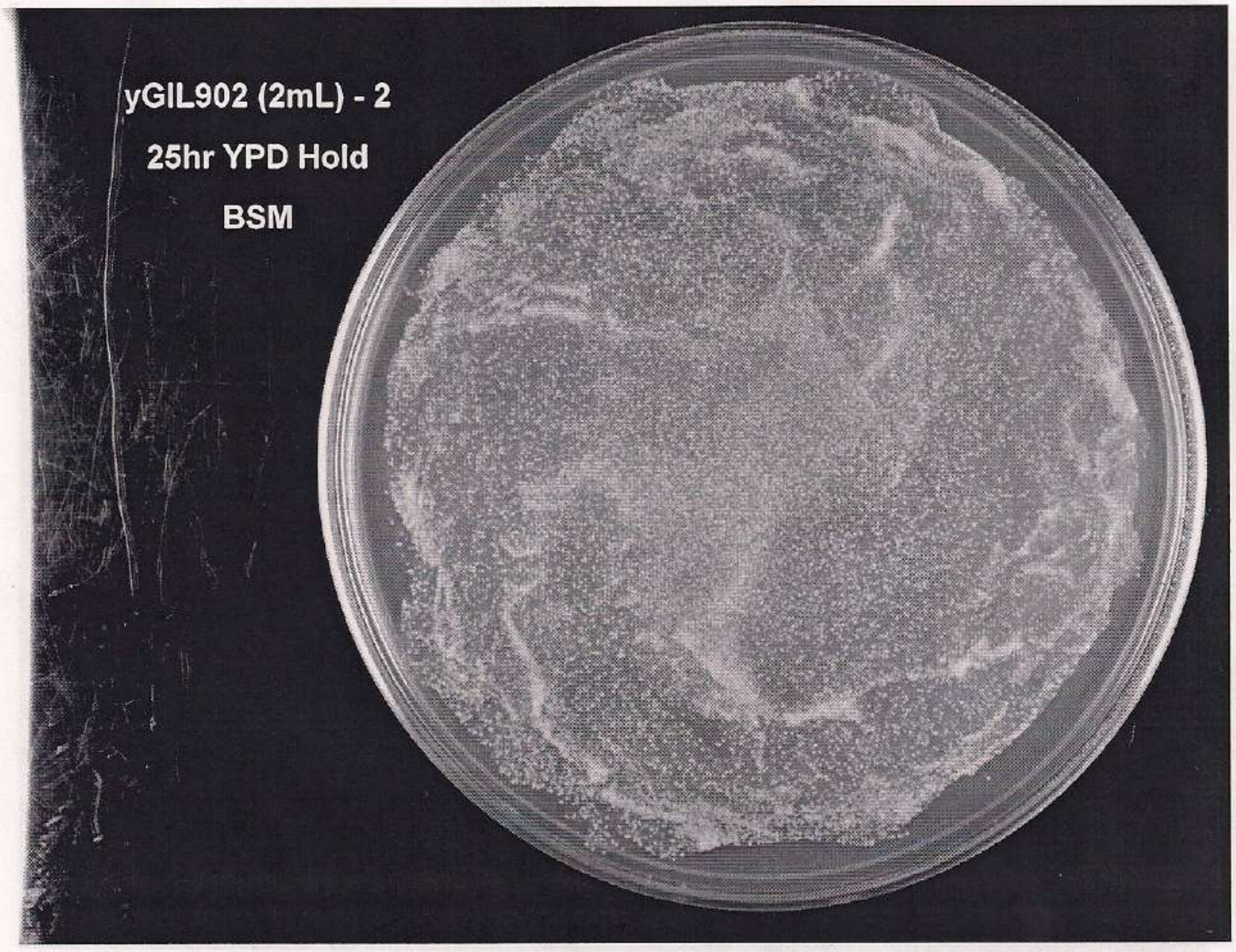

yGIL898 (2mL) - 1
25hr YPD Hold
BSM


yGIL898 (2mL) - 2
25hr YPD Hold
BSM


yGIL902 (1mL) - 1
25hr YPD Hold
BSM


yGIL902 (1mL) - 2
25hr YPD Hold
BSM


yGIL902 (2mL) - 1
25hr YPD Hold
BSM


yGIL902 (2mL) - 2
25hr YPD Hold
BSM

Day 1 BSP $\boxed{} \xrightarrow{8\mu L}$ Sm $\boxed{} \xrightarrow{4\mu L}$ Gen 0 $\boxed{}$

4/19/15

- Began BSP propagation w/ own YPD tips, YPD+A+T, and new transfer tips per generation.
  - Dispense @ 5PM (*Began thaw @ 4:30 PM)
  - Transfer @ 5:10 PM
  - Incubator @ 5:26 PM (1) 5:30 (2)

* Transfer tips for BSP 1 did not unload before moving to BSP 2 and dipped into BSP 2 & sucked up some of the pools before I got to the light curtain. It did not get to push liquid back into BSP 2 plate & had been diluted twice already so we ~~kep~~ fixed the program and got the contaminated tips ejected before continuing BSP 2 propagation. Contamination should be minimal, but still very upsetting :(

4/20/15

- Gen 0 → 10: Dispense: 5PM
  Transfer: 5:10 PM
  Incubator: 5:15 PM
  } pools = ~<1.5 mL pelleted in freezer

4/21/15: Gen 10 → 20 Incubator: 5:05 PM

4/22/15: Gen 20 → 30 Incubator: 5:04 PM

4/23/15: Gen 30 → 40 Incubator: 5:02 pm

4/24: Gen 40 → 50 @ 5:03 PM

4/25: Gen 50 → 60 @ 5:04 PM

4/26: Gen 60 → 70 @ 5:04 PM.

4/29: Gen 90 → 100 @ 5:04 PM * pooled later due to symposium @ 5:50 PM (plates were in fridge)

4/30/15

- Greg forgot to do an ~~RNASE~~ RNase step like we've done in past genomic preps, ~~but we only ha~~ (pg 45)
    - all samples are 50uL and we usually add 5uL RNase to 250uL and we only have ~250uL RNase 100 mg/mL ~~80~~ for 70 samples, so we will add that to 11mL water and add ~~150m~~ 150uL of that to each 50uL sample
    - added 20uL 3M sodium acetate before phenol chloroform

5/1/15

- diluted all gDNA to 2.5 ng/uL (see pg. 148)

5/2/15

- Did Library Preps (pg 149 - 152) on pools 3-16 @ 0, 20, 40, 60, & 80 generations

5/4/15

- Quantified the DNA in our preps by Qubit and Bioanalyzer (summary on pg. 163).

VEBO

Quick yeast genomic preparation (12/18/08)

Grow overnight culture

Spin down 1 ml of saturated overnight culture. *Optional: wash once with 1 ml H_2O.*

Resuspend cells in 200 ul of Lysis buffer.

Add ~0.2 g Glass beads

Add 200 ul of Phenol/Chloroform

Vortex for 10 min.

Spin 5 min at top speed. *Alternative: Dump contents into a phase lock tube (Light) to aid in separation of organic and aqueous layers.*

Remove ~~150 ul~~ ~200 of aqueous layer and add to ~~300 ul~~ 400 of ethanol. Precipitate DNA in –20° C freezer for at least 30 min.

Pellet DNA by spinning for 10 minutes at top speed. Decant or aspirate ethanol.

Add 1 ml of 70% ethanol, vortex, and spin for 2 min at top speed. Decant or aspirate ethanol.

Dry DNA pellet in speed-vac for ~10 min. Resuspend pellet in 50 ul of ~~TE~~. ddH_2O.

Set 1: 3-00 → 16-00
(24) 3-20 → 11-20

Set 2: 12-20 → 16-20
(46) 3-40 → 16-40
3-60 → 16-60
3-80 → 16-80

Lysis Buffer
2% Triton X-100
1% SDS
100 mM NaCl
10 mM Tris-Cl, pH 8.0
1 mM EDTA

To make 50 ml of Lysis Buffer
40.9 ml H_2O
5 ml of 20% Triton X-100
2.5 ml of 20% SDS
1 ml of 5 M NaCl
500 ul of 1 M Tris-Cl, pH 8.0
100 ul of 0.5 M EDTA

Set 1 in -20 @ 9:20 am → 1:30 pm ; Set 2 in -20 @ 11:00 am → 2:00 pm

11-40 leaked, I added additional 50 λ Lysis Buffer & 50 λ P/C before vortex

MA

gDNA dilutions

Pool-Generation	Before RNase Nanodrop (ng/ul)	After RNase Nanodrop (ng/ul)	After RNase Qbit (ng/ul)	Sample Vol.	Volume of H2O	Conc. (ng/ul)
03-00	2430.2	948.3	6.84	5	8.7	2.5
04-00	2986.2	1260.7	6	5	7	2.5
05-00	2882.3	1266.9	5.7	5	6.4	2.5
06-00	2970.3	1137.6	8.72	5	12.4	2.5
07-00	3907.1	1435.8	6.66	5	8.3	2.5
08-00	2963.6	1392.1	9	5	13	2.5
09-00	3325.4	1320.4	7.58	5	10.2	2.5
10-00	3390.7	1628.2	5.84	5	6.7	2.5
11-00	2991.5	1487.8	7.88	5	10.8	2.5
12-00	3073.9	1440.6	5.8	5	6.6	2.5
13-00	3016.9	1505.0	4.68	5	4.4	2.5
14-00	3153.2	1192.2	5.5	5	6	2.5
15-00	3346.1	1702.3	5.8	5	6.6	2.5
16-00	3411.4	1742.7	5.46	5	5.9	2.5
03-20	2891.2	1428.5	11.44	5	17.9	2.5
04-20	4002.5	1660.7	7.98	5	11	2.5
05-20	2990.6	1538.8	9.9	5	14.8	2.5
06-20	2706.2	1332.2	11.04	5	17.1	2.5
07-20	3071.4	1655.1	9.44	5	13.9	2.5
08-20	2857.3	1363.9	13.54	5	22.1	2.5
09-20	3026.1	1504.8	8.44	5	11.9	2.5
10-20	3037.3	1348.0	6.54	5	8.1	2.5
11-20	2858.4	1427.3	10.76	5	16.5	2.5
12-20	3422.8	1608.2	6	5	7	2.5
13-20	2314.2	966.9	5.42	5	5.8	2.5
14-20	2320.4	966.3	5.04	5	5.1	2.5
15-20	2290.4	945.4	3	5	1	2.5
16-20	2507.8	935.8	3.34	5	1.7	2.5
03-40	2206.3	887.4	5.52	5	6	2.5
04-40	2949.5	1297.7	3.92	5	2.8	2.5
05-40	2393.5	1041.1	4.64	5	4.3	2.5
06-40	2190	918.0	5.66	5	6.3	2.5
07-40	2391.9	949.6	3.82	5	2.6	2.5
08-40	1940.1	695.9	5.64	5	6.3	2.5
09-40	2352.7	1048.1	4.34	5	3.7	2.5
10-40	2806.4	1213.1	4.54	5	4.1	2.5
11-40	1370.3	428.8	1.386	5	0	1.4
12-40	2844.4	1337.7	3.54	5	2.1	2.5
13-40	2256.4	909.0	3.98	5	3	2.5
14-40	2318.2	1071.7	4.9	5	4.8	2.5
15-40	2377.5	1045.7	2.56	5	0	2.6
16-40	2650.9	1163.8	2.74	5	0.5	2.5
03-60	2107.6	978.1	5.04	5	5.1	2.5
04-60	2560.3	1174.9	2.58	5	0	2.6
05-60	2041.6	999.0	2.38	5	0	2.4
06-60	1974.8	865.6	3.68	5	2.4	2.5
07-60	2387.6	1083.3	3.26	5	1.5	2.5
08-60	2098.2	952.6	5.16	5	5.3	2.5
09-60	2155	912.4	2.92	5	0.8	2.5
10-60	1916.6	822.9	1.954	5	0	2.0
11-60	2249.7	883.4	4.54	5	4.1	2.5
12-60	2622.8	1132.9	2.44	5	0	2.4
13-60	2316	1056.0	4.78	5	4.6	2.5
14-60	1915.4	815.6	2.94	5	0.9	2.5
15-60	2916.6	1261.5	3.38	5	1.8	2.5
16-60	2327.6	1079.7	2.52	5	0	2.5
03-80	2236.9	1076.7	5.66	5	6.3	2.5
04-80	2344.2	1083.6	2.4	5	0	2.4
05-80	2642.4	1262.4	3.46	5	1.9	2.5
06-80	1920.7	785.1	2.86	5	0.7	2.5
07-80	2652.8	1339.7	4.84	5	4.7	2.5
08-80	2094.1	920.6	4.9	5	4.8	2.5
09-80	2119.1	1028.0	3.14	5	1.3	2.5
10-80	2441.3	1099.9	2.96	5	0.9	2.5
11-80	2034.7	840.6	4.84	5	4.7	2.5
12-80	2676.7	1024.9	2.88	5	0.8	2.5
13-80	2054.4	914.5	3.76	5	2.5	2.5
14-80	2152.7	965.3	4.34	5	3.7	2.5
15-80	2576.7	1267.5	3.24	5	1.5	2.5
16-80	2887.3	1119.8	3.62	5	2.2	2.5

ILLUMINA SEQUENCING LIBRARY PREPARATION

14 pools at 5 time points (Gen 0, 20, 40, 60, and 80)= 70 samples

703 → 712

N5's	N7's			(Pools 3-9)		N7's		(Pools 10-16)				
502											X	X
											X	X
											X	X
											X	X
											X	X
											X	X
508											X	X
	X	X	X	X	X	X	X	X	X	X	X	X

TAGMENTATION

Tagmentation Master Mix (TMM):
- 70 x 1.05 x 1.25λ = **91.88λ TD Buffer**
- 70 x 1.05 x 0.25λ = **18.38λ TDE1**
- Mix thoroughly by gently pipetting 20 times
- Distribute into 7 PCR tubes (or 1 PCR strip) with 10 x 1.2 x 1.5λ = **18λ into each tube**
- Use a multichannel to distribute each TMM tube into the first 10 columns with **1.5λ per well** ("tagmentation plate)

Transfer 1λ of gDNA (at or below 2.5 ng/λ) into the tagmentation plate/strips
**Dot gDNA above the tagmentation mix, then vortex right before thermocycler

Cover with Microseal 'B' and do a quick spin to get all liquid to bottom

Thermocycler: ("Nextera 1")
- 55°C for 10 mins
- Hold at 4°C

PCR & RECONDITIONING

Thaw KAPA polymerase mix and indices at room temp and vortex to mix. (Recommended to store the indices in strips so they are accessible with a multichannel)

Make 7 Row Master Mixes (RMMs):
- 10 x 1.2 x 1.88λ = **22.56λ 2x KAPA master mix**
- 10 x 1.2 x 0.625λ = **7.5λ of each of the N5xx indices** in each of the 7 PCR tubes

**Note which tube gets which index
- Mix thoroughly by gently pipetting 20 times

Make 10 Column Master Mixes (CMMs):
- 7 x 1.2 x 1.88λ = **15.8λ 2x KAPA master mix**
- 7 x 1.2 x 0.625λ = **5.25λ of each of the N7xx indices** in each of the 10 PCR tubes
  **Note which tube gets which index
- Mix thoroughly by gently pipetting 20 times

Use a multichannel to transfer **2.5λ of CMMs in each well** of the plate so that each column receives the same N7xx index

Use a multichannel to transfer **2.5λ of RMMs in each well** of the plate so that each row receives the same N5xx index
**** Total volume = 7.5λ**

Cover plate with Microseal 'A' and do a quick spin to get all liquid to bottom

Thermocycler: ("Nextera 2")
- 72°C for 3 mins
- 98°C for 5 mins
- 98°C for 10 sec
- 62°C for 30 sec
- 72°C for 30 sec
- Repeat steps (3-5) 8 times
- 72°C for 5 mins
- Hold at 4°C

Make Reconditioning PCR Master Mix (RPMM)
- 70 x 1.1 x 8.5λ = **654.5λ 2x KAPA master mix**
- 70 x 1.1 x 0.5λ = **38.5λ primer P1**
- 70 x 1.1 x 0.5λ = **38.5λ primer P2**
  **1:10 dilution of primers P1 and P2 before use
- Mix thoroughly by gently pipetting 20 times

Distribute RPMM into 7 tubes with 10 x 1.1 x 9.5λ = **104.5λ in each tube**

Use a multichannel to transfer **9.5λ of RPMM into each** well of the plate and mix thoroughly by gently pipetting 10 times
****Final PCR volume: 17λ**

Cover plate with Microseal 'A' and do a quick spin to get all liquid to bottom

Thermocycler: ("Nextera 3")
- 95°C for 5 mins
- 98°C for 20 secs
- 62°C for 20 secs

- 72°C for 30 secs
- Repeat steps (2-4) 4 times
- 72°C for 30 secs
- Hold at 4°C

PCR CLEAN UP & SIZE SELECTION

Bring AMPure XP beads to room temp and prepare 100mL of 70% EtOH and give the strips a quick spin to get the liquid to the bottom

Vortex the beads for 30 sec to ensure they are evenly dispersed

Transfer **17λ of beads** into new 96-well plate

Add the PCR product to corresponding wells and mix thoroughly by gently pipetting 20 times

**Use a P20 with a 1-2λ on a 20-200λ tip in order to get as much of the PCR product as possible

Incubate at room temp for 5 minutes. DNA is now on the beads

START BIOMEK:

Place the plate on the magnetic stand and incubate for about 1 minute to separate beads from solution

Aspirate the clear solution from the plate and discard

** Do not disturb the beads! If beads are accidentally pipetted, resuspend them back and wait for solution to clear up and repeat.

While the plate is still on the magnetic stand, dispense 200λ **70% EtOH (100mL in reservoir)** into each well and incubate for 30 seconds at room temp

Aspirate out EtOH with out disturbing the beads and discard

Repeat for a total of 2 washes

Remove any remaining EtOH with a P10 pipette

Let the plate air dry for approximately 5 mins, do not over dry the beads

Take the plate off the magnetic stand and add 33λ **10 mM Tris-HCl (pH8) (50mL in reservoir)** to each well of the plate

Carefully resuspend the beads by mixing 10-15 times and incubate at room temp for 2 min, the DNA is now in solution

Place the plate back on the magnetic stand and incubate for about 1 min to separate beads from solution, wait for solution to become clear

While the plate is on the magnetic stand, aspirate the clear solution from the plate and transfer to a fresh plate

** Do not disturb the beads! If beads are accidentally pipetted, resuspend them back and wait for solution to clear up and repeat.

Assay Class: High Sensitivity DNA Assay
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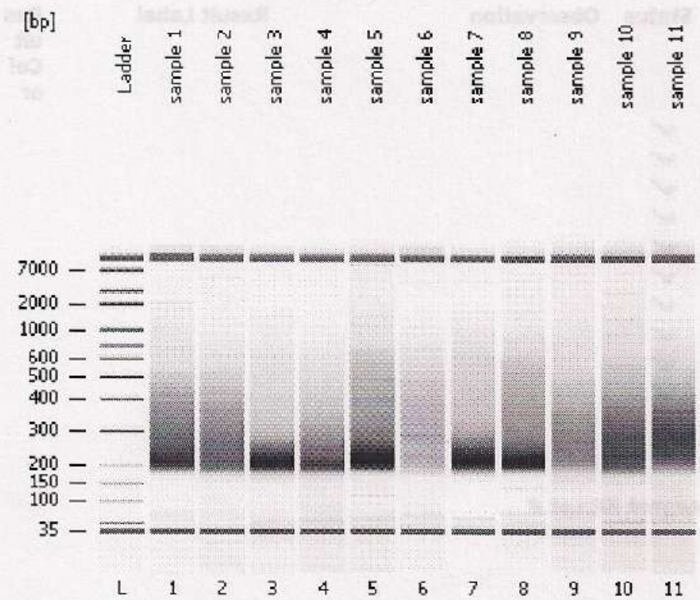
Created: 5/4/2015 4:48:09 PM
Modified: 5/4/2015 5:29:29 PM

Electrophoresis File Run Summary



Instrument Information:

Instrument Name: DE34903215
Firmware: C.01.069
Serial#: DE34903215
Type: G2938C

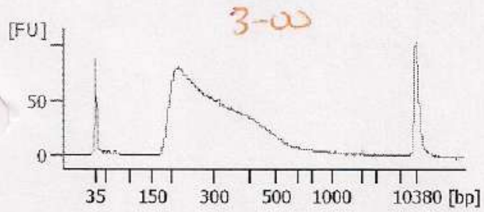
Assay Information:

Assay Origin Path: C:\Program Files\Agilent\2100 bioanalyzer\2100 expert\assays\dsDNA\High Sensitivity DNA.xsy
Assay Class: High Sensitivity DNA Assay
Version: 1.03
Assay Comments: Copyright © 2003-2010 Agilent Technologies

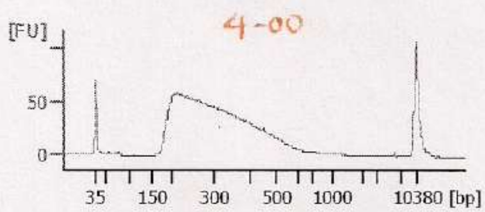
Chip Information:

Chip Lot #:
Reagent Kit Lot #:
Chip Comments:

sample 1



sample 2



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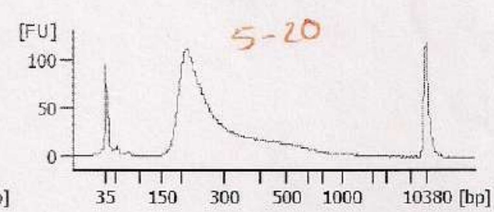

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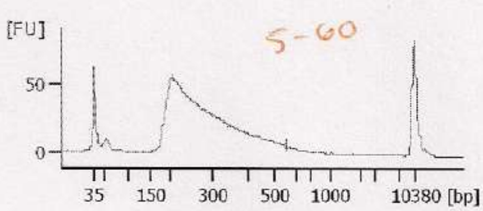

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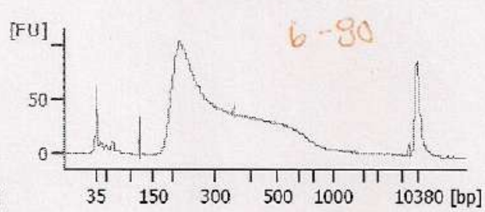

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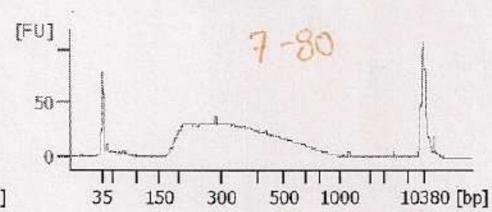

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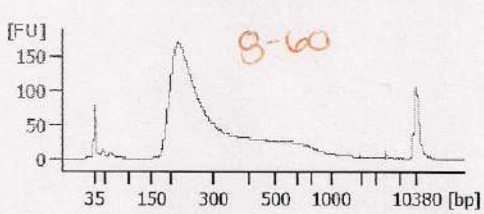

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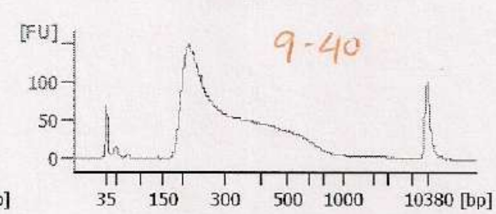
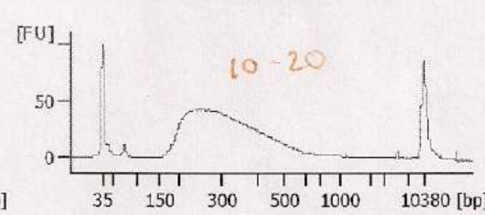
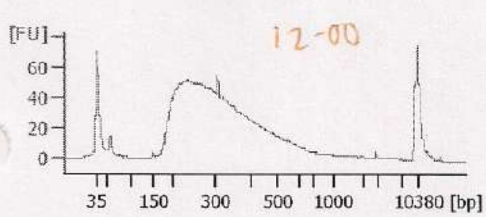


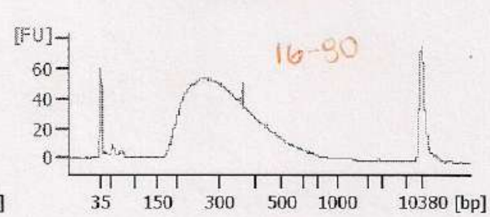
sample 9



sample 10



sample 11



Assay Class: High Sensitivity DNA Assay
Data Path: C:\...gh Sensitivity DNA Assay_DE34903215_2015-05-04_16-48-09.xad

Created: 5/4/2015 4:48:09 PM
Modified: 5/4/2015 5:29:29 PM

Electrophoresis File Run Summary (Chip Summary)

Sample Name	Sample Comment	Rest. Digest	Status	Observation	Result Label	Result Color
sample 1		☐	✓			
sample 2		☐	✓			
sample 3		☐	✓			
sample 4		☐	✓			
sample 5		☐	✓			
sample 6		☐	✓			
sample 7		☐	✓			
sample 8		☐	✓			
sample 9		☐	✓			
sample 10		☐	✓			
sample 11		☐	✓			
Ladder		☐	✓			

Chip Lot # **Reagent Kit Lot #**

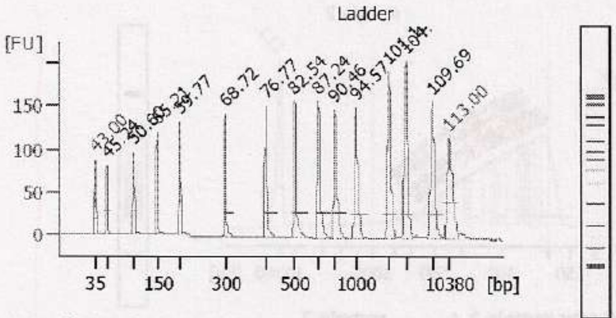
Chip Comments :

184

Assay Class: High Sensitivity DNA Assay
Data Path: C:\...gh Sensitivity DNA Assay_DE34903215_2015-05-04_16-48-09.xad

Created: 5/4/2015 4:48:09 PM
Modified: 5/4/2015 5:29:29 PM

Electropherogram Summary



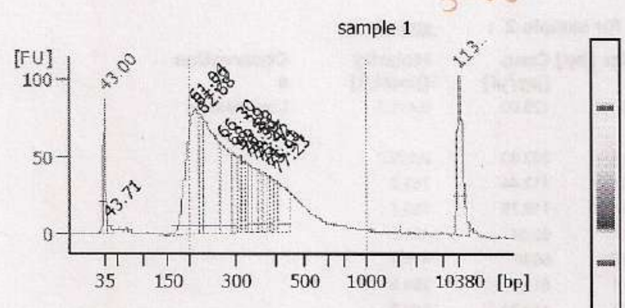
Overall Results for Ladder

Noise: 0.2

Peak table for Ladder

Peak	Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]	Observations
1	35	125.00	5,411.3	Lower Marker
2	50	150.00	4,545.5	Ladder Peak
3	100	150.00	2,272.7	Ladder Peak
4	150	150.00	1,515.2	Ladder Peak
5	200	150.00	1,136.4	Ladder Peak
6	300	150.00	757.6	Ladder Peak
7	400	150.00	568.2	Ladder Peak
8	500	150.00	454.5	Ladder Peak
9	600	150.00	378.8	Ladder Peak
10	700	150.00	324.7	Ladder Peak
11	1,000	150.00	227.3	Ladder Peak
12	2,000	150.00	113.6	Ladder Peak
13	3,000	150.00	75.8	Ladder Peak
14	7,000	150.00	32.5	Ladder Peak
15	10,380	75.00	10.9	Upper Marker

3-00



Overall Results for sample 1 : sample 1

Number of peaks found: 14
Noise: 0.3
Corr. Area 1: 1,838.0

Region table for sample 1 : sample 1

From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]	Color
200	1,000	1,838.0	88	333	36.6	1,789.20	9,316.7	

185

Assay Class: High Sensitivity DNA Assay
Data Path: C:\...gh Sensitivity DNA Assay_DE34903215_2015-05-04_16-48-09.xad
Created: 5/4/2015 4:48:09 PM
Modified: 5/4/2015 5:29:29 PM

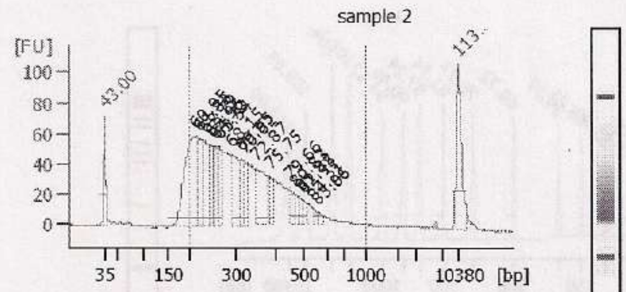
Electropherogram Summary Continued ...

3-00

Peak table for sample 1 : **sample 1**

Peak	Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]	Observations
1	35	125.00	5,411.3	Lower Marker
2	40	14.14	539.2	
3	214	417.96	2,956.7	
4	223	123.42	840.1	
5	232	345.97	2,254.9	
6	273	162.45	901.6	
7	295	88.61	454.7	
8	314	43.51	209.6	
9	326	36.54	169.8	
10	333	44.94	204.4	
11	344	61.83	272.2	
12	358	33.92	143.4	
13	374	30.88	125.0	
14	390	24.27	94.3	
15	408	87.78	326.1	
16	10,380	75.00	10.9	Upper Marker

4-00



Overall Results for sample 2 : **sample 2**

Number of peaks found: 17
Noise: 0.3
Corr. Area 1: 1,542.9

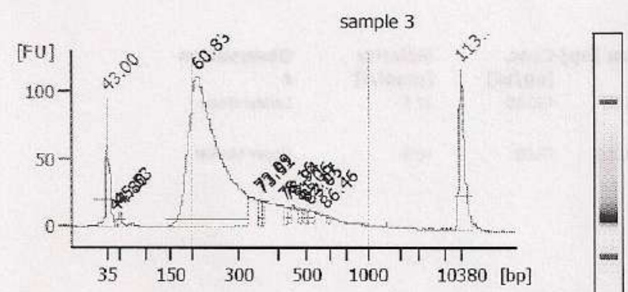
Region table for sample 2 : **sample 2**

From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]	Color
200	1,000	1,542.9	87	338	35.3	1,580.40	8,084.5	

Peak table for sample 2 : **sample 2**

Peak	Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]	Observations
1	35	125.00	5,411.3	Lower Marker
2	213	352.83	2,508.0	
3	223	112.44	763.2	
4	234	118.76	769.7	
5	247	68.61	421.1	
6	255	69.97	415.1	
7	264	51.47	294.9	
8	294	116.72	602.3	
9	315	38.40	184.5	
10	324	41.83	195.8	
11	352	111.85	482.0	
12	387	30.31	118.6	
13	450	32.22	108.4	
14	470	20.44	65.8	
15	488	12.14	37.7	
16	499	13.33	40.5	
17	536	8.20	23.2	
18	554	9.43	25.8	
19	10,380	75.00	10.9	Upper Marker

5-20



Overall Results for sample 3 : **sample 3**

Number of peaks found: 11
Noise: 0.2
Corr. Area 1: 1,521.9

Region table for sample 3 : **sample 3**

From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]	Color
200	1,000	1,521.9	83	314	41.2	1,361.41	7,641.8	

186

Assay Class: High Sensitivity DNA Assay
Data Path: C:\...gh Sensitivity DNA Assay_DE34903215_2015-05-04_16-48-09.xad

Created: 5/4/2015 4:48:09 PM
Modified: 5/4/2015 5:29:29 PM

Electropherogram Summary Continued ...

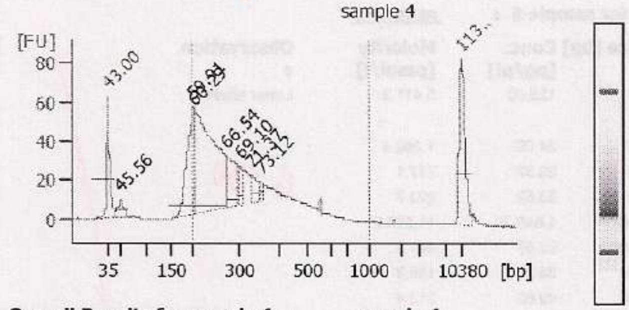
5-60

Peak table for sample 3 : sample 3

Peak	Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]	Observations
1	35	125.00	5,411.3	Lower Marker
2	47	8.83	284.4	
3	53	20.55	589.9	
4	212	1,304.86	9,331.9	
5	354	14.15	60.5	
6	359	12.24	51.6	
7	424	7.95	28.4	
8	438	8.06	27.9	
9	474	8.38	26.8	
10	494	7.88	24.2	
11	511	9.61	28.5	
12	583	3.06	7.9	
13	10,380	75.00	10.9	Upper Marker

5-20

sample 4



Overall Results for sample 4 : sample 4

Number of peaks found:	7
Noise:	0.2
Corr. Area 1:	1,007.0

Region table for sample 4 : sample 4

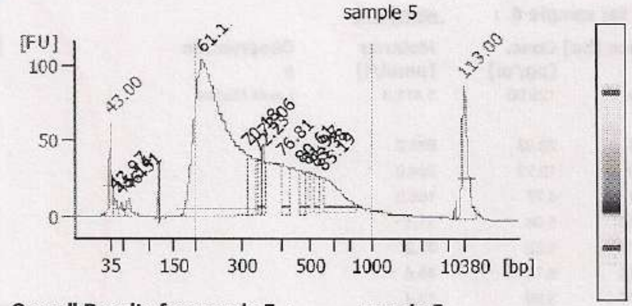
From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]	Color
200	1,000	1,007.0	82	318	35.5	1,192.76	6,432.0	

6-90

Peak table for sample 4 : sample 4

Peak	Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]	Observations
1	35	125.00	5,411.3	Lower Marker
2	53	47.43	1,356.0	
3	202	219.51	1,650.5	
4	206	534.59	3,936.9	
5	276	95.29	523.8	
6	305	23.80	118.4	
7	333	36.32	165.3	
8	355	10.45	44.7	
9	10,380	75.00	10.9	Upper Marker

sample 5



Overall Results for sample 5 : sample 5

Number of peaks found:	12
Noise:	0.2
Corr. Area 1:	2,022.6

Region table for sample 5 : sample 5

From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]	Color
200	1,000	2,022.6	87	354	40.8	2,283.75	11,741.8	

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Assay Class: High Sensitivity DNA Assay
Data Path: C:\...gh Sensitivity DNA Assay_DE34903215_2015-05-04_16-48-09.xad

Created: 5/4/2015 4:48:09 PM
Modified: 5/4/2015 5:29:29 PM

Electropherogram Summary Continued ...

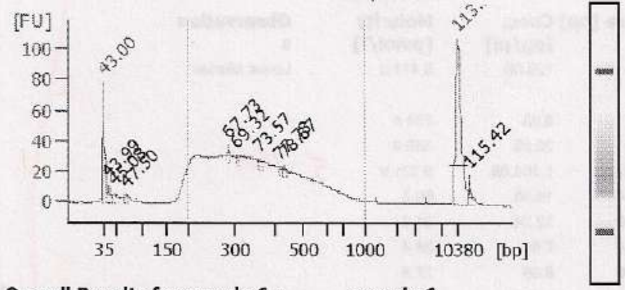
Peak table for sample 5 : sample 5

6-80

Peak	Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]	Observations
1	35	125.00	5,411.3	Lower Marker
2	42	34.00	1,240.4	
3	49	23.37	717.1	
4	62	33.62	823.7	
5	216	1,649.76	11,593.2	
6	318	92.67	441.3	
7	344	35.21	155.3	
8	354	49.60	212.4	
9	401	72.96	275.9	
10	466	32.51	105.6	
11	490	29.77	92.0	
12	518	34.15	99.9	
13	556	109.09	297.1	
14	10,380	75.00	10.9	Upper Marker

7-80

sample 6



Overall Results for sample 6 : sample 6

Number of peaks found:	9
Noise:	0.2
Corr. Area 1:	1,065.1

Region table for sample 6 : sample 6

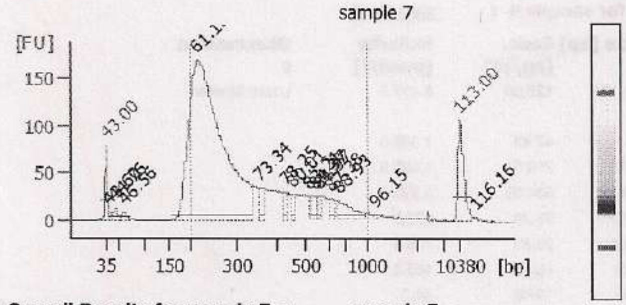
From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]	Color
200	1,000	1,065.1	86	370	36.1	1,000.46	4,785.1	

8-60

Peak table for sample 6 : sample 6

Peak	Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]	Observations
1	35	125.00	5,411.3	Lower Marker
2	42	23.03	838.2	
3	49	12.73	394.0	
4	71	8.77	186.8	
5	289	5.94	31.1	
6	307	5.52	27.2	
7	360	8.71	36.6	
8	417	3.69	13.4	
9	433	5.64	19.7	
10	10,380	75.00	10.9	Upper Marker
11	12,855	0.00	0.0	

sample 7



Overall Results for sample 7 : sample 7

Number of peaks found:	14
Noise:	0.2
Corr. Area 1:	2,570.5

Region table for sample 7 : sample 7

From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]	Color
200	1,000	2,570.5	86	334	44.2	2,441.98	13,281.7	

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Assay Class: High Sensitivity DNA Assay
Data Path: C:\...gh Sensitivity DNA Assay_DE34903215_2015-05-04_16-48-09.xad

Created: 5/4/2015 4:48:09 PM
Modified: 5/4/2015 5:29:29 PM

Electropherogram Summary Continued ...

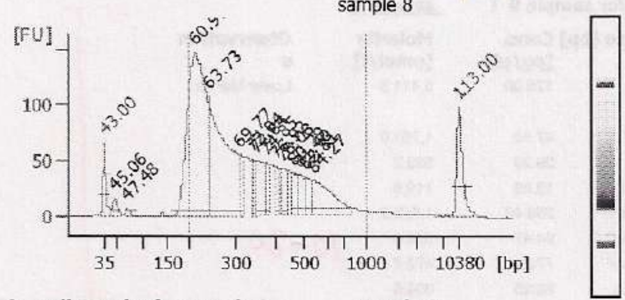
9-40

Peak table for sample 7 : sample 7

Peak		Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]	Observations
1	◀	35	125.00	5,411.3	Lower Marker
2		39	14.65	568.7	
3		47	35.45	1,148.4	
4		62	27.94	679.2	
5		215	2,218.45	15,644.7	
6		357	50.54	214.3	
7		426	24.65	87.7	
8		456	18.39	61.1	
9		514	15.77	46.4	
10		539	23.26	65.4	
11		552	19.17	52.6	
12		604	18.08	45.3	
13		653	58.01	134.7	
14		1,242	2.53	3.1	
15	▶	10,380	75.00	10.9	Upper Marker
16		13,606	0.00	0.0	

8-60

sample 8



Overall Results for sample 8 : sample 8

Number of peaks found: 14
Noise: 0.2
Corr. Area 1: 2,602.2

Region table for sample 8 : sample 8

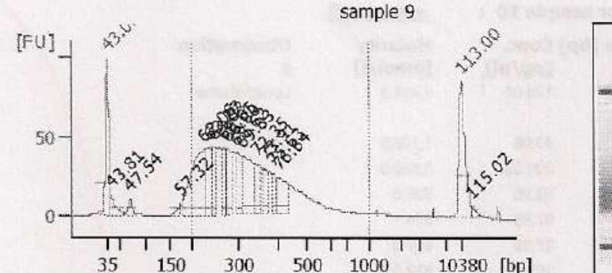
From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]	Color
200	1,000	2,602.2	88	344	39.9	2,639.87	13,783.0	

10-20

Peak table for sample 8 : sample 8

Peak		Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]	Observations
1	◀	35	125.00	5,411.3	Lower Marker
2		49	53.05	1,645.9	
3		71	21.01	449.0	
4		213	1,128.97	8,018.4	
5		244	748.80	4,644.6	
6		313	79.41	384.3	
7		341	45.24	200.9	
8		351	121.01	522.1	
9		376	50.44	203.1	
10		403	39.07	147.0	
11		423	63.60	228.0	
12		461	44.79	147.2	
13		480	53.31	168.2	
14		506	48.50	145.3	
15		537	148.97	420.5	
16	▶	10,380	75.00	10.9	Upper Marker

sample 9



Overall Results for sample 9 : sample 9

Number of peaks found: 15
Noise: 0.3
Corr. Area 1: 1,179.7

Region table for sample 9 : sample 9

From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]	Color
200	1,000	1,179.7	85	341	35.2	1,324.98	6,684.7	

Assay Class:	High Sensitivity DNA Assay	Created:	5/4/2015 4:48:09 PM
Data Path:	C:\...gh Sensitivity DNA Assay_DE34903215_2015-05-04_16-48-09.xad	Modified:	5/4/2015 5:29:29 PM

Electropherogram Summary Continued ...

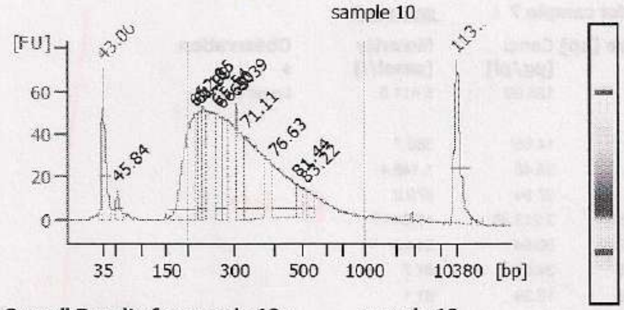
12-00

Peak table for sample 9 : sample 9

Peak		Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]	Observations
1	◀	35	125.00	5,411.3	Lower Marker
2		40	47.50	1,781.0	
3		71	28.26	599.2	
4		173	13.66	119.6	
5		226	268.49	1,803.2	
6		239	94.47	598.6	
7		248	77.24	472.7	
8		270	60.55	339.6	
9		279	72.78	395.8	
10		299	77.47	392.7	
11		314	138.47	668.6	
12		345	45.12	198.1	
13		365	40.60	168.7	
14		386	30.81	121.0	
15		398	63.03	239.7	
16	▶	10,380	75.00	10.9	Upper Marker
17		12,440	0.00	0.0	

10-20

sample 10



Overall Results for sample 10 : sample 10

Number of peaks found:	11
Noise:	0.2
Corr. Area 1:	1,373.8

Region table for sample 10 : sample 10

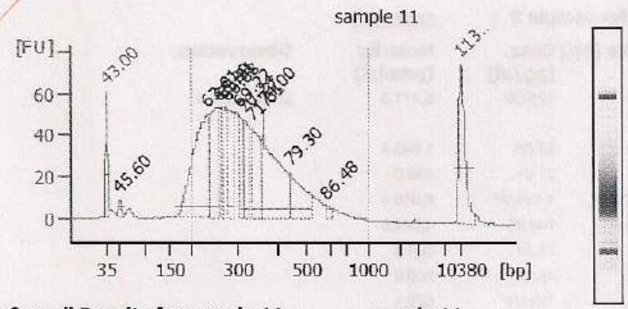
From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]	Color
200	1,000	1,373.8	86	343	36.7	1,760.96	8,933.0	

16-80

Peak table for sample 10 : sample 10

Peak		Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]	Observations
1	◀	35	125.00	5,411.3	Lower Marker
2		56	43.96	1,198.3	
3		215	336.33	2,368.0	
4		225	92.90	626.0	
5		232	87.98	574.1	
6		264	97.28	557.3	
7		275	105.72	582.0	
8		308	135.87	667.7	
9		330	230.39	1,058.7	
10		398	143.37	545.4	
11		481	24.72	77.9	
12		514	25.01	73.7	
13	▶	10,380	75.00	10.9	Upper Marker

sample 11



Overall Results for sample 11 : sample 11

Number of peaks found:	11
Noise:	0.1
Corr. Area 1:	1,381.3

Region table for sample 11 : sample 11

From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]	Color
200	1,000	1,381.3	91	335	32.1	1,624.99	8,198.0	

Peak table for sample 11 : sample 11

Peak		Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]	Observations
1	◀	35	125.00	5,411.3	Lower Marker
2		53	22.74	645.8	
3		236	374.17	2,400.7	
4		259	190.54	1,112.6	
5		269	70.54	397.9	
6		279	110.45	598.9	

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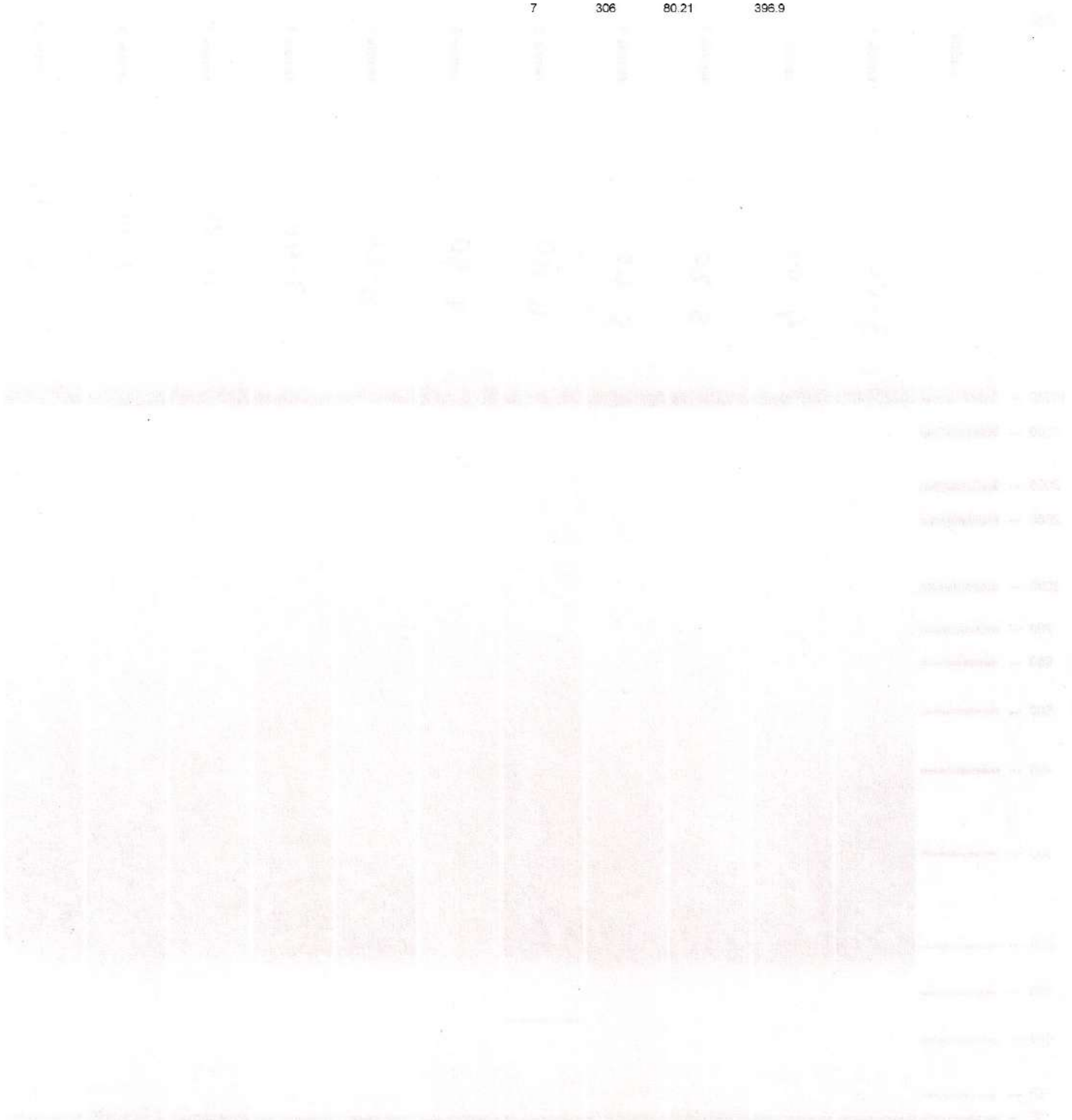
Assay Class:	High Sensitivity DNA Assay	Created:	5/4/2015 4:48:09 PM
Data Path:	C:\...gh Sensitivity DNA Assay_DE34903215_2015-05-04_16-48-09.xad	Modified:	5/4/2015 5:29:29 PM

Gel Image

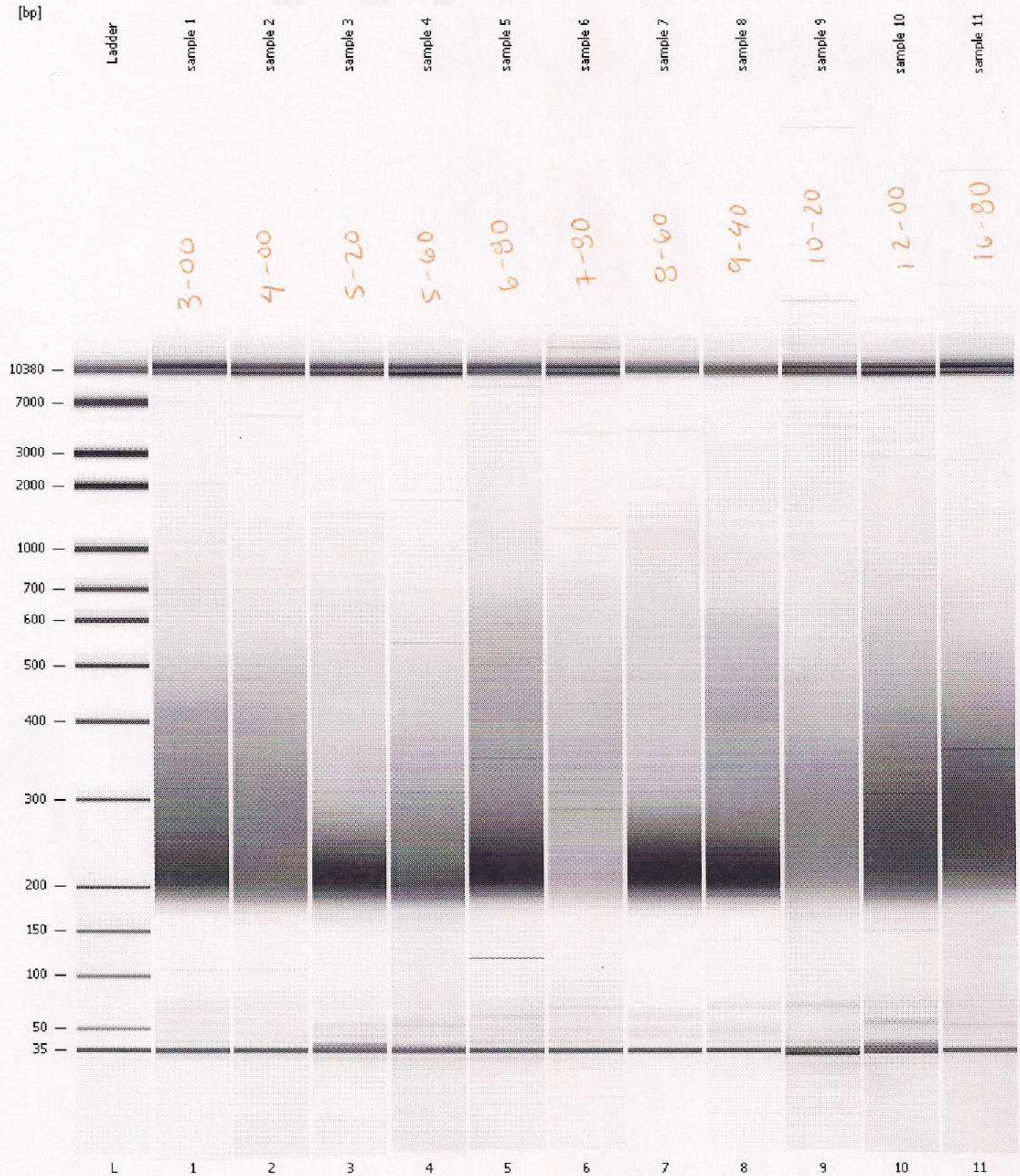
7 306 80.21 396.9



191

Assay Class: High Sensitivity DNA Assay
Data Path: C:\...gh Sensitivity DNA Assay_DE34903215_2015-05-04_16-48-09.xad
Created: 5/4/2015 4:48:09 PM
Modified: 5/4/2015 5:29:29 PM

Gel Image



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		Qubit (ng/ml)				Bioanalyzer				
	Pool-Generation	Read 1	Read 2	Average	Qubit converted (ng/ul)	Fragment Length	Conc. (ng/ul)	Vol. to add		
Sample 1	03-00	15.4	15.9	15.65	1.565	333	1.79	3.07	Total Volume (ul)	215
	03-20	28.1	29.3	28.7	2.87			1.68	Pool Conc. (ng/ul)	1.57
	03-40	17.4	18.2	17.8	1.78			2.70		
	03-60	22.8	25.1	23.95	2.395			2.01		
	03-80	23.7	24.9	24.3	2.43			1.98		
Sample 2 -	04-00	47	49.2	48.1	4.81	338	1.58	1.00		
	04-20	18.2	19	18.6	1.86			2.59		
	04-40	8.66	9.05	8.855	0.8855			5.43		
	04-60	15.6	16.2	15.9	1.59			3.03		
	04-80	18.3	19.2	18.75	1.875			2.57		
	05-00	6.81	7.11	6.96	0.696			6.91		
Sample 3 -	05-20	11.4	11.8	11.6	1.16	314	1.36	4.15		
	05-40	10.3	10.7	10.5	1.05			4.58		
Sample 4 -	05-60	5.25	5.55	5.4	0.54	318	1.19	8.91		
	05-80	11.3	11.8	11.55	1.155			4.16		
	06-00	12.1	12.6	12.35	1.235			3.89		
	06-20	16.3	17.1	16.7	1.67			2.88		
	06-40	11.6	12.2	11.9	1.19			4.04		
	06-60	9.21	9.62	9.415	0.9415			5.11		
sample 5 -	06-80	20.5	21.1	20.8	2.08	354	2.28	2.31		
	07-00	30.8	32.2	31.5	3.15			1.53		
	07-20	14.8	15.3	15.05	1.505			3.20		
	07-40	43.5	46	44.75	4.475			1.07		
	07-60	13	13.8	13.4	1.34			3.59		
Sample 6 -	07-80	17.2	17.9	17.55	1.755	370	1	2.74		
	08-00	5.84	6.01	5.925	0.5925			8.12		
	08-20	28.2	29.4	28.8	2.88			1.67		
	08-40	37	37.9	37.45	3.745			1.28		
Sample 7 -	08-60	8.62	8.99	8.805	0.8805	334	2.44	5.46		
	08-80	11.1	11.4	11.25	1.125			4.28		
	09-00	8.64	8.89	8.765	0.8765			5.49		
	09-20	25.7	26.3	26	2.6			1.85		
Sample 8 -	09-40	11.7	12	11.85	1.185	344	2.64	4.06		
	09-60	13.1	13.4	13.25	1.325			3.63		
	09-80	30.3	30.2	30.25	3.025			1.59		
	10-00	18.9	19.5	19.2	1.92			2.51		
Sample 9	10-20	29.2	30.1	29.65	2.965	341	1.32	1.62		
	10-40	28.6	29.5	29.05	2.905			1.66		
	10-60	24.1	24.8	24.45	2.445			1.97		
	10-80	22.1	22.8	22.45	2.245			2.14		
	11-00	20.8	21.3	21.05	2.105			2.29		
	11-20	26.1	26.7	26.4	2.64			1.82		
	11-40	21.9	22.3	22.1	2.21			2.18		
	11-60	16.7	17.4	17.05	1.705			2.82		
	11-80	19.6	19.8	19.7	1.97			2.44		
sample 10 -	12-00	10	10.2	10.1	1.01	343	1.76	4.76		
	12-20	17.6	18	17.8	1.78			2.70		
	12-40	12.7	12.8	12.75	1.275			3.77		
	12-60	10.3	11.2	10.75	1.075			4.47		
	12-80	17.9	18.2	18.05	1.805			2.66		
	13-00	21	21.9	21.45	2.145			2.24		
	13-20	21.4	22.4	21.9	2.19			2.20		
	13-40	31.8	33.1	32.45	3.245			1.48		
	13-60	23.2	23.7	23.45	2.345			2.05		
	13-80	18.2	18.8	18.5	1.85			2.60		
	14-00	24.3	25.4	24.85	2.485			1.94		
	14-20	13.3	13.8	13.55	1.355			3.55		
	14-40	6.42	6.67	6.545	0.6545			7.35		
	14-60	15.2	15.5	15.35	1.535			3.13		
	14-80	34.3	35.1	34.7	3.47			1.39		
	15-00	21.5	22.2	21.85	2.185			2.20		
	15-20	18.5	18.8	18.65	1.865			2.58		
	15-40	31.7	32.3	32	3.2			1.50		
	15-60	16.4	16.5	16.45	1.645			2.92		
	15-80	18.8	19	18.9	1.89			2.54		
	16-00	16.4	16.5	16.45	1.645			2.92		
	16-20	20.6	20.7	20.65	2.065			2.33		
	16-40	21.6	22.4	22	2.2			2.19		
	16-60	16.5	16.5	16.5	1.65			2.92		
Sample 11 -	16-80	17.7	18.1	17.9	1.79	335	1.62	2.69		

8.907407407

194

5/3/15

FITNESS ASSAYS ON SEGREGANTS

Thawing streams for the 1st round of fitness assays.

Last week I picked a single colony of yGIL519 (BY Reference) & yGIL521 (RM Ref), started an o/N culture, diluted the o/N 1:50 and distributed across 6 96 well plates. I grew the plates o/N and added glycerol/freezedown.

Today I will do a 1:32 dilution of the BY Ref Plate #1 into 4 96-well plates.

I will also do a 1:32 of segregants from yGIL871 & yGIL890, 2 plates each.

→ These are the BY control cross and the BYS1-A08 evolved cross.

In incubator @ 3:25pm

5/5/15

Yesterday I started the competition setup and dilutions, unfortunately as the first transfers were being done the z-axis motor gave out.

- I will have to wait until the Bravo is fixed and start again.
- I still ran FACS on the 0 time point of plate 871-1 mixed w/ BY Ref. (this was the only one that successfully transferred.)

5/7/15

Ok, let's try this again.

Dilute yGIL871 & 890 segregants and BY Ref 1:32 → In incubator @ 2:45pm

5/8/15

- Mix each experimental plate 50:50 w/ Ref. → Back in incubator @ 9:45
- Transfer → in incubator @ 11:40

5/9/15

- Transfer started @ 11:15, in incubator @ 11:34
- Running FACS on Gen. 0 — Lots of "Waste Buffer Tank Full" errors

5/10/15

- In incubator @ 11:35

5/11/15

- I did the rest of the dilutions at the same time, out to 50 gen.

5/19/15

DISCUSSION OF BSI FITNESS ASSAYS

- Overall, the fitness assays look great. This is some of the best looking fitness-assay data I have. Certainly the best one-color data.

· I found a new way to gate in FlowJo that works well — magnetic gating. Basically I draw a narrow elliptical gate ~~at~~ and fit that to reference population. It automatically adjusts to maximize the points under the gate in each subsequent ~~pop~~ sample. → I used the same gate to analyze all of the FACS data.

→ Examples on p. 197 — for some I also drew gates by autogating to see how ratio changes differs w/ magnetic gating. As far as I can tell, it is the same.

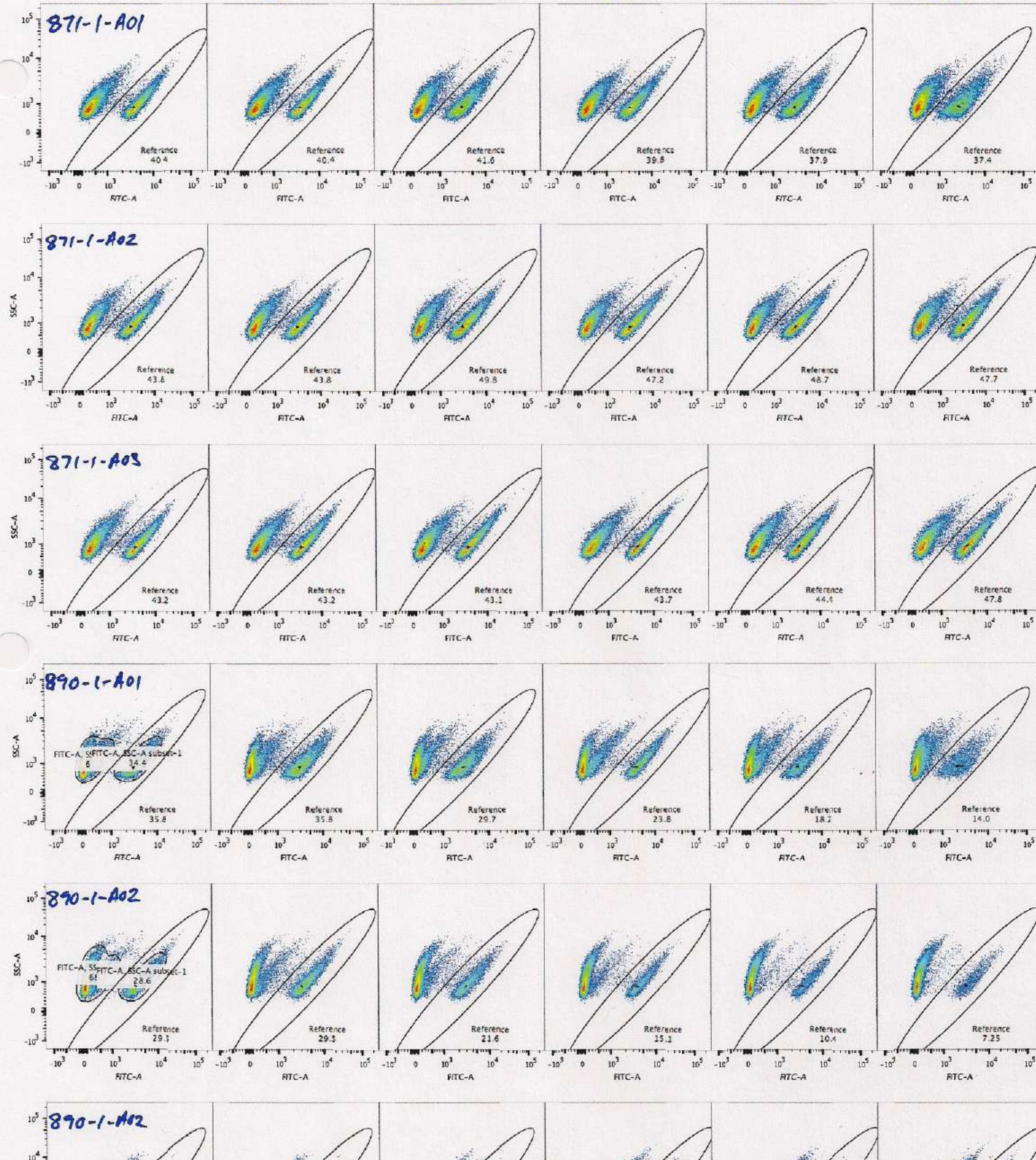
· For some of the evolved (see 890-1-A01 & A02 on p. 197) the experimental population appears to have two subpopulations. The one tends to disappear as the competition proceeded.

· I don't think I need to go out to 50 gen. The data on p. 199 & 201 are from gen 0 → 40.

→ For some reason the ~~d~~ FACS data for a few Gen 50 populations do not look great (poor separation between Exp & Ref)

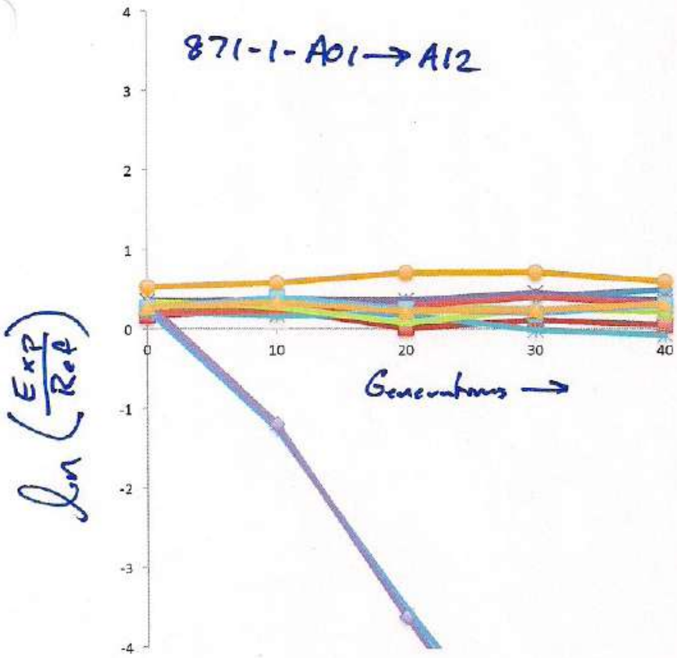
5/19/15 ANALYSIS OF FITNESS DATA

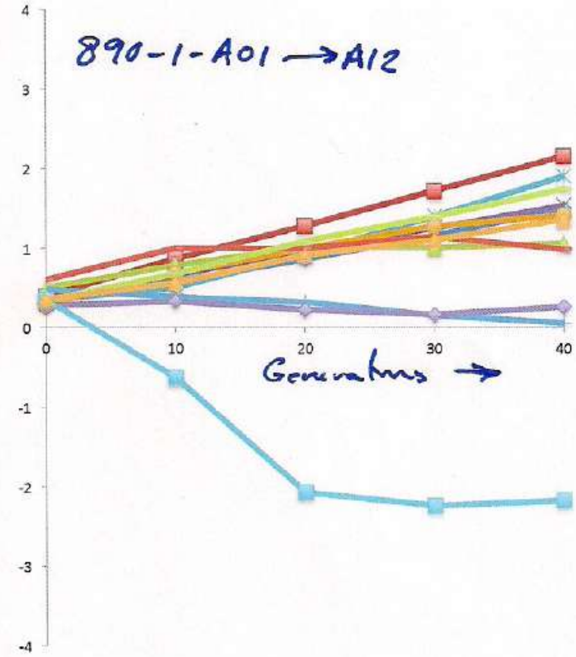
10 20 30 40 50

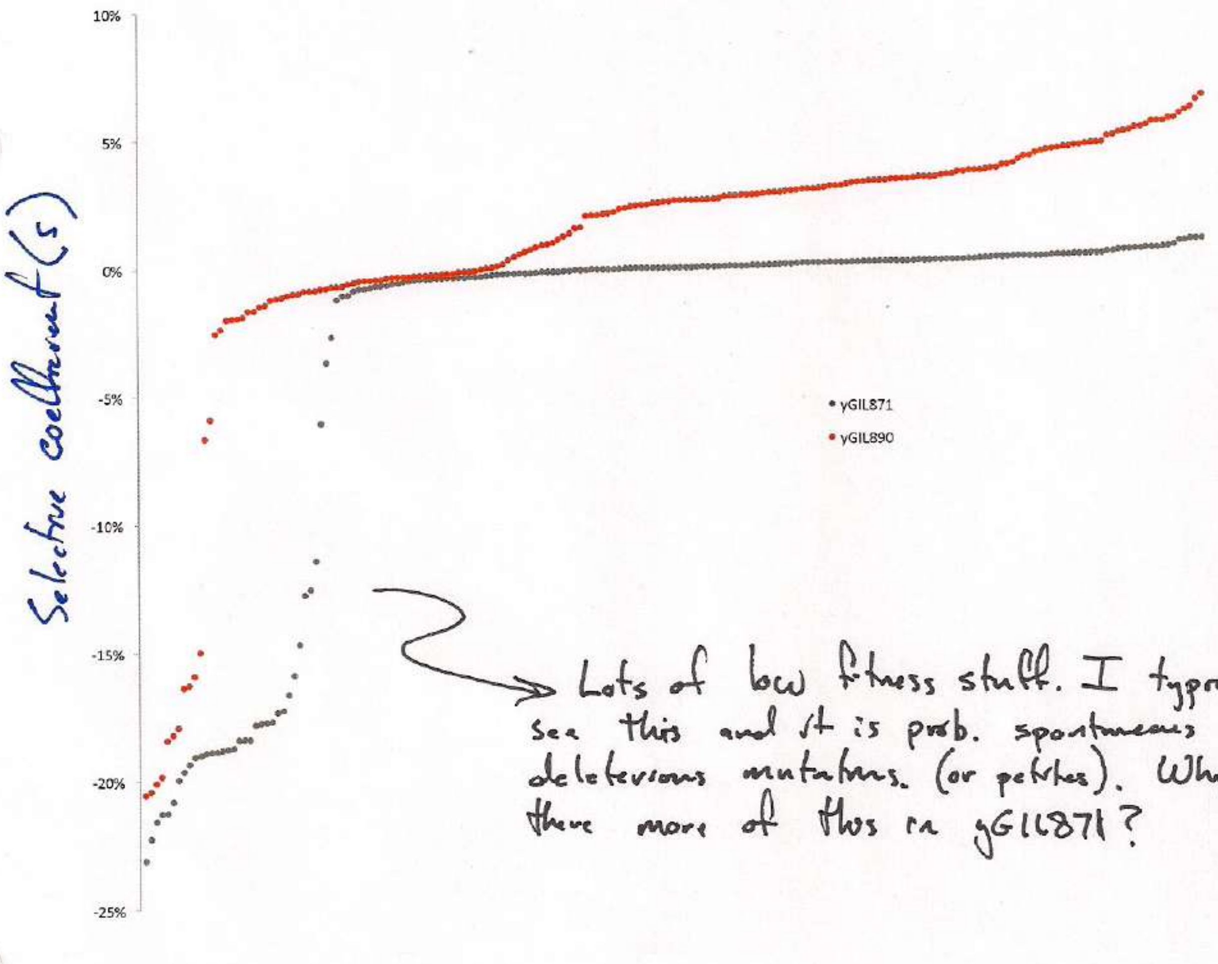


This was supposed to be Gen 0, but I repeated Gen 10.

ANALYSIS OF FITNESS DATA CONT.







Lots of low fitness stuff. I typically see this and it is prob. spontaneous deleterious mutations. (or petites). Why is there more of this in yGIL871?