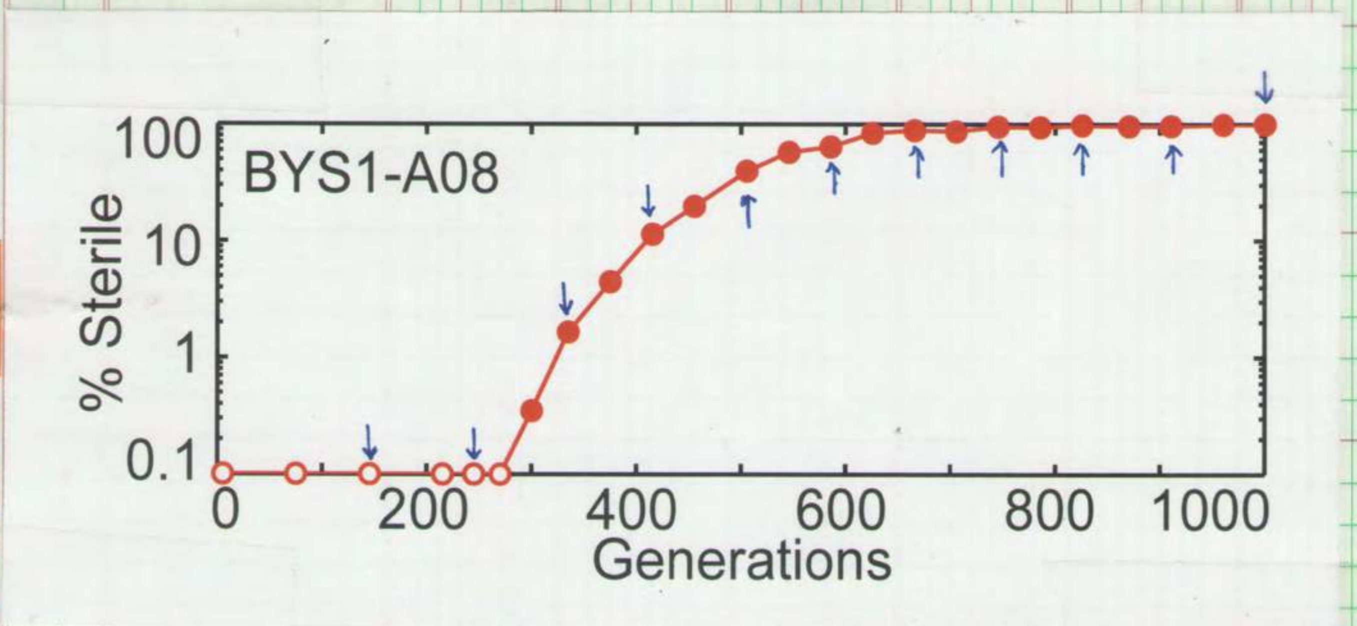
	Initials	Date
Prepared By		
Approved By		

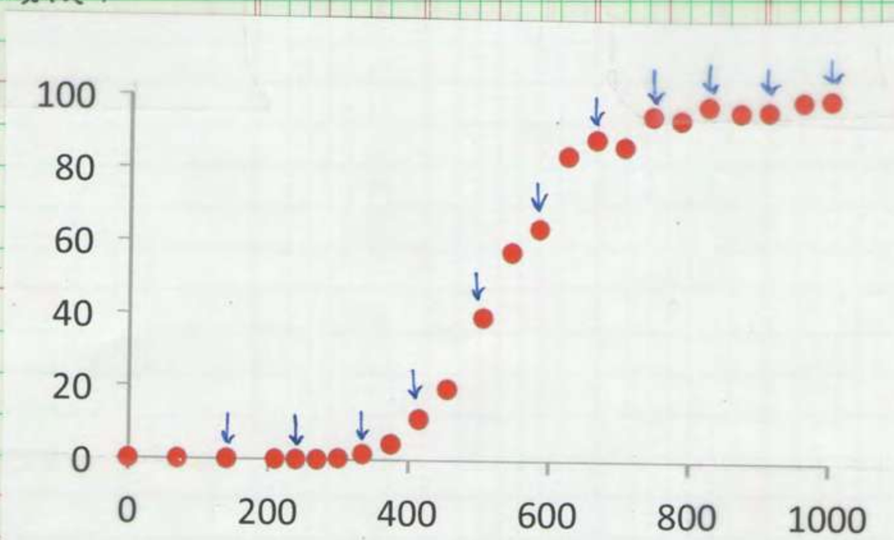
1/13/11

We have started a collaboration w/ George Weinstock's group at Wash U to sequence a number of yeast strains including population time-course sequencing from our long term evolution project.

As a pilot we will sequence 11 time points from the sweep population BYS1-A08. We will multiplex time points 140, 240, 335, 415, 505, 585, 665, 745, 825, 910, & 1000 (~ every 80 generations). In addition we will include a duplicate of generation 140 prepped by the glass bead lysis protocol rather than the QIAGEN column.



linear scale:



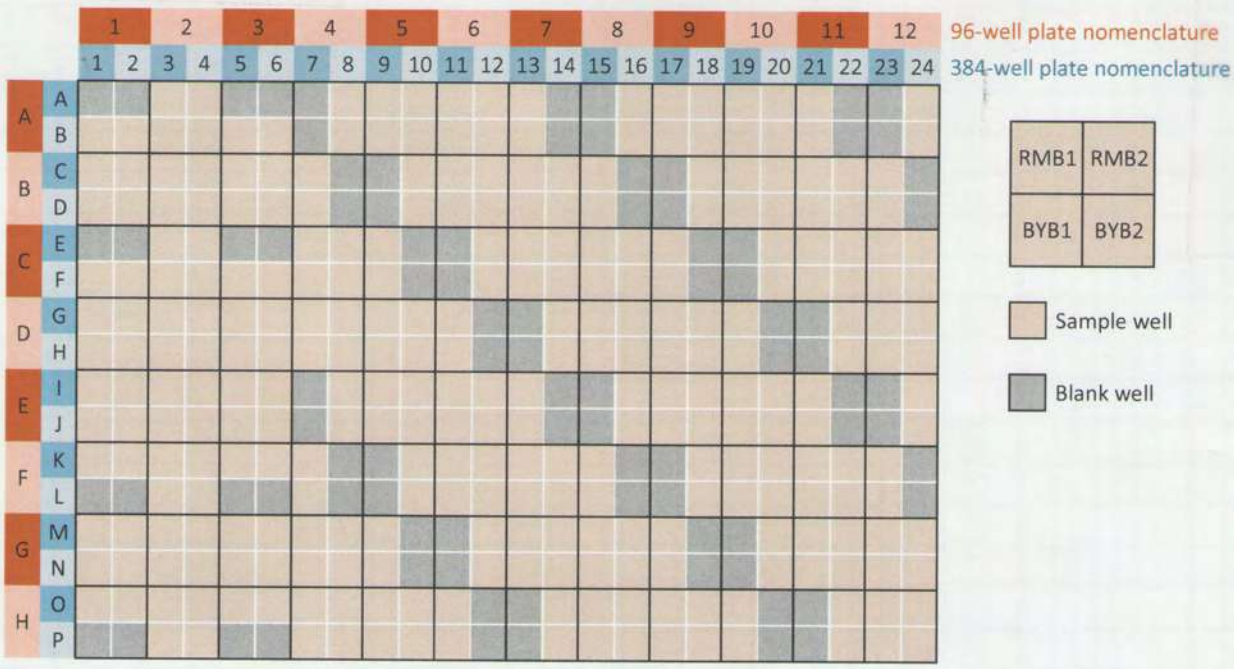
1/13/11

REVIVING POPULATIONS FROM FREEZER

Yesterday I took 8 1 ~~ul~~ of BYS1-A08 for each point and inoculated 10 ml of YPD + amp & tet

The layout of the 384 well plates from the long-term evolution is as follows:

Large population size cultures



Small population size cultures

	Initials	Date
Prepared By		
Approved By		

1/13/11

GENOMIC PREPS

For the sequencing, Wash U wants ~20 μg of sample. Given the yield that Pat got (and based upon my earlier gDNA preps for Tiling Arrays, see Evolution 1, p) I will need to do 20 mL cultures (at least)

- Take my 10 mL cultures and transfer to a large bore tube
  ↳ add 11 mL of YPD+A&T to ensure that I have 20 ml for tomorrow.

  ↳ These cells will have undergone a large amplification — from 8 λ (~ 10^6 cells) to 20 mL (~ 10^{10} cells), and for this initial pilot they will have been in YPD tubes for ~40h.

- I want to be sure that these samples we are submitting are what we think. I did two things with the cultures:

  (1) Add 4 λ of each culture to YPD + 2x αF (old stuff from fridge)

  (2) Streak each culture for singles → I will later pick singles & test for αF^R.

1/14/11

- Today I did the genomic preps

NOTES

- Started around 9:15, break for 1h Proteinase K incubation @ 11:45, finish 3:15
- Both Proteinase K & zymolase incubations for 1h
- Resuspend pellet in 200 λ H_2O

1/14/11

COULTER COUNTS

Bkg		360^{000}
Gen	140	1.34 E8
	240	1.16
	335	1.447
	415	1.557
	505	1.532
	585	1.7
	665	1.989
	745	2.209
	825	2.181
	910	2.041
	1000	2.162
Post Bkg		6.506 E6

10λ into 20mL

- During the 1 h. zymolase incubation I counted the original cultures
- I started w/ ~ 10^{10} cells

NANODROP

BYS1-A08 Generation	ng/mL
140	239.5
240	207.7
335	172.3
415	192.4
505	96.1, 123.1, 157.8
585	139.7
665	480.4, 515.0
745	233.5
825	147.8
910	160.6
1000	175.6

counted outliers again

lots of variation - perhaps the nanodrop is not working properly

I have between 20 & 50 μg total

	Initials	Date
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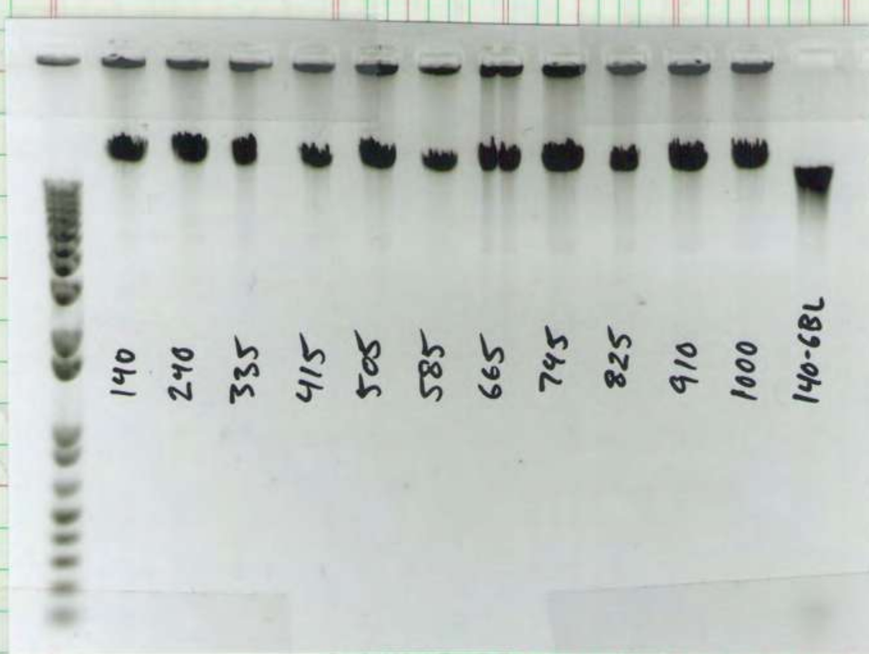
1/20/11

· On 1/17 we checked the concentration of the gDNA preps on the fluorimeter

(COPIED FROM PAT'S NOTES)

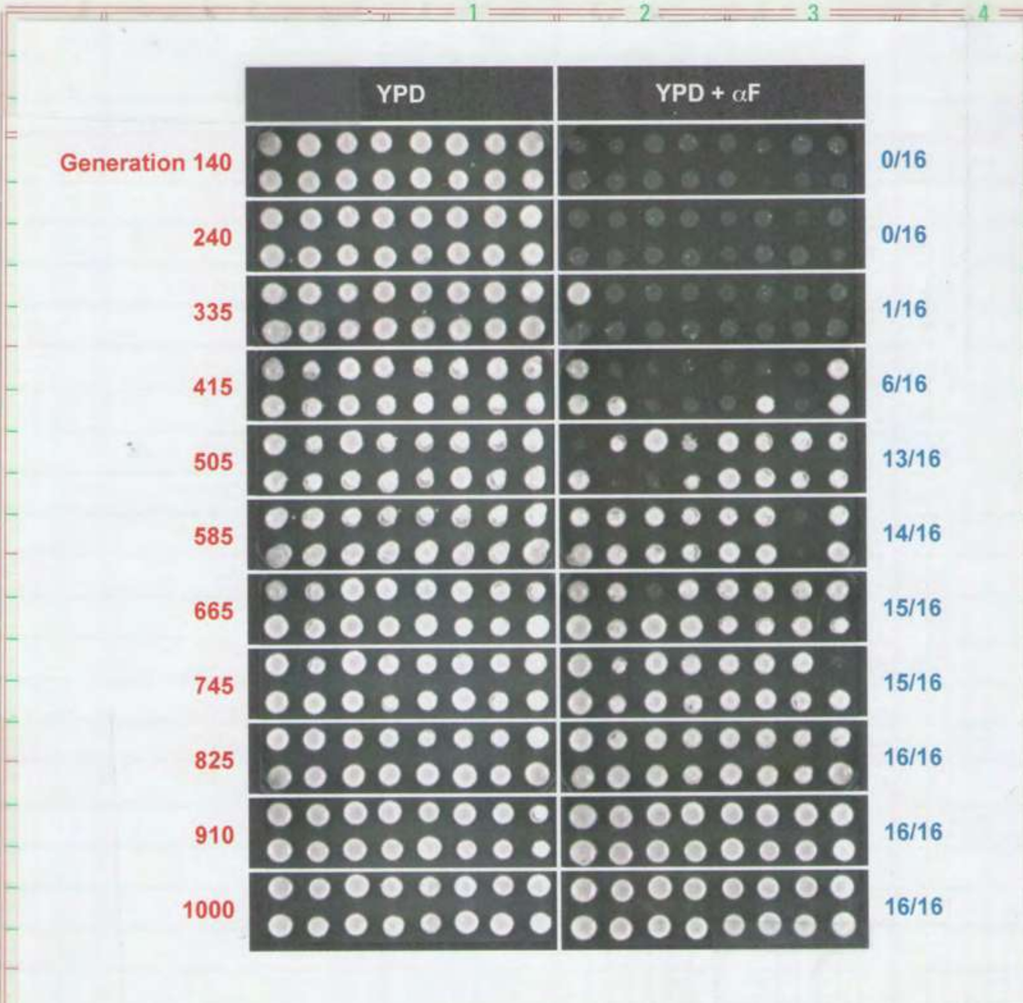
SAMPLE	ng/µL 1:50	True ng/µL
140	17.7	88.5
240	27.0	135
335	27.0	135
415	23.3	116.5
505	26.9	134.5
585	24.5	122.5
665	22.5	112.5
745	25.7	128.5
825	23.5	117.5
910	28.1	140.5
1000	34.8	174.0
140-GBL	42.4	212.0 ← Glass Bead Lysis preparation

RUN GEL (< 1%) 50 V, 1.5 h



1/20/11

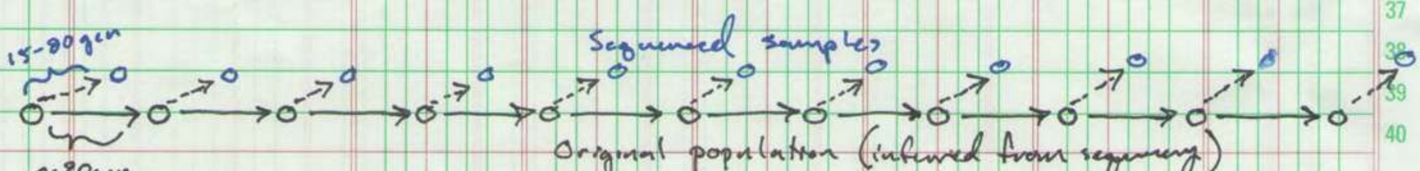
TESTING ~~384~~ SINGLE CLONES FOR αF^R



· For each of the 11 time points I struck for singles, picked 16 for each, and pinned to YPD and YPD + ~~[illegible]~~ αF (see p. 3)

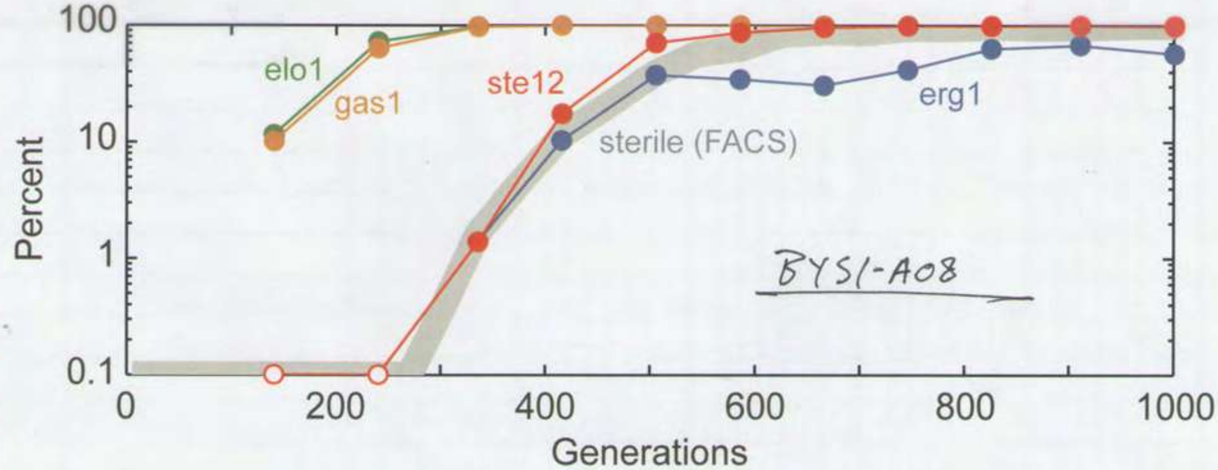
NOTES

- Notice how by pinning, the 50% sterile point occurs between generations 415 and 505, yet in the experiment the halfway point is around 550 (see p. 1)
  → accordingly our cultures are shifted about 80 gen — also look at the 10% mark (gen 335 above vs. 415 on p. 1)
- The amplification to 20 ml culture accounts for 10 gen. & the construction of the 384 well format, perhaps another 5 → where ~~[illegible]~~ does the other 55 come from?



5/20/11

RESULTS FROM WASH U. SEQUENCING



Gene	Mutation	Biological Process	Coverage
STE12	GGC CAG → -GC CAA	Mating	76 ± 9
ELO1	UAU, Tyr ⟶ CAU, His	Fatty acid elongation	113 ± 17
GAS1	CCA, Pro ⟶ CUA, Leu	Chromatin silencing	106 ± 11
ERG1	Promoter mutation	Ergosterol biosynthesis	31 ± 6

We have results back on population BYS1-A08

- The sterile mutation is in ste12 → Q: Do ste12 cells arrest as well as others or may we be underestimating these frequencies by FACS?

- We found 2 other fixed mutations (elo1 & gas1) which were ~~gash~~ in the same bkg. We will need to do reconstruction expts to determine which one (if either one) of these increases fitness and which is a hitchhiker.

→ the ste12 mutation occurred in this sweeping bkg - giving it the initial boost that allowed it to initially increase in fitness faster than expected given the effect of a sterile alone.

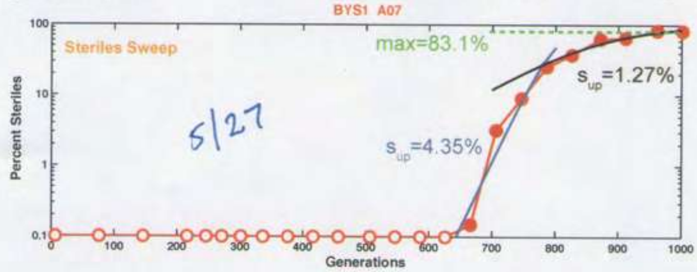
- I am not sure about the erg1 mutation - It was a BPS in a nearly-homopolymer run; and it was in a region of low coverage.

5/20/11

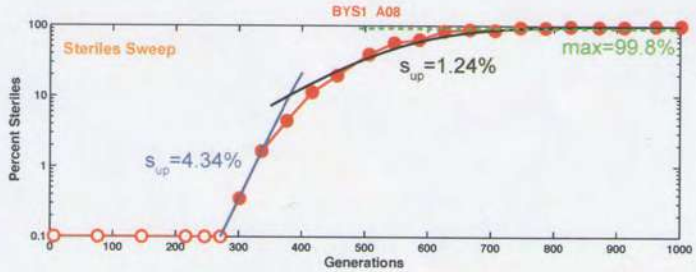
NOTES ON SEQUENCING ANALYSIS

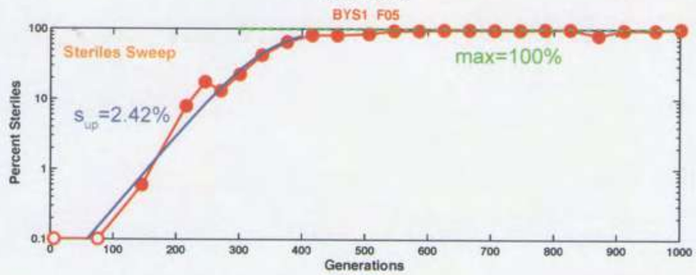
- Our experimental strain is in the W303 background, which differs from S288c by 8-9000 SNPs — we need to make a good W303 reference strain for alignment. We did this using only the 140 Gen. time point — but the right thing to do is to pool all of our data.
- For the ~~A~~ subsequent gDNA preps we will use Pat's modified glass-bead lysis protocol.
- We need to come up with a good way for determining how well we can detect low frequency polymorphisms — to do this I will mix various strains ~~at~~ → for instance 90% W303, 9% S288c, 0.9% RM11, 0.09% Sigma
- I should do this in triplicate (at least)
  → we can also pool the data and adjust the coverage by sampling ~~at~~ ~~by looking at how~~ and generate a relation for % known SNPs detected versus coverage.
- We need a way of detecting Indel mutations
- We need to figure out if we have any way of determining rearrangements

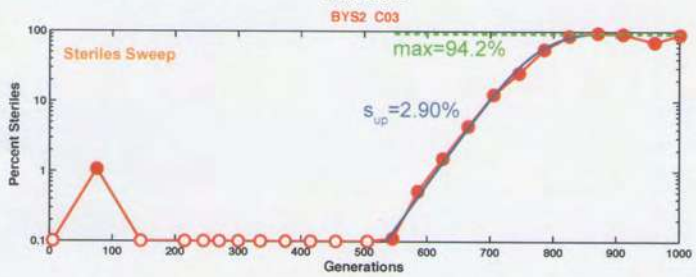
5/24/11 Cultures for sequencing

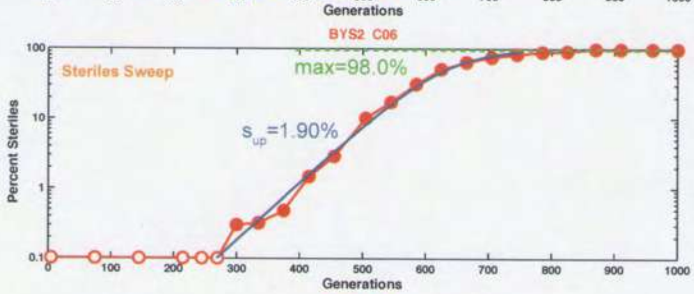


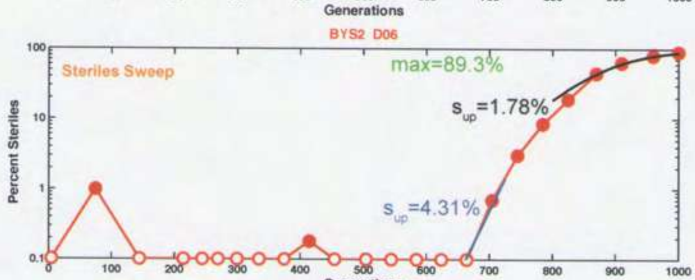
5/27

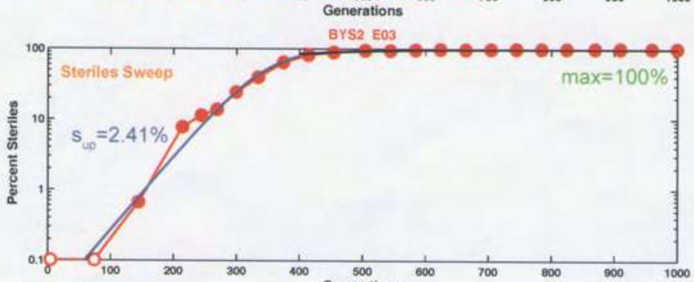


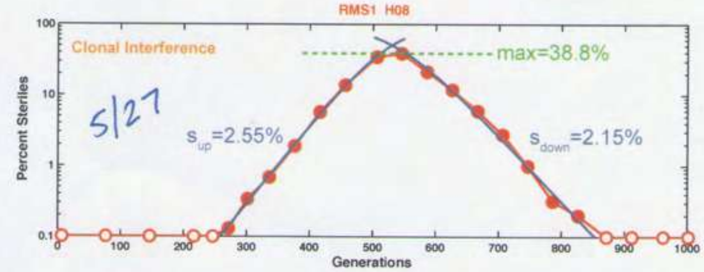












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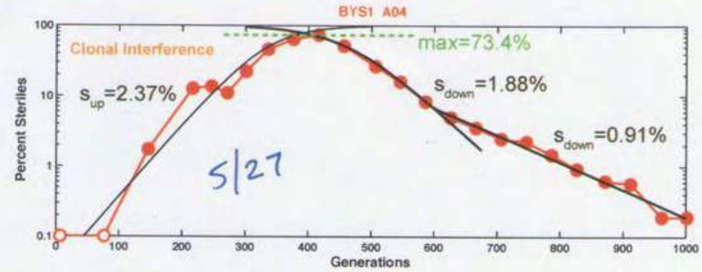
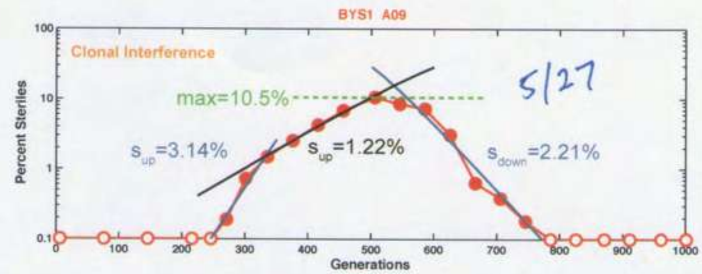
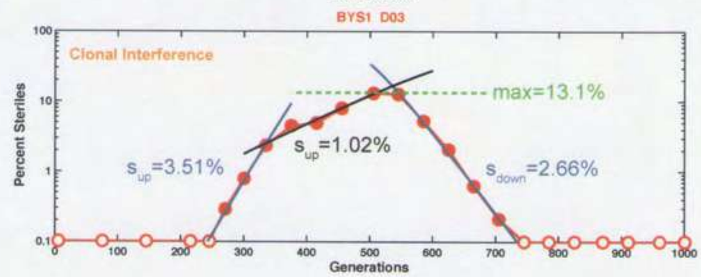
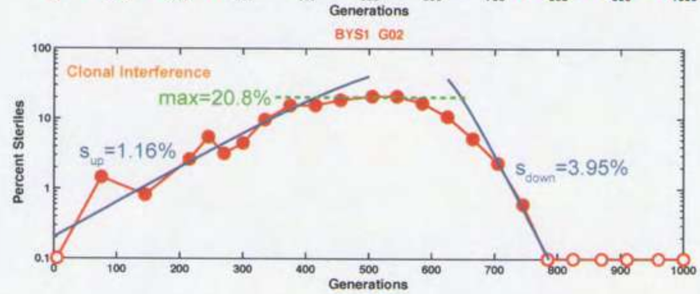


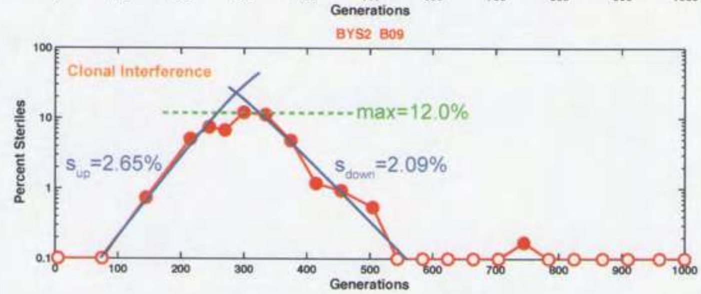
5/27

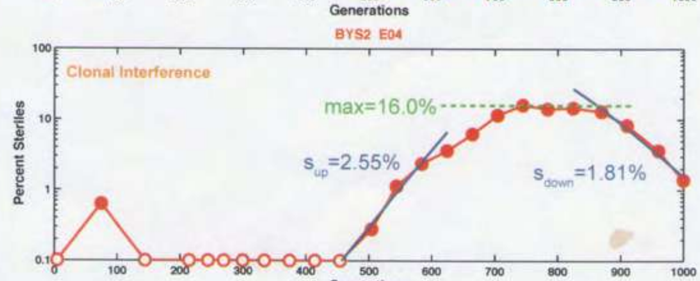


5/27

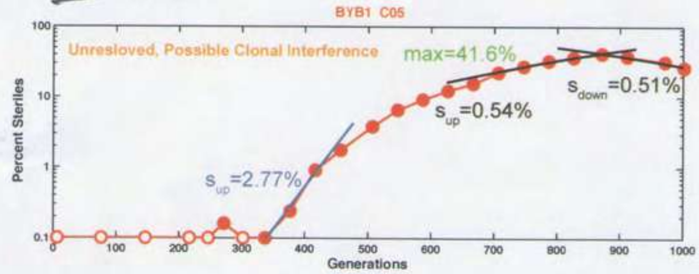


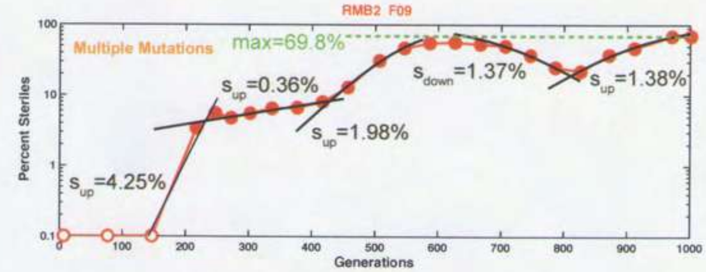


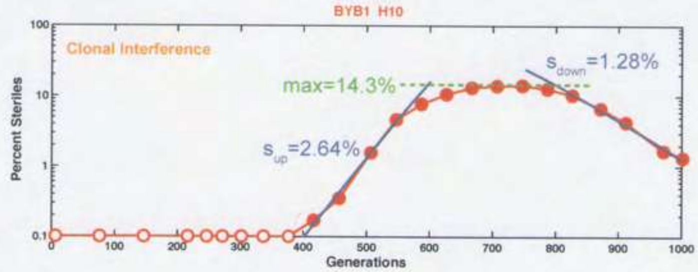


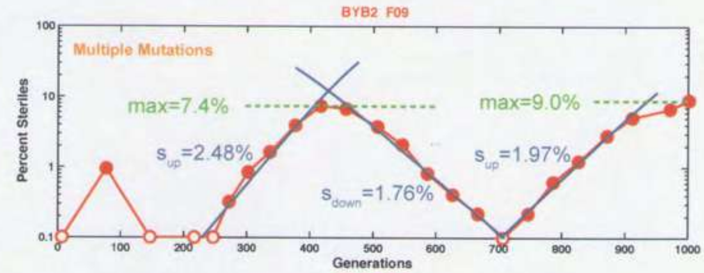


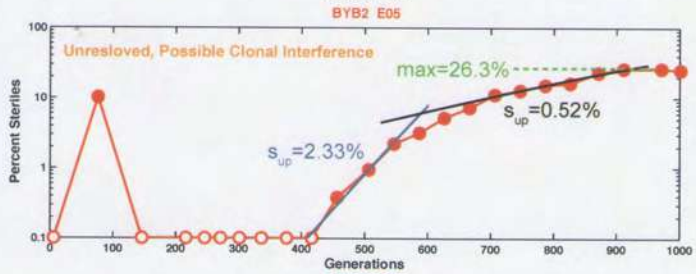
5/24/11

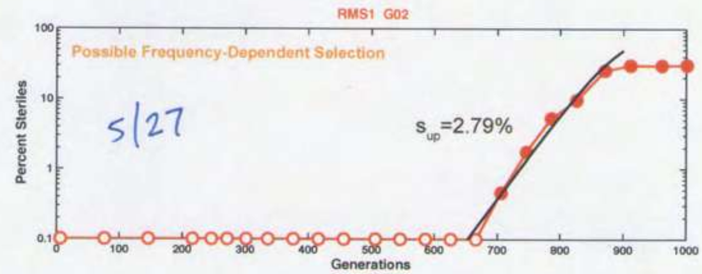

BYB1 C05
Unresloved, Possible Clonal Interference
max=41.6%
s_{down}=0.51%
s_{up}=0.54%
s_{up}=2.77%
Percent Steriles
Generations

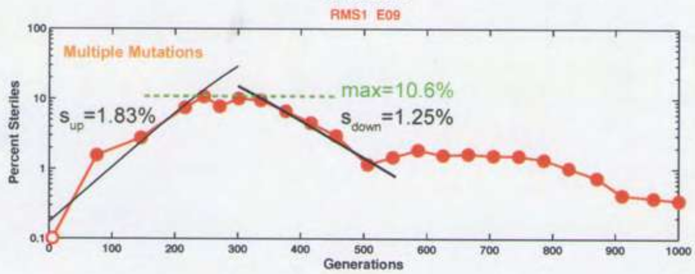

RMB2 F09
Multiple Mutations
max=69.8%
s_{up}=0.36%
s_{down}=1.37%
s_{up}=1.38%
s_{up}=1.98%
s_{up}=4.25%
Percent Steriles
Generations

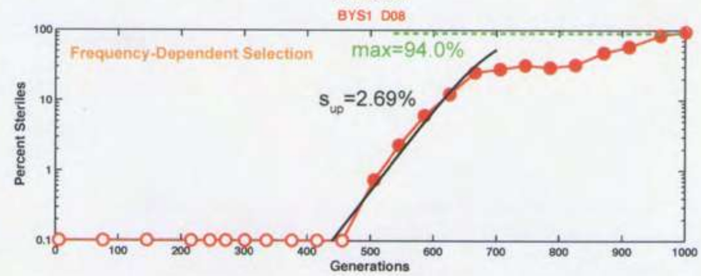

BYB1 H10
Clonal Interference
max=14.3%
s_{down}=1.28%
s_{up}=2.64%
Percent Steriles
Generations

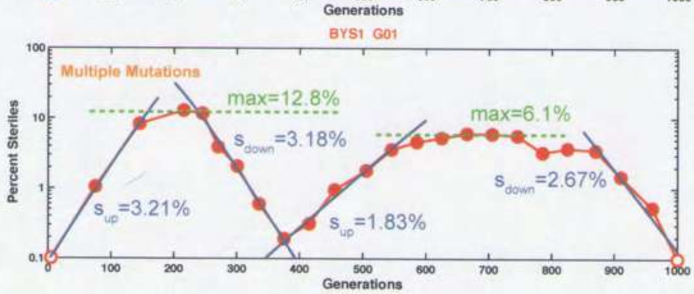

BYB2 F09
Multiple Mutations
max=7.4%
max=9.0%
s_{up}=2.48%
s_{up}=1.97%
s_{down}=1.76%
Percent Steriles
Generations

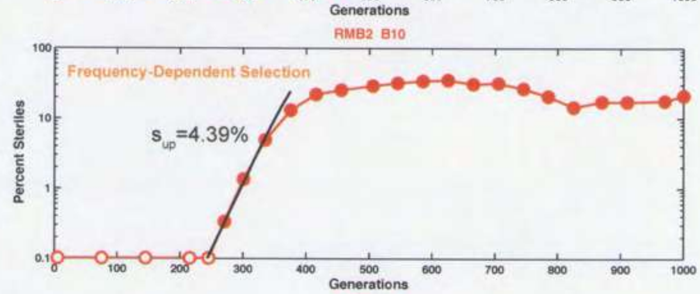

BYB2 E05
Unresloved, Possible Clonal Interference
max=26.3%
s_{up}=0.52%
s_{up}=2.33%
Percent Steriles
Generations

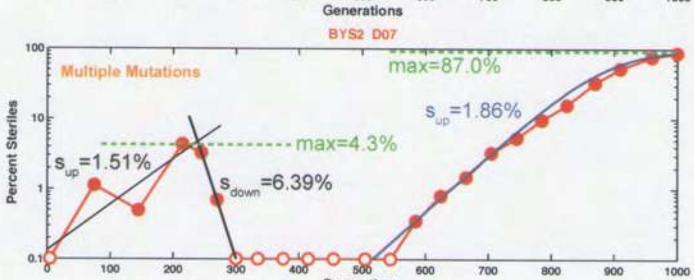

RMS1 G02
Possible Frequency-Dependent Selection
5|27
s_{up}=2.79%
Percent Steriles
Generations

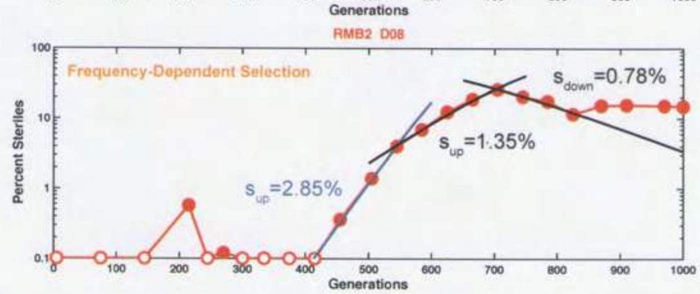

RMS1 E09
Multiple Mutations
max=10.6%
s_{up}=1.83%
s_{down}=1.25%
Percent Steriles
Generations

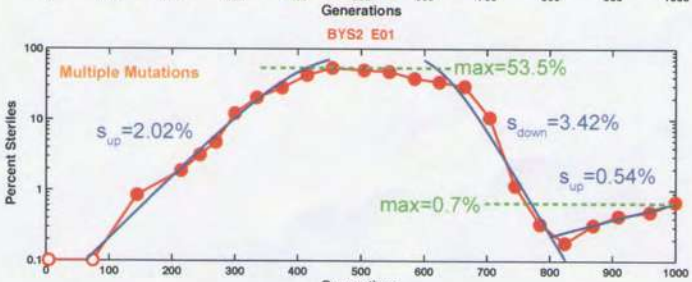

BYS1 D08
Frequency-Dependent Selection
max=94.0%
s_{up}=2.69%
Percent Steriles
Generations

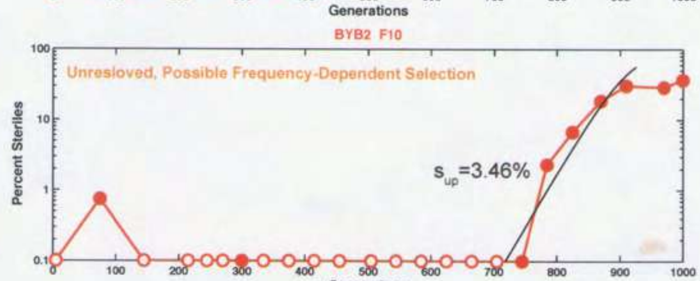

BYS1 G01
Multiple Mutations
max=12.8%
max=6.1%
s_{down}=3.18%
s_{down}=2.67%
s_{up}=3.21%
s_{up}=1.83%
Percent Steriles
Generations


RMB2 B10
Frequency-Dependent Selection
s_{up}=4.39%
Percent Steriles
Generations

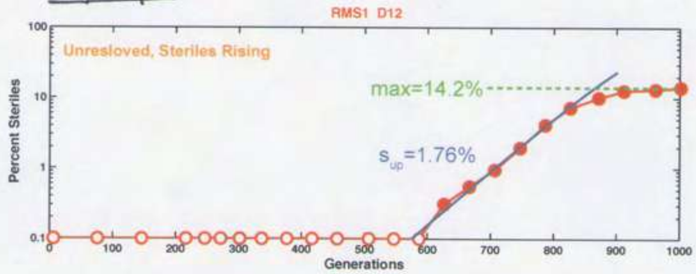

BYS2 D07
Multiple Mutations
max=87.0%
s_{up}=1.86%
max=4.3%
s_{up}=1.51%
s_{down}=6.39%
Percent Steriles
Generations

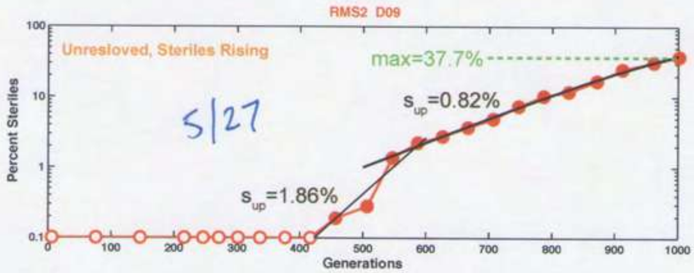

RMB2 D08
Frequency-Dependent Selection
s_{down}=0.78%
s_{up}=1.35%
s_{up}=2.85%
Percent Steriles
Generations

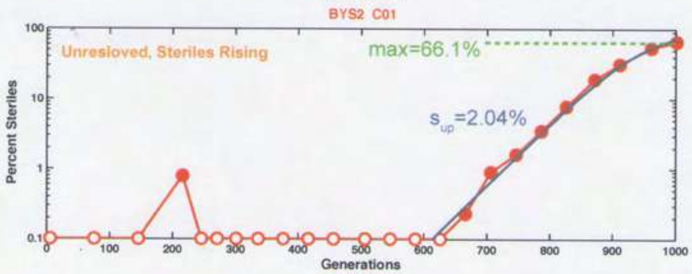

BYS2 E01
Multiple Mutations
max=53.5%
s_{up}=2.02%
s_{down}=3.42%
s_{up}=0.54%
max=0.7%
Percent Steriles
Generations

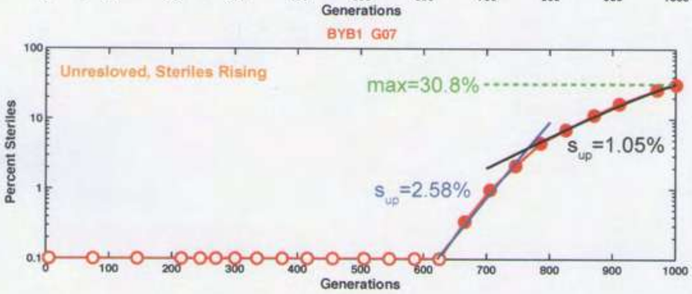

BYB2 F10
Unresloved, Possible Frequency-Dependent Selection
s_{up}=3.46%
Percent Steriles
Generations

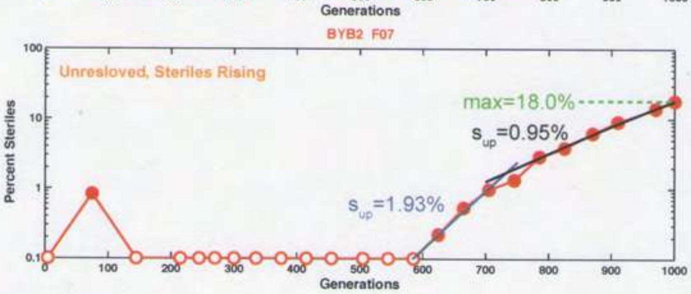
5/24/11

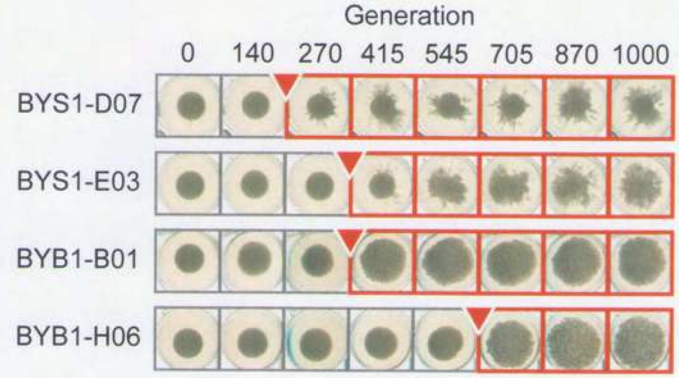






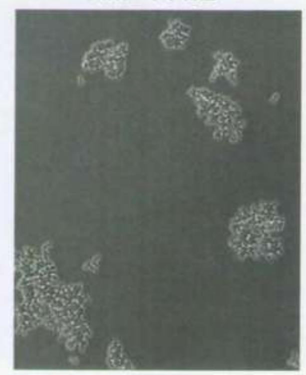


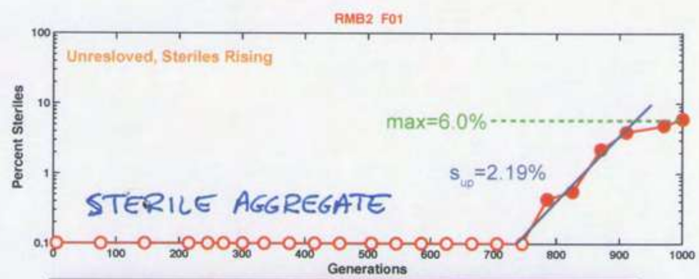


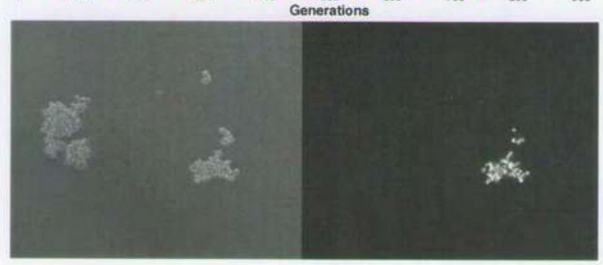


BYS2-A11

RMB1-A02



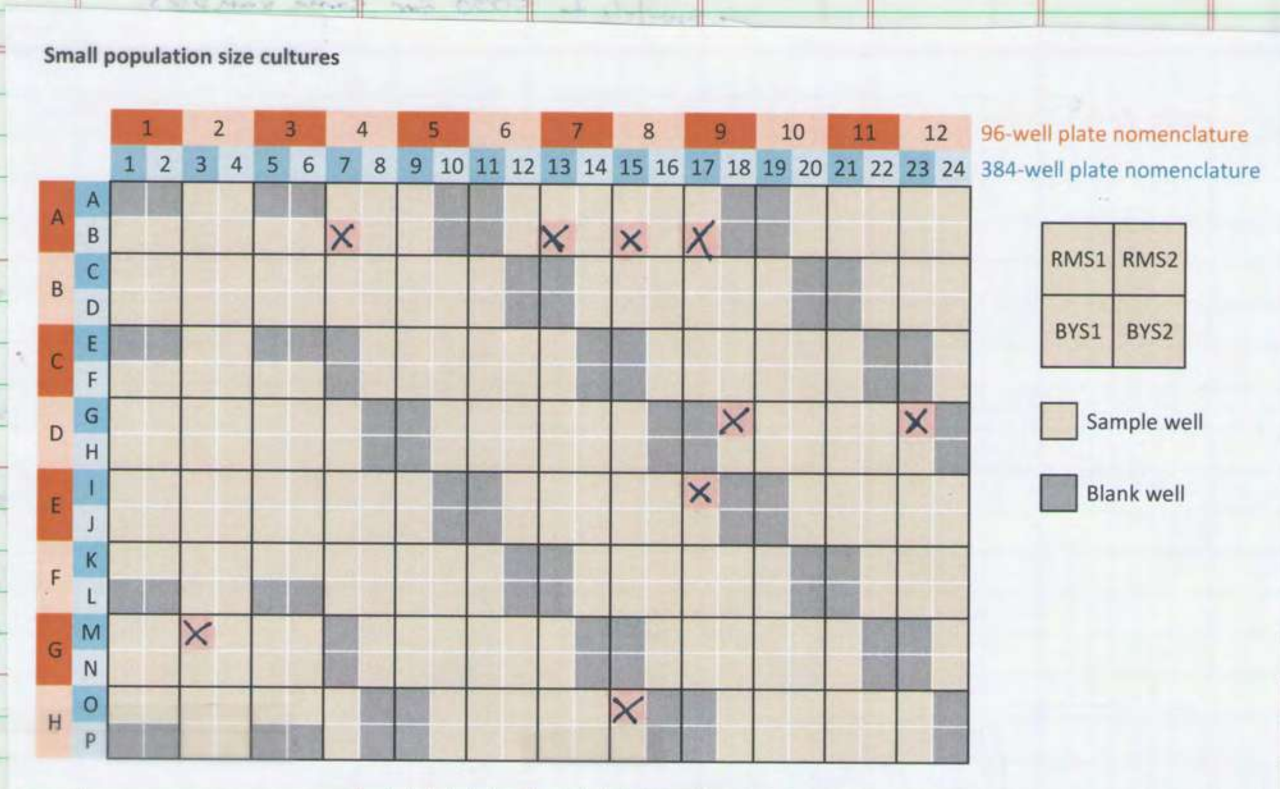




	Initials	Date
Prepared By		
Approved By		

5/25/11

START OF SEQUENCING PREPS



RMS1 D12	Steriles Rising
RMS1 E09	Multiple Mutations
RMS1 G02	FDS
RMS1 H08	Clonal Interference
RMS2 D09	Steriles Rising
BYS1 A04	Clonal Interference
BYS1 A07	Sweep
BYS1 A08	Sweep (1/13/11)
BYS1 A09	Clonal Interference
BYS1 D03	Clonal Interference
BYS1 D07	Abnormal Pellets
BYS1 D08	FDS
BYS1 E03	Abnormal Pellets
BYS1 F05	Sweep
BYS1 G01	Multiple Mutations
BYS1 G02	Clonal Interference
BYS2 A11	Aggregates
BYS2 B09	Clonal Interference
BYS2 C01	Steriles Rising
BYS2 C03	Sweep
BYS2 C06	Sweep
BYS2 D06	Sweep
BYS2 D07	Multiple Mutations/Sweep
BYS2 E01	Multiple Mutations
BYS2 E03	Sweep
BYS2 E04	Clonal Interference
RMB1 A02	Aggregates
RMB2 B10	FDS
RMB2 D08	FDS
RMB2 F01	Sterile Aggregates
RMB2 F09	Multiple Mutations
BYB1 B01	Abnormal Pellets
BYB1 C05	Clonal Interference
BYB1 G07	Steriles Rising
BYB1 H06	Abnormal Pellets
BYB1 H10	Clonal Interference
BYB2 E05	Clonal Interference
BYB2 F07	Steriles Rising
BYB2 F09	Multiple Mutations
BYB2 F10	FDS

} 5/25/11

- I have selected 40 populations for sequencing (left, & p. 9-13)
- On 5/23 I started 30ml YPD+A&T cultures for RMS1-D12 & RMS1-E09 for the timepoints listed on p. 1
  → w/ 8λ from plate
  → 30° rollerdrum @ 4:40pm
- The cultures took 2 days to get to saturation.

NOTE

Looking under the scope, RMS1-E09 is an aggregate.

5/25/11

gDNA Preps on RMS1-D12 & RMS1-E09

switch to 5000 for some samples

Yeast Genomic DNA Preparation: Glass Bead Lysis Method

1. Grow a culture overnight in ~~30 mL~~ of medium.
2. Spin cells at ~~3,000~~ RPM for 3 minutes. Wash pellet with ~~1 mL~~ 0.8 mL of ddH_2O and transfer to a sterile eppendorf tube. Pellet cells and aspirate all supernatant. 2 mL
3. Resuspend cells with 600 µL of DNA extraction buffer by vortexing.
4. Add 300 µL of glass beads (Sigma, 425-600 µm, acid-washed).
5. Add 300 µL of phenol:chloroform:isoamyl alcohol (25:24:1; Tris saturated) mix and vortex 4 minutes. Spin at max. speed for 5 minutes.
6. Remove supernatant to a new tube. (transferred 800-900 λ)
7. Add 5 µL of RNAse A (100 mg/mL) and incubate at 37°C for 30 minutes.
8. Phenol/Chloroform extract once more (with 300 µL of phenol:chloroform:isoamyl alcohol) and remove supernatant to a new tube (at this step, if you would like to use a phase-lock gel to ensure removal of organic solvent, it would be appropriate).
9. Add 1 mL of ice-cold 95-100% EtOH. Spin 5 minutes at full speed to pellet the DNA.
10. Wash pellet in 80% EtOH, dry, and finally resuspend in 100 µL of ddH_2O.
11. Check and record DNA concentration using Qubit or Nanodrop.

DNA Extraction Buffer

2%	TX-100
1%	SDS
100 mM	NaCl
10 mM	Tris (pH = 8.0)
1 mM	EDTA

NOTE: This DNA works just as well as DNA prepared using a Qiagen column for use in Illumina-based sequencing methods

NOTES

- We had lots of cells.
  - 30 mL is a little too much – switch to 25 mL
- We need 2 mL tubes for the 1st step and for EtOH ppt.
- To & I ran the preps & split the samples at the end. Some were pelleted immediately after EtOH ppt; others were o/n in freezer.

START NEXT SET OF CULTURES

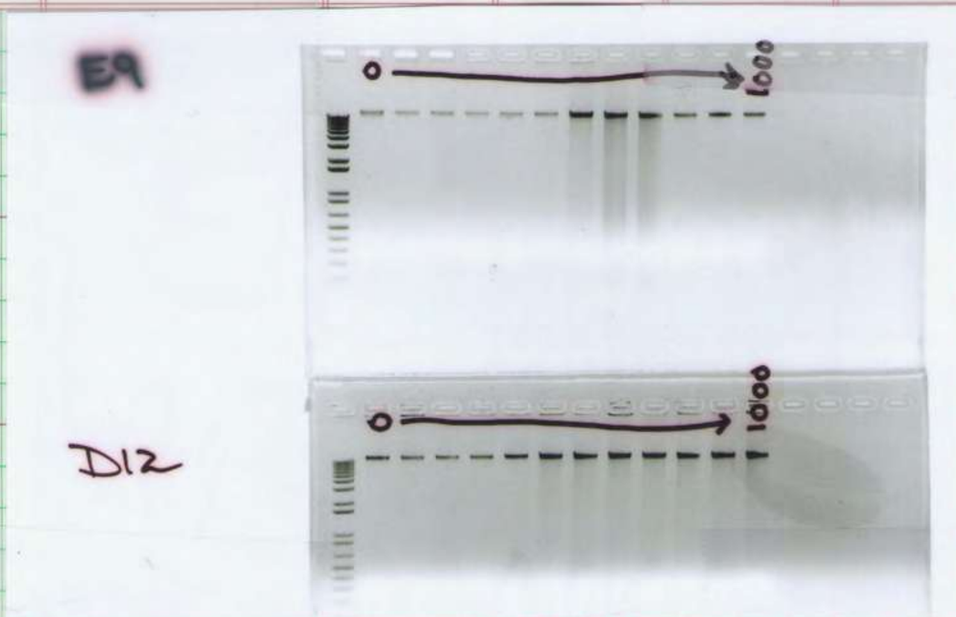
- I started cultures for RMS1-G2 → BYS1-A09 (p. 15)
  - on 30° rollerdrum ~6:30 pm
  - as before I transferred 8λ of culture; this time I put it in 25 mL YPD (+A+T)

	Initials	Date
Prepared By		
Approved By		

5/26/11

QUALITY CONTROL

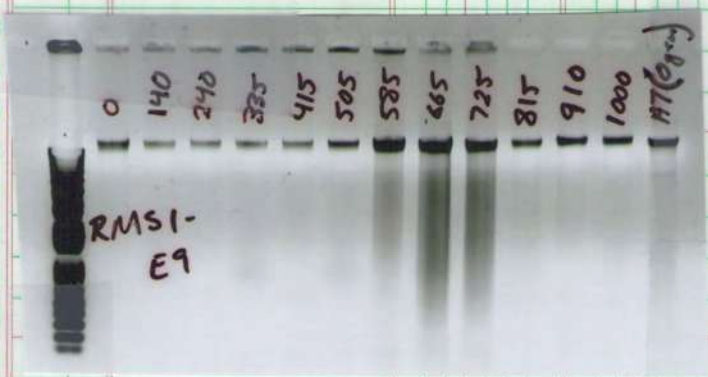
J. ran the 24 preps on a gel → look good

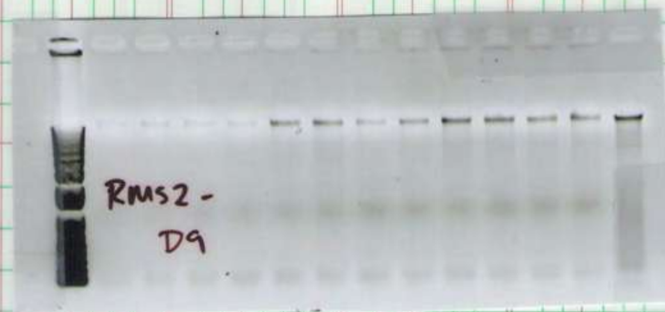


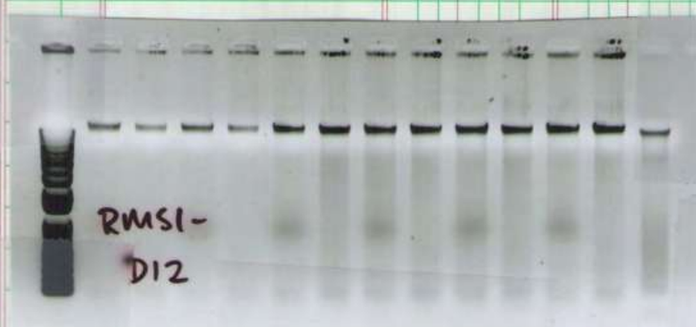
Individual Name	Concentration (Qbit)
LTE_Gen_140	88.5
LTE_Gen_240	135
LTE_Gen_335	135
LTE_Gen_415	116.5
LTE_Gen_505	134.5
LTE_Gen_585	122.5
LTE_Gen_665	112.5
LTE_Gen_725	128.5
LTE_Gen_815	117.5
LTE_Gen_910	140.5
LTE_Gen_1000	174
LTE_Gen_140gbl	212
RMS1-D12_0	68.4
RMS1-D12_140	40.8
RMS1-D12_240	60
RMS1-D12_335	43.2
RMS1-D12_415	69.8
RMS1-D12_505	80
RMS1-D12_585	82
RMS1-D12_665	73.6
RMS1-D12_745	110.6
RMS1-D12_825	59.8
RMS1-D12_910	102.4
RMS1-D12_1000	69
RMS1-E09_0	34.2
RMS1-E09_140	62.4
RMS1-E09_240	32.8
RMS1-E09_335	52.2
RMS1-E09_415	33.6
RMS1-E09_505	51.8
RMS1-E09_585	70.8
RMS1-E09_665	208
RMS1-E09_745	160
RMS1-E09_825	41
RMS1-E09_910	22.4
RMS1-E09_1000	32

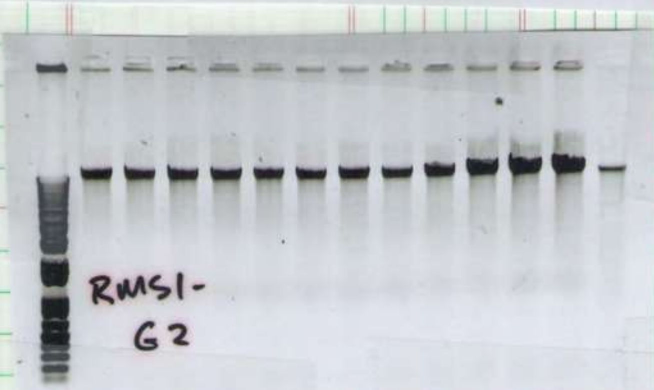
6/9/11

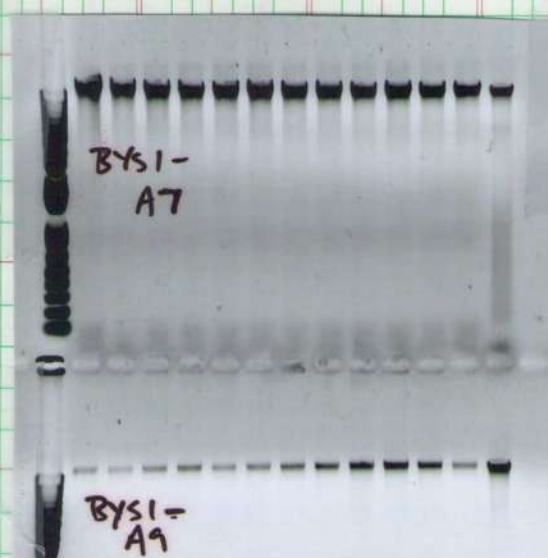
Over the last few weeks, Pat, Jo, & I have been doing gDNA preps with inconsistent yields

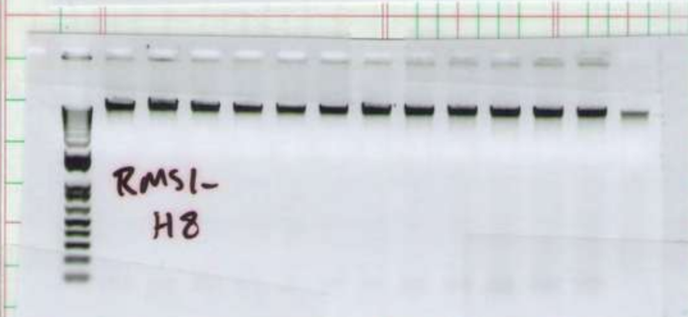








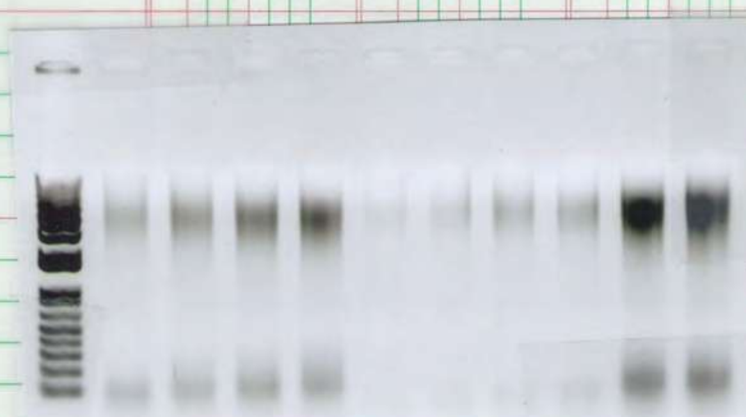




	Initials	Date
Prepared By		
Approved By		

	ng/ul (Q-bit)	ng/ul (Sample)	Total ug
RMS1-G02_0	0.0556	5.56	0.556
RMS1-G02_140	0.105	10.5	1.05
RMS1-G02_240	0.351	35.1	3.51
RMS1-G02_335	0.112	11.2	1.12
RMS1-G02_415	0.065	6.5	0.65
RMS1-G02_505	0.172	17.2	1.72
RMS1-G02_585	0.123	12.3	1.23
RMS1-G02_665	0.112	11.2	1.12
RMS1-G02_745	0.263	26.3	2.63
RMS1-G02_825	0.0801	8.01	0.801
RMS1-G02_910	0.866	86.6	8.66
RMS1-G02_1000	0.225	22.5	2.25
RMS1-H08_0	0.166	16.6	1.66
RMS1-H08_140	0.305	30.5	3.05
RMS1-H08_240	0.127	12.7	1.27
RMS1-H08_335	0.152	15.2	1.52
RMS1-H08_415	0.21	21	2.1
RMS1-H08_505	0.198	19.8	1.98
RMS1-H08_585	0.132	13.2	1.32
RMS1-H08_665	0.851	85.1	8.51
RMS1-H08_745	0.444	44.4	4.44
RMS1-H08_825	0.894	89.4	8.94
RMS1-H08_910	0.32	32	3.2
RMS1-H08_1000	0.517	51.7	5.17
RMS2-D09_0	0.0451	4.51	0.451
RMS2-D09_140	0.0748	7.48	0.748
RMS2-D09_240	0.162	16.2	1.62
RMS2-D09_335	0.167	16.7	1.67
RMS2-D09_415	0.186	18.6	1.86
RMS2-D09_505	0.335	33.5	3.35
RMS2-D09_585	0.116	11.6	1.16
RMS2-D09_665	0.112	11.2	1.12
RMS2-D09_745	0.122	12.2	1.22
RMS2-D09_825	0.163	16.3	1.63
RMS2-D09_910	0.195	19.5	1.95
RMS2-D09_1000	0.14	14	1.4
BYS1-A04_0	0.171	17.1	1.71
BYS1-A04_140	0.104	10.4	1.04
BYS1-A04_240	0.122	12.2	1.22
BYS1-A04_335	0.138	13.8	1.38
BYS1-A04_415	0.407	40.7	4.07
BYS1-A04_505	0.0407	4.07	0.407
BYS1-A04_585	0.109	10.9	1.09
BYS1-A04_665	0.136	13.6	1.36
BYS1-A04_745	0.22	22	2.2
BYS1-A04_825	0.12	12	1.2
BYS1-A04_910	0.133	13.3	1.33
BYS1-A04_1000	0.141	14.1	1.41
BYS1-A07_0	0.156	15.6	1.56
BYS1-A07_140	0.157	15.7	1.57
BYS1-A07_240	0.256	25.6	2.56
BYS1-A07_335	0.661	66.1	6.61
BYS1-A07_415	0.155	15.5	1.55
BYS1-A07_505	0.156	15.6	1.56
BYS1-A07_585	0.121	12.1	1.21
BYS1-A07_665	0.169	16.9	1.69
BYS1-A07_745	0.244	24.4	2.44
BYS1-A07_825	0.208	20.8	2.08
BYS1-A07_910	0.0949	9.49	0.949
BYS1-A07_1000	0.187	18.7	1.87
BYS1-A09_0	0.149	14.9	1.49
BYS1-A09_140	0.398	39.8	3.98
BYS1-A09_240	0.171	17.1	1.71
BYS1-A09_335	0.0724	7.24	0.724
BYS1-A09_415	0.0598	5.98	0.598
BYS1-A09_505	0.091	9.1	0.91
BYS1-A09_585	0.0776	7.76	0.776
BYS1-A09_665	0.247	24.7	2.47
BYS1-A09_745	0.163	16.3	1.63
BYS1-A09_825	0.107	10.7	1.07
BYS1-A09_910	0.125	12.5	1.25
BYS1-A09_1000	0.213	21.3	2.13

These #s I suspect are off by a factor of 10 - I rechecked a few to verify this.



1) 600μ glass beads, 30s bead beat
2) 60
3) 90
4) 120
5) 200μ glass beads, 30s bead beat
6) 60
7) 90
8) 120
9) 600μ glass beads 150
10) " 150

6/30/11

We have decided to use the 2.5 min bead beating protocol for gDNA preps. We have sent a sample set to WashU.

Saccharomyces cerevisiae

Yeast Genomic DNA Preparation: Glass Bead Lysis Method

For genomic DNA preparations to be used as part of the sequencing collaboration with Washington University in St. Louis

DAY BEFORE

1. Prepare 2-mL screw-cap tubes to be used for preparations (one tube for each preparation):
    a. 600 μL of acid washed glass beads (425-600 μm, acid-washed; Sigma)
    b. 400 μL of phenol:chloroform:isoamyl alcohol (25:24:1, Tris saturated)
2. Place 2-mL tubes at 4°C until use.
3. Prepare (unless already done) a cell pellet from a 20 mL overnight culture. Store at -20°C in a microcentrifuge tube.

DAY OF

1. Resuspend frozen cell pellet with 400 μL of DNA Extraction Buffer using a combination of pipetting and/or vortexing.
2. Pipette cell suspension into 2-mL tube containing glass beads and phenol:chloroform:isoamyl alcohol.
3. Insert samples into Bead Beater (in cold room) and bead beat for 2.5 minutes (the bead beater holds a maximum of 16 tubes).
4. Spin at maximum speed in a microcentrifuge for 5 minutes.
    While this sample is spinning prepare a set of microcentrifuge tubes each containing 5 L of RNAse A (100 mg/mL).
5. Remove supernatant to tube containing RNAse A (there will be approximately 500-600 μL of supernatant).
6. Incubate at 37°C for 60 minutes (could do this incubation for longer, if it is convenient).
    At this point, another round of 16 samples could be started and brought up to the RNAse A incubation... allows for parallel preparation of samples).
7. Add 300 μL of phenol:chloroform:isoamyl alcohol to the sample, vortex for 15 seconds, and spin at full speed for 5 minutes. Then remove ~350 μL supernatant to a new tube (at this step, if you would like to use a phase-lock gel to ensure removal of organic solvent, it would be appropriate).
8. Add 1 mL of ice-cold 95-100% ethanol. Chill samples for at least 1 hour (can go overnight) at -20°C.
9. Spin 5 minutes at full speed to pellet the DNA. Aspirate or decant the supernatant.
10. Wash pellet with 1 mL of ice-cold 70% ethanol (vortex briefly, spin at full speed 15 seconds, decant ethanol, spin at full speed 15 seconds, then aspirate remaining ethanol) and resuspend in 200 μL of ddH_2O.
11. Check and record DNA concentration using Qubit fluorometer.

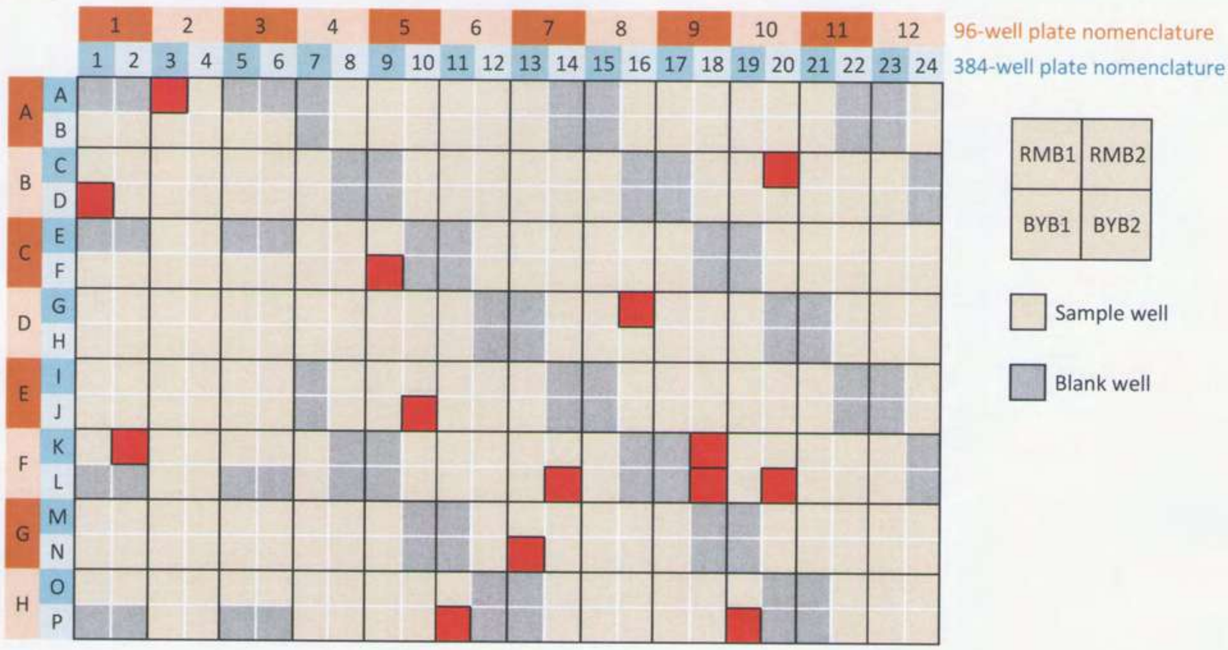
DNA Extraction Buffer		for 100 mL
2%	TX-100	2 mL of 100% Triton X-100
1%	SDS	10 mL of 10% SDS
100 mM	NaCl	2 mL of 5 M NaCl
10 mM	Tris (pH = 8.0)	1 mL of 1 M Tris (pH = 8.0)
1 mM	EDTA	200 μL of 500 mM EDTA

NOTE: Whenever performing a phenol/chloroform extraction, it is always better to leave some sample behind than to bring some of the interphase (cell debris) into subsequent steps.

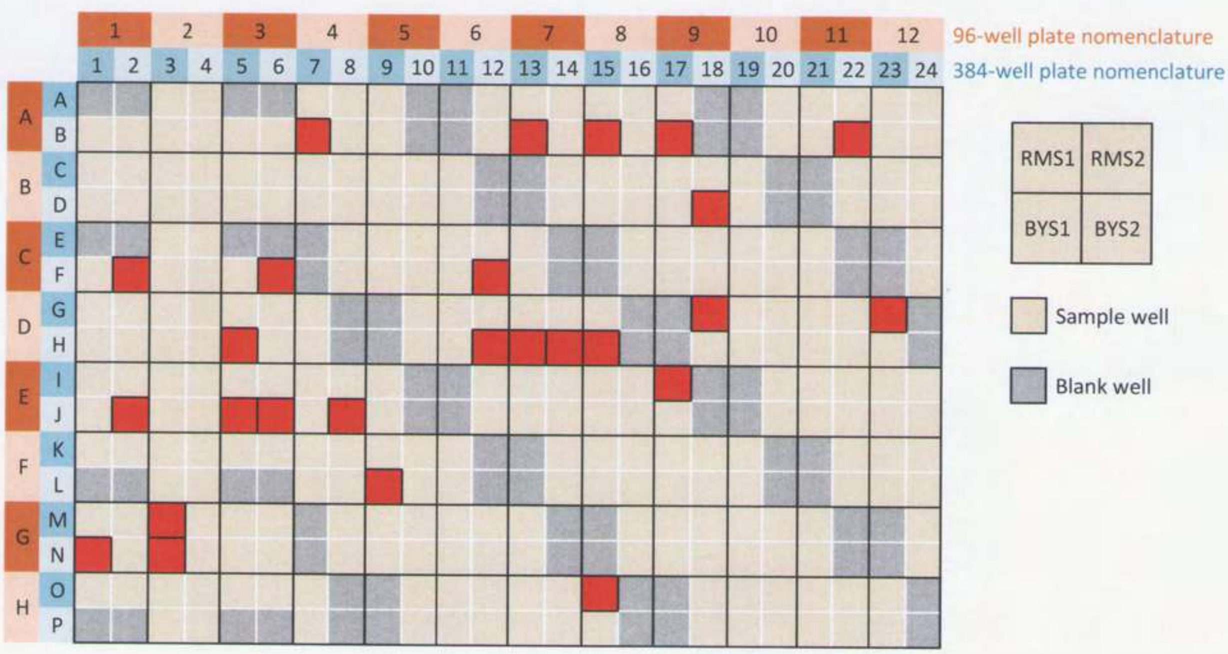
NOTE: This DNA works just as well as DNA prepared using a Qiagen column for use in Illumina-based sequencing methods

6/30/11 ~~[illegible]~~ Populations to sequence

Large population size cultures



Small population size cultures



While we await word from Wash U regarding the glass bead lysis method, Jo & I have been making cell pellets for all of the LTE populations we are planning to sequence.

6/30/11

	Initials	Date
Prepared By		
Approved By		

Sequencing strain mixtures

- On Monday (6/27) I streaked the following strains:

W303 - yGIL121
S288C - DBY12000
RM11 - DBY13000
Sigma - DBY8931

- Yesterday I picked a single colony of S288C, RM11, & Sigma and inoculated 10 ml YPD. I inoculated 100 mL YPD (in flask) w/ a single colony of W303.

Coulter Counts

Bkg		118000	106000	124000
W303	1	$1.052E^8$	$1.06E^8$	$1.066E^8$
	2	$1.073E^8$	$1.074E^8$	$1.07E^8$
	3	$2.665E^8$	$2.65E^8$	$2.649E^8$
S288C	1	$1.344E^8$	$1.358E^8$	$1.339E^8$
	2	$1.417E^8$	$1.398E^8$	$1.405E^8$
	3	$1.337E^8$	$1.333E^8$	$1.313E^8$
RM11	1	$2.838E^7$	$2.831E^8$	$2.912E^8$
	2			
	3			
Sigma	1			
	2			
	3			
Post Bkg				

X } 3 ~~diff~~ independent dilutions

10 λ into 20 mL

I made 3 mixes using only W303 & S288C

Percent	Strain	cells/ml	ml	ul	cells
90.0%	W303	2.146E+08	25	25000	5.365E+09
10.0%	S288c	2.668E+08	2.0	2010.9	5.365E+08
99.0%	W303	2.146E+08	25	25000	5.365E+09
1.0%	S288c	2.668E+08	0.2	201.1	5.365E+07
99.9%	W303	2.146E+08	25	25000	5.365E+09
0.1%	S288c	2.668E+08	0.0	20.1	5.365E+06

10/10/11

	Initials	Date
Prepared By		
Approved By		

UPDATE ON SEQUENCING PROJECT

Before Jo left she did gDNA preps on ~200 of the cell pellets. (1-013 → 1-210)

Some of the pellets were missing from the set (these were the BYS2 gen 665 cultures; Michael dropped this plate a while back). For some of the others something messed up.

I remade the cell pellets for the ones listed below (for the BYS2 gen. 665 cultures, I used gen 705 instead)

S-705	1-059	LTE_BYS2_C03-665		Missing from set
S-705	1-071	LTE_BYS2_C06-665		Missing from set
S-705	1-083	LTE_BYS2_D06-665		Missing from set
S-705	1-095	LTE_BYS2_E03-665	705	Missing from set
S-705	1-167	LTE_BYS2_B09-665		Missing from set
S-705	1-179	LTE_BYS2_E04-665		Missing from set
S-585	1-022	LTE_BYS1_A07-585		Need to be re-grown
S-665	1-023	LTE_BYS1_A07-665		Need to be re-grown
S-140	1-089	LTE_BYS2_E03-140		Need to be re-grown
S-240	1-090	LTE_BYS2_E03-240		Need to be re-grown
S-415	1-032	LTE_BYS1_A08-415		Need to be re-grown, something was wrong with the pellet. After bead beating and spin down there was no top phase.
S-825	1-037	LTE_BYS1_A08-825		Need to be re-grown, something was wrong with the pellet. After bead beating and spin down there was no top phase.
S-1000	1-039	LTE_BYS1_A08-1000		Need to be re-grown, something was wrong with the pellet. After bead beating and spin down there was no top phase.
S-140	1-041	LTE_BYS1_F05-140		Need to be re-grown, something was wrong with the pellet. After bead beating and spin down there was no top phase.

My Mistakes

While I remade the pellets for the BYS2 Gen. 665 for the six above, I forgot to do the other BYS2 gen 665 (the ones that Jo didn't get to).

I have started making a list of the other missing or screwed up ones (I had some issues with the bead beater).

11/17/11

Update on sequencing project

Pat & I finished all of the gDNA preps. Once done, we quantified them in the fluorometer. ~~From that we decided to reprep all of those that were too low (< 5 ng/µL) and w~~

~~After~~ We decided to reprep the following:

(1) Everything that ~~w~~ had a low yield (< 5ng/µL)
(2) Everything that we screwed up
(3) The four populations w/ abnormal pellets

→ Given what we now know about the importance of spatial structure in these populations we decided to grow them in 96-well plates

- ~~inoculate 15 mL YPD+A&T w/ 8 λ cult~~
- inoculate 1 well (130 λ YPD+A&T) with 8 λ of culture (essentially ~~a big~~ double a big dilution from the original expt).
- Grow o/n then take the 130 λ & inoculate 15 mL YPD+A&T
- Distribute over a 96-well plate
- At this point I got the flu and was home for a week, so these plates grew for longer than I would have liked.
- Pool & gDNA prep as usual.

All samples were sent to Wash U on 11/10

11/17/11

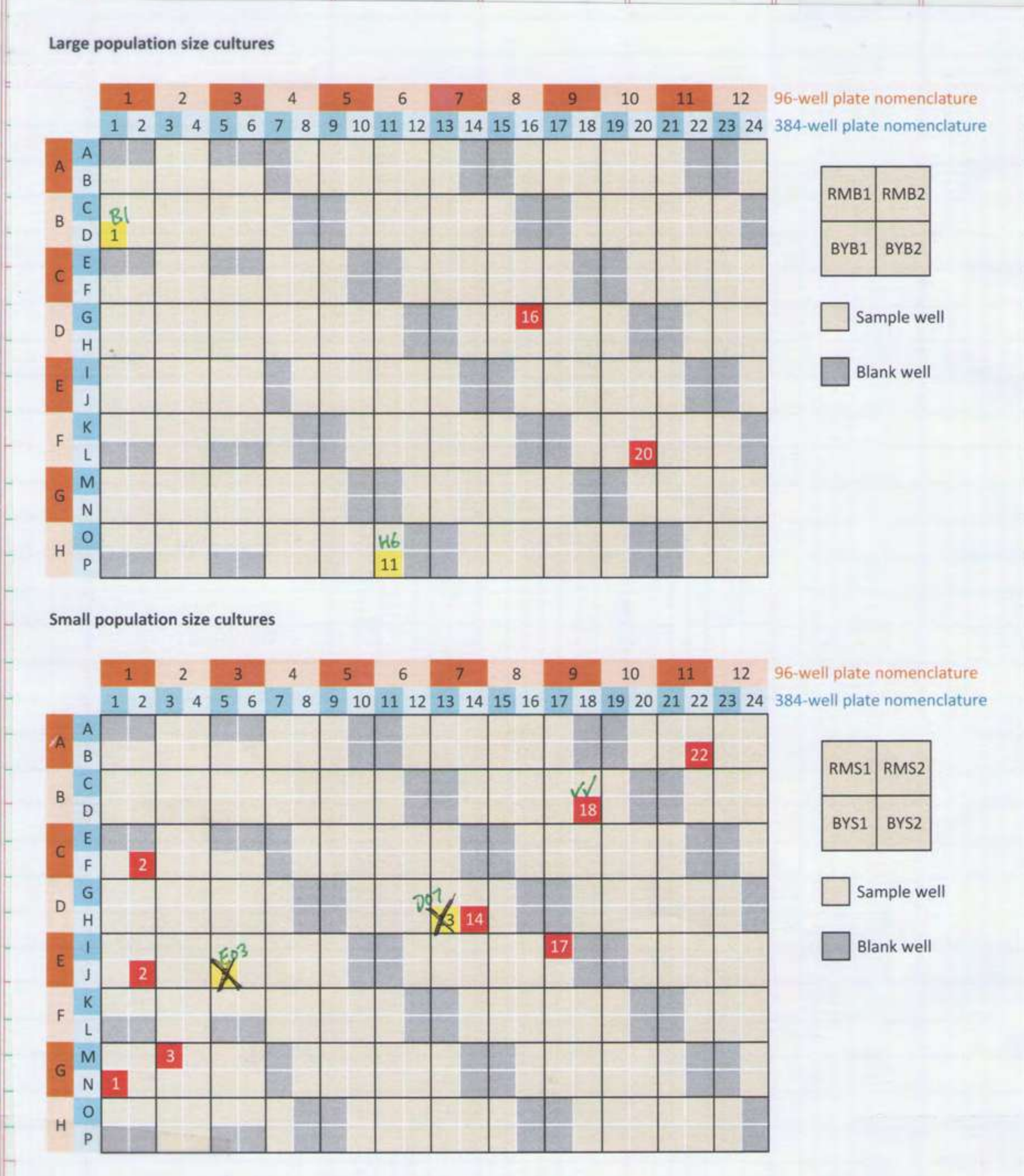
	Initials	Date
Prepared By		
Approved By		

227 did not grow up - omit

	Individual Name	Sample Sul	Processed	Notes	
	LTE_BYS1_A07-585	1-022	x	Sweep	
	LTE_BYS1_A07-665	1-023	x	Sweep	
	LTE_BYS1_A08-415	1-032	x	Sweep	
	LTE_BYS1_A08-825	1-037	x	Sweep	
	LTE_BYS1_A08-1000	1-039	x	Sweep	
	LTE_BYS1_F05-140	1-041	x	Sweep	
	LTE_BYS2_C03-665	1-059	x	Sweep	
	LTE_BYS2_C06-665	1-071	x	Sweep	
	LTE_BYS2_D06-665	1-083	x	Sweep	
	LTE_BYS2_E03-140	1-089	x	Sweep	
	LTE_BYS2_E03-240	1-090	x	Sweep	
	LTE_BYS2_E03-665	1-095	x	Sweep	
	LTE_BYS2_B09-665	1-167	x	Clonal Interference	
	LTE_BYS2_B09-745	1-168		Clonal Interference	1
	LTE_BYS2_B09-910	1-170		Clonal Interference	2
	LTE_BYS2_B09-1000	1-171		Clonal Interference	3
	LTE_RMS1_E09-665	1-227		Multiple Mutations	4
	LTE_RMS1_E09-825	1-229		Multiple Mutations	5
	LTE_BYS1_G01-910	1-242		Multiple Mutations	6
	LTE_BYS2_D07-140	1-245		Multiple Mutations/Sweep	7
	LTE_BYS2_D07-240	1-246		Multiple Mutations/Sweep	8
	LTE_BYS2_D07-335	1-247		Multiple Mutations/Sweep	9
	LTE_BYS2_D07-415	1-248		Multiple Mutations/Sweep	10
	LTE_BYS2_D07-505	1-249		Multiple Mutations/Sweep	11
	LTE_BYS2_D07-585	1-250		Multiple Mutations/Sweep	12
705	LTE_BYS2_D07-665	1-251		Multiple Mutations/Sweep	13
	LTE_BYS2_D07-745	1-252		Multiple Mutations/Sweep	14
	LTE_BYS2_D07-825	1-253		Multiple Mutations/Sweep	15
705	LTE_BYS2_E01-665	1-263		Multiple Mutations	16
	LTE_RMS1_G02-665	1-299		FDS	17
	LTE_RMS1_G02-745	1-300		FDS	18
B.y	LTE_RMB2_D08-585	1-334		FDS	19
B.y	LTE_RMB2_D08-665	1-335		FDS	20
B.y	LTE_RMB2_D08-745	1-336		FDS	21
B.y	LTE_RMB2_D08-825	1-337		FDS	22
B.y	LTE_RMB2_D08-910	1-338		FDS	23
B.y	LTE_RMB2_D08-1000	1-339		FDS	24
B.y	LTE_BYB2_F10-0	1-340		FDS	25
705	LTE_BYS2_C01-665	1-383		Steriles Rising	26
705	LTE_BYS2_A11-665	1-467		Aggregates	27
	NI_18	1-506		From Schulte grove soil	28
	NI_23	1-512		From Van Horne wild plum leaf	29
	GSY509	2-001		Described in 2009 Genome Research I	30
	GSY501	2-003		Described in 2009 Genome Research Du	31
	GSY508	2-017		Described in 2009 Genome Research Du	32

LTE_BYS1_D07-0	~~1-412~~ 517	Abnormal Pellets
LTE_BYS1_D07-140	1-413	Abnormal Pellets
LTE_BYS1_D07-240	1-414	Abnormal Pellets
LTE_BYS1_D07-335	1-415	Abnormal Pellets
LTE_BYS1_D07-415	1-416	Abnormal Pellets
LTE_BYS1_D07-505	1-417	Abnormal Pellets
LTE_BYS1_D07-585	1-418	Abnormal Pellets
LTE_BYS1_D07-665	1-419	Abnormal Pellets
LTE_BYS1_D07-745	1-420	Abnormal Pellets
LTE_BYS1_D07-825	1-421	Abnormal Pellets
LTE_BYS1_D07-910	1-422	Abnormal Pellets
LTE_BYS1_D07-1000	1-423	Abnormal Pellets
LTE_BYS1_E03-0	1-424	Abnormal Pellets
LTE_BYS1_E03-140	1-425	Abnormal Pellets
LTE_BYS1_E03-240	1-426	Abnormal Pellets
LTE_BYS1_E03-335	1-427	Abnormal Pellets
LTE_BYS1_E03-415	1-428	Abnormal Pellets
LTE_BYS1_E03-505	1-429	Abnormal Pellets
LTE_BYS1_E03-585	1-430	Abnormal Pellets
LTE_BYS1_E03-665	1-431	Abnormal Pellets
LTE_BYS1_E03-745	1-432	Abnormal Pellets
LTE_BYS1_E03-825	1-433	Abnormal Pellets
LTE_BYS1_E03-910	1-434	Abnormal Pellets
LTE_BYS1_E03-1000	1-435	Abnormal Pellets
LTE_BYB1_B01-0	1-436	Abnormal Pellets
LTE_BYB1_B01-140	1-437	Abnormal Pellets
LTE_BYB1_B01-240	1-438	Abnormal Pellets
LTE_BYB1_B01-335	1-439	Abnormal Pellets
LTE_BYB1_B01-415	1-440	Abnormal Pellets
LTE_BYB1_B01-505	1-441	Abnormal Pellets
LTE_BYB1_B01-585	1-442	Abnormal Pellets
LTE_BYB1_B01-665	1-443	Abnormal Pellets
LTE_BYB1_B01-745	1-444	Abnormal Pellets
LTE_BYB1_B01-825	1-445	Abnormal Pellets
LTE_BYB1_B01-910	1-446	Abnormal Pellets
LTE_BYB1_B01-1000	1-447	Abnormal Pellets
LTE_BYB1_H06-0	1-448	Abnormal Pellets
LTE_BYB1_H06-140	1-449	Abnormal Pellets
LTE_BYB1_H06-240	1-450	Abnormal Pellets
LTE_BYB1_H06-335	1-451	Abnormal Pellets
LTE_BYB1_H06-415	1-452	Abnormal Pellets
LTE_BYB1_H06-505	1-453	Abnormal Pellets
LTE_BYB1_H06-585	1-454	Abnormal Pellets
LTE_BYB1_H06-665	1-455	Abnormal Pellets
LTE_BYB1_H06-745	1-456	Abnormal Pellets
LTE_BYB1_H06-825	1-457	Abnormal Pellets
LTE_BYB1_H06-910	1-458	Abnormal Pellets
LTE_BYB1_H06-1000	~~1-459~~ 564	Abnormal Pellets

11/17/11

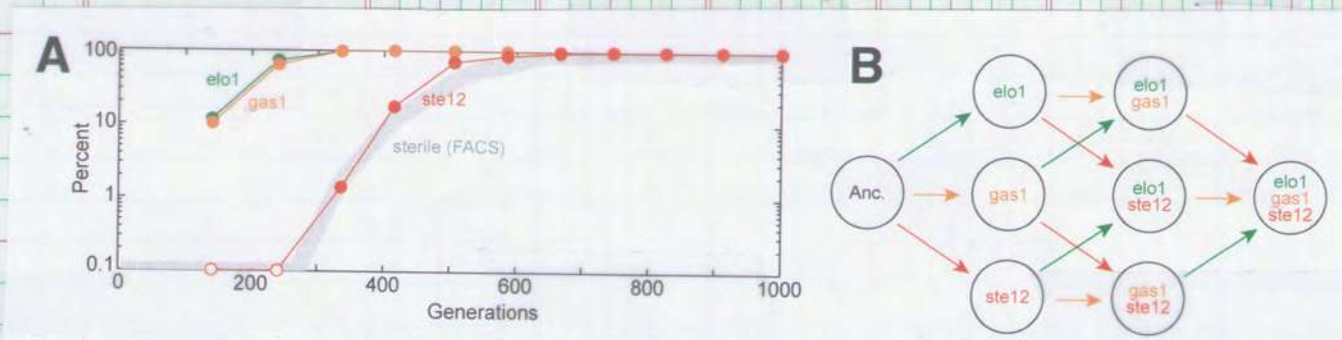

Large population size cultures
96-well plate nomenclature
384-well plate nomenclature
RMB1 RMB2
BYB1 BYB2
Sample well
Blank well
B1
H6
Small population size cultures
96-well plate nomenclature
384-well plate nomenclature
RMS1 RMS2
BYS1 BYS2
Sample well
Blank well
D07
E03

11/17/11

	Initials	Date
Prepared By		
Approved By		

Now that the gDNA preps have been sent to Wash U I can turn my focus to the reconstruction experiment.

We found previously that the BY51-A08 population fixed three mutations (p. 7 and below). I now want to construct all possible combinations of these three mutations.



Each allele swap will be done by first replacing the WT allele with URA3MX (heterologous URA3), then replacing URA3 with the evolved allele.

PCR URA3MX CASSETTE

280 λ H_2O
35 λ 10× Buffer
10.5 λ dNTPs
3.5 λ pAG60
3.5 λ Taq

→ 96 λ + 2 λ each Primer

59969713 IDT
G.LANG
98978397 349 24-Jun-2011
STE12_URA3Forward
5'- TTA CCC AAG AAT ACC GCT AAG GGC AG
Tm=69.1°C MW=18,599.1
13.5 OD_{260} = 22.3nm = 0.41mg

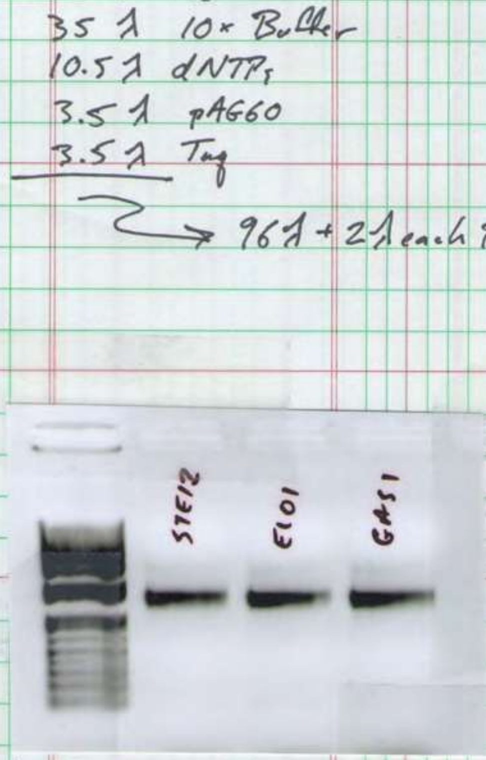
59969714 IDT
G.LANG
98978398 350 24-Jun-2011
STE12_URA3Reverse
5'- GGG TAT TGA TTT ATA TTT TGA GAA TAA
Tm=63.4°C MW=18,670.2
14.5 OD_{260} = 23.6nm = 0.44mg

59969717 IDT
G.LANG
98978399 353 24-Jun-2011
ELO1_URA3Forward
5'- TCT TGG CTT ACC CGC GTC GGC TTT AC
Tm=71.1°C MW=18,473.0
13.3 OD_{260} = 23.6nm = 0.44mg

59969718 IDT
G.LANG
98978400 354 24-Jun-2011
ELO1_URA3Reverse
5'- GCT AAT AAA TTA TAT GTT TTT GTC CTG
Tm=66.2°C MW=18,454.0
14.4 OD_{260} = 24.8nm = 0.46mg

59969721 IDT
G.LANG
98978401 357 24-Jun-2011
GAS1_URA3Forward
5'- TGG TTG TTT TCC TCG AAC AAA TTA GAT
Tm=65.8°C MW=18,502.1
14.5 OD_{260} = 24.5nm = 0.45mg

59969722 IDT
G.LANG
98978402 358 24-Jun-2011
GAS1_URA3Reverse
5'- ATT TGT TAG TCT CTT CGA AGT ACA TGT
Tm=65.0°C MW=18,407.0
12.9 OD_{260} = 21.8nm = 0.40mg



11/18/11

TRANSFORMATION OF yGIL432 (BY ANCESTOR)

· Yesterday I started an o/n culture of yGIL432

· ~9am → Dilute o/N 1:100 & 1:50 into 20 mL YPD

12/1/11

The -ura medium I used was not very good - It was made from an old mix (which must have been low for his and/or trp.) I made new -ura medium and replica plated the transformation plates.

→ Once replica plated, everything grew fine

I picked 4 putative transformants & restreaked to YPD (two days ago)

→ Today I picked a single colony off of each plate & inoculated 5 mL of YNB + his & trp.

PCR OF EVOLVED ALLELES

100λ HiFi

Using the following primers I amplified the evolved alleles off of a template from BY51-A08-1000.

59969715 IDT
G.LANG
98977989 24-Jun-2011
STE12_EvoForward
5'- ACA AAA TCA GAT AAT TCG CC -3'
Tm=48.5°C MW=6,078.0
6.4OD260 = 31.4nm = 0.19mg

59969719 IDT
G.LANG
98977991 24-Jun-2011
ELO1_EvoForward
5'- AAC ACA ACA AAT CGC AAG CC -3'
Tm=54.7°C MW=6,057.0
7.6OD260 = 37.4nm = 0.23mg

59969723 IDT
G.LANG
98977993 24-Jun-2011
Gas1Evo_Forward
5'- TTT TCG TGC CGC AAA CGT GG -3'
Tm=59.6°C MW=6,124.0
6.0OD260 = 32.6nm = 0.20mg

59969716 IDT
G.LANG
98977990 24-Jun-2011
STE12_EvoReverse
5'- TGA GCA GAA TCT TCG TCA CC -3'
Tm=54.6°C MW=6,077.0
6.3OD260 = 33.1nm = 0.20mg

59969720 IDT
G.LANG
98977992 24-Jun-2011
ELO1_EvoReverse
5'- GGT GTG ACA CTA GAA TCG CC -3'
Tm=55.5°C MW=6,142.0
7.4OD260 = 38.0nm = 0.23mg

59969724 IDT
G.LANG
98977994 24-Jun-2011
Gas1Evo_Reverse
5'- GAA CAC AAG CCT CCG TTT GG -3'
Tm=56.7°C MW=6,102.0
6.5OD260 = 34.3nm = 0.21mg

ALLELE	EXPECTED SIZE (bp)
STE12	1268
ELO1	1518
GAS1	1427

→ Qiagen Prep → elute 50 µl

12/5/11

	Initials	Date
Prepared By		
Approved By		

TRANSFORMATION w/ EVOLVED ALLELES

On Friday (12/2) I transformed the allele-replacement intermediates (Δs::URA3MX) with the evolved alleles.

↳ On ~~Thursday~~ Friday I noted that all 12 putative transformants (4 each for the three strains) grew in -ura.

~~Fri~~ ↳ On Thursday (12/1) I started these overnights by picking single colonies off the restreaked YPD plates.

12/2 @ 9:30 - Dilute 2 o/Ns each 1:50 into 10 ml YPD

@ 3:30 - Start transformation

• Pick 2 colonies off of each plate and restreak onto YPD

12/7/11

This morning I picked colonies off of the restreaked plate and inoculated 5 ml YPD.

NOTE: All four of the plates from the ELO1* transformations show a mixture of big & small colonies. This is something I noticed after the 1st round of transformation as well. The initial colonies off of the transformation are normal size suggesting that the ELO1* strain produces small colonies at a high rate. I should find a glycerol plate to check if these are petites. → 12/9/11: they are indeed petites.

12/9/11

Yesterday I phenotyped the 12 putative transformants. All are ura- 5FOA[R] but unfortunately the 4 ste12* strains all mate just fine and still have the fluorescent reporter. So where did URA3MX go? I should check all of the strain construction materials. And I should go back to the URA3MX ~~strain~~ transformation and streak some more putative transformants.

12/15/11

Yesterday I went back to the original transformation plate (the URA3MX replacements) and I replica plated the ste12Δ::URA3MX and gas1Δ::URA3MX to -ura and YPD+αF

{ - 13% (11 of 85) colonies of ste12Δ::URA3 are αF^R
{ - 0% (0 of 49) " " gas1Δ " " "

I picked three αF^R ste12Δ::URA3 and ~~replica p~~ restreaked on YPD.

→ This suggests that I should test ~10 putative transformants per transformation.

12/17/11

On 12/15 I replica plated the three transformation plates to YPD+Nat.

→ I realized that the URA3MX is likely getting integrated at the Nat marker near GPA1.

- The Nat^R perfectly correlates w/ αF^R on the ste12Δ plates.

- There were a several Nat^R colonies on the ~~exp.~~ gas1Δ and elo1Δ plates
→ pick 3 each (for elo1Δ, one of the three picked colonies was small)

1/5/12

TRANSFORMATION OF EVOLVED ALLELES (TAKE TWO)

Although I was able to isolate phenotypically correct (URA$^+$, Nat^R, & sterile in the case of ste12) transformants, given that the strains have been propagated and replica plated several times, I have decided to go back and restart the strain construction.

- On Saturday (12/31) I streaked yGIL432 from frozen stock.
- On Tuesday (12/3) I started a 5 mL o/n from single colony
- On Wed (yesterday) morning I diluted 1:50 & 1:100 in YPD (10 mL) ~ 9:30 am

↳ I ~~pulled~~ spun down & pooled the cells ~ 2pm & transformed with URA3MX cassette targeted to ste12, elo1, & gas1 (see p. 30)

~~Today~~ ↳ I plated the transformations onto YPD + Nat

- This morning I replica plated to -ura.

1/9/12

There are between 50-100 colonies on each transformation plate. Given that I first plated on Nat, these putative transformants should be devoid of URA3 integration at the GPA1::Nat locus.

↳ I picked 4 putative transformants off of the ste12 plate and 2 each off of the elo1 & gas1 plates ~ 10 am

I checked the strains for mating. As expected the 2 elo1s & 2 gas1s respond to αF; surprisingly the 4 ste12s do as well suggesting that these putative transformants are incorrect.

↳ I replica plated the -ura plates to YPD, & YPD+Nat, & YPD+αF (for ste12 only) to see if, like last time, only a fraction of the URA$^+$ ~~cell~~ colonies are Nat^R.

↳ · The result on Nat was messy (perhaps the lawn on the -ura plate was still viable & gave a high bkg).
 · On the αF plate it appears that only a few of the ~~x~~ URA prototrophic colonies are sterile

1/16/12

TRANSFORMATION OF EVOLVED ALLELES (TAKE 3)

This time I will ~~transform~~ plate onto -ura + Nat plates.

400 mL H_2O	100 mL H_2O
10 g Agar	10 g Dextrose
AUTOCLAVE	1 g CSM-URA
	1 g YNB w/o amino acids and ammonium sulfate
	0.5 g Monosodium Glutamate
	FILTER STERILIZE

Mix & add Nat

- On Friday (1/13) I streaked yGIL432 from the freezer.
- On Sunday (1/15) I started o/n
- This morning I diluted 1:50 & 1:100 in YPD ~ 8:20 am

Coulter Count (12:30)		Coulter Count (2:30)	
Bkg	1.404 E6	Bkg	404000
1:50	1.336 E7	1:50	2.826 E7
1:100	9.108 E6	1:100	1.663 E7
Post Bkg	698000	Post Bkg	1.124 E6

@2:30 Pool 1:50 & 1:100 dilutions and start transformations

↳ I added
- 100 λ LiOAc (Resuspension)
- 20 λ ssssDNA
- I used 60 λ per transformation
- 12 λ PCRs
- 420 λ PEG Mix
- 54 λ DMSO

	Initials	Date
Prepared By		
Approved By		

1/24/12

Testing putative transformants for αF^R

- It took a while for putative transformants to grow on Synthetic Glutamate plates.
- There are ~16 colonies on the ste12Δ::URAMX plate and ~35 # on each the elo1Δ::URA3MX and gas1Δ::URA3MX plates ~~~~ respectively. The colonies on ste12Δ are larger than on elo1Δ, which in turn are larger than ones on gas1Δ, which are quite small.
- Pick 4 putative transformants off of the ste12Δ plate and 2 each off of elo1Δ & gas1Δ and inoculate 100 μl YPD+αF ~ 11 am

→ I only picked 1 off of gas1 → the 8th sample: I picked "background" lawn off of the ste12Δ plate.

		PLATE		
A	A	ste12Δ	→	STERILE
B	B	"	→	αF-sens.
C	C	"	→	STERILE
D	D	"	→	STERILE
E	E	~~ELO~~ elo1Δ	→	αF-sens
F	F	"	→	"
H	G	gas1Δ	→	"
	H	background ste12Δ	→	large vacuole cells, didn't grow

1/25/12

I picked 3 colonies off of each plate & inoculated 5 mL YPD (on 1/23)

→ Labeled (confusingly diff. from above)

- ste12Δ A, C, D
- elo1Δ E, F, G
- gas1Δ H, I, J

The gas1Δs grew slowly → on ~~ee~~ 1/24 start gDNA preps → 1 mL each, 2 mL from gas1Δ

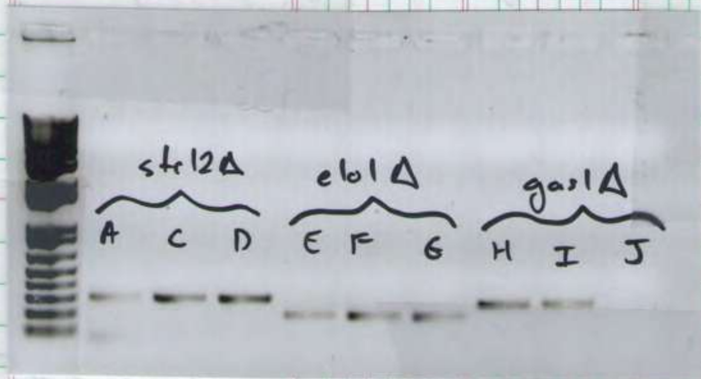
This morning I made gDNA preps & ran a PCR to check the strains.

1/25/12

PCR VERIFICATION OF STRAINS

Using the "EvoForward" primers (p. 30) and

78137290 IDT
G.LANG
99154246 05-Jul-2011
URA3MX_intR1
5'- GGC AAG CTA AAC AGA TCT GG -3'
Tm=53.6°C MW=6,175.1
5.4OD = 26.7nm = 0.16mg



EXPECTED PRODUCTS (bp)

ste12Δ	300
elo1Δ	195
gas1Δ	271

TRANSFORM EVOLVED ALLELES

I took the 9 saturated YPD cultures and diluted into SC-ura.

ste12Δ & elo1Δ 50λ into 5mL } ~2pm
gas1Δ 200λ into 5mL

1/26/12

Coulter count -ura cultures

Bkg	4.322 E6	
ste12A	6.75 E7	dilute 1:20 to 3E6 → 500λ into 9.5mL YPD (A,C,E,F)
elo1G	6.706 E7	
gas1H	2.547 E7	dilute 1:5 to 5E6 → 2mL into 8mL YPD (H,I)
Post Bkg	2.438 E6	

Dilutions @ 9:15am

Coulter Counts 2:15

Bkg	1.44 E6
ste12A	9.662 E6
elo1E	9.644 E6
gas1	9.296 E6
Post Bkg	1.164 E6

Transformation Started
@ 3:30pm → Plate to YPD

	Initials	Date
Prepared By		
Approved By		

1/27/12

Today I put the YPD plates in the fridge & froze down the strains from the 1st round:

yGIL618 –	ste12Δ::URA3MX	clone A
619 –	″	C
620 –	elo1Δ:: ″	E
621 –	″	F
622 –	gas1Δ:: ″	H
623 –	″	I

2/20/12

I pulled the YPD plates from the fridge and replica plated to 5FOA

3/19/12

For some reason, the gas1* transformations were unsuccessful — I had a lawn on the 5FOA plates. I replica plated again to YPD then back to 5FOA but, still, a weak lawn

The ste12* & elo1* by comparison looked good → pick 10 putative transformants

I made gDNA preps for each & PCR OVER →

I picked eight transformants and restreaked for singles (the strains were stored at -4° while I was away)

Yesterday I picked a single purified colony and inoculated 5 mL YPD

Today I will freeze down & do gDNA preps so that I can verify the mutations by sequencing.

Freeze down transformants

ste12*	A1 →	yGIL624
	A6 →	625
	C1 →	626
	C2 →	627
elo1*	E1 →	628
	E6 →	629
	F1 →	630
	F2 →	631

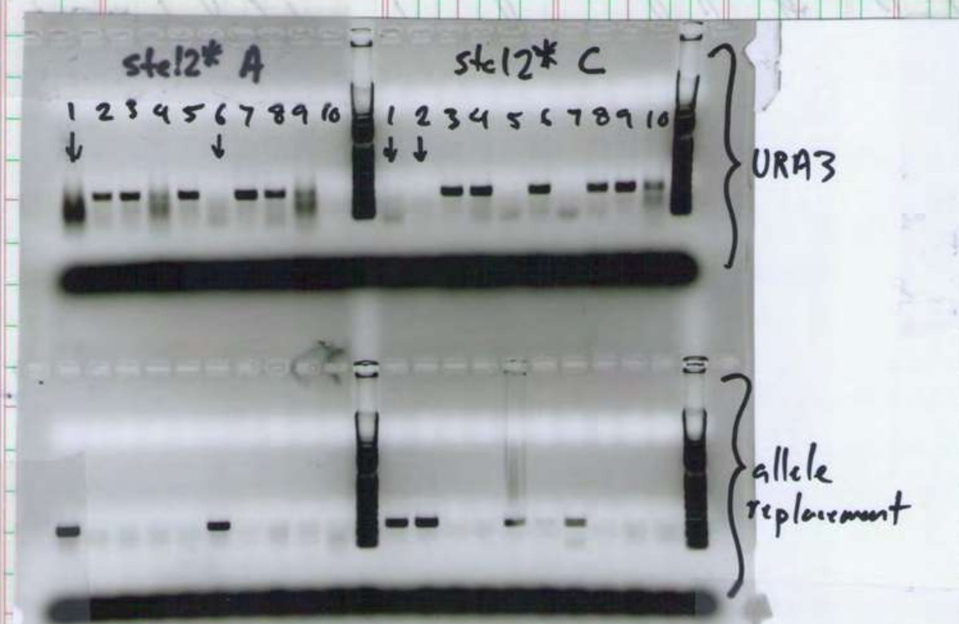
PCR VERIFICATION OF ste12* & elo1* transformants

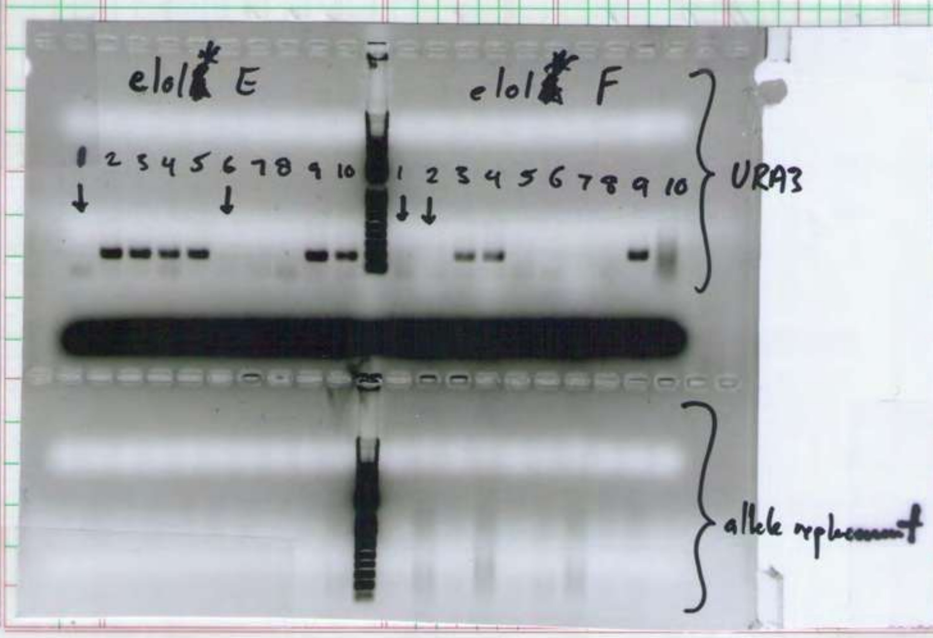
URA3 - same primers as page 36

allele replacement - ~~EvoForward Primers~~

EvoReverse Primers (p. 30) &

oGIL373	STE12Evo_New_Check_FOR	GGAACACTGCTGTTTCACCT
oGIL372	ELO1Evo_New_Check_FOR	GGCTATTGCTGCTGGTGCAG





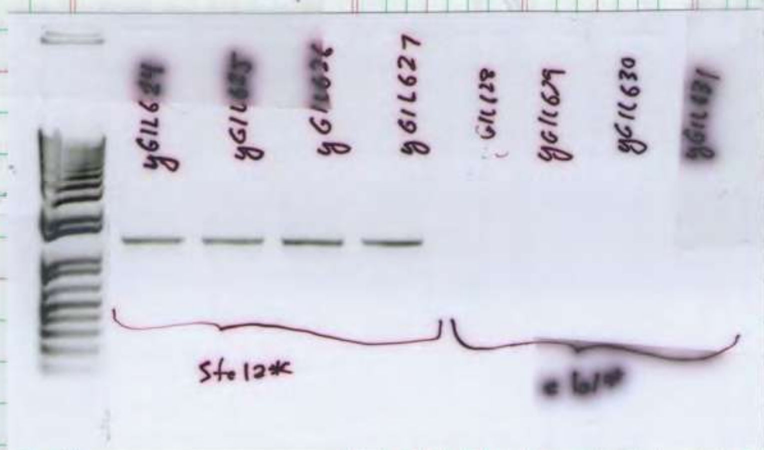
	Initials	Date
Prepared By		
Approved By		

3/20/12

PCR EVOLVED ALLELES

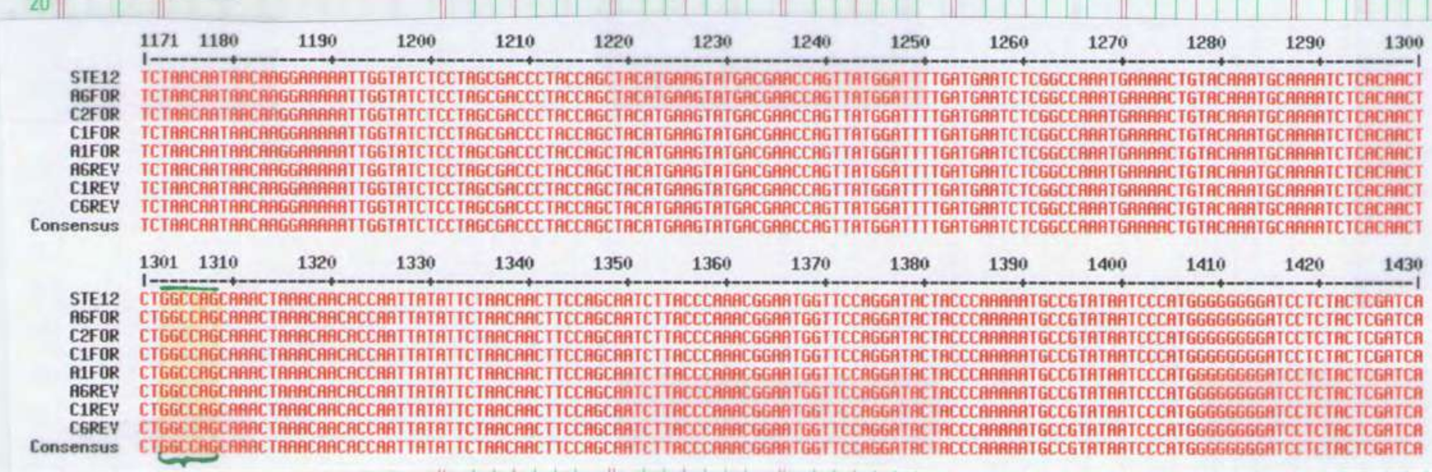
- I amplified the ste12* alleles off of the gDNA preps from yesterday
- The elo1* PCRs failed

↳ primers on p. 30



PCR cleanup & submit ste12* for sequencing.

3/23/12



```
          1171 1180      1190      1200      1210      1220      1230      1240      1250      1260      1270      1280      1290      1300
STE12     TCTAACAATAACAAGGAAAAAATTGGTATCTCCTAGCGACCCTACCAGCTACATGAAGTATGACGAACCAGTTATGGATTTTGATGAATCTCGGCCAAATGAAAACTGTACAAATGCAAAATCTCACAACT
A6FOR     TCTAACAATAACAAGGAAAAAATTGGTATCTCCTAGCGACCCTACCAGCTACATGAAGTATGACGAACCAGTTATGGATTTTGATGAATCTCGGCCAAATGAAAACTGTACAAATGCAAAATCTCACAACT
C2FOR     TCTAACAATAACAAGGAAAAAATTGGTATCTCCTAGCGACCCTACCAGCTACATGAAGTATGACGAACCAGTTATGGATTTTGATGAATCTCGGCCAAATGAAAACTGTACAAATGCAAAATCTCACAACT
C1FOR     TCTAACAATAACAAGGAAAAAATTGGTATCTCCTAGCGACCCTACCAGCTACATGAAGTATGACGAACCAGTTATGGATTTTGATGAATCTCGGCCAAATGAAAACTGTACAAATGCAAAATCTCACAACT
A1FOR     TCTAACAATAACAAGGAAAAAATTGGTATCTCCTAGCGACCCTACCAGCTACATGAAGTATGACGAACCAGTTATGGATTTTGATGAATCTCGGCCAAATGAAAACTGTACAAATGCAAAATCTCACAACT
A6REV     TCTAACAATAACAAGGAAAAAATTGGTATCTCCTAGCGACCCTACCAGCTACATGAAGTATGACGAACCAGTTATGGATTTTGATGAATCTCGGCCAAATGAAAACTGTACAAATGCAAAATCTCACAACT
C1REV     TCTAACAATAACAAGGAAAAAATTGGTATCTCCTAGCGACCCTACCAGCTACATGAAGTATGACGAACCAGTTATGGATTTTGATGAATCTCGGCCAAATGAAAACTGTACAAATGCAAAATCTCACAACT
C6REV     TCTAACAATAACAAGGAAAAAATTGGTATCTCCTAGCGACCCTACCAGCTACATGAAGTATGACGAACCAGTTATGGATTTTGATGAATCTCGGCCAAATGAAAACTGTACAAATGCAAAATCTCACAACT
Consensus TCTAACAATAACAAGGAAAAAATTGGTATCTCCTAGCGACCCTACCAGCTACATGAAGTATGACGAACCAGTTATGGATTTTGATGAATCTCGGCCAAATGAAAACTGTACAAATGCAAAATCTCACAACT

          1301 1310      1320      1330      1340      1350      1360      1370      1380      1390      1400      1410      1420      1430
STE12     CTGGCCAGCAAACTAAACAACACCAATTATATTCTAACAACTTCCAGCAATCTTACCCAAACGGAATGGTTCCAGGATACTACCCAAAAAATGCCGTATAATCCCATGGGGGGGGATCCTCTACTCGATCA
A6FOR     CTGGCCAGCAAACTAAACAACACCAATTATATTCTAACAACTTCCAGCAATCTTACCCAAACGGAATGGTTCCAGGATACTACCCAAAAAATGCCGTATAATCCCATGGGGGGGGATCCTCTACTCGATCA
C2FOR     CTGGCCAGCAAACTAAACAACACCAATTATATTCTAACAACTTCCAGCAATCTTACCCAAACGGAATGGTTCCAGGATACTACCCAAAAAATGCCGTATAATCCCATGGGGGGGGATCCTCTACTCGATCA
C1FOR     CTGGCCAGCAAACTAAACAACACCAATTATATTCTAACAACTTCCAGCAATCTTACCCAAACGGAATGGTTCCAGGATACTACCCAAAAAATGCCGTATAATCCCATGGGGGGGGATCCTCTACTCGATCA
A1FOR     CTGGCCAGCAAACTAAACAACACCAATTATATTCTAACAACTTCCAGCAATCTTACCCAAACGGAATGGTTCCAGGATACTACCCAAAAAATGCCGTATAATCCCATGGGGGGGGATCCTCTACTCGATCA
A6REV     CTGGCCAGCAAACTAAACAACACCAATTATATTCTAACAACTTCCAGCAATCTTACCCAAACGGAATGGTTCCAGGATACTACCCAAAAAATGCCGTATAATCCCATGGGGGGGGATCCTCTACTCGATCA
C1REV     CTGGCCAGCAAACTAAACAACACCAATTATATTCTAACAACTTCCAGCAATCTTACCCAAACGGAATGGTTCCAGGATACTACCCAAAAAATGCCGTATAATCCCATGGGGGGGGATCCTCTACTCGATCA
C6REV     CTGGCCAGCAAACTAAACAACACCAATTATATTCTAACAACTTCCAGCAATCTTACCCAAACGGAATGGTTCCAGGATACTACCCAAAAAATGCCGTATAATCCCATGGGGGGGGATCCTCTACTCGATCA
Consensus CTGGCCAGCAAACTAAACAACACCAATTATATTCTAACAACTTCCAGCAATCTTACCCAAACGGAATGGTTCCAGGATACTACCCAAAAAATGCCGTATAATCCCATGGGGGGGGATCCTCTACTCGATCA
```

I sequenced the 4 putative allele replacement strains → unfortunately all are WT. Today I will submit for sequencing the PCRs of the evolved alleles → p. 30.

3/27/12

CONFUSING RESULTS

- Last week I sequenced four putative allele swaps for ste12 and all were WT. (p. 39).

- My suspicion was that ~~the~~ I streaked the wrong strain from the freezer when preping gDNA for my allele swaps.

- Last Friday I sequenced the PCRs I used for the transformations. (see p. 30).

- To my surprise these have the mutation. → at least ste12 (I have yet to check the others).

→ so what happened? and where should I go from here?

I verified each step by PCR. Perhaps I switched up the ste12* and the elo1* putative allele swaps. I was considering abandoning these strains any way and starting over.

3/27/12

	Initials	Date
Prepared By		
Approved By		

Backcrossing: Strategy for making allele combinations:

Rather than constructing individual allele-swaps I am considering doing a backcrossing approach.

I first need to generate a MATα version of the ancestor strain → this is difficult since there is no HMLα cassette in the strain and since it ~~was~~ was a spore from a diploid that likely had some heterozygosities.

→ I pulled the parent strain (yGIL103) from the mg collection and I will pick an α spore with the correct genotype and backcross this to the ~~pure~~ ancestral strain(s) several times

Some issues

(1) I will need to complement the steriles to get some of these to mate

(2) There could be things in the bkg that the sequencing missed → i.e. other segregating mutations.

(3) I will need to make α-versions of both the RM & BY markers.

(4) There could be mutations in the α-strains that backcrossing did not get rid of.

(5) Because ~~##~~ mating goes through a bottleneck of 1, I ~~am~~ could potentially fix low frequency mutations that are in the evolution expt. → I may need to do ~3 independent diploids per backcross.

Advantages

(1) Just like QTL mapping → pick random spores, genotype at known ~~###~~ SNPs, and do fitness assays.

(2) Fast → can generate all combinations at once

4/2/12

GENERATING MATα VERSIONS OF ANCESTRAL STRAINS BY BACKCROSSING

Last week I sporulated yGIL103 by letting the 5mL YPD culture saturate for 3 days @ 30°C; ~~we~~ spin 1mL; wash once w/ H_2O; resuspend in 1mL of 2% KAc (supp w/ His & Trp); and add to 2 mL KAc (total of 3mL volume); RT rollerdrum for 4 days.

Today I dissected 10 tetrads.

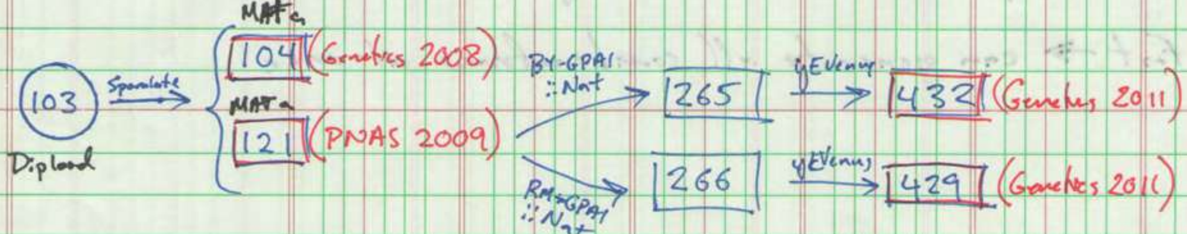
Your Name	Greg Lang	**Chromosomal Genotype**	URA3
			ade2-1
Strain Name	yGIL103		his3-11,15
			leu2-3,112
			trp1-1
Previous Name of Strain			CAN1/can1-100
			BAR1/bar1Δ::ADE2
Lab of Origin			HMLα/hmlαΔ::LEU2
Strain Background	W303		
Notebook Reference	Mutation Rate 5		
Date Entered	9/6/2007		
Mating Type	MATa/MATα	**Replicating Plasmid**	
		Integrated Plasmid	
		Plasmid Marker	
Comments	Cross between GIL102 and GIL087		

Sort by Background | Sort by Entry Date | Condensed List | Sort by Your Name

4/4/12

I ~~dissected~~ replica plated the 10 tetrads (plus one that Matt dissected) to YPD, -ade, -leu, -arg+CAN, and to mating type testers

I also streaked yGIL121, yGIL432, and yGIL429 to backcross to.



	Initials	Date
Prepared By		
Approved By		

4/5/12

Phenotyping spores of yGIL103

Dissection of yGIL103

Tetrad	Spore	-ade	-leu	-arg+CAN
	1 A		x	
	B	x		
	C	x	x	x
	D			x
	2 A			
	B		x	x
	C	x		x
	D	x	x	
	3 A			
	B	x		
	C		x	x
	D	x	x	x
	4 A	x	x	
	B	x	x	x
	C			
	D			x
	5 A			
	B	x	x	x
	C		x	
	D	x		x
	6 A	x		x
	B			x
	C		x	
	D	x	x	
	7 A	x		
	B		x	x
	C	x	x	x
	D			
	8 A	x		
	B	x	x	x
	C			
	D		x	x
	9 A	x		x
	B		x	
	C	x	x	x
	D			
	10 A	x		x
	B	x	x	x
	C			
	D		x	
	11 A		x	x
	B			
	C	x	x	x
	D	x		

I want to isolate a MATα spore w/ bar1Δ::ADE2, hml1Δ::LEU2 and CAN1. I will mating type them tomorrow, but three spores genotype correctly.

↳ Cross yGIL121 to these three spores 11:20am

I also crossed yGIL121 to all 4 #11 spores. as a control for mating

~4pm - I can see that all three phenotypically correct spores are MATα
- Although there were plenty of shmoos there were very few (if any) good zygotes - probably because these spores are bar1Δ

↳ ultimately I didn't pick any zygotes

~~I strea~~

4/6/12

~~I took the overnight matings and streaked onto -ura + Nat since the spores are URA3, Nat^S and yGIL~~ Nat^S as well

↳ This is not correct (yGIL121 is URA3, Nat^S; the derived ancestral strains yGIL429 3432 are ura3-, Nat^R)

4/6/12

CROSSING yGIL121 & yGIL103 - spore 2D:

Dissection of yGIL103

Tetrad	Spore	-ade	-leu	-arg+CAN	+MATa	+MATalpha
1	A		x			x
	B	x			x	
	C	x	x	x		x
	D			x	x	
2	A					x
	B		x	x	x	
	C	x		x		x
	D	x	x		x	
3	A				x	
	B	x				x
	C		x	x		x
	D	x	x	x	x	
4	A	x	x		x	
	B	x	x	x	x	
	C					x
	D			x		x
5	A					x
	B	x	x	x		x
	C		x		x	
	D	x		x	x	
6	A	x		x	x	
	B			x		x
	C		x			x
	D	x	x		x	
7	A	x			x	
	B		x	x	x	
	C	x	x	x		x
	D					x
8	A	x			x	
	B	x	x	x		x
	C					x
	D		x	x	x	
9	A	x		x		x
	B		x		x	
	C	x	x	x	x	
	D					x
10	A	x		x		x
	B	x	x	x	x	
	C					x
	D		x		x	
11	A		x	x		x
	B				x	
	C	x	x	x		x
	D	x			x	

- I set up 12 cell-to-cell matings of these two strains ~ 10:20 am
- @ 12:45

MATING

1	8 - center bud zygote (CB)
2	No change (NC)
3	CB
4	NC
5	CB
6	oo8 - side bud zygote? (SB)
7	SB
8	NC
9	SB
10	SB (but linear)
11	SB
12	CB

4/13/12

- On ~~Wednesday~~ Saturday (4/7) I picked the ~~10~~ 12 colonies (zygotes or mixed colonies) and inoculated 5 ml YPD.
  - Colony # 5 smaller than rest
  - All colonies were small
- On Wednesday (4/11) I spun 1 ml, washed once w/ H_2O and resuspended in 3 ml 2% KAc + His & Trp

	Initials	Date
Prepared By		
Approved By		

4/14/12

DISSECTION OF 1ST BACKCROSS

- Analysis of 12 putative zygotes: 3 & 5 - no tetrads, lots of ~~tetrads in the rest.~~
- Perhaps a few too many diads in #1; I will therefore dissect #2.
- Yesterday I struck yGIL121 from the freezer.
- Pick a single colony of yGIL121 and streak onto YPD
- Digest 4 tetrads of ~~2D on~~ 2D #2 onto the same plate and place a single cell of yGIL121 next to each spore. 2ND BACKCROSS

4/23/12

~~On Friday (4/19) I spun the 1~~

- On Monday (4/16) Sandy Inoculated each of the 16 colonies into YPD
- On Wednesday (4/19) I spun, washed, and resuspended 1 ml of each culture in 2% KAc.
- Today I looked at each under the scope. Only 1 tetrad showed two-to-two segregation of tetrads

TETRADS

1A	2A	3A	4A
1B	2B	3B	4B
1C	2C	3C	4C
1D	2D	3D	4D

→ Dissect 2D & place a single cell of yGIL121 next to each spore. 3RD BACKCROSS

10 tetrads (numbered 1-10, see p. 49)

4/25/12

Inoculate each of the 40 colonies into 3 ml YPD

4/29/12

SPORULATING THE 3RD BACKCROSS

- Today I will take the 40 YPD cultures and · spin 1ml · wash once in H_2O · resuspend 3 ml 2% KAc + his & trp.

- Streak yGIL432 (BY·ancestor) and yGIL429 (RM Ancestor) from freezer.

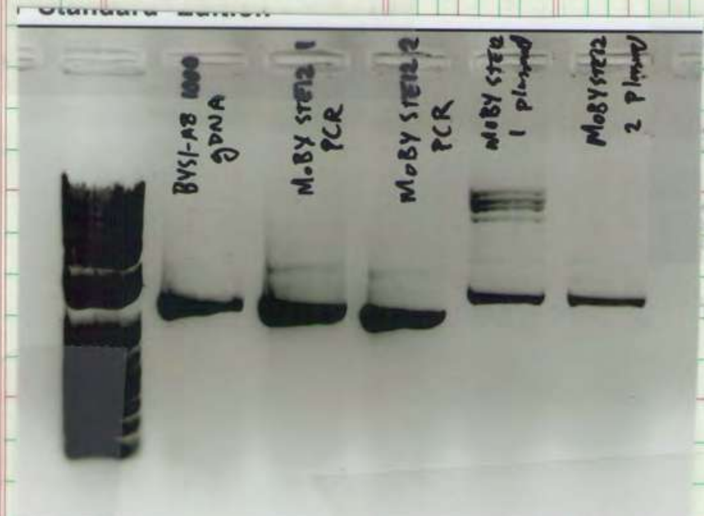
4/29/12

Transforming evolved (ste12*) mutant w/ STE12-plasmid

· In order to backcross the evolved strain I first need to compliment the ste12 mutation.

· On Thursday (4/26) I streaked the STE12-containing plasmid from the MoBY collection. This plasmid is a CEN-based plasmid containing the STE12 driven by its endogenous promoter - and contains both the KanMX and URA3 reporters.

→ The instructions for the MoBY collection suggest growing the bacteria in Tetracycline (25 mg/mL), Chloramphenicol (30 mg/mL) and Kanamycin (100 mg/mL). I went straight from liquid into 2 tubes - one containing all three drugs (#1) and one containing just Kan (#2)



· PCR off plasmids (and gDNA as a control) using STE12 external primers (p. 30)

· STE12 amplifies off of both plasmids. Plasmid #1 has some additional higher MW bands (supercoiling? concatamers?)

· On Friday (4/27) I streaked BY51-A08-545 from the freezer.
→ I chose generation 545 because it is the first time point where ste12* mutation is above 50%

· Pick 20 clones of BY51-A08-545 and inoculate 5 mL YPD

4/30/12

TRANSFORMATION OF pMoBY-STE12

- Dilute all 20 cultures 1:100 into ~~#~~ 10mL YPD ~ 9:45 am
- I need to figure out which of the 20 clones I picked contain the ~~ste~~ ste12** mutation.

↳ In a 96-well plate add 5λ of each culture to 100λ of YPD + αF → ~4h @ 30°C.

↳ Only clones #5 & #7 were mating-competent (shmoos in αF)

- I was expecting only about half to be sterile, but given that these cultures were propagated forward (in making the 384-well stocks, restreaking, etc) the fraction sterile enriched to ~~nearly~~ ~ 90%

I want to transform several clones → pick #s 1 → 4 and transform w/ pMoBY-STE12.

- @ ~3pm → #1: 2.905E7
- When resuspending in LiOAc mix, I resuspended in 50λ (instead of the usual 70) and I did not split (I transformed ~~the whole~~ all of the cells).
- Transform w/ 5λ of plasmid pMoBY-STE12 #1
- Plate on −URA.

5/2/12

Each of the four plates have tens- (if not hundreds-) of thousands of transformants

↳ I should have used far less plasmid (perhaps 0.1 λ)

- Pick 2 colonies each (1.1 − 4.2) and inoculate 5mL YPD.

	Initials	Date
Prepared By		
Approved By		

5/2/12

This morning I took the 40 sporulating cultures and checked microscopically for tetrads

		Cultures w/ tetrads
Original tetrad # 1 (see p. 45)	–	C
" 2 "	–	No tetrads
3	–	D
4	–	No tetrads
5	–	B & D
6	–	A & D
7	–	B & C
8	–	A & B
9	–	A & D
10	–	A & D

(5–10) ~~All~~ 2:2 segregation

- I saw 2:2 segregation of tetrads (i.e. mating type) in 6 of 10 tetrads
  - → the likely reason I don't see 2:2 in all cases is that one of the two original cells presumably dies.
- All of the sporulations had comparable efficiencies, but to my eye 7B and 9A may be slightly better
- Dissect 5 tetrads each of 7B and 9A.

5/3/12

- I took the 8 putative gMoBY-STE12 transformants (o/n cultures from yesterday) and exposed them to YPD+αF to see if the ste12* ~~m~~ mutation is complemented. → 10:30 am
- 2 pm: I looked for shmoos in the putative transformants.
  - → surprisingly the positive control (yGIL121) did not work (perhaps I used too many or old cells since I used a colony from a plate in the fridge). Nevertheless 5 of the 8 putative transformants show shmoos:

1.1 - shmoo → freezedown as yGIL640
1.2 - shmoo
2.1 - shmoo → " " yGIL641
2.2 - shmoo
3.1 - no shmoo
3.2 - no shmoo
4.1 - no shmoo
4.2 - shmoo → " " yGIL642

I also made gDNA preps of these 3 strains

5/4/12

Isolation of a MATα version of yGIL121 and crossing to yGIL429 & 432

- 4 of 5 tetrads of 7B (see p. 49) produced 4-spore tetrads
- All 5 " " 9A " " " "

- I picked all of the 4-spore tetrads and put each colony into 150λ YPD in a 96-well plate (same for a single colony of 429 & 432)

↳ Transfer 5λ each to 100λ YPD+αF ~ 9:30 am

@ 2:30 pm look for shmoos – all 9 tetrads show 2:2 seg. of αF^{S}

7B · 1A, $\boxed{1B}$, 1C, 1D
" · 2A, 2B, 2C, 2D
" · 3A, 3B, 3C, 3D
" · 4A, 4B, 4C, 4D
9A · $\boxed{1A}$, 1B, 1C, 1D
" · 2A, 2B, 2C, 2D
" · 3A, 3B, 3C, 3D
" · 4A, 4B, 4C, 4D
" · 5A, 5B, 5C, 5D

MATα
MATa (shmoo)

7B·1B ⟶ start o/n culture for freezedown
9A·1A ⟶ and cross each to 429 & 432

→ I set up the following crosses (10 each)

7B·1B × 429
7B·1B × 432
9A·1A × 432

5/5/12

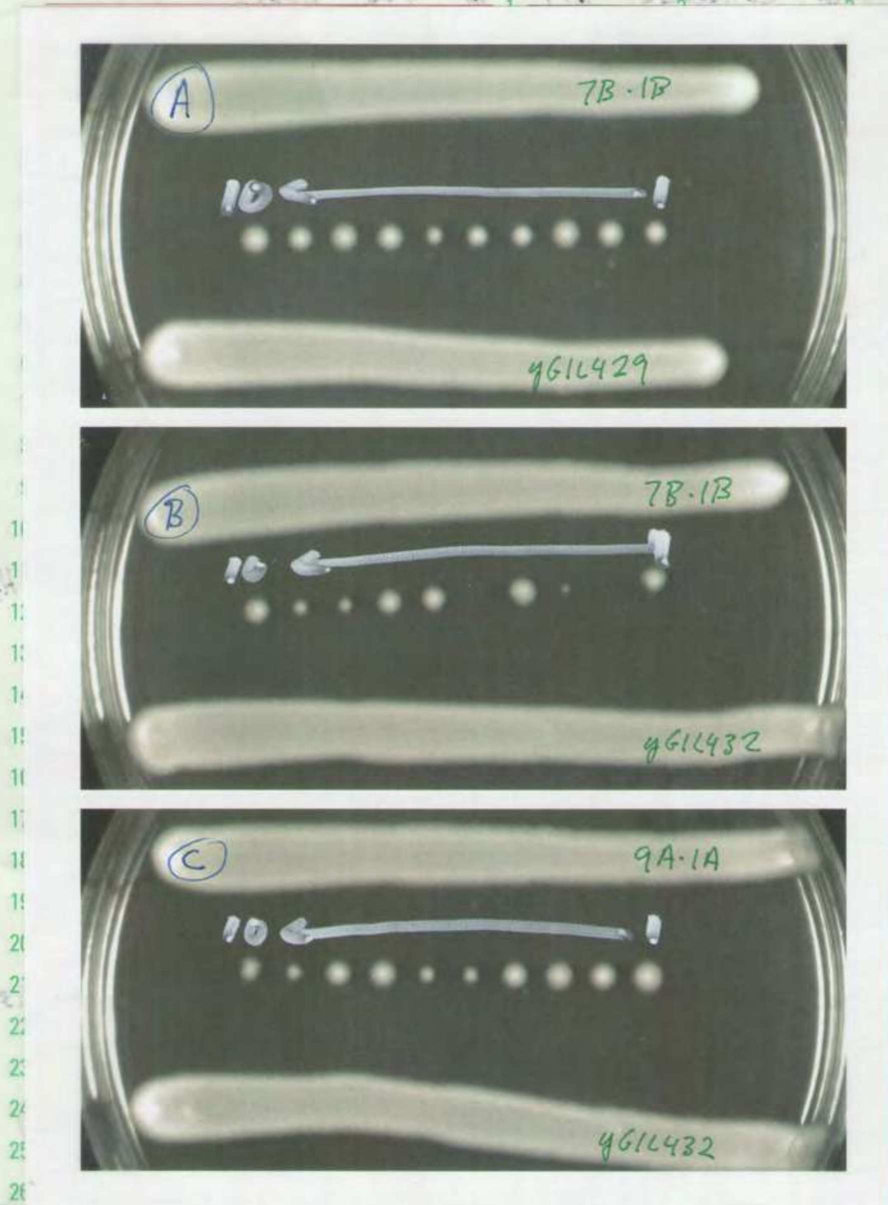
Freezedown Strains

7B·1B ⟶ yGIL643
9A·1A ⟶ yGIL644

	Initials	Date
Prepared By		
Approved By		

5/7/12

EVALUATION / SPORULATION OF CROSSES



• On Friday I set up crosses between the MATα-versions of yGIL121 and the Ancestral strains from the evolution experiment.

• This morning I noticed more size variation than I would like in the colonies. There are perhaps five types of colonies:

(1) Large (i.e. A10) - probably a good mating.

(2) Small (i.e. B8) - perhaps 1 of the two initial cells died. - These would therefore be haploid (something I could check)

(3) Pinprick (only 1: B3). Either 1 cell died & one sick, or they formed a diploid but that cell is sick.

(4) No colony (only two: B2, B5) - Both founding cells died

(5) Odd morphology (sectored, dead cells; only 1: C10)

Not counting the pinprick or the odd morphology, I would say that of the 28 colonies, 20 are large (2 viable cells & mating), 6 are small (only 1 cell alive), and 2 are no colonies (both cells died. This is close to expectation if the probability of a cell being alive is 0.82:

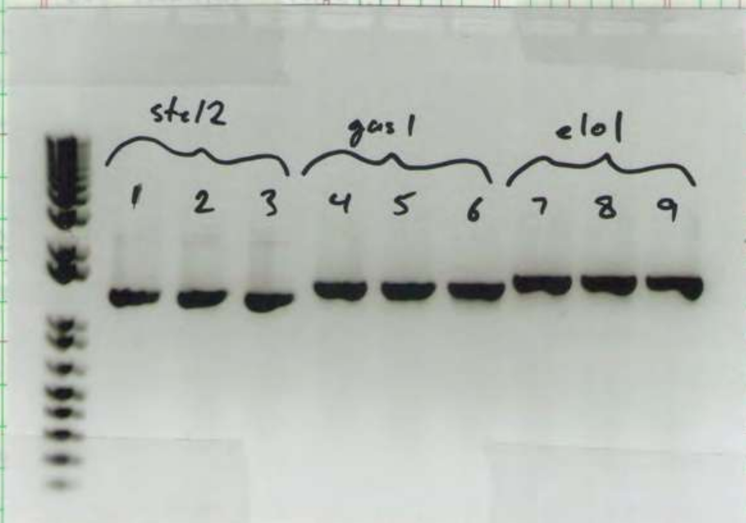
Expected: 19, 8, 1
Observed: 20, 6, 2

→ I am going to replica plate these plates to -URA & Nat to genotype the colonies.

5/8/12

• Yesterday I picked the colonies off of the cross plates and inoculated 5 mL YPD

PCR off of yGIL640, 641, & 642 (see p. 49)



Columns 1, 4, 7 → yGIL640
" 2, 5, 8 → " 641
" 3, 6, 9 → " 642

HSF: (Standard Protocol)

→ Qiagen prep → elute 50 μL H_2O

Sequence 1 Y

PHENOTYPING THE CROSSES

Colony	Size	-Ura	Clonat	
A1	Big		+	Haploid
A2	Big	+		Haploid
A3	Big	+	+	Diploid
A4	Small		+	Haploid
A5	Small		+	Haploid
A6	Small		+	Haploid
A7	Big	+		Haploid
A8	Big	+	+	Diploid
A9	Big	+		Haploid
A10	Big	+	+	Diploid
B1	Big	+	+	Diploid
B2	None	N/A	N/A	N/A
B3	Pinprick	N/A	N/A	N/A
B4	Big	+	+	Diploid
B5	None	N/A	N/A	N/A
B6	Big	+		Haploid
B7	Big	+		Haploid
B8	Small	N/A	N/A	N/A
B9	Small	N/A	N/A	N/A
B10	Big	N/A	N/A	N/A
C1	Big	+	+	Diploid
C2	Big	+		Haploid
C3	Big	+	+	Diploid
C4	Big	+		Haploid
C5	Small		+	Haploid
C6	Small		+	Haploid
C7	Big	+	+	Diploid
C8	Big	+		Haploid
C9	Small		+	Haploid
C10	Odd Morphology	+	+	Diploid

→ All small colonies are haploid MATa (either yGIL429 or 432)

Big colonies are either Diploid or haploid MATα (yGIL643 or 644) with one exception (A1) which may be a small colony classified as Big (see p. 51).

Overall it seems that colony size may be due to either Mating type or URA3. More likely URA3.

5/10/12

I started sporulations on the 8 diploid/Big colony cultures, as before (see p. 46 for example): A3, A8, A10, B1, B4, C1, C3, & C7

	Initials	Date
Prepared By		
Approved By		

5/9/12

SEQUENCING yGIL640, yGIL641, & yGIL642 TO VERIFY EVOLVED ALLELES

- Sequencing using evolved alleles (p. 30)
- Sequencing in both directions confirmed the gas1* and ec61* mutations; the ste12* is also confirmed but it is slightly complicated by the fact that the ~~URA~~ STE12* mutation is covered by the wild-type STE12 on the plasmid.

↳ Both ste12* and STE12 were amplified. Below is the chromatogram from yGIL642 (the other two are the same)

~~STE12 · · · TCTGG~~
~~ste12* · · · CTCCTGC~~

STE12 · · · CTCTG GCCAGCAA
ste12* · · · CTCTG CCAACAAA

5/10/12

FITNESS ASSAYS ON SINGLE & DOUBLE MUTANTS

- Matt Remillard (a rotation student who has been working with me) has succeeded in constructing the three single mutants and the three double mutants (p. 29) ~~using~~ by transformation using URA3MX::KanMX cassette from the pCORE plasmid as the intermediate.

→ His rotation is ending soon, so we will do the fitness assays next week before he can make the triple.

He made the same observation that I had before — that gas1Δ (URA3-disruption) grows slowly (p. 35); however, Matt noticed that when the gas1* mutation is put in the cells grow even better than wild-type.

→ Going into these fitness assays, I am expecting to find that gas1* provides an advantage and that elo1* is neutral.

→ see p. 61

3/14/12

	Initials	Date
Prepared By		
Approved By		

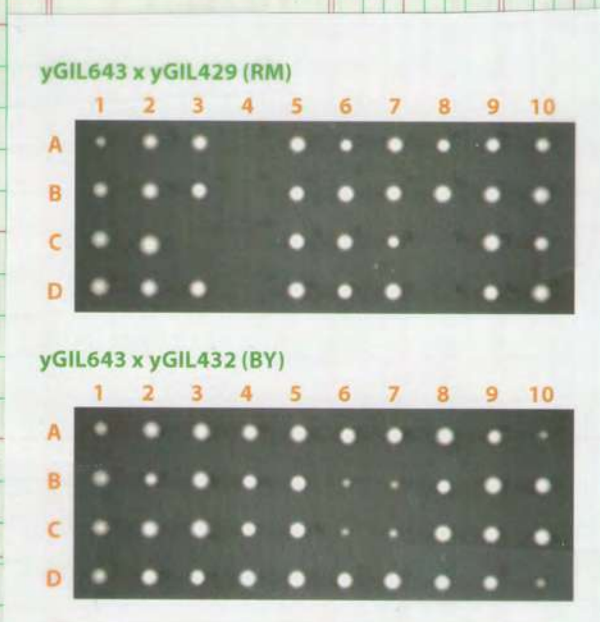
DISSECTING THE 4th BACKCROSS

- I looked at the 8 sporulations (p. 52) and all 8 show lots of tetrads.

→ Dissect 10 tetrads each of A3 (yGIL643×yGIL429, p50) and B4 (yGIL643×yGIL432, p 50).

5/16/12

Possible Translocation — I was expecting all 4-spore viable; however, the 643×429 cross shows a 0:4, a ~~[illegible]~~ 2:2, and a 3:1 viable:dead. And the 643×432 shows three 2:2 fitness defects



To complicate matters both URA3 and NatMX are segregating as well which leads to fitness differences.

→ I am therefore less concerned with the small differences between the colonies

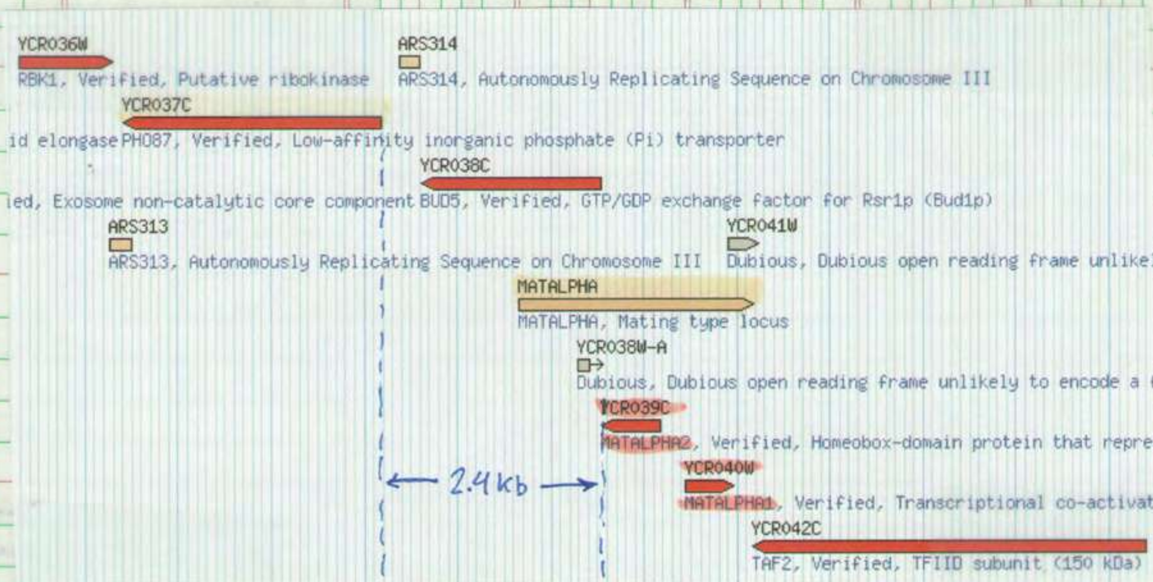
- Replica plate to -Ura, 5FOA, Nat, and mating testers (yGIL354 & 355).

3/14/12

LINKING URA3 TO MATα AS A PROXY FOR MATING TYPE

I want to scale up the backcrossing strategy (see p. 41); however, since ste mutations will be segregating I will have trouble assigning mating type.

→ ste* - containing segregants can only be used if they segregate 2:2 with ~~ma~~ MATa.



The method above will require doing many dissections ~~of~~ in which half the spores will be thrown out (because half will be MATα) and more will be thrown out because their mating type will be ambiguous.

If I tightly link URA3 to mating type, I will solve both of these issues. Selection of random spores will produce almost exclusively MATa.

- To do this I will first take the pho87Δ::KanMX strain from the Δ collection and replace KanMX w/ URA3 using the plasmid pGIL001 (Mutation Rate 3, p. 22).

→ • I will then amplify pho87Δ::URA3 from this strain and transform the strains that come out of the 4th backcross.

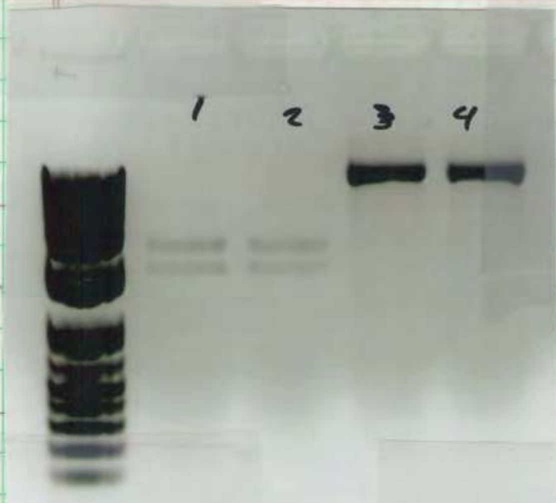
→ PHO87 was picked simply because it is the closest gene to MATalpha included in the deletion collection (2.36 kb) and there ~~are no~~ should be no effect on mating/sporulation/growth of the diploid.

5/~~16~~ 18/12

	Initials	Date
Prepared By		
Approved By		

TRANSFORMATION OF pho87Δ::KanMX w/ URA3 FROM pGIL001

• ~~[illegible]~~ On 5/16 I miniprepped two clones containg pGIL001.



• Digest o/N w/ EcoRI & BamHI

38.5 λ	H_2O
5	EcoRI Buffer
5	miniprep plasmid
0.5	BSA
0.5	EcoRI
0.5	BamHI
50	

LANE	
1	plasmid A - E/B digest
2	" B - "
3	" A - miniprep
4	" B - miniprep

• ~~EU~~ PCR cleanup of o/N digests → elute in 30λ EB

• Yesterday morning I diluted o/N culture of pho87Δ::KanMX 1:50 & 1:100 → @ 9:30 am

• @ 2:40pm - Coulter Count

Bkg	6.812 E6
1:100	2.979 E7 → transform 1:100 dilution
1:50	4.411 E7
Post Bkg	2.228 E6

• I did the normal transformation protocol and transformed the split culture w/ the two doubly-digested plasmids.

5/21/12

There are about 50 transformants on the A plate & 100 on B. There seem to be two colony sizes as well.

→ I replica plated each to YPD & YPD+G418. → In fridge (5/22)

5/28/12

No colonies grew on G418 → pick two colonies each of A&B. A1&B1 big colonies; A2&B2 - small colonies and inoculate 5 mL YPD.

5/18/12

ANALYSIS OF 4th BACKCROSS

yGIL429 Spore	-Ura	5FOA	ClonNat	+MATa	+MATalpha
1 A		x			x
B	x		x		x
C		x		x	
D	x		x	x	
2 A	x			x	
B		x	x	x	
C	x		x		
D		x	x		x
3 A	x		x	x	
B	x		x		x
C					
D		x			x
4 A					
B					
C					
D					
5 A		x		x	
B	x		x		x
C	x		x		x
D		x		x	
6 A	x		x		x
B		x		x	
C		x		x	
D	x		x		x
7 A		x		x	
B		x		x	
C	x		x		x
D	x		x		x
8 A		x			x
B	x		x	x	
C					
D					
9 A	x		x		x
B		x		x	
C	x		x	x	
D		x			x
10 A	x				x
B	x		x	x	
C		x			x
D		x	x	x	

yGIL432 Spore	-Ura	5FOA	ClonNat	+MATa	+MATalpha
1 A	x				x
B		x	x	x	
C	x			x	
D		x	x		x
2 A		x		x	
B	x		x		x
C		x		x	
D	x		x		x
3 A	x			x	
B	x			x	
C	x		x		
D		x	x		x
4 A	x			x	
B		x	x		x
C		x	x		x
D	x			x	
5 A		x		x	
B	x		x		x
C	x		x		x
D		x		x	
6 A		x			x
B	x		x	x	
C	x		x	x	
D		x			x
7 A		x	x		x
B	x			x	
C	x			x	
D		x	x		x
8 A		x		x	
B		x			x
C	x		x	x	
D	x		x		x
9 A		x			x
B	x		x	x	
C	x		x	x	
D		x			x
10 A	x		x	x	
B	x				x
C		x			x
D		x	x	x	

Dead

Low Fitness

→ I was able to find only one correct spore from each cross that came from a normal looking 4-spore tetrad.

I restreaked ~~A4.10D~~ A3.10D and B4.1B to YPD. → these should be nearly-isogenic MATα versions of yGIL429 & yGIL432, respectively.

→ I am still a little apprehensive because of spore-viability issues in this cross.

I struck yGIL429 & 432 from freezer and will set up the following crosses in the next week:

429 ⟷ A3.10D

432 ⟷ B4.1B

58

5/21/12

	Initials	Date
Prepared By		
Approved By		

SETTING UP CROSSES

On Friday I struck the following:

yGIL429 onto YPD from freezer
yGIL432 " " " "
yGIL640 " YPD+G418 " "
yGIL641 " " " "
yGIL642 " " " "
A3.10D " YPD " tetrad dissection plate
B4.1B " " " "

Today I set up matings by micromanipulating individual cells for the following crosses:

MATa		MATα
429	×	10D (645)
429	×	1B (646)
432	×	10D (645)
432	×	1B (646)
640	×	1B "
641	×	1B "
642	×	1B "

This arranged mating setup was much faster this morning. Rather than streak a small colony onto a YPD plate, I inoculated a small colony (or fraction of a big colony) into 5 mL YPD and used 10λ of this.

5/22/12

- Yesterday afternoon I looked at each of the matings under the scope to determine if zygotes were formed — I marked those where it looked pretty clear that a zygote had formed.

- This morning, very small colonies had formed. I picked 3 putative diploid colonies from each cross (4 from 642×1B just to be balanced).

- Freezedown: A3.10D → yGIL645
  B4.7B → yGIL646

5/28/12

The tubes have been on the 30° drum for 6 days (3 is my usual)
↳ prep in 2% KAc as before (p. 42).

5/22/12

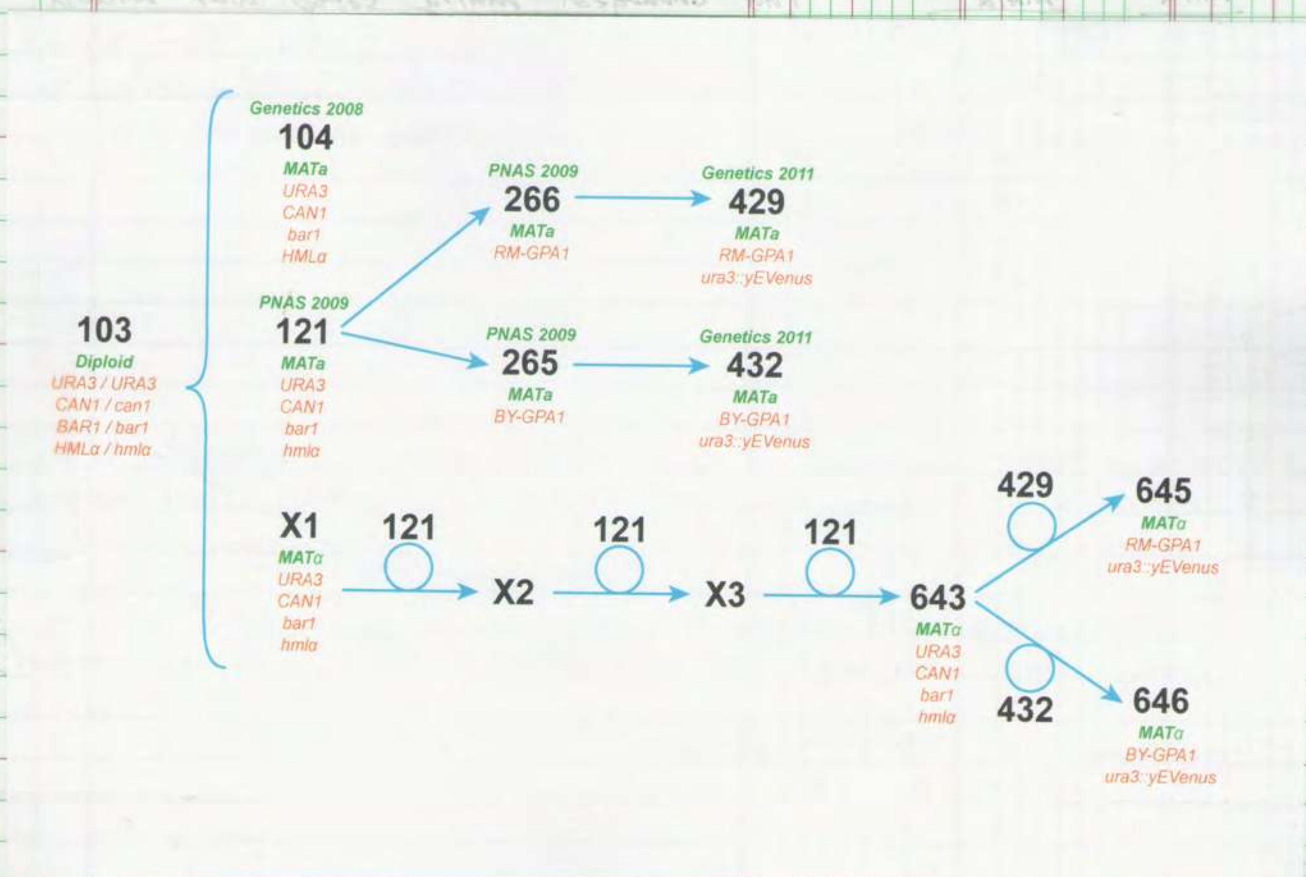
NOTES ON MATα VERSIONS OF ANCESTRAL STRAINS

On the dissection plates on p. 55 I see two types of 2:2 segregation of colony size:

(1) very small colonies in 643×432 cross. In this case the small colony phenotype is linked to MATα (see p. 58)

(2) somewhat smaller colonies in both crosses and linked to MATa — I suspect that it may be mating-type itself since MATa's are hypersensitive to αF. If this is the reason I do not need to worry.

Summary of Strain Construction:

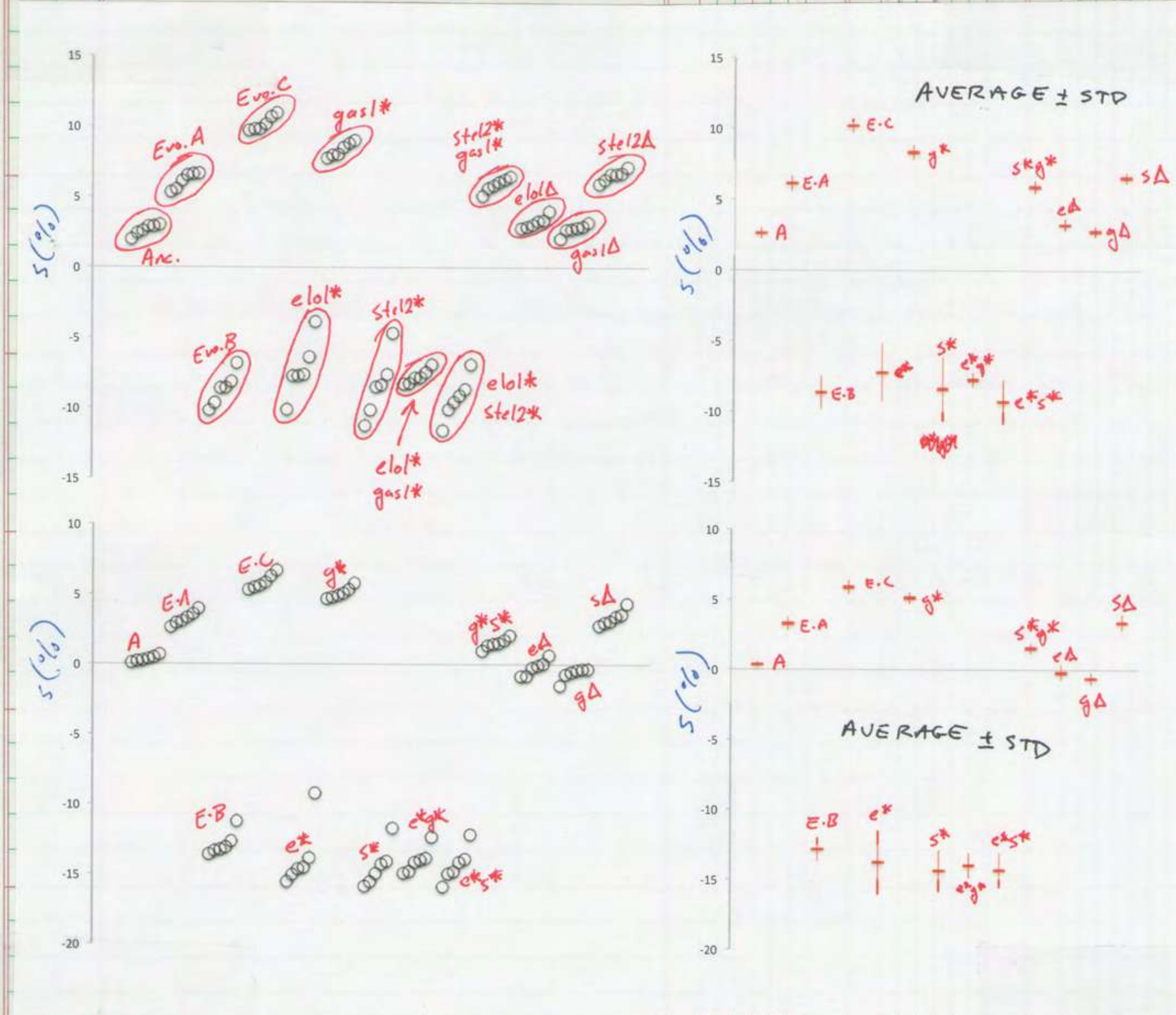


5/22/12

	Initials	Date
Prepared By		
Approved By		

FITNESS ASSAYS ON RECONSTRUCTED STRAINS

continued from p. 54 ...



· Top panels are based on gen 10, 20, 30, 40
· Bottom panels are based on gen 40 and the estimated initial mixture (40:60, Exp:Ref except for gas1Δ, 60:40, Exp:Ref).

6/8/12

TRANSFORMATION OF yGIL645 & yGIL646 w/ pho87Δ::URA3

· I made gDNA preps of putative pho87Δ::URA3 transformants A1, A2, B1, B2 (p. 57).

PCR using primers:

85558656 IDT
G.LANG
106236253 29-May-2012
PHO87_extF1
5'- GGT TTA AGA TTG TGC ATA GG -3'
Tm=48.8°C MW=6,227.1
$6.3\,OD_{260}$ = 31.2nm = 0.19mg

85558657 IDT
G.LANG
106236254 29-May-2012
PHO87_extR1
5'- GTT ACT CAA TTG TAT CAA GG -3'
Tm=45.9°C MW=6,131.0
$5.6\,OD_{260}$ = 28.0nm = 0.17mg

6/9/12

PCR failed. I will try again w/ the following modifications

(1) Drop annealing temp to 46° (these primers have a low Tm)
(2) Extend extension time to 2½ min (expected size is 2262 bp)

6/10/12

Again this PCR failed — I will put this transformation on hold for now.

6/8/12

	Initials	Date
Prepared By		
Approved By		

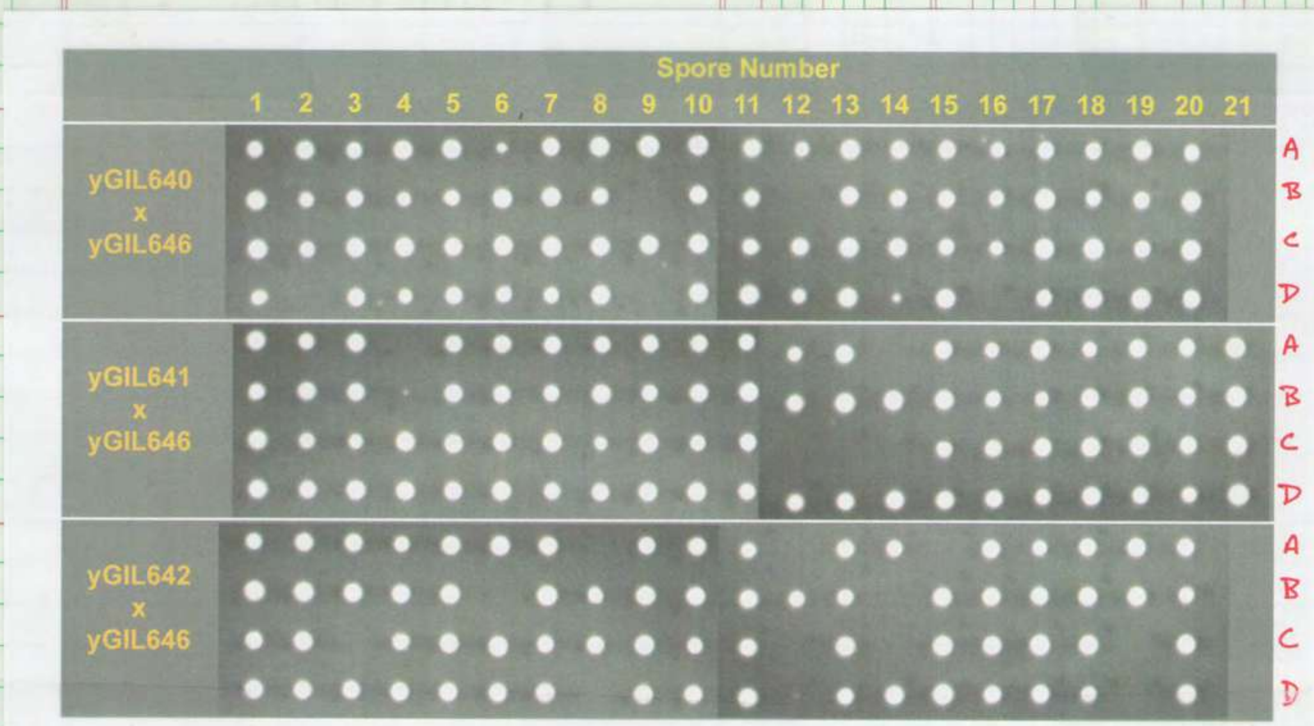
DISSECTING BACKCROSSED EVOLVED STRAINS

I really overdid this sporulation (p. 59)
· Strains in YPD 5/22 – 5/28 (6 days)
· " " 2% KAc 5/28 – 6/7 (10 days)

Yesterday I looked under the scope at the 3 replicates of each of the three backcrossed evolved strains:
· yGIL640 × yGIL646
· " 641 × "
· " 642 × "

I picked the ones w/ the best sporulation efficiency (640-1, 641-1, 642-3) and dissected 20 tetrads each.

6/9/12



VIABILITY

CROSS	4:0	3:1	2:2	1:3	TOTAL
yGIL646 × yGIL640	16	3	1		20
" " 641	17	2	2		21
" " 642	13	3	3	1	20
Overall	46	8	6	1	61

6/9/12

NOTES ON BACKCROSS DISSECTION

- Overall this is reasonable. Spore viability of 91% (221/244)

- Given the high spore viability it is unlikely that there is a translocation in the background, which is something I was worried about previously (p. 55 & 58)

- Do I need to do more dissections?

  - I have 46 4-spore viable tetrads → I want only MATa's (92) however mating-type testing will be unambiguous only ~~when~~ for tetrads that segregate mating type and the ste12* mutation as PD or NPD.
  - Half of the tetrads should be TT → ~ 46 MATa's will come out of this.

  - I could increase these numbers in 3 ways:
    (1) Dissect more tetrads
    (2) PCR genotype the MAT locus in TTs
    (3) If the plasmid is still retained I could mating-type the ste12* spores

  → On a related note I need to ~~genotype~~ test the spores on -ura and 5FOA to see if the plasmid is still present and to isolate plasmid-free spores for the fitness assays.

- Replica (each dissection plate) plate to YPD, -Ura, 5FOA, YPD+a-tester, & YPD+α-tester

6/10/12

- Today I replica plated the tester matings to minimal

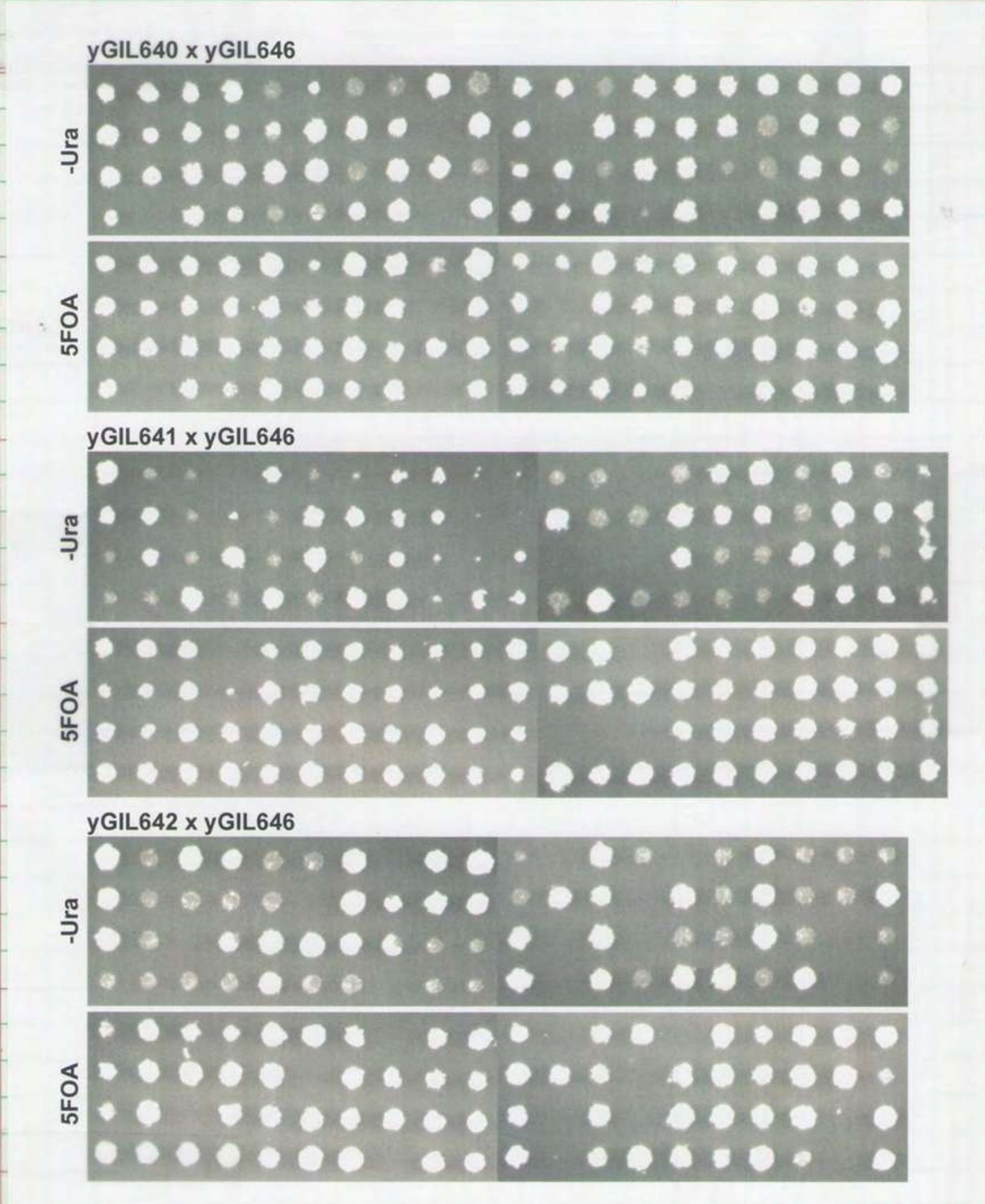
- Yesterday I dissected an additional 20 tetrads from the three backcrosses (19 from yGIL641×646, since I did 21 last time).

  → this should give me plenty of segregants for the fitness assays.

6/10/12

	Initials	Date
Prepared By		
Approved By		

PHENOTYPING SEGREGANTS: PLASMID RETENTION



PLASMID LOSS RATE

(15/75)
20%

(29/78)
37%

(35/68)
51%

NOTES:

- Everything grew on 5FOA suggesting that the plasmid was either lost ~~or~~ in the spore or is lost at a high rate, making ~~a~~ plasmid-minus segregants easy to acquire.
- Different plasmid-loss rates probably reflect copy # of the plasmid in the original diploid
- The high rate of retention (64% overall) increases the chance that I can mating type test even the ste12* segregants

6/11/12

MATING-TYPE TESTING OF SEGREGANTS

yGIL640 x yGIL646

		+a	+α	
1	A		x	
	B	x		
	C	x		
	D		x	
2	A	x		
	B		x	
	C		x	
	D			
3	A		x	
	B		x	
	C	x		
	D	x		
4	A	x		
	B		x	
	C			
	D		x	
5	A			ste12*
	B		x	
	C	x		
	D	x		
6	A		x	
	B	x		
	C	x		
	D		x	
7	A		x	STE12
	B	x		
	C			
	D		x	
8	A			ste12*
	B		x	
	C	x		
	D	x		
9	A			
	B			
	C	x		
	D			
10	A			
	B		x	
	C			
	D	x		
11	A	x		
	B		x	
	C		x	
	D	x		
12	A		x	
	B			
	C	x		
	D		x	
13	A			
	B		x	
	C			
	D	x		
14	A	x		
	B		x	
	C	x		
	D		x	fitness ?
15	A	x		
	B		x	
	C		x	
	D	x		
16	A		x	
	B		x	
	C			
	D			
17	A		x	ste12* ~~STE12~~
	B	x		
	C	x		
	D		x	ste12* ~~STE12~~
18	A		x	
	B		x	
	C	x		
	D	x		
19	A	x		
	B		x	
	C		x	
	D	x		
20	A		x	
	B	x		
	C			
	D		x	

yGIL641 x yGIL646

		+a	+α	
1	A		x	ste12*
	B		x	ste12*
	C	x		
	D	x		
2	A		x	STE12
	B	x		
	C	x		
	D			ste12*
3	A			
	B			
	C		x	
	D			
4	A			
	B			
	C	x		
	D			
5	A	x		
	B			ste12*
	C			ste12*
	D	x		
6	A	x		
	B		x	
	C		x	
	D			
7	A			
	B		x	
	C			
	D		x	
8	A	x		
	B	x		
	C		x	
	D		x	
9	A		x	
	B		x	
	C	x		
	D			
10	A	x		
	B			ste12*
	C		x	
	D	x		
11	A		x	
	B			
	C		x	
	D	x		
12	A		x	
	B	x		
	C			
	D	x		
13	A			
	B			
	C			
	D		x	
14	A			
	B			
	C			
	D		x	
15	A		x	STE12
	B	x		
	C		x	
	D			
16	A		x	
	B		x	
	C			
	D	x		
17	A	x		
	B		x	
	C	x		
	D			ste12*
18	A		x	STE12
	B			ste12*
	C	x		
	D	x		
19	A	x		
	B		x	
	C			
	D		x	
20	A		x	STE12
	B		x	
	C			
	D	x		
21	A			
	B		x	
	C		x	
	D			

yGIL642 x yGIL646

		+a	+α	
1	A		x	
	B			
	C		x	
	D	x		
2	A	x		
	B			
	C		x	STE12
	D			
3	A	x		
	B	x		
	C			
	D			
4	A		x	ste12*
	B	x		
	C		x	ste12*
	D	x		
5	A			ste12*
	B	x		
	C	x		
	D			
6	A			
	B			
	C			
	D			
7	A	x		
	B			
	C		x	
	D			
8	A			
	B		x	
	C	x		
	D			
9	A		x	
	B	x		
	C	x		
	D			ste12*
10	A	x		
	B	x		
	C		x	STE12
	D			ste12*
11	A	x		
	B			
	C		x	
	D		x	
12	A			
	B		x	
	C			
	D			
13	A	x		
	B		x	
	C	x		
	D		x	
14	A		x	
	B			
	C			
	D			
15	A			
	B	x		
	C	x		
	D			
16	A			
	B	x		
	C			
	D		x	
17	A		x	
	B	x		
	C			
	D		x	STE12
18	A			
	B			
	C	x		
	D		x	
19	A			
	B	x		
	C			
	D			
20	A			
	B		x	
	C	x		
	D			

- I was able to determine mating type for more segregants than I expected.
- Looking only at four-spore viables:

yGIL640-646	29
" 641 "	33
" 642 "	21
Total	83

- Based upon plasmid loss patterns (p.65), I was able to tentatively genotype the STE12 locus.
- I guess I didn't need to do the second round of tetrad dissections.

6/11/12 PICKING SEGREGANTS

yGIL640 x yGIL646							
		+a	+α	MATa	-U		
1	A		x	MATa	x		1
	B	x			x		
	C	x			x		
	D		x	MATa	x		2
2	A	x			x		
	B		x	MATa	x		
	C		x	MATa	x		
	D						
3	A		x	MATa	x		3
	B		x	MATa	x		4
	C	x			x		
	D	x			x		
4	A	x			x		
	B		x	MATa	x		2
	C				x		
	D		x	MATa	x		6 -
5	A			MATa		ste12	7
	B		x	MATa	x		8
	C	x			x		
	D	x					
6	A		x	MATa	x		9 -
	B	x			x		
	C	x			x		
	D		x	MATa	x		10
7	A		x	MATa		STE12	11
	B	x			x		
	C				x		
	D		x	MATa			12 -
8	A			MATa		ste12	13
	B		x	MATa	x		14
	C	x			x		
	D	x			x		
9	A				x		
	B						
	C	x			x		
	D						
10	A						
	B		x	MATa	x		15
	C						
	D	x			x		
11	A	x			x		
	B		x	MATa	x		16
	C		x	MATa	x		17
	D	x			x		
12	A		x	MATa	x		
	B						
	C	x			x		
	D		x	MATa	x		
13	A						
	B		x	MATa	x		18
	C						
	D	x			x		
14	A	x			x		
	B		x	MATa	x		
	C	x			x		
	D		x	MATa			
15	A	x			x		
	B		x	MATa	x		19
	C		x	MATa	x		20
	D	x			x		
16	A		x	MATa	x		
	B		x	MATa	x		
	C						
	D						
17	A		x	MATa	x	ste12	21
	B	x					
	C	x					
	D		x	MATa	x	ste12	22
18	A		x	MATa	x		23
	B		x	MATa	x		24
	C	x			x		
	D	x			x		
19	A	x			x		
	B		x	MATa	x		25
	C		x	MATa	x		26
	D	x			x		
20	A		x	MATa	x		27
	B	x					
	C						
	D		x	MATa	x		28

yGIL641 x yGIL646							
		+a	+α	MATa	-U		
1	A		x	MATa	x	ste12	29
	B		x	MATa	x	ste12	30
	C	x					
	D	x					
2	A		x	MATa		STE12	31
	B	x			x		
	C	x			x		
	D			MATa		ste12	32
3	A						
	B						
	C		x	MATa		STE12	33
	D				x		
4	A						
	B						
	C	x			x		
	D						
5	A	x			x		
	B			MATa		ste12	34
	C			MATa		ste12	35
	D	x			x		
6	A	x					
	B		x	MATa	x		36
	C		x	MATa	x		37
	D						
7	A						
	B		x	MATa	x		38
	C						
	D		x	MATa	x		39
8	A	x			x		
	B	x			x		
	C		x	MATa	x		40
	D		x	MATa	x		41
9	A		x	MATa	x		42
	B		x	MATa	x		43
	C	x			?		
	D				?		
10	A	x			?		
	B			MATa	?		44
	C		x	MATa	?		45
	D	x			x		
11	A		x	MATa	?		46
	B				?		
	C		x	MATa	x		47
	D	x			x		
12	A		x	MATa			
	B	x			x		
	C						
	D	x					
13	A						
	B						
	C						
	D		x	MATa	x		
14	A						
	B						
	C						
	D		x	MATa			
15	A		x	MATa		STE12	48
	B	x			x		
	C		x	MATa	x		49
	D						
16	A		x	MATa	x		50
	B		x	MATa	x		51
	C						
	D	x					
17	A	x			x		
	B		x	MATa	x		52
	C	x					
	D			MATa		ste12	53
18	A		x	MATa		STE12	54
	B			MATa		ste12	55
	C	x			x		
	D	x			x		
19	A	x			x		
	B		x	MATa	x		56
	C				x		
	D		x	MATa	x		57
20	A		x	MATa		STE12	58
	B		x	MATa	x		59
	C						
	D	x			x		
21	A						
	B		x	MATa	x		60
	C		x	MATa	x		61
	D				x		

yGIL642 x yGIL646							
		+a	+α	MATa	-U		
1	A		x	MATa	x		62
	B				x		
	C		x	MATa	x		63
	D	x					
2	A	x					
	B						
	C		x	MATa		STE12	64
	D						
3	A	x			x		
	B	x					
	C						
	D			MATa			
4	A		x	MATa	x		65
	B	x					
	C		x	MATa	x		66
	D	x					
5	A			MATa		ste12	67
	B	x					
	C	x			x		
	D			MATa	x		68
6	A						
	B						
	C				x		
	D						
7	A	x			x		
	B				x		
	C		x	MATa	x		69
	D						
8	A						
	B		x	MATa	x		
	C	x			x		
	D						
9	A		x	MATa	x		70
	B	x			x		
	C	x					
	D			MATa		ste12	71
10	A	x			x		
	B	x			x		
	C		x	MATa		STE12	72
	D			MATa		ste12	73
11	A	x					
	B						
	C		x	MATa	x		74
	D		x	MATa	x		75
12	A						
	B		x		x		
	C						
	D						
13	A	x			x		
	B		x	MATa	x		76
	C	x			x		
	D		x	MATa	x		77
14	A		x	MATa			
	B						
	C						
	D						
15	A						
	B	x			x		
	C	x					
	D				x		
16	A						
	B	x					
	C						
	D		x	MATa	x		78
17	A		x	MATa	x		79
	B	x			x		
	C				x		
	D		x	MATa		STE12	80
18	A						
	B						
	C	x					
	D		x	MATa	x		81 -
19	A						
	B	x					
	C						
	D						
20	A						
	B		x	MATa	x		82 -
	C	x					
	D						

NOTE: Some segregants grew weakly on 5FOA (indicated by "-"). See p. 65

6/12/12 PICKING SEGREGANTS CONT.

- Last night I picked 80 segregants (I omitted #s 81 & 82, p. 67).
- I also picked 8 clones each of the ancestor & BYS1-A08-1000 from plates in fridge.
  - Ancestor plate from 5/11/12
  - BYS1-A8-1000 plate from 4/27/12

NOTE: Cells were picked off of the 5FOA plates

- Plate setup:

1 → 10	Ancestor
11 → 20	
21 → 30	
31 → 40	
41 → 50	Evolved
51 → 60	
61 → 70	
71 → 80	

This morning, the Anc. & Evolved pellets looked more diffuse than the segregants. Old ~~the~~ colonies?

- I also set up a Ref. plate by picking a single colony of yGIL from a plate in fridge (5/11/12), inoculating 20 mL YPD and aliquoting 130 λ in each well of 96-well plate.

SETTING UP FITNESS ASSAYS

- I used the Biomek to write several programs for fitness assays.
  - "SetupCompetition" - mix Exp & Ref plate 50:50 (60 λ each)
  - "dispense" - dispense 125 λ YPD into 4 96-well plates and dispense 60 λ PBST into a 384-well plate
  - "transferAndFACS" - transfer 4 λ into a new YPD plate and transfer 4 λ into 384-well plate and inject air into 384-well plate
  - "transfer" - transfer 4 λ into 96-well plate.

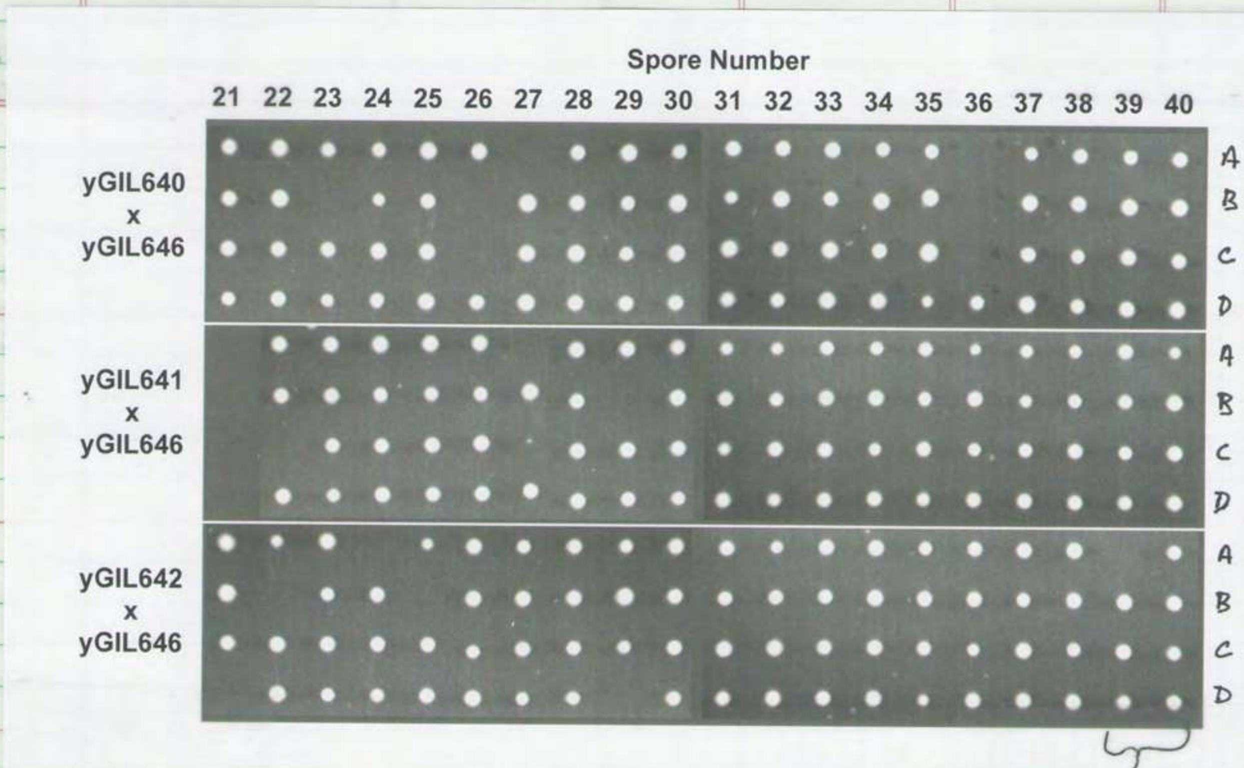
→ these programs were written for maintaining 4 plates.

- Dilutions @ 9am (I was aiming for 8:30).
- NOTE: I will do the competitions at the Big bottleneck (12 h dilutions). I debated this quite a bit. I think the fitnesses of the evolved alleles will be quite similar (if not identical) at both bottlenecks. For instance, ste12 is 1.5% at both bottlenecks (and in tubes on a roller drum.) In other words the physiology is the same — just different bottlenecks... and the smaller the bottleneck, the more error in measurement.

6/12/12

	Initials	Date
Prepared By		
Approved By		

SECOND ROUND OF TETRAD DISSECTION



NOTE: There was some confusion when I was dissecting the tetrads and I may have switched yGIL642×646 39D & 40D.

I will only be able to tell if I see 3:1 segregation of mating type. I might not be able to assign mating type on these tetrads.

Viable Spores

	4:0	3:1	2:2	1:3	TOTAL
yGIL640×646	16	2	1	1	20
" 641× "	16	2	1		19
" 642× "	14	6			20
Overall	46	10	2	1	59

INCLUDING 1st ROUND (p. 63)

Viable Spores

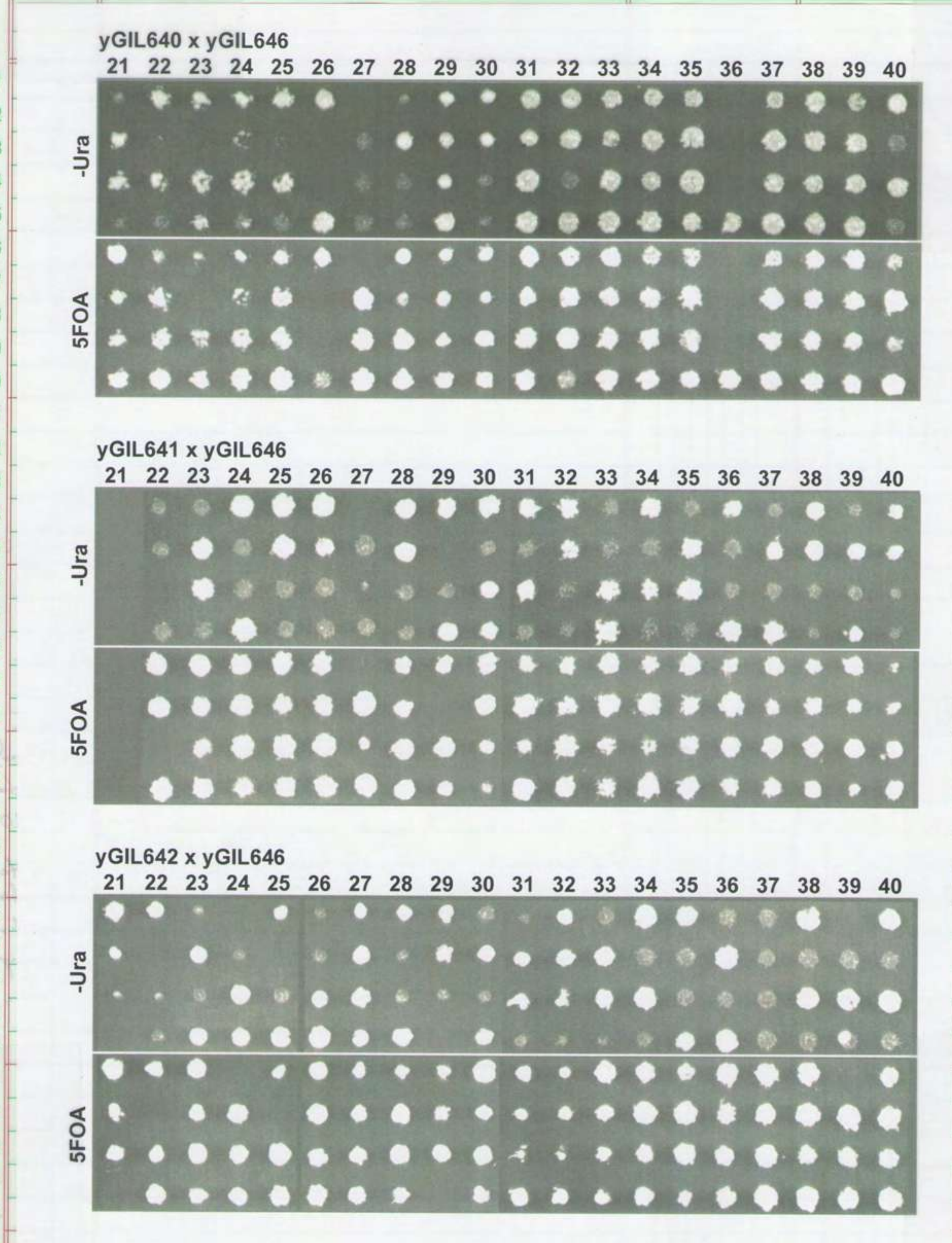
	4:0	3:1	2:2	1:3	TOTAL
yGIL640×646	32	5	2	1	40
" 641× "	33	4	3		40
" 642× "	27	9	3	1	40
Overall	92	18	8	2	

Is there something else segregating in the 642×646 cross?

• Yesterday I replica plated the dissection plates as before (p. 64)

6/12/12

PLASMID RETENTION IN SECOND ROUND OF DISSECTION



PLASMID LOSS RATE
26% (19/73)
52% (38/73)
46% (34/74)

6/15/12

PICKING SEGREGANTS - SECOND ROUND

yGIL640 x yGIL646

		+a	+α	MATa	-U		
21	A			MATa		ste12	81
	B	x			x		
	C	x			x		
	D		x	MATa		STE12	82
22	A				x		
	B						
	C	x			x		
	D		x	MATa		STE12	83
23	A	x			x		
	B						
	C	x			x		
	D		x	MATa	x		
24	A	x			x		
	B		x	MATa		STE12	84
	C	x			x		
	D		x	MATa		STE12	85
25	A	x			x		
	B		x	MATa		STE12	86
	C	x			x		
	D			MATa		ste12	87
26	A		x	MATa	x		
	B						
	C						
	D	x			x		
27	A						
	B						
	C						
	D						
28	A		x	MATa		STE12	88
	B	x			x		
	C						
	D						
29	A	x			x		
	B		x	MATa	x		89
	C		x	MATa	x		90
	D	x			x		
30	A		x	MATa	x		91
	B	x			x		
	C	x					
	D			MATa		ste12	92
31	A		x	MATa	x		93
	B		x	MATa	x		94
	C	x			x		
	D	x			x		
32	A		x		x		
	B		x		x		
	C						
	D		x		x		
33	A		x	MATa	x		95
	B		x	MATa	x		96
	C	x			x		
	D	x			x		
34	A		x	MATa	x		97
	B	x			x		
	C		x	MATa	x		98
	D	x			x		
35	A		x	MATa	x		99
	B	x			x		
	C				x		
	D		x	MATa	x		100
36	A						
	B						
	C						
	D		x	MATa	x		
37	A		x	MATa	x		101
	B	x			x		
	C		x	MATa	x		102
	D	x			x		
38	A	x			x		
	B	x			x		
	C		x	MATa	x		103
	D		x	MATa	x		104
39	A		x	MATa	x		105
	B	x			x		
	C	x			x		
	D		x	MATa	x		106
40	A	x			x		
	B						
	C		x	MATa	x		107
	D						

yGIL641 x yGIL646

		+a	+α	MATa	-U		
22	A						
	B		x	MATa			
	C						
	D						
23	A		x	MATa		STE12	108
	B				x		
	C		x	MATa	x		109
	D						
24	A				x		
	B		x	MATa		STE12	110
	C						
	D	x			x		
25	A	x		MATa	x		111
	B	x		MATa	x		112
	C						
	D						
26	A	x			x		
	B		x	MATa	x		113
	C		x	MATa		STE12	114
	D						
27	A						
	B						
	C						
	D		x				
28	A	x			x		
	B		x	MATa	x		115
	C			MATa		STE12	116
	D	x					
29	A	x			x		
	B						
	C						
	D		x		x		
30	A	x			x		
	B						
	C		x	MATa	x		117
	D		x	MATa	x		118
31	A	x			x		
	B		x	MATa		STE12	119
	C	x			x		
	D			MATa		ste12	120
32	A	x			x		
	B		x	MATa	x		121
	C						
	D						
33	A		x	MATa		STE12	122
	B			MATa		ste12	123
	C	x			x		
	D	x			x		
34	A		x	MATa	x		124
	B	x					
	C		x	MATa	x		
	D						
35	A			MATa		ste12	125
	B		x	MATa	x	STE12	126
	C	x			x		
	D	x					
36	A		x	MATa	x	ste12	127
	B	x					
	C		x	MATa		STE12	128
	D	x			x		
37	A			MATa		ste12	129
	B	x			x		
	C		x	MATa		STE12	130
	D	x			x		
38	A		x	MATa	x		131
	B		x	MATa	x		132
	C	x					
	D						
39	A			MATa		ste12	133
	B	x			x		
	C		x	MATa		STE12	134
	D	x			x		
40	A	x			x		
	B				x		
	C		x	MATa		ste12	135
	D						

yGIL642 x yGIL646

		+a	+α	MATa	-U		
21	A				x		
	B				x		
	C				x		
	D						
22	A	x		MATa	x		
	B						
	C						
	D	x		MATa			
23	A	x					
	B		x	MATa	x	ste12	136
	C	x					
	D		x	MATa	x	ste12	137
24	A						
	B						
	C		x	MATa	x		
	D	x			x		
25	A	x			x		
	B						
	C		x	MATa			
	D	x			x		
26	A			MATa		ste12	138
	B	x					
	C		x	MATa	x		139
	D	x			x		
27	A		x	MATa	x		140
	B	x			x		
	C	x			x		
	D		x	MATa	x		141
28	A	x			x		
	B			MATa		ste12	142
	C		x	MATa		STE12	143
	D	x			x		
29	A	x		MATa	x		
	B				x		
	C						
	D						
30	A			MATa		ste12	144
	B	x			x		
	C		x	MATa		STE12	145
	D	x			x		
31	A	x					
	B		x	MATa	x		146
	C	x			x		
	D		x	MATa		STE12	147
32	A	x			x		
	B		x	MATa	x		148
	C				x		
	D						
33	A						
	B		x	MATa	x		149
	C	x			x		
	D		x	MATa		STE12	150
34	A		x	MATa	x	STE12	151
	B						
	C	x			x		
	D						
35	A		x	MATa	x		152
	B	x					
	C			MATa		ste12	153
	D	x			x		
36	A						
	B	x			x		
	C		x	MATa		STE12	154
	D		x	MATa	x		155
37	A						
	B		x	MATa		STE12	156
	C	x					
	D						
38	A	x			x		
	B	x					
	C		x	MATa	x		157
	D			MATa		ste12	158
39	A						
	B		x	MATa			
	C		x	MATa	x		
	D	x			x		
40	A	x			x		
	B			MATa		ste12	159
	C	x			x		
	D		x	MATa		STE12	160

6/15/12 PICKING SEGREGANTS – 2nd ROUND

- On ~~6/14/12~~ 6/14 (AM) I picked segregants from the second tetrad dissections — #s ~~[illegible]~~ 81–160 (p. 71).
- Yesterday I made 4 copies of this plate w/ the Bioma ~ 8pm

→ Setup similar to Plate 1 (p. 68). The Anc. & Evo. are 8 independent colonies, different from plate 1, but picked off the same YPD plates.

↳ NOTE: For both Plate 1 & 2 the BYS1-A08 evolved colonies are from Generation 545 (see p. 47). — most, but not all, will have the ste12* mutation ⇒ I could mating-type test them.

- On 6/13 I made 4 copies of Plate 1 (which was in fridge)
- This morning I froze down a copy of Plates 1 & 2.

6/15/12

	Initials	Date
Prepared By		
Approved By		

TESTING SNP-SPECIFIC PCR

SNP-Specific primers:

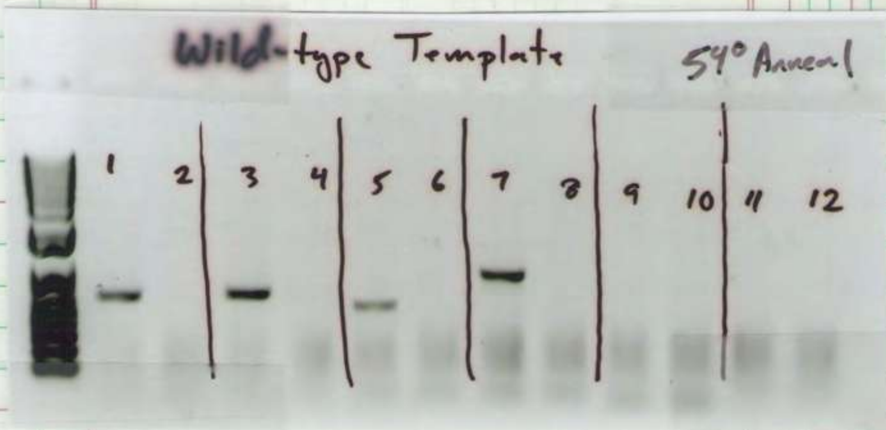
		Tm		LANE (next page)
oGIL352	STE12_WT_Reverse	54.6	ATTGGTGTTGTTTAGTTTGC	
oGIL382	STE12_WT_Forward	54.8	AATCTCACAACTCTGGCCAG	1
oGIL383	STE12_Mutant_Forward	52.1	AAATCTCACAACTCTGCCAA	2
oGIL351	STE12_EvoForward	48.5	ACAAAATCAGATAATTCGCC	
oGIL384	STE12_WT_Reverse	49.4	ATTGGTGTTGTTTAGTTTGC	3
oGIL385	STE12_Mutant_Reverse	47.9	ATTGGTGTTGTTTAGTTTGT	4
oGIL360	GAS1_EvoReverse	56.7	GAACACAAGCCTCCGTTTGG	
oGIL386	GAS1_WT_Forward	45.5	AAGAACTACAGAAAAATTCC	5
oGIL387	GAS1_Mutant_Forward	44.3	AAGAACTACAGAAAAATTCT	6
oGIL359	GAS1_EvoForward	59.6	TTTTCGTGCCGCAAACGTGG	
oGIL388	GAS1_WT_Reverse	52.2	ATTGGAAGAGTAGCCAACTG	7
oGIL389	GAS1_Mutant_Reverse	50.5	ATTGGAAGAGTAGCCAACTA	8
oGIL356	ELO1_EvoReverse	55.5	GGTGTGACACTAGAATCGCC	
oGIL390	ELO1_WT_Forward	54.1	TGCTACAGCTTTACTGTGCT	9
oGIL391	ELO1_Mutant_Forward	55.2	TGCTACAGCTTTACTGTGCC	10
oGIL355	ELO1_EvoForward	54.7	AACACAACAAATCGCAAGCC	
oGIL392	ELO1_WT_Reverse	43.9	TAACCAACCAATTGATTATA	11
oGIL393	ELO1_Mutant_Reverse	45.6	TAACCAACCAATTGATTATG	12

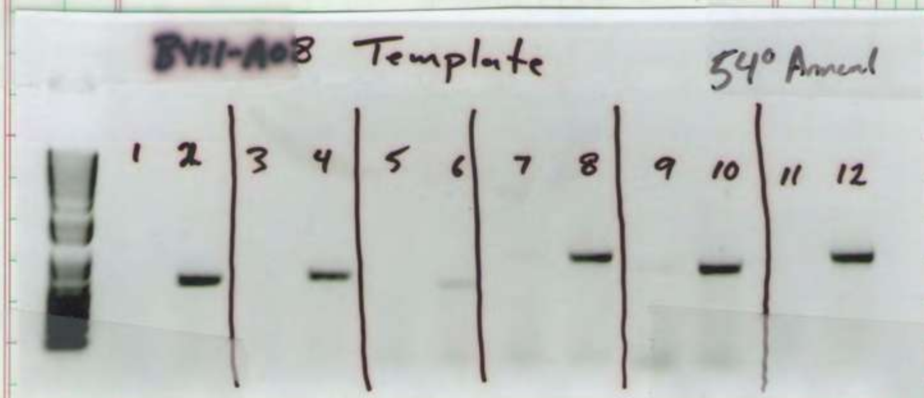
- Test these primers using standard Taq Protocol w/ 54° Annealing temp.

6/15/12

RESULTS OF SNP PCRs

· Each #'d lane is a unique primer pair (see previous page).





Overall pretty good — I have two sets of primers for distinguishing STE12 & ste12* (1/2 & 3/4) and one set for GAS1 & gas1* (7/8). I have positive primers for elo1* (10 & 12) but neither ELO1 set worked.

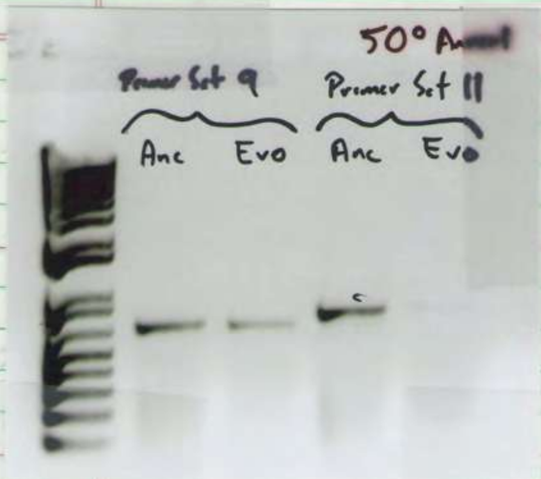
→ I could try dropping the annealing temp., but Primer 9 39° has a reasonable temp.

6/16/12

	Initials	Date
Prepared By		
Approved By		

SNP- PCRs CONTINUED

Rerun EL01 primer sets 9 & 11 w/ 50° Annealing temp.



- @ 54° no products were formed, but at 50° both the Anc. & Evo alleles are amplified w/ Primer Set 9.
- Primer Set 11 shows a good discrimination

I will use Primer Sets:

- 1 & 2 for STE12/ste12*
- 7 & 8 for GAS1/gas1*
- 11 & 12 for ELO1/elo1*

6/26/12 SNP PCR ON SEGREGANTS

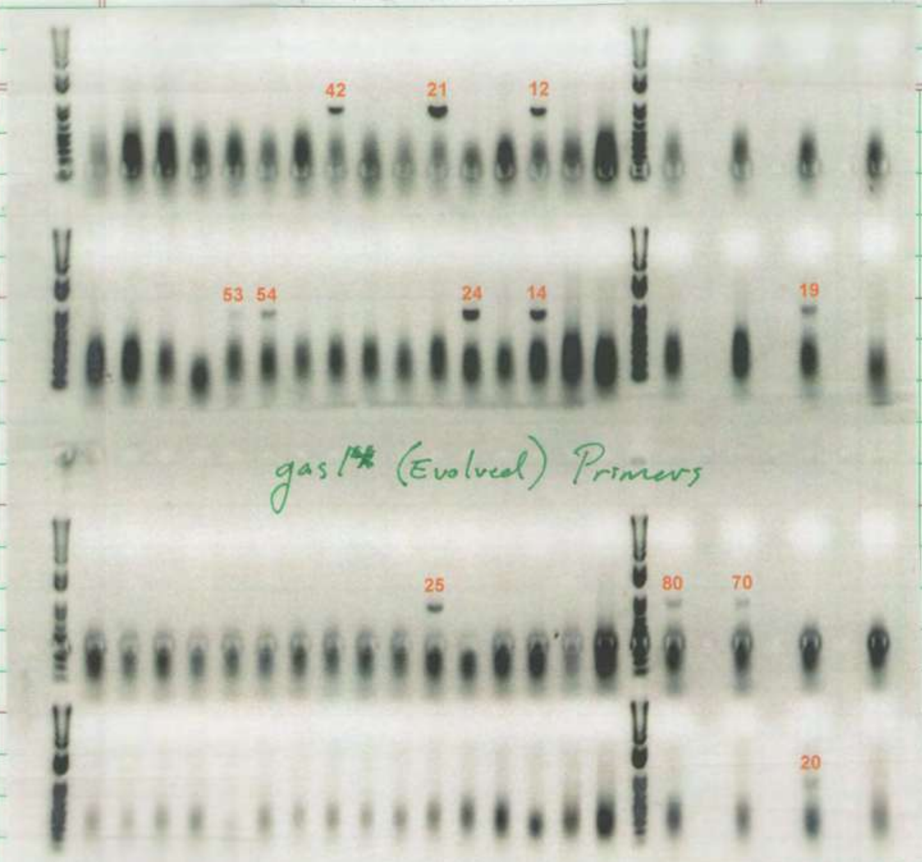
- Last week I made gDNA preps for all 160 BYS1-A08 backcross segregants using a modification of the standard protocol.
  - pool 3 wells of segregant (~ 400 λ) and add to 1 mL H_2O, spin, aspirate, follow std. prep.
- Scaled down to 20λ PCRs for 96-well plates:

H_2O	6.2	(1240)	← (100×)
10× Buffer	2	(400)	
dNTPs	0.6	(120)	
Primer 1 (359)	0.4	(80)	
Primer 2 (389)	0.4	(80)	
Template	10	← 1:20 dilution, added by robot	
PafTaq	0.4	(80)	
Total	20		

- I tested the PCR program using the Evolved-allele primer set for gas1 (Primers 359 & 389, p. 73)
  - I used the old PCRX thermal cycler for Plate 1-80 and PX2 for 81-160.
  - Neither seal was on as well as it should have been → leakage, especially around edges.

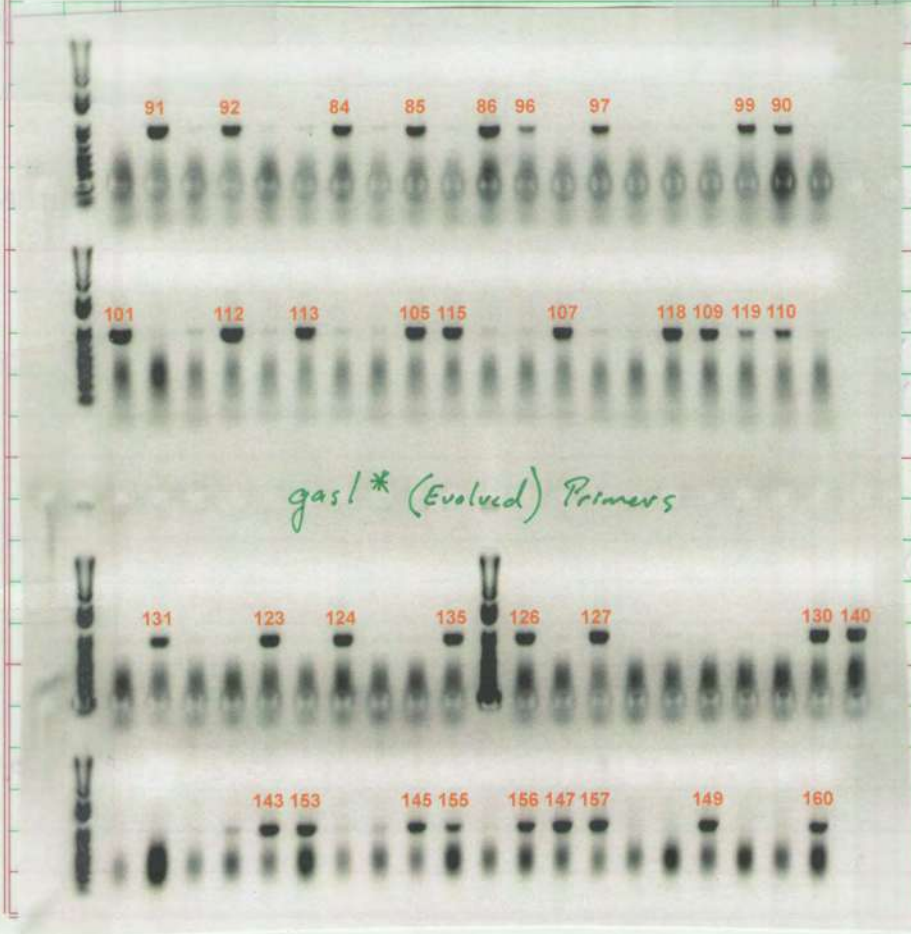
6/26/12

SNP GENOTYPING CONTINUED



Row 1	71	72	61	62	51	52	41	42	31	32	21	22	11	12	1	2	79	69	59	49
Row 2	73	74	63	64	53	54	43	44	33	34	23	24	13	14	3	4	39	29	19	9
Row 3	75	76	65	66	55	56	45	46	35	36	25	26	15	16	5	6	80	70	60	50
Row4	77	78	67	68	57	58	47	48	37	38	27	28	17	18	7	8	40	30	20	10

I was expecting more positive hits (only 12; expected ~40)

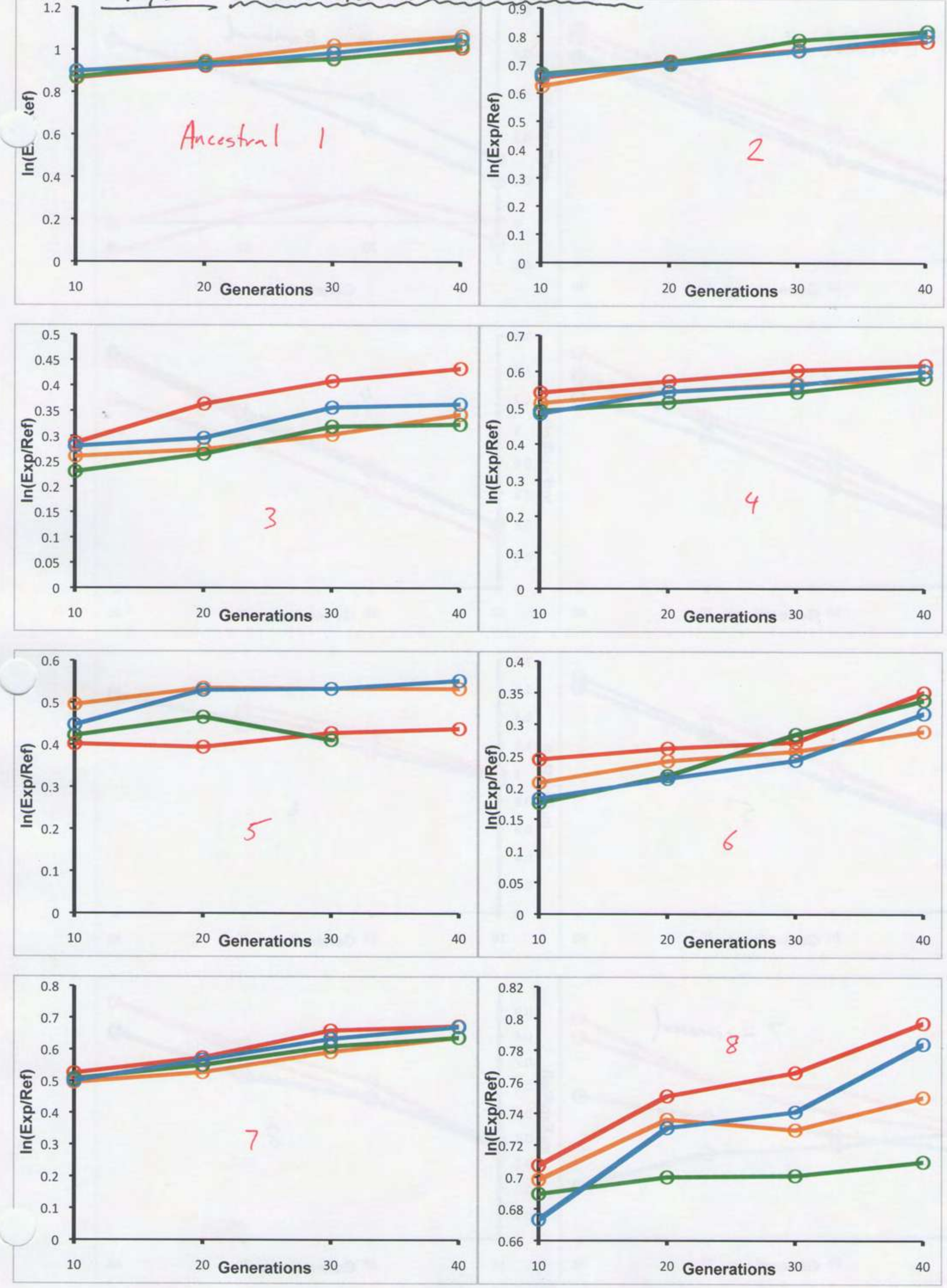


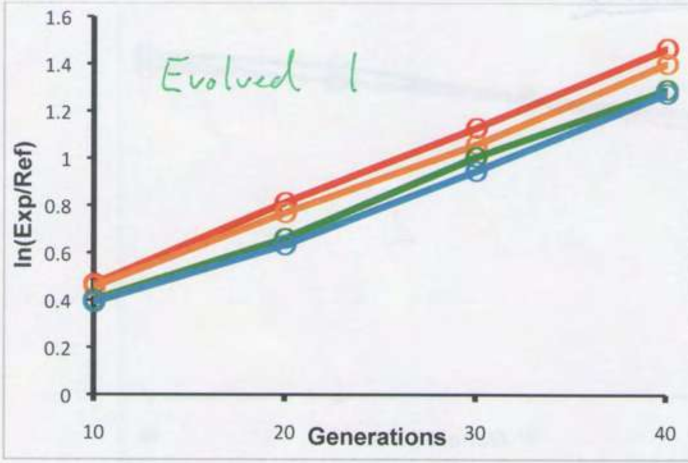
Row 1	81	91	82	92	83	93	84	94	85	95	86	96	87	97	88	98	89	99	90	100
Row 2	101	111	102	112	103	113	104	114	105	115	106	116	107	117	108	118	109	119	110	120
Row 3	121	131	122	132	123	133	124	134	125	135	126	136	127	137	128	138	129	139	130	140
Row4	141	151	142	152	143	153	144	154	145	155	146	156	147	157	148	158	149	159	150	160

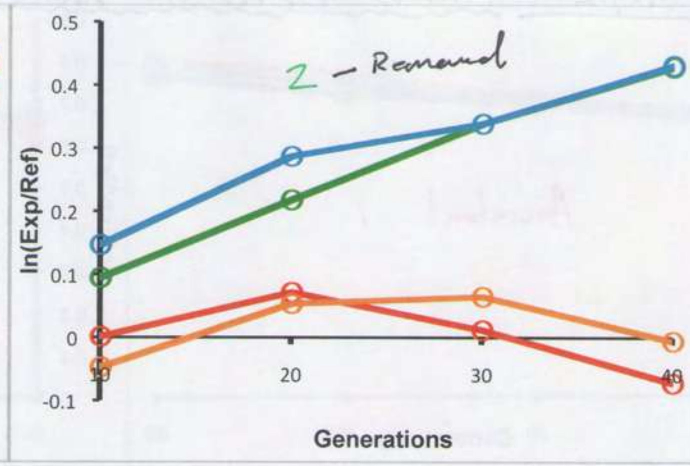
Looks good (36 hits, expected ~60)

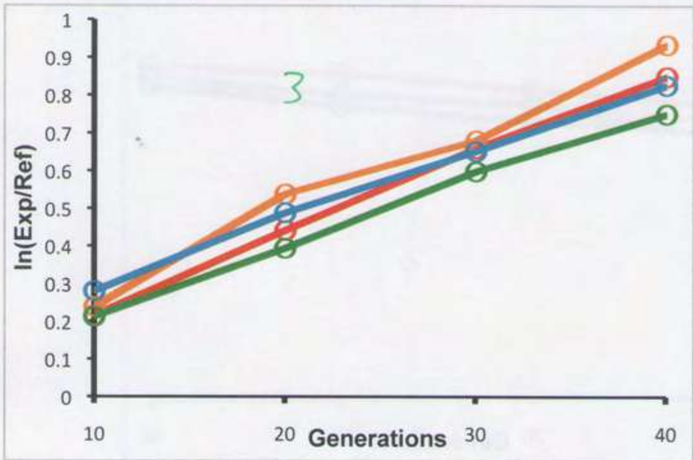
some still ambiguous (leakage?)
Running WT primers will clarify.

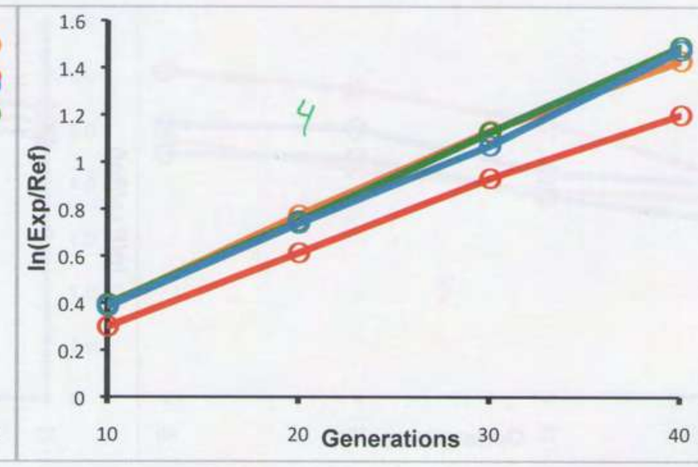
6/20/12 → FITNESS ASSAYS ON SEGREGANTS

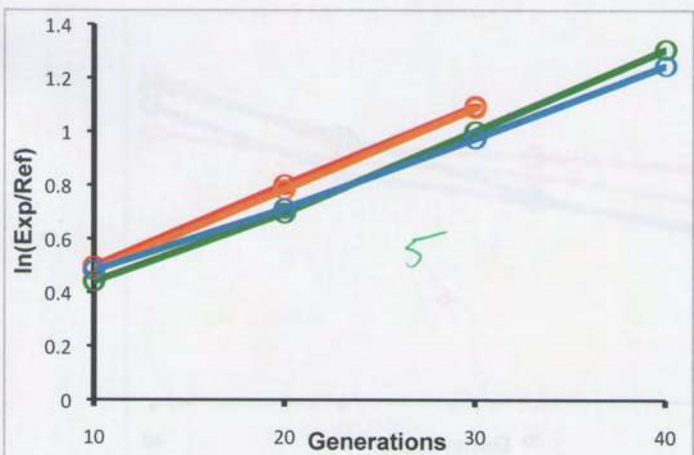


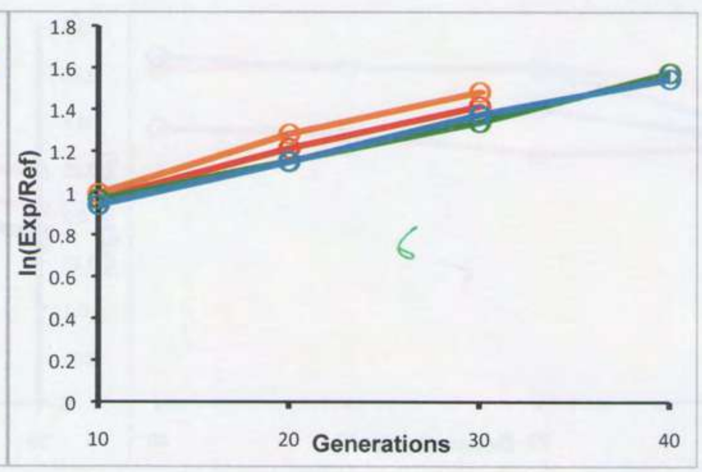

Evolved 1
ln(Exp/Ref)
Generations
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
10
20
30
40


2 – Remaned
ln(Exp/Ref)
Generations
-0.1
0
0.1
0.2
0.3
0.4
0.5
10
20
30
40

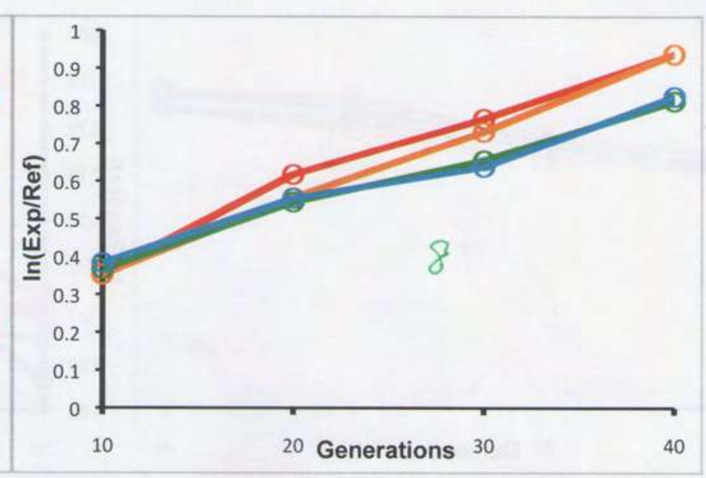

3
ln(Exp/Ref)
Generations
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
10
20
30
40

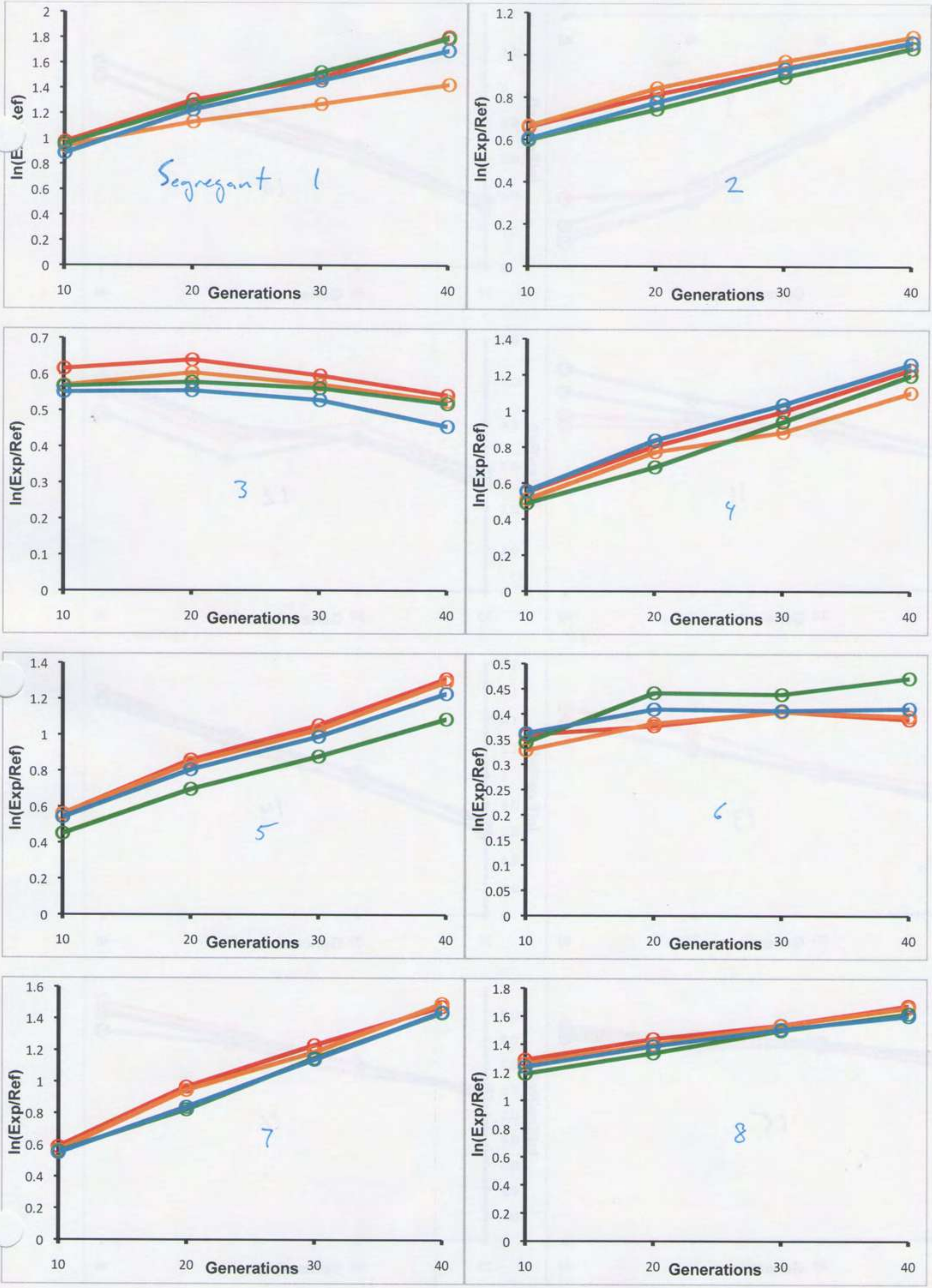

4
ln(Exp/Ref)
Generations
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
10
20
30
40

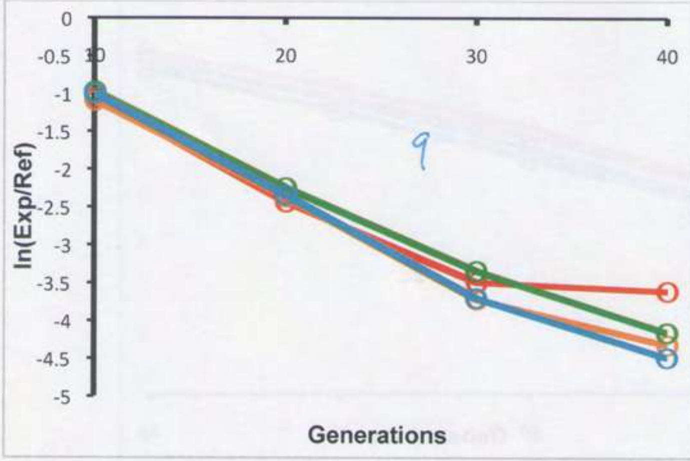

5
ln(Exp/Ref)
Generations
0
0.2
0.4
0.6
0.8
1
1.2
1.4
10
20
30
40

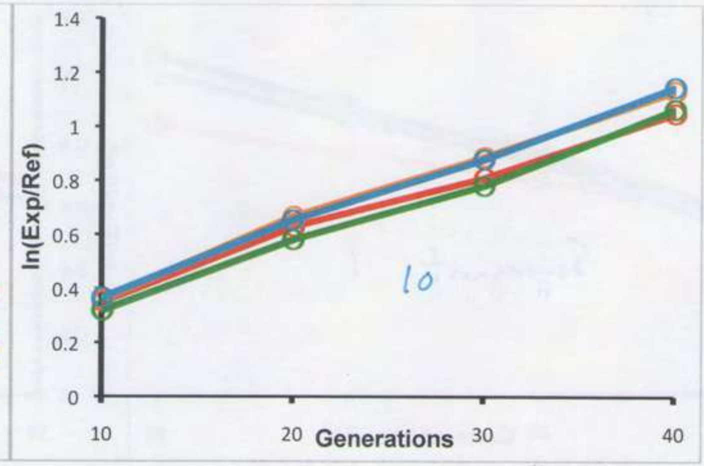

6
ln(Exp/Ref)
Generations
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
10
20
30
40

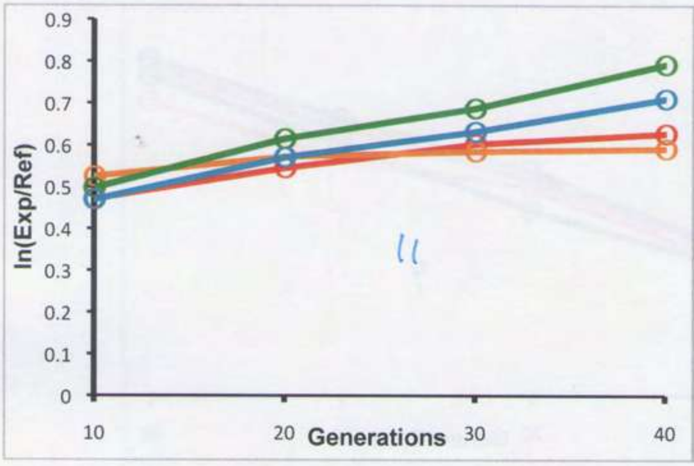

7 – remared
ln(Exp/Ref)
Generations
0
0.1
0.2
0.3
0.4
0.5
0.6
10
20
30
40

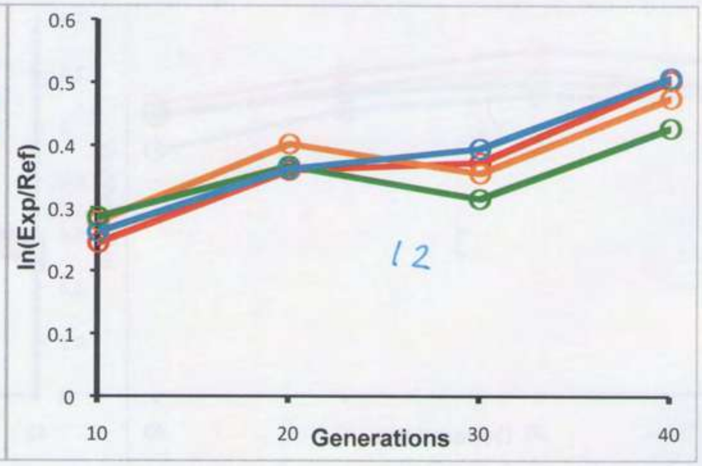

8
ln(Exp/Ref)
Generations
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
10
20
30
40

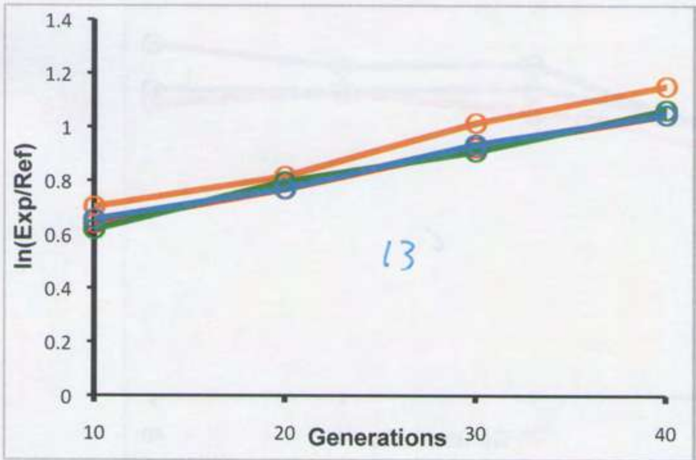

Segregant 1
2
3
4
5
6
7
8
ln(Exp/Ref)
Generations
10
20
30
40

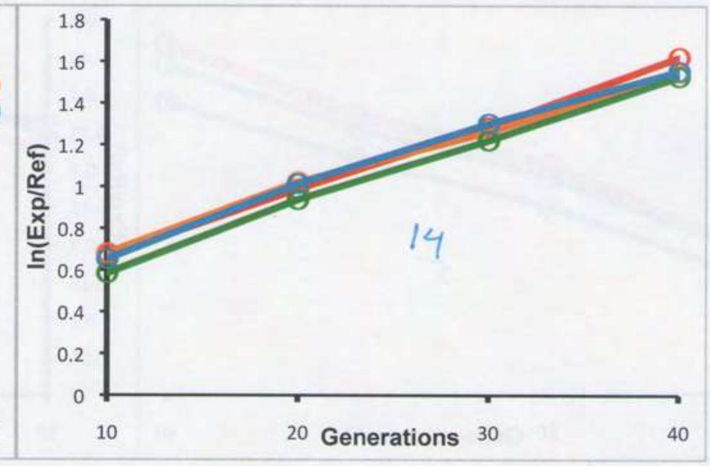

9
ln(Exp/Ref)
Generations
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
10
20
30
40

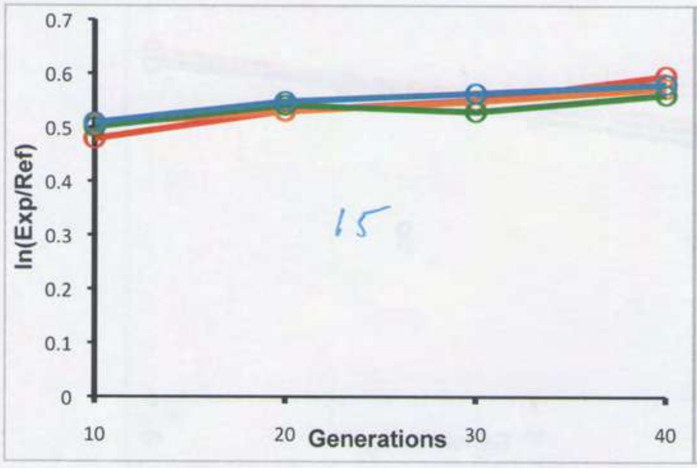

10
ln(Exp/Ref)
Generations
0
0.2
0.4
0.6
0.8
1
1.2
1.4
10
20
30
40

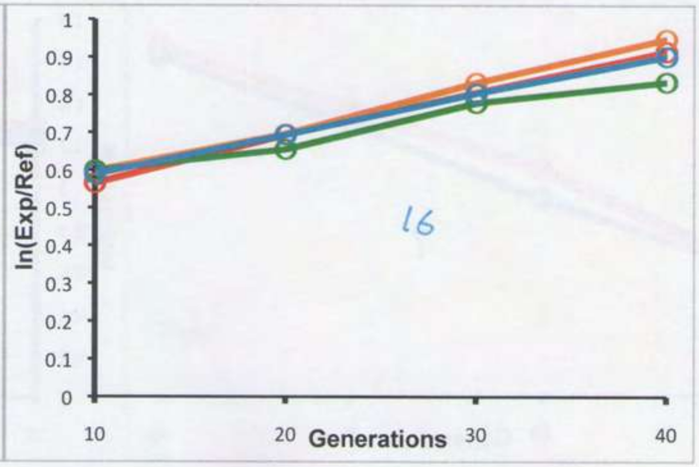

11
ln(Exp/Ref)
Generations
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
10
20
30
40

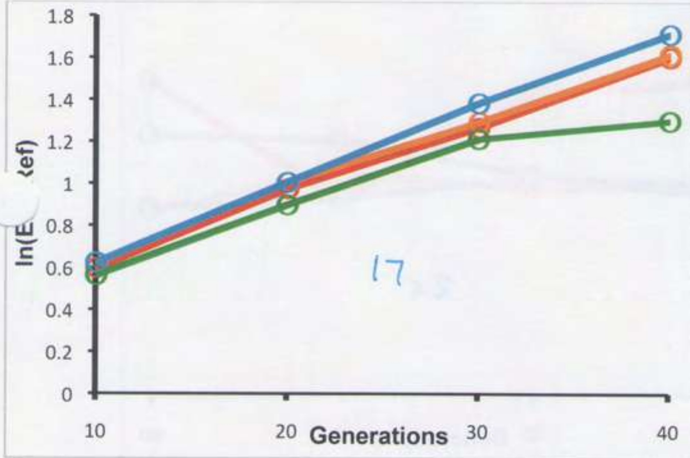

12
ln(Exp/Ref)
Generations
0
0.1
0.2
0.3
0.4
0.5
0.6
10
20
30
40

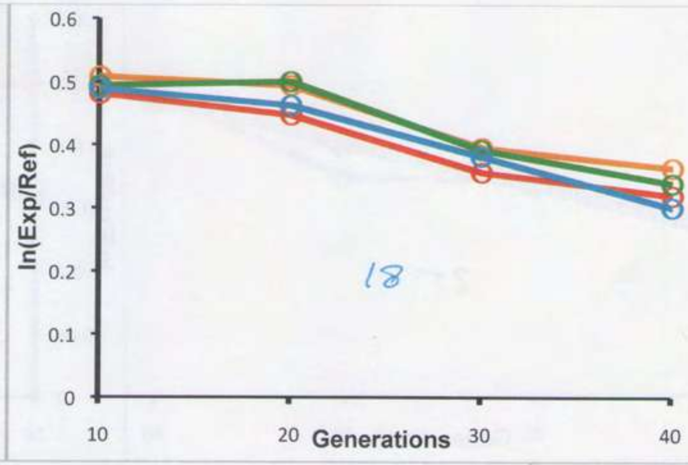

13
ln(Exp/Ref)
Generations
0
0.2
0.4
0.6
0.8
1
1.2
1.4
10
20
30
40

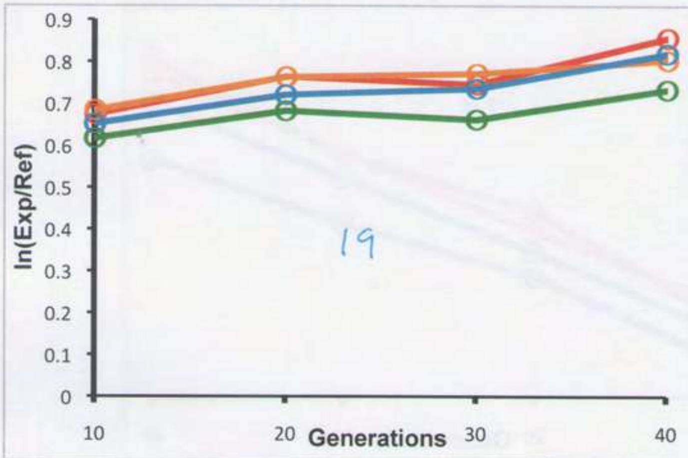

14
ln(Exp/Ref)
Generations
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
10
20
30
40

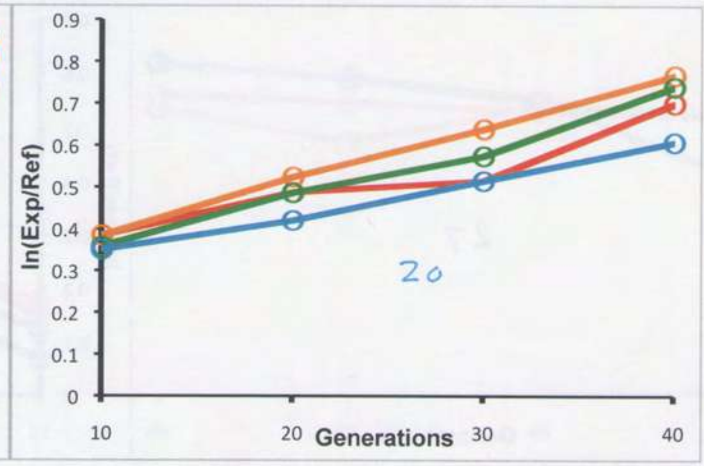

15
ln(Exp/Ref)
Generations
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
10
20
30
40


16
ln(Exp/Ref)
Generations
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
10
20
30
40


ln(Exp/Ref)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
10
20
30
40
Generations
17


ln(Exp/Ref)
0.6
0.5
0.4
0.3
0.2
0.1
0
10
20
30
40
Generations
18


ln(Exp/Ref)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10
20
30
40
Generations
19


ln(Exp/Ref)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10
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30
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Generations
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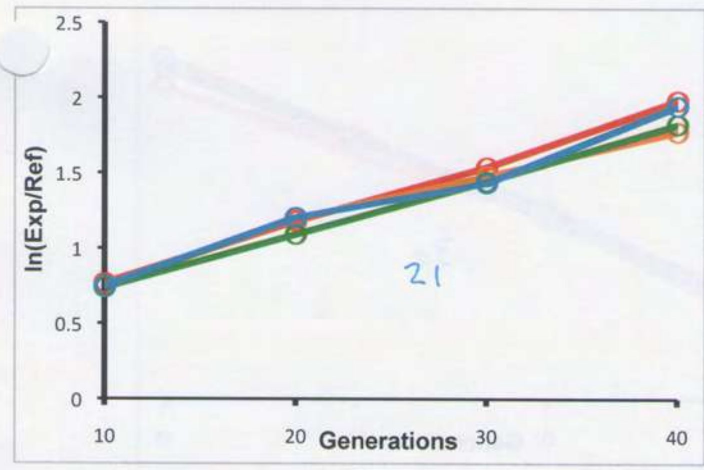
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Generations
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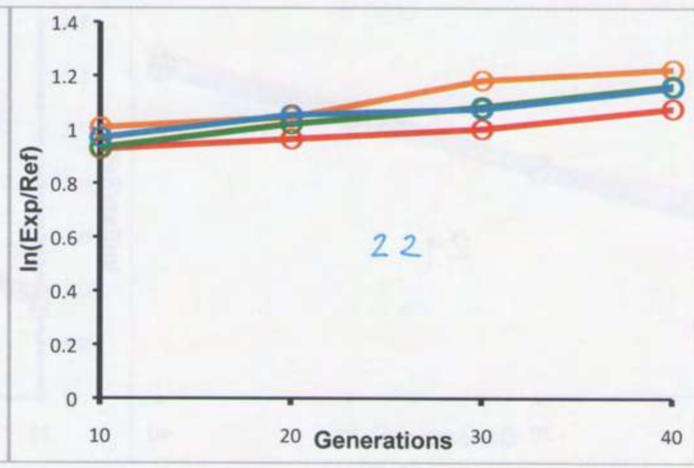
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0.2
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20
30
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Generations
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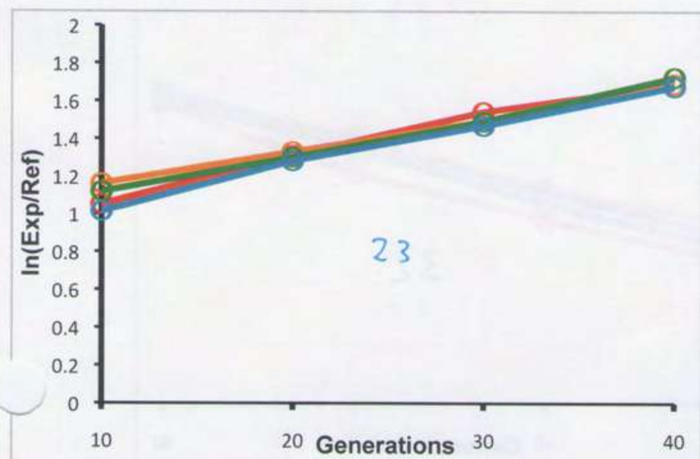
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1.8
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0.4
0.2
0
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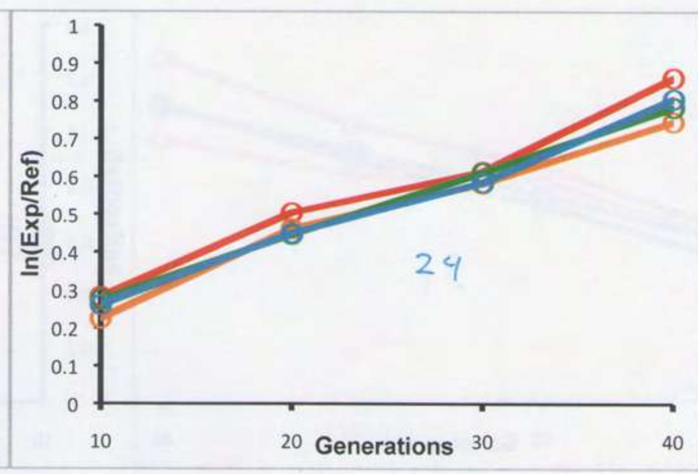
20
30
40
Generations
23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0.1
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20
30
40
Generations
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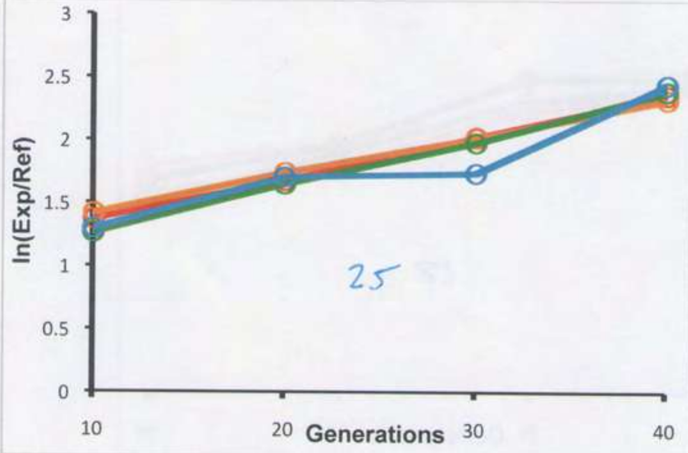
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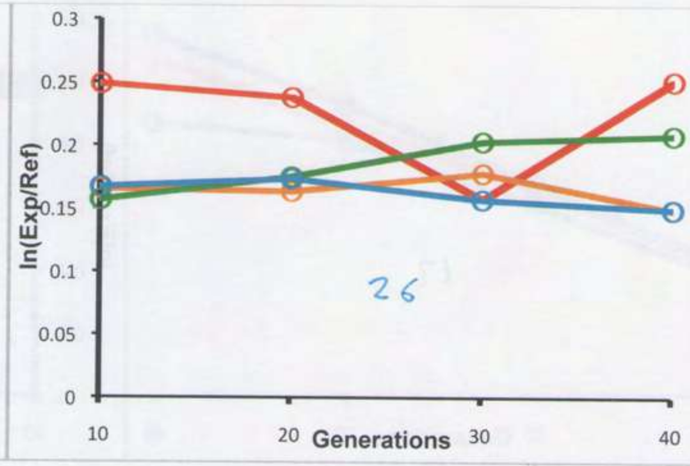
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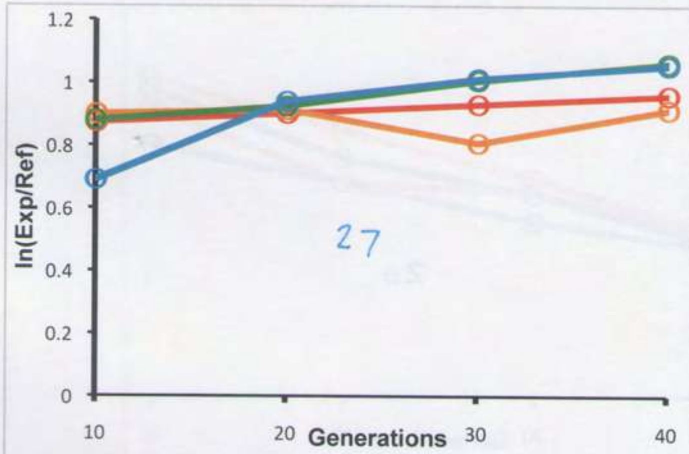
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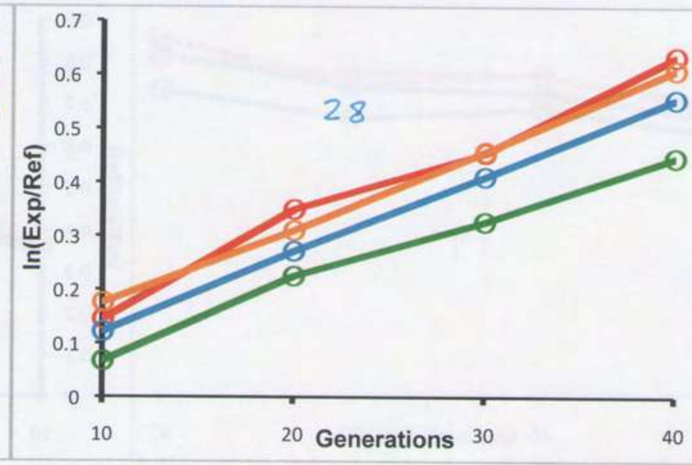
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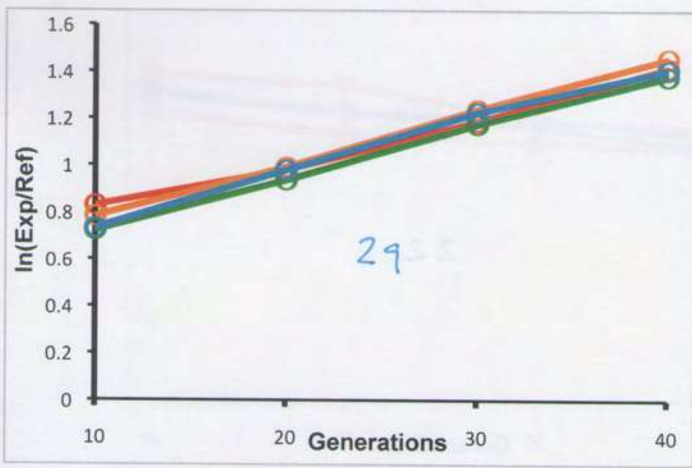
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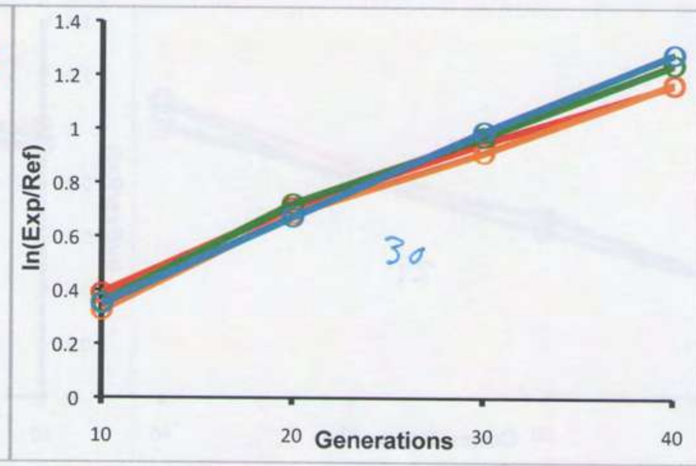
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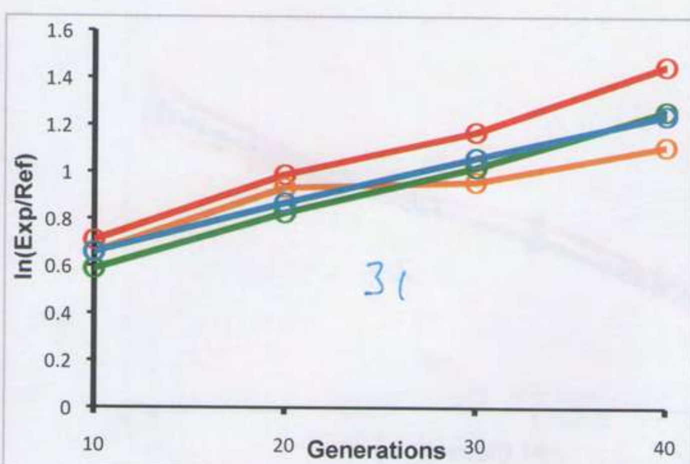
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Generations
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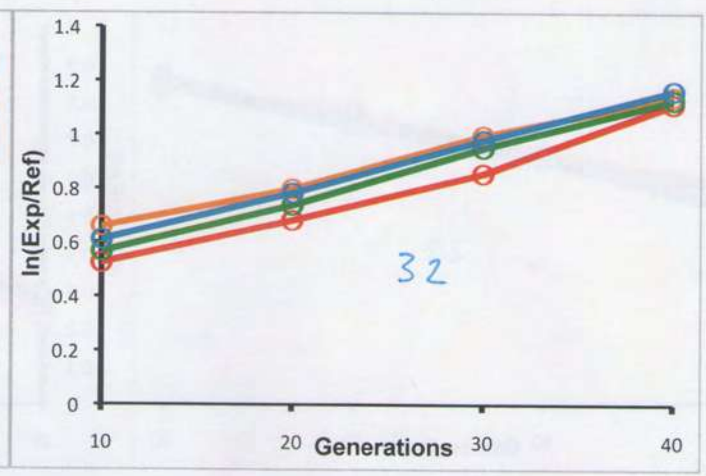
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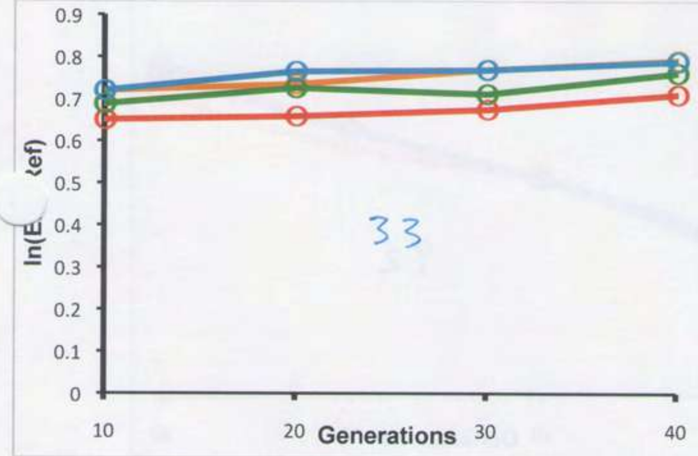
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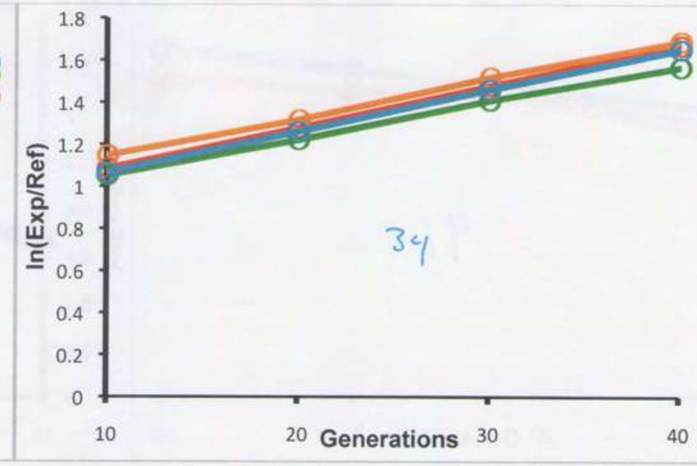
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Generations
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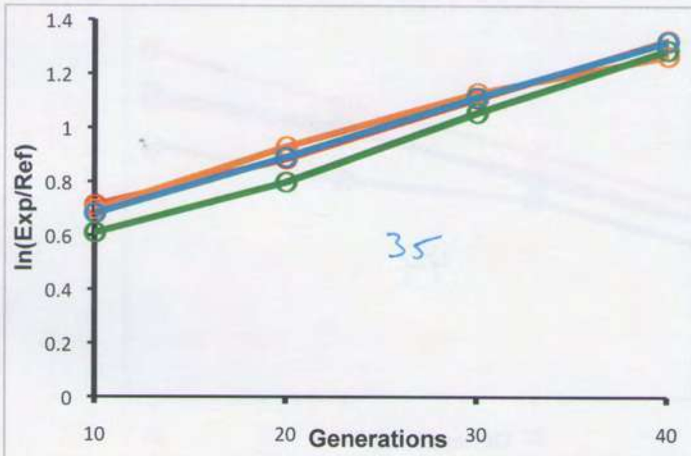
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Generations
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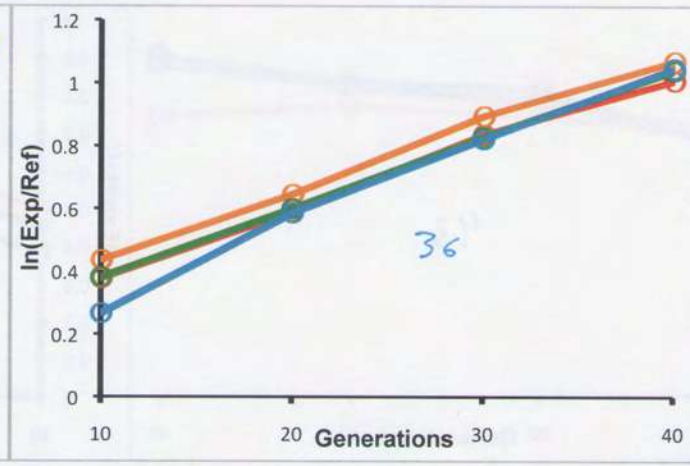
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Generations
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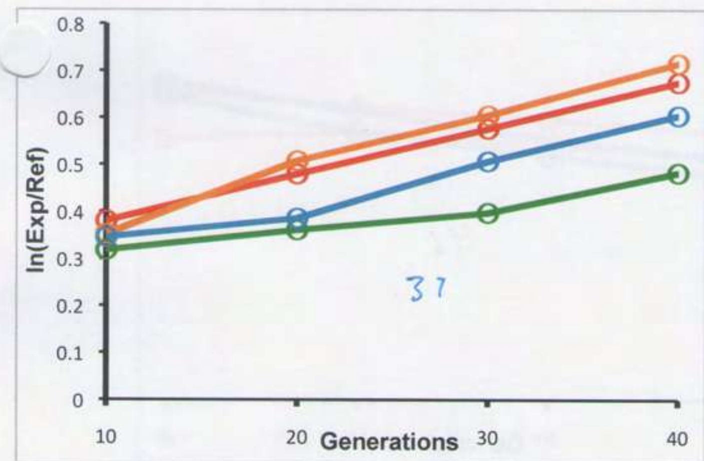
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Generations
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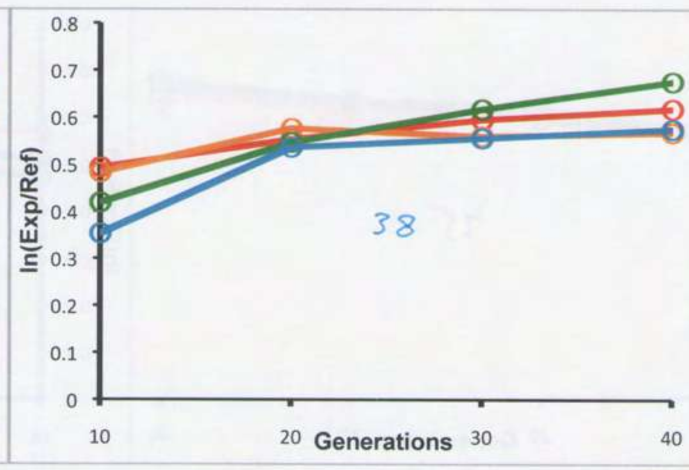
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Generations
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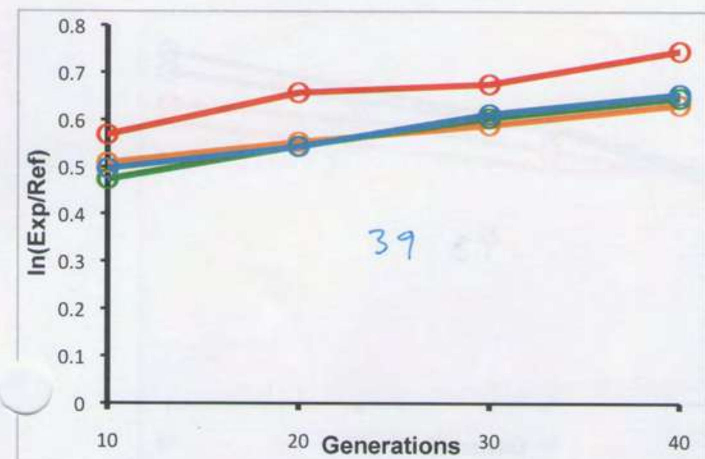
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Generations
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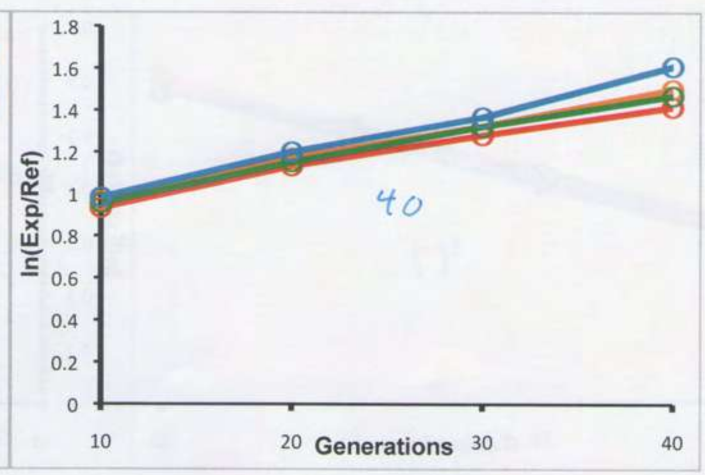
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Generations
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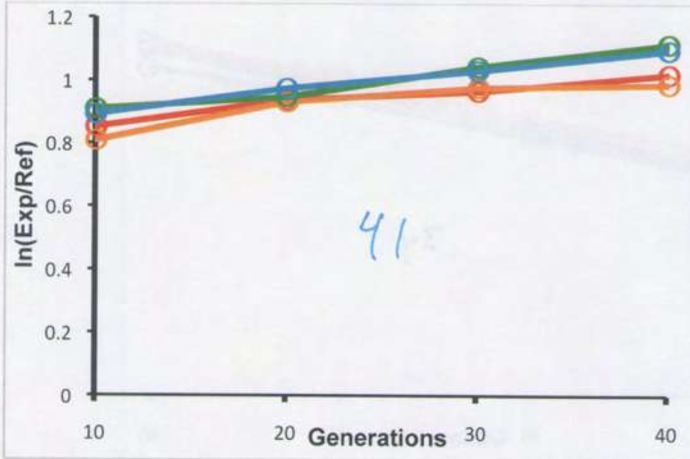
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10 20 30 40
Generations
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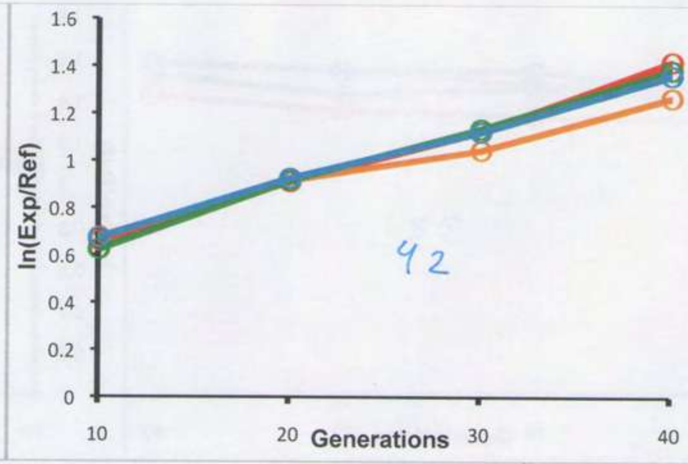
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10 20 30 40
Generations
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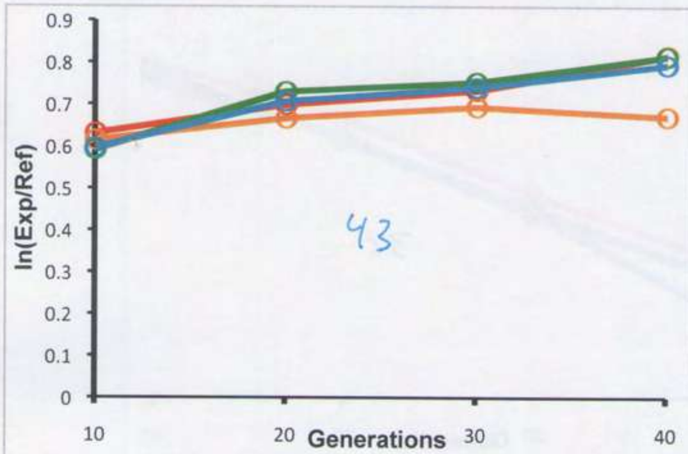
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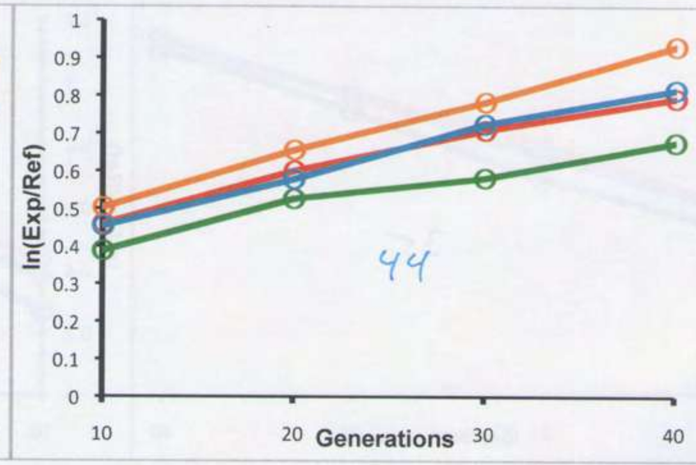
10 20 30 40
Generations
43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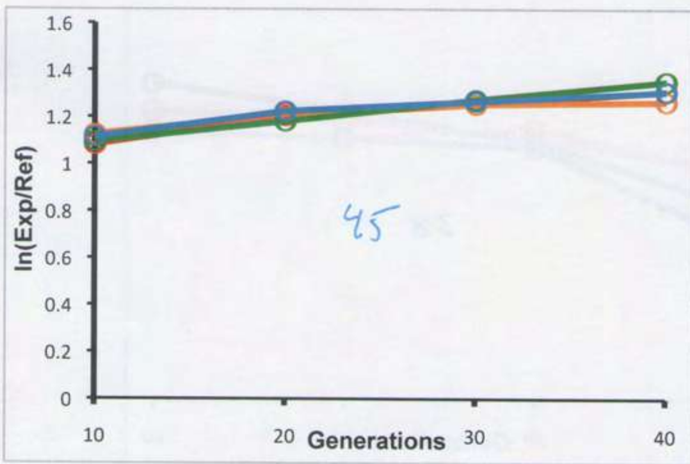
10 20 30 40
Generations
44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10 20 30 40
Generations
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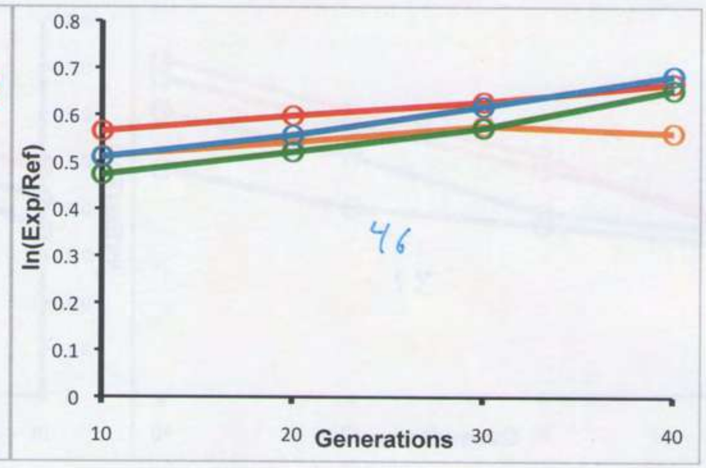
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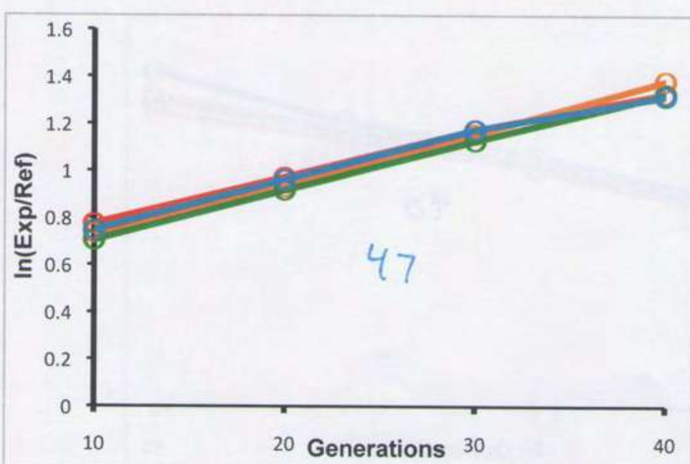
10 20 30 40
Generations
46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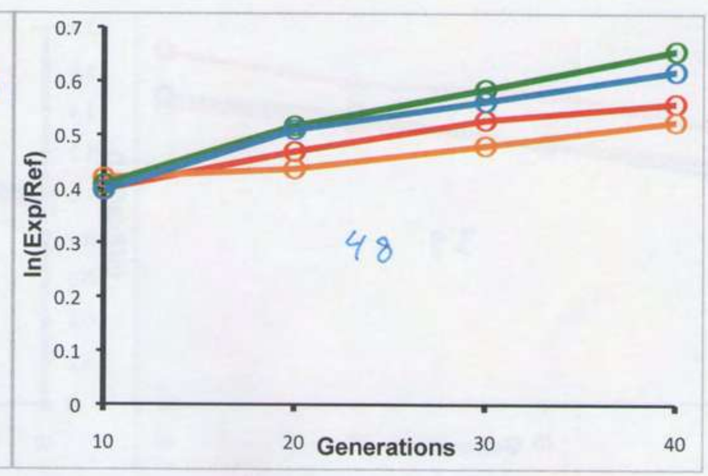
10 20 30 40
Generations
47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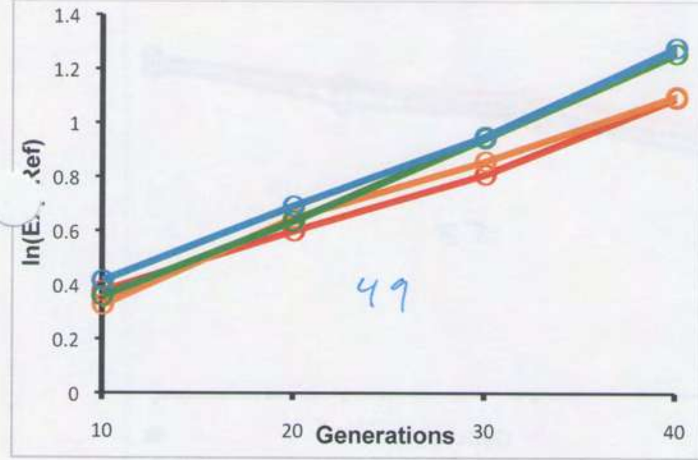
Generations
48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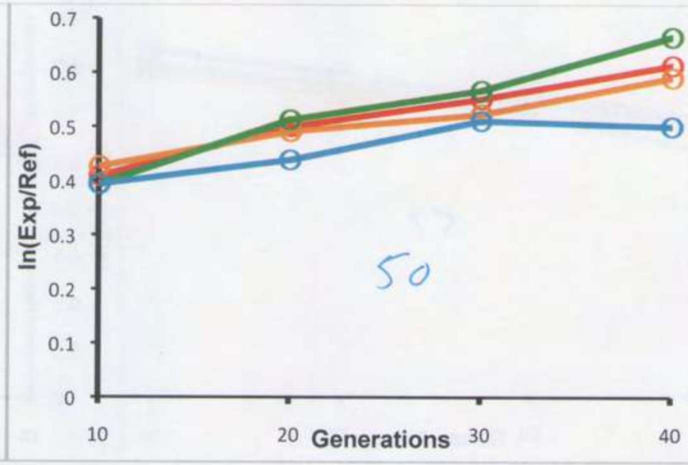
1.2
1
0.8
0.6
0.4
0.2
0
10
20
Generations
30
40
49

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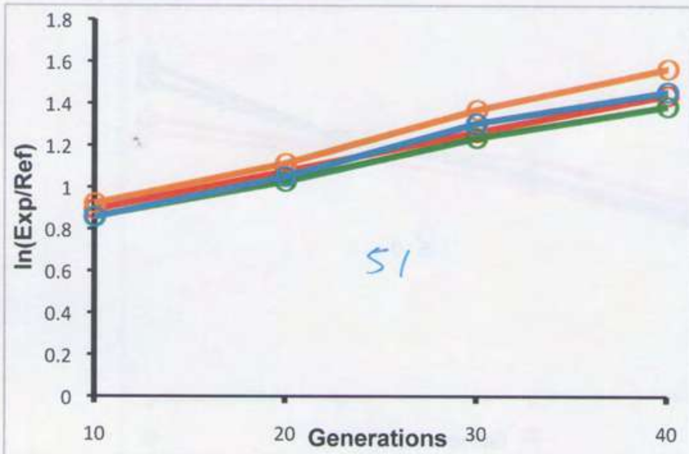
0.2
0.1
0
ln(Exp/Ref)
10
20
Generations
30
40
50

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1.6
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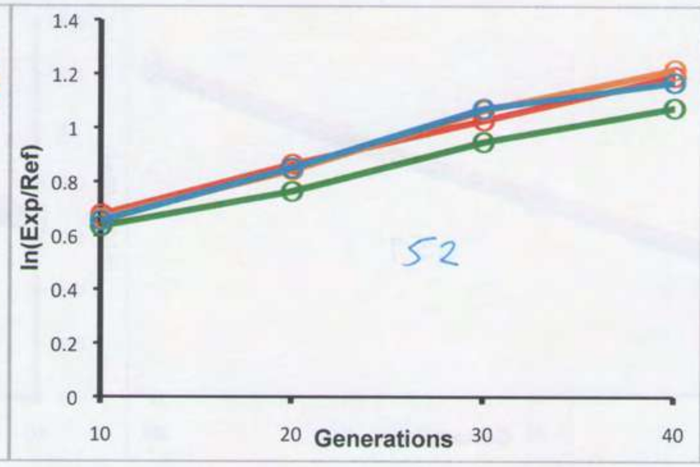
0.4
0.2
0
ln(Exp/Ref)
10
20
Generations
30
40
51

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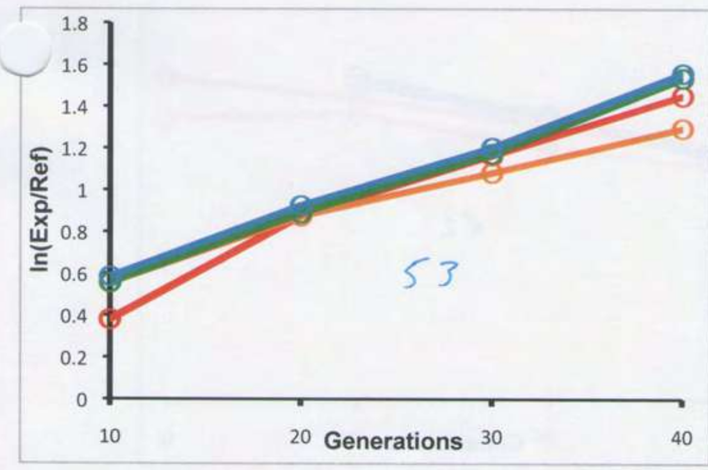
0.6
0.4
0.2
0
ln(Exp/Ref)
10
20
Generations
30
40
52

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1.6
1.4
1.2
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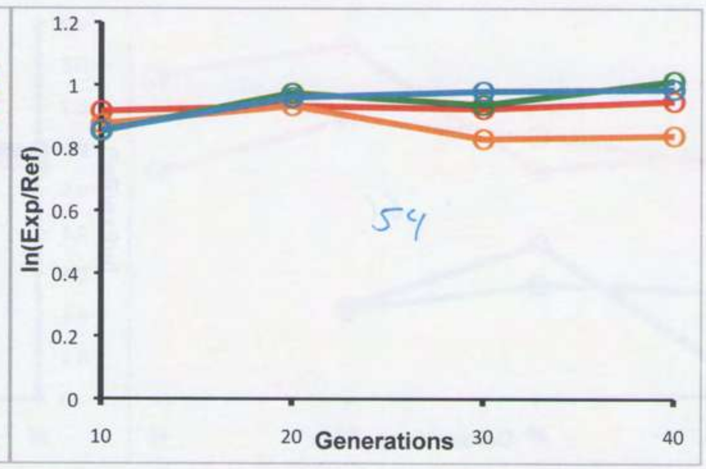
0.8
0.6
0.4
0.2
0
ln(Exp/Ref)
10
20
Generations
30
40
53

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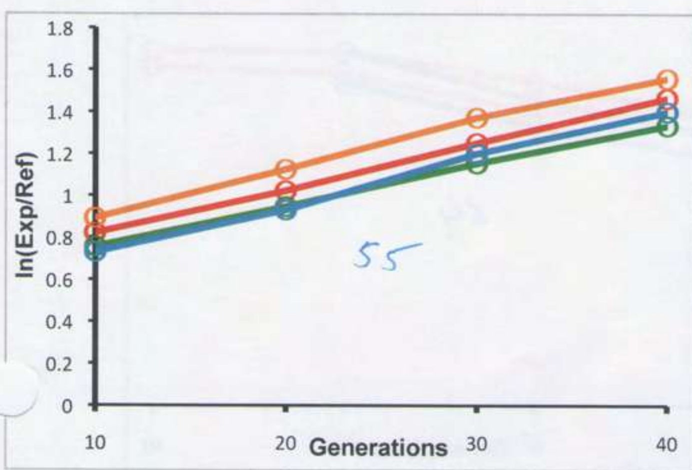
0.6
0.4
0.2
0
ln(Exp/Ref)
10
20
Generations
30
40
54

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1.6
1.4
1.2
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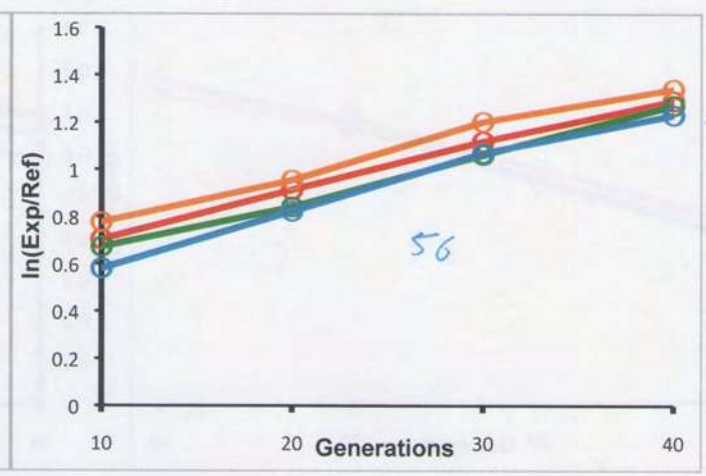
0.8
0.6
0.4
0.2
0
ln(Exp/Ref)
10
20
Generations
30
40
55

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1.2
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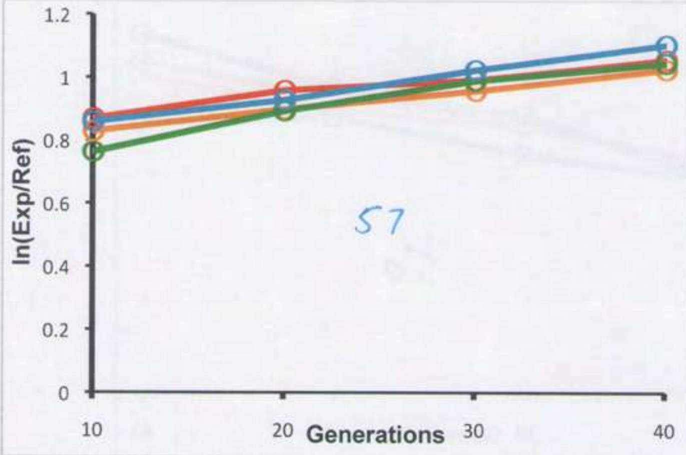
0.8
0.6
0.4
0.2
0
ln(Exp/Ref)
10
20
Generations
30
40
56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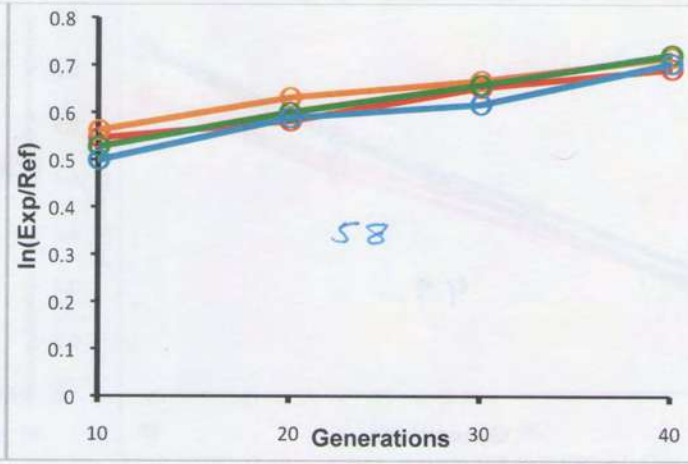
0.6
0.4
0.2
0
ln(Exp/Ref)
57
10
20 Generations 30
40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0.4
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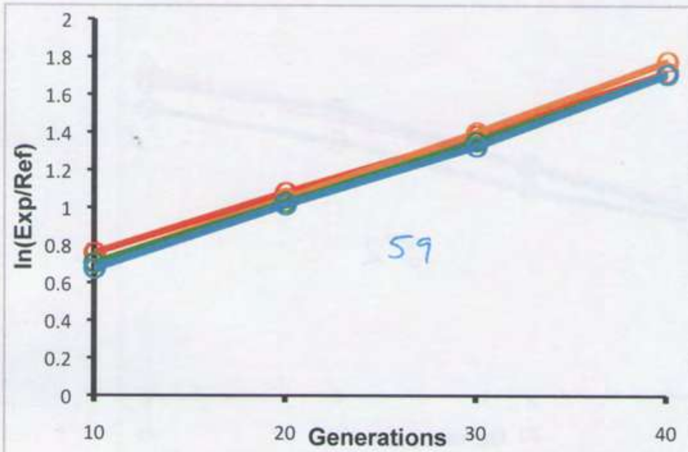
0.2
0.1
0
ln(Exp/Ref)
58
10
20 Generations 30
40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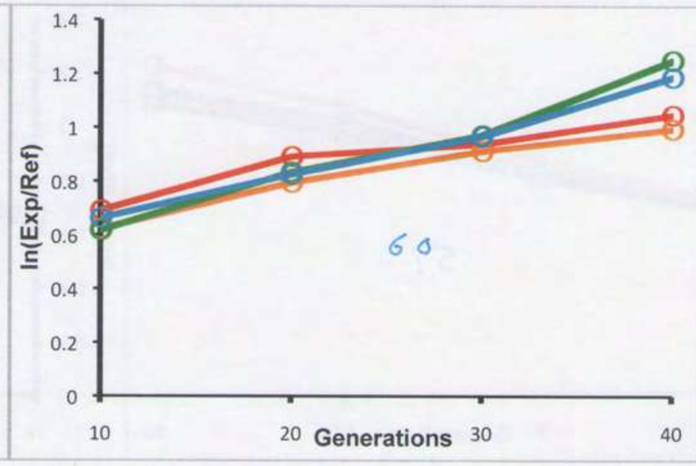
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
ln(Exp/Ref)
59
10
20 Generations 30
40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0.4
0.2
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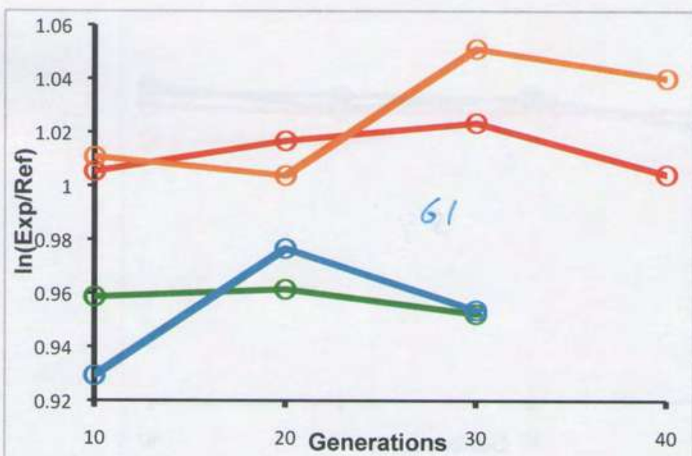
ln(Exp/Ref)
60
10
20 Generations 30
40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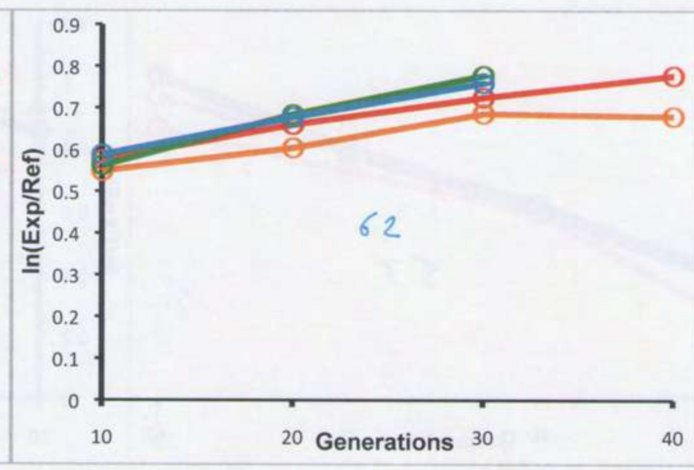
0.94
0.92
ln(Exp/Ref)
61
10
20 Generations 30
40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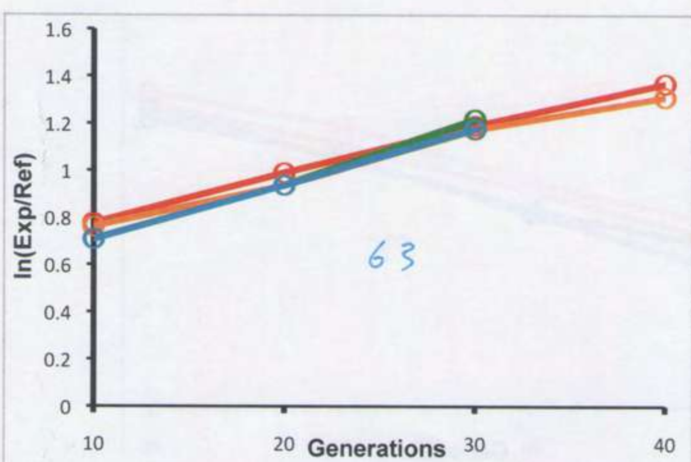
0.4
0.3
0.2
0.1
0
ln(Exp/Ref)
62
10
20 Generations 30
40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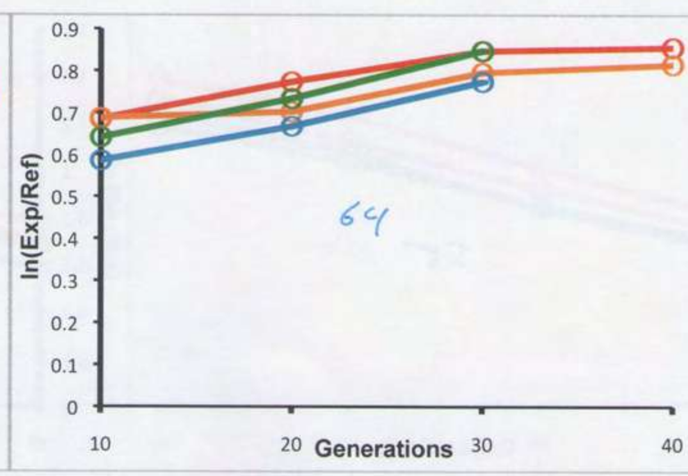
0.8
0.6
0.4
0.2
0
ln(Exp/Ref)
63
10
20 Generations 30
40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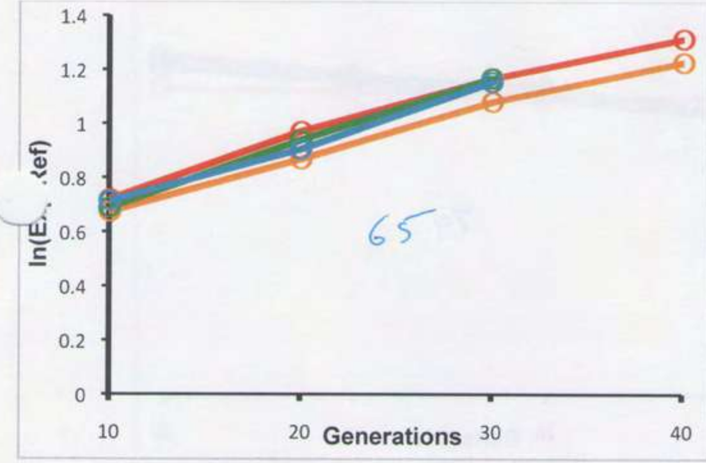
0.1
0
ln(Exp/Ref)
64
10
20 Generations 30
40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20
Generations
30
40
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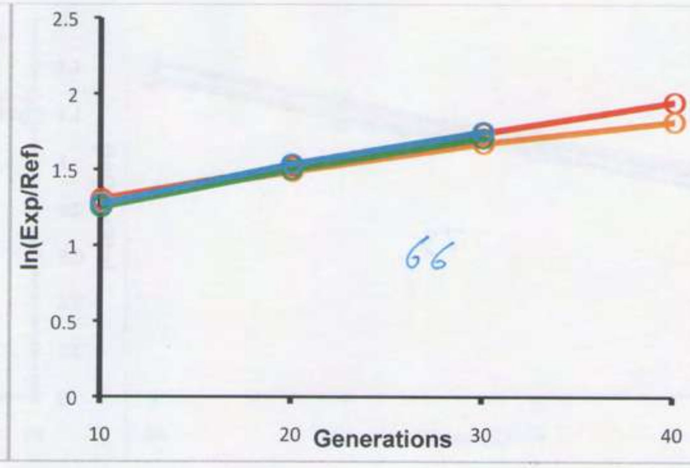
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10
20
Generations
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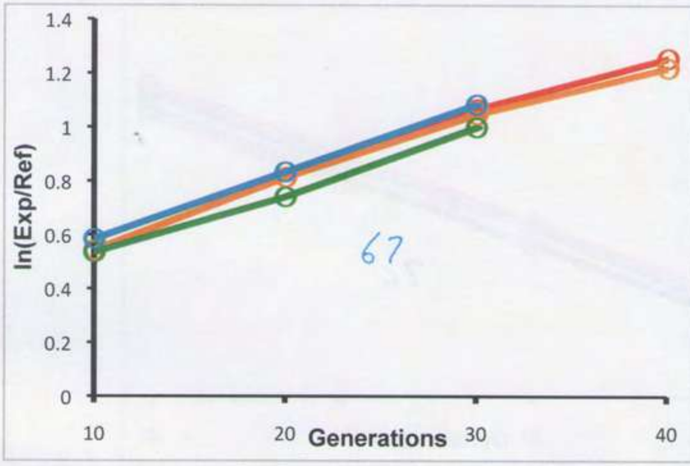
40
66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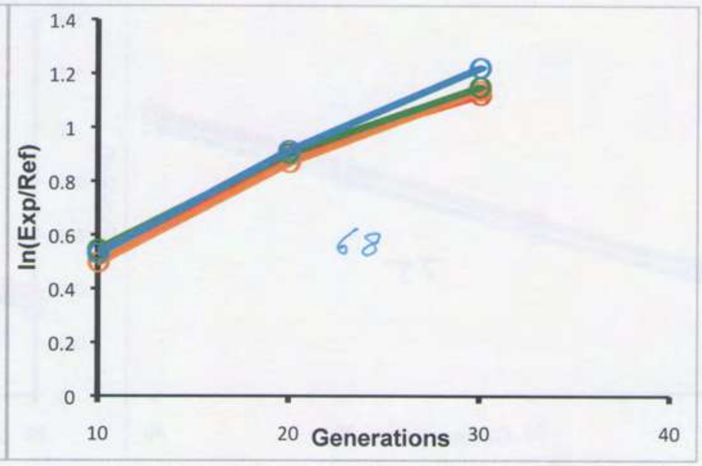
ln(Exp/Ref)
10
20
Generations
30
40
67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20
Generations
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40
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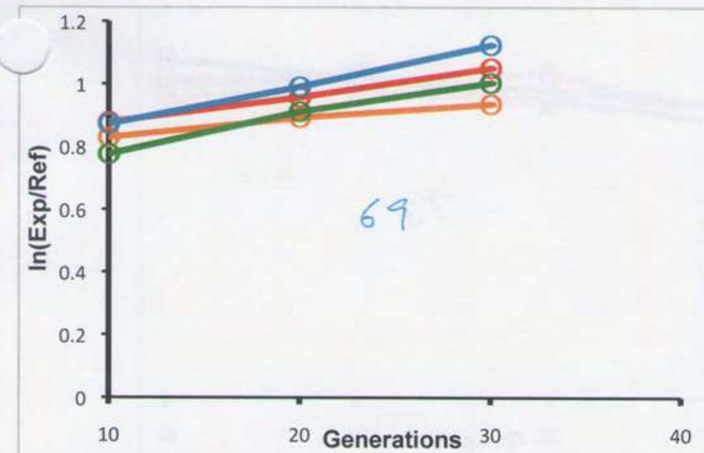
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0.6
0.4
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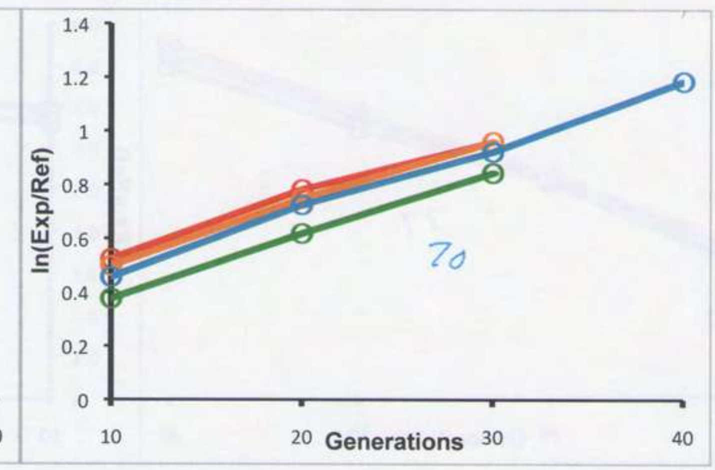
0
ln(Exp/Ref)
10
20
Generations
30
40
69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0.4
0.2
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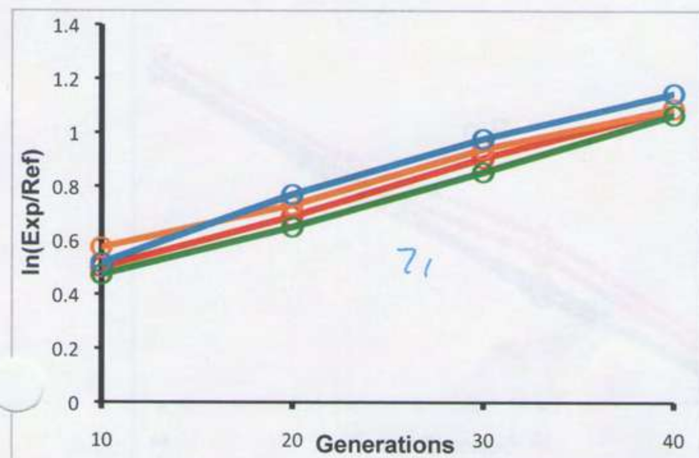
10
20
Generations
30
40
70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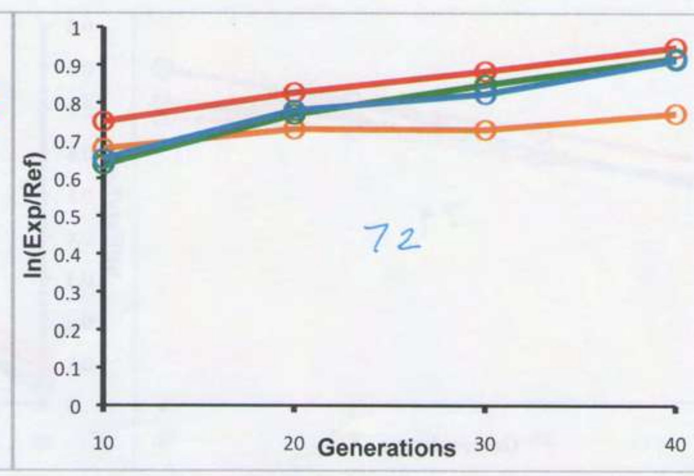
0.4
0.2
0
ln(Exp/Ref)
10
20
Generations
30
40
71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0.3
0.2
0.1
0
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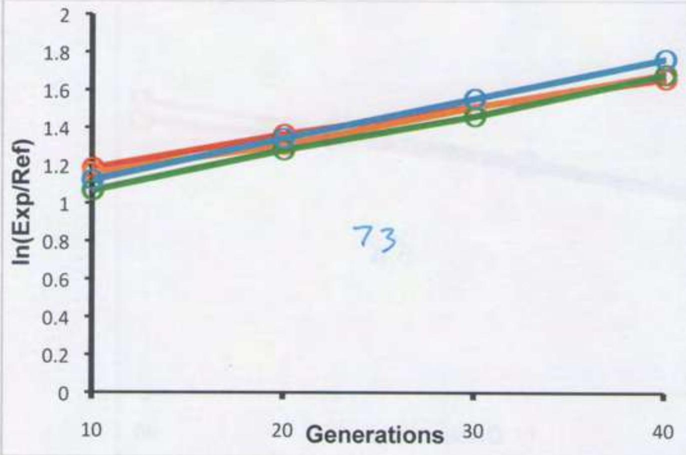
20
Generations
30
40
72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0.4
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0
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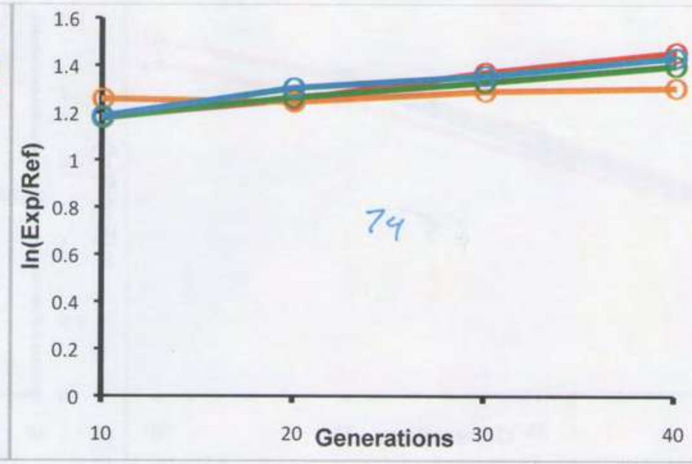
20
30
40
Generations
73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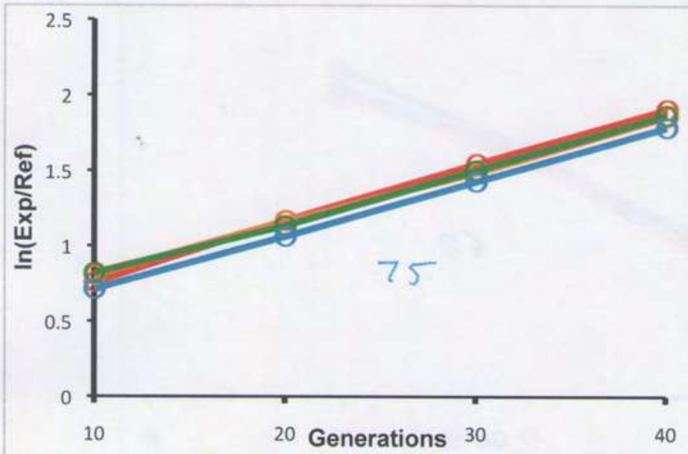
1.4
1.2
1
0.8
0.6
0.4
0.2
0
10
20
30
40
Generations
74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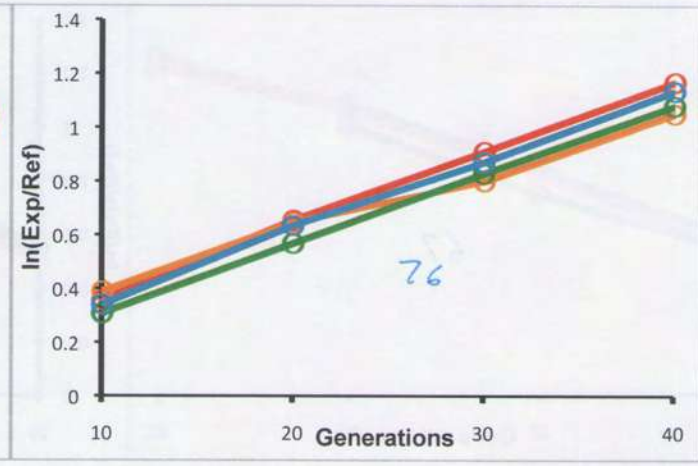
2
1.5
1
0.5
0
10
20
30
40
Generations
75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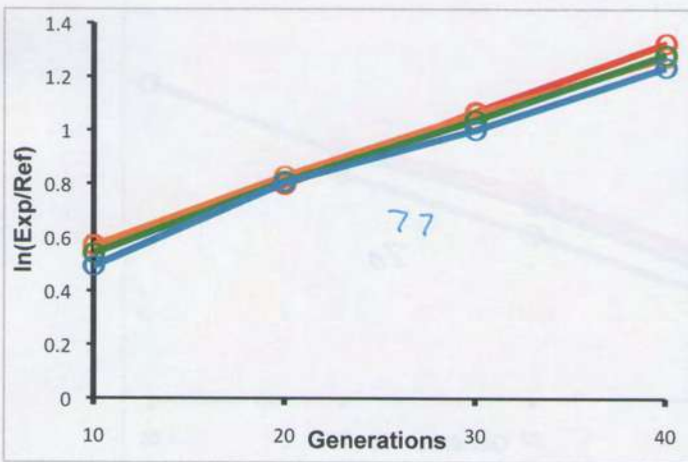
1.2
1
0.8
0.6
0.4
0.2
0
10
20
30
40
Generations
76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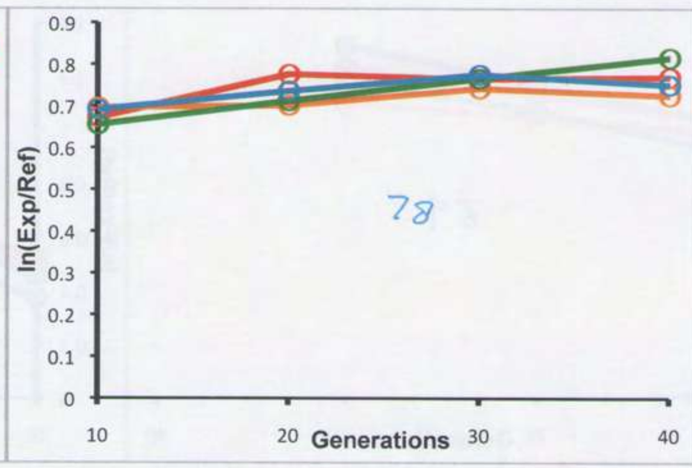
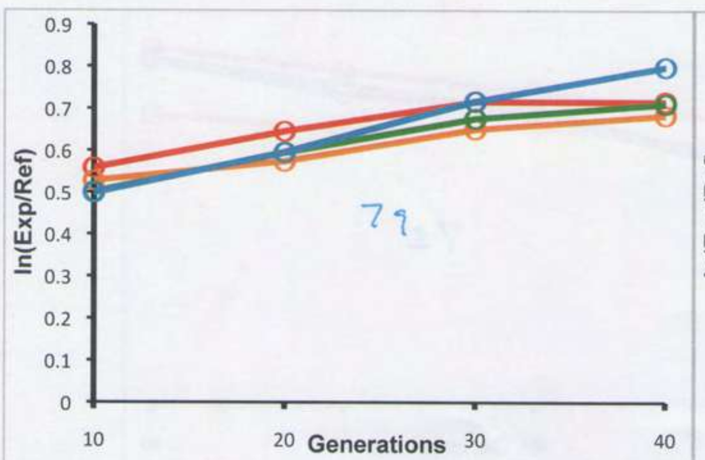
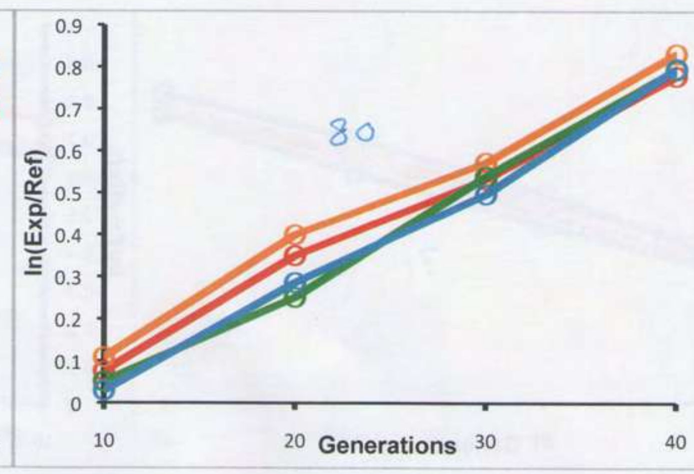
1.2
1
0.8
0.6
0.4
0.2
0
10
20
30
40
Generations
77


ln(Exp/Ref)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10
20
30
40
Generations
78

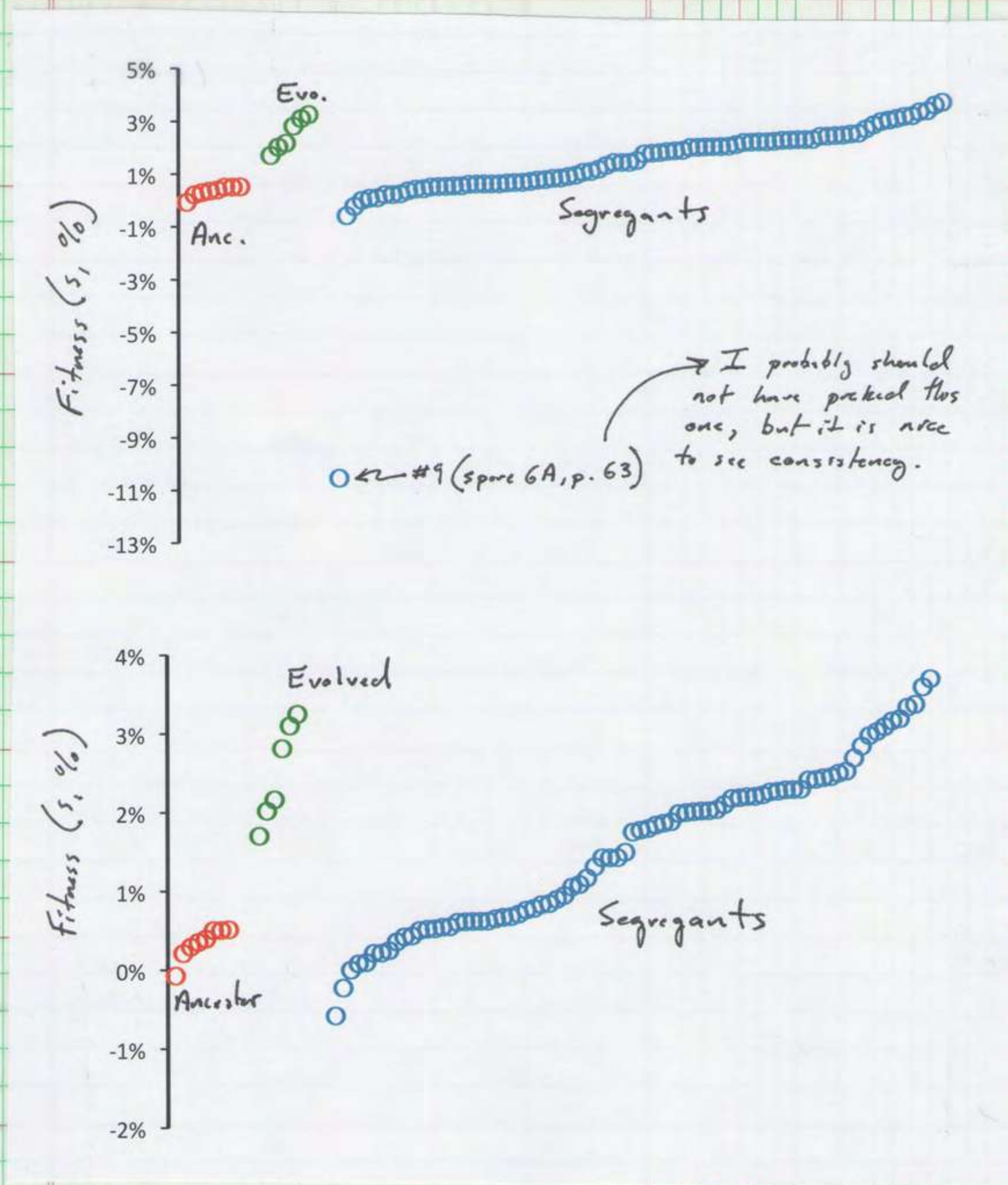

ln(Exp/Ref)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10
20
30
40
Generations
79


ln(Exp/Ref)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10
20
30
40
Generations
80

6/20/12

	Initials	Date
Prepared By		
Approved By		

ANALYZING FITNESS DATA



NOTES

(1) Ancestor has low fitness — but not 0, which I was expecting

(2) Evolved has high fitness — but not 4.5%, which I was expecting

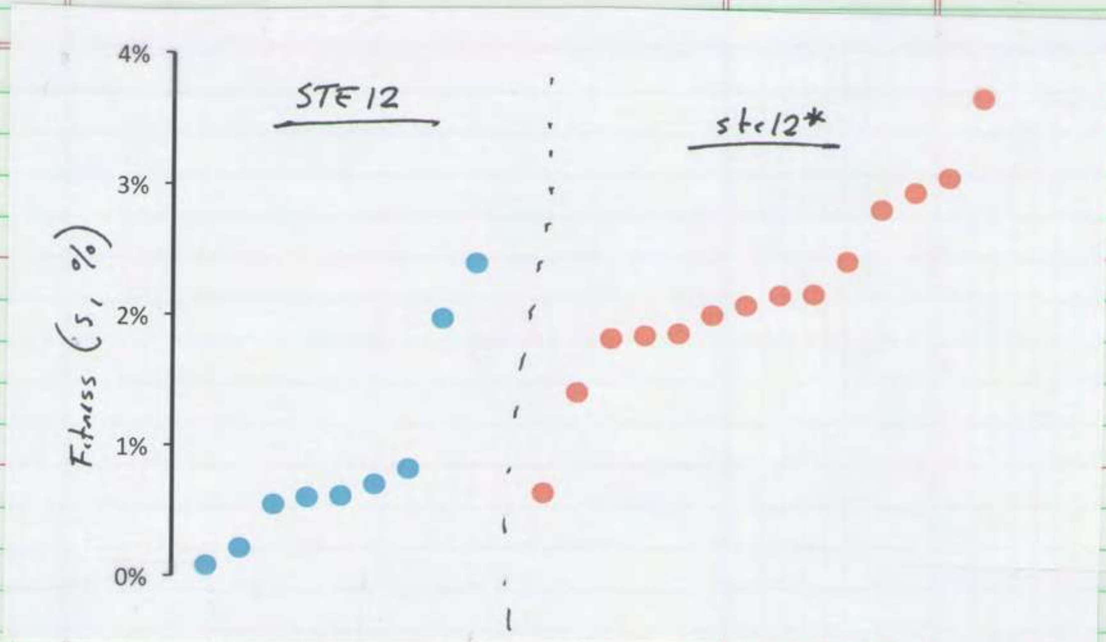
(3) The fitness range is slightly smaller than expected

Why? → often the fluorescent reporter gives a fitness disadvantage, so (1) is not that surprising. Also, I could have picked a bad clone — the plates were old (see p. 68)

But Why (2)? → I did notice that the incubator was set ~~at~~ closer to 32° than 30° → but would it make this much of a diff
↳ also the propagation regimen.

6/20/12

Inferring the fitness of the ste12* mutation



STE12 Ancestral Allele	
Seg. 054	0.08%
Seg. 033	0.21%
Seg. 058	0.55%
Seg. 048	0.61%
Seg. 011	0.62%
Seg. 072	0.71%
Seg. 064	0.83%
Seg. 031	1.98%
Seg. 080	2.41%
ste12 Evolved Allele	
Seg. 022	0.65%
Seg. 013	1.42%
Seg. 032	1.84%
Seg. 034	1.86%
Seg. 073	1.88%
Seg. 071	2.02%
Seg. 035	2.10%
Seg. 029	2.17%
Seg. 055	2.18%
Seg. 067	2.44%
Seg. 030	2.84%
Seg. 007	2.97%
Seg. 053	3.08%
Seg. 021	3.69%

- I took those segregants for which I can infer the genotype of STE12, and plotted their fitness.

	Median Fitness
STE12	0.62
ste12*	2.14

} Diff. = 1.52%

This recapitulates the expected fitness advantage of the ste12 mutation, and gives me hope that this will work well once I genotype the segregants at the other loci.

6/21/12

	Initials	Date
Prepared By		
Approved By		

IDENTIFYING STERILE MUTATIONS IN SEQUENCING DATA

- From our 40 sequenced populations I took the 34 where steriles were observed and I looked at the BAM files in IGV & (using the timepoint where steriles peak) to find the underlying ste mutation.

Population	Gene	WT	Mutation
BYS1 A07	STE11	ACATTGT	ACATTG**A**
BYS1 A08	STE12	TGGCCAG	TG-CCA**A**
BYS1 F05	STE4	GAGTTTC	GAGTTT**A**
BYS2 C03	STE5	CTGTACA	CTGTAC**G**
BYS2 C06	STE5	TACGTTA	TACGTT**C**
BYS2 D06	STE5	GATTGGA	GATTGG**T**
BYS2 E03	STE12	AAAGTA(T6)	AAAGTA(**T7**)
RMS1 H08	STE11	ATCCATG	ATCCAT**C**
BYS1 A04	STE4	ACCGCCC	ACCGCC**T**
BYS1 A09	?		
BYS1 D03	?		
BYS1 G02	?		
BYS2 B09	?		
BYS2 E04	?		
BYB1 C05	STE11	ACCGGTA	ACCGGT**T**
BYB1 H10	STE4	GAAAGAG	GAAAGA**T**
BYB2 E05	STE5	CTATTTC	CTATTT**T**
RMS1 E09	?		
BYS1 G01	?		
BYS2 D07	STE4	TTATATG	TTATAT**T**
BYS2 E01	?		
RMB2 F09	?		
BYB2 F09	?		
RMS1 G02	?		
BYS1 D08	?		
RMB2 B10	STE11	CAGGAGG	CAGGAG**T**
RMB2 D08	STE4	TGGACTCG	TGGACT**AA**
BYB2 F10	?		
RMS1 D12	?		
RMS2 D09	STE18	CAACAGC	CAACAG**T**
BYS2 C01	?		
BYB1 G07	STE4	AAAGGAC	AAAGGA**G**
BYB2 F07	?		
RMB2 F01	STE11	CACAGAG	CACAGA**T**

- I looked for mutations in the following genes:

STE 2, 4, 18, 50, 20, 11, 7, 5, FUS3, STE12, APC1 } to look for sterile mutations

GAS1, ELO1, MSH2 } just to look.

- I found mutations in 18/~~[illegible]~~34

- In looking at the data in IGV, I have noticed that the sterile frequencies as measured from the seq. reads is greater than from the FACS data.

→ This is something I didn't see w/ BYS1-A08 (p. 7) but I was predicting to see (p. 6)

- NOTE ON RMB2 F01: This is an interesting population, which evolved sterile aggregates. The sterile is predicted to be only 6% by FACS — although estimating the fraction sterile in an ~~aggregate~~ aggregate population is difficult. I was not only able to find the sterile, but I found another mutation in PSE1. I only found this since GAS1 is the neighboring gene.

		1	2	3

6/20/12

	Initials	Date
Prepared By		
Approved By		

MORE NOTES ON FITNESS ASSAYS

PROPAGATION REGIME: During the last run of fitness assays, I ran the following regime

	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6
AM		Mix, D/P	D/P	D/P	D/P	P
DAYTIME		FACS(0)	FACS(10)	FACS(20)	FACS(30)	FACS(40)
PM	o/N	D	D	D	D	

D = Dilute
P = PBS FACS setup.

This time I will do the following:

	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6	DAY 7
AM		Mix, D	D	D	D	D	
DAYTIME			FACS(5)	FACS(15)	FACS(25)	FACS(35)	FACS(45)
PM	o/N	D/P	D/P	D/P	D/P	D/P	

I will put the FACS plates in the fridge o/N → this will help in two ways:

(1) mCherry will be brighter and more consistent. After transferring to PBS (and getting dO_2) the protein takes hours to mature. I did some tests last week and o/N in the fridge works well.

(2) I can do my FACS time earlier — less competition for the instrument.

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SECOND ROUND OF FITNESS ASSAYS – SEGREGANTS 81-160

- Yesterday morning I picked a single colony off of the mCherry Reference plate (streaked on) and inoculated 20 mL YPD.
  ↳ Distribute 130λ into each well of a 96-well plate. → 30° @ 9:30am
- Last night @ 8:20pm I ~~diluted the~~ transferred 4λ into 125 of the mCherry Ref as well as a copy of the Segregant 1 (1-80) plate and two copies of the segregant 2 (81-160) plate.

NOTE: I will run two fitness assays simultaneously:
(1) Fitness Assays on Segregants 81-160
(2) Bulk Segregant assays on pooled segregants 1-160

- This morning I started the fitness assays (1) → transfer @ 8:15.

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FITNESS ASSAYS 1 (QTL APPROACH): Last night I set up the first FACS plate, which stayed in the fridge o/n and is running now. I also put a tip wash in the program. I will use the same 4 250λ tips for YPD, Water, EtOH, & PBS and the same 3 ~~25λ~~ 20λ tips for the three replicate plates for the duration of the experiment.

- Last night I started the programs @ 8:10 and got the plates in the 30° @ 8:30 → since being serviced the robot was at 50% speed – I changed it back this morning

- This morning I got the plates @ 30° at 8:20.

FITNESS ASSAYS 2 (BULK SEGREGANT APPROACH): Last night I pooled the segregants ~~10 mL YPD~~
- I removed by aspirating the last two columns of the Segregant plates (which contain the controls)
- I added 10 mL YPD to an upside down tip box lid
- Add 100λ of each of 160 segregants to lid
- Pour into a 50 mL conical (total 25 mL)
- Coulter count

6/22/12

	Initials	Date
Prepared By		
Approved By		

FITNESS ASSAYS 2 (BULK ~~SEGRANT~~ SEGREGANT APPROACH) CONT. :

· Coulter Count on Segregant Pool (from last night)

3.042E7
3.075E7 } 60 λ into 20 mL

SAMPLE 0

↳ The segregant pool is ~6E7

· Last night I set up three pools for propagation (A, B, C) by diluting 15 λ into 10 mL of prewarmed ~~YPD~~ YPD
↳ dilution @ 8:38 pm.

· This morning ~ 8:30 - Test Coulter count Pool A

Bkg 802000
Pool A 2.325E7 (60 λ into 10 mL)

· Dilute 15 λ into 10 mL Prewarmed YPD → 8:38 am

· Coulter Count (8:45 AM)

Bkg	1.148E6	974000	998000
Pool A	2.606E7	2.632E7	2.677E7
Pool B	2.773E7	2.79E7	2.768E7
Pool C	2.8E7	2.635E7	2.698E7
Post Bkg	1.908E6	1.434E6	1.396E6

SAMPLE 1

· I also took 1 mL culture, spin, wash 1 mL H_2O, aspirate, freeze cell pellet for gDNA prep later

Coulter Count (8:20 PM)

Bkg	1.126E6	752000	566000
Pool A	2.57E7	2.513E7	2.496E7
Pool B	2.503E7	2.494E7	2.398E7
Pool C	2.642E7	2.609E7	2.639E7
Post Bkg	1.732E6	1.276E6	1.07E6

SAMPLE 2

· Dilute 15 λ into 10 mL Prewarmed YPD → 8:30 pm

· Sample as above.

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FITNESS ASSAYS - BULK SEGREGANT APPROACH CONTINUED

Dilute 15 λ into 10 mL → 8:30 am

Coulter Counts (8:30 am)

Bkg	1.072 E6	682000	590000
Pool A	3.153 E7	3.169 E7	3.199 E7
Pool B	3.472 E7	3.395 E7	3.386 E7
Pool C	3.199 E7	3.156 E7	3.195 E7
Post Bkg	1.512 E6	864000	744000

SAMPLE 3

Coulter Counts (8:15 pm)

Bkg	624000	540000	548000
Pool A	3.053 E7	3.164 E7	3.185 E7
Pool B	3.552 E7	3.607 E7	3.593 E7
Pool C	3.073 E7	3.021 E7	3.026 E7
Post Bkg	2.9 E6	1.07 E6	922000

SAMPLE 4

Dilute 15 λ into 10 mL → 8:30 pm

6/24/12

Dilute 15 λ into 10 mL → 8:50 am

Coulter Count (8:45 am)

Bkg	1.034 E6	546000	544000
Pool A	4.099 E7	4.189 E7	4.152 E7
Pool B	4.227 E7	4.142 E7	4.192 E7
Pool C	3.464 E7	3.377 E7	3.367 E7
Post Bkg	1.796 E6	1.104 E6	720000

SAMPLE 5

Coulter Count (8:15 pm)

Bkg	956000	686000	584000
Pool A	3.841 E7	3.727 E7	3.797 E7
Pool B	4.167 E7	4.101 E7	4.079 E7
Pool C	3.144 E7	3.213 E7	3.122 E7
Post Bkg	1.84 E6	1.044 E6	990000

SAMPLE 6

Dilute 15 λ into 10 mL → 8:30 pm

	Initials	Date
Prepared By		
Approved By		

6/25/12

FITNESS ASSAYS CONT.

· Dilute 15λ into 10 mL → 8:30 am

· Coulter Count (8:35 am)

Bkg	922000	582000	540000
Pool A	5.029E7	5.049E7	5.075E7
Pool B	5.316E7	5.204E7	5.237E7
Pool C	4.004E7	4.002E7	3.972E7
Post Bkg	1.906E6	868000	810000

SAMPLE 7

· Coulter Count (8:15 pm)

Bkg	514000	428000	382000
Pool A	4.744E7	4.87E7	4.857E7
Pool B	4.821E7	4.816E7	4.794E7
Pool C	3.741E7	3.709E7	3.679E7
Post Bkg	2.076E6	1.114E6	948000

SAMPLE 8

· Dilute 10λ into 10 mL → 8:30 pm

6/26/12

· Coulter Count 8:20 am

Bkg	1.138E6	556000	428000
Pool A	3.246E7	3.268E7	3.236E7
Pool B	3.631E7	3.655E7	3.647E7
Pool C	2.909E7	2.956E7	2.918E7
Post Bkg	1.324E6	886000	870000

SAMPLE 9

· Dilute 15λ into 10 mL → 8:30 am

Coulter Count 8:15 pm

Bkg	534000	464000	430000
Pool A	3.487E7	3.476E7	3.387E7
Pool B	3.907E7	3.854E7	3.898E7
Pool C	3.467E7	3.401E7	3.568E7
Post Bkg	1.646E6	1.246E6	858000

SAMPLE 10

· Dilute 15λ into 10 mL → 8:30

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SAMPLE 11

Coulter Count (8:25 am)

Bkg	$1.172E^6$	626000	564000
Pool A	$4.248E^7$	$4.251E^7$	$4.339E^7$
Pool B	$4.77E^7$	$4.758E^7$	$4.699E^7$
Pool C	$4.067E^7$	$4.079E^7$	$4.102E^7$
Post Bkg	$1.742E^6$	$1.058E^6$	$1.006E^6$

6/26/12

NOTES ON FITNESS ASSAYS

QTL Approach

- The new propagation regime worked well. The last plate ran today – mCherry was bright and consistent from experiment to experiment.
- All dilutions were done within a 10 min window (8:10 - 8:20)
- I noticed last night that during the air injection into PBS step some of the outer tips were not mixing. I don't know why – they mixed and transferred fine from/to YPD in 96 well plates.
  ↳ Wet filters from washing tips?

Bulk Segregant Approach

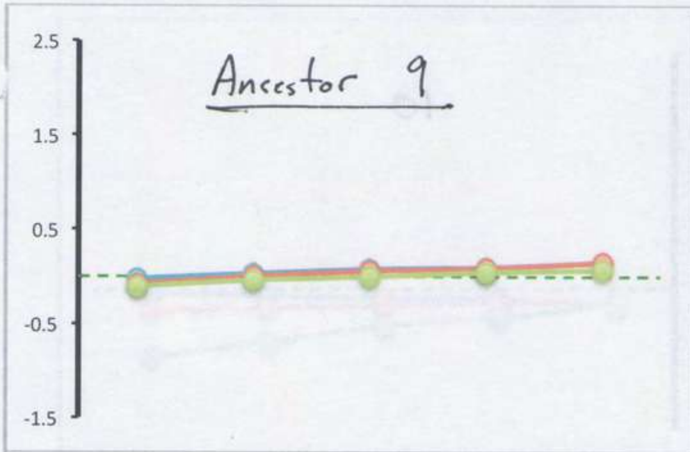
- At each sample time point I froze cell pellets. — I have twelve time points (including original pool).
- I screwed up sample 3 — I froze the 1mL culture in YPD rather than spinning, aspirating, washing, aspirating, and freezing cell pellets.

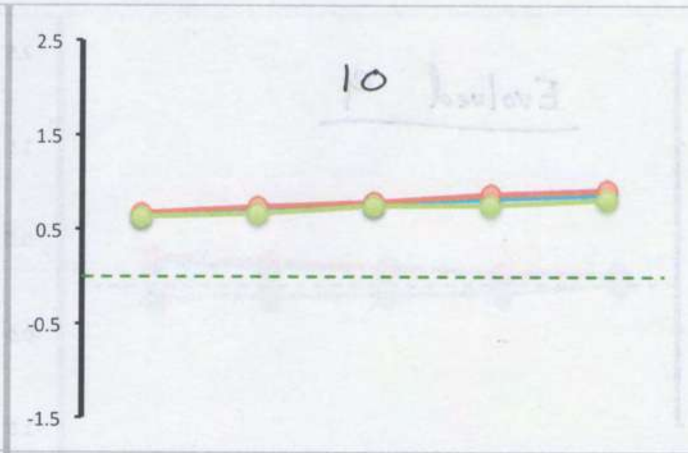
↳ I realized this when I went to prep sample #4 (6/23 PM). I did two things: (1) thaw & prep sample 2
(2) resample tubes from bench (labeled 3*). These cells clearly grew some more at RT. I would prefer to use the original sample.

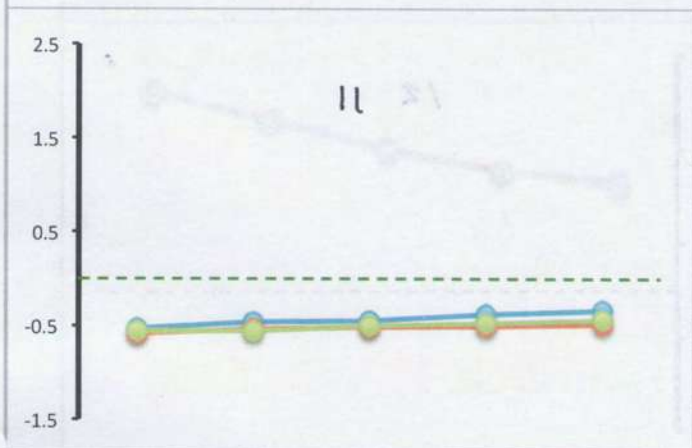
- All of the dilutions were 15 λ into 10 mL, except 6/25 PM (10 λ).

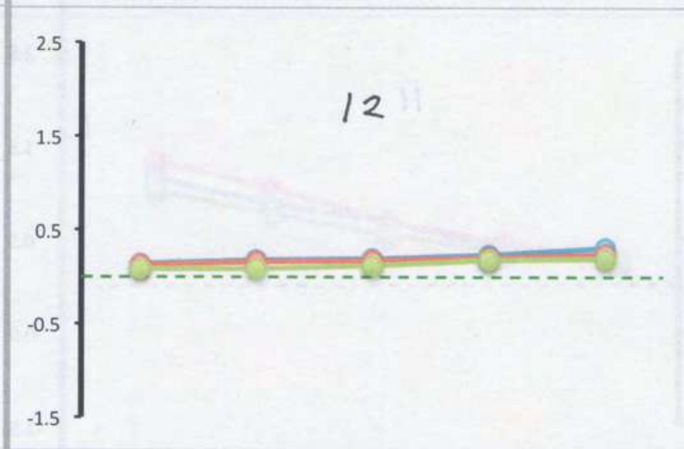
	1	2	3

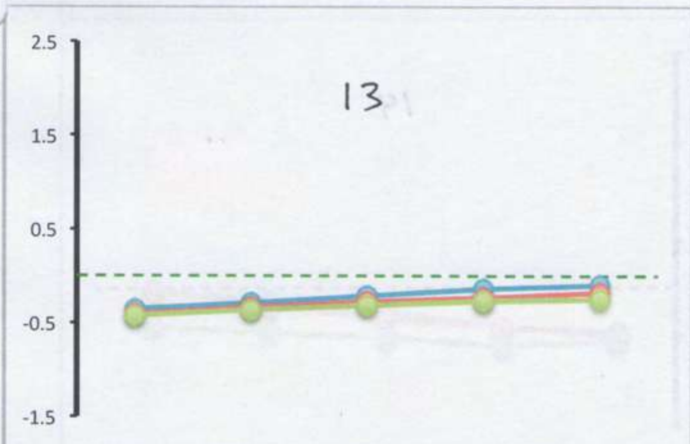
6/27/12 RESULTS OF FITNESS ASSAYS ON SEGREGANTS 81-160

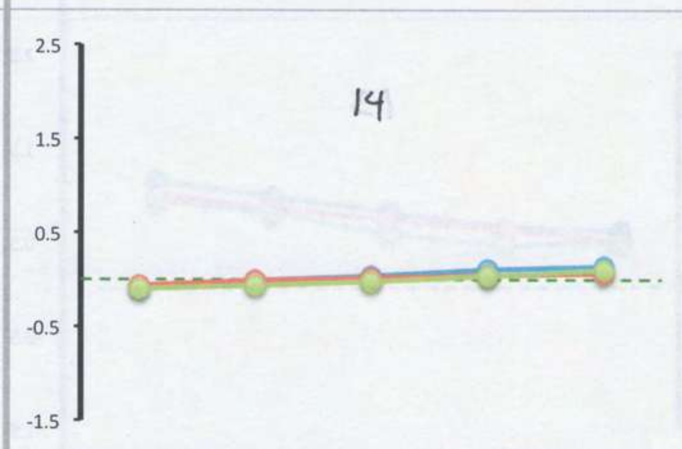


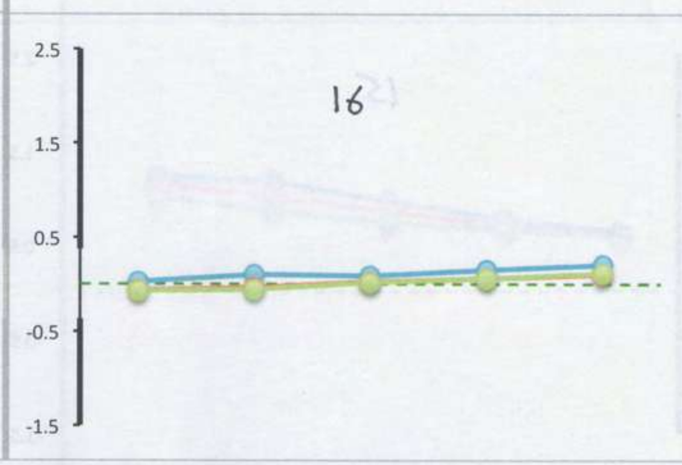




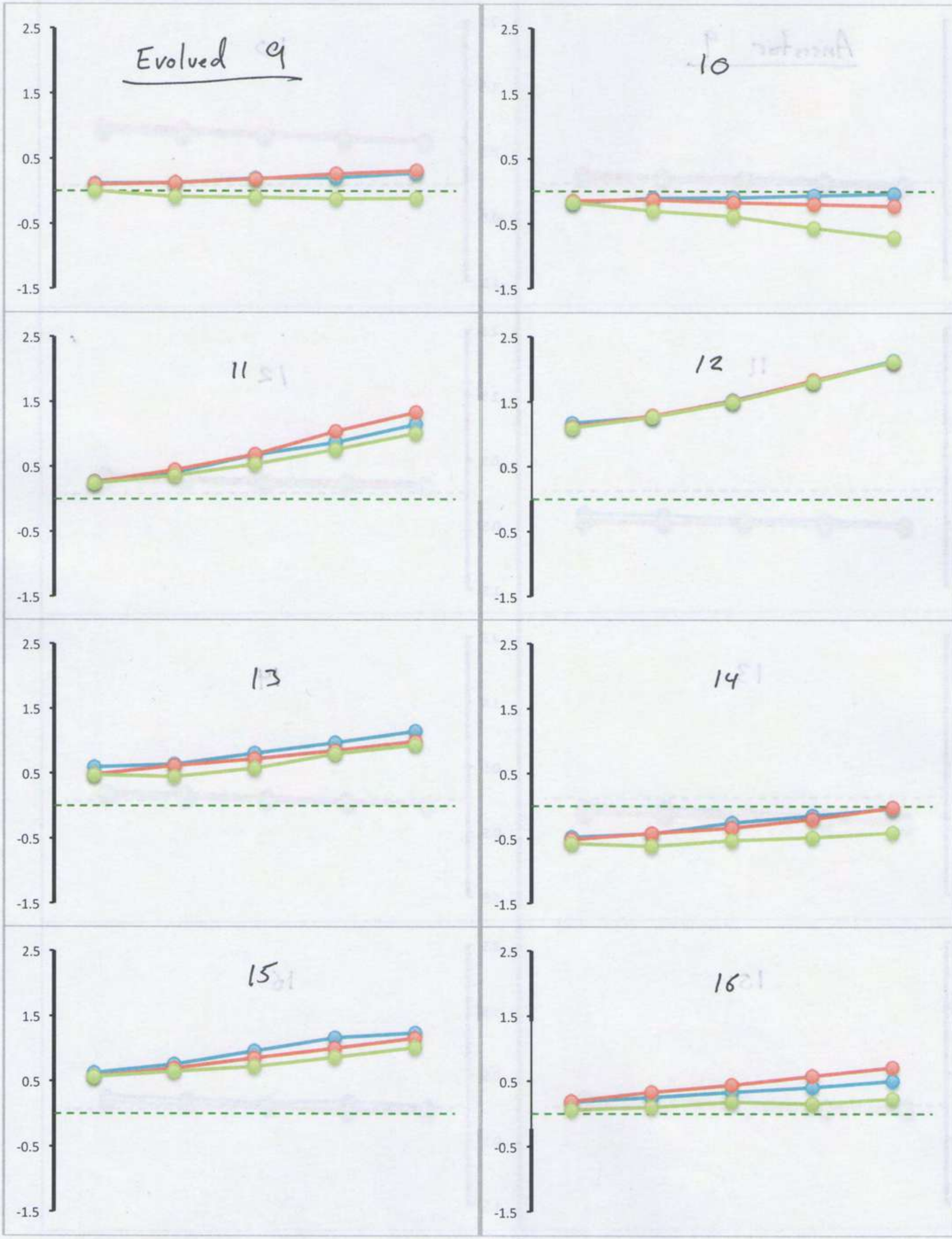




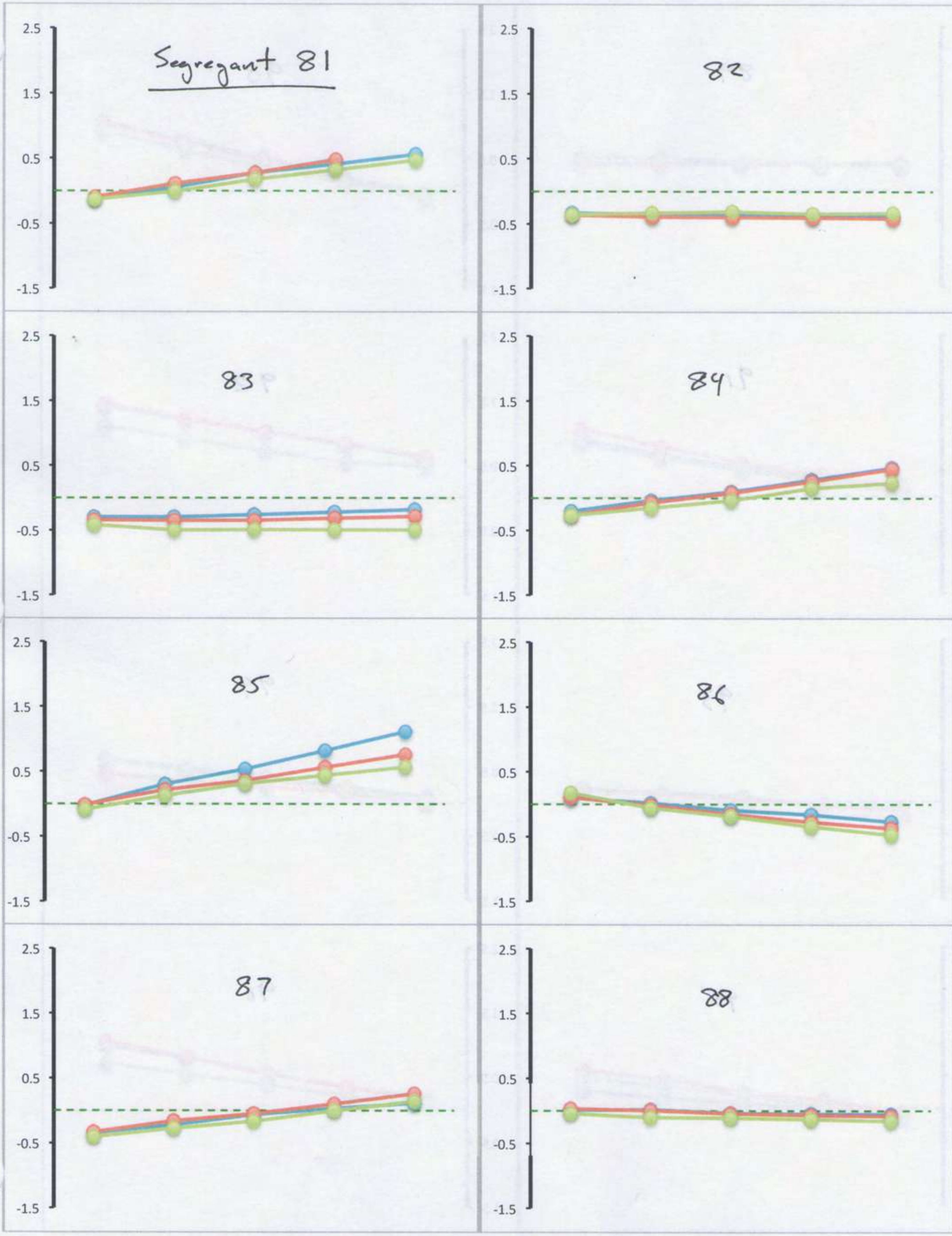




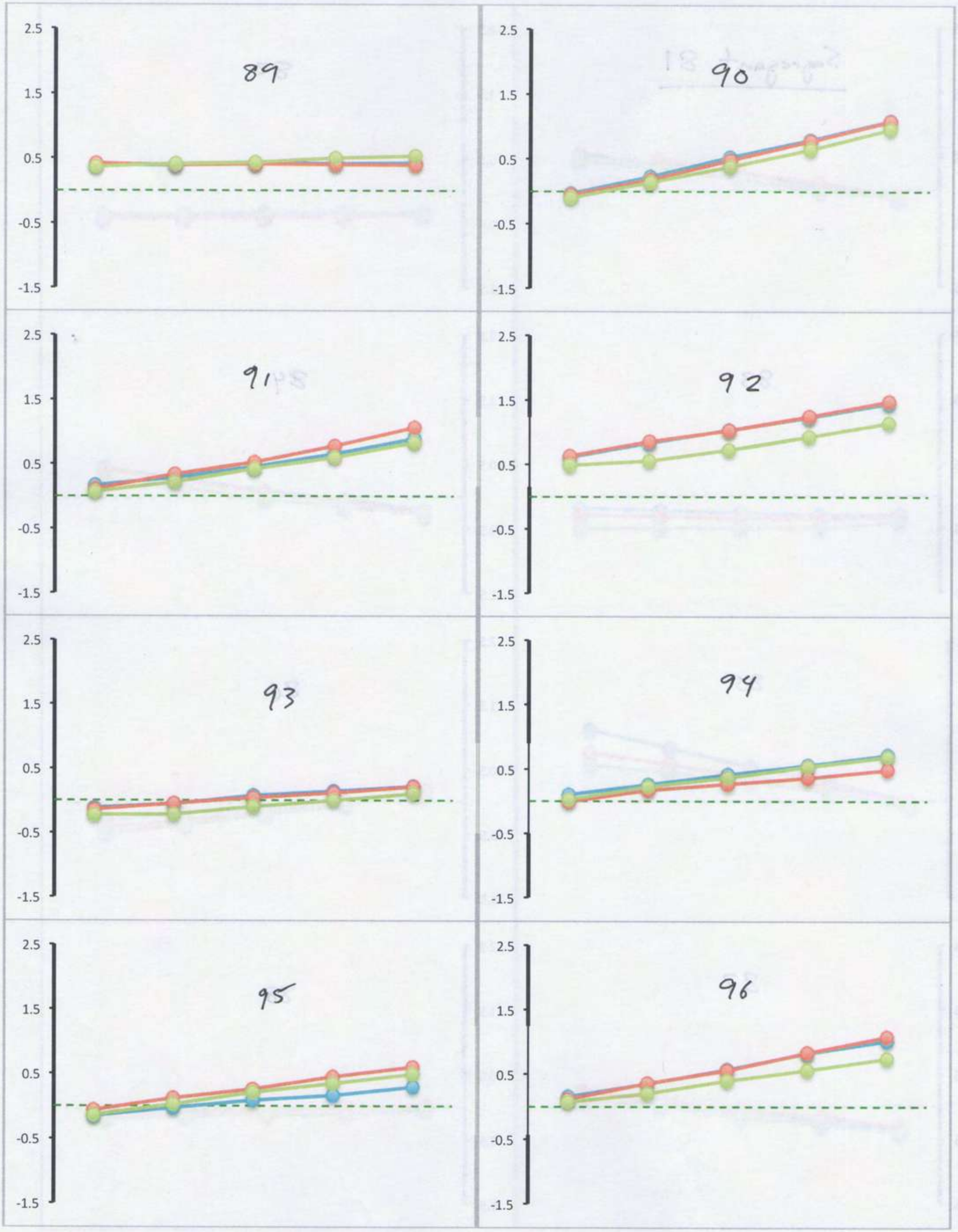
6/27/12



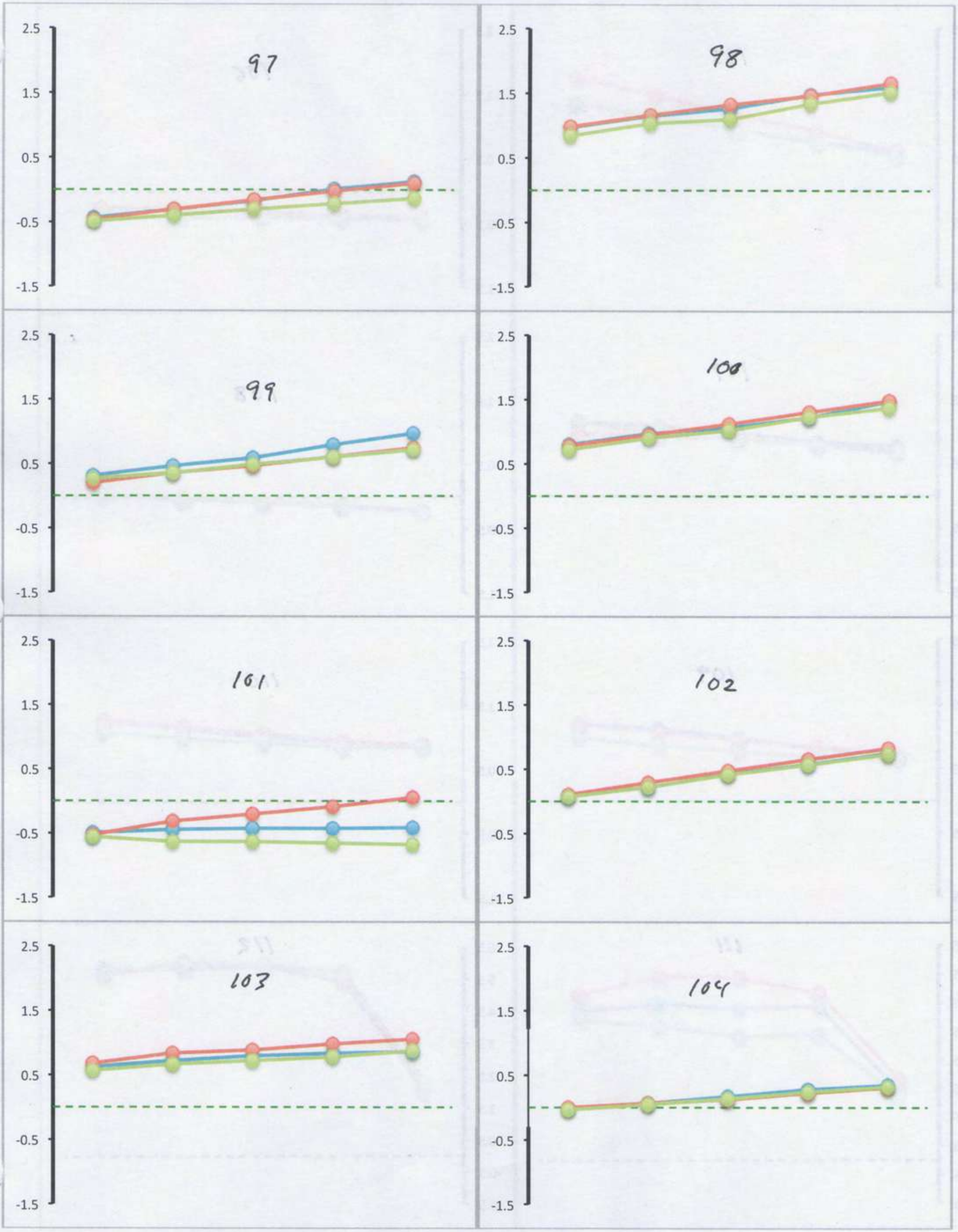
6/27/12



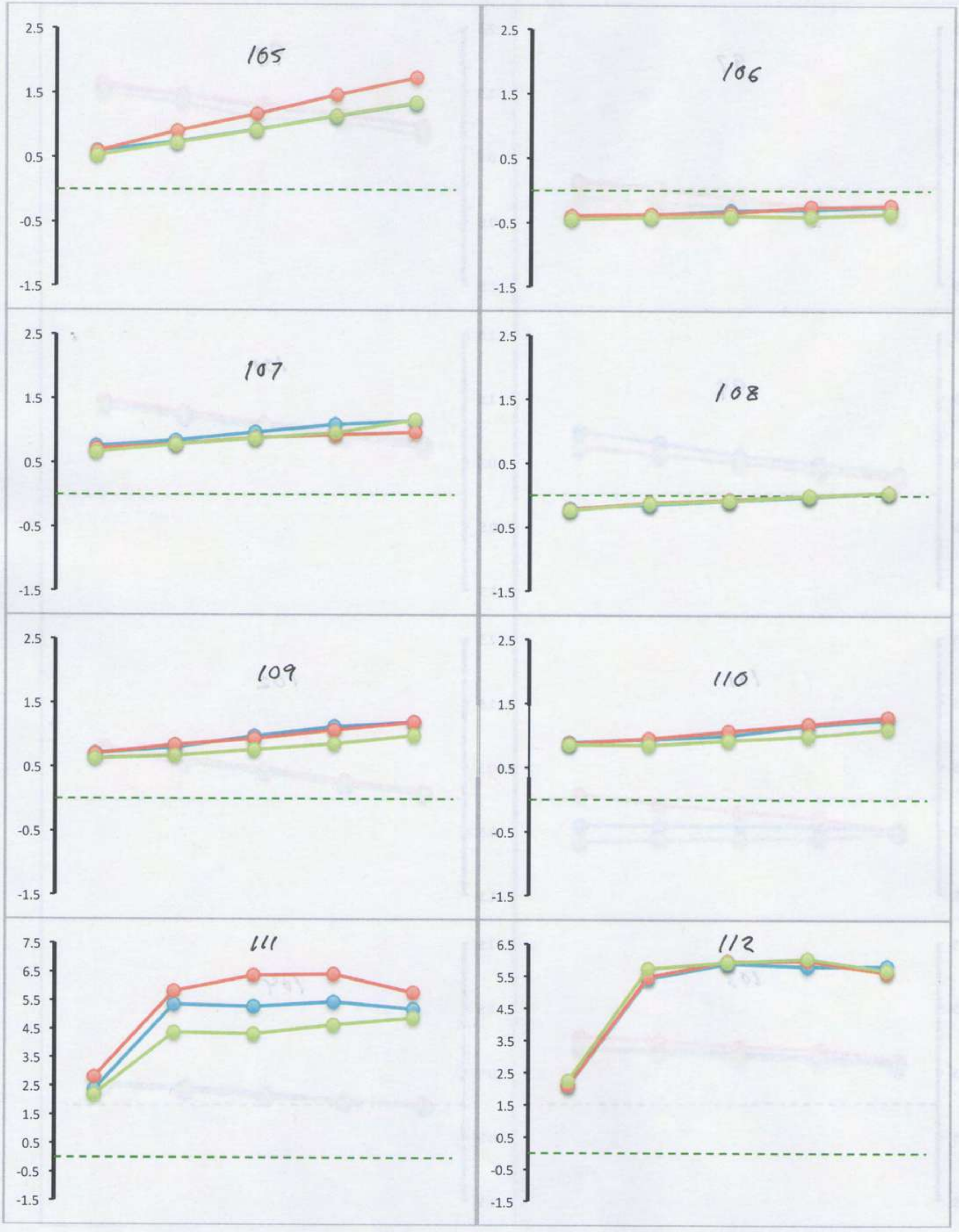
6/27/12



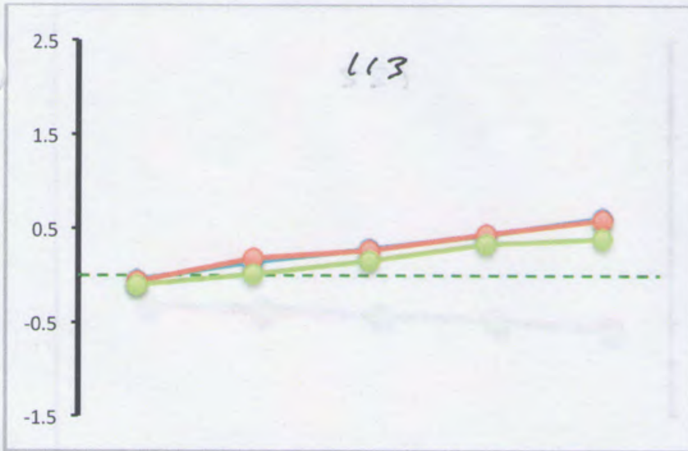
6/27/12



6/27/12



6/27/12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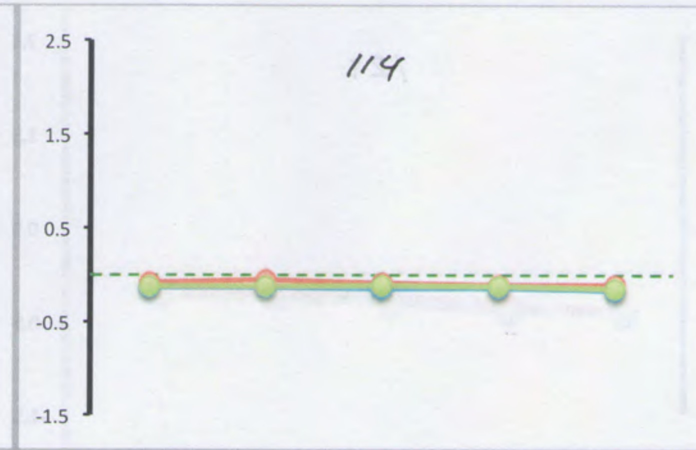
1.5
0.5
-0.5
-1.5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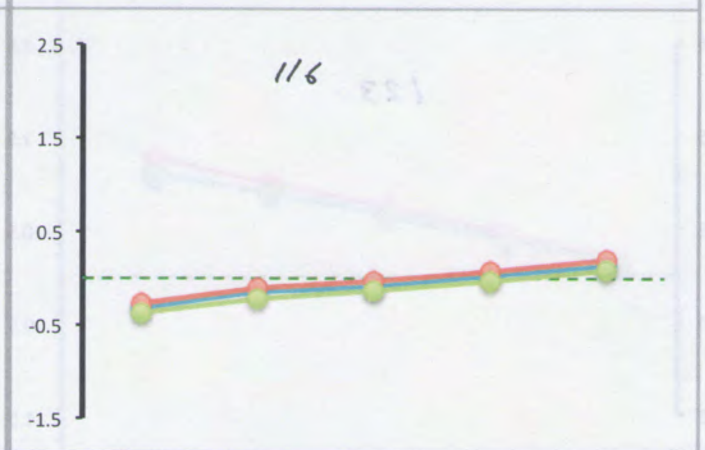
2.5
1.5
0.5
-0.5
-1.5


115
2.5
1.5
0.5
-0.5
-1.5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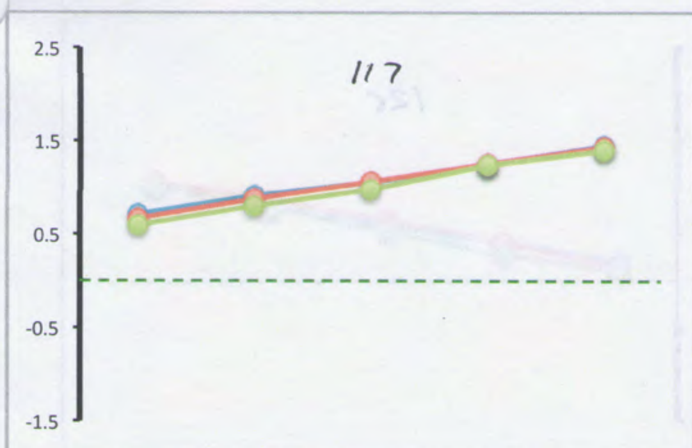
-1.5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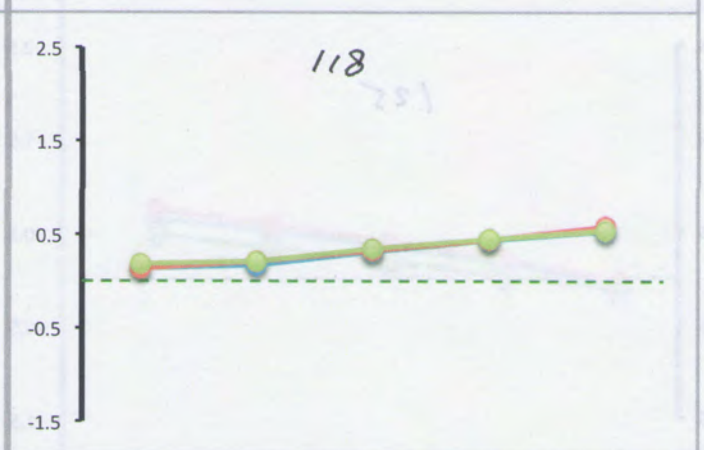
-1.5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0.5
-0.5
-1.5


119
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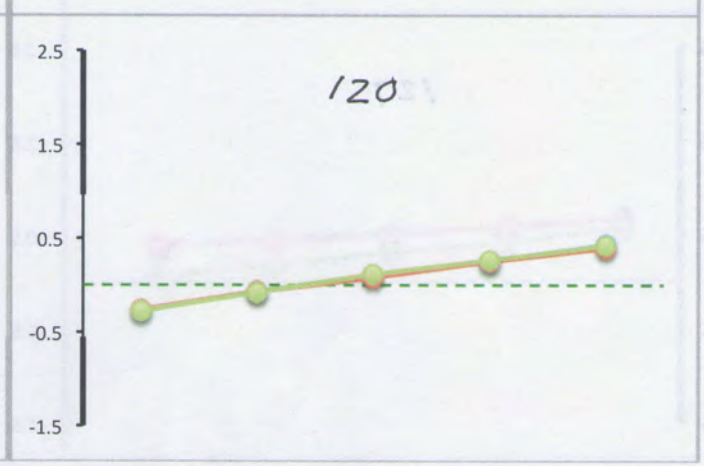
1.5
0.5
-0.5
-1.5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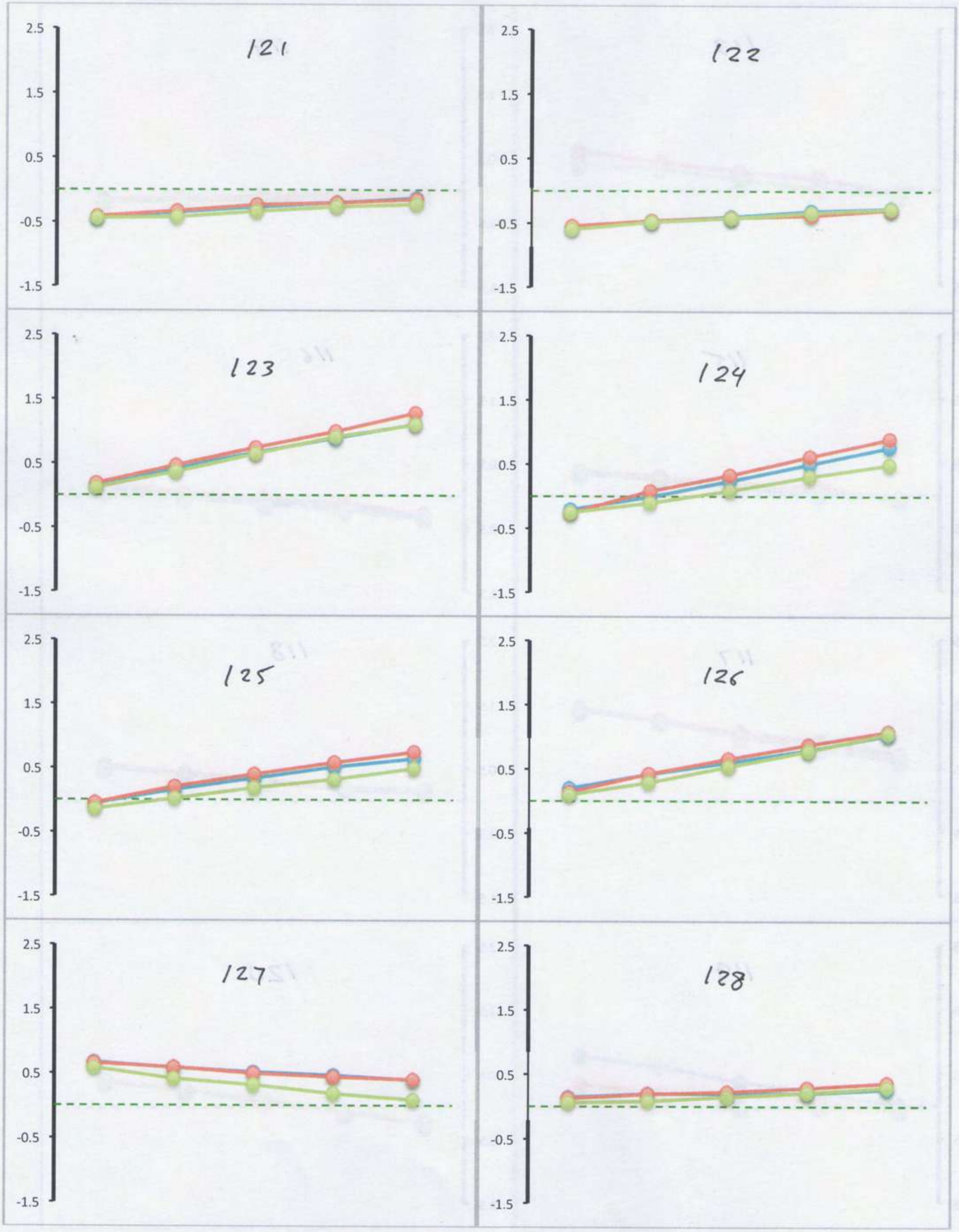
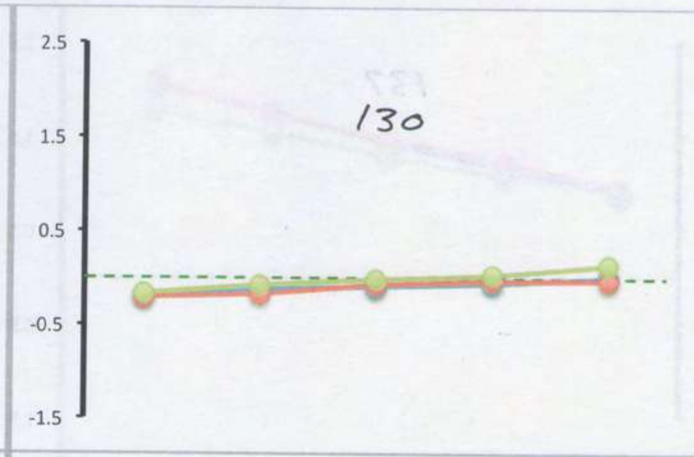
1.5
0.5
-0.5
-1.5

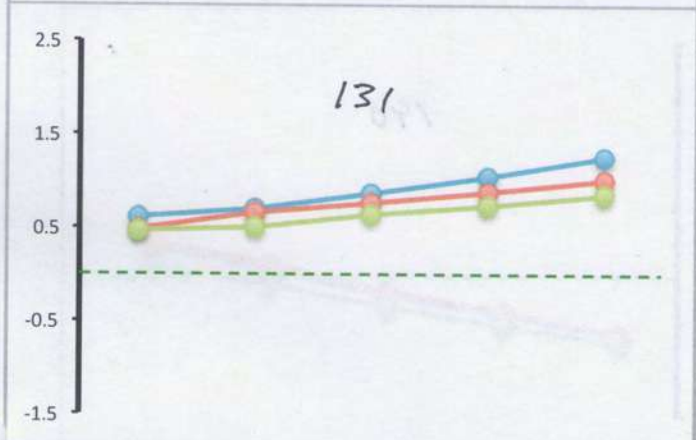
6/27/12



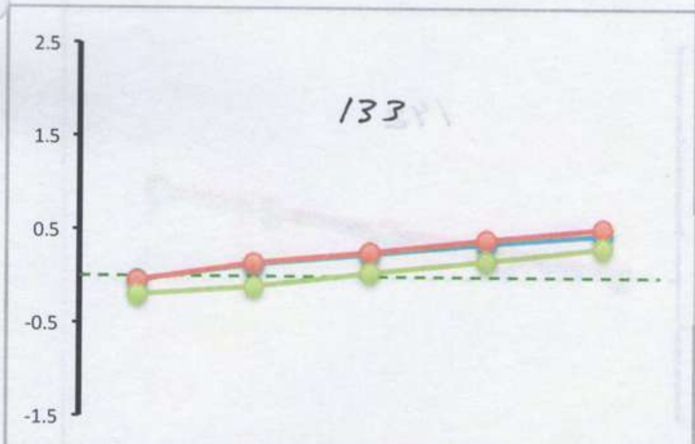
6/27/12


129
2.5
1.5
0.5
-0.5
-1.5


130
2.5
1.5
0.5
-0.5
-1.5


131
2.5
1.5
0.5
-0.5
-1.5


132
2.5
1.5
0.5
-0.5
-1.5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1.5
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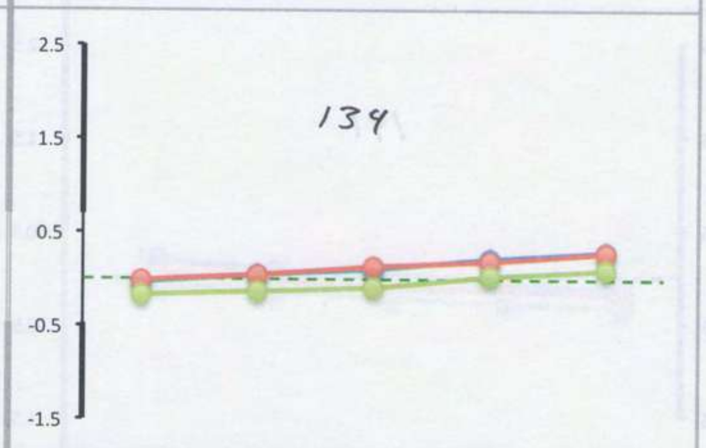
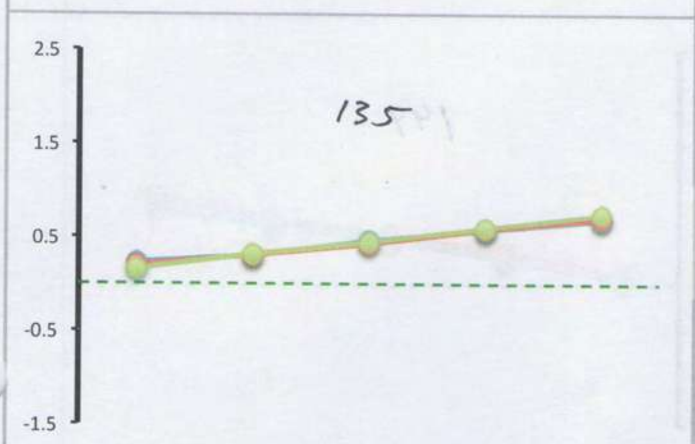

134
2.5
1.5
0.5
-0.5
-1.5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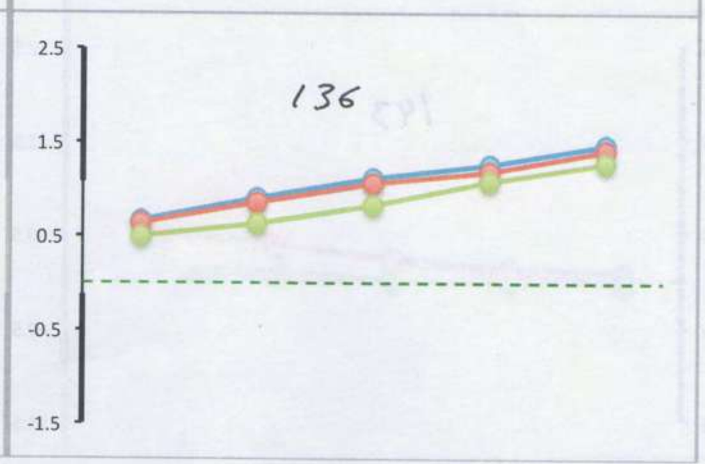
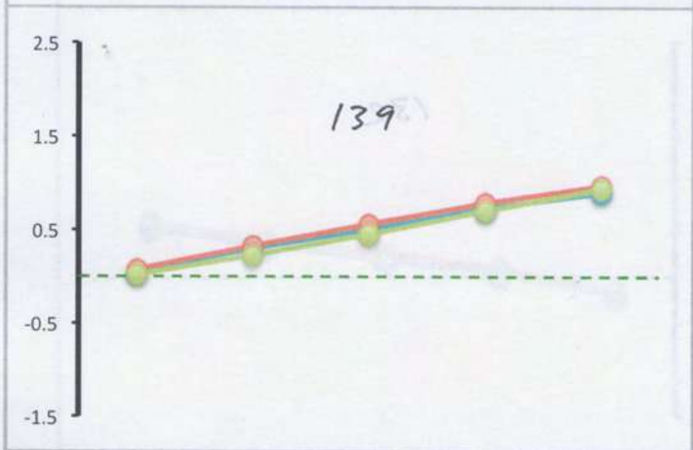
2.5
1.5
0.5
-0.5
-1.5


136
2.5
1.5
0.5
-0.5
-1.5

6/27/12


2.5
1.5
0.5
-0.5
-1.5
137


2.5
1.5
0.5
-0.5
-1.5
138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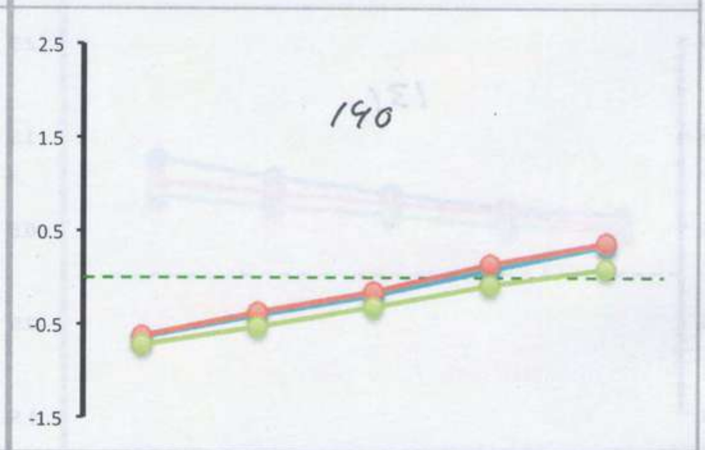
1.5
0.5
-0.5
-1.5
139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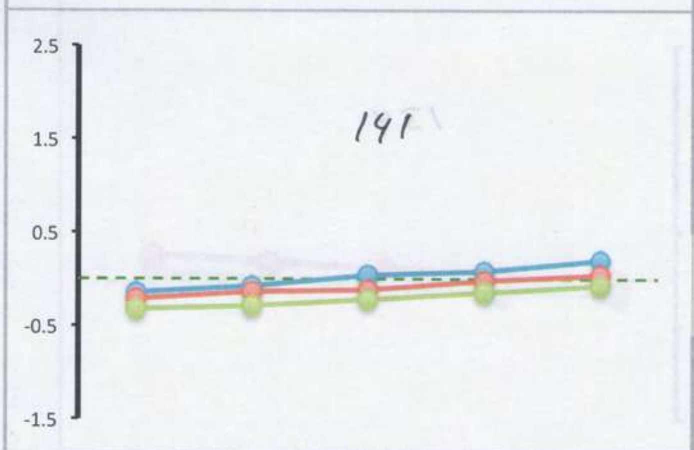
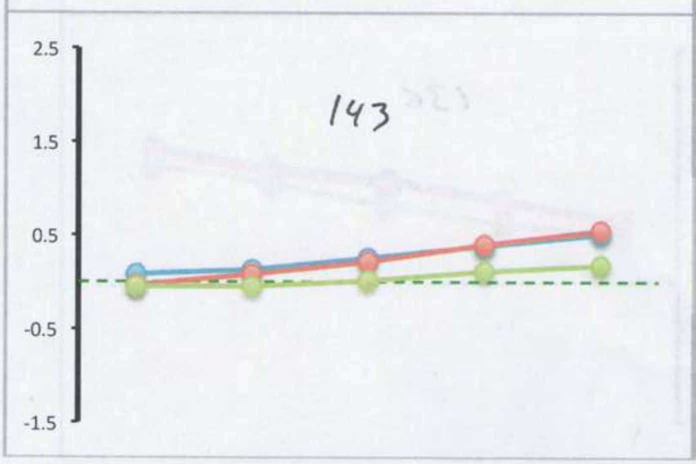
-0.5
-1.5
140


2.5
1.5
0.5
-0.5
-1.5
141


2.5
1.5
0.5
-0.5
-1.5
142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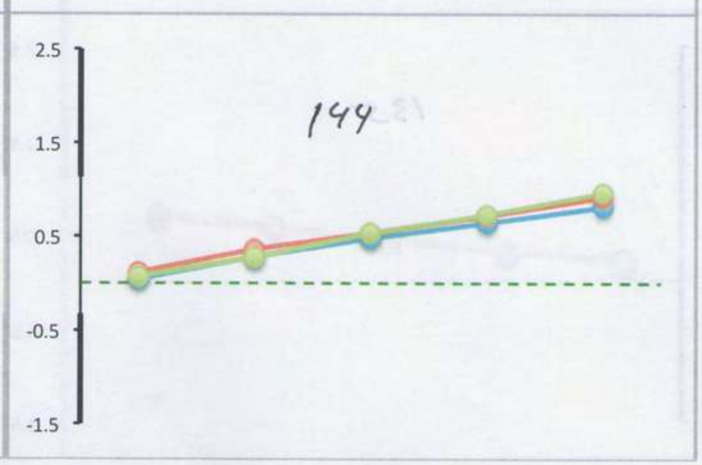
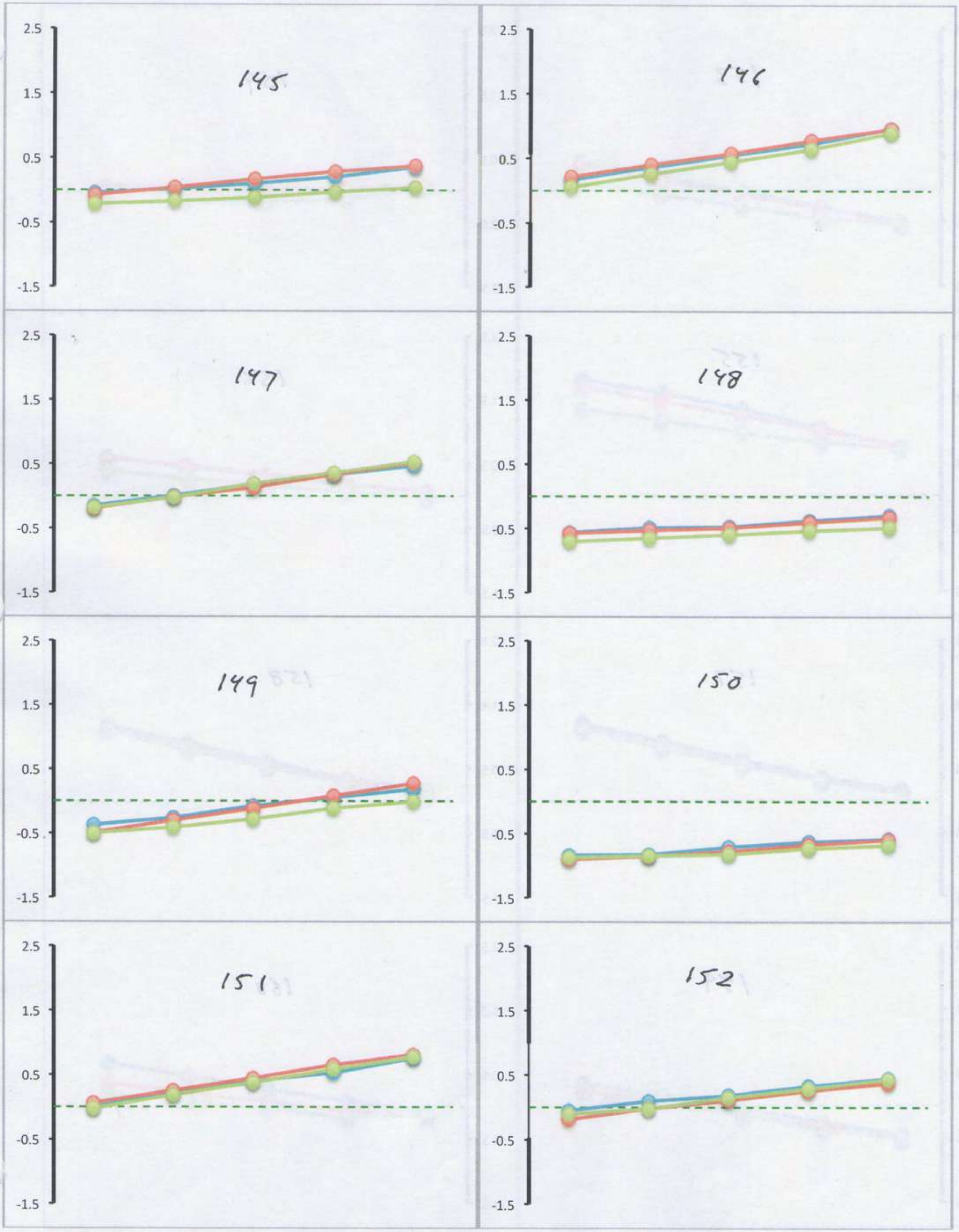
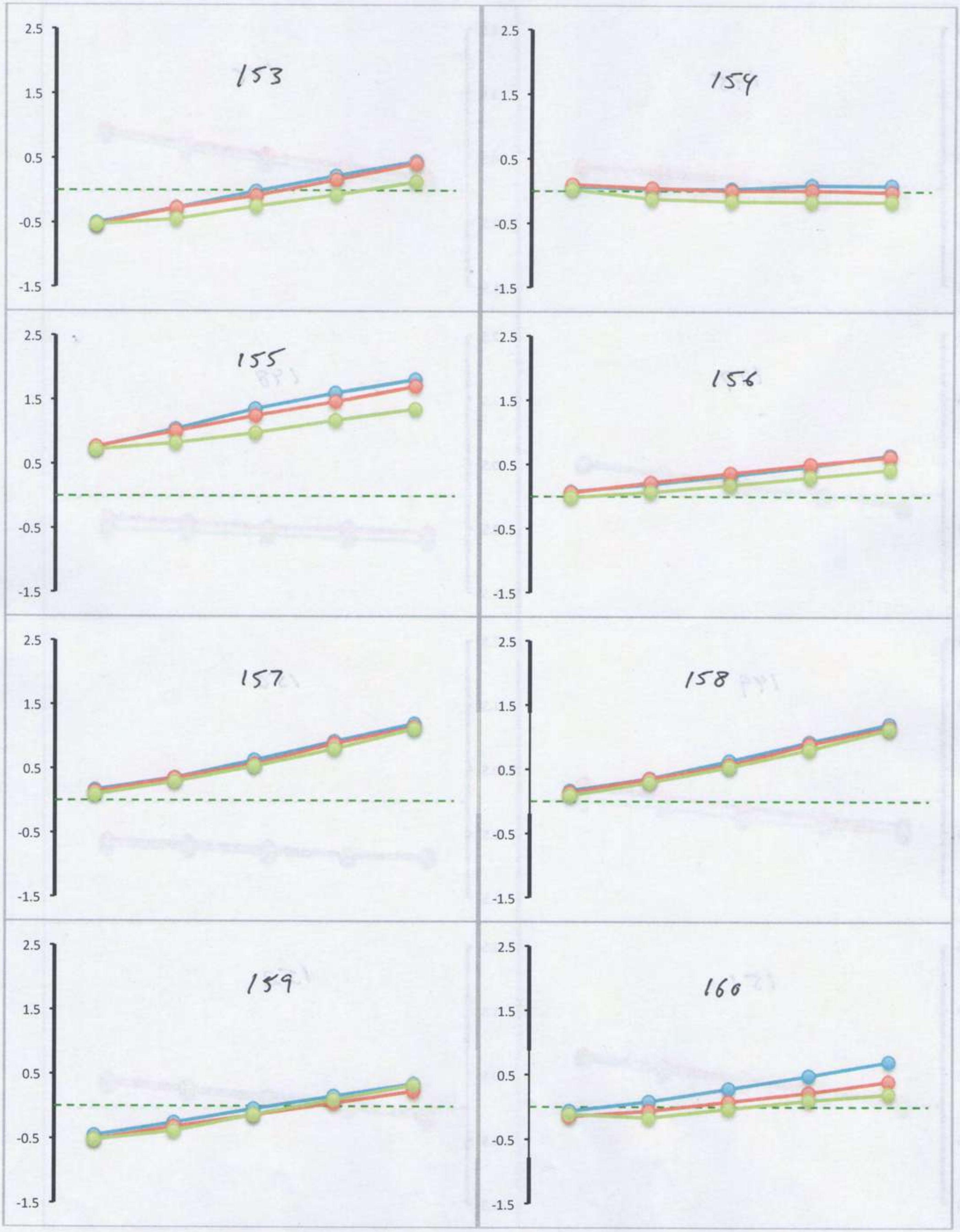
1.5
0.5
-0.5
-1.5
143


2.5
1.5
0.5
-0.5
-1.5
144

6/27/12



6/27/12



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Initials	Date
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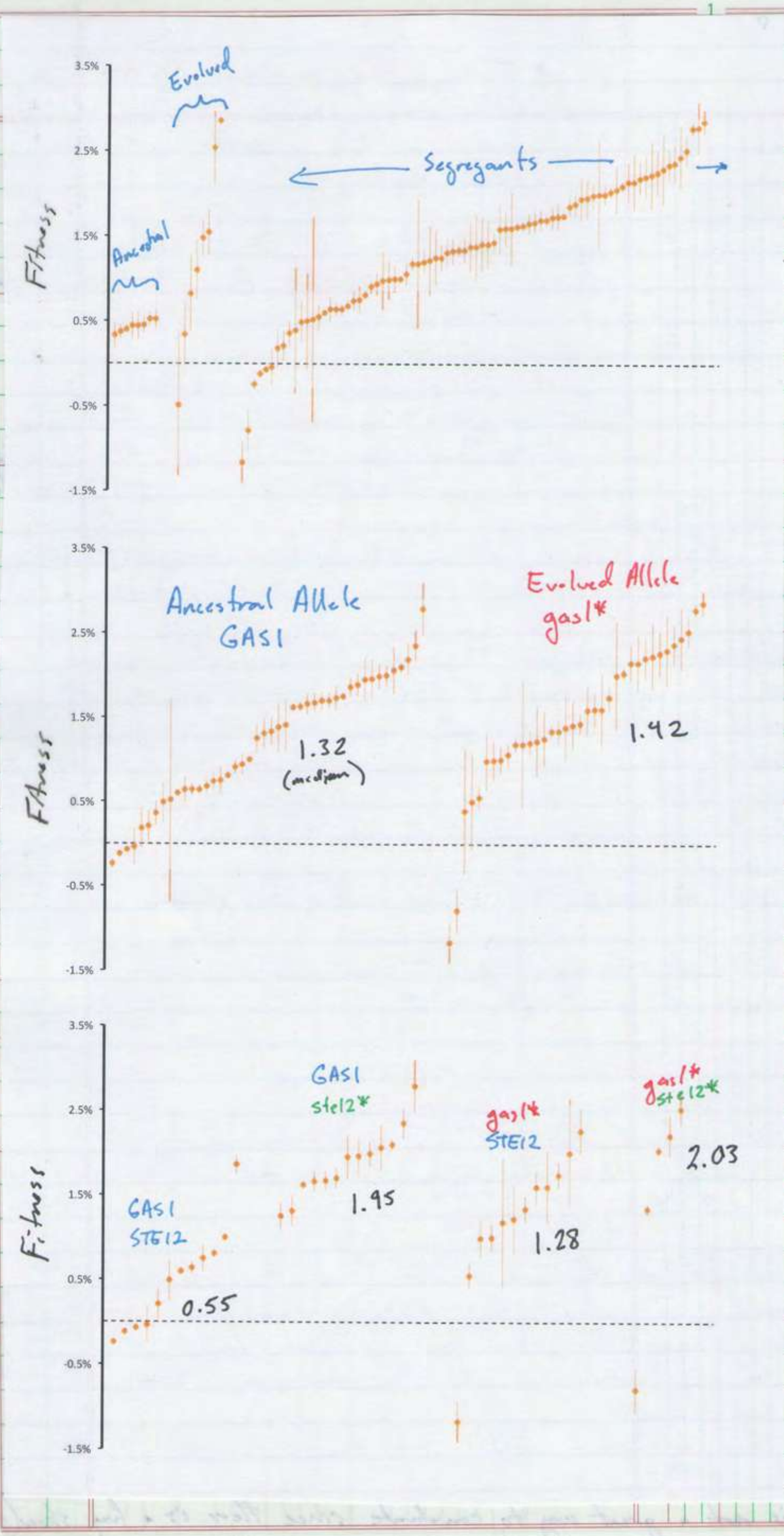
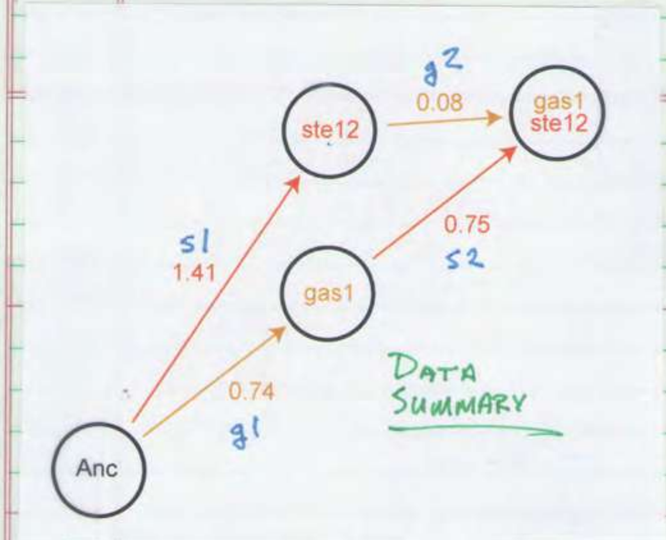
Approved By	

PRELIMINARY ANALYSIS OF FITNESS ASSAYS ON SEGREGANTS 81-160



7/3/12

NOTES & MORE PRELIMINARY ANALYSIS



- Taking the medians (p. 113) as the actual values, there is one expected result and two surprises:

  Expected: The fitness advantage conferred by the ste12* mutation is 1.41%, close to expected.

  Not Expected #1: The advantage of gas1* is only 0.74% — I was expecting ~3%, w/ the combined gas1* ste12* having ~4.5% over Ancestor.

Not Expected #2: The figure above shows strong negative epistasis. The null expectation is that $s1 = s2 = 1.5\%$ and that $g1 = g2$.

- There is one big inconsistency between the data from the original experiment (p 1) and the reconstructed fitnesses. — That is the ~~fitness~~ highest fitness (of both the evolved clones and the segregants) is lower than expected. I was expecting the evolved clones and the fittest segregants to have a fitness of 4.5%. The highest I observe is about 3-3.5% (p. 89 & 113)

  → At one level it could be said that this is due to ~~focus~~ a combination of:
  (1) Lower than expected fitness of gas1*, and
  (2) Negative epistasis of gas1* and ste12*

Possible Explanations:

(1) This data does not account for elo1*. This is true but elo1* cannot make up the diff since no segregants or evolved clones have the expected 4.5%.

(2) Our original estimates of the % sterile (and thus changes in % sterile) are ~~a~~ wrong. I don't buy this either since we calibrated carefully using the standard curves, and the frequencies seem to be corroborated by the sequencing data.
→ This comes w/ two caveats:
(A) We know that several special cases have changed their response to αF (false FDS)
(B) Sequencing is not a great way to corroborate since there is a freq. skew (p. 6 & 91).

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NOTES & MORE PRELIMINARY ANALYSIS CONT.

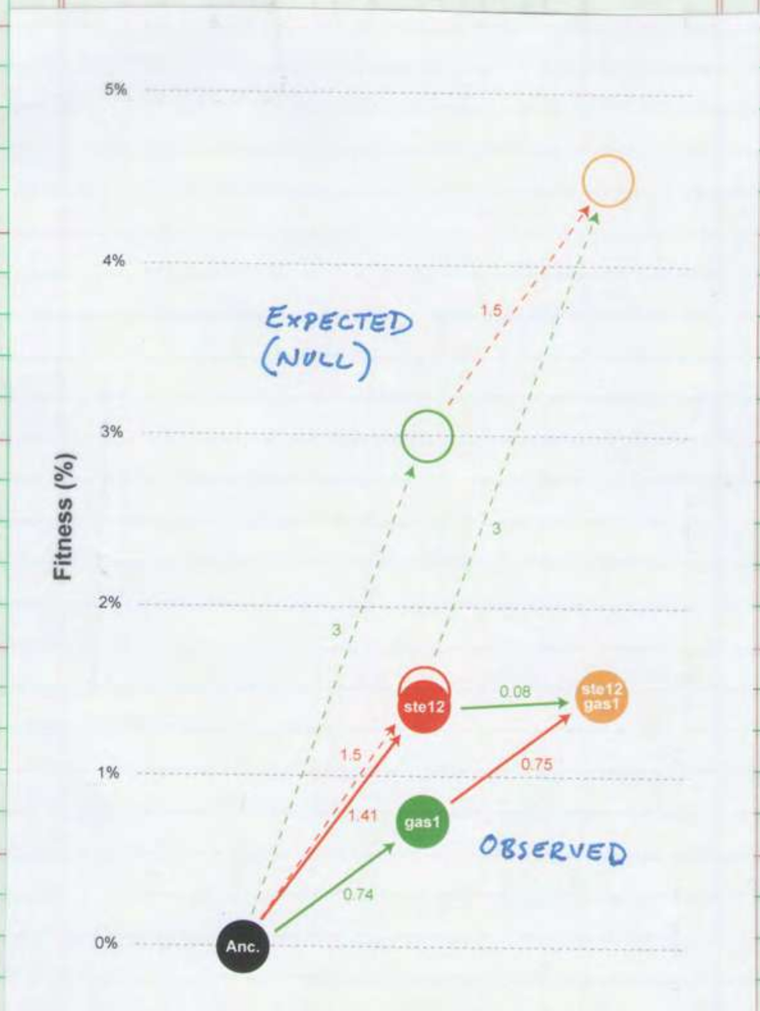
Possible Explanations Continued:

(3) The data are correct, but because the evolution of BYS1-A08 was done at the small bottleneck and the fitness assays at the big, the fitness of gas1# is different.

→ I will do these fitness assays at the small bottleneck.

(4) The necessity of going through a single clone fixes deleterious mutations which segregate in the cross

→ I do see spread in the fitnesses of the evolved clones, but (unless many deleterious mutations are segregating) I would expect to see some segregants with 4.5% fitness.



	Initials	Date
Prepared By		
Approved By		

		1	2	3

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	Initials	Date
Prepared By		
Approved By		

PREPARATION FOR SEQUENCING BULK-SEGREGANT SAMPLES

Strategy: I will amplify & sequence the 4 genes (STE12, GAS1, ELO1, & ERG1) from the pooled gDNA samples (p. 95-98), multiplex both by time point and by experiment and sequence on 1/10 of an Illumina lane.

- 1/10 lane ~ 10 M reads
- 12 time points * 4 genes * 3 experiments → 144 samples
- Coverage per sample ~ $10^7/144$ → ~ 70K reads/sample

I could conceivably multiplex ~~all~~ all of my populations in one lane.

The sequencing will be single-end, 99 bp. ~~ent~~ including 6bp "Experiment" indices on each end.

```
GAS1   GACGTCAGACAATACATCAG CGACAAGAACTACAGAAAAATTC [C] AGTTGGCTACTCTTCCAATGATG ACGAAGATACCAGAGTTAAG
ELO1   TTTCCTACATACATACCATC ATGGTGCTACAGCTTTACTGTGC [T] ATAATCAATTGGTTGGTTACACA GCAGTTACATGGGTTCCTGT
STE12  GGCCAAATGAAAACTGTACA AATGCAAAATCTCACAACTCT [GGCCAG] CAAACTAAACAACACCAATT ATATTCTAACAACTTCCAGC
ERG1   TTGTTTGCTTTTCCTTTTCC TTATTTCTTTCTAGCTTCTAATT [T] TCTTTCTTTTTTTTTTTTTC ATTGAAAATTATATATATAT
```

Forward Primer

Mutation

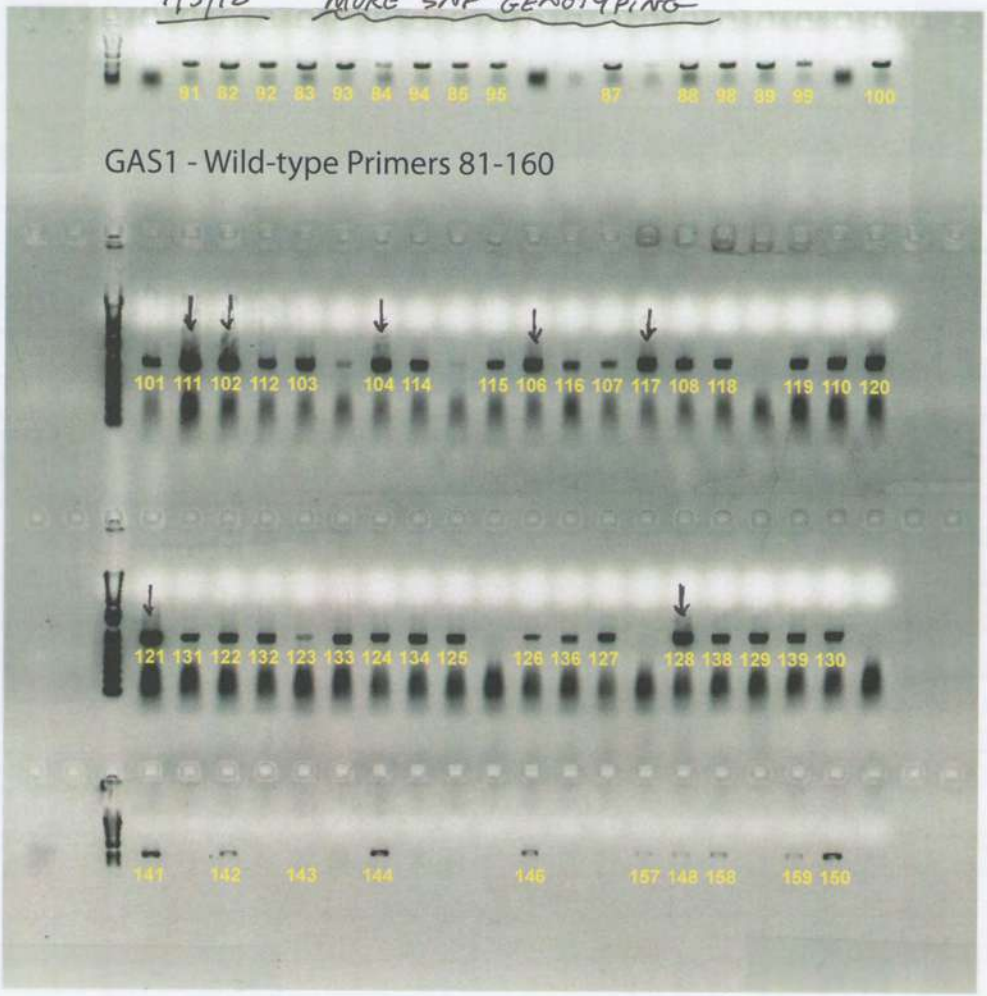
Reverse Primer

|← Amplified Sequence →|

	BASE 1	2	3	4	5	6	Total
Barcode A	G	T	A	T	A	G	Exp. 1 Forward
Barcode B	A	C	G	G	C	T	" 1 Reverse
Barcode C	T	A	A	A	T	T	" 2 Forward
Barcode D	A	C	T	C	G	A	" 2 Reverse
Barcode E	C	G	T	T	C	G	" 3 Forward
Barcode F	G	A	C	C	G	C	" 3 Reverse
Count G	2	1	1	1	2	2	9
Count A	2	2	2	1	1	1	9
Count T	1	1	2	2	1	2	9
Count C	1	2	1	2	2	1	9

		1	2	3

7/5/12 MORE SNP GENOTYPING



	Initials	Date
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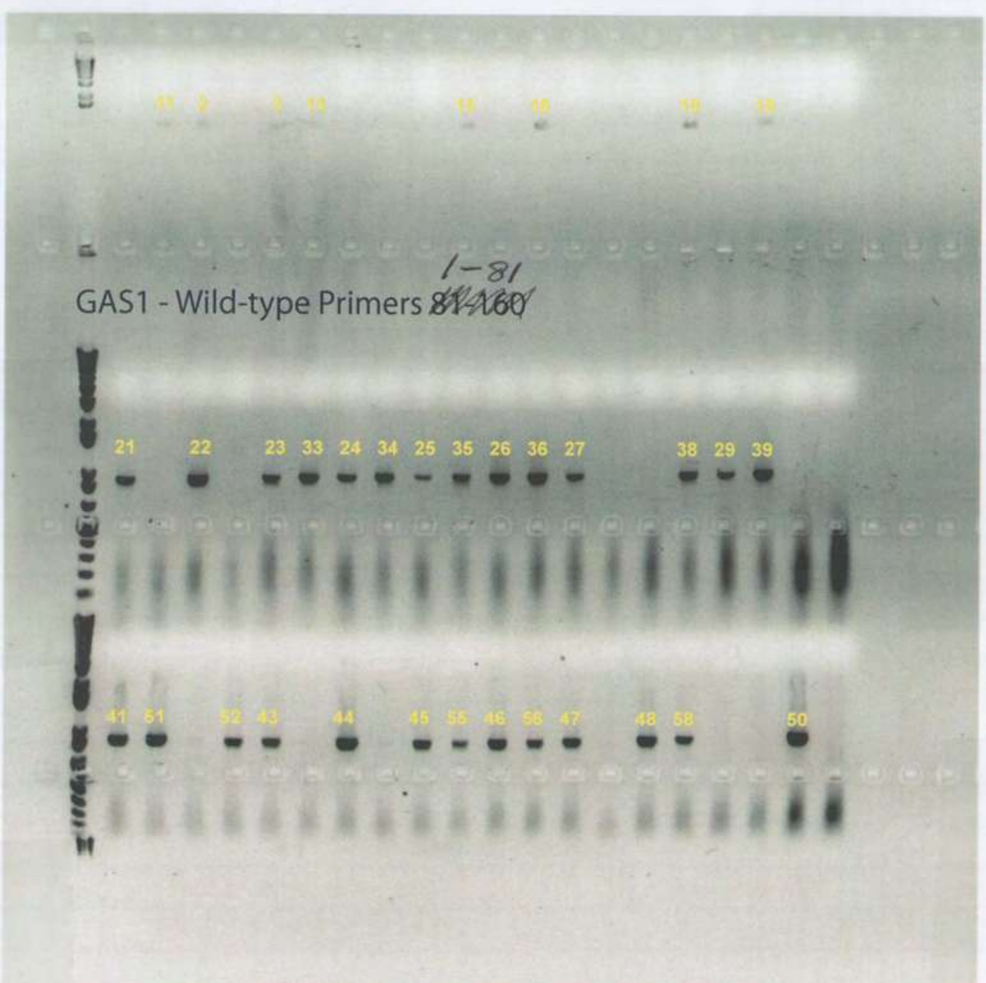
Approved By		

→ Many of these are probably false positives. Those ones w/ arrows are more believable for two reasons: (1) the bands are more intense, and (2) they are corroborated by the SNP PCR using the Mutant primers (p 76).



7/5/12

NOTES ON SNP GENOTYPING

- I have been having issues w/ the SNP PCRs. Basically the plates are not sealing, resulting in little volume around the edges.

→ This means that there is likely also mixing of adjacent wells and a number of false positives.

- I ran the gel w/o the solid base and it caused the bands on the top and bottom to run off (out) of the gel.

→ The dye was noticeably retarded.

- I will probably need to redo these PCRs

- I am running the PCRs w/ the EL01 mutant primer (#12, p74), with the modifications: (1) I doubled the volume to 40λ, (2) I really pressed the seal as well using an eraser.

H_2O	4480 λ
10× Buffer	800
dNTPs (10mM)	240
Primer 355	160
Primer 393	160
PiTaq	160
	6000

→ add 30λ to 10λ template (1:20 dilution)

7/14/12

- 1-80 were run in the PCRx thermalcycler
- 81-160 " " " PX2 "

- I am still having leakage issues & it seems to be worse around the edges and in PX2.

- I ran a test of just water & loading dye using the rubber holes — that seemed to better overall, but there were still problems w/ PX2

	Initials	Date
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Approved By		

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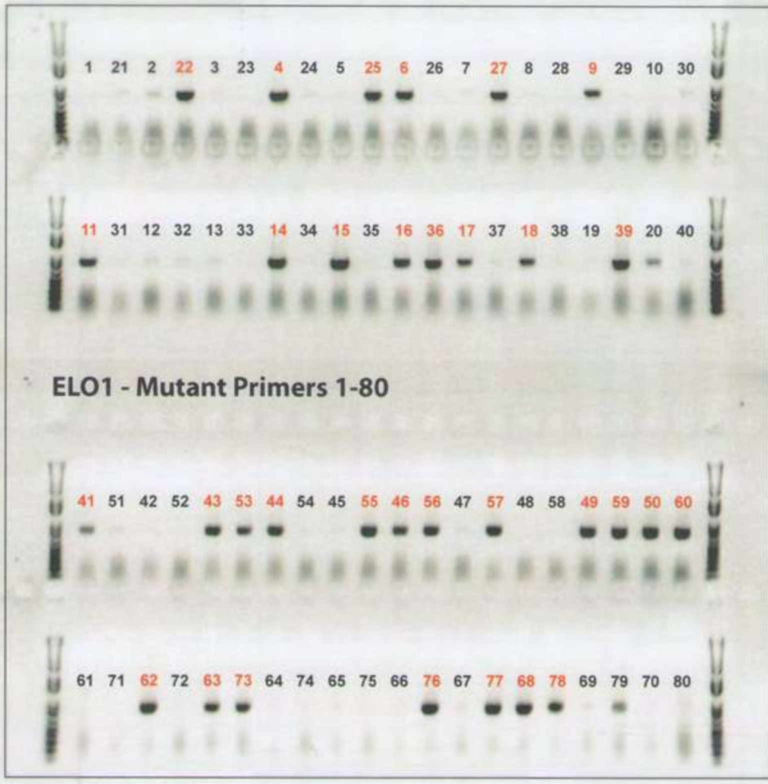
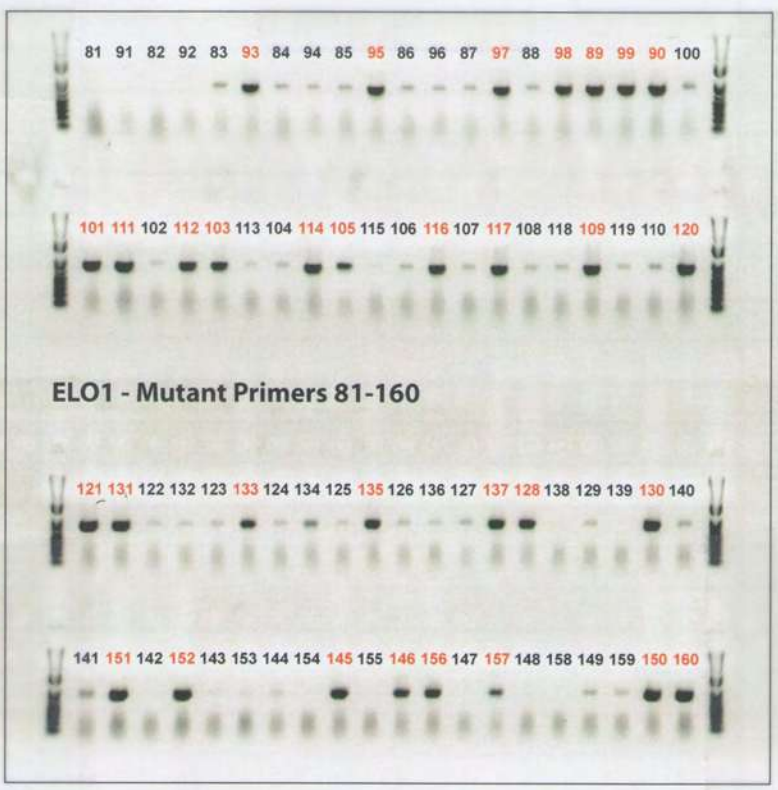
RESULTS OF PCR GENOTYPING W/ ELO1 MUTANT PRIMERS



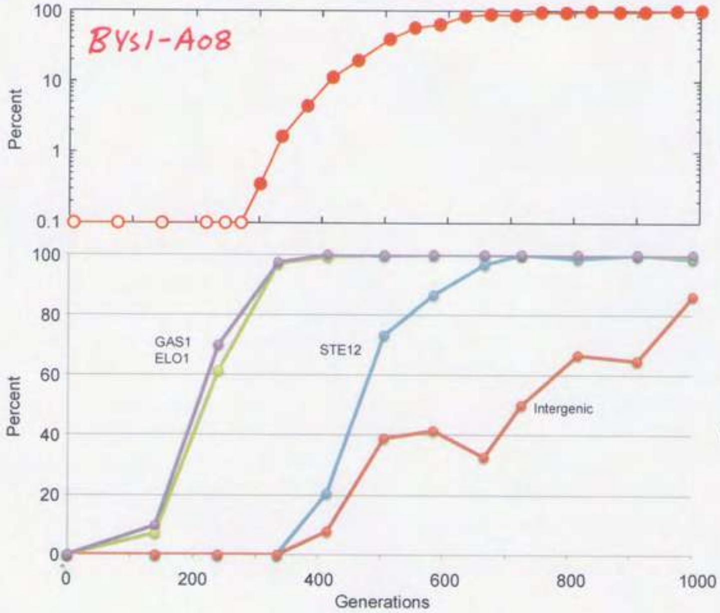
33/80

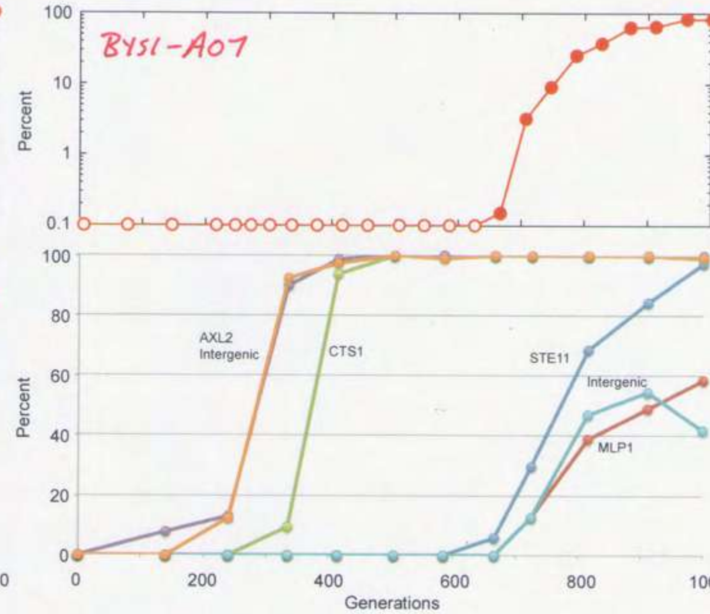


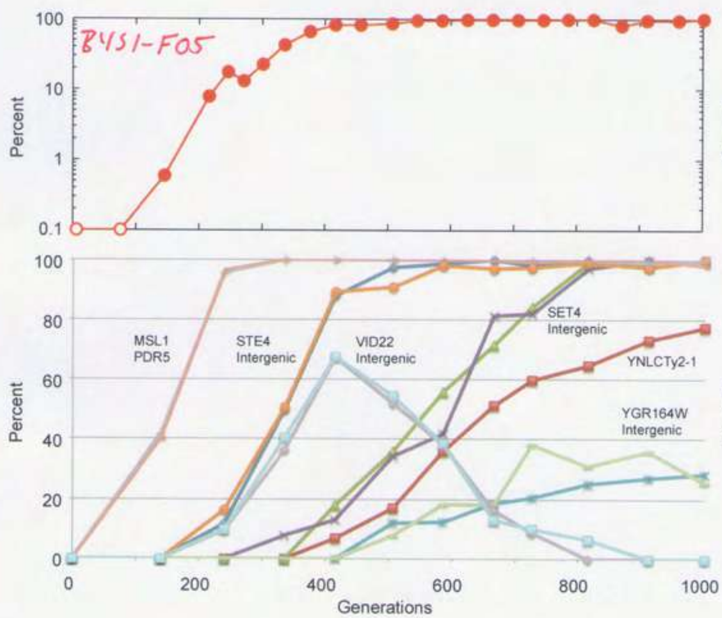
32/80

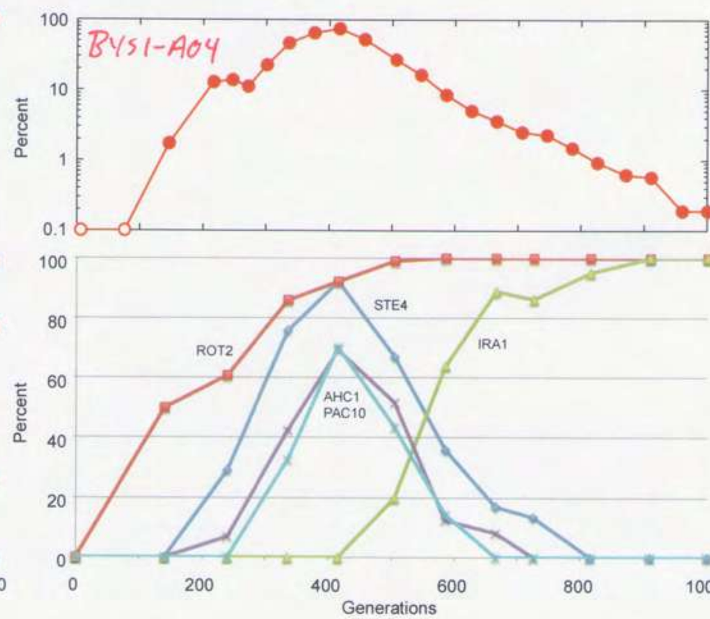
		1	2	3

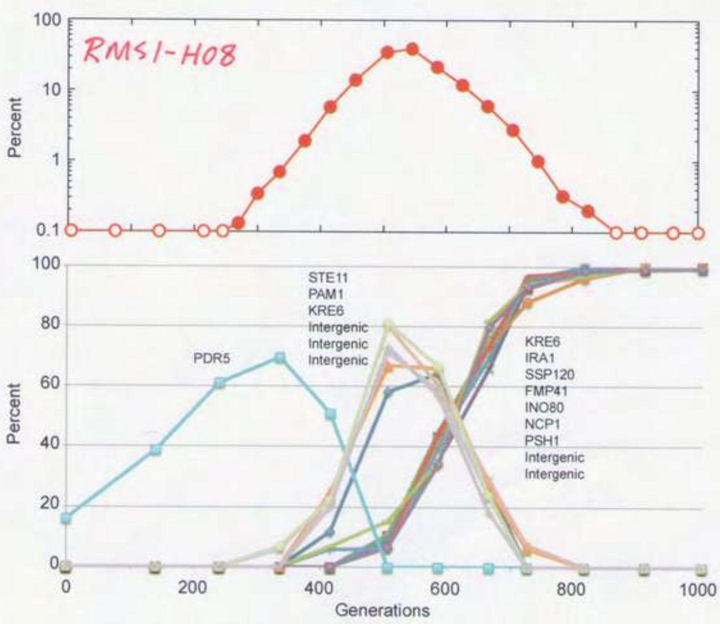
7/14/12 PRELIMINARY ANALYSIS OF SEQUENCING DATA







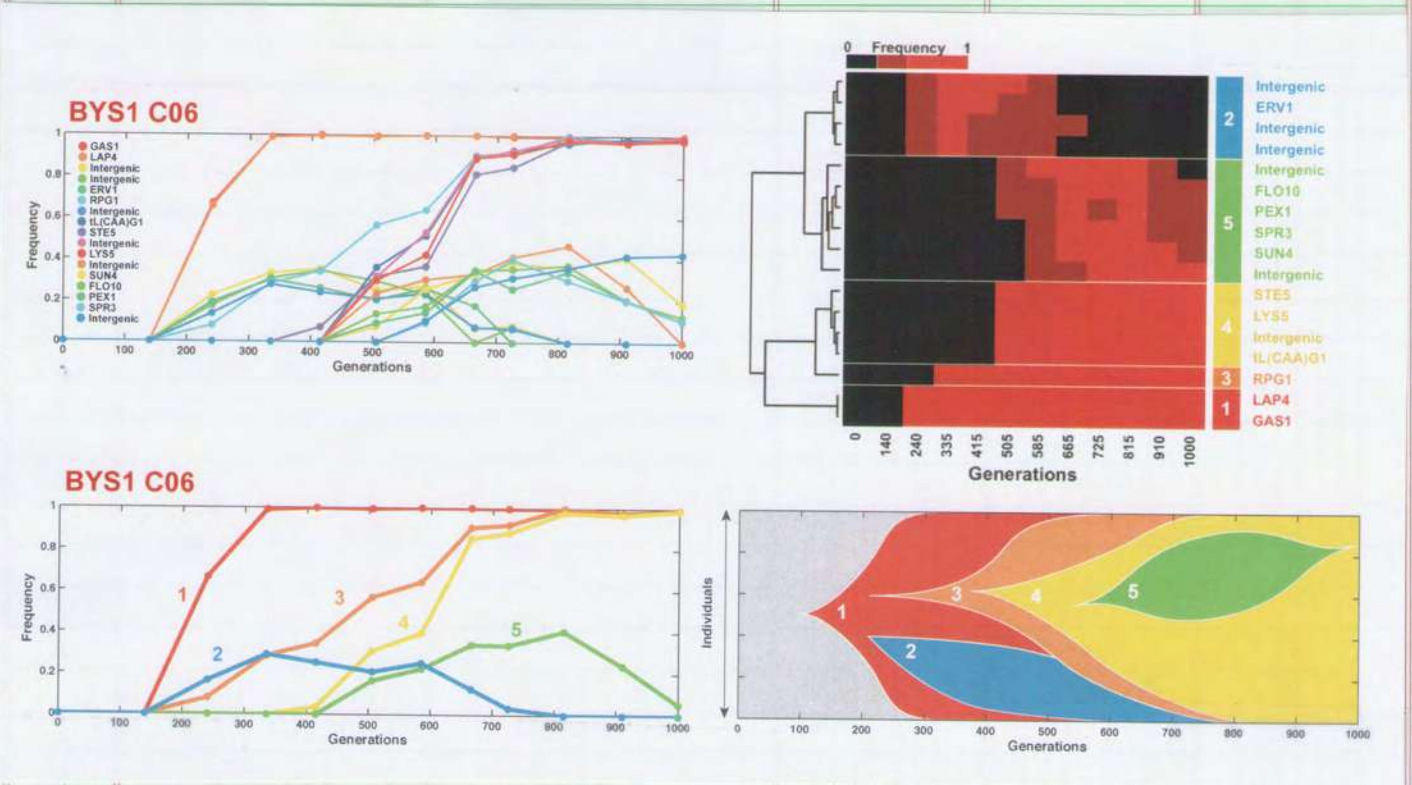




NOTES

- The preliminary analysis looks pretty good.
- This was done using Mark's scripts, ranking his output by autocorrelation, then looking through the first 50 or so trajectories by eye.
- Just these five populations show a range of interesting trajectories

CLUSTERING TO ASSIGN HAPLOTYPES



- In order to assign mutations to haplotypes, I clustered ~~them~~ the trajectories using Euclidean distance and non-mean-centered.
  - ↳ I played around with various other types of clustering as well as SVD and this seemed to work best.

7/14/12

	Initials	Date
Prepared By		
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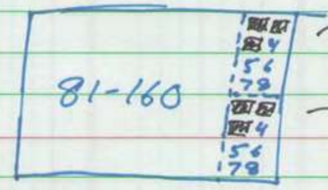
FITNESS ASSAYS AT THE SMALL BOTTLENECK

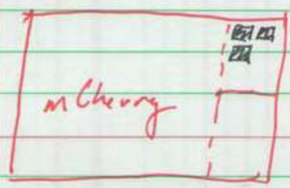
Given that when I did fitness assays on BY51-A08 segregants at the ~~small~~ large bottleneck I got smaller fitnesses than expected, I have ~~gone~~ started those assays again at the small bottleneck. → see "(3)" on p. 115.

I decided to use Plate 2 (Segregants 81-160) since the SNP genotyping is better for these.



→ remove Anc 9-11 and replace w/ pooled segregant population (p. 94) from freezer

→ remove Evo 9-11 and replace w/ 640, 641, 642 (p. 49)



→ 3 empty wells

- On 7/7 I struck yGIL520 (mCherry Ref) from freezer
- On 7/9 I struck yGIL640-642 onto 5FOA (to select against plasmid)
- Mixed plates and propagate in triplicate at the small bottleneck.
- After transfers I take the bulk segregant wells from plate A add 500 λ of H_2O, spin, aspirate, 3' freeze pellet.

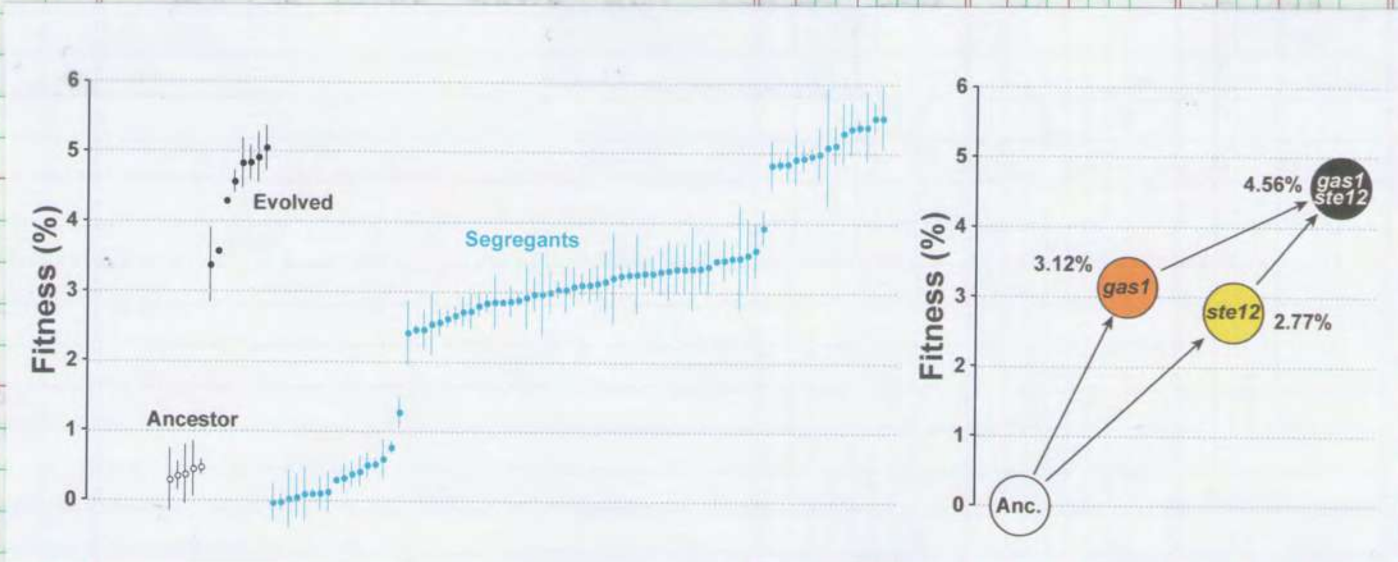
8/16/12

In retrospect I did not need to put the pooled segregant population into 3 wells since I ran ~~the~~ 3 plates.

8/16/12

RESULTS OF FITNESS ASSAYS AT THE SMALL BOTTLENECK.

Repeating the fitness assays at the small bottleneck made all the difference:



- Expected fitness of Evolved clones (By measuring sterile slope) is 4.34%

The evolved clones are at 4.5% (with two lower outliers) — I should check to see if these are STE12. WT. (I suspect they are).

- The segregants fall into discrete clusters. — Compare to the continuous distribution (p. 113) at the big bottleneck.

- Using the current state of the genotyping I can assign the genotypes (at GAS1 & STE12) to these strains. This now falls in line with what I expected (see p. 115). The fitness of the ste12* mutation is slightly higher than expected (2.77%); thus this still shows diminishing returns epistasis.

I need to fully genotype these strains.

NOTES & QUESTIONS

(1) Why are the fittest segregants fitter than the evolved clones?
- Could there be a fixed deleterious mutation? ELO1? something else?
- Could the selection of individual clones to backcross have fixed low frequency deleterious mutations — something I speculated about on p. 41.

8/16/12

	Initials	Date
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NOTES & QUESTIONS CONTINUED

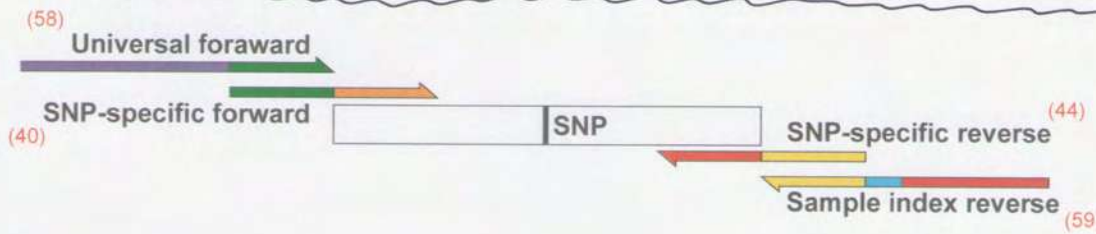
(2) One might have expected the fitness of the ancestor relative to the ancestor to be higher than the ~~measured~~ fitness estimated from the slope. We in fact see that (4.34% vs. 4.56%) but it is not clear how we could assign significance to this.

(3) Why is the fitness of ste12* 2.72%? Perhaps this will change once I fully genotype these strains. Or perhaps there is an interaction w/ elo1*

(4) It is interesting to see that gas1* has a 3% effect at the small bottleneck but only 0.74% at the big bottleneck
↳ it follows that gas1* must exert its advantage at low density or when coming out of lag.
↳ It is interesting to note that gas1 was only hit in the small population size cultures.

(5) Since I now know that it is extremely important to do the fitness assays ~~on the~~ under the conditions as the original experiment, I discarded the samples from the first Bulk Segregant experiment (p. 95-98).

8/16/12 NEW PCR STRATEGY FOR SEQUENCING BULK-SEGREGANT EXPERIMENT



FORWARD PRIMER DESIGN -------------->

After consulting w/ Wei, I decided to abandon my previous scheme (p.117) in favor of this one.

oGIL421 Universal_forward (58 bp)
AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT

oGIL422 GAS1_forward_1 (40 bp)
ACACGACGCTCTTCCGATCTGACGTCAGACAATACATCAG
oGIL423 ELO1_forward_~~2~~1 (40 bp)
ACACGACGCTCTTCCGATCTTTTCCTACATACATACCATC
oGIL424 STE12_forward_~~3~~1 (40 bp)
ACACGACGCTCTTCCGATCTGGCCAAATGAAAACTGTACA
oGIL425 ERG1_forward_~~4~~1 (40 bp)
ACACGACGCTCTTCCGATCTTTGTTTGCTTTTCCTTTTCC
oGIL426 GAS1_forward_~~1~~2 (40 bp)
ACACGACGCTCTTCCGATCTGTCAGACAATACATCAGCGA
oGIL427 ELO1_forward_2 (40 bp)
ACACGACGCTCTTCCGATCTCCTACATACATACCATCATG
oGIL428 STE12_forward_~~3~~2 (40 bp)
ACACGACGCTCTTCCGATCTCAAATGAAAACTGTACAAAT
oGIL429 ERG1_forward_~~4~~2 (40 bp)
ACACGACGCTCTTCCGATCTTTTGCTTTTCCTTTTCCTTA

The second set of Forward primers is shifted by 3 bp allowing multiplexing (I hope).

REVERSE PRIMER DESIGN <--------------

oGIL430 Sample_Index01 (59 bp)
GATCGGAAGAGCACACGTCTGAACTCCAGTCACATCACGATCTCGTATGCCGTCTTCTG
oGIL431 Sample_Index02 (59 bp)
GATCGGAAGAGCACACGTCTGAACTCCAGTCACCGATGTATCTCGTATGCCGTCTTCTG
oGIL432 Sample_Index03 (59 bp)
GATCGGAAGAGCACACGTCTGAACTCCAGTCACTTAGGCATCTCGTATGCCGTCTTCTG
oGIL433 Sample_Index04 (59 bp)
GATCGGAAGAGCACACGTCTGAACTCCAGTCACTGACCAATCTCGTATGCCGTCTTCTG
oGIL434 Sample_Index05 (59 bp)
GATCGGAAGAGCACACGTCTGAACTCCAGTCACACAGTGATCTCGTATGCCGTCTTCTG
oGIL435 Sample_Index06 (59 bp)
GATCGGAAGAGCACACGTCTGAACTCCAGTCACGCCAATATCTCGTATGCCGTCTTCTG
oGIL436 Sample_Index07 (59 bp)
GATCGGAAGAGCACACGTCTGAACTCCAGTCACCAGATCATCTCGTATGCCGTCTTCTG
oGIL437 Sample_Index08 (59 bp)
GATCGGAAGAGCACACGTCTGAACTCCAGTCACACTTGAATCTCGTATGCCGTCTTCTG
oGIL438 Sample_Index09 (59 bp)
GATCGGAAGAGCACACGTCTGAACTCCAGTCACGATCAGATCTCGTATGCCGTCTTCTG
oGIL439 Sample_Index10 (59 bp)
GATCGGAAGAGCACACGTCTGAACTCCAGTCACTAGCTTATCTCGTATGCCGTCTTCTG
oGIL440 Sample_Index11 (59 bp)
GATCGGAAGAGCACACGTCTGAACTCCAGTCACGGCTACATCTCGTATGCCGTCTTCTG
oGIL441 Sample_Index12 (59 bp)
GATCGGAAGAGCACACGTCTGAACTCCAGTCACCTTGTAATCTCGTATGCCGTCTTCTG

oGIL442 GAS1_reverse (44 bp)
ATCTCGTATGCCGTCTTCTGCTTGCTTAACTCTGGTATCTTCGT
oGIL443 ELO1_reverse (44 bp)
ATCTCGTATGCCGTCTTCTGCTTGACAGGAACCCATGTAACTGC
oGIL444 STE12_reverse (44 bp)
ATCTCGTATGCCGTCTTCTGCTTGGCTGGAAGTTGTTAGAATAT
oGIL445 ERG1_reverse (44 bp)
ATCTCGTATGCCGTCTTCTGCTTGATATATATATAATTTTCAAT

8/16/12

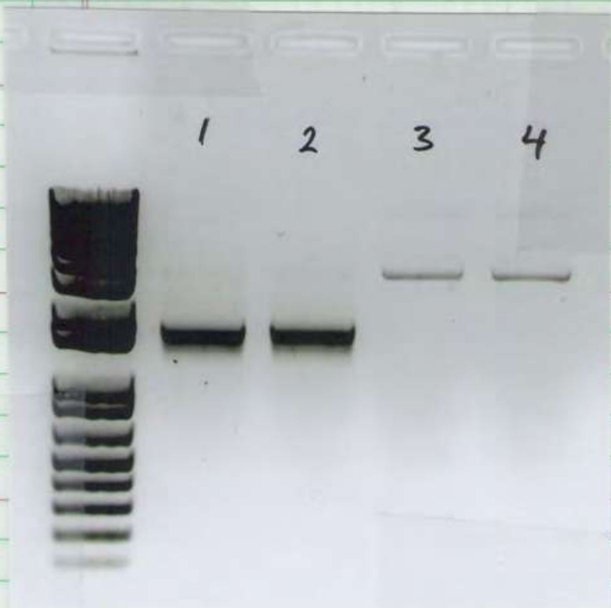
	Initials	Date
Prepared By		
Approved By		

TRANSFORMATION OF URA3 NEAR MATα IN yGIL646

· Yesterday I amplified the URA3MX cassette off of pAG60 using the following:

446 →	URA3_MATalpha_Forward	AATTAATATTCATGTTTAGTACTGAAAATTAAGAATACTTGATCCCCGGGTTAATTAAGG
447 →	URA3_MATalpha_Reverse	CTGGTTTCTTATCAAAAAATATTATCTACTGTAGTTATGCTAATACGACTCACTATAGGG

These primers integrate URA3MX between BUD5 and PHO87, tightly linking URA3 to MATα



LANE	
1	PCR off prep 1
2	PCR off prep 2
3	pAG60 prep. 1
4	pAG60 prep. 2

→ Elute 30λ EB; transform 10λ

8/20/12

· Too many (small) colonies & too much bkg. on -Ura plates.

↳ I replica plated to another -Ura plate (this time a minimal plate supplemented w/ his & trp. — the transformation plates were CSM-Ura).

8/20/12

	Initials	Date
Prepared By		
Approved By		

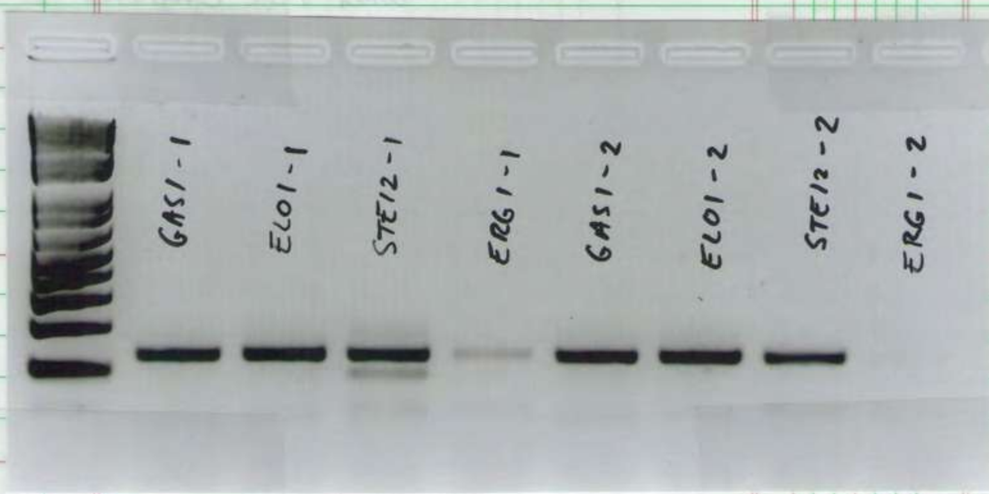
PILOT EXPERIMENT OF BULK SEGREGANT PCRs

· I made gDNA preps of the 18 Bulk Segregant samples (p. 127)

Experiment	Generation					
A	0	10	20	30	40	50
B			"			
C			"			

· I amplified the 4 SNPs genes (GAS1, ELO1, STE12, ERG1) from gDNA-A0 using both versions of the forward primers. (p. 131).

→ STANDARD PCR CONDITIONS
50λ PCR
30 rounds
only change – 1 min. extension



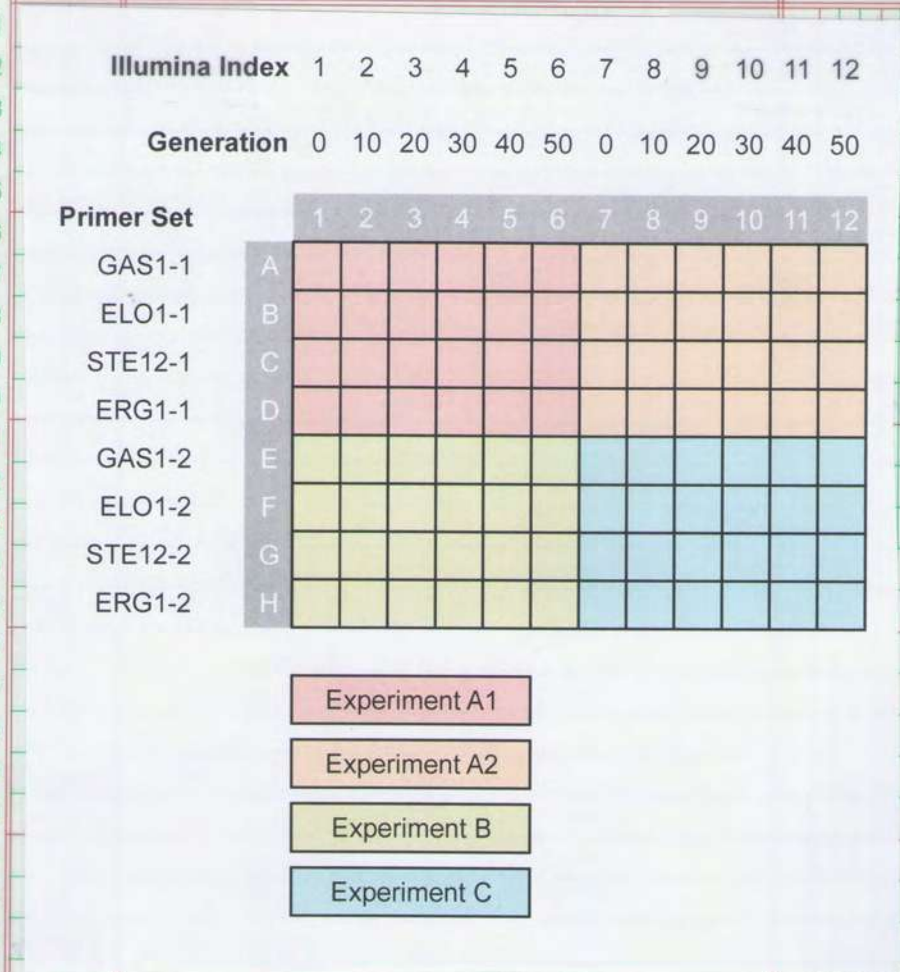
← expected ~1406p

→ I used 0.5λ of the above PCRs as template for the second round; in this case I amplified w/ 421 & 430 (p. 131)

8/21/12

PCR AMPLIFICATION FOR ILLUMINA SEQUENCING

PCR Plate Setup:



8 mixes (one for each primer pair)

1st Round Setup

H_2O	560
10×Buff.	70
dNTPs	21
Primer 1	14
Primer 2	14
Taq	7

→ 49 λ each

- same PCR as p. 135
- When PCR completed I added 150 λ H_2O to each well.

2nd Round setup

H_2O	5070 λ
10×Buffer	650
dNTPs	195
Universal F primer	130
Taq	65

· split into 12 tubes

Mix 470 λ
Reverse Primer 10 λ

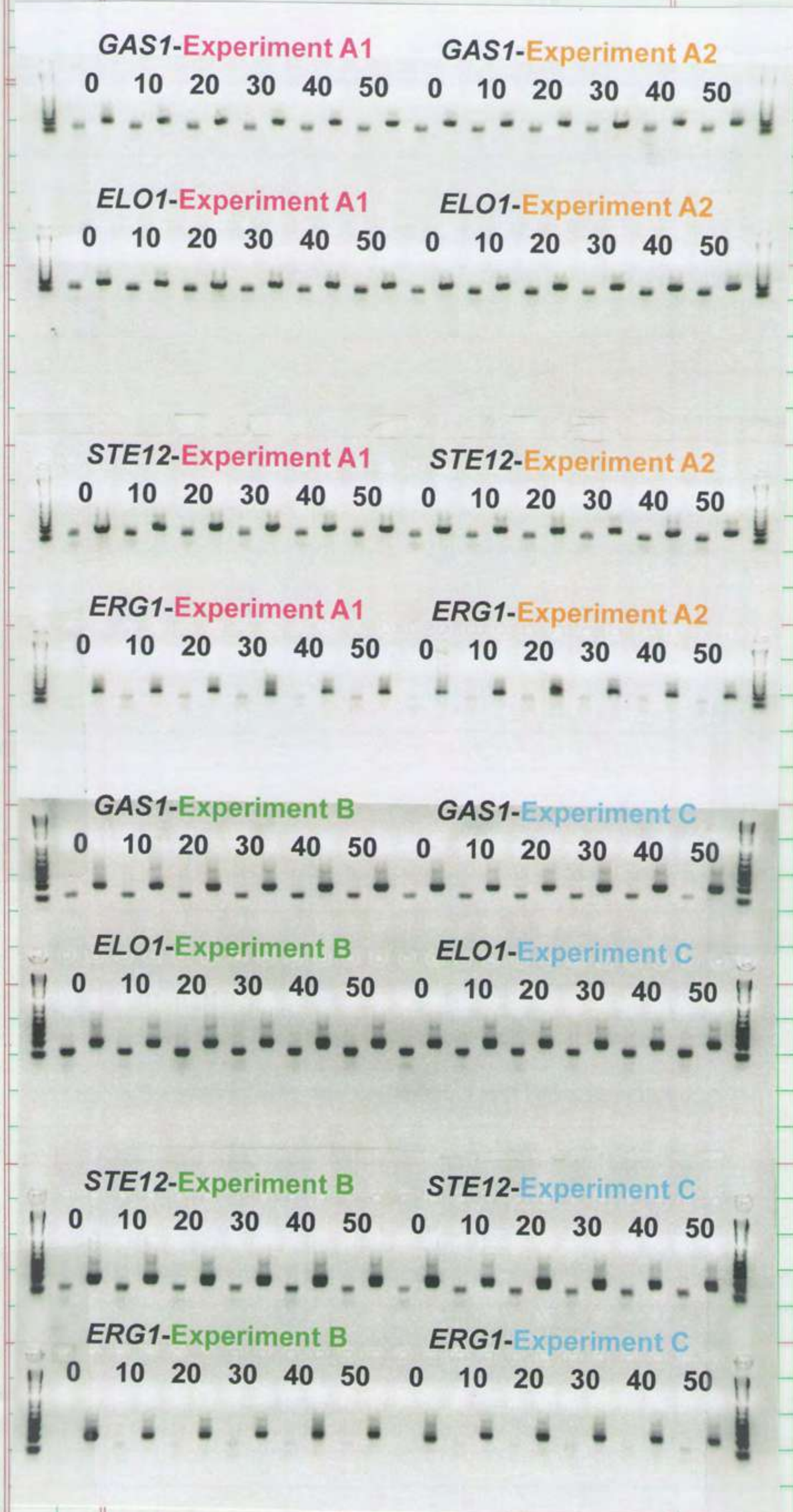
→ 48 λ into each well + 2 λ of diluted 1st round.

	Initials	Date
Prepared By		
Approved By		

8/22/12

PREPARATION OF SEQUENCING POOL

PCRs from Round 1 & 2:

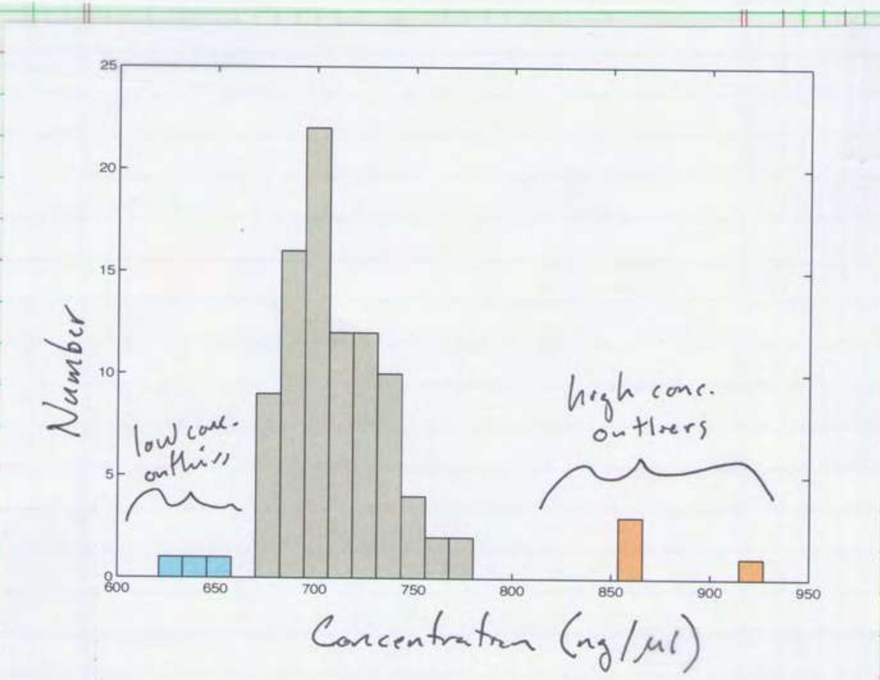


It looks like all of the PCRs worked. I need to quantify.

8/22/12 QUANTIFICATION OF PCRs

Primers	Experiment	ng/ul
GAS1 - 1	A1	689
GAS1 - 1	A1	678
GAS1 - 1	A1	743
GAS1 - 1	A1	670
GAS1 - 1	A1	643
GAS1 - 1	A1	687
GAS1 - 1	A2	708
GAS1 - 1	A2	706
GAS1 - 1	A2	698
GAS1 - 1	A2	863
GAS1 - 1	A2	689
GAS1 - 1	A2	704
ELO1 - 1	A1	702
ELO1 - 1	A1	687
ELO1 - 1	A1	854
ELO1 - 1	A1	718
ELO1 - 1	A1	709
ELO1 - 1	A1	692
ELO1 - 1	A2	761
ELO1 - 1	A2	732
ELO1 - 1	A2	726
ELO1 - 1	A2	709
ELO1 - 1	A2	772
ELO1 - 1	A2	754
STE12 - 1	A1	862
STE12 - 1	A1	673
STE12 - 1	A1	727
STE12 - 1	A1	706
STE12 - 1	A1	728
STE12 - 1	A1	738
STE12 - 1	A2	720
STE12 - 1	A2	696
STE12 - 1	A2	697
STE12 - 1	A2	697
STE12 - 1	A2	751
STE12 - 1	A2	679
ERG1 - 1	A1	696
ERG1 - 1	A1	687
ERG1 - 1	A1	710
ERG1 - 1	A1	751
ERG1 - 1	A1	723
ERG1 - 1	A1	712
ERG1 - 1	A2	700
ERG1 - 1	A2	698
ERG1 - 1	A2	773
ERG1 - 1	A2	733
ERG1 - 1	A2	716
ERG1 - 1	A2	740

Primers	Experiment	ng/ul
GAS1 - 2	B	681
GAS1 - 2	B	727
GAS1 - 2	B	716
GAS1 - 2	B	691
GAS1 - 2	B	738
GAS1 - 2	B	722
GAS1 - 2	C	696
GAS1 - 2	C	694
GAS1 - 2	C	691
GAS1 - 2	C	749
GAS1 - 2	C	716
GAS1 - 2	C	743
ELO1 - 2	B	736
ELO1 - 2	B	702
ELO1 - 2	B	739
ELO1 - 2	B	707
ELO1 - 2	B	766
ELO1 - 2	B	694
ELO1 - 2	C	724
ELO1 - 2	C	738
ELO1 - 2	C	689
ELO1 - 2	C	621
ELO1 - 2	C	705
ELO1 - 2	C	698
STE12 - 2	B	689
STE12 - 2	B	678
STE12 - 2	B	718
STE12 - 2	B	709
STE12 - 2	B	723
STE12 - 2	B	701
STE12 - 2	C	724
STE12 - 2	C	706
STE12 - 2	C	698
STE12 - 2	C	675
STE12 - 2	C	704
STE12 - 2	C	690
ERG1 - 2	B	927
ERG1 - 2	B	652
ERG1 - 2	B	725
ERG1 - 2	B	690
ERG1 - 2	B	722
ERG1 - 2	B	688
ERG1 - 2	C	703
ERG1 - 2	C	703
ERG1 - 2	C	699
ERG1 - 2	C	680
ERG1 - 2	C	687
ERG1 - 2	C	676



~~All of the~~

- I measured the concentration by nanodrop.

All are ~700 ng/μL and there is less than 2-fold diff. between the highest and lowest.

I will therefore pool equal volumes of each.

	Initials	Date
Prepared By		
Approved By		

8/22/12

POOLING & PURIFYING PCR PRODUCTS

- I transferred 10λ of each PCR to a trough (~1mL) and transferred to a microfuge tube.
- I purified 2× 100λ of the pooled PCR using a Qiagen PCR purification kit
  - → eluted in 50λ H_2O each & ~~pooled~~ combined (100λ total)

← 200 bp

8/23/12 Quantification

Nanodrop: $\boxed{90\ ng/\mu L}$

→ I ran twice (90.2 & 89.8)

Q-Bit: 2λ into 198λ : 0.770 ng/µL → $\boxed{77\ ng/\mu L}$

10λ into 190λ : 3.19 ng/µL → $\boxed{63.8\ ng/\mu L}$

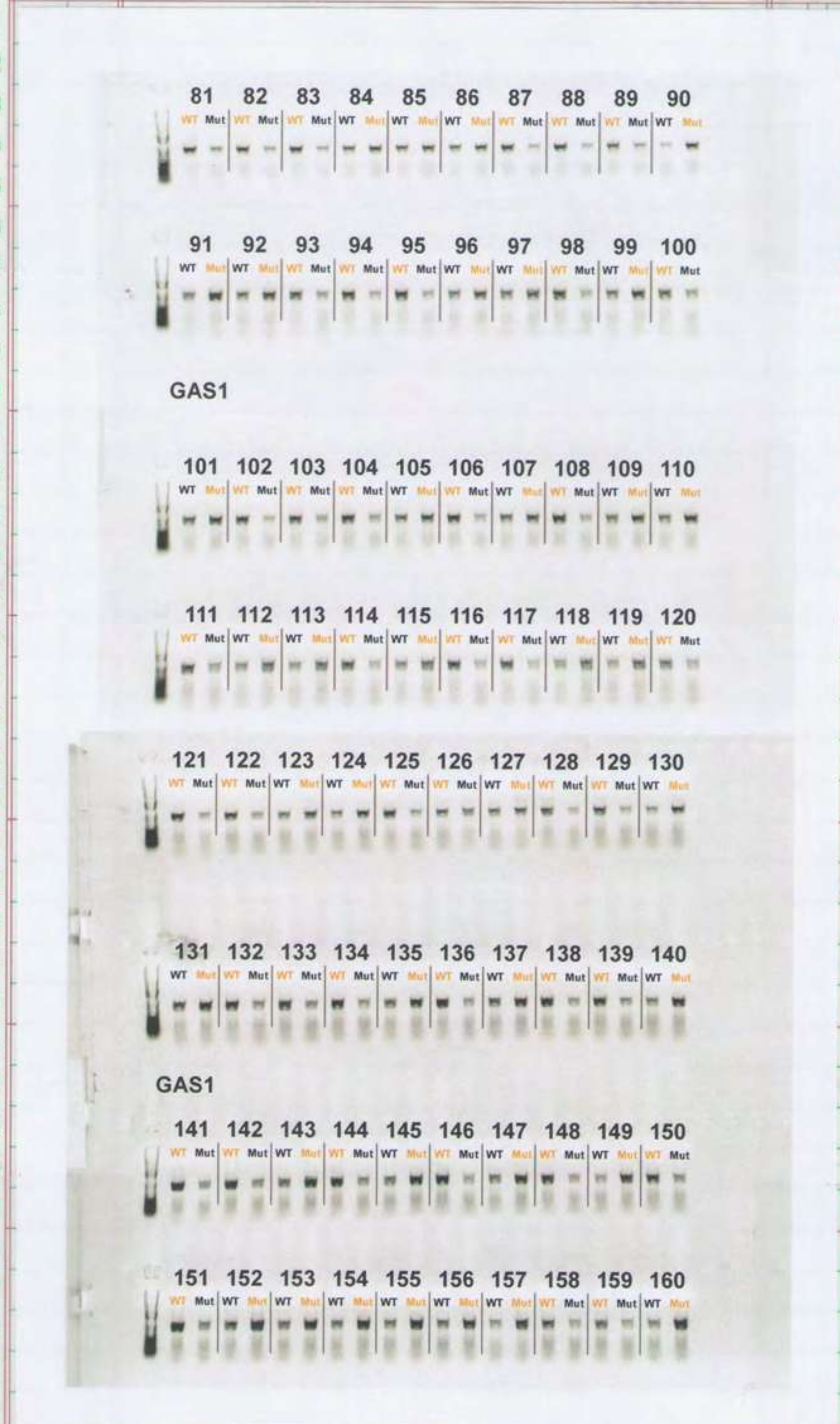
DILUTING FOR SEQUENCING

- I will submit 10nM pool.
- Size 202 bp
  M.W. ~1.25E^5 ng/nMol } → Conc. of 10nM is ~1.25 ng/µL
- Since the conc. is ~75ng/µL I should dilute 1:60 (10λ into 590λ H_2O)

8/27/12

SNP GENOTYPING

Last week I reran all of the SNP PCRs



· I took the plate w/ the 1:20 gDNA preps from the freezer
→ 10 λ onto 6 plates

To each plate I added 30 λ of the following master mix:

H_2O	2240
10x Buffer	400
dNTPs	120
Primer 1	80
Primer 2	80
Taq	80

→ 30 λ each

· Primers - see p. 73

· Annealing temp 54° except Primerset H (50°)

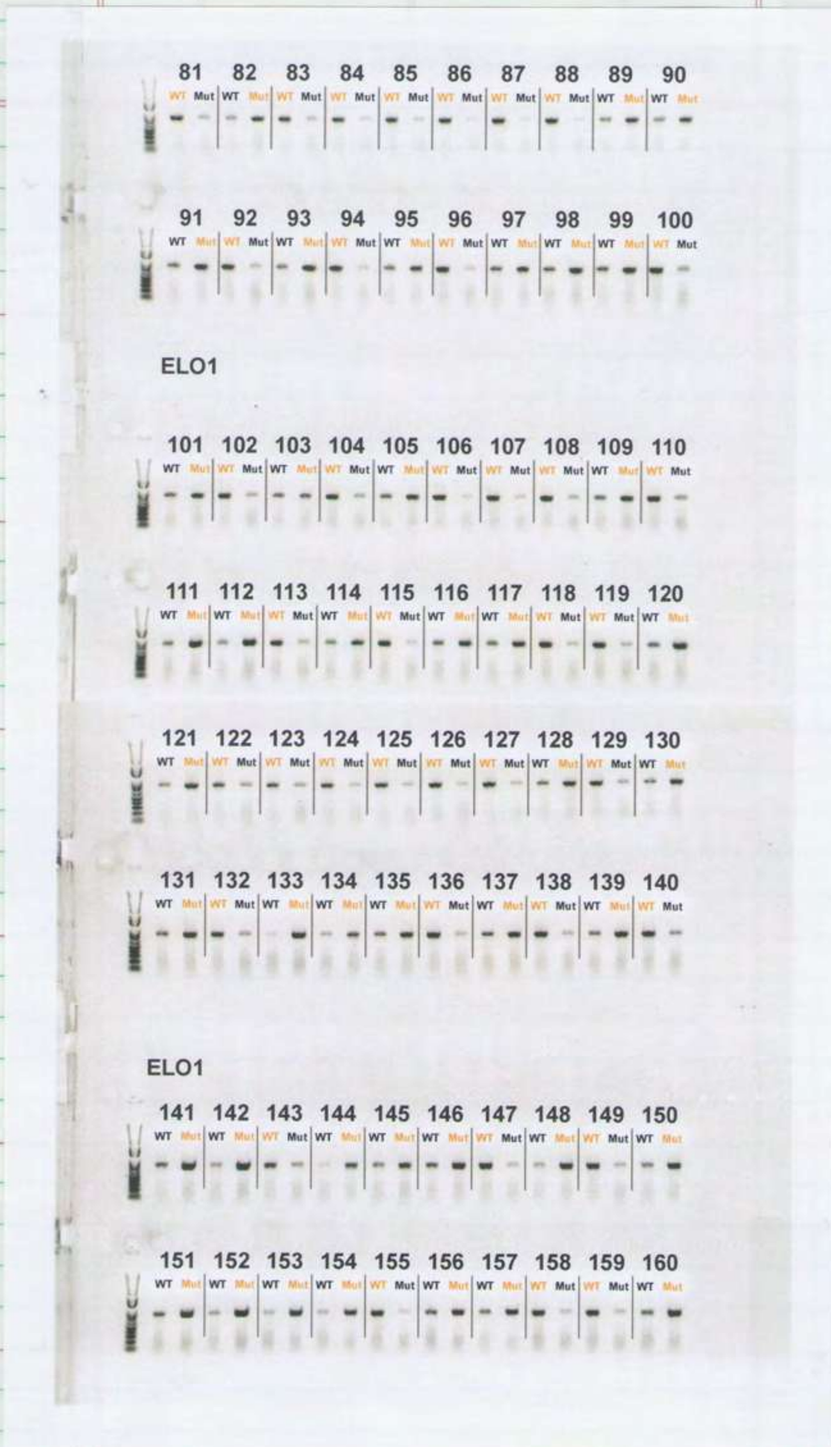
· Run on MJ Tetrads.

→ This is not as clear as before (p. 76); nevertheless, I called mutations here and found a (nearly) perfect match with my previous calls.

8/27/12

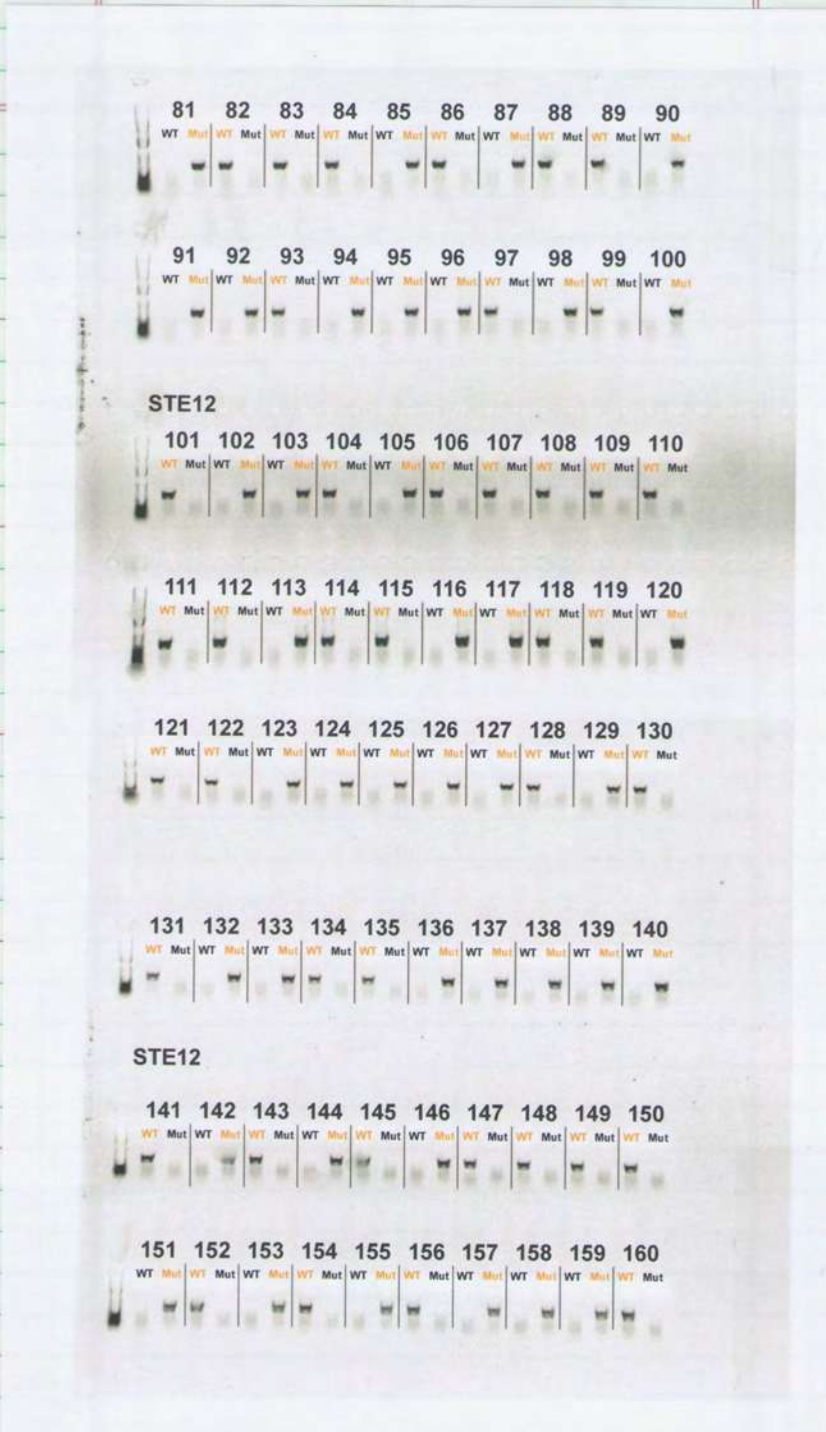
	Initials	Date
Prepared By		
Approved By		

SNP Genotyping continued.



8/27/12

SNP Genotyping Continued.



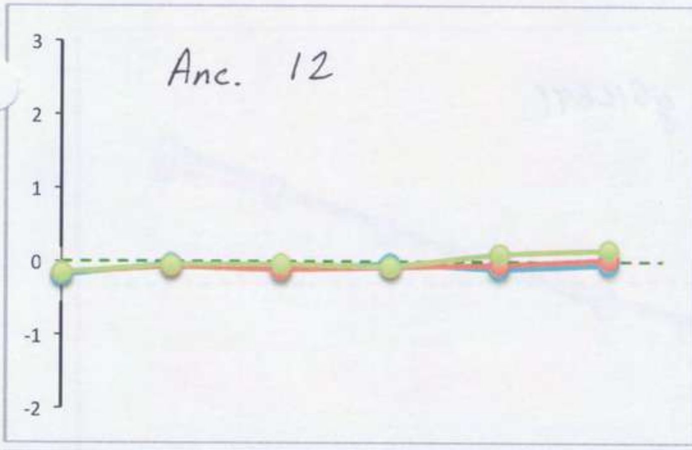
8/27/12 COMPARISON W/ SNP GENOTYPING ROUND 1 & 2

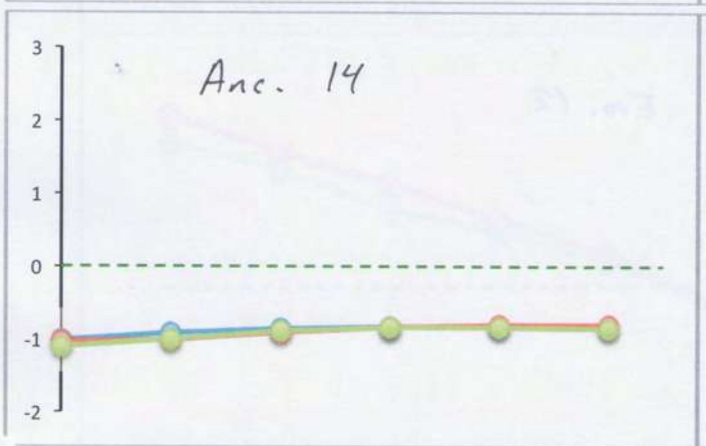
Genotype: 0 = WT, 1 = Mut

Comparison with Previous Data

	p140 GAS1	p142 STE12	~~p142~~ p141 ELO1			p.76 Pervious SNP PCR		p.71 Phenotype	
81	0	1	0		Seg. 081	0	WT-GAS1	1	ste12
82	0	0	1	Conflicting	Seg. 082	0	WT-GAS1	0	WT-STE12
83	0	0	0	Corrected	Seg. 083	0	WT-GAS1	0	WT-STE12
84	1	0	0		Seg. 084	1	gas1	0	WT-STE12
85	1	1	0		Seg. 085	1	gas1	1	WT-STE12
86	1	0	0		Seg. 086	1	gas1	0	WT-STE12
87	0	1	0		Seg. 087	0	WT-GAS1	1	ste12
88	0	0	0		Seg. 088	0	WT-GAS1	0	WT-STE12
89	0	0	1		Seg. 089	0	WT-GAS1	0	
90	1	1	1		Seg. 090	1	gas1	1	
91	1	1	1		Seg. 091	1	gas1	1	
92	1	1	0		Seg. 092	1	gas1	1	ste12
93	0	0	1		Seg. 093	0	WT-GAS1	0	
94	0	1	0		Seg. 094	0	WT-GAS1	1	
95	0	1	1		Seg. 095	0	WT-GAS1	1	
96	1	1	0		Seg. 096	1	gas1	1	
97	1	0	1		Seg. 097	1	gas1	0	
98	0	1	1		Seg. 098	0	WT-GAS1	1	
99	1	0	1		Seg. 099	1	gas1	0	
100	0	1	0		Seg. 100	0	WT-GAS1	1	
101	1	0	1		Seg. 101	1	gas1	0	
102	0	1	0		Seg. 102	0	WT-GAS1	1	
103	0	1	1		Seg. 103	0	WT-GAS1	1	
104	0	0	0		Seg. 104	0	WT-GAS1	0	
105	1	1	1		Seg. 105	1	gas1	1	
106	0	0	0		Seg. 106	0	WT-GAS1	0	
107	1	0	0		Seg. 107	1	gas1	0	
108	0	0	0		Seg. 108	0	WT-GAS1	0	WT-STE12
109	1	0	1		Seg. 109	1	gas1	0	
110	1	0	0		Seg. 110	1	gas1	0	WT-STE12
111	0	0	1		Seg. 111	0	WT-GAS1	0	
112	1	0	1		Seg. 112	1	gas1	0	
113	1	1	0		Seg. 113	1	gas1	1	
114	0	0	1		Seg. 114	0	WT-GAS1	0	WT-STE12
115	1	0	0		Seg. 115	1	gas1	0	
116	0	1	1		Seg. 116	0	WT-GAS1	1	WT-STE12
117	0	1	1		Seg. 117	0	WT-GAS1	1	
118	1	0	0		Seg. 118	1	gas1	0	
119	1	0	0		Seg. 119	1	gas1	0	WT-STE12
120	0	1	1		Seg. 120	0	WT-GAS1	1	ste12
121	0	0	1		Seg. 121	0	WT-GAS1	0	
122	0	0	0		Seg. 122	0	WT-GAS1	0	WT-STE12
123	1	1	0		Seg. 123	1	gas1	1	ste12
124	1	1	0		Seg. 124	1	gas1	1	
125	0	1	0		Seg. 125	0	WT-GAS1	1	ste12
126	1	1	0		Seg. 126	0	gas1	1	WT-STE12
127	1	1	0		Seg. 127	1	gas1	1	ste12
128	0	0	1		Seg. 128	0	WT-GAS1	0	WT-STE12
129	0	1	0		Seg. 129	0	WT-GAS1	1	ste12
130	1	0	1		Seg. 130	1	gas1	0	WT-STE12
131	1	0	1		Seg. 131	1	gas1	0	
132	0	1	0		Seg. 132	0	WT-GAS1	1	
133	0	1	1		Seg. 133	0	WT-GAS1	1	ste12
134	0	0	1		Seg. 134	0	WT-GAS1	0	WT-STE12
135	1	0	1		Seg. 135	1	gas1	0	ste12
136	0	1	0		Seg. 136	0	WT-GAS1	1	ste12
137	1	1	1		Seg. 137	1	WT-GAS1	1	ste12
138	0	1	0		Seg. 138	0	WT-GAS1	1	ste12
139	0	1	1		Seg. 139	0	WT-GAS1	1	
140	1	1	0		Seg. 140	1	gas1	1	
141	0	0	1		Seg. 141	0	WT-GAS1	0	
142	0	1	1		Seg. 142	0	WT-GAS1	1	ste12
143	1	0	0		Seg. 143	1	gas1	0	WT-STE12
144	0	1	1		Seg. 144	0	WT-GAS1	1	ste12
145	1	0	1		Seg. 145	1	gas1	0	WT-STE12
146	0	1	1		Seg. 146	0	WT-GAS1	1	
147	1	0	0		Seg. 147	1	gas1	0	WT-STE12
148	0	0	1		Seg. 148	0	WT-GAS1	0	
149	1	0	0		Seg. 149	1	gas1	0	
150	0	0	1		Seg. 150	0	WT-GAS1	0	WT-STE12
151	0	1	1		Seg. 151	0	WT-GAS1	1	WT-STE12
152	1	0	1		Seg. 152	1	WT-GAS1	0	
153	1	1	1		Seg. 153	1	gas1	1	ste12
154	1	0	1		Seg. 154	1	WT-GAS1	0	WT-STE12
155	1	1	0		Seg. 155	1	gas1	1	
156	1	0	1		Seg. 156	1	gas1	0	WT-STE12
157	1	1	1		Seg. 157	1	gas1	1	
158	0	1	0		Seg. 158	0	WT-GAS1	1	ste12
159	0	1	0		Seg. 159	0	WT-GAS1	1	ste12
160	1	0	1		Seg. 160	1	gas1	0	WT-STE12

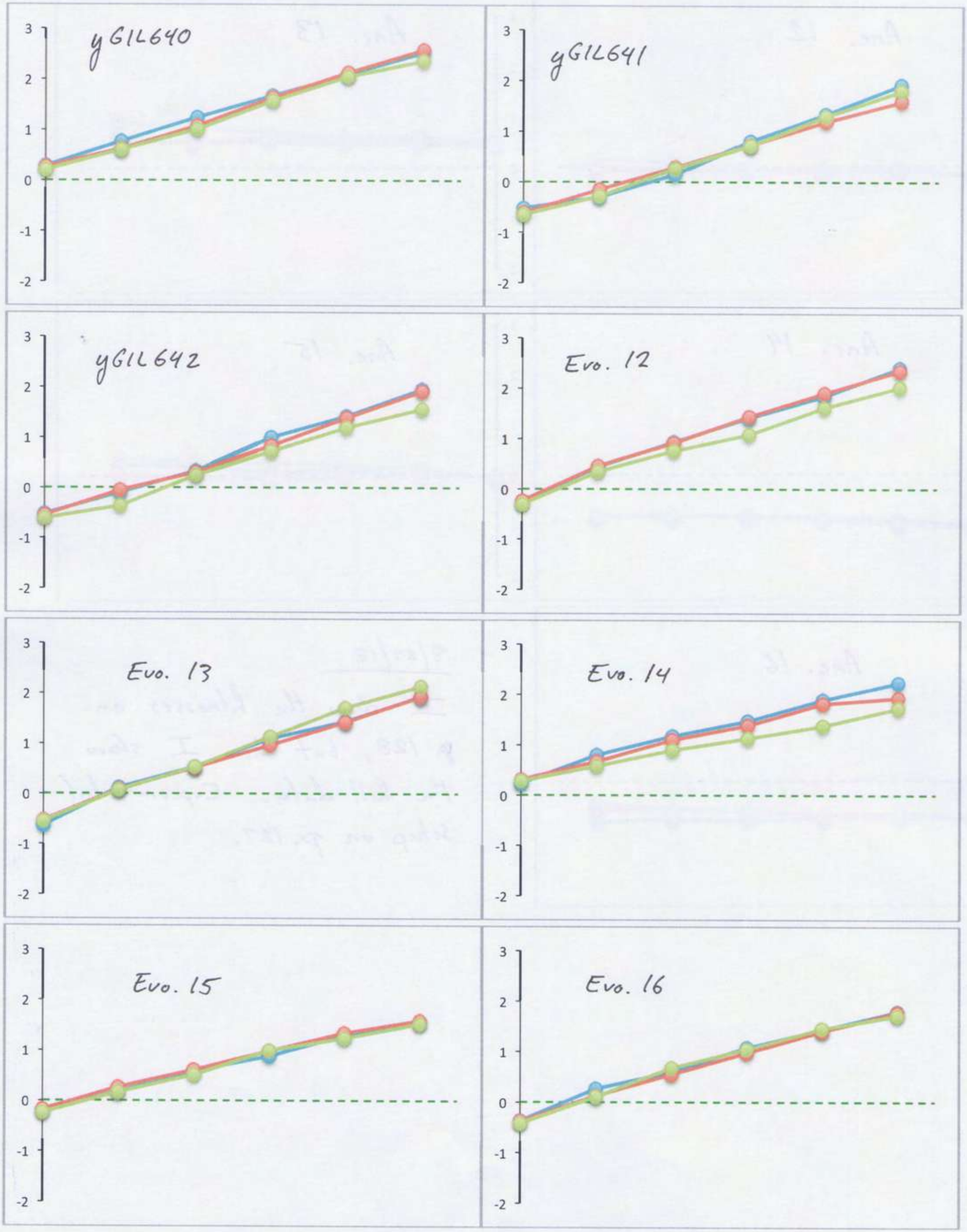
8/27/12 FITNESS ASSAYS ON SEGREGANTS 81-160 AT THE SMALL BOTTLENECK





8/27/12

I show the fitnesses on p. 128, but here I show the full data. Experimental setup on p. 127.


yGIL640
yGIL641
yGIL642
Evo. 12
Evo. 13
Evo. 14
Evo. 15
Evo. 16
3
2
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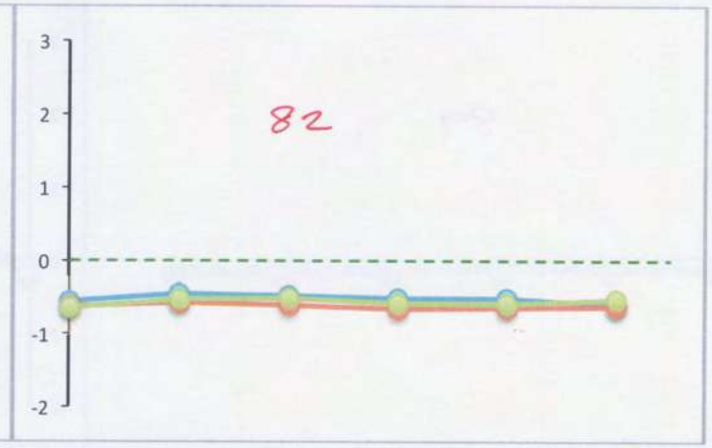
0
-1
-2


Segregant 81
3
2
1
0
-1
-2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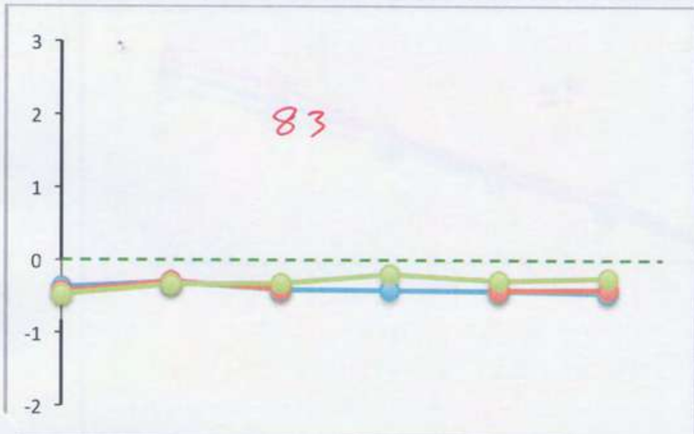
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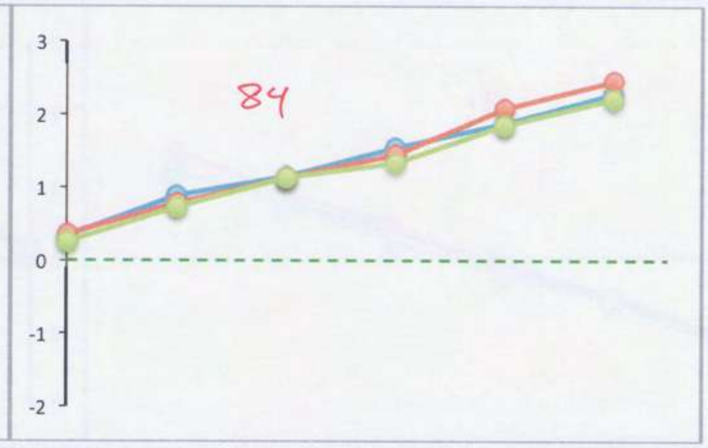
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-1
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85
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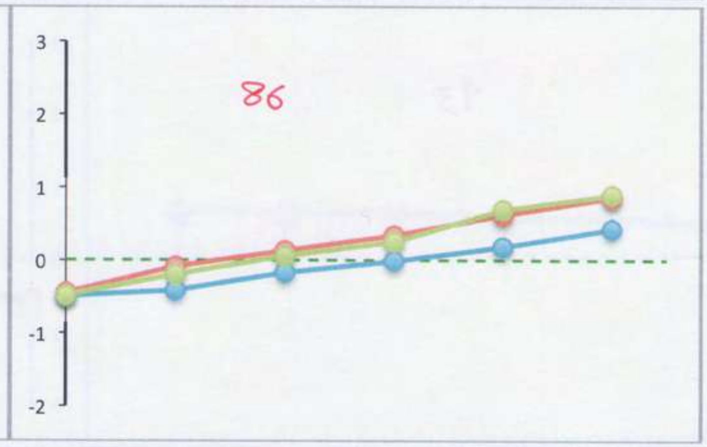
0
-1
-2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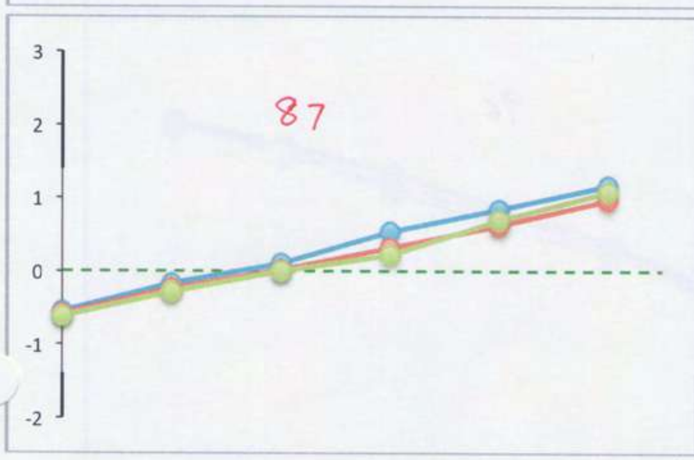
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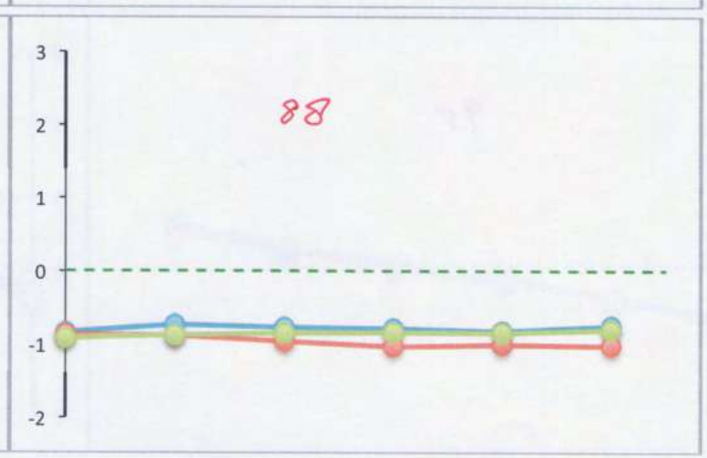
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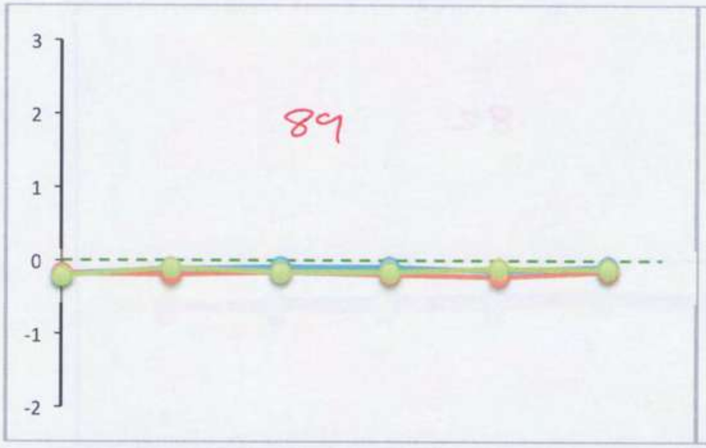
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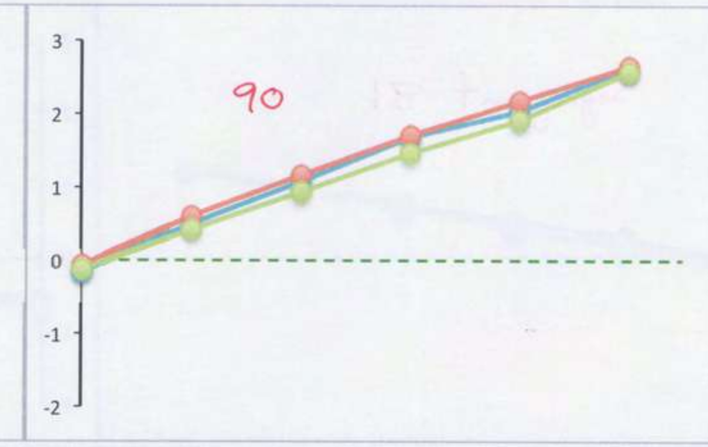
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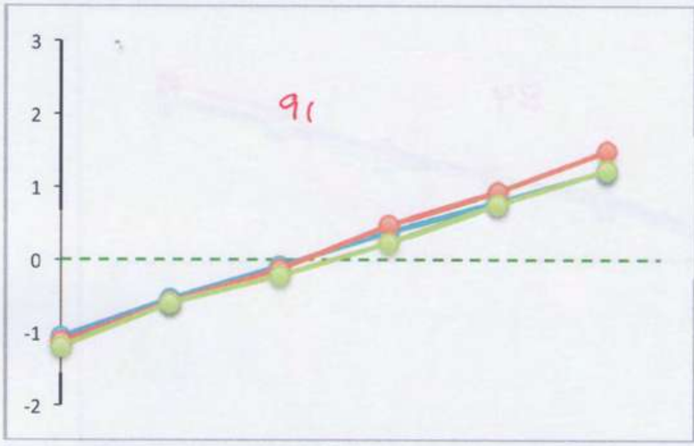
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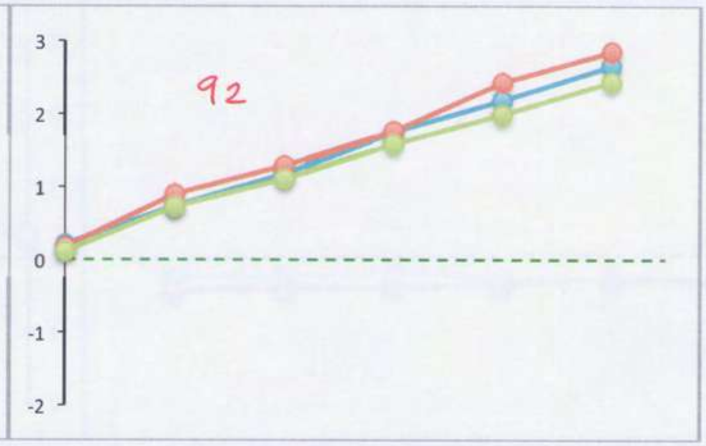
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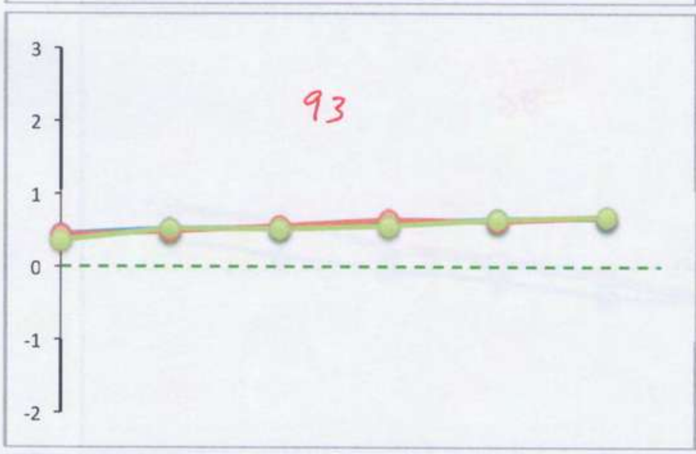
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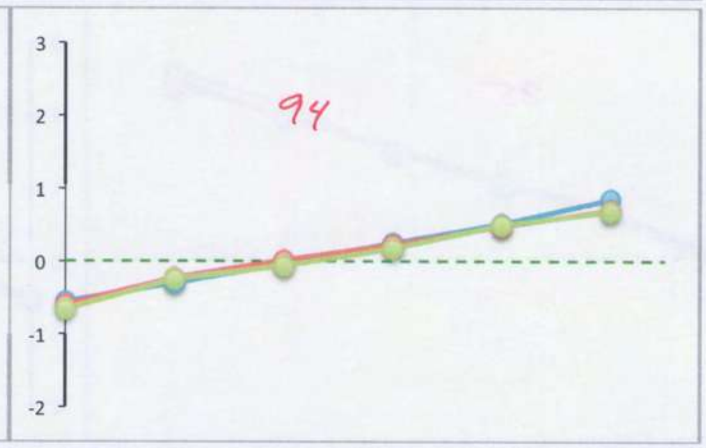
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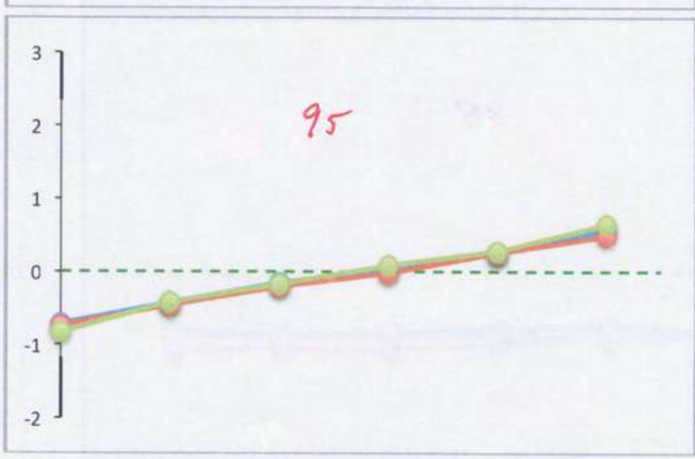
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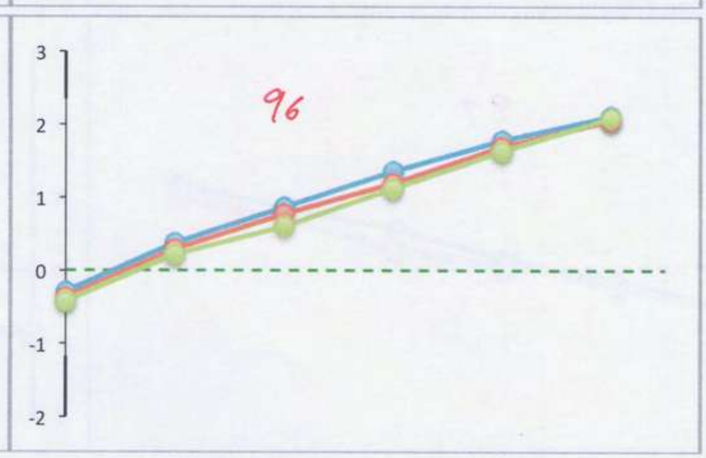
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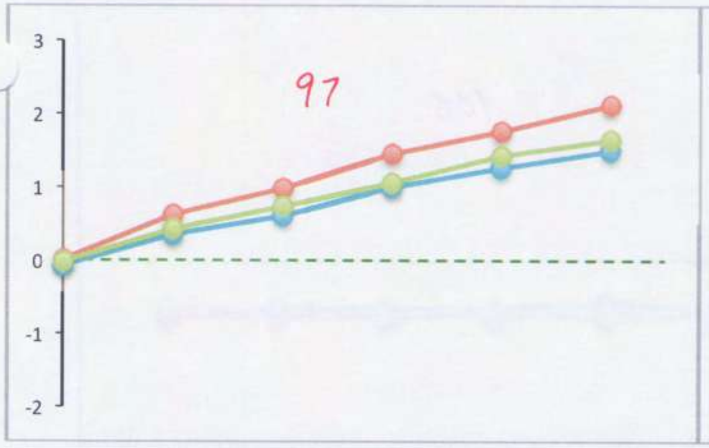
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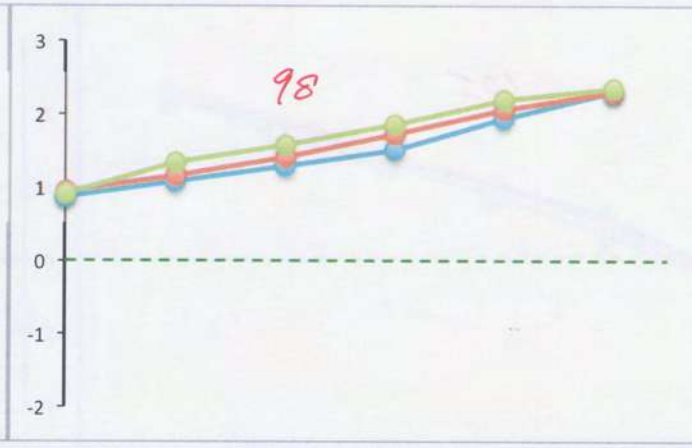
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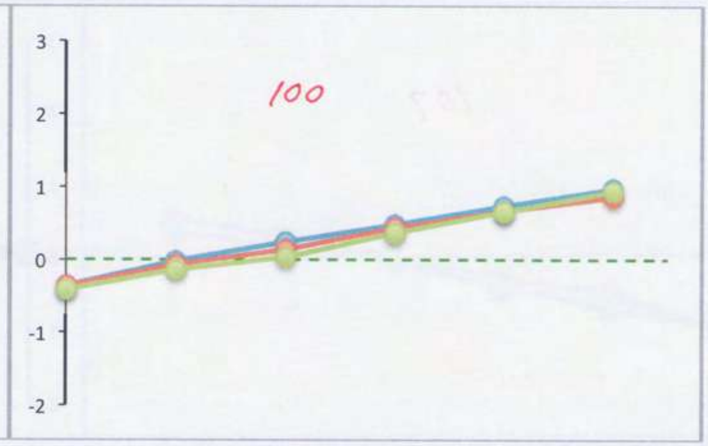
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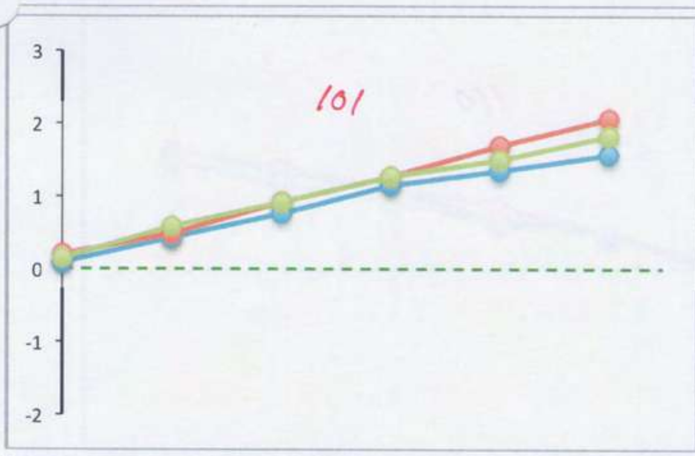
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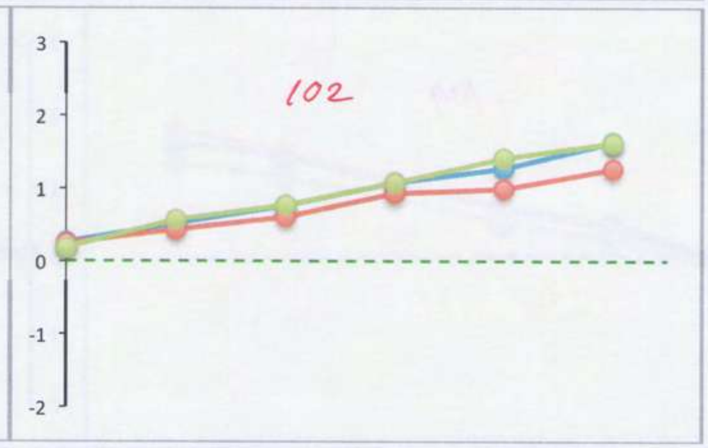
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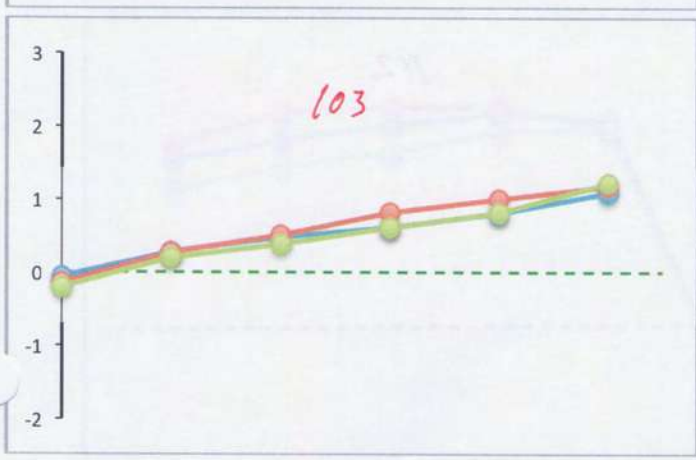
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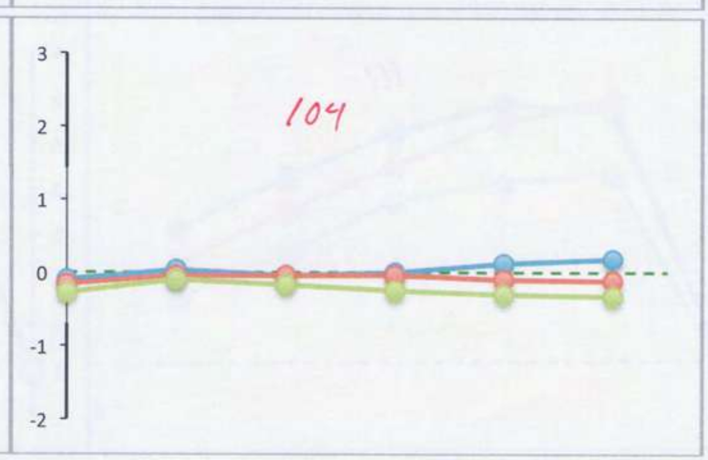
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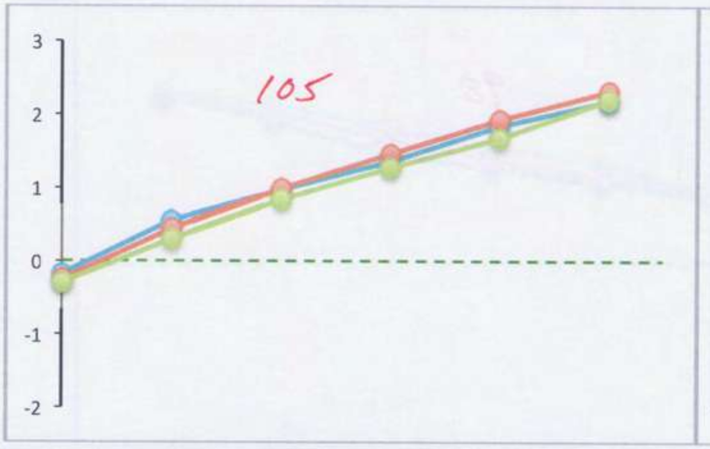
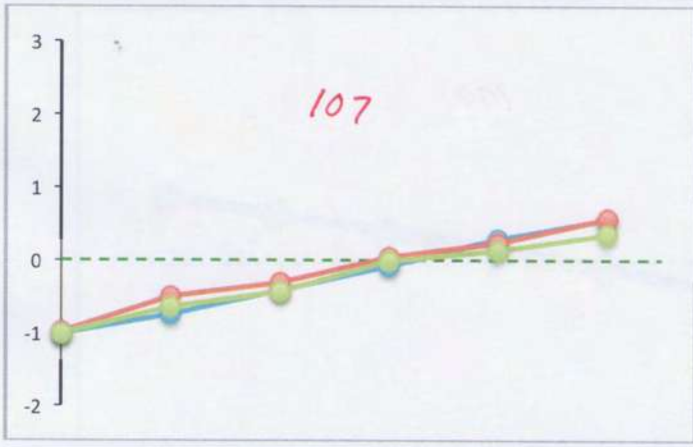
-2


105


106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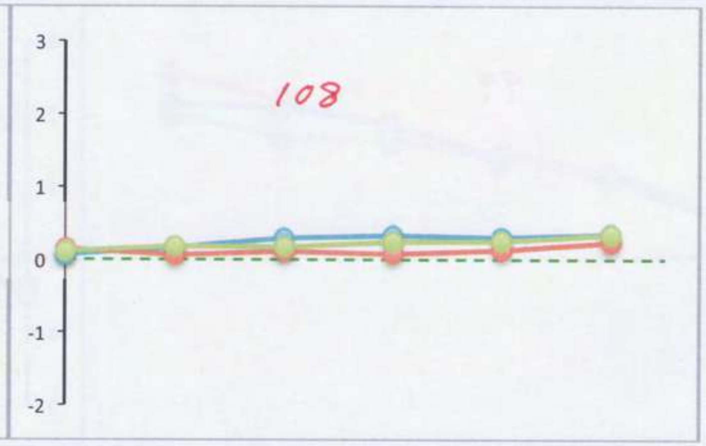
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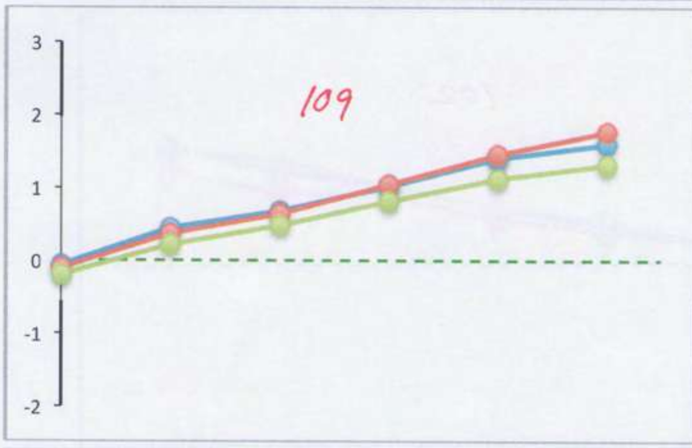
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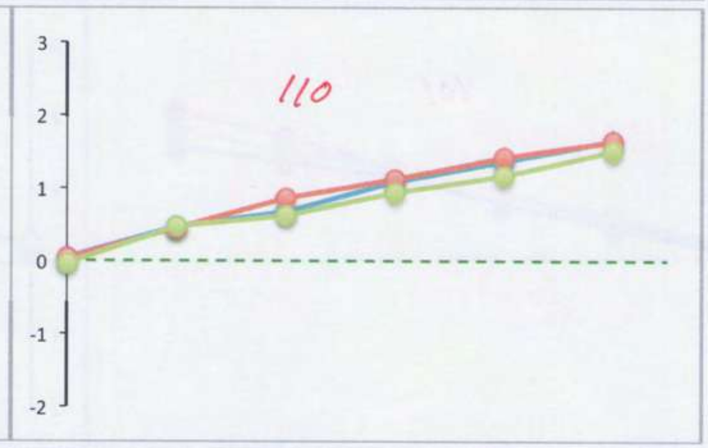
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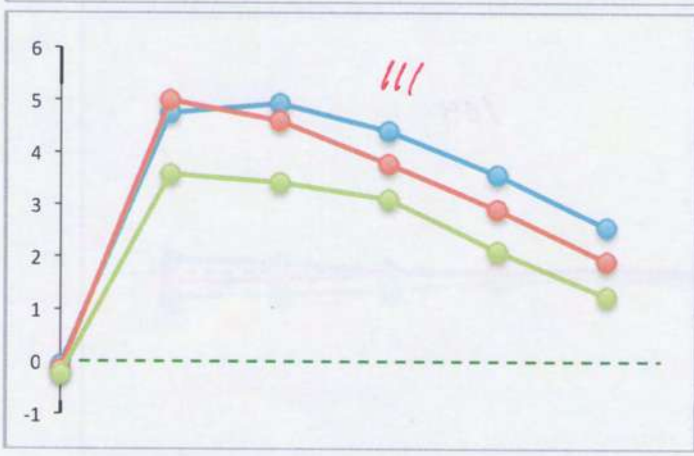
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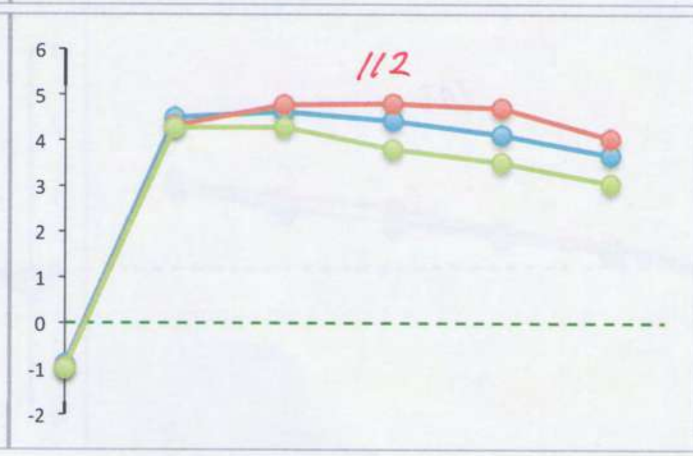
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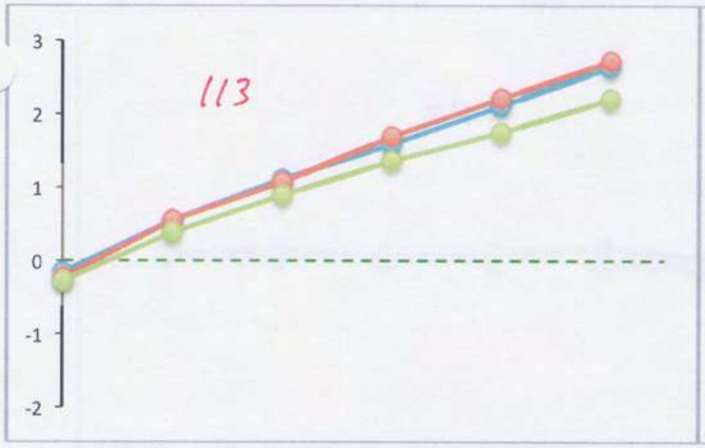
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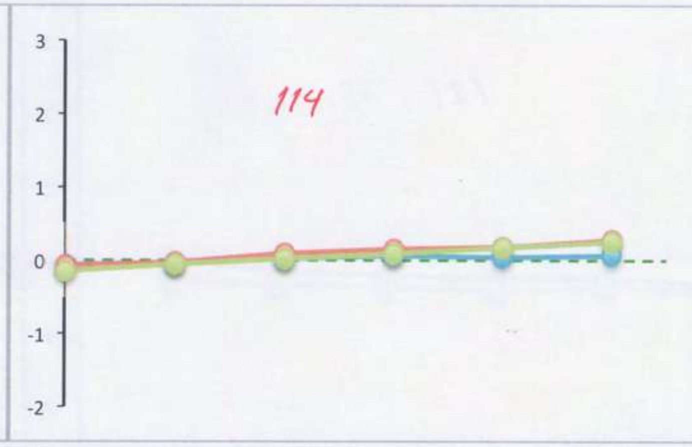
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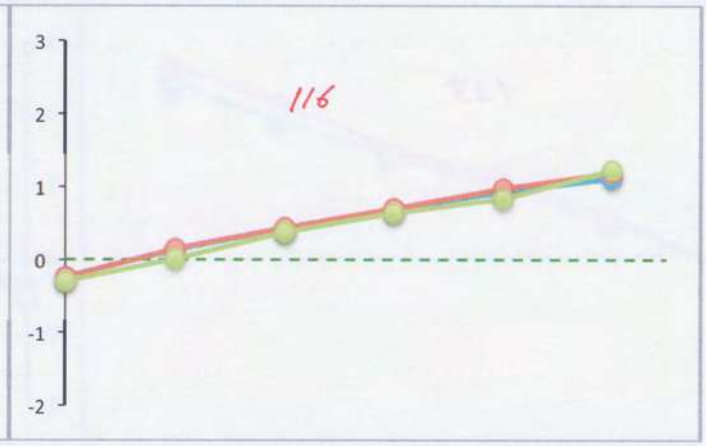
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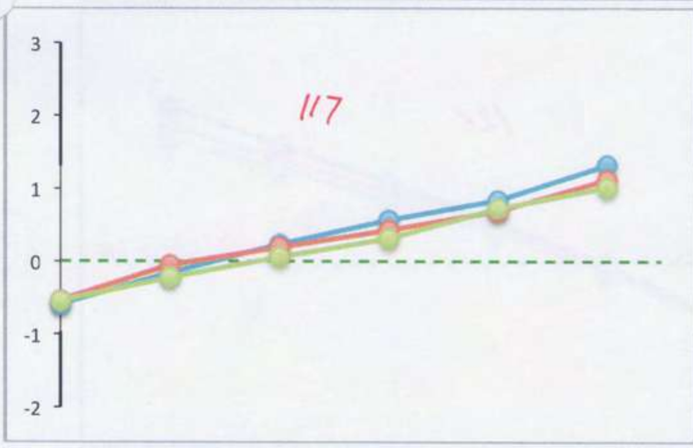
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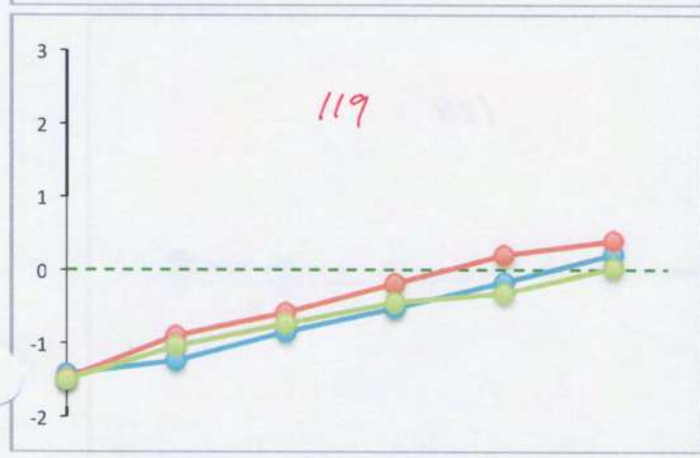
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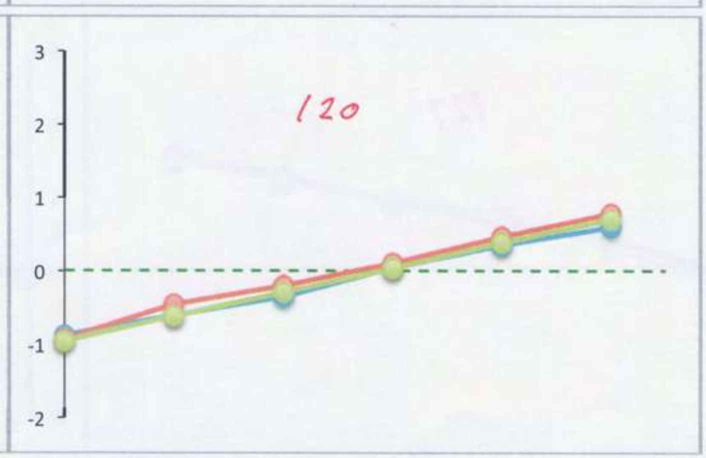
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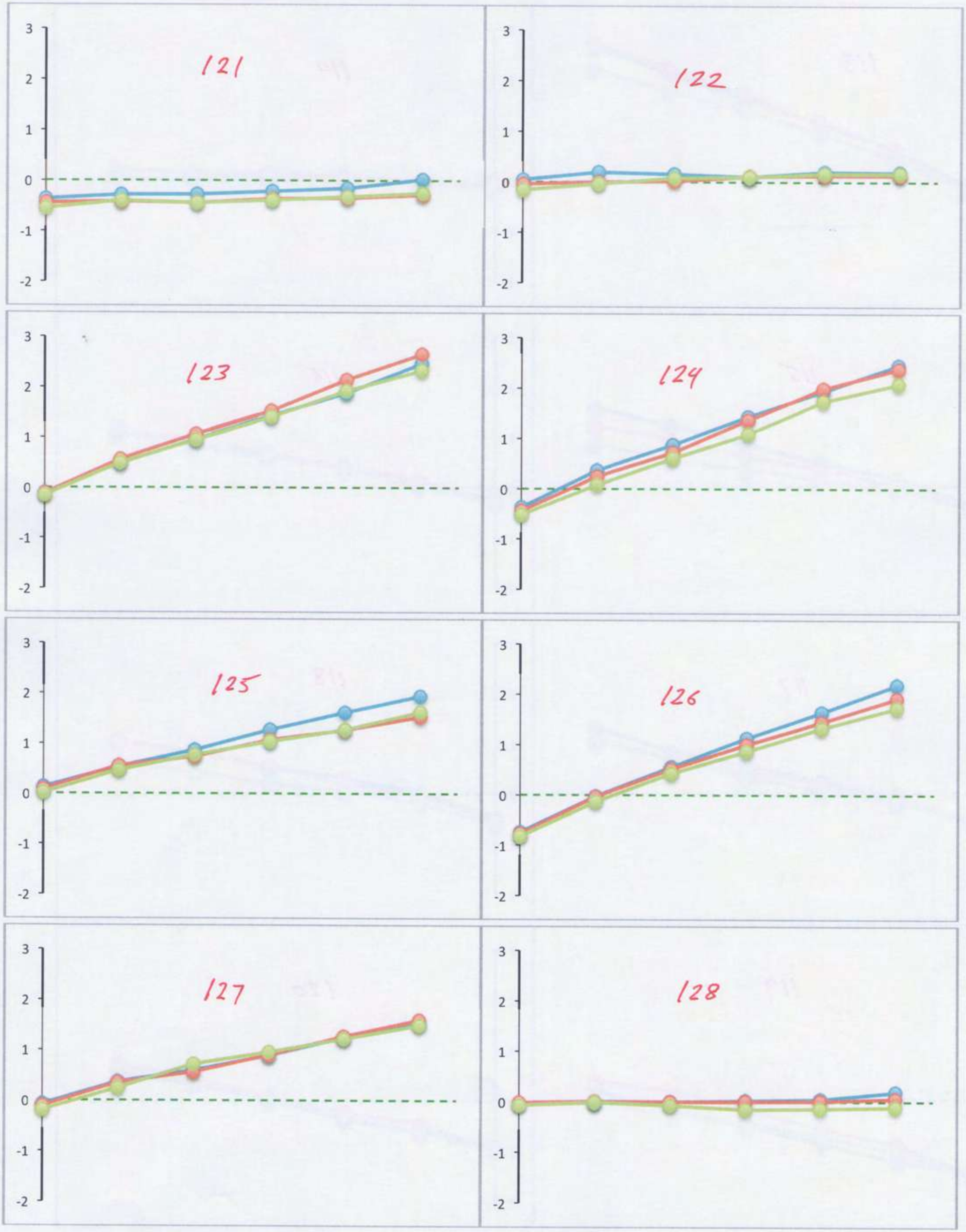
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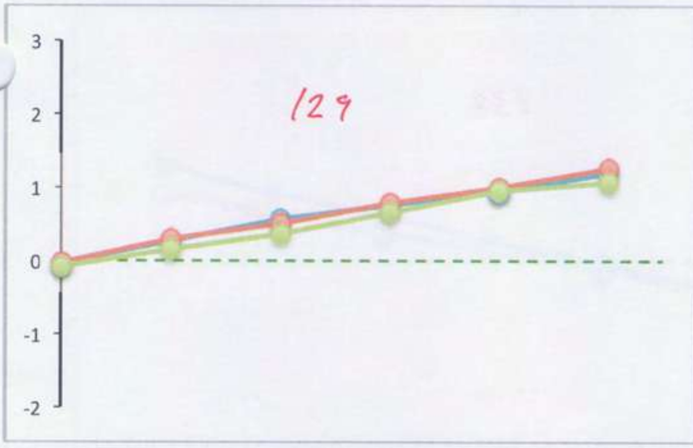
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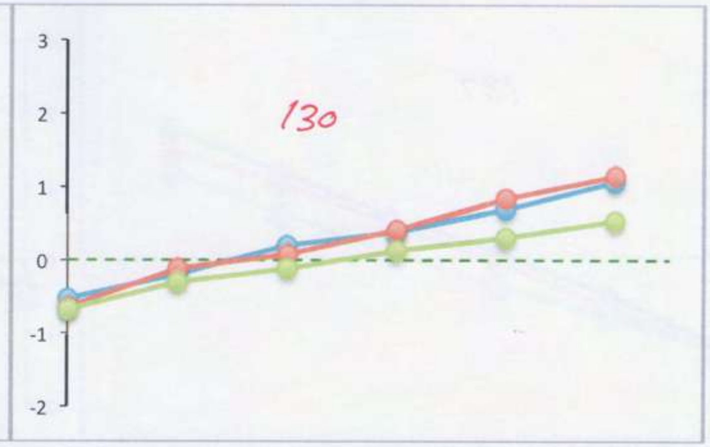
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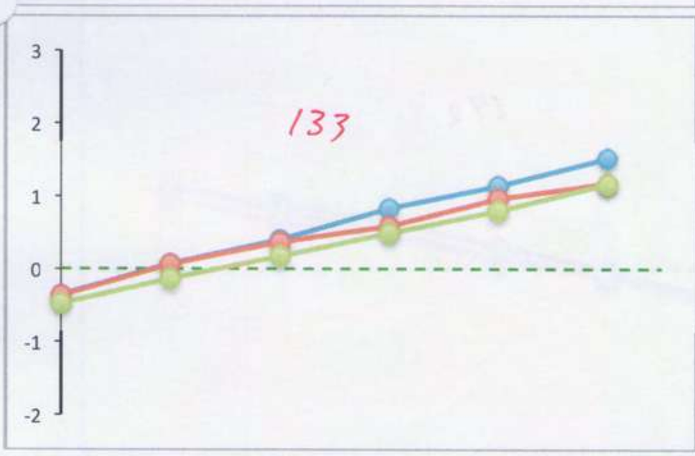
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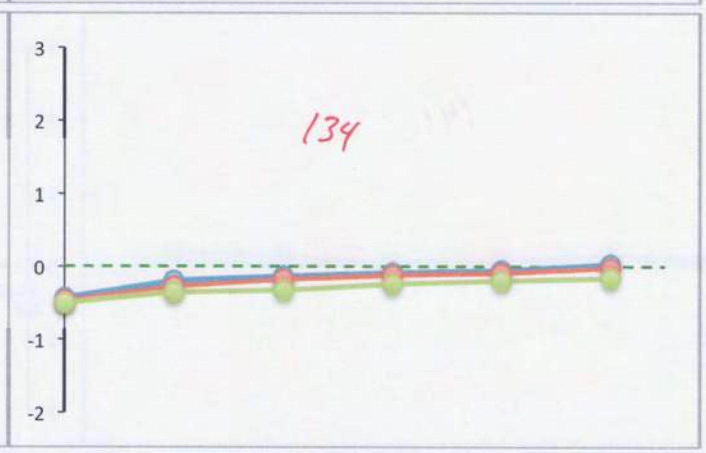
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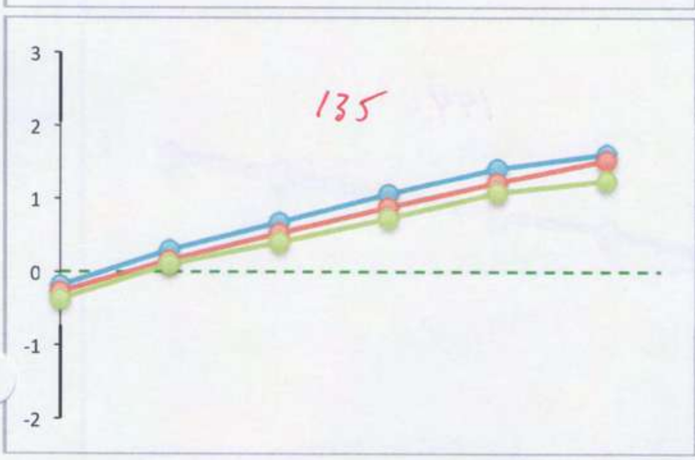
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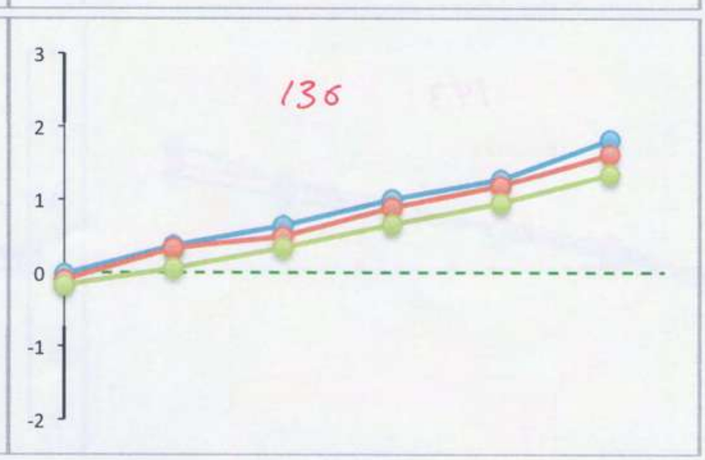
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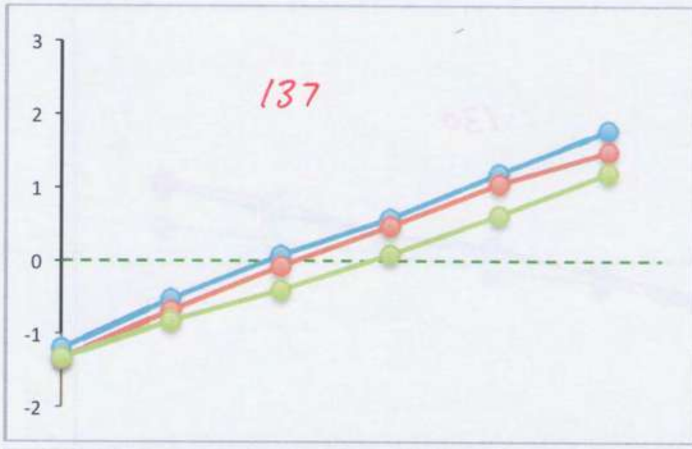
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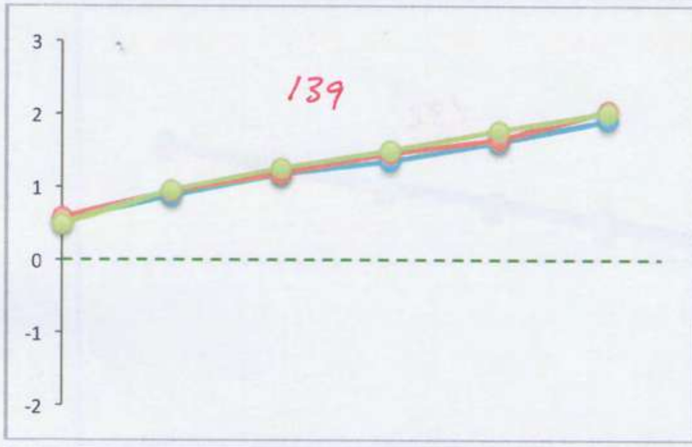
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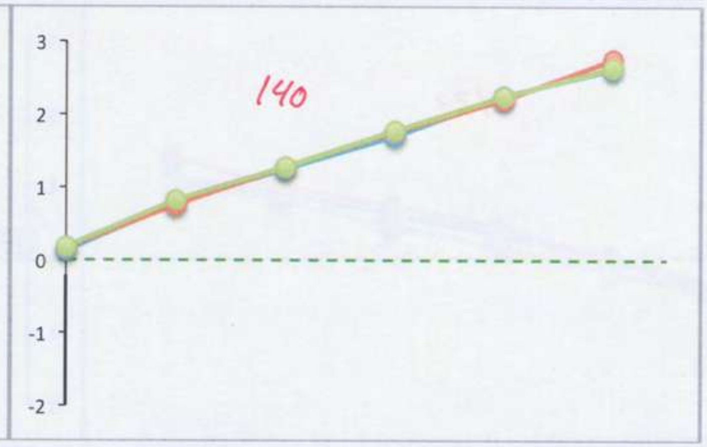
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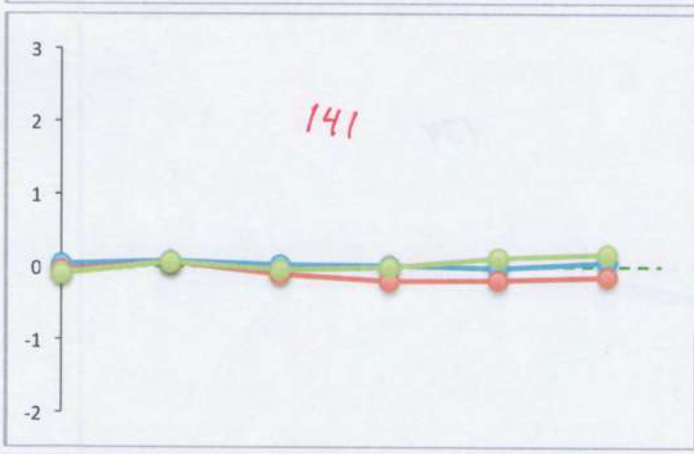
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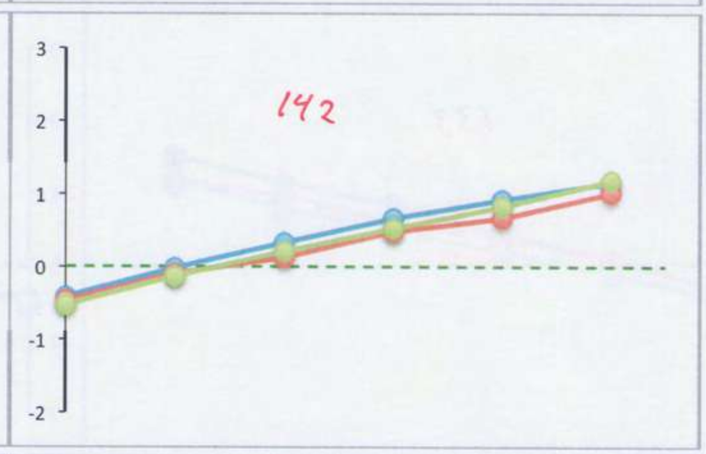
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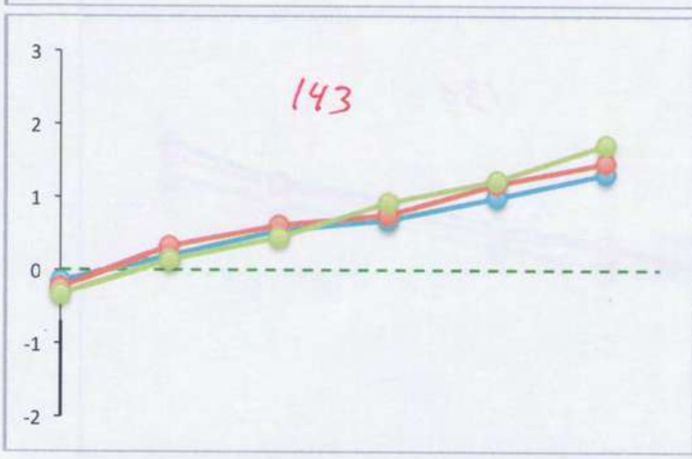
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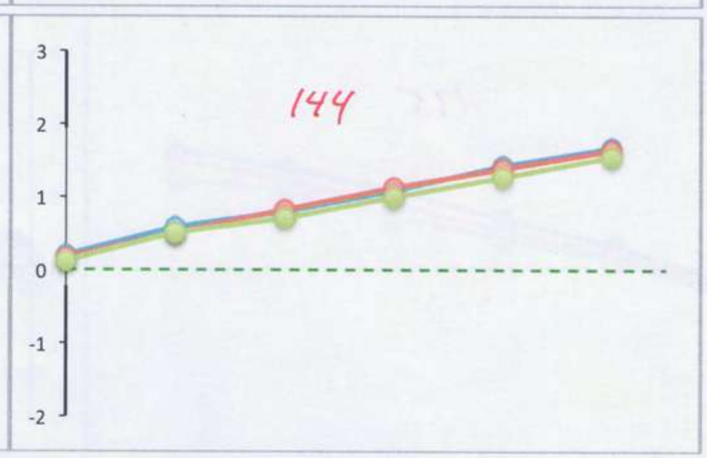
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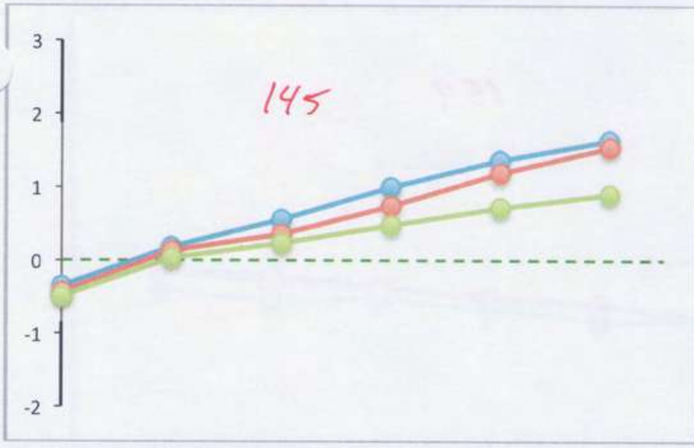
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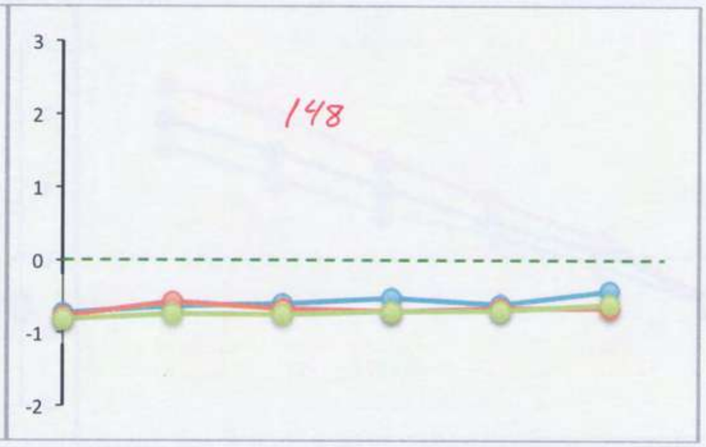
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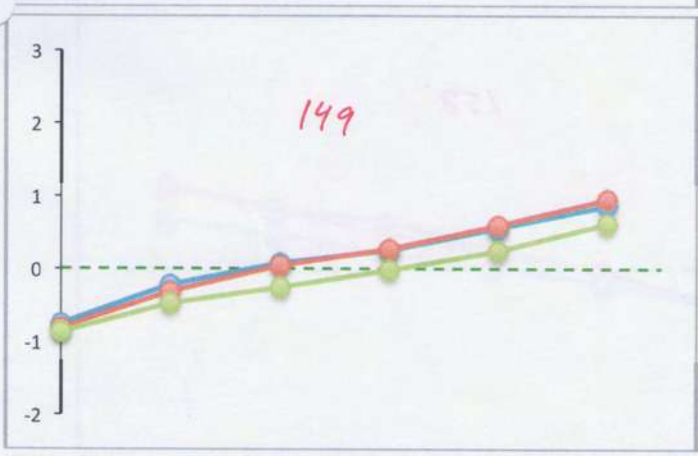
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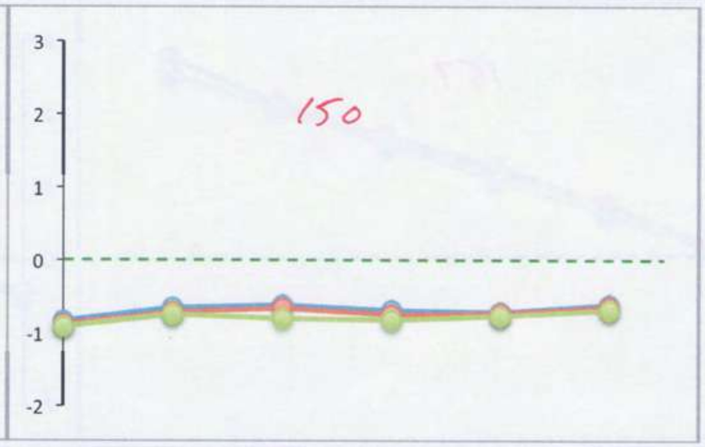
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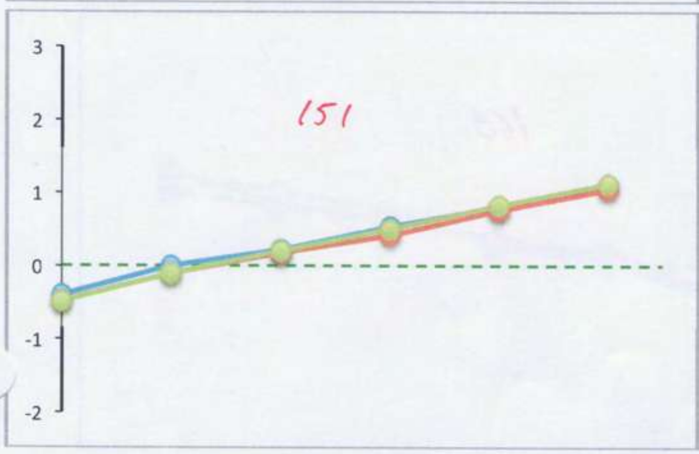
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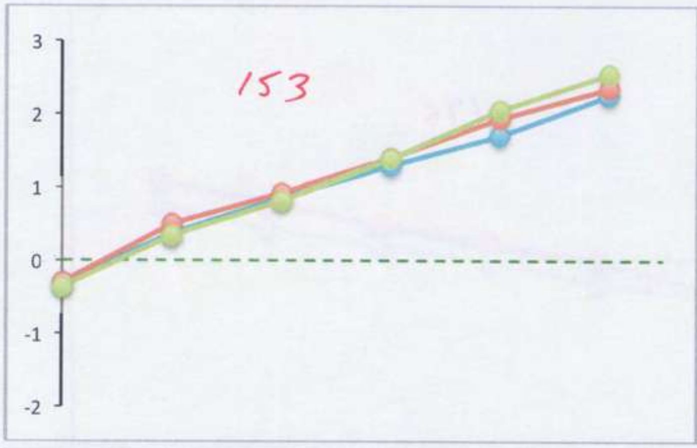
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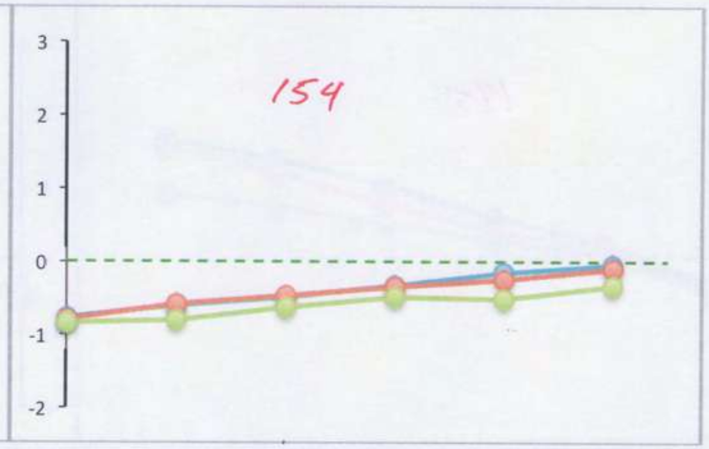
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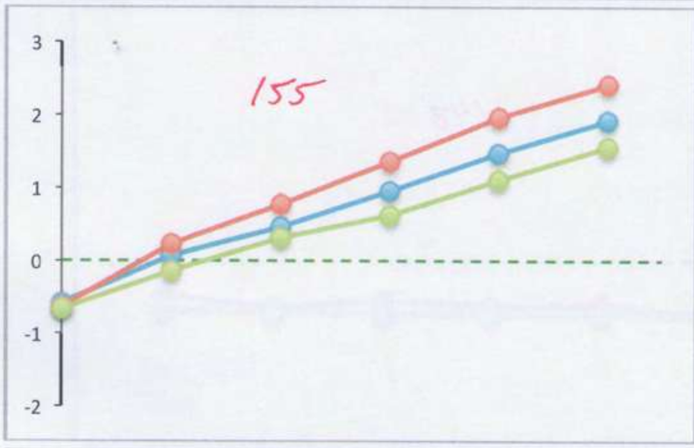
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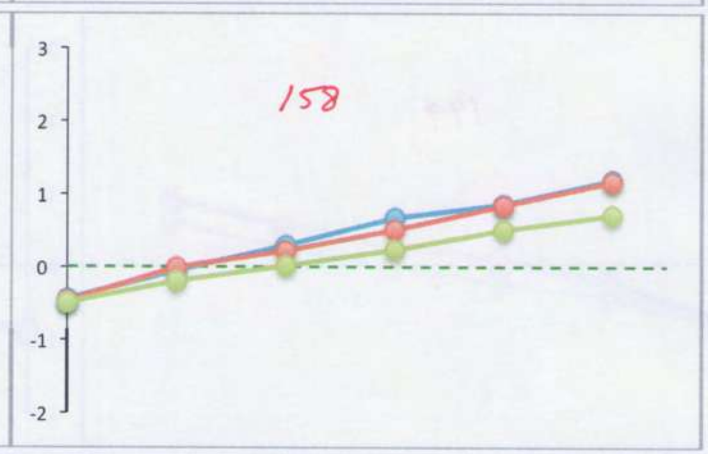
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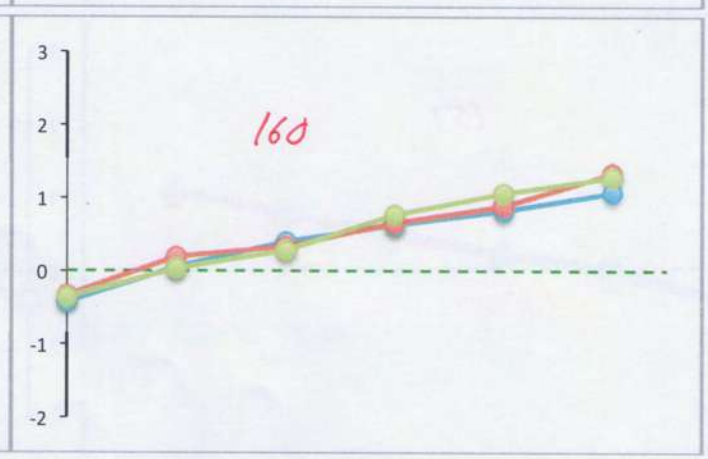
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8/27/12

GENETIC DISSECTION OF BYS1-A08

	Initials	Date
Prepared By		
Approved By		

NOTES

· The evolved fitness (vs. Ancestor) is close to expectation, but the range of fitnesses of the segregants is greater

↳ low freq. mutations that were fixed by bottlenecking during backcross?

· I should check the two low fitness evolved clones to see if they have the ste12 mutation (my guess is that they don't)