6/11/07

In these last few months in Boston I have decided to start a new experiment:

Determine the fitness of αF resistant clones.

I suspect that αF resistant clones may be more fit than non-selected clones. There are two reasons for this

(1) αF resistance has been observed during experimental evolution (need ref. - Jun Yi, Dawn, or Michael). This could mean that the is a large target for αF^R or that αF^R provides a selective benefit.

(2) Data from fluctuation assays conferring αF^R do not follow the Luria-Delbrück dist. and are not modeled any better using a combined Luria-Delbrück/Poisson distribution.

There a several reasons why I want to know if αF mutants have a fitness advantage.

(1) It could help to answer the question What is the nature of beneficial mutations in yeast? Is it simply the loss of unnecessary protein production? Loss of pathways?

(2) It could serve as a method for isolating beneficial mutations — and learning something about their distribution.

(3) The evolution of αF^R could serve as a good model system to study adaptive evolution. We would know all of the possible targets, the rate, and dist. of fitness effects.

(4) We could find the mutations by complementation and determine where the fall on the pathway.

6/11/07

Streak strains from freezer:

GIL050 (YFP reference)

Spores of GIL103

6C

13B } URA3, CAN1, Mata, bar1Δ, hmlαΔ (Notebook MR5 pg. 5)

16A

GIL104 (from fridge)

6/20/07

- Grew GIL104, 6C, 13B, & 16A in 2xSC-ura
- Plated on 5FOA - Q&D to determine relative mut. rate. - I know that mut. rate differences were segregating in this cross - It probably doesn't matter but I would prefer low mut. rate.

STRAIN	5FOA^R COLONIES
GIL104	4
6C	8
13B	4
16A	2 ← use this strain for expt.

7/2/07

- I want to test run a fitness assay.
  - Inoculate 3ml YPD+ADE/TRP with single colony off of plate
    - 16A
    - GIL050

7/11/07

I quit the previous attempt. Retry today.

- Dilute sat. o/N cultures 16A, GIL050 1:10 in YPD (10:15)
- Dilute 1:10 again @ 2:30
- OD_{600} at 6:00

16A: 0.7 → Dilute 1:4 (2.5 ml into 7.5 ml)
GIL050: 1.0 → Dilute 1:5.5 (2 ml into 9 ml)
} should be OD_{600} of 0.175

- At 9:00: Mix samples in the following ratios:

GIL050(Ref)		16A	OD_{600}
1	:	0	0.89
1	:	1	0.97
2	:	1	0.95
0	:	1	1.04

7/11/07

.... contnued from previous page.

- each mixture was diluted into 10ml YPD (20 λ, 1:500 dilution)
  - YPD prewarmed @ 30° in incubator.

- 1ml of each mixture → transferred to microcentrifuge tube → spin 1 min → aspirate → resuspend in 1ml PBST. (.1% tween)

- Coulter count each mixture

Bkg		82	150	200
050	1	12730	12652	12526
	2	12822	12764	12908
	3	15848	15504	15602
1:1	1	14140	14212	15794
	2	18284	17520	17604
	3	14512	13912	14278
1:2	1	18092	18434	18160
	2	24786	23424	23882
	3	14078	13344	13412
16A	1	32544	32334	32808
	2	18064	17312	16932
	3	20474	20544	28280
Post Bkg		902	686	538
1:2	4	24684	24378	24252
16A	4	34372	34416	34652

→ should have used 10mL Coulter sol'n instead of 20.

This is the shittiest data I have seen w/ the Coulter counter. It suggests that I can't dilute consistently. It also ~~doesn't~~ isn't consistent w/ the ODs.

7/12/07

@ 9:00am – Repeat dilutions, prep. for FACS, Coulter counting as above.

OD_{600}:

~~GIL~~ 16A : GIL050	OD_{600}
0 : 1	0.70
1 : 1	0.85
1 : 2	0.79
1 : 0	1.02

7/12/07

10 λ into 10 mL

Bkg		602	364	288
050	1	25006	25406	24486
	2	26938	26460	26996
	3	26322	25942	25724
1:1	1	47484	47308	47048
	2	44862	44262	44356
	3	48128	48040	47966
1:2	1	39830	39674	39156
	2	40544	39932	39568
	3	34142	34076	34460
16A	1	74982	74836	74650
	2	74124	74194	73900
	3	69802	69512	68476
Post Bkg		1204	1530	962

9:00 pm

Bkg		66	152	186
050	1	14608	14346	14200
	2	18222	17906	18290
	3	18712	18354	18156
1:1	1	39296	38684	38102
	2	43226	42832	42728
	3	40582	40112	39794
1:2	1	30004	30598	29710
	2	28652	28518	28108
	3	29798	29718	30378
16A	1	61300	61218	60324
	2	60132	59682	60756
	3	70518	69850	69626
Post Bkg		650	562	398

20 λ into 10 mL

38122	37176	38004
38978	38386	37640
37008	36510	36600

~~Gt~~ 16A:GIL050	OD_{600}
0 : 1	0.59
1 : 1	0.87
1 : 2	0.76
1 : 0	1.10

7/13/07

9:10 am

OD_{600}:

16A : G11050	OD_{600}
0 : 1	0.56
1 : 1	0.97
1 : 2	0.83
1 : 0	1.25

Bkg		122	136	174
050	1	33284	32912	33518
	2	33268	33126	32776
	3	33972	33578	33440
1:1	1	53274	52710	52264
	2	48834	48508	48020
	3	51536	51200	50742
1:2	1	38908	38964	38640
	2	36578	36064	35852
	3	40094	39380	40012
16A	1	94724	94056	93360
	2	81740	81836	81604
	3	67504	66234	66244
Post Bkg		1100	1130	1098

7/19/07

FACS DATA USING LSRII

		FREQ	NUMBER	
	~~Specimen_001_Tube_001.fcs~~		~~22753~~	
	~~YFP 530_30-A, FSC-A subset~~	~~100.00~~	~~22753~~	
	~~YFP 530_30-A, FSC-A subset-1~~	~~0.00~~	~~0~~	
1:1 Gated	Specimen_001_1,3a,1_001.fcs		20000	
	YFP 530_30-A, FSC-A subset	69.83	13966	← 16A
	YFP 530_30-A, FSC-A subset-1	30.18	6036	← Ref.
	Specimen_001_1,3a,1_002.fcs		20000	
	YFP 530_30-A, FSC-A subset	76.13	15226	
	YFP 530_30-A, FSC-A subset-1	23.88	4775	
	Specimen_001_1,3a,1_003.fcs		20000	
	YFP 530_30-A, FSC-A subset	80.80	16160	
	YFP 530_30-A, FSC-A subset-1	19.20	3840	
	Specimen_001_1,3a,1_004.fcs		20000	
	YFP 530_30-A, FSC-A subset	85.38	17076	
	YFP 530_30-A, FSC-A subset-1	14.62	2924	
1:2 Gated	Specimen_001_1,3a,2_001.fcs		20000	
	YFP 530_30-A, FSC-A subset	52.40	10481	
	YFP 530_30-A, FSC-A subset-1	47.60	9519	
	Specimen_001_1,3a,2_002.fcs		20000	
	YFP 530_30-A, FSC-A subset	58.70	11739	
	YFP 530_30-A, FSC-A subset-1	41.32	8263	
	Specimen_001_1,3a,2_003.fcs		20000	
	YFP 530_30-A, FSC-A subset	66.60	13319	
	YFP 530_30-A, FSC-A subset-1	33.41	6682	
	Specimen_001_1,3a,2_004.fcs		20000	
	YFP 530_30-A, FSC-A subset	73.22	14644	
	YFP 530_30-A, FSC-A subset-1	26.79	5358	
All 16A Gated	Specimen_001_16A_001.fcs		20000	
	YFP 530_30-A, FSC-A subset	100.00	20000	
	YFP 530_30-A, FSC-A subset-1	0.00	0	
	Specimen_001_16A_002.fcs		20000	
	YFP 530_30-A, FSC-A subset	100.00	20000	
	YFP 530_30-A, FSC-A subset-1	0.00	0	
	Specimen_001_16A_003.fcs		20000	
	YFP 530_30-A, FSC-A subset	100.00	20000	
	YFP 530_30-A, FSC-A subset-1	0.00	0	
	Specimen_001_16A_004.fcs		20000	
	YFP 530_30-A, FSC-A subset	100.00	19999	
	YFP 530_30-A, FSC-A subset-1	0.00	1	
	Specimen_001_Ref_001.fcs		20000	

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	YFP 530_30-A, FSC-A subset	2.15	430
	YFP 530_30-A, FSC-A subset-1	97.86	19571
All Ref	Specimen_001_Ref_002.fcs		20000
	YFP 530_30-A, FSC-A subset	2.78	556
Gated	YFP 530_30-A, FSC-A subset-1	97.24	19447
	Specimen_001_Ref_003.fcs		20000
	YFP 530_30-A, FSC-A subset	2.46	493
	YFP 530_30-A, FSC-A subset-1	97.54	19507
	Specimen_001_Ref_004.fcs		20000
	YFP 530_30-A, FSC-A subset	4.10	819
	YFP 530_30-A, FSC-A subset-1	95.92	19184
	Specimen_001_1,3a,2(2)_001.fcs		20000
1:2	YFP 530_30-A, FSC-A subset	52.72	10543
No Gate	YFP 530_30-A, FSC-A subset-1	47.30	9459
	Specimen_001_1,3a,2(2)_002.fcs		20000
✓	YFP 530_30-A, FSC-A subset	59.74	11947
	YFP 530_30-A, FSC-A subset-1	40.27	8054
	Specimen_001_1,3a,2(2)_003.fcs		20000
	YFP 530_30-A, FSC-A subset	66.47	13294
	YFP 530_30-A, FSC-A subset-1	33.54	6709
	Specimen_001_1,3a,2(2)_004.fcs		20000
	YFP 530_30-A, FSC-A subset	72.74	14547
	YFP 530_30-A, FSC-A subset-1	27.27	5454
	Specimen_001_1,3a,1(2)_001.fcs		20000
1:1	YFP 530_30-A, FSC-A subset	68.16	13631
No Gate	YFP 530_30-A, FSC-A subset-1	31.85	6369
	Specimen_001_1,3a,1(2)_002.fcs		20000
✓	YFP 530_30-A, FSC-A subset	75.31	15062
	YFP 530_30-A, FSC-A subset-1	24.70	4939
	Specimen_001_1,3a,1(2)_003.fcs		20000
	YFP 530_30-A, FSC-A subset	80.98	16195
	YFP 530_30-A, FSC-A subset-1	19.02	3805
	Specimen_001_1,3a,1(2)_004.fcs		20000
	YFP 530_30-A, FSC-A subset	84.91	16982
	YFP 530_30-A, FSC-A subset-1	15.10	3019

NOTES:

- Since the haploid 16A and the diploid Reference strains are different sizes, I will not gate the counts.
- I manually set the windows to determine YFP / No YFP
  ↳ these were applied equally to each sample.

7/19/07

ANALYSIS OF COMPETITION

1:1 Mixture of 16A:Reference

	OD600	Counts	Population Size: Initial	Population Size: Final	Total Gen.	Fract. of Population: 16A	Fract. of Population: Ref.	log Ratio
Start	0.89	28556	----	473080000	0	68.16	31.85	0.7608205
12 h	0.85	47308	946160	473080000	8.96578428	75.31	24.7	1.11480968
24 h	0.87	40112	946160	401120000	17.6935183	80.98	19.02	1.44871115
36 h	0.97	51200	802240	512000000	27.0114125	84.91	15.1	1.72689713

1:2 Mixture of 16A:Reference

	OD600	Counts	Population Size: Initial	Population Size: Final	Total Gen.	Fract. of Population: 16A	Fract. of Population: Ref.	log Ratio
Start	0.95	36320	----	399320000	0	52.72	47.3	0.10848459
12 h	0.79	39932	798640	399320000	8.96578428	59.74	40.27	0.39439504
24 h	0.76	29798	798640	297980000	17.5092387	66.47	33.54	0.68401196
36 h	0.83	38908	595960	389080000	26.8598743	72.74	27.27	0.98110424

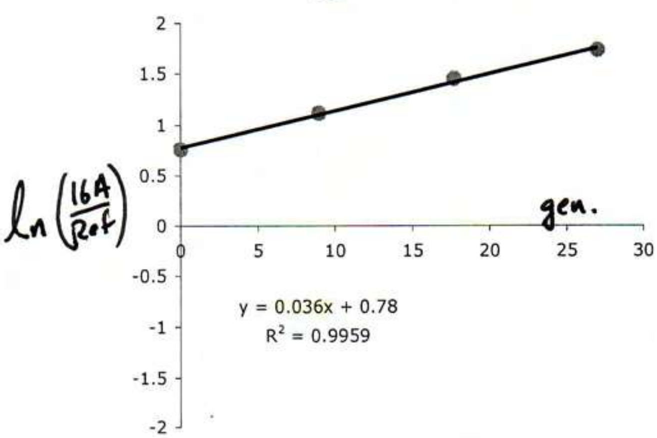
All 16A

	OD600	Counts	Population Size: Initial	Population Size: Final	Total Gen.	Fract. of Population: 16A	Fract. of Population: Ref.	log Ratio
Start	1.04	40940	----	741240000	0	100	0	----
12 h	1.02	74124	1482480	741240000	8.96578428	100	0	----
24 h	1.1	61218	1482480	612180000	17.6555837	100	0	----
36 h	1.25	81740	1224360	817400000	27.0384544	100	0	----

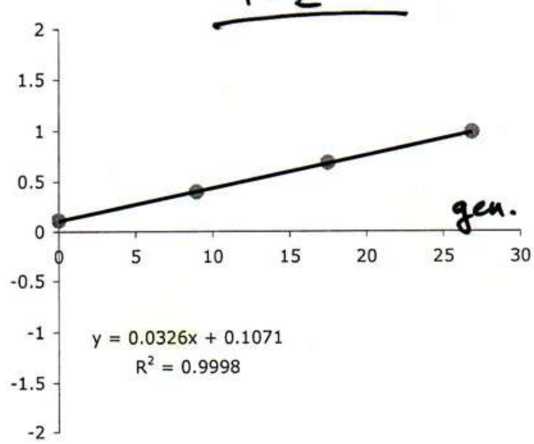
All Reference

	OD600	Counts	Population Size: Initial	Population Size: Final	Total Gen.	Fract. of Population: 16A	Fract. of Population: Ref.	log Ratio
Start	0.89	25644	----	259420000	0	2.15	97.86	-3.81807004
12 h	0.7	25942	518840	259420000	8.96578428	2.78	97.24	-3.55473122
24 h	0.59	19002	518840	190020000	17.4824301	2.46	97.54	-3.6801012
36 h	0.56	16642	380040	166420000	26.256892	4.1	95.92	-3.15252754

1:1



1:2

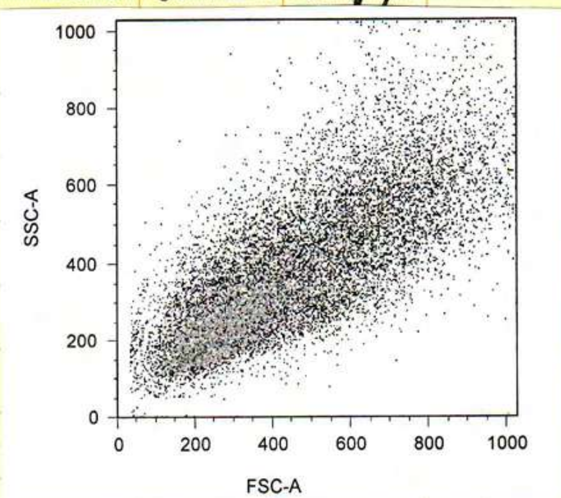


- The linearity is excellent.
- The Ref. has a 3% fitness diff. compared to 16A.

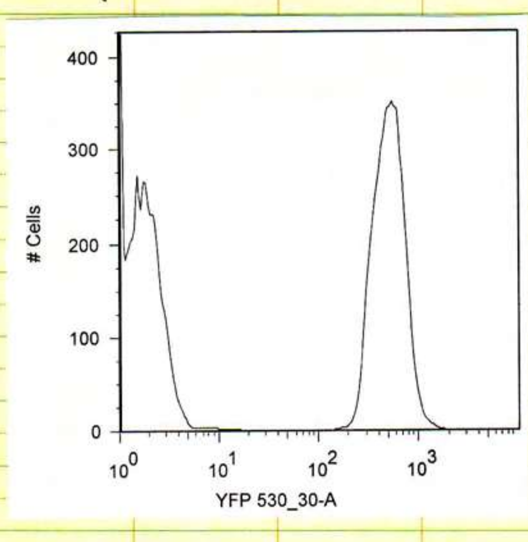
7/19/07

MORE NOTES ON FITNESS ASSAYS

- Although the cells were less clumpy in PBST than they were when I resuspended in just H_2O, they were still clumpy.



This might not matter however. I sonicated ~~and~~ ~~w~~ for 2 min in PBST. Under the scope they seem fine to me. I may ignore this issue — the signal from YFP is sufficiently separated.



7/19/07

~~[illegible]~~ STRAINS FOR ASSAY

- I will get strains from 2 sources
  (1) Spontaneous αF^R mutations of 16A (hereafter GIL121).
  (2) From the deletion collection.

METHOD 1: Spontaneous αF^R

Perform a fluctuation assay on GIL121 in order to measure the mutation rate to αF^R, the distribution of mutants per culture and to isolate independent αF^R mutations.

- Dilute an O/N culture of GIL121 1:10 in H_2O
  ↳ 10λ of dilution into 10 ml 2×SC (only 0.2% glucose)
  ↳ 10λ into each well of 96-well plate

  done on 7/18

- Today I will add 90λ H_2O to each well and spot onto overdried αF plates.

METHOD 2: Strains from deletion collection

- Isolate strains from deletion collection that are reported to be sterile — some of these may not be αF^R. Also Isolate some unrelated to mating in a-cells (i.e. STE3). Also isolate some dubious ORFS — these will serve as "wild-type" controls to which I will normalize the data.

7/19/07

STERILE?

				PLATE	ROW	COLUMN
STE2 R S	YFL026W	BY4741	06_1	31	D	6
STE4 I S	YOR212W	BY4741	15_3	20	E	3
STE5 B S	YDR103W	BY4741	04_4	11	C	5
STE7 U S	YDL159W	BY4741	04_2	40	F	6
STE11 L S	YLR362W	BY4741	12_5	22	A	6
~~STE12~~	~~YHR084W~~	-----	-----	-----	-----	-----
STE20 E S	YHL007C	BY4741	08_1	14	G	10
STE50 G	YCL032W	BY4741	03_1	16	C	9
FAR1 K S	YJL157C	BY4741	10_1	21	E	4
FAR3 Y	YMR052W	BY4741	chr00_5	50	B	3
FAR7 S	YFR008W	BY4741	06_1	31	F	9
FAR8 A	YMR029C	BY4741	13_2	3	H	7
VPS64 N	YDR200C	BY4741	04_5	23	F	2
FAR10 M	YLR238W	BY4741	12_4	22	E	8
FAR11 V	YNL127W	BY4741	14_3	42	F	9
FUS1 F	YCL027W	BY4741	03_1	16	C	5
FUS3 Q	YBL016W	BY4741	02_1	27	B	1
DIG1 O S	YPL049C	BY4741	16_3	25	B	3
DIG2 W	YDR480W	BY4741	chr4_8	48	D	2
STE3 H	YKL178C	BY4741	11_2	18	H	10
~~STE6~~	~~YKL209C~~	-----	-----	-----	-----	-----
STE13 J	YOR219C	BY4741	15_3	20	E	8
STE14 C S	YDR410C	BY4741	04_7	12	B	12
STE23 D	YLR389C	BY4741	12_5	70	A	6
STE24 T	YJR117W	BY4741	00_12	38	A	8
AXL1 P	YPR122W	BY4741	16_5	25	F	3
RAM1 X S	YDL090C	BY4741	chr4_1	48	A	3
YEL014C 1	YEL014C	BY4741	05_2	12	E	5
YGR139W 2	YGR139W	BY4741	07_5	13	H	1
YLR255C 3	YLR255C	BY4741	12_4	22	F	7
~~YLR184W~~	~~YLR184W~~	~~BY4741~~	~~12_3~~	~~17~~	~~B~~	~~12~~ ← fitness defect
YDR029W 4	YDR029W	BY4741	04_3	39	A	4

Not in deletion collection

→ dubious ORFS that do not overlap w/ another ORF — will be used as WT.

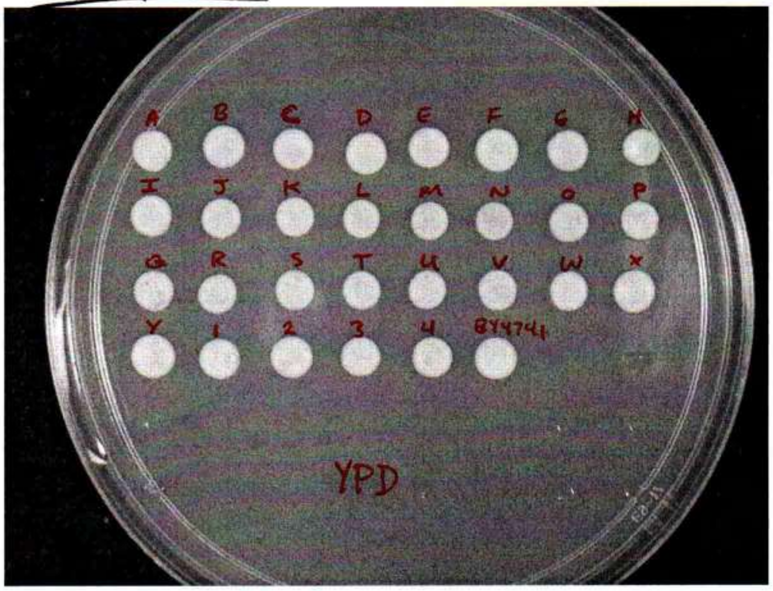
• 3
• 11
• • 12 – 2
• 13
• 14
• • 16 – 2
• 17
• 18
• • 20 – 2
• 21
• • • 22 – 3
• 23
• • 25 – 2
• 27
• • 31 – 2
• 38
• 39
• 40
• 42
• • 48 – 2
50
70

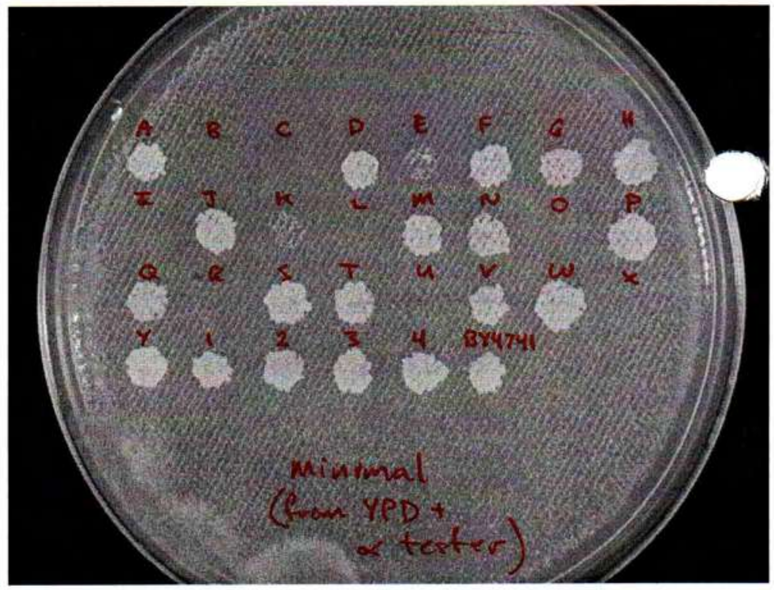
7/20/07

NOTES:

- Each strain was coded with a letter or number (if WT) – shown above
- YPS64Δ has a fitness defect obvious from plating
- YLR184WΔ (one of the WT strains) has a severe fitness defect and will not be used – instead the 5th WT strain will be BY4741

7/24/07


A B C D E F G H
I J K L M N O P
Q R S T U V W X
Y 1 2 3 4 BY4741
YPD


A B C D E F G H
I J K L M N O P
Q R S T U V W X
Y 1 2 3 4 BY4741
Minimal
(from YPD +
α tester)

7/20/07

Coulter count αF fluct. assay

Bkg	8	10	12
1	9168	9228	9346
2	10282	9948	9868
3	10350	10342	10938
Post Bkg	164	288	170

Plate Counts (7/21/07)

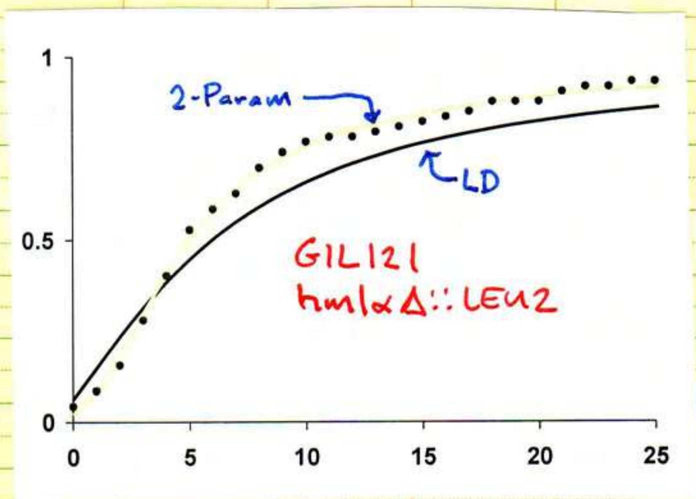
The colonies were a little too small to count last night. Today the plates are a little overgrown. I will need to use a large colony-size threshold to distinguish between αF^R colonies arising from ~~mu~~ post-plating mutations.

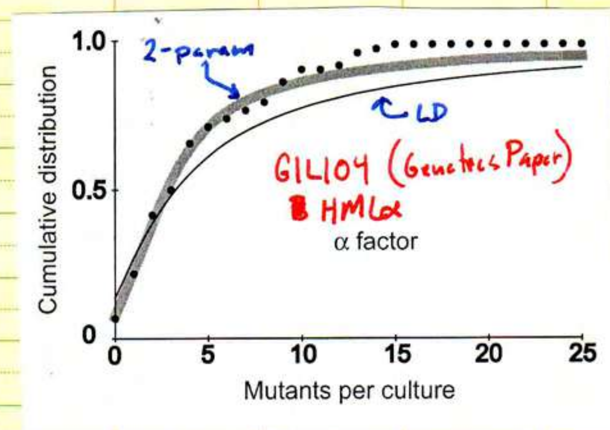
	1	2	3	4	5	6	7	8	9
1	17(3)	10(3)	2(4)	5(9)	4(3)	2(0)	0(1)	7(3)	1(5)
2	~150	4(9)	34(3)	3(5)	5(0)	4(3)	11(1)	6(3)	21(3)
3	2(1)	5(4)	5(1)	39(0)	3(3)	1(3)	14(2)	6(0)	7(10)
4	4(2)	10(0)	22(8)	1(6)	5(2)	3(~100)	18(1)	5(1)	2(1)
5	3(6)	8(2)	3(2)	4(2)	3(0)	4(1)	4(2)	9(3)	24(0)
6	5(0)	3(2)	16(1)	6(6)	5(1)	8(6)	8(2)	0(5)	4(4)
7	0(1)	8(0)	5(2)	6(2)	3(2)	18(0)	4(1)	7(6)	21(0)
8	13(2)	2(2)	9(1)	15(1)	~150	9(0)	29(0)	8(0)	3(2)

Innoculate 3 ml YPD w/ a single colony from each culture that produced mutants.

In order to prevent myself from being biased when picking colonies I consistently chose the colony closest to "12 o'clock".

7/26/07





ANALYSIS OF FLUCTUATION ASSAYS

I had commented earlier that fluct. assays to αF^R do not fit well to either the Luria-Delbruck dist or a combined (two-param.) LD/Poisson distribution (see p. 1). However that was for a strain that could switch mating types. I thought that this dist looked like a sub-Luria-Delbruck dist. – indicating that mutants are less fit.

↳ With a strain that cannot switch (hmlαΔ::LEU2) the data fit well to the 2-param. model

↳ Of course αF^R mutants resulting from switching will grow slowly – they ~~are~~ would find themselves as a single α cell in a tube full of a's.

7/21/07

START FITNESS ASSAYS FOR DELETION COLLECTION MUTANTS

- I will perform assays as before (see p. 3-9) in 10 mL cultures.

→ The large tube racks hold 24 tubes – therefore it makes sense to do multiples of 24.

- VPS64Δ has a known fitness defect which is obvious on YPD (see p. 13). Therefore I will not use this strain.
- For the WT I will do 6 replicates of each WT strain – I will not do BY4741 – it would be nice to know if the KanMX cassette conferred a fitness disadvantage, but the real control is the four strains with this cassette in a dubious ORF.

- Yesterday I started O/N cultures from a single colony in 3mL YPD (all YPD is supplemented w/ ADE).
- ~~Today I diluted back all~~. I also started cultures of the reference strain (GIL050) and BY4741 from a cold culture.
- This morning I diluted all cultures back 1:300 (10 λ into 3 mL YPD) & I diluted Ref. back 1:300 (33.3 λ in to each of 4 10 mL tubes).

→ I hope to mix the cultures ~ 9:00pm tonight.

Preparation of YPD tubes

During this expt I will need 48 × 3 = 144 ~~tes~~ 10 mL YPD tubes. To aliquot the media I prepared 2 L of YPD, aliquotted it into 10mL culture tubes using the automatic dispenser (from Coulter counter) the autoclaving the 6 racks of tubes.

→ I supplemented Ade before autoclaving – hopefully enough is there so as not to further handicap the Ref. strain which is ade2.

Testing αF^R and Sterility

To determine which of the deletion strains are αF^R and/or sterile, I will spot each strain (using the pin transfer) onto αF, YPD, and YPD containing a lawn of ~~an~~ α-mating tester.

→ 7/22/07 – everything grew on YPD and YPD+αF (because everything is BAR1)
- I will rep. onto minimal media today.

7/21/07

SETTING UP COMPETITIONS

Get a rough estimate of cell number in the ref. strain and one of the mutant strains:

- No sonication

Bkg		986	790	
Ref	1	10218	10308	→ ~ 10^7
	2	10036	10086	
A	1	16802	16484	→ ~ $2 \cdot 10^7$
	2	19812	19838	
Post Bkg		1566	—	

- I want ~1:2 experimental : Reference → mix 700λ Ref w/ 300λ of each strain in microfuge tube
- Dilute each strain 1:500 into YPD (20λ into 10mL)
- Spin Down → resuspend in 1mL PBST.
- Coulter count the unmixed Reference and the 4 WT strains (next page) ⇒

NOTES

- Culture "Q" looked less dense than the others → fitness defect?
- Each of the 4 WT strains were diluted 1:500 into 6 independent cultures (labeled 1·1 - 4·6).

7/21/07

Coulter Count Pure Cultures (Before mixing)

Bkg		30	36	14			
WT1	1	34694	33996	34998			
	2	25920	25440	38776	38250	38332	
	3	29950	30194	29794			
WT2	1	23638	23592	23924			
	2	27910	27732	26896			
	3	29970	29888	29480			
WT3	1	34794	35218	34616			
	2	20566	19782	20680	28330	28388	26756
	3	34022	33710	33676			
WT4	1	38048	38138	37744			
	2	25300	25628	25470			
	3	37306	37196	36982			
Ref.	1	24404	24226	23802			
	2	25568	25958	25312			
	3	24356	23720	24222			
Post. Bkg.		342	380	326			

Something is still strange with my counting ??

7/22/07

- Dilute all cultures back 1:500 (20λ into 10mL) → 9:30
- Transfer 1mL of each culture to microfuge → spin → resus. in PPST
  ↳ put in fridge w/ yesterdays samples.
- Coulter Count the WT cultures 1.1, 2.1, 3.1, & 4.1
  ↳ - transfer 1mL to glass culture tube
  - sonicate 2 min.

7/22/07

Count Mixed cultures (11:00) am

Bkg		36	60	54
1.1	1	19540	19610	19608
	2	23134	22604	22704
	3	17068	17064	17106
2.1	1	21876	20118	19608
	2	19572	19760	19334
	3	20878	20420	20624
3.1	1	18468	18172	18184
	2	20256	20402	20890
	3	18066	17608	17744
4.1	1	22952	22734	22500
	2	20322	21132	21288
	3	25470	24778	24992
Post Bkg.		332	372	258

NOTES:

- By the time I get around to counting an hour and a half has gone by since I diluted. Also it is not practical to count each competition. Therefore, ~~I will~~ for the calculations I will assume that 8 generations pass between dilutions.
  - I will continue to monitor counts for these cultures so that I can estimate bottlenecks and actual generations.

7/22/07

Count Cultures (11:00 pm)

Bkg		82	236	232
1.1	1	13970	12604	12296
	2	14300	14580	14542
	3	14132	13672	13520
2.1	1	14596	14436	14840
	2	14522	14216	14160
	3	14052	13732	13552
3.1	1	15378	15490	15652
	2	15116	15064	14684
	3	13780	13932	13930
4.1	1	16228	16406	16072
	2	14824	14066	14144
	3	16612	15546	16050
Post Bkg		346	318	320

7/23/07

Count Cultures (10:30 am) – 20 λ into 10 mL

Bkg		26	42	50
1.1	1	13440	13398	13418
	2	16342	15770	15844
	3	15092	14736	14672
2.1	1	13532	15246	13506
	2	16208	16198	16288
	3	15558	15576	15362
3.1	1	15304	14616	14470
	2	15086	15006	14932
	3	15032	15162	14922
4.1	1	17052	17032	16862
	2	15826	16156	16100
	3	19680	19238	19160
Post Bkg		452	276	174

7/23/07

Deletion collection fitness assays cont.

Last night (@ 9:30) and this morning (@ 9:30) I diluted (last night only), counted, and prepped for FACS as before.

NOTES:

- I dropped tube 1.1(4) before innoculation → lost some vol → shouldn't matter since the tubes are not saturating.
- Tube "T" had autoclave tape in it - sterile of course, but I should look carefully at the tubes in the next round. There was a lot of crap in them.

~~6/24/07~~ 7/24/07

NOTES ON FACS

- Yesterday I ran all of my samples through the LSRII FACS analyzer.
- Preparation for FACS
  - (1) Vortex → transfer to FACS tube
  - (2) Sonicate 1 min (8 at a time)
  - (3) FACS
- Preparing the samples took longer than expected ~2h
- I realized during my FACS run that I should have adjusted the FSC — this shouldn't really matter
- Two problems w/ S288C
  - (1) It was not as ~~east~~ easy to distinguish between YFP and no YFP. All S288C showed a little autofluorescence
    ↳ this may be partly due to not adjusting FSC (?)
  - (2) All strains had slight disadvantage compared to Ref.
    ↳ this is bad because I assumed the opposite and put the reference at a disadvantage!

7/22/07

FITNESS ASSAYS ON SPONTANEOUS αF^R MUTANTS

- Yesterday I picked 69 spontaneously occurring αF^R mutants off of a fluct. assay (see p. 15).
- I will take 24 of these and compare their fitness with 24 unselected clones of GIL121.
- Yesterday I plated GIL121 to single colonies starting from a cold culture.
- Today I plan to passage 24 αF^R cultures and pick 24 off of the GIL121 plates so that I can start this assay tomorrow.

CONCERNS:

(1) I should have plated GIL121 to singles at the same time as the fluct. assay — I worry that the passaged αF^R cultures and the 24 unselecteds will be at diff. densities tomorrow when I go to start this assay.

↳ If I want to compare fit. dist^ns of these two sets of 24 I want them to be treated in the same (i.e. no systematic diffs in starting ratios)

(2) I did not restreak my αF^R strains. This could be a problem for strains with fitness disadvantages. — If I picked any bkg WT (αF^s) cells off of the FA plates these could overtake the αF^R.

↳ This is not a problem for αF^R clones with fitness equal or greater than WT.

7/23/07

- Last night I picked 24 colonies of GIL121 off of YPD and innoc. 3 mL cultures
- Last night I ~~diluted~~ passaged the 1st 24 αF^R mutants (5 λ into 3 mL)

} @ 9:00pm

- This morning — dilute all 48 cultures 1:300 (10 λ into 3 mL)

↳ except α14 — this was growing slowly → dilute 1:100.

} 11:30am

- Start a 1:50 & 1:100 dilution of cold Ref. culture → shaker - 12:00 noon.

7/23/07

- 9:30 - estimate the # of cells per culture:

Bkg	210	212
Ref.	19812	18290 → 4E7
α1	24222	24548 → 5E7
1	18966	18336 → 4E7
Post. Bkg	334	

do 600λ : 400λ
Ref : exp.

- Dilute cultures back (10:00pm)
- Prep. for FACS
- Coulter count

7/24/07

Today I spotted the 24 αF[R] and 24 control strains onto YPD, YPD+αF, & YPD + α-tester as before (pg. 17)

7/23/07

Counts on Pure cultures (11:00pm)

Bkg		36	38	30
α1	1	98494	97512	96034
	2	100132	100098	99258
	3	97520	99506	96738
α2	1	84322	83238	83076
	2	75408	75484	76400
	3	90376	89852	90544
1	1	87044	85578	84900
	2	85476	85516	84988
	3	79232	79624	78892
2	1	92216	91558	92296
	2	88872	87604	87524
	3	100050	100300	100284
Ref	1	50244	50280	48430
	2	53948	53804	53796
	3	55154	54334	55294
Post Bkg		514	526	370

NOTES: (~~8/24~~ 7/24/07)

- I mixed strains 600λ Ref : 400λ Exp. This was intended to give slightly more Ref; however, judging from the counts above I probably have more Exp. in each ~~culture~~ mix.

- The cultures were pretty dense when I mixed them. I would not have wanted any cultures above 10^8. This was just a result of the FACS taking too long yesterday afternoon.

7/24/07

Count Mixed Cultures (11:15 am)

Bkg		176	194	196
α1	1	50632	50976	51682
	2	62230	61682	60914
	3	57608	59678	57920
α2	1	51176	50484	50056
	2	64260	64084	64142
	3	68962	69502	68414
1	1	47100	45996	45034
	2	41446	41734	— ← spilled
	3	49570	49462	49330
2	1	47548	46470	45492
	2	49340	48486	47386
	3	40188	39754	39632
Post Bkg		660	576	434

Count Cultures (11:00 pm)

Bkg		66	26	26
α1	1	51848	52370	52874
	2	54206	54540	53954
	3	59976	58890	59328
α2	1	49888	49748	49286
	2	45904	45714	45288
	3	50826	50722	51534
1	1	36902	37242	37344
	2	33196	33310	32948
	3	42698	42540	43240
2	1	42116	41076	42496
	2	42262	44088	43998
	3	44438	44134	43842
Post Bkg		666	788	530

7/25/07

Count Cultures (11:00)

Bkg		36	68	72
α1	1	63582	63238	63646
	2	66158	65328	65218
	3	65034	65532	64990
α2	1	46416	45782	45246
	2	50468	48522	48278
	3	50832	49802	51022
1	1	37016	36228	36032
	2	35972	35332	36140
	3	38538	38202	38950
2	1	43536	44088	43170
	2	46604	45690	46232
	3	42240	41576	41846
Post Bkg		262	386	296

NOTES:

- I did all of the dilutions (for the spontaneous αF^R cultures) on the 10s.
- Today I will do FACS on all of these samples → I will prepare as before
  - ↳ this time I will adjust the FSC before I get started
- I started analyzing the FACS data from the deletion collection and it looks a bit messy. → not very linear.
  - ↳ In many cultures the ratio of $\frac{E}{R}$ dropped very low after the 1st transfer → this is because I started the Exp. at a disadvantage and they all had fitness defect compared to Ref.
  - ↳ I will need to set a threshold above or below which I will consider the culture fixed for one of the two strains
    - ↳ look in Downs paper
    - ↳ ~~[illegible]~~ this range looks good: $-2 \leq \log_2\left(\frac{E}{R}\right) \leq 2$

$$\frac{1}{4} \leq \frac{E}{R} \leq 4$$

7/28/07

↳ I am using $\left|\ln\left(\frac{E}{R}\right)\right| \leq 2.5$ as cutoff (see p. 32)

7/27/07

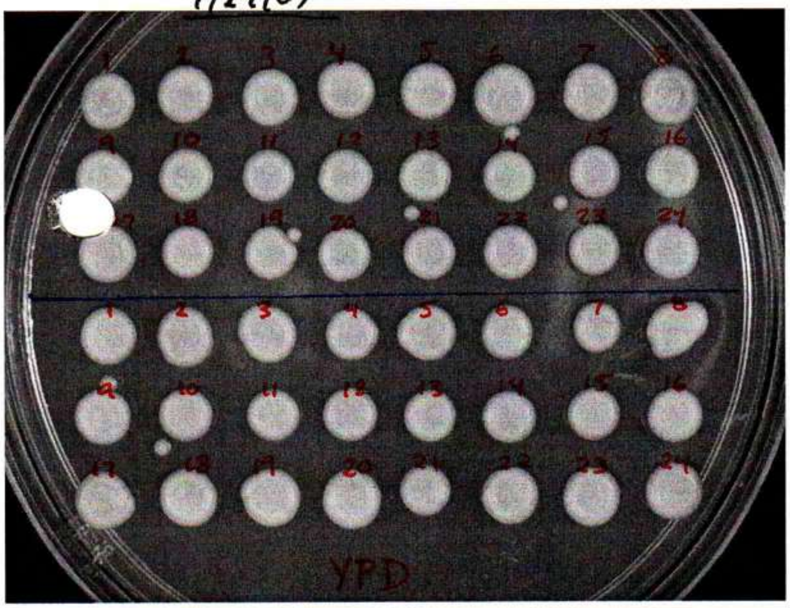
PERFORM FACS ON SAMPLES

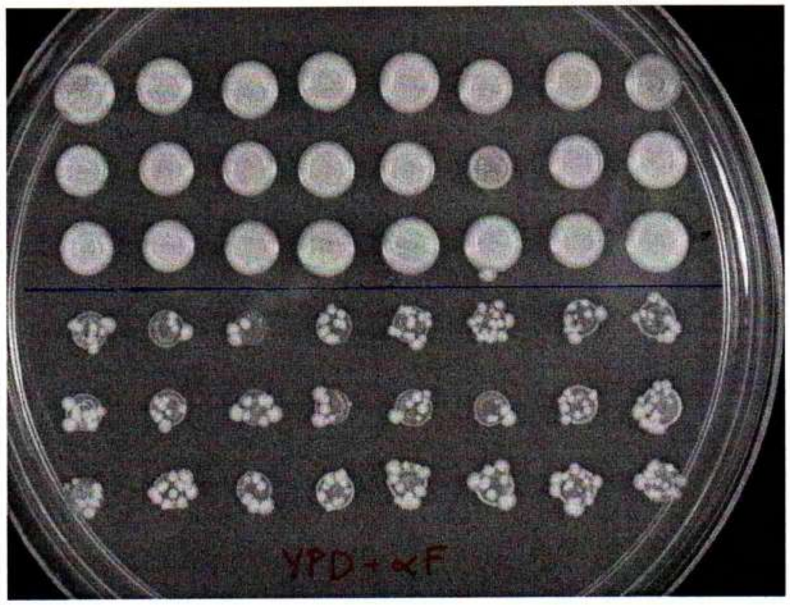
- Prepare samples as before (p. 22)
- This time I adjusted the FSC and SSC voltgage

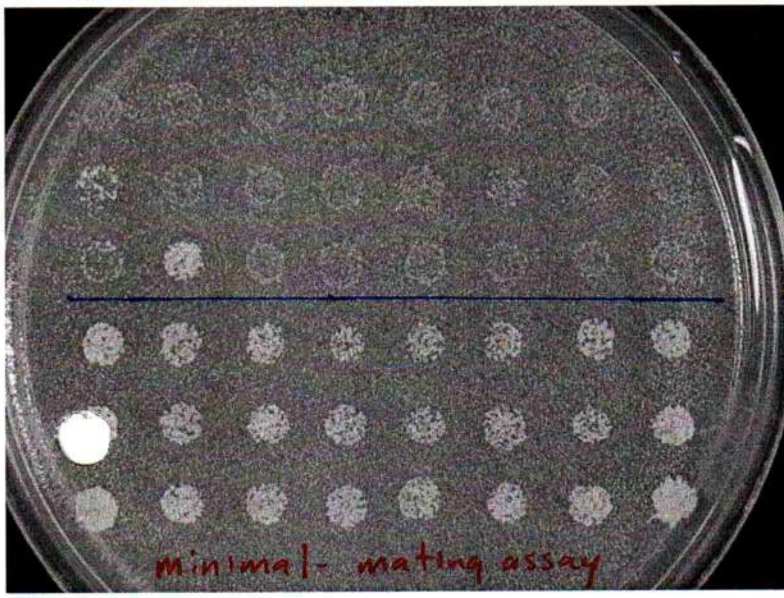
INSTRUMENT SETTINGS

	voltage
SSC	324
YFP	500

7/27/07







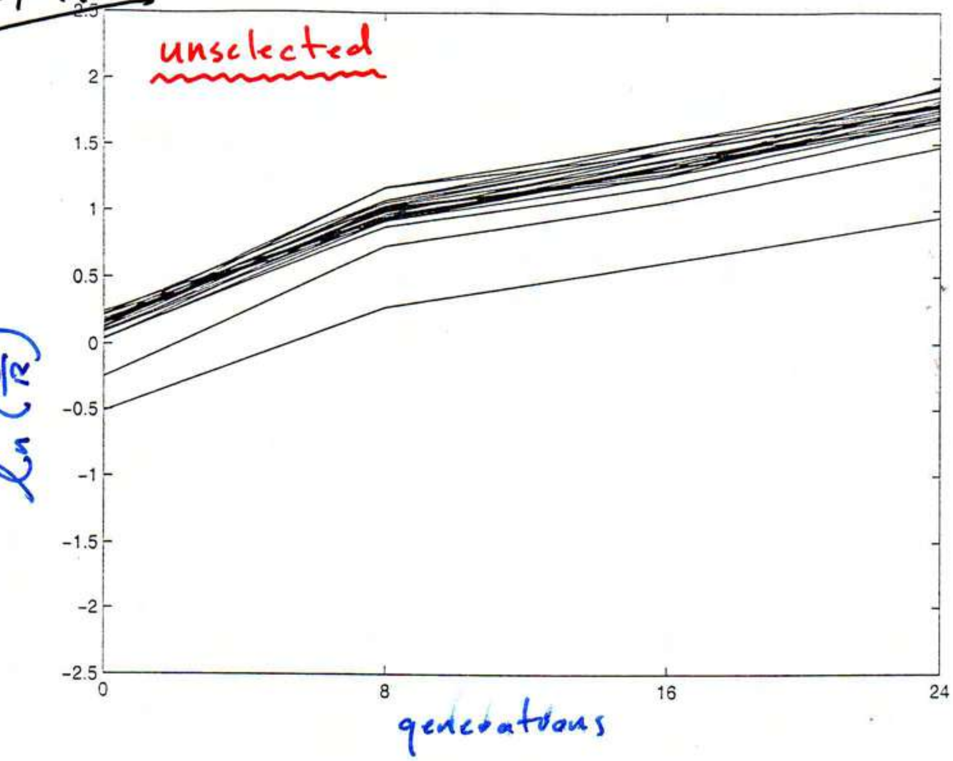
αF^R
1-24

unselected
1-24

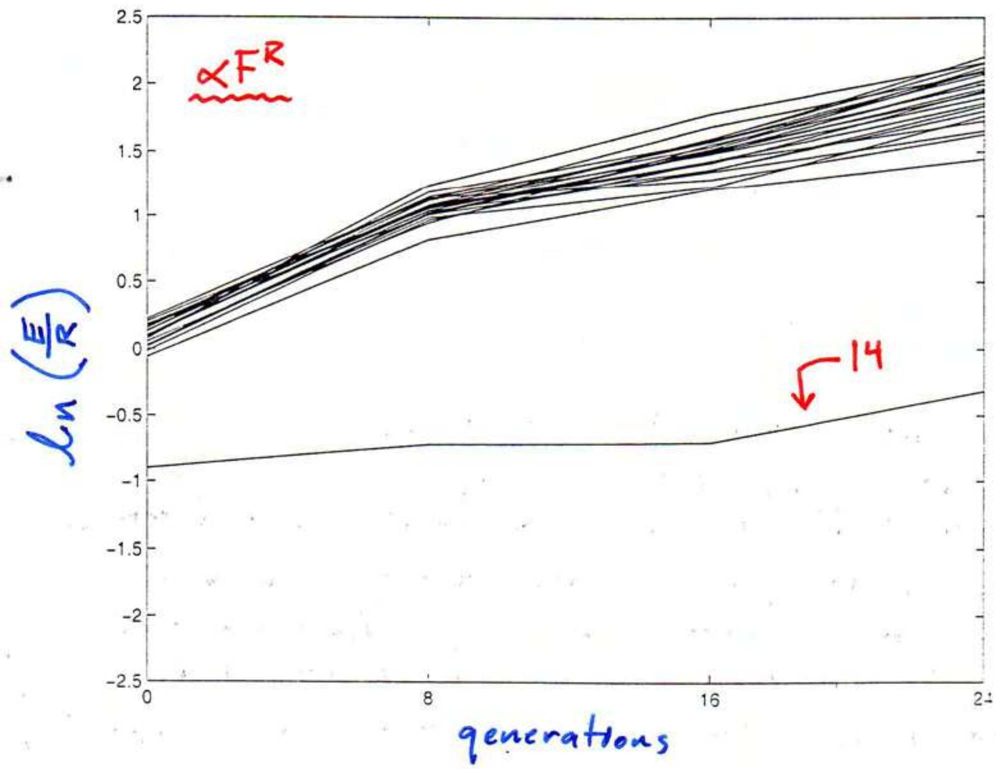
NOTES

- As expected all unselected clones are αF^S and mate.
- Interestingly, αF^R strain 18 mates → simple explanation is that this is a mixed culture → I will restreak to singles and retest
- αF resistant strain 14 seems to grow normally ~~bu~~ on YPD but not on YPD+αF → suggests modest sensitivity. However one would expect weakly αF^R strains to mate → 14 does not. Interestingly, this strain has the lowest fitness (see p.23 & p.30)

7/27/07



strain	sel. coeff.
unselected	-0.94%
unselected	-0.85%
unselected	-0.58%
unselected	-0.57%
unselected	-0.37%
unselected	-0.36%
unselected	-0.14%
unselected	-0.12%
unselected	-0.12%
unselected	-0.06%
unselected	-0.03%
unselected	-0.02%
unselected	0.02%
unselected	0.03%
unselected	0.05%
unselected	0.18%
unselected	0.25%
unselected	0.43%
unselected	0.49%
unselected	0.59%
unselected	0.91%
unselected	1.02%
unselected	1.16%
unselected	1.30%
average	0.09%
stdev.	0.59%



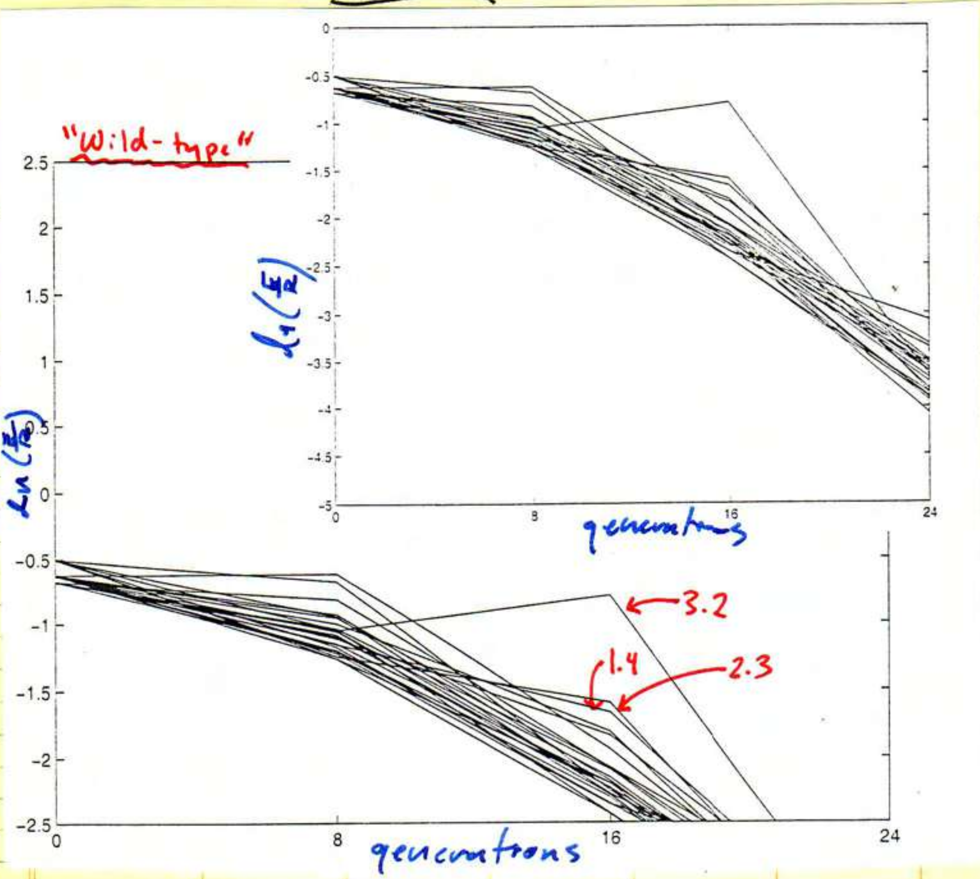
strain	sel. coeff.
14	-2.29%
3	-1.75%
2	-0.88%
8	-0.84%
20	-0.68%
18	-0.17%
17	0.08%
7	0.17%
5	0.50%
9	0.87%
23	0.91%
24	1.04%
16	1.06%
6	1.07%
12	1.07%
22	1.31%
1	1.33%
15	1.44%
13	1.56%
10	1.73%
21	1.77%
19	1.96%
4	2.06%
11	2.50%
average	0.66%
stdev.	1.23%

NOTES

- Generations assumed to be 8 per 12 hours → I could calculate this more accurately using the coulter counts on p. 25-27.
- In all cases the Experimental strain was greatly favored in the 1st 8 gen. A simple explaination for this is that the Reference strain went through a lag phase during this time (this was a concern I had see p. 25). Overall culture growth was less during this period (compare counts p 25-27). Why the lag? → The Ref. was grown in 50 ml flask culture (cell density not too high p. 25). → Next time use mutiple tubes.

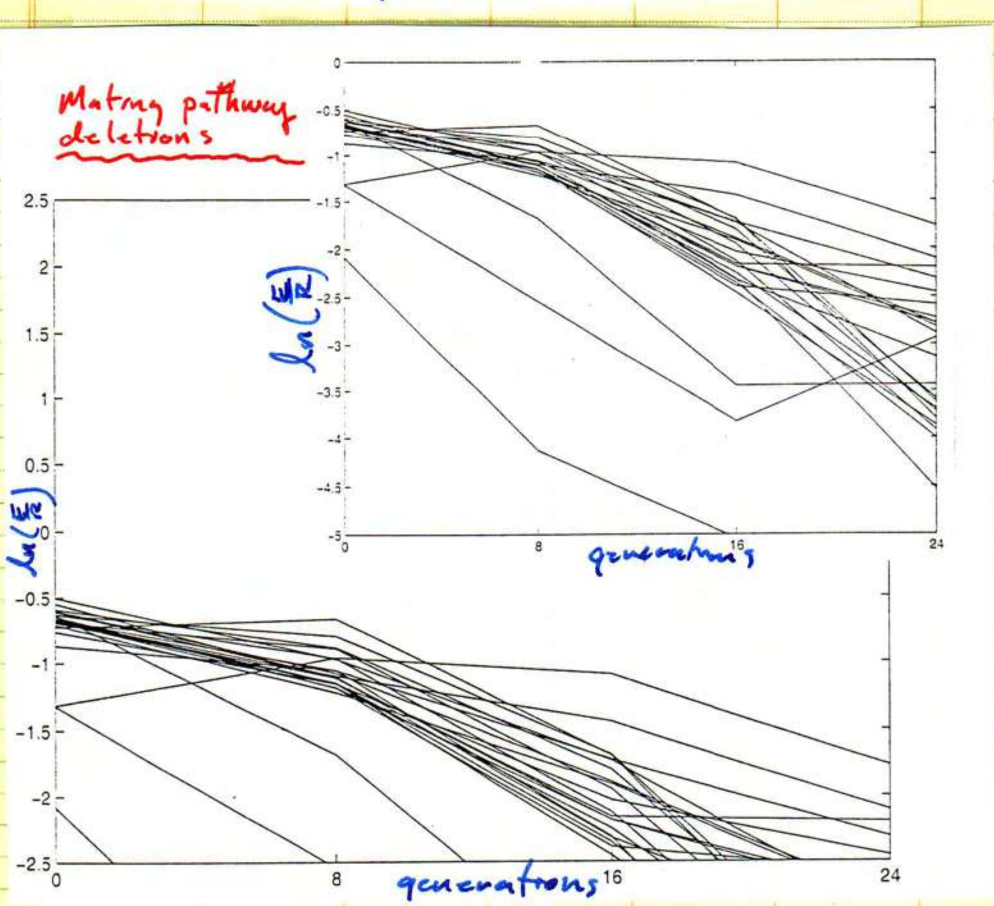
7/27/07

"Wild-type"



strain	sel. coeff.
1.1	-1.45%
1.5	-0.75%
1.2	-0.73%
1.6	-0.54%
1.3	-0.52%
1.4	2.53%
2.2	-2.58%
2.1	-2.58%
2.5	-2.14%
2.4	-1.70%
2.6	-1.57%
2.3	2.23%
3.4	-0.89%
3.3	-0.78%
3.1	-0.48%
3.6	0.34%
3.5	0.40%
3.2	7.78%
4.3	-0.46%
4.6	-0.31%
4.2	0.12%
4.5	0.91%
4.4	1.50%
4.1	1.67%
average	0.00%
stdev.	2.16%

Mating pathway deletions



strain	sel. coeff.
T	-3.50%
E	-1.73%
F	-1.50%
K	-1.34%
C	-1.23%
D	-0.53%
H	-0.12%
W	-0.10%
R	-0.08%
G	0.19%
S	1.07%
U	1.24%
A	1.42%
L	1.55%
P	1.60%
Y	1.60%
V	1.64%
M	2.04%
I	2.42%
B	2.92%
J	3.14%
O	6.74%
Q	----
X	----
average	0.79%
stdev.	2.14%

7/27/07

CALCULATION OF SELECTIVE COEFFICIENTS (s) FROM COMPETITIONS

STEP 1: Calculating slope from competition

Since I suspect that the Ref. strain went through a lag phase during the 1st 12 h. of the competition w/ αF^R, I disregarded the 1st point when calculating the slope in this expt.

↳ ∴ Slope determined w/ 3 points

Calculating slope for strains from the deletion collection was more difficult. Many log ratios were ~~or~~ below −2.5 → this is what I used as a cutoff:

$$e^{2.5} \approx 12:1$$

Two strains (Q - fus3Δ & X - ram1Δ) were below the cutoff after the 1st time point, therefore I could not calculate a slope.

↳ in no case were all 4 points above the cutoff ⇒ therefore all slopes were determined from ~~4 points or~~ 2 or 3 points.

STEP 2: Normalize slope to wild type

All selective coefficients (slopes) are relative to the Ref. strain. To normalize to wild-type.

$s_{E/R}$ - selective coefficient of experimental to Ref.

$s_{W/R}$ - " " " wild-type " "

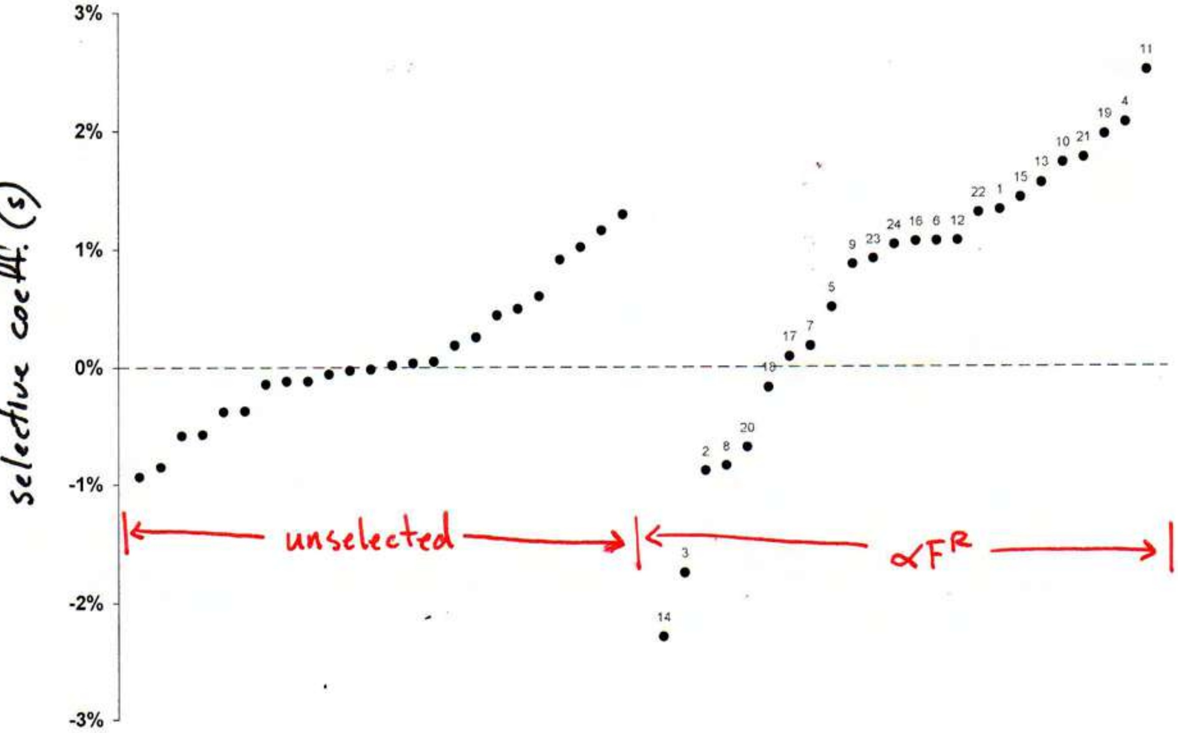
$s_{E/W}$ - " " " experimental to wild-type

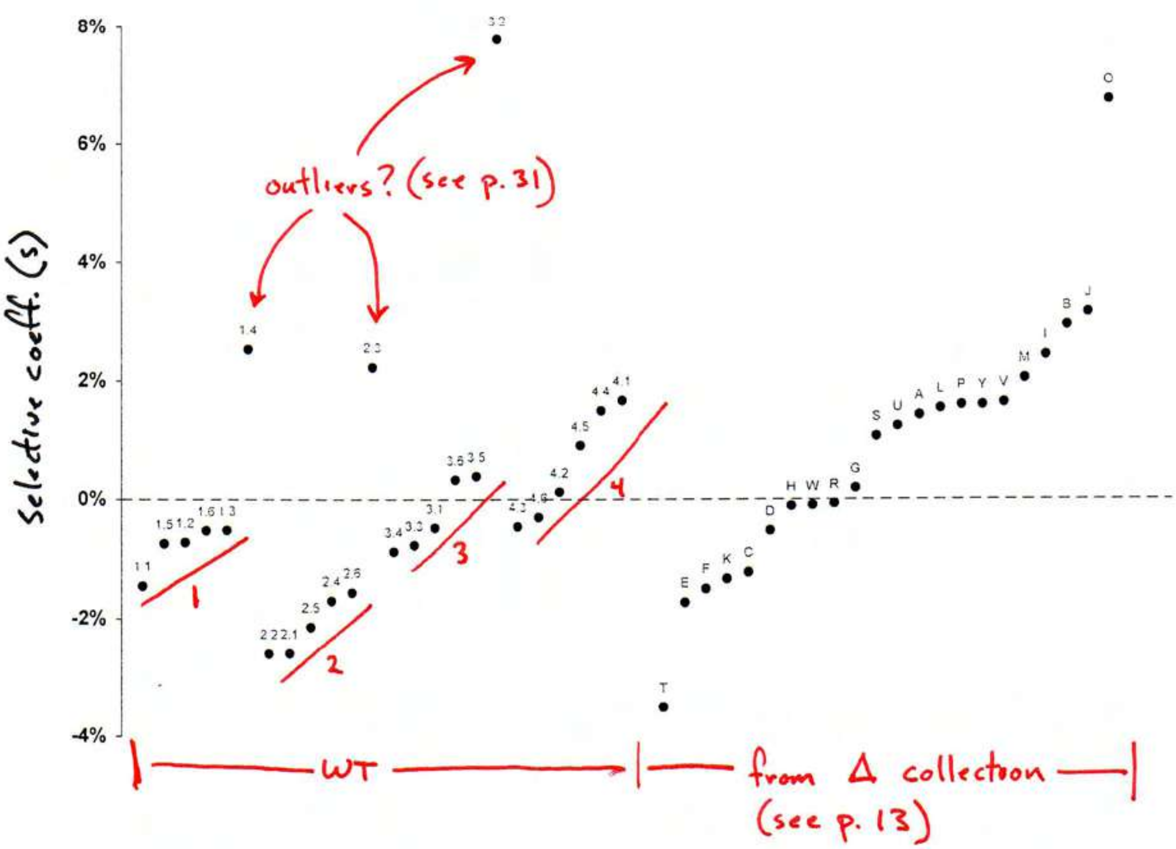
w - relative fitness ($w = 1 - s$)

$$s_{E/W} = (1 - w_{E/W}) = 1 - \frac{w_{E/R}}{w_{W/R}} = 1 - \frac{1 - s_{E/R}}{1 - s_{W/R}}$$

- For the αF^R expt. I normalized everything to the median "s" for the unselected ~~mutants~~ clones.
- For the deletion collection expt. I normalized to the mean of all "wild-type" clones

7/28/07


selective coeff. (s)
3%
2%
1%
0%
-1%
-2%
-3%
unselected
αF^R


selective coeff. (s)
8%
6%
4%
2%
0%
-2%
-4%
outliers? (see p. 31)
1
2
3
4
WT
from Δ collection
(see p. 13)

7/28/07

DISCUSSION OF FITNESS ASSAYS

- Even though there were some issues with the assays, overall I am very pleased w/ the results.

- The error in the measurements (0.59, see p 30) is similar to what Michael found by measuring the same clone multiple times.

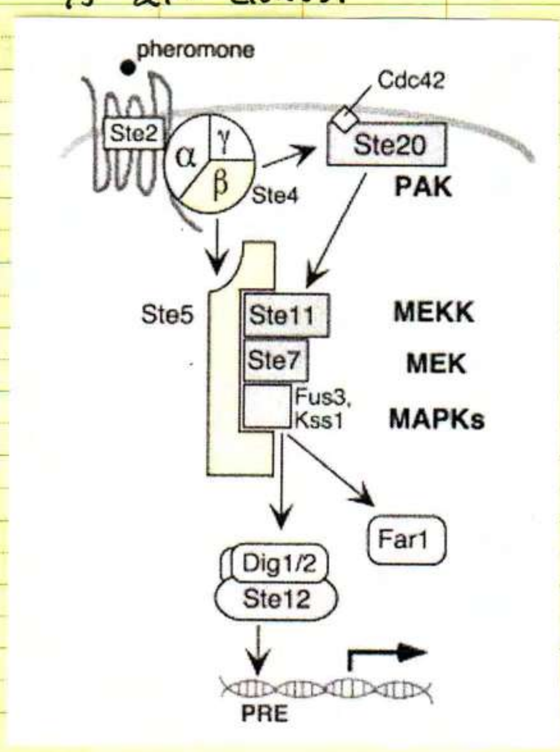
- It is clear from my results that there is greater variation in fitness in αF^R clones than for unselected clones

↳ One possible explanation (still interesting, but unlikely, I think) is that the αF^R clones carry additional mutations which increase the variance in the fitness dist. This could result from transient mutators — If by selecting for αF^R, I enriched for strains with an elevated MR I may expect to see an increased variance in other traits (i.e. fitness)

  - I find this explanation unlikely because the dist. of fitnesses would be expected to follow the dist. of fit. effects of all mutation → In other words, I would expect most of the strains to have a decreased fitness.

  - I could eliminate this possible explanation by using as a control can^R mutants instead of unselected clones.

- The big challenge now is to find the mutations in these strains → it is unlikely that I will be able to make much progress before I move to Princeton. Now I will concentrate on determining the fitnesses of the other 45 αF^R clones.



7/31/07

Although I think the deletion collection experiment was a failure, there is one interesting observation ~~— the~~ — looking just at the top for fitnesses, I see STE4 and STE5 — two upstream (and interacting) components

7/31/07

MORE DISCUSSION

FINDING MUTATIONS

It would be nice if I can make some progress in finding these mutations. After looking a Thea's paper I may try some similar essays

(1) Determine if the strains are salt sensitive – this would indicate shared components with the HOG pathway (i.e. STE11, STE20, STE50)

(2) I could also see if these mutants can block arrest due to overexpression of particular mating genes. Thea had made overexpression plasmids w/ STE4, STE5, STE7, STE11, FAR1-22, DIG1, and DIG2. I cannot find STE5, DIG1 & DIG2 use LEU2 as a marker – so I can't use them, and I cannot get STE4 or STE7 to regrow from perm.

→ the STE11 & FAR1-22 plasmids I can use but I first need to find out what the FAR1-22 allele is and also to find out if STE11 overexpression arrests cells.

→ Also these vectors use URA3 as a marker.

DISTINGUISHING BETWEEN ARREST AND PROTEIN PRODUCTION

There are two explanations for why losing the mating pathway could be beneficial – (1) It shuts off unnecessary protein production (pheromone?) or (2) Inappropriate signaling (which must be tolerated in any switchlike signaling pathway) transiently stalls the cell cycle.

→ How to test?

(1) Grow in media lacking a.a.'s – this will put additional cost on prot. production but will not effect signaling.

(2) Find mutants that separate the function of arresting and gratuitous protein production.

- Do ~~F~~ far1Δ cells still have normal transcriptional response?
- Do ste12Δ cells still arrest?

FITNESS ASSAYS ON THE REMAINDER OF THE αF^R STRAINS

7/30/07

Counts on Pure Cultures

Bkg		52	72	76
α25	1	36452	36388	—
	2	31086	30790	—
	3	—	—	—
α26	1	31040	27366	—
	2	32460	32060	—
	3	—	—	—
25	1	26530	26678	—
	2	27900	28328	—
	3	—	—	—
26	1	24800	24014	—
	2	30872	30540	—
	3	—	—	—
Ref	1	8772	8900	8676
	2	7752	7726	7588
	3	7628	7042	7126
Post Bkg		1260	158	64

- Mix 800λ Ref : 200λ Exp

7/31/07: NOTES

- O/N cultures of αF^R strains 25–69, 3 more unselected clones (25–27), and Ref. were diluted back 1:300 @ 12:45.
  → 10μL into 3mL (except Ref – 200λ into 60mL → put into 6 10mL tubes)
- Ref was too dilute → next time do a 1:100.
- I counted before I set up cultures – since I was running short on time, I only counted 2 samples of each strain (and only twice).
- Assay I is being performed as before (see p 18).

7/31/07

Count Mixed Cultures (11:45) am

Bkg		14	32	50
α25	1	24070	23350	23204
	2	26620	26666	26294
	3	24610	24510	24422
α26	1	27376	26720	26164
	2	29282	28556	28438
	3	26472	26210	25926
25	1	24760	24330	25674
	2	26894	26608	26802
	3	23042	23252	23006
26	1	22904	23538	23034
	2	20058	20082	20162
	3	22878	22850	22860
Post Bkg		426	320	264

Count again (11:30 pm)

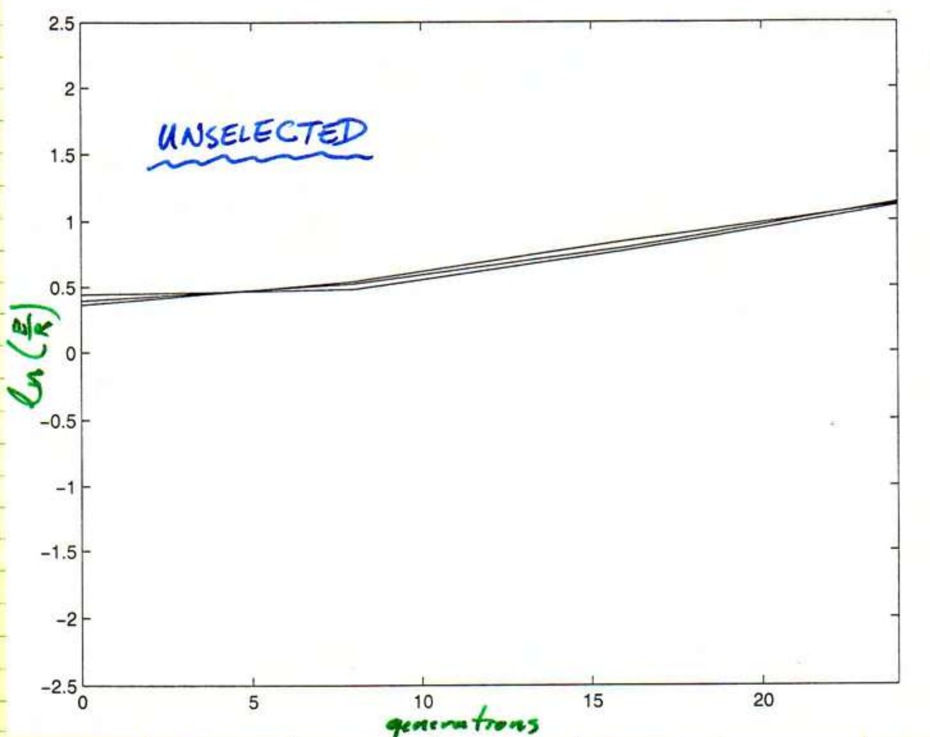
Bkg		66	194	84
α25	1	32006	31638	32818
	2	27262	27350	27950
	3	35002	34294	34084
α26	1	35238	34634	34996
	2	35532	35248	34900
	3	35266	35644	33764
25	1	27772	28228	27964
	2	27904	27866	28092
	3	29538	29974	29262
26	1	30652	30522	30632
	2	29130	31616	31402
	3	28786	28430	28948
Post Bkg		720	690	570

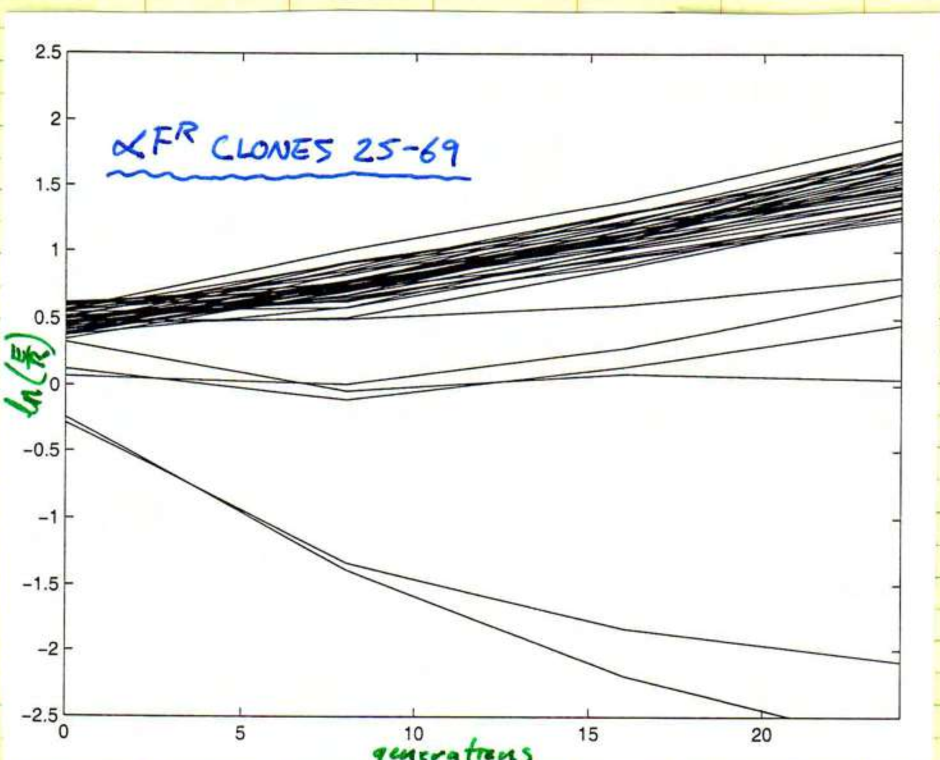
8/1/07

Count again (11:30)

Bkg		10	24	22
α 25	1	34808	34422	34596
	2	38980	37842	38742
	3	38696	38814	38410
α 26	1	46790	45348	44654
	2	42944	43460	43306
	3	45396	44332	43866
26	1	28188	28504	28222
	2	29416	29176	29296
	3	25914	24444	24516
Post Bkg		610	282	318

8/4/07

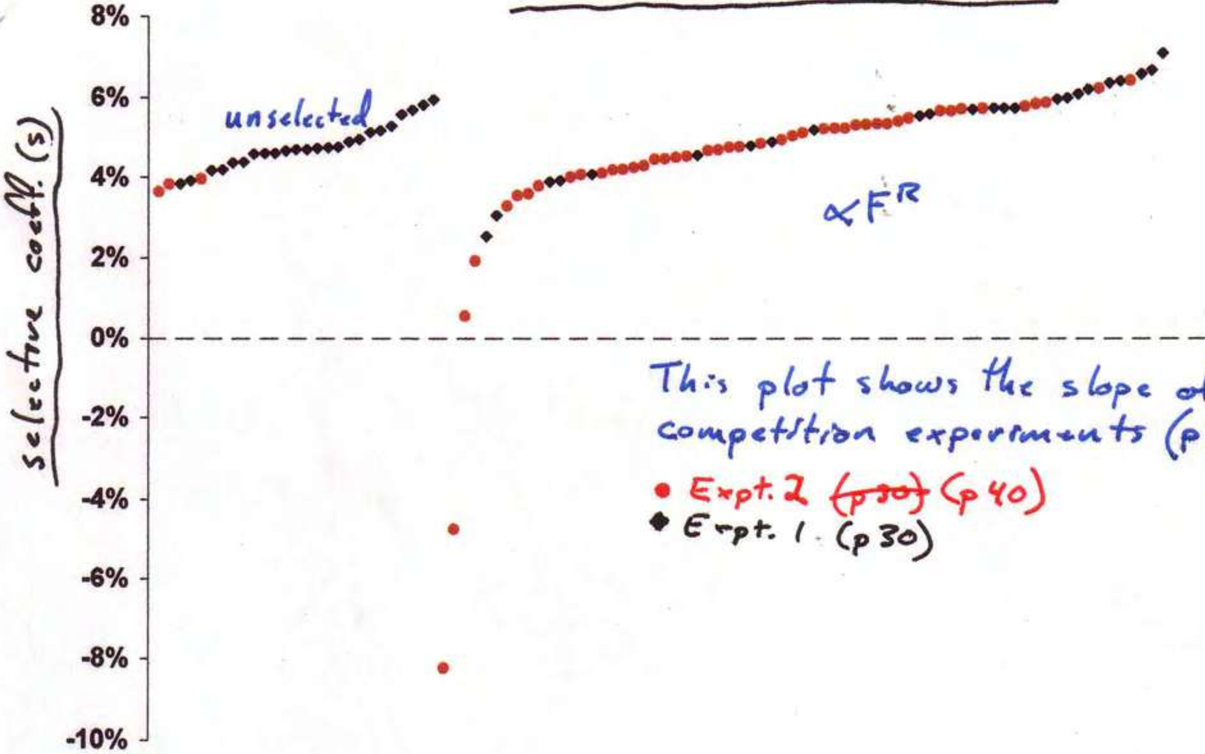




strain	sel. coeff.
52	-12.53%
43	-8.90%
38	-3.38%
62	-1.96%
67	-0.56%
68	-0.30%
47	-0.25%
50	-0.05%
30	0.19%
49	0.25%
59	0.29%
56	0.37%
63	0.39%
41	0.44%
60	0.47%
64	0.65%
65	0.66%
53	0.70%
25	0.73%
34	0.89%
36	0.89%
45	0.97%
46	0.98%
57	1.06%
31	1.16%
33	1.26%
44	1.34%
61	1.45%
39	1.46%
35	1.47%
28	1.55%
51	1.57%
27	1.57%
32	1.58%
26	1.65%
42	1.74%
55	1.91%
66	1.92%
29	1.96%
48	1.98%
54	2.03%
69	2.09%
58	2.13%
40	2.50%
37	2.70%
unselected	-0.20%
unselected	0.00%
unselected	0.12%

8/4/07

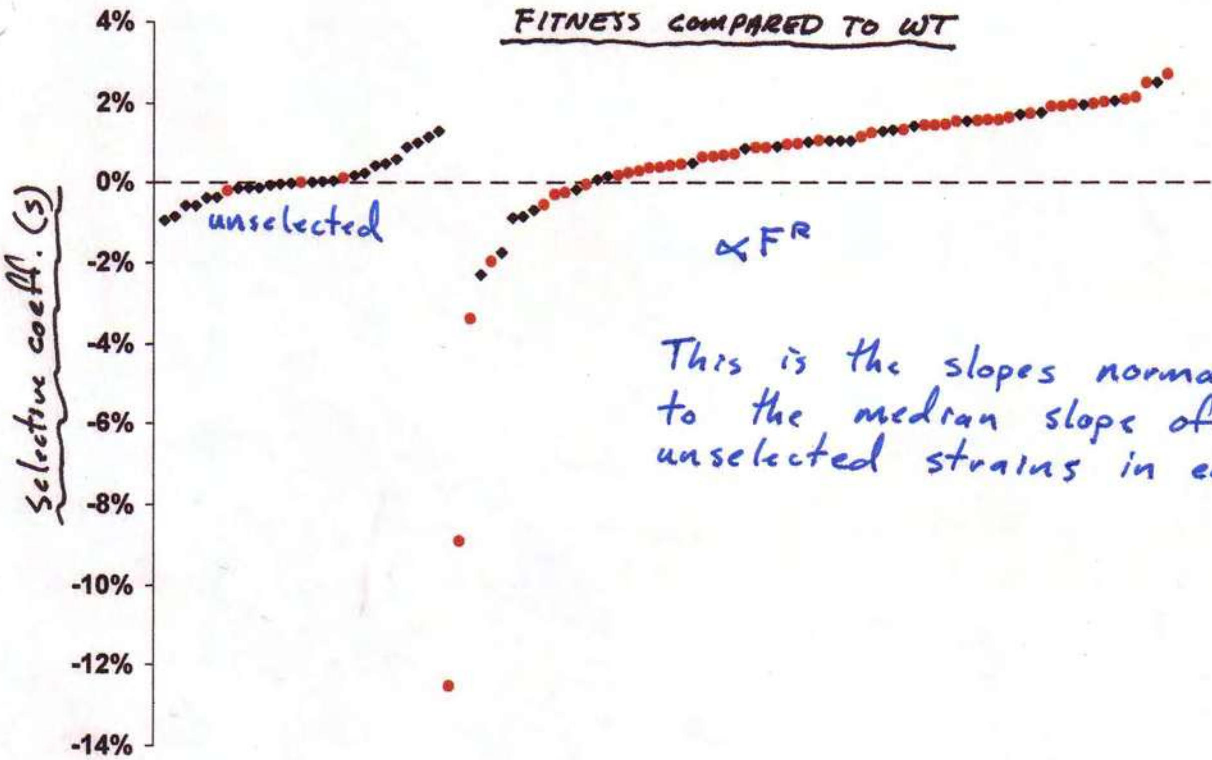
FITNESS COMPARED TO REF.



This plot shows the slope of the competition experiments (p 30 & 40)

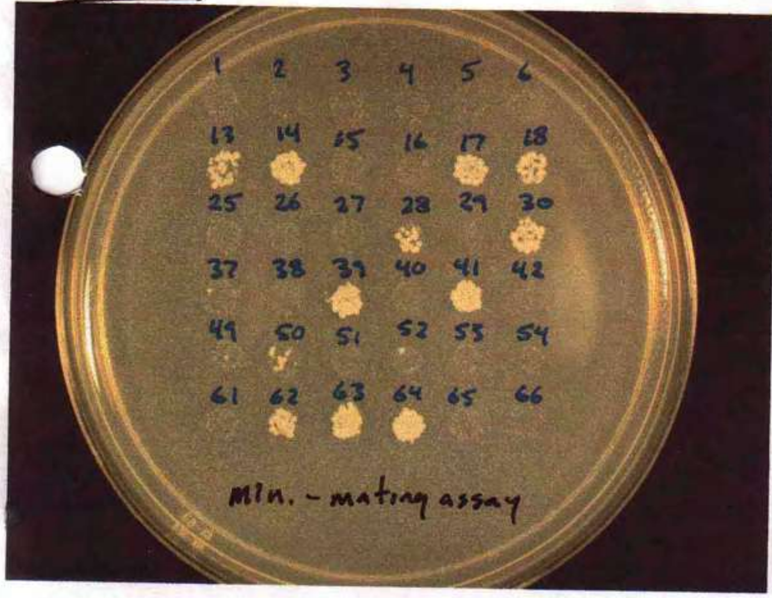
• Expt. 2 ~~(p 30)~~ (p 40)
◆ Expt. 1 (p 30)

FITNESS COMPARED TO WT



This is the slopes normalized to the median slope of the unselected strains in each experiment.

8/4/07



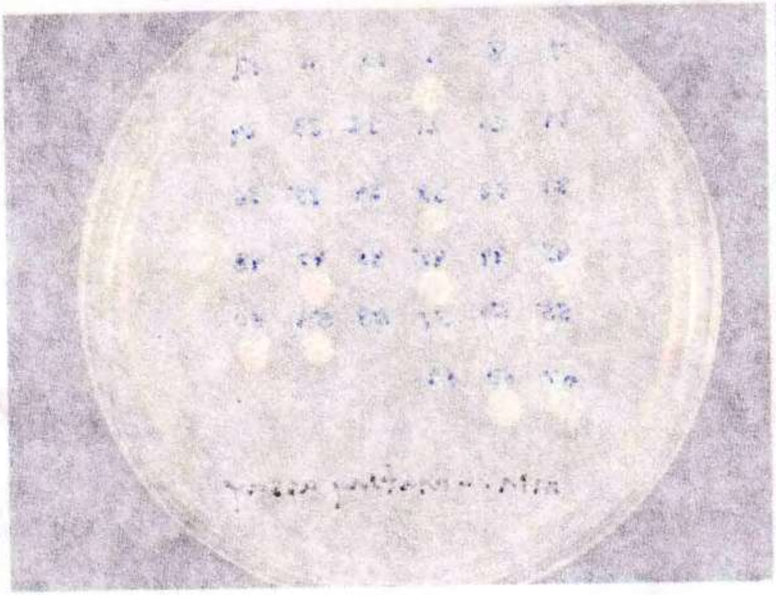
SALT RESISTANCE & MATING IN αF^R STRAINS

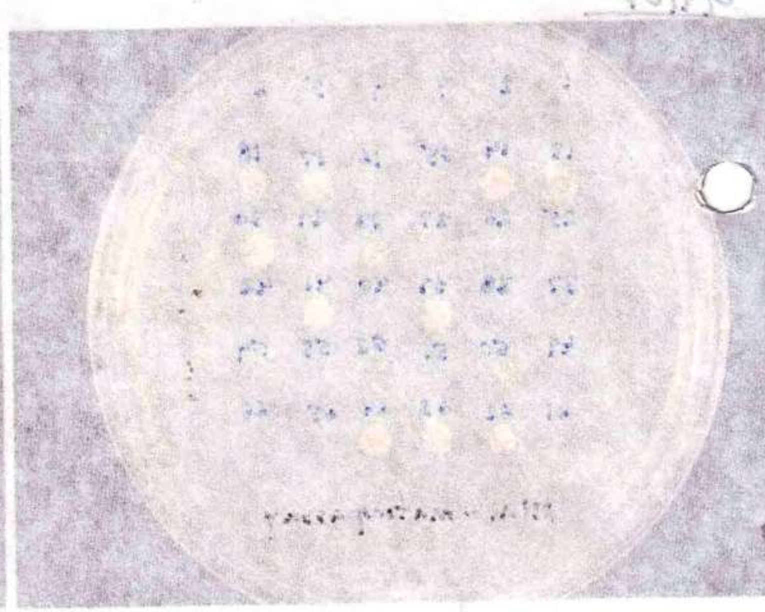
While I was setting up the last set of ~~fluctuation~~ fitness assays I put the 69 αF^R strains into duplicate 96-well plates and froze the down — also I ~~sp~~ pin transferred onto αF, YPD, YPD + 1M NaCl, and ~~α~~ YPD + α-tester.

- again I noticed that #14 grows fine on YPD but slowly on αF.
- surprisingly all strains grew on 1M NaCl
- also surprisingly, I see lots of mating (even in strains that did not mate previously. (see p. 29)

STRONG MATERS (Many Colonies)	WEAK MATERS (a few colonies)
9	2
13	12
14	31
17	34
18 ← mated previously (see p. 29)	36
28	37
30	43
33	49
39	50
41	52
45	
47	
59	
60	
62	
63	
64	
67	
68	

There are two explanations for this: mixed cultures and back mutation - since I am now seeing more maters in these cultures (even those with fitness advantages) I suspect that back mutation is the likely explanation.





SALT RESISTANCE & MATING IN cgPP STRAINS

While I was setting up the last set of fitness assays I put the 69 cgPP strains into duplicate 96-well plates and froze them down — also I pin transformed onto cgF, YPD, YPD+1M NaCl and YPD+α-factor.

- again I noticed that #14 grows fine on YPD but slowly on cgF.
- surprisingly all strains grew on 1M NaCl
- also surprisingly, I see lots of mating (even in strains that did not mate previously (see p. 29)

STRONG MATERS (many colonies)	WEAK MATERS (a few colonies)
9	8
13	15
14	31
17	34
18 ← mated previously (see p. 29)	36
28	37
30	43
33	44
39	50
41	52
45	
47	
53	
60	
62	
63	
65	
67	
68	

There are two explanations for this: mixed cultures and back-mutation — since I am now seeing more matings in these cultures (even those with fitness advantages) I suspect that back mutation is the likely explanation.

8/4/07

DISCUSSION OF CONFUSING OBSERVATIONS

There are a few observations I have made that require an explanation.

(1) Why are none of the αF^R strains salt-sensitive?

→ In selecting 69 αF^R mutants I would expect some of the mutations to be in STE11, 20, or 50.

Possible explanations

- mixed cultures
- back mutation
- biased picking of αF^R strains - even though I tried to be unbiased (p15) I may have avoided these muts if they formed v. small colonies.

8/7/07

(2) The 1st 12 hours of the fitness assays:

In the 1st fitness assay I performed the slope of $\ln(\frac{E}{R})$ vs. gen was linear throughout (see p. 9). However, ~~Th~~ in both fit. assays on αF^R strains the slope in the 1st 12 hours is different.

Page 30 - In all cultures Expt. strains enrich

→ explanation: Reference was in stationary phase when the expt. began

Page 40 - In most cultures Expt. strains grew more slowly in 1st 12 hours

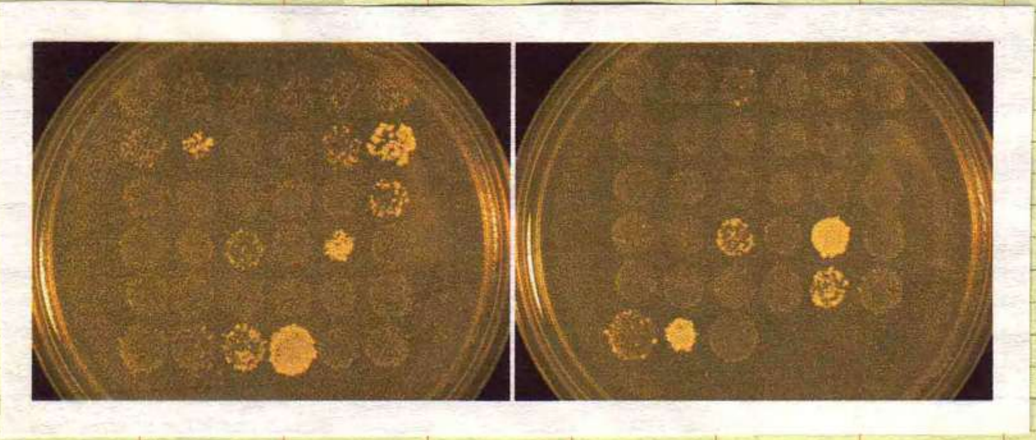
→ explanation: Perhaps many Expt. strains were in stationary

→ Should I rework the protocol so that the 1st time point is 6h. after mixing to ensure that all strains are exponential throughout? I think the answer is yes, but I do not want to switch the protocol in the middle of this project → Perhaps when Nilay or Hannah do the assays they should tweak the protocol.

8/7/07

MORE DISCUSSION OF CONFUSING RESULTS

(3) Mating of αF^R strains: As shown previously (pgs 29 & 43), some of the αF^R strains mate. I have mentioned two possible explanations: back mutation and mixed cultures — another possibility is that the αF^R strains really do mate. To determine if this is a possibility I replica plated these strains from an αF plate ~~with~~ to a plate w/ a lawn of α-tester and replica plated to mm after 1 days growth.



- Most of the strains that ~~grew~~ mated previously did not mate here (compare these plates w/ p 43).
- Still some strains showed significant mating — or no diminishment of mating: 18, 47, 64, & 68

↳ I will streak to singles and test mating of these.

(4) Why are there differences in the fitness of WT and Ref variable from expt. to expt.?

- On p. 9 the fitness was ~~2.~~ 3.3% and 3.6% (1st expt)
- 2nd expt. (pg. 30) - fitness ~ 4.5%
- 3rd expt (pg. 40) - fitness ~ 4%

} compared on pg 41, top

↳ fitnesses are consistent within expt. but not between expts. Why?

↳ Explanations:

(1) Diff. # of generations have passed in each expt. (i.e. due to #2 below)

(2) Media/culture conditions effect relative fitness

→ This shouldn't matter since I normalize, but this is irritating.

8/11/07

FITNESS ASSAYS ON Can^R STRAINS

In this experiment I will address to concerns from earlier:

(1) The increased variance I see in fitnesses of αF^R strains may be due to selection enriching for mutators (see p. 35)

→ I will measure the fitnesses of Can^R strains which I would not predict to be beneficial.

(2) In the previous expts, the unselected clones were single colonies from the same culture and may have shared ~~[illegible]~~ a common ancestor more recently than colonies selected from independent cultures of the fluct. assay (see p. 23)

→ To address this I performed a 72-tube fluct. assay and streaked the remaining 24 cultures to YPD

→ ∴ the Can^R and unselected strains all share the same MRCA.

~~On Sunday (8/5): single colony inoculated into 3ml~~

- On Monday (8/6): Pick single colony off of cold plate of GIL121
  - resuspend in 1ml H_2O
  - dilute 1:10,000 into ~~H_2O~~ 2×SC
  - put 30λ into each well of a 96 well plate.
  - plate seal → 30° incubator
- On Wednesday (8/8): ~~spot plate 72 cultures onto -arg+CAN~~
  - add 70λ H_2O to each well
  - spot 72 cultures onto -arg+CAN
  - for the remaining 24, streak 20λ to singles on YPD
- Yesterday (8/10): Start 3 mL YPD o/N cultures for fitness assays
  - for -arg+CAN - pick a single colony from the 1st 24 spots w/ at least 1 colony

    → to be unbiased, I picked the colony closest to 12-o'clock (like on p. 15) (labeled c1 - c24)
  - for unselected, I blindly placed a mark on each of the 24 plates and picked the colony closest to the spot. (labeled 28-51)

NOTE: Some colonies were noticeably smaller - these could be colonies which started growth later - for -arg+CAN, these could be ~~not~~ the result of mutations occurring post plating.

→ small colonies: C5, C14, C15, C19, 39, 48

8/11/07

- Today I am setting up the fitness assays
- Previously I have mentioned the 1st 12-hour problem (p 45).
  ↳ one possible explanation could be that the media used to get the strains growing exponentially ~~is~~ was different from the media used afterward in the previous 3 large scale assays (pgs. 17, 23, & 37). In the pilot expt. (where there was no 1st 12 h problem) this was not the case (p. 3)
  ↳ Here I will grow everything in 10-ml tubes using the same media (apart from o/N)
- Dilute all cultures ~1:300 (30λ into 10ml) (except C8, 39, 48 which had not saturated → dilute 1:100, 100λ into 10ml)
- Like an idiot I forgot to start an o/N of the Ref again.
  ↳ I started 4 1:100 and 4 1:200 dilutions of a cold Ref culture
- → all dilutions @ 12:30

Coulter Count Pure Cultures

Bkg		326	338
C1	1	33500	32596
	2	36700	26580
C2	1	30792	~~30600~~ 30606
	2	16752	16674
28	1	33770	33630
	2	22616	22634
29	1	36290	36256
	2	34928	34490
Ref	1	17962	17882
	2	14912	16508
Post Bkg		568	632

600 λ Ref. : 200 Exp.
~~750~~ λ Ref. : ~~250~~ Exp.
↳ I did not make enough Ref. to use 750λ per mix.

- The Bkg and the 1's were counted before I set up the expt. (~ 9:00pm)
- The 2's and Post Bkg were counted afterwards (~ 11:00pm)
- I may have mixed up C1 & C2 – I noticed after sonication that they were both labeled "C1".

8/11/07

- Mix strains 600λ Ref : 200 λ Exp. I wanted to do 750:250 but I didn't grow enough Reference

↳ For strains: 39 → 600 : 400

C8, 48 } → 600: 600

- Dilutions @ 9:30

8/12/07

Coulter Count Mixed Cultures (11:00 am)

Bkg		132	80	90
c1	1	46338	45640	45368
	2	46076	45896	45084
	3	47458	46554	46510
C2	1	42332	41482	42158
	2	35450	35320	34202
	3	40928	39818	40624
28	1	31742	31244	31464
	2	33750	33524	33572
	3	31010	33462	30688
29	1	45600	45390	45454
	2	41938	41396	41606
	3	43974	43890	44100
Post Bkg		2852	1004	846

8/12/07

Count again (11:00pm)

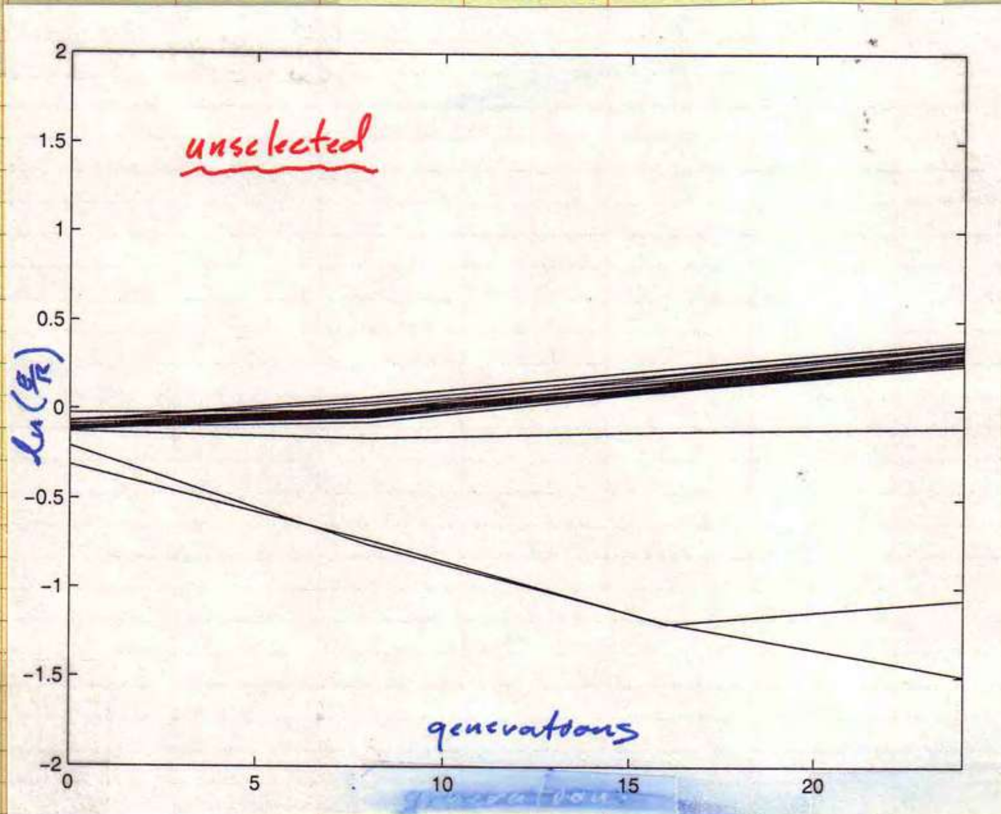
Bkg		96	130	128
c1	1	30394	30540	31404
	2	39212	39148	39286
	3	38024	37952	37998
c2	1	35590	35222	35236
	2	36476	35170	35066
	3	38634	37682	37576
28	1	22448	22552	22544
	2	20464	20192	20716
	3	21198	20576	20802
29	1	37214	37264	37172
	2	40400	39702	39662
	3	33644	33708	32990
Post Bkg		368	464	316

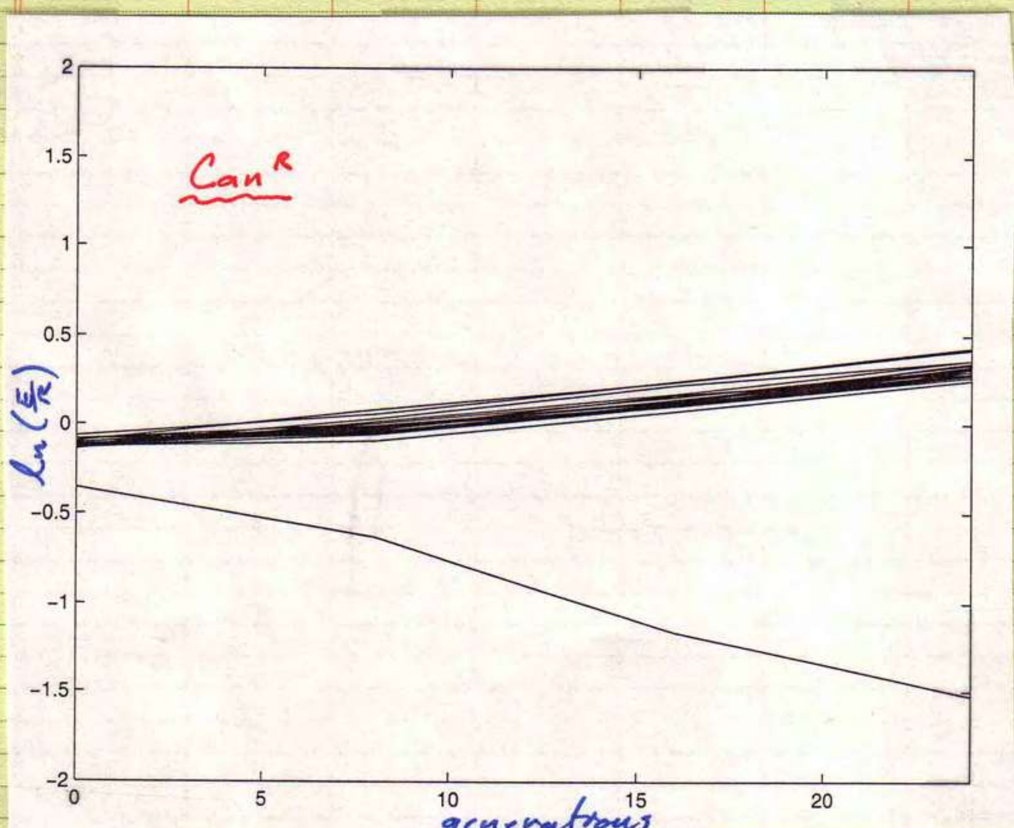
8/13/07

Count again (11:45am)

Bkg		46	64	100
c1	1	46048	~~45336~~ 45336	45080
	2	39978	38560	39270
	3	46262	46998	46540
c2	1	44596	43430	44310
	2	43238	42948	43182
	3	46542	46154	46318
28	1	21228	20998	21694
	2	23666	23826	23882
	3	23816	23256	23544
29	1	41378	41222	41120
	2	42502	42016	41690
	3	44118	43908	43618
Post Bkg		1008	864	658

8/15/07





uns_39	-7.24%
uns_48	-5.25%
uns_28	-1.61%
uns_33	-0.40%
uns_46	-0.17%
uns_38	-0.16%
uns_35	-0.12%
uns_47	-0.11%
uns_36	-0.10%
uns_43	-0.02%
uns_44	-0.01%
uns_37	0.00%
uns_32	0.00%
uns_50	0.02%
uns_45	0.02%
uns_42	0.03%
uns_40	0.05%
uns_34	0.09%
uns_29	0.09%
uns_41	0.11%
uns_30	0.11%
uns_49	0.12%
uns_31	0.20%
uns_51	0.24%
CanR_08	-6.92%
CanR_24	-0.08%
CanR_19	-0.06%
CanR_18	-0.06%
CanR_12	-0.04%
CanR_14	-0.03%
CanR_16	-0.02%
CanR_01	0.01%
CanR_04	0.02%
CanR_20	0.03%
CanR_13	0.03%
CanR_02	0.04%
CanR_22	0.08%
CanR_11	0.10%
CanR_15	0.11%
CanR_21	0.11%
CanR_17	0.17%
CanR_09	0.20%
CanR_03	0.22%
CanR_07	0.25%
CanR_10	0.35%
CanR_23	0.38%
CanR_05	0.39%
CanR_06	0.42%

8/21/07

DISCUSSION OF FITNESS ASSAYS ON Can^R MUTANTS

(1) Little variation in fitnesses among Can^R mutants

- This suggests that the variation observed in αF^R mutants is due to the mutations causing αF^R and not to unlinked mutations. In other words, I did not just enrich for mutators which would be expected to show more variation in fitness.

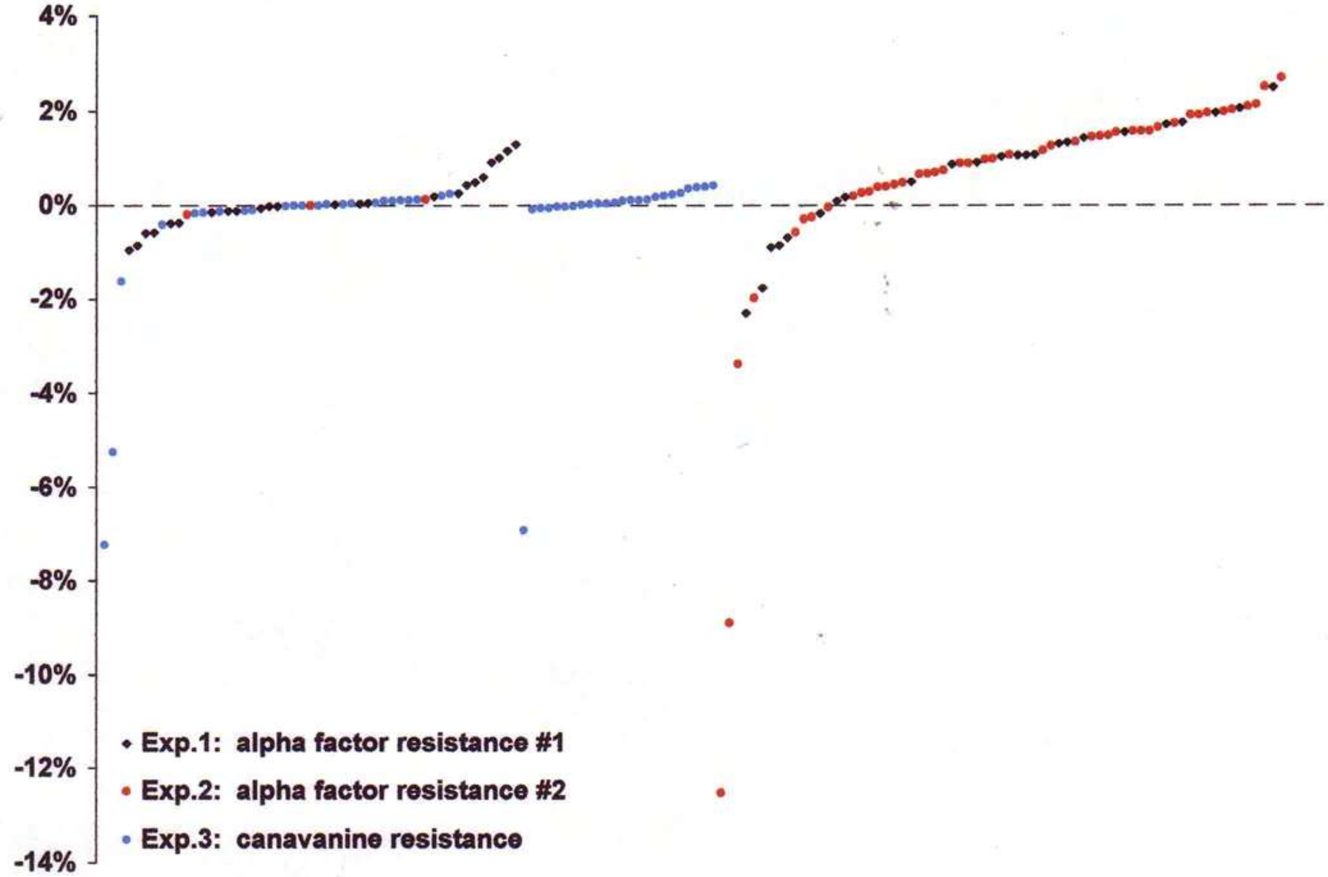
(2) Can^R mutants have the same fitness as WT

- This shows that there is no loss of fitness (or gain) that results from the loss of CAN1

(3) Interestingly I have been observing several ~~strains~~ clones (of αF^R, Can^R, and WT) with very low fitnesses (see p. 53)

(4) I am not sure what to make of the tails on the fitness dist. of WT clones from Exp. 1. I suspect they are spurious — perhaps if condition upon particular correlation coeffs. I can filter these out.

8/15/07


4%
2%
0%
-2%
-4%
-6%
-8%
-10%
-12%
-14%
Exp.1: alpha factor resistance #1
Exp.2: alpha factor resistance #2
Exp.3: canavanine resistance

8/11/07

FITNESS ASSAYS ON SELECTED DELETION STRAINS

I want to measure the fitness of particular deletions in the mating pathway. I chose the most upstream component (STE2), STE4, a shared component (STE11), STE7, the transcription factor (STE12) and FAR1. As a control I will measure the fitness of a CAN1 delete.

~~[illegible]~~
~~Last week~~

- I made gDNA preps from the above strains from the yeast deletion collection (all from the haploid collection except STE12 which is from heterozygous diploid collection)
- PCR using the following primers:

31957805 IDT
B.NEUGEBOREN
33808631 8/6/2007
STE2extF1
5'- ATT TAA GCA GGC CAA CGT CC -3'
Tm= 55.9 ºC , MW= 6,086.0
7.1 OD_{260} 36.70 nmol 0.22 mg

31957803 IDT
B.NEUGEBOREN
33808629 8/6/2007
STE2extR1
5'- CTG AGA GTT CTA GAT CAT GG -3'
Tm= 49.1 ºC , MW= 6,172.1
7.0 OD_{260} 35.30 nmol 0.22 mg

31957800 IDT
B.NEUGEBOREN
33808626 8/6/2007
STE4extF1
5'- CTT CAC ATG ACT TGA ATC CC -3'
Tm= 51.0 ºC , MW= 6,012.0
7.5 OD_{260} 41.10 nmol 0.25 mg

31957809 IDT
B.NEUGEBOREN
33808635 8/6/2007
STE4extR1
5'- TAG ATG ATT CTA GCA AGT TGG -3'
Tm= 49.5 ºC , MW= 6,500.3
6.1 OD_{260} 28.60 nmol 0.19 mg

31957812 IDT
B.NEUGEBOREN
33808839 8/6/2007
STE11extF1
5'- TTT CTT TAT GCT GTC CTC ACC -3'
Tm= 52.6 ºC , MW= 6,289.1
6.8 OD_{260} 37.90 nmol 0.24 mg

31957799 IDT
B.NEUGEBOREN
33808625 8/6/2007
STE11extR1
5'- TAT CAC CTC GCA AAG AAA GG -3'
Tm= 52.4 ºC , MW= 6,119.0
6.0 OD_{260} 29.70 nmol 0.18 mg

31957810 IDT
B.NEUGEBOREN
33808837 8/6/2007
STE7extF1
5'- AGT TCT AAG ATT GTG TTG TCC -3'
Tm= 50.1 ºC , MW= 6,442.2
6.9 OD_{260} 34.20 nmol 0.22 mg

31957808 IDT
B.NEUGEBOREN
33808634 8/6/2007
STE7extR1
5'- GGG TTA TTA ATC GCC TTC GG -3'
Tm= 53.4 ºC , MW= 6,139.0
7.0 OD_{260} 36.90 nmol 0.23 mg

31957807 IDT
B.NEUGEBOREN
33808633 8/6/2007
STE12extF1
5'- ACA TAG TAC CAC TAC GTT CC -3'
Tm= 51.0 ºC , MW= 6,021.0
6.1 OD_{260} 31.70 nmol 0.19 mg

31957802 IDT
B.NEUGEBOREN
33808628 8/6/2007
STE12extR1
5'- CGA TCA TGT AGT TTT GGA GG -3'
Tm= 50.7 ºC , MW= 6,203.1
7.2 OD_{260} 36.50 nmol 0.23 mg

31957806 IDT
B.NEUGEBOREN
33808632 8/6/2007
FAR1extF1
5'- CAA ATG CGA AGG TAC CTT GG -3'
Tm= 54.1 ºC , MW= 6,166.1
6.9 OD_{260} 34.70 nmol 0.22 mg

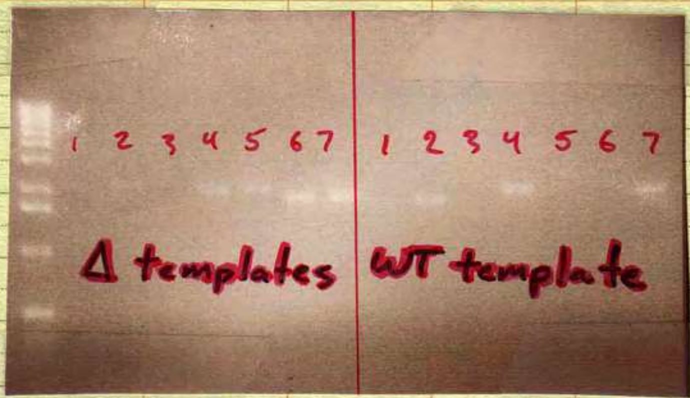
31957811 IDT
B.NEUGEBOREN
33808838 8/6/2007
FAR1extR1
5'- GCC AAT AGG TTC TTT CTT AGG -3'
Tm= 50.8 ºC , MW= 6,427.2
6.8 OD_{260} 34.30 nmol 0.22 mg

31957804 IDT
B.NEUGEBOREN
33808630 8/6/2007
CAN1extF3
5'- TAA ACC GAA TCA GGG AAT CC -3'
Tm= 52.0 ºC , MW= 6,119.0
6.4 OD_{260} 31.30 nmol 0.19 mg

31957801 IDT
B.NEUGEBOREN
33808627 8/6/2007
CAN1extR3
5'- CGG GAG CAA GAT TGT TGT GG -3'
Tm= 56.6 ºC , MW= 6,253.1
8.0 OD_{260} 40.10 nmol 0.25 mg

8/11/07

PCR OFF OF DELETION COLLECTION



Lane	Primers
1	STE 2
2	" 4
3	" 11
4	" 7
5	" 12
6	FAR 1
7	CAN 1

NOTES

- I had trouble getting PCR to work for STE2 & STE11.
- I tried multiple annealing temps and extension times.
- In the end I pooled all of my attempts and PCR purified from the whole mix.

PCR purifications: lanes same as above

- I may not have STE11

← Primer dimer in my two troublesome PCRs

- Transform these purified products in the usual way.
- Today I replica plated to G418

8/14/07

- No colonies on STE11 plates
- 10-100 colonies on all other plates

8/14/07

START OF FITNESS ASSAYS FOR DELETION STRAINS

- Last night I picked 7 independent colonies of each transformation
  - Except for STE11, since there were no colonies.
- Also, when I plated the transformation, I streaked 20λ of GIL121 onto triplicate YPD plates from the transformation flask — this will serve as a WT control.

↳ I picked 6 single colonies.

- All o/N in YPD — also passage Ref.
- This morning I diluted all 48 cultures back 1:300 (30λ into 10 mL) except Ref (1:50, 200λ into 10 mL) — 12:30

Coulter count Pure Cultures (9:15pm)

Bkg	78	140	30
can1 A 1	29842	26710	
2	27122	27160	
can1 B 1	25792	24990	
2	27428	26838	
WT A 1	29586	29914	
2	29246	29094	
WT B 1	29344	30036	
2	30094	29348	
~~Bkg~~			
Ref. 1	37120	36940	
2	37324	37354	
Post Bkg	536	614	

500λ Exp. : 500λ Ref.

- Dilutions @ 9:45 pm

8/15/07

- Today I noticed that the cultures looked a little dense. I decided to dilute 15λ into 10 mL (instead of 20λ)

↳ dilutions @ 9:45

8/15/07

Coulter Count Mixed Cultures (11:00 am)

Bkg	32	6	54	
Can1 A 1	40050	39920	39182	
2	47230	41204	41342	41244
3	42452	42246	42110	
Can1 B 1	50428	49680	49334	
2	50622	50696	50636	
3	52386	51016	51030	
WT A 1	54408	53620	54032	
2	52570	51626	51488	
3	53872	53044	53050	
WT B 1	54240	54142	54172	
2	55108	54698	54482	
3	45834	45708	46490	
Post Bkg	846	698	622	

Coulter count again (11:00 pm)

Bkg	22	52	38	
Can1 A 1	20082	18824	18696	
2	19012	18532	18552	
3	16858	16464	16614	
Can1 B 1	24506	22740	~~2274~~	22724
2	17238	16136	15962	
3	25224	25252	25386	
WT A 1	22920	22790	22818	
2	24692	24420	25178	
3	21836	21588	21934	
WT B 1	24384	23514	23722	
2	23312	23454	23258	
3	26184	25916	25990	
Post Bkg	374	340	244	

8/16/07

Count again (11:00am)

Bkg		58	46	32
Canl A	1	14364	14108	14246
	2	13672	13266	13438
	3	12928	12268	12534
Canl B	1	16470	15984	16400
	2	13802	14136	13962
	3	15604	15552	15600
WT A	1	15752	16184	16150
	2	22048	21936	21622
	3	19396	18818	18980
WT B	1	19670	19756	19358
	2	21402	20972	21164
	3	20358	20068	20042
Post Bkg		256	198	170

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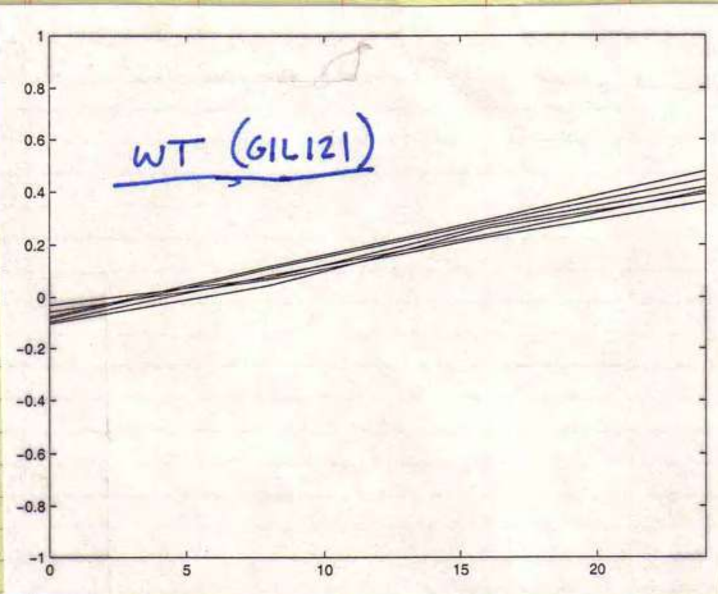
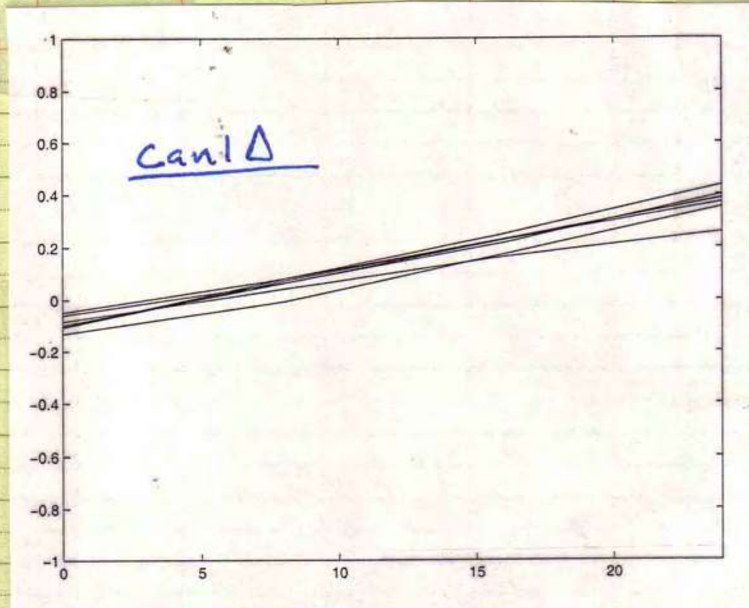
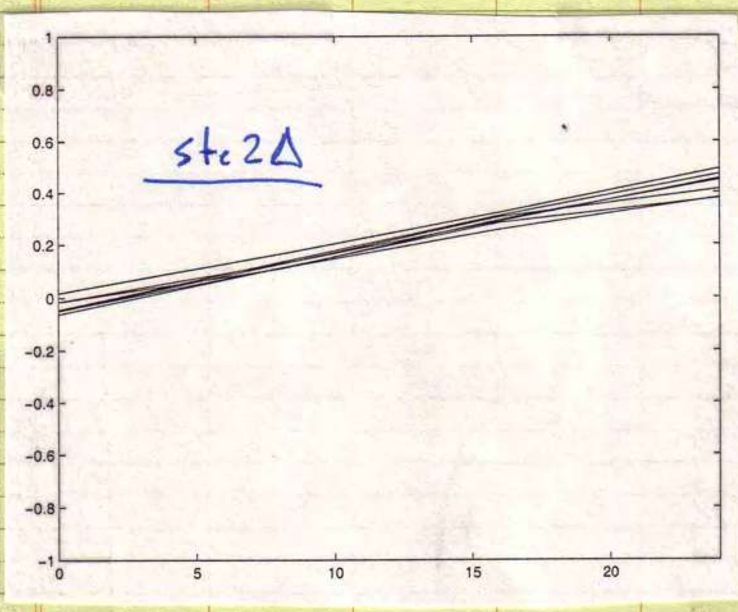
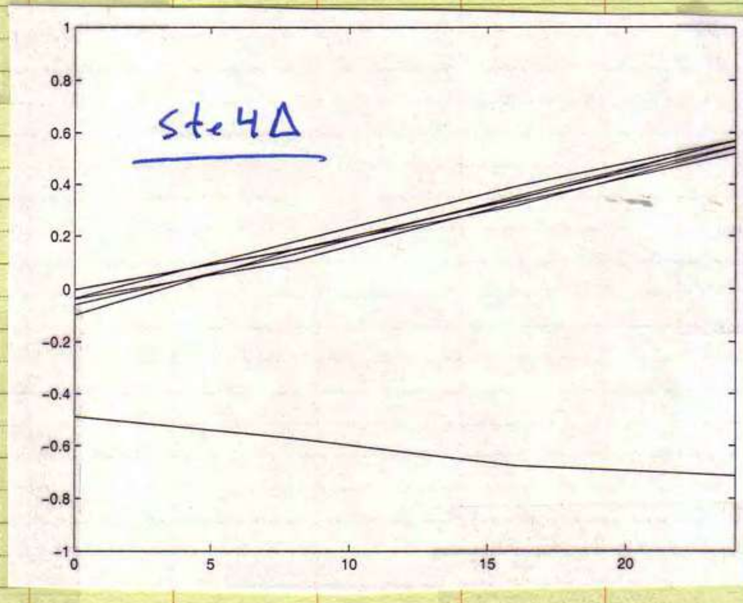
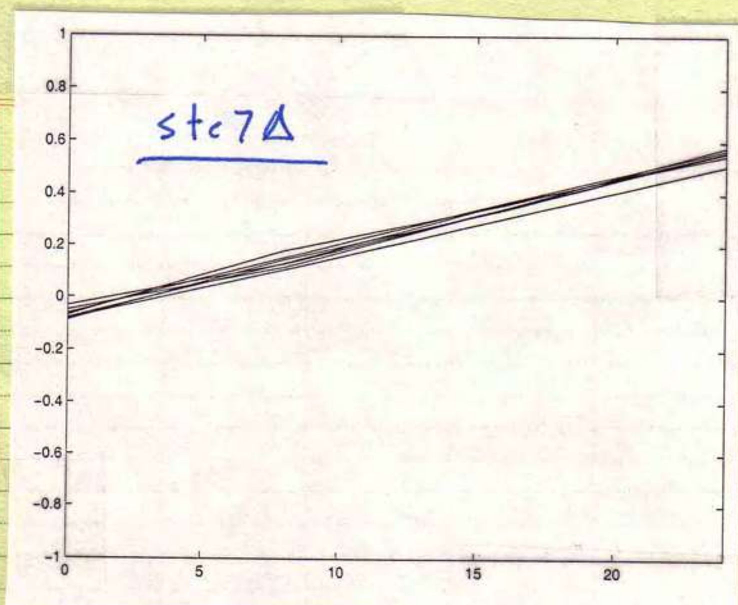
8/20/07

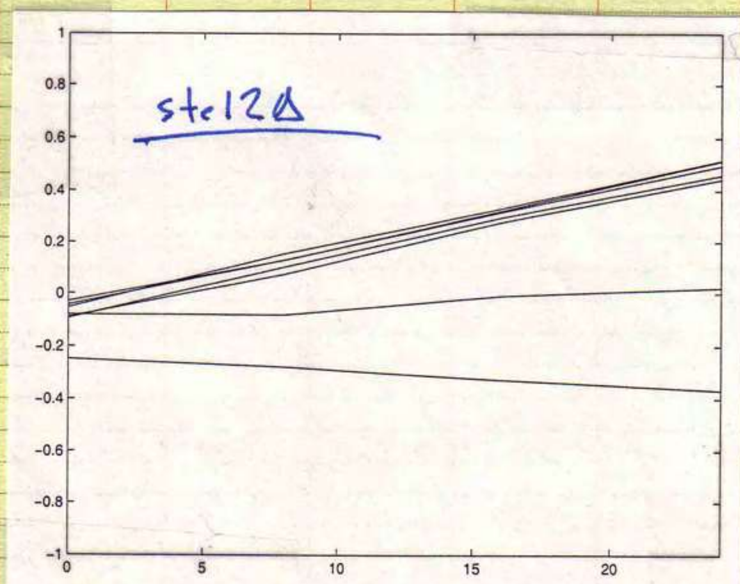

WT (GIL121)
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
0
5
10
15
20

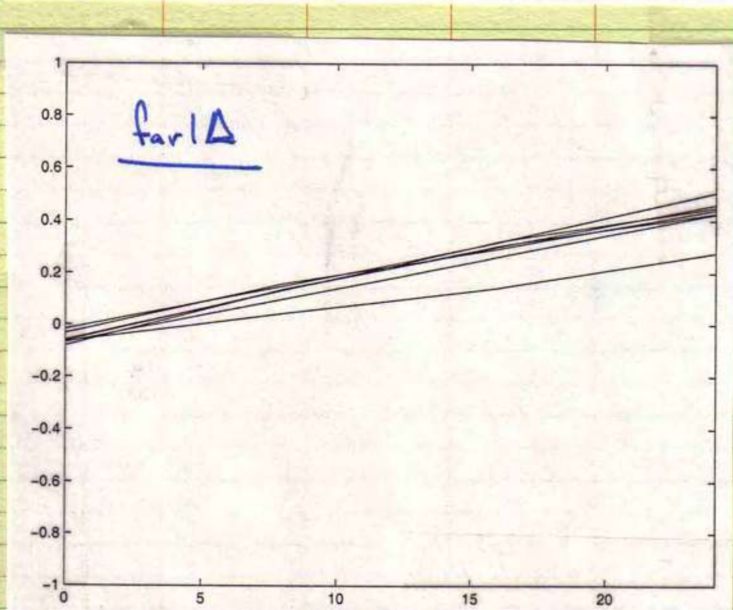

can1Δ
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
0
5
10
15
20


ste2Δ
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
0
5
10
15
20


ste4Δ
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
0
5
10
15
20







WT	D	-0.25%
	B	-0.09%
	C	0.05%
	E	0.12%
	A	0.19%
	F	0.28%
can1	A	-0.60%
	E	-0.11%
	B	-0.06%
	C	0.00%
	D	0.01%
	F	0.02%
	G	0.02%
ste2	C	-0.21%
	G	-0.18%
	F	-0.06%
	D	-0.06%
	B	-0.05%
	A	-0.04%
	E	0.15%
ste4	D	-3.06%
	C	0.25%
	G	0.35%
	A	0.37%
	E	0.53%
	F	0.63%
	B	0.66%
ste7	B	0.46%
	D	0.48%
	G	0.58%
	A	0.63%
	C	0.64%
	F	0.65%
	E	0.68%
ste12	A	-2.58%
	D	-1.57%
	G	0.24%
	F	0.24%
	E	0.27%
	B	0.31%
	C	0.34%
far1	D	-0.62%
	C	-0.08%
	E	0.03%
	F	0.04%
	A	0.12%
	B	0.15%
	G	0.22%

8/20/07

NOTES REGARDING RECENT FITNESS ASSAYS

SOLUTION TO THE FIRST 12-HOUR PROBLEM:

→ Earlier I noted that the first 12 hours of the fitness assays often have a different slope compared to the ~~end~~ slope during hours 12-48 (see p 45).

One explanation is that this effect could result from acclimating the strains in media from a diff. batch than the media used in the subsequent expt (see p 48)

In the most recent two fit. assays I used the same media for the expt. and the growth to exponential phase following the 1:300 dilution.

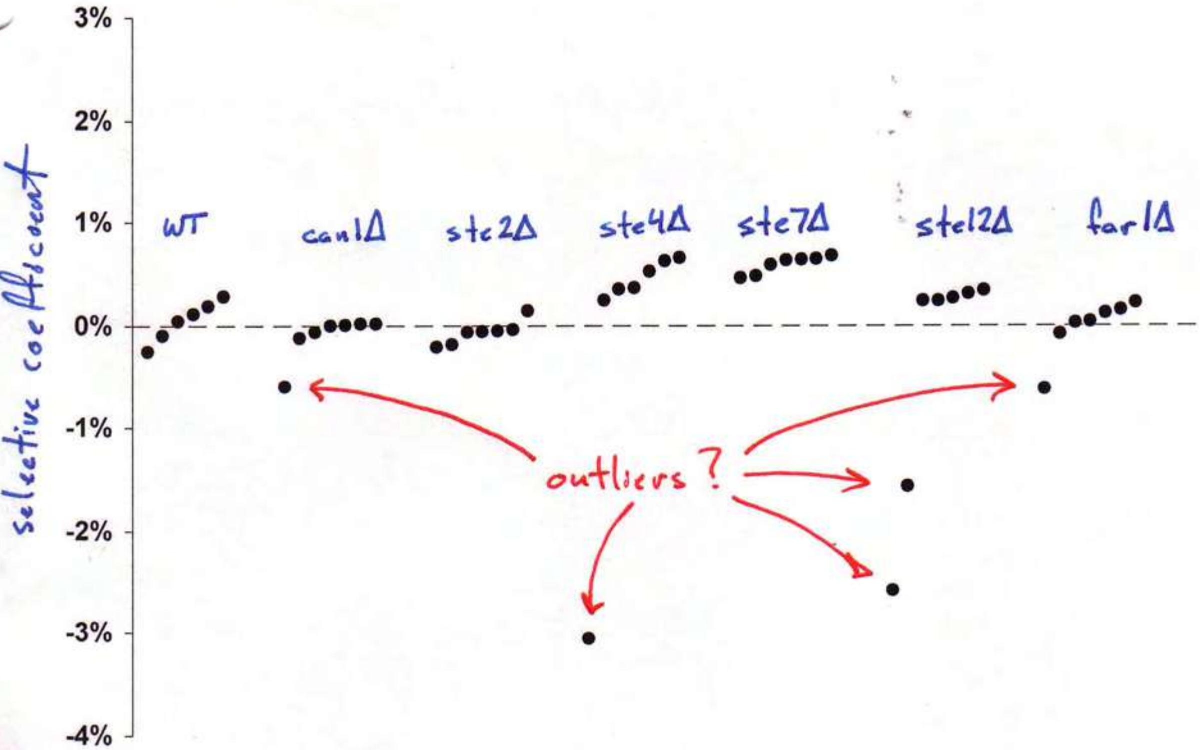
The slopes seem to be constant throughout (compare competitions on pages 51, 60, & 61 to those on pgs 30 & 40.)

8/21/07

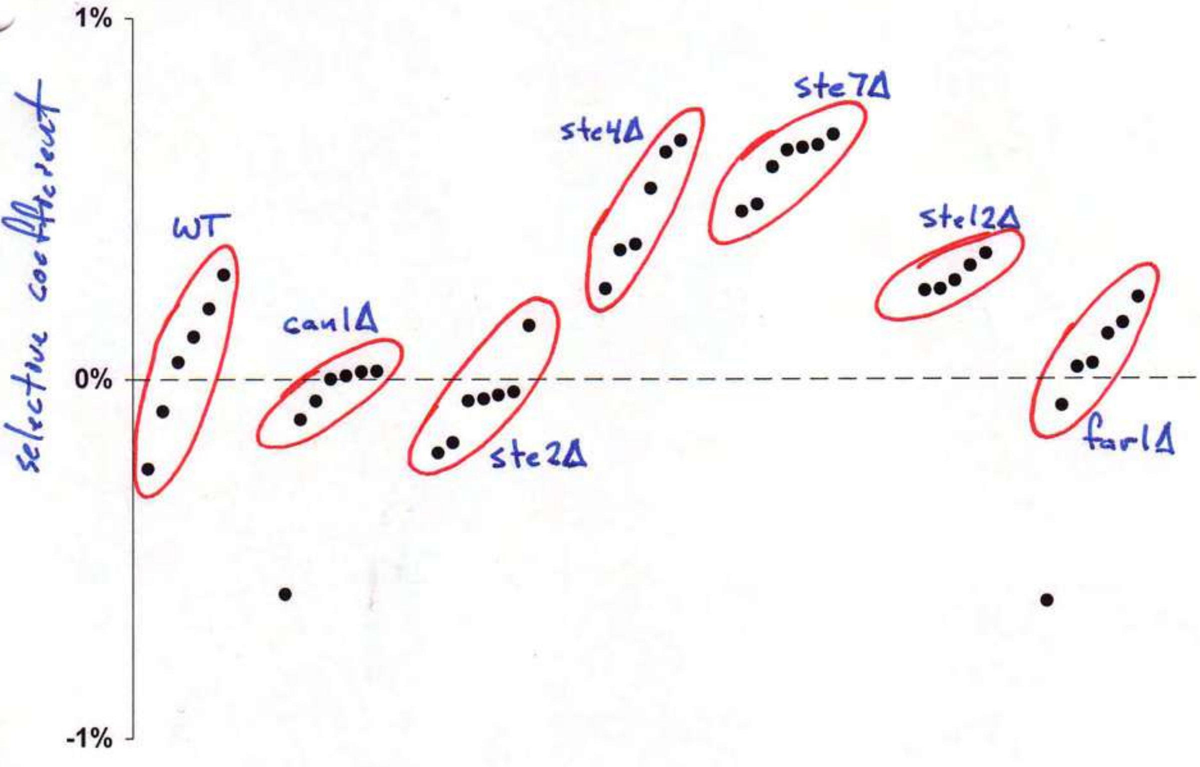
It is interesting to note that the fitness increase from deleting ste4, ste7, or ste12 (although significantly diff from can1) is less than the fitness increase observed in some spontaneous αF^R clones (pg. 33)

→ Could this mean that partial loss of function of the pathway is better than complete loss?

8/20/07

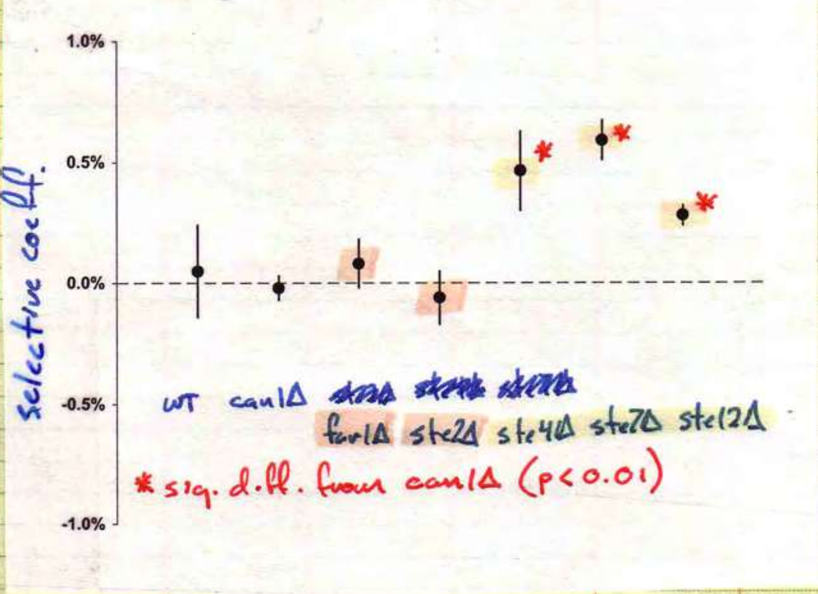


3%
2%
1%
0%
-1%
-2%
-3%
-4%
selective coefficient
WT
can1Δ
ste2Δ
ste4Δ
ste7Δ
ste12Δ
far1Δ
outliers ?

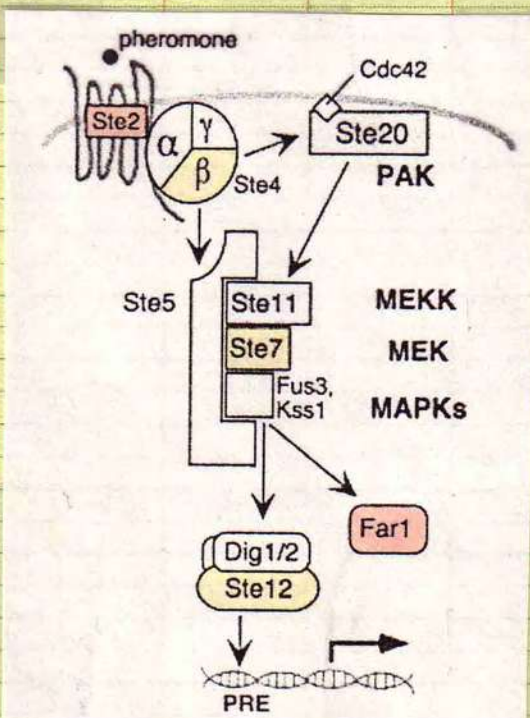


1%
0%
-1%
selective coefficient
WT
can1Δ
ste2Δ
ste4Δ
ste7Δ
ste12Δ
far1Δ

8/20/07



averages of the data on p. 63 (excluding outliers)

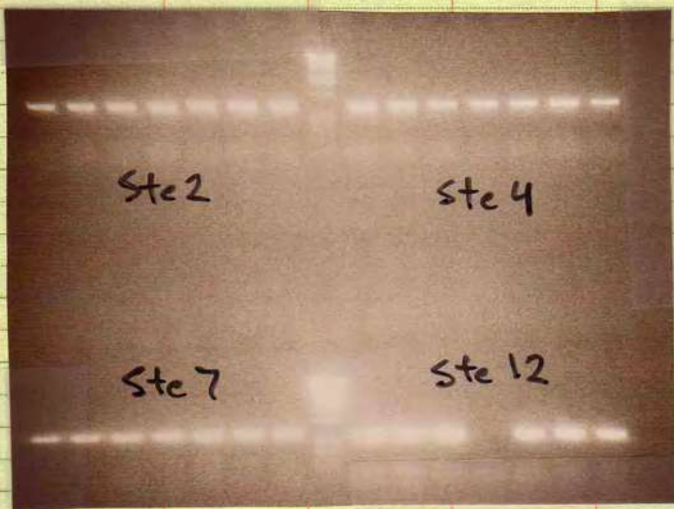


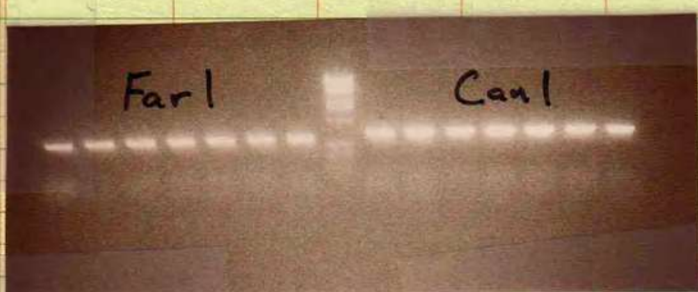
CONCLUSIONS

- The fitness cost of mating competence is the result of Ste12 transcription, not transient arrest
- The receptor (Ste2) is not involved — therefore this looks like basal signaling imposes a fitness cost.
- can1Δ::KanMX4 looks like wt — therefore there is no detectable cost to expressing KanMX4

9/10/07

VERIFICATION OF DELETIONS





Verify deletions using primers

KanMX1ntR

and :

32325526 IDT
B.NEUGEBOREN
34181155 8/22/2007
STE2extF2
5'- GTC TCG TGC ATT AAG ACA GG -3'
Tm= 53.2 ºC , MW= 6,157.0
5.9 OD_{260} 29.70 nmol 0.18 mg

32325527 IDT
B.NEUGEBOREN
34181156 8/22/2007
STE4extF2
5'- GTT TTC AAC TGA CAC TTG CC -3'
Tm= 52.4 ºC , MW= 6,043.0
6.1 OD_{260} 33.90 nmol 0.20 mg

32325528 IDT
B.NEUGEBOREN
34181157 8/22/2007
STE7extF2
5'- GTG GTC TTA AAA GAA TGT GG -3'
Tm= 48.9 ºC , MW= 6,236.1
6.5 OD_{260} 31.70 nmol 0.20 mg

32325525 IDT
B.NEUGEBOREN
34181154 8/22/2007
STE12extF2
5'- ACA ACT CTT CGC GGT CAG G -3'
Tm= 57.5 ºC , MW= 5,788.8
5.5 OD_{260} 30.50 nmol 0.18 mg

32325530 IDT
B.NEUGEBOREN
34181159 8/22/2007
FAR1extF2
5'- GCC ATT TCA CCG AAA ATA CC -3'
Tm= 52.1 ºC , MW= 6,030.0
7.3 OD_{260} 38.00 nmol 0.23 mg

32325529 IDT
B.NEUGEBOREN
34181158 8/22/2007
CAN1extF4
5'- AAT CTA GGG TTT CTG TGT GG -3'
Tm= 51.9 ºC , MW= 6,194.1
7.2 OD_{260} 37.10 nmol 0.23 mg

This expt was done the day before I left Boston - so it took me a while to put this in the notebook.

~~I remember~~

- The only incorrect strain is Ste12-D, one of the outliers. I remember that several of the strains were able to mate (and these were also outliers). Interesting - deletion and duplication?

8/8/07

PHENOTYPING αF^R STRAINS

· I isolated two of Thea's plasmids pTCN112 and pTCN113 both are Gal overexpression plasmids w/ His marker.

- pTCN112 overexpresses STE4
- pTCN113 overexpresses FAR1-22

} both induce arrest on Gal

· In order to determine where in the pathway these mutations lie I will determine if these strains (mutations) can overcome each of these two arrests.

· Last weekend I Mini prepped these plasmids

pTCN112: 1341 ng/μl

pTCN113: 143 ng/μl

· Today I will transform all 69 αF^R strains + WT with each plasmid

TRANSFORMATION:

· O/N cultures ⟹ dilute 1:100 (30 λ into 3 mL) into 5 mL falcon tubes (11:30)

· OD_{600} of WT @ 4:00 = 0.29 ⟶ start btw 5:30 & 6

@ 5:45 = 0.67

· Make new PEG Mix – combine w/ old PEG mix – filter sterilize – add 8 mL DMSO to ~ 70 mL PEG Mix

· LiOAc Mixes:

pTCN112: ~~4000 λ~~ 4800 λ LiOAc Mix
1000 λ ssDNA
200 λ Plasmid
6000 λ ⟹ 60 λ per rxn

pTCN113: 4500 λ LiOAc
1000 λ ssDNA
500 λ Plasmid
6000 ⟹ 60 λ per rxn

8/11/07 NOTES ON TRANSFORMATIONS

Bodo's Lithium Acetate Transformation

LiOAc mix 100mM LiOAc → filter sterilize
10mMTris pH7.5
1mM EDTA

PEG mix 40%PEG → membrane filtration. → filter sterilize
100mM LiOAc
10mM Tris pH 7.5
1mM EDTA

~~25 ml culture~~ 3 mL culture (~~at start~~

1) Grow culture to OD 0.5-0.7 in YPD.

→5~ for minutes 3-4×

2) Spin, transfer to Eppendorf tube and wash $\boxed{\text{twice}}$ with sterile water 20
↳ setting 5 on centrifuge (in 50 ml falcon tube)

3) Wash twice in 1 ml LiAc mix. ← During second LiOAc wash I split into 2 500λ tubes so that I could transform both plasmids by resuspending in LiOAc Mix w/ plasmid

~~120~~ 60λ ~~25~~ 3 ~~20~~

4) Resuspend in ~~360µl~~ LiAc mix per ~~50~~ ml culture ~~and 40µl carrier DNA (0°C).~~ w/ plasmid & 30 DNA

5) ~~use 10 µl cells/plasmid transformation and 50µl for integrative transformation.~~

more volume possible

6) ~~Add thawed cells to DNA (maximally 2µl DNA per 10µl competent cells) and mix.~~
1λ per 10λ above (3λ for 30λ)

7) Add sixfold volume of 40% PEG, mix and incubate 30', ~~30°C~~ $\boxed{\text{(room temp if ts).}}$
(360λ)

8) ~~Add DMSO to final concentration of 10%.~~ → helps cells survive shock.
↳ not necessary

9) Heat shock 15', 42°C (37°C if ts).

10) Sediment cells for 2-3 min at 2000rpm in Eppendorf centrifuge.

11) Resuspend in ~~200 µl~~ 100λ YPD and plate out on selective medium.

8/11/07

RESULTS OF TRANSFORMATIONS

- The efficiency was excellent: thousands of colonies per plate
- I let the plates incubate for 2 days.
- Yesterday I inoculated 2 colonies from each plate into 150λ of 2+SC-his in 3 96-well plates.

↳ · Today I will pin transfer to YPD (liq.) so that I can save these strains
· Also I will pin transfer to solid media:

YPD, YPD+NaCl, YPD+αF+NaCl, YPD+αF, -his, -his Gal/Raf, YPD+Matα

NOTES:

- I accidently inoculated α23-113-2 into the well of α22-113-2.
- For two strains (α43, α52) there were clearly 2 sizes of colonies — in this case the 1st colony picked was Big, the second small

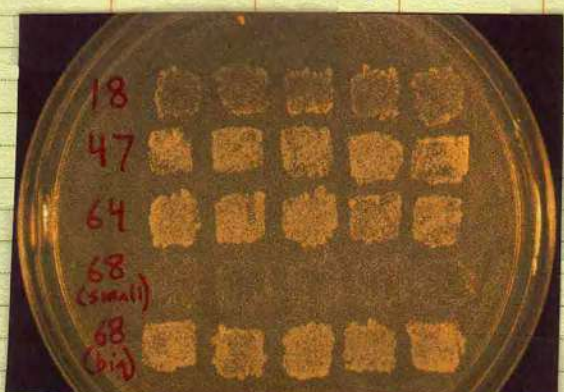
↳ maybe α59 as well.

8/14/07

Are some of these strains reverting?

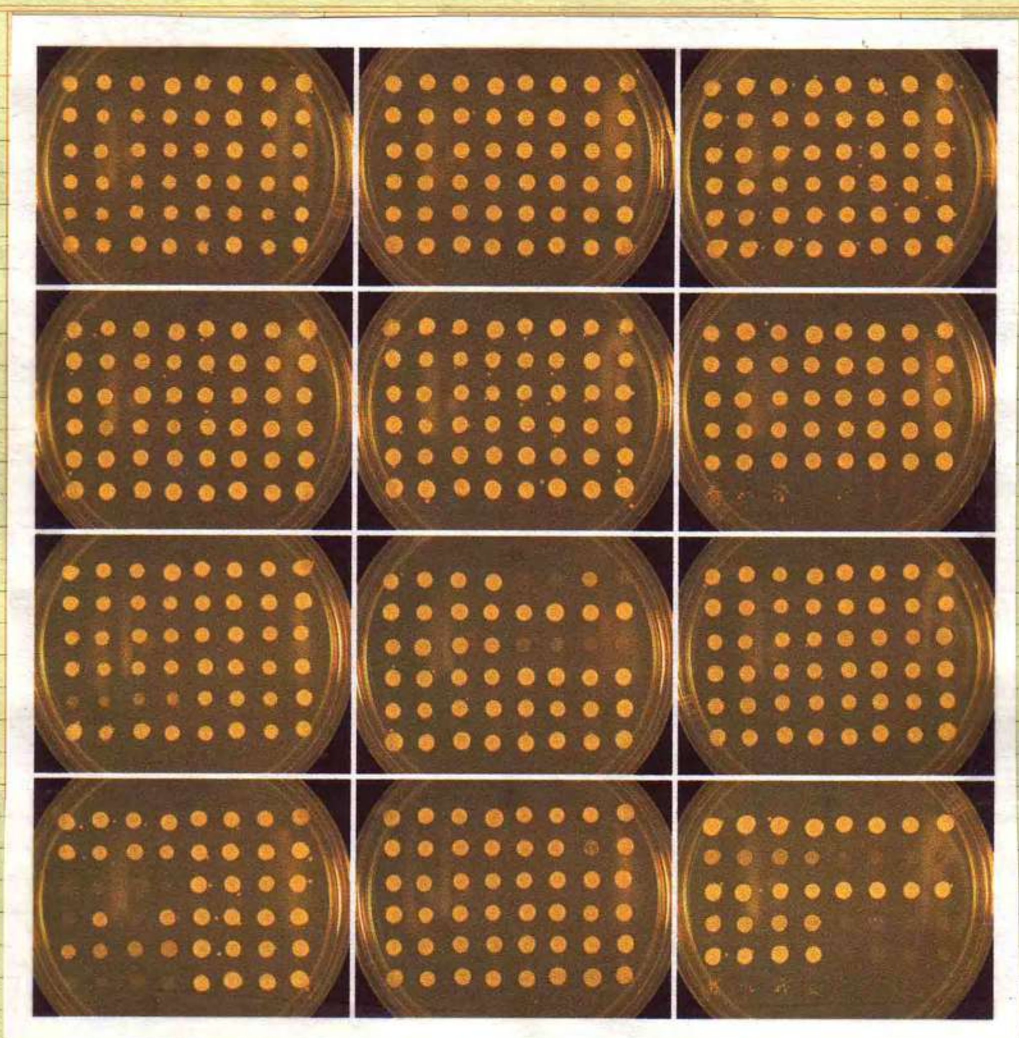
- On 8/7 (p 46) I ~~plat~~ streaked to singles 4 strains I suspected of reverting (18, 47, 64, & 68).
- I picked 5 colonies of each (for 68, I noticed two colony sizes, so I picked 5 of each) and patched them onto plates containing the mating tester.
- After replica plating to ~~at~~ min. I see that none of the 18s mated, all of the 47s and 64s mated, and for 68, all of the big colonies mated but none of the small colonies did.

↳ This could be a culture in the process of being over taken by revertants (or contaminating WT)



- In order to distinguish btw. contaminating WT vs. revertants I picked 3 small 68 colonies and 3 large 68 colonies and grew them in YPD for two days.
- Today I, streaked to singles on YPD to see if I can find big colonies in what were pure cultures of small colony cells.

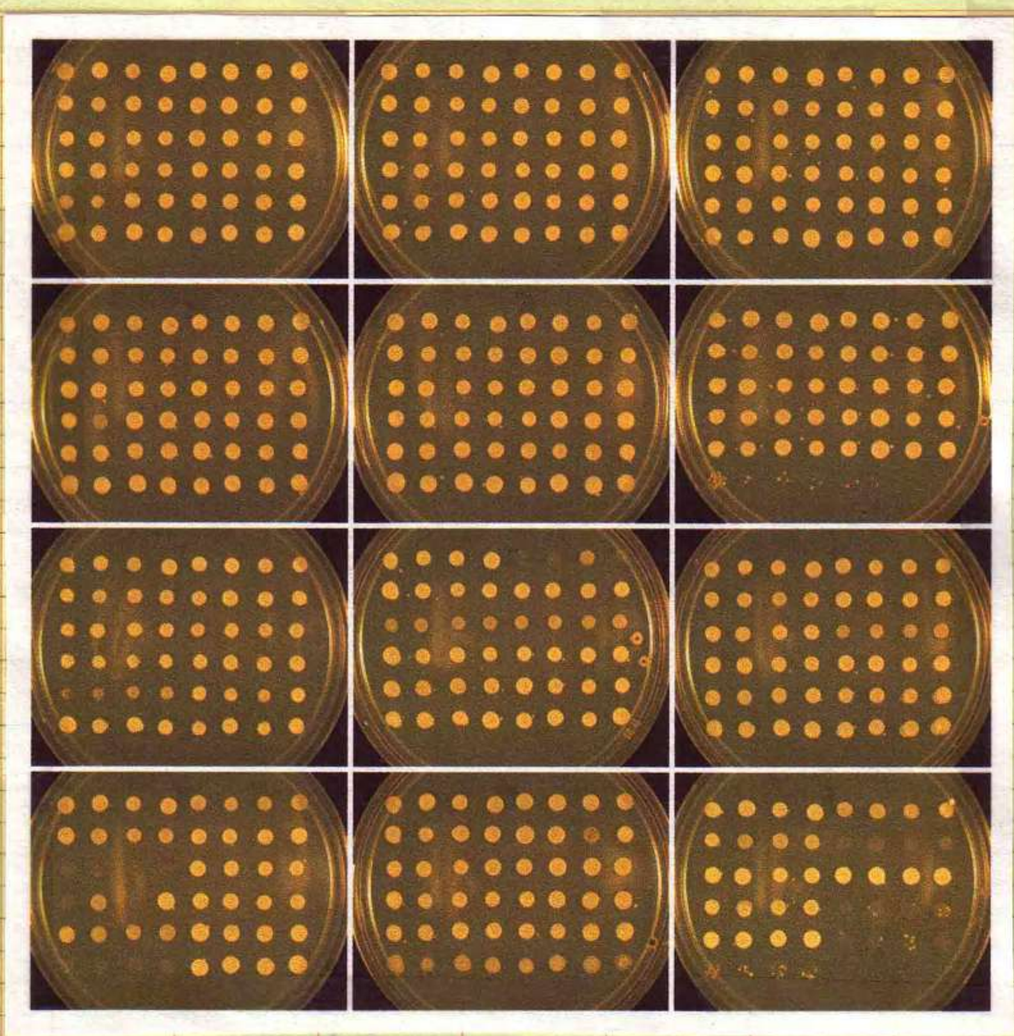
8/21/07



YPD
day 2

YPD + αF
day 2

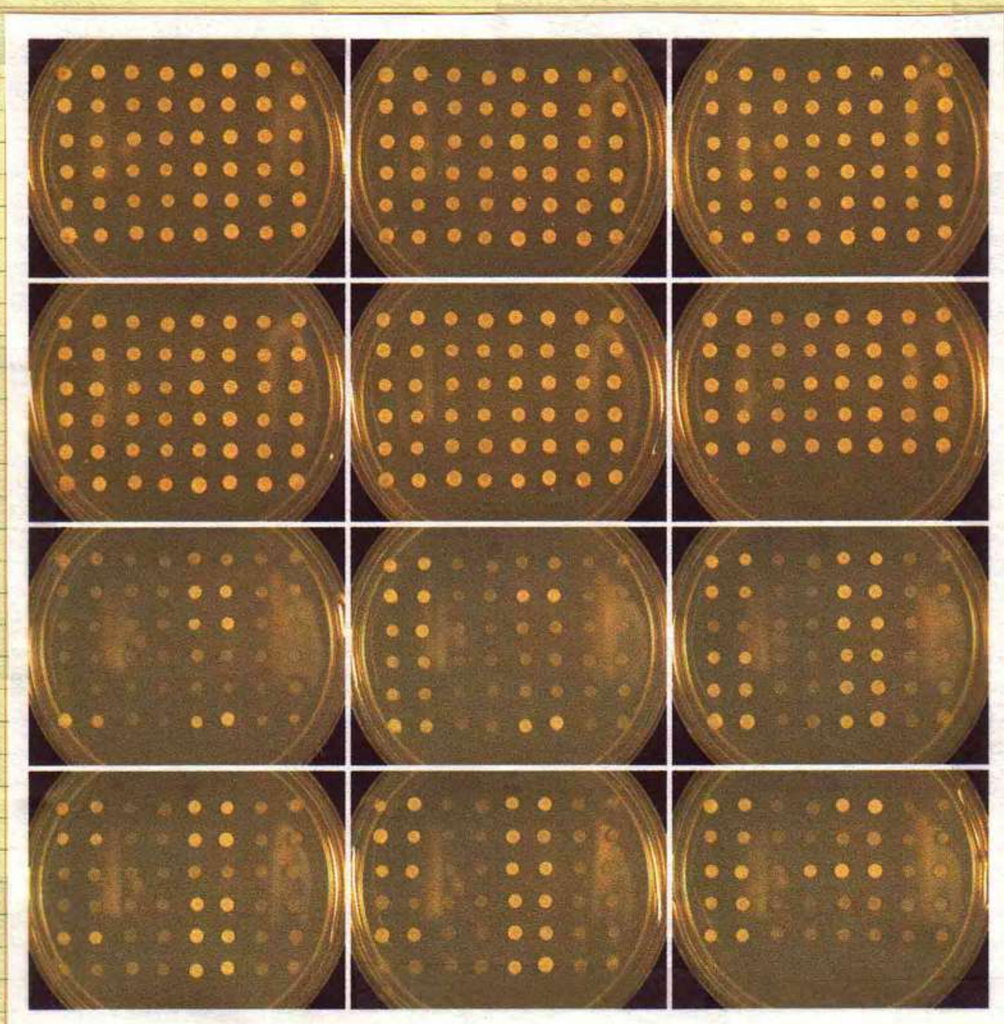
8/21/07



YPD + NaCl
(day 4)

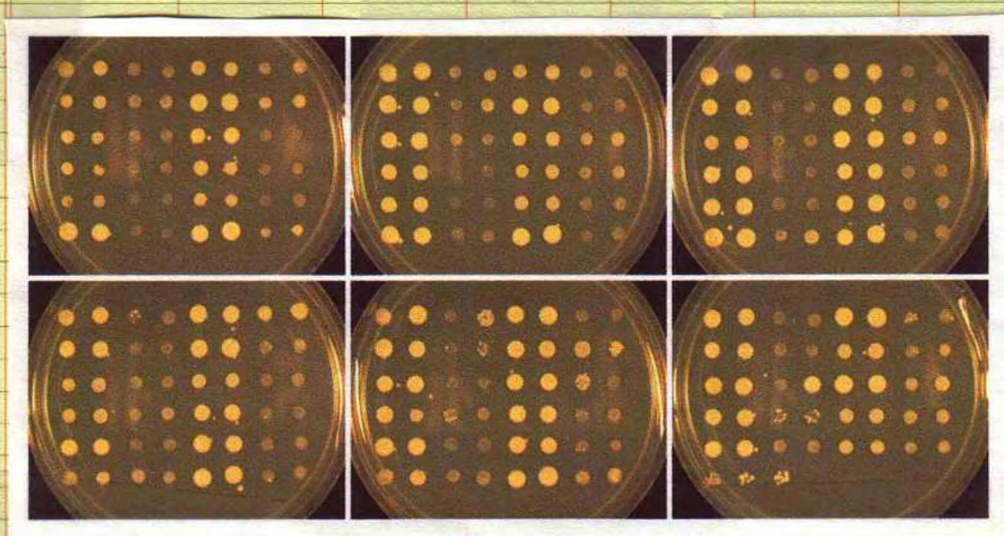
YPD + NaCl
+ KF
(day 4)

8/21/07



-his
(day 2)

-his +Gal/Raf
(day 2)



-his + Gal/Raf
(day 4)

8/21/07

STRAIN	Fitness	Gal-STE4	Gal-FAR1-22	aF+NaCl	alphaF	Mating
37	2.70%					
11	2.50%					
40	2.50%					
58	2.13%					
69	2.09%					
4	2.06%					
54	2.03%					
48	1.98%					
19	1.96%					
29	1.96%	-				
66	1.92%					
55	1.91%	-				
21	1.77%					
42	1.74%	-				
10	1.73%	-				
26	1.65%					
32	1.58%					
27	1.57%					
51	1.57%					
13	1.56%					
28	1.55%					
35	1.47%					
39	1.46%					+
61	1.45%					
15	1.44%					
44	1.34%					
1	1.33%	-				
22	1.31%	-				
33	1.26%					
31	1.16%					
12	1.07%					
6	1.07%					
16	1.06%					
57	1.06%					
24	1.04%					
46	0.98%					
45	0.97%					+
23	0.91%					
36	0.89%					
34	0.89%					
9	0.87%	- (WT)				+
25	0.73%					
53	0.70%					
65	0.66%		+			
64	0.65%	- (WT)			-	+
5	0.50%	-				
60	0.47%			- (WT)		+
41	0.44%	- (WT)			-	+
63	0.39%					+
56	0.37%					
59	0.29%	-		- (WT)		+
49	0.25%					
30	0.19%			- (WT)		+
7	0.17%	-				
17	0.08%			- (WT)		+
50	-0.05%					
18	-0.17%	- (WT)		- (WT)	-	+
47	-0.25%	- (WT)			-	+
68	-0.30%	- (WT)			-	+
67	-0.56%					+
20	-0.68%	-				
8	-0.84%	-				
2	-0.88%	-				
3	-1.75%	-				
62	-1.96%			- (WT)		
14	-2.29%	- (WT)			1 of 4	+
38	-3.38%					
43	-8.90%	-			2 of 4	+
52	-12.53%					1 of 4
WT		- (WT)		- (WT)		+

8/27/07

Some strains I had trouble phenotyping because they reverted to WT. For 8 strains (14, 18, 41, 43, 47, 52, 64, & 68) I replica plated both transformation plates to αF plates. I then picked 3 αF^R colonies off of each plate and grew them in 2x SC-his + αF and streaked 5 λ onto YPD, YPD+NaCl+αF, 2x SC-his, 2x SC-his + Gal/Raf. The results are on p. 77.

There is still some ambiguity between independent clones. Much of this difficulty may have arisen from the fact that I didn't restreak and purify the original αF mutants. I suspect that what I call "revertants" may actually be WT cells that came along when I picked colonies, and not true revertants. To demonstrate this I picked 3 single colonies (both big and small) of Δ68 (see p. 69) and found that they seem to propagate as pure cultures — although this was not done rigorously.

8/22/07

			αF + Salt sens.	Growth on Gal	Fitness defects on YPD & -his
1		++ 1			Yes
2		++ 2		+	
3	14	++ 3			Yes
4		–			
5		–			
6		++ 4			
7		+ 5		+	
8		++ 6	Yes	+	
9	18	++ 7		+	
10		+ 8	Yes		
11		++ 9			
12		+ 10	Yes		
13		–			
14		–			
15	41	++ 11		+	
16		++ 12			
17		–			
18		–			
19		++ 13		+	
20		++ 14		+	
21	43	++ 15		+	
22		++ 16			
23		++ 17			
24		++ 18			
25		+ 19		+	
26		++ 20		+ (No)	
27	47	++ 21		+	
28		++ 22			Yes
29		++ 23	Yes - very sens		
30		–			
31		++ 24		+	
32		++ 25		+	
33	52	++ 26		+	
34		++ 27			
35		++ 28			
36		++ 29			
37		++ 30		+	
38		++ 31		+	
39	64	++ 32		+	
40		–			
41		+ 33	Yes		
42		–			
43		++ 34		+	
44		–			
45	68	++ 35	Yes	+	
46		–			
47		++ 36			
48		–			

8/27/07

DISCUSSION OF PHENOTYPIC CHARACTERIZATIONS

Overall there was a lot of ambiguity in my phenotypic characterizations as seen on pages 75 & 77. Many times independent transformants did not behave the same way. In addition there was lots of reversion to wild-type and several places where it was difficult to score growth versus no growth (especially on high salt plates).

I will need to look through these results more carefully once I get to Princeton.

FUTURE DIRECTIONS

- I could try to publish what I have or I could continue working on this project. I may want to redo the original αF expt and purify the cultures before phenotyping. Likewise I could phenotype more carefully with other overexpression expts (such as STE7, or something else lower in the pathway).

other expts:

- Delete Mfα1 and Mfα2: measure fitness of these individual and double deletes in both Mata & Matα strains.
- Backcross spontaneous mutations by Far1-22 overexpression. This would show that the fitness benefit is due to a single mutation and from the same mutation as αFR. Also could be used to find mutation itself.
- Delete and fitness assays on shared components (Ste11, Ste20)
- Look at basal signaling from Fus1 promoter in both constructed and spontaneous mutants — show that spontaneous mut.s w/ fitness benefit is due to loss of basal signaling
- Find mutations in evolved αF^R strains or monitor the timecourse of evolution of αF^R.
- Simulations to determine under which αF^R should evolve.
  ↳ we know the mut. rate the target size and fitness benefit

11/8/07

Now that I am at Princeton I have decided to resurrect this project. Specifically there are two experiments I would like to do.

(1) Find αF^R mutations using Affymetrix tiling arrays

(2) Show that αF^R mutations, which increase fitness, do so by decreasing basal expression downstream of Ste12.

For both of these experiments I will use the top 7 spontaneous mutants with the highest selective coefficients from experiment 2. These are αF^R strains 37, 40, 58, 69, 54, 48, 29.

A few weeks ago I went up to Boston to get my strain collection, including the 96-well plates containing these αF^R mutants.

At that time I streaked the 7 strains, as well as the parental strain (GIL121), a ste7Δ (GIL), and a ste12Δ (onto YPD — let the strains grow for two days — parafilm and put in 4° fridge.

Yesterday I started 5 mL O/N cultures of GIL121, ste7, ste12, and αF^R29.

This morning I diluted the cultures 1:10 into YPD+αF. Since I had problems with picking WT colonies during the Gal-Overexpression expt. (see p. 75-78), I want to verify that the single colonies I pick for the arrays are really αF^R. This should not be a problem for these beneficial αF^R mutations, but just a precaution.

→ After 2.5 h I looked at the cells under the scope. Many shmoos in the GIL121 culture but only vegetatively growing cells in the other 3 as expected.

Since I am in a new lab, new media, new culture conditions, etc. I wanted to coulter count to see how dense the cultures were saturating.

Bkg	$1.01E^6$	$1.076E^6$
GIL121	$3.538E^8$	$3.541E^8$
ste7Δ	$3.57E^8$	$3.554E^8$
ste12Δ	$3.68E^8$	$3.707E^8$
αF^R29	$3.599E^8$	$3.617E^8$
Post Bkg	$1.096E^7$	$4.398E^6$

11/12/07

YEAST TILING ARRAYS TO UNCOVER αF^R MUTATIONS

gDNA preps

· Tonight I am going to inoculate 25mL of YPD with single colonies of GIL121, and the 7 fittest αF^R strains — 7:30pm.

11/13/07

· @ 12:00 - 100λ of each culture into 900λ of YPD+αF
@ 3:15 - looked at each culture - only GIL121 had shmoos as expected.

· QIAGEN gDNA preps
Klett @ start of prep.

GIL121	475
α29	450
α37	450
α40	450
α48	475
α54	450
α58	450
α69	425

· Used 20 mL of each culture for preps.
· All incubations were done for 1h.
· Resuspend pellet in 1mL TE (pH 8.0)

11/14/07

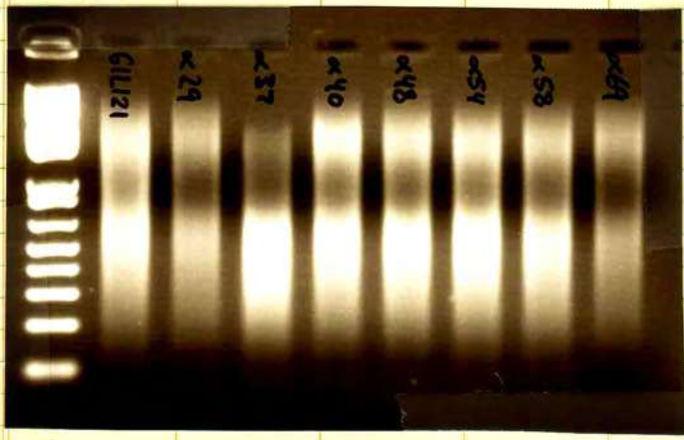
Nanodrop to determine concentration of gDNA preps

GIL121	56.1 ng/µl
α29	32.0
α37	39.5
α40	43.1
α48	41.7
α54	43.7
α58	40.1
α69	29.3

11/14/07

· Sonicate DNA (α37 was sonicated twice)

· Run on 2% gel



· Clean sample

↳ using Zymo Research clean kit → elute 25 λ H_2O

· Quantify using fluorometer

GIL121	~~25 ng/μl~~ 25.1 ng/μl	→	20 λ +	16 λ H_2O for labeling
α29	39.2		13	23
α37	50.0		10	26
α40	58.3		9	27
α48	36.4		14	22
α54	39.4		13	23
α58	52.5		10	26
α69	49.1		11	25

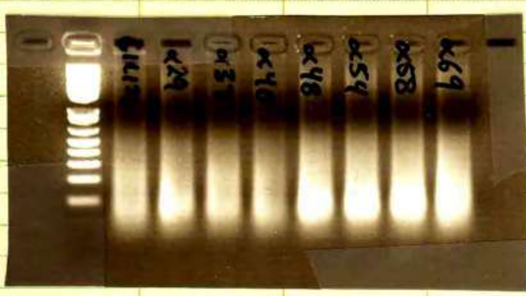
· Labeling started ~ 6:00pm

~~[illegible]~~ 11/15/07

- EtOH ppt labeled DNA → resuspend in 50λ H_2O
- Quantify on the fluorometer

GIL121	78.3 ng/μl	→	25.5 λ +	107.5 λ H_2O
α29	113		17.7	115.3
α37	90.7		22.1	110.9
α40	71.7		27.9	105.1
α48	141		14.2	118.8
α54	156		12.8	120.2
α58	151		13.2	119.8
α69	130		15.4	117.6

- Hybridization started ~ 4:30



11/20/07

Analysis of tiling array data

Overall this expt. seems to have been successful. I have been analyzing the data using the Affmetrix viewer IGB. Gresham helped with the data processing by running SNPscanner on my array data.

NOTES

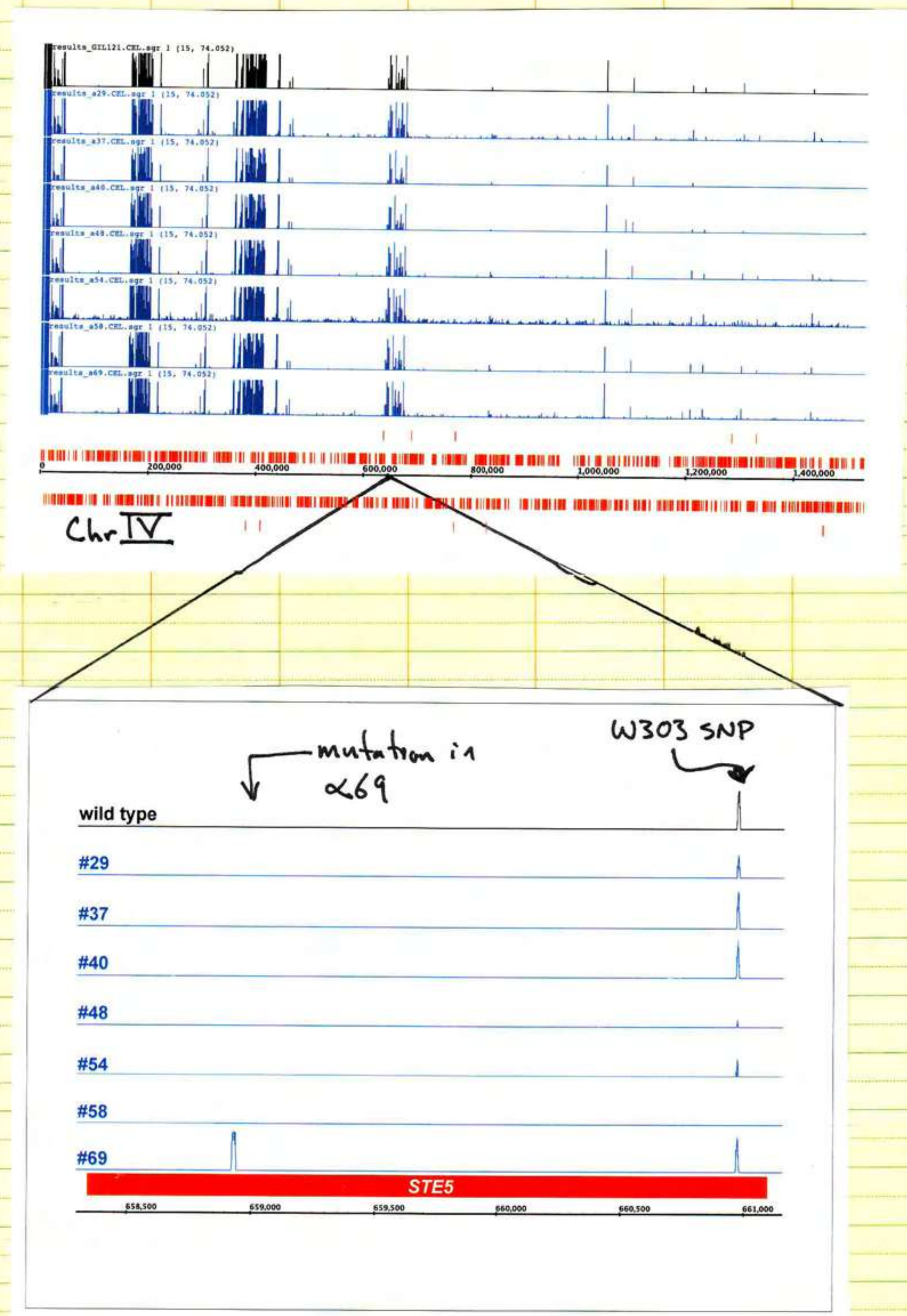
- α54 in particular is a bit noisy making it difficult to identify peaks
- As of today I have found a total of 10 SNPs ~~in my~~ between the 8 strains:

STRAIN	MUTATIONS	
alpha29		
alpha37	*APC1*	*EDS1*
alpha40	*SUM1*	
alpha48	*STE7*	*MEF2*
alpha54	*CPD1*	
alpha58	*STE11*	
alpha69	*STE5*	*IKS1*
WT	intragenic on Chr10 (~200 kb)	

11/20/07

Analysis of Array data continued

- It is apparent (as was known) that W303 is a mosaic strain with the majority of the genome having very good similarity to S288c with a few regions of great divergence:



11/30/07

Analysis of tiling array data (continued)

· I have tried a number of methods to find additional mutations; however I have not been able to find more than the 10 listed on page 82.

· Order primers to sequence these SNPS. I used SGD to design primers in a window 300 bp up/down stream of the predicted SNPs.

· I was surprised to find a SNP in Ste11 — I had tested all of these strains for salt sensitivity and found that all seven of these αF^R strains are salt-tolerant.

↳ I decided to retest these strains — I streaked GIL121, α29, α54, & α58 onto YPD + 1M NaCl

- To my surprise, one of these strains did not grow — α29
- I am currently retesting these 7 αF^R strains for salt-tolerance and the ability to sporulate.
- I may have to go back and retest all of the strains by first streaking to singles on YPD then assaying for αF^R and salt-tolerance and sporulation.

12/3/07

I am a little confused about this salt-sensitivity. I restreaked all ~~onto~~ of the strains onto 1M NaCl plates (on 11/30, see above) and all of the strains grew, including $\alpha F^R 29$ which I recognized as ~~~~ salt-sensitive last week. These were two independent colonies — both picked off of the same ~~~~ YPD plate and tested for αF^R — the first of these strains went onto the tiling array (which found nothing) and the second ~~~~ is being used in the expression analysis → I am not sure if this is true — they could be the same cultures

Possible explanations:

(1) Cell cycle - In the first expt. the cultures were very saturated — the salt sensitivity of $\alpha F^R 29$ could occurr only when the culture has become saturated — the second expt. cells were in exponential growth when streaked onto NaCl

(2) Supressor mutation

I also tested the following strains for sporulation (spin 1 mL sat. culture → resuspend in 3 mL 2% KAc): GIL121, αF^R 29, 37, 40, 48, 54, 58, 69, ste7Δ, GIL103 (diploid)

↳ only GIL103 sporulates.

12/5/07

SEQUENCING SNPs

- I ordered primers for each of the 10 SNPs
  - primers amplify ~300 bp up/downstream of each SNP.

34323842 IDT
G.LANG
36157586 11/28/2007
SNP1_APC1_F 021
5'- GTT TCA AAA TCT TGG GCT TGT -3'
Tm= 51.9 ºC , MW= 6,442.2
6.8OD$_{260}$ 34.50nmol 0.22mg

34323843 IDT
G.LANG
36157587 11/28/2007
SNP1_APC1_R 022
5'- TGG GCA TAT TCT GTC TTG GTG -3'
Tm= 54.9 ºC , MW= 6,474.2
6.4OD$_{260}$ 33.10nmol 0.21mg

34323852 IDT
G.LANG
36157596 11/28/2007
SNP6_CPD1_F 031
5'- TGT AGG CGT GAT CAA GTG CTT -3'
Tm= 56.7 ºC , MW= 6,492.3
8.4OD$_{260}$ 41.50nmol 0.27mg

34323853 IDT
G.LANG
36157597 11/28/2007
SNP6_CPD1_R 032
5'- CAC TCG AGC ACA ATT TTC TTG -3'
Tm= 52.4 ºC , MW= 6,356.2
6.7OD$_{260}$ 34.70nmol 0.22mg

34323844 IDT
G.LANG
36157588 11/28/2007
SNP2_EDS1_F 023
5'- CGG TTC CGC TCA ATT TTA TC -3'
Tm= 51.8 ºC , MW= 6,034.0
6.8OD$_{260}$ 37.80nmol 0.23mg

34323845 IDT
G.LANG
36157589 11/28/2007
SNP2_EDS1_R 024
5'- ACA CTG CCC CTT TTT TTT GC -3'
Tm= 54.8 ºC , MW= 5,984.9
7.3OD$_{260}$ 43.30nmol 0.26mg

34323854 IDT
G.LANG
36157598 11/28/2007
SNP7_STE11_F 033
5'- GGC AAA TAC TGA TTG GGG TT -3'
Tm= 53.1 ºC , MW= 6,212.1
6.5OD$_{260}$ 32.80nmol 0.20mg

34323855 IDT
G.LANG
36157599 11/28/2007
SNP7_STE11_R 034
5'- CCA GGG GTA TGA GAA TCA AAT -3'
Tm= 52.1 ºC , MW= 6,503.3
7.7OD$_{260}$ 35.00nmol 0.23mg

34323846 IDT
G.LANG
36157590 11/28/2007
SNP3_SUM1_F 025
5'- GCC TGC CTC TTT TTC TTT TG -3'
Tm= 52.5 ºC , MW= 6,006.9
5.9OD$_{260}$ 36.50nmol 0.22mg

34323847 IDT
G.LANG
36157591 11/28/2007
SNP3_SUM1_R 026
5'- CAC ACA ACC AAT CAG TTT [illegible] -3'
Tm= 52.6 ºC , MW= 6,374.2
6.0OD$_{260}$ 29.60nmol 0.19mg

34323856 IDT
G.LANG
36157600 11/28/2007
SNP8_STE5_F 035
5'- TAC ACC TTC TAC GGT TTC CA -3'
Tm= 53.0 ºC , MW= 6,002.9
7.3OD$_{260}$ 40.20nmol 0.24mg

34323857 IDT
G.LANG
36157601 11/28/2007
SNP8_STE5_R 036
5'- TCG TTT GTC TTT CAA CAG G -3'
Tm= 50.4 ºC , MW= 5,784.8
5.9OD$_{260}$ 33.80nmol 0.20mg

34323848 IDT
G.LANG
36157592 11/28/2007
SNP4_STE7_F 027
5'- GGG CTA AAA GTA AGA ATT TTC -3'
Tm= 47.2 ºC , MW= 6,493.3
8.2OD$_{260}$ 37.80nmol 0.25mg

34323849 IDT
G.LANG
36157593 11/28/2007
SNP4_STE7_R 028
5'- ACG GGG ATT GTG ATA GAG AA -3'
Tm= 52.9 ºC , MW= 6,270.1
7.6OD$_{260}$ 35.40nmol 0.22mg

34323858 IDT
G.LANG
36157602 11/28/2007
SNP9_IKS1_F 037
5'- GTG GTT CAA AGG GCA ATT CA -3'
Tm= 53.8 ºC , MW= 6,181.1
7.5OD$_{260}$ 37.10nmol 0.23mg

34323859 IDT
G.LANG
36157603 11/28/2007
SNP9_IKS1_R 038
5'- TAC AAC AGT ATT GCA GTG GCG -3'
Tm= 55.5 ºC , MW= 6,470.3
6.1OD$_{260}$ 29.00nmol 0.19mg

34323850 IDT
G.LANG
36157594 11/28/2007
SNP5_MEF2_F 029
5'- CAA CAA AAA TCT TTG CAT TGC -3'
Tm= 49.7 ºC , MW= 6,373.2
8.7OD$_{260}$ 43.10nmol 0.27mg

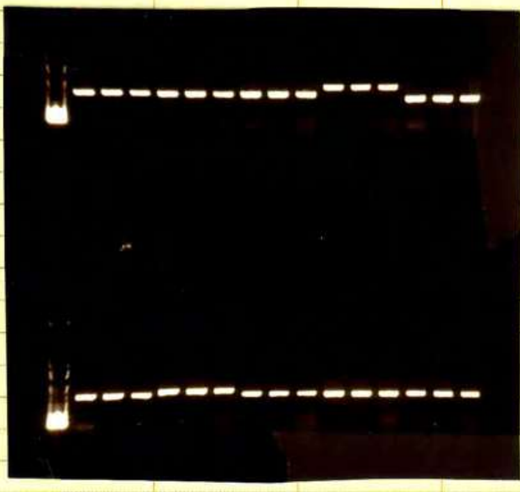
34323851 IDT
G.LANG
36157595 11/28/2007
SNP5_MEF2_R 030
5'- CGT TTG GCC AGT CTC ATC ATT -3'
Tm= 55.6 ºC , MW= 6,363.2
7.1OD$_{260}$ 37.50nmol 0.24mg

34323860 IDT
G.LANG
36157604 11/28/2007
SNP10_intragenicChrX_F
5'- TCG GAG AAA CCA TTG AC[illegible] -3' 039
Tm= 55.0 ºC , MW= 6,488.3
6.6OD$_{260}$ 30.60nmol 0.20mg

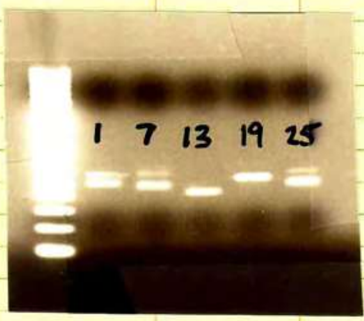
34323861 IDT
G.LANG
36157728 11/28/2007
SNP10_intragenicChrX_R
5'- GAT TAG GAC AAT CTT GGC AG -3' 040
Tm= 50.8 ºC , MW= 6,181.1
6.0OD$_{260}$ 29.80nmol 0.18mg

- I made gDNA preps from the cultures used for expression analysis
  ↳ this gives me gDNA preps from independent colonies - this will allow me to determine if any of the SNPs arose during sample processing
- PCR - standard protocol (50° anneal, 2 min extension)
- Qiagen Cleanup - elute 30λ
- Submit for sequencing to Genewiz.

After PCR:



After QIAGEN Cleanup:



LANE	gDNA	Primer Set
1	GIL121-1	SNP 1
2	α37-1	
3	α37-2	
4	GIL121-1	SNP 2
5	α37-1	
6	α37-2	
7	GIL121-1	SNP 3
8	α40-1	
9	α40-2	
10	GIL121-1	SNP 4
11	α48-1	
12	α48-2	
13	GIL121-1	SNP 5
14	α48-1	
15	α48-2	
16	GIL121-1	SNP 6
17	α54-1	
18	α54-2	
19	GIL121-1	SNP 7
20	α58-1	
21	α58-2	
22	GIL121-1	SNP 8
23	α69-1	
24	α69-2	
25	GIL121-1	SNP 9
26	α69-1	
27	α69-2	
28	α29-1	SNP 10
29	GIL121-1	
30	GIL121-2	

I looks like I picked up an extra band in 3 of these 5 that I looked at. Perhaps there is a contaminant in my Qiagen buffers. Hopefully this does not effect sequencing.

APC1

```
           2251                                                                                                         2340
      APC1 CTC GTA CAC TCT CCA AAT TTT TTA CCG GAT TAT GTT TTA GGC ATC CTT TCC AGC TTT AAA GTG GAT AAA GAC GAG TTA CAA ACA TAT CCC
    37_2_f   N NNN NNN NNN NNN NNN TTT TTA CCG GAT TAT GTT TTA GGC ATC CTT TCC AGC TTT AAA GTG GAT AAA GAC GAG TTA CAA ACA TAT CCC
  GIL121_f   N NNN NNN NNN NTC NNT TTT TTA CCG GAT TAT GTT TTA GGC ATC CTT TCC AGC TTT AAA GTG GAT AAA GAC GAG TTA CAA ACA TAT CCC
    37_1_f      NN NNN NNN NTC NAT TTT TTA CCG GAT TAT GTT TTA GGC ATC CTT TCC AGC TTT AAA GTG GAT AAA GAC GAG TTA CAA ACA TAT CCC
  GIL121_r CTC GTA CAC TCT CCA AAT TTT TTA CCG GAT TAT GTT TTA GGC ATC CTT TCC AGC TTT AAA GTG GAT AAA GAC GAG TTA CAA ACA TAT CCC
    37_1_r CTC GTA CAC TCT CCA AAT TTT TTA CCG GAT TAT GTT TTA GGC ATC CTT TCC AGC TTT AAA GTG GAT AAA GAC GAG TTA CAA ACA TAT CCC
    32_2_r CTC GTA CAC TCT CCA AAT TTT TTA CCG GAT TAT GTT TTA GGC ATC CTT TCC AGC TTT AAA GTG GAT AAA GAC GAG TTA CAA ACA TAT CCC
 Consensus ctc gta cac tct cca aat TTT TTA CCG GAT TAT GTT TTA GGC ATC CTT TCC AGC TTT AAA GTG GAT AAA GAC GAG TTA CAA ACA TAT CCC

           2341                                                                                                         2430
      APC1 TTA GGC ATT TTG GTG CCT TTA CAA AAC ATT TTA AAA ATT CTG GAA GAT AAG CTA TCT GAA GTA AGA GAC AAC TTG GAG TTA TTA GAT AGG
    37_2_f TTA GGC ATT TTG GTG CCT TTA CAA AAC ATT TTA AAA ATT CTG GAA GAT AAG CTA TCT GAA GTA AGA GAC AAC TTG GAG TTA TTA GAT AGG
  GIL121_f TTA GGC ATT TTG GTG CCT TTA CAA AAC ATT TTA AAA ATT CTG GAA GAT AAG CTA TCT GAA GTA AGA GAC AAC TTG GAG TTA TTA GAT AGG
    37_1_f TTA GGC ATT TTG GTG CCT TTA CAA AAC ATT TTA AAA ATT CTG GAA GAT AAG CTA TCT GAA GTA AGA GAC AAC TTG GAG TTA TTA GAT AGG
  GIL121_r TTA GGC ATT TTG GTG CCT TTA CAA AAC ATT TTA AAA ATT CTG GAA GAT AAG CTA TCT GAA GTA AGA GAC AAC TTG GAG TTA TTA GAT AGG
    37_1_r TTA GGC ATT TTG GTG CCT TTA CAA AAC ATT TTA AAA ATT CTG GAA GAT AAG CTA TCT GAA GTA AGA GAC AAC TTG GAG TTA TTA GAT AGG
    32_2_r TTA GGC ATT TTG GTG CCT TTA CAA AAC ATT TTA AAA ATT CTG GAA GAT AAG CTA TCT GAA GTA AGA GAC AAC TTG GAG TTA TTA GAT AGG
 Consensus TTA GGC ATT TTG GTG CCT TTA CAA AAC ATT TTA AAA ATT CTG GAA GAT AAG CTA TCT GAA GTA AGA GAC AAC TTG GAG TTA TTA GAT AGG

           2431                                                                                                         2520
      APC1 GCC GAT TTA CAA AGG TGT AGT GCC ATT ATC AAT AGT ATA AGA AGT GAT TCT AAA GAA GTT CTC AAA AGA GGC CAA AGA GAT AGT TAT ATG
    37_2_f GCC GAT TTA CAA AGG TGT AGT GCC ATT ATC AAT AGT ATA AGA AGT GAT TCT AAA GAA GTT CTC AAA AGA GGC CAA AGA GAT ATT TAT ATG
  GIL121_f GCC GAT TTA CAA AGG TGT AGT GCC ATT ATC AAT AGT ATA AGA AGT GAT TCT AAA GAA GTT CTC AAA AGA GGC CAA AGA GAT AGT TAT ATG
    37_1_f GCC GAT TTA CAA AGG TGT AGT GCC ATT ATC AAT AGT ATA AGA AGT GAT TCT AAA GAA GTT CTC AAA AGA GGC CAA AGA GAT ATT TAT ATG
  GIL121_r GCC GAT TTA CAA AGG TGT AGT GCC ATT ATC AAT AGT ATA AGA AGT GAT TCT AAA GAA GTT CTC AAA AGA GGC CAA AGA GAT AGT TAT ATG
    37_1_r GCC GAT TTA CAA AGG TGT AGT GCC ATT ATC AAT AGT ATA AGA AGT GAT TCT AAA GAA GTT CTC AAA AGA GGC CAA AGA GAT ATT TAT ATG
    32_2_r GCC GAT TTA CAA AGG TGT AGT GCC ATT ATC AAT AGT ATA AGA AGT GAT TCT AAA GAA GTT CTC AAA AGA GGC CAA AGA GAT ATT TAT ATG
 Consensus GCC GAT TTA CAA AGG TGT AGT GCC ATT ATC AAT AGT ATA AGA AGT GAT TCT AAA GAA GTT CTC AAA AGA GGC CAA AGA GAT AtT TAT ATG
```

AGT → ATT
S I

```
           2521                                                                                                         2610
      APC1 TTA TGC AAA GTG CCA CTC GCC AAG AAC AGA AGC TCT TTG TCT AAG AAG CCC AGT GAC ATA TAT TCA ATA TTA TCC GAG ATT GTG AAA AGT
    37_2_f TTA TGC AAA GTG CCA CTC GCC AAG AAC AGA AGC TCT TTG TCT AAG AAG CCC AGT GAC ATA TAT TCA ATA TTA TCC GAG ATT GTG AAA AGT
  GIL121_f TTA TGC AAA GTG CCA CTC GCC AAG AAC AGA AGC TCT TTG TCT AAG AAG CCC AGT GAC ATA TAT TCA ATA TTA TCC GAG ATT GTG AAA AGT
    37_1_f TTA TGC AAA GTG CCA CTC GCC AAG AAC AGA AGC TCT TTG TCT AAG AAG CCC AGT GAC ATA TAT TCA ATA TTA TCC GAG ATT GTG AAA AGT
  GIL121_r TTA TGC AAA GTG CCA CTC GCC AAG AAC AGA AGC TCT TTG TCT AAG AAG CCC AGT GAC ATA TAT TCA ATA TTA TCC GAG ATT GTG AAA AGT
    37_1_r TTA TGC AAA GTG CCA CTC GCC AAG AAC AGA AGC TCT TTG TCT AAG AAG CCC AGT GAC ATA TAT TCA ATA TTA TCC GAG ATT GTG AAA AGT
    32_2_r TTA TGC AAA GTG CCA CTC GCC AAG AAC AGA AGC TCT TTG TCT AAG AAG CCC AGT GAC ATA TAT TCA ATA TTA TCC GAG ATT GTG AAA AGT
 Consensus TTA TGC AAA GTG CCA CTC GCC AAG AAC AGA AGC TCT TTG TCT AAG AAG CCC AGT GAC ATA TAT TCA ATA TTA TCC GAG ATT GTG AAA AGT

           2611                                                                                                         2700
      APC1 GCA AGT CAA GTT CCA CTG GAT GGA TCA GCT ATG AGG ATG TCG AAT ATT CAG GAT GAT GAA GAT ATT GAT GAA GGT CGA TCT CTC AAA TTA
    37_2_f GCA AGT CAA GTT CCA CTG GAT GGA TCA GCT ATG AGG ATG TCG AAT ATT CAG GAT GAT GAA GAT ATT GAT GAA GGT CGA TCT CTC AAA TTA
  GIL121_f GCA AGT CAA GTT CCA CTG GAT GGA TCA GCT ATG AGG ATG TCG AAT ATT CAG GAT GAT GAA GAT ATT GAT GAA GGT CGA TCT CTC AAA TTA
    37_1_f GCA AGT CAA GTT CCA CTG GAT GGA TCA GCT ATG AGG ATG TCG AAT ATT CAG GAT GAT GAA GAT ATT GAT GAA GGT CGA TCT CTC AAA TTA
  GIL121_r GCA AGT CAA GTT CCA CTG GAT GGA TCA GCT ATG AGG ATG TCG AAT ATT CAG GAT GAT GAA GAT ATT GAT GAA GGT CGA TCT CTC AAA TTA
    37_1_r GCA AGT CAA GTT CCA CTG GAT GGA TCA GCT ATG AGG ATG TCG AAT ATT CAG GAT GAT GAA GAT ATT GAT GAA GGT CGA TCT CTC AAA TTA
    32_2_r GCA AGT CAA GTT CCA CTG GAT GGA TCA GCT ATG AGG ATG TCG AAT ATT CAG GAT GAT GAA GAT ATT GAT GAA GGT CGA TCT CTC AAA TTA
 Consensus GCA AGT CAA GTT CCA CTG GAT GGA TCA GCT ATG AGG ATG TCG AAT ATT CAG GAT GAT GAA GAT ATT GAT GAA GGT CGA TCT CTC AAA TTA

           2701                                                                                                         2790
      APC1 AAT GCA GGA CTG ATT TTT TCA GAA GAT AAA AGA TTT ACT CAT GTT GTA TCA CTA TTA GCT TAT TAC AGG CCC ACA AAA ACT CAA TTT TTT
    37_2_f AAT GCA GGA CTG ATT TTT TCA GAA GAT AAA AGA TTT ACT CAT GTT GTA TCA CTA TTA GCT TAT TAC AGG CCC ACA AAA ACT CAA TTT TTT
  GIL121_f AAT GCA GGA CTG ATT TTT TCA GAA GAT AAA AGA TTT ACT CAT GTT GTA TCA CTA TTA GCT TAT TAC AGG CCC ACA AAA ACT CAA TTT TTT
    37_1_f AAT GCA GGA CTG ATT TTT TCA GAA GAT AAA AGA TTT ACT CAT GTT GTA TCA CTA TTA GCT TAT TAC AGG CCC ACA AAA ACT CAA TTT TTT
  GIL121_r AAT GCA GGA CTG ATT TTT TCA GAA GAT AAA AGA TTT ACT CAT GTT GTA TCA CTA TTA GCT TAT AC
    37_1_r AAT GCA GGA CTG ATT TTT TCA GAA GAT AAA AGA TTT ACT CAT GTT GTA TCA CTA TTA GCN TAT AC
    32_2_r AAT GCA GGA CNG ATT TTT TCA GAA GAT AAA AGA TTT ACT CAT GTN GTA TCA CTA TTA GCT TAT AC
 Consensus AAT GCA GGA CtG ATT TTT TCA GAA GAT AAA AGA TTT ACT CAT GTt GTA TCA CTA TTA GCt TAT tac agg ccc aca aaa act caa ttt ttt
```

EDS1

```
           181                                                                                                          270
      EDS1                                                                                              ATG TCA CAC CAT GTT
  GIL121_f CTT TAT AGC TTT GGA ACC TTT CGT AGC TGT AGC CCG CTA CGT AGA AAT ACA TAA CAA TAG AAT AAT CTT TCA AAA ATG TCA CAC CAT GTT
    37_1_f CTT TAT AGC TTT GGA ACC TTT CGT AGC TGT AGC CCG CTA CGT AGA AAT ACA TAA CAA TAG AAT AAT CTT TCA AAA ATG TCA CAC CAT GTT
    37_2_f CTT TAT AGC TTT GGA ACC TTT CGT AGC TGT AGC CCG CTA CGT AGA AAT ACA TAA CAA TAG AAT AAT CTT TCA AAA ATG TCA CAC CAT GTT
    37_1_r CTT TAT AGC TTT GGA ACC TTT CGT AGC TGT AGC CCG CTA CGT AGA AAT ACA TAA CAA TAG AAT AAT CTT TCA AAA ATG TCA CAC CAT GTT
    32_2_r CTT TAT AGC TTT GGA ACC TTT CGT AGC TGT AGC CCG CTA CGT AGA AAT ACA TAA CAA TAG AAT AAT CTT TCA AAA ATG TCA CAC CAT GTT
  GIL121_r CTT TAT AGC TTT GGA ACC TTT CGT AGC TGT AGC CCG CTA CGT AGA AAT ACA TAA CAA TAG AAT AAT CTT TCA AAA ATG TCA CAC CAT GTT
 Consensus ctt tat agc ttt gga acc ttt cgt agc tgt agc ccg cta cgt aga aat aca taa caa tag aat aat ctt tca aaa ATG TCA CAC CAT GTT

           271                                                                                                          360
      EDS1 CCT AAT TTA TAT GGT ACA CCT ATT CGC GAC CCT CAC GAA CGC AAA CGC AAT TCA GCG TCA ATG GGC GAA GTA AAT CAG TCA GTT TCT TCG
  GIL121_f CCT AAT TTA TAT GGT ACA CCT ATT CGC GAC CCT CAC GAA CGC AAA CGC AAT TCA GCG TCA ATG GGC GAA GTA AAT CAG TCA GTT TCT TCG
    37_1_f CTT AAT TTA TAT GGT ACA CCT ATT CGC GAC CCT CAC GAA CGC AAA CGC AAT TCA GCG TCA ATG GGC GAA GTA AAT CAG TCA GTT TCT TCG
    37_2_f CTT AAT TTA TAT GGT ACA CCT ATT CGC GAC CCT CAC GAA CGC AAA CGC AAT TCA GCG TCA ATG GGC GAA GTA AAT CAG TCA GTT TCT TCG
    37_1_r CTT AAT TTA TAT GGT ACA CCT ATT CGC GAC CCT CAC GAA CGC AAA CGC AAT TCA GCG TCA ATG GGC GAA GTA AAT CAG TCA GTT TCT TCG
    32_2_r CTT AAT TTA TAT GGT ACA CCT ATT CGC GAC CCT CAC GAA CGC AAA CGC AAT TCA GCG TCA ATG GGC GAA GTA AAT CAG TCA GTT TCT TCG
  GIL121_r CCT AAT TTA TAT GGT ACA CCT ATT CGC GAC CCT CAC GAA CGC AAA CGC AAT TCA GCG TCA ATG GGC GAA GTA AAT CAG TCA GTT TCT TCG
 Consensus CtT AAT TTA TAT GGT ACA CCT ATT CGC GAC CCT CAC GAA CGC AAA CGC AAT TCA GCG TCA ATG GGC GAA GTA AAT CAG TCA GTT TCT TCG
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CCT → CTT
P L

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           361                                                                                                          450
      EDS1 AGG AAT TGT GAA AGA GGA AGT GAA AAG GGC ACG AAG CAA AGG AAG AAA GCC TCT CAC GCT TGT GAC CAA TGT AGA AGG AAA AGA ATC AAG
  GIL121_f AGG AAT TGT GAA AGA GGA AGT GAA AAG GGC ACG AAG CAA AGG AAG AAA GCC TCT CAC GCT TGT GAC CAA TGT AGA AGG AAA AGA ATC AAG
    37_1_f AGG AAT TGT GAA AGA GGA AGT GAA AAG GGC ACG AAG CAA AGG AAG AAA GCC TCT CAC GCT TGT GAC CAA TGT AGA AGG AAA AGA ATC AAG
    37_2_f AGG AAT TGT GAA AGA GGA AGT GAA AAG GGC ACG AAG CAA AGG AAG AAA GCC TCT CAC GCT TGT GAC CAA TGT AGA AGG AAA AGA ATC AAG
    37_1_r AGG AAT TGT GAA AGA GGA AGT GAN AAG GGC ACG AAG CAA AGG AAG AAA GCC TCT CAC GCT TGT GAC CAA TGT AGA AGG AAA AGA ATC AAG
    32_2_r AGG AAT TGT GAA AGA GGA AGT GAA AAG GGC ACG AAG CAA AGG AAG AAA GCC TCT CAC GCT TGT GAC CAA TGT AGA AGG AAA AGA ATC AAG
  GIL121_r AGG AAT TGT GAA AGA GGA AGT GAA AAG GGC ACG AAN CNA AGG AAG AAA GCC TCT CAC GCT TGT GAC CNA NGT AGA AGG AAA AGA ATC AAG
 Consensus AGG AAT TGT GAA AGA GGA AGT GAa AAG GGC ACG AAg CaA AGG AAG AAA GCC TCT CAC GCT TGT GAC CaA tGT AGA AGG AAA AGA ATC AAG

           451                                                                                                          540
      EDS1 TGC AGG TTT GAT AAA CAT ACC GGC GTA TGT CAA GGG TGC TTA GAA GTC GGT GAA AAG TGT CAA TTT ATT AGA GTT CCA TTG AAA CGT GGT
  GIL121_f TGC AGG TTT GAT AAA CAT ACC GGC GTA TGT CAA GGG TGC TTA GAA GTC GGT GAA AAG TGT CAA TTT ATT AGA GTT CCA TTG AAA CGT GGT
    37_1_f TGC AGG TTT GAT AAA CAT ACC GGC GTA TGT CAA GGG TGC TTA GAA GTC GGT GAA AAG TGT CAA TTT ATT AGA GTT CCA TTG AAA CGT GGT
    37_2_f TGC AGG TTT GAT AAA CAT ACC GGC GTA TGT CAA GGG TGC TTA GAA GTC GGT GAA AAG TGT CAA TTT ATT AGA GTT CCA TTG AAA CGT GGT
    37_1_r TGC AGG TTT GAT AAA CAT ACC GGC GTA TGT CAA GGG TGC TTA GAA GTC GGN NAA AAG NGT CAA TTA TAG A
    32_2_r TGC AGG TTT GAT AAA CAT ACC GGC GTA TGT CAA GGG TGC TTA GAA GTC GGN GAA AAG TGT CAA TTA TAG A
  GIL121_r TGC AGG TTT GAT AAA CAT ACC GGC GTA TGT CAA GGG TGC TTA GAA GTC GG
 Consensus TGC AGG TTT GAT AAA CAT ACC GGC GTA TGT CAA GGG TGC TTA GAA GTC GGt gaa aag tgt caa ttt att aga gtt cca ttg aaa cgt ggt
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SUMI

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           451                                                                                                                                    540
      SUM1 AAG AAT CAA GTA AAT ACT GAC ATA TTG GTC AAT AAT TTG AAT GAA CGA TTG AAC AAA TTA TCG ACA ATG TTG CAA AAT ACT TCG AAA GTA
    40_2_r TGN NNT CAA NTA AAT ACT GAC ATA TTG GTC AAT AAT TTG AAT GAA CGA TTG AAC AAA TTA TCG ACA ATG TTG CAA AAT ACT TCG AAA GTA
  GIL121_r NNN NGA NNN NTA AAT ACT GAC ATA TTG GTC AAT AAT TTG AAT GAA CGA TTG AAC AAA TTA TCG ACA ATG TTG CAA AAT ACT TCG AAA GTA
    40_1_r TNA NAN CAA GTA AAT ACT GAC ATA TTG GTC AAT AAT TTG AAT GAA CGA TTG AAC AAA TTA TCG ACA ATG TTG CAA AAT ACT TCG AAA GTA
  GIL121_f AAG AAT CAA GTA AAT ACT GAC ATA TTG GTC AAT AAT TTG AAT GAA CGA TTG AAC AAA TTA TCG ACA ATG TTG CAA AAT ACT TCG AAA GTA
    40_2_f AAG AAT CAA GTA AAT ACT GAC ATA TTG GTC AAT AAT TTG AAT GAA CGA TTG AAC AAA TTA TCG ACA ATG TTG CAA AAT ACT TCG AAA GTA
    40_1_f AAG AAT CAA GTA AAT ACT GAC ATA TTG GTC AAT AAT TTG AAT GAA CGA TTG AAC AAA TTA TCG ACA ATG TTG CAA AAT ACT TCG AAA GTA
 Consensus aag aat caa gTA AAT ACT GAC ATA TTG GTC AAT AAT TTG AAT GAA CGA TTG AAC AAA TTA TCG ACA ATG TTG CAA AAT ACT TCG AAA GTA

           541                                                                                                                                    630
      SUM1 AAT CAC TCT AAC CTT CTC ATA GAA AAT TCA TCC AAC AAT ACC AGT TCA CAG CAT AAT ACC TCT TCT TCT CGA AGG GGA CCT GGC AGG CCA
    40_2_r AAT CAC TCT AAC CTT CTC ATA GAA AAT TCA TCC AAC AAT ACC AGT TCA CAG CAT AAT ACC TCT TCT TCT CGA AGG GGA CCT GGC AGG CCA
  GIL121_r AAT CAC TCT AAC CTT CTC ATA GAA AAT TCA TCC AAC AAT ACC AGT TCA CAG CAT AAT ACC TCT TCT TCT CGA AGG GGA CCT GGC AGG CCA
    40_1_r AAT CAC TCT AAC CTT CTC ATA GAA AAT TCA TCC AAC AAT ACC AGT TCA CAG CAT AAT ACC TCT TCT TCT CGA AGG GGA CCT GGC AGG CCA
  GIL121_f AAT CAC TCT AAC CTT CTC ATA GAA AAT TCA TCC AAC AAT ACC AGT TCA CAG CAT AAT ACC TCT TCT TCT CGA AGG GGA CCT GGC AGG CCA
    40_2_f AAT CAC TCT AAC CTT CTC ATA GAA AAT TCA TCC AAC AAT ACC AGT TCA CAG CAT AAT ACC TCT TCT TCT CGA AGG GGA CCT GGC AGG CCA
    40_1_f AAT CAC TCT AAC CTT CTC ATA GAA AAT TCA TCC AAC AAT ACC AGT TCA CAG CAT AAT ACC TCT TCT TCT CGA AGG GGA CCT GGC AGG CCA
 Consensus AAT CAC TCT AAC CTT CTC ATA GAA AAT TCA TCC AAC AAT ACC AGT TCA CAG CAT AAT ACC TCT TCT TCT CGA AGG GGA CCT GGC AGG CCA

           631                                                                                                                                    720
      SUM1 AGA AAA GAT GCC TCG ACC TCC ACA ATG AAT AAG TTA GTG TCA AAC GCA GCC TCG GTC AAT CTC AAA AGT GCA TCC AAT CAG GGT GCT CCT
    40_2_r AGA AAA GAT GCC TCG ACC TCC ACA ATG AAT AAG TTA GTG TCA AAC GCA GCC TCG GTC AAT CTC AAA AGT GCA TCC AAT CAG GGT GCT CCT
  GIL121_r AGA AAA GAT GCC TCG ACC TCC ACA ATG AAT AAG TTA GTG TCA AAC GCA GCC TCG GTC AAT CTC AAA AGT GCA TCC AAT CAG GGT GCT CCT
    40_1_r AGA AAA GAT GCC TCG ACC TCC ACA ATG AAT AAG TTA GTG TCA AAC GCA GCC TCG GTC AAT CTC AAA AAT GCA TCC AAT CAG GGT GCT CCT
  GIL121_f AGA AAA GAT GCC TCG ACC TCC ACA ATG AAT AAG TTA GTG TCA AAC GCA GCC TCG GTC AAT CTC AAA AGT GCA TCC AAT CAG GGT GCT CCT
    40_2_f AGA AAA GAT GCC TCG ACC TCC ACA ATG AAT AAG TTA GTG TCA AAC GCA GCC TCG GTC AAT CTC AAA AGT GCA TCC AAT CAG GGT GCT CCT
    40_1_f AGA AAA GAT GCC TCG ACC TCC ACA ATG AAT AAG TTA GTG TCA AAC GCA GCC TCG GTC AAT CTC AAA AAT GCA TCC AAT CAG GGT GCT CCT
 Consensus AGA AAA GAT GCC TCG ACC TCC ACA ATG AAT AAG TTA GTG TCA AAC GCA GCC TCG GTC AAT CTC AAA AgT GCA TCC AAT CAG GGT GCT CCT
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AGT → AAT

S N

in only 1 clone

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           721                                                                                                                                    810
      SUM1 TTC TCA CCA GTA AAT ATT ACT TTA CCG ACT GCC GTA GTA CAA ACG TCT AAA TCC AAA AGG TAT TTT GTG GAA CCA TCA ACG AAA CAA GAG
    40_2_r TTC TCA CCA GTA AAT ATT ACT TTA CCG ACT GCC GTA GTA CAA ACG TCT AAA TCC AAA AGG TAT TTT GTG GAA CCA TCA ACG AAA CAA GAG
  GIL121_r TTC TCA CCA GTA AAT ATT ACT TTA CCG ACT GCC GTA GTA CAA ACG TCT AAA TCC AAA AGG TAT TTT GTG GAA CCA TCA ACG AAA CAA GAG
    40_1_r TTC TCA CCA GTA AAT ATT ACT TTA CCG ACT GCC GTA GTA CAA ACG TCT AAA TCC AAA AGG TAT TTT GTG GAA CCA TCA ACG AAA CAA GAG
  GIL121_f TTC TCA CCA GTA AAT ATT ACT TTA CCG ACT GCC GTA GTA CAA ACG TCT AAA TCC AAA AGG TAT TTT GTG GAA CCA TCA ACG AAA CAA GAG
    40_2_f TTC TCA CCA GTA AAT ATT ACT TTA CCG ACT GCC GTA GTA CAA ACG TCT AAA TCC AAA AGG TAT TTT GTG GAA CCA TCA ACG AAA CAA GAG
    40_1_f TTC TCA CCA GTA AAT ATT ACT TTA CCG ACT GCC GTA GTA CAA ACG TCT AAA TCC AAA AGG TAT TTT GTG GAA CCA TCA ACG AAA CAA GAG
 Consensus TTC TCA CCA GTA AAT ATT ACT TTA CCG ACT GCC GTA GTA CAA ACG TCT AAA TCC AAA AGG TAT TTT GTG GAA CCA TCA ACG AAA CAA GAG

           811                                                                                                                                    900
      SUM1 TCG CTT TTA TTA TCT GCC CCT TCA TCA TCA AGA GAT GAT GCT GAT ATG TCC TTG ACT TCC GTA CCG CAA AGA ACG AAC AAT GAA AAT GGT
    40_2_r TCG CTT TTA TTA TCT GCC CCT TCA TCA TCA AGA GAT GAT GCT GAT ATG TCC TTG ACT TCC GTA CCG CAA AGA ACG AAC AAT GAA AAT GGT
  GIL121_r TCG CTT TTA TTA TCT GCC CCT TCA TCA TCA AGA GAT GAT GCT GAT ATG TCC TTG ACT TCC GTA CCG CAA AGA ACG AAC AAT GAA AAT GGT
    40_1_r TCG CTT TTA TTA TCT GCC CCT TCA TCA TCA AGA GAT GAT GCT GAT ATG TCC TTG ACT TCC GTA CCG CAA AGA ACG AAC AAT GAA AAT GGT
  GIL121_f TCG CTT TTA TTA TCT GCC CCT TCA TCA TCA AGA GAT GAT GCT GAT ATG TCC TTG ACT TCC GTA CCG CAA AGA ACG AAC AAT GAA AAN NGT
    40_2_f TCG CTT TTA TTA TCT GCC CCT TCA TCA TCA AGA GAT GAT GCT GAT ATG TCC TTG ACT TCC GTA CCG CAA AGA ACG AAC AAT GAA AAT GGT
    40_1_f TCG CTT TTA TTA TCT GCC CCT TCA TCA TCA AGA GAT GAT GCT GAT ATG TCC TTG ACT TCC GTA CCG CAA AGA ACG AAC AAT GAA AAT GGT
 Consensus TCG CTT TTA TTA TCT GCC CCT TCA TCA TCA AGA GAT GAT GCT GAT ATG TCC TTG ACT TCC GTA CCG CAA AGA ACG AAC AAT GAA AAt gGT

           901                                                                                                                                    990
      SUM1 AAA GAG CGA CCA TCC ACT GCT AAT TCT AGC TCC ATC ACA CCA ACA CCT GTT ACG CCG AAC AAC TTA ATT CAA ATC AAA AGA AAA AGA GGC
    40_2_r AAA GAG CGA CCA TCC ACT GCT AAT TCT AGC TCC ATC ACA CCA ACA CCT GTT ACG CCG AAC AAC TTA ATT CAA ATC AAA AGN NNA NNN NGN
  GIL121_r AAA GAG CGA CCA TCC ACT GCT AAT TCT AGC TCC ATC ACA CCA ACA CCT GTT ACG CCG AAC AAC TTA ATT CAA ATC AAA AGA NNA NNN NNN
    40_1_r AAA GAG CGA CCA TCC ACT GCT AAT TCT AGC TCC ATC ACA CCA ACA CCT GTT ACG CCG AAC AAC TTA ATT CAA ATC AAA AGA NAN NNN NNN
  GIL121_f AAA GAG CGA CCA TCC ACT GCT AAT TCT AGC TCC NTC ACA CCA ACA C
    40_2_f AAA GAG CGA CCA TCC ACT GCT AAT TCT AGC TCC NTC ACA CCA ACA CC
    40_1_f AAA GAG CGA CCA TCC ACT GCT AAT TCT AGC TCC ATC ACA CCA ACA CC
 Consensus AAA GAG CGA CCA TCC ACT GCT AAT TCT AGC TCC aTC ACA CCA ACA Cct gtt acg ccg aac aac tta att caa atc aaa ag. ... ... ...
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STE7

TTA CAG → TAA -AG

L Q *

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           361                                                                                                                                    450
      STE7
  GIL121_f ACC TAC AGG TTA ATG TGA AGC GAC ACT AAT GAT AAG AAA ACT GCA TCG GAT CAT ATC TGT TTT TGC AGC GTG GTA TAT TGG TTG TTG GTC
    48_1_f ACC TAC AGG TTA ATG TGA AGC GAC ACT AAT GAT AAG AAA ACT GCA TCG GAT CAT ATC TGT TTT TGC AGC GTG GTA TAT TGG TTG TTG GTC
    48_2_f ACC TAC AGG TTA ATG TGA AGC GAC ACT AAT GAT AAG AAA ACT GCA TCG GAT CAT ATC TGT TTT TGC AGC GTG GTA TAT TGG TTG TTG GTC
    48_1_r ACC TAC AGG TTA ATG TGA AGC GAC ACT AAT GAT AAG AAA ACT GCA TCG GAT CAT ATC TGT TTT TGC AGC GTG GTA TAT TGG TTG TTG GTC
    48_2_r ACC TAC AGG TTA ATG TGA AGC GAC ACT AAT GAT AAG AAA ACT GCA TCG GAT CAT ATC TGT TTT TGC AGC GTG GTA TAT TGG TTG TTG GTC
  GIL121_r ACC TAC AGG TTA ATG TGA AGC GAC ACT AAT GAT AAG AAA ACT GCA TCG GAT CAT ATC TGT TTT TGC AGC GTG GTA TAT TGG TTG TTG GTC
 Consensus acc tac agg tta atg tga agc gac act aat gat aag aaa act gca tcg gat cat atc tgt ttt tgc agc gtg gta tat tgg ttg ttg gtc

           451                                                                                                                                    540
      STE7 ATG TTT CAA CGA AAG ACT TTA CAG AGA AGG AAC TTG AAA GGG CTC AAT CTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG
  GIL121_f ATG TTT CAA CGA AAG ACT TTA CAG AGA AGG AAC TTG AAA GGG CTC AAT CTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG
    48_1_f ATG TTT CAA CGA AAG ACT TAA -AG AGA AGG AAC TTG AAA GGG CTC AAT CTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG
    48_2_f ATG TTT CAA CGA AAG ACT TAA -AG AGA AGG AAC TTG AAA GGG CTC AAT CTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG
    48_1_r ATG TTT CAA CGA AAG ACT TAA -AG AGA AGG AAC TTG AAA GGG CTC AAT CTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG
    48_2_r ATG TTT CAA CGA AAG ACT TAA -AG AGA AGG AAC TTG AAA GGG CTC AAT CTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG
  GIL121_r ATG TTT CAA CGA AAG ACT TTA CAG AGA AGG AAC TTG AAA GGG CTC AAT CTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG
 Consensus ATG TTT CAA CGA AAG ACT TaA .AG AGA AGG AAC TTG AAA GGG CTC AAT CTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG

           541                                                                                                                                    630
      STE7 GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT CGA ATA GAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT
  GIL121_f GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT CGA ATA GAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT
    48_1_f GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT CGA ATA GAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT
    48_2_f GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT CGA ATA GAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT
    48_1_r GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT CGA ATA GAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT
    48_2_r GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT CGA ATA GAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT
  GIL121_r GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT CGA ATA GAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT
 Consensus GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT CGA ATA GAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT

           631                                                                                                                                    720
      STE7 TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CAA
  GIL121_f TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CAA
    48_1_f TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CAA
    48_2_f TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CAA
    48_1_r TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CAA
    48_2_r TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CAA
  GIL121_r TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CAA
 Consensus TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CAA

           721                                                                                                                                    810
      STE7 CCG CAA AAT GAA CCT GTT TTA GTT CTG TCT TCT CTA TCA CAA TCC CCG TGT GTA TCA TCA TCA TCA TCT TTG TCC ACG CCA TGC ATT ATA
  GIL121_f CCG CAA AAT GAA CCT GTT TTA GTT CTG TCT TCT NNN NNN NNN NCC NNN NNN
    48_1_f CCG CAA AAT GAA CCT GTT TTA GTT CTG TCT TCN NNN NNN NNN CCC NAN NNN NNN
    48_2_f CCG CAA AAT GAA CCT GTT TTA GTT CTG TCT TNN NNN NNN NNN CCC CNA NNN
    48_1_r CCG CAA
    48_2_r CCG CAA
  GIL121_r CCG CAA
 Consensus CCG CAA aat gaa cct gtt tta gtt ctg tct t.. ... ... ... .cc ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
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MEF2

48_1_f
48_1_r
48_2_f
48_2_r

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          1081                                                                                                              1170
     MEF2 CTT GCA TTC AAA GTG ATT ACG GAT CCA ATC AGG GGT AAA CAA ATA TTC ATT AGA ATT TAT TCA GGT ACA CTA AAC AGT GGC AAT ACC GTT
   41_2_f         NNN NNN NNN NNN TAN GGA NCC ATC NGG GGT AAA CAA ATA TTC NTT AGA ATT TAT TCA GGT ACA CTA AAC AGT GGC AAT ACC GTT
   48_1_f       N NNN NNN NNN NNN NNN GGA NCC ATC NGG GGT AAA CAA ATA TTC ATT AGA ATT TAT TCA GGT ACA CTA AAC AGT GGC AAT ACC GTT
 GIL121_f     NNN NNN NNN NNN NNN NNN NNN NNN NTC NGG GGT AAA CAA ATA TTC ATT AGA ATT TAT TCA GGT ACA CTA AAC AGT GGC AAT ACC GTT
 GIL121_r CTT GCA TTC AAA GTG ATT ACG GAT CCA ATC AGG GGT AAA CAA ATA TTC ATT AGA ATT TAT TCA GGT ACA CTA AAC AGT GGC AAT ACC GTT
   40_2_r CTT GCA TTC AAA GTG ATT ACG GAT CCA ATC AGG GGT AAA CAA ATA TTC ATT AGA ATT TAT TCA GGT ACA CTA AAC AGT GGC AAT ACC GTT
   40_1_r CTT GCA TTC AAA GTG ATT ACG GAT CCA ATC AGG GGT AAA CAA ATA TTC ATT AGA ATT TAT TCA GGT ACA CTA AAC AGT GGC AAT ACC GTT
Consensus ctt gca ttc aaa gtg att acg gat cca aTC aGG GGT AAA CAA ATA TTC aTT AGA ATT TAT TCA GGT ACA CTA AAC AGT GGC AAT ACC GTT

          1171                                                                                                              1260
     MEF2 TAT AAT TCT ACT ACT GGG GAA AAA TTT AAA TTG GGT AAA TTA CTG ATA CCC CAT GCG GGA ACA TCA CAA CCT GTA AAT ATT TTA ACT GCG
   41_2_f TAT AAT TCT ACT ACT GGG GAA AAA TTT AAA TTG GGT AAA TTA CTG ATA CCC CAT GCG GGA ACA TCA CAA CCT GTA AAT ATT TTA ACT GCG
   48_1_f TAT AAT TCT ACT ACT GGG GAA AAA TTT AAA TTG GGT AAA TTA CTG ATA CCC CAT GCG GGA ACA TCA CAA CCT GTA AAT ATT TTA ACT GCG
 GIL121_f TAT AAT TCT ACT ACT GGG GAA AAA TTT AAA TTG GGT AAA TTA CTG ATA CCC CAT GCG GGA ACA TCA CAA CCT GTA AAT ATT TTA ACT GCG
 GIL121_r TAT AAT TCT ACT ACT GGG GAA AAA TTT AAA TTG GGT AAA TTA CTG ATA CCC CAT GCG GGA ACA TCA CAA CCT GTA AAT ATT TTA ACT GCG
   40_2_r TAT AAT TCT ACT ACT GGG GAA AAA TTT AAA TTG GGT AAA TTA CTG ATA CCC CAT GCG GGA ACA TCA CAA CCT GTA AAT ATT TTA ACT GCG
   40_1_r TAT AAT TCT ACT ACT GGG GAA AAA TTT AAA TTG GGT AAA TTA CTG ATA CCC CAT GCG GGA ACA TCA CAA CCT GTA AAT ATT TTA ACT GCG
Consensus TAT AAT TCT ACT ACT GGG GAA AAA TTT AAA TTG GGT AAA TTA CTG ATA CCC CAT GCG GGA ACA TCA CAA CCT GTA AAT ATT TTA ACT GCG

          1261                                                                                                              1350
     MEF2 GGC CAA ATC GGA TTG CTT ACT GGC TCT ACA GTC GAA AAC AAC ATA TCT ACT GGA GAT ACA CTA ATA ACG CAT TCG TCG AAG AAG GAT GGG
   41_2_f GGC CAA ATC GGA TTG CTT ACT GGC TCT ACA GTC GAA AAC AAC ATA TCT ACT GGA GAT ACA CTA ATA ACG CAT TCG TCG AAG AAG GAT GGG
   48_1_f GGC CAA ATC GGA TTG CTT ACT GGC TCT ACA GTC GAA AAC AAC ATA TCT ACT GGA GAT ACA CTA ATA AAG CAT TCG TCG AAG AAG GAT GGG
 GIL121_f GGC CAA ATC GGA TTG CTT ACT GGC TCT ACA GTC GAA AAC AAC ATA TCT ACT GGA GAT ACA CTA ATA ACG CAT TCG TCG AAG AAG GAT GGG
 GIL121_r GGC CAA ATC GGA TTG CTT ACT GGC TCT ACA GTC GAA AAC AAC ATA TCT ACT GGA GAT ACA CTA ATA ACG CAT TCG TCG AAG AAG GAT GGG
   40_2_r GGC CAA ATC GGA TTG CTT ACT GGC TCT ACA GTC GAA AAC AAC ATA TCT ACT GGA GAT ACA CTA ATA ACG CAT TCG TCG AAG AAG GAT GGG
   40_1_r GGC CAA ATC GGA TTG CTT ACT GGC TCT ACA GTC GAA AAC AAC ATA TCT ACT GGA GAT ACA NTA ATA AAG CAT TCG TCG AAG AAG GAT GGG
Consensus GGC CAA ATC GGA TTG CTT ACT GGC TCT ACA GTC GAA AAC AAC ATA TCT ACT GGA GAT ACA cTA ATA AcG CAT TCG TCG AAG AAG GAT GGG

          1351                                                                                                              1440
     MEF2 TTG AAA TCA CTT GAT AAA AAA AAG GAG TTG ACG TTA AAA ATC AAT TCT ATC TTC ATT CCC CCA CCA GTT TTT GGT GTT TCC ATC GAA CCA
   41_2_f TTG AAA TCA CTT GAT AAA AAA AAG GAG TTG ACG TTA AAA ATC AAT TCT ATC TTC ATT CCC CCA CCA GTT TTT GGT GTT TCC ATC GAA CCA
   48_1_f TTG AAA TCA CTT GAT AAA AAA AAG GAG TTG ACG TTA AAA ATC AAT TCT ATC TTC ATT CCC CCA CCA GTT TTT GGT GTT TCC ATC GAA CCA
 GIL121_f TTG AAA TCA CTT GAT AAA AAA AAG GAG TTG ACG TTA AAA ATC AAT TCT ATC TTC ATT CCC CCA CCA GTT TTT GGT GTT TCC ATC GAA CCA
 GIL121_r TTG AAA TCA CTT GAT AAA AAA AAG GAG TTG ACG TTA AAA ATC AAT TCT ATC TTC ATT CCC CCA CCA GTT TTT GGT GTT TCC ATC GAA CCA
   40_2_r TTG AAA TCA CTT GAT AAA AAA AAG GAG TTG ACG TTA AAA ATC AAT TCT ATC TTC ATT CCC CCA CCA GTT TTT GGT GTT TCC ATC GAA CCA
   40_1_r TTG AAA TCA CTT GAT AAA AAA AAG GAG TTG ACG TTA AAA ATC AAT TNT NTC TTC ATT CCC CCA CCA GTT TTT GGT GTT TCC ATC GNN NNN
Consensus TTG AAA TCA CTT GAT AAA AAA AAG GAG TTG ACG TTA AAA ATC AAT TcT aTC TTC ATT CCC CCA CCA GTT TTT GGT GTT TCC ATC Gaa cca

          1441                                                                                                              1530
     MEF2 AGG ACT TTA AGT AAT AAA AAA TCG ATG GAA GAA GCT TTA AAC ACT TTA ATT ACT GAA GAT CCC AGT CTT TCA ATT TCT CAA AAT GAT GAG
   41_2_f AGG ACT TTA AGT AAT AAA AAA TCG ATG GAA GAA GCT TTA AAC ACT TTA ATT ACT GAA GAT CCC AGT CTT TCA ATT TCT CAA AAT GAT NAN
   48_1_f AGG ACT TTA AGT AAT AAA AAA TCG ATG GAA GAA GCT TTA AAC ACT TTA ATT ACT GAA GAT CCC AGT CTT TCA ATT TCT CAA AAT GAT NNN
 GIL121_f AGG ACT TTA AGT AAT AAA AAA TCG ATG GAA GAA GCT TTA AAC ACT TTA ATT ACT GAA GAT CCC AGT CTT TCA ATT TCT CAA AAT GAT NNN
 GIL121_r AGG ACT TTA AGT AAT AAA AAA TCG ATG GAA GAA GCT TTA ANC ACT TTA ATT
   40_2_r AGG ACT TTA AGT AAT AAA AAA TCG ATG GAA GAA GCT TTA A
   40_1_r NGG NCT TTA AGT AAT AAA AAA TCG ATG GAA GAA GCT TTA A
Consensus aGG aCT TTA AGT AAT AAA AAA TCG ATG GAA GAA GCT TTA Aac act tta att act gaa gat ccc agt ctt tca att tct caa aat gat ...
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ACG → AAG
T K
only in 1 clone

CPD1

CAC → CAG
H Q
only in 1 clone

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          181                                                                                                               270
     CPD1                                                                                  A TGG CAA TAG CAT TAT GGT ATT GTC
 GIL121_f TTG TAA TTA TTC TAG TTT GGG AAT TCG CGA TAC TTC AAT TTG CTA ATA TCA GTA ACC ACA GAA AAA TGG CAA TAG CAT TAT GGT ATT GTC
   54_1_f TTG TAA TTA TTC TAG TTT GGG AAT TCG CGA TAC TTC AAT TTG CTA ATA TCA GTA ACC ACA GAA AAA TGG CAA TAG CAT TAT GGT ATT GTC
   54_2_f TTG TAA TTA TTC TAG TTT GGG AAT TCG CGA TAC TTC AAT TTG CTA ATA TCA GTA ACC ACA GAA AAA TGG CAA TAG CAT TAT GGT ATT GTC
 GIL121_r TTG TAA TTA TTC TAG TTT GGG AAT TCG CGA TAC TTC AAT TTG CTA ATA TCA GTA ACC ACA GAA AAA TGG CAA TAG CAT TAT GGT ATT GTC
   54_2_r TTG TAA TTA TTC TAG TTT GGG AAT TCG CGA TAC TTC AAT TTG CTA ATA TCA GTA ACC ACA GAA AAA TGG CAA TAG CAT TAT GGT ATT GTC
   54_1_r TTG TAA TTA TTC TAG TTT GGG AAT TCG CGA TAC TTC AAT TTG CTA ATA TCA GTA ACC ACA GAA AAA TGG CAA TAG CAT TAT GGT ATT GTC
Consensus ttg taa tta ttc tag ttt ggg aat tcg cga tac ttc aat ttg cta ata tca gta acc aca gaa aaA TGG CAA TAG CAT TAT GGT ATT GTC

          271                                                                                                               360
     CPD1 CAC CAC AAG GAT CAG TTG CTT ACG AGA CAT TAC AGA TGC TTA TAT TTT CGT TTC AAA CGT TGT TCC CAG ATT CAC CAG TTT TTG AAC CTC
 GIL121_f CAC CAC AAG GAT CAG TTG CTT ACG AGA CAT TAC AGA TGC TTA TAT TTT CGT TTC AAA CGT TGT TCC CAG ATT CAC CAG TTT TTG AAC CTC
   54_1_f CAC CAG AAG GAT CAG TTG CTT ACG AGA CAT TAC AGA TGC TTA TAT TTT CGT TTC AAA CGT TGT TCC CAG ATT CAC CAG TTT TTG AAC CTC
   54_2_f CAC CAC AAG GAT CAG TTG CTT ACG AGA CAT TAC AGA TGC TTA TAT TTT CGT TTC AAA CGT TGT TCC CAG ATT CAC CAG TTT TTG AAC CTC
 GIL121_r CAC CAC AAG GAT CAG TTG CTT ACG AGA CAT TAC AGA TGC TTA TAT TTT CGT TTC AAA CGT TGT TCC CAG ATT CAC CAG TTT TTG AAC CTC
   54_2_r CAC CAC AAG GAT CAG TTG CTT ACG AGA CAT TAC AGA TGC TTA TAT TTT CGT TTC AAA CGT TGT TCC CAG ATT CAC CAG TTT TTG AAC CTC
   54_1_r CAC CAG AAG GAT CAG TTG CTT ACG AGA CAT TAC AGA TGC TTA TAT TTT CGT TTC AAA CGT TGT TCC CAG ATT CAC CAG TTT TTG AAC CTC
Consensus CAC CAc AAG GAT CAG TTG CTT ACG AGA CAT TAC AGA TGC TTA TAT TTT CGT TTC AAA CGT TGT TCC CAG ATT CAC CAG TTT TTG AAC CTC

          361                                                                                                               450
     CPD1 ATG TGA CGG TAA CGT CCC ATT TGG TGT GCA ATA GTA AAG ATG ATG TCA ACA AAA TCT TGA CCT CCT GTG TTG CTG CCA TCC AAT CGA TAA
 GIL121_f ATG TGA CGG TAA CGT CCC ATT TGG TGT GCA ATA GTA AAG ATG ATG TCA ACA AAA TTT TGA CCT CCT GTG TTG CTG CCA TCC AAT CGA TAA
   54_1_f ATG TGA CGG TAA CGT CCC ATT TGG TGT GCA ATA GTA AAG ATG ATG TCA ACA AAA TTT TGA CCT CCT GTG TTG CTG CCA TCC AAT CGA TAA
   54_2_f ATG TGA CGG TAA CGT CCC ATT TGG TGT GCA ATA GTA AAG ATG ATG TCA ACA AAA TTT TGA CCT CCT GTG TTG CTG CCA TCC AAT CGA TAA
 GIL121_r ATG TGA CGG TAA CGT CCC ATT TGG TGT GCA ATA GTA AAG ATG ATG TCA ACA AAA TTT TGA CCT CCT GTG TTG CTG CCA TCC AAT CGA TAA
   54_2_r ATG TGA CGG TAA CGT CCC ATT TGG TGT GCA ATA GTA AAG ATG ATG TCA ACA AAA TTT TGA CCT CCT GTG TTG CTG CCA TCC AAT CGA TAA
   54_1_r ATG TGA CGG TAA CGT CCC ATT TGG TGT GCA ATA GTA AAG ATG ATG TCA ACA AAA TTT TGA CCT CCT GTG TTG CTG CCA TCC AAT CGA TAA
Consensus ATG TGA CGG TAA CGT CCC ATT TGG TGT GCA ATA GTA AAG ATG ATG TCA ACA AAA TtT TGA CCT CCT GTG TTG CTG CCA TCC AAT CGA TAA

          451                                                                                                               540
     CPD1 GGT CAC ACC AAA CAG CAA AGA AAG GAC GTA AGG GAC AGG TAT CCC ATG CTG TTG CAG CTC CTT TGG TAT CTT TCA ATG GAT GCA GTG TAG
 GIL121_f GGT CAC ACC AAA CAG CAA AGA AAG GAC GTA AGG GAC AGG CAT CCC ATA CTG TTG CAG CTC CTT TGG TAT CTT TCA ATG GAT GCA GTG TAG
   54_1_f GGT CAC ACC AAA CAG CAA AGA AAG GAC GTA AGG GAC AGG CAT CCC ATA CTG TTG CAG CTC CTT TGG TAT CTT TCA ATG GAT GCA GTG TAG
   54_2_f GGT CAC ACC AAA CAG CAA AGA AAG GAC GTA AGG GAC AGG CAT CCC ATA CTG TTG CAG CTC CTT TGG TAT CTT TCA ATG GAT GCA GTG TAG
 GIL121_r GGT CAC ACC AAA CAG CAA AGA AAG GAC GTA AGG GAC AGG CAT CCC ATA CTG TTG CAG CTC CTT
   54_2_r GGT CAC ACC AAA CAG CAA AGA AAG GAC GTA AGG GAC AGG CAT CCC ATA CTG TTG CAG CTC CTT
   54_1_r GGT CAC ACC AAA CAG CAA AGA AAG GAC GTA AGG GAC AGG CAT CCC ATA CTG TTG CAG CTC CTT TGG T
Consensus GGT CAC ACC AAA CAG CAA AGA AAG GAC GTA AGG GAC AGG cAT CCC ATa CTG TTG CAG CTC CTT tgg tat ctt tca atg gat gca gtg tag
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STE11

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           1711                                                                                                                                                                                  1800
STE11      CAT AAG AAG AAC ATT ATT CAC AGA GAT ATC AAG GGT GCA AAT ATT TTG ATT GAT ATC AAA GGT TGC GTA AAA ATT ACT GAT TTT GGT ATT
GIL121_f     N NNN NNN NNN ANT TAT TNN NNA GAN ATC AAG GGT GCA AAT ATT TTG ATT GAT ATC AAA GGT TGC GTA AAA ATT ACT GAT TTT GGT ATT
58_1_f       N NNN NNN NNN NNT TAT TCN NGA GAN NTC NAG GGT GCA AAT ATT TTG ATT GAT ATC AAA GGT TGC GTA AAA ATT ACT GAT TTT GGT ATT
58_2_f     NNN NNN NNN NNN ANT TAN TNN NNA GAN ATC AAG GGT GCA AAT ATT TTG ATT GAT ATC AAA GGT TGC GTA AAA ATT ACT GAT TTT GGT ATT
GIL121_r   CAT AAG AAG AAN NTT ATT CAC AGA GAT ATC AAG GGT GCA AAT ATT TTG ATT GAT ATC AAA GGT TGC GTA AAA ATT ANT GAT TTT GGT ATT
58_1_r     CAT AAG AAG AAC ATT ATT CAC AGA GAT ATC AAG GGT GCA AAT ATT TTG ATT GAT ATC AAA GGT TGN GTA AAA ATT ACT GAT TTT GGT ATT
58_2_r     CAT AAG AAG AAC ATT ATT CAC AGA GAT ATC AAG GGT GCA AAT ATT TTG ATT GAT ATC AAA GGT TGC GTA AAA ATT ANT GAT TTT GGT ATT
Consensus  cat aag aag aan atT att cac agA GAt aTC aAG GGT GCA AAT ATT TTG ATT GAT ATC AAA GGT TGc GTA AAA ATT AcT GAT TTT GGT ATT

           1801                                                                                                                                                                                  1890
STE11      TCA AAA AAA TTA TCA CCT TTG AAT AAA AAA CAA AAT AAG AGA GCT TCT TTG CAA GGT TCC GTA TTC TGG ATG TCA CCA GAG GTG GTC AAA
GIL121_f   TCA AAA AAA TTA TCA CCT TTG AAT AAA AAA CAA AAT AAG AGA GCT TCT TTG CAA GGT TCC GTA TTC TGG ATG TCA CCA GAG GTG GTC AAA
58_1_f     TCA AAA AAA TTA TCA CCT TTG AAT AAA AAA CAA AAT AAG AGA GCT TCT TTG CAA GGT TCC GTA TTC TGG ATG TCA CCA GAG GTG GTC AAA
58_2_f     TCA AAA AAA TTA TCA CCT TTG AAT AAA AAA CAA AAT AAG AGA GCT TCT TTG CAA GGT TCC GTA TTC TGG ATG TCA CCA GAG GTG GTC AAA
GIL121_r   TCA AAA AAA TTA TCA CCT TTG AAT AAA AAA CAA AAT AAG AGA GNT TNT TTG CAA GGT TCC GTA TTT TGG ATG TCA CCA GAG GTG GTC AAA
58_1_r     TCA AAA AAA TTA TCA CCT TTG AAT AAA AAA CAA AAT AAG AGA GNT TNT TTG CAA GGT TCC GTA TTT TGG ATG TCA CCA GAG GTG GTC AAA
58_2_r     TCA AAA AAA TTA TCA CCT TTG AAT AAA AAA CAA AAT AAG AGA GNT TNT TTG CAA GGT TCC GTA TTT TGG ATG TCA CCA GAG GTG GTC AAA
Consensus  TCA AAA AAA TTA TCA CCT TTG AAT AAA AAA CAA AAT AAG AGA GcT TcT TTG CAA GGT TCC GTA TTc TGG ATG TCA CCA GAG GTG GTC AAA

           1891                                                                                                                                                                                  1980
STE11      CAG ACC GCT ACT ACT GCT AAA GCG GAT ATA TGG TCT ACA GGA TGT GTT GTC ATT GAA ATG TTT ACC GGT AAG CAT CCT TTC CCA GAT TTT
GIL121_f   CAG ACC GCT ACT ACT GCT AAA GCG GAT ATA TGG TCT ACA GGA TGT GTT GTC ATT GAA ATG TTT ACC GGT AAG CAT CCT TTC CCA GAT TTT
58_1_f     CAG ACC GCT ACT ACT GCT AAA GCG GAT ATA TGG TCT ACA GGA TGT GTT GTC ATT GAA ATG TTT ACC GGT AAG CAT CAT TTC CCA GAT TTT
58_2_f     CAG ACC GCT ACT ACT GCT AAA GCG GAT ATA TGG TCT ACA GGA TGT GTT GTC ATT GAA ATG TTT ACC GGT AAG CAT CAT TTC CCA GAT TTT
GIL121_r   CAG ACC GCT ACT ACT GCT AAA GCG GAT ATA TGG TTT ACA GGA TGT GTT GTC ATT GAA ATG TTT ACC GGT AAG CAT CCT TTC CCA GAT TTT
58_1_r     CAG ACC GCT ACT ACT GCT AAA GCG GAT ATA TGG TTT ACA GGA TGT GTT GTC ATT GAA ATG TTT ACC GGT AAG CAT CAT TTC CCA GAT TTT
58_2_r     CAG ACC GCT ANT ACT GNT AAA GCG GAT ATA TGG TTT ACA GGA TGT GTT GTC ATT GAA ATG TTT ACC GGT AAG CAT CAT TTC CCA GAT TTT
Consensus  CAG ACC GCT AcT ACT GcT AAA GCG GAT ATA TGG TcT ACA GGA TGT GTT GTC ATT GAA ATG TTT ACC GGT AAG CAT CaT TTC CCA GAT TTT
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CCT → CAT

P H

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           1981                                                                                                                                                                                  2070
STE11      TCT CAA ATG CAA GCG ATC TTC AAA ATA GGC ACA AAC ACG ACC CCC GAG ATA CCT TCC TGG GCT ACG TCA GAA GGA AAG AAT TTC TTA AGA
GIL121_f   TCT CAA ATG CAA GCG ATC TTC AAA ATA GGC ACA AAC ACG ACC CCC GAG ATA CCT TCC TGG GCT ACG TCA GAA GGA AAG AAT TTC TTA AGA
58_1_f     TCT CAA ATG CAA GCG ATC TTC AAA ATA GGC ACA AAC ACG ACC CCC GAG ATA CCT TCC TGG GCT ACG TCA GAA GGA AAG AAT TTC TTA AGA
58_2_f     TCT CAA ATG CAA GCG ATC TTC AAA ATA GGC ACA AAC ACG ACC CCC GAG ATA CCT TCC TGG GCT ACG TCA GAA GGA AAG AAT TTC TTA AGA
GIL121_r   TTT CAA ATG CAA GCG ATT TTC AAA ATA GGC ACA AAC ACG ACC CCC GAG ATA CCT TCC TGG GNT ACG TCA GAA GGA AAG AAT TTT TTA AGA
58_1_r     TNT CAA ATG CAA GCG ATT TTC AAA ATA GGC ACA AAC ACG ACC CCC GAG ATA CCT TCC TGG GNT ACG TCA GAA GGA AAG AAT TTN TTA AGA
58_2_r     TTT CAA ATG CAA GCG ATT TTC AAA ATA GGC ACA AAC ACG ACC CCC GAG ATA CCT TCC TGG GNT ANG TCA GAA GGA AAG AAT TTT TTA AGA
Consensus  TcT CAA ATG CAA GCG ATc TTC AAA ATA GGC ACA AAC ACG ACC CCC GAG ATA CCT TCC TGG GcT AcG TCA GAA GGA AAG AAT TTc TTA AGA

           2071                                                                                                                                                                                  2160
STE11      AAG GCA TTT GAG TTG GAT TAT CAA TAC AGG CCT AGT GCC CTT GAA TTG CTG CAG CAT CCA TGG CTG GAT GCA CAC ATA ATT TGA
GIL121_f   AAG GCA TTT GAG TTG GAT TAT CAA TAC AGG CCT AGT GCC CTT GAA TTG CTG CAG CAT CCA TGG CTG GAT GCA CAC ATA ATT TGA AAA AGG
58_1_f     AAG GCA TTT GAG TTG GAT TAT CAA TAC AGG CCT AGT GCC CTT GAA TTG CTG CAG CAT CCA TGG CTG GAT GCA CAC ATA ATT TGA AAA AGG
58_2_f     AAG GCA TTT GAG TTG GAT TAT CAA TAC AGG CCT AGT GCC CTT GAA TTG CTG CAG CAT CCA TGG CTG GAT GCA CAC ATA ATT TGA AAA AGG
GIL121_r   AAG GCA TTT GAG TTG GAT TAT CAA TAC AGG CCT AGT GCC CTT GAA TTG CTG CAG CAT CCA TGG CTG GAT GCA CAC ATA ATT TGA AAA AGG
58_1_r     AAG GCA TTT GAG TTG GAT TAT CAA TAC AGG CCT AGT GCC CTT GAA TTG CTG CAG CAT CCA TGG CTG GAT GCA CAC ATA ATT TGA AAA AGG
58_2_r     AAG GCA TTT GAG TTG GAT TAT CAA TAC AGG CCT AGT GCC CTT GAA TTG NTG CAG CAT CCA TGG CTG GAT GCA CAC ATA ATT TGA AAA AGG
Consensus  AAG GCA TTT GAG TTG GAT TAT CAA TAC AGG CCT AGT GCC CTT GAA TTG cTG CAG CAT CCA TGG CTG GAT GCA CAC ATA ATT TGA aaa agg

           2161                                                                                                                                                                                  2250
STE11
GIL121_f   GCT ACT TAT TAA TTC TTT TTA TGG CAC TAA AGT GCT CTG GCC GAT TTT TTA TCA AAT AAT ACA TAT ATA TCA TAT TTA GTG ACA TTT ATT
58_1_f     GCT ACT TAT TAA TTC TTT TTA TGG CAC TAA AGT GCT CTG GCC GAT TTT TTA TCA AAT AAT ACA TAT ATA TCA TAT TTA GTG ACA TTT ATT
58_2_f     GCT ACT TAT TAA TTC TTT TTA TGG CAC TAA AGT GCT CTG GCC GAT TTT TTA TCA AAT AAT ACA TAT ATA TCA TAT TTA GTG ACA TTT ATT
GIL121_r   GCT ACT TAT TAA TTC TTT TTA TGG CAN TAA AGT GCT CTG GCC GAT TTT TTA TCA AAT AAT ACA TAT ATA TCA TAT TTA GTG ACA TTT ATT
58_1_r     GCT ACT TAT TAA TTC TTT TTA TGG CAN TAA AGT GCT CTG GCC GAT TTT TTA TCA AAT AAT ACA TAT ATA TCA TAT TTA GTG ACA TTT ATT
58_2_r     GNT ACT TAT TAA TTN TTT TTA TGG CAN TAA AGT GCT NTG GCC GAT TTT TTA TCA AAT AAT ACA TAT ATA TCA TAT TTA GTG ACA TTT ATT
Consensus  gct act tat taa ttc ttt tta tgg ca. taa agt gct ctg gcc gat ttt tta tca aat aat aca tat ata tca tat tta gtg aca ttt att
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STE5

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           361                                                                                                                                                                                   450
STE5       TTG CCA AGA CCT AAT TCC AAT CTC TTT CAC GCA AGT AAT AGT AAC CTA TCC CGA GCA AAT GAG CCC CCA AGG GCC GAA AAT TTA TCA GAT
69_2_f     TTT GNN NNA CCT AAT TCC AAT CTC TTT CAC GCA AGT AAT ANT ANC CTA TCC CGA GCA AAT GAG CCC CCA AGG GCC GAA AAT TTA TCA GAT
69_1_f     TTG CCN AGA CCT AAT TCC AAT CTC TTT CAC GCA AGT AAT AGT AAC CTA TCC CGA GCA AAT GAG CCC CCA AGG GCC GAA AAT TTA TCA GAT
GIL121_f   TTT GCC AGA CCT AAT TCC NAT CTC TTT CAC GCA AGT AAT AGT AAC CTA TCC CGA GCA AAT GAG CCC CCA AGG GCC GAA AAT TTA TCA GAT
GIL121_r   TTG CCA AGA CCT AAT TCC AAT CTC TTT CAC GCA AGT AAT AGT AAC CTA TCC CGA GCA AAT GAG CCC CCA AGG GCC GAA AAT TTA TCA GAT
69_1_r     TTG CCA AGA CCT AAT TCC AAT CTC TTT CAC GCA AGT AAT AGT AAC CTA TCC CGA GCA AAT GAG CCC CCA AGG GCC GAA AAT TTA TCA GAT
69_2_r     TTG CCA AGA CCT AAT TCC AAT CTC TTT CAC GCA AGT AAT AGT AAC CTA TCC CGA GCA AAT GAG CCC CCA AGG GCC GAA AAT TTA TCA GAT
Consensus  TTg cca agA CCT AAT TCC aAT CTC TTT CAC GCA AGT AAT AgT AaC CTA TCC CGA GCA AAT GAG CCC CCA AGG GCC GAA AAT TTA TCA GAT

           451                                                                                                                                                                                   540
STE5       AAT ATA CCA CCC AAG GTC GCT CCA TTT GGC TAT CCA ATA CAA AGA ACC TCT ATT AAA AAA TCC TTT TTG AAT GCT TCT TGT ACG TTA TGT
69_2_f     AAT ATA CCA CCC AAG GTC GCT CCA TTT GGC TAT CCA ATA CAA AGA ACC TCT ATT AAA AAA TCC TTT TTG AAT GCT TCT TGT ACG TTA TGT
69_1_f     AAT ATA CCA CCC AAG GTC GCT CCA TTT GGC TAT CCA ATA CAA AGA ACC TCT ATT AAA AAA TCC TTT TTG AAT GCT TCT TGT ACG TTA TGT
GIL121_f   AAT ATA CCA CCC AAG GTC GCT CCA TTT GGC TAT CCA ATA CAA AGA ACC TCT ATT AAA AAA TCC TTT TTG AAT GCT TCT TGT ACG TTA TGT
GIL121_r   AAT ATA CCA CCC AAG GTC GCT CCA TTT GGC TAT CCA ATA CAA AGA ACC TCT ATT AAA AAA TCC TTT TTG AAT GCT TCT TGT ACG TTA TGT
69_1_r     AAT ATA CCA CCC AAG GTC GCT CCA TTT GGC TAT CCA ATA CAA AGA ACC TCT ATT AAA AAA TCC TTT TTG AAT GCT TCT TGT ACG TTA TGT
69_2_r     AAT ATA CCA CCC AAG GTC GCT CCA TTT GGC TAT CCA ATA CAA AGA ACC TCT ATT AAA AAA TCC TTT TTG AAT GCT TCT TGT ACG TTA TGT
Consensus  AAT ATA CCA CCC AAG GTC GCT CCA TTT GGC TAT CCA ATA CAA AGA ACC TCT ATT AAA AAA TCC TTT TTG AAT GCT TCT TGT ACG TTA TGT
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TGT → TCT

C S

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           541                                                                                                                                                                                   630
STE5       GAC GAG CCT ATT TCT AAC AGA AGA AAG GGA GAG AAA ATT ATA GAG CTT GCA TGT GGC CAC TTA AGT CAC CAA GAA TGT CTT ATT ATC TCT
69_2_f     GAC GAG CCT ATT TCT AAC AGA AGA AAG GGA GAG AAA ATT ATA GAG CTT GCA TCT GGC CAC TTA AGT CAC CAA GAA TGT CTT ATT ATC TCT
69_1_f     GAC GAG CCT ATT TCT AAC AGA AGA AAG GGA GAG AAA ATT ATA GAG CTT GCA TCT GGC CAC TTA AGT CAC CAA GAA TGT CTT ATT ATC TCT
GIL121_f   GAC GAG CCT ATT TCT AAC AGA AGA AAG GGA GAG AAA ATT ATA GAG CTT GCA TGT GGC CAC TTA AGT CAC CAA GAA TGT CTT ATT ATC TCT
GIL121_r   GAC GAG CCT ATT TCT AAC AGA AGA AAG GGA GAG AAA ATT ATA GAG CTT GCA TGT GGC CAC TTA AGT CAC CAA GAA TGT CTT ATT ATC TCT
69_1_r     GAC GAG CCT ATT TCT AAC AGA AGA AAG GGA GAG AAA ATT ATA GAG CTT GCA TCT GGC CAC TTA AGT CAC CAA GAA TGT CTT ATT ATC TCT
69_2_r     GAC GAG CCT ATT TCT AAC AGA AGA AAG GGA GAG AAA ATT ATA GAG CTT GCA TCT GGC CAC TTA AGT CAC CAA GAA TGT CTT ATT ATC TCT
Consensus  GAC GAG CCT ATT TCT AAC AGA AGA AAG GGA GAG AAA ATT ATA GAG CTT GCA TcT GGC CAC TTA AGT CAC CAA GAA TGT CTT ATT ATC TCT

           631                                                                                                                                                                                   720
STE5       TTT GGC ACC ACT TCA AAG GCA GAC GTT CGT GCG CTA TTT CCT TTT TGT ACC AAA TGT AAA AAA GAT ACT AAC AAA GCC GTT CAA TGC ATT
69_2_f     TTT GGC ACC ACT TCA AAG GCA GAC GTT CGT GCG CTA TTT CCT TTT TGT ACC AAA TGT AAA AAA GAT ACT AAC AAA GCC GTT CAA TGC ATT
69_1_f     TTT GGC ACC ACT TCA AAG GCA GAC GTT CGT GCG CTA TTT CCT TTT TGT ACC AAA TGT AAA AAA GAT ACT AAC AAA GCC GTT CAA TGC ATT
GIL121_f   TTT GGC ACC ACT TCA AAG GCA GAC GTT CGT GCG CTA TTT CCT TTT TGT ACC AAA TGT AAA AAA GAT ACT AAC AAA GCC GTT CAA TGC ATT
GIL121_r   TTT GGC ACC ACT TCA AAG GCA GAC GTT CGT GCG CTA TTT CCT TTT TGT ACC AAA TGT AAA AAA GAT ACT AAC AAA GCC GTT CAA TGC ATT
69_1_r     TTT GGC ACC ACT TCA AAG GCA GAC GTT CGT GCG CTA TTT CCT TTT TGT ACC AAA TGT AAA AAA GAT ACT AAC AAA GCC GTT CAA TGC ATT
69_2_r     TTT GGC ACC ACT TCA AAG GCA GAC GTT CGT GCG CTA TTT CCT TTT TGT ACC AAA TGT AAA AAA GAT ACT AAC AAA GCC GTT CAA TGC ATT
Consensus  TTT GGC ACC ACT TCA AAG GCA GAC GTT CGT GCG CTA TTT CCT TTT TGT ACC AAA TGT AAA AAA GAT ACT AAC AAA GCC GTT CAA TGC ATT

           721                                                                                                                                                                                   810
STE5       CCA GAA AAT GAT GAA CTA AAG GAT ATT CTA ATT TCT GAT TTT TTG ATT CAT AAG ATT CCT GAT TCT GAG TTA TCA ATC ACA CCT CAG TCC
69_2_f     CCA GAA AAT GAT GAA CTA AAG GAT ATT CTA ATT TCT GAT TTT TTG ATT CAT AAG ATT CCT GAT TCT GAG TTA TCA ATC ACA CCT CAG TCC
69_1_f     CCA GAA AAT GAT GAA CTA AAG GAT ATT CTA ATT TCT GAT TTT TTG ATT CAT AAG ATT CCT GAT TCT GAG TTA TCA ATC ACA CCT CAG TCC
GIL121_f   CCA GAA AAT GAT GAA CTA AAG GAT ATT CTA ATT TCT GAT TTT TTG ATT CAT AAG ATT CCT GAT TCT GAG TTA TCA ATC ACA CCT CAG TCC
GIL121_r   CCA GAA AAT GAT GAA CTA AAG GAT ATT CTA ATT TCT GAT TTT TTG ATT CAT AAG ATT CCT GAT TCT GAG TTA TCA ATC ACA CCT CAG TCC
69_1_r     CCA GAA AAT GAT GAA CTA AAG GAT ATT CTA ATT TCT GAT TTT TTG ATT CAT AAG ATT CCT GAT TCT GAG TTA TCA ATC ACA CCT CAG TCC
69_2_r     CCA GAA AAT GAT GAA CTA AAG GAT ATT CTA ATT TCT GAT TTT TTG ATT CAT AAG ATT CCT GAT TCT GAG TTA TCA ATC ACA CCT CAG TCC
Consensus  CCA GAA AAT GAT GAA CTA AAG GAT ATT CTA ATT TCT GAT TTT TTG ATT CAT AAG ATT CCT GAT TCT GAG TTA TCA ATC ACA CCT CAG TCC
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IKS1

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           811                                                                                                                  900
     IKS1  TTC ATT TTA CAA CAG TAT TGC AGT GGC GGC AAT TTA GAA GAC TGT ATT CTA AGA AAA GTG TTC AAT AGG TTC TCA GAT ACC GAA TCA CCT
   69_2_r                                                 NN NNN NNN NNN NTT CTA NNN NNG NTC NAT AGG TTC TCA GAT ACC GAA TCA CCT
   69_1_r                                                 NN NNN NNN NNN NTT CTA NNN NTG TTC NAT AGG TTC TCA GAT ACC GAA TCA CCT
 GIL121_r                                                  N NNN NNN NNN NNT TCT ANN NNN NNN NNT AGG TTC TCA GAT ACC GAA TCA CCT
 GIL121_f                              GT GGC GGC AAT TTA GAA GAC TGT ATT CTA AGA AAA GTG TTC AAT AGG TTC TCA GAT ACC GAA TCA CCT
   69_2_f                           C AGT GGC GGC AAT TTA GAA GAC TGT ATT CTA AGA AAA GTG TTC AAT AGG TTC TCA GAT ACC GAA TCA CCT
   69_1_f                              GT GGC GGC AAT TTA GAA GAC TGT ATT CTA AGA AAA GTG TTC AAT AGG TTC TCA GAT ACC GAA TCA CCT
Consensus  ... ... ... ... ... ... ... .gt ggc ggc aat tta gaa gac tgt att cta aga aaa gtg ttc aaT AGG TTC TCA GAT ACC GAA TCA CCT

           901                                                                                                                  990
     IKS1  GAA GAG AGG AAA AAA AAA TTT AGA ACT CGC AAG AAA AAT CAC GGT AAA TCA GGA GAA GTT GGC CTA TCT ACG GAA CAG CTC GTA TCC ATT
   69_2_r  GAA GAG AGG AAA AAA AAA TTT AGA ACT CGC AAG AAA AAT CAC GGT AAA TCA GGA GAA GTT GGC CTA TCT ACG GAA CAG CTC GTA TCC ATT
   69_1_r  GAA GAG AGG AAA AAA AAA TTT AGA ACT CGC AAG AAA AAT CAC GGT AAA TCA GGA GAA GTT GGC CTA TCT ACG GAA CAG CTC GTA TCC ATT
 GIL121_r  GAA GAG AGG AAA AAA AAA TTT AGA ACT CGC AAG AAA AAT CAC GGT AAA TCA GGA GAA GTT GGC CTA TCT ACG GAA CAG CTC GTA TCC ATT
 GIL121_f  GAA GAG AGG AAA AAA AAA TTT AGA ACT CGC AAG AAA AAT CAC GGT AAA TCA GGA GAA GTT GGC CTA TCT ACG GAA CAG CTC GTA TCC ATT
   69_2_f  GAA GAG AGG AAA AAA AAA TTT AGA ACT CGC AAG AAA AAT CAC GGT AAA TCA GGA GAA GTT GGC CTA TCT ACG GAA CAG CTC GTA TCC ATT
   69_1_f  GAA GAG AGG AAA AAA AAA TTT AGA ACT CGC AAG AAA AAT CAC GGT AAA TCA GGA GAA GTT GGC CTA TCT ACG GAA CAG CTC GTA TCC ATT
Consensus  GAA GAG AGG AAA AAA AAA TTT AGA ACT CGC AAG AAA AAT CAC GGT AAA TCA GGA GAA GTT GGC CTA TCT ACG GAA CAG CTC GTA TCC ATT

           991                                                                                                                 1080
     IKS1  ATA AGG GAT ATT GCA AGA GGT TTA CAC GAA CTG CAC AGT ATT GGC CTA ATA CAT AGG GAC TTG AAA CCA TCC AAC TGT TTA TTA TTG ACT
   69_2_r  ATA AGG GAT ATT GCA AGA GGT TTA CAC GAA CTG CAG AGT ATT GGC CTA ATA CAT AGG GAC TTG AAA CCA TCC AAC TGT TTA TTA TTG ACT
   69_1_r  ATA AGG GAT ATT GCA AGA GGT TTA CAC GAA CTG CAG AGT ATT GGC CTA ATA CAT AGG GAC TTG AAA CCA TCC AAC TGT TTA TTA TTG ACT
 GIL121_r  ATA AGG GAT ATT GCA AGA GGT TTA CAC GAA CTG CAC AGT ATT GGC CTA ATA CAT AGG GAC TTG AAA CCA TCC AAC TGT TTA TTA TTG ACT
 GIL121_f  ATA AGG GAT ATT GCA AGA GGT TTA CAC GAA CTG CAC AGT ATT GGC CTA ATA CAT AGG GAC TTG AAA CCA TCC AAC TGT TTA TTA TTG ACT
   69_2_f  ATA AGG GAT ATT GCA AGA GGT TTA CAC GAA CTG CAG AGT ATT GGC CTA ATA CAT AGG GAC TTG AAA CCA TCC AAC TGT TTA TTA TTG ACT
   69_1_f  ATA AGG GAT ATT GCA AGA GGT TTA CAC GAA CTG CAG AGT ATT GGC CTA ATA CAT AGG GAC TTG AAA CCA TCC AAC TGT TTA TTA TTG ACT
Consensus  ATA AGG GAT ATT GCA AGA GGT TTA CAC GAA CTG CAg AGT ATT GGC CTA ATA CAT AGG GAC TTG AAA CCA TCC AAC TGT TTA TTA TTG ACT
```

→ CAC → CAG
H Q

```
           1081                                                                                                                1170
     IKS1  CCA TTT AAA AGT GAC AAT GAG AAT GAC AAC GTA TAC GAC AGG GAA CAC AAT TCA GAT GAG TTT TTC CCA TCA ATT GTT ATT GGC GAT TTG
   69_2_r  CCA TTT AAA AGT GAC AAT GAG AAT GAC AAC GTA TAC GAC AGG GAA CAC AAT TCA GAT GAG TTT TTC CCA TCA ATT GTT ATT GGC GAT TTG
   69_1_r  CCA TTT AAA AGT GAC AAT GAG AAT GAC AAC GTA TAC GAC AGG GAA CAC AAT TCA GAT GAG TTT TTC CCA TCA ATT GTT ATT GGC GAT TTG
 GIL121_r  CCA TTT AAA AGT GAC AAT GAG AAT GAC AAC GTA TAC GAC AGG GAA CAC AAT TCA GAT GAG TTT TTC CCA TCA ATT GTT ATT GGC GAT TTG
 GIL121_f  CCA TTT AAA AGT GAC AAT GAG AAT GAC AAC GTA TAC GAC AGG GAA CAC AAT TCA GAT GAG TTT TTC CCA TCA ATT GTT ATT GGC GAT TTG
   69_2_f  CCA TTT AAA AGT GAC AAT GAG AAT GAC AAC GTA TAC GAC AGG GAA CAC AAT TCA GAT GAG TTT TTC CCA TCA ATT GTT ATT GGC GAT TTG
   69_1_f  CCA TTT AAA AGT GAC AAT GAG AAT GAC AAC GTA TAC GAC AGG GAA CAC AAT TCA GAT GAG TTT TTC CCA TCA ATT GTT ATT GGC GAT TTG
Consensus  CCA TTT AAA AGT GAC AAT GAG AAT GAC AAC GTA TAC GAC AGG GAA CAC AAT TCA GAT GAG TTT TTC CCA TCA ATT GTT ATT GGC GAT TTG

           1171                                                                                                                1260
     IKS1  GGG GAA AGT CAG TTG GAA GGA GAG TCC AGA TTG GGT ACT GGT TGC ACA GGA ACA TTA GAG TTT ACA GCA CCA GAC CTT ATT ATC CAG GGG
   69_2_r  GGG GAA AGT CAG TTG GAA GGA GAG TCC AGA TTG GGT ACT GGT TGC ACA GGA ACA TTA GAG TTT ACA GCA CCA GAC CTT ATT ATC CAG GGG
   69_1_r  GGG GAA AGT CAG TTG GAA GGA GAG TCC AGA TTG GGT ACT GGT TGC ACA GGA ACA TTA GAG TTT ACA GCA CCA GAC CTT ATT ATC CAG GGG
 GIL121_r  GGG GAA AGT CAG TTG GAA GGA GAG TCC AGA TTG GGT ACT GGT TGC ACA GGA ACA TTA GAG TTT ACA GCA CCA GAC CTT ATT ATC CAG GGG
 GIL121_f  GGG GAA AGT CAG TTG GAA GGA GAG TCC AGA TTG GGT ACT GGT TGC ACA GGA ACA TTA GAG TTT ACA GCA CCA GAC CTT ATT ATC CAG GGG
   69_2_f  GGG GAA AGT CAG TTG GAA GGA GAG TCC AGA TTG GGT ACT GGT TGC ACA GGA ACA TTA GAG TTT ACA GCA CCA GAC CTT ATT ATC CAG GGG
   69_1_f  GGG GAA AGT CAG TTG GAA GGA GAG TCC AGA TTG GGT ACT GGT TGC ACA GGA ACA TTA GAG TTT ACA GCA CCA GAC CTT ATT ATC CAG GGG
Consensus  GGG GAA AGT CAG TTG GAA GGA GAG TCC AGA TTG GGT ACT GGT TGC ACA GGA ACA TTA GAG TTT ACA GCA CCA GAC CTT ATT ATC CAG GGG

           1261                                                                                                                1350
     IKS1  AGA CCA GTT TCT TCT TCT ACG TTG CCT TCA AGA TCG AGT CAC ACA TAC AAT GAA TAC ACC TTT GCT TCC GAT ATG TAT TCT CTA GGA ATG
   69_2_r  AGA CCA GTT TCT TCT TCT ACG TTG CCT TCA AGA TCG AGT CAC ACA TAC AAT GAA TAC ACC TTT GCT TCC GAT ATG TAT TCT CTA GGA ATG
   69_1_r  AGA CCA GTT TCT TCT TCT ACG TTG CCT TCA AGA TCG AGT CAC ACA TAC AAT GAA TAC ACC TTT GCT TCC GAT ATG TAT TCT CTA GGA ATG
 GIL121_r  AGA CCA GTT TCT TCT TCT ACG TTG CCT TCA AGA TCG AGT CAC ACA TAC AAT GAA TAC ACC TTT GCT TCC GAT ATG TAT TCT CTA GGA ATG
 GIL121_f  AGA CCA GTT TCT TCT TCT ACG TTG CCT TCA AGA TCG AGT CAC ACA TAC AAT GAA TAC ACC TTT GCT TCC GAT ATG
   69_2_f  AGA CCA GTT TCT TCT TCT ACG TTG CCT TCA AGA TCG AGT CAC ACA TAC AAT GAA TAC ACC TTT GCT TCC GAT ATG
   69_1_f  AGA CCA GTT TCT TCT TCT ACG TTG CCT TCA AGA TCG AGT CAC ACA TAC AAT GAA TAC ACC TTT GCT TCC GAT AT
Consensus  AGA CCA GTT TCT TCT TCT ACG TTG CCT TCA AGA TCG AGT CAC ACA TAC AAT GAA TAC ACC TTT GCT TCC GAT ATg tat tct cta gga atg
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intragenic on ChrX

```
           1                                                                                                                      90
   29_1_f   NN NNN NNN NGT NNN NNN GTT NNN NNN NAA TTA TTG GAA ACA AAC GAG AAC AAT AAT ATA TTA CAT GAA CAT ATA GAT GCT ACA GGG ACT
GIL121_2_f GNN NNN NNN NNG GNN CTA GTT NNN NNN NAA TTA TTG GAA ACA AAC GAG AAC AAT AAT ATA TTA CAT GAA CAT ATA GAT GCT ACA GGG ACT
GIL121_1_f NNN NNN NNN GTG NNN NNG TTG NNN ANA CNA TTA TTG GAA ACA AAC GAG AAC AAT AAT ATA TTA CAT GAA CAT ATA GAT GCT ACA GGG ACT
 Consensus .NN NNN NNN n.g nNN nn. gTt NNN nNn naA TTA TTG GAA ACA AAC GAG AAC AAT AAT ATA TTA CAT GAA CAT ATA GAT GCT ACA GGG ACT

           91                                                                                                                    180
   29_1_f  TAC TAT TTA TTC GAT ACG TTA ACT TCA ACC AAC AAA AGA TTC TAC CCT AAG GAT TGT GTT TTT GAT TAT AGG ACT AAT AAT GTC GAG AAC
GIL121_2_f TAC TAT TTA TTC GAT ACG TTA ACT TCA ACC AAC AAA AGA TTC TAC CCT AAG GAT TGT GTT TTT GAT TAT AGG ACT AAT AAT GTC GAG AAC
GIL121_1_f TAC TAT TTA TTC GAT ACG TTA ACT TCA ACC AAC AAA AGA TTC TAC CCT AAG GAT TGT GTT TTT GAT TAT AGG ACT AAT AAT GTC GAG AAC
 Consensus TAC TAT TTA TTC GAT ACG TTA ACT TCA ACC AAC AAA AGA TTC TAC CCT AAG GAT TGT GTT TTT GAT TAT AGG ACT AAT AAT GTC GAG AAC

           181                                                                                                                   270
   29_1_f  ATT CCT ATT CTC TTA AAC AAT TTT AAA AAA TTC ATC AAG AAA TAT CAA TTT GAT GAT GTC TTT GAA AAT GAT ATC ATA GAA ATC GAT CCT
GIL121_2_f ATT CCT ATT CTC TTA AAC AAT TTT AAA AAA TTC ATC AAG AAA TAT CAA TTT GAT GAT GTC TTT GAA AAT GAT ATC ATA GAA ATC GAT CCT
GIL121_1_f ATT CCT ATT CTC TTA AAC AAT TTT AAA AAA TTC ATC AAG AAA TAT CAA TTT GAT GAT GTC TTT GAA AAT GAT ATC ATA GAA ATC GAT CCT
 Consensus ATT CCT ATT CTC TTA AAC AAT TTT AAA AAA TTC ATC AAG AAA TAT CAA TTT GAT GAT GTC TTT GAA AAT GAT ATC ATA GAA ATC GAT CCT

           271                                                                                                                   360
   29_1_f  CGT GAA AAT GAA ATC TTG TGC AAG ATA ATC AAA GAA GGA CTC GGT GAA AGT TTA GAT ATC ATG AAC ACA AAT ACA ACT GAC ATT TTT AGG
GIL121_2_f CGT GAA AAT GAA ATC TTG TGC AAG ATA ATC AAA GAA GGA CTC GGT GAA AGT TTA GAT ATC ATG AAC ACA AAT ACA ACT GAC ATT TTT AGG
GIL121_1_f CGT GAA AAT GAA ATC TTG TGC AAG ATA ATC AAA GAA GGA CTC GGT GAA AGT TTA GAT ATC ATG AAC ACA AAT ACA ACT GAC ATT TTT AGG
 Consensus CGT GAA AAT GAA ATC TTG TGC AAG ATA ATC AAA GAA GGA CTC GGT GAA AGT TTA GAT ATC ATG AAC ACA AAT ACA ACT GAC ATT TTT AGG

           361                                                                                                                   450
   29_1_f  ATA ATC GAT GGT TTA AAA AAN NAA NNN NNA AAN TTN NNN GGN AAA AAN NNN NNA ANT NNN ANC NGG NAA AAN GNT TTN GNT NNN NNN NNN
GIL121_2_f ATA ATC GAT GGT TTA AAA AAA CAA NNA TNA AAN TTT GNN GGN AAA AAA NGN NNA ANT TAA ANC CGG GAA AAA GGT TTG GNT NNN NNN NNN
GIL121_1_f ATA ATC GAT GGT TTA AAA AAA NAA NNN NNA AAN TTN NNN NGN AAA ANN NNN NNA ANT NNN ANC NGG GAA AAN GGT TTG GNT NNN NNN ANN
 Consensus ATA ATC GAT GGT TTA AAA AAa nAA NNn nNA AAN TTn nNN gGN AAA Aan NnN NNA ANT nnn ANC nGG gAA AAn GgT TTg GNT NNN NNN nNN

           451                                                                                                                   540
   29_1_f  NNN NNN AAN TNC NNN NTN NNN ANN NAN NAA NNT NNN AAN NTN GNN NNN ANG GNA AAA NGG NNT TTT TNN NNN NNN NNN CNN NNN NNN NNC
GIL121_2_f NNG NNN AAN TNC NNN NTN NNN ANG GAN NAA NCT NNA AAN NTN GNN NNN ANG GNA AAA NNG NAT TTT TNN NNN NNG NNN CNN NNN NNN NCN
GIL121_1_f NGG NNA AAN TNC NNN NTN NNN ANN NNN NAA NNT NNA AAN NTN GNN NNN ANG GNA AAA NNG NNT TTT TNN NNN NGG NNN CNN NNN NNN NCN
 Consensus Nng NNn AAN TNC NNN NTN NNN ANn naN NAA NnT NNa AAN NTN GNN NNN ANG GNA AAA NnG NnT TTT TNN NNN Nng NNN CNN NNN NNN Ncn

           541         551
   29_1_f  NNN NNN AAN NN
GIL121_2_f NNN NNN NNN N
GIL121_1_f NNN NNA AAN AN
 Consensus NNN NNn aaN nn
```

11/30/07

EXPRESSION ARRAYS TO CHARACTERIZE αF^R MUTANTS

- From the same plates as the Yeast tiling arrays started from (in fridge) I picked a single colony
  ↳ O/N culture (11/27)
- 11/28 - @ 2:15 - dilute saturated culture 1:200 (25λ into 5 mL)
  - @ 9:30 - dilute 1:1000 (10λ into 10 mL)
- 11/29 - @ 9:15 - coulter count GIL121 → ~4 E7
  - dilute 1:100 (100λ into 10 mL prewarmed YPD)
  - @ ~2:30 - ~~begin RNA prep~~ Harvest cells, begin RNA prep

Harvesting for RNA

Filtering the cells is the quickest way to collect them for later RNA processing. I don't recommend spinning cells because of the chance you'll get a stress response or a temperature shock.

The following protocol is intended for ~108_1010 cells. If the filtering seems slow, harvest less culture or divide the cells over multiple filters. If you're harvesting less than ~108 cells, you should use 25 mm filters in 2 ml tubes with a smaller filter support (09-753G, Fisher).

Plug the filter support (K953805-0000, Fisher) in a sidearm flask hooked to vacuum. Center a 0.45 micron nylon filter (R04SP04700, GE Osmonics) on the filter support. Place the funnel on top and carefully clamp it all together. Start the vacuum. Listen for any whistling noises that may indicate a leak in the seal. If you do get whistling, make sure that the filter is centered and free of wrinkles. Some fraction of the filters have cracks or holes that will interfere with the harvest. Replace the filter with a new one if this seems to be the case.

Remove the top from a labeled 15 ml Falcon tube. Put the tube in a bucket of liquid nitrogen.

Once everything is ready, remove the culture from the shaker. Turn on the vacuum. Pour desired amount of culture into the funnel of the filter apparatus. Watch to make sure it is filtering properly and that no cells are making it into the flask. You can refilter if you have this problem, but try to avoid it.

Once the culture has completely filtered through, remove the clamp and then the funnel. Pull the vacuum hose off the sidearm. This order is important to keep cells from sticking to the funnel. Use forceps or a spatula to lift the edge of the filter, avoiding the cells in the center. Carefully roll up the filter. Dump out the liquid nitrogen in the 15 ml tube, pop the rolled up filter inside, loosely cap it, and dunk it back into the liquid nitrogen. Store the tubes at _80.

11/30/07

Coulter counts at the beginning of RNA Harvesting from 11/30

Bkg	172000	
GIL121	1.66E7	1.656E7
α29	1.826E7	1.756E7
α37	1.579E7	1.899E7
α40	1.913E7	1.844E7
α48	1.641E7	1.617E7
α54	1.671E7	1.656E7
α58	1.553E7	1.55E7
α69	1.867E7	1.656E7
ste7Δ	1.53E7	1.515E7
ste12Δ	1.615E7	1.567E7
Post Bkg	1.716E6	386000

RNA prep

Hybrid of the DeRisi protocol (www.microarrays.org) and a standard acid-phenol prep circulating around the Brown/Botstein labs circa 2001

Use the small prep if you've used 25 mm filters and the large prep for 47 mm filters.

Use RNase free reagents and glass-/plastic-ware throughout! Remember to use glass pipets with chloroform.

Lysis buffer
(100 ml)

2 ml	0.5 M EDTA
5 ml	10% SDS
1 ml	1 M Tris pH 7.5
92 ml	RNase-free water

Small RNA prep

Remove a manageable set of samples from the _80. They should be in 2 ml eppendorf tubes.
Before they thaw, add 750 μl lysis buffer. Vortex, trying to get all the cells off the membrane.
Add 750 μl acid phenol. Vortex.
Incubate 1 hour 65C, vortexing every 20 minutes.
Fish out the filter and discard.
Ice 10 min.
While they are incubating, spin the 2 ml heavy phase lock gel tubes (made by Eppendorf, sold lots of places) for 30 sec full speed in a room temperature microcentrifuge. Set aside.
Spin lysate 5 min.
With a pipet, transfer the top aqueous layer to the PLG tube.
Add 750 μl chloroform. Invert to mix. Do not vortex!
Spin 5 min.
Pour aqueous layer into a new 15 ml Falcon tube.
Add 75 μl (or 1/10 volume if you lost some) 3 M sodium acetate. Mix.
Add 1.5 ml (or 2 volumes) ethanol. Mix.
Incubate _20C >30 min (preferably overnight).
Spin 3000 rpm 10 min.
Wash pellet 2X with 70% ethanol, with 2 min 3000 rpm spins between washes.
Air dry inverted on the bench 30 min.
Dissolve pellet in 25 μl water. You can speed up the dissolution by heating the sample or by pipetting the pellet up and down, but I prefer a gentler room-temperature-with-frequent-flicking approach.
Measure the concentration with the spectrophotometer or nanodrop.
Check the quality of the RNA on the Bioanalyzer or a gel.

11/30

Nanodrop Quantification of crude RNA

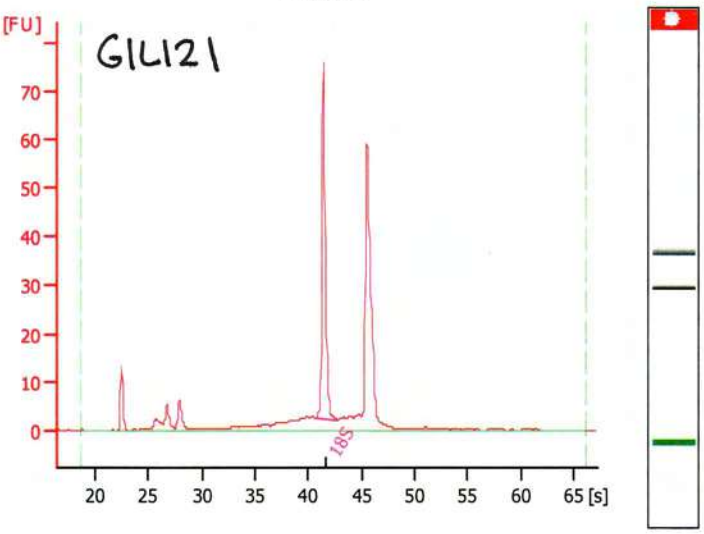
GIL121	4103.1	μg/mL
α29	4189.4	↓
α37	4121.4	
α40	4196.3	
α48	4174.0	
α54	4127.6	
α58	4175.6	
α69	4190.4	
ste7Δ	4120.0	
ste12Δ	3782.3	

Assay Class: EukaryoteTotal RNA Nano
Data Path: C:\... EukaryoteTotal RNA Nano DE24802677 2007-12-03 15-28-37.xad

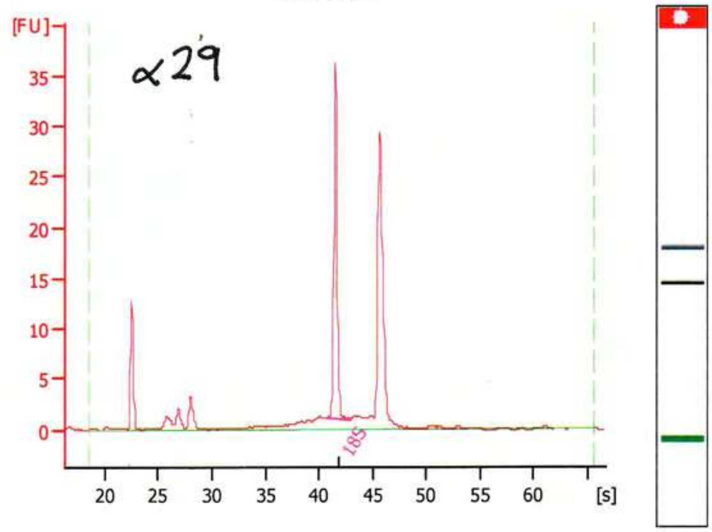
Created: 12/3/2007 3:28:37 PM
Modified: 12/3/2007 3:49:50 PM

Electropherogram Summary

Sample 1



Sample 2



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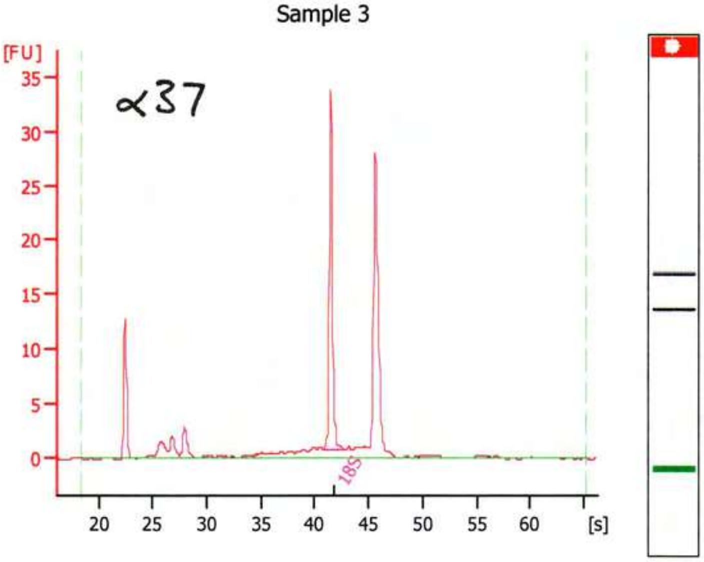

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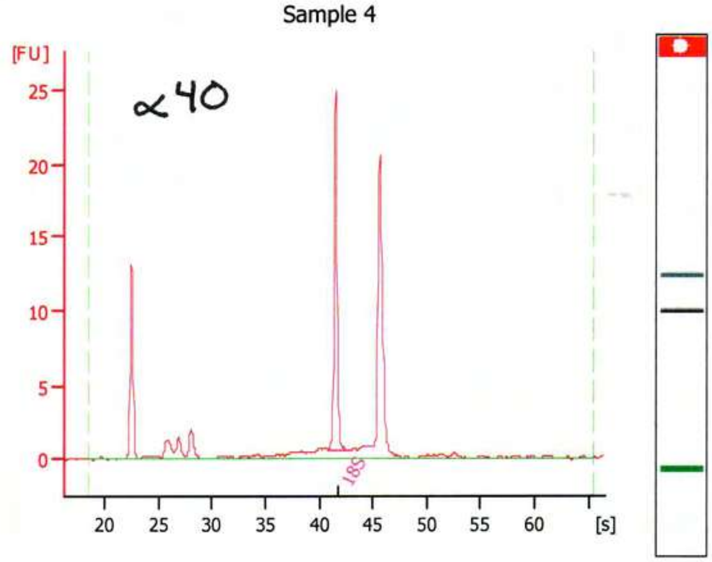
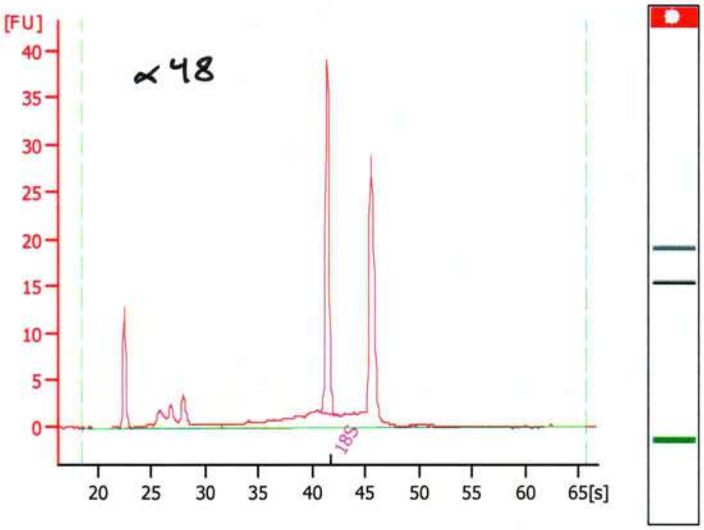


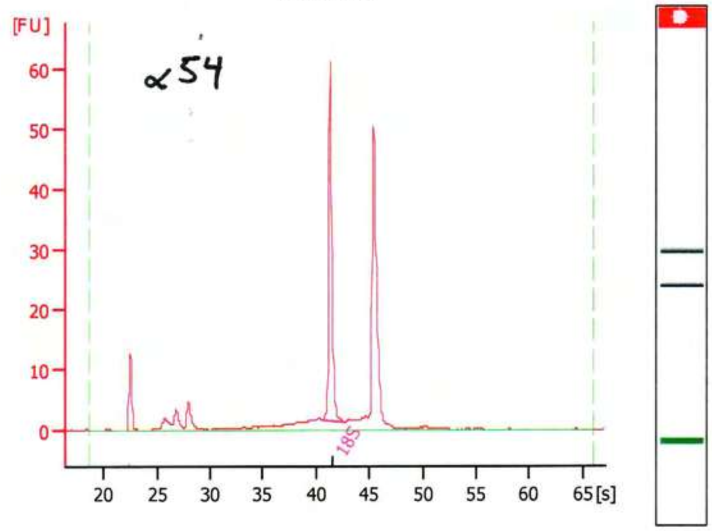
Assay Class:	EukaryoteTotal RNA Nano	Created:	12/3/2007 3:28:37 PM
Data Path:	C:\... EukaryoteTotal RNA Nano DE24802677 2007-12-03 15-28-37.xad	Modified:	12/3/2007 3:49:50 PM

Electropherogram Summary Continued ...

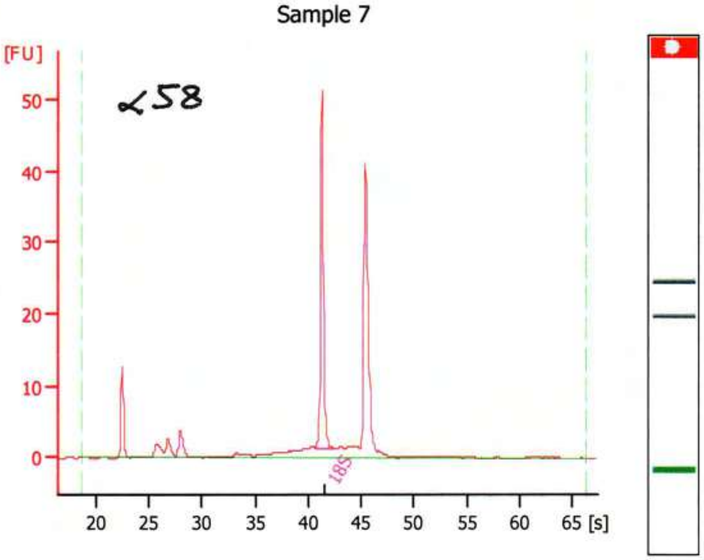
Sample 5



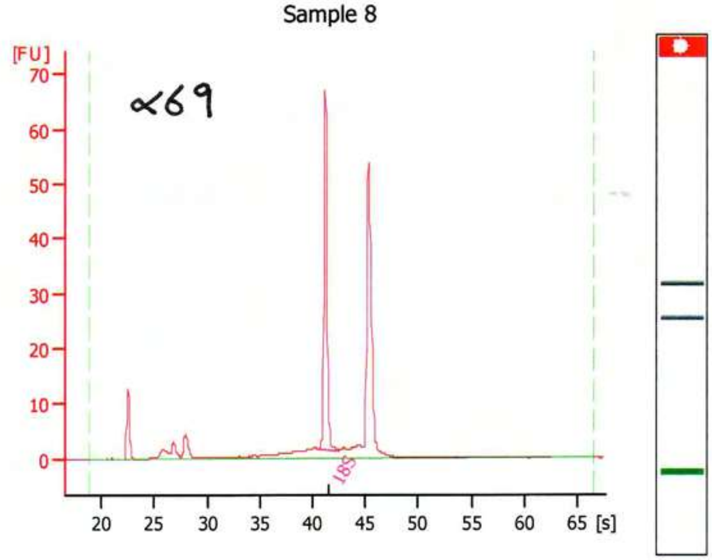
Sample 6



Sample 7



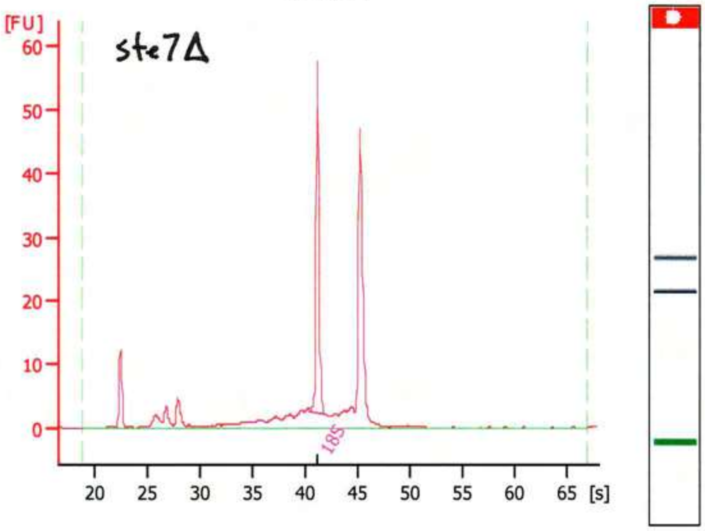
Sample 8



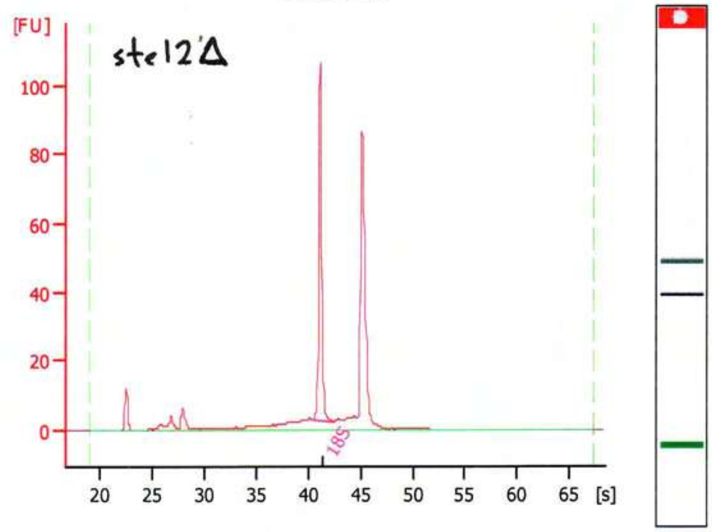
Assay Class:	EukaryoteTotal RNA Nano	Created:	12/3/2007 3:28:37 PM
Data Path:	C:\... EukaryoteTotal RNA Nano DE24802677 2007-12-03 15-28-37.xad	Modified:	12/3/2007 3:49:50 PM

Electropherogram Summary Continued ...

Sample 9

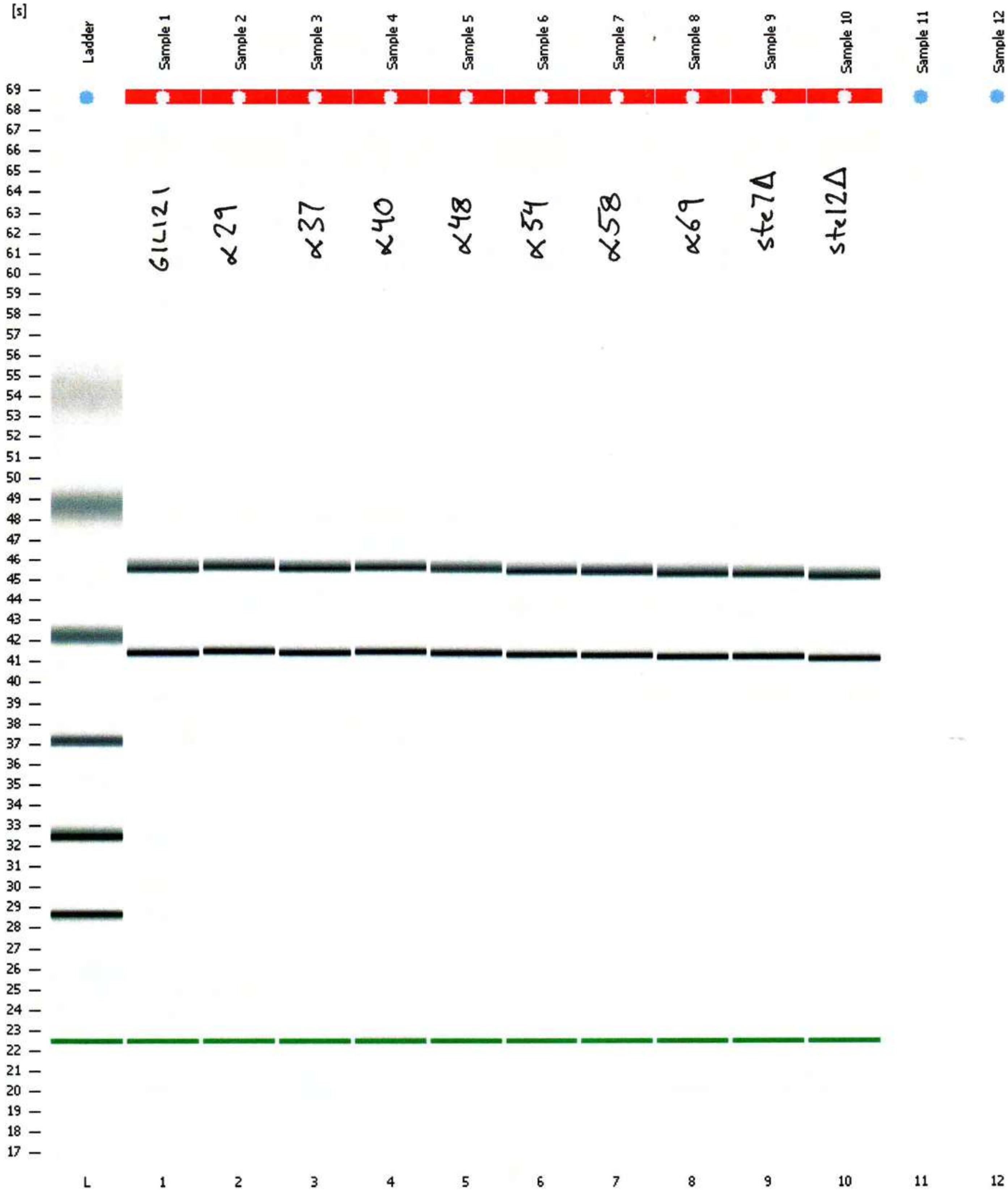


Sample 10



Assay Class:	EukaryoteTotal RNA Nano	Created:	12/3/2007 3:28:37 PM
Data Path:	C:\... EukaryoteTotal RNA Nano DE24802677 2007-12-03 15-28-37.xad	Modified:	12/3/2007 3:49:50 PM

Gel Image



12/5/07

Labeling RNA using Agilent Low RNA linear amplification kit.

- Thaw RNA samples
- Re-quantify using the nanodrop

GIL121	1367.8	→	3.7 λ	+ 46.3 λ H_2O
α29	573.3	→	8.7	41.3
α37	403.1	→	12.4	37.6
α40	403.8	→	12.4	37.6
α48	737.0	→	6.8	43.2
α54	752.1	→	6.6	43.4
α58	604.8	→	8.3	41.7
α69	924.2	→	5.4	44.6
ste7Δ	767.5	→	6.5	43.5

Label RNA

- 3 reference (GIL121)

RNA labeling
The amplification/labeling is done per Agilent instructions with half volume reactions and a quarter amount of recommended dye.

Make a 100 ng/μl stock of total RNA.

Aliquot 3.25 μl (325 ng) total RNA into a PCR tube.
Add 2.5 μl T7 Promoter Primer.
65C 10 min
ice 5 min

Warm 5X first strand buffer at 100C, with occasional vortexing, until it completely dissolves (~1 min).
Prepare cDNA master mix, in this order at RT:

1X	
2 μl	5X first strand buffer
1 μl	0.1 M DTT
0.5 μl	10 mM dNTP mix
0.5 μl	MMLV RT
0.25 μl	RNaseOUT

Add 4.25 μl to each reaction.
40C 2 hours
65C 15 min
ice 5 min

Add 0.8 μl Cy3 CTP to each reference reaction.
Add 0.6 μl Cy5 CTP to each experimental reaction.

↑ in the lab
↓ in the microarray facility

Warm 50% PEG at 40C until it's resuspended and easy to pipet.
Prepare transcription master mix, in this order at RT:

1X	
8.25 μl	water
10 μl	4X transcription buffer
3 μl	0.1 M DTT
4 μl	NTP mix
3.2 μl	50% PEG
0.25 μl	RNaseOUT
0.3 μl	inorganic pyrophosphatase
0.4 μl	T7 RNA polymerase

Mix by pipetting.

Add 29.4 μl to each reaction. Mix by pipetting.
40C 2 hours in the dark

Purify with an RNeasy column.
Nanodrop 1 ul to check yield and dye incorporation.
Store RNA at -80C.

12/5/07

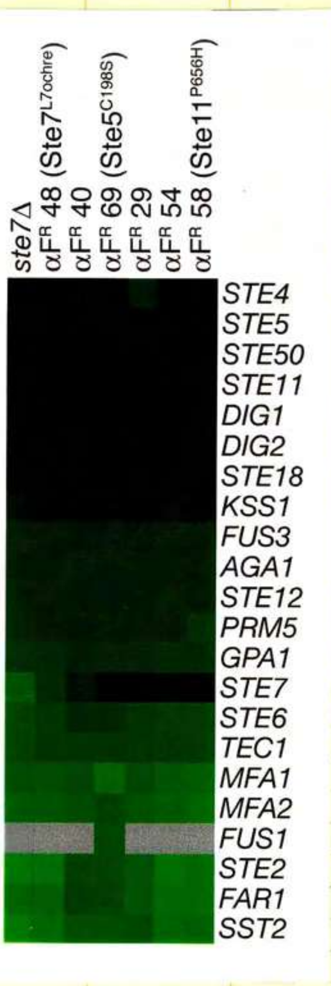
Quantification of dye incorporation using the nanodrop.

	RNA ng/μL	Cy3 pmol/μL	Cy5 pmol/μL
E11121 1	253.4	1.6	0.0
" 2	273.3	1.8	0.1
" 3	258.2	1.4	0.1
α29	225.0	0.1	1.5
α37	52.7	0.0	0.2
α40	203.8	0.0	0.9
α48	137.6	0.1	0.7
α54	192.6	0.1	1.1
α58	206.4	0.1	1.0
α69	233.2	-0.4	1.0
ste7Δ	225.8	0.1	1.2
α37 (2nd)	1.0	0.0	0.1

12/12/07

RESULTS OF EXPRESSION ARRAYS

The figure below shows that all of the beneficial mutations do, as expected, downregulate basal expression in the mating pathway



- Interestingly you can see downregulation of STE7 in the two STE7 mutants – the Ste7^{ochre} transcript may be being degraded by NMD.
- I also see downregulation of STE7 in αF^{R}40 – a strain where the YTA did not find a mutation. – Looking back at the YTA data I see what might be a real SNP in STE7 – fortunately it lies within ~~in~~ the region covered by the SNP primers (see p. 85). I have submitted these samples for sequencing.
- I also see downregulation of STE4 in αF^{R}29. αF^{R}29 is phenotypically consistent w/ a STE4 mutation – it arrests following STE4 overexpression (see p75). There are no obvious SNPs in STE4. I have designed primers to sequence STE4 and the 200bp upstream region.

12/13/07

αF[R] 40 contains an Ochre mutation in Ste7

```
          361                                                                                                                                   450
     STE7
GIL121_f  ACC TAC AGG TTA ATG TGA AGC GAC ACT AAT GAT AAG AAA ACT GCA TCG GAT CAT ATC TGT TTT TGC AGC GTG GTA TAT TGG TTG TTG GTC
  40_1Q_f  ACC TAC AGG TTA ATG TGA AGC GAC ACT AAT GAT AAG AAA ACT GCA TCG GAT CAT ATC TGT TTT TGC AGC GTG GTA TAT TGG TTG TTG GTC
  40_1E_f  ACC TAC AGG TTA ATG TGA AGC GAC ACT AAT GAT AAG AAA ACT GCA TCG GAT CAT ATC TGT TTT TGC AGC GTG GTA TAT TGG TTG TTG GTC
  40_2E_f  ACC TAC AGG TTA ATG TGA AGC GAC ACT AAT GAT AAG AAA ACT GCA TCG GAT CAT ATC TGT TTT TGC AGC GTG GTA TAT TGG TTG TTG GTC
  40_2Q_f  ACC TAC AGG TTA ATG TGA AGC GAC ACT AAT GAT AAG AAA ACT GCA TCG GAT CAT ATC TGT TTT TGC AGC GTG GTA TAT TGG TTG TTG GTC
GIL121_r  ACC TAC AGG TTA ATG TGA AGC GAC ACT AAT GAT AAG AAA ACT GCA TCG GAT CAT ATC TGT TTT TGC AGC GTG GTA TAT TGG TTG TTG GTC
  40_2E_r  ACC TAC AGG TTA ATG TGA AGC GAC ACT AAT GAT AAG AAA ACT GCA TCG GAT CAT ATC TGT TTT TGC AGC GTG GTA TAT TGG TTG TTG GTC
  40_2Q_r  ACC TAC AGG TTA ATG TGA AGC GAC ACT AAT GAT AAG AAA ACT GCA TCG GAT CAT ATC TGT TTT TGC AGC GTG GTA TAT TGG TTG TTG GTC
  40_1E_r  ACC TAC AGG TTA ATG TGA AGC GAC ACT AAT GAT AAG AAA ACT GCA TCG GAT CAT ATC TGT TTT TGC AGC GTG GTA TAT TGG TTG TTG GTC
  40_1Q_r  ACC TAC AGG TTA ATG TGA AGC GAC ACT AAT GAT AAG AAA ACT GCA TCG GAT CAT ATC TGT TTT TGC AGC GTG GTA TAT TGG TTG TTG GTC
Consensus  ACC TAC AGG TTA ATG TGA AGC GAC ACT AAT GAT AAG AAA ACT GCA TCG GAT CAT ATC TGT TTT TGC AGC GTG GTA TAT TGG TTG TTG GTC

          451                                                                                                                                   540
     STE7  ATG TTT CAA CGA AAG ACT TTA CAG AGA AGG AAC TTG AAA GGG CTC AAT CTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG
GIL121_f  ATG TTT CAA CGA AAG ACT TTA CAG AGA AGG AAC TTG AAA GGG CTC AAT CTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG
  40_1Q_f  ATG TTT CAA CGA AAG ACT TTA CAG AGA AGG AAC TTG AAA GGG CTC AAT CTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG
  40_1E_f  ATG TTT CAA CGA AAG ACT TTA CAG AGA AGG AAC TTG AAA GGG CTC AAT CTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG
  40_2E_f  ATG TTT CAA CGA AAG ACT TTA CAG AGA AGG AAC TTG AAA GGG CTC AAT CTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG
  40_2Q_f  ATG TTT CAA CGA AAG ACT TTA CAG AGA AGG AAC TTG AAA GGG CTC AAT CTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG
GIL121_r  ATG TTT CAA CGA AAG ACT TTA CAG AGA AGG AAC TTG AAA GGG CTC AAT CTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG
  40_2E_r  ATG TTT CAA CGA AAG ACT TTA CAG AGA AGG AAC TTG AAA GGG CTC AAT CTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG
  40_2Q_r  ATG TTT CAA CGA AAG ACT TTA CAG AGA AGG AAC TTG AAA GGG CTC AAT NTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG
  40_1E_r  ATG TTT CAA CGA AAG ACT TTA CAG AGA AGG AAC TTG AAA GGG CTC AAT CTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG
  40_1Q_r  ATG TTT CAA CGA AAG ACT TTA CAG AGA AGG AAC TTG AAA GGG CTC AAT CTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG
Consensus  ATG TTT CAA CGA AAG ACT TTA CAG AGA AGG AAC TTG AAA GGG CTC AAT CTT AAC CTG CAC CCA GAT GTG GGC AAT AAT GGC CAA TTG CAG

                                                             E → Ochre
          541                                                                                                                                   630
     STE7  GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT CGA ATA GAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT
GIL121_f  GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT CGA ATA GAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT
  40_1Q_f  GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT CGA ATA TAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT
  40_1E_f  GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT CGA ATA TAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT
  40_2E_f  GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT CGA ATA TAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT
  40_2Q_f  GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT CGA ATA TAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT
GIL121_r  GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT CGA ATA GAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT
  40_2E_r  GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT NGA ATA TAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT
  40_2Q_r  GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT NGA ATA TAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT
  40_1E_r  GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT NGA ATA TAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT
  40_1Q_r  GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT NGA ATA TAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT
Consensus  GAA AAG ACA GAG ACT CAC CAG GGA CAA TCT cGA ATA tAA GGC CAC GTG ATG TCT AAC ATT AAT GCA ATA CAG AAT AAT AGC AAC CTG TTT

          631                                                                                                                                   720
     STE7  TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CAA
GIL121_f  TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CAA
  40_1Q_f  TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CAA
  40_1E_f  TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CAA
  40_2E_f  TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CAA
  40_2Q_f  TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CAA
GIL121_r  TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CAA
  40_2E_r  TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CAA
  40_2Q_r  TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CAA
  40_1E_r  TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CAA
  40_1Q_r  TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CA
Consensus  TTG CGA AGA GGC ATA AAA AAA AAA CTG ACG TTG GAT GCG TTT GGT GAT GAC CAA GCT ATA TCG AAA CCA AAC ACT GTG GTA ATA CAG CAA
```

→ Results from sequencing shows that α40 has a nonsense mutation in Ste7

12/21/07

αF^R 29 has a frameshift mutation in ste4

· I amplified and sequenced ste4 from αF^R29_1 & αF^R29-2 using the following primers:

34562054 IDT	34562055 IDT	34562056 IDT	34562057 IDT
G.LANG	G.LANG	G.LANG	G.LANG
36414062 12/11/2007	36414063 12/11/2007	36414110 12/11/2007	36414064 12/11/2007
STE4_extF1	STE4_extR1	STE4_intF1	STE4_intR1
5'- GTT TTC TTC CCC TTT TTG TAG -3'	5'- GCA TCC ATT GAT AAT TAC TGC -3'	5'- CAT ATA TTG ACA GCA AGT GG -3'	5'- AGA AAT ATA GCA AGT ATG TCC -3'
Tm= 49.5 °C , MW= 6,335.1	Tm= 49.6 °C , MW= 6,380.2	Tm= 48.5 °C , MW= 6,165.1	Tm= 47.1 °C , MW= 6,462.3
6.2OD260 34.70nmol 0.22mg	7.3OD260 36.40nmol 0.23mg	5.3OD260 25.80nmol 0.16mg	7.5OD260 33.80nmol 0.22mg

```
            901                                                                                                                      990
       STE4 TTC GCT AGC TGT GGA TCA GAC GGG TAT ACT TAC ATA TGG GAT AGC AGA TCT CCG TCC GCT GTA CAA AGC TTT TAC GTT AAC GAT AGT GAT
 29_1_extR1 TTC GCT AGC TGT GGA TCA GAC GGG TAT ACT TAC ATA TGG GAT AGC AGA TCT CCG TCC GCT GTA CAA AGC TTT TAC GTT AAC GAT AGT GAT
 GIL121_ext TTC GCT AGC TGT GGA TCA GAC GGG TAT ACT TAC ATA TGG GAT AGC AGA TCT CCG TCC GCT GTA CAA AGC TTT TAC GTT AAC GAT AGT GAT
 29_2_extR1 TTC GCT AGC TGT GGA TCA GAC GGG TAT ACT TAC ATA TGG GAT AGC AGA TCT CCG TCC GCT GTA CAA AGC TTT TAC GTT AAC GAT AGT GAT
 GIL121_int TTC GCT AGC TGT GGA TCA GAC GGG TAT ACT TAC ATA TGG GAT AGC AGA TCT CCG TCC GCT GTA CAA AGC TTT TAC GTT AAC GAT AGT GAT
 29_1_intF1 TTC GCT AGC TGT GGA TCA GAC GGG TAT ACT TAC ATA TGG GAT AGC AGA TCT CCG TCC GCT GTA CAA AGC TTT TAC GTT AAC GAT AGT GAT
 29_2_intF1 TTC GCT AGC TGT GGA TCA GAC GGG TAT ACT TAC ATA TGG GAT AGC AGA TCT CCG TCC GCT GTA CAA AGC TTT TAC GTT AAC GAT AGT GAT
 GIL121_ext --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 29_1_extF1 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 29_2_extF1 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 GIL121_int
 29_1_intR1
 29_2_intR1
  Consensus ttc gct agc tgt gga tca gac ggg tat act tac ata tgg gat agc aga tct ccg tcc gct gta caa agc ttt tac gtt aac gat agt gat
```

ΔG @ position 830

```
            991                                                                                                                     1080
       STE4 ATT AAT GCA CTT CGT TTT TTC AAA GAC GGG ATG TCG ATT GTT GCA GGA AGT GAC AAT GGT GCG ATA AAT ATG TAT GAT TTA AGG TCG GAC
 29_1_extR1 ATT AAT GCA CTT C-T TTT TTC AAA GAC GGG ATG TCG ATT GTT GCA GGA AGT GAC AAT GGT GCG ATA AAT ATG TAT GAT TTA AGG TCG GAC
 GIL121_ext ATT AAT GCA CTT CGT TTT TTC AAA GAC GGG ATG TCG ATT GTT GCA GGA AGT GAC AAT GGT GCG ATA AAT ATG TAT GAT TTA AGG TCG GAC
 29_2_extR1 ATT AAT GCA CTT C-T TTT TTC AAA GAC GGG ATG TCG ATT GTT GCA GGA AGT GAC AAT GGT GCG ATA AAT ATG TAT GAT TTA AGG TCG GAC
 GIL121_int ATT AAT GCA CTT CGT TTT TTC AAA GAC GGG ATG TCG ATT GTT GCA GGA AGT GAC AAT GGT GCG ATA AAT ATG TAT GAT TTA AGG TCG GAC
 29_1_intF1 ATT AAT GCA CTT C-T TTT TTC AAA GAC GGG ATG TCG ATT GTT GCA GGA AGT GAC AAT GGT GCG ATA AAT ATG TAT GAT TTA AGG TCG GAC
 29_2_intF1 ATT AAT GCA CTT C-T TTT TTC AAA GAC GGG ATG TCG ATT GTT GCA GGA AGT GAC AAT GGT GCG ATA AAT ATG TAT GAT TTA AGG TCG GAC
 GIL121_ext --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 29_1_extF1 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 29_2_extF1 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 GIL121_int
 29_1_intR1
 29_2_intR1
  Consensus att aat gca ctt c.t ttt ttc aaa gac ggg atg tcg att gtt gca gga agt gac aat ggt gcg ata aat atg tat gat tta agg tcg gac

            1081                                                                                                                    1170
       STE4 TGT TCT ATT GCT ACT TTT TCT CTT TTT CGA GGT TAT GAA GAA CGT ACC CCT ACC CCT ACT TAT ATG GCA GCT AAC ATG GAG TAC AAT ACC
 29_1_extR1 TGT TCT ATT GCT ACT TTT TCT CTT TTT CGA GGT TAT GAA GAA CGT ACC CCT ACC CCT ACT TAT ATG GCA GCT AAC ATG GAG TAC AAT ACC
 GIL121_ext TGT TCT ATT GCT ACT TTT TCT CTT TTT CGA GGT TAT GAA GAA CGT ACC CCT ACC CCT ACT TAT ATG GCA GCT AAC ATG GAG TAC AAT ACC
 29_2_extR1 TGT TCT ATT GCT ACT TTT TCT CTT TTT CGA GGT TAT GAA GAA CGT ACC CCT ACC CCT ACT TAT ATG GCA GCT AAC ATG GAG TAC AAT ACC
 GIL121_int TGT TCT ATT GCT ACT TTT TCT CTT TTT CGA GGT TAT GAA GAA CGT ACC CCT ACC CCT ACT TAT ATG GCA GCT AAC ATG GAG TAC AAT ACC
 29_1_intF1 TGT TCT ATT GCT ACT TTT TCT CTT TTT CGA GGT TAT GAA GAA CGT ACC CCT ACC CCT ACT TAT ATG GCA GCT AAC ATG GAG TAC AAT ACC
 29_2_intF1 TGT TCT ATT GCT ACT TTT TCT CTT TTT CGA GGT TAT GAA GAA CGT ACC CCT ACC CCT ACT TAT ATG GCA GCT AAC ATG GAG TAC AAT ACC
 GIL121_ext --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 29_1_extF1 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 29_2_extF1 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 GIL121_int
 29_1_intR1
 29_2_intR1
  Consensus tgt tct att gct act ttt tct ctt ttt cga ggt tat gaa gaa cgt acc cct acc cct act tat atg gca gct aac atg gag tac aat acc

            1171                                                                                                                    1260
       STE4 GCG CAA TCG CCA CAA ACT TTA AAA TCA ACA AGC TCA AGC TAT CTA GAC AAC CAA GGC GTT GTT TCT TTA GAT TTT AGT GCA TCT GGA AGA
 29_1_extR1 GCG CAA TCG CCA CAA ACT TTA AAA TCA ACA AGC TCA AGC TAT CTA GAC AAC CAA GGC GTT GTT TCT TTA GAT TTT AGT GCA TCT GGA AGA
 GIL121_ext GCG CAA TCG CCA CAA ACT TTA AAA TCA ACA AGC TCA AGC TAT CTA GAC AAC CAA GGC GTT GTT TCT TTA GAT TTT AGT GCA TCT GGA AGA
 29_2_extR1 GCG CAA TCG CCA CAA ACT TTA AAA TCA ACA AGC TCA AGC TAT CTA GAC AAC CAA GGC GTT GTT TCT TTA GAT TTT AGT GCA TCT GGA AGA
 GIL121_int GCG CAA TCG CCA CAA ACT TTA AAA TCA ACA AGC TCA AGC TAT CTA GAC AAC CAA GGC GTT GTT TCT TTA GAT TTT AGT GCA TCT GGA AGA
```

The sequencing results show that αF^R29 contains a frameshift mutation in ste4 — this mutation ~~was co~~ is completely invisible to SNP scanner, but clearly showed up on the expression array — possibly due to nonsense-mediated decay.

12/12/07

NOTES

· Now that I have finished the array analysis I have just about enough data to write up a paper. There are clearly a number of experiments I could do and a few that I should do before writing up.

Experiments that need to be done

· Assay salt sensitivity - previously when I ~~did~~ tested for growth on 1M NaCl there was some ambiguity (p). I need to redo this expt. by streaking from stock onto YPD - picking 2 colonies - assaying for αF^R and streaking onto 1M NaCl.

· Expression analysis on strains without fitness benefit - I need to do another round of Agilent arrays. I need to ~~relabe~~ relabel αF^R 37 which didn't label well last time. In addition, I will prep RNA from the following strains:

ste2Δ
Ste4Δ
Far1Δ
α67 } no fitness advantage (see p. 40)
α68
α47

12/13/07

· On Monday (12/10) I streaked strains α25-α69 from frozen stock onto YPD - I also streaked Ste2Δ, Ste4Δ, and Far1Δ

· Yesterday I picked a single colony off of Ste2Δ, Ste4Δ, and Far1Δ YPD plates. I picked 3 single colonies off of α47, α67, & α68 YPD plates → inoc. 5 ml YPD

· Today I tested for αF^R (~~10 λ culture →~~ 10 λ culture into 90 λ YPD + αF)

STRAIN	SHMOO
Ste2Δ	
Ste4Δ	
Far1Δ	Yes
α47-1	Yes
2	Yes
3	Yes
α67-1	
2	
3 ← I picked a small colony	
α68-1	Yes
2	Yes
3	

· Dilute Ste2, Ste4, & Far1, α67-1, & α68-3 1:200 (25 λ into 5 ml) @ 3:00 pm

12/14/07

10:00 - Coulter Count

Bkg	34000	
Ste 2	3.676 E7	Dilute 1:100 (10λ into 10ml prewarmed YPD)
Ste 4	4.057 E7	
Far 1	3.662 E7	
α67	3.018 E7	
α68	3.286 E6	α68-1 is the dilution from this morning
α68(orig.)	1.729 E8	α68-2 is the 1:1000 dilution from last night
Post Bkg	4.028 E6	1.78E6

· α68 seems to be v. dilute so I made a 1:100 dilution of the culture from yesterday which was still in the rollerdrum.

· I tested to see if they were still αF[R]

↳ α67 now has a large # of shmoos
↳ α68 is still completely αF[R]

· 5:30 - Harvest cells by filtration as before (p 93) - store in -80

· Coulter Count @ Harvest

Bkg	616000	
~~a~~ Ste 2	2.715 E7	
Ste 4	2.9E7	
Far 1	3.153 E7	
α67	2.527 E7	
α68-1	2.717 E7	
α68-2	3.886 E7	
Post Bkg	2.028E6	1.506 E6

12/19/07

Quantification of crude RNA from nanodrop.

· I prepared crude RNA from ste2Δ, ste4Δ, far1Δ, and α68-1 (see p.) as before (p 94) except that I mistakenly resuspended in 100λ H_2O instead of 25λ.

ste2Δ	2512.6	ng/μL
ste4Δ	2792.4	↓
far1Δ	2672.4	
α68	1842.2	

· Clean up w/ RNeasy

- 50λ of ste2Δ, ste4Δ, far1Δ, & α68
- for α37 and ste12, I pooled the crude RNA and the cleaned up RNA from the previous expt. and cleaned up all of that.

Requantify following cleanup

Ste2Δ	59.1	ng/μL
ste4Δ	77.0	↓
far1Δ	64.4	
α68	42.5	
α37	29.1	
ste12Δ	40.5	

· put cleaned RNA in -20. Store crude RNA in -80.

12/20/07

The concentration of RNA that I got after cleanup is much lower than last time (pg. 103) One possibility is that the RNA degraded — another possibility is that the RNeasy didn't work right — yet another possibility is that ~~th~~ I did something wrong w/ the nanodrop. I will requantify today.

Ste2Δ	59.2	same as it was yesterday
ste4Δ	76.5	
far1Δ	68.0	
α68	40.7	
α37	28.9	
ste12Δ	40.4	

· Looking at the Bioanalyzer results (p111-117) the RNA is not degraded

· I will label the RNA using full volume rxns & dye (instead of half rxns & half dye as before)

↳ I added 1.2 μL of Cy5 to each of the samples — except ste12Δ because I ran out of Cy5 — ste12Δ probably got ~.8λ.

12/20/07

Nanodrop to check dye incorporation

	RNA ng/µL	Cy3 pmol/µL	Cy5 pmol/µL
ste2Δ	120.8	0.0	0.3
ste4Δ	102.0	0.0	0.2
far1Δ	111.9	0.0	0.3
α68	145.8	-0.2	0.2
α37	145.5	0.1	0.5
ste12Δ	~~129~~ 129.8	0.1	0.2
GIL121-1	248.9	1.6	0.0
GIL121-2	270.9	1.8	0.0
GIL121-3	259.7	1.4	0.0

12/21/07

I have decided not to hybe the RNA since I had poor dye incorporation. I will retry after the new year.

Below is a summary of the amplification and dye incorporation from the previous two expts (p 104 & 110)

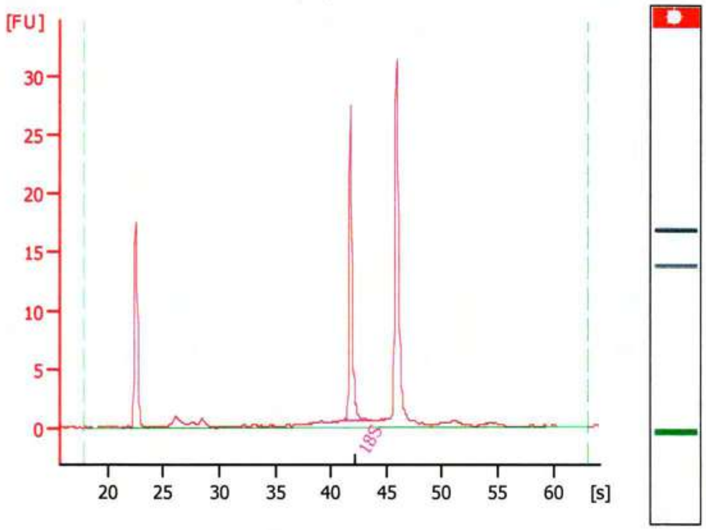
sample	RNA conc. ng/ul	input ng	output ng/ul	output ng	amplification fold	Cy pmol/ul	incorporation pmol/ng
GIL121-1	100	325	253	7602	23	1.6	6.31E-03
GIL121-2	100	325	273	8199	25	1.8	6.59E-03
GIL121-3	100	325	258	7746	24	1.4	5.42E-03
29	100	325	225	6750	21	1.5	6.67E-03
37	100	325	53	1581	5	0.2	3.80E-03
40	100	325	204	6114	19	0.9	4.42E-03
48	100	325	138	4128	13	0.7	5.09E-03
54	100	325	193	5778	18	1.1	5.71E-03
58	100	325	206	6192	19	1.0	4.84E-03
69	100	325	233	6996	22	1.0	4.29E-03
ste7	100	325	226	6774	21	1.2	5.31E-03
ste2	59	118	121	3624	31	0.3	2.48E-03
ste4	77	153	102	3060	20	0.2	1.96E-03
far1	68	136	112	3357	25	0.3	2.68E-03
68	41	81	146	4374	54	0.2	1.37E-03
37	29	58	146	4365	76	0.5	3.44E-03
ste12	40	81	130	3894	48	0.2	1.54E-03

Assay Class: EukaryoteTotal RNA Nano
Data Path: C:\... EukaryoteTotal RNA Nano DE24802677 2007-12-20 13-18-56.xad

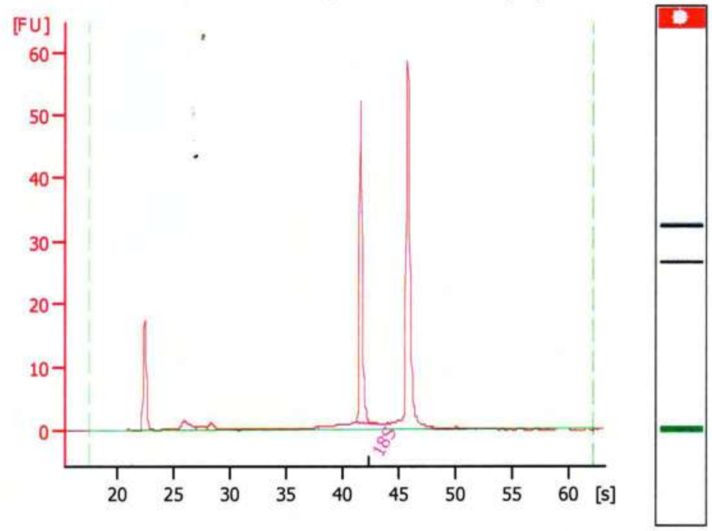
Created: 12/20/2007 1:18:56 PM
Modified: 12/20/2007 1:42:55 PM

Electropherogram Summary

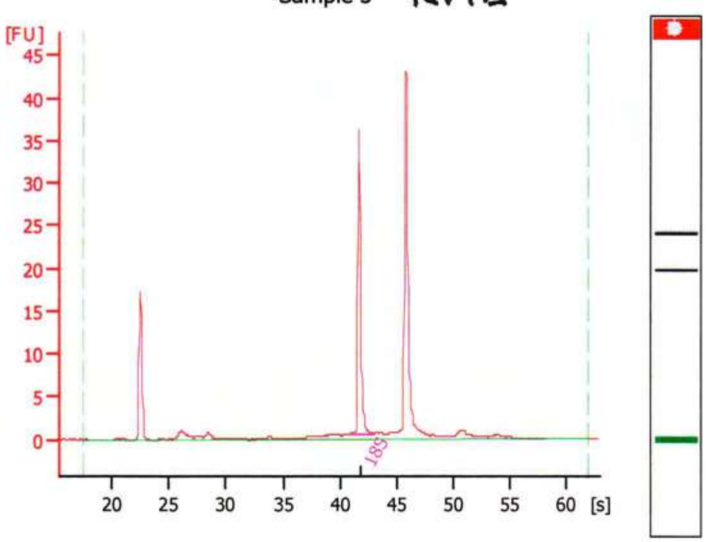
~~Sample 1~~ ste2Δ



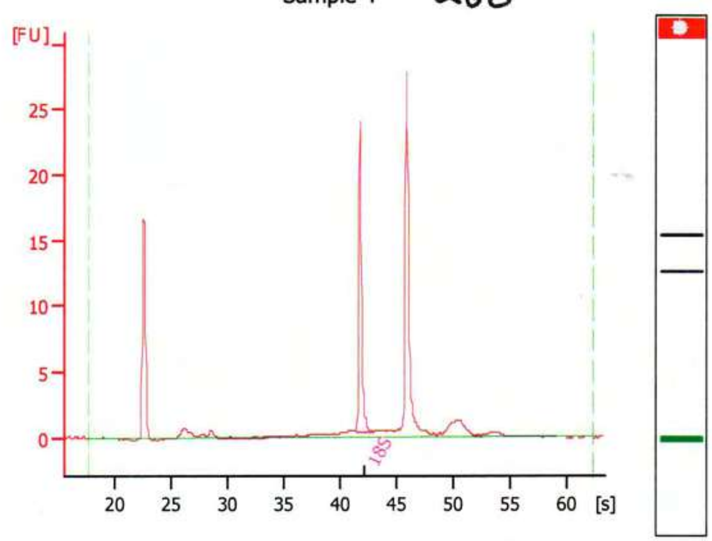
~~Sample 2~~ ste4Δ



~~Sample 3~~ far1Δ



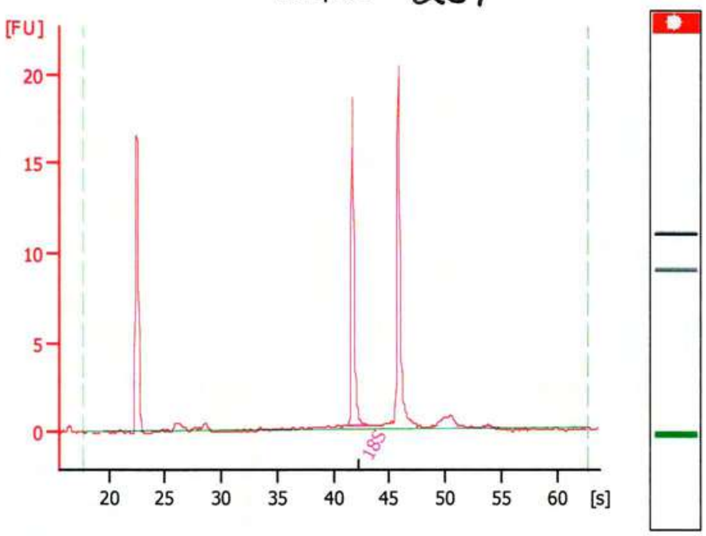
~~Sample 4~~ α68



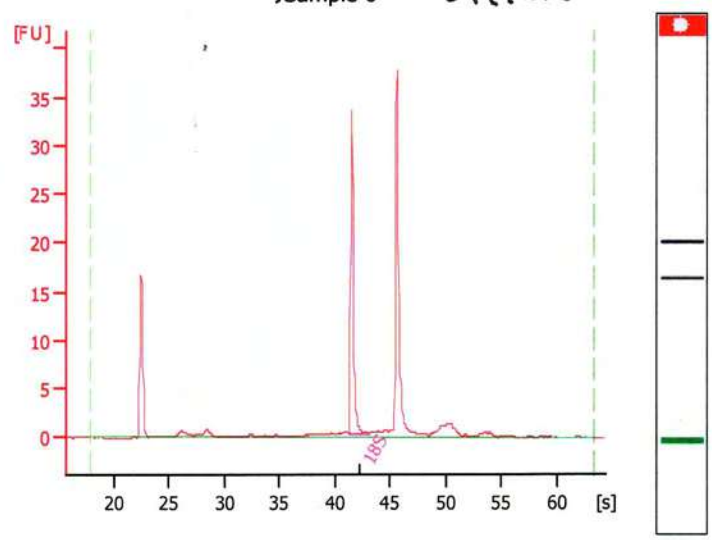
Assay Class:	EukaryoteTotal RNA Nano	Created:	12/20/2007 1:18:56 PM
Data Path:	C:\... EukaryoteTotal RNA Nano DE24802677 2007-12-20 13-18-56.xad	Modified:	12/20/2007 1:42:55 PM

Electropherogram Summary Continued ...

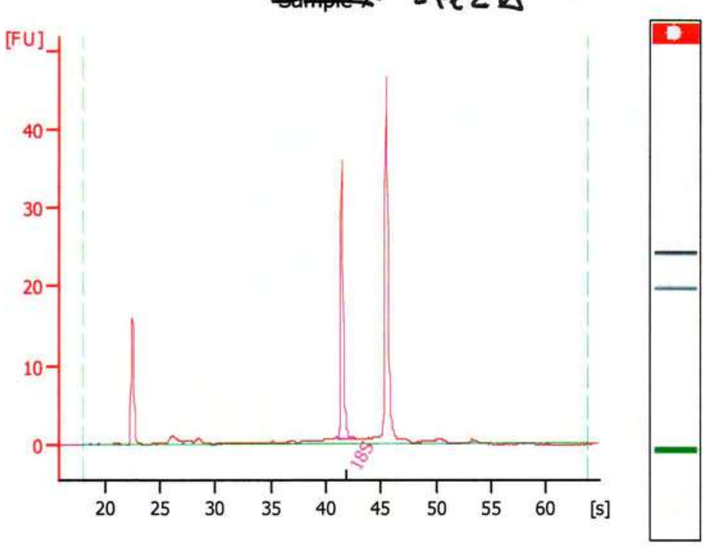
~~Sample 5~~ α37



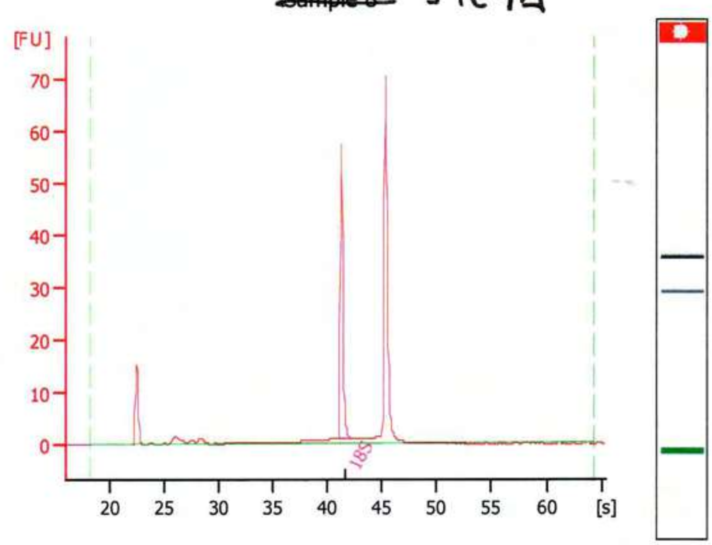
~~Sample 6~~ stc12Δ



~~Sample 7~~ stc2Δ



~~Sample 8~~ stc4Δ

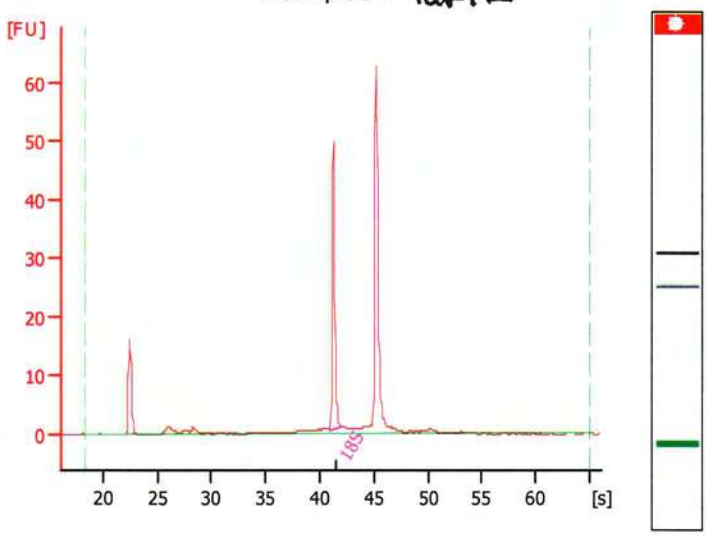


Assay Class: EukaryoteTotal RNA Nano
Data Path: C:\... EukaryoteTotal RNA Nano DE24802677 2007-12-20 13-18-56.xad

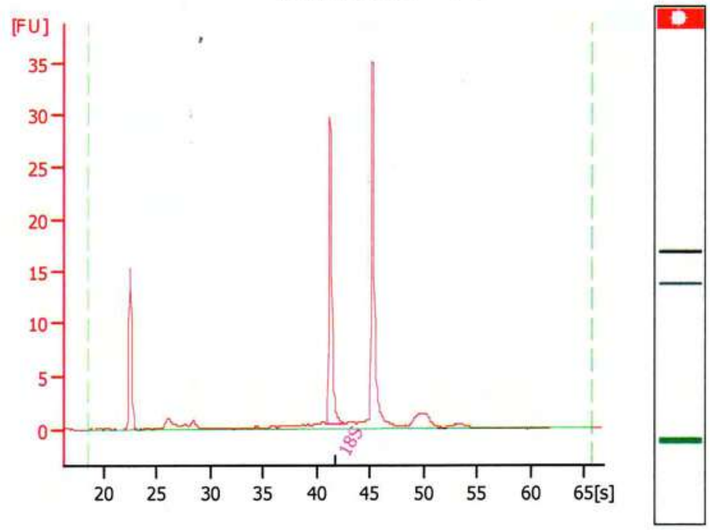
Created: 12/20/2007 1:18:56 PM
Modified: 12/20/2007 1:42:55 PM

Electropherogram Summary Continued ...

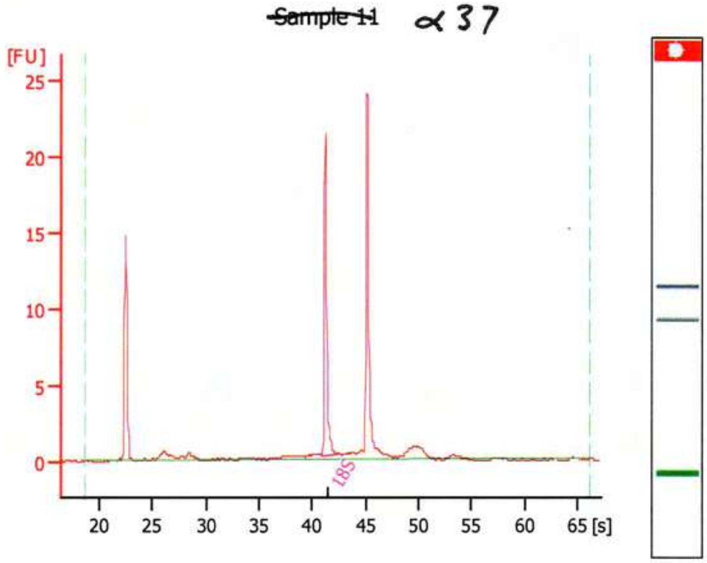
~~Sample 9~~ far1Δ



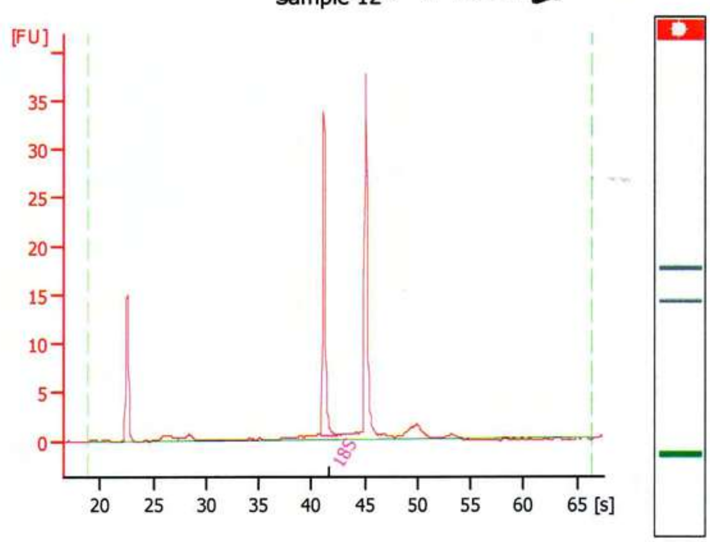
~~Sample 10~~ α68



~~Sample 11~~ α37



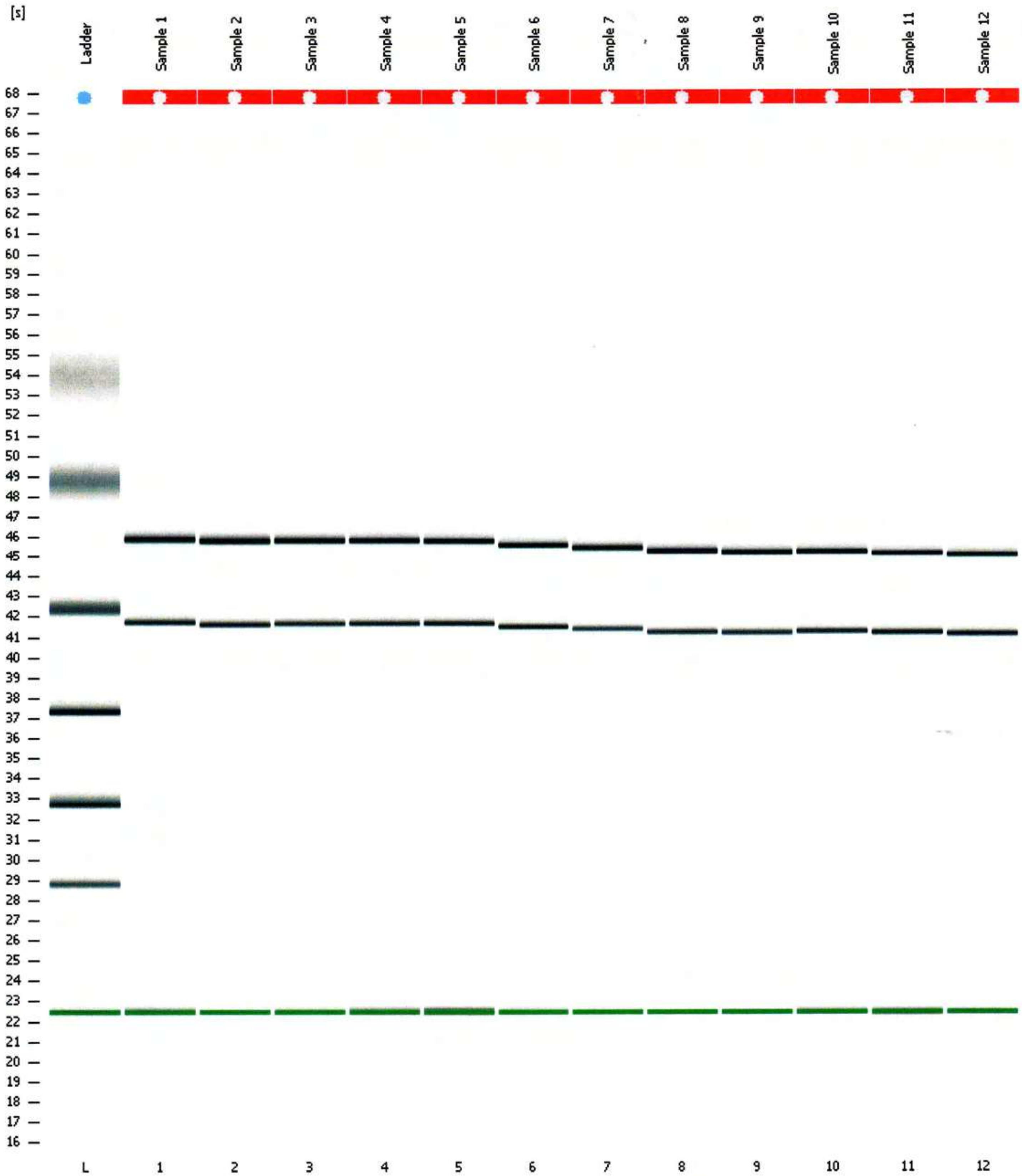
~~Sample 12~~ ste12Δ



Assay Class: EukaryoteTotal RNA Nano
Data Path: C:\... EukaryoteTotal RNA Nano DE24802677 2007-12-20 13-18-56.xad

Created: 12/20/2007 1:18:56 PM
Modified: 12/20/2007 1:42:55 PM

Gel Image



12/13/07

I have noticed that some strains acquire suppressors that restore mating (p 76). Here I am characterizing the shmoo morphology after 3h exposure to αF for 2 clones from each strain

TEST αF^R of my strains ~~SALT SENSITIVITY OF αF^R STRAINS~~

STRAIN		αF^R	STRAIN		αF^R
25	1		49	1	
	2			2	
26	1		50	1	
	2			2	
27	1		51	1	
	2			2	
28	1		52	1	
	2			2	
29	1		53	1	
	2			2	
30	1	No	54	1	
	2	No		2	
31	1		55	1	
	2			2	
32	1		56	1	
	2			2	
33	1		57	1	
	2			2	
34	1		58	1	
	2			2	
35	1		59	1	} small # of shmoos
	2			2	
36	1		60	1	} ″
	2			2	
37	1		61	1	
	2			2	
38	1	(a few shmoos)	62	1	
	2			2	
39	1		63	1	} small # of shmoos
	2			2	
40	1		64	1	} like 41, 47
	2			2	
41	1	No } >> shmoos than WT	65	1	
	2			2	
42	1		66	1	
	2			2	
43	1		67	1	} a few shmoos
	2			2	
44	1		68	1	} like 41, 47, 63
	2			2	
45	1	No } some shmoos	69	1	
	2	No		2	
46	1		41 small	1	
	2			2	
47	1	No } abnormal shmoos (like 41)			
	2	No			
48	1				
	2				

41 had two diff. colony sizes on YPD – I picked two of the small ones. It appears that the small colonies are αF^R and the large ones ~~are ...~~ have picked up a suppressor

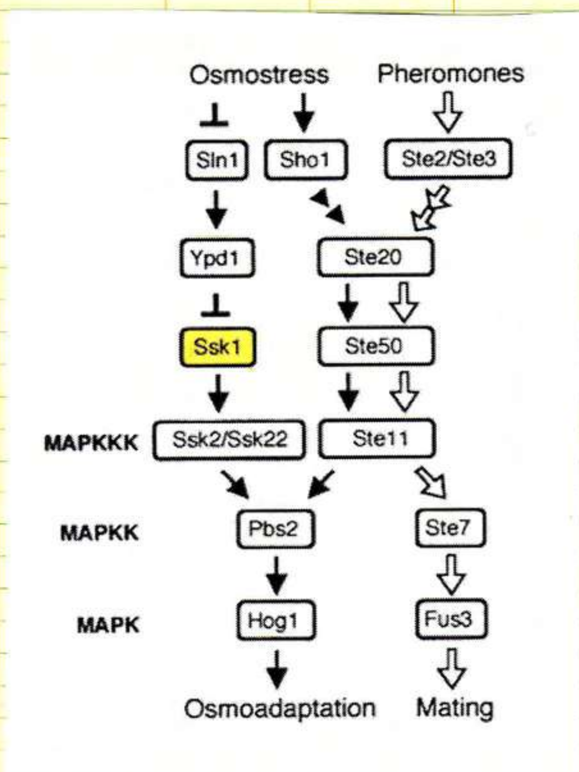
12/14/07

30 1
2 very strange morphology - some budding, some w/ multiple shmoos, some look like zygotes
3
4
38 1 - budding; maybe a few shmoos
2 "
3 " maybe arrested in G0 but no shmoo
4 "
41 1 "
2 "
3 "
4 "
45 1 v. small # of shmoos
2 "
3 a few long, narrow (abnormal) shmoos
4 "
47 1 large # of shmoos (abnormal, multiple shmoos)
2 "
3 "
4 "
59 1 "
2 lots of shmoos, but also budding cells - shmoos look more normal
3 "
4 mostly budding, some normal-looking shmoos
60 1 mostly large-budded cells - some shmoos
2 like αF 30
3 "
4 "
63 1 large budded cells
2 " , some unbudded cells
3 some shmoos (abnormal)
4 "
64 1 like 30/47/60
2 "
3 "
4 "
67 1 budding
2 "
3 "
4 " a few shmoos
68 1 like 30/47/60/64
2 mostly budding w/ a few shmoos
3 like 30/47/60/64
4 "

12/18/07

NOTES

· I have been spending time picking single colonies ~~off~~ of each of the αF^R strains and testing for salt sensitivity only again to realize that everything grows on 1M NaCl. This was a stupid mistake on my part. There are two pathways leading to Pbs2 and Hog1 — one of which is Ste20 - Ste50 - Ste11. The other is Sln1 - Ypd1 - Ssk1 - Ssk2/Ssk22



Therefore to test for salt-sensitivity I need to disrupt the Sln1 pathway. Then did her peptomer assay in an ssk1Δ strain. I have ordered primers to delete ssk1.

1/18/08

PREPARATION OF $\alpha F^R 47$ & $\alpha F^R 67$ FOR EXPRESSION MICROARRAY

· Previously when I have grown up $\alpha F^R 47$ & $\alpha F^R 67$ I noticed that the form shmoos - this has made me concerned that they are picking up ~~mutations and~~ suppressor mutations or reversions.

· Instead of growing the cultures O/N and diluting in the morning, yesterday I picked ~~4~~ single colonies of each strain and inoculated 10 ml YPD. - probably ~11am.

· Last night ~8:30pm I filtered the cells for expression analysis

· Coulter count

Bkg: 296000 316000

α 47	1	2.636 E7
	2	4.303 E7
	3	3.298 E7
	4	5.759 E7
α67	1	2.538 E7
	2	3.054 E7
	3	3.851 E7
	4	3.945 E7

Post Bkg 2.138 E6 1.184 E6 810000

· In addition, I diluted each culture 1:10 (~~to~~ 10 λ into 90 λ) YPD + αF

· Today I looked at the αF cultures under the microscope
↳ all 4 of the α47 cultures ~~had multiple~~ looked the same: multiple shmoos. All ~~8~~ 4 α67 cultures look the same as well: all devoid of shmoos - I saw only 1 or 2 shmoos after viewing sever fields of ~100 cells.

Coulter count αF cultures to determine amount of growth:
1:30

Bkg 112000

α 47	1	5.102 E6
	2	6.304 E6
	3	4.924 E6
	4	7.652 E6
α67	1	9.354 E7
	2	1.191 E8
	3	9.599 E7
	4	9.836 E7

Post. Bkg. 4.604 E6 2.142 E6 1.488 E6

1/22/08

PREPARATION OF $\alpha F^R 37$, $\alpha F^R 68$, & ste12Δ FOR EXPRESSION ARRAYS

- Last night I came in and inoculated 10 ml of YPD w/ a single colony of $\alpha F^R 37$, $\alpha F^R 68$, & ste12Δ – ~~these is are the only~~

↳ 12:00 midnight

- This morning (9:30) – filter for RNA prep
- 10λ of each into 90λ YPD + αF to test for αF^R

↳ 1:00pm: α68 produced WT-like shmoos (probably revertant)
α37 looks αF^R

Coulter count – 9:30 am

Bkg	142000	138000	272000
α37	1.965E7	1.922E7	1.973E7
α68	3.162E7	3.206E7	3.241E7
ste12Δ	4.439E7	4.491E7	4.452E7
Post Bkg	2.034E6	1.16E6	666000

NOTES

I have crude RNA and cleaned-up RNA for strains ste2Δ, ste4Δ, far1Δ, and α68 (p. 109)

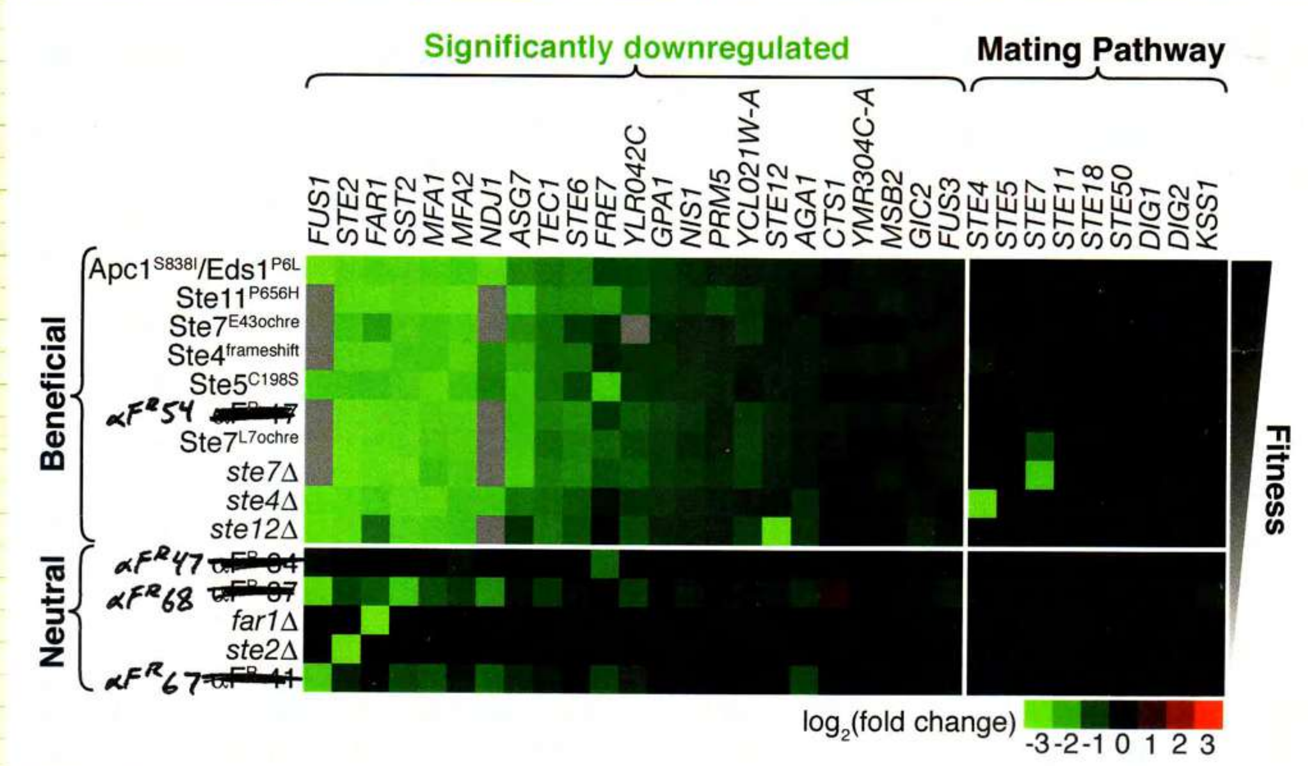
I have cleaned-up RNA for α37 & ste12Δ

For all of these the conc. is lower than I would like, so I decided to prepare more samples. Unfortunately this was last minute and the only ones I had on plates were α37, α68, & ste12Δ.

3/10/08

NOTES ON MICROARRAY EXPRESSION EXPERIMENTS

- The details of the expression array expts. are in the "microarray experiments" notebook
- I have done 3 sets of microarray experiments:
  (1) α29, α37, α40, α48, α54, α58, & α69 & ste7Δ vs. GIL121 (p. 93-104)
  (2) α37, α47, α67, ste2Δ, ste4Δ, far1Δ, α68 vs. GIL121 (p. 123 & microarray notebook)
  (3) α37, α47, α67, ste2Δ, ste4Δ, far1Δ, α68 vs. GIL121 (p. 124 & microarray notebook)
- I used the same labeled reference for expt. 1 & 2. Because the second set of arrays looked noiser I relabeled all samples and the reference for experiment 3. However, Expt. 3 also looked noisy. According to Alex the arrays in expts 2 & 3 look typical and the arrays in expt. 1 look more like dye-swap controls. The noise in expts 2 & 3 is most likely due to slight difference in growth conditions ~~[illegible]~~ or variation in the RNA preps. — All of the reference RNA was prepared during expt. 1. — If I want cleaner arrays I will need to ~~[illegible]~~ grow all the ~~cell~~ strains in the same media and do all of the RNA preps in parallel.
- From the arrays I can show that strains w/ a growth advantage downregulate the mating pathway whereas sterile strains without a growth advantage do not.



3/7/08

Discussion of experiments

· I have written a draft of a paper describing this work.

· There are some additional experiments I would like to do for this manuscript.

· I don't think it is necessary to do any additional phenotypic characterization (i.e. shmoo morphologies or salt sensitivity)

· The Kruglyak lab has shown that the Gpa1 in laboratory strains contains a SNP that results in an upregulation of basal signaling through the pathway. It seems likely that this mutation was selected for in the laboratory because it allows for faster mating or a more efficient mating response. However there should be a tradeoff in that the laboratory strain should grow slower since it has upregulated the mating pathway.

· I plan on reconstructing the wild-isolate Gpa1 allele in my W303 background. I will do 3 expts.

(1) Expression array to determine the difference in expression of the mating genes between these two strains.

(2) Fitness assays. I will at first try competing each strain against the common reference. The fitness difference between the strains may be too small to see any difference this way. I may then compete these strains directly against one another by labeling them w/ GFP and Tomato. Also I could try a PCR based method using the single SNP difference - either Maitreya's method or quantative PCR.

(3) Mating assays. I will do the traditional mating assay — mix a 3 α 1:10, ~~filter~~ mate on filters, plate to determine the # of mated a-cells and unmated a-cells. I might also try a FACS-based mating assay. I expect this would produce better data and allow for more frequent sampling.

· In order to do the above experiments I will need to construct several strains. For the expression analysis and the fitness assays I will need:

- yGIL121 w/ the RM allele of Gpa1 marked w/ Nat
- yGIL121 w/ the lab allele of Gpa1 marked w/ Nat

· For the mating assay I will need a Matα W303 with auxotrophies that can complement auxotrophies in yGIL121. I probably also want to put the RM allele of Gpa1 in this background.

→ It could be that a mating advantage will only be revealed if both partners have the same allele →

3/7/08

→ this may not be that big of a deal, ~~#~~ since I will be looking for a reduction in mating efficiency when I put the RM allele in W303.

~~I will~~ If I want to develop a FACS-based mating assay, I will need to transform GFP and Tomato into the Mata and Matα strains.

STRAIN CONSTRUCTION I ~ REPLACING GPA1 w/ THE RM-GPA1 ALLELE

· Here I describe the construction of two W303 strains expressing either the wild- or lab- versions of GPA1.

· From Josh in the Kruglyak lab I acquired the strain JW1 which is a BY strain with the RM-Gpa1 allele marked with ClonNAT.

→ From this strain I will amplify the C-terminus of Gpa1 and ClonNAT and clone it into pFA6 in the BamH1 and SacII sites.

· Prep plasmid pFA6a-GFP-~~Nat~~ ~~KanMX~~ NatMX (pGIL021) from my plasmid collection and digest:

w/ BamHI - overnight - 10:30 am
· clean up w/ Zymo kit
w/ SacII - 11:30 - 5:15 → @ 5:15 I added CIP

· PCR off of JW1 using GPA1_intF1_BamH1 & GPA1_extR1_SacII

36062271 IDT
G.LANG
37866741 3/5/2008 063
GPA1_intF1_BamHI
5'- AGT TTT ATT GGA TCC AGC AAT GAG TGA ATA CGA CC
Tm= 61.2 ºC , MW= 10,794.1
12.1 OD_{260} 34.60 nmol 0.37 mg

36062272 IDT
G.LANG
37866742 3/5/2008 064
GPA1_extR1_SacII
5'- TAC CAG GGA CCG CGG TTC GAG ATA ATA CCC TG TCC
Tm= 67.7 ºC , MW= 10,717.0
12.6 OD_{260} 37.80 nmol 0.40 mg

· Set up double digest of PCR products

- elute PCRs in 22 λ H_2O
- add 2.5 λ NEBuffer4, 0.25 λ BSA, 0.25 λ BamHI, 0.25 λ SacII
- digest o/N

3/8/08

LANE

1 Undigested prep of pGIL201 A
2 ——— " ——— B
3 BamHI/SacII/CIP pGIL201 A
4 ——— " ——— B
5 PCR off of JWI clone A
6 BamHI/SacI digested PCR A
7 ——— " ——— B

Two problems:

(1) My overnight double digest resulted in star activity

(2) My plasmid prep was not very concentrated.

I will address both of these issues before continuing.

· Redo PCR as before - put in freezer

3/10/08

· Today I remade a miniprep of the plasmid pGIL023 (pFA6a-GFP-Nat) using more amp. to ensure a better prep.

· Purify PCRs (2 - 100λ) using zymoresearch kit → elute 11λ each → pool

· Digest pGIL023 & GPA1::Nat PCR w/ BamHI

12 ~~10~~ λ	H_2O		
10 λ	pGIL023	22 λ	eluted PCR
2.5 λ	BamHI Buffer	2.5	BamHI Buffer
0.25 λ	BSA	0.25	BSA
0.25 λ	BamHI	0.25	BamHI
25 λ	Total	25 λ	

· 37° ~ 3:00 pm

· BamI digest went for 2.5 h → clean up w/ Zymo kit → elute 22λ

· Digest pGIL023 & GPA1:NAT PCR with ~~Cl~~ SacII/ClaI & SacII respectively

22λ elution (pGIL023 or GPA1 cut w/ BamHI)
2.5 NEB4
0.25 BSA
0.25 SacII
0.25 ClaI (pGIL023 only)

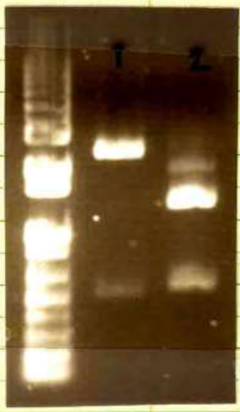
· 37° for 4.5 h (after 3.25 h I added 0.25 λ CIP to plasmid digest)

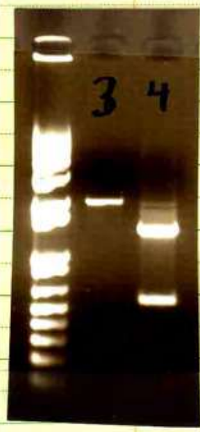
· Put in freezer overnight.

3/11/08

STAR ACTIVITY OF BamHI

- I am still getting some cutting in the middle of my PCR product — not complete, but the majority is cut:





LANE	
1	pGIL023 cut w/ BamHI, SacII, ClaI & CIP'd
2	GAPI::~~Kan~~NatMX PCR cut w/ BamHI, SacII
3	uncut GAPI::~~Kan~~NatMX PCR
4	same as 2

Near NatMX there are two "near consensus" BamHI sequences. There may be other near consensus sequences — I have not looked exhaustively — but the latter of these would yield a fragment consistent w/ the ~600 bp fragment above.

NatMX

```
CCGGGTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTTGTCCCCGCCGGGTCACCCGGCCAGCGACAT
GGAGGCCCAGAATACCCTCCTTGACAGTCTTGACGTGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGT
ACATTTAGCCCATACATCCCCATGTATAATCATTTGCATCCATACATTTTGATGGCCGCACGGCGCGAAGC
AAAAATTACGGCTCCTCGCTGCAGACCTGCGAGCAGGGAAACGCTCCCCTCACAGACGCGTTGAATTGTCC
CCACGCCGCGCCCCTGTAGAGAAATATAAAAGGTTAGGATTTGCCACTGAGGTTCTTCTTTCATATACTTC
CTTTTAAAATCTTGCTAGGATACAGTTCTCACATCACATCCGAACATAAACAACCATGGGTACCACTCTTG
ACGACACGGCTTACCGGTACCGCACCAGTGTCCCGGGGGACGCCGAGGCCATCGAGGCACTGGATGGGTCC
TTCACCACCGACACCGTCTTCCGCGTCACCGCCACCGGGGACGGCTTCACCCTGCGGGAGGTGCCGGTGGA
CCCGCCCCTGACCAAGGTGTTCCCCGACGACGAATCGGACGACGAATCGGACGACGGGGAGGACGGCGACC
CGGACTCCCGGACGTTCGTCGCGTACGGGGACGACGGCGACCTGGCGGGCTTCGTGGTCGTCTCGTACTCC
GGCTGGAACCGCCGGCTGACCGTCGAGGACATCGAGGTCGCCCCGGAGCACCGGGGGCACGGGGTCGGGCG
CGCGTTGATGGGGCTCGCGACGGAGTTCGCCCGCGAGCGGGGCGCCGGGCACCTCTGGCTGGAGGTCACCA
ACGTCAACGCACCGGCGATCCACGCGTACCGGCGGATGGGGTTCACCCTCTGCGGCCTGGACACCGCCCTG
TACGACGGCACCGCCTCGGACGGCGAGCAGGCGCTCTACATGAGCATGCCCTGCCCCTAATCAGTACTGAC
AATAAAAAGATTCTTGTTTTCAAGAACTTGTCATTTGTATAGTTTTTTTATATTGTAGTTGTTCTATTTTA
ATCAAATGTTAGCGTGATTTATATTTTTTTTCGCCTCGACATCATCTGCCCAGATGCGAAGTTAAGTGCGC
AGAAAGTAATATCATGCGTCAATCGTATGTGAATGCTGGTCGCTATACTGCTGTCGATTCGATACTAACGC
CGCCATCCAGTGTCGAAAAC
```

Inhibiting Star Activity (from NEB)

If you are concerned about star activity, we recommend the following guidelines.

1. Use as few units as possible to get a complete digestion. This avoids overdigestion and reduces the final glycerol concentration in the reaction.
2. Make sure the reaction is free of any organic solvents such as alcohols which might be present in the DNA preparation.
3. Raise the ionic strength of the reaction buffer to 100?150 mM (provided the enzyme is not inhibited by high salt).
4. Lower the pH of the reaction buffer to pH 7.0.
5. Use Mg++ as the divalent cation.

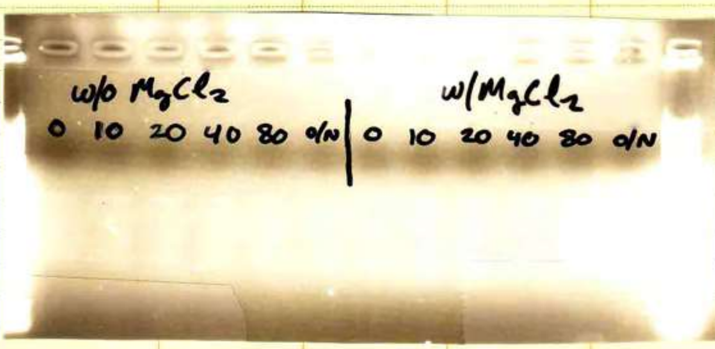
3/11/08

TEST FOR OPTIMAL DIGESTION CONDITIONS.

- I reran 5 100λ PCRs of the GPA1:: NatMX
- I cleaned up 1 PCR w/ Zymo kit → elute in 22λ H_2O
- I will set up 2 digests – one with less enzyme and higher ionic strength and the other the same as before.

12 λ	H_2O		9.6	H_2O
10 λ	PCR		10λ	PCR
2.5	BamHI Buffer		2.5	BamHI Buffer
0.25	BSA		2.5	50 mM $MgCl_2$
0.25	BamHI		0.25	BSA
25 λ			0.15	BamHI
			25λ	

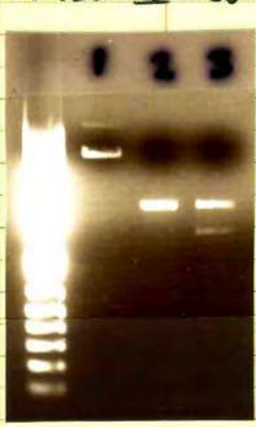
- Take 3λ from each – add to 9λ H_2O and put in freezer at t=0, t=10, t=20, t=40, t=80, & t=overnight (15.5h)



→ This doesn't tell me much. The smaller fragment is present in all lanes and the larger fragment is almost absent in the o/N digestion
↳ The DNA was too dilute.

3/12 - I think I will design a new primer. I should be able to clone into the BglII site instead of the BamHI site
↳ I will digest pG1023 & the GPA1::Nat PCR product w/ BglII to make sure that the pt plasmid only cuts once and that there is not an internal BglII site in the PCR product.

- Also I will submit the purified PCR for sequencing



3/13/08

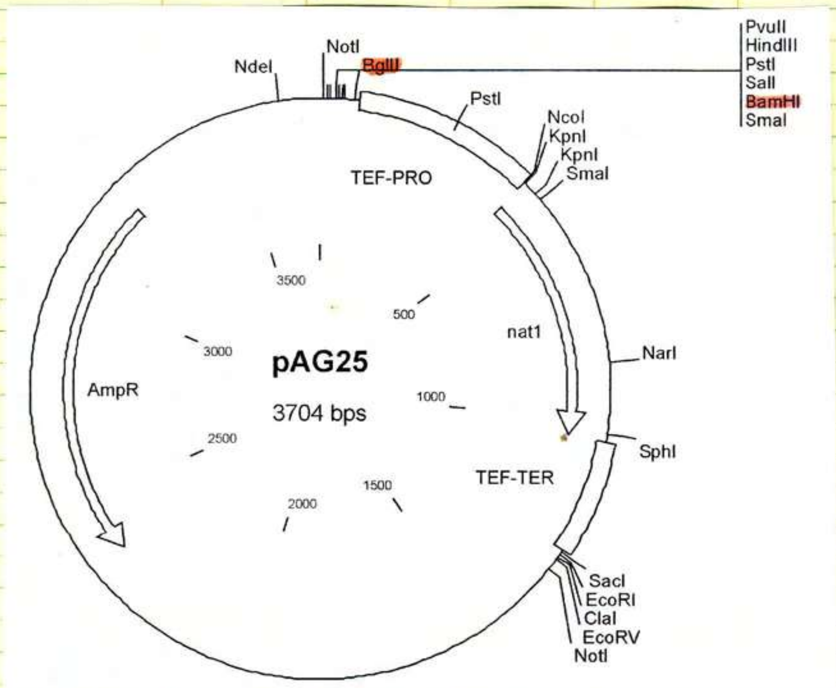
LANE	
1	pG1023 - cut w/ BglII ~2h
2	GPA1::Nat PCR - uncut
3	GPA1::Nat PCR – cut w/ BglII ~2h

→ ∴ The GPA1::Nat PCR also has an internal BglII site

3/13/08

NOTES ON GPA1 CLONING EFFORT

· When the Kruglyak lab cloned GPA1 into pAG25 they must have left ~~much o~~ some of the multicloning site ~~in tan~~ intact. At least the BamHI site and the BglII site are between GPA1 and Nat. I need to wait until the sequencing comes back to redesign my forward primer. I will probably use HindIII, SalI, or NotI.



3/14/08

SEQUENCING OF THE GPA1::NAT PCR FROM JW1

GPA1::NatMX PCR

```
AGCAATGAGTGAATACGACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAATAATGCTATTTG
ACACGTTATTGAACTCTAAGTGGTTCAAAGATACACCGTTTATTTTGTTTTTAAATAAAATTGATTTGTTCGAGGAA
AAGGTAAAAAGCATGCCCATAAGAAAGTACTTTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAA
ATATTTTGAGAAGATATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCGATA
CCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACCTTAAAAAAAGTGGTATTATA
TGAAGGAACTGTATAATTAAAGTAGTGTTTAGATACGTAAATTCTGTTTCCGAAGATGCAAGAAGGAGCAGCAGCAC
CAGAAAAAAATTGCTATTTTTCTTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTTTGTTCAACAG
TTCTTGATCTCGTTAAATCGTTGGATCCCCGGGTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTCCCCGC
CGGGTCACCCGGCCAGCGACATGGAGGCCCAGAATACCCTCCTTGACAGTCTTGACGTGCGCAGCTCAGGGGCATGA
TGTGACTGTCGCCCGTACATTTAGCCCATACATCCCCATGTATAATCATTTGCATCCATACATTTTGATGGCCGCAC
GGCGCGAAGCAAAAATTACGGCTCCTCGCTGCAGACCTGCGAGCAGGGAAACGCTCCCCTCACAGACGCGTTGAATT
GTCCCCACGCCGCGCCCCTGTAGAGAAATATAAAAGGTTAGGATTTGCCACTGAGGTTCTTCTTTCATATACTTCCT
TTTAAAATCTTGCTAGGATACAGTTCTCACATCACATCCGAACATAAACAACCATGGGTACCACTCTTGACGACACG
GCTTACCGGTACCGCACCAGTGTCCCCCGGGGGACGCCGAGGCCATCGAGGCACTGGATGGGTCCTTCACCACCGAC
ACCGTCTTCCGCGTCACCGCCACCGGGGACGGCTTCACCCTGCGGGAGGTGCCGGTGGACCCGCCCCTGACCAAGGT
GTTCCCCGACGACGAATCGGACGACGAATCGGACGACGGGGAGGACGGCGACCCGGACTCCCGGACGTTCGTCGCGT
ACGGGGACGACGGCGACCTGGCGGGCTTCGTGGTCGTCTCGTACTCCGGCTGGAACCGCCGGCTGACCGTCGAGGAC
ATCGAGGTCGCCCCGGAGCACCGGGGGCACGGGGTCGGGCGCGCGTTGATGGGGCTCGCGACGGAGTTCGCCCGCGA
GCGGGGCGCCGGGCACCTCTGGCTGGAGGTCACCAACGTCAACGCACCGGCGATCCACGCGTACCGGCGGATGGGGT
TCACCCTCTGCGGCCTGGACACCGCCCTGTACGACGGCACCGCCTCGGACGGCGAGCAGGCGCTCTACATGAGCATG
CCCTGCCCCTAATCAGTACTGACAATAAAAAGATTCTTGTTTTCAAGAACTTGTCATTTGTATAGTTTTTTTATATT
GTAGTTGTTCTATTTTAATCAAATGTTAGCGTGATTTATATTTTTTTTCGCCTCGACATCATCTGCCCAGATGCGAA
GTTAAGTGCGCAGAAAGTAATATCATGCGTCAATCGTATGTGAATGCTGGTCGCTATACTGCTGTCGATTCGATACT
AACGCCGCCATCCAGTGTCGAAAACGAGCTCGAATTCATCGATGATATCAGATCCACTAGTGGCCTATGCCCGGGTT
TTCAATTGAAAAACAAGGGTAATAAAATCGCATGAGAAAAAAAAGGTCCAGACACTTTTTTAGAAGAAAAACTCTTG
AAAACAATTGATTCTTCTACGTAAGGACAGGGTATTATCTCGAA
```

black = primer binding sites & SNP (G)

yellow = RE sites

GGATCC = BamHI
TACGTA = SnaBI

gray = homology to GPA1 region of the genome

red = GPA1 partial coding sequence

blue = NatMX coding sequence

- From the sequencing I can tell that GPA1 was cloned into the BamHI site
- I have verified that this is the RM version of GPA1
- I have found that I have sufficient downstream homology
  - 128 bp
  - 104 bp after SnaBI digest
- I have ordered a variation of the GPA1 forward primer with a HindIII site instead of a BamHI site.

3/19/08

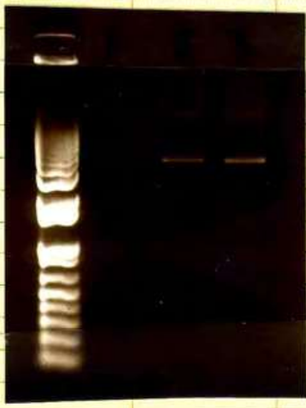
CLONING OF GPA1::NATMX

- Yesterday I streaked pGIL023 from frozen stock.
- This morning I picked 2 single colonies and inoculated 5 mL LB amp in order to do minipreps this afternoon.
- Miniprep → elute in 30 λ H_2O

- Set up PCR using GPA1_extR1-SacII (p 128) &

→ Zymo cleanup → elute in 20 λ H_2O

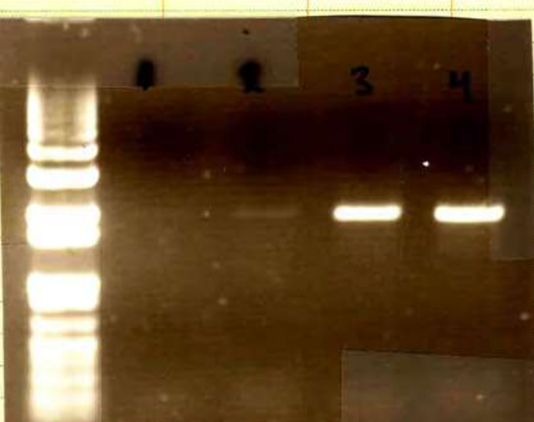
36237397 IDT
G.LANG
38028671 3/14/2008 068
GPA1_intF1_HindIII
5'- AGT TTT ATT AAG CTT AGC AAT GAG TGA ATA CGA
Tm= 58.6 ºC , MW= 10,793.1
9.4 OD_{260} 26.60 nmol 0.29 mg



LANE	
1	PCR — No Product
2	Miniprep #1
3	Miniprep #2

- Rerun PCR o/N

3/20/08



LANE	FORWARD RESITE	TEMP	
1	BamHI	JW1 clone	A
2	"	"	B
3	HindIII	"	A
4	"	"	B

→ for some reason now the PCRs with the old primer didn't work well but the PCRs with the new (HindIII) primer works well — ???

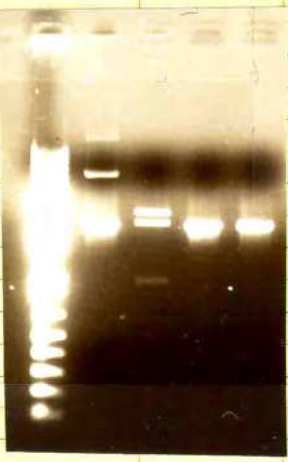
3/20/08

· Set up RE digest of pGIL023 and the GPA1::NatMx PCR

· I am digesting "miniprep #1" and the PCR off of "JW1 clone B" (see the gels on p. 134).

· 25 λ double digest w/ HindIII and SacII in NEB 2 – 11:30 am

· @ 2:30 pm – add 0.25 λ CIP to vector digest



LANE
1 pGIL023 uncut
2 pGIL023 cut w/ HindIII & SacII
3 GPA1::NatMx PCR uncut
4 GPA1::NatMx PCR cut w/ HindIII & SacII

→ HindIII cuts pGIL023 twice – once in the MCS
– once in KanMX

→ SacII cuts pGIL023 once

Expected product sizes: 2401 bp – vector
2049
983

· Gel purify the empty vector using Qiagen kit – gel run @ ~~6~~ 5:15 → elute 30 λ H_2O
- Use Zymo kit to purify PCR digest. → elute 10 λ H_2O (5:30)

· Nanodrop purified vector & insert

pGIL023 vector   4.4 ng/μL → concentrate using Zymo kit → elute 10 λ → 13.5 ng/μL
GPA1::NatMx PCR   150 ng/μL

5 λ	H_2O
8 λ	Vector
4 λ	Insert
2 λ	10× Ligase Buffer
1 λ	T4 DNA Ligase
20 λ	TOTAL

· ligate 20 min → transform DH5α cells

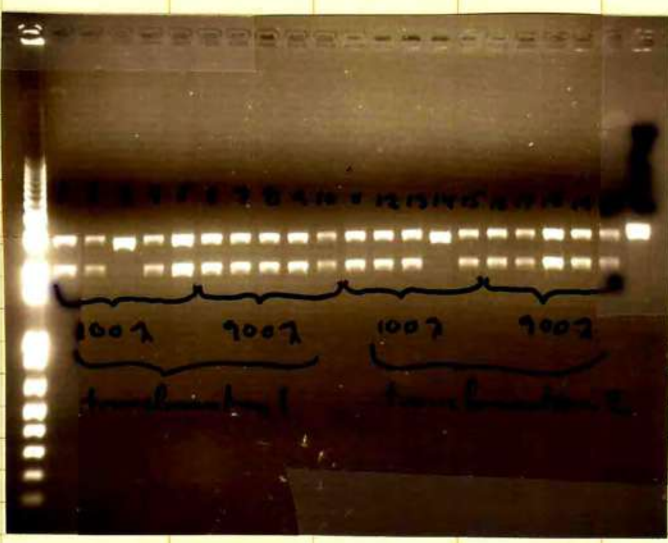
3/21/08

TRANSFORMATION OF DH5α w/ LIGATION

- Last night I set up ~~four~~ two transformations: both were 5λ of ligation. I did not do an ~~no~~ empty vector control because I used all of the prepared vector in the ligation. Since I used two REs, CIP'd and gel purified I am not very worried about the vector coming back together
- When I plated the ligations I spread 100λ of each onto 1 plate and spun down the other ~900mL and plated ~~?~~ onto another plate.
- Today I see a lawn around the edge of all 4 plates — this is probably from my spreading of amp on the plates. LBA plates were old.
- In the center of the plates I see single colonies — many more on the 900λ plates as expected
- Pick 5 colonies off of each plate → inoculate 2mL LBA (11:00am)
- Miniprep the 20 plasmids (~5:30pm)
- RE digest w/ EcoRV & PvuII

368.5λ	H_2O
55	NEBuffer 3
5.5	BSA
5.5	EcoRV
5.5	PvuII
440λ	

440λ → 20λ + 5λ plasmid → digest from ~~?~~ 6:30-8



EXPECTED PRODUCT SIZES

correct: 2550, 1827

vector only: 2401

pGIL023: 2751, 2667

→ 18 of 20 are correct, 2 are vector only

- Inoculate 3mL LBA w/ 5λ of 1, 2, 4, 5, 11, 12, 13, 15 ↗ 3/22/08 · Freeze down 1mL of each in duplicate.

3/22/08

SEQUENCING OF PLASMIDS

· To get the range of plasmid concentrations I will nanodrop #2 & #5 (p136)

#2 - 14.5 ng/μL } sequence 2λ of each plasmid
#5 - 57.0 ng/μL

· Sequence plasmids 1, 2, 4, 5, 11, 12, 13, & 15 using:

- GPA1_intF1_HindIII	8 pmol / ~~??~~ 26.6 pmol/μL	= 0.30	⇒ 0.3λ per rxn
- GPA1-extR1_SacII	8 / 37.8	= 0.21	⇒ 0.2
- KanMX/NatMX_R1	8 / 37.0	= 0.22	⇒ 0.2

3/25/08

Looking at the sequencing results ~~??~~ (p 139-141) for the GPA1 gene on the plasmids I see only 1 mutation in plasmid #13 resulting in Y→H substitution at position 409.

4/9/08

~~I put labeled~~

· On 3/22 (p. 136) I froze down the 8 sequenced plasmids. Yesterday I labeled them:

1
2 } ~~#~~ pGIL025
4
5
11 } discarded
12
13 - pGIL026
15 - discarded

· Yesterday (4/8) I streaked pGIL025 from freezer. This morning I started 2 5mL cultures in LB+Amp with single colonies.

· From each 5mL culture → 3 minipreps → elute 30λ EB

Miniprep #1
2 } same clone
3

4
5 } same clone
6

4/10/08

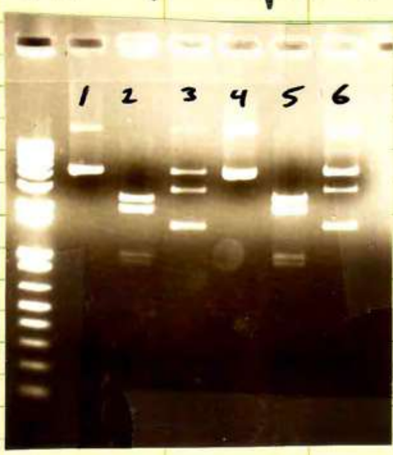
TRANSFORM yGIL121 w/ GPA1^{S469I}::NatMX AND GPA1::NatMX

· Digest minipreps from yesterday:

#1 & #4 – uncut
#2 & #5 – add 3λ NEBuffer 2, .3λ SacII, .3λ HindIII
#3 & #6 – add 3λ NEBuffer 4, .3λ BSA, .3λ SnaBI

4/11/08

After o/N digestion:



PREDICTED SIZES

SacI, HindIII: 2402, 1975
SnaBI: 2853, 1524

GPA1::NatMX fragments

· Gel purify GPA1::NatMX fragments

← 1975
← 1524
} see p. 133 for sequences of these fragments.

· Transform yGIL121 w/ the two fragments above

- On Wednesday (4/9) I streaked yGIL121 from frozen stock
- Yesterday I picked 2 single colonies and inoculated 5 mL YPD
- This morning I diluted back ~~1:20~~ 1:100 (100λ into 10mL) ~9:30am
- Start transformations @ 3:00 (~1.5 e7 cells/mL)

3/25/08

```
          541                                                        600
GPA1_NAT  GAGTGAATACGACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAAT
      03                      TGTTGTTTGAGGAGAAGAGTGAACAGAATGCATGAATCAAT
      01                           TTTGAGGAGAAGAGTGAACAGAATGCATGAATCAAT
      04                 AGAGTTGTTTGAGGATGAAGAGTGAACAGAATGCATGAATCAAT
      05                         AGTTTGAGGAGAAGAGTGAACAGAATGCATGAATCAAT
      02                         GGGATGAGGAGAAGAGTGAACAGAATGCATGAATCAAT
      07                       GGGGTTTGAGGAGAAGAGTGAACAGAATGCATGAATCAAT
      06                            TTGAGGAGAAGAGTGAACAGAATGCATGAATCAAT
      08                   GAGATGTTTGAGGAGAAGAGTGAACAGAATGCATGAATCAAT
      17  GAGTGAATACGACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAAT
      20  GAGTGAATACGACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAAT
      22  GAGTGAATACGACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAAT
      21  GAGTGAATACGACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAAT
      23  GAGTGAATACGACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAAT
      19  GAGTGAATACGACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAAT
      18  GAGTGAATACGACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAAT
      24  GAGTGAATACGACCNGATGTTGTTTGAGGATGAAAGAGTGTACAGAATGCANGAATCAAT
Consensus                        T TgaGGatgaAAGAGTGAACAGAATGCATGAATCAAT

          601                                                        660
GPA1_NAT  AATGCTATTTGACACGTTATT-GAACTCTAAGTGGTTCAAAGATACACCGTTTATTTTGT
      03  AATGCTATTTGACACGTTATN-GNTCTCTNAGTGGTTCAAAGATACACCGTTTATTTTGT
      01  AATGCTATTTGACACGTTATN-GNTCTCTNAGTGGTTCAAAGATACACCGTTTATTTTGT
      04  AATGCTATTTGACACGTTATT-GANCTCTAAGTGGTTCAAAGATACACCGTTTATTTTGT
      05  AATGCTATTTGACACGTTATN-GNTCTCTAAGTGGTTCAAAGATACACCGTTTATTTTGT
      02  AATGCTATTTGACACGTTANC-GTTTTCGGGGTGGTTCAAAGATACACCGTTTATTTTGT
      07  AATGCTATTTGACACGTTATCTGNTCTCNAAGTGGTTCAAAGATACACCGTTTATTTTGT
      06  AATGCTATTTGACACGTTANC-GNTNTCTAAGTGGTTCAAAGATACACCGTTTATTTTGT
      08  AATGCTATTTGACACGTTANC-GTTCTCNAAGTGGTTCAAAGATACACCGTTTATTTTGT
      17  AATGCTATTTGACACGTTATT-GAACTCTAAGTGGTTCAAAGATACACCGTTTATTTTGT
      20  AATGCTATTTGACACGTTATT-GAACTCTAAGTGGTTCAAAGATACACCGTTTATTTTGT
      22  AATGCTATTTGACACGTTATT-GAACTCTAAGTGGTTCAAAGATACACCGTTTATTTTGT
      21  AATGCTATTTGACACGTTATT-GAACTCTAAGTGGTTCAAAGATACACCGTTTATTTTGT
      23  AATGCTATTTGACACGTTATT-GAACTCTAAGTGGTTCAAAGATACACCGTTTATTTTGT
      19  AATGCTATTTGACACGTTATT-GAACTCTAAGTGGTTCAAAGATACACCGTTTATTTTGT
      18  AATGCTATTTGACACGTTATT-GAACTCTAAGTGGTTCAAAGATACACCGTTTATTTTGT
      24  ANTGCTNTNTGACACGTTATT-GAACTCTAAGTGGTTCAAAGATACACCGTTTATTTTGT
Consensus AATGCTATTTGACACGTTATT GAaCTCTAAGTGGTTCAAAGATACACCGTTTATTTTGT

          661                                                        720
GPA1_NAT  TTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACT
      03  TTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACT
      01  TTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACT
      04  TTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACT
      05  TTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACT
      02  TTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACT
      07  TTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACT
      06  TTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACT
      08  TTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACT
      17  TTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACT
      20  TTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACT
      22  TTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACT
      21  TTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACT
      23  TTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACT
      19  TTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACT
      18  TTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACT
      24  TTTTAAANAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTNCT
Consensus TTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACT
```

Plasmid	Traces
1	1, 17
2	2, 18
4	3, 19
5	4, 20
11	5, 21
12	6, 22
13	7, 23
15	8, 24

3/25/08

```
           721                                                         780
GPA1_NAT   TTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGA
03         TTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGA
01         TTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGA
04         TTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGA
05         TTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGA
02         TTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGA
07         TTCCTGATCACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGA
06         TTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGA
08         TTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGA
17         TTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGA
20         TTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGA
22         TTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGA
21         TTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGA
23         TTCCTGATCACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGA
19         TTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGA
18         TTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGA
24         TTCCTGATTCCCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTCAAATATTTTGAGAAGA
Consensus  TTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGA

           781                                                         840
GPA1_NAT   TATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCG
03         TATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCG
01         TATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCG
04         TATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCG
05         TATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCG
02         TATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCG
07         TATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCG
06         TATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCG
08         TATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCG
17         TATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCG
20         TATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCG
22         TATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCG
21         TATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCG
23         TATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCG
19         TATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCG
18         TATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCG
24         TATTTTTGAGCTTGAATAAGACAAACAAACCAATCNACGTGAANCGAACCTGCGCTACCG
Consensus  TATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCG

           841                                                         900
GPA1_NAT   ATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACC
03         ATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACC
01         ATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACC
04         ATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACC
05         ATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACC
02         ATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACC
07         ATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACC
06         ATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACC
08         ATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACC
17         ATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACC
20         ATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACC
22         ATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACC
21         ATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACC
23         ATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACC
19         ATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACC
18         ATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACC
24         ANACCCNAACTATGAAGTTCGTATTGAGNGCAGTCACCNATCTAATCATCCAGCANANCC
Consensus  ATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACC
```

3/25/08

SNP stop

```
          901                                                        960
GPA1_NAT  TTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA-GTGTTTAGATACGTA
      03  TTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA-GTGTTTAGATACGTA
      01  TTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA-GTGTTTAGATACGTA
      04  TTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA-GTGTTTAGATACGTA
      05  TTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA-GTGTTTAGATACGTA
      02  TTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA-GTGTTTAGATACGTA
      07  TTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA-GTGTTTAGATACGTA
      06  TTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA-GTGTTTAGATACGTA
      08  TTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA-GTGTTTAGATACGTA
      17  TTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA-GTGTTTAGATACGTA
      20  TTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA-GTGTTTAGATACGTA
      22  TTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA-GTGTTTAGATACGTA
      21  TTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA-GTGTTTAGATACGTA
      23  TTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA-GTGTTTAGATACGTA
      19  TTAAAAAAAGTGGTATTATATGAAGGAACNGTATAATTAAAGTA-GTGTTTAGATACGTA
      18  TTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA-GTGTTTAGATACGTA
      24  TTNNAAAAAGTGGTATTCTATGAAGGANCTGTATNATTAAAGTACGTGTTTAGATACGTA
Consensus TTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA GTGTTTAGATACGTA

          961                                                       1020
GPA1_NAT  AATTCTGTTTCCGAAGATGCAAGAAGGAGCAG-CAGCACCAGAAAAAATTGCTATTTTTC
      03  AATTCTGTTTCCGAAGATGCAAGAAGGAGCAG-CAGCACCAGAAAAAATTGCTATTTTTC
      01  AATTCTGTTTCCGAAGATGCAAGAAGGAGCAG-CAGCACCAGAAAAAATTGCTATTTTTC
      04  AATTCTGTTTCCGAAGATGCAAGAAGGAGCAG-CAGCACCAGAAAAAATTGCTATTTTTC
      05  AATTCTGTTTCCGAAGATGCAAGAAGGAGCAG-CAGCACCAGAAAAAATTGCTATTTTTC
      02  AATTCTGTTTCCGAAGATGCAAGAAGGAGCAG-CAGCACCAGAAAAAATTGCTATTTTTC
      07  AATTCTGTTTCCGAAGATGCAAGAAGGAGCAG-CAGCACCAGAAAAAATTGCTATTTTTC
      06  AATTCTGTTTCCGAAGATGCAAGAAGGAGCAG-CAGCACCAGAAAAAATTGCTATTTTTC
      08  AATTCTGTTTCCGAAGATGCAAGAAGGAGCAG-CAGCACCAGAAAAAATTGCTATTTTTC
      17  AATTCTGTTTCCGAAGATGCAAGAAGGAGCAG-CAGCACCAGAAAAAATTGCTANTTNTC
      20  AATTCTGTTTCCGAAGATGCAAGAAGGAGCAG-CAGCACCAGAAAAAATTGCTATTTTTC
      22  AATTCTGTTTCCGAAGATGCAAGAAGGAGCAG-CAGCACCAGAAAAAATNGCTATTTNTC
      21  AATTCTGTTTCCGAAGATGCAAGAAGGAGCAG-CAGCACCAGAAAAAATTGCTATTTNTC
      23  AATTCTGTTTCCGAAGATGCAAGAAGGAGCAG-CAGCACCAGAAAAAATTGCTATTTNTC
      19  AATTCTGTTTCCGAAGATGCAAGAAGGAGCAG-CAGCACCAGAAAAAATT
      18  AATTCTGTTTCCGAAGATGCAAGAAGGAGCAG-CAGCACCAGAAAAAA
      24  AANTCTGTTTCCGAAGATGCAAGAAGGAGCANGCAGCTCCAGAAANANNNGCTANTGGTG
Consensus AATTCTGTTTCCGAAGATGCAAGAAGGAGCAG CAGCACCAGAAAAAATTGCTATTT TC

          1021                                                      1080
GPA1_NAT  TTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTTTGTTCAACAGTTCT
      03  TTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTTTGTTCAACAGTTCT
      01  TTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTTTGTTCAACAGTTCT
      04  TTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTTTGTTCAACAGTTCT
      05  TTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTTTGTTCAACAGTTCT
      02  TTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTTTGTTCAACAGTTCT
      07  TTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTTTGTTCAACAGTTCT
      06  TTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTTTGTTCAACAGTTCT
      08  TTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTTTGTTCAACAGTTCT
      17  TTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTTGTCAACAGTCTG
      20  TTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAA
      22  TTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTTGTC
      21  TTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTTGTCAACATCTG
      23  TTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTTGTCAACATC
      19
      18
      24  TTCTCCCTTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTTGTCAACTGT
Consensus TTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTT
```

4/15/08

TRANSFORMATION OF yGIL121 CONTINUED

· The transformation of yGIL121 ~~##~~ in order to generate ~~#~~ isogenic strains w/ differing GPA1 alleles did not work. There were two ~~pt~~ problems:

- low transformation efficiency:

yGIL121-1-RM allele	2 colonies	→ restreak onto YPD (labeled D, E)
yGIL121-1-W303 allele	~~3~~ ~~on 4/14~~	
yGIL121-2-RM allele	3 colonies	→ restreak onto YPD (labeled A-C)
yGIL121-2-W303 allele	0 colonies	

- contamination: All four plates had some bacterial contamination. the plate yGIL121-1-W303 was overrun with contamination.

There are three possible reasons why this transformation didn't work:

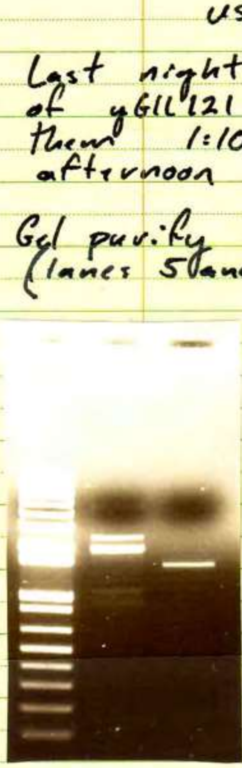
(1) Poor efficiency - I don't think this is the reason, but to be careful, I will use new carrier DNA next time

(2) Liquid Holding - Usually I would plate onto YPD and replicate plate onto Nat the following day - this time I did a 1.5h liquid hold in YPD then plated directly onto Nat. Next time I will replica plate.

(3) Bad Plasmid - ~~##~~ Since I cloned the GPA1::Nat fragment by PCR, it could contain a mutation. I did verify the Nat by sequencing. Next time I will use a different plasmid (see p. 136).

· Last night I inoculated 5 ml YPD w/ two single colonies of yGIL121 (labeled yGIL121-3 & 4). This morning I diluted them 1:100 (100 λ into 10 ml). I will transform these this afternoon → 9:00 am

· Gel purify the ~~##~~ SacI/HindIII & SnaBI digestions of pGIL025 (lanes 5 and 6 on p. 138)



· Clearly my gel purification of this first lane was terrible - I did not run the gel very long so I did not get good separation of the bands. (compare to gel on p. 138)

· Transform yGIL121-3&4 w/ the purified digests (at left)

↳ cell counts yGIL121-3 ~ 1.55E7
yGIL121-4 ~ 2.55E7

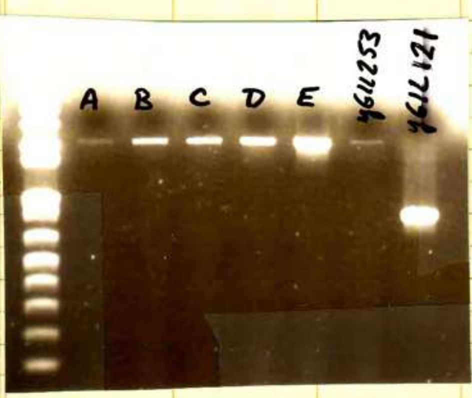
· 4/16/08 - replica plate onto YPD + ClonNat

4/17/08

- On Tuesday (4/15) I started o/n cultures of the 5 putative GPA1 transformants (A–E). ~~ws~~
- Yesterday (4/16) I made gDNA preps of these cultures ~~W~~ and I streaked onto YPD and YPD + ClonNat.
- PCR using the following primers:

36680342 IDT
G.LANG
38433036 4/9/2008
GPA1_intF2 069
5'- AAT TTA ACA TCG GCT CGT CC -3'
Tm= 53.0 ºC , MW= 6,052.0
7.0OD$_{260}$ 37.10nmol 0.23mg

36680343 IDT
G.LANG
38433037 4/9/2008
GPA1_extR2 070
5'- ATA TAT CCC GAG TAT TTA CC -3'
Tm= 45.6 ºC , MW= 6,051.0
7.0OD$_{260}$ 35.50nmol 0.21mg



It's obvious that these integrations worked. I will need to sequence to determine if the W303 allele or the RM allele is present in these strains.

4/23/08
- Relabel: B ⇒ yGIL265
  C ⇒ yGIL266
  D ⇒ yGIL267
- Discard: A, E

I froze down these 5 transformants (A–E). I will relabel them once ~~it~~ they are sequenced.

4/21/08

- On Thursday (4/17) I submitted the putative transformants A–E for sequencing using primers GPA1_intF1_HindIII & GPA1_ext_R1_SacII (I should have used KanMX/NatMX_RI instead)
- On pages 145 & 146 is the sequencing reads from primer GPA1_intF1_HindIII
  - Transformants A, C–E have the "RM-wild" allele. Transformant B has the "W303-1ab" allele.
  - The sequencing of E was not great – many ambiguities, poor quality
  - The called ambiguities and errors in A are artefacts of software interpretation of trace files. Upon visual inspection of the trace files, A is WT with the RM allele.
  - The traces ~~###~~ over the polymorphism is unambiguous. I am confident in these predictions even from just this one trace.

4/21/08 **SEQUENCING GPA1::NAT TRANSFORMANTS**

```
            1                                                           60
GPA1_NAT    AAGCTTAGCAATGAGTGAATACGACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAAT
GPA1-04-Pr                                  AGAGTGTTGAGAGAAGAGTGAACAGAAT  ← transformant A
GPA1-02-Pr                                AGAGTTGTTGAGGAGAAGAGTGAACAGAAT  ←  "  B
GPA1-03-Pr                                AGAGTTGTTGAGGAGAAGAGTGAACAGAAT  ←  "  C
GPA1-01-Pr                            NAANNGTTGTTTGAGGATGAAGAGTGAACAGAAT  ←  "  D
GPA1-05-Pr                            GNNNAGNGGGATTGAGGAGAAGAGTGAACAGAAT  ←  "  E
Consensus   ..............................agagtttttgaggagAAGAGTGAACAGAAT
            61   ┌ not a real insertion                                 120
GPA1_NAT    GCATG-AATCAATAATGCTATTTGACACGTTATTGAACTCT---AAGTGGTTCAAAGATA
GPA1-04-Pr  GCATG-AATCAATAATGCTATTTGACACGTTATTGAACTCT---AAGTGGTTCAAAGATA
GPA1-02-Pr  GCATG-AATCAATAATGCTATTTGACACGTTATTGAACTCT---AAGTGGTTCAAAGATA
GPA1-03-Pr  GCATG-AATCAATAATGCTATTTGACACGTTATTGAACTCT---AAGTGGTTCAAAGATA
GPA1-01-Pr  GCATGGAATCAATAATGCTATTTGACACGTTNCTGTNCTCT---AAGTGGTTCAAAGATA
GPA1-05-Pr  GCATG-AATCAATAATGCTATTTGACANGNCCCCCTNNNNAGGGNAGTGGTTCAAAGATA  ← poor sequencing
Consensus   GCATG.AATCAATAATGCTATTTGACAcGttattgaactct...aAGTGGTTCAAAGATA
            121                                                         180
GPA1_NAT    CACCGTTT-ATTTTGTTTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATG
GPA1-04-Pr  CACCGTTT-ATTTTGTTTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATG
GPA1-02-Pr  CACCGTTT-ATTTTGTTTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATG
GPA1-03-Pr  CACCGTTT-ATTTTGTTTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATG
GPA1-01-Pr  CACCGTTT-ATTTTGTTTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATG
GPA1-05-Pr  CACCGNTTTATTTTGTNCCCTTNTANAANTGATTTGTTCGAGGAAAAGGTAAAAAGCATG  ← poor sequencing
Consensus   CACCGtTT.ATTTTGTttttaaaTAaAAtTGATTTGTTCGAGGAAAAGGTAAAAAGCATG
            181                                                         240
GPA1_NAT    CCCATAAGAAAGTACTTTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTA
GPA1-04-Pr  CCCATAAGAAAGTACTTTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTA
GPA1-02-Pr  CCCATAAGAAAGTACTTTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTA
GPA1-03-Pr  CCCATAAGAAAGTACTTTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTA
GPA1-01-Pr  CCCATAAGAAAGTACTTTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGGTCTA
GPA1-05-Pr  CCCATAAGAAAGTACTTTCCTGATTACCAGGGACNTGTCGGCGATGCAGAAGCGGGTCTA
Consensus   CCCATAAGAAAGTACTTTCCTGATTACCAGGGACgTGTCGGCGATGCAGAAGCGGGTCTA
                                               → "G"
            241                                                         300
GPA1_NAT    AAATATTTTGAGAAGATATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAA
GPA1-04-Pr  AAATATTTTGAGAAGATATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAA
GPA1-02-Pr  AAATATTTTGAGAAGATATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAA
GPA1-03-Pr  AAATATTTTGAGAAGATATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAA
GPA1-01-Pr  AAATATTTTGAGAAGATATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAA
GPA1-05-Pr  AAATATTTTGAGAAGATATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAA
Consensus   AAATATTTTGAGAAGATATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAA
            301                                                         360
GPA1_NAT    CGAACCTGCGCTACCGATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTA
GPA1-04-Pr  CGAACCTGCGCTACCGATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTA
GPA1-02-Pr  CGAACCTGCGCTACCGATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTA
GPA1-03-Pr  CGAACCTGCGCTACCGATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTA
GPA1-01-Pr  CGAACCTGCGCTACCGATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTA
GPA1-05-Pr  CGAACCTGCGCTACCGATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTA
Consensus   CGAACCTGCGCTACCGATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTA
            361                    ┌ SNP   STOP                         420
GPA1_NAT    ATCATCCAGCAAAACCTTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA
GPA1-04-Pr  ATCATCCAGCAAAACCTTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA
GPA1-02-Pr  ATCATCCAGCAAAACCTTAAAAAAATTGGTATTATATGAAGGAACTGTATAATTAAAGTA
GPA1-03-Pr  ATCATCCAGCAAAACCTTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA
GPA1-01-Pr  ATCATCCAGCAAAACCTTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA
GPA1-05-Pr  ATCATCCAGCAAAACCTTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA
Consensus   ATCATCCAGCAAAACCTTAAAAAAAgTGGTATTATATGAAGGAACTGTATAATTAAAGTA
            421                                                         480
GPA1_NAT    GTGTTTAGATACGTAAATTCTGTTTCCGAAGATGCAAGAAGGAGCAGCAGCACCAGAAAA
GPA1-04-Pr  GTGTTTAGATACGTAAATTCTGTTTCCGAAGATGCAAGAAGGAGCAGCAGCACCAGAAAA
GPA1-02-Pr  GTGTTTAGATACGTAAATTCTGTTTCCGAAGATGCAAGAAGGAGCAGCAGCACCAGAAAA
GPA1-03-Pr  GTGTTTAGATACGTAAATTCTGTTTCCGAAGATGCAAGAAGGAGCAGCAGCACCAGAAAA
GPA1-01-Pr  GTGTTTAGATACGTAAATTCTGTTTCCGAAGATGCAAGAAGGAGCAGCAGCACCAGAAAA
GPA1-05-Pr  GTGTTTAGATACGTAAATTCTGTTTCCGAAGATGCAAGAAGGAGCAGCAGCACCAGAAAA
Consensus   GTGTTTAGATACGTAAATTCTGTTTCCGAAGATGCAAGAAGGAGCAGCAGCACCAGAAAA
```

```
            481                                                         540
 GPA1_NAT   AATTGCTATTTTTCTTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTT
GPA1-04-Pr  AATTGCTATTTTTCTTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTT
GPA1-02-Pr  AATTGCTATTTTTCTTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTT
GPA1-03-Pr  AATTGCTATTTTTCTTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTT
GPA1-01-Pr  AATTGCTATTTTTCTTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTT
GPA1-05-Pr  AATTGCTATTTTTCTTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTT
 Consensus  AATTGCTATTTTTCTTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTT
            541                                                         600
 GPA1_NAT   TGTTCAACAGTTCTTGATCTCGTTAAATCGTTGGATCCCCGGGTTAATTAAGGCGCGCCA
GPA1-04-Pr  TGTTCAACAGTTCTTGATCTCGTTAAATCGTTGGATCCCCGGGTTAATTAAGGCGCGCCA
GPA1-02-Pr  TGTTCAACAGTTCTTGATCTCGTTAAATCGTTGGATCCCCGGGTTAATTAAGGCGCGCCA
GPA1-03-Pr  TGTTCAACAGTTCTTGATCTCGTTAAATCGTTGGATCCCCGGGTTAATTAAGGCGCGCCA
GPA1-01-Pr  TGTTCAACAGTTCTTGATCTCGTTAAATCGTTGGATCCCCGGGTTAATTAAGGCGCGCCA
GPA1-05-Pr  TGTTCAACAGTTCTTGATCTCGTTAAATCGTTGGATCCCCGGGTTAATTAAGGCGCGCCA
 Consensus  TGTTCAACAGTTCTTGATCTCGTTAAATCGTTGGATCCCCGGGTTAATTAAGGCGCGCCA
            601                                                         660
 GPA1_NAT   GATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGACATGGAGGCCCAGA
GPA1-04-Pr  GATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGACATGGAGGCCCAGA
GPA1-02-Pr  GATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGACATGGAGGCCCAGA
GPA1-03-Pr  GATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGACATGGAGGCCCAGA
GPA1-01-Pr  GATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGACATGGAGGCCCAGA
GPA1-05-Pr  GATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGACATGGAGGCCCAGA
 Consensus  GATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGACATGGAGGCCCAGA
            661                                                         720
 GPA1_NAT   ATACCCTCCTTGACAGTCTTGACGTGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGT
GPA1-04-Pr  ATACCCTCCTTGACAGTCTTGACGTGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGT
GPA1-02-Pr  ATACCCTCCTTGACAGTCTTGACGTGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGT
GPA1-03-Pr  ATACCCTCCTTGACAGTCTTGACGTGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGT
GPA1-01-Pr  ATACCCTCCTTGACAGTCTTGACGTGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGT
GPA1-05-Pr  ATACCCTCCTTGACAGTCTTGACGTGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGT
 Consensus  ATACCCTCCTTGACAGTCTTGACGTGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGT
            721                                                         780
 GPA1_NAT   ACATTTAGCCCATACATCCCCATGTATAATCATTTGCATCCATACATTTTGATGGCCGCA
GPA1-04-Pr  ACATTTAGCCCATACATCCCCATGTATAATCATTTGCATCCATACATTTTGATGGCCGCA
GPA1-02-Pr  ACATTTAGCCCATACATCCCCATGTATAATCATTTGCATCCATACATTTTGATGGCCGCA
GPA1-03-Pr  ACATTTAGCCCATACATCCCCATGTATAATCATTTGCATCCATACATTTTGATGGCCGCA
GPA1-01-Pr  ACATTTAGCCCATACATCCCCATGTATAATCATTTGCATCCATACATTTTGATGGCCGCA
GPA1-05-Pr  ACATTTAGCCCATACATCCCCATGTATAATCATTTGCATCCATACATTTTGATGGCCGCA
 Consensus  ACATTTAGCCCATACATCCCCATGTATAATCATTTGCATCCATACATTTTGATGGCCGCA
            781                                                         840
 GPA1_NAT   CGGCGCGAA-GCAAAAATTACGGCTCCTCGCTGCAGACCTGCGAGCAGGGAAACGCTCCC
GPA1-04-Pr  CGGCGCGAA-GCAAAAATTACGGCTCCTCGCTGCAGACCTGCGAGCAGGGAAACGCTCCC
GPA1-02-Pr  CGGCGCGAA-GCAAAAATTACGGCTCCTCGCTGCAGACCTGCGAGCAGGGAAACGCTCCC
GPA1-03-Pr  CGGCGCGAA-GCAAAAATTACGGCTCCTCGCTGCAGACCTGCGAGCAGGGAAACGCTCCC
GPA1-01-Pr  CGGCGCGAA-GCAAAAATTACGGCTCCTCGCTGCAGACCTGCGAGCAGGGAAACGCTCCC
GPA1-05-Pr  CGGCGCGNAAGCAAAAATTANGGCTCCTCGCTGCAGACCTGCGAGCAGGGAAACGCTCCC
 Consensus  CGGCGCGaA.GCAAAAATTAcGGCTCCTCGCTGCAGACCTGCGAGCAGGGAAACGCTCCC
            841                                                         900
 GPA1_NAT   CTCACAGACGCGTTG-AATTGTCCCCACGCCGCGCCCCTGTAGAGAA---ATATAAAA--
GPA1-04-Pr  CTCACAGACGCGTTG-AATTGTCCCCACGCCGCGCCCCTGTAGAGAA---ATATAAAA--
GPA1-02-Pr  CTCACAGACGCGTTG-AATTGTCCCCACGCCGCGCCCCTGTAGAGGA---AATATAAAAG
GPA1-03-Pr  CTCACAGACGCGTTG-AATTGTCCCCACGCCGCGCCCCTGTAGAGAA---ATATAAAA--
GPA1-01-Pr  CTCACAGACGCGTTGAAATTGTCCCCACGCCGCGCCCCTGTAGAGAA----ATATANAAG
GPA1-05-Pr  CTCANAGACGCGTTGGAATTGNCCCCACGCCGGGGCCCCCTGNAGAAGGAAATATAAAAG
 Consensus  CTCAcAGACGCGTTG.AATTGtCCCCACGCCGcGcCCCtgTagAGaA...aaaaaAaA..
            901                                                         960
 GPA1_NAT   GGTTAGGATTTGCCACTGAGGTTCTTCTTT-CATAT-ACTTCCTTTTAAAAT-CTTGCTA
GPA1-04-Pr  GGTTAGGATTTGCCACTGAGGTNCTTCTTT-CATAT-ACTTCCTTTTAAAAT-CTTGCTA
GPA1-02-Pr  GGTTAGGATTTGCCACTGAGGGTCTTCTTT-CATAT-ACTTCCCTTTTAAAATCTTGCTA
GPA1-03-Pr  GGTTAGGATTTGCCACTGAGGTTCTTCNTTTCATAT-ACTTCCNTTTAAAATCCTTGCTA
GPA1-01-Pr  GTTAGGGATTTGCCACTGAGGTTCTTCTTTCATAN--ACTTCCTTTTAAAAATCTTGCCT
GPA1-05-Pr  GTTAGGATTTGCCNNTGNAGGTTCTTCTTTTNANATANTTTCCTTTTNAAAATCNTGGCT
 Consensus  GgTtaGgaTTtgCcactgAGGttCTTCtTT.catat.acTTCCtTTTaAAAatCtTGcta
```

4/25/08

· On 4/15 (p. 143) I retransformed yGIL121 w/ SacI/HindIII and SnaBI digests of pGIL025.

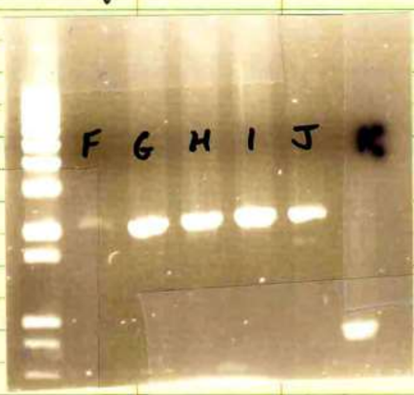
~~On monday (4/11) I~~ · Again I had poor transformation efficiency: 2 colonies on yGIL121-3 w/ SacII/HindIII (labeled J & K); 4 colonies on yGIL121-4 w/ SacII/HindIII (labeled F-I); no colonies on either transformation plate w/ SnaBI.

· On monday (4/21) I streaked putative transformants F-K on YPD

· On wednesday (4/23) I picked a single colony and inoculated 5 mL YPD

· Yesterday I froze down these six putative transformants (to be relabeled later)

· Yesterday I made gDNA preps of F-K, and performed PCR using primers GPA1-intF2 & GPA1-extR2 (p. 144).



→ F-J look correct by PCR. K is not a proper integration.

· Yesterday I submitted F-J for sequencing using primers GPA1_intF2 (p. 144) & KanMX/NatMX_R1 (p. 137)

4/28/08

Analysis of GPA1::NAT sequences

- Today I got back the sequencing from last week
- Transformants G & H have the lab allele; F, I, & J have the wild allele. → relabel G → yGIL268, H → yGIL269, F → yGIL270, I → yGIL271, J → yGIL272
- In these sequences I see two additional SNPs in G&H (see p. 150 & 151). These lie beyond the stop codon. Looking in SGD, these two SNPs are in S288c these two SNPs are present. Therefore the plasmids I constructed (all isogenic except for 1 mutation, p. 139–141) contain ~~3~~ at least 3 SNP differences from S288c — the one of interest (coding) and the two noncoding

→ ~~Strain yGIL265 ("8" p. 144) got just the all 3 "wild" SNP~~

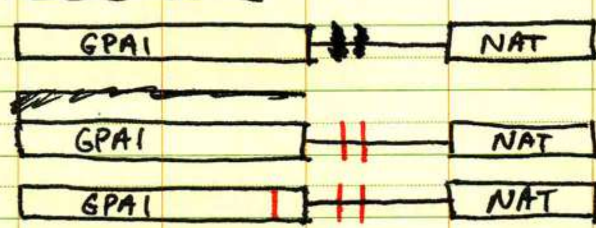
STRAINS

yGIL268
yGIL269

yGIL265

yGIL266
" 267
" 270
" 271
" 272

STRUCTURE



| = RM, "wild" SNP

5/9/08

I looked at the available RM sequence to determine if the noncoding SNPs are in the RM sequence or if ~~they ar~~ were introduced during construction of the JW1 strain I got from the Kruglyak lab (p. 128)

RM11-1a
AAATATTTTGAGAAGATATTTTTGAGCTTGAATAAGACAAACAAACCAATCTACGTGAAA
CGAACCTGCGCTACCGATACCCAAACTATGAAGTTCGTATTGAGTGCAGTCACCGATCTA
ATCATCCAGCAAAACCTTAAAAAAAGTGGTATTATATGAAGGAACTGTATAATTAAAGTA
GTGTTTAGATACGTAAATTCTGTTTCCGAAGATGCAAGAAGGAGCAGCAGCACCAGAAAA
AATTACTATTTTTCTTCTCCATTAGAGTCTATGACGGAATGCCAAATGAAAAAGCCATTT
TGTTCAACAGTTCTTGATCTCGTTAAATCGTTCCGGGTTTTCAATTGAAAAACAAGGGTA
ATAAAATCGCATGAGAAAAAAAAAGGTCCAGACACTTTTTTAGAAGAAAAACTCTTGAAAA

BLUE – Real RM SNPs
RED – False RM SNP (probably occurred during construction of JW1, p. 128)

4/28/08 SEQUENCING GPA1::NAT TRANSFORMANTS - SECOND ROUND

```
             1                                                         60
GPA1NAT
GPA1-11-pr                         AGTCCGNGCGNGNGGGC-GCGTTCTGAACGTAAGAAGT
GPA1-12-pr                          GNNCGCGC-NGNGGGC-GCGTTCTGA-CGTAG--AGT
GPA1-13-pr                               GNCC-CGGGGGC-GCGTTCTGA-CGTAAGAAGT
GPA1-14-pr                         AGNCCGCGC-GGNGGGC-GCGTTCTGA-CGTAAGAAGT
GPA1-15-pr                         AGTCCGCGCTGGGGGGC-GCGTTCTGA-CGTAAGAAGT
16           TCGGCTCGTCCAAATTCAAGGTTCTCGACGCTGGTGGGCAGCGTTCTGAACGTAAGAAGT
17             GGCTCGTCCAAATTCAAGGTTCTCGACGCTGGTGGGCAGCGTTCTGAACGTAAGAAGT
18             GGCTCGTCCAAATTCAAGGTTCTCGACGCTGGTGGGCAGCGTTCTGAACGTAAGAAGT
19             GCTCGTCCCAAATTCAAGGTTCTCGACGCTGGTGGGCAGCGTTCTGAACGTAAGAAGT
20             GCTCGTCCCAAATTCAAGGTTCTCGACGCTGGTGGGCAGCGTTCTGAACGTAAGAAGT
Consensus    ........................tc..cgCtgG.GGGC.GCGTTCTGAaCGTAagaAGT
             61                                                       120
GPA1NAT                                         AAGCTTAGCAATGAGTGAATACG
GPA1-11-pr   GGATTCATTGTTTCGNAGGANTTACAGCAGTTTTATTTGTTTTAGCNATGAGTGAATACG
GPA1-12-pr   GGATTCATTGTTTCGAAGGAATTACAGCAGTTTTATTTGTTTTAGCAATGAGTGAATACG
GPA1-13-pr   GGATTCATTGTTTCGAAGGAATTACAGCAGTTTTATTTGTTTTAGCAATGAGTGAATACG
GPA1-14-pr   GGATTCATTGTTTCGAAGGAATTACAGCAGTTTTATTTGTTTTAGCAATGAGTGAATACG
GPA1-15-pr   GGATTCATTGTTTCGAAGGAATTACAGCAGTTTTATTTGTTTTAGCAATGAGTGAATACG
16           GGATTCATTGTTTCGAAGGAATTACAGCAGTTTTATTTGTTTTAGCAATGAGTGAATACG
17           GGATTCATTGTTTCGAAGGAATTACAGCAGTTTTATTTGTTTTAGCAATGAGTGAATACG
18           GGATTCATTGTTTCGAAGGAATTACAGCAGTTTTATTTGTTTTAGCAATGAGTGAATACG
19           GGATTCATTGTTTCGAAGGAATTACAGCAGTTTTATTTGTTTTAGCAATGAGTGAATACG
20           GGATTCATTGTTTCGAAGGAATTACAGCAGTTTTATTTGTTTTAGCAATGAGTGAATACG
Consensus    GGATTCATTGTTTCGaAGGAaTTACAGCAGTTTTATTTGTTTTAGCAATGAGTGAATACG
             121                                                      180
GPA1NAT      ACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAATAATGCTATTTG
GPA1-11-pr   ACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCNTGAATCAATAATGCTATTTG
GPA1-12-pr   ACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAATAATGCTATTTG
GPA1-13-pr   ACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAATAATGCTATTTG
GPA1-14-pr   ACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAATAATGCTATTTG
GPA1-15-pr   ACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAATAATGCTATTTG
16           ACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAATAATGCTATTTG
17           ACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAATAATGCTATTTG
18           ACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAATAATGCTATTTG
19           ACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAATAATGCTATTTG
20           ACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAATAATGCTATTTG
Consensus    ACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAATAATGCTATTTG
             181                                                      240
GPA1NAT      ACACGTTATTGAACTCTAAGTGGTTCAAAGATACACCGTTT-ATTTTGTTTTTAAATAAA
GPA1-11-pr   ACACGTTATTGAACTCTANGTGGTTCANAGATACACCGTTT-ATTTTGTTTTTAAATAAN
GPA1-12-pr   ACACGTTATTGAACTCTAAGTGGTTCAAAGATACACCGTTT-ATTTTGTTTTTAAATAAA
GPA1-13-pr   ACACGTTATTGAACTCTAAGTGGTTCAAAGATACACCGNTTTATTTTGTTTTTAAATAAA
GPA1-14-pr   ACACGTTATTGAACTCTAAGTGGTTCAAAGATACACCGTTT-ATTTTGTTTTTAAATAAA
GPA1-15-pr   ACACGTTATTGAACTCTAAGTGGTTCAAAGATACACCGTTT-ATTTTGTTTTTAAATAAA
16           ACACGTTATTGAACTCTAAGTGGTTCAAAGATACACCGTTT-ATTTTGTTTTTAAATAAA
17           ACACGTTATTGAACTCTAAGTGGTTCAAAGATACACCGTTT-ATTTTGTTTTTAAATAAA
18           ACACGTTATTGAACTCTAAGTGGTTCAAAGATACACCGTTT-ATTTTGTTTTTAAATAAA
19           ACACGTTATTGAACTCTAAGTGGTTCAAAGATACACCGTTT-ATTTTGTTTTTAAATAAA
20           ACACGTTATTGAACTCTAAGTGGTTCAAAGATACACCGTTT-ATTTTGTTTTTAAATAAA
Consensus    ACACGTTATTGAACTCTAAGTGGTTCAAAGATACACCGTTT ATTTTGTTTTTAAATAAA
             241                                                      300
GPA1NAT      ATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACTTTCCTGATTAC
GPA1-11-pr   ATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACTTTCCTGATTAC
GPA1-12-pr   ATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACTTTCCTGATTAC
GPA1-13-pr   ATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACTTTCCTGATTAC
GPA1-14-pr   ATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACTTTCCTGATTAC
GPA1-15-pr   ATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACTTTCCTGATTAC
16           ATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACTTTCCTGATTAC
17           ATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACTTTCCTGATTAC
18           ATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACTTTCCTGATTAC
19           ATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACTTTCCTGATTAC
20           ATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACTTTCCTGATTAC
```

TRANSFORMANT

~~F - 11, 16~~
~~G - 12, 17~~
~~H - 13, 18~~
~~I - 14, 19~~
~~J - 15, 20~~

F - 11, 16
G - 12, 17
H - 13, 18
I - 14, 19
J - 15, 20

```
Consensus    ATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCATAAGAAAGTACTTTCCTGATTAC
             301                                                       360
GPA1NAT      CAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGATATTTTTGAGC
GPA1-11-pr   CAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGATATTTTTGAGC
GPA1-12-pr   CAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGATATTTTTGAGC
GPA1-13-pr   CAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGATATTTTTGAGC
GPA1-14-pr   CAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGATATTTTTGAGC
GPA1-15-pr   CAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGATATTTTTGAGC
        16   CAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGATATTTTTGAGC
        17   CAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGATATTTTTGAGC
        18   CAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGATATTTTTGAGC
        19   CAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGATATTTTTGAGC
        20   CAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGATATTTTTGAGC
Consensus    CAGGGACGTGTCGGCGATGCAGAAGCGGGTCTAAAATATTTTGAGAAGATATTTTTGAGC
             361                                                       420
GPA1NAT      TTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCGATACCCAAACT
GPA1-11-pr   TTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCGATACCCAAACT
GPA1-12-pr   TTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCGATACCCAAACT
GPA1-13-pr   TTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCGATACCCAAACT
GPA1-14-pr   TTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCGATACCCAAACT
GPA1-15-pr   TTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCGATACCCAAACT
        16   TTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCGATACCCAAACT
        17   TTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCGATACCCAAACT
        18   TTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCGATACCCAAACT
        19   TTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCGATACCCAAACT
        20   TTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCGATACCCAAACT
Consensus    TTGAATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCGATACCCAAACT   SNP
             421                                                       480
GPA1NAT      ATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACCTTAAAAAAAGT
GPA1-11-pr   ATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACCTTAAAAAAAGT
GPA1-12-pr   ATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACCTTAAAAAAATT
GPA1-13-pr   ATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACCTTAAAAAAATT
GPA1-14-pr   ATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACCTTAAAAAAAGT
GPA1-15-pr   ATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACCTTAAAAAAAGT
        16   ATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACCTTAAAAAAAGT
        17   ATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACCTTAAAAAAATT
        18   ATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACCTTAAAAAAATT
        19   ATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACCTTAAAAAAAGT
        20   ATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACCTTAAAAAAAGT
Consensus    ATGAAGTTCGTATTGAGTGCAGTCACCGATCTAATCATCCAGCAAAACCTTAAAAAAAgT
             481           STOP                                        540
GPA1NAT      GGTATTATATGAAGGAACTGTATAATTAAAGTAGTGTTTAGATACGTAAATTCTGTTTCC
GPA1-11-pr   GGTATTATATGAAGGAACTGTATAATTAAAGTAGTGTTTAGATACGTAAATTCTGTTTCC
GPA1-12-pr   GGTATTATATGAAGGAACTGTATAATTAAAGTAGTGTTTAGATACGTAAATTCTGTTTCC
GPA1-13-pr   GGTATTATATGAAGGAACTGTATAATTAAAGTAGTGTTTAGATACGTAAATTCTGTTTCC
GPA1-14-pr   GGTATTATATGAAGGAACTGTATAATTAAAGTAGTGTTTAGATACGTAAATTCTGTTTCC
GPA1-15-pr   GGTATTATATGAAGGAACTGTATAATTAAAGTAGTGTTTAGATACGTAAATTCTGTTTCC
        16   GGTATTATATGAAGGAACTGTATAATTAAAGTAGTGTTTAGATACGTAAATTCTGTTTCC
        17   GGTATTATATGAAGGAACTGTATAATTAAAGTAGTGTTTAGATACGTAAATTCTGTTTCC
        18   GGTATTATATGAAGGAACTGTATAATTAAAGTAGTGTTTAGATACGTAAATTCTGTTTCC
        19   GGTATTATATGAAGGAACTGTATAATTAAAGTAGTGTTTAGATACGTAAATTCTGTTTCC
        20   GGTATTATATGAAGGAACTGTATAATTAAAGTAGTGTTTAGATACGTAAATTCTGTTTCC
Consensus    GGTATTATATGAAGGAACTGTATAATTAAAGTAGTGTTTAGATACGTAAATTCTGTTTCC
             541                                                       600
GPA1NAT      GAAGATGCAAGAAGGAGCAGCAGCACCAGAAAAAAATTGCTATTTTTCTTCTCCATTAGAG
GPA1-11-pr   GAAGATGCAAGAAGGAGCAGCAGCACCAGAAAAAAATTGCTATTTTTCTTCTCCATTAGAG
GPA1-12-pr   GAAGATGCAAGAAGGAGCAGCAGCACCAGAAAAAAATTACTATTTTTCTTCTCCATTAGAG
GPA1-13-pr   GAAGATGCAAGAAGGAGCAGCAGCACCAGAAAAAAATTACTATTTTTCTTCTCCATTAGAG
GPA1-14-pr   GAAGATGCAAGAAGGAGCAGCAGCACCAGAAAAAAATTGCTATTTTTCTTCTCCATTAGAG
GPA1-15-pr   GAAGATGCAAGAAGGAGCAGCAGCACCAGAAAAAAATTGCTATTTTTCTTCTCCATTAGAG
        16   GAAGATGCAAGAAGGAGCAGCAGCACCAGAAAAAAATTGCTATTTTTCTTCTCCATTAGAG
        17   GAAGATGCAAGAAGGAGCAGCAGCACCAGAAAAAAATTACTATTTTTCTTCTCCATTAGAG
        18   GAAGATGCAAGAAGGAGCAGCAGCACCAGAAAAAAATTACTATTTTTCTTCTCCATTAGAG
        19   GAAGATGCAAGAAGGAGCAGCAGCACCAGAAAAAAATTGCTATTTTTCTTCTCCATTAGAG
```

Noncoding SNP 1

```
20          GAAGATGCAAGAAGGAGCAGCAGCACCAGAAAAAATTGCTATTTTTCTTCTCCATTAGAG
Consensus   GAAGATGCAAGAAGGAGCAGCAGCACCAGAAAAAATTgCTATTTTTCTTCTCCATTAGAG
            601          Noncoding SNP 2                                660
GPA1NAT     TCTATGACGGAATGCCAAATGAAAAAGCCATTTTGTTCAACAGTTCTTGATCTCGTTAAA
GPA1-11-pr  TCTATGACGGAATGCCAAATGAAAAAGCCATTTTGTTCAACAGTTCTTGATCTCGTTAAA
GPA1-12-pr  TCTATGATGGAATGCCAAATGAAAAAGCCATTTTGTTCAACAGTTCTTGATCTCGTTAAA
GPA1-13-pr  TCTATGATGGAATGCCAAATGAAAAAGCCATTTTGTTCAACAGTTCTTGATCTCGTTAAA
GPA1-14-pr  TCTATGACGGAATGCCAAATGAAAAAGCCATTTTGTTCAACAGTTCTTGATCTCGTTAAA
GPA1-15-pr  TCTATGACGGAATGCCAAATGAAAAAGCCATTTTGTTCAACAGTTCTTGATCTCGTTAAA
16          TCTATGACGGAATGCCAAATGAAAAAGCC
17          TCTATGATGGAATGCCAAATGAAAAAGCC
18          TCTATGATGGAATGCCAAATGAAAAAGCC
19          TCTATGACGGAATGCCAAATGAAAAAGCC
20          TCTATGACGGAATGCCAAATGAAAAAGCC
Consensus   TCTATGAcGGAATGCCAAATGAAAAAGCCatttgttcaacagttcttgatctcgttaaa
            661                                                       720
GPA1NAT     TCGTTGGATCCCCGGGTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTCCCCGC
GPA1-11-pr  TCGTTGGATCCCCGGGTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTCCCCGC
GPA1-12-pr  TCGTTGGATCCCCGGGTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTCCCCGC
GPA1-13-pr  TCGTTGGATCCCCGGGTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTCCCCGC
GPA1-14-pr  TCGTTGGATCCCCGGGTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTCCCCGC
GPA1-15-pr  TCGTTGGATCCCCGGGTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTCCCCGC
16
17
18
19
20
Consensus   tcgttggatccccgggttaattaaggcgcgccagatctgtttagcttgcctcgtccccgc
            721                                                       780
GPA1NAT     CGGGTCACCCGGCCAGCGACATGGAGGCCCAGAATACCCTCCTTGACAGTCTTGACGTGC
GPA1-11-pr  CGGGTCACCCGGCCAGCGACATGGAGGCCCAGAATACCCTCCTTGACAGTCTTGACGTGC
GPA1-12-pr  CGGGTCACCCGGCCAGCGACATGGAGGCCCAGAATACCCTCCTTGACAGTCTTGACGTGC
GPA1-13-pr  CGGGTCACCCGGCCAGCGACATGGAGGCCCAGAATACCCTCCTTGACAGTCTTGACGTGC
GPA1-14-pr  CGGGTCACCCGGCCAGCGACATGGAGGCCCAGAATACCCTCCTTGACAGTCTTGACGTGC
GPA1-15-pr  CGGGTCACCCGGCCAGCGACATGGAGGCCCAGAATACCCTCCTTGACAGTCTTGACGTGC
16
17
18
19
20
Consensus   cgggtcacccggccagcgacatggaggcccagaataccctccttgacagtcttgacgtgc
            781                                                       840
GPA1NAT     GCAGCTCAGGGGCATGATGTGACTGTCGCCCGTACATTTAGCCCATACATCCCCATGTAT
GPA1-11-pr  GCAGCTCAGGGGCATGATGTGACTGTCGCCCGTACATTTAGCCCATACATCCCCATGTAT
GPA1-12-pr  GCAGCTCAGGGGCATGATGTGACTGTCGCCCGTACATTTAGCCCATACATCCCCATGTAT
GPA1-13-pr  GCAGCTCAGGGGCATGATGTGACTGTCGCCCGTACATTTAGCCCATACATCCCCATGTAT
GPA1-14-pr  GCAGCTCAGGGGCATGATGTGACTGTCGCCCGTACATTTAGCCCATACATCCCCATGTAT
GPA1-15-pr  GCAGCTCAGGGGCATGATGTGACTGTCGCCCGTACATTTAGCCCATACATCCCCATGTAT
16
17
18
19
20
Consensus   gcagctcaggggcatgatgtgactgtcgcccgtacatttagcccatacatccccatgtat
            841                                                       900
GPA1NAT     AATCATTTGCATCCATACATTTTGATGGCCGCACGGCGCGAAGCAAAAATTACGGCTCCT
GPA1-11-pr  AATCATTTGCATCCATACATTTTGATGGCCGCACGGCGCGAAGCAAAAATTACGGCTCCT
GPA1-12-pr  AATCATTTGCATCCATACATTTTGATGGCCGCACGGCGCGAAGCAAAAATTACGGCTCCT
GPA1-13-pr  AATCATTTGCATCCATACATTTTGATGGCCGCACGGCGCGAAGCAAAAATTACGGCTCCT
GPA1-14-pr  AATCATTTGCATCCATACATTTTGATGGCCGCACGGCGCGAAGCAAAAATTACGGCTCCT
GPA1-15-pr  AATCATTTGCATCCATACATTTTGATGGCCGCACGGCGCGAAGCAAAAATTACGGCTCCT
16
17
18
```

```
         19
         20
  Consensus  aatcatttgcatccatacattttgatggccgcacggcgcgaagcaaaaattacggctcct
             901                                                       960
    GPA1NAT  CGCTGCAGACCTGCGAGCAGGGAAACGCTCCCC-TCACAGACGCGTTGAATTGTCCCCAC
 GPA1-11-pr  CGCTGCAGACCTGCGAGCAGGGAAACGCTCCCCCTCACAGACGCGTTGAANTGTCCCCAC
 GPA1-12-pr  CGCTGCAGACCTGCGAGCAGGGAAACGCTCCCC-TCACAGACGCGTTGAATTGTCCCCCC
 GPA1-13-pr  CGCTGCAGACCTGCGAGCAGGGAAACGCTCCCC-TCACAGACGCGTTGAATTGTCCCCAC
 GPA1-14-pr  CGCTGCAGACCTGCGAGCAGGGAAACGCTCCCC-TCACAGACGCGTTGAATTGTCCCCAC
 GPA1-15-pr  CGCTGCAGACCTGCGAGCAGGGAAACGCTCCCC-TCACAGACGCGTTGAATTGTCCCCAC
         16
         17
         18
         19
         20
  Consensus  cgctgcagacctgcgagcagggaaacgctcccc tcacagacgcgttgaa.tgtcccc.c
             961                                                      1020
    GPA1NAT  GCCGCGCCCCTGTAGAGAAATATAAAAGGTTAGGA---TTTGCCACTGAGGTTCTTCTTT
 GPA1-11-pr  CCCGCGCCCCTGTAGAGAAATATAAAAGGTTAGGAATTTGNCCNCTGANGGTTCTTCTTT
 GPA1-12-pr  GCCGCGCCCCTGTAGAGAAATATNAAAGGTTAGGA--TTTGCCNCTGGAGGTTCTCCTTT
 GPA1-13-pr  GCCGCGCCCCTGTAGAGAAATATAAAAGGTTAGGA--TTGCCACT--GAGGTTCTTCNTT
 GPA1-14-pr  GCCGCGCCCCTGTAGAGAAATATAAA-GGTTAGGA--TTGCCACT--GAGGTTCTTCNTT
 GPA1-15-pr  GCCGCGCCCCTGTAGAGAAATATAAAAGGTTAGGA--TTTGCCACTGGAGGTTCTTCTTT
         16
         17
         18
         19
         20
  Consensus  .ccgcgcccctgtagagaaatat.aa.ggttagga  .t..........ggttct.c.tt
             1021                                                     1080
    GPA1NAT  C-ATATACTTCCTTTT-AAAATCTTGCTA---GGATACAGTTCTCACATCACATCCGAAC
 GPA1-11-pr  CCATATACCTNCCTTTTAAAATCCTTGGCTAGGGANACNGGTTCCTCACCATCNCA
 GPA1-12-pr  C-ATATACTTCCTTTT--AAATCTNGCTA---GGATACAGTTCTCNCATCNCATCCGAAC
 GPA1-13-pr  C-ATATACTTCCTTTT--AAANCTTGCTA---GGATACAGTTCTCACATCNCATCCGAAC
 GPA1-14-pr  C-NTATACTNCCTTTT--AAATCTTGCTA---GGATACAGTTCTCNCATCNCATCCGAAC
 GPA1-15-pr  C-ATATACTTCCCTTTTAAAATCTTGCTA---GGATACAGTTCTCACATCACATCCN
         16
         17
         18
         19
         20
  Consensus  c .tatac...c.ttt..aaa.c......   gga.ac.g.t..c.ca.c....c.....
             1081                                                     1140
    GPA1NAT  ATAAACAACCATGGGTACCACTCTTGACGACACGGCTTACCGGTACCGCACCAGTGTCCC
 GPA1-11-pr
 GPA1-12-pr  ATAAACANCCNNN
 GPA1-13-pr  ATAAACCACCNTGGN
 GPA1-14-pr  ATAAACAACCNTGGGTNCC-----------------------------------------
 GPA1-15-pr
         16
         17
         18
         19
         20
  Consensus  ....................
```

4/23/08

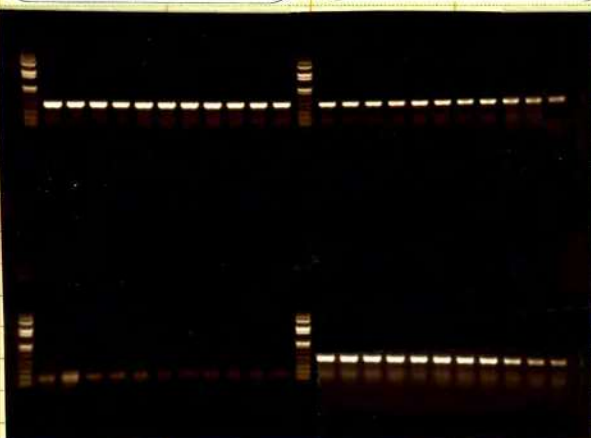
SNP PCR TO DISTINGUISH GPA1 ALLELES

The fitness difference btw the two GPA1 alleles may be too small to detect by competing each against a common reference strain (error rate ~ 0.5%).

If I am to compete these strains directly I will need a way of distinguishing btw the alleles. I will try SNP based PCR using primer GPA1_intF2 (p. 144) and SNP-specific reverse primers:

36680344 IDT
G.LANG
38433038 4/9/2008
GPA1_intR1_SNP_RM
5'- GTT CCT TCA TAT AAT ACC AC -3' 071
Tm= 45.2 ºC , MW= 6,011.0
7.6 OD_{260} 39.80 nmol 0.24 mg

36680345 IDT
G.LANG
38433039 4/9/2008
GPA1_intR1_SNP_W303
5'- GTT CCT TCA TAT AAT ACC AA -3' 072
Tm= 44.2 ºC , MW= 6,035.0
7.5 OD_{260} 38.20 nmol 0.23 mg



I ran 12 annealing temps ranging from 45°–58°. I ~~only~~ ~~ran~~ did not run the lowest temp. on this gel because of space.

SET	ALLELE	PRIMERS
A	RM (wild)	RM
B	RM	W303
C	W303	RM
D	W303 (lab)	W303

A	C
B	D

- The primer w/ the W303 (lab) SNP is discriminatory – it can distinguish between the two templates
- The primer w/ the RM (wild) SNP is non-discriminatory – it ~~cannot~~ amplifies from both templates equally well.

This could be explained if a steric clash is needed at the 3' end to prevent primer amplification. The ~~table~~ table below shows the base pair at the 3' end of the primer

		ALLELE wild	ALLELE lab
PRIMER	wild	G:C	T:C
PRIMER	lab	G:A	T:A

- If I design primers to use the other strand as a template I ~~can~~ might be able to make the wild SNP primer discriminatory.

4/25/08

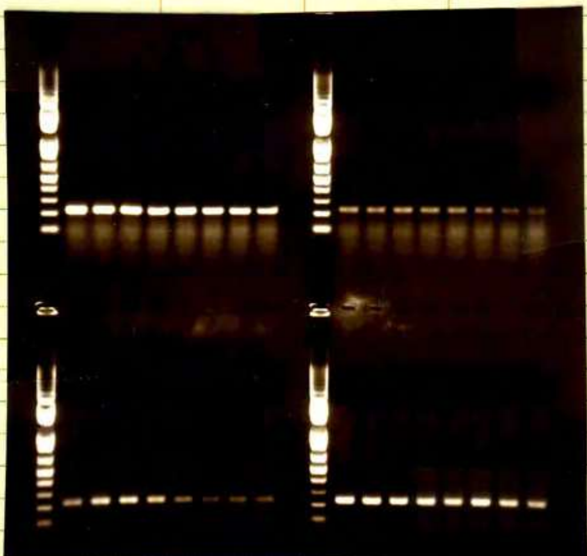
DISTINGUISHING GPA1 ALLELES CONT.

· PCR using SNP-based primers recognizing the non-coding strand as a template, Reverse primer: KanMX/NatMX_R1

36926797 IDT
G.LANG
38652598 4/23/2008
GPA1_intF3_SNP_RM
5'- CCA GCA AAA CCT TAA AAA AAG -3' 073
Tm= 48.6 ºC , MW= 6,409.3
7.1OD260 32.50nmol 0.21mg

36926798 IDT
G.LANG
38652599 4/23/2008
GPA1_intF3_SNP_W303
5'- CCA GCA AAA CCT TAA AAA AAT -3' 074
Tm= 47.9 ºC , MW= 6,384.3
7.8OD260 35.90nmol 0.23mg

Here I ran 8 annealing temps. ranging from 49° - 57°



Labels same as p. 153

Neither of these forward primers were particularly discriminatory.

↳ I could try increasing the annealing temp. further.

I could also use two SNP primers - ~~two of~~ there are two additional SNP differences in the RM strain (see p. 148)

5/9/08

SNP-Based PCR using Pairs of SNP Primers

FORWARD PRIMERS: GPA1_intF3_SNP_RM & W303 (above)

NEW REVERSE PRIMERS:

37098342 IDT
G.LANG
38817959 5/2/2008
GPA1_extR3_SNP_RM
5'- TAA TGG AGA AGA AAA ATA GC -3' 077
Tm= 45.1 ºC , MW= 6,231.1
6.9OD260 31.20nmol 0.19mg

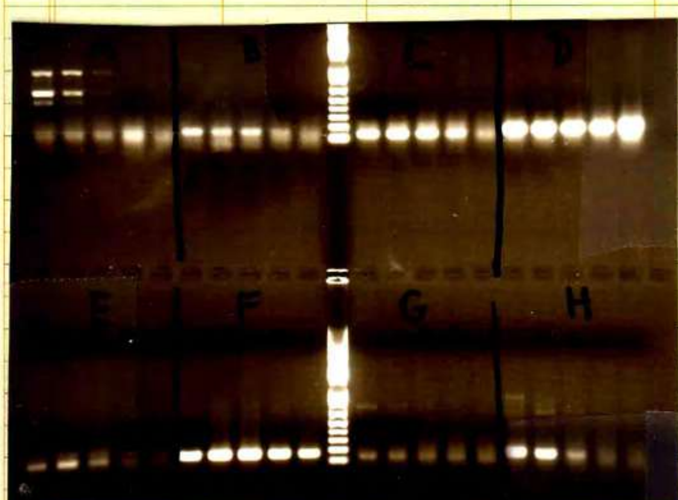
37098343 IDT
G.LANG
38817960 5/2/2008
GPA1_extR3_SNP_W303
5'- TAA TGG AGA AGA AAA ATA GT -3' 078
Tm= 43.5 ºC , MW= 6,246.2
5.7OD260 25.40nmol 0.16mg

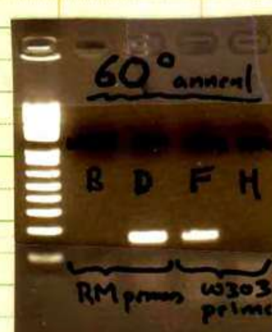
37098344 IDT
G.LANG
38818208 5/2/2008
GPA1_extR4_SNP_RM
5'- CTT TTT CAT TTG GCA TTC CG -3' 079
Tm= 50.6 ºC , MW= 6,040.0
5.3OD260 30.50nmol 0.18mg

37098345 IDT
G.LANG
38817961 5/2/2008
GPA1_extR4_SNP_W303
5'- CTT TTT CAT TTG GCA TTC CA -3' 080
Tm= 49.5 ºC , MW= 6,024.0
6.0OD260 33.90nmol 0.20mg

Annealing temps = [46°, 49°, 54°, 57°, 60°]

	PRIMERS	TEMP.
A)	RM (extR3)	W303
B)	RM (R4)	W303
C)	RM (R3)	RM
D)	RM (R4)	RM
E)	W303 (extR3)	W303
F)	W303 (R4)	W303
G)	W303 (R3)	RM
H)	W303 (R4)	RM





To distinguish ~~EXT~~ GPA1 alleles use:

GPA1_intF3_SNP_RM & GPA1_ext~~R~~R4_SNP_RM

" W303 " W303

extension: 2 m

anneal: 60°C

3/8/08

STRAIN CONSTRUCTION II - CREATING A MATING TESTER

I will start w/ the strain yGIL122 with genotype:

W303 - Matα URA3 ade2-1 his3-11,15 leu2-3,112 trp1-1 can1-100

For the first step I will revert the ~~trp1-1~~ mutation

- Inoculate 5 mL of YPD with a single colony of yGIL122
- Overlay onto 4 YNB plates

300 λ His (1g/100mL)
300 λ Leu (1g/100mL)
300 ~~60~~ λ Ade ~~(2g/100mL)~~ (0.2g/100mL)
60 λ Trp (1g/100mL) $\longrightarrow$ add to only 1 plate.

3/12/08

- There is a lawn of cells on the control (His, Leu, Ade, Trp) plate. On the 3 -Trp plates there are about 20-30 colonies per plate.

$\hookrightarrow$ I picked a single colony off of each of these 3 plates and restreaked onto -Trp

3/14/08

- All three colonies restreaked on -Trp - put in fridge until next week.

3/19/08

- Yesterday I inoculated a single colony off of ~~each~~ two of the -trp plates into YPD.
- Today I froze down the two TRP1 revertants (now yGIL257 & yGIL258).
- I made a 1:100 dilution of yGIL257 & yGIL258 (100λ into 10 mL YPD) for transformation - 11:00 am

[redacted]

3/19/08

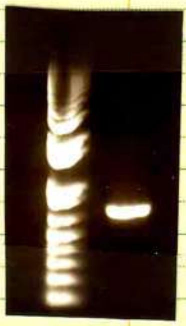
TRANSFORMATION OF yGIL257 & yGIL258 w/ HIS3

- I set up a 200λ PCR using a gDNA prep from yGIL255 (FY diploid) and primers:

36078308 IDT
G.LANG
37882350 3/6/2008
HIS3_extR1 065
5'- TGC AGC TTT AAA TAA TCG GTG -3'
Tm= 51.3 ºC , MW= 6,460.3
6.1OD260 29.40nmol 0.19mg

36078307 IDT
G.LANG
37882962 3/6/2008
HIS3_extF1 066
5'- AGG CAA GAT AAA CGA AGG CA -3'
Tm= 54.5 ºC , MW= 6,217.1
6.7OD260 30.70nmol 0.19mg

- Zymo cleanup → elute in 20λ H_2O



- Coulter count ~6:00pm

Bkg	150000	112000
yGIL257	2.869 E7	2.895 E7
yGIL258	3.031 E7	2.951 E7
Post Bkg	1.36 E6	608000

3/22

The transformation appears to have worked although not very efficiently

- On the yGIL257 transformation plate I see ~14 colonies
- On the yGIL258 —— " —— ~4 —— " ——
- On the control plate I see none.

Pick 2 colonies off of each plate and restreak onto -his (i.e. Min + ADE + LEU)

3/24/08

- All four His+ transformants restreaked on -his.
- I picked single colonies off of yGIL257-1 & yGIL258-1 and inoculated 5 mL YPD.

→ 3/25/08
- Freeze down → yGIL257 His+ = yGIL259
→ yGIL258 His+ = yGIL260

4/10/08

TRANSFORMATION OF yGIL216 & yGIL218 w/ KANMX

In order to do the mating assay by FACS I will need to put GFP & Tomato into my mating tester strain and experimental strain respectively. I will be using NatMX to mark the GPA1 locus; therefore, I will need to use KanMX to mark the fluorophore.

I have strains w/ GFP (or Tomato) at YEL014C marked w/ NatMX:

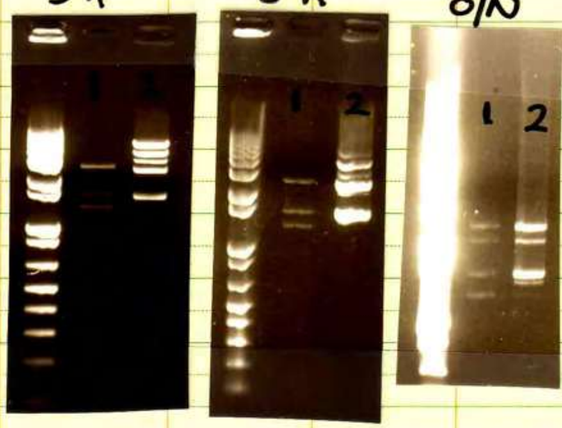
yGIL216 - FY4 w/ dTomato::NatMX @ YEL014C

yGIL218 - FY4 w/ GFP::NatMX @ YEL014C

I will transform both of these strains w/ the KanMX fragment from plasmids. pGIL023, pGIL024 (see "fitness in chemostat Notebook" p.3 & 39)

· Yesterday I digested pGIL023 & pGIL024 w/ PvuII & ~~SacII~~ SacI

3 h 5 h O/N



LANE		PREDICTED FRAGMENTS
1	pGIL023	2412, 1497, 1254, 270
2	pGIL024	2412, 1497, 1512

? - first of all, I don't know why I see more frag. than expected - star activity of PvuII?

- also, this ~~shows that pGIL024~~ is consistent w/ previous observation that pGIL024 is screwed up - see "fitness in chemostat" notebook p.9

· Transform yGIL216 & yGIL218 with the digested plasmids pGIL023 & pGIL024.

4/15/08

· Transformation efficiency was not as high as I expected:

STRAIN	PLASMID	COLONIES
yGIL216	—	1
"	pGIL023	0
"	pGIL024	14
yGIL218	—	0
"	pGIL023	3
"	pGIL024	7

· I picked 3 putative transformants off of the two plates that were transformed with pGIL024 → labeled yGIL216 A ··· yGIL218 C → restreak onto YPD

~~4~~ 4/17/08

- Yesterday I inoculated single colonies of ~~of~~ the putative transformants yGIL216A - yGIL218c in 5 mL YPD
- Today I streaked each onto YPD, YPD+ClonNat, and YPD+G418 to verify that the KanMX cassette was integrated in place of the NatMX cassette.

- I also looked under the scope to verify that the yGIL216-derived putative transformants are red and the .218-derived green.

4/21/08

- All six putative transformants grew on YPD & YPD+G418.
- yGIL216 B is the only to also grow on YPD+ClonNat

4/23/08

- Relabel transformants:

yGIL216 A ⟹ yGIL 261
yGIL216 C ⟹ yGIL 262
yGIL218 A ⟹ yGIL 263
yGIL218 B ⟹ yGIL 264

- discard yGIL216 B & yGIL218 C

4/29/08

- Make gDNA preps from yGIL261 & yGIL263
- Yesterday I restreaked the following strains from frozen stock:

yGIL265 } p148
yGIL266
yGIL268
yGIL270
yGIL121 - p12
yGIL259 } p156
yGIL260

⟹ tomorrow I will transform w/ ~~GFP~~ GFP/Tom::KAN

5/1/08

→ PCR didn't work - need to verify strains

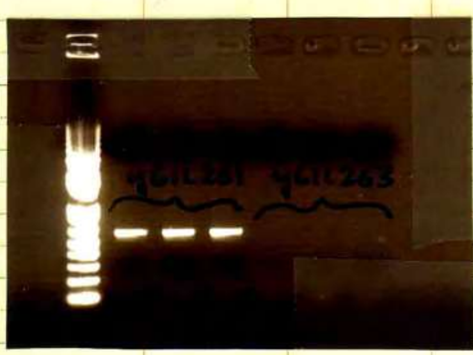
5/1/08

VERIFICATION OF yGIL261 & yGIL263

· In order to transform strains with the GFP/Tomato :: KanMX cassette I amplified off of yGIL261 & 263 using primers:

36926799 IDT
G.LANG
38653072 4/23/2008
YEL014CextF2 3 075
5'- TCA GTT CCT GTC TTC GTA GG -3'
Tm= 53.5 ºC , MW= 6,090.0
6.5OD260 35.50nmol 0.22mg

36926800 IDT
G.LANG
38653073 4/23/2008
YEL014CextR2 3 076
5'- GCT GTT TTG ATT GAT ATA GG -3'
Tm= 46.3 ºC , MW= 6,193.1
7.0OD260 35.60nmol 0.22mg



→ This didn't work as expected.

It appears that yGIL261 is WT at YEL014C.

I can't get a product off of yGIL263 (3.5 min extension → too short)

I wanted to determine which drug marker was present in each strain and at which locus.

PRIMER PAIR

A	YEL014_extF2	&	KanMx_intR1
B	"	&	NatMx_intR1
C	YER255_extF2	&	KanMx_intR1
D	"	&	NatMx_intR1

· yGIL263 is correct

· I can't determine yGIL261

· I want to go back and test the genotype of all of my Fluorescent:: Drug marked strains.

· Streaks from frozen stock:

- yGIL261-yGIL264 & yGIL205-yGIL220

5/5/08

· Last night I started o/n cultures of yGIL205-220 & yGIL261-264
· Today I made gDNA preps of these strains.

These ~~row~~ columns are the same as p.

{ A-YEL014C_extF2 / KanMX_intR1
B- " / NatMX_intR1
C- YER255C_extF2 / KanMX_intR1
D- " / NatMX_intR1

5/8/08

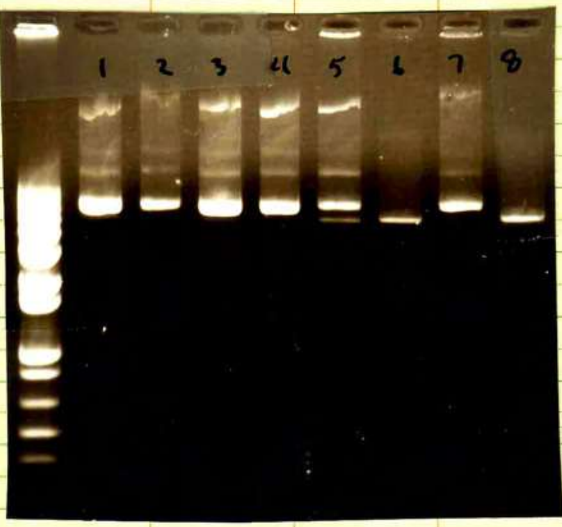
· In addition to determining the ~~drug~~ drug marker/locus I examined them under the scope to determine the fluorophore and I tested mating type by looking for shmoo/zygote formation in the presence of yGIL121 (Mat a) and yGIL260 (Mat α)

STRAIN	MARKER	LOCUS	FLUOROPHORE	MATING TYPE
yGIL 205	NatMX	014	Tomato	α ·
206	?	?	Tomato	α
207	NatMX	255	GFP	α ·
208	NatMX	255	GFP	α
209	NatMX	014	Tomato	α
210	NatMX	014	Tomato	α
211	NatMX	014	GFP	α ·
212	NatMX	255	Dark	a
213	NatMX	255	Tomato	a ·
214	NatMX	255	GFP	a ·
215	?	?	GFP	a
216	NatMX	255	Tomato	a
217	NatMX	014	Tomato	a ·
218	NatMX	014	GFP	?
219	NatMX	014	GFP	? - mating in both crosses?
220	NatMX	255	Tomato	α ·
261	?	?	Tomato	a
262	?	?	Tomato	a
263	KanMX	014	GFP	? > no mating in either cross
264	KanMX	014	GFP	?

5/14/08

TRANSFORMATION OF yGIL210 & yGIL211 w/ KANMX.

- Since transformation of KanMX into yGIL216 & 218 was not correct (p. 157-160), I will transform to verified strains (isogenic except for the fluorophore) w/ KanMX
- On Monday (5/12) I streaked pGIL023 & pGIL024 from frozen stock
- Yesterday (5/13) I miniprepped 2 colonies from each plate and digested the plasmids o/n w/ EcoRI



LANE	
1	pGIL023-1
2	— -2
3	pGIL024-1
4	— -2
5	pGIL023-1
6	— -2
7	pGIL024-1
8	— -2

Lanes 5–8: linearized w/ EcoRI

→ pGIL023-1 & pGIL024-1 did not digest completely w/ EcoRI

∴ use pGIL023-2 & pGIL024-2 for transformations.

- Last night I started o/n cultures of yGIL210 & yGIL211 from the same single colony used for the gDNA preps on p. 160.
- Dilute the saturated o/n cultures 1:100 (100λ into 10 mL) ~10:45 am
- At ~4:15 pm → coulter counts ~3E7 ⟹ start transformation.

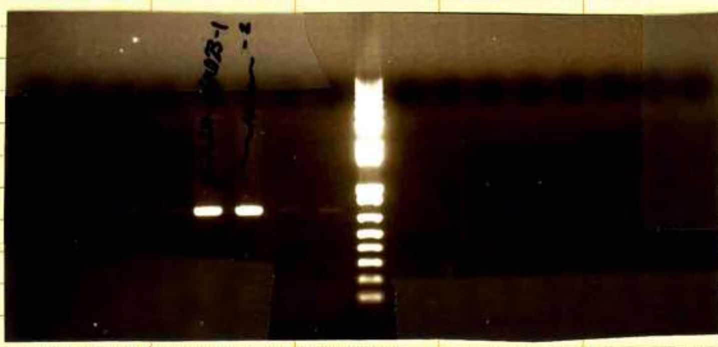
5/15/08
- Today I replica plated the YPD transformation plates to YPD+2xG418.
- Transformation efficiency was again low — and similar to before (see p. 157)

STRAIN	PLASMID	COLONIES	
yGIL210	pGIL023	14	→ Restreak 3 onto YPD
"	pGIL024	1	→ " 1 "
yGIL211	pGIL023	5	→ " 2 "
"	pGIL024	8	→ " 2 "

→ gDNA preps of these 8 strains

5/23/08

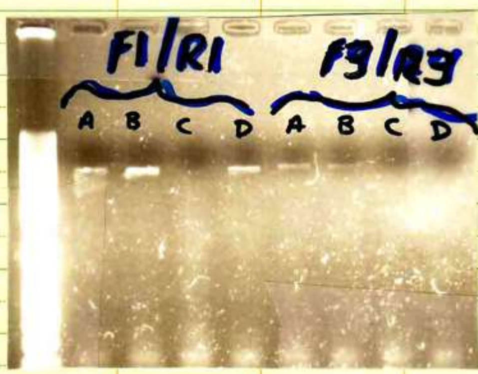
- Of the 8 putative transformants
  - All 8 grew on YPD & G418
  - Only the two yGIL211-pGIL023 putative transformants failed to grow on ClonNat
  - → these are the only 2 correct ones
- PCR using
  - YEL014C_extF2 & KanMX_intR1 (see p. 160)
  - YEL014C_extF3 & YEL014C_extR3 (see p. 159)



- None of the external PCRs worked
- PCR confirmation for the yGIL211-pGIL023 putative transformants
  - → both are green under scope
  - → Freeze down
    - yGIL211-pGIL023-1 = yGIL273
    - yGIL211-pGIL023-2 = yGIL274

I don't know why these PCRs do not work. I have another set of external primers — the ones used to construct these strains (see Fitness in Chemostat Notebook) — YEL014C_extF1 / R1.

Run PCR using both primer sets – anneal: 50°; extension: 3m



LANE	TEMPLATE
A	~~pGIL211~~ pGIL211-pGIL023 transformant 1
B	yGIL211-pGIL023 transformant 2
C	yGIL210
D	yGIL211

→ surprisingly the F3/R3 pcr worked

Zymo clean lanes 1,2,5,6

5/5/08

FITNESS ASSAYS ON GPA1 STRAINS

- On Friday (5/2) I streaked yGIL268, 269, 265, 266, 270, & 271 (see p. 148) from frozen stock onto YPD.

- Last night I inoculated 3 ml YPD w/ 3 colonies off of each plate (labeled x, y, & z) ⟶ 10:00pm

- On Friday I received 2 copies of my yGIL050 reference strain from Harvard — my tube is apparently missing from my freezer box so I got 050 from Hannah's collection and Beverly's collection. I Friday I restreaked each of these to single colonies. Last night I started 3 ml o/Ns with single colonies off of these two plates. — I supplemented the YPD for Ref strain w/ ADE.

- On Saturday I prepared the media for this expt. I made 1 L of YPD (supplemented w/ ADE) and alloquotted 10 ml into 87 tubes then autoclaved the tubes. Yesterday I put all of the tubes in the 30° room.

- I looked under the scope at the yGIL050 ~~strains~~ strains I recieved from Harvard. Both still express GFP. Lab lore suggested that the CWP2::GFP was cytoplasmicly localized not cell-wall as was hoped when the strain was constructed. However looking under the microscope it seems to really be cell-wall localized.

- Dilute all overnight experimental strains 1:300 (30λ into 10 ml)
  Make 2 dilutions of Reference strain 1:20 (500λ into 10 ml)
  ⟶ 11:45 am – I used Ref. #1 – the one from Hannah's collection.

- 6:15 – The cultures appear to be saturating earlier than I expected.

COULTER COUNT (6:45pm)

Bkg		414000	416000	464000
yGIL265x	1	1.504E7	1.527E7	1.539E7
	2	1.774E7	1.757E7	1.731E7
yGIL266x	1	1.698E7	1.713E7	1.701E7
	2	1.864E7	1.857E7	1.868E7
yGIL268x	1	1.589E7	1.669E7	1.611E7
	2	1.777E7	1.778E7	1.801
yGIL270x	1	1.647E7	1.6 E7	1.606E7
	2	1.684E7	1.689E7	1.671E7
Ref.	1	3.314E7	3.34 E7	3.362E7
	2	3.696E7	3.653E7	3.635E7
Post Bkg		2.61E6	1.354E6	974000

250 ~~500~~ λ Exp.: 250λ Ref.

5/6/08

NOTES ON SET-UP OF FITNESS ASSAYS

· I set up 21 fitness assays:

- All 18 strains v. yGIL050 reference
- Direct competitions: yGIL265 v. yGIL266, yGIL268 v. yGIL270, yGIL269 v. yGIL271

· I am only spinning down 500λ and resuspending in 1ml PBST — this is so the cells are not too concentrated for the FACS.

· ~~After I~~ Dilutions (20 λ into 10 ml) - 7:30pm.

· After I did the dilutions - I ~~filtered~~ harvested the "x" and "y" strains for mRNA by filtration.

↳ the cells may have been too dense - at that point they would have been ~3E7 — I finished filtration @ 8:45pm.

This morning I did the second dilution - the cultures looked too dense - I did 15λ into 10mL.

Coulter Count

Bkg		306000	330000	338000
265x	1	5.647E7	5.759E7	5.752E7
	2	6.492E7	6.475E7	6.472E7
266x	1	6.157E7	6.11E7	6.057E7
	2	6.781E7	6.922E7	6.796E7
268x	1	5.989E7	5.783E7	5.812E7
	2	6.63E7	6.778E7	6.783E7
270x	1	5.41E7	5.396E7	5.521E7
	2	6.757E7	6.712E7	6.776E7
265v.266	1	7.066E7	7.032E7	7.078E7
	2	7.799E7	7.899E7	7.847E7
Post Bkg		6.502E6	1.768E6	992000

@ 7:50pm - Dilute all cultures 15λ into 10ml

5/6/08

Coulter Count (~8:00 ~~7:30~~ pm)

Bkg	396000	384000	390000
265 × 1	5.834E7	5.888E7	5.901E7
2	6.681E7	6.738E7	6.788E7
266 × 1	5.951E7	6.082E7	6.163E7
2	6.692E7	6.797E7	6.83 E7
268 × 1	5.677E7	5.739E7	5.635E7
2	6.318E7	6.208E7	6.251E7
270 × 1	5.343E7	5.429E7	5.37E7
2	6.08E7	6.216E7	6.18E7
265 v. 266 1	7.981E7	7.9 E7	8.061E7
2	8.157E7	8.361E7	8.403E7
Post Bkg	5.08E6	2.408E6	1.726E6

5/7/08

Coulter Count ~~[illegible]~~ (8:15 am)

Bkg	274000	254000	264000
265 × 1	5.791E7	5.826E7	5.997E7
2	6.243E7	6.238E7	6.164E7
266 × 1	6.305E7	6.317E7	6.293E7
2	6.855E7	6.739E7	6.762E7
268 × 1	5.94E7	5.854E7	5.922E7
2	6.202E7	6.237E7	6.326E7
270 × 1	5.482E7	5.424E7	5.431E7
2	5.691E7	5.598E7	5.557E7
265 v. 266 1	8.801E7	8.801E7	8.809E7
2	9.29E7	9.252E7	9.393E7
Post Bkg	3.222E6	1.9E6	1.038E7

· @ 7:30am I collected 500λ of each competition — spin down — resuspend in 1 ml PBST.

5/7/08

NOTES ON FITNESS ASSAYS

In this run of fitness assays the culture densities were higher than past assays (compare for example to p 57-59).

Other than the first (original) dilution all dilutions were 15λ into 10ml rather than 20λ into 10ml. — The need to do bigger dilutions suggests that the cells are growing faster as well. Why? — Is this just do to slight differences in media or growth conditions — I used narrower tubes — rollerdrum speed?

↳ I put the cultures back into the incubator — I want to determine the final culture density.

In this run, I only spun down 500λ of cells at each ~~#~~ sample — for FACS here at Princeton the desired concentration is ~10^6 (whereas at Harvard I routinely ran @ 10^7). I may also want to dilute the cells further before running the FACS this afternoon.

Interestingly, when I coulter counted the second dilution was, (without exception) more concentrated. I don't understand how this could be. Perhaps particles stick to the tube walls and w/ each vortex more are released into the liquid. I don't know how likely this is but I have no other good explanation.

As expected the direct competitions (i.e. yGIL265 v. yGIL266) ~~satur~~ grew to higher densities — this is because these cultures do not include the reference which has a fitness disadvantage.

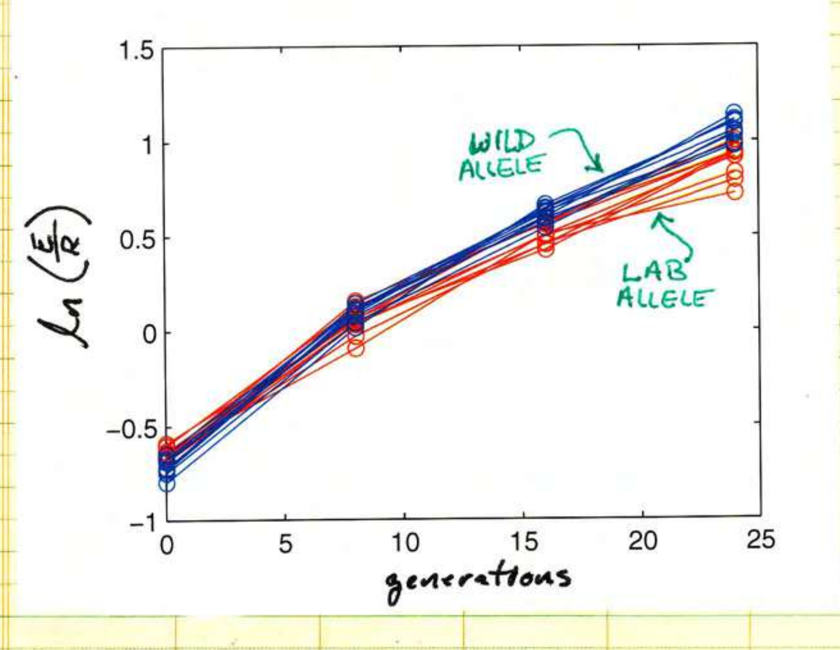
5/8/08

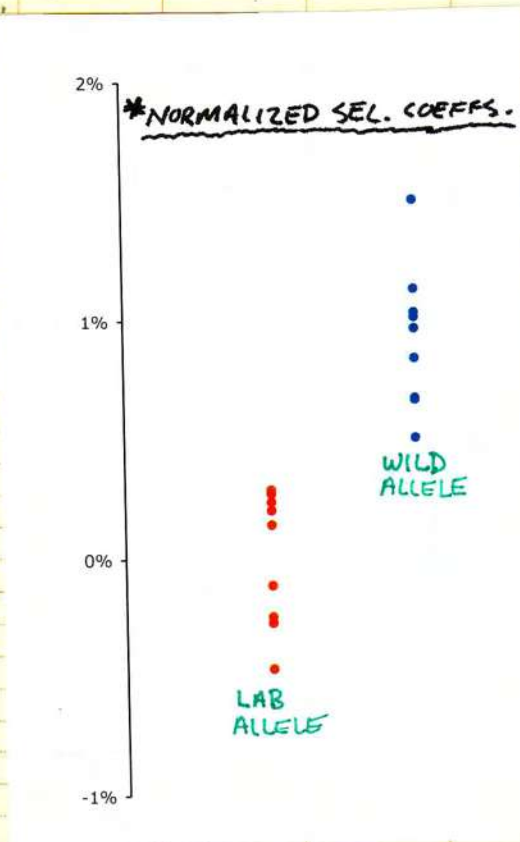
Coulter Count Saturated Cultures (4:30pm)

Bkg		400000	380000	480,000
~~255~~x	1	2.564E9	2.604E9	2.582E9
	2	2.767E9	2.765E9	2.763E9
266 x	1	2.678E9	2.691E9	2.697E9
	2	2.687E9	2.677E9	2.672E9
268 x	1	2.536E9	2.539E9	2.516E9
	2	2.489E9	2.472E9	2.459E9
270 x	1	2.606E9	2.618E9	2.6 E9
	2	2.652E9	2.644E9	2.68 E9
265 v. 266	1	3.053E9	2.976E9	2.961E9
	2	3.011E9	2.978E9	3.01 E9
Post Bkg		7.918E8	3.798E7	2.248E7

5/8/07

RESULTS FROM GPA1 FITNESS ASSAYS





	R^2	sel. coeff. raw	sel. coeff. normalized	mean	stdev
yGIL265-X	0.98	5.83%	-0.47%		
Y	0.98	6.47%	0.14%	-0.15%	0.30%
Z	1.00	6.20%	-0.12%		
yGIL266-X	0.99	7.02%	0.65%		
Y	0.98	7.03%	0.66%	0.78%	0.21%
Z	0.99	7.40%	1.02%		
yGIL268-X	0.99	6.57%	0.23%		
Y	0.99	6.62%	0.28%	0.24%	0.04%
Z	0.99	6.53%	0.20%		
yGIL269-X	0.99	6.03%	-0.27%		
Y	0.99	6.06%	-0.25%	-0.09%	0.31%
Z	0.99	6.61%	0.27%		
yGIL270-X	0.99	6.85%	0.49%		
Y	0.99	7.33%	0.95%	0.76%	0.24%
Z	0.99	7.20%	0.83%		
yGIL271-X	0.99	7.51%	1.12%		
Y	0.99	7.38%	1.00%	1.20%	0.26%
Z	0.99	7.91%	1.49%		

* I normalized the sel. coeffs. to the mean of the LAB expts (yGIL265, 268, 269)

- The wild (RM) allele has an ~1% ~~fitness~~ growth rate advantage over the lab (w303) allele.
- Like usual I assumed 8 generation per 12h – I know that the strains underwent more generations in this experiment (see note on p.
  ↳ Here the reference strain has a greater fitness disadvantage – btw 6 & 7% (compare to 3% previously, p. 9). This could be because of the additional generations or because I had to reacquire this strain from Harvard.
- There is no fitness effect of the two noncoding mutations (see p. 148) – compare fitness of yGIL265 w/ yGIL268 & yGIL269.

5/9/08

ANALYSIS OF DIRECT COMPETITIONS BETWEEN GPA1 ALLELES

· In order to isolate single colonies ~~#~~ from the direct competitions (yGIL265 v. yGIL266, yGIL268 v. yGIL270, & yGIL269 v. yGIL271) I serially diluted the cultures (~10^7) 1:10,000 by 4x 1:10 dilutions (100λ into 900λ H_2O)

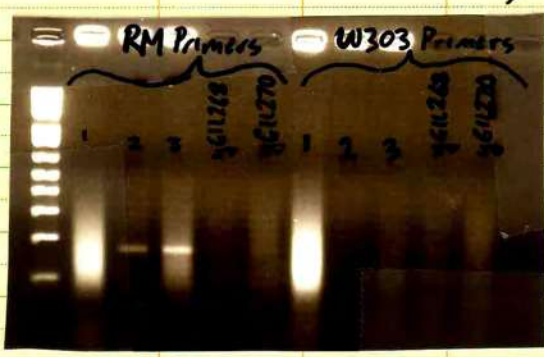
↳ plate 100λ of 10^{-4} dilution (~10^3 cells/ml) onto duplicate YPD plates

↳ On Sunday (5/11) I put plates in fridge

5/14/08

ATTEMPT AT COLONY PCR

· I picked 3 colonies off of the yGIL268 v. yGIL267 (timepoint 4) plate and smeared cells inside duplicate PCR tubes. — Heat 94° for 10 min — add PCR mix following ~~#~~ program on p. 154



→ This did not work well.

PROBLEMS:
(1) Controls didn't work ???
(2) Too much nucleic acid in lanes 1
↳ probably too many cells

I will give up on colony PCR and do regular gDNA preps.

GENOMIC PREPS ON SAMPLES FROM THE yGIL268 v. yGIL270 DIRECT COMPETITION

· Last night I picked 48 single colonies off of the yGIL268 v. yGIL270 plate 1-1 and 4-1 (timepoints 1 & 4 from the experiment) and inoculated 1 ml YPD in microfuge tubes. I incubated these o/N in the 30° room with the lids open (I covered ~~of the~~ the entire rack w/ foil)

· This morning I did gDNA preps following the standard glass bead lysis method. → only did gDNA preps on 48 timepoint 1 cultures.

NOTES

· I used the same tube for all aspirations — shouldn't matter — I avoided the cells.

· I saw (and picked) several small colonies — I assume these are petites — probably the same that show reduced fitness (see p. 53 & 63 — outliers)

↳ I marked these tubes with "p"

5/15/08

· Last night I put the 48 microfuge tubes from timepoint 4 in the fridge.

· Last night I inoculated 48 cultures from timepoints 2 & 3 as before

· Today I did gDNA preps on the cultures from timepoints 2-4.
↳ it takes 1.5 h. to do 48 preps - from cultures in microfuge tubes to EtOH ppt.

· All 192 gDNA preps are precipitating in EtOH in the -20. Tomorrow I will finish them.

5/16/08

· Today I finished all 192 gDNA preps - spin 10 min, wash w/ 75% EtOH. ~~Resuspend~~ Speed Vac for 5 min - Resuspend in 50λ TE.

5/17/08

· PCR on the 192 gDNA preps - 4 96-well plates
- 20λ PCRs
3280 H_2O
80 Forward Primer
80 Reverse Primer
400 10x Buffer
40 dNTPs
40 Taq
→ Add 18λ to each Rxn + 1λ template

· Large 2% gel - 14g Agarose + 700 mL 0.5x TAE + 25λ EtBr
- cool in 42° waterbath

5/20/08

· Unfortunately these PCRs didn't work - the hotlid on the thermal cycler was turned down to 60°. I turned the lid back up to 100° and reran the same plates with no luck. I tried running a few PCRs on the downstairs thermalcycler and I haven't been able to get results like I did previously (p. 154).

↳ I will have to re-optimize (annealing temp, etc) for the downstairs thermalcycler - this is dissapointing.

5/20/08

SOME NOTES ON STRAIN CONSTRUCTION

• As I am preparing to do the mating assays on the strains carrying different GPA1 alleles I realized that integrating the alleles into yGIL121 was not a good idea since this strain is bar1Δ — dammit.

I have several options:

(1) Transform a BAR1 ~~XXXX~~ Mat a W303 strain (such as yGIL087 - the original Rothstein W303-1a w/ URA3).

PROS: Probably ~~that~~ what I should have done to begin with.

CONS: ~~Would need to~~. I would not have complementary auxotrophies in this strain - I was relying on LEU2 & ADE2 in yGIL121 in the mating assay - I would need to transform this strain w/ LEU2 first. ADE2 is not sufficient because it reverts at high frequency.

(2) Put BAR1 back into strains yGIL265, 266, 268, 270, 289, & 271. There are two methods to do this:

(a) replace bar1Δ::ADE2 w/ BAR1 - screen on αF plates - look for red colonies - only αF mutants or BAR1 transformants will grow on αF - BAR1 transformants will appear red (loss of ADE2)

PROS:: Clever - would produce the exact strains I want.

CONS: Not sure it will work. I will lose ADE2 as nutritional marker.

(b) Integrate BAR1 @ URA3.

PROS: Should work but will have bkg. from URA3 pt. mutation. Maintains ADE2 as nutritional marker

CONS: Need to make sure that BAR1 works - αF plates

(3) Cross to a BAR1 strain such as yGIL122 (see p. 155)

PROS: I know this will work - can get GPA1 alleles in both mating types

CONS: yGIL121 & yGIL122 are not strictly isogenic - yGIL122 has one of Matthew's strains as a grandparent - therefore there is the possibility of introducing other crap. If I use this method I may be obliged to redo the fitness assays.

At this point I like option (2) the best - order primers.

5/23/08

TRANSFORMATION OF GPA1-ALLELE STRAINS w/ GFP::Kan & BAR1

· These fluorophore/drug marked strains have been pretty resistant to PCR. (For example see p. 162)

· Yesterday I attempted to amplify GFP::Kan from yGIL273 (p. 162) using primers YEL014C-extF1/R1 & F3/R3 (p. 162)

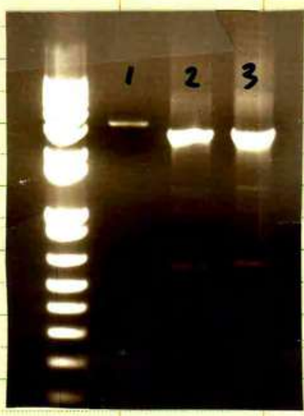
↳ unfortunately only the F1/R1 PCR worked → Zymo cleanup → elute 10 λ.

· In addition, as one (long-shot) attempt to resolve the bar1Δ issue I amplified BAR1 off of yGIL210 & yGIL211 using primers:

37385473 IDT
G.LANG
39085098 5/20/2008
BAR1_extF1 081
5'-CTT AAG AAG GCC GTT GAA GG-3'
Tm= 53.5 ºC , MW= 6,206.1
6.7OD260 33.30nmol 0.21mg

37385474 IDT
G.LANG
39085099 5/20/2008
BAR1_extR1 082
5'-ACT AAA TGG TGA AAG CGC GG-3'
Tm= 56.0 ºC , MW= 6,215.1
6.9OD260 33.60nmol 0.21mg

→ cleanup using zymoclean → 10 λ



LANE

1 - GFP::KanMX
2 - BAR1 from yGIL210
3 - BAR1 — " — 211

} pool and add 20 λ H_2O

· Yesterday I transformed strains yGIL265, 266, 268, 270, & 121 with the coctail of GFP::KanMX / BAR1.

· Today I replica plated the YPD plates first to G418 then to αF.

5/29/08

· The transformations appeared to work well - many colonies on each plate.

· On Tuesday (5/27) I picked 3 off of each plate (both αF & G418) and restreaked to YPD

· All 15 αF restreaks are white - therefore they are spontaneous αF^R not ~~BAR1~~.

· Today I started cultures of the 15 $G418^R$ strains. Looking under the scope I see a range of GFP intensities - from dark to dull to very bright.

↳ I think tomorrow I will need to screen many putative transformants for GFP.

6/2/08 Only one of ~50 putative transformants is actually showing reasonable fluorescence
Freeze down this strain (yGIL266 #2) as yGIL278.

5/25/08

NOTES ON PROJECT

· Visiting Boston - Discussed this project w/ Andrew & Joanna. A few thoughts:

· Joanna has found that W303 produces very high levels of mating pheromone - I should send her my strains (isogenic except for the GPA1 allele) and check the amount of pheromone produced by these strains.

→ Also I should get her protocol for the mating assay.

· I have been rationalizing the increase in mating pathway expression in lab-derived strains as the lazy (or anxious) graduate student hypothesis — That the upregulated mating pathway increases mating kinetics - making strains harboring this allele the first to form zygotes.

→ It is important to realize that mating in the wild is predominantly intra-ascus whereas mating in the lab is done typically btw haploid lines.

· Although the RM version of GPA1 confers a growth advantage, it may never be realized in the wild since haploids are short lived — It would therefore be useful to do fitness assays on diploids.

· My plan now is to do mating assays using a Matα strain w/ the lab GPA1 allele and varying the allele in the Mata Bkg.
→ it would also be useful to do this expt. with the wild-GPA1 allele in the Matα strain - This is an expt. I should really do but may be difficult since I am having trouble getting a ~~##~~ dTomato::KanMX strain.

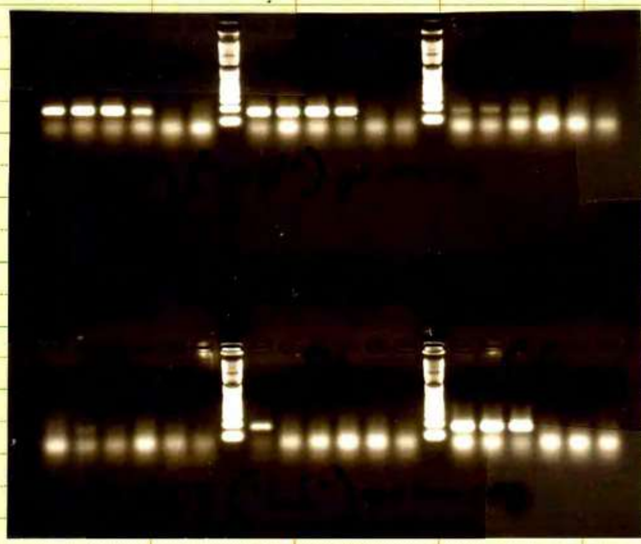
· I should do some competitive mating assays by marking the strains carrying diff. GPA1 alleles with diff nutritional markers.

5/27/08

~~[illegible]~~ ALLELE-SPECIFIC PCR ON GPA1 COMPETITIONS

- Last week I attemped Allele-specific PCRs on individual colonies from the GPA1 competitions (p. 169). These PCRs didn't work as I had hoped. I reset the hot lid to 100° and reran the same plates to no avail. I then tried amplifying a select few and also failed.

- I decided to re-optimize the annealing temp. using the downstairs thermal cycler using 10λ volume PCRs. On thursday (5/22) I ran the first three wells from the timepoint 1 plate (labeled below as A1, B1, C1, see p. 168 & 169) using both sets of primers.



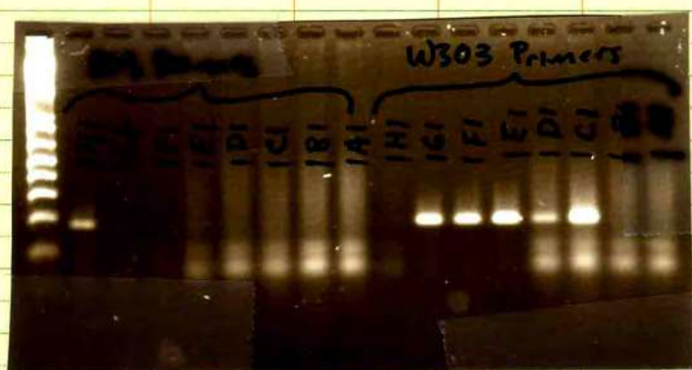
Annealing temp	Lane
45.0	1
48.2	2
53.4	3
59.6	4
63.4	5
65.0	6

It seems like I should try using 54° annealing (instead of 60° - see p. 154)

5/29/08

I reran the 4 96-well plate PCRs in the Microarray facility yesterday. - 10λ PCR w/ 54° annealing temp.

To check that the PCR worked I ran out columns 1 & 2 of Plate 1



- Of the 8 gDNA preps 1 was wild allele, 5 lab allele, and 2 no product

- I labeled the gDNA preps 1A1 - 1H1 meaning time-point 1 well A1 - H1.

- I know from the gel above that the A1 & B1 no-products are actually both wild alleles

5/30/08

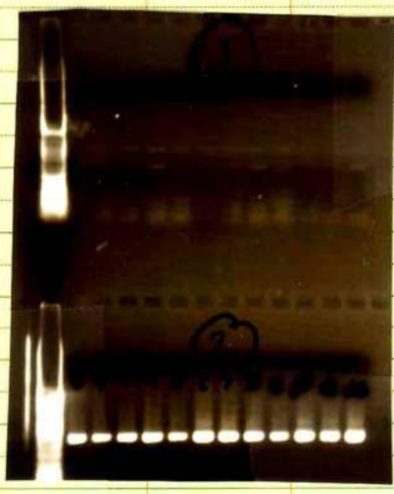
QUANTATATIVE SEQUENCING ON GPA1 COMPETITIONS.

· Maitreya has had success determining the ratios of strains in a mixed culture by sequencing. The idea is just to read all the ratios of the SNPs from the chromatogram. I will try this with my competitions.

· I made gDNA preps of the direct-competition mixes (see p 164)

· PCR using two primer sets:

1. GPA1_intF2 (p.144) & GPA1_extR2 (p144)
2. " & KanMx/NatMx_R1 (p. 137)



LANE	STRAINS	TIME PT.
1	265 v. 266	1
2	"	2
3	"	3
4	"	4
5	268 v. 270	1
6	"	2
7	"	3
8	"	4
9	269 v. 271	1
10	"	2
11	"	3
12	"	4

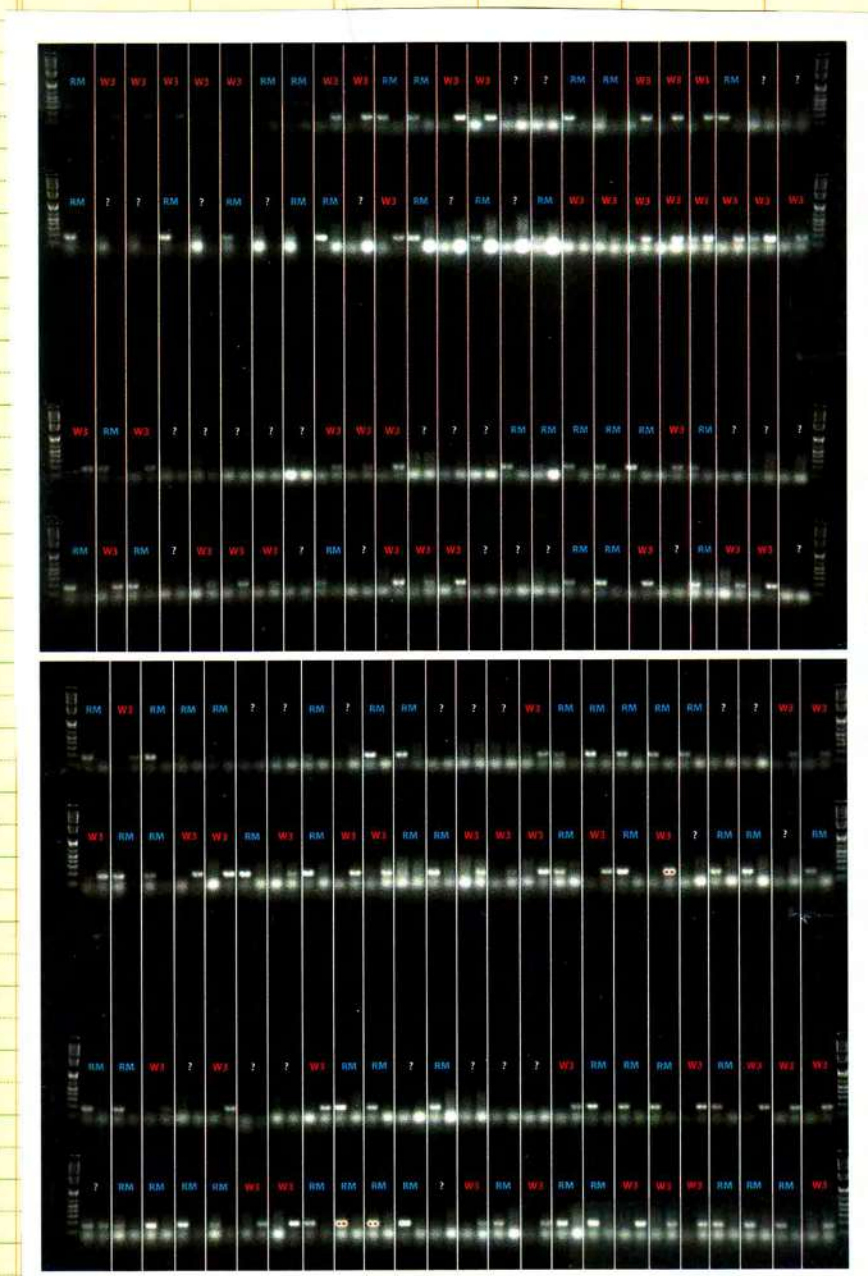
· External PCR did not work as before (see p. 147)

· ExoSAP PCRs w/ Kan/Nat_R1 → submit for sequencing

6/2/08

ANALYSIS OF ALLELE-SPECIFIC PCR ON DIRECT COMPETITIONS

- On Saturday (5/31) I ran the rest of the 4 96-well plate PCR (16 were run on 5/29 — see p.173). The results are below
- The 1st 8 calls were made based upon the gels on p.173



Timepoint 1

Timepoint 2

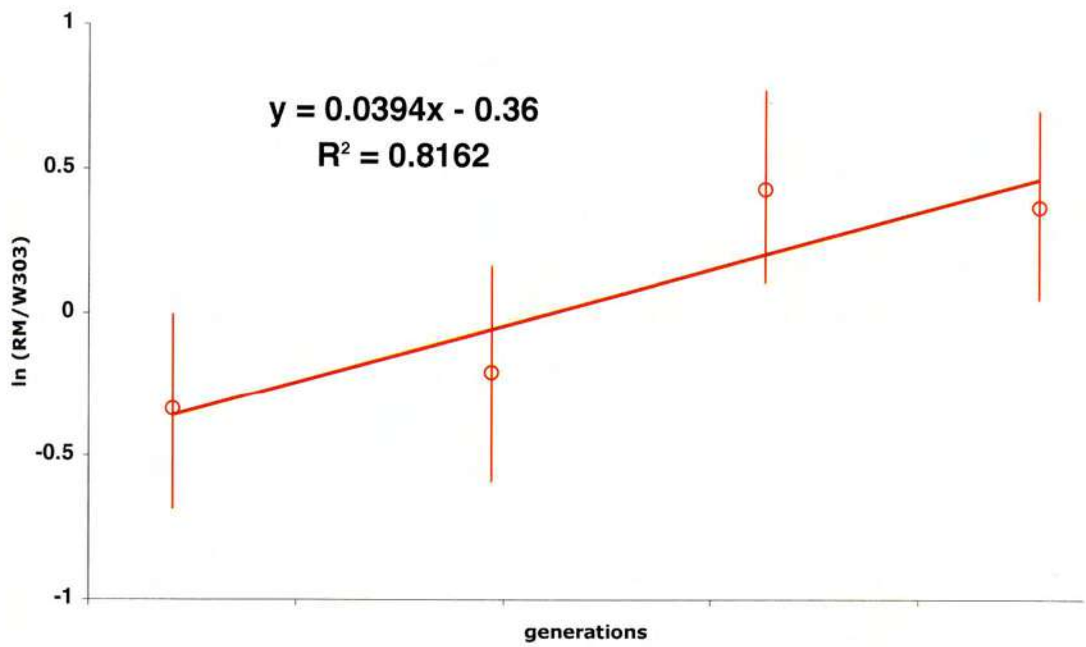
Timepoint 3

Timepoint 4

6/2/08

CALCULATING SEL. COEFF. FROM PCR DATA

TIME POINT	NUMBER RM	W303	?	GENERATIONS	FRACT-RM (p)	BINOMIAL ERROR	ln(RM/W303)	low error	high error
1	15	21	12	0.00	0.42	0.08	-0.34	0.35	0.33
2	13	16	19	7.69	0.45	0.09	-0.21	0.39	0.37
3	23	15	10	14.28	0.61	0.08	0.43	0.32	0.35
4	23	16	9	20.88	0.59	0.08	0.36	0.32	0.34



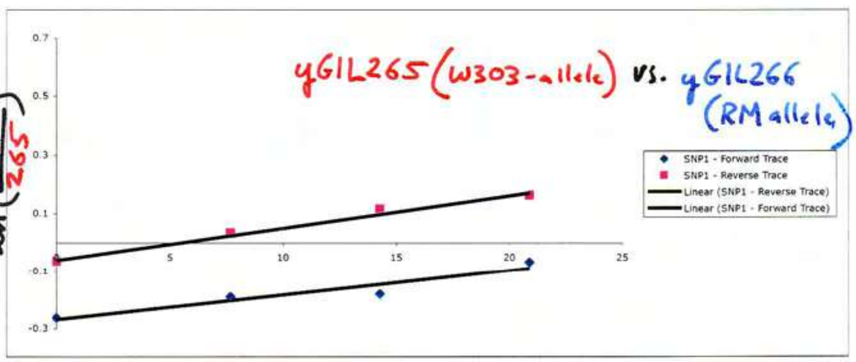
- Error bars were calculated from expected error due to binomial sampling
- The estimated sel. coeff. calculated here is higher than expected (see p. 167).

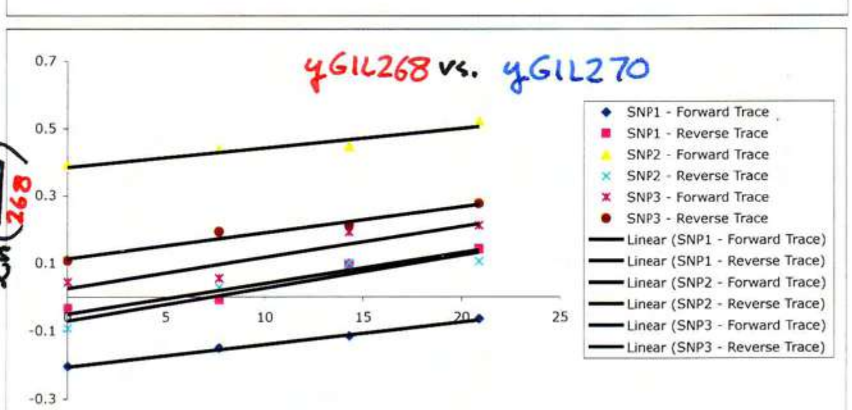
→ However a slope of 0.01 will fit within these error bars.

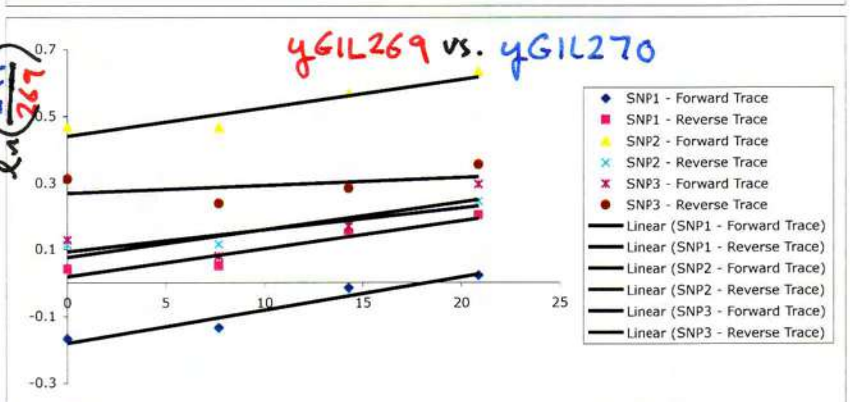
6/3/08

ANALYSIS OF QUANTATATIVE SEQUENCING ON GPA1 DIRECT COMPETITIONS

- I ~~sequenced the~~ amplified GPA1 off of the mixed-population gDNA preps from the GPA1 direct competitions and submitted them for sequencing (p.174).
- All the chromatograms look good.
- Using the "view trace" java applet on GENEWIZ's web site I extracted the peak heights for the SNPs in each trace and used these to calculate ratios of the two strains in each mixed population.







yGIL265 vs. yGIL266		
Trace	Sel. Coeff.	R^2
SNP1 - Forwar	0.86%	0.90
SNP1 - Revers	1.10%	0.99
AVERAGE	**0.98%**	
STDEV	**0.18%**	

yGIL268 vs. yGIL270		
Trace	Sel. Coeff.	R^2
SNP1 - Forwar	0.66%	1.00
SNP1 - Revers	0.90%	0.94
SNP2 - Forwar	0.57%	0.92
SNP2 - Revers	0.97%	0.89
SNP3 - Forwar	0.92%	0.86
SNP3 - Revers	0.77%	0.96
AVERAGE	**0.80%**	
STDEV	**0.16%**	

yGIL269 vs. yGIL271		
Trace	Sel. Coeff.	R^2
SNP1 - Forwar	0.98%	0.93
SNP1 - Revers	0.84%	0.91
SNP2 - Forwar	0.85%	0.87
SNP2 - Revers	0.66%	0.88
SNP3 - Forwar	0.84%	0.65
SNP3 - Revers	0.24%	*0.18
AVERAGE	**0.83%**	
STDEV	**0.12%**	

OVERALL	
AVERAGE	**0.84%**
STDEV	**0.15%**

*I excluded SNP3-Reverse in the averages of yGIL269 v. 271 & the overall average

6/2/08

TRANSFORMATION OF GPA1 STRAINS w/ ura3Δ::BAR1 PCR

- For now I have given up on transforming the GPA1-allele strains w/ GFP - I had little success with this (see p. 171).
- Of the options I discussed for reintroducing BAR1 into my strains (p. 170) the selection on αF did not work (p 171)
- I will therefore now try to put BAR1 @ the URA3 locus.
- On Friday (5/30) I amplified BAR1 off of the yGIL210 gDNA prep using primers BAR1_extF1/BAR1_extR1 as before (p171). In addition I used primers ⟶ ⟹ extension: 3m, 100λ PCRs

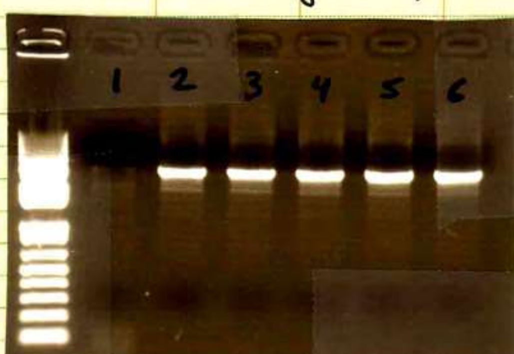
URA3_BAR1_extF1 - ACTGCACAGAACAAAAACCTGCAGGAAACGAAGATAAATCCTTAAGAAGGCCGTTGAAGG
URA3_BAR1_extR1 - GTGAGTTTAGTATACATGCATTTACTTATAATACAGTTTTACTAAATGGTGAAAGCGCGG

LANE	PRIMERS
1	BAR1_extF1/R1
2	URA3_BAR1_extF1/R1

NOTE: these primers bind ~500bp up/downstream of bar1

⟹ weak amplification using URA3_BAR1-extF1/R1

Reamplify using lane 2 (above) as template using URA3-BAR1-extF1/R1
- increase annealing temp to 60° - 5 × 100λ PCR



LANE	
1	- lane 2 from above
2	5 100λ PCRs →
3	
4	
5	
6	

6/3/08
- Repeat these 5 PCRs
- Zymo cleanup all 10 PCRs → elute each in 10λ H_2O → pool

6/3/08

- Start 5ml YPD o/N cultures of yGIL265, 266, 268, 269, 270, 271, 121, 278
- Make 5FOA plates: 500ml YNB4 media, 0.5g 5FOA, 1ml 500× His, 1ml 500× Trp, 2.5ml 100× Ura.

↳ this only made 26 plates ~~→ I need 32 — pour more tomorrow.~~

6/4/08

6/~~6~~5/08

· Since I am integrating BAR1 @ URA3 and selecting on 5FOA, I will need to control for the possibility of ura3 mutants overwhelming my actual transformants — In particular I must avoid the jackpot problem.

↳ For this reason I did not do the transformations yesterday. Instead I started o/N cultures of the 8 strains in -URA media — YNB supplemented w/ 2x His & 2xTrp.

· This morning I diluted the cultures 1:100 into 10 mL YPD. ~11:00

↳ I expect to transform the cultures btw 4&5 (~~~~ 6 generations) ∴ The jackpot is not as much of an issue with so few generations.

7pm - Start transformation

Coulter count

Bkg	558000	652000
265	$3.283e^7$	
270	$2.687e^7$	
Post Bkg	$2.512e^6$	

6/7/08

NOTES ON TRANSFORMATION

· Cells grew for 8h (~ 7-8 generations) unselected.

· Incubated @ 30° → heat shock @ 42°

· I did not do a liq. recovery — URA3 protein perdurance could be an issue — hopefully not.

6/24/08

· This transformation was unsuccessful — same # of colonies on 5FOA control & expt. plates.

↳ This was almost certainly a perdurance issue — looking at other protocols, 5FOA ~~should~~ selection requires an o/N incubation to eliminate Ura3 protein.

6/24/08

CROSSING GPA1 STRAINS TO y61L259

- I have decided that transforming BAR1 into my GPA1 strains is not ideal because I would need to sequence a number of clones from each strain to find a WT BAR1. The right way is to put ura3Δ::BAR1 on a plasmid and integrate this @ URA3, but even then there is no guarantee that these strains would mate properly. I have therefore decided to cross my strains to y61L259 (p. 156)
  - → Using this method I can get my GPA1 alleles in both the exp. strain and the mating tester.
- On 6/12 I crossed the following strains to y61L259: y61L265, 266, 268, 269, 270, 271, $ 278. → incubate on YPD plate o/n
- On 6/13 I streaked the patches onto minimal.
- On 6/18 → pick single colony off each plate and inoculate 5 ml YPD.
- 6/19 → freezedown:

y61L259	×	y61L265	→	y61L279
	×	266	→	280
	×	268	→	281
	×	269	→	282
	×	270	→	283
	×	271	→	284
	×	278	→	285

7/3/08

This is wrong - see p. 189 for correct descriptions.

~~and sporulate : spindown~~

- 6/21 → sporulate the now 3-day old cultures
  - spin down 1 ml
  - wash w/ 1 ml H_2O
  - resuspend 5 ml 2% KAc
- 6/24 → Coulter count $ determine sporulation efficiency for y61L279 $ 280

Bkg	54000	46000	56000
y61L279	1.023E8	1.055E8	1.056E8
y61L280	1.055E8	1.066E8	1.065E8
Post Bkg	5.388E6	2.22E6	1.328E6

	#tetrads	#veg. cells
y61L279	53	105
y61L280	53	115

6/24/08

The genotype of all these diploids is:

URA3/URA3
ade2/ade2
his3/HIS3
leu2/leu2
trp1/TRP1
CAN1/can1
bar1Δ::ADE2/BAR1
hmlαΔ::LEU2/HMLα
GPA1::Nat/GPA1
Mata/Matα
yel014cΔ::GFP::Kan/YEL014C ← only in yGIL285

~~////~~ For the random spore I will need to make plates that are YNB + CAN + LEU + ADE & YNB + CAN + HIS + TRP + ADE

→ Ideally I want low ADE so I can distinguish bar1Δ::ADE2 from BAR1.

6/26/08

· Yesterday I dissected 72 tetrads of ~~yGIL0~~ yGIL285

→ This was the easiest time I have had dissecting. The sporulation protocol I used resulted in good efficiency.

Preparation for dissection went as follows:

· To 50λ of Zymolyase (150 μg/mL) add 5 λ of sporulated culture.
· 30° Rollerdrum for 10 min
· Add 150λ H_2O → ice
· Place 10 λ on a dry YPD plate and allow cells to spread by gravity

6/27/08

Fuck! Someone used a large amount of bleach in the 30° room and sterilized all of my plates. I had several strains streaked and my 3 dissection plates. In all cases only a few colonies near the center of the plate are present → consistent with gas diffusing in from the edges.

→ I should redo the dissections today.

6/27/08

→ Did 24 more this afternoon.

- This morning I redid some of the dissections (48 so far; perhaps I will do another plate this afternoon).
- The annoying part is not that I wasted a few hours under the scope, but that I keep getting further behind on this project. → I have been trying to put BAR1 back into these strains for a month. This is getting ridiculous.
- I also started a random spore protocol on the 7 sporulated strains (from CSH manual)
  - spin down 1 ml sporulated culture
  - aspirate
  - resuspend in 200 λ H_2O
  - add 5 λ B-
  - incubate 1 h on 30° rollerdrum
  - add 0.1 ml 0.5 mm glass beads
  - incubate 1 h on 30° rollerdrum
  - add 1 ml H_2O
  - Vortex 1 min
- Dilute 10^{-1} 10^{-2} & 10^{-3} → plate 100 λ onto YNB+CAN+LEU & low ADE
  YNB+CAN+HIS+TRP low ADE

7/1/08

Notes on strain construction

This strain construction is becomming a pain.

- On Sunday (6/29) I replica plated the 3 dissection plates to the following: minimal, -his, -trp, -leu, -ade, -arg+CAN, Nat, G418, YPD+Mata (met6Δ), YPD+Matα (met6Δ), and YPD

↳ the met6Δ strains came from Allegra and make good testers

- On Monday I realized that the strain labeled as "yGIL285" (in sporulation tube) is not yGIL285 — This strain should have GFP. I screened all 7 of the sporulated cultures — the one labeled "yGIL284" was green

↳ I must have mislabeled my cultures somewhere along the way. — This was probably done while picking off of the mating plate due to the mirror image issue of writing on the back of the petri dish.

↳ This would suggest that the strain I dissected is actually yGIL284, and should still contain the RM version of GPA1 marked w/ NatMX — but I will need to sequence to verify this.

7/1/08

· The good news (other than that I've gotten much better at tetrad dissection) is that I have the strains I need to start mating assays. These of course do not have GFP so I will need to do diluting-and-plating-based assays.

7/3/08

MORE NOTES ON STRAIN CONSTRUCTION

· Mislabeled strains – As I mentioned on p. 183 I must have mislabeled the strains which I picked off of the cross plate.
I plated them as follows:

→ However I must have picked from left-to-right therefore I mixed up 259×266 & 259×265, 259×270 & 259×268, and 259×278 & 259×271. 259×269 would still be correct.

If this is true I dissected 259× 271, and the RM-GPA1 allele should still be segregating marked by ClonNat. Therefore I can still do the mating assays with strains genotyped from this cross.

The corrected strain designations for yGIL279 – yGIL285 are ...

yGIL279	=	yGIL259	×	yGIL 266
280	=		×	265
281	=		×	270
282	=		×	269
283	=		×	268
284	=		×	278
~~285~~				
285	=		×	271

· Yesterday I selected strains from the spores of yGIL285 that can be used in the mating assay. I picked these strains off of the YPD plate and restreaked to YPD to get a single colony for the assay. (p185)

· Random spore – the random spore protocol worked well (p. 183). For now I am putting most of these plates aside to focus on the mating assays. However I to the 10^{-1}, 10^{-2}, 10^{-3} plates of yGIL284 spores on -leu and replica plated to G418 and G418 + ClonNat. This identifies strains with the GFP reporter w/ or w/o the RM-GPA1 allele. Today I will restreak several red (ade⁻) colonies that are G418ᴿ Natˢ and G418ᴿ Natᴿ so that I can find ones that are ~~RRA~~ trp⁻ his⁻. These ~~will be~~ strains could be used for FACS-based mating assays should that prove necessary.

7/2/08 PHENOTYPING SPORES OF yGIL285

Tetrad	Spore	inv.	fit.	red	MIN	his	trp	leu	ade	Can	Nat	+ a	+ alpha	
1	A			x		+	+	+		+			+	
	B			x				+		+	+		+	
	C								+	+	+	+		
	D					+	+		+			+		
2	A			x							+		+	
	B						+	+	+				+	
	C					+	+		+	+		+		yGIL290
	D			x		+		+		+	+	+		
3	A	X												
	B			x				+		+	+		+	
	C					+	+		+			+		
	D			x						+	+		+	
4	A				+	+	+	+	+			+	+	
	B			x		+				+	+		+	
	C			x				+			+	+		
	D						+		+	+			+	
5	A						+	+	+	+	+	+		
	B					+			+		+		+	
	C							+	+	+	+		+	
	D			x		+	+			+		+		
6	A							+	+		+	+		
	B							+	+		+		+	
	C			x		+	+						+	
	D			x		+	+			+		+		
7	A		x	x			+	+		+	+	+		
	B					+			+	+		+		
	C			x			+				+		+	
	D					+		+	+				+	
8	A								+			+		
	B			x		+	+	+		+	+		+	
	C			x			+	+			+		+	
	D					+			+	+		+		
9	A			x		+	+	+		+	+		+	
	B			x		+	+				+	+		
	C							+	+	+		+		
	D								+				+	
10	A					+	+		+				+	
	B	X												
	C			x				+			+	+		
	D			x						+	+		+	
11	A			x		+		+				+		
	B						+		+	+	+	+		
	C						+	+	+	+	+		+	
	D			x		+							+	
12	A					+	+	+	+	+	+	+	+	
	B			x									+	
	C	X												
	D							+	+			+		
13	A			x			+	+		+	+		+	
	B			x		+	+	+			+		+	
	C					+			+	+		+		
	D								+			+		
14	A					+			+	+		+		
	B							+	+				+	
	C			x			+				+		+	
	D			x		+	+	+		+	+	+		
15	A				+	+	+	+	+		+	+	+	
	B	X												
	C	X												
	D			x			+				+		+	
16	A			x		+	+				+		+	
	B					+			+	+		+		
	C							+	+			+		
	D			x			+	+		+	+		+	
17	A					+	+		+	+	+	+		yGIL291
	B			x			+	+		+			+	
	C				+	+	+	+	+		+	+	+	
	D			x		+						+		
18	A			x								+		
	B			x		+	+			+	+		+	
	C							+	+	+		+		
	D				+	+	+	+	+		+	+	+	
19	A				+	+	+	+	+			+	+	
	B			x				+		+	+	+		
	C	X												
	D			x						+	+		+	
20	A						+		+		+		+	
	B					+		+	+			+		
	C			x				+		+			+	yGIL286
	D			x		+	+			+	+	+		yGIL292
21	A	X												
	B	X												
	C			x		+					+		+	
	D							+	+	+	+	+		
22	A								+	+	+	+		
	B		x	x		+	+	+				+		
	C			x			+	+					+	
	D					+			+	+	+		+	
23	A								+	+	+	+		
	B			x			+	+				+		
	C			x		+	+	+					+	
	D					+			+	+	+		+	
24	A					+		+	+		+	+		
	B				+	+	+	+	+		+	+	+	
	C			x			+			+		+		
	D			x			+			+			+	

Tetrad	Spore	inv.	fit.	red	MIN	his	trp	leu	ade	Can	Nat	a	alpha	
25	A					+	+		+	+	+		+	
	B						+	+	+	+	+		+	
	C			x				+				+		
	D			x		+						+		
26	A							+	+	+		+		
	B			x		+	+				+		+	
	C			x			+			+	+	+		
	D					+		+	+				+	
27	A					+			+	+			+	
	B	X												
	C							+	+	+			+	
	D	X												
28	A								+	+	+	+		
	B			x		+	+	+					+	
	C			x			+	+		+			+	
	D	X												
29	A						+	+	+			+		
	B						+		+	+			+	
	C			x		+				+	+		+	
	D			x		+		+			+	+		
30	A			x						+	+		+	
	B					+	+		+			+		
	C					+		+	+	+	+		+	
	D			x			+	+				+		
31	A					+		+	+			+		
	B			x				+		+			+	yGIL287
	C			x			+				+	+		
	D					+	+		+	+	+		+	
32	A			x			+				+	+		
	B			x		+		+					+	
	C					+		+	+	+		+		
	D						+		+	+	+		+	
33	A								+			+		
	B			x		+	+	+		+	+		+	
	C					+		+	+	+		+		
	D			x			+				+		+	
34	A								+		+		+	
	B					+			+	+	+	+		
	C			x		+	+	+		+			+	
	D			x			+	+				+		
35	A						+		+	+	+		+	
	B				+	+	+	+	+		+	+	+	
	C			x				+				+		
	D			x		+				+		+		
36	A							+	+	+		+		
	B					+			+			+		
	C			x			+	+			+		+	
	D			x		+	+			+	+		+	
37	A			x		+							+	
	B						+	+	+		+	+		
	C			x		+	+	+		+	+	+		
	D								+	+			+	
38	A				+	+	+	+	+		+	+	+	
	B			x			+						+	
	C			x			+	+					+	
	D					+		+	+	+	+	+		
39	A						+		+	+	+		+	
	B			x		+		+					+	
	C				+	+	+	+	+		+	+	+	
	D				+	+	+	+	+		+	+	+	
40	A			x			+	+				+		
	B								+	+	+		+	
	C					+			+		+		+	
	D			x		+	+	+		+		+		
41	A			x		+		+			+		+	
	B						+		+	+		+		
	C			x		+				+	+	+		
	D						+	+	+				+	
42	A					+	+		+		+		+	
	B	X												
	C					+	+		+		+	+		
	D			x				+		+			+	
43	A			x		+	+				+	+		
	B							+	+	+			+	
	C								+			+		
	D			x		+	+	+		+	+		+	
44	A					+		+	+	+			+	
	B			x			+	+			+	+		
	C								+				+	
	D			x		+	+			+	+	+		yGIL293
45	A					+			+	+	+	+		
	B					+	+		+			+		
	C			x				+			+		+	yGIL289
	D			x			+	+		+			+	
46	A			x			+	+					+	
	B			x			+	+					+	
	C					+			+	+	+	+		
	D								+		+	+		
47	A			x		+		+		+	+	+		
	B					+	+		+				+	
	C						+	+	+	+		+		
	D			x							+		+	
48	A			x				+		+			+	
	B			x		+	+			+	+		+	
	C					+			+			+		
	D						+	+	+		+	+		

Tetrad	Spore	inv.	fit.	red	MIN	his	trp	leu	ade	Can	Nat	a	alpha	
49	A			x		+				+	+	+		
	B						+		+	+		+		
	C				+	+	+	+	+			+	+	
	D			x				+			+		+	
50	A			x						+	+		+	
	B				+	+	+	+	+	+		+	+	
	C			x		+	+						+	
	D				+	+	+	+	+		+	+	+	
51	A								+	+	+	+		
	B					+		+	+	+	+	+		
	C			x		+	+	+					+	
	D			x			+						+	
52	A			x		+	+					+		
	B			x			+	+		+		+		
	C					+		+	+		+		+	
	D								+	+	+		+	
53	A			x		+	+			+			+	
	B			x		+	+	+		+		+		
	C							+	+		+		+	
	D								+		+	+		
54	A						+	+	+			+		
	B			x		+	+	+		+			+	
	C			x						+	+		+	
	D					+			+		+	+		
55	A					+			+	+		+		
	B			x			+	+		+	+	+		
	C			x		+		+					+	
	D						+		+		+		+	
56	A					+	+		+			+		
	B			x		+		+		+	+		+	
	C						+	+	+	+			+	
	D			x							+	+		
57	A						+		+	+		+		
	B			x		+		+		+	+	+		
	C					+	+		+				+	
	D			x				+			+		+	yGIL288
58	A								+	+	+	+		
	B					+		+	+		+	+		
	C			x		+	+	+		+			+	
	D			x			+						+	
59	A			x		+	+						+	
	B							+	+	+	+	+		
	C					+		+	+	+	+	+		
	D			x			+						+	
60	A	X												
	B	X												
	C			x			+	+		+		+		
	D	X												
61	A			x		+		+					+	
	B			x		+				+		+		
	C						+		+		+		+	
	D						+	+	+	+	+	+		
62	A						+		+				+	
	B			x		+	+			+		+		
	C			x		+		+			+	+		
	D							+	+	+	+		+	
63	A				+	+	+	+	+	+		+	+	
	B			x		+					+		+	
	C			x				+		+	+	+		
	D						+		+				+	
64	A				+	+	+	+	+		+	+	+	
	B			x			+			+			+	
	C			x				+			+	+		
	D	X												
65	A				+	+	+	+	+	+		+	+	
	B					+	+		+				+	
	C			x		+		+			+	+		
	D			x		+				+	+		+	
66	A			x			+					+		
	B						+	+	+	+			+	
	C			x		+				+	+		+	
	D					+		+	+		+	+		
67	A			x			+			+	+	+		
	B					+			+	+		+		
	C	X												
	D					+		+	+				+	
68	A				+	+	+	+	+			+	+	
	B			x		+		+		+	+		+	
	C						+		+	+		+		
	D			x		+					+	+		
69	A			x		+	+	+		+			+	
	B			x		+	+	+				+		
	C								+		+	+		
	D								+	+	+		+	
70	A				+	+	+	+	+	+	+	+	+	
	B				+	+	+	+	+		+	+	+	
	C				+	+	+	+	+		+	+	+	
	D				+	+	+	+	+		+	+	+	
71	A					+			+		+	+		
	B			x		+	+	+					+	
	C	X												
	D	X												
72	A						+		+	+	+	+		
	B			x		+							+	
	C			x		+		+		+			+	
	D						+	+	+		+	+		

Strains to be used in the experiment

- GPA1-W303. his. trp. LEU. ade. CanR. Mat a
- GPA1-RM::NatR. his. trp. LEU. ade. CanS. Mat a
- GPA1-W303. HIS. TRP. leu. ADE. CanR. Mat alpha
- GPA1-RM::NatR. HIS. TRP. leu. ADE. CanR. Mat alpha

7/16/08

GPA1-RM:NatR, HIS, TRP, leu, ade, CanR, Matα

7/3/08

~~MATING~~ MATING ASSAYS ON GPA1 MUTANTS

- I want to test if the lab-derived GPA1 allele ~~ra~~ has higher mating efficiency or faster kinetics than the RM allele.
- I will use two assays:
  - standard mating assay to determine the kinetics and efficiency of each strain separately
  - competitive mating assay where Mat a strains carrying either GPA1 allele are mixed and mated to Matα in order to calc. the % advantage in mating.

STRAINS: the strains to be used are on p. 185. Because of the confusion in strains (see p. 183, 184) I will need to verify that the ~~[illegible]~~ GPA1 allele is what I expect in these strains. I would have had to do this anyway because it is possible (but unlikely) that a crossover would occur btw GPA1 and the surrogate NatMX.

- I picked a single colony off of each plate (for yGIL286-291, p. 185)
  ↳ Colonies were still small (especially for yGIL286-289 since I did not add Ade to the plates) since I just streaked them out yesterday.
  ↳ innoc. into 5 mL YPD+Ade → rollerdrum 5:15 pm.
- Today I also prepared all of the media needed for both the competitive growth assay (which I will do tomorrow) and the standard mating assay (which I will do on Saturday).

7/4/08

COULTER COUNT

~~Bkg~~			
~~yGIL286~~	226^{000}	216^{000}	Bkg
~~287~~	$1.791E^8$	$1.826E^8$	yGIL286
~~288~~	$1.797E^8$	$1.807E^8$	287
~~289~~	$1.782E^8$	$1.807E^8$	288
~~290~~	$1.714E^8$	$1.736E^8$	289
~~291~~	$2.468E^8$	$2.464E^8$	290
~~Post Bkg~~	$2.631E^8$	$2.644E^8$	291
	$8.61E^6$	$5.846E^6$	Post Bkg

28 λ ~ 5 E^6 cells

Mat a ~ $1.8E^8$ → ~~[illegible]~~

Mat α ~ $2.5E^8$ → ~~[illegible]~~

~~20 λ ~ 5 E^6 cells~~

8 λ ~ 2 E^6 cells

↳ Freeze down yGIL286-291 for permanent stock.

7/4/08

Competitive Mating Assay

For comparing the mating advantage in *MAT***a**

*MAT***a** and *MAT*α strains should have complementary auxotrophies and the diploids formed by the two *MAT***a** strains must be distinguishable. For instance:

Experimental strains:
E1 *MAT***a**, GPA1-W303, *his3, trp1, LEU2, ade2, can1-100*
E2 *MAT***a**, GPA1-RM::*NatMX, his3, trp1, LEU2, ade2, CAN1*

Tester strains:
T1 *MAT*α, GPA1-W303, *HIS3, TRP1, leu2, ADE2, can1-100*
T2 *MAT*α, GPA1-RM::*NatMX, HIS3, TRP1, leu2, ADE2, can1-100*

Diploids can be selected on minimal media. E1 diploids are Can^R Nat^S; E2 diploids are Can^S Nat^R.

Grow overnight cultures of both *MAT***a** strains and the tester *MAT*α in YPD + Ade (extra adenine is necessary since experimental haploids are *ade2*).

Coulter count. Mix $5x10^6$ E1 cells, $5x10^6$ E2 cells in 10 ml YPD + Ade.

Dilute cells 1:500 in H_2O. Plate 200 λ onto –leu (~400 cells/plate). Label plates "pre-mating."

Add $2x10^6$ Tester cells. Filter onto a 25 mm 0.45 micron nylon filter. Place filter onto a YPD + Ade plate (four filters per plate).

Incubate 30° C for 5 hours.

Remove filter from plate and wash off cells with 1 ml H_2O (Concentration of diploids should be ~10^6).

Dilute cells $1:10^3$ in H_2O. Plate 200 λ onto and minimal. Label plates "post-mating."

Incubate plates 30° C for two days.

Replica plate "pre-mating" –leu plates and "post-mating" minimal plates to Canavanine and ClonNat.

Incubate plates 30° C overnight.

Count colonies on Canavainine and ClonNat plates. Determine the ratio of Can^R Nat^S to Can^S Nat^R pre- and post-mating.

· Set up six assays:
(1) yGIL286/yGIL288 × yGIL290
(2) yGIL287/yGIL289 × "
(3) yGIL286/yGIL289 × "
(4) yGIL286/yGIL288 × yGIL291
(5) yGIL287/yGIL289 × "
(6) yGIL286/yGIL289 × "

7/5/08

QUANTITATIVE MATING ASSAY

· Yesterday I picked single colonies off of yGIL286-291 plates and inoc 5 ml YPD+Ade ~ 7pm

· I will only do the Q mating assay with four strains:

- yGIL286 w/ yGIL290
- yGIL288 "
- yGIL286 w/ yGIL291
- yGIL288 "

Quantitative Mating Assay

For determining the efficiency and kinetics of mating in *MAT***a**

*MAT***a** and *MAT*α strains should have complementary auxotrophies and the diploids formed by the two *MAT***a** strains must be distinguishable. For instance:

Experimental strains:
E1 *MAT***a**, GPA1-W303, *his3, trp1, LEU2, ade2, can1-100*
E2 *MAT***a**, GPA1-RM::*NatMX, his3, trp1, LEU2, ade2, CAN1*

Tester strains:
T1 *MAT*α, GPA1-W303, *HIS3, TRP1, leu2, ADE2, can1-100*
T2 *MAT*α, GPA1-RM::*NatMX, HIS3, TRP1, leu2, ADE2, can1-100*

Diploids can be selected on minimal media.

Grow overnight cultures of both *MAT***a** strains and the tester *MAT*α in YPD + Ade (extra adenine is necessary since experimental haploids are *ade2*).

Coulter count. Mix $2x10^7$ Experimental cells with 10^8 Tester cells in 100 ml YPD + Ade. Put 1 ml into a 1.5 ml microfuge tube—this will be time point 1.

Dilute cells 1:10^{2} in H_2O. Plate 200 λ onto and minimal and -leu.

Filter 10 ml of cells onto eight 25 mm 0.45 micron nylon filters. Place filters onto YPD + Ade plates (four filters per plate).

Incubate 30° C

At 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 3.5 h, 4 h, and 5 h remove a filter from the plate and wash off cells with 1 ml H_2O (Concentration of diploids should be ~10^6). Dilute and plate as above.

Incubate plates 30° C for two days. 1:10^3

Count colonies to determine the mating efficiency.

Coulter count

Bkg	252000	210000	
yGIL286	1.667E8	1.703E8	Mat a ⇒ 118 λ ~ 2E7 cells
288	1.706E8	1.726E8	
290	2.021E8	2.027E8	Mat α ⇒ 500 λ ~ E8 cells
291	2.036E8	2.336E8	
Post Bkg	7.374E6	3.434E6	

7/5/08

- As I expected filtering to take a while, I recorded the time when I started and ended each flask.

yGIL286 w/ yGIL290	Start -	0 min
	End -	11 min
yGIL288 w/ yGIL290	Start -	13 min
	End -	22 min
yGIL286 w/ yGIL291	Start -	24 min
	End -	33 min
yGIL288 w/ yGIL291	Start -	35 min
	End -	44 min

7/6/08

- The plates from the competitive mating assay have grown for ~2 days.

→ On the "pre-mating" plates, I see the ~400 colonies/plate that I expected.
On the 3 "post-mating" plates ~~from the~~ where yGIL291 is the Matα, I do not see any colonies. I see ~400 colonies/plate where yGIL290 is the Matα. Yesterday as the Q mating assay was progressing I saw mating to yGIL291 under the scope - but poor mating. I also rechecked the plates used to phenotype these strains (p. 185). It appears that the effect of this GPA1 mutation on mating is more severe than I originally suspected.

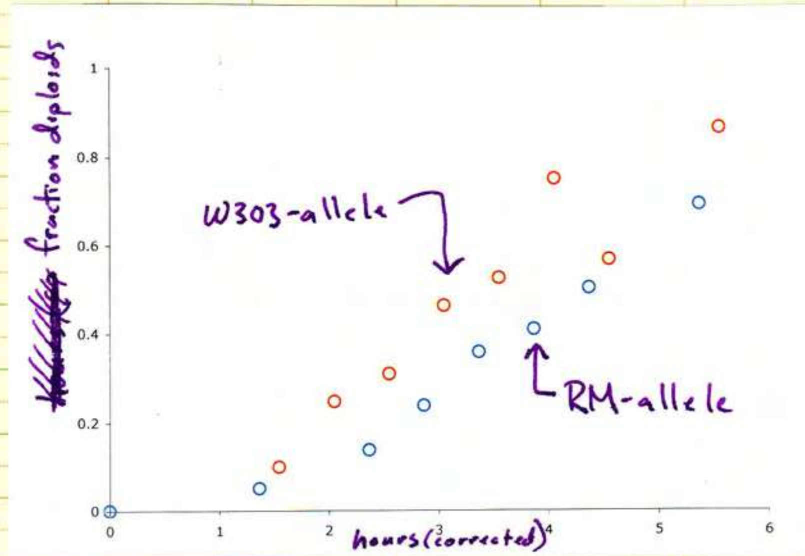
- Replica plate the plates to -arg+CAN $ ClonNat.

7/16/08

RESULTS FROM MATING ASSAYS

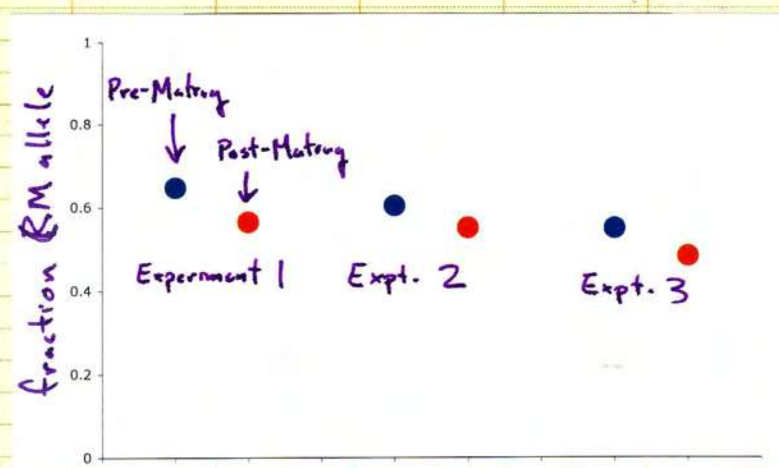
Quantitative Mating Assay

Time (h) Raw	Time (h) Corrected	Colonies on minimal	Colonies on -leu	Mating Efficiency
yGIL286 (MATa, W303) x yGIL290 (MATalpha, W303)				
0		0	249	0%
1	1.55	40	400	10%
1.5	2.05	95	383	25%
2	2.55	105	339	31%
2.5	3.05	198	429	46%
3	3.55	306	584	52%
3.5	4.05	509	680	75%
4	4.55	528	933	57%
5	5.55	1060	1230	86%
yGIL288 (MATa, RM) x yGIL290 (MATalpha, W303)				
0		0	355	0%
1	1.37	17	333	5%
1.5	1.87	43	0	
2	2.37	65	473	14%
2.5	2.87	118	495	24%
3	3.37	234	654	36%
3.5	3.87	320	784	41%
4	4.37	474	947	50%
5	5.37	861	1248	69%



Competitive Mating Assay

Colonies on -leu	Replica plate ClonNat	Fraction RM Pre mating	Colonies on minimal	Replica plate ClonNat	Fraction RM Post mating
yGIL286 (MATa, W303) & yGIL288 (MATa, RM) x yGIL290 (MATalpha, W303)					
263	170	65%	510	288	56%
yGIL287 (MATa, W303) & yGIL289 (MATa, RM) x yGIL290 (MATalpha, W303)					
330	199	60%	639	352	55%
yGIL286 (MATa, W303) & yGIL289 (MATa, RM) x yGIL290 (MATalpha, W303)					
328	180	55%	655	317	48%



NOTES

- Overall I was a successful expt. There is a clear mating advantage in strains carrying the W303 allele of GPA1.
- I will need to repeat these experiments to get a better quantitative answer.
- Some issues:
  - Strain yGIL291 (MATα, RM-allele) mated (I saw zygotes under the microscope) but nothing grew on minimal — I must have scored the spore incorrectly (there was not 2:2 segregation, see p. 185).
    → I picked two more MATα RM-allele strains (yGIL292 & yGIL293). Both are ade⁻ — that will be fine but I will have to supplement w/ Ade (see p. 185). I will use these 2 strains when I repeat the assay.
  - Too many colonies: I need plate fewer cells (and account for cell division during the 5h mating). Also I could not count the Canavanine plates which I replica plated to in the Competitive Assay — I should plate directly to canavanine — Also I need to supplement w/ Leu for the pre-mating plates.

7/22/08

COMPETITIVE MATING ASSAYS

Today I am repeating the competitive mating assays (see p. 188)

Protocol changes:

(1) My MATα-RM strain is ade2 so I will supplement everything w/ Adenine

(2) I will plate half the cells from pre-mating mix and one quarter of the cells from the post-mating mix compared to previous.

(3) Add sonication step

(4) In addition to replica plating I will directly plate onto discriminating media

(5) All platings in duplicate.

STRAINS

E1 - yGIL286
E2 - yGIL288
T1 - yGIL290
T2 - yGIL292

see p. 185

Competitive Mating Assay (7/22/08)

For comparing the mating advantage in *MAT***a**

*MAT***a** and *MAT*α strains should have complementary auxotrophies and the diploids formed by the two *MAT***a** strains must be distinguishable. For instance:

Experimental strains:
E1 *MAT***a**, GPA1-W303, *his3, trp1, LEU2, ade2, can1-100*
E2 *MAT***a**, GPA1-RM::*NatMX, his3, trp1, LEU2, ade2, CAN1*

Tester strains:
T1 *MAT*α, GPA1-W303, *HIS3, TRP1, leu2, ADE2, can1-100*
T2 *MAT*α, GPA1-RM::*NatMX, HIS3, TRP1, leu2, ade2, can1-100*

Diploids can be selected on minimal media = adenine.
E1/T1 diploids are Can^R Nat^S; E2/T1 diploids are Can^S Nat^R.
E1/T2 diploids are Can^R Nat^R; E2/T2 diploids are Can^S Nat^R.

Grow overnight cultures of both *MAT***a** strains and the tester *MAT*α in YPD + Ade (extra adenine is necessary since experimental haploids are *ade2*).

Coulter count. Mix $5x10^6$ E1 cells, $5x10^6$ E2 cells in 10 ml YPD + Ade. Sonicate.

Dilute cells 1:500 in H_2O. Plate 100 λ onto –leu (~200 cells), -leu+canavanine, and ClonNat. Label plates "pre-mating."

Add $2x10^6$ Tester cells. Filter onto a 25 mm 0.45 micron nylon filter. Place filter onto a YPD + Ade plate (four filters per plate).

Incubate 30° C for 5 hours.

Remove filter from plate and wash off cells with 1 ml H_2O (Concentration of diploids should be ~10^6). Sonicate.

Dilute cells $1:2x10^3$ in H_2O. Plate 100 λ onto and minimal+ade, and minimal+ade+can. Label plates "post-mating."

Incubate plates 30° C for two days.

Replica plate "pre-mating" –leu plates and "post-mating" minimal plates to Canavanine and ClonNat.

Incubate plates 30° C overnight.

Count colonies on Canavainine and ClonNat plates. Determine the ratio of Can^R Nat^S to Can^S Nat^R pre- and post-mating.

7/22/08

· Yesterday I started o/N cultures of yGIL286, 288, 290, & 292.
· Dilute 1:50 (200 λ into 10 mL YPD+ADE) ~ 11am
· Coulter Count (2:30pm)

Bkg	1.992E6	2.63E6
yGIL286 1	1.382E7	1.33E7
" 2	1.782E7	1.526E7
yGIL288 1	1.432E7	1.393E7
" 2	1.443E7	1.394E7
yGIL290 1	1.912E7	1.924E7
" 2	2.245E7	2.233E7
yGIL292 1	1.472E7	1.448E7
" 2	1.387E7	1.293E7
Post Bkg	2.484E6	2.088E6

Experiments 1 & 2 { 332 λ yGIL286 + 353 λ yGIL288 + 96 λ yGIL290 in 9.22ml YPD+ADE

Experiments 3 & 4 { —— " —— + 142 λ yGIL292 In ~~9.11~~ 9.2 ml YPD+ADE

Plate counts from second attempt at competitive mating assay (7/29/08)

Expt.plate	-leu	-leu CAN	-leu Nat	-leu Nat (rep)	min	min CAN
1.1	182	22	118	110	340	32
1.2	137	30	112	90	412	32
2.1	176	23	70	94	378	31
2.2	186	22	107	104	371	1
3.1	167	22		96	239	18
3.2	235	12	127	127	251	14
4.1	*166*	1	91		220	17
4.2	*194*	23	123		230	20

8/4/08

LONG-TERM PROPAGATION OF yGIL121

I want to set up a long-term evolution experiment to look explicitly for the fixation of sterile mutations.

Mutation Rate to $\alpha F^R \sim 3E^{-6}$

P{Mutation arises & survives drift} = $N\mu s = 0.03$ (assuming $N_e \sim 10^6, s \sim 2\%$)

→ ∴ waiting time for mutation to occur & survive drift $\sim \frac{1}{p} = 30$ generations

An αF^R mutation surviving drift will sweep in $t = \ln\left(\frac{f_t}{f_0}\right)/s = \left(\frac{.99}{.02}\right)/.02 \sim 200$ gen

∴ I expect too see αF^R sweeping in ~300 generations.

~~Today I estab~~

· On Friday (8/1) I streaked yGIL121 from frozen stock.

· Today I established 20 lineages (A-T) each from a single colony. ⟶ these tubes were labeled A1 - T1

- I plan to propagate these lines by diluting 1:2000 (5λ into 10mL) each day periodically freezing 1mL samples.

Generations / day = ln(2000) = 7.6

Days to 300 generations = 40

~~Satur~~ N_e ≈ 10^7 ← assuming 2E7 saturated cultures (p. 166)

8/5/08

· 12:00 – dilute all 20 cultures 1:2000 (5λ into 10mL)

· Coulter count to determine saturation density (10λ into 20mL)

Bkg	1.42E6	1.35E6
A ~~#~~1	1.116E8	1.127E8
B ~~#~~1	1.106E8	1.114E8
C ~~#~~1	1.086E8	1.079E8
D ~~#~~1	1.044E8	1.04E8
Post Bkg	5.506E6	3.374E6

8/6/08

· Again dilute 1:2000 – ~~@~~ 12:00

· Coulter count (10λ into 20mL)

Bkg	1.034E6	906000
A2	5.306E7	5.418E7
B2	5.376E7	5.433E7
C2	5.484E7	5.44E7
D2	5.179E7	5.189E7
Post Bkg	3.176E6	1.978E6

8/7/08

- 12:00 - again dilute 1:2000 and count (10 λ in 20 ml)

Bkg	916000	886000
A3	1.05 E8	1.072E8
B3	1.097E8	1.097E8
C3	1.076 E8	1.066E8
D3	1.071E8	1.076E8
Post BKg	5.372E6	~~2.696E6~~

8/8/08

NOTES ON LONG-TERM PROPAGATION EXPERIMENT

- For the first two transfers I used YPD made by Lublea (Tubes A1-T1 & A2-T2)
- On Monday (8/4) I prepared 2 L of YPD supplemented w/ additional Ade & Trp. - This media was aliquotted into tubes and autoclaved. I intend to prepare the media in this way for the remainder of the experiment.

↳ During autoclaving I lose some liquid - the caps were not pushed down therefore I lost more than usual - the volume may be closer to 9 ml than to 10 ml

Transfer 1 **Media** Batch 1
Date 8/4/08 **Time** ~5pm
Sample No

Coulter Count
Bkg
A
B
C
D
Post

Notes
20 lines each started from a single colony of yGIL121 (see p 195)

Transfer 2 **Media** 1
Date 8/5/08 **Time** 12:00pm
Sample No

Coulter Count
Bkg 1.42E6 1.35E6
A1 1.16E8 1.29E8
B1 1.106E8 1.114E8
C1 1.086E8 1.079E8
D1 1.044E8 1.04E8
Post 5.506E6 3.374E6

10λ into 20 mL

Notes

Transfer 3 **Media** 2
Date 8/6/08 **Time** 12 pm
Sample No

Coulter Count
Bkg 1.034E6 706000
A2 5.306E~~8~~7 5.418E~~8~~7
B2 5.376E~~8~~7 5.433E~~8~~7
C2 5.484E7 5.44E7
D2 5.179E7 5.189E7
Post 3.176E6 1.978E6

10λ into 20 mL

Notes

Transfer 4 **Media** 2
Date 8/7/08 **Time** 12 pm
Sample Yes (A3-T3)

Coulter Count
Bkg 916000 886000
A3 1.05E8 1.072E8
B3 1.097E8 1.047E8
C3 1.076E8 1.066E8
D3 1.071E8 1.076E8
Post 5.372E6 2.696E6

10λ into 20 mL

Notes

Transfer 5 **Media** 2
Date 8/8/08 **Time** 12 pm
Sample No

Coulter Count
Bkg
A
B
C
D
Post

Notes

Transfer 6 **Media** 2
Date 8/9/08 **Time** 10:15am
Sample No

Coulter Count
Bkg
A
B
C
D
Post

Notes

Transfer 7 **Media** 2
Date 8/10/08 **Time** 12 pm
Sample Yes (A6-T6)

Coulter Count
Bkg 414000 442000
A6 1.132E8 1.146E8
B6 1.199E8 1.195E8
C6 1.191E8 1.191E8
D6 1.179E8 1.165E8
Post 4.346E6 2.968E6

10λ into 20 mL

Notes

Transfer Media Coulter Count Notes
8 2
Bkg
A
Date Time
B
8/11/08 12pm
C
D
Sample No
Post

Transfer Media Coulter Count Notes
9 2
Bkg
A
Date Time
B
8/12/08 12pm
C
D
Sample No
Post

Transfer Media Coulter Count Notes
10 2
Bkg 628000 560000
A9 1.13E8 1.154E8
Date Time
B9 1.117E8 1.117E8
8/13/08 12pm
C9 1.111E8 1.114E8
D9 1.112E8 1.108E8
10 λ into 20 mL
Sample Yes (A9-T9)
Post 3.856E6 2.612E6

Tested for αF^R-4
· 2 λ into 20 λ YPD+αF
· After 4 h there were plenty of shmoos in all 20 cultures

Transfer Media Coulter Count Notes
11 2
Bkg
A
Date Time
B
8/14/08 12pm
C
D
Sample No
Post

Transfer Media Coulter Count Notes
12 2
Bkg
A
Date Time
B
8/15/08 12pm
C
D
Sample
Post

Transfer Media Coulter Count Notes
13 3
Bkg 644000 596000
A 1.112E8 1.125E8
Date Time
B 1.143E8 1.15 E8
8/16/08 12pm
C 1.088E8 1.1 E8
D 1.103E8 1.094E8
10 λ into 20mL
Sample Yes (A12-T12)
Post 3.754E6 2.72 E6

Someone moved my tubes around in the rollerdrum. I noticed that I & J were inverted — I assume that this happened when my tubes were moved, but it is possible that I inverted these lines — I will start dating my tubes.

Transfer Media Coulter Count Notes
14 3
Bkg
A
Date Time
B
8/17/08 3:30 pm
C
D
Sample No
Post

Transfer 15 **Media** 3

Date 8/18/08 **Time** 12 pm

Sample No

Coulter Count

Bkg
A
B
C
D
Post

Notes

Tested for αF^R (same as on 8/13)

↳ All 20 strains still respond.

Transfer 16 **Media** 3

Date 8/19/08 **Time** 12pm

Sample Yes (A15-T15)

Coulter Count

Bkg 800000 748000
A 1.065E8 1.082E8
B 1.023E8 1.02 E8
C 1.057E8 1.052E8
D 1.022E8 1.032E8
10λ into 20mL
Post 3.796E6 2.61E6

Notes

Transfer 17 **Media** 3

Date 8/20/08 **Time** 12pm

Sample No

Coulter Count

Bkg
A
B
C
D
Post

Notes

Transfer 18 **Media** 3

Date 8/21/08 **Time** 12pm

Sample No

Coulter Count

Bkg
A
B
C
D
Post

Notes

Transfer 19 **Media** 3

Date 8/22/08 **Time** 12 pm

Sample Yes (A18-T18)

Coulter Count

Bkg 1.382E6 1.482E6
A 1.086E8 1.103E8
B 1.147E8 1.165E8
C 1.1E8 1.102E8
D 1.108E8 1.096E8
10λ into 20ml
Post 5.65E6 3.922E6

Notes

Tested for αF^R as before

↳ Vast majority in all cultures respond to αF, but for the 1st time I can see budding cells—although they are very rare.

Transfer 20 **Media** 3

Date 8/23/08 **Time** 12 pm

Sample No

Coulter Count

Bkg
A
B
C
D
Post

Notes

Transfer 21 **Media** 3

Date 8/24/08 **Time** 12 pm

Sample No

Coulter Count

Bkg
A
B
C
D
Post

Notes

Transfer 22 **Media** 3

Date 8/25/08 **Time** 12pm

Sample Yes (A21-T21)

Coulter Count
Bkg $1.092E^6$ $1.208E^6$
A $1.099E^8$ $1.113E^8$
B $1.084E^8$ $1.079E^8$
C $1.129E^8$ $1.125E^8$
D $1.122E^8$ $1.134E^8$
10λ into 20mL
Post $4.592E^6$ $3.356E^6$

Notes

Transfer 23 **Media** 4

Date 8/26/08 **Time** 12pm

Sample No

Coulter Count
Bkg
A
B
C
D
Post

Notes

Transfer 24 **Media** 4

Date 8/27/08 **Time** 12pm

Sample No

Coulter Count
Bkg
A
B
C
D
Post

Notes

Transfer 25 **Media** 4

Date 8/28/08 **Time** 12pm

Sample Yes (A24-T24)

Coulter Count
Bkg $1.568E^6$ $1.614E^6$
A $1.112E^8$ $1.135E^8$
B $1.147E^8$ $1.143E^8$
C $1.094E^8$ $1.099E^8$
D $1.132E^8$ $1.147E^8$
Post $5.636E^6$ $3.814E^6$

Notes

Transfer 26 **Media** 4

Date 8/29/08 **Time** 12pm

Sample No

Coulter Count
Bkg
A
B
C
D
Post

Notes

Transfer 27 **Media** 4

Date 8/29/08 **Time** 12pm

Sample No

Coulter Count
Bkg
A
B
C
D
Post

Notes

Transfer 28 **Media** 4

Date 8/30/08 **Time** 12pm

Sample Yes (A27-T27)

Coulter Count
Bkg $1.432E^6$ $1.374E^6$
A $1.145E^8$ $1.14E^8$
B $1.106E^8$ $1.1E^8$
C $1.124E^8$ $1.129E^8$
D $1.113E^8$ $1.106E^8$
10λ into 20mL
Post $4.888E^6$ $3.472E^6$

Notes

Transfer 29 Media 4 | Coulter Count | Notes
Bkg
A
Date 9/1/08 Time 12pm
B
C
D
Sample No | Post

Transfer 30 Media 4 | Coulter Count | Notes
Bkg
A
Date 9/2/08 Time 12pm
B
C
D
Sample No | Post

Transfer 31 Media 4 | Coulter Count | Notes
Bkg 838000 904000
A 1.11E8 1.111E8
Date 9/3/08 Time 12pm
B 1.122E8 1.111E8
C 1.101E8 1.109E8
D 1.103E8 1.104E8
10λ into 20mL
Sample Yes (A30-T30) | Post 4.002E6 3.056E6

Transfer 32 Media 5 | Coulter Count | Notes
Bkg
A
Date 9/4/08 Time 12pm
B
C
D
Sample No | Post

Transfer 33 Media 5 | Coulter Count | Notes
Bkg
A
Date 9/5/08 Time 12pm
B
C
D
Sample No | Post

Transfer 34 Media 5 | Coulter Count | Notes
Bkg 1.06E6 1.304E6
A 1.316E8 1.346E8
Date 9/11/08 Time ~~12pm~~ 11:30am
B 1.384E8 1.393E8
C 1.364E8 1.369E8
D 1.302E8 1.318E8
10λ into 20mL
Sample Yes (A33-T33) | Post 6.366E6 4.004E6

Transfer 35 Media 5 | Coulter Count | Notes
Bkg
A
Date 9/12/08 Time 11:30am
B
C
D
Sample No | Post

Transfer Media Coulter Count Notes
36 5
Bkg
A
Date Time
B
9/13/08 11:30am
C
D
Sample No.
Post

Transfer Media Coulter Count Notes
37 5
Bkg 1.954E6 2.052E6
A 1.11E8 1.12E8
Date Time
B 1.11E8 1.116E8 10λ into 20 ml
9/14/08 11:30am
C 1.123E8 1.118E8
D 1.124E8 1.114E8
Sample Yes (A36-T36)
Post 5.976E6 4.122E6

Transfer Media Coulter Count Notes
38 5
Bkg
A
Date Time
B
9/15/08 11:30am
C
D
Sample No
Post

Transfer Media Coulter Count Notes
39 5
Bkg
A
Date Time
B
9/16/08 12:15pm
C
D
Sample No
Post

Transfer Media Coulter Count Notes
40 5
Bkg ~~9.6E6~~ ~~1.426E7~~ 976000 1.04E6
A 8.303E7 8.408E7
Date Time
B 9.514E7 9.542E7 10λ into 20ml
9/17/08 12pm
C 1.028E8 1.025E8
D 9.269E7 9.288E7
Sample Yes (A39-T39)
Post 3.334E6 2.544E6

Transfer Media Coulter Count Notes
Bkg
A
Date Time
B
C
D
Sample
Post

Transfer Media Coulter Count Notes
Bkg
A
Date Time
B
C
D
Sample
Post