A FORAY INTO EVOLUTIONARY DYNAMICS

For a while I have been interested in doing some long-term evolution experiments to watch sterile strains sweep through a population — convinced that this could inform us about important parameters — certainly the beneficial mutation rate and perhaps the distribution of fitness effects of beneficial mutations.

Typically with long-term evolution experiments, beneficial mutations are inferred by noting the increase in fitness of a culture or observing the purging of neutral markers (i.e. canavanine resistance) that accompanies a selective sweep. ~~Values for~~ Estimates for the beneficial mutation rate and dist. of fitness effects are obtained by model fitting the data.

In such experiments, the identity of the beneficial mutations is usually unknown or is determined post hoc. With αF^R we have an expectation that, if given enough time, these beneficial mutations will arise and fix in the populations. Moreover, with αF^R we have an easily measurable phenotype and the ability to carefully ~~m~~ determine the mutation rate and fitness advantage of these mutants. A simple experiment to start with is to propagate a large # of parallel cultures and watch for αF^R strains to sweep. Under the hypothesis that these are the only available beneficial mutations, one should be able to calculate the distribution of times until αF^R mutants sweep.

Previously I started a long-term propagation of strain yGIL121 (see "Evolution I" Notebook p. 195-202) for 40 transfers (~ 300 generations). To my eye I ~~could~~ thought I could see αF^R cells in the populations

⟶ I may at some point try to analyze these better (by FACS?)

STRAIN CONSTRUCTION

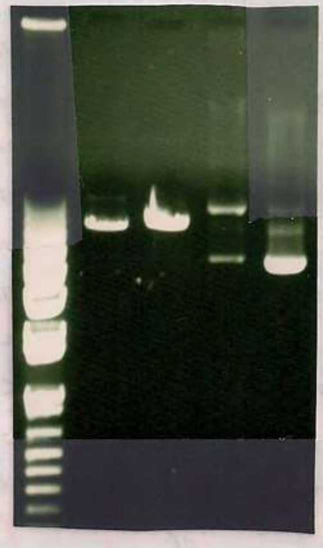
The base strains for this experiment will be:

- yGIL265 } both are MATa, hmlαΔ, bar1Δ ; 265 has S288c version of GPA1
- yGIL266 } ; 266 " RM — 〃 —

↳ see Evolution 1, p148

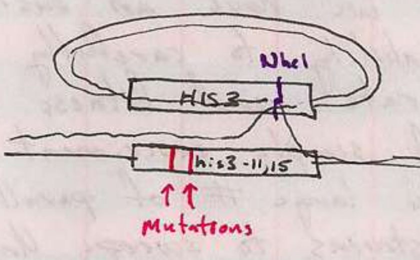
In order to better distinguish steriles from WT cells I integrated P_{FUS1}-yEVenus at HIS3.

- I obtained pNT134 from the Murray lab. pNT134 is a pRS403-based integrating vector containing P_{FUS1}-yEVenus-$CLN2^{PEST}$-$Term_{ADH}$ which is a destabilized fluorescent readout of the mating pathway.
- I miniprepped two clones containing pNT134 (A & B) and cut w/ NheI for 3h. (on 1/23)



LANE

1 pNT134 A } cut w/ NheI
2 — 〃 — B } cut w/ NheI
3 — 〃 — A } uncut
4 — 〃 — B } uncut



- Also on 1/23 I transformed the following strains w/ the linearized plasmid
  - yGIL126 (yGIL121 ste2Δ::KanMX)
  - 128 (— 〃 — ste4Δ::KanMX)
  - 134 (— 〃 — far1Δ::KanMX)
  - 265 (S288c-GPA1::NatMX)
  - 266 (RM11-GPA1::NatMX)

- On Monday (1/26) I picked two putative transformants off of each plate (thousands of colonies per plate) and restreaked to YPD.

- Yesterday I picked a single colony off of each YPD plate and inoculated 3mL YPD.

- This morny I streaked o/N cultures to -his plates.

1/30/09

- All of the putative transformants grew on -his plates. We do not have the proper filters to check yEVenus on the microscope so I will check the fluorescence by ~~F~~ FACS in two weeks.

- I froze down the following strains:

yGIL126	putative transformant	1	→	yGIL379
"	—— " ——	2	→	380
yGIL128	—— " ——	1	→	381
"	—— " ——	2	→	382
yGIL134	—— " ——	1	→	383
"	—— " ——	2	→	384
yGIL265	—— " ——	1	→	385
"	—— " ——	2	→	386
yGIL266	—— " ——	1	→	387
"	—— " ——	2	→	388

3/27/09

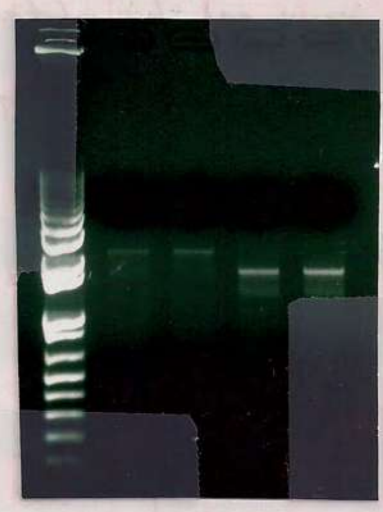
None of the previous transformants phenotyped correctly – I could not see fluorescence either by microscopy or FACS. I probably just restored the WT HIS3. I have chosen a different strategy — I will amplify the insert from the vectors pNT1·34 (pGIL035) and pNT1·37 (pGIL036).

PCR off of pGIL035 A, B and pGIL036 A, B using primers:

oGIL133	pRS403_URA3_extF	ACTGCACAGAACAAAAACCTGCAGGAAACGAAGATAAATCCTCACTATAGGGCGAATTGG
oGIL134	pRS403_URA3_extR	GTGAGTTTAGTATACATGCATTTACTTATAATACAGTTTGCAATTAACCCTCACTAAAGG

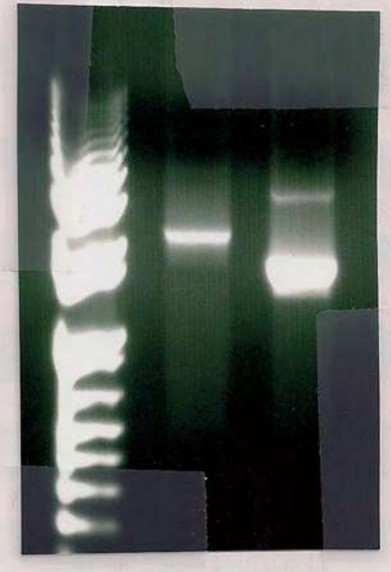
pRS403_URA3_extF and pRS403_URA3_extR will PCR amplify over the MCS of pRS403 and contain 40 bp of homology to URA3

these primers target the insert to URA3



LANE	PLASMID PCR TEMP.	
1	pGIL035	A
2	"	B
3	pGIL036	A
4	"	B

PCR purify: elute in 30 λ EB



LANE	
1	pGIL035
2	pGIL036

→ The higher bands may correspond to loops around the circular plasmid.

4/5/09

	Initials	Date
Prepared By		
Approved By		

SCREENING PUTATIVE TRANSFORMANTS FOR αF-ARREST & YFP

- Despite growing the overnight culture in -Ura media I seem to have approx. the same number of colonies on the experimental plates and the negative control.

- I picked 6 putative transformants off of each plate and restreaked to YPD

→ I restreaked 6 per YPD plate - but for many restreaks it is difficult to get single colonies

	PLATE	NOTATION	
- yGIL265	w/ pGIL035 PCR	A	1-6
- "	control	E	1-6
- "	w/ pGIL036 PCR	B	1-6
- "	control	F	1-6
- yGIL266	w/ pGIL035 PCR	C	1-6
- "	control	G	1-6
- "	w/ pGIL036 PCR	D	1-6
- "	control	H	1-6

- Yesterday I picked a patch of cells off of each plate (A1, B1, ..., H1) and inoculated 100λ YPD + αF

→ Only in B1 could I see YFP expression
- streak out B1 from both αF culture & restreak plate onto clean YPD to get single colonies for freezedown.

- It appears that I have isolated yGIL265 w/ the P_{FUS1}-YFP ~~reported~~ reporter

- Today I picked cells from D1-D6 & H1-H6 and inoculated 100λ YPD + αF

4/7/09

- Two weeks ago I did this same transformation as above - I initially assumed it was unsuccessful because it appeared that the positive and neg. plates had equal #s of colonies. I went back and screened 6 colonies off of each of those plates as well:

I: yGIL265 w/pGIL035
J: " ↓ 6
K: yGIL266 5
L: " 6

4/7/09

- Cursory screening of putative transformants found positive hits in B1, J1 (yGIL265 w/ pGIL036) and D6, L3, & L6 (yGIL266 w/ pGIL035)

- I restreaked the above putative transformants to YPD - Pick 3 colonies each (B1A, B1B, B1C, ..., L6A, L6B, L6C) - inoculate 3mL YPD

- This morning I added 10λ each culture to 100λ YPD+αF

- After 4h, screen for YFP
  - Only B1A & B1C were fluorescent; B1C was a small colony
    ↳ freezedown B1B as yGIL428.

4/9/09

- Yesterday I screened additional putative transformants for YFP expression

  ↳ It looks like the reporter takes longer to induce in the yGIL266 bkg
  Freeze down putative transformant D6D → yGIL429
  D6F → yGIL430

- Today I am going to look at the induced cells in the LSRII
  - last night I added 100λ of saturated culture of yGIL428 - 430 to 100λ of YPD+2×αF or YP+2×αF
  ↳ this morning I did the same

4/16/09

- I struck yGIL428 (BY-GPA1) & yGIL429 (RM-GPA1) from freezer. - yGIL428 appeared to grow slowly - two possibilities → deleterious mutation
  → reporter more costly in BY-GPA1 strain

  ↳ I picked 3 more colonies off plates B & J from bench (Bx, By, Bz, Jx, Jy, Jz)
  - I checked the reporter under the microscope - all showed GFP upon induction but Bz was exceptionally bright
  - Freeze down:

Bz - yGIL431
Bx - 432
By - 433
Jx - 434
Jy - 435
Jz - 436

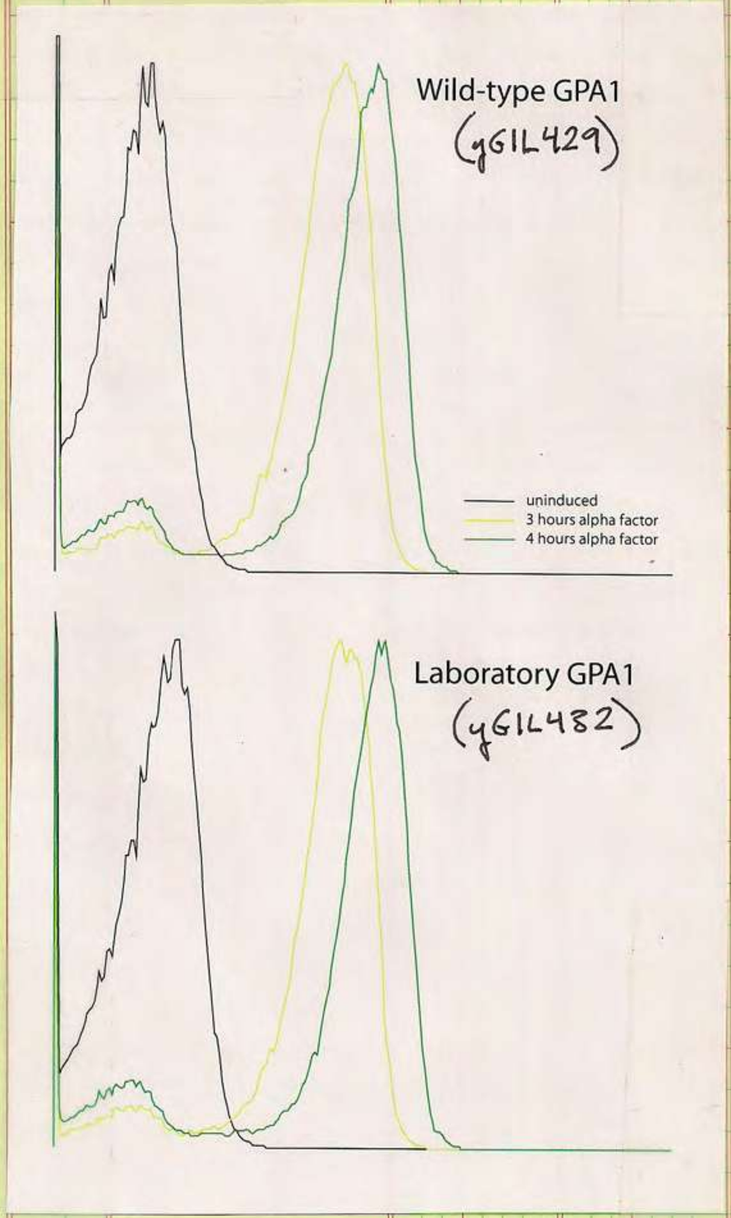
4/17/09 NOTE

- I recall that Bz may have been a small colony from the "B" plate. Both Bz (yGIL431) and yGIL428 are bright w/ a slow growth rate. Perhaps these strains have integrated two copies of the reporter (upper band p 4). I could backcross to sort this out.

5/6/09

	Initials	Date
Prepared By		
Approved By		

- On Monday 5/3 I streaked yGIL428 - yGIL436 to YPD
- Yesterday I started 3 ml o/N cultures in YPD
- Today I will run all 9 potential strains through FACS following 3 or 4 hours in αF
  - It is clear from the growth on YPD that yGIL428 & yGIL431 grow slowly and should not be used.
- Add 10λ o/N culture to 90λ YPD+αF ~ 10:40



5/7/09

ANALYSIS OF INDUCTION EXPT.

- On p. 9-11 are the histograms for the 9 potential strains
- Consistent w/ the hypothesis of multiple copy of reporter, strains yGIL428 & 431 have both higher basal & induced fluorescence.
- All other strains appear to be of equal quality - I will plan to use yGIL429 & yGIL432 for the experiment
- The laboratory G1406T-allele of GPA1 results in higher basal expression of the FUS1 reporter as expected

  ↳ This difference is diminished upon induction.
- A significant fraction of the population remains dark following induction
  10-15% after 3 h
  15-20% after 4 h

5/7/09

NOTES AND DISCUSSION OF INDUCTION EXPERIMENT

- For this experiment we used a different filter — yEVenus is maximally excited ~ 530. The standard GFP filter is 525/50. Previously we used this filter, but here we switched to the narrower band 535/30 — I could try counting the same tubes w/ each filter to determine how best to distinguish ~~GFP~~ positive from negative.

- What is the origin of the dark cells in the induced population?

  Possibilities:
  - Dead cells
  - Uninduced cells
  - Steriles

- I do not think they are uninduced because the ~~[illegible]~~ extra hour of induction does not shift this population — the pop. gets larger.
- Dead cells are a possibility — I could vital stain
- I suspect that these may be steriles — they would undergo ~2 gen. therefore 3% steriles will result in 12% dark after 3 h.

The correct answer is prob. a combination of dead & sterile

How small of a fraction of steriles do we need to detect?
→ we can always use other methods on small scale (plating, microscopy)

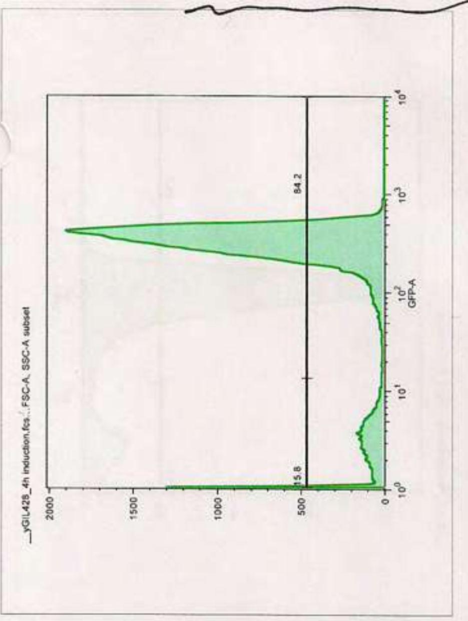
WHAT NEXT?

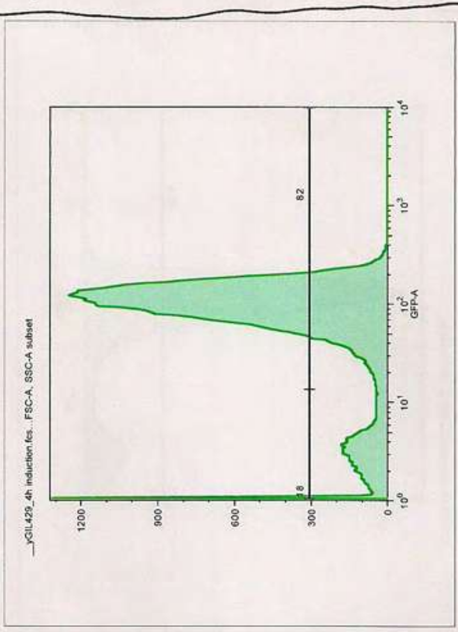
- Optimize induction time/conditions. → trade-off between separation of distributions & amplification of ~~[illegible]~~ steriles.

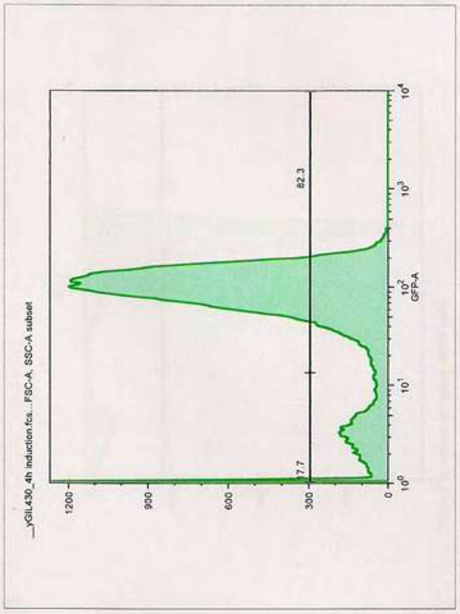
- Generate a standard curve by seeding in steriles and plotting observed fraction non-fluorescent versus fraction seeded.

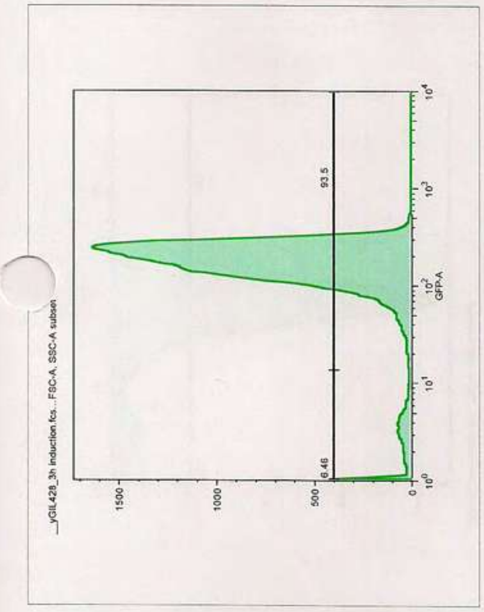
- Move to 96-well format

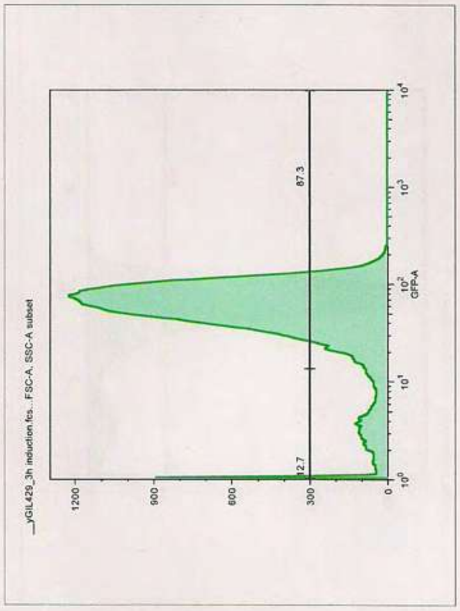
5/7/09 RESULTS FROM INDUCTION EXPERIMENT

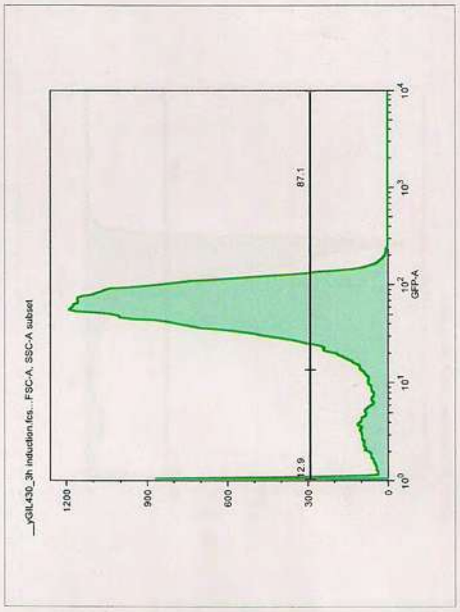


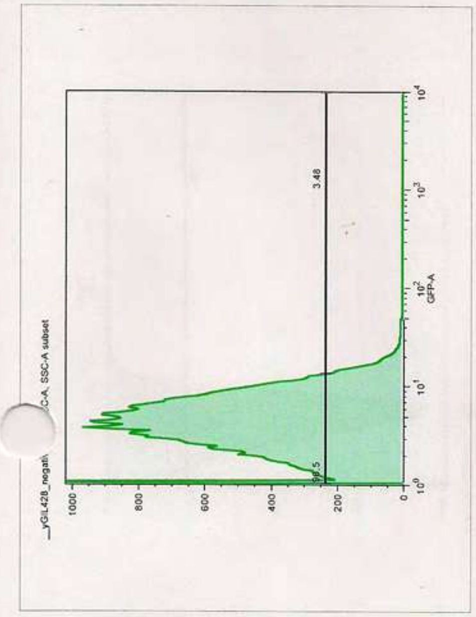


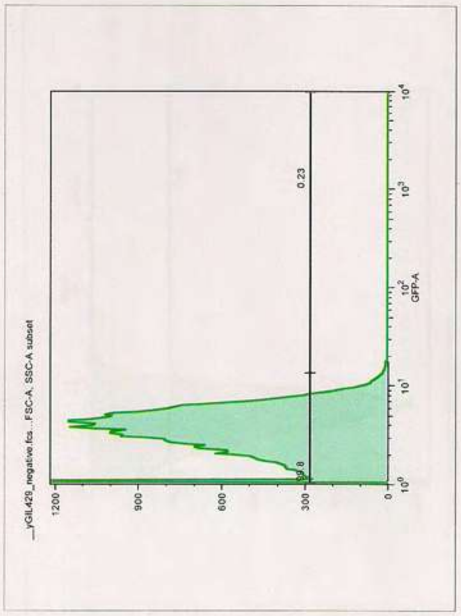


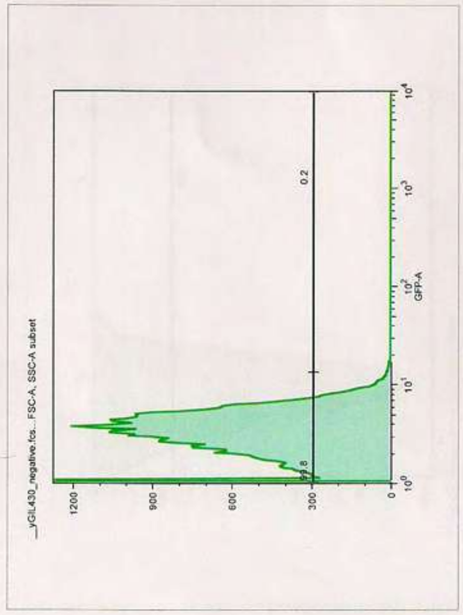




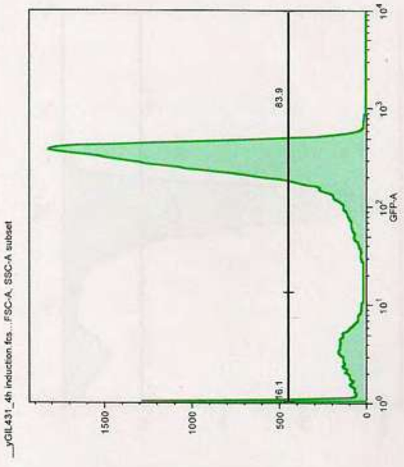


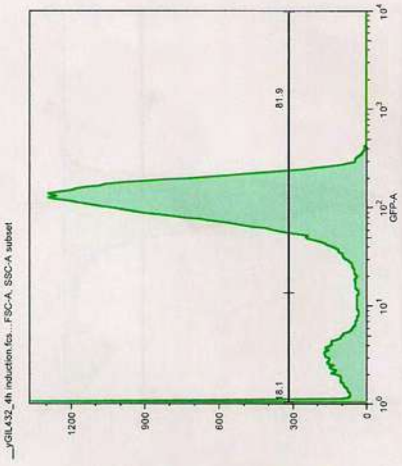


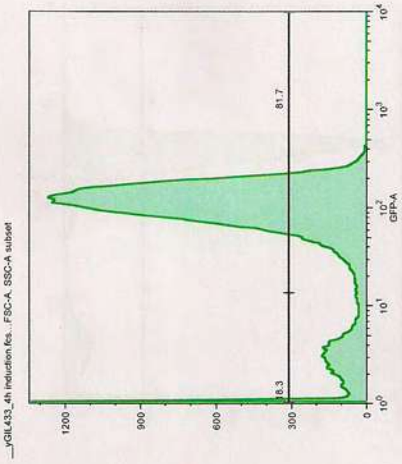


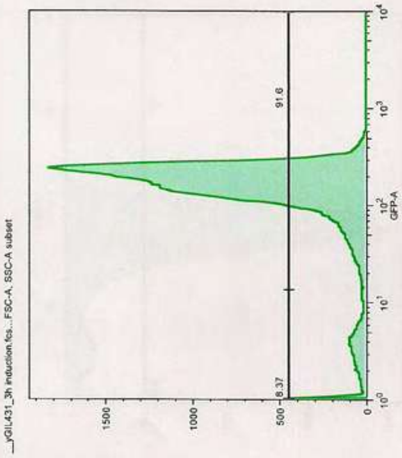


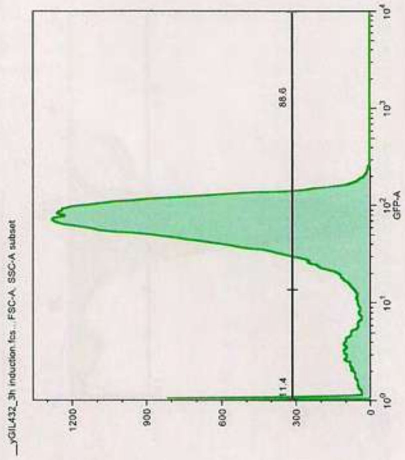
5/7/09 RESULTS CONT.

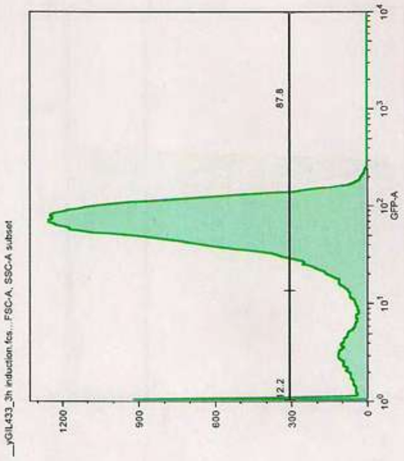


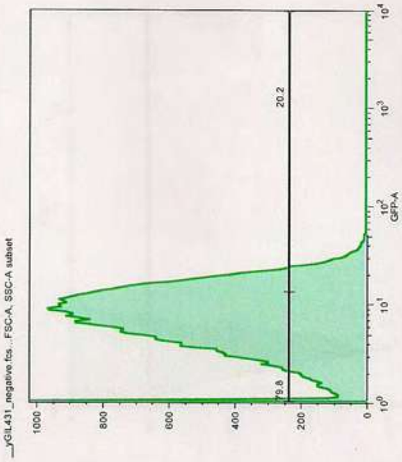


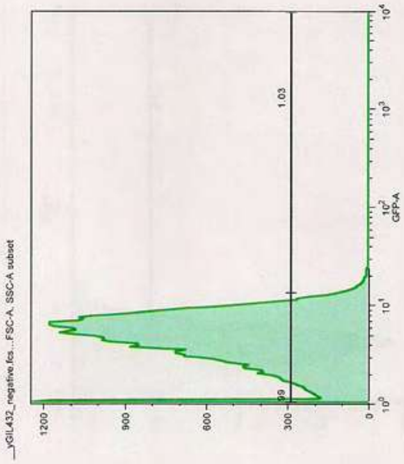


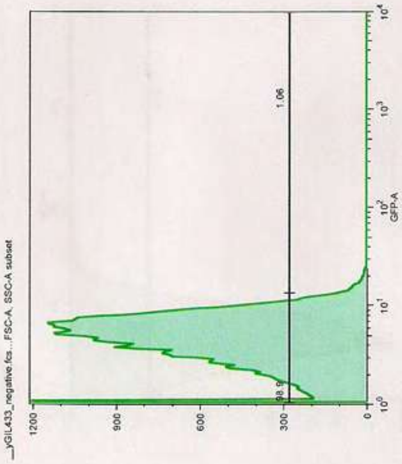




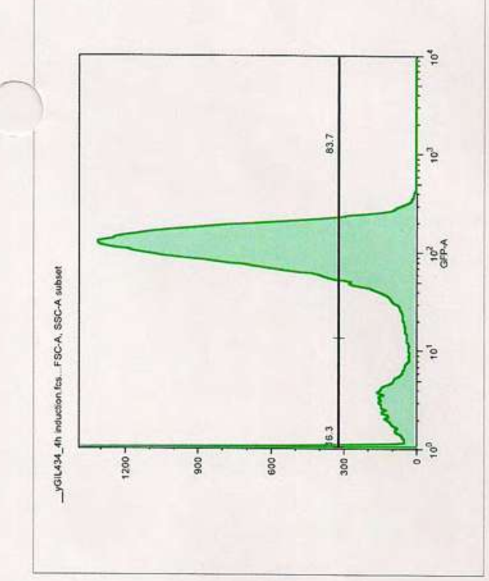


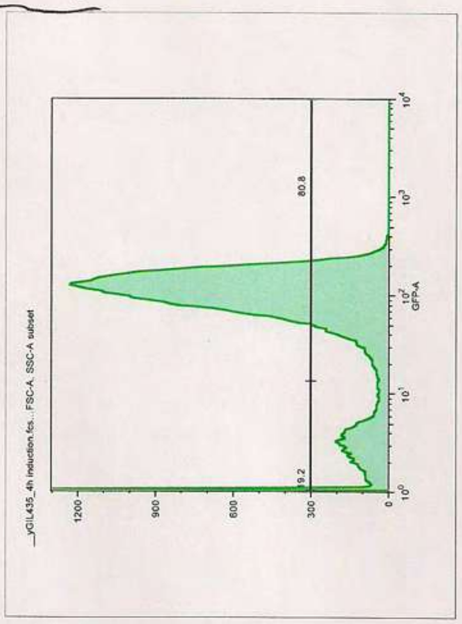


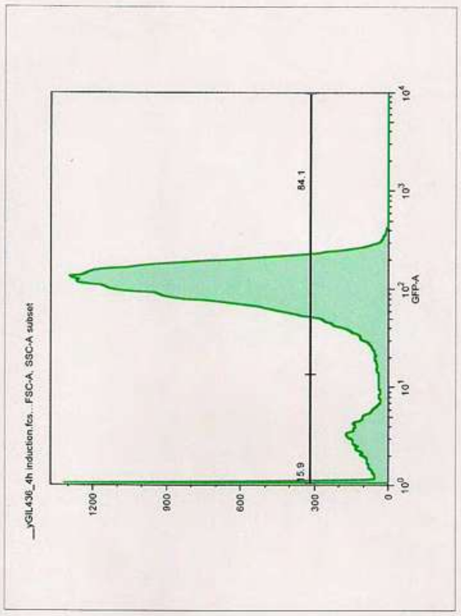


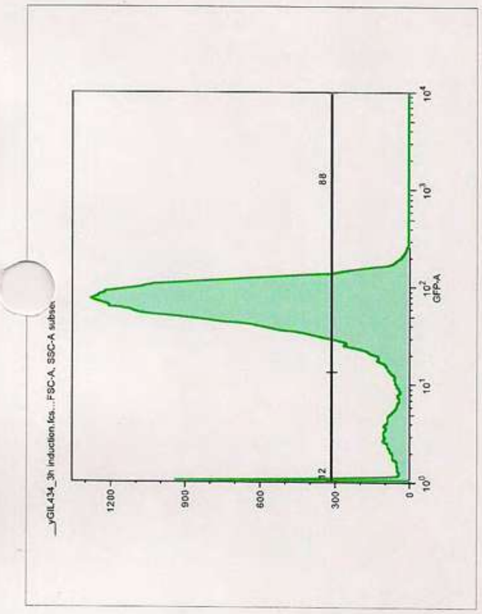


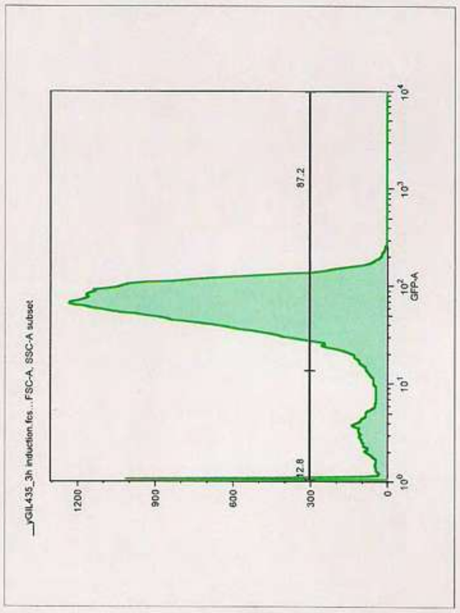
5/7/09 RESULTS CONT.

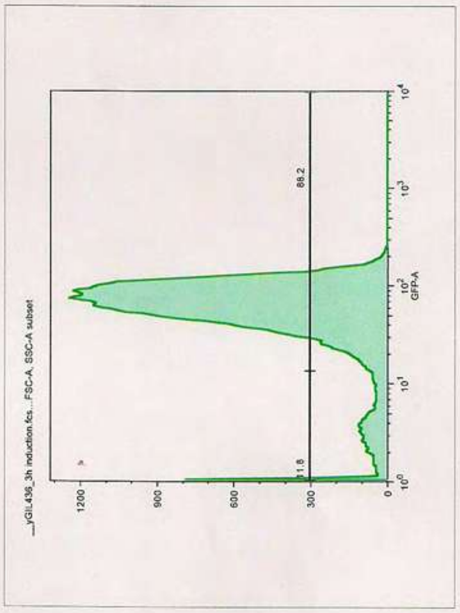


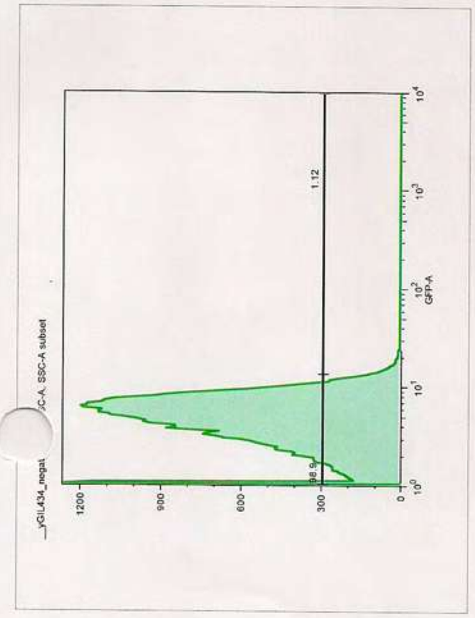


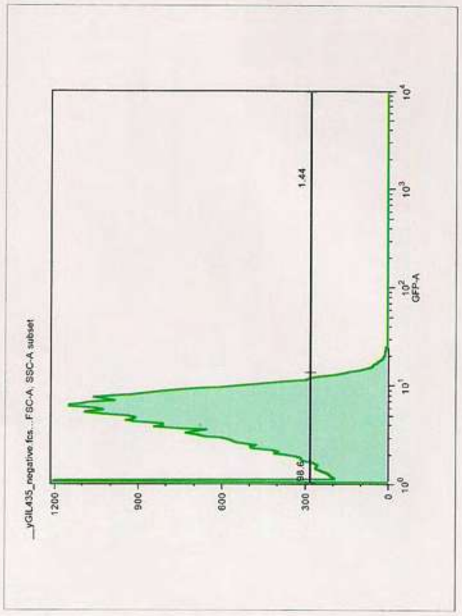


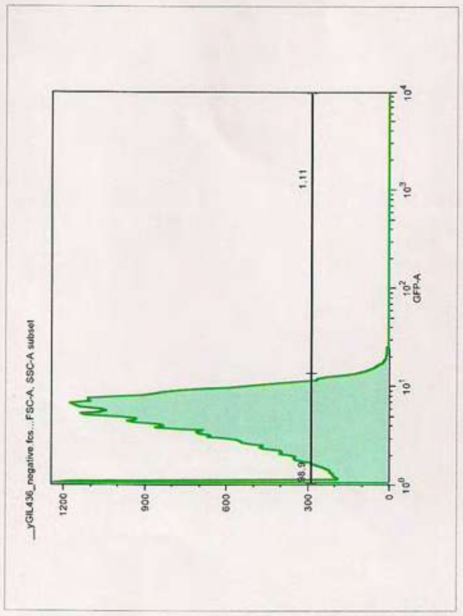












6/5/09

	Initials	Date
Prepared By		
Approved By		

INDUCTION TIME-COURSE IN 96-WELL PLATES

- Last night I started o/n cultures of the following in 5ml YPD (filtered):
  - yGIL265 - GPA1-G1406T } untransformed
  - " 266 - WT-GPA1 }
  - " 432 - GPA1-G1406T } w/ P_{FUS1}-YEVenus reporter
  - " 429 - WT-GPA1 }

- Set up induction expt
  - all rows identical
  - media sterile filtered
  - each column → either 100λ uninduced culture (columns 1,2,7,8) or 10λ culture + 90λ YPD+αF

COLUMN		
1	yGIL265	
2	yGIL429	
3	↓	1 h induction
4		2 h "
5		3 h "
6		4 h "
7	yGIL265	
8	yGIL432	
9	↓	1 h induction
10		2 h "
11		3 h "
12		4 h "

6/9/09

	Initials	Date
Prepared By		
Approved By		

INDUCTION IN 96-WELL PLATES, SECOND ATTEMPT

In the previous induction experiment (p 13) there were two obvious problems:

(1) cells were far too dense – I diluted saturated cultures 10λ into 90λ YPD+αF. Assuming $3E^8$ cells/saturated culture the density was ~ $3E^7$/ml ($3E^6$ total cells). This time I will try for 10-fold lower: 2λ into 200λ YPD+αF

(2) Induction was not so good and was inconsistent among replicates This time I will induce for longer (1.5-hour intervals):

0, 1.5h, 3h, 4.5h, o/n, untransformed
B C D E F A ⟵ column (yGIL266/yGIL429)
H I J K L G ⟵ column (yGIL~~266~~ 265/yGIL432)

- This morning I started 5 ml cultures from single colony:
  - yGIL265
  - yGIL266
  - yGIL432
  - yGIL429

- Start o/n inductions of yGIL429 & yGIL432 ~ 5pm

6/16/09

	Initials	Date
Prepared By		
Approved By		

SETUP OF LONG-TERM PROPAGATION

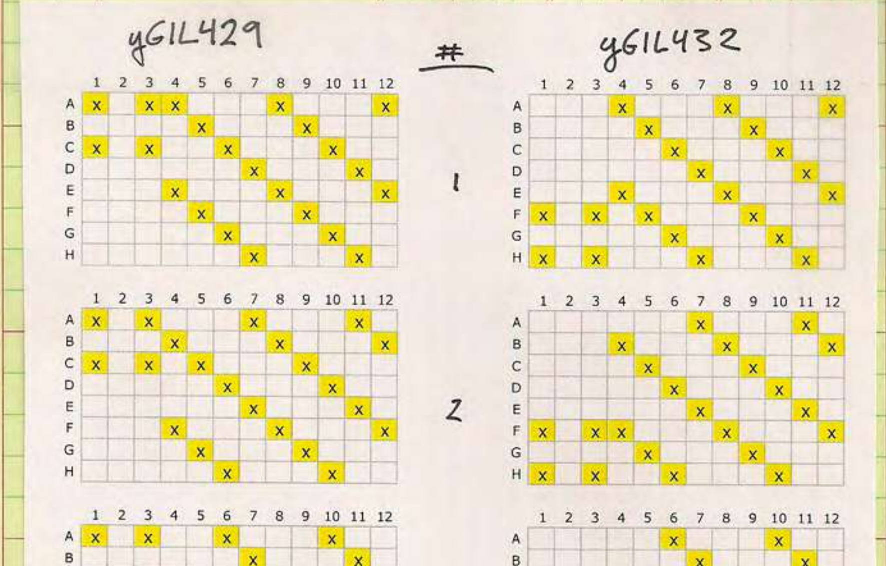
- On Saturday (6/13) I struck yGIL429 & yGIL432 from -80.
- Yesterday (6/15) I started 5mL YPD cultures from a single colony of each strain.
- This morning I diluted the o/N cultures 1:30 (1 mL into 30mL YPD)

↳ Coulter Count Diluted cultures (~9:30am)

Bkg	160000	304000
yGIL429	$1.681E^{7}$	$1.736E^{7}$
yGIL432	$1.348E^{7}$	$1.347E^{7}$
Post Bkg	$6.558E^{6}$	$2.302E^{6}$

NOTE: I forgot that I was making duplicate plates → ∴ I made a second 1:30 dilution of each o/N culture.

- I set up the plates by aliquotting 125 λ of the diluted culture into 76 wells → 4 plates per strain.



yGIL429 # yGIL432

1

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

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

2

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

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

3

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

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

4

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

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

NOTES ON PLATE SETUP

- Each Plate has 22 blank wells. Each plate has a different pattern of the 22 blank wells.
- The 1st 3 columns identify the strain
  - yGIL429, A1, A3, C1, C3 blank
  - " 432, F1, F3, H1, H3 blank
- ~~The~~ Columns 4-12 have blanks on the diagonal
  - Plate 1, 1 blank in "upper right"
  - " 2, 2 — " —
  - " 3, 3 — " —
  - " 4, 4 — " —
- Plates put in incubator @ 11:00am

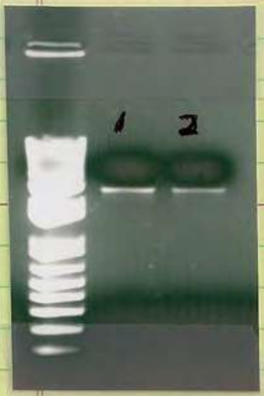
6/24/09

	Initials	Date
Prepared By		
Approved By		

TRANSFORMATION OF yGIL429 & yGIL432 w/ ste2Δ & ste7Δ cassettes

In order to do fitness assays to determine the selective coefficients of sterile strains under the conditions of our experiment, I need to derive steriles from our experimental strains

PCR Δ cassettes



1) ste2Δ::KanMX
2) ste7Δ::KanMX } → Zymo purify → elute 10λ

- On Monday (6/22) I streaked yGIL429 & yGIL432 from freezer.
- Yesterday I started 3ml YPD cultures of these strains.
- This morning the strains were not too saturated
  ↳ @ 9 I added add'l 7ml to each to bring up to 10mL

COULTER COUNTS		(10am)
Bkg	94000	118000
yGIL429	2.666E7	2.665E7
yGI432	2.474E7	2.442E7
Post Bkg	812000	462000

- 10:50 → start transformations.

6/25/09

- Replica plate transformations to YPD+G418

6/26/09

- Transformation efficiency looks good — pick two single colonies from each plate and restreak to YPD.

6/28/09

- Pick single colony from restreaked plates → inoc. 5ml YPD

6/30/09

- Yesterday I restreaked the 8 putative transformants to YPD & G418.
  ↳ All regrew on both plates
  - yGIL432 ste7Δ A formed small colonies (fitness defecT)
    ↳ also low sat. density in liquid.

- Yesterday I tested for αF^R → All 8 are αF^R

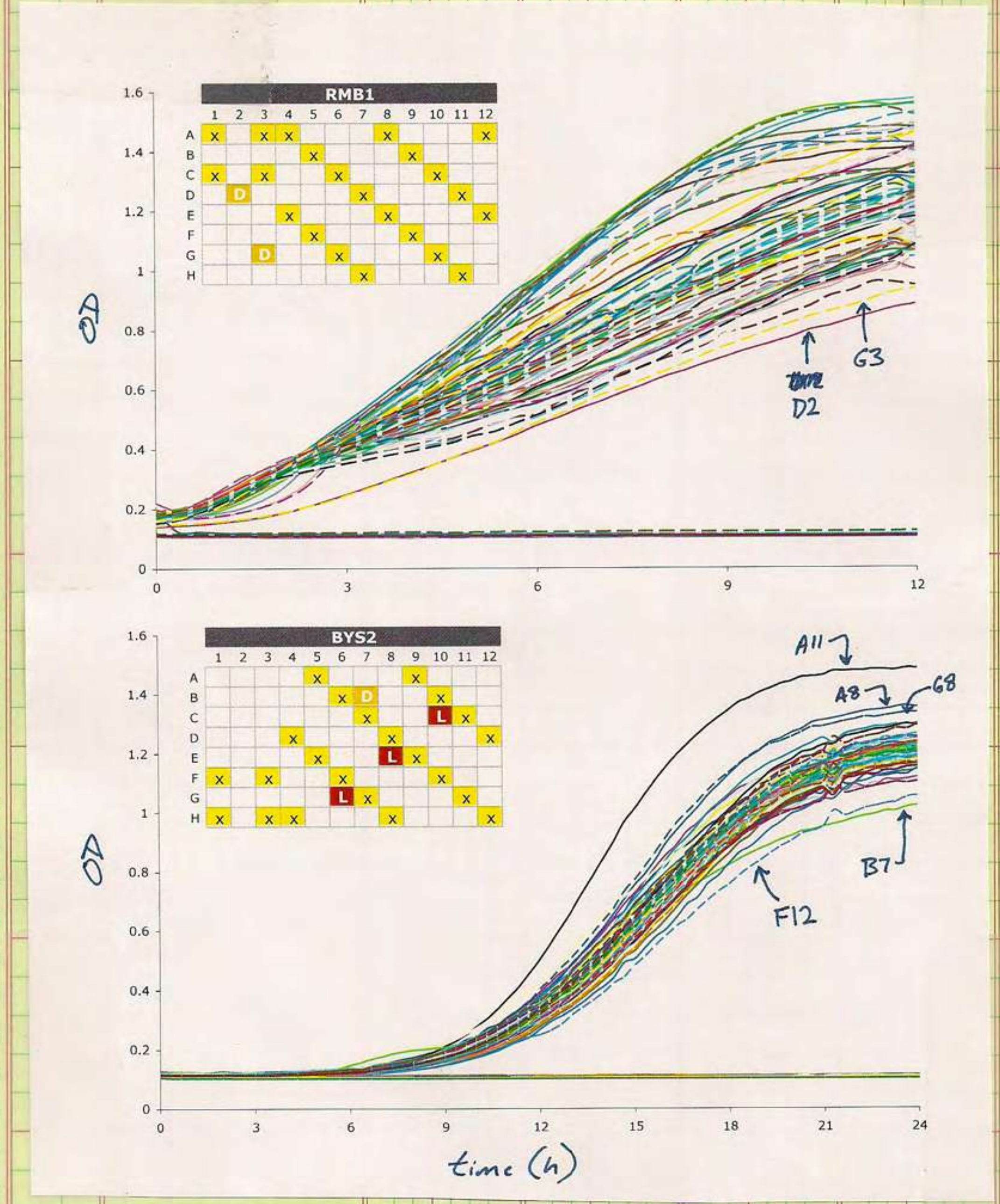
- Freezedown strains:
  - yGIL429 ste2Δ A → yGIL491
  - " " B → " 492
  - " ste7Δ A → " 493
  - " " B → " 494
  - yGIL432 ste2Δ A → " 495
  - " " B → " 496
  - " ste7Δ A → " 497 (fitness defect)
  - " " B → " 498

7/8/2009

	Initials	Date
Prepared By		
Approved By		

TECAN GROWTH CURVES

- Over the last two days we ran growth curves in the Tecan on copies of RMB1-40 & BYS2-21



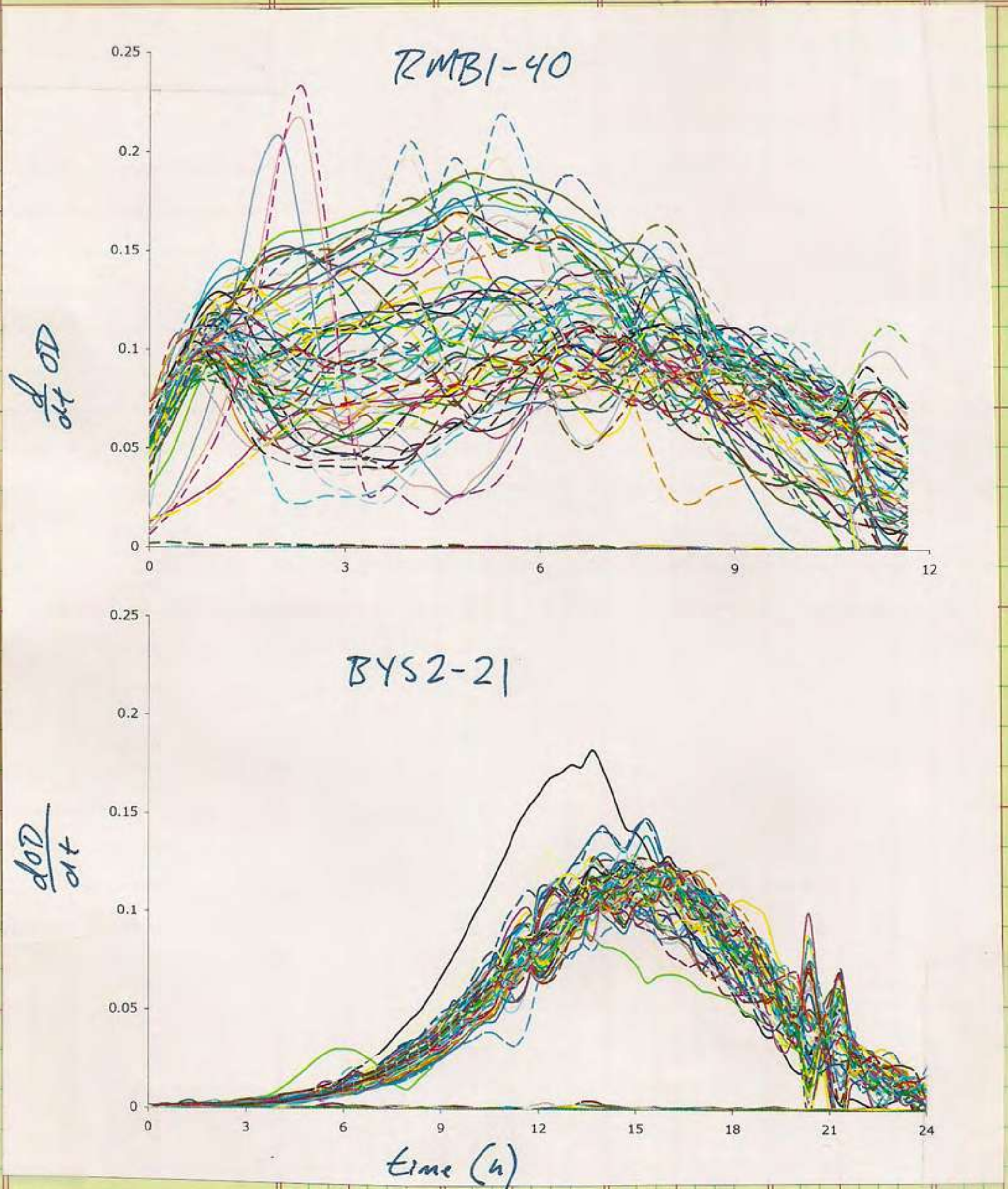
RMB1

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

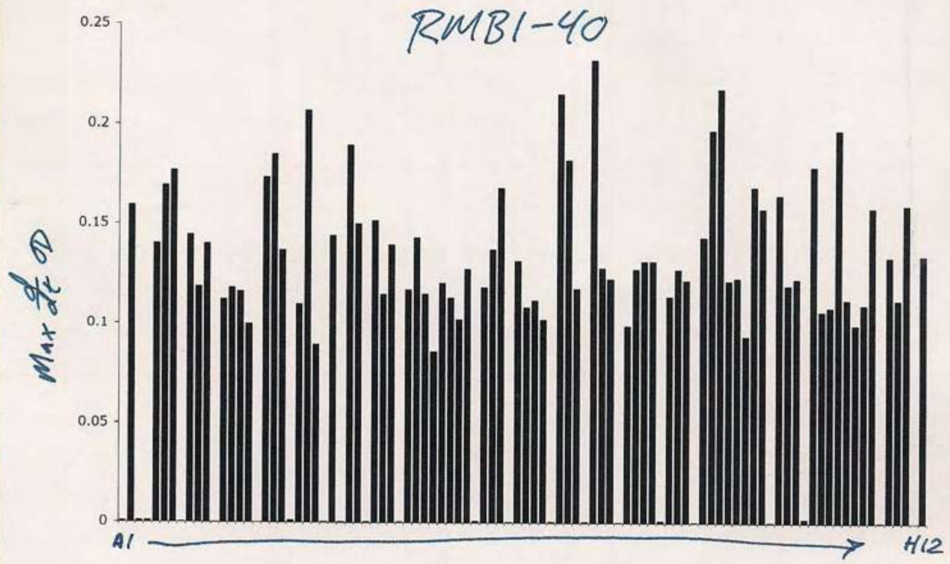
BYS2

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

7/9/09 MORE ANALYSIS OF TECAN DATA

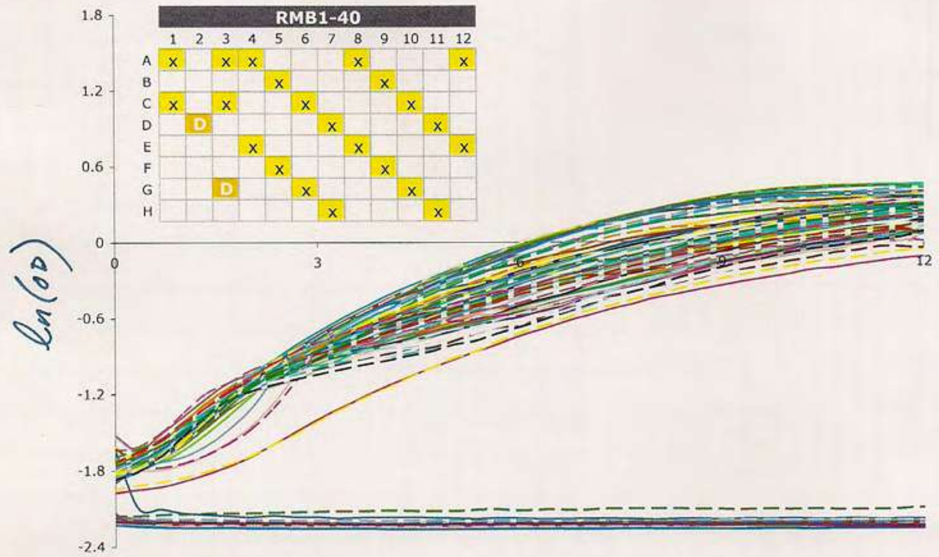


	Initials	Date
Prepared By		
Approved By		


RMB1-40

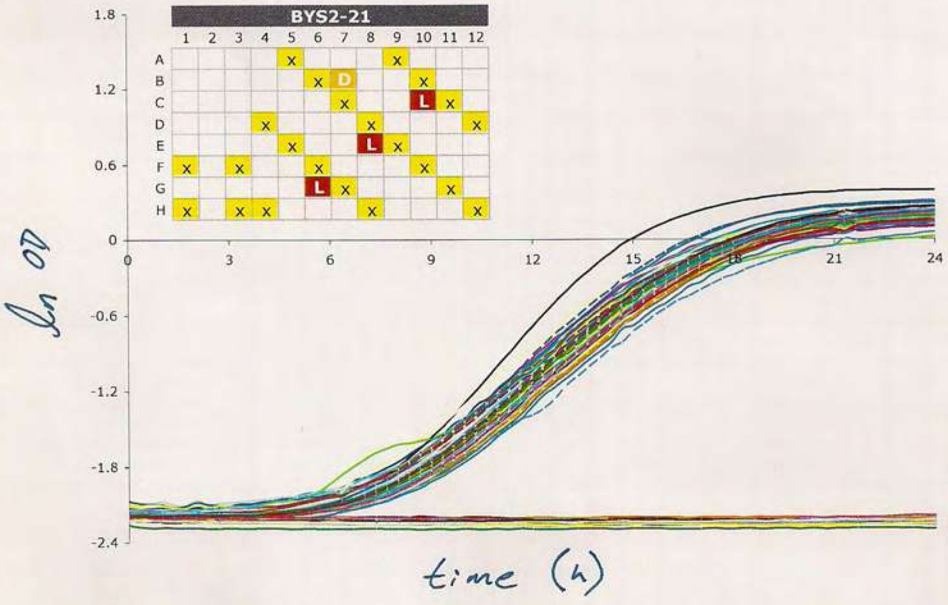

BYS2-21

RMB1-40



RMB1-40

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

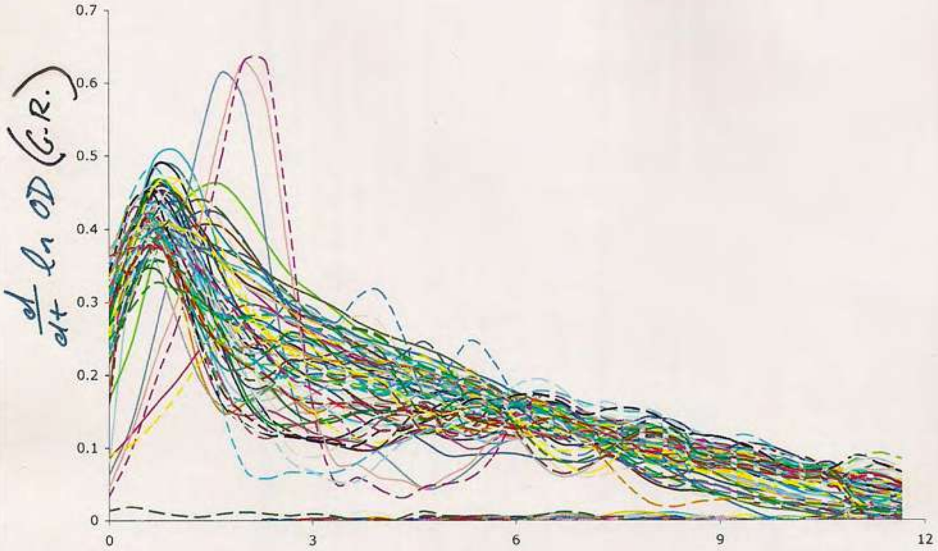


BYS2-21

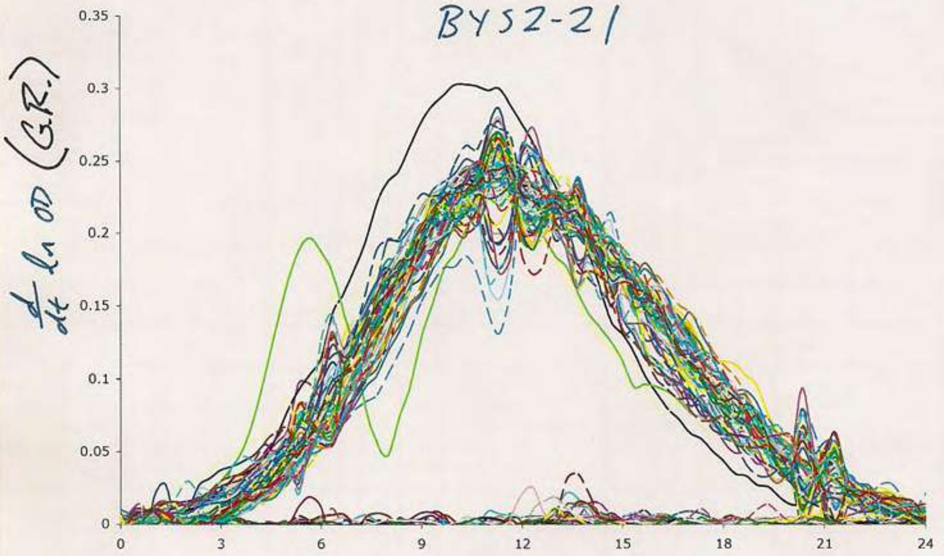
	1	2	3	4	5	6	7	8	9	10	11	12
A					X				X			
B						X	D			X		
C							X			L	X	
D				X				X				X
E					X			L	X			
F	X		X			X				X		
G						L	X				X	
H	X		X	X				X				X

	Initials	Date
Prepared By		
Approved By		

RMB1-40

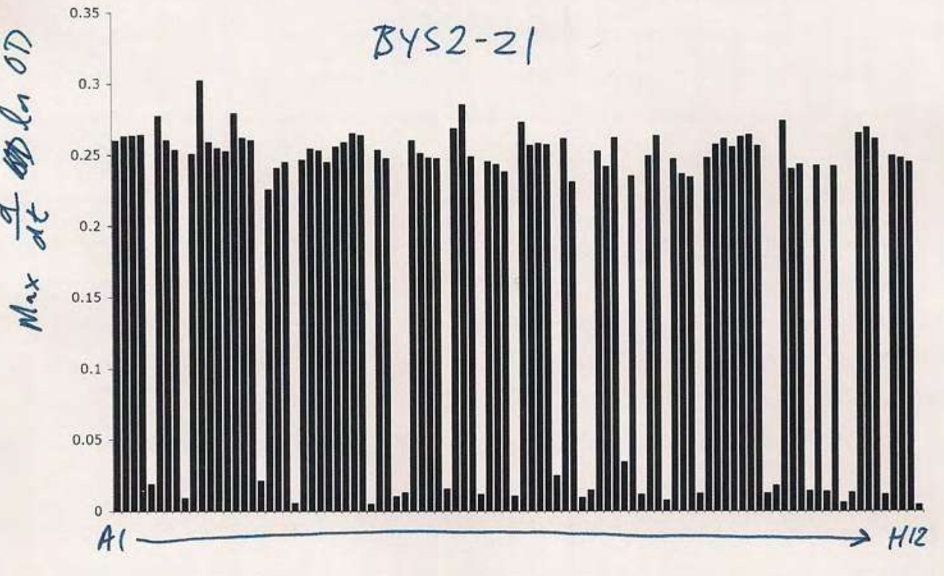


BY52-21

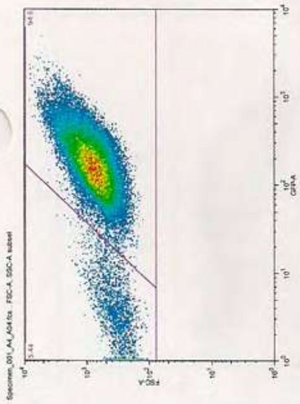


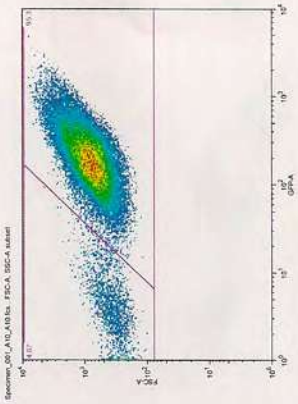
RMB1-40

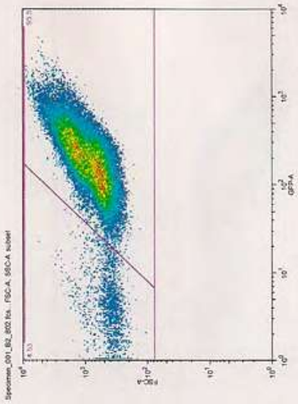
Max Growth Rate

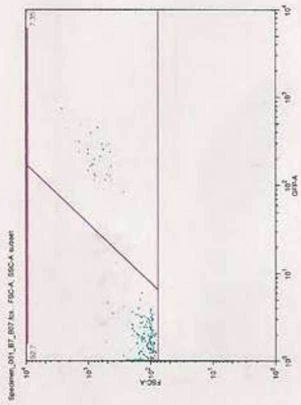


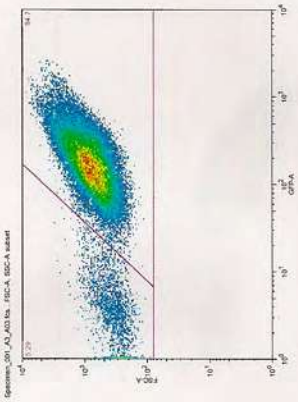
7/11/09 FACS ON PLATE BYS2-23

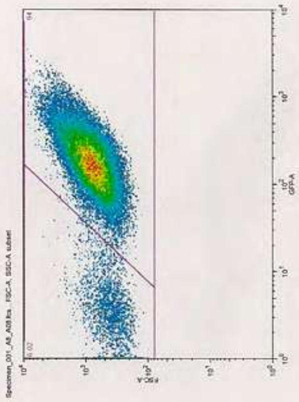


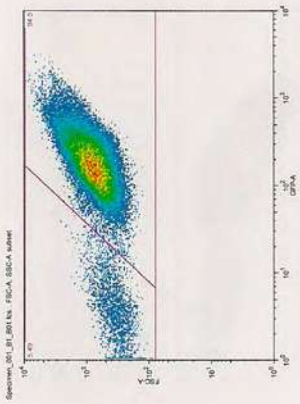


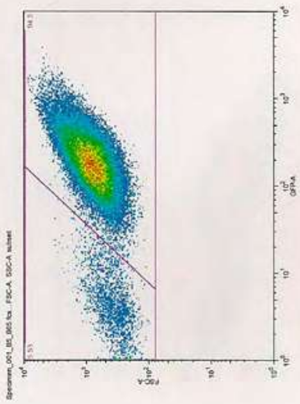


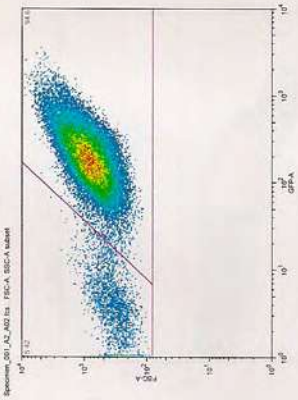


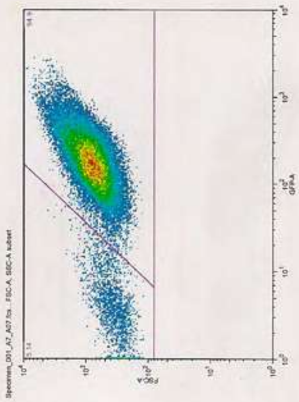


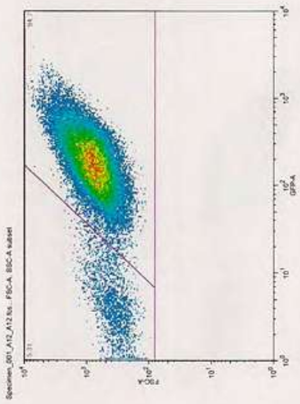


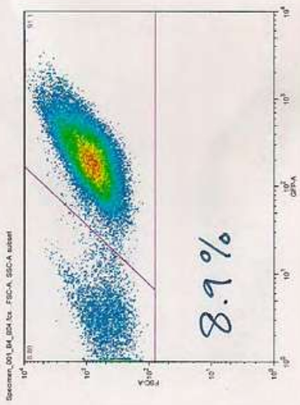




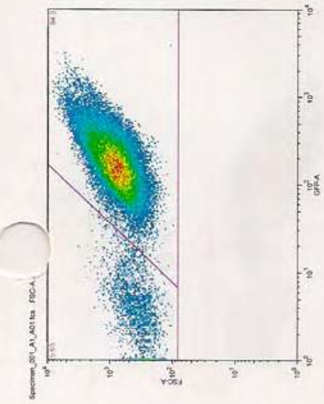


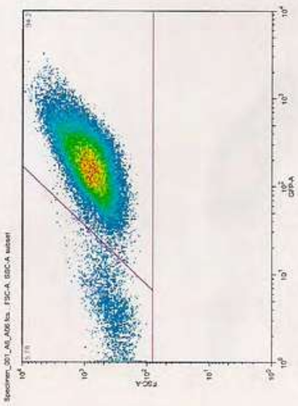


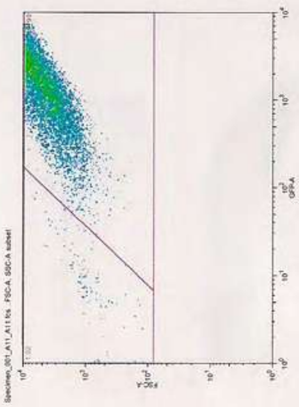


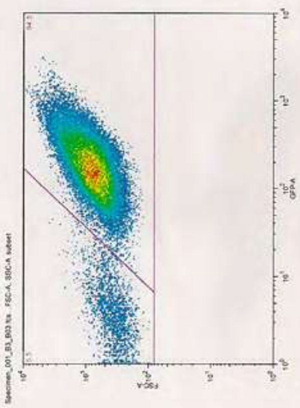


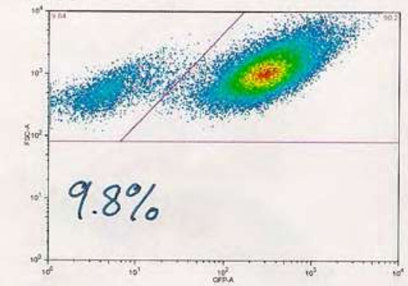
8.9%



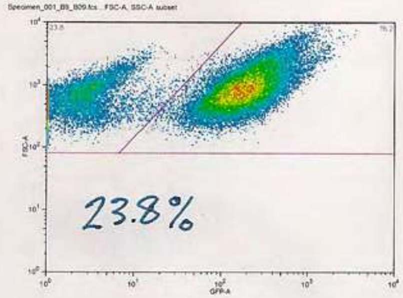




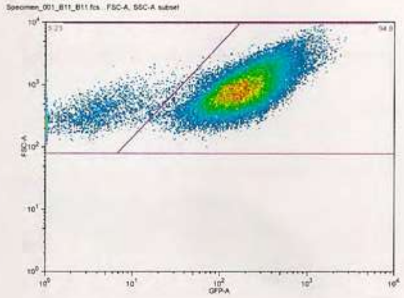


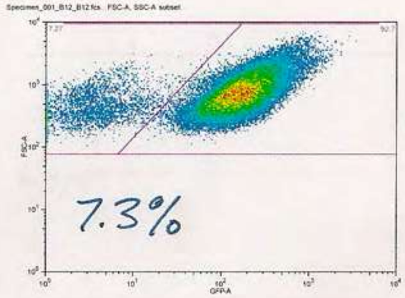

Specimen_001_B8_B08.fcs...FSC-A, SSC-A subset

9.8%

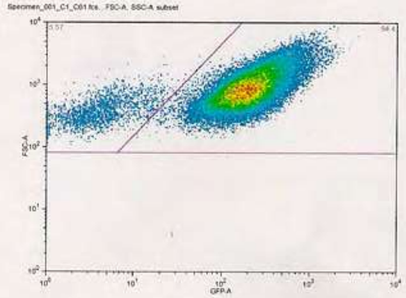

Specimen_001_B9_B09.fcs...FSC-A, SSC-A subset

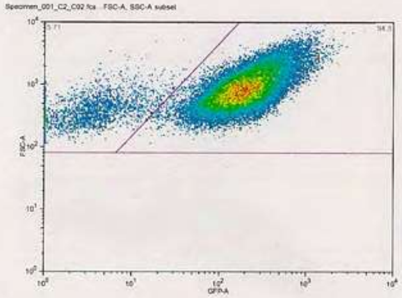
23.8%

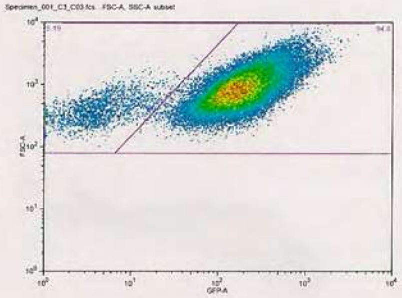

Specimen_001_B11_B11.fcs...FSC-A, SSC-A subset

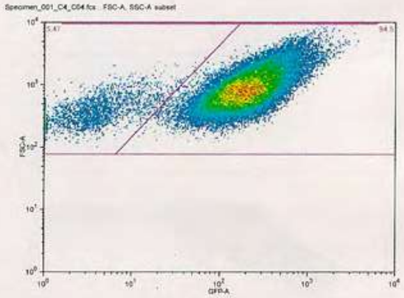

Specimen_001_B12_B12.fcs...FSC-A, SSC-A subset

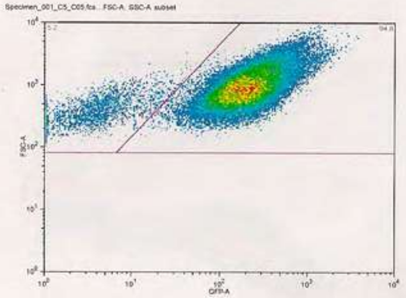
7.3%

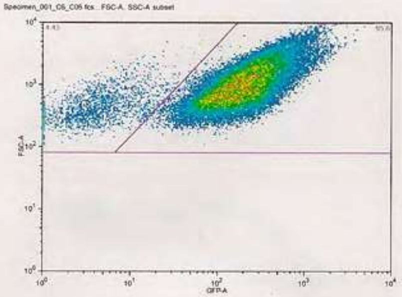

Specimen_001_C1_C01.fcs...FSC-A, SSC-A subset

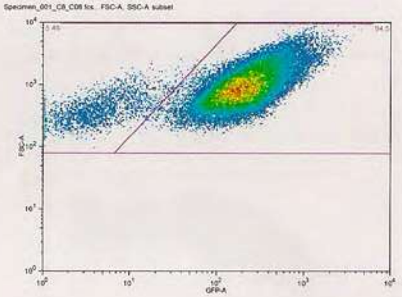

Specimen_001_C2_C02.fcs...FSC-A, SSC-A subset

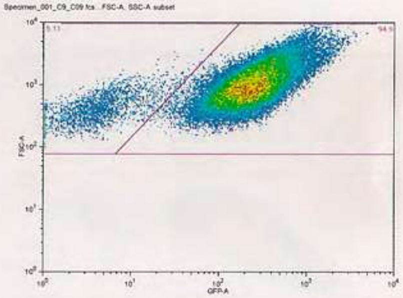

Specimen_001_C3_C03.fcs...FSC-A, SSC-A subset

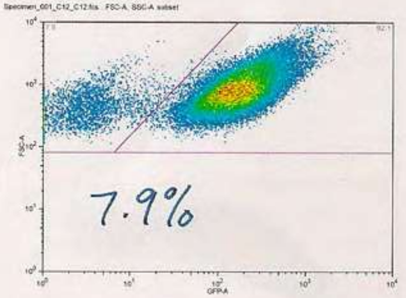

Specimen_001_C4_C04.fcs...FSC-A, SSC-A subset


Specimen_001_C5_C05.fcs...FSC-A, SSC-A subset

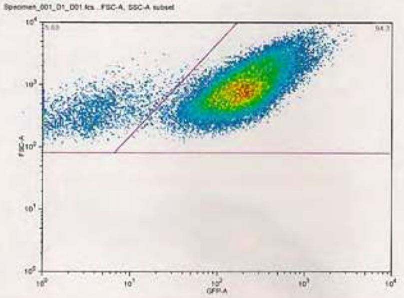

Specimen_001_C6_C06.fcs...FSC-A, SSC-A subset

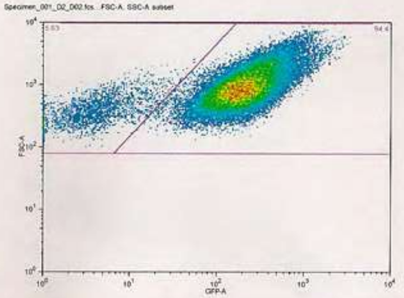

Specimen_001_C8_C08.fcs...FSC-A, SSC-A subset

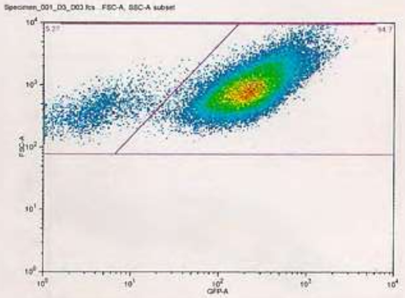

Specimen_001_C9_C09.fcs...FSC-A, SSC-A subset


Specimen_001_C12_C12.fcs...FSC-A, SSC-A subset

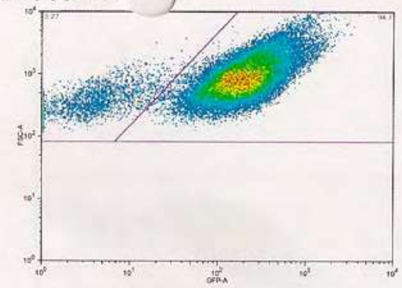
7.9%


Specimen_001_D1_D01.fcs...FSC-A, SSC-A subset

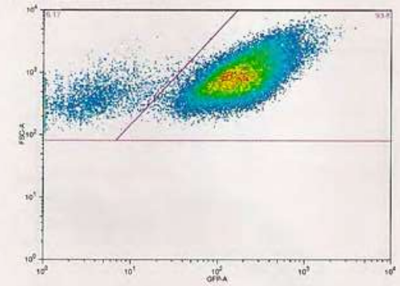

Specimen_001_D2_D02.fcs...FSC-A, SSC-A subset


Specimen_001_D3_D03.fcs...FSC-A, SSC-A subset

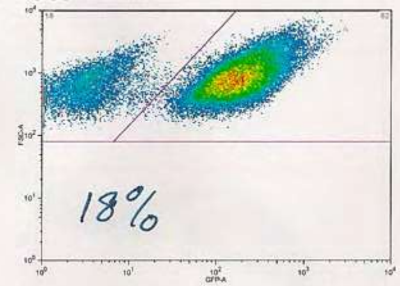
Specimen_001_D5_D05.fcs...FSC-A



Specimen_001_D6_D06.fcs...FSC-A, SSC-A subset

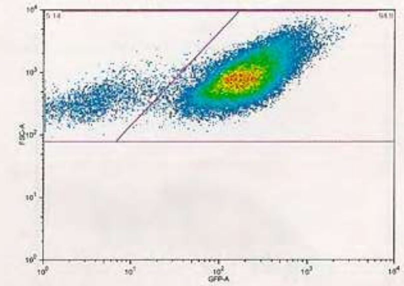


Specimen_001_C7_D07.fcs...FSC-A, SSC-A subset

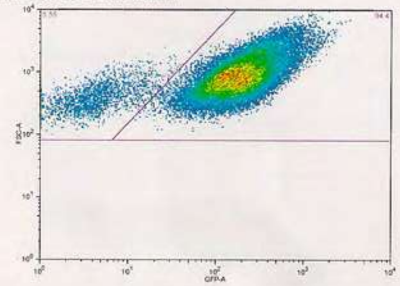


18%

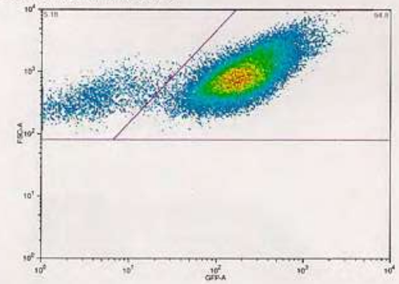
Specimen_001_D9_D09.fcs...FSC-A, SSC-A subset



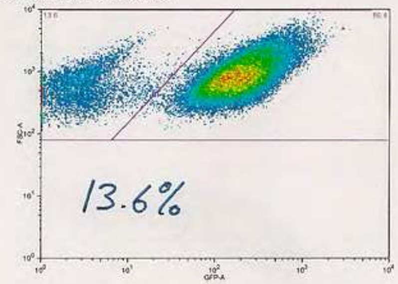
Specimen_001_D10_D10.fcs...FSC-A, SSC-A subset



Specimen_001_D11_D11.fcs...FSC-A, SSC-A subset

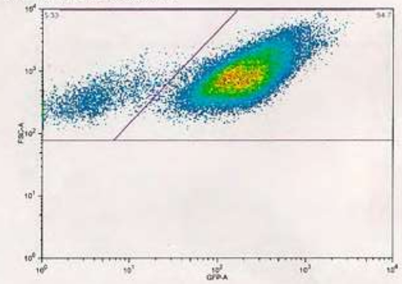


Specimen_001_E1_E01.fcs...FSC-A, SSC-A subset

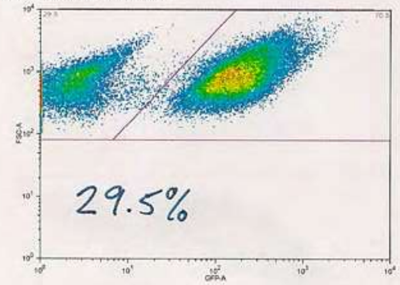


13.6%

Specimen_001_E2_E02.fcs...FSC-A, SSC-A subset

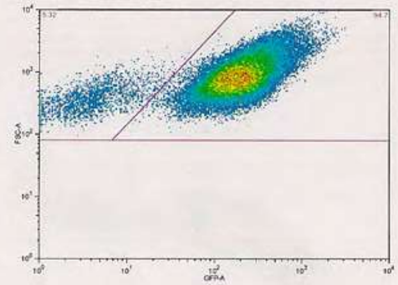


Specimen_001_E3_E03.fcs...FSC-A, SSC-A subset

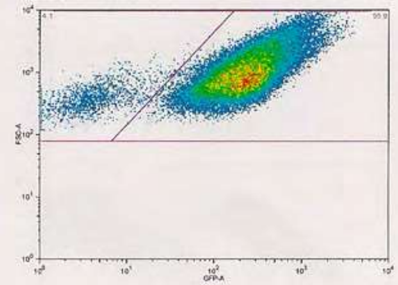


29.5%

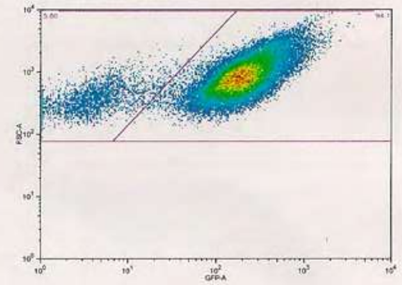
Specimen_001_E4_E04.fcs...FSC-A, SSC-A subset



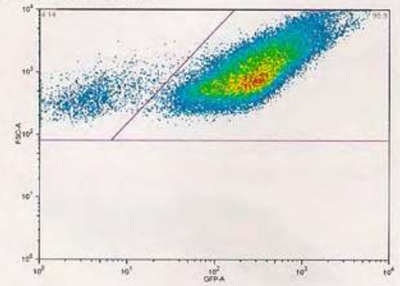
Specimen_001_E6_E06.fcs...FSC-A, SSC-A subset



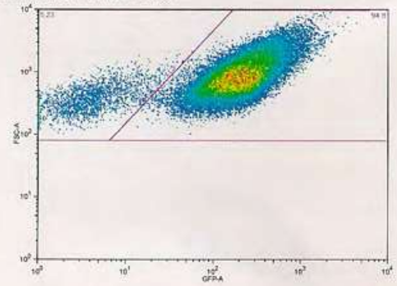
Specimen_001_E7_E07.fcs...FSC-A, SSC-A subset



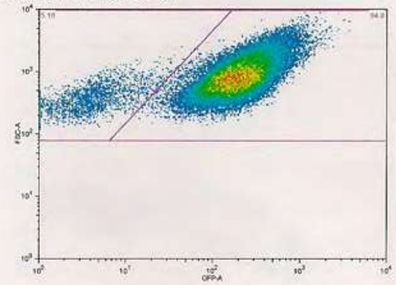
Specimen_001_E10_E10.fcs...FSC-A, SSC-A subset



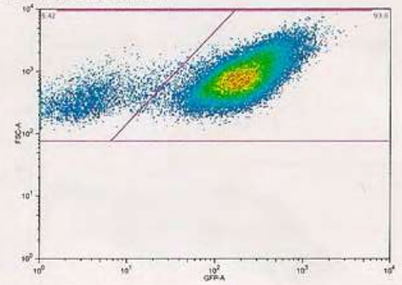
Specimen_001_E11_E11.fcs...FSC-A, SSC-A subset



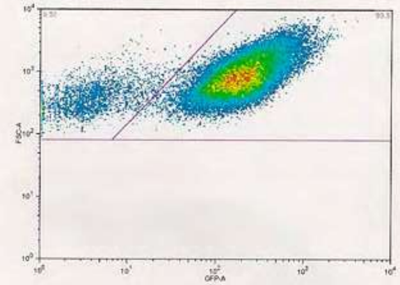
Specimen_001_E12_E12.fcs...FSC-A, SSC-A subset



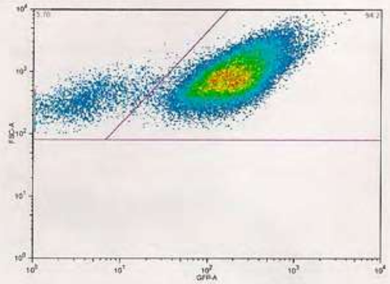
Specimen_001_F2_F02.fcs...FSC-A, SSC-A subset



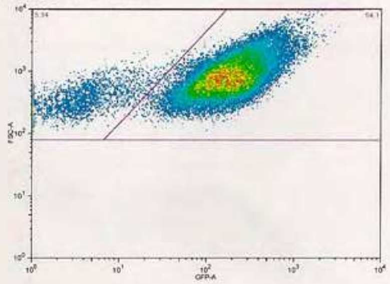
Specimen_001_F4_F04.fcs...FSC-A, SSC-A subset



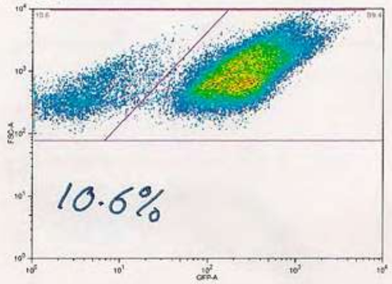
Specimen_001_F5_F05.fcs...FSC-A, SSC-A subset



Specimen_001_F7_F07.fcs...FSC-A, SSC-A subset

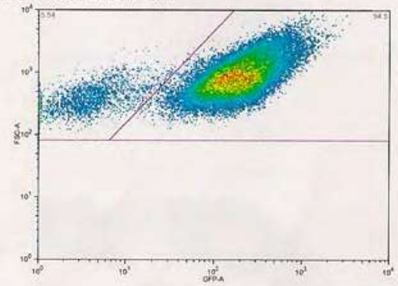


Specimen_001_F8_F08.fcs...FSC-A, SSC-A subset

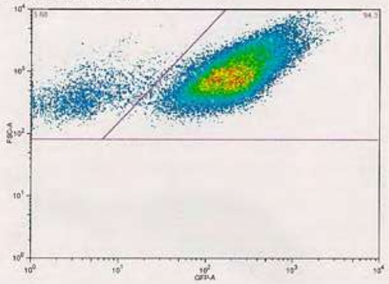


10.6%

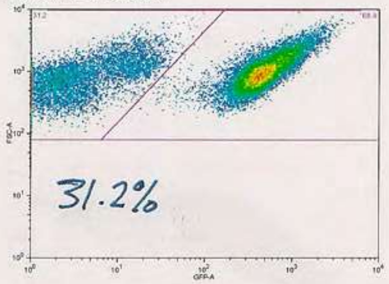
Specimen_001_F9_F09.fcs...FSC-A, SSC-A subset



Specimen_001_F11_F11.fcs...FSC-A, SSC-A subset

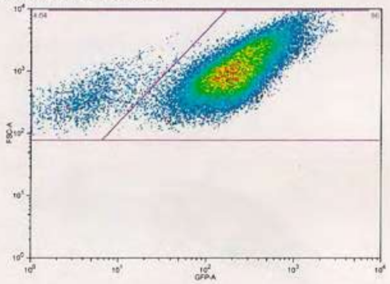


Specimen_001_F12_F12.fcs...FSC-A, SSC-A subset

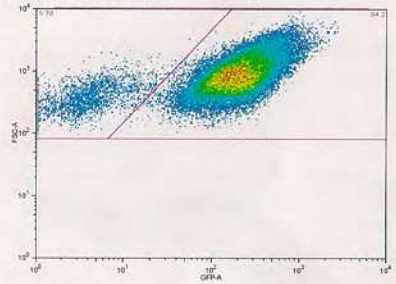


31.2%

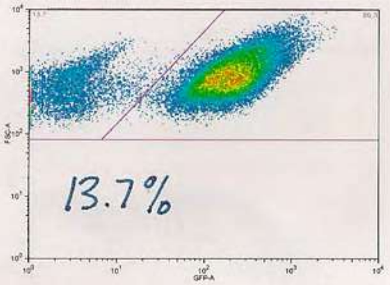
Specimen_001_G1_G01.fcs...FSC-A, SSC-A subset



Specimen_001_G2_G02.fcs...FSC-A, SSC-A subset

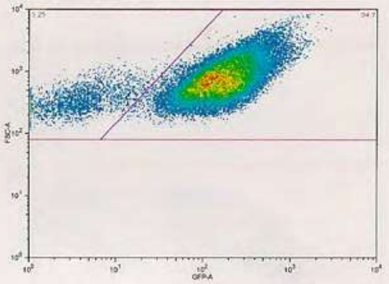


Specimen_001_G3_G03.fcs...FSC-A, SSC-A subset

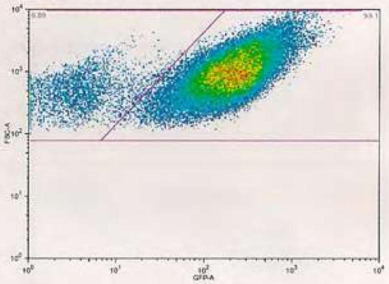


13.7%

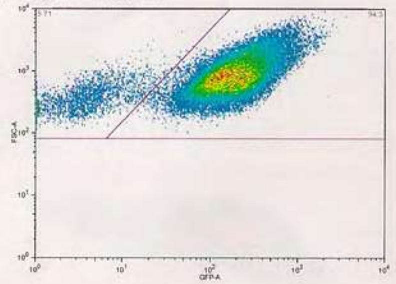
Specimen_001_G4_G04.fcs...FSC-A, SSC-A subset



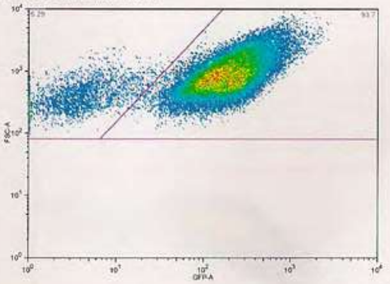
Specimen_001_G5_G05.fcs...FSC-A, SSC-A subset



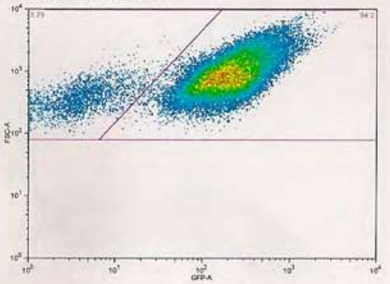
Specimen_001_G6_G06.fcs...FSC-A, SSC-A subset



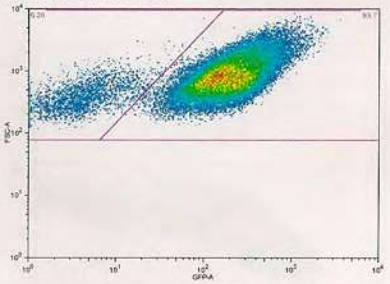
Specimen_001_G9_G09.fcs...FSC-A, SSC-A subset



Specimen_001_G10_G10.fcs...FSC-A, SSC-A subset



Specimen_001_G12_G12.fcs...FSC-A, SSC-A subset

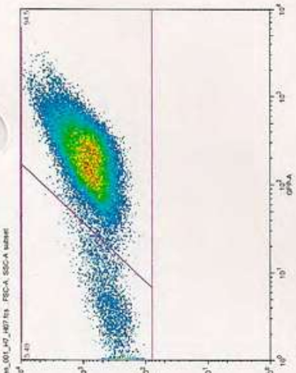


7/11/07 NOTES ON INDUCTION OF BYS2-23

We changed the induction protocol as follows:

- At the same time as we do the evening dilutions, transfer 4λ into 100λ YPD+αF (for this expt we used 2×αF) in PCR plate
- 30° for 4h → 4° forever in thermal cycler
- spin down 2 min 5000 ~~RPM~~ RPM
- Aspirate off media, resuspend in 125 PBST; transfer to 96 well plate

↳ this induction protocol worked very well.

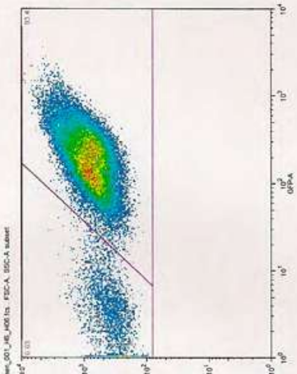


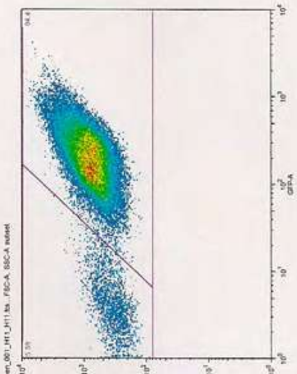
- Background non-induced cells was 5-6% for most cultures.
- 10 ~~9~~ cultures appeared to have above background non-fluorescent cells (presumably steriles): B4, B8, B9, D7, E1, E3, F8, F12, G3, H5
- 2 others may have a modest increase: B12, C12.
- A11 (the only one that appeared to grow faster & saturate at a higher density — see p21) is larger — thus brighter as well. Perhaps 2n?
  ↳ but still MATa
- B7 ~~as~~ has v. small cells (bacteria?)
- B2 has two populations of induced cells — possibly another size diff?

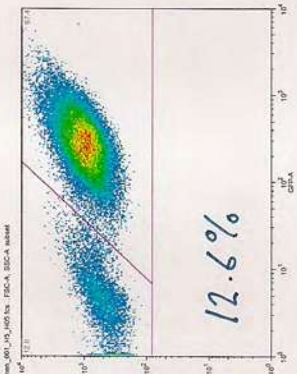
↳ I should get size dist.ⁿ for A11 & B2

- Something is not quite right w/ F12 — it looks slow growing/dilute via Tecan data. It has a high fraction of non-inducers, but they look diff. in FSC v. GFP than the others.

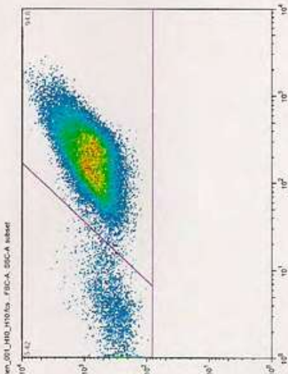
↳ perhaps a contaminant. I should look at these under the microscope.

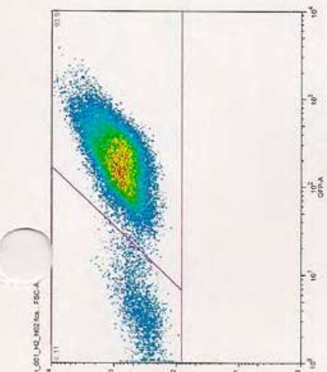


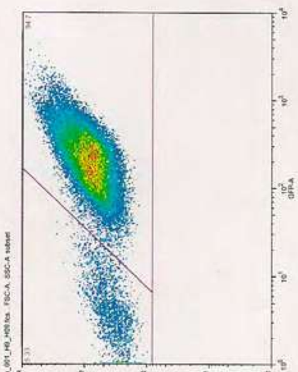




12.6%







	Initials	Date
Prepared By		
Approved By		

7/12/09

STANDARD CURVE EXPERIMENT

Now that we have worked out an induction protocol we need to generate standard curves relating the fraction of observed non-induced cells after induction to the fraction of steriles present before induction — this is necessary since steriles will continue to grow during the induction period.

- Last night I started 5 mL YPD cultures of the following
  - 4612429 (RM)
  - " 432 (BY)
  - " 493 (RM ste7Δ)
  - " 498 (BY ste7Δ)

these plates were originally streaked from stock on   and were at my benchtop.

- This morning I aliquotted the saturated cultures into a 96-well plate:

Rows A & B - RM
" C & D - BY
" E & F - RM ste7Δ
" G & H - BY ste7Δ

incubator @ 9:50am

- After the morning dilutions I diluted the plate — same dilution as the Bsg bottleneck cultures — this will get the cultures growing under the same conditions as the expt. cultures
- I will sacrifice columns 1, 2, 11, & 12 to the coulter counter.
- I will ~~mix~~ transfer columns 3-10 to a new plate (mixing WT & respective steriles such that the fraction of steriles is:

COLUMN

ROW	3	4	5	6	7	8	9	10	
A	0	1	5	10	20	30	40	50	RM
B	50	60	70	80	90	95 ~~100~~	99	100	
C	0	1	5	10	20	30	40	50	BY
D	50	60	70	80	90	95	99	100	

- I will transfer the cultures 8 times into 4 PCR plates
  - The 1st time into 1x dF
  - The 2nd time into 2x dF in Rows H-E (plate inverted)

→ The 4 PCR plates will be induced for 4, 6, 8, and 12 h.

7/12/09

Coulter Count (9:20 pm)

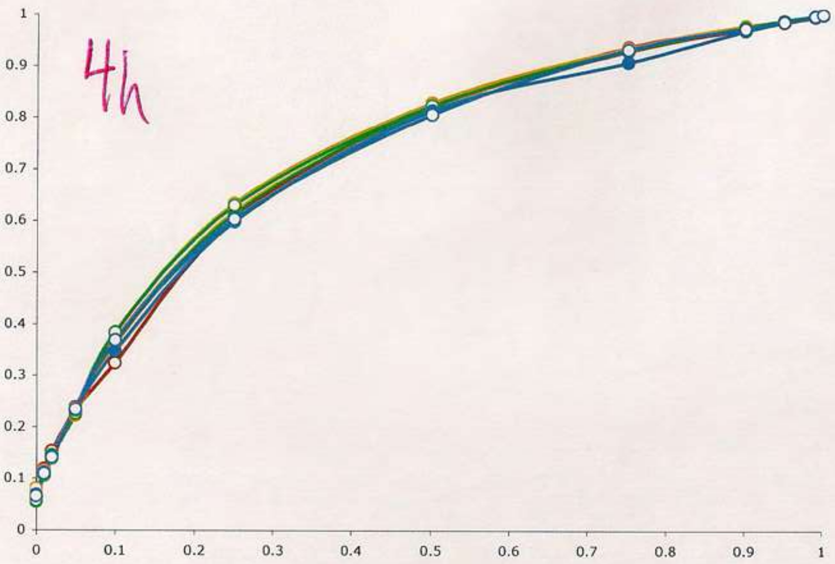
Bkg	54000	270000
RM	5.626 E7	5.702 E7
RM	5.508 E7	5.507 E7
BY	4.919 E7	4.895 E7
BY	5.078 E7	5.028 E7
RMste7	4.333 E7	4.338 E7
RMste7	4.677 E7	4.762 E7
BYste7	5.019 E7	5.09 E7
BYste7	5.598 E7	5.547 E7
PostBkg	1.802 E6	1.542 E6

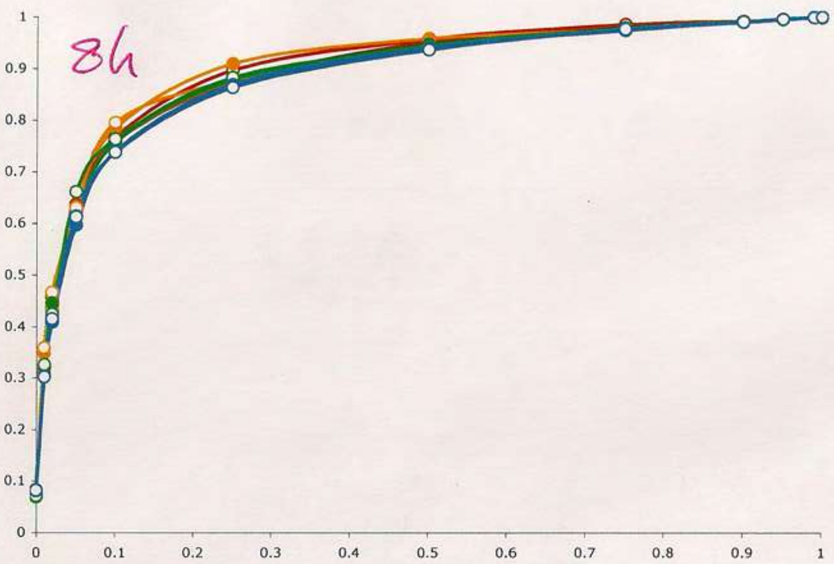
} 10 λ into 20 ml

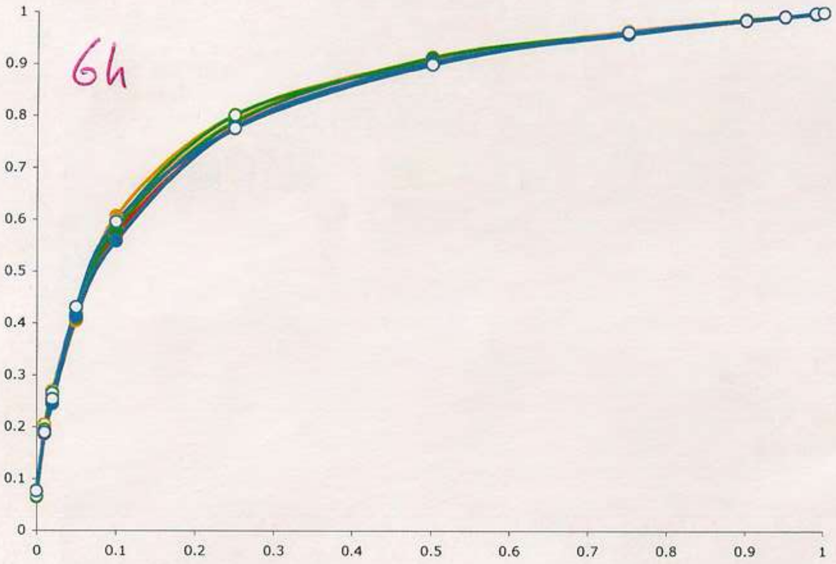
- Set up master plate — new setup:

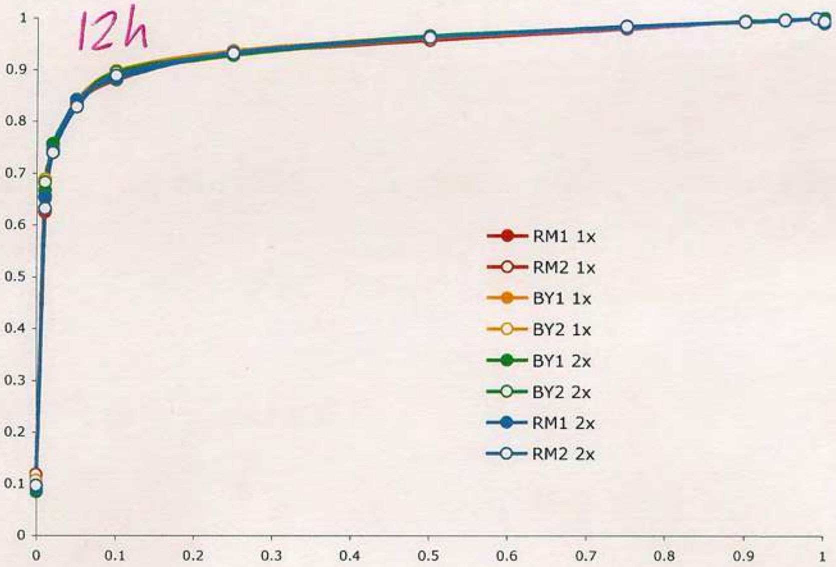
Column	Ste7Δ	WT
1	0 λ	100 λ
2	1	99
3	2	98
4	5	95
5	10	90
6	25	75
7	50	50
8	75	25
9	90	10
10	95	5
11	99	1
12	100	0

Rows & αF conc. same as p.


4h
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1


8h
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1


6h
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1


12h
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
RM1 1x
RM2 1x
BY1 1x
BY2 1x
BY1 2x
BY2 2x
RM1 2x
RM2 2x

8/18/09

PROJECT UPDATE

We have been propagating our cultures through 500 generations — corresponding to 50 ~~#~~ transfers for the small population size and 100 transfers for the big population size.

~~the~~ We have been inducing and running FACS on the cultures approximately every 40 gen. We have 12 time points between generation 0 and 500:

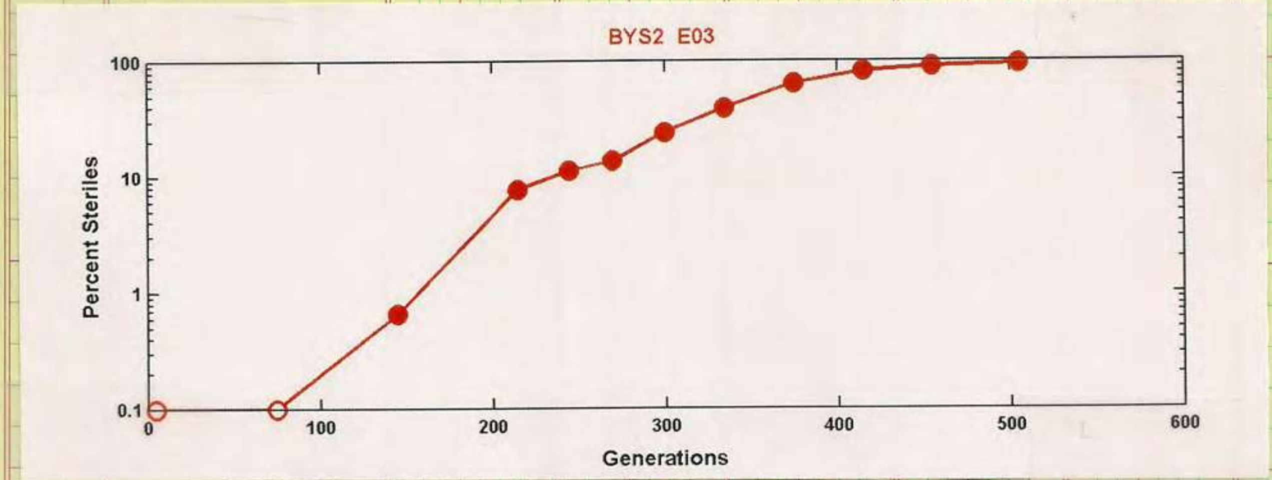
5, 75, 145, 215, 245, 270, 300, 335, 375, 415, 445, & 505

from frozen samples The 5 gen. shift is from a 12h growth period prior to induction

from real time samples. The 5 gen shift after 330 gen is from a 12h recovery after our weeklong hiatus.

The experiment seems to be working quite well; however the results are different from what we expected. What follows is a cursary summary of this expt. through 500 gen.

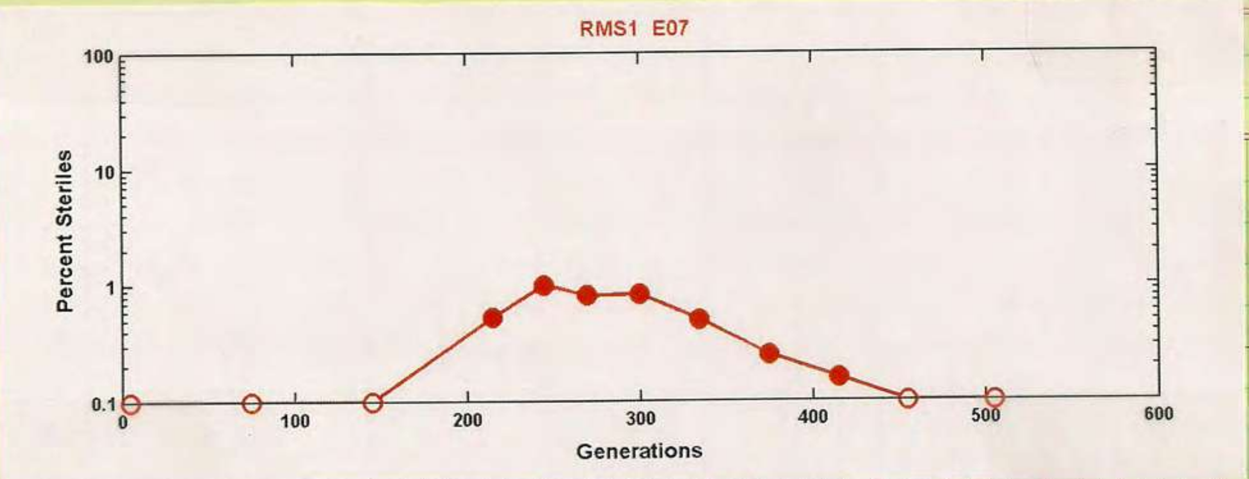
- We do see a few cultures ~~that~~ where sterile strains have fixed. Only in two cultures I am confident that they will not be outcompeted. For example:



- Many other cultures show steriles rising linearly (on a log plot), however I suspect that the majority of these will be outcompeted. This is presumably because a second ~~muta~~ beneficial mutation arises in the ancestral population and ~~out~~ sweeps to fixation, thereby eliminating the sterile ~~pop~~ population

8/18/09

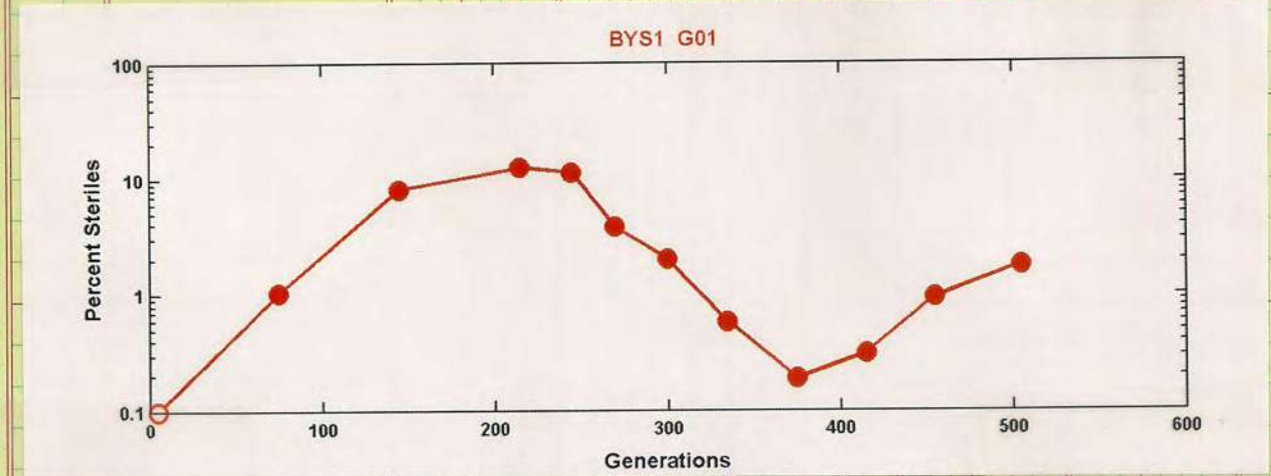
EXAMPLE OF A STERILE BEING OUTCOMPETED:



- In the majority of our ~600 cultures we see nothing happening — presumably, because other mutations are sweeping, thus preventing steriles from reaching high frequency.

- When we do see steriles arise, the majority of those populations eventually get outcompeted. (such as RMS1-E07)

- In a couple cases we see more complicated trajectories:



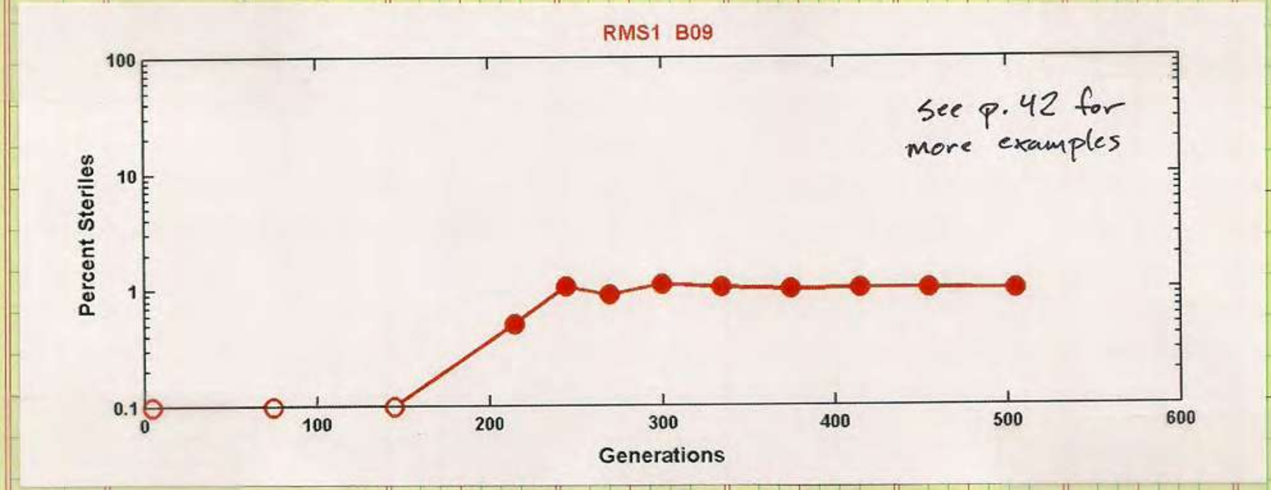
↳ In the case of BYS1-G01, steriles arose early and increased to ~10%, then began to decrease in abundance due to clonal interference. However, by generation 400 the steriles are on the rise again. There are two ways this could happen: the most likely explanation is that a second sterile mutation occurred in the sweeping population. It is also possible (although less likely) that a second beneficial mutation occurred in the dwindling sterile population. When the expt. is done, BYS1-G01 will be a prime candidate for a detailed case study.

8/18/09

	Initials	Date
Prepared By		
Approved By		

A SURPRISING TRAJECTORY

We also see some trajectories for which we do not have a satisfactory explanation



see p. 42 for more examples

We can see 17 cultures in which sterile strains arise, then flatten out

RMS - 7 : RMS1 - B9, C5, G5, H10, H12, RMS2 - F1, G1
BYS - 6 : BYS1 - D1, E11, H10, BYS2 - B8, C4, H5
RMB - 1 : RMB2 - F9
BYB - 3 : BYB1 - B2, B12, BYB2 - H8

8/19
between 17 and 30 depending how they are counted

→ The equilibrium for these is somewhere between 0.2 & 3%

I can think of two possible explanations (neither of which I am satisfied with) for these trajectories:

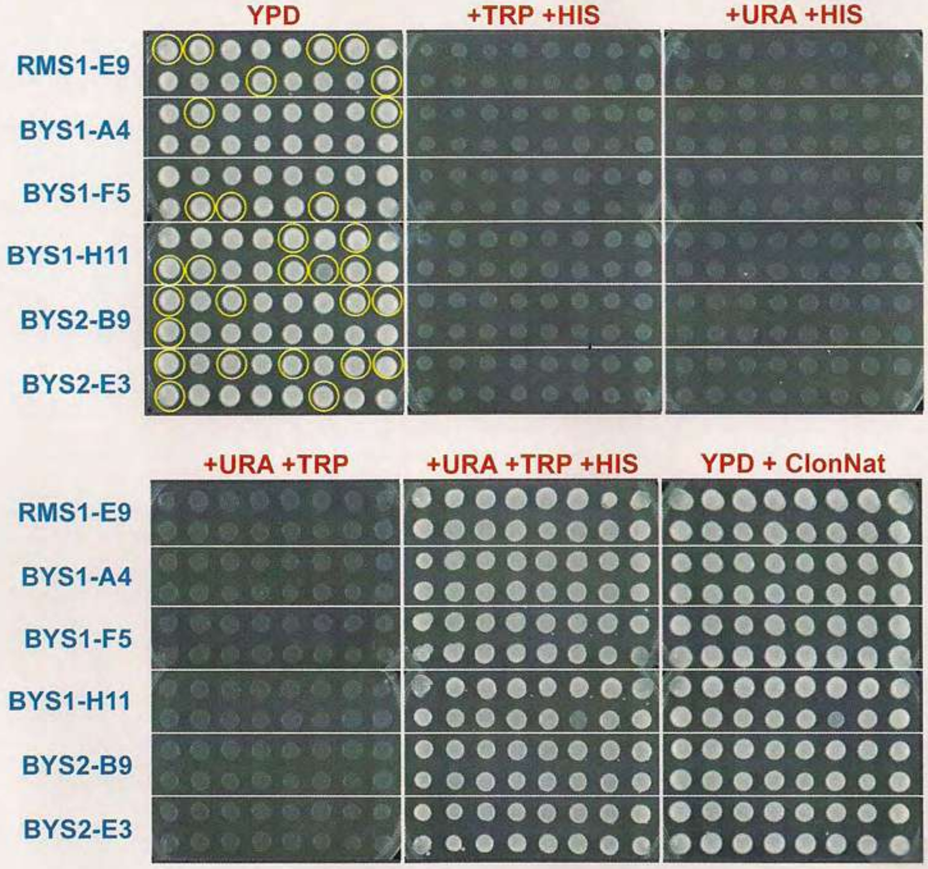
(1) They are neutral sterile mutations (i.e. STE2) that have drifted to high frequency. This is supported by two observations: these steriles seem to "jump" from nothing to their equilibrium frequency, and they are more common in the small populations (13 to 4). However, the observation that some of these are ~3% makes it seem unlikely that drift can provide a comforting explanation. Either we don't understand how drift works here or we (inadvertently) subjected our cultures to a v. small bottleneck.

(2) The other possibility is that the ancestral genotype is gone and the steriles are coexisting in the population with another genotype that exhibits frequency-dependent selection.

8/18/09

- We needed to show that the sterile strains in our populations are derived from our strain background, which is ura3, trp1, his3, Nat^R.
  - ↳ at generation 270 we took the six cultures w/ the highest frequency of steriles and struck for single colonies.
    - ↳ pick 16 colonies each and pin transfer to -ura, -his, -trp, YPD, & Nat
      - circled clones are mating-proficient
      - un-circled clones are sterile

Sterile strains are derived from WT background (Generation 270)

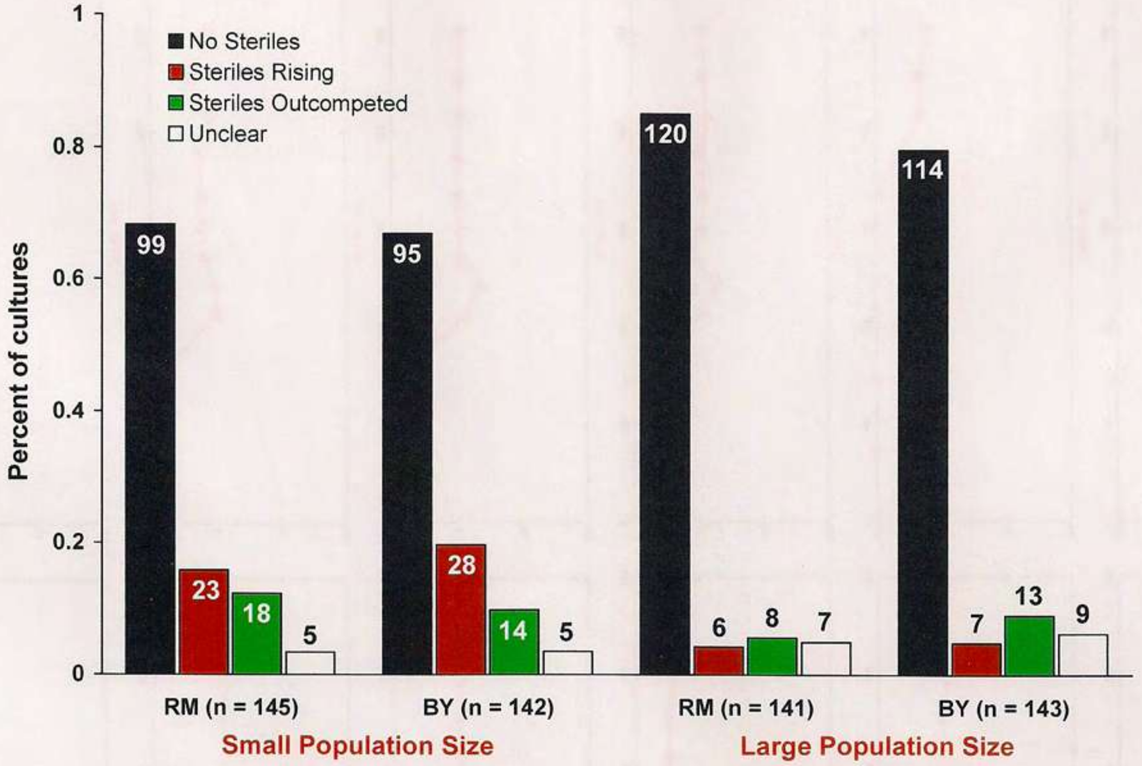


SUMMARY STATISTICS

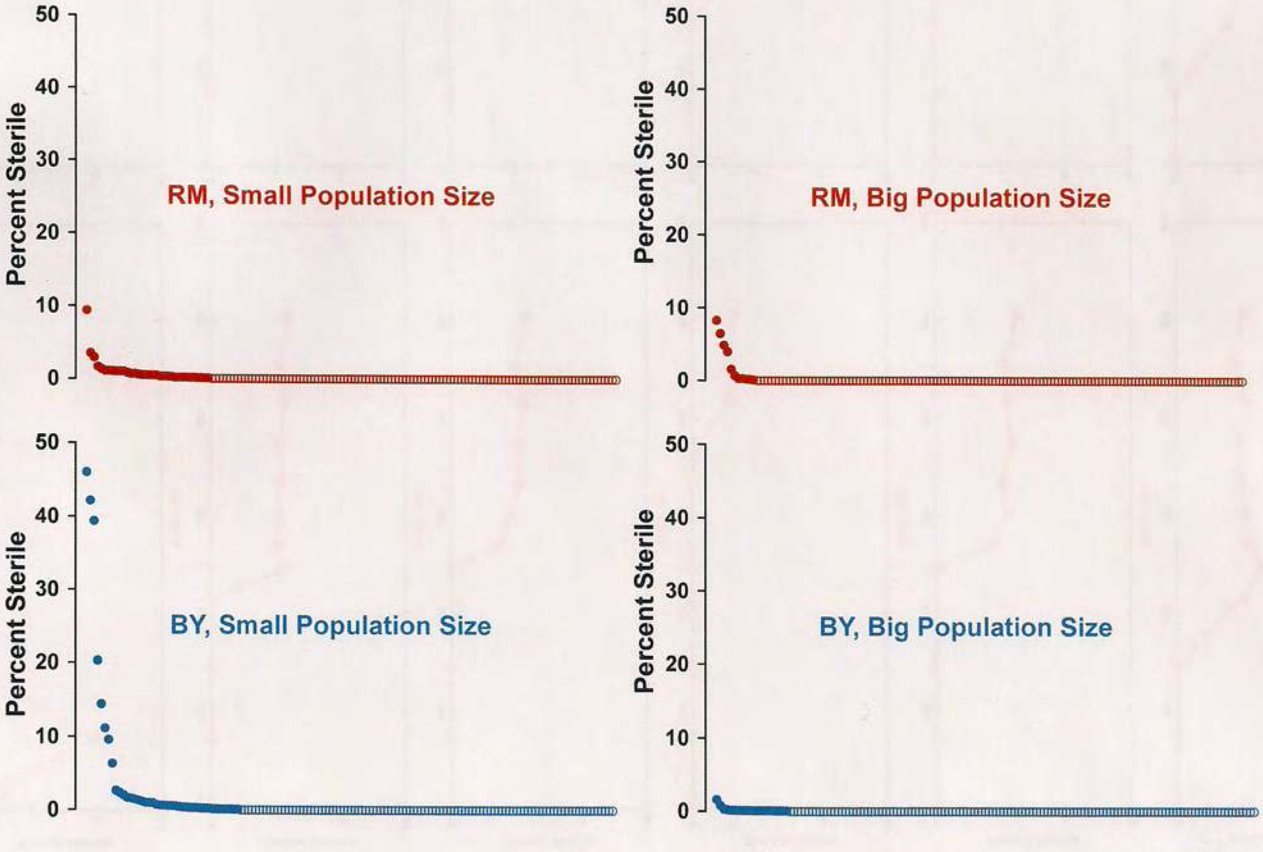
On page 41 are summary statistics which I prepared for lab meeting on 8/3.

↳ these statistics are outdated — many of the cultures where steriles were increasing later appeared to be outcompeted by non-steriles.

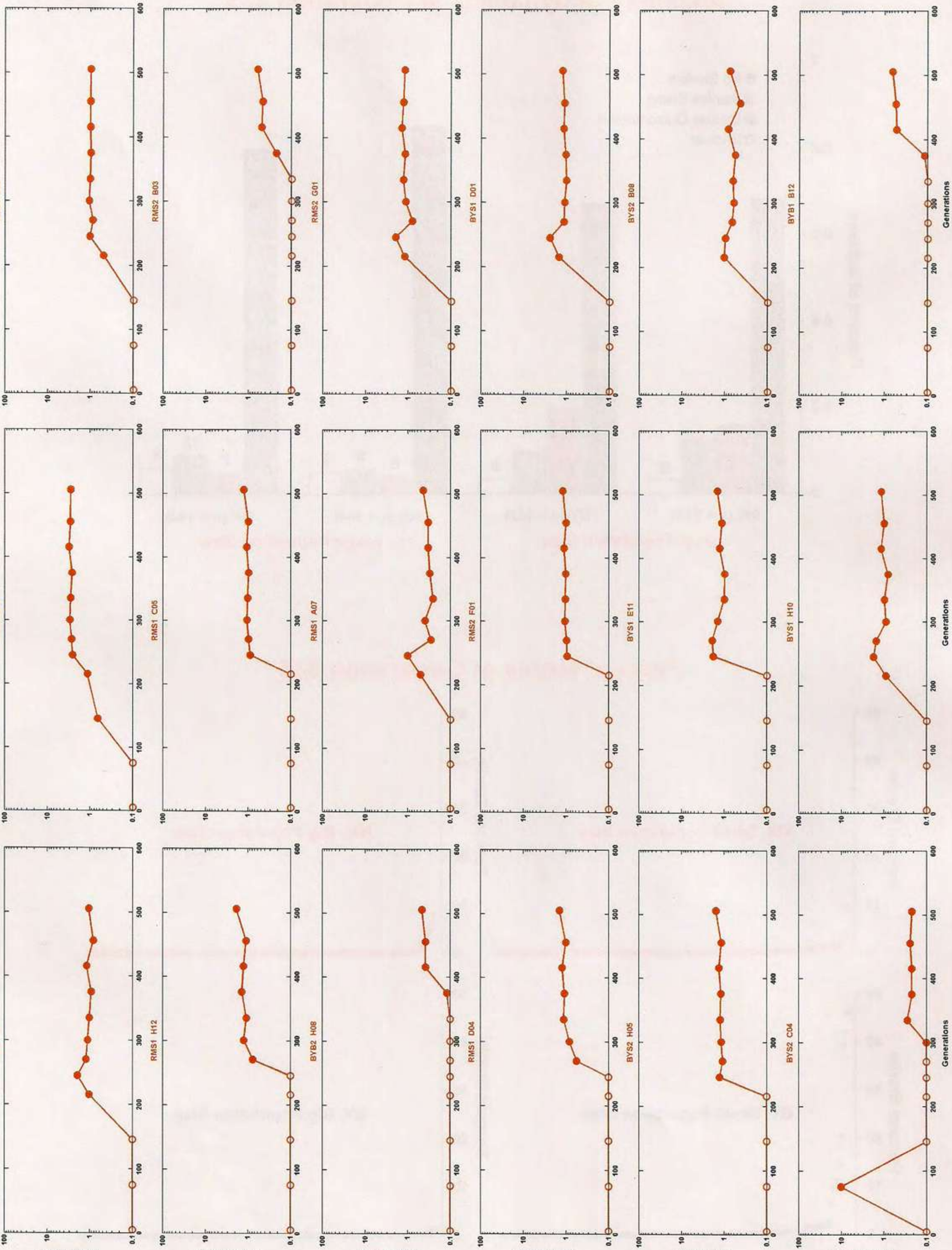
Summary of dynamics at Generation 335



Percent sterile at Generation 335



8/19/09 18 EXAMPLES OF STERILES AT EQULIBRIUM



8/19/09

	Initials	Date
Prepared By		
Approved By		

TRANSFORMATION OF yGIL121, BY4741, & BY4742 w/ dTomato & GFP

- In order to do fitness assays of both ~~ent~~ mating competent and sterile strains against a common reference we need to construct an adequate reference strain. For this I will transform yGIL121 (W303, MATa, ade2, his3, leu2, trp1, bar1Δ::ADE2, hmlαΔ::LEU2) w/ dTomato::Nat or GFP::Nat at ~~ZEL~~ YLR255C.

- On 8/10 I streaked the following:
  pGIL022 - pFA6a, dTomato::Nat
  pGIL021 - " GFP::Nat

- Last week I miniprepped two clones each.

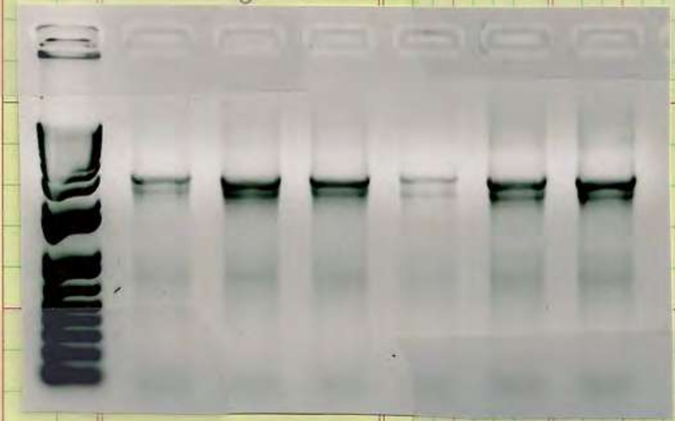
- Over the weekend (8/15) I ran PCR on pGIL021-A & pGIL022-A using

003
32944977 IDT
G.LANG
34796300 9/21/2007
YLR255CextF1
5'- CTA AAC GAG GCC GCG AGG GTG GAT TTC GCC CG
Tm= 73.5 ºC , MW= 18,647.1
13.3OD260 22.80nmol 0.43mg

004
32944978 IDT
G.LANG
34796301 9/21/2007
YLR255CextR1
5'- GAA TCT ATA CAC ACA GAA ATC GTA CTT CAA GAA
Tm= 63.1 ºC , MW= 18,475.1
15.2OD260 24.60nmol 0.46mg

50° Anneal, 3 m extension

- On monday (8/17) I ran PCR on all 4 plasmids:



LANE	TEMPLATE
1	pGIL021 A from 8/15
2	" A from 8/17
3	" B "
4	pGIL022 A from 8/15
5	" A from 8/17
6	" B "

→ pool lanes 1&2 & 4&5. elute in 40λ from zymo. kit.

- On Saturday 8/15 I streaked yGIL121, BY4741, & BY4742 from freezer
- Last night I started o/n of a single clone each
- This morning I diluted each o/n 1:100 into duplicate 10 mL YPD tubes
- Coulter Counts (~2 pm)
  Bkg 84^{000} 31^{000}
  yGIL121 $2.392E^{7}$ $2.317E^{7}$ $2.383E^{7}$
  Post Bkg ~~[illegible]~~ $1.128E^{6}$ 696^{000}

- On 8/20 I replica plated the 6 plates to YPD+Nat

11203
11507

8/26/09

TRANSFORMATION CONTINUED

· 50 - 100 putative transformants grew on each Nat plate

↳ pick 3 colonies from each and restreak to YPD.

9/2/09

· On 8/31 I picked a single colony off of each restreaked plate and inoculated 3 ml of YPD. Today I screened the cultures under the scope to look for fluorescence

Putative transformant	Fluorescence	Freeze Down
yGIL121 - GFP 1	Yes	yGIL 504
" 2	Yes	" 505
" 3	Yes	" 506
dTomato 1	Yes	" 507
" 2	No	
" 3	No	
BY4741 - GFP 1	Yes (but may be dimmer than others)	" 508
" 2	No	
" 3	No	
dTomato 1	No	
" 2	Yes	" 509
" 3	No	
BY4742 - GFP 1	No	
" 2	No	
" 3	No	
dTomato 1	No	
" 2	No	
" 3	Yes	" 510

8/19/09

	Initials	Date
Prepared By		
Approved By		

INVESTIGATING LARGER CELLS

We have seen a number of cultures that have fixed larger cells. Some of these fixations occurred early (see BYS2-210-A11 on p. 21 or 20 generations later on p. 27). These cells have an advantage over steriles since they often occur in populations w/o steriles and, in a few cases, we have witnessed the decline of steriles and the concomitant rise of larger cells.

In addition to being larger the cells also appear to be hypersensitive to αF and show very low background of non-fluorescent cells (and low cell number) following induction.

A likely explanation for these changes is that the cells are 2n (although still MATa since they induce). This has been seen before by others - Gresham, Sally Otto, etc.

I want characterize these larger cells to determine if they are indeed 2n. The 1st test is to show that they are derived from our strain background - they almost certainly are since they induce YFP in the presence of αF, but I will phenotype them, just as I did for the steriles after generation 270 (p. 40)

- After generation 395, I took the 10 populations that had fixed larger cells (and 1 population that appeared to have fixed smaller cells) and streaked to singles. ~~After growing~~
- After growing up these plates sat on my bench then in my fridge for a few days.
- Yesterday I picked 8 colonies off of each plate and inoculated 100λ YPD.
- Today I pin transferred to the same plates as on p. 40.

8/20/09

SIZE DISTRIBUTIONS FOR POPULATIONS FIXED FOR LARGER CELLS

- As of generation 505, 31 populations appear to have fixed larger cells

COULTER COUNT - all are from generation 535

Bkg	86000	86^{000}
Control 1: RMS1-A5	$6.99E^7$	
" 2: BYS1-A2	$7.168E^7$	
" 3: RMB1-B3	$6.531E^7$	
" 4: BYB1-A1	$7.095E^7$	
RMS1-A11	~~$1.292E^7$~~	~~$4.769E^7$~~ — $9.724E^6$
B2	$2.164E^7$	
D6	$1.731E^7$	
D7	$2.203E^7$	
RMS2-C5	$1.847E^7$	
C10	$1.977E^7$	
E12	$2.248E^7$	
F5	$1.138E^7$	
BYS1-F2	$2.964E^7$	
H6	$3.552E^7$	
H11	$1.811E^7$	
BYS2-A11	$1.817E^7$	
RMB1-A2	$1.318E^7$	
B11	$2.136E^7$	
C7	$1.673E^7$	
D10	$9.606E^6$	
E5	$1.625E^7$	
H8	$7.542E^7$	
RMB2-A6	$9.706E^6$	
D2	$1.339E^7$	
F1	$8.96E^6$	
H5	$8.16E^6$	
H8	$1.083E^7$	
BYB1-D1	$1.117E^7$	
D10	$1.338E^7$	
F2	$7.772E^6$	
H8	$1.124E^7$	
BYB2-A1	$1.119E^7$	
A12	$8.192E^6$	
G2	$1.289E^7$	
G6	$1.232E^7$	
Post Bkg	$1.1E^6$	418^{000}

8/26/09

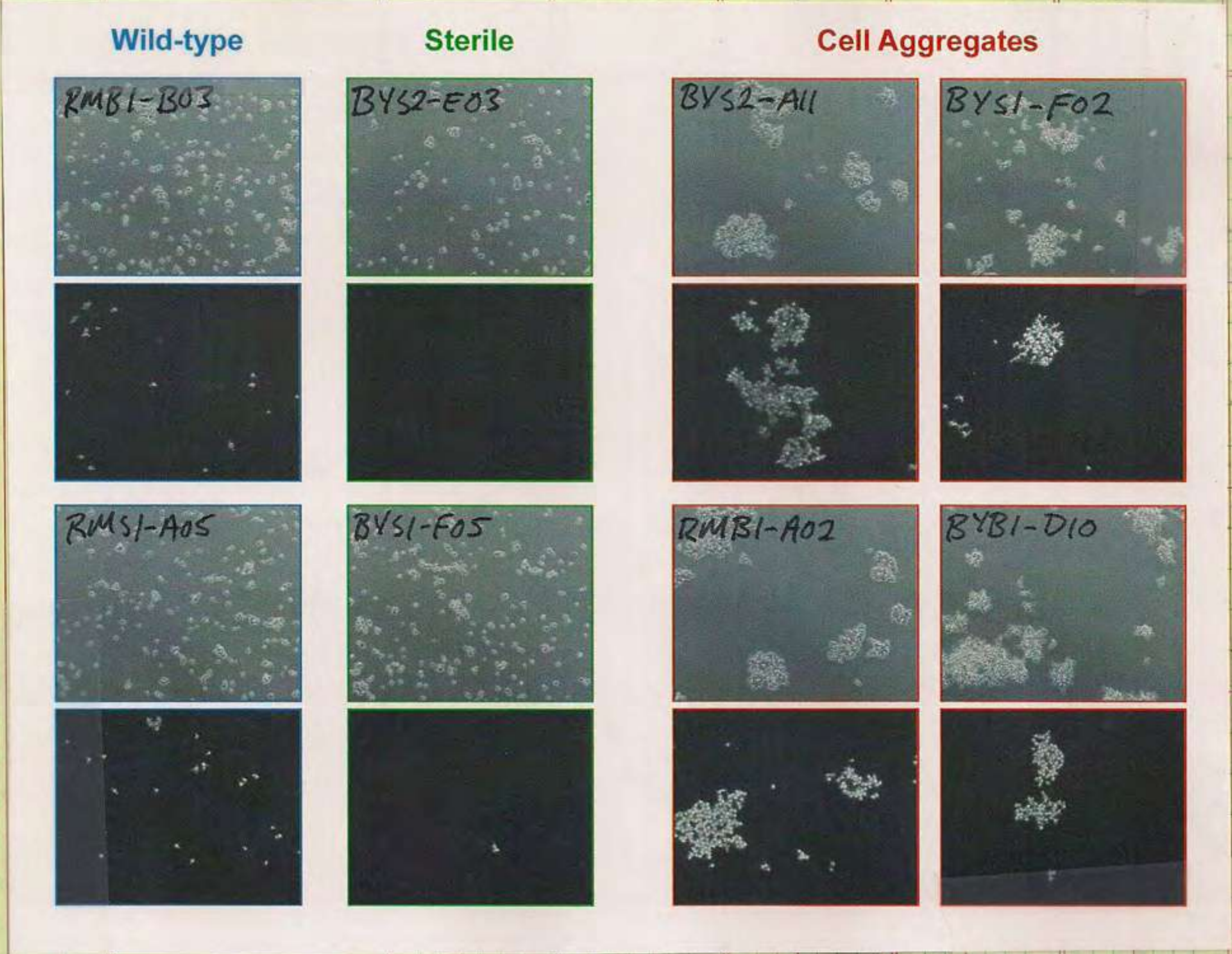
All of this coulter counting was pointless

→ Our "big" cells are not really big cells per se, but are cell aggregates. All of the counts appear to be dilute and the distributions (not shown since they don't make sense) are flat.

→ see p. 48 →

8/26/09

MICROSCOPY REVEALS CELL AGGREGATION



← DIC

← yEVenus after 6h αF induction

← DIC

← yEVenus after 6h αF induction

- I took pictures of the following strains from ngen. 555 (DIC) & 545 (αF)

1 WT-RMS1-A05
2 WT-BYS1-A02
3 WT-RMB1-B03
4 WT-BYB1-A01
5 S-BYS1-F05
6 S-BYS2-E03
7 B-RMS1-A11
8 B-RMS1-B02
9 B-RMS1-D06
10 B-RMS1-D07
11 B-RMS2-C05
12 B-RMS2-C10
13 B-RMS2-E12
14 B-RMS2-F05
15 B-BYS1-F02
16 B-BYS1-H06
17 B-BYS1-H11
18 B-BYS2-A11
19 B-RMB1-A02
20 B-RMB1-B11
21 B-RMB1-C07
22 B-RMB1-D10
23 B-RMB1-E05
24 B-RMB1-H08
25 B-RMB2-A06
26 B-RMB2-D02
27 B-RMB2-F01
28 B-RMB2-H05
29 B-RMB2-H08
30 B-BYB1-D01
31 B-BYB1-D10
32 B-BYB1-F02
33 B-BYB1-H08
34 B-BYB2-A01
35 B-BYB2-A12
36 B-BYB2-G02
37 B-BYB2-G06

These include 4 "WT" evolved strains (1 from each plate), the two cultures where steriles have fixed, and the 31 cultures that have fixed cell aggregates

8/26/09 → TWO SIMPLE EXPERIMENTS

	Initials	Date
Prepared By		
Approved By		

INVESTIGATING THE BENEFIT OF CELL AGGREGATES

At first pass one may assume that cell aggregates provide a benefit during the transfer — for instance, cells that stick together could be more likely to be sucked up during the dilution. However the Tecan growth curves on p.21 suggests an alternative explanation. Well BYS2-A11 fixed cell aggregates early on — this culture shows a significant increase in growth rate and saturation density suggesting that the competitive advantage of these aggregates is in the growth phase, not the transfer.

→ Tomorrow night Michael will set up a Tecan run to ask if this is a general property of these aggregates.

DISTINGUISHING BTW FREQ.-DEPENDENT SELECTION VS. HITCHHIKING

- In 19 of our cultures sterile strains are clearly present but have taken the somewhat surprising trajectory of increasing but then reaching an equilibrium (see p. 39 & 42).
- In order to distinguish between frequency-dependent selection and hitchhiking as a mechanism I took 10λ of αF-arrested cultures at generation 585 and transferred the cultures at left to the media used for general propagation

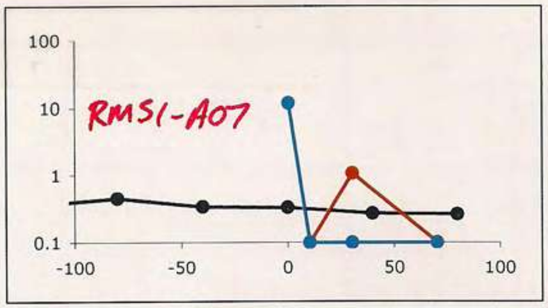
A01	RMS1-A07
A04	RMS1-B09
A07	RMS1-C05
A10	RMS1-D04
B01	RMS1-G05
B04	RMS1-G10
B07	RMS1-H10
B10	RMS1-H12
C01	**RMS2-B03**
C04	**RMS2-F01**
C07	**RMS2-G01**
C10	BYS1-D01
D01	BYS1-E11
D04	BYS1-H10
D07	**BYS2-B08**
D10	**BYS2-C04**
E01	**BYS2-H05**
E04	BYB1-B12
E07	**BYB2-H08**

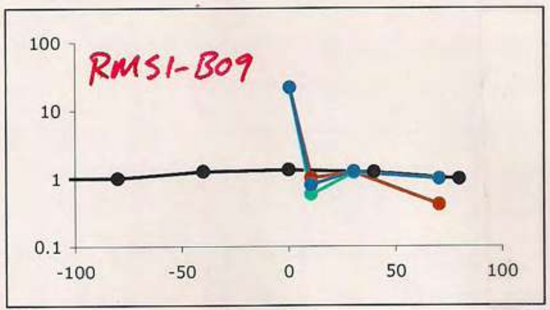
→ 5:00 pm on 8/25

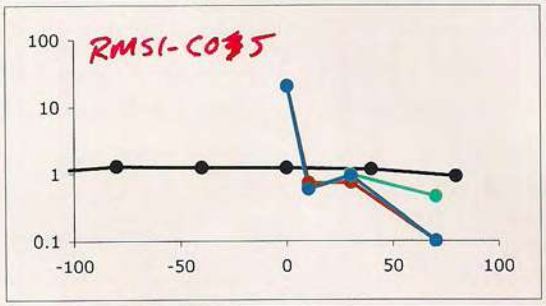
- Tonight Michael will induce these cultures to see the fraction sterile in each culture
- We will propagate this plate as a small-bottleneck culture and watch the change in fraction sterile

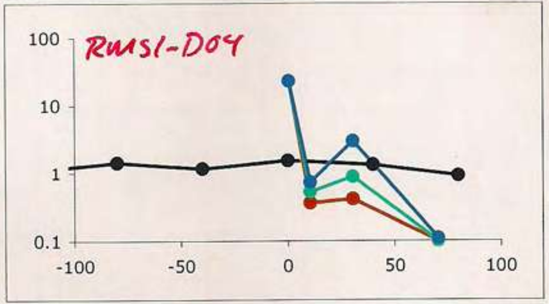
9/3/09

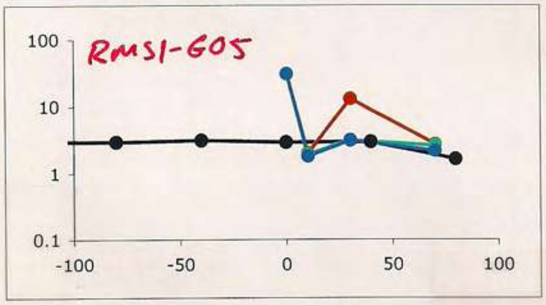
TEST FOR FREQUENCY-DEPENDENCE

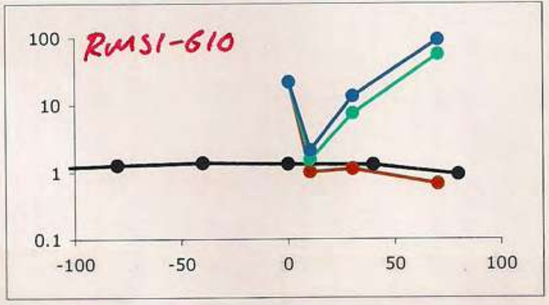


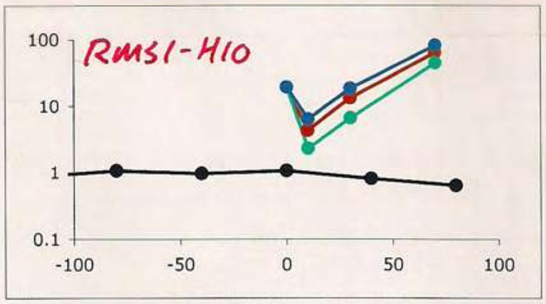


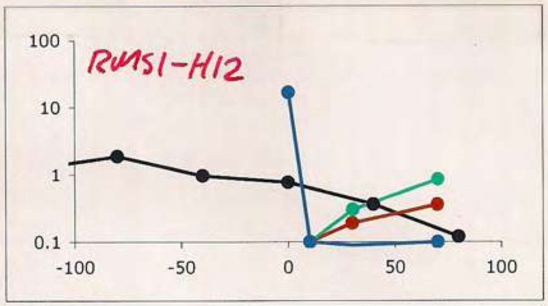


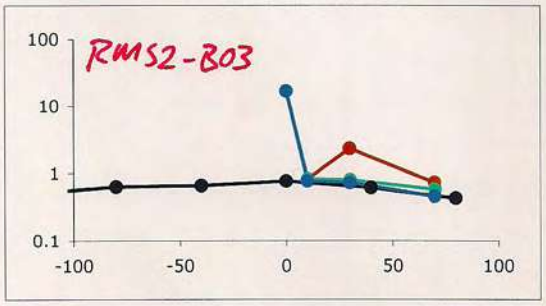


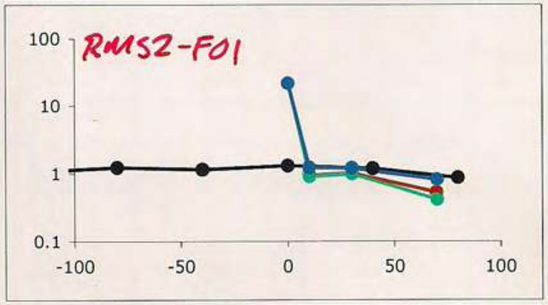


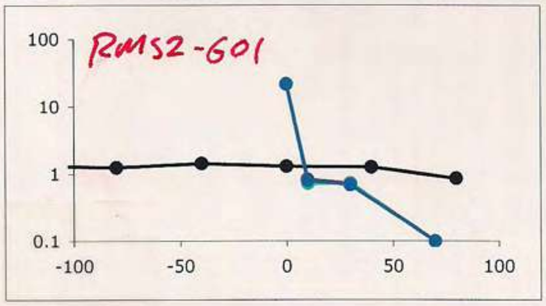


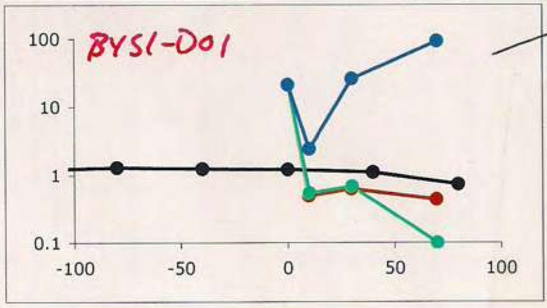








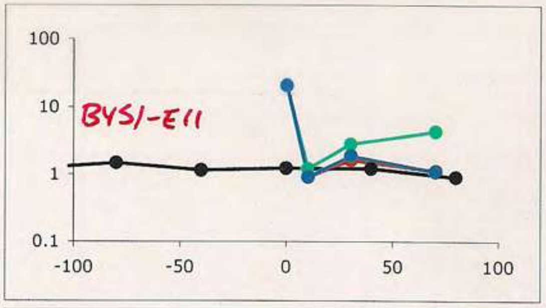


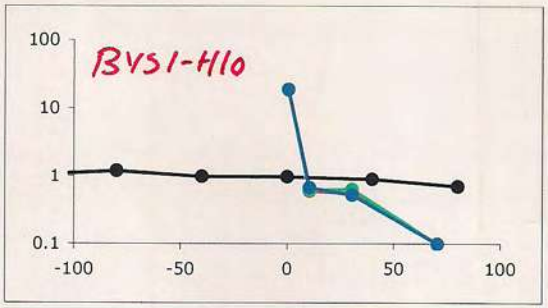


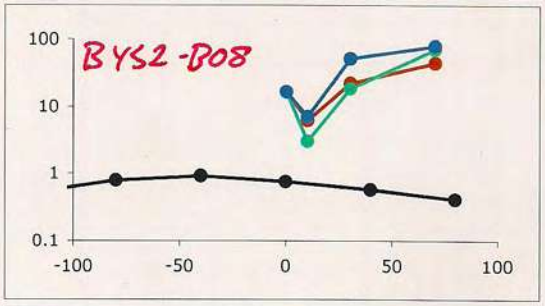
The FACS data for BYS1-D01 does not look right — it looks like one distribution → we shouldn't look too much into this one.

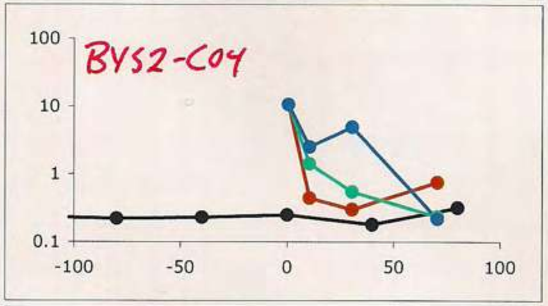
9/3/09

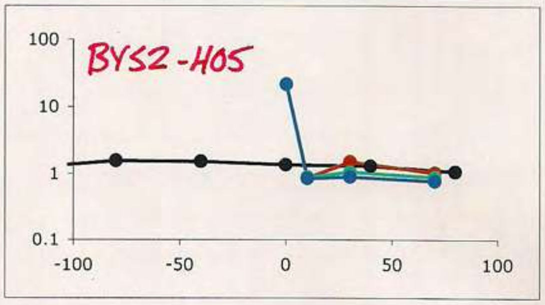
	Initials	Date
Prepared By		
Approved By		

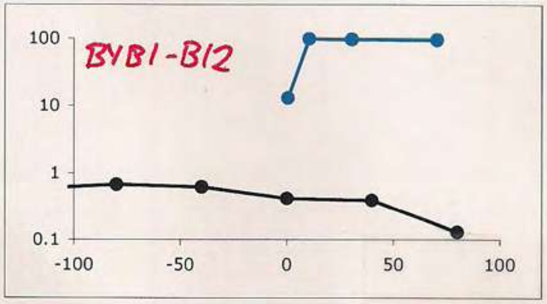


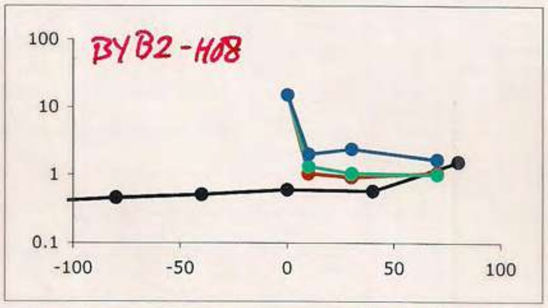












- "0" is time of αF shift. (generation 585)
- black curve is the experimental population
- [illegible] red, green, & blue are the 3 replicates released from αF arrest.

→ This experiment was expected to produce one of two possible outcomes:
  - steriles return to post-shift equilibrium
  - steriles stay at new equilibrium

We did not expect to see
(1) Steriles fixing or increasing
(2) [illegible] Nonsteriles fixing
(3) Different trajectories for the 3 replicates
(4) Oscillations (if there are really any in this data)

11/2/09

PROJECT UPDATE

We have been quite busy for the last two months but I have not updated this notebook in awhile.

- We reached 1,000 generations and are not actively propagating
- We reran the standard curve expt (p33-35) this time including more points at the lower end. Also we ran this twice – once from fresh culture and once from frozen.
- Most recently we have been trying to measure fitness using the ~~Biotek~~ BioTek & Tecan plate readers, but this was met with little success.

I am now starting on two things:

(1) Construct fluorescent reference strains so that we can perform co-culture competition fitness assays on our plates.

(2) Measure mutation rate to αF^R in our experimental strains

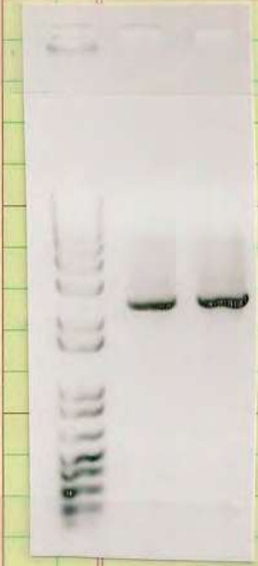
	Initials	Date
Prepared By		
Approved By		

11/2/09

CONSTRUCTION OF REFERENCE STRAINS FOR COMPETITION

I will transform yGIL265 & yGIL266 with ymCitrine & mCherry @ URA3 like I had done previously with other strains (see Evolution 5, p.43)

- On Friday (10/30) I streaked yGIL265 & yGIL266 from stock
- —— " —— I amplified ymCitrine & mCherry from pGIL039 & pGIL040



LANE	
1	ymCitrine
2	mCherry

11/7/09

I screwed up – forgot to supplement the 5FOA w/ His & Trp.

→ Today I restreaked yGIL265 & yGIL266 from stock
- Set up PCR again.

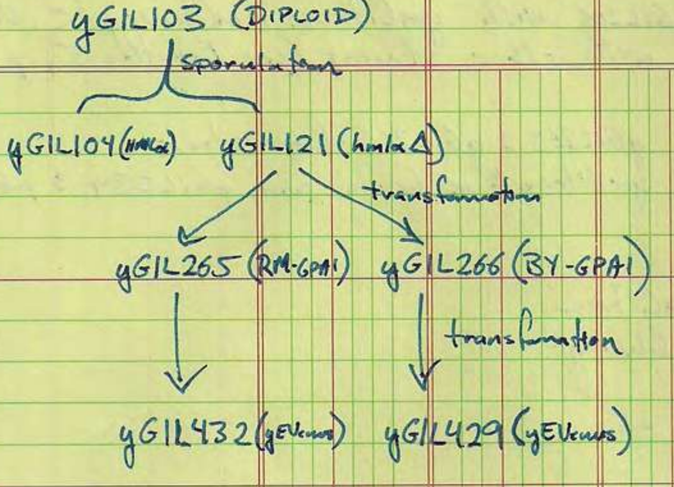
11/18/09

- I succeeded in ~~constructing~~ integrating ymCitrine & ymCherry @ URA3 in both yGIL265 & yGIL266. I verified that I could see the reporter by both microscopy & FACS
- Yesterday I froze down these strains:

- yGIL265	Citrine	→	yGIL519
- "	Cherry	→	520
- yGIL266	Citrine	→	521
- "	Cherry	→	522

11/2/09

MEASURE MUTATION RATE TO αF^R IN yGIL429 & yGIL432



I have previously measured $\mu_{\alpha F^R}$ very accurately for yGIL104.
This strain differs critically from our strains in that it is HMLα

I plan to measure the rate to αF^R in yGIL432 and yGIL429

- On Friday (10/30) I streaked yGIL432 & yGIL429 from stock.
- Also on Friday I poured αF plates

0.7 L H_2O
20 g Dextrose
20 g Peptone
10 g Yeast Extract
25 g Bacto Agar
—— STIR ——
0.3 L H_2O
—— AUTOCLAVE (P1 ; 15 m exposure) ——
After Cool:
250 mL YPD + 5×αF (50 μg/mL)
⟶ Take 250 mL YPD + αF (10 μg/mL)
—— Filter Sterilize ——
Add 10 mg αF (2 × 5 mg vials)

I poured 64 plates — exactly enough for 8 96-well plates

11/2/09

	Initials	Date
Prepared By		
Approved By		

- Made – YNB + URA+HIS+TRP + 2% Glucose
  – YNB + URA+HIS+TRP + 0.1% Glucose

↳ I supplemented the auxotrophes 2x

- Pick 4 colonies each off of yGIL429 & yGIL432 and inocalate 5mL YNB (2% glucose) → ↳ rollerdrum @ 5pm

11/3/09

Set up 96-well plates

↳ for each of the 8 cultures (yGIL429 A-D & yGIL432 A-D)
- dilute 1:10 (100λ into 900λ) in o/n media
- dilute the 1:10 dilution 1:1000 into 0.1% Glucose (10λ into 10mL)
- using multi-channel aliquot 10λ into 96-well plate
  ↳ 30° incubator @ 11:00 am

Put filters on plates

11/3/09

Plate fluctuation assays

- Add H_2O – for yGIL429-A – 80λ – many spot fusions
  yGIL432-A – 70λ – some spot fusions (large spots)
  the rest – 60λ – very few spot fusions
- Started spot plating ~ 11 ; ended ~3 – plates in 30° incubator ~3:30pm

11/3/09

Coulter Counts

		1	2	3	4	5	6
Bkg		606^{000}	552^{000}	574^{000}			
yGIL429	A 1	1.371E^7	1.215E^7	1.2 E^7	100λ into 10ml		
	2	1.197E^7	1.186E^7	1.189E^7			
	3	1.215E^7	1.199E^7	1.216E^7			
	B 1	2.029E^7	1.969E^7	1.959E^7			
	2	1.88E^7	1.897E^7	1.941E^7			
	3	1.878E^7	1.906E^7	1.887E^7			
	C 1	2.263E^7	2.308E^7	2.278E^7			
	2	2.211E^7	2.271E^7	2.216E^7			
	3	2.357E^7	2.281E^7	2.253E^7			
	D 1	2.799E^7	2.579E^7	2.587E^7			
	2	2.703E^7	2.699E^7	2.738E^7			
	3	2.657E^7	2.704E^7	2.639E^7			
yGIL432	A 1	1.237E^7	1.228E^7	1.233E^7			
	2	1.277E^7	1.252E^7	1.237E^7			
	3	1.232E^7	1.27E^7	1.202E^7			
	B 1	1.896E^7	1.891E^7	1.931E^7			
	2	1.907E^7	1.908E^7	1.88E^7			
	3	1.909E^7	1.901E^7	1.927E^7			
	C 1	2.401E^7	2.316E^7	2.33E^7			
	2	2.294E^7	2.282E^7	2.324E^7			
	3	2.398E^7	2.342E^7	2.342E^7			
	D 1	2.535E^7	2.522E^7	2.516E^7			
	2	2.485E^7	2.475E^7	2.519E^7			
	3	2.556E^7	2.488E^7	2.469E^7			
Post Bkg		1.528E^6	630^{000}	428^{000}			

429 A

	1	2	3	4	5	6	7	8	9
1	1	1(8)	0	0	1	3	0	2	3
2	3	0	0	0	1	1	0	~~3~~ 0	1
3	6(1)	0	0(1)	0	0	0	1	1	0
4	1(2)	0	1	14	0	0	0	2	0
5	0	1	0	1	1	0	0	0	0
6	1	0	3	0	0	1	0	0	0
7	2	1	1	0	0	0	1(4)	0	2(1)
8	1	20	2	3	1	0	0	0	0

429B

	1	2	3	4	5	6	7	8	9
1	0	0	0	3(1)	0	0	0	3	0
2	0	0	0	0	0	2	0	0	0
3	3	0	0	1	0(1)	0(~40)	0	0	0(2)
4	0	1	1(2)	1	1	1	0	1	1
5	2(1)	1	0	0	2	3	0	1	0
6	2(2)	0	0	0	0	0	2	3	0
7	0	2	0	~~3~~ 0	~~3~~ 0	1	1	1	0
8	0	0	0	2	1	0	7	3	1

429 C

	1	2	3	4	5	6	7	8	9
1	19	0	~~3~~ 2	1	2(1)	0	1	0	0(1)
2	0	0	0	1	1	1	1	1	1
3	60(1)	0(1)	0	1	0	0(1)	7	0	1
4	0	0	1	1	2(1)	0	1	1	3
5	0(2)	0	1	1	0	0	3	1	0
6	1	3	0	0	1	0	1	0	0
7	0	1	0	1	0(~60)	0	4	1	1
8	1	0	1	0	0(1)	8	14	0	1

429D

	1	2	3	4	5	6	7	8	9
1	1	1	12	0	4	13	1	0	0
2	2	1	0	0	1	2	0	3	1
3	7	0	0	0	2	3	3	1(1)	0(1)
4	1	0	0	0(1)	0(1)	15	0	8	2
5	4	3(~50)	1	0	3	0	0(1)	1	4
6	1	0(1)	0	0	2(5)	7	~139	0(~40)	2(1)
7	0	2(2)	4(3)	1	0	0	0	0	6
8	2	2	1	2	3	0	1	2	0

432 A

	1	2	3	4	5	6	7	8	9
1	2	0	2	0	2	1	0	0	0
2	0	2	0	2(~40)	0	0	0	1	0
3	2	1	1	0	0	0	0	0	0
4	0	2	0	1	1(1)	3	1	0(16)	0
5	1	0	0	0	0	2	0(1)	1	0
6	0	1	1	0	4	0	0	1(1)	1
7	1	0	0	0	1	0	0	0	1
8	0	1	1	0	2	2	0(1)	1	0

432B

	1	2	3	4	5	6	7	8	9
1	1	0	1	3	0	1	0(1)	0	2
2	3	3	0(1)	8	6(1)	0	2	0	3(1)
3	0	0	2	0	7	0	0	1	2
4	3	0	2(1)	3	0	2(2)	1	0	0
5	0	0	0	1	1	3	1(1)	1	2
6	2	1	1	1	0	2(1)	0	3	2
7	0	14	0	0	2(1)	0	1	0	0
8	3	0	0	4	~150	14	3	3	0

432C

	1	2	3	4	5	6	7	8	9
1	0	0	2	22	0	1	2	4	2
2	0	1	2	1(1)	0	3(~50)	1(1)	8(1)	0
3	22	0	19	0(15)	2	1	0	1	1
4	5	5	1	0	1	0	1(2)	1	91
5	0	1	0	0(1)	0(1)	7	2	1	0
6	2	1(10)	1	1	8	0	1	1	0
7	2(1)	1	1	0	0	3	0	1(2)	4
8	1	0	1	0	0	0	0	0	1

432D

	1	2	3	4	5	6	7	8	9
1	1	2	0	4	0(1)	0	0	1	0(1)
2	9	0	0	0	0(2)	2	2	1	6
3	0	2	1	1	0	6	0	4	0(2)
4	1	4	0	0	0	1	2	4	1
5	4	8	0	0	0	28	17	0	0
6	1	10(1)	0	8	41	1	2	0(1)	0
7	0	45	0	1	35	7	0	0	2
8	0	4	6	0(1)	1	0	9	0	2

	1	2	3	4	5	6	7	8	9
1									
2									
3									
4									
5									
6									
7									
8									

	1	2	3	4	5	6	7	8	9
1									
2									
3									
4									
5									
6									
7									
8									

11/18/09

ANALYSIS OF FLUCTUATION ASSAYS

DATA SUMMARY

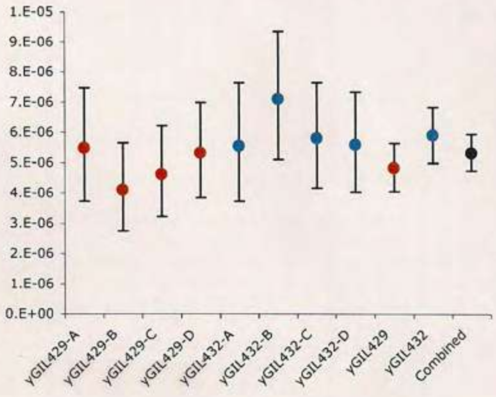
CLONE	yGIL429-A	yGIL429-B	yGIL429-C	yGIL429-D	yGIL432-A	yGIL432-B	yGIL432-C	yGIL432-D	yGIL429	yGIL432	Combined	Average	yGIL121
Experiment data													
C	72	72	72	72	72	72	72	72	288	288	576		720
N	9.72E+04	1.14E+05	1.37E+05	1.59E+05	8.63E+04	1.14E+05	1.40E+05	1.49E+05	1.27E+05	1.22E+05	1.25E+05		3.74E+05
Mutation rate from the ML method using the one-parameter model													
m	0.533	0.467	0.633	0.849	0.479	0.813	0.811	0.834	0.611	0.721	0.664		2.19
-	0.363	0.312	0.440	0.612	0.321	0.583	0.582	0.600	0.513	0.612	0.590		1.73
+	0.726	0.644	0.849	1.113	0.659	1.070	1.068	1.096	0.715	0.837	0.741		2.69
u	5.48E-06	4.10E-06	4.63E-06	5.32E-06	5.55E-06	7.10E-06	5.80E-06	5.59E-06	4.82E-06	5.89E-06	5.33E-06	5.38E-06	5.86E-06
-	1.75E-06	1.36E-06	1.41E-06	1.49E-06	1.83E-06	2.01E-06	1.64E-06	1.57E-06	7.70E-07	8.95E-07	5.93E-07	8.78E-07	1.24E-06
+	1.99E-06	1.55E-06	1.59E-06	1.66E-06	2.08E-06	2.24E-06	1.84E-06	1.75E-06	8.18E-07	9.48E-07	6.19E-07	8.78E-07	1.34E-06
Mutation rate from the ML method using the two-parameter model													
m	-0.98	-0.85	-0.77	-1.19	-0.78	-1.17	-1.10	0.83	-1.25	-1.16	-1.16		1.15
d	0.09	0.00	0.31	0.33	0.10	0.24	0.24	0.00	0.00	0.18	0.11		1.66
u	-1.01E-05	-7.49E-06	-5.60E-06	-7.47E-06	-8.98E-06	-1.03E-05	-7.89E-06	5.59E-06	-9.87E-06	-9.46E-06	-9.33E-06		3.07E-06
Mutation rate from the P0 method													
P(0)	0.542	0.597	0.458	0.403	0.569	0.417	0.375	0.444	0.500	0.451	0.476		0.050
m	0.613	0.515	0.780	0.909	0.563	0.875	0.981	0.811	0.693	0.795	0.743		9.575
u	6.31E-06	4.53E-06	5.71E-06	5.70E-06	6.52E-06	7.65E-06	7.02E-06	5.43E-06	5.47E-06	6.50E-06	5.96E-06	6.04E-06	1.93E-05

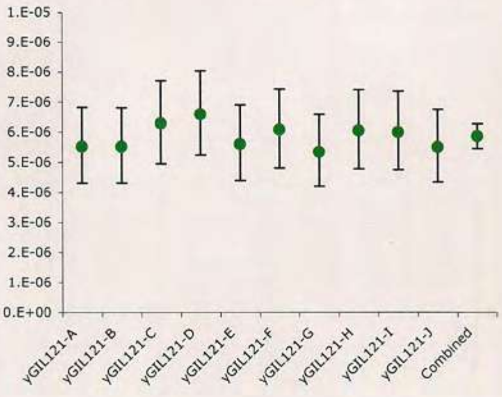
ONE PARAMETER MODEL

	yGIL429-A	yGIL429-B	yGIL429-C	yGIL429-D	yGIL432-A	yGIL432-B	yGIL432-C	yGIL432-D	yGIL429	yGIL432	Combined
m	0.533	0.467	0.633	0.849	0.479	0.813	0.811	0.834	0.611	0.721	0.664
-	0.363	0.312	0.440	0.612	0.321	0.583	0.582	0.600	0.513	0.612	0.590
+	0.726	0.644	0.849	1.113	0.659	1.070	1.068	1.096	0.715	0.837	0.741
N	9.72E+04	1.14E+05	1.37E+05	1.59E+05	8.63E+04	1.14E+05	1.40E+05	1.49E+05	1.27E+05	1.22E+05	1.25E+05
u	5.48E-06	4.10E-06	4.63E-06	5.32E-06	5.55E-06	7.10E-06	5.80E-06	5.59E-06	4.82E-06	5.89E-06	5.33E-06
-	1.75E-06	1.36E-06	1.41E-06	1.49E-06	1.83E-06	2.01E-06	1.64E-06	1.57E-06	7.70E-07	8.95E-07	5.93E-07
+	1.99E-06	1.55E-06	1.59E-06	1.66E-06	2.08E-06	2.24E-06	1.84E-06	1.75E-06	8.18E-07	9.48E-07	6.19E-07

	yGIL121-A	yGIL121-B	yGIL121-C	yGIL121-D	yGIL121-E	yGIL121-F	yGIL121-G	yGIL121-H	yGIL121-I	yGIL121-J	Combined
m	1.97	2.00	2.24	2.42	2.07	2.30	2.13	2.28	2.31	2.23	2.19
-	1.54	1.56	1.77	1.93	1.63	1.82	1.68	1.80	1.83	1.76	2.04
+	2.43	2.47	2.75	2.96	2.56	2.82	2.62	2.80	2.83	2.74	2.35
N	3.57E+05	3.62E+05	3.57E+05	3.68E+05	3.71E+05	3.78E+05	3.98E+05	3.77E+05	3.84E+05	4.06E+05	3.74E+05
u	5.51E-06	5.51E-06	6.28E-06	6.58E-06	5.60E-06	6.07E-06	5.35E-06	6.05E-06	6.00E-06	5.50E-06	5.86E-06
-	1.20E-06	1.20E-06	1.32E-06	1.35E-06	1.20E-06	1.26E-06	1.14E-06	1.26E-06	1.25E-06	1.15E-06	4.04E-07
+	1.31E-06	1.30E-06	1.43E-06	1.46E-06	1.30E-06	1.37E-06	1.24E-06	1.37E-06	1.35E-06	1.25E-06	4.15E-07

} from Genetics paper





⟹

It seems like there are at least two problems with the previous fluctuation assays:

(1) The m was too low (always less than 1 by the one param model) → ∴ the $P(0)$ class was ~ 0.5

→ I suspect that the low $P0$ may be the explanation for the nonsensical values I got from the two-param model.

(2) The cells had not saturated when I started plating. Because of this the ~~tht~~ later assays had higher m & N.

	Initials	Date
Prepared By		
Approved By		

11/18/09

FITNESS ASSAYS – PILOT EXPERIMENT

We plan to use competitive fitness assays for three things:

(1) To determine the fitness advantage of sterile strains in our two propagation regimes

(2) To characterize the change in mean fitness throughout the course of the evolution experiment

→ Do we need to do all 8 plates × 5 time points (generations) × 2 or 3 time points (fit. assay) ~ 80 plates of FACS or should we just pick a few interesting plates for further characterization?

(3) To determine the fraction of spontaneous steriles that have an advantage (ie. ste7) versus the neutral ones (ie. ste2)

I have not decided which strains should be included in this experiment or how the plates will be laid out, but yesterday I streaked the following strains:

yGIL522 (RM) } ymCherry Ref
520 (BY) }
266 (RM) } Ancestor to exp. strains
265 (BY) }
429 (RM) } Experimental ancestor
432 (BY) }
491 (RM) } " ste2Δ::KanMX
493 (BY) }
495 (RM) } " ste7Δ::KanMX
498 (BY) }
BYS2-A11-99 }
BYS1-F02-99 } aggregates
RMB1-A02-198 }
RMB1-D10-198 }

In addition, I have spontaneous αF^R strains in the fridge
→ these are from the fluctuation assays

11/30/09

	Initials	Date
Prepared By		
Approved By		

SECOND ROUND OF FLUCTUATION ASSAYS

Make YPD + αF plates

- ✓ 0.7 L H_2O
- ✓ 30 g Dextrose
- ✓ 30 g Peptone
- ✓ 15 g Yeast Extract
- ✓ 30 g Agar
- ✓ — STIR →
- ✓ 0.3 L H_2O
- ✓ — AUTOCLAVE — (P1, 15m exposure)
- ✓ After cool
  0.5 L 3×αF (30 μg/mL)

→ 500 mL H_2O
— Filter Sterilize —
Add 15 mg αF (3 × 5mg vials)

→ I was able to pour 80 plates → this would be sufficent for 10 96-well plates; however, I will probably need a few extras — some always get contaminated — also some are pretty thin.

I streaked yGIL429 & yGIL432 from freezer (~ 4pm)

12/2/09

Yesterday I started o/n cultures of yGIL429 & yGIL432 in 5 mL Min + His + Trp + URA.

→ I made new His + Trp stock & made new Min + HTU media supplementing the auxotrophes 2×

78 mL H_2O
10 mL 20% Dextrose
10 mL 10× YNB w/o a.a.
2 mL Uracil (2 mg/mL)
0.4 mL His + Trp (1g/100 mL)
100 λ

yGIL429 K
L
M
N
O
↓
yGIL432 P
Q
R
S
T
↓

12/2/09

Make new MIN + HTU (0.1% Glucose)

87 ml	H_2O
10 ml	10× YNB (w/o a.a.)
500 λ	20% Dextrose
2 mL	Uracil (2mg/mL) - 100×
400 λ	His + Trp (1g/100 mL each) - 500×
100 mL	

Set up fluctuation assays

- Dilute each culture 1:10,000 → Dilute 1:10 (100λ into 900λ MIN+HTU@0.1%Glc) → 9λ of 1:10 into 9 mL media
- Distribute 10λ into each well of 96-well plate

↳ Plates in 30° incubator @ 8:45pm (plate seal)

12/3/09

I will coulter count the o/n cultures from yesterday to get an approximation for the bottleneck ~ these were on my bench o/n

Bkg		930^{000}	706^{000}	
yGIL429	K	$5.689E^{7}$	$5.725E^{7}$	
"	L	$5.977E^{7}$	$5.952E^{7}$	
"	M	$5.657E^{7}$	$5.714E^{7}$	
"	N	$5.584E^{7}$	$5.673E^{7}$	
"	O	$5.219E^{7}$	$5.264E^{7}$	
yGIL432	P	$5.712E^{7}$	$5.687E^{7}$	
"	Q	$6.651E^{7}$	$6.522E^{7}$	
"	R	$5.509E^{7}$	$5.464E^{7}$	
"	S	$6.309E^{7}$	$6.162E^{7}$	
"	T	$6.054E^{7}$	$5.968E^{7}$	
Post Bkg		$4.972E^{6}$	$1.838E^{6}$	$1.616E^{6}$

- Filters on plates ~ 5:45pm

12/4/09

- Remove filters ~ 8:30am
- Plate fluctuation assays 10
  - @ 8:45am put all 96-well plates in fridge
  - plating from 8:45 - 1 (~4 hours)
  - for most of the plates the plate seals did not seal column 12 → for these I only removed 12 wells for Coulter counts, so that I would have at least 72 cultures for plating → added 60λ H_2O to each well

PLATE ORDER	PLATE SEAL FAIL
K	?
P	Yes
L	?
Q	Yes
M	Yes
R	Yes
N	Yes
S	Yes
O	Yes
T	No

Coulter Counts

Test count yGIL432-T (200λ into 10ml) 8.305E7 ← too high use 100λ per 10ml

Bkg		1.248E6	1.088E6	1.086E6
yGIL429	K 1	4.032E7.	3.928E7	4.117E7
	2	4.324E7	4.261E7.	4.194E7
	3	4.348E7	4.346E7.	4.333E7
	L 1	4.407E7	4.363E7.	4.296E7
	2	4.678E7	4.259E7	4.279E7.
	3	4.357E7	4.319E7.	4.22E7
	M 1	4.427E7	4.49E7	4.464E7.
	2	4.364E7.	4.301E7	4.397E7
	3	4.53E7	4.516E7.	4.486E7
	N 1	4.198E7	4.181E7	4.182E7.
	2	4.179E7	4.065E7.	4.054E7
	3	4.393E7	4.324E7.	4.261E7
	O 1	4.468E7	4.407E7	4.432E7.
	2	4.359E7	4.164E7	4.338E7.
	3	4.331E7	4.321E7.	4.266E7

100λ into 10 ml

yGIL432	P	1	$4.129E^{7}$	$3.92E^{7}$	$3.997E^{7}$ ·	
↓	↓	2	$4.901E^{7}$	$4.045E^{7}$ ·	$4.024E^{7}$	$4.193E^{7}$
		3	$4.408E^{7}$	$4.385E^{7}$ ·	$4.231E^{7}$	
	Q	1	$4.166E^{7}$	$4.193E^{7}$	$4.192E^{7}$ ·	
	↓	2	$4.02E^{7}$	$4.172E^{7}$ ·	$4.257E^{7}$	
		3	$3.748E^{7}$	$4.249E^{7}$	$4.159E^{7}$ ·	$4.138E^{7}$
	R	1	$4.179E^{7}$	$4.216E^{7}$ ·	$4.248E^{7}$	
	↓	2	$4.03E^{7}$	$4.043E^{7}$ ·	$4.047E^{7}$	
		3	$4.136E^{7}$	$4.14E^{7}$ ·	$4.204E^{7}$	
	S	1	$4.472E^{7}$	$4.327E^{7}$	$4.352E^{7}$ ·	
	↓	2	$5.264E^{7}$	$4.808E^{7}$ ·	$4.597E^{7}$	$4.86E^{7}$
		3	$4.535E^{7}$	$4.756E^{7}$	$4.647E^{7}$ ·	
	T	1	$4.031E^{7}$	$3.825E^{7}$	$3.908E^{7}$ ·	
	↓	2	$3.771E^{7}$	$3.827E^{7}$	$3.799E^{7}$ ·	
		3	$3.972E^{7}$	$3.972E^{7}$ ·	$3.991E^{7}$	
Post Bkg			$1.602E^{6}$	$1.134E^{6}$	$1.064E^{6}$ ·	

COUNTING FLUCTUATION ASSAYS 12/5/09 1pm

K

	1	2	3	4	5	6	7	8	9
1	2	1	0	0	0	2	7	0	2
2	0	0	0	1	1	0	2	12	2
3	0	1	0	0 ~~13(8)~~	0	13(3)	0	0	1
4	0	0	1	0	3	2	0	0	0
5	1	1	59	1(1)	1	2	2	4	1
6	1(28)	0	1	2	3	0	0	0	0
7	1	0	2	0	1	0	0	0	0
8	3(1)	2	0	1	1	2(8)	2	7	0

L

	1	2	3	4	5	6	7	8	9
1	0	0	1	0	2	1	3	0	0
2	0	0	30	1	2(1)	6	4	2(1)	0
3	2	3	3	0	0	6	0	0	1
4	0	0	3	7	0	1	0	2	0
5	0	0	1	4	0	0	0	0	17
6	0	1(1)	1	6	0(1)	0	0	4	3
7	3	0	1	3	0	0	0	0	0
8	0	2	2(5)	1	1	0	0	1	0

M

	1	2	3	4	5	6	7	8	9
1	0	4	0	9	5	0(1)	1	0	1
2	0	1	0	1(1)	0	3	0	1	2
3	0	3	3	1	1(1)	0	0 ~~(1)~~	0 ~~(1)~~	0
4	0	0	1	0	1	0(1)	1	0	0
5	1	0(1)	1	0	47	3	0	0	0
6	0	0	2	3	1	0	0	6	41
7	3	0	3	0	0	0	7	0	0
8	3	0	1	0	1	0	0	2	0

N

	1	2	3	4	5	6	7	8	9
1	6	0	0	0	1	0(2)	1	2(1)	0
2	0	0	12	1	0(1)	0	1(1)	1(2)	0(1)
3	0	36	0	2	1	0	0	5(1)	2
4	0	0	~~45~~ 0	~~0~~ 45	~~22~~ 3	~~0~~ 22	~~7~~ 0	2	0
5	0	1	0	5	1	0	0	0	3
6	3	0	0	0	0	1	7	3	0
7	0	0	0	1(3)	0	0	1	0(3)	2
8	0	1	1(3)	0(4)	0	0	0	0	34

O

	1	2	3	4	5	6	7	8	9
1	0	10	1	1	1	0	2	0	8
2	0	0	0	0	1	0	2	1	2
3	1	0	0	0	0	4	1	0	2
4	3	11	0	0(1)	3	0	0	2	0
5	2	0	11	3(1)	4	2	1	0	0(1)
6	0	1	0	0	0	0	0	0	3(3)
7	3	0	0	0	0	0(1)	1	0	0
8	0	1	2	1(3)	0	1	3	1	0

P

	1	2	3	4	5	6	7	8	9
1	1	1	0	0	2(2)	2	1	1	0
2	4	30	3(1)	0	0	~1000	0	9	5(3)
3	0	0	0	1	0	1	0(1)	0(1)	0
4	1	6	0	4	1	0	0	0(1)	34
5	1	46	1	0(1)	12	0(1)	0(1)	1	0
6	0	6	1	1(1)	1(3)	0(2)	2(1)	1	0
7	0	7	0	0	0	2	1	0	0(1)
8	2	3	0	0	1	0	0	2	1

Q

	1	2	3	4	5	6	7	8	9
1	24	0	0	0	0	2	4	0	1
2	3	0	0	0	7(2)	7	0	12	0
3	0(1)	0	0	0	3	1	3(1)	0(1)	1
4	0	2	1	1	0	0(2)	0	3(1)	5(1)
5	0 [illegible]	8	5	0(~100)	3	4	0	0	0
6	0	2	0	0	1	8	4	0	0(1)
7	0	2(1)	1	3	0	0	0	1(1)	1
8	1	0(1)	0(1)	2(4)	4(1)	0(1)	0	0	0

R

	1	2	3	4	5	6	7	8	9
1	0	63	1	1(4)	1	3	0(1)	0(2)	22
2	0	0	0	3	0(6)	0	1	0	0
3	0	5(1)	0	1(1)	0	0	0	3	0(2)
4	0	0	0	0(3)	1	2	0	0(1)	3
5	2	0	0	1	0	23	0	0	1
6	0(1)	1(1)	0	0(1)	0(1)	0	0	0	13
7	0	7	1	4(5)	0	0	1	0	3(1)
8	3	0	0	2	0	0	0	0	0

S

	1	2	3	4	5	6	7	8	9
1	0	0	0	0	0	1	0(1)	4	4
2	3	6	0	2	2	0	0	0	1
3	7	0	0	1	0(1)	2	2	18	1(1)
4	0(3)	0	1	0	0	0	0	1	0
5	0	0	1	1(5)	0	0	0	1	0
6	0	2	0(1)	0(2)	21	0	0	0	0
7	1	1	1	0	1	4	0	0	1(1)
8	18	2	2(2)	3	0	1	5	2	1(1)

T

	1	2	3	4	5	6	7	8	9
1	1	0(1)	0	22	1	1(2)	0	1	0
2	3	2	1	1	2(17)	10(2)	0	0	0
3	0	1(2)	0	0	0	0	3(1)	0	0(2)
4	0	0	4	2	1	0	21	0	1
5	0(1)	0	1	0	0	47	0	0	0
6	0	0	4	0	0(~17)	0	0	0(1)	0
7	0	0(1)	0	2	11	1	1(5)	0(1)	0
8	0	0	7	0(1)	0	0	0	0	2

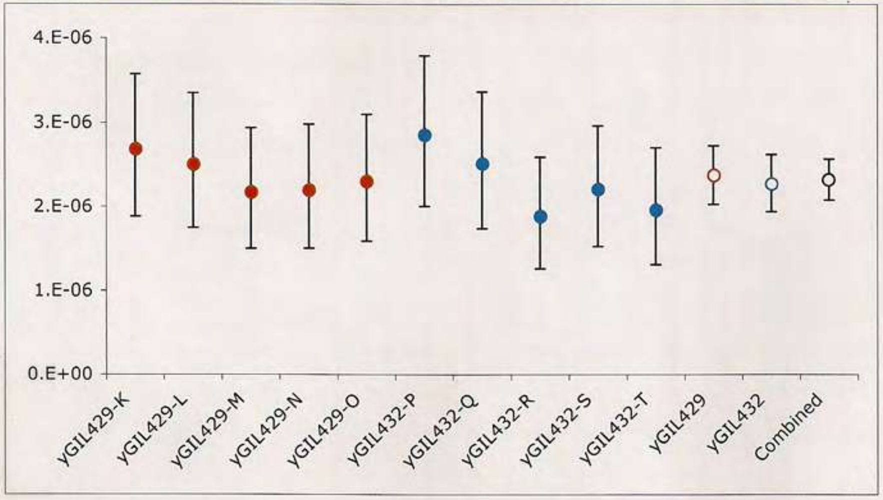
12/8/09

ANALYSIS OF FLUCTUATION ASSAYS

	Initials	Date
Prepared By		
Approved By		

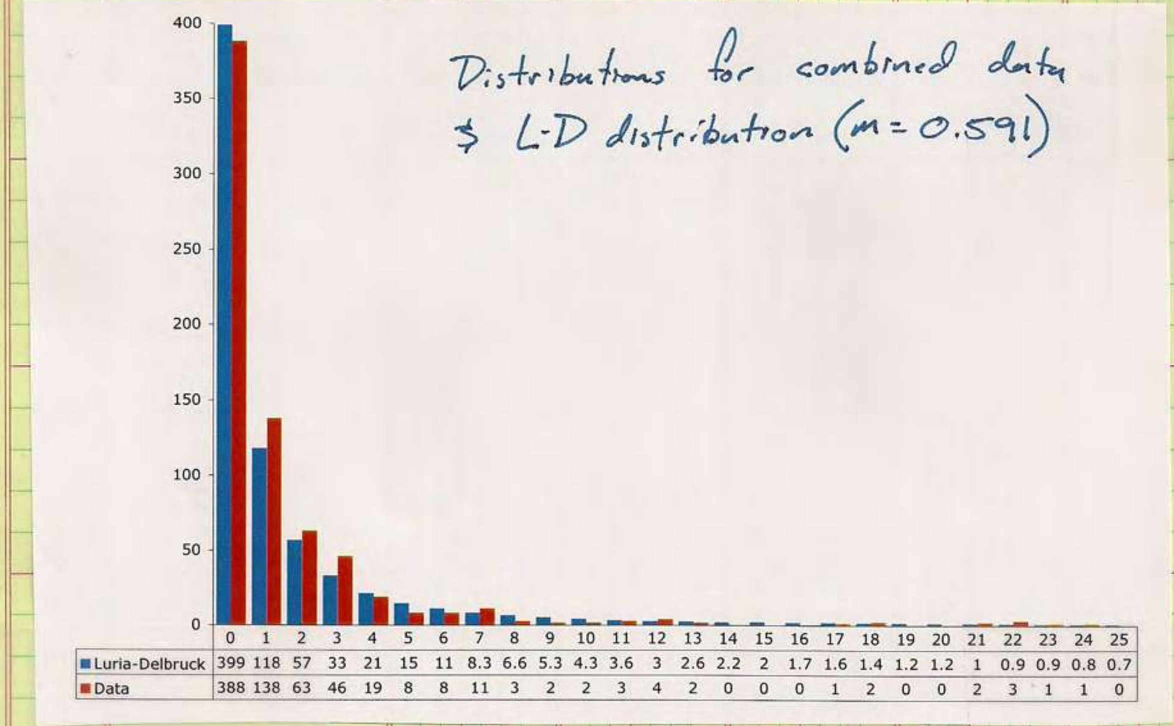
Bold indicates which model is favored by AIC

CLONE	yGIL429-K	yGIL429-L	yGIL429-M	yGIL429-N	yGIL429-O	yGIL432-P	yGIL432-Q	yGIL432-R	yGIL432-S	yGIL432-T	yGIL429	yGIL432	Combined	Average	yGIL104	yGIL121
Experiment data																
C	72	72	72	72	72	72	72	72	72	72	360	360	720		720	72
N	2.56E+05	2.59E+05	2.68E+05	2.51E+05	2.60E+05	2.43E+05	2.50E+05	2.48E+05	2.79E+05	2.34E+05	2.59E+05	2.51E+05	2.55E+05	2.55E+05	3.74E+05	994800
Mutation rate from the P0 method																
P(0)	0.458	0.514	0.542	0.569	0.528	0.472	0.542	0.625	0.514	0.625	0.522	0.556	0.539		0.050	0.042
m	0.780	0.666	0.613	0.563	0.639	0.750	0.613	0.470	0.666	0.470	0.650	0.588	0.618		2.996	3.178
u	3.05E-06	2.57E-06	2.29E-06	2.24E-06	2.46E-06	3.09E-06	2.45E-06	1.89E-06	2.39E-06	2.00E-06	2.51E-06	2.34E-06	2.43E-06	2.44E-06	8.02E-06	3.19E-06
Mutation rate from the ML method using the one-parameter model																
m	0.686	0.649	0.581	0.551	0.598	0.691	0.628	0.467	0.614	0.459	0.613	0.570	0.591		2.19	2.775
-	0.482	0.453	0.400	0.377	0.414	0.487	0.437	0.312	0.426	0.306	0.525	0.486	0.530		2.039	2.230
+	0.915	0.869	0.786	0.748	0.807	0.922	0.844	0.644	0.827	0.634	0.706	0.658	0.655		2.345	3.363
u	2.68E-06	**2.50E-06**	**2.17E-06**	**2.19E-06**	2.30E-06	2.85E-06	**2.51E-06**	**1.88E-06**	2.20E-06	**1.96E-06**	2.37E-06	**2.27E-06**	**2.32E-06**	2.32E-06	5.86E-06	2.79E-06
-	7.97E-07	7.55E-07	6.76E-07	6.94E-07	7.10E-07	8.44E-07	7.64E-07	6.24E-07	6.75E-07	6.53E-07	3.39E-07	3.33E-07	2.40E-07	3.08E-07	4.04E-07	5.48E-07
+	8.96E-07	8.52E-07	7.65E-07	7.88E-07	8.03E-07	9.49E-07	8.62E-07	7.12E-07	7.63E-07	7.46E-07	3.59E-07	3.52E-07	2.50E-07	3.08E-07	4.15E-07	5.92E-07
Mutation rate from the ML method using the two-parameter model																
m	0.2889	0.5906	0.4775	0.5055	0.4111	0.5293	0.6279	0.4598	0.4173	0.4115	0.47	0.51	0.49		1.15	1.62
d	1.88	0.14	0.28	0.11	0.64	0.38	0.00	0.02	0.62	0.14	0.41	0.16	0.26		1.66	1.42
u	**1.13E-06**	2.28E-06	1.78E-06	2.01E-06	**1.58E-06**	**2.18E-06**	2.51E-06	1.85E-06	**1.50E-06**	1.75E-06	**1.82E-06**	2.02E-06	1.94E-06	1.86E-06	**3.07E-06**	**1.63E-06**



P0	
yGIL429 & 432	**2.43E-06**
yGIL121	**3.19E-06** PNAS
yGIL104	**8.02E-06** Genetics
One parameter	
yGIL429 & 432	**2.32 (2.08 - 2.57) x 10-6**
yGIL121	**2.79 (2.24 - 3.38) x 10-6**
yGIL104	**5.86 (5.46 - 6.28) x 10-6**
Two parameter	
yGIL429 & 432	**1.94 x 10-6**
yGIL121	**1.63 x 10-6**
yGIL104	**3.07 (2.65 - 3.62) x 10-6**

Distributions for combined data & L-D distribution (m = 0.591)



	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
🟦 Luria-Delbruck	399	118	57	33	21	15	11	8.3	6.6	5.3	4.3	3.6	3	2.6	2.2	2	1.7	1.6	1.4	1.2	1.2	1	0.9	0.9	0.8	0.7
🟥 Data	388	138	63	46	19	8	8	11	3	2	2	3	4	2	0	0	0	1	2	0	0	2	3	1	1	0

12/8/09

DISCUSSION OF FLUCTUATION ASSAYS

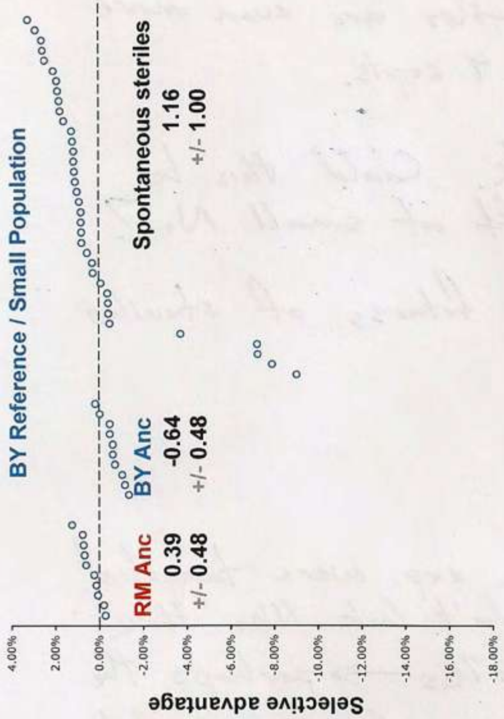
Overall the fluctuation assays look very good.

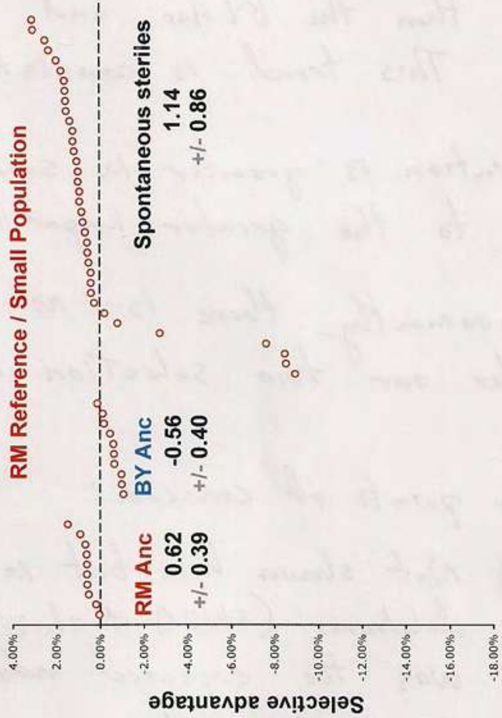
↳ range is good (only 2 less than m = 0.5 and none above m = 2)
- Coulter counts consistent from plate to plate (see p.)
- 6 of 10 are best fit w/ one-param. model — the combined 720-tube assay is best fit by one-param ⇒ thus the 95% CIs can be determined empirically
  - ↳ I don't see it as a problem that the Genetics expt was analyzed w/ the two-param. model. I should still quote 3.07 for HMLα and 2.32 for hmlαΔ. This would indicate that ~25% αF^R mutants are not in the mating pathway in HMLα cells
- Importantly there is no difference btw the RM & BY strains

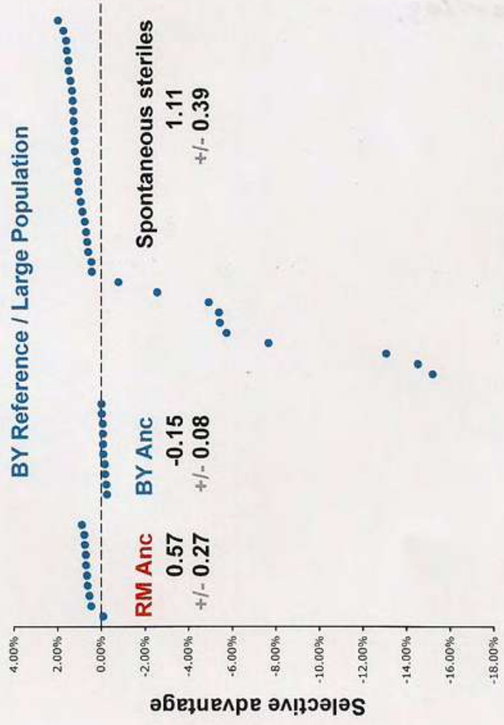
- NOTE: I had to modify the two-param program in Matlab to prohibit negative values of 'm' or 'd'.

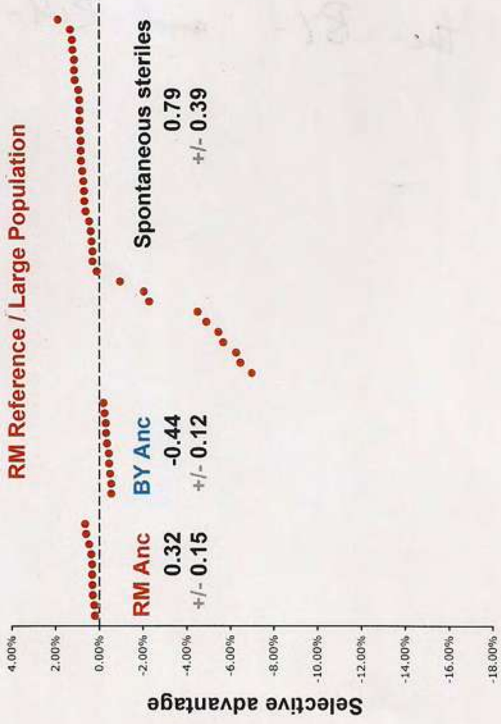
- I should ~~calculate~~ generate SSDscore distributions for 720 tube fluct. assays @ m = 0.5913

12/16/09 FITNESS ASSAYS









12/16/09

ANALYSIS OF FITNESS ASSAYS

- The results look good — We see that the RM Anc is more fit than the BY Anc, and that the steriles are even more fit. This trend is consistent in all 4 expts.

- Variation is greater in small bottleneck. Could this be due to the greater importance of drift at small N_e?

- Conveniently there is no diff. in the fitness of steriles under our two selection regimes.

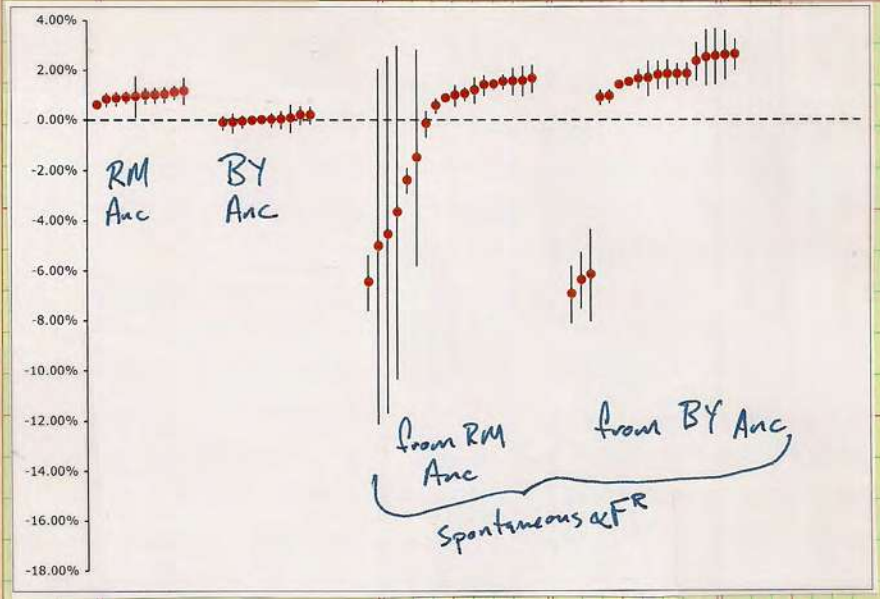
- Two points of concern:
  (1) Not shown here but included in the exp. were targeted deletions (ste2Δ & ste7Δ) → it didn't look like there was the expected advantage of ste7Δs → perhaps the KanMX cassette is more deleterious under our conditions.

  (2) The steriles derived from the BY strain seem to have higher fitness than those derived from the RM Anc.
  ↳ This can be clearly seen on p. 73 where I separated the BY- and RM- derived steriles.

12/16/09

	Initials	Date
Prepared By		
Approved By		

FITNESS ASSAYS



← These data are mean ± stdev of the four assays on p. 71 normalized to mean BY Anc

I have been neglecting this notebook lately. Basically the 1st round of fitness assays (p. 61) were a bust – we could not clearly distinguish the ymCherry positive cells. We suspect the problem to be dissolved oxygen and we have changed ~~a~~ our FACS preparations accordingly.

- For the assay we keep all solutions (YPD+A&T & PBST) in the fridge – cold liquids hold more dissolved oxygen.
- When we transfer at night we do a pseudo-"induction" where we dilute cells and grow 3h in the thermalcycler.

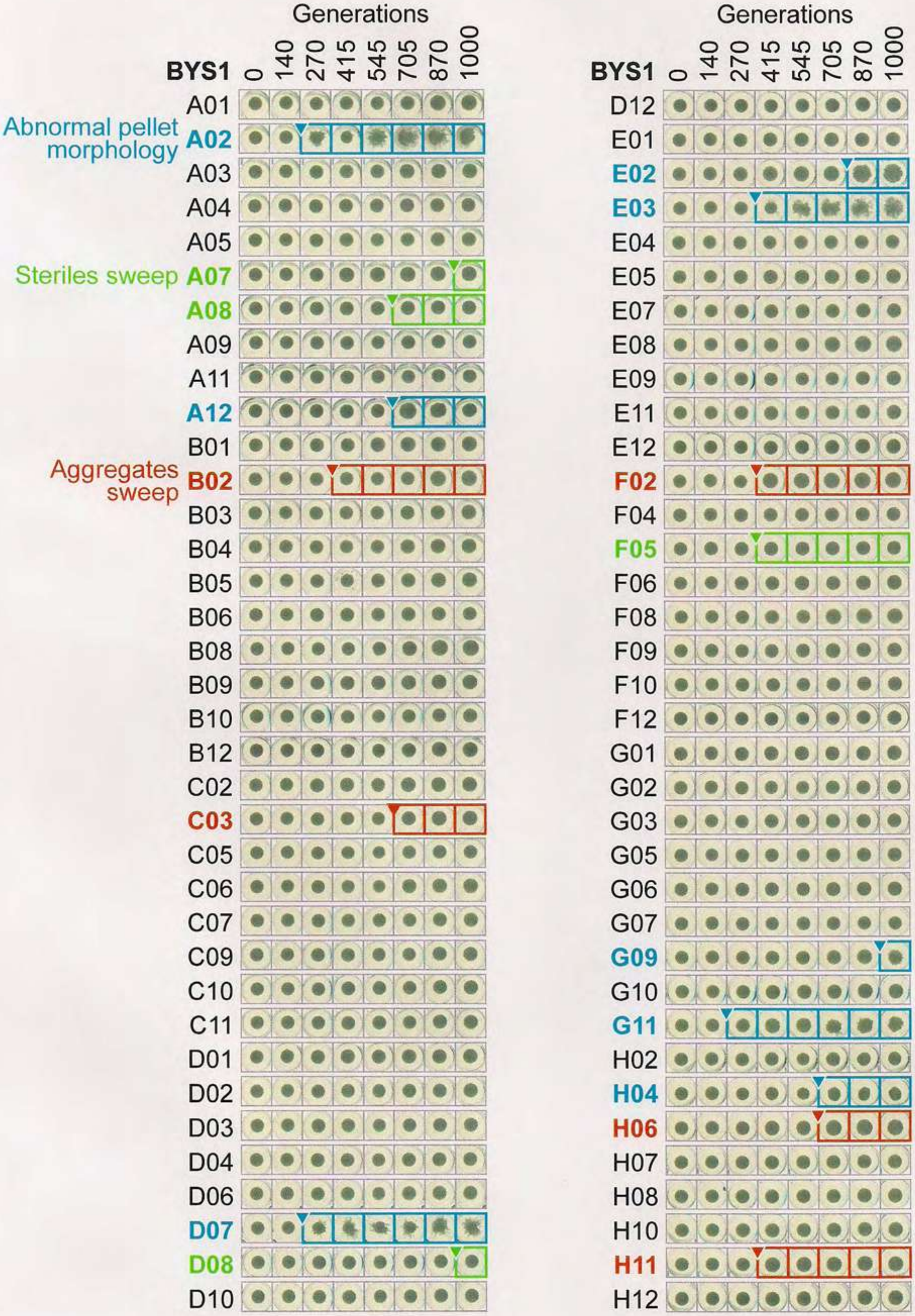
We are currently running fitness assays on 8 time points from the BYS1 plates (~140 generation spacing)

12/22/09

	Initials	Date
Prepared By		
Approved By		

Coulter Counts

		1	2	3	4	5	6
10λ into 20 mL					Resonicate		
Bkg		434^{000}	384^{000}	360^{000}			
Gen. 0	1	$3.784E^{7}$	$3.848E^{7}$	$3.78E^{7}$	$4.029E^{7}$		
	2	$2.292E^{7}$	$2.171E^{7}$	$2.256E^{7}$	$4.304E^{7}$		
	3	$4.845E^{7}$	$4.064E^{7}$	$4.095E^{7}$	$4.198E^{7}$		
Gen. 140	1	$2.063E^{7}$	$2.025E^{7}$	$2.112E^{7}$	$3.635E^{7}$		
	2	$3.248E^{7}$	$3.192E^{7}$	$3.192E^{7}$	$3.588E^{7}$		
	3	$2.945E^{7}$	$2.97E^{7}$	$2.912E^{7}$	$3.597E^{7}$		
Gen 270	1	$3.415E^{7}$	$3.451E^{7}$	$3.449E^{7}$	$3.837E^{7}$		
	2	$2.96E^{7}$	$2.785E^{7}$	$2.776E^{7}$	$3.677E^{7}$		
	3	$1.41E^{7}$	$1.34E^{7}$	$1.376E^{7}$	$3.866E^{7}$		
Gen. 415	1	$1.703E^{7}$	$1.67E^{7}$	$1.673E^{7}$	$3.698E^{7}$		
	2	$1.44E^{7}$	$1.451E^{7}$	$1.463E^{7}$	$3.741E^{7}$		
	3	$3.008E^{7}$	$3.051E^{7}$	$3.075E^{7}$	$3.369E^{7}$		
Gen. 545	1	$3.478E^{7}$	$3.375E^{7}$	$3.386E^{7}$	$3.987E^{7}$		
	2	$1.219E^{7}$	$1.18E^{7}$	$1.321E^{7}$	$4.171E^{7}$		
	3	$1.741E^{7}$	$1.776E^{7}$	$1.828E^{7}$	$4.097E^{7}$		
Gen. 705	1	$2.207E^{7}$	$2.242E^{7}$	$2.148E^{7}$	$3.712E^{7}$		
	2	$7.942E^{6}$	$6.784E^{6}$	$6.574E^{7}$	$3.772E^{7}$		
	3	$2.234E^{7}$	$2.277E^{7}$	$2.308E^{7}$	$3.657E^{7}$		
Gen. 870	1	$9.97E^{6}$	$1.007E^{7}$	$1.088E^{7}$	$4.061E^{7}$		
	2	$2.124E^{7}$	$1.998E^{7}$	$1.968E^{7}$	$3.9E^{7}$		
	3	$3.111E^{7}$	$3.108E^{7}$	$3.184E^{7}$	$3.793E^{7}$		
Gen. 1000	1	$3.299E^{7}$	$3.38E^{7}$	$3.349E^{7}$	$3.64E^{7}$		
	2	$1.871E^{7}$	$1.816E^{7}$	$1.779E^{7}$	$3.813E^{7}$		
	3	$1.948E^{7}$	$2.023E^{7}$	$2.003E^{7}$	$3.86E^{7}$		
Post Bkg		$1.638E^{6}$	$1.102E^{6}$	616^{000}			


Generations
BYS1
0
140
270
415
545
705
870
1000
A01
Abnormal pellet morphology
A02
A03
A04
A05
Steriles sweep
A07
A08
A09
A11
A12
B01
Aggregates sweep
B02
B03
B04
B05
B06
B08
B09
B10
B12
C02
C03
C05
C06
C07
C09
C10
C11
D01
D02
D03
D04
D06
D07
D08
D10
Generations
BYS1
0
140
270
415
545
705
870
1000
D12
E01
E02
E03
E04
E05
E07
E08
E09
E11
E12
F02
F04
F05
F06
F08
F09
F10
F12
G01
G02
G03
G05
G06
G07
G09
G10
G11
H02
H04
H06
H07
H08
H10
H11
H12

	Initials	Date
Prepared By		
Approved By		

1/19/10

In order to measure mean population fitness and photograph cell pellets we have unfrozen BYBI populations from generation 0, 140, 270, 415, 545, 705, 820, & 1000.

In order to determine the final cell densities (thus N_e) I will coulter count six sample wells from BYBI generation 0

Coulter Counts

Bkg	494^{000}	474^{000}	472^{000}
BYBI-Gen0 - B4	$9.389E^7$	$9.637E^7$	$9.582E^7$
C5	$9.155E^7$	$9.219E^7$	$9.136E^7$
D6	$8.604E^7$	$8.41E^7$	$8.485E^7$
E7	$9.983E^7$	$9.963E^7$	$9.929E^7$
F8	$8.68E^7$	$8.635E^7$	$8.604E^7$
G9	$8.819E^7$	$8.744E^7$	$8.767E^7$
Post Bkg	$5.44E^6$	$2.694E^6$	$2.412E^6$

10λ into 20 mL

As of 07/02/09

Big N

RMB1

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

RMB2

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

BYB1

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

↑ lost 8/24/09

BYB2

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

Small N

RMS1

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

RMS2

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

BYS1

lost 8/24/09

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

BYS2

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

X = Originally skipped
L = Subsequently lost
D = Dilute

START OF PROPAGATION NOTEBOOK

SETUP (6/16/09)

yGIL429

RMB1

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

RMB2

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

RMS1

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

RMS2

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

yGIL432

BYB1

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

BYB2

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

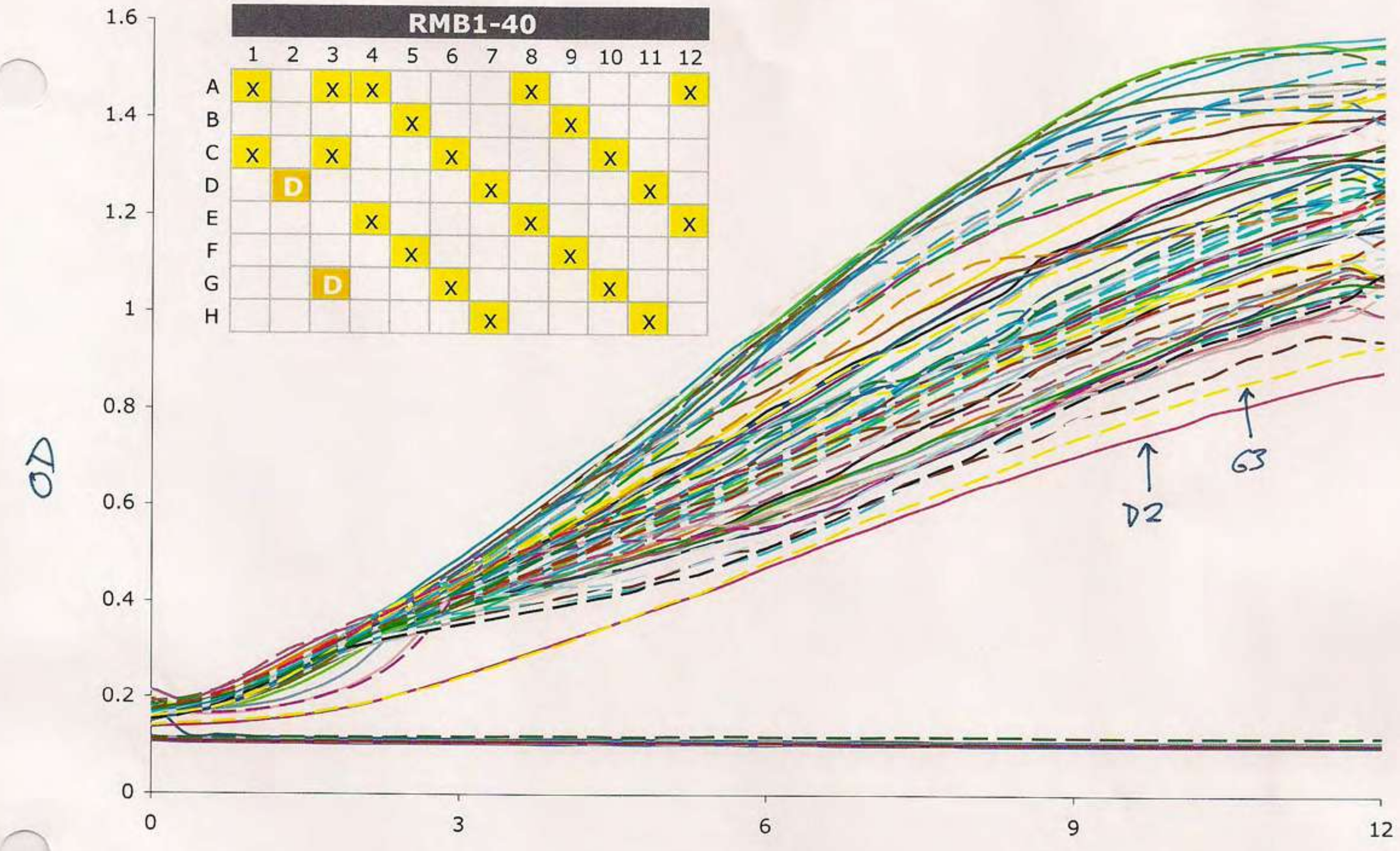
BYS1

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

BYS2

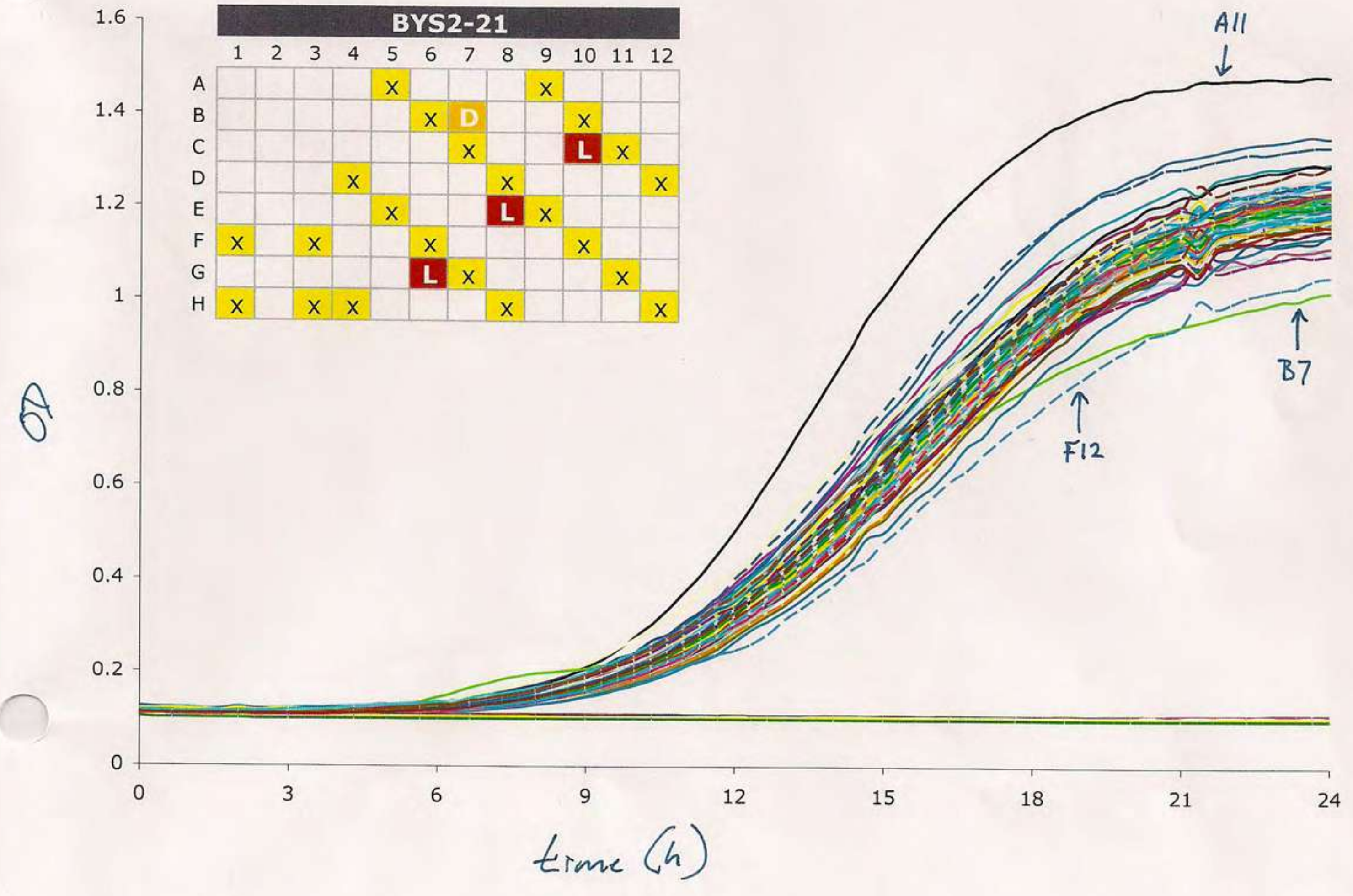
	1	2	3	4	5	6	7	8	9	10	11	12
A					X				X			
B						X				X		
C							X				X	
D				X				X				X
E					X				X			
F	X		X			X				X		
G							X				X	
H	X		X	X				X				X

TECAN GROWTH CURVES



RMB1-40

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



BYS2-21

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

BIG BOTTLENECK CULTURES

Transfer Setup
Date 6/16/09
Notes
Plates All (B&S)
Time 11am
Glycerol Y [✓ N]

Transfer 1
Date 6/16/09
Notes
Plates B&S
Time ~~9:40(S)~~ ~~Small: 9:40pm~~ Big: 9:40pm Small: 9:50
Glycerol [✓ Y] N

Transfer 2
Date 6/17/09
Notes Done by 9:55
Plates B
Time 9:30am
Glycerol Y [✓ N]

Transfer 3
Date 6/17/09
Notes Everything looks good.
Finished 9:40pm
Plates B
Time 9:30pm
Glycerol Y [✓ N]

Transfer 2
Date 6/17/09
Notes Started 9:45pm
Everything looks good!
Finished 10pm
Plates S
Time 9:45pm
Glycerol Y [✓ N]

Transfer 4
Date 6/18/09
Notes Done by 9:55am
Everything looks good.
We are running low on P20 barriers.
Plates B
Time 9:40am
Glycerol Y [✓ N]

Transfer 5
Date 6/18/09
Notes Done by 9:55pm
Everything looks really good.
Densities appear same as before (just by turbidity)
Plates B
Time 9:40pm
Glycerol Y [✓ N]

Transfer 6
Date 6/19/09
Plates B
Time 9:45am
Glycerol Y [✓ N]
Notes
Done by 9:55am
Everything looks good
Densities OK by turbidity

Transfer 7
Date 6/19/09
Plates B
Time 9:35pm
Glycerol Y [✓ N]
Notes
Everything looks good
Densities OK by turbidity

Transfer 8
Date 6/20/09
Plates B
Time 9:55am
Glycerol Y [✓ N]
Notes
ELG
DOBT.

Transfer 9
Date 6/20/09
Plates B
Time 9:40pm
Glycerol Y [✓ N]
Notes
The tip that was to transfer RMB1-D6 was clogged – transfer done by hand

Transfer 10
Date 6/21/09
Plates B
Time 9:40am
Glycerol Y [✓ N]
Notes
No problems

Transfer 11
Date 6/21/09
Plates B
Time 9:40pm
Glycerol Y [✓ N]
Notes
BYB2-10 has 2 x-contam events: F3, B4
Otherwise everything looks good.

Transfer 12
Date 6/22/09
Plates B
Time 9:40am
Glycerol Y [✓ N]
Notes
BYB1-11-B3: small diffuse pellet ~ prob. OK
BYB2-11: F3, B4 still look contaminated
No contamination in RM, but a few diffuse pellets:
RMB1-11 - D2 ✓, D10, G3 ✓, RMB2-11 - A8 ✓, D2, C4, B5, E8, F6 ✓, H5

Transfer 13 **Date** 6/22/09
Plates B **Time** 9:40pm
Glycerol Y [✓ N]

Notes
RMB1- D2, G3 small others OK
RMB2 .A8, F6 small others OK
BYB1 - C10 x-c, B3 small other OK.
BYB2 _ F3, B4 still x-c, fixed

Transfer 14 **Date** 6/23/09
Plates B **Time** 9:40am
Glycerol Y [✓ N]

Notes
RMB1 - x/C in F5, H7 ; Diffuse in B7, D2, D10, G3
RMB2 - No x/c ; Diffuse in A2, A8, B5, C4, D2, F6, H5
BYB1 - x/C in C10 ; Diffuse in B2, H5
BYB2 - x/c in H1 ; Diffuse in C6
↑
*B12

Transfer 15 **Date** 6/23/09
Plates B **Time** 9:42pm
Glycerol [✓ Y] N

Notes
RMB1 - x/C F5, H7
RMB2 - No x/c
BYB1 - x/C C10
BYB2 - x/c B12, H1
} reset empty wells by removing tips corresponding to these wells.

Transfer 16 **Date** 6/24/09
Plates B **Time** 9:40am
Glycerol Y [✓ N]

Notes
No new x/c

Transfer 17 **Date** 6/24/09
Plates B **Time** 9:40pm
Glycerol Y [✓ N]

Notes
RMB1-OK
RMB2-OK
BYB1-x/c C6
BYB2-OK

Transfer 18 **Date** 6/25/09
Plates B **Time** 9:30am
Glycerol Y [✓ N]

Notes
· No new x/c
· Reset BYB1-C6 by removing tip

Transfer 19 **Date** 6/25/09
Plates B **Time** 9:35pm
Glycerol Y [✓ N]

Notes
RMB1 - OK
RMB2 - OK, E9 small
BYB1 - OK, H5, B3 small
BYB2 - OK, E9 small

→ & wipe down workspace w/ EtOH

→ Add ampicillin to media to see if this prevents/cures contamination

Transfer	**Date**	**Notes**
20	6/26/09	RMB1 - x/c C1 everything else looks good I Checked wells RMB1: A2, B1, C1 (contaminant), D1, E1, F1, H1 by microscopy after ~4h αF. The contaminant in C1 is bacterial. This bact. is also mixed in w/ yeast in D1. All others are still completely mating proficient
Plates	**Time**	
B	9:40am	
Glycerol	Y [✓ N]	

Transfer	**Date**	**Notes**
21	6/26/09	RMB1-D6 did not transfer (clogged tip) - transfer by hand ; ~~E8~~ C1 still cont. RMB2 - ~~A8~~ A2 contaminated (yellow color) ; E9 dilute BYB1 - H5 dilute BYB2 - H2 dilute
Plates	**Time**	
B	9:40pm	
Glycerol	Y [✓ N]	

Transfer	**Date**	**Notes**
~~21~~ 22	6/27/09	Contamination in RMB1-C1 cleared up w/ Amp in media RMB1-E9 still v. dilute RMB2 - F6 well empty (and A8) - transfer these 2 wells by hand from RMB2-20 (both wells were dilute in this plate) RMB2 - A2 still looks contaminated → under microscope - looks like all bacteria (no yeast). I will remove this tip next dilution BYB1 - B6 & H5 are v. dilute BYB2 - E9 is v. dilute
Plates	**Time**	
B	9:40 am	
Glycerol	Y [✓ N]	

Transfer	**Date**	**Notes**
23	6/27/09	Removed tip for RMB2-A2 RMB1 - looks fine RMB2 - in addition to A2, we lost 3 ~~entries~~ wells: F6, A8, E9 BYB1 - we lost B5 BYB2 - looks fine
Plates	**Time**	
B	9:35pm	
Glycerol	Y [✓ N]	

Transfer	**Date**	**Notes**
24	6/28/09	RMB1 - C2 dilute RMB2 - looks OK ; still some cells in E9 BYB1 - H5 dilute BYB2 - looks good
Plates	**Time**	
B	9:~~30~~40am	
Glycerol	Y [✓ N]	

Transfer	**Date**	**Notes**
25	6/28/09	RMB1 - looks good RMB2 - same as last transfer BYB1 - same as last transfer BYB2 - looks good
Plates	**Time**	
B	9:47pm	
Glycerol	Y [✓ N]	

Transfer	**Date**	**Notes**
26	6/29/09	Everything same as last transfer
Plates	**Time**	
B	9:37am	
Glycerol	Y [✓ N]	

Transfer 27
Date 6/29/09
Plates B
Time 9:30pm
Glycerol Y [✓ N]

Notes
RMB1- OK. looks good
RMB2 - OK, same as before, but E9 is returning.
BYB1 - Ok, same as before
BYB2 - looks good

Transfer 28
Date 6/30/09
Plates B
Time 9:35am
Glycerol Y [✓ N]

Notes
Everything same

Transfer 29
Date 6/30/09
Plates B
Time 9:40pm
Glycerol [✓ Y] N

Notes Put plates from B-28 into -80 freezer after glycerol.
Everything looks the same as before.

Transfer 30
Date 7/1/09
Plates B
Time 9:48am
Glycerol Y [✓ N]

Notes
Everything same

Transfer 31
Date 7/1/09
Plates B
Time 9:30pm
Glycerol Y [✓ N]

Notes Everything same. To summarize, this means:
RMB1- Dilute: D2, G3
RMB2 - Lost: A2, A8, F6 Dilute: E9
BYB1- Lost: B3. Dilute: H5
BYB2- OK

Transfer 32
Date 7/2/09
Plates B
Time 9:36am
Glycerol Y [✓ N]

Notes
Everything same
BYB1-H5 looks more dilute than before

Transfer 33
Date 7/2/09
Plates B
Time 9:40pm
Glycerol Y [✓ N]

Notes
Everything same.

Transfer	Date	Notes
34	7/3/09	Everything same
Plates	**Time**	
B	9:35am	
Glycerol	Y [✓ N]	

Transfer	Date	Notes
35	7/3/09	Everything same.
Plates	**Time**	
B	9:50pm	
Glycerol	Y [✓ N]	

Transfer	Date	Notes
36	7/4/09	Everything same
Plates	**Time**	
B	9:55am	
Glycerol	Y [✓ N]	

Transfer	Date	Notes
37	7/4/09	Everything same
Plates	**Time**	
B	9:40pm	
Glycerol	Y [✓ N]	

Transfer	Date	Notes
38	7/5/09	Everything same
Plates	**Time**	
B	9:55am	
Glycerol	Y [✓ N]	

Transfer	Date	Notes
39	7/5/09	Everything same
Plates	**Time**	
B	9:45pm	
Glycerol	Y [✓ N]	

Transfer	Date	Notes
40	7/6/09	Everything same
Plates	**Time**	
B	9:35am	
Glycerol	Y [✓ N]	

↑ Part above he

New boxes tracked below

Transfer 41
Date 7/6/09
Notes Everything same
Plates B
Time 9:45pm
Glycerol Y [✓ N]

~~New boxes tracked below~~

Transfer 42
Date 7/7/09
Notes Everything same

New tip boxes
250 - 2
20B - 1

Plates B
Time 9:38am
Glycerol Y [✓ N]

Transfer 43
Date 7/7/09
Notes Everything same. Froze #42.
Plates B
Time 9:35pm
Glycerol [✓ Y] N

Transfer 44
Date 7/8/09
Notes Everything same

New Tip Boxes
250 - 1

Plates B
Time 9:45am
Glycerol Y [✓ N]

Transfer 45
Date 7/8/09
Notes Everything same.

New Tip Boxes
250 - 1

Plates B
Time 9:25pm
Glycerol Y [✓ N]

Transfer 46
Date 7/9/09
Notes Everything same

New Tip Boxes
250 - 1
20B - 5

Plates B
Time 9:38am
Glycerol Y [✓ N]

Transfer 47
Date 7/9/09
Notes Everything same.

New Tip Boxes
250B - 1
250 - 1
20B - 15

Plates B
Time 10:00pm
Glycerol Y [✓ N]

Transfer 48
Date 7/10/09
Notes Everything Same
New Tips
250 - 1
Plates B
Time 9:35am
Glycerol Y [✓ N]

Transfer 49
Date 7/10/09
Notes Everything same.
Froze #48
New Tips
250 - 1
250B - 8
Plates B
Time 9:10pm
Glycerol [✓ Y] N

Transfer 50
Date 7/11/09
Notes Everything same
New Tips
250 - 1
Plates B
Time 9:34am
Glycerol Y [✓ N]

Transfer 51
Date 7/11/09
Notes Everything same
New Tips
250 - 1
Plates B
Time 9:35am
Glycerol Y [✓ N]

Transfer 52
Date 7/12/09
Notes Everything same
New Tips
250 - 1
20B - 5
Plates B
Time 9:35am
Glycerol Y [✓ N]

Transfer 53
Date 7/12/09
Notes Everything Same
New Tips
250 - 2
20B - 14
Plates B
Time 9:35pm
Glycerol Y [✓ N]

Transfer 54
Date 7/13/09
Notes RMB2-E9 is officially lost (was dilute)
Everything else same
New Tips
250 - 6
Plates B
Time 9:27am
Glycerol Y [✓ N]

Transfer 55 **Date** 7/13/09
Notes Everything same
Set up for inductions
Froze #54
Plates B **Time** 10:05 pm
Glycerol [✓ Y] N **New Tips** 250 - 2 **250B** - 8 **20B** -

Parted above the ↑

Transfer 56 **Date** 7/14/09
Notes Everything same
Plates B **Time** 9:35am
Glycerol Y [✓ N] **New Tips** 250 - [illegible] 10 **250B** - **20B** -

Transfer 57 **Date** 7/14/09
Notes Everything same
Plates B **Time** 9:30pm
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** - 1

Transfer 58 **Date** 7/15/09
Notes Everything same
Plates B **Time** 9:30am
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - 4 **20B** -

Transfer 59 **Date** 7/15/09
Notes Everything same.
Plates B **Time** 9:35pm
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** -

Transfer 60 **Date** 7/16/09
Notes Everything same
Plates B **Time** 9:35am
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** -

Transfer 61 **Date** 7/16/09
Notes Everything same
Set up inductions
Froze #60
Plates B **Time** 9:35pm
Glycerol [✓ Y] N **New Tips** 250 - 6 **250B** - 4 **20B** -

Transfer 62
Date 7/17/09
Notes Everything same.
Plates B
Time 9:35am
Glycerol Y [✓ N]
New Tips 250 - 9 **250B -** **20B -**

Transfer 63
Date 7/17/09
Notes Everything same.
Plates ~~[illegible]~~B
Time 9:30pm
Glycerol Y [✓ N]
New Tips 250 - 1 **250B -** **20B -** 12

Transfer 64
Date 7/18/09
Notes Everything same
Plates B
Time 9:26am
Glycerol Y [✓ N]
New Tips 250 - **250B -** **20B -**

Transfer X
Date 7/18/09
Notes Everything same.
Froze #64
Did not xfer
Plates B
Time 9:35pm
Glycerol [✓ Y] N
New Tips 250 - 8 **250B -** **20B -**

Transfer X
Date 7/28/09
Notes INDUCTIONS
Plates B&S
Time X
Glycerol Y N
New Tips 250 - 9 **250B -** **20B -** 9

Transfer X
Date 7/29/09
Notes RESTART
Plates B
Time 9:00am
Glycerol Y [✓ N]
New Tips 250 - 1 **250B -** **20B -** 8

Transfer 65
Date 7/29/09
Notes Everything looks good, same as before [illegible]
Plates B
Time 9:50pm
Glycerol Y [✓ N]
New Tips 250 - 2 **250B -** **20B -** 13

Transfer 66 **Date** 7/30/09
Plates B **Time** 9:53am
Notes Everything same
Glycerol Y [✓ N] **New Tips** 250 - 1 250B - 20B -

Transfer 67 **Date** 7/30/09
Plates B **Time** 9:50pm
Notes Everything same.
Froze down # 66
Began Inductions
Glycerol [✓ Y] N **New Tips** 250 - 3 250B - 20B -

Transfer 68 **Date** 7/31/09
Plates B **Time** 9:36
Notes Everything same
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 69 **Date** 7/31/09
Plates B **Time** 9:35pm
Notes Everything same
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 70 **Date** 8/1/09
Plates B **Time** 9:30am
Notes Everything same
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 71 **Date** 8/1/09
Plates B **Time** 9:45pm
Notes Everything same
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 72 **Date** 8/2/09
Plates B **Time** 9:26am
Notes Everything same
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer	Date	Notes
73	8/2/09	Everything same.
Plates	**Time**	
B	9:45pm	

Glycerol Y [✓ N] **New Tips** 250 - 1 250B - 20B - 10

Transfer	Date	Notes
74	8/3/09	Everything same
Plates	**Time**	
B	9:34am	

Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B - 4

Transfer	Date	Notes
75	8/3/09	Everything same. Froze #74 Set up inductions
Plates	**Time**	
B	9:35pm	

Glycerol [✓ Y] N **New Tips** 250 - 10 250B - 20B -

Transfer	Date	Notes
76	8/4/09	Everything Same
Plates	**Time**	
B	9:30am	

Glycerol Y [✓ N] **New Tips** 250 - 8 250B - 20B - 4

Transfer	Date	Notes
77	8/4/09	Everything same
Plates	**Time**	
B	9:50pm	

Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer	Date	Notes
78	8/5/09	Everything same
Plates	**Time**	
B	9:40am	

Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B - 3

Transfer	Date	Notes
79	8/5/09	Everything same
Plates	**Time**	
B	9:30pm	

Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B - 12

Pad above [illegible]
↑

Transfer 80
Date 8/6/09
Notes Everything same
Plates B
Time 9:32am
Glycerol Y [✓ N] **New Tips** **250 -** **250B -** **20B -** 4

Transfer 81
Date 8/6/09
Notes Everything same.
Plates B
Time 9:30pm
Glycerol Y [✓ N] **New Tips** **250 -** **250B -** **20B -** 6

Transfer 82
Date 8/7/09
Notes Everything same
Plates B
Time 9:28am
Glycerol Y [✓ N] **New Tips** **250 -** **250B -** **20B -** 4

Transfer 83
Date 8/7/09
Notes Everything same.
Froze #82
Set up inductions
Plates B
Time 9:30pm
Glycerol [✓ Y] N **New Tips** **250 -** 1 **250B -** **20B -**

Transfer 84
Date 8/8/09
Notes Everything same.
Plates B
Time 9:50am
Glycerol Y N **New Tips** **250 -** 9 **250B -** **20B -**

Transfer 85
Date 8/8/09
Notes Everything same.
Plates B
Time 9:40pm
Glycerol Y N **New Tips** **250 -** 1 **250B -** **20B -**

Transfer 86
Date 8/9/09
Notes Everything same
Oy vey tip problem
Plates B
Time 10:00am
Glycerol Y [✓ N] **New Tips** **250 -** 1 **250B -** **20B -** 5

Transfer 87 **Date** 8/9/09
Notes Everything same
Plates B **Time** 9:45pm
Glycerol Y [✓ N] **New Tips** **250 -** 2 **250B -** **20B -** 20

Transfer 88 **Date** 8/10/09
Notes Everything same
Plates B **Time** 9:40am
Glycerol Y [✓ N] **New Tips** **250 -** 8 **250B -** **20B -** 12

Transfer 89 **Date** 8/10/09
Notes Everything same
Plates B **Time** 9:25pm
Glycerol Y [✓ N] **New Tips** **250 -** 2 **250B -** **20B -** 20

Transfer 90 **Date** 8/11/09
Notes Everything same
Plates B **Time** 9:31am
Glycerol Y [✓ N] **New Tips** **250 -** **250B -** **20B -**

Transfer 91 **Date** 8/11/09
Notes Everything same
Froze #90
Set up inductions.
Plates B **Time** 9:55pm
Glycerol [✓ Y] N **New Tips** **250 -** 10 **250B -** **20B -**

Transfer 92 **Date** 8/12/09
Notes Everything same
Plates B **Time** 9:39am
Glycerol Y [✓ N] **New Tips** **250 -** 8 **250B -** **20B -**

Transfer 93 **Date** 8/12/09
Notes Everything same
Plates B **Time** 9:45pm
Glycerol Y N **New Tips** **250 -** 2 **250B -** **20B -**

Transfer 94 **Date** 8/13/09 **Notes** Everything same

Plates B **Time** 9:43am

Glycerol Y [✓ N] **New Tips** 250 - 9 **250B** - **20B** -

Transfer 95 **Date** 8/13/09 **Notes** Everything same

Plates B **Time** 9:30pm

Glycerol Y [✓ N] **New Tips** 250 - 2 **250B** - **20B** - 13

Transfer 96 **Date** 8/14/09 **Notes** Everything same

Plates B **Time** 9:31 am

Glycerol Y [✓ N] **New Tips** 250 - ~~8~~ 10 **250B** - **20B** -

Transfer 97 **Date** 8/14/09 **Notes** Everything same

Plates B **Time** 9:35pm

Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** - 12

Transfer 98 **Date** 8/15/09 **Notes** Everything same

Plates B **Time** 9:25am

Glycerol Y [✓ N] **New Tips** 250 - **250B** - **20B** -

Transfer 99 **Date** 8/15/09 **Notes** Everything same

Plates B **Time** 9:45pm

Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** - 12

Transfer 100 **Date** 8/16/09 **Notes** Everything same

Plates B **Time** 9:20am

Glycerol Y [✓ N] **New Tips** 250 - **250B** - **20B** -

Transfer 101 **Date** 8/16/09 **Notes** Everything same
Froze #100
Set up inductions
Plates B **Time** 10:00pm
Glycerol [✓ Y] N **New Tips** 250 - ~~[illegible]~~ 10 **250B** - **20B** -

Transfer 102 **Date** 8/17/09 **Notes** Everything same
Plates B **Time** ~~10:~~ 9:26am
Glycerol Y [✓ N] **New Tips** 250 - **250B** - **20B** -

Transfer 103 **Date** 8/17/09 **Notes** Everything same
BYB1 labeled #101, not 102. Was this just a typo?
Anyway. I labeled these all #103.
Plates B **Time** 9:35pm
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** -

Transfer 104 **Date** 8/18/09 **Notes** Everything same
Plates B **Time** 9:25am
Glycerol Y [✓ N] **New Tips** 250 - **250B** - **20B** -

Transfer 105 **Date** 8/18/09 **Notes** Everything same
Plates B **Time** 9:24pm
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** -

Transfer 106 **Date** 8/19/09 **Notes** Everything same
Plates B **Time** 9:23am
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** -

Transfer 107 **Date** 8/19/09 **Notes** Everything same
Plates B **Time** 9:26pm
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** -

Transfer 108 **Date** 8/20/09 **Notes** Everything same
Plates B **Time** 9:25am
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** -

Transfer 109 **Date** 8/20/09 **Notes** Everything same
Froze #108
Set up inductions
Plates B **Time** 9:25pm
Glycerol [✓ Y] N **New Tips** 250 - 10 **250B** - **20B** -

Transfer 110 **Date** 8/21/09 **Notes** Everything same
Plates B **Time** 9:24am
Glycerol Y [✓ N] **New Tips** 250 - 9 **250B** - **20B** -

Transfer 111 **Date** 8/21/09 **Notes** Everything same. After the small dilutions I noticed a loose tip on the POD; however, all tips from the small bottleneck were accounted for — I will have to check tomorrow morning to make sure all wells transferred.
Plates B **Time** 9:32pm
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** -

Transfer 112 **Date** 8/22/09 **Notes** Everything same. Despite finding a stray tip last night all cultures transferred — the tip must have fallen off after the transfer.
Plates B **Time** 9:43am
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** -

Transfer 113 **Date** 8/22/09 **Notes** Everything same
Plates B **Time** 9:25pm
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** -

Transfer 114 **Date** 8/23/09 **Notes** Everything same
Plates B **Time** 9:25
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** -

Transfer 115 **Date** 8/23/09
Notes Everything same
Plates B **Time** 9:20pm
Glycerol Y [✓ N] **New Tips** 250 - 1 250B - 20B - 12

Transfer 116 **Date** 8/24/09
Notes Everything same
Plates B **Time** 9:26
Glycerol Y [✓ N] **New Tips** 250 - 1 250B - 20B - 4

Transfer 117 **Date** 8/24/09
Notes Skipped RMB1-D2, G3 3— contained only bacteria
Froze #116
Set up inductions
Plates B **Time** 9:29pm
Glycerol [✓ Y] N **New Tips** 250 - 10 250B - 20B -

Transfer 118 **Date** 8/25/09
Notes Everything same
Plates B **Time** 9:19am
Glycerol Y [✓ N] **New Tips** 250 - 9 250B - 20B -

Transfer 119 **Date** 8/25/09
Notes Everything same.
Plates B **Time** 9:35pm
Glycerol Y [✓ N] **New Tips** 250 - 1 250B - 20B - 12

Read above here ↑

Transfer 120 **Date** 8/26/09
Notes Everything same
Plates B **Time** 9:45am
Glycerol Y N **New Tips** 250 - 1 250B - 20B - 4

Transfer 121 **Date** 8/26/09
Notes Everything same
Set up induction for αF shift xpt.
Did small dilution on that plate
Plates B **Time** 9:55pm
Glycerol Y [✓ N] **New Tips** 250 - 3 250B - 20B - 14

Transfer 122 **Date** 8/27/09
Plates B **Time** 9:32am
Notes Everything same
Glycerol Y [✓ N] **New Tips** 250 - 2 250B - 20B - 4

Transfer 123 **Date** 8/27/09
Plates B **Time** 9:45pm
Notes Everything same except: RMB2 - C5 - x/c

Set up Tecan growth curve expt.

xferred αF shift xpt (small x farn)
Glycerol Y [✓ N] **New Tips** 250 - 2 250B - 20B - 16

Transfer 124 **Date** 8/28/09
Plates B **Time** 9:34am
Notes Everything same
Glycerol Y [✓ N] **New Tips** 250 - 1 250B - 20B -

Transfer 125 **Date** 8/28/09
Plates B **Time** 10:55pm
Notes Everything same.
I fucked up 1st time though, had to redo.
Hence later time.

Froze #124
Set up inductions. Induced αF-shift + small x farnd. Tecan plate to fridge
Glycerol [✓ Y] N **New Tips** 250 - 12 250B - 20B - 1

Transfer 126 **Date** 8/29/09
Plates B **Time** 9:38am
Notes Everything same
Glycerol Y [✓ N] **New Tips** 250 - 10 250B - 20B -

Transfer 127 **Date** 8/29/09
Plates B **Time** 9:55pm
Notes Everything same
Glycerol Y [✓ N] **New Tips** 250 - 1 250B - 20B -

Transfer 128 **Date** 8/30/09
Plates B **Time** 9:37am
Notes Note RMB2-C5 x/c cont - reset well pls.
⟵⟶ Removed ~~[illegible]~~ tip for RMB2-C5
Everything else same
Glycerol Y [✓ N] **New Tips** 250 - 1 250B - 20B -

Transfer 129 **Date** 8/30/09 **Notes** Everything same
Plates B **Time** 9:50pm
Glycerol Y [✓ N] **New Tips** 250 - 1 250B - 20B - 3

Transfer 130 **Date** 8/31/09 **Notes** Everything same
Plates B **Time** 9:34am
Glycerol Y [✓ N] **New Tips** 250 - 1 250B - 20B - 5

Transfer 131 **Date** 8/31/09 **Notes** Everything same
Plates B **Time** 9:35pm
Glycerol Y [✓ N] **New Tips** 250 - 19 250B - 20B - 3

Transfer 132 **Date** 9/1/09 **Notes** Everything same
Plates B **Time** 9:31am
Glycerol Y [✓ N] **New Tips** 250 - 1 250B - 20B - 4

Transfer 133 **Date** 9/1/09 **Notes** Everything same
Froze #132
Set up inductions
Plates B **Time** 9:50pm
Glycerol [✓ Y] N **New Tips** 250 - 11 250B - 20B -

Transfer 134 **Date** 9/2/09 **Notes** Everything same
Plates B **Time** 9:41am
Glycerol Y [✓ N] **New Tips** 250 - 10 250B - 20B -

Transfer 135 **Date** 9/2/09 **Notes** Everything same
Plates B **Time** 9:40pm
Glycerol Y [✓ N] **New Tips** 250 - 2 250B - 20B -

Transfer 136 **Date** 9/3/09 **Notes** Everything same
Plates B **Time** 9:25am
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** - 4

Transfer 137 **Date** 9/3/09 **Notes** Everything same
Plates B **Time** 9:35am
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** -

Transfer 138 **Date** 9/4/09 **Notes** Everything same
Plates B **Time** 9:22am
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** - 4

Transfer 139 **Date** 9/4/09 **Notes** Everything same
Plates B **Time** 9:26pm
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** - 14

Transfer 140 **Date** 9/5/09 **Notes** Everything same
Plates B **Time** 9:24 am
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** -

Transfer 141 **Date** 9/5/09 **Notes** Everything same
Froze # 140
Set up inductions
Plates B **Time** 9:30pm
Glycerol [✓ Y] N **New Tips** 250 - 10 **250B** - **20B** -

Transfer 142 **Date** 9/6/09 **Notes** Everything same
Plates B **Time** 9:22am
Glycerol Y [✓ N] **New Tips** 250 - 10 **250B** - **20B** -

Transfer 143 **Date** 9/6/09
Notes Everything same
Plates B **Time** 9:22 pm
Glycerol Y [✓ N] **New Tips** 250 - 1 250B - 20B -

Transfer 144 **Date** 9/7/09
Notes I may see a little cross contamination coming up in RMB2-A3 & A7 – I will have to monitor these wells over the next few transfers.
Everything else is the same
Plates B **Time** 9:18am
Glycerol Y [✓ N] **New Tips** 250 - 1 250B - 20B -

Transfer 145 **Date** 9/7/09
Notes I looked at the plates prior to transfer to unfortunately find every blank well w/ some low level of contamination
↳ I reset all empty wells on all 8 plates (B & S)
Plates B **Time** 10:20pm
Glycerol Y [✓ N] **New Tips** 250 - 1 250B - 20B - 7

Transfer 146 **Date** 9/8/09
Notes The reset wells are still empty indicating that the contaminant is not from the current media bottle
Plates B **Time** 9:31am
Glycerol Y [✓ N] **New Tips** 250 - 1 250B - 20B - 4

Transfer 147 **Date** 9/8/09
Notes Everything same
Plates B **Time** 9:36pm
Glycerol Y [✓ N] **New Tips** 250 - 1 250B - 20B - 12

Transfer 148 **Date** 9/9/09
Notes Everything same
Plates B **Time** 9:25am
Glycerol Y [✓ N] **New Tips** 250 - 1 250B - 20B - 4

Transfer 149 **Date** 9/9/09
Notes RMB2-E9 is back from lost
Everything else same
Froze #148
Set up inductions

Unfroze RMB-116, 124, 132, 140. Did 16µL xfer, put result in incubator to localize time of contam event
Plates B **Time** 9:50pm
Glycerol [✓ Y] N **New Tips** 250 - [illegible] 250B - 10 20B -

Transfer 150 **Date** 9/10/09 **Notes** Everything same
Plates B **Time** 9:26am
Glycerol Y [✓ N] **New Tips** **250 -** 8 **250B -** 1 **20B -** 4

Transfer 151 **Date** 9/10/09 **Notes** Everything same

Checked RMB1-116, 124, 132, 140 set up last night. RMB1-140 has small contam pellets in E4, E12. Others all look clean so far.

Plates B **Time** 9:45pm
Glycerol Y [✓ N] **New Tips** **250 -** **250B -** 1 **20B -** 1

Transfer 152 **Date** 9/11/09 **Notes** Everything same
Plates B **Time** 9:26am
Glycerol Y [✓ N] **New Tips** **250 -** **250B -** 1 **20B -**

Transfer 153 **Date** 9/11/09 **Notes** Everything same.

Checked RMB1-116, 124, 132, 140
Everything same as last night.
Conclusion → actually only 2 contam. in RMB1-140
Suggests problem was contaminated media.
Left in incubator to be sure.

Plates B **Time** 9:50pm
Glycerol Y [✓ N] **New Tips** **250 -** **250B -** 1 **20B -** 12

Transfer 154 **Date** 9/12/09 **Notes** Everything same.
Campus - total CF getting in - hence later than usual.
Plates B **Time** 10:30am
Glycerol Y [✓ N] **New Tips** **250 -** **250B -** 1 **20B -**

Transfer 155 **Date** 9/12/09 **Notes** Everything same

Checked RMB1-116, 124, 132, 140.
All same as before
Earlier conclusion seems reasonable.

Plates B **Time** 9:55pm
Glycerol Y [✓ N] **New Tips** **250 -** **250B -** 1 **20B -** 12

Transfer 156 **Date** 9/13/09 **Notes** Everything same
Plates B **Time** 9:50am
Glycerol Y [✓ N] **New Tips** **250 -** **250B -** 1 **20B -**

Transfer 157 **Date** 9/13/09
Plates B **Time** 9:50pm
Notes Everything same
Froze #156
Set up reductions
Spotted 8μL of all to G418
(exc. RMB1 - 12μL of that)

RMB1 - 116, 124, 132, 140
expt. - same as before
Removed from incubator now.

Set up 4 pos + 2 neg controls for spotting expt.

Glycerol [✓ Y] N **New Tips** 250 - **250B** - 10 **20B** - 4

Transfer 158 **Date** 9/14/09
Plates B **Time** 9:55am
Notes Everything same
Glycerol Y [✓ N] **New Tips** 250 - 8 **250B** - 2 **20B** -

Transfer 159 **Date** 9/14/09
Plates B **Time** 9:45pm
Notes Everything same
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 1 **20B** -

Transfer 160 **Date** 9/15/09
Plates B **Time** 9:45am
Notes Everything same
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 1 **20B** -

Transfer 161 **Date** 9/15/09
Plates B **Time** 9:45pm
Notes Everything same

Checked plates from spotting expt. @ 3pm. All look pretty OK - only few contam wells, and those mostly pretty low level

Glycerol Y [✓ N] **New Tips** 250 - 3 **250B** - 1 **20B** - 7

Transfer 162 **Date** 9/16/09
Plates B **Time** 9:55am
Notes Everything same
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 1 **20B** -

Pardelise here ↑

Transfer 163 **Date** 9/16/09
Plates B **Time** 9:45pm
Notes Everything same.
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 1 **20B** - 6

Transfer	**Date**	**Notes**			
164	9/17/09	Everything same			
Plates	**Time**				
B	9:45am				
Glycerol	Y [✓ N]	**New Tips**	**250 -** '	**250B -** 1	**20B -** 4

Transfer	**Date**	**Notes**			
165	9/17/09	Everything same Froze #164 Set up induction			
Plates	**Time**				
B	9:45pm				
Glycerol	[✓ Y] N	**New Tips**	**250 -**	**250B -** 11	**20B -**

Transfer	**Date**	**Notes**			
166	9/18/09	Everything same			
Plates	**Time**				
B	9:50am				
Glycerol	Y [✓ N]	**New Tips**	**250 -**	**250B -**	**20B -**

Transfer	**Date**	**Notes**			
167	9/18/09	Everything same			
Plates	**Time**				
B	9:35pm				
Glycerol	Y [✓ N]	**New Tips**	**250 -** 1	**250B -**	**20B -** 2

Transfer	**Date**	**Notes**			
168	9/19/09	Everything same.			
Plates	**Time**				
B	9:30am				
Glycerol	Y [✓ N]	**New Tips**	**250 -** 1	**250B -**	**20B -** 4

Transfer	**Date**	**Notes**			
169	9/19/09	Everything same			
Plates	**Time**				
B	9:35pm				
Glycerol	Y [✓ N]	**New Tips**	**250 -** 1	**250B -**	**20B -** 12

Transfer	**Date**	**Notes**			
170	9/20/09	Everything same			
Plates	**Time**				
B	9:30am				
Glycerol	Y [✓ N]	**New Tips**	**250 -**	**250B -** 1	**20B -** 4

Transfer 169-B **Date** 9/20/09
Plates B **Time** 10:00pm
Notes This is a repeat of the 9/19 PM dilution.
↳ see the note for S-85-B.
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 1 **20B** - 12

Transfer 170-B **Date** 9/21/09
Plates B **Time** 9:20am
Notes Looking at the S-84 & B-168 plates that were left @ 30°
all of the S-84 plates are free of this contaminant but
the B-168 plates show some (RMB1-E8, H11, BYB1-B9, D11, G10, & BYB2-E7)
therefore the cont. first appeared at the 9/19 AM dilution.
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 1 **20B** - 4

Transfer restart **Date** 9/21/09
Plates B **Time** 9:34am
Notes Because of the above contamination I am restarting
from the frozen 164 (from 9/17)
Glycerol Y [✓ N] **New Tips** 250 - **250B** - **20B** - 8

Transfer 165 **Date** 9/21/09
Plates B **Time** 9:35pm
Notes From the restarted plates,
Everything looks good w/them
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** - 12

Transfer 166 **Date** 9/22/09
Plates B **Time** 9:51am
Notes Everything same
I had to use non-barrier tips for BYB1 & BYB2
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 2 **20B** - 2 + 2 NB

Transfer 167 **Date** 9/22/09
Plates B **Time** 9:50pm
Notes Everything same.
Glycerol Y [✓ N] **New Tips** 250 - 1 **250B** - **20B** - 12

Transfer **Date**
Plates **Time**
Notes Note tip Issues! (see my email).
Glycerol Y N **New Tips** 250 - **250B** - **20B** -

112

Transfer 168 **Date** 9/23/09 **Notes** Everything same
Plates B **Time** 9:28am
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 1 **20B** - 4

☆ Note Skipping to designate gens!!

Transfer 171 **Date** 9/23/09 **Notes** Everything same
Plates B **Time** 9:35pm
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 1 **20B** - 12

Transfer 172 **Date** 9/24/09 **Notes** Everything same
Plates B **Time** 9:23am
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 1 **20B** - 4

Transfer 173 **Date** 9/24/09 **Notes** Everything same
Plates B **Time** 9:50pm
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 1 **20B** - 12

Transfer 174 **Date** 9/25/09 **Notes** Everything same
Plates B **Time** 9:24am
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 1 **20B** - 4

Transfer 175 **Date** 9/25/09 **Notes** Everything same
Froze #174
Set up inductions
Plates B **Time** 9:50pm
Glycerol [✓ Y] N **New Tips** 250 - **250B** - 10 **20B** - 12

Transfer 176 **Date** 9/26/09 **Notes** Everything same
Plates B **Time** 9:36am
Glycerol Y [✓ N] **New Tips** 250 - 8 **250B** - 1 **20B** - 4

Transfer 177
Date 9/26/09
Notes Everything same
Plates B
Time 9:35pm
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 1 **20B** - 12

Transfer 178
Date 9/27/09
Notes Everything same
Plates B
Time 9:18am
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 1 **20B** - 4

Transfer 179
Date 9/27/09
Notes Everything same
Plates B
Time 9:22pm
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 1 **20B** - 12

Transfer 180
Date 9/28/09
Notes Everything same
Plates B
Time 9:23am
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 1 **20B** - 4

Transfer 181
Date 9/28/09
Notes Everything same
Plates B
Time 9:35pm
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 1 **20B** -

Transfer 182
Date 9/29/09
Notes Everything same
Plates B
Time 9:26am
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 1 **20B** -

Transfer 183
Date 9/29/09
Notes Everything same
Froze #182
Set up inductions
Plates B
Time 9:55pm
Glycerol [✓ Y] N **New Tips** 250 - 2 **250B** - 10 **20B** -

Transfer 184 **Date** 9/30/09 **Notes** Everything same
Plates B **Time** 9:26am
Glycerol Y [✓ N] **New Tips** 250 - 8 250B - 1 20B -

Transfer 185 **Date** 9/30/09 **Notes** Everything same
Plates ~~[illegible]~~ B **Time** 9:35pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 2 20B -

Transfer 186 **Date** 10/1/09 **Notes** Everything same
Plates ~~A~~ B **Time** 9:29am
Glycerol Y [✓ N] **New Tips** 250 - 250B - 1 20B -

Transfer 187 **Date** 10/1/09 **Notes** Everything same
~~[illegible] lost tip for B~~
Plates B **Time** 9:46pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 1 20B -

Transfer 188 **Date** 10/2/09 **Notes** Everything Same
Plates B **Time** 9:27am
Glycerol Y [✓ N] **New Tips** 250 - 250B - 1 20B -

Transfer 189 **Date** 10/2/09 **Notes** Everything same
Plates B **Time** 9:45pm
Glycerol Y [✓ N] **New Tips** 250 - – 250B - 1 20B -

Transfer 190 **Date** 10/3/09 **Notes** Everything same
Plates B **Time** 9:31am
Glycerol Y [✓ N] **New Tips** 250 - 250B - 1 20B -

Transfer 191 **Date** 10/3/09
Plates B **Time** 9:40pm
Notes Everything same
Glycerol Y [✓ N] **New Tips** **250 -** **250B -** 1 **20B -**

Transfer 192 **Date** 10/4/09
Plates B **Time** 9:28am
Notes Everything same
↳ except RMB2-E09 should be a lost well. Perhaps I never reset this well after the contamination.
→ Nope, you didn't. Let's leave it as a pos. control in future 6418 plts.
Glycerol Y [✓ N] **New Tips** **250 -** **250B -** 1 **20B -**

Transfer 193 **Date** 10/4/09
Plates B **Time** 9:45pm
Notes Everything same.
Froze #192
Set up inductions.
Glycerol [✓ Y] N **New Tips** **250 -** **250B -** 2 **20B -**

Transfer 194 **Date** 10/5/09
Plates B **Time** 9:30am
Notes Everything same
Glycerol Y [✓ N] **New Tips** **250 -** 8 **250B -** 1 **20B -**

Transfer 195 **Date** 10/5/09
Plates B **Time** 9:30pm
Notes Everything same
Set up inductions bc of dropped plate in FACS room
Glycerol Y [✓ N] **New Tips** **250 -** **250B -** 2 **20B -**

Transfer 196 **Date** 10/6/09
Plates B **Time** 9:27am
Notes Removed tip for RMB2-E09 (see note from transfer 192)
Everything same
Glycerol Y [✓ N] **New Tips** **250 -** 4 **250B -** 1 **20B -**

Transfer 197 **Date** 10/6/09
Plates B **Time** 9:45pm
Notes Everything same
Froze #196
Glycerol [✓ Y] ~~N~~ **New Tips** **250 -** **250B -** 9 **20B -**

Transfer 198 **Date** 10/7/09 **Notes** Everything same
Plates B **Time** 9:34am
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 1 **20B** -

Transfer 199 **Date** 10/7/09 **Notes** Everything same
Froze #198
Plates B **Time** 9:40pm
Glycerol [✓ Y] N **New Tips** 250 - **250B** - 9 **20B** -

Transfer 200 **Date** 10/8/09 **Notes** Everything same
Plates B **Time** 9:30am
Glycerol Y [✓ N] **New Tips** 250 - **250B** - 1 **20B** -

Transfer 201 **Date** 10/8/09 **Notes** Everything same.
Set up inductions.
Froze #200.
Spotted to 6418 for
contam. test.
Plates B **Time** 9:35pm
Glycerol [✓ Y] N **New Tips** 250 - **250B** - 9 **20B** -

Transfer **Date** **Notes** Final FACs setup
Plates **Time**
Glycerol Y N **New Tips** 250 - 8 **250B** - **20B** -

Paused thing here

Transfer **Date** 10/15/09 **Notes** Tecan setup
Plates **Time**
Glycerol Y N **New Tips** 250 - ~~1~~ 2 **250B** - **20B** -

Transfer **Date** 10/21/09 **Notes** Standard curves & Growth curves
Plates **Time**
Glycerol Y N **New Tips** 250 - 5 **250B** - **20B** -

Transfer X	**Date** 10/28/09	**Notes** Std. Crv.	
Plates	**Time**		
Glycerol Y N	**New Tips** 250 - ~~4~~ 5	**250B -**	**20B -**

Transfer	**Date**	**Notes**	
Plates	**Time**		
Glycerol Y N	**New Tips** 250 -	**250B -**	**20B -**

Transfer	**Date**	**Notes**	
Plates	**Time**		
Glycerol Y N	**New Tips** 250 -	**250B -**	**20B -**

Transfer	**Date**	**Notes**	
Plates	**Time**		
Glycerol Y N	**New Tips** 250 -	**250B -**	**20B -**

Transfer	**Date**	**Notes**	
Plates	**Time**		
Glycerol Y N	**New Tips** 250 -	**250B -**	**20B -**

Transfer	**Date**	**Notes**	
Plates	**Time**		
Glycerol Y N	**New Tips** 250 -	**250B -**	**20B -**

Transfer	**Date**	**Notes**	
Plates	**Time**		
Glycerol Y N	**New Tips** 250 -	**250B -**	**20B -**

SMALL BOTTLENECK CULTURES

Transfer 3
Date 6/18/09
Plates S
Time 9:55pm
Glycerol Y [✓ N]
Notes Done by 10:10pm
Everything looks good
Densities appear OK by turbidity.

Transfer 4
Date 6/19/09
Plates S
Time 9:55pm
Glycerol Y [✓ N]
Notes Problems: RMS1-3 has 1 c-c event (well C12)
RMS2 is OK
BYS1 OK except one well noticeably lower density (D11)
BYS2 apparent contamination in well D6
Several middles have same issues except BYS2

Transfer ~~3-B~~
Date ~~6/19/09~~
Plates ~~S~~
Time ~~10:55pm~~
Glycerol Y N
Notes Went back to plates from 9/17/09 (xfer 2)
Redid all 4 xfers.

X - going back, same problem w/ c-c back to RMS1-1
Same problem w/ BYS1 back to BYS1-2

Transfer
Date
Plates
Time
Glycerol Y N
Notes Seems like no point going all the way back to RMS1-1, etc.
So let's just keep going, destroy the offending wells

Transfer 5
Date 6/20/09
Plates S
Time 10:00pm
Glycerol Y [✓ N]
Notes Same as before, no further problems

Transfer 6
Date 6/21/09
Plates S
Time 10:00pm
Glycerol Y [✓ N]
Notes RMS1 - OK
RMS2 - G10 small, G1 not offered well?
BYS1 - D11 small
BYS2 - OK, few smallish wells: G6, B7, C10
Apparent D6 contam. is gone.

Transfer 7
Date 6/22/09
Plates S
Time 10:00pm
Glycerol Y [✓ N]
Notes RMS1 - B6 small
RMS2 - G10 small, G1 small
BYS1 - D11 small
BYS2 - x-c G11, F10(?), small: G6, C10, B7
also in several middle

Transfer 8
Date 6/23/09
Plates S
Time 10:04 pm
Glycerol [✓ Y] N

Notes
RMS1 - x/c B7
RMS2 - No x/c
BYS1 - No x/c
BYS2 - x/c in A5, B6 F10, G11
reset empty wells by removing tips

Transfer 9
Date 6/24/09
Plates S
Time 10:05 pm
Glycerol Y [✓ N]

Notes
RMS1 - OK
RMS 2 - OK
BYS1 - OK
BYS2 - OK.

Transfer 10
Date 6/25/09
Plates S
Time 9:55 pm
Glycerol Y [✓ N]

Notes
RMS1 - OK
RMS2 - OK
BYS1 - x/c C12, small D11
BYS2 - OK

Transfer 11
Date 6/26/09
Plates S
Time 10:05 pm
Glycerol Y [✓ N]

Notes
BYS1 - C12 still cont.

Transfer 12
Date 6/27/09
Plates S
Time 10:00 pm
Glycerol Y [✓ N]

Notes
RMS1 - lost E12
RMS2 - OK
BYS1 - lost C1, D11
BYS2 - lost C10, G6

Transfer 13
Date 6/28/09
Plates S
Time 10:10 pm
Glycerol Y [✓ N]

Notes
RMS1 - same as last transfer
RMS2 - "
BYS1 - C1 was not lost (prob. just v. dilute) - growth now in C1
BYS2 - same as last transfer

Transfer 14
Date 6/29/09
Plates S
Time 9:45 pm
Glycerol Y [✓ N]

Notes
RMS1 - Same as last
RMS2 - "
BYS1 - "
BYS2 - lost H4, G6, E8, C10

Transfer 15 **Date** 6/30/09

Notes Same as before.

Froze down xfer #14.

Plates S **Time** 10:00pm

Glycerol [✓ Y] N

Transfer 16 **Date** 7/1/09

Notes Same as before. To summarize, this means:

RMS1 - Lost: E12

RMS2 - Dilute: H3

BYS1 - Lost: D11 Dilute: C1

BYS2 - Lost: C10, E8, G6, H4. Dilute: B7

Not lost - this was originally skipped

Plates S **Time** 9:48pm ~~10:00pm~~

Glycerol Y [✓ N]

Transfer 17 **Date** 7/2/09

Notes Everything same.

Plates S **Time** 9:55pm

Glycerol Y [✓ N]

Transfer 18 **Date** 7/3/09

Notes Everything same.

Plates S **Time** 10:05pm

Glycerol Y N

Transfer 19 **Date** 7/4/09

Notes Everything same.

Plates S **Time** 9:50pm

Glycerol Y N

Paid from above ↑

Transfer 20 **Date** 7/5/09

Notes BYS1 - possible x/c in A6, not yet clear (serial middle OK (not x/c there).

Otherwise everything same.

Plates S **Time** 10:05pm

Glycerol Y N

New Boxes from below

Transfer 21 **Date** 7/6/09

Notes x/c in BYS1 confirmed, well A6

Removed tip to reset well.

Otherwise everything same.

Later: A6 x/c is bacterial, not real x/c.

New Tip Boxes

250B - 1

20B - 13

Plates S **Time** 10:05pm

Glycerol Y [✓ N]

Transfer 22 **Date** 7/7/09
Plates S **Time** 9:50pm
Glycerol [✓ Y] N
Notes Everything same. Froze #21

<u>New Tip Boxes</u>
P250 - 1
P250B - 8

Transfer 23 **Date** 7/8/09
Plates S **Time** 9:45pm
Glycerol Y [✓ N]
Notes Everything same

Transfer 24 **Date** 7/9/09
Plates S **Time** 10:10pm
Glycerol Y [✓ N]
Notes RMS2 - we lost B7 (also gone in serial middle)
Otherwise everything same.

Transfer 25 **Date** 7/10/09
Plates S **Time** 9:20pm
Glycerol [✓ Y] N
Notes ~~B~~ BYS2 - F12 slick
Otherwise everything same

Froze #24

Transfer 26 **Date** 7/11/09
Plates S **Time** 9:45pm
Glycerol Y [✓ N]
Notes Everything same.

Transfer 27 **Date** 7/12/09
Plates S **Time** 9:45pm
Glycerol Y [✓ N]
Notes Everything same.

Paid when here
↑

Transfer 28 **Date** 7/13/09
Plates S **Time** 10:15pm
Glycerol [✓ Y] N
Notes F12 slick - BYS2 - poss bact?
Otherwise everything same
Set up selection
Froze #27

Transfer 29
Date 7/14/09
Notes Everything same
Plates S
Time 9:45pm
Glycerol Y [✓ N] **New Tips** **250 -** **250B -** **20B -**

Transfer 30
Date 7/15/09
Notes Everything same.
Plates S
Time 9:55pm
Glycerol Y [✓ N] **New Tips** **250 -** **250B -** **20B -**

Transfer 31
Date 7/16/09
Notes Everything same
Set up inductions
Froze #30
Plates S
Time 9:50pm
Glycerol [✓ Y] N **New Tips** **250 -** **250B -** **20B -**

Transfer 32
Date 7/17/09
Notes Everything same.
Plates S
Time 9:45pm
Glycerol Y [✓ N] **New Tips** **250 -** **250B -** **20B -**

Transfer X
Date 7/18/09
Notes Everything same
Froze # 32
Did not xfer
Plates S
Time 9:35pm
Glycerol [✓ Y] N **New Tips** **250 -** **250B -** **20B -**

Transfer X
Date 7/29/09
Notes RESTART – we are restarting the small dilutions
w/ a big dilution in the am before bottlenecking
Plates S
Time 9:12am
Glycerol Y [✓ N] **New Tips** **250 -** **250B -** **20B -**

Transfer 33
Date 7/29/09
Notes Everything same as before xfer except:
BYS2 - F12 looks ~~was~~ strange + dilute.
Plates S
Time 10:15pm
Glycerol Y [✓ N] **New Tips** **250 -** **250B -** **20B -**

Transfer 34
Date 7/30/09
Notes Everything same.
Froze #33
Started inductions.
Plates S
Time 10:15pm
Glycerol [✓ Y] N
New Tips 250 - 250B - 20B -

Transfer 35
Date 7/31/09
Notes Everything same.
Plates S
Time 10:00pm
Glycerol Y [✓ N]
New Tips 250 - 250B - 20B -

Transfer 36
Date 8/1/09
Notes Everything same.
Plates S
Time 10:05pm
Glycerol Y [✓ N]
New Tips 250 - 250B - 20B -

Transfer 37
Date 8/2/09
Notes Everything same.
Plates S
Time 10:15pm
Glycerol Y [✓ N]
New Tips 250 - 250B - 20B -

Transfer 38
Date 8/3/09
Notes Everything same
Froze #37
Set up inductions
Plates S
Time 9:55pm
Glycerol [✓ Y] N
New Tips 250 - 250B - 20B -

Transfer 39
Date 8/4/09
Notes Everything same.
Plates S
Time 10:10pm
Glycerol Y [✓ N]
New Tips 250 - 250B - 20B -

↑
Past also new

Transfer 40
Date 8/5/09
Notes Everything same
Plates S
Time 9:50pm
Glycerol Y [✓ N]
New Tips 250 - 250B - 20B -

Transfer 41 **Date** 8/6/09 **Notes** Everything same
Plates S **Time** 9:45pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 42 **Date** 8/7/09 **Notes** Everything same
Froze #41
Set up inductions.
Plates S **Time** 9:52pm
Glycerol [✓ Y] N **New Tips** 250 - 250B - 20B -

Transfer 43 **Date** 8/8/09 **Notes** Everything same
Tips falling off all over the place (1-slash)
Plates S **Time** 9:50pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 44 **Date** 8/9/09 **Notes** Everything same
(used new tips)
Plates S **Time** 9:55pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 45 **Date** 8/10/09 **Notes** Everything same
Plates S **Time** 9:35pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 46 **Date** 8/11/09 **Notes** Everything same
Froze #45
Set up inductions
Plates S **Time** ~~9~~10:05pm
Glycerol [✓ Y] N **New Tips** 250 - 250B - 20B -

Transfer 47 **Date** 8/12/09 **Notes** Everything same
Plates S **Time** 10:05pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 48 **Date** 8/13/09 **Notes** Everything same
Plates S **Time** 9:40pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 49 **Date** 8/14/09 **Notes** Everything same
Plates S **Time** 9:45pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 50 **Date** 8/15/09 **Notes** Everything same
Plates S **Time** 9:55pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 51 **Date** 8/16/09 **Notes** Everything same
Froze #50
Set up induction
Plates S **Time** 10:10pm
Glycerol [✓ Y] N **New Tips** 250 - 250B - 20B -

Transfer 52 **Date** 8/17/09 **Notes** Everything same
Plates S **Time** 9:45pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 53 **Date** 8/18/09 **Notes** Everything same
Plates S **Time** 9:45pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 54 **Date** 8/19/09 **Notes** Everything same
Plates S **Time** 9:42pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 55
Date 8/20/09
Notes Everything same
Froze #54
Set up inductions
Plates S
Time 9:35pm
Glycerol [✓ Y] N
New Tips 250 - 250B - 20B -

Transfer 56
Date 8/21/09
Notes Everything same
Plates S
Time 9:40pm
Glycerol Y [✓ N]
New Tips 250 - 250B - 20B -

Transfer 57
Date 8/22/09
Notes Everything same
Plates S
Time 9:47pm
Glycerol Y [✓ N]
New Tips 250 - 250B - 20B -

Transfer 58
Date 8/23/09
Notes Everything same
Plates S
Time 9:42
Glycerol Y [✓ N]
New Tips 250 - 250B - 20B -

Transfer 59
Date 8/24/09
Notes Skipped RMS2-H3 & BYS2-B7 — contained only bacteria
Froze #58
Set up inductions
Plates S
Time 9:45pm
Glycerol [✓ Y] N
New Tips 250 - 250B - 20B -

Paid above here ↑

Transfer 60
Date 8/25/09
Notes Everything same
Plates S
Time 9:55pm
Glycerol Y [✓ N]
New Tips 250 - 250B - 20B -

Transfer 61
Date 8/26/09
Notes Everything same
Plates S
Time 10:10pm
Glycerol Y [✓ N]
New Tips 250 - 250B - 20B -

Transfer 62 **Date** 8/27/09 **Notes** Everything same
Plates S **Time** 10:00pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 63 **Date** 8/28/09 **Notes** Everything same
I fucked up, had to redo. Hence later time.
Froze #62
Set up inductions
Plates S **Time** 11:05pm
Glycerol [✓ Y] N **New Tips** 250 - 250B - 20B -

Transfer 64 **Date** 8/29/09 **Notes** Everything same
Plates S **Time** 10:15pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 65 **Date** 8/30/09 **Notes** Everything same.
Plates S **Time** 10:05pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 66 **Date** 8/31/09 **Notes** Everything same
Plates S **Time** 9:50pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 67 **Date** 9/1/09 **Notes** Everything same
Froze #66
Set up inductions
Froze dF-shift expt. from 8/31
Set up that induction
Plates S **Time** 10:10pm
Glycerol [✓ Y] N **New Tips** 250 - 250B - 20B -

Transfer 68 **Date** 9/2/09 **Notes** Everything same
Plates S **Time** 9:55pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 69
Date 9/3/09
Notes Everything same
Plates S
Time 9:50pm
Glycerol Y [✓ N]
New Tips **250 -** **250B -** **20B -**

Transfer 70
Date 9/4/09
Notes Everything same
Plates S
Time 9:54pm
Glycerol Y [✓ N]
New Tips **250 -** **250B -** **20B -**

Transfer 71
Date 9/5/09
Notes Everything same
Froze #70
Set up inductions
Plates S
Time 9:53pm
Glycerol [✓ Y] N
New Tips **250 -** **250B -** **20B -**

Transfer 72
Date 9/6/09
Notes Everything same
Plates S
Time 9:50pm
Glycerol Y [✓ N]
New Tips **250 -** **250B -** **20B -**

Transfer 73
Date 9/7/09
Notes Reset empty wells (see note from Big transfer #145)
Plates S
Time 10:35
Glycerol Y [✓ N]
New Tips **250 -** **250B -** **20B -**

Transfer 74
Date 9/8/09
Notes Everything same
Plates S
Time 9:58pm
Glycerol Y [✓ N]
New Tips **250 -** **250B -** **20B -**

Transfer 75
Date 9/9/09
Notes Everything same
Froze #74
Set up inductions.
Plates S
Time 10:10pm
Glycerol [✓ Y] N
New Tips **250 -** **250B -** **20B -**

Transfer 76
Date 9/10/09
Plates S
Time 10:10pm
Notes Everything same.
Glycerol Y [✓ N]
New Tips 250 - 250B - 20B -

Transfer 77
Date 9/11/09
Plates S
Time 10:05pm
Notes Everything same.
Glycerol Y N
New Tips 250 - 250B - 20B -

Transfer 78
Date 9/12/09
Plates S
Time 10:10pm
Notes Everything same
Glycerol Y [✓ N]
New Tips 250 - 250B - 20B -

Transfer 79
Date 9/13/09
Plates S
Time 10:05pm
Notes Everything same
Froze #78
Set up inductions
Spotted all to G418 (8μL exc. RMS1 - that 8-16μL dry on well).
Glycerol [✓ Y] N
New Tips 250 - 250B - 20B -

Transfer 80
Date 9/14/09
Plates S
Time 10:00pm
Notes Everything same
Glycerol Y [✓ N]
New Tips 250 - 250B - 20B -

Transfer 81
Date 9/15/09
Plates S
Time 10:00pm
Notes Everything same.
Glycerol Y [✓ N]
New Tips 250 - 250B - 20B -

Paused after here ↑

Transfer 82
Date 9/16/09
Plates S
Time 10:00pm
Notes Everything same
Glycerol Y [✓ N]
New Tips 250 - 250B - 20B -

Transfer 83 **Date** 9/17/09
Plates S **Time** 10:00pm
Notes Everything same
Froze #82
Set up inductions
Glycerol [✓ Y] N **New Tips** 250 - 250B - 20B -

Transfer 84 **Date** 9/18/09
Plates S **Time** 9:45pm
Notes Everything same
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 85 **Date** 9/19/09
Plates S **Time** 9:50pm
Notes Everything same
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 85-B **Date** 9/20/09
Plates S **Time** 10:25pm
Notes This is a repeat of the 9/19 dilution - plates 85 were diluted as well as the SM from last night & the media bottle; however, the SMs from 9/18 & 9/17 are fine. plates ~~84~~ S-84 & B-168 all look fine as well.
Glycerol Y [✓ N] **New Tips** 250 - ~~[illegible]~~ 250B - 20B -

Transfer ~~[illegible]~~ restart **Date** 9/21/09
Plates S **Time** 9:47am
Notes See note from B-170-B & B-165-restart. Because we have had additional contamination we have decided to restart from frozen S-82 (from 9/17)
This restart was a nonserial transfer
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B - ~~[illegible]~~

Transfer (again) 83 **Date** 9/21/09
Plates S **Time** 9:55pm
Notes From restart.
All looks OK then.
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 84 **Date** 9/22/09
Plates S **Time** 10:05pm
Notes Everything same.
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

☆ Note Remember to correct w/ gens. 86 = 860 gens

Transfer 86	**Date** 9/23/09	**Notes** Everything same
Plates S	**Time** 9:50pm	
Glycerol Y [✓ N]	**New Tips** 250 -	250B - 20B -

Transfer 87	**Date** 9/24/09	**Notes** Everything same.
Plates S	**Time** 10:05pm	
Glycerol Y [✓ N]	**New Tips** 250 -	250B - 20B -

Transfer 88	**Date** 9/25/09	**Notes** Everything same. Froze #87 Set up inductions
Plates S	**Time** 10:05pm	
Glycerol [✓ Y] N	**New Tips** 250 -	250B - 20B -

Transfer 89	**Date** 9/26/09	**Notes** Everything same.
Plates S	**Time** 9:55pm	
Glycerol Y [✓ N]	**New Tips** 250 -	250B - 20B -

Transfer 90	**Date** 9/27/09	**Notes** Everything same
Plates S	**Time** 9:44pm	
Glycerol Y [✓ N]	**New Tips** 250 -	250B - 20B -

Transfer 91	**Date** 9/28/09	**Notes** Everything same
Plates S	**Time** 9:50pm	
Glycerol Y [✓ N]	**New Tips** 250 -	250B - 20B -

Transfer 92	**Date** 9/29/09	**Notes** Everything same Froze #91 Set up inductions
Plates S	**Time** 10:10pm	
Glycerol [✓ Y] N	**New Tips** 250 -	250B - 20B -

Transfer 93 **Date** 9/30/09 **Notes** Everything same.
Plates S **Time** 9:50pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 94 **Date** 10/1/09 **Notes** Everything same
We lost the tip for BYS2-B3 after the transfer to the SM.
Plates S **Time** 10:15pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 95 **Date** 10/2/09 **Notes** Everything same
BYS2-B3 OK
lost tip for BYS2-E9 after xfer to SM, but that's an empty well anyway.
Plates S **Time** 10:05pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 96 **Date** 10/3/09 **Notes** Everything same.
Plates S **Time** 10:00pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 97 **Date** 10/4/09 **Notes** Everything same.
Set up inductions
Froze #96.
Plates S **Time** 9:55pm
Glycerol [✓ Y] N **New Tips** 250 - 250B - 20B -

Transfer 98 **Date** 10/5/09 **Notes** Everything same.
Plates S **Time** 9:45pm
Glycerol Y [✓ N] **New Tips** 250 - 250B - 20B -

Transfer 99 **Date** 10/6/09 **Notes** Everything same
Froze #98
Plates S **Time** 10:00pm
Glycerol [✓ Y] N **New Tips** 250 - 250B - 20B -

133

Transfer 100 **Date** 10/7/09 **Notes** Everything same.

Plates S **Time** 9:55pm

Glycerol [✓ Y] N **New Tips** 250 - 250B - 20B -

that
Part here
↑

Transfer 101 **Date** 10/8/09 **Notes** Everything same. Froze #100. Set up inductions.

Spotted to 6418 for contam. test.

Plates S **Time** 9:55pm

Glycerol [✓ Y] N **New Tips** 250 - 250B - 20B -

Transfer **Date** **Notes**

Plates **Time**

Glycerol Y N **New Tips** 250 - 250B - 20B -

Transfer **Date** **Notes**

Plates **Time**

Glycerol Y N **New Tips** 250 - 250B - 20B -

Transfer **Date** **Notes**

Plates **Time**

Glycerol Y N **New Tips** 250 - 250B - 20B -

Transfer **Date** **Notes**

Plates **Time**

Glycerol Y N **New Tips** 250 - 250B - 20B -

Transfer **Date** **Notes**

Plates **Time**

Glycerol Y N **New Tips** 250 - 250B - 20B -

	Initials	Date
Prepared By		
Approved By		

Billing code: ~~346-60[illegible]~~ 346-6079

6/27/09

PROTOCOL CHANGES

- It seems that our contamination is bacterial (not cross-contamination)
  ↳ added Amp to the YPD
  - The 1st transfers to use Amp ~~[illegible]~~ were S-11 & B-21

- We have been having some clogged-tip issues. The current tips have been used too many times (3 or 4 runs). The clogged tips usually look like they contain dried YPD. Normally we have been using the tips then washing everything at the end — this means putting dirty tips back in the boxes.
  ↳ Modified the programs to wash the tips before placing back in tip box.
  - First used on transfers S-12 & B-23

6/29/09

Protocol Change: Set wash protocols to include the air-wash (with tip-touch) in the 96-well spin plates. Otherwise kept Greg's modifications.

We have adopted a system for reusing tips:
Fresh tips, the 1-slash = autoclaved once, then 2-slashes = autoclaved 2x, then throw them away.

7/2/09

Coulter Count

Counted in reverse direction

Bkg	64000	112000	
RMB1(31) - B2	1.687E8	1.718E8	The cultures are saturating between 1 & 2 E8 → the coulter was probably out of range for more accurate should have diluted
RMB2(31) - B2	1.929E8	1.938E8	
BYB1(31) - B2	1.647E8	1.658E8	
BYB2(31) - B2	1.725E8	1.732E8	
RMB1(31) - D2	1.576E7	1.582E7	these cultures are "dilute"
RMB1(31) - G3	2.546E6	2.512E6	
RMB2(31) - E9	2.469E7	2.471E7	
BYB1(31) - H5	1.967E7	1.818E7	
Post Bkg	6.062E6	2.732E6	

	Initials	Date
Prepared By		
Approved By		

7/3/09

The new tips appear to perform a little worse after being autoclaved. I'm noticing more liquid in the tips after ~~after~~ and after washing. And a couple of times a tip or few have fallen off during the washing. Got to watch this more carefully w/ these tips.

7/4/09

As I do the transfer, I am overcome by a crashing wave of patriotism.

7/8/09

Submitted billing based on usage through 7/5/09.
From now on will submit billing every Monday based on usage previous week, as tracked in log.

Comp. plates afered at: Small: 10:00pm
Big : 10:05pm

7/9/09

Comp plates afered at: Small: 10:50pm
Big = 10:35pm

7/10/09

PROTOCOL CHANGE:

Since adding amp to media we have had only 1 case of bact. contamination — BYS1-21-A6. We have now added Tetracycline to the media.

concentrations

Ampicillin - 100 µg/mL
Tetracycline - 25 µg/mL

→ 1st transfers w/ both drugs: B-48, S-25

9/10/09

Protocol Changes

- Given the recent contamination, we switched to using 250-barrier tips for dispensing YPD.
- Also we switched (~~or will switch~~) the induction protocol such that the tips do not go back into the experimental plates after they have been in YPD+αF

→ Both changes took place last night (B-149, S-75)

	Initials	Date
Prepared By		
Approved By		

8/31/09

Setting up fitness assays:

Grow up 96-well plate of ref.

xfer to 2 96-well plates (big/small xfer)

grow 24h & (1 dilution for big xfer plate)

Mixing strains:

Fill 8 plates w/40μL media

Add 20μL of ref (big to 4 plates, small to 4) to each plate.

Add 20μL of each evolved to plates.

Xfer from mixed plates (8μL rather than 4μL) as usual.

Put in incubator for competition

Spin down mixed plate. Resuspend in PBS.

→ Do FACS.

August 2009

🟧 Seminars
🟥 Other
🟩 Work
🟦 Home

July 2009						
S	M	T	W	T	F	S
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30	31	

August 2009						
S	M	T	W	T	F	S
						1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23	24	25	26	27	28	29
30	31					

September 2009						
S	M	T	W	T	F	S
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30			

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
26	27	28	29 FACS	30	31 FACS	1
2	3	4 FACS	5 Greg out	6	7	8 Greg out
9 Greg out	10	11 ← Week of FACS →	12 *	13	14	15
16	17 *	18 Mont	19 Mat	20 Mont	21 Mont *	22 Mont
23 Mart	24 Mont	25 *	26	27	28	29 *
30	31	1	2	3	4	5

September 2009

🟧 Seminars
🟥 Other
🟩 Work
🟦 Home

August 2009						
S	M	T	W	T	F	S
						1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23	24	25	26	27	28	29
30	31					

September 2009						
S	M	T	W	T	F	S
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30			

October 2009						
S	M	T	W	T	F	S
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	31

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
30	31	1	2 *	3	4 Mont	5 Mont
6 Mont *	7 Mont *	8 Mart	9	10 ~~708~~ 745 *	11	12 Greg out
13	14 785 ~~[illegible]~~ *	15	16	17	18 ~~[illegible]~~ 825 *	19 Greg out
20	21	22 *	23	24	25	26 *
27	28	29	30 *	1	2	3

September 2009

August 2009

S	M	T	W	T	F	S
						1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23	24	25	26	27	28	29
30	31					

September 2009

S	M	T	W	T	F	S
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30			

October 2009

S	M	T	W	T	F	S
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	31

🟦 Home

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
30	31	1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23 850	24 860	25 870	26 880 FACS on 870
27 890	28 900	29 910	30 920 FACS on 910	1	2	3

October 2009

September 2009

S	M	T	W	T	F	S
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30			

October 2009

S	M	T	W	T	F	S
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	31

November 2009

S	M	T	W	T	F	S
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30					

🟦 Home

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
27	28	29	30	1 930	2 940	3 950
4 960 [redacted]	5 970 FACS on 960	6 980	7 990	8 1000	9 FACS on 1000	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	31

lost or Agg

RMS1 A02	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 A04	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 A05	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 A07	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 A08	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 A09	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 A11	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 A12	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 B01	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 B02	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 B03	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 B04	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 B05	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 B06	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 B08	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 B09	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 B10	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 B12	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 C02	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 C05	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 C06	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 C07	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 C09	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 C10	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 C11	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 D01	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 D02	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 D03	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 D04	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 D06	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 D07	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 D08	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 D10	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 D11	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 D12	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 E01	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 E02	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 E03	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 E04	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 E05	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 E07	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 E08	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 E09	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 E11	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 F01	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 F02	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 F03	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 F04	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 F05	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 F06	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 F08	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 F09	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 F10	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 F12	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 G01	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 G02	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 G03	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 G05	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 G06	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 G07	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 G09	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 G10	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 G11	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 H01	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 H02	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 H03	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 H04	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 H06	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 H07	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 H08	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 H10	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 H11	SWP	CI	MM	FDS	AGG	UNC	NS
RMS1 H12	SWP	CI	MM	FDS	AGG	UNC	NS

UNC ?CI

10/14/09
- DIC - Gain: 1 Exposure: 2s
- YFP - Gain: 1 Exposure: 0.02s

FDS ?CI

TM

RMS2 A02	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 A04	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 A06	SWP	CI	MM	FDS	AGG	UNC	~~NS~~
RMS2 A07	SWP	CI	MM	FDS	AGG	UNC	~~NS~~
X **RMS2 A08**	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 A10	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 A11	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 A12	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 B01	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 B02	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 B03	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 B04	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 B05	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 B08	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 B09	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 B11	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 B12	SWP	~~CI~~	MM	FDS	AGG	UNC	NS
RMS2 C02	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 C04	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 C05	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 C06	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 C08	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 C09	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 C10	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 C12	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 D01	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 D02	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 D03	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 D05	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 D06	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 D07	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 D09	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 D10	SWP	CI	MM X	FDS	AGG	UNC	NS
RMS2 D11	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 E01	SWP	~~CI~~	MM	FDS	AGG	UNC	NS
RMS2 E02	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 E03	SWP	CI	~~MM~~	FDS	AGG	UNC	NS
RMS2 E04	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 E06	SWP	CI	MM	FDS	AGG	UNC	~~NS~~
RMS2 E07	SWP	CI	MM	FDS	AGG	~~UNC~~ UNC	NS
RMS2 E08	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 E10	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 E11	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 E12	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 F01	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 F02	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 F03	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 F04	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 F05	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 F07	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 F08	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 F09	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 F11	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 F12	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 G01	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 G02	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 G03	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 G04	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 G05	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 G06	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 G08	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 G09	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 G10	SWP	CI	MM	~~FDS~~	AGG	UNC	NS
RMS2 G12	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 H01	SWP	CI	MM	FDS	AGG	UNC	~~NS~~
RMS2 H02	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 H05	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 H06	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 H07	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 H09	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 H10	SWP	CI	MM	FDS	AGG	UNC	NS
RMS2 H11	SWP	CI	MM	FDS	AGG	UNC	NS

small cells

remove

UNC ?CI,, DS

sweeping

UNC. S.R.

sweeping

sweeping

?UNC CI

sweeping

UNC ?MM

sweeping

CI?.

?CI

? FDS/CI

M 146

BYS1 A01	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 A02	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 A03	SWP	CI	MM	FDS	AGG	UNC	NS	[illegible]
BYS1 A04	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 A05	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 A07	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 A08	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 A09	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 A11	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 A12	SWP	CI X	MM	FDS	AGG	UNC	NS	
BYS1 B01	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 B02	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 B03	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 B04	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 B05	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 B06	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 B08	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 B09	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 B10	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 B12	SWP	CI	MM	FDS	AGG	UNC	NS	
~~**BYS1 C01**~~	~~SWP~~	~~CI~~	~~MM~~	~~FDS~~	~~AGG~~	~~UNC~~	~~NS~~	
BYS1 C02	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 C03	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 C05	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 C06	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 C07	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 C09	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 C10	SWP	CI	MM	FDS	AGG	UNC	NS	S.I.
BYS1 C11	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 D01	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 D02	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 D03	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 D04	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 D06	SWP	CI X	MM	FDS	AGG	UNC	NS	
BYS1 D07	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 D08	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 D10	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 D12	SWP	CI	MM	FDS	AGG	UNC	NS	CI?
BYS1 E01	SWP	CI	MM	FDS	AGG	UNC	NS	S.R
BYS1 E02	SWP	CI	MM	FDS	AGG	UNC	NS	[illegible]
BYS1 E03	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 E04	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 E05	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 E07	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 E08	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 E09	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 E11	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 E12	SWP	CI	MM	FDS	AGG	UNC	NS	CI?
BYS1 F02	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 F04	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 F05	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 F06	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 F08	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 F09	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 F10	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 F12	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 G01	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 G02	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 G03	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 G05	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 G06	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 G07	SWP	CI	MM	FDS ?CI	AGG	UNC	NS	
BYS1 G09	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 G10	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 G11	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 H02	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 H04	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 H06	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 H07	SWP	CI	MM	FDS	AGG	UNC	NS	S.R.
BYS1 H08	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 H10	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 H11	SWP	CI	MM	FDS	AGG	UNC	NS	
BYS1 H12	SWP	CI	MM	FDS	AGG	UNC	NS	

	BYS2 A01	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 A02	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 A03	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 A04	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 A06	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 A07	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 A08	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 A10	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 A11	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 A12	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 B01	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 B02	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 B03	SWP	CI	MM	FDS	AGG	UNC	NS	
Overgrowth X	**BYS2 B04**	SWP	CI	MM X	FDS	AGG	UNC	NS	
	BYS2 B05	SWP	CI	MM	FDS	AGG	UNC	NS	
	~~**BYS2 B07**~~	~~SWP~~	~~CI~~	~~MM~~	~~FDS~~	~~AGG~~	~~UNC~~	~~NS~~	
remove	**BYS2 B08**	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 B09	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 B11	SWP	CI	MM	FDS	AGG	UNC	NS	
X	**BYS2 B12**	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 C01	SWP	CI	MM	FDS	AGG	UNC	NS	S.R.
	BYS2 C02	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 C03	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 C04	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 C05	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 C06	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 C08	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 C09	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 C12	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 D01	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 D02	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 D03	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 D05	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 D06	SWP	CI	MM	FDS	AGG	UNC	NS	
X	**BYS2 D07**	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 D09	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 D10	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 D11	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 E01	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 E02	SWP	CI X	MM	FDS	AGG	UNC	NS	
	BYS2 E03	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 E04	SWP	CI	MM	FDS	AGG	UNC	NS	?CI
	BYS2 E06	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 E07	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 E10	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 E11	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 E12	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 F02	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 F04	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 F05	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 F07	SWP	CI	MM	FDS	AGG	UNC	NS	?CI
	BYS2 F08	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 F09	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 F11	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 F12	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 G01	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 G02	SWP	CI	MM	FDS	AGG	UNC	NS	S.R.
	BYS2 G03	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 G04	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 G05	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 G08	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 G09	SWP	CI	MM	FDS CI	AGG	UNC	NS	
	BYS2 G10	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 G12	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 H02	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 H05	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 H06	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 H07	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 H09	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 H10	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYS2 H11	SWP	CI	MM	FDS	AGG	UNC	NS	

RMB1 A02	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 A05	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 A06	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 A07	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 A09	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 A10	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 A11	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 B01	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 B02	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 B03	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 B04	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 B06	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 B07	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 B08	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 B10	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 B11	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 B12	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 C02	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 C04	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 C05	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 C07	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 C08	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 C09	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 C11	SWP	CI X	MM	FDS	AGG	UNC	NS	
RMB1 C12	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 D01	SWP	CI	MM	FDS	AGG	UNC	NS	
~~RMB1 D02~~	~~SWP~~	~~CI~~	~~MM~~	~~FDS~~	~~AGG~~	~~UNC~~	~~NS~~	
RMB1 D03	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 D04	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 D05	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 D06	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 D08	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 D09	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 D10	SWP	CI	MM	FDS	AGG	UNC	NS	→ Steriles from Aggs
RMB1 D12	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 E01	SWP	CI	MM	FDS	AGG	UNC	NS	A→S
RMB1 E02	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 E03	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 E05	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 E06	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 E07	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 E09	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 E10	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 E11	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 F01	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 F02	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 F03	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 F04	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 F06	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 F07	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 F08	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 F10	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 F11	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 F12	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 G01	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 G02	SWP	CI	MM	FDS	AGG	UNC	NS	
~~RMB1 G03~~	~~SWP~~	~~CI~~	~~MM~~	~~FDS~~	~~AGG~~	~~UNC~~	~~NS~~	
RMB1 G04	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 G05	SWP	CI	MM	FDS	AGG	UNC	NS	→ Sterile then Agg
RMB1 G07	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 G08	SWP	CI	MM	FDS	AGG	UNC	NS	S→A
RMB1 G09	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 G11	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 G12	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 H01	SWP	CI	MM	FDS	AGG	UNC	NS	→ S.R.
RMB1 H02	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 H03	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 H04	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 H05	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 H06	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 H08	SWP	CI	MM	FDS	AGG	UNC	NS	→ S.R.
RMB1 H09	SWP	CI	MM	FDS	AGG	UNC	~~NS~~	
RMB1 H10	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB1 H12	SWP	CI	MM	FDS	AGG	UNC	NS	

ML

RMB2 A04	SWP	CI	MM	FDS	AGG	~~UNC~~	NS	
RMB2 A05	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 A06	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 A09	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 A10	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 A12	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 B01	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 B02	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 B03	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 B05	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 B06	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 B07	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 B09	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 B10	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 B11	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 C02	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 C04	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 C06	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 C07	SWP	CI	MM	FDS	AGG	UNC	NS	← St. Agg
RMB2 C08	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 C10	SWP	CI	MM	FDS	AGG	UNC	NS	A → S
RMB2 C11	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 C12	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 D01	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 D02	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 D03	SWP	CI	MM	FDS	AGG	UNC	NS	→ CI?
RMB2 D04	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 D05	SWP	CI	MM	FDS	AGG	UNC	NS	→ S.R?
RMB2 D07	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 D08	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 D09	SWP	~~CI~~	MM	FDS	AGG	UNC	NS	
RMB2 D11	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 D12	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 E01	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 E02	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 E03	SWP	CI	MM ~~MM~~	FDS	AGG	UNC	~~NS~~	
RMB2 E04	SWP	CI ~~CI~~	~~MM~~	FDS	AGG	UNC	NS	
RMB2 E05	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 E06	SWP	~~CI~~	MM	FDS	AGG	UNC	NS	
RMB2 E08	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 E10	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 E12	SWP	CI	MM	FDS	AGG	UNC	NS	✓
RMB2 F01	SWP	CI	MM	FDS	AGG	UNC	NS	← St. Agg Gbest ea.
RMB2 F02	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 F03	SWP	CI	MM	FDS	AGG	UNC	NS	A → S
RMB2 F05	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 F07	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 F09	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 F10	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 F11	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 G01	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 G02	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 G03	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 G04	SWP	CI	MM	FDS	AGG	~~UNC~~	NS	
RMB2 G06	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 G07	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 G08	SWP	CI	MM	FDS	AGG	UNC	NS	✓
RMB2 G10	SWP	CI	MM	FDS	AGG	UNC	NS	← St. agg
RMB2 G11	SWP	CI	MM	FDS	AGG	UNC	NS	S → A
RMB2 G12	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 H01	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 H02	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 H03	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 H04	SWP	CI	MM	FDS	AGG	UNC	~~NS~~	S.R
RMB2 H05	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 H07	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 H08	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 H09	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 H11	SWP	CI	MM	FDS	AGG	UNC	NS	
RMB2 H12	SWP	CI	MM	FDS	AGG	UNC	NS	

BYB1 A01	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 A02	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 A03	SWP	CI	MM	FDS	AGG	UNC	NS	→ SR
BYB1 A05	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 A06	SWP	CI	MM	FDS	~~AGG~~	UNC	NS	
BYB1 A07	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 A09	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 A10	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 A11	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 B01	SWP	CI	MM	FDS	AGG	UNC	~~NS~~	→ CI
BYB1 B02	SWP	CI	MM	FDS	AGG	UNC	NS	x
BYB1 B04	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 B06	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 B07	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 B08	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 B10	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 B11	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 B12	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 C01	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 C02	SWP	CI	MM	FDS	AGG	UNC	NS	→ SR
BYB1 C03	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 C04	SWP	CI	MM	FDS	AGG	UNC	~~NS~~	
BYB1 C05	SWP	CI	MM	FDS	AGG	UNC	NS	→ CI
BYB1 C07	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 C08	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 C09	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 C11	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 C12	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 D01	SWP	CI	MM	FDS	AGG	UNC	NS	→ SR
BYB1 D02	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 D03	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 D04	SWP	CI	MM	FDS	AGG	UNC	~~NS~~	
BYB1 D05	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 D06	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 D08	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 D09	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 D10	SWP	CI	MM	FDS	AGG	UNC	NS	← Sl. Agg
BYB1 D12	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 E01	SWP	CI	MM	FDS	AGG	UNC	NS	A → S
BYB1 E02	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 E03	SWP	CI	MM	FDS	AGG	UNC	NS	→ CI
BYB1 E05	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 E06	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 E07	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 E09	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 E10	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 E11	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 F02	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 F04	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 F06	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 F07	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 F08	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 F10	SWP	CI	MM	FDS	AGG	UNC	NS	→ SR
BYB1 F11	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 F12	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 G01	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 G02	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 G03	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 G04	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 G05	SWP	CI	MM	FDS	AGG	UNC	NS	→ S.R
BYB1 G07	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 G08	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 G09	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 G11	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 G12	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 H02	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 H04	SWP	CI	MM	FDS	AGG	UNC	NS	
x ~~**BYB1 H05**~~	~~SWP~~	~~CI~~	~~MM~~	~~FDS~~	~~AGG~~	~~UNC~~	~~NS~~	
BYB1 H06	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 H08	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 H09	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 H10	SWP	CI	MM	FDS	AGG	UNC	NS	
BYB1 H12	SWP	CI	MM	FDS	AGG	UNC	NS	

TWA

	BYB2 A01	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 A02	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 A03	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 A04	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 A05	SWP	CI	MM	FDS	AGG	UNC	NS	→ SR
	BYB2 A06	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 A08	SWP	CI	MM	FDS	AGG	UNC	~~NS~~	
	BYB2 A09	SWP	CI	MM	FDS	AGG	UNC	NS	→ CI
	BYB2 A10	SWP	~~CI~~	MM	FDS	AGG	UNC	NS	
	BYB2 A12	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 B01	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 B02	SWP	CI	MM	FDS	AGG	UNC	~~NS~~	
	BYB2 B03	SWP	CI	MM	FDS	AGG	UNC	NS	→ SR
	BYB2 B05	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 B06	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 B07	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 B09	SWP	~~CI~~	MM	FDS	AGG	UNC	NS	
	BYB2 B10	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 B11	SWP	CI	MM	FDS	AGG	UNC	NS	→ CI
	BYB2 C01	SWP	CI	MM	FDS	AGG	UNC	~~NS~~	→ SR
	BYB2 C02	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 C03	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 C04	SWP	CI	MM	FDS	AGG	UNC	~~NS~~	→ SR
	BYB2 C06	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 C07	SWP	CI	MM	FDS	AGG	UNC	~~NS~~	
	BYB2 C08	SWP	~~CI~~	MM	FDS	AGG	UNC	NS	
	BYB2 C10	SWP	CI	MM	FDS	AGG	UNC	~~NS~~	→ SR
	BYB2 C11	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 C12	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 D01	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 D02	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 D03	SWP	CI	MM	FDS	AGG	UNC	~~NS~~	
	BYB2 D04	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 D05	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 D07	SWP	CI	MM	FDS	AGG	UNC	NS	→ SR
	BYB2 D08	SWP	CI	MM	FDS	AGG	UNC	~~NS~~	→ SR
	BYB2 D09	SWP	CI	MM	FDS	AGG	UNC	~~NS~~	
	BYB2 D11	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 D12	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 E01	SWP	CI	MM	FDS	AGG	UNC	NS	→ SR
	BYB2 E02	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 E03	SWP	~~CI~~	MM	FDS	AGG	UNC	NS	
Spread over gate ×	**BYB2 E04**	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 E05	SWP	CI	MM	FDS	AGG	UNC	NS	→ CI
	BYB2 E06	SWP	CI	MM	FDS	AGG	UNC	~~NS~~	→ SR
	BYB2 E08	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 E09	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 E10	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 E12	SWP	CI	MM	FDS	AGG	UNC	NS	→ CI
	BYB2 F02	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 F05	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 F06	SWP	CI	MM	FDS	AGG	UNC SR	NS	
	BYB2 F07	SWP	CI	MM	FDS	AGG	UNC SR	NS	
	BYB2 F09	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 F10	SWP	CI	MM	FDS	AGG	UNC FDS?	NS	
	BYB2 F11	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 G01	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 G02	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 G03	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 G04	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 G06	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 G07	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 G08	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 G10	SWP	CI	~~MM~~	FDS	AGG	UNC	NS	
	BYB2 G11	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 G12	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 H02	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 H04	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 H05	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 H07	SWP	CI	MM	FDS	AGG	UNC	NS	
Spread over gate ×	**BYB2 H08**	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 H09	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 H11	SWP	CI	MM	FDS	AGG	UNC	NS	
	BYB2 H12	SWP	CI	MM	FDS	AGG	UNC SR	~~NS~~	

152