	Or		NAME	POSITION		GAP		PLATE	COL	ROW
TELOMERE			TEL06L	1	5530					
1	YFL063W		hypothetical	5066	5521	-464	00_18	51	A	1
2	YFL056C		AAD6	14793	15431	9272	06_1	31	F	5
3	YFL055W		AGP3	17004	18680	1573	06_1	31	F	4
4	YFL054C		hypothetical	20847	22787	2167	06_1	31	F	3
5	YFL052W		hypothetical	28232	29629	5445	06_1	31	F	1
6	YFL050C		ALR2	33272	35848	3643	06_1	31	E	11
7	YFL049W		hypothetical	36803	38674	955	06_1	31	E	10
8	YFL047W		RGD2	40421	42565	1747	06_1	31	E	8
9	YFL044C		YOD1	44655	45560	2090	06_1	31	E	6
10	YFL041W		FET5	49139	51007	3579	06_1	31	E	4
11	YFL036W		RPO41	58781	62836	7774	06_1	31	E	2
12	YFL034W		uncharacterized	65475	68696	2639	06_1	31	D	12
13	YFL032W		hypothetical	74870	75190	6174	06_1	31	D	11
14	YFL027C		GYP8	80417	81910	5227	06_1	31	D	7
15	YFL025C	1	BST1	84143	87232	2233	06_1	31	D	5
16	YFL023W	2	BUD27	90984	93374	3752	06_1	31	D	4
17	YFL021W	3	GAT1	95964	97496	2590	06_1	31	D	3
18	YFL019C		hypothetical	100246	100599	2750	00_15	46	B	9
19	YFL015C		hypothetical	106463	106957	5864	06_1	31	D	1
20	YFL012W		hypothetical	110641	111087	3684	00_17	50	G	7
21	YFL011W		HXT10	112339	113979	1252	06_1	31	C	12
22	YFL010C	4	WWM1	115102	115737	1123	00_17	50	G	5
23	YFL007W	5	BLM3	123474	129905	7737	00_17	50	G	4
24	YFL004W		VTC2	131805	134291	1900	00_17	50	G	3
25	YFL003C		MSH4	134516	137152	225	00_17	50	G	2
26	YFL001W	6	DEG1	147126	148454	9974	00_17	50	G	1
			CDEI	148505	148514	51				
CENTROMERE			CDEII	148515	148597	1				
			CDEIII	148598	148622	1				
27	YFR001W	7	LOC1	149105	149719	483	06_1	31	F	6
28	YFR006W		hypothetical	156139	157746	6420	06_1	31	F	7
29	YFR007W		hypothetical	159293	160354	1547	06_1	31	F	8
30	YFR009W		GCN20	162482	164740	2128	06_1	31	F	10
31	YFR012W		hypothetical	167881	168489	3141	06_1	31	F	12
32	YFR014C		CMK1	172529	173869	4040	06_1	31	G	1
33	YFR016C		hypothetical	177034	180735	3165	06_1	31	G	3
34	YFR017C		hypothetical	182262	182849	1527	06_1	31	G	4
35	YFR019W		FAB1	184490	191326	1641	00_15	46	B	11
36	YFR021W		ATG18	194800	196302	3474	06_1	31	G	7
37	YFR023W		PES4	199862	201697	3560	06_1	31	G	9
38	YFR026C		hypothetical	205736	206245	4039	06_1	31	G	11
39	YFR030W	8	MET10	213300	216407	7055	00_15	46	C	2
40	YFR032C	9	hypothetical	222078	222947	5671	00_2	26	E	3
41	YFR035C		hypothetical	226109	226453	3162	00_2	26	E	7
42	YFR039C		hypothetical	231999	233531	5546	00_14	44	A	12
43	YFR043C		hypothetical	239101	239814	5570	00_2	26	E	12
44	YFR045W		hypothetical	242129	242986	2315	00_2	26	F	2
45	YFR049W		YMR31	248510	248881	5524	00_2	26	F	6
46	YFR053C		HXK1	253579	255036	4698	00_2	26	F	7
47	YFR054C		hypothetical	258842	259420	3806	00_2	26	F	8
48	YFR055W		hypothetical	264191	265213	4771	00_2	26	F	9
49	YFR057W		hypothetical	269048	269503	3835	00_2	26	F	11
	TELOMERE		TEL06R	269718	270148	215				

NOTES

1 decreased drug resistance (rapamycin)
normal drug resistance (wortmannin)
grows well on -met, no growth on -lys, no growth on drop-in media
mates like alpha

2 reduced fitness in rich medium (YPD)

3 increased drug resistance (rapamycin)
normal drug resistance (wortmannin)

4 overlaps with a gene necessary for GAP1 repression:
may cause problems for CAN1 counterselection

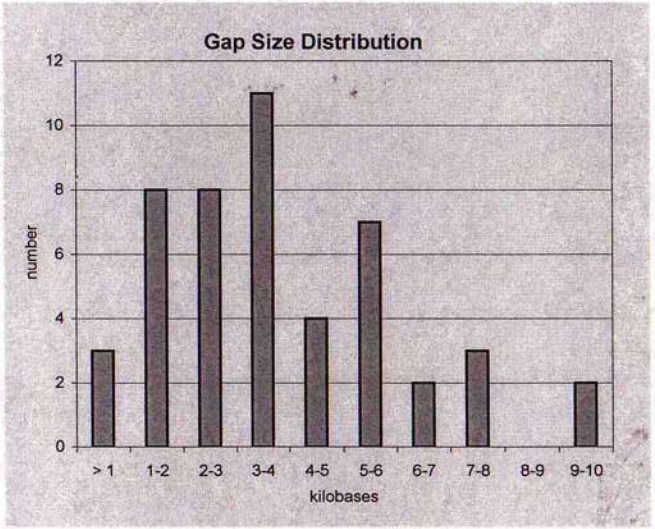
5 temperature sensitive

6 slow growth rate (but i believe that this is only at 37)

7 reduced fitness in rich medium (YPD)

8 methionine auxotroph

9 Exhibits sensitivity at 5 gene in SC - thr medium



7/26
~~8/26~~

The purpose of this expt is to produce a map of ChrVI showing the variation (or lack of variation) in mutation rate across its length

Three reasons for choosing ChrVI:

(1) it is the second smallest chromosome: 270 kb (ChI is 230 kb)

(2) DEG1 - the closest gene to a CEN - is on ChrVI

(3) sup11 & sup6 - tRNA-Tyr genes which show ~2-fold differences in mut. freq - are on ChrVI (Ito-Harashima, McCusker 2002).

(4) None of the ~30 known mutator alleles (according to Huang and Kolodner et al, 2003) are on ChrVI

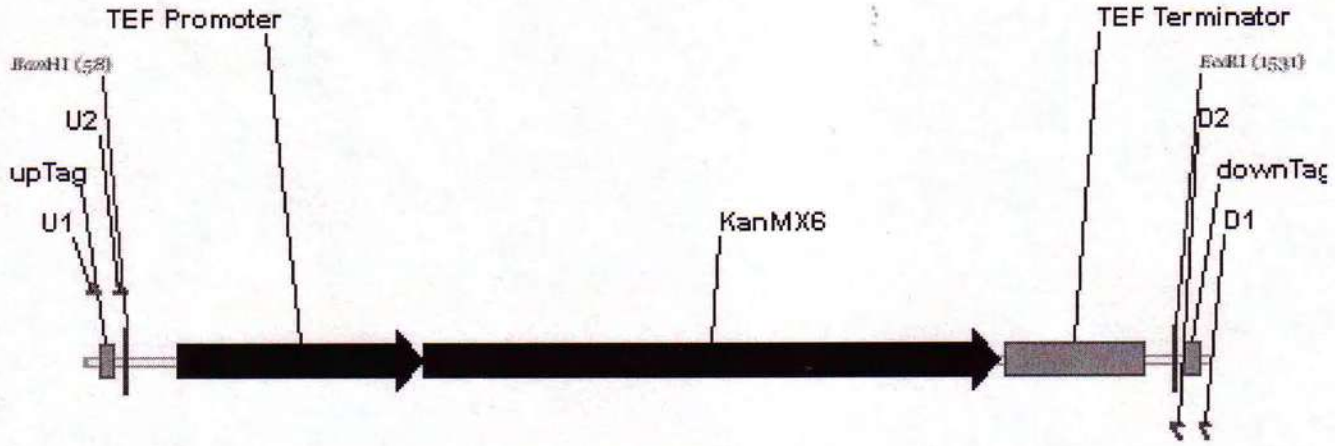
LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 692 10VG

7/26
~~8/26~~

Pulling strains from the Δ collection:

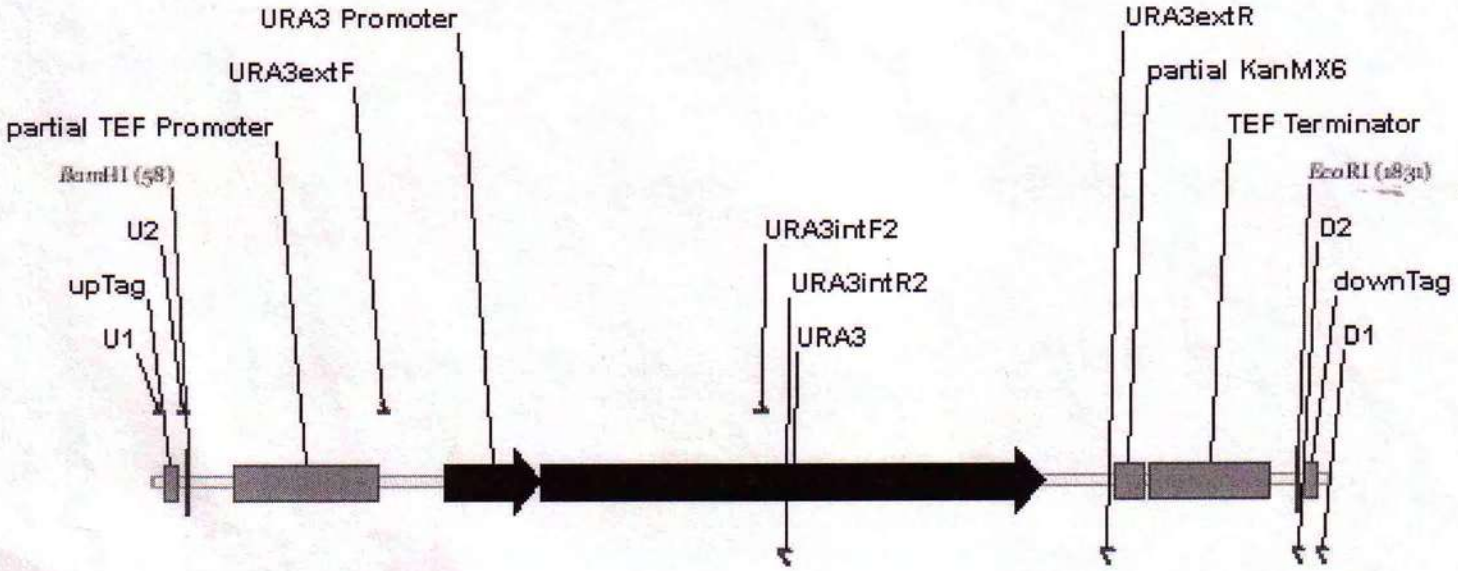
Today I pulled the 49 strains on p.1 from the deletion collection and streaked them on YPD + 2× G418

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 692 10VG



KANMX6 deletion cassette

1578 bp



KANMX6delta::URA3 deletion cassette

1878 bp

7/26
~~8/26~~

PCR of URA3 from W303 and transformation into GIL004 (YEL020C Δ::KANMX6)

I decided to do the initial integration of URA3 into the locus YEL020C — this is the neighboring locus to URA3 (which in the Δ collection is absent). This will be useful if I wish to determine if the new context of the reporter — in the middle of the KANMX6 cassette changes the mut rate.

· Yesterday I picked a single colony off of a YEL020CΔ YPD plate that has been in the fridge for quite a while and inoc. ~30 ml YPD + ADE.

· Grew o/n — I had to dilute once during the day to keep cells in exp. growth.

· Yesterday I performed PCR on a gDNA prep of GIL066 using primers:

17066072 IDT
V.INDJEIAN
18330653 7/8/2005
URA3extF
5 ATC AAA GAA GGT TAA TGT GG -3
Tm= 47.8 °C , MW= 6,229.1
4.2 OD_{260} 19.72 nmol 0.12 mg

17066073 IDT
V.INDJEIAN
18330654 7/8/2005
URA3extR
5 TCA TTA TAG AAA TCA TTA CG -3
Tm= 42.1 °C , MW= 6,099.0
3.8 OD_{260} 18.55 nmol 0.11 mg

94 :	30s	← I meant to do 5 min.
94	30s	30x
45	30s	30x
72	1.5 m	30x
72	5 m	

Today I verified that the PCR worked and purified the product.

PCR the gDNA prep and PCR w/

Sequence - URA3extFintegration — 100 nmole DNA oligo, 80 bases

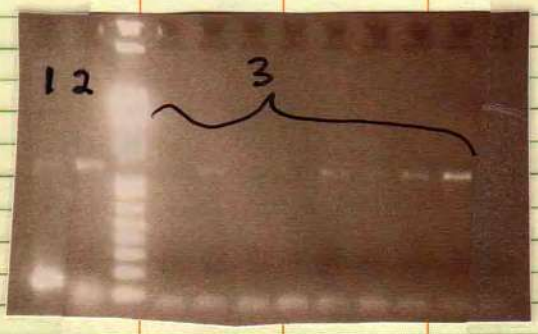
5 AGA CCT GCG AGC AGG GAA ACG CTC CCC TCA CAG ACG CGT TGA ATT GTC CCC ACG CCG CGC|ATC AAA GAA GGT TAA TGT GG -3

← KanMX6 homology

Sequence - URA3extRintegration — 100 nmole DNA oligo, 80 bases

5 TGT CAG TAC TGA TTA GAA AAA CTC ATC GAG CAT CAA ATG AAA CTG CAA TTT ATT CAT ATC|TCA TTA TAG AAA TCA TTA CG -3

← KanMX6 homology



1) PCR w/ a previous PCR as temp.

2) PCR w/ gDNA as temp.

3) PCRs for another expt (amplified w/ URA3extF & URA3extR) to give a size comparison.

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

~~8/26~~ 7/26

OD of 0.7

Prep YEL020CΔ strain for transformation according to protocol on next page

~~transform with~~

PCR purify lane 2 (previous page) & resus. in 30λ EB.
Transform 50λ YEL020C w/ 10λ PCR or H_2O (neg cont.)

~~8/29~~ 7/29

About 20 colonies on transformed plate
Ø colonies on neg. control plate.

Pick 6 colonies and restreak on -URA.

7/30

Replica plate -URA plates onto YPD+G418

7/31

All 6 strains are G418 sensitive

Start YPD+ADE cultures of each of the 6 strains.

Bodo's Lithium Acetate Transformation

LiOAc mix

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

PEG mix

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

~~25 mL culture~~

1) Grow culture to OD 0.5-0.7 in YPD. (0.7) (~~at stationary~~
→ 5" for minimum
2) Spin, transfer to Eppendorf tube and wash ~~twice~~ ~~3-4x~~ 3x cold with sterile water Spin 10 mL
↳ ~~setting 5 on centrifuge (in 50 mL falcon tube)~~ 5min @ ~~2000~~ 2,000 ~~20 sec full~~
3) Wash twice in 1 ml LiAc mix.
4) Resuspend in ~~360~~ ~~120~~ 90λ ~~360μl~~ LiAc mix per ~~50~~ ~~25~~ 10 mL ml culture and ~~40μl~~ ~~20~~ 10λ carrier DNA (0°C).
5) use 10 μl cells/plasmid transformation and ~~50μl~~ ~~30~~ 50λ for integrative transformation.
6) Add thawed cells to DNA (maximally 2μl DNA per 10μl competent cells) and mix.
~~1λ per 10λ above~~ ~~(3λ for 30λ)~~ 10λ DNA + 50λ Cells
7) Add sixfold volume of 40% PEG, mix and incubate 30', ~~30°C (room temp if ts)~~ @ room temp.
360λ
8) Add DMSO to final concentration of 10%. → helps cells survive temperature shock.
↳ not necessary 47λ DMSO
9) Heat shock 15', ~~42°C (37°C if ts)~~.
10) Sediment cells for 2-3 min at 2000rpm in Eppendorf centrifuge.
11) Resuspend in 200 μl YPD and plate out on selective medium.

Stock Sol'ns

45% PEG
1 M Tris (pH 7.5)
.5 M EDTA
1 M LiOAc

PEG mix: 100ml:
10ml 1M LiOAc
1ml 1M ~~Tris~~ pH 7.5
0.2ml 0.5M ~~EDTA~~
88.8ml 45% PEG

Bode's Lithium Acetate Transformation

LiOAc mix
100mM LiOAc
10mM Tris pH 7.5
1mM EDTA

PEG mix
40%PEG
100mM LiOAc
10mM Tris
1mM EDTA

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

2) Spin, transfer to Eppendorf tube and wash with sterile water

3) Wash twice in 1 ml LiAc mix.

4) Resuspend in 360µl LiAc mix per 50ml culture and 40µl carrier DNA (0°C).

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

6) Add thawed cells to DNA (maximally 2µl DNA per 10µl competent cells) and mix

7) Add sterile volume of 40% PEG, mix and incubate 30' 30°C (room temp is ok)

8) Add DMSO to final concentration of 10%.

9) Heat shock 15' 42°C

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

11) Resuspend in 200 µl YPD and plate on selective medium.

Stock Solutions

40% PEG
1M Tris (pH 7.5)
5M EDTA
1M LiOAc

PEG mix: 10ml:
10ml 1M LiOAc
1ml 1M Tris pH 7.5
0.2ml 0.5M EDTA
8.8ml 50% PEG

8/2

· gDNA preps of the 6 transformants

I screwed up the last two — I added them to the same tube of EtOH during gDNA prep.

Set up PCR using primers URA3 u1 & URA3 d1 — 100λ

17314227
V.INDJEIAN
18570433 7/22/2005
URA3u1
5 GAT GTC CAC GAG GTC TCT -3
Tm= 53.4 °C , MW= 5,490.6
5.4 OD_{260} 31.99 nmol 0.18 mg

17314231
V.INDJEIAN
18572943 7/22/2005
URA3d1
5 CGG TGT CGG TCT CGT AG -3
Tm= 54.8 °C , MW= 5,233.4
4.8 OD_{260} 30.32 nmol 0.16 mg

94	2:00 m	
94	30 s	30 cycles
53	30 s	
72	2:30	
72	~~5:00~~ 5:00	

8/3

LANE	TEMPLATE
1	gDNA prep of transformant 1
2	" 2
3	" 3
4	" 4
5	whole cell PCR from cold culture of x-form 5
6	" 6
7	gDNA prep of mixed x-formants 5 & 6

← Post PCR cleanup (versus in 30λ EB)

· Submit lanes 1-4 (2λ) for MCB sequencing w/ — see next page →

SET UP DIGESTIONS

33.5 λ	H_2O
5 λ	10× EcoRI Buffer
10 λ	PCR Elution
.5 λ	100× BSA
.5 λ	EcoRI
.5 λ	BamHI
50 λ	

↑ for PCR products

77 λ	H_2O
10 λ	10× EcoRI Buffer
10 λ	pFA6 (Vectorology #1)
1 λ	100× BSA
1 λ	EcoRI
1 λ	BamHI
100 λ	

↑ for Plasmid

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 692 10VG

Sequencing continued.

Primers used:

A	URA3u1
B	URA3d1
C	URA3extF3
D	URA3extR
E	URA3intF2
F	URA3intR2

Templates: KanMXΔ::URA3 1
〃 2
〃 3
〃 4

STRIP #	Position 1	2	3	4	5	6	7	8
1	A1	A2	A3	B1	A4	B2	B3	B4
2	C1	C2	C3	C4	D1	D2	D3	D4
3	E1	E2	E3	E4	F1	F2	F3	F4

8/5 Sequences of 4 transformants

```
           1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
trans_1    CCCCG GGTTAATTAA GGCGCGCCAG ATCTGTTTAG CTTGCCTCGT CCCCGCCGGG TCACCCGGCC
trans_2      CCCCG GGTTAATTAA GGCGCGCCAG ATCTGTTTAG CTTGCCTCGT CCCCGCCGGG TCACCCGGCC
trans_4  CGGATCCCCG GGTTAATTAA GGCGCGCCAG ATCTGTTTAG CTTGCCTCGT CCCCGCCGGG TCACCCGGCC
trans_3      TCCCCG GGTTAATTAA GGCGCGCCAG ATCTGTTTAG CTTGCCTCGT CCCCGCCGGG TCACCCGGCC
Consensus  .....CCCCG GGTTAATTAA GGCGCGCCAG ATCTGTTTAG CTTGCCTCGT CCCCGCCGGG TCACCCGGCC

           71                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
trans_1    AGCGACATGG AGGCCCAGAA TACCCTCCTT GACAGTCTTG ACGTGCGCAG CTCAGGGGCA TGATGTGACT
trans_2    AGCGACATGG AGGCCCAGAA TACCCTCCTT GACAGTCTTG ACGTGCGCAG CTCAGGGGCA TGATGTGACT
trans_4    AGCGACATGG AGGCCCAGAA TACCCTCCTT GACAGTCTTG ACGTGCGCAG CTCAGGGGCA TGATGTGACT
trans_3    AGCGACATGG AGGCCCAGAA TACCCTCCTT GACAGTCTTG ACGTGCGCAG CTCAGGGGCA TGATGTGACT
Consensus  AGCGACATGG AGGCCCAGAA TACCCTCCTT GACAGTCTTG ACGTGCGCAG CTCAGGGGCA TGATGTGACT

           141                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
trans_1    GTCGCCCGTA CATTTAGCCC ATACATCCCC ATGTATAATC ATTTGCATCC ATACATTTTG ATGGCCGCAC
trans_2    GTCGCCCGTA CATTTAGCCC ATACATCCCC ATGTATAATC ATTTGCATCC ATACATTTTG ATGGCCGCAC
trans_4    GTCGCCCGTA CATTTAGCCC ATACATCCCC ATGTATAATC ATTTGCATCC ATACATTTTG ATGGCCGCAC
trans_3    GTCGCCCGTA CATTTAGCCC ATACATCCCC ATGTATAATC ATTTGCATCC ATACATTTTG ATGGCCGCAC
Consensus  GTCGCCCGTA CATTTAGCCC ATACATCCCC ATGTATAATC ATTTGCATCC ATACATTTTG ATGGCCGCAC

           211                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
trans_1    GGCGCGAAGC AAAAATTACG GCTCCTCGCT GCAGACCTGC GAGCAGGGAA ACGCTCCCC- TCACAGACGC
trans_2    GGCGCGAAGC AAAAATTACG GCTCCTCGCT GCAGACCTGC GAGCAGGGAA ACGCTCCCC- TCACAGACGC
trans_4    GGCGCGAAGC AAAAATTACG GCTCCTCGCT GCAGACCTGC GAGCAGGGAA ACGCTCCCC- TCACAGACGC
trans_3    GGCGCGAAGC AAAAATTACG GCTCCTCGCT GCAGACCTGC GAGCAGGGAA ACGCTCCCCC TCACAGACGC
Consensus  GGCGCGAAGC AAAAATTACG GCTCCTCGCT GCAGACCTGC GAGCAGGGAA ACGCTCCCC. TCACAGACGC

           281                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
trans_1    GTTGAATTGT CCCCACGCCG CGCATCAAAG AAGGTTAATG TGGCTGTGGT TTCAGGGTCC ATAAAGCTTT
trans_2    GTTGAATTGT CCCCACGCCG CGCATCAAAG AAGGTTAATG TGGCTGTGGT TTCAGGGTCC ATAAAGCTTT
trans_4    GTTGAATTGT CCCCACGCCG CGCATCAAAG AAGGTTAATG TGGCTGTGGT TTCAGGGTCC ATAAAGCTTT
trans_3    GTTGAATTGT CCCCACGCCG CGCATCAAAG AAGGTTAATG TGGCTGTGGT TTCAGGGTCC ATAAAGCTTT
Consensus  GTTGAATTGT CCCCACGCCG CGCATCAAAG AAGGTTAATG TGGCTGTGGT TTCAGGGTCC ATAAAGCTTT
```

URA3extFintegration →

```
           351                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
trans_1    TCAATTCATC TTTTTTTTTT TTATTCTTTT TTTTGATTTC GGTTTCCTTG AAATTTTTTT GATTCGGTAA
trans_2    TCAATTCATC TTTTTTTTTT TTATTCTTTT TTTTGATTTC GGTTTCCTTG AAATTTTTTT GATTCGGTAA
trans_4    TCAATTCATC TTTTTTTTTT TTATTCTTTT TTTTGATTTC GGTTTCCTTG AAATTTTTTT GATTCGGTAA
trans_3    TCAATTCATC TTTTTTTTTT TTATTCTTTT TTTTGATTTC GGTTTCCTTG AAATTTTTTT GATTCGGTAA
Consensus  TCAATTCATC TTTTTTTTTT TTATTCTTTT TTTTGATTTC GGTTTCCTTG AAATTTTTTT GATTCGGTAA

           421                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
trans_1    TCTCCGAACA GAAGGAAGAA CGGAGGAAGG AGCACAGACT TAGATTGGTA TATATACGCA TATGTAGTGT
trans_2    TCTCCGAACA GAAGGAAGAA CGAAGGAAGG AGCACAGACT TAGATTGGTA TATATACGCA TATGTAGTGT
trans_4    TCTCCGAACA GAAGGAAGAA CGAAGGAAGG AGCACAGACT TAGATTGGTA TATATACGCA TATGTAGTGT
trans_3    TCTCCGAACA GAAGGAAGAA CGAAGGAAGG AGCACAGACT TAGATTGGTA TATATACGCA TATGTAGTGT
Consensus  TCTCCGAACA GAAGGAAGAA CGaAGGAAGG AGCACAGACT TAGATTGGTA TATATACGCA TATGTAGTGT

           491                                                                                                                                                                                                                                                                                                                                                                                                                                                
trans_1    TGAAGAAACA TGAAATTGCC CAGTATTCTT AACCCAACTG CACAGAACAA AAACATGCAG GAAACGAAGA
trans_2    TGAAGAAACA TGAAATTGCC CAGTATTCTT AACCCAACTG CACAGAACAA AAACATGCAG GAAACGAAGA
trans_4    TGAAGAAACA TGAAATTGCC CAGTATTCTT AACCCAACTG CACAGAACAA AAACATGCAG GAAACGAAGA
trans_3    TGAAGAAACA TGAAATTGCC CAGTATCCTT AACCCAACTG CACAGAACAA AAACATGCAG GAAACGAAGA
Consensus  TGAAGAAACA TGAAATTGCC CAGTATtCTT AACCCAACTG CACAGAACAA AAACATGCAG GAAACGAAGA

           561                                                                                                                                                                                                                                                                                                                                                                                                                                                
trans_1    TAAATCATGT CGAAAGCTAC ATATAAGGAA CGTGCTGCTA CTCATCCTAG TCCTGTTGCT GCCAAGCTAT
trans_2    TAAATCATGT CGAAAGCTAC ATATAAGGAA CGTGCTGCTA CTCATCCTAG TCCTGTTGCT GCCAAGCTAT
trans_4    TAAATCATGT CGAAAGCTAC ATATAAGGAA CGTGCTGCTA CTCATCCTAG TCCTGTTGCT GCCAAGCTAT
trans_3    TAAATCATGT CGAAAGCTAC ATATAAGGAA CGTGCTGCTA CTCATCCTAG TCCTGTTGCT GCCAAGCTAT
Consensus  TAAATCATGT CGAAAGCTAC ATATAAGGAA CGTGCTGCTA CTCATCCTAG TCCTGTTGCT GCCAAGCTAT
```

URA3 START

```
           631                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 700
trans_1    TTAATATCAT GCACGAAAAG CAAACAAACT TGTGTGCTTC ATTGGATGTT CGTACCACCA AGGAATTACT
trans_2    TTAATATCAT GCACGAAAAG CAAACAAACT TGTGTGCTTC ATTGGATGTT CGTACCACCA AGGAATTACT
trans_4    TTAATATCAT GCACGAAAAG CAAACAAACT TGTGTGCTTC ATTGGATGTT CGTACCACCA AGGAATTACT
trans_3    TTAATATCAT GCACGAAAAG CAAACAAACT TGTGTGCTTC ATTGGATGTT CGTACCACCA AGGAATTACT
Consensus  TTAATATCAT GCACGAAAAG CAAACAAACT TGTGTGCTTC ATTGGATGTT CGTACCACCA AGGAATTACT

           701                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 770
trans_1    GGAGTTAGTT GAAGCATTAG GTCCCAAAAT TTGTTTACTA AAAACACATG TGGATATCTT GACTGATTTT
trans_2    GGAGTTAGTT GAAGCATTAG GTCCCAAAAT TTGTTTACTA AAAACACATG TGGATATCTT GACTGATTTT
trans_4    GGAGTTAGTT GAAGCATTAG GTCCCAAAAT TTGTTTACTA AAAACACATG TGGATATCTT GACTGATTTT
trans_3    GGAGTTAGTT GAAGCATTAG GTCCCAAAAT TTGTTTACTA AAAACACATG TGGATATCTT GACTGATTTT
Consensus  GGAGTTAGTT GAAGCATTAG GTCCCAAAAT TTGTTTACTA AAAACACATG TGGATATCTT GACTGATTTT

           771                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 840
trans_1    TCCATGGAGG GCACAGTTAA GCCGCTAAAG GCATTATCCG CCAAGTACAA TTTTTTACTC TTCGAAGACA
trans_2    TCCATGGAGG GCACAGTTAA GCCGCTAAAG GCATTATCCG CCAAGTACAA TTTTTTACTC TTCGAAGACA
trans_4    TCCATGGAGG GCACAGTTAA GCCGCTAAAG GCATTATCCG CCAAGTACAA TTTTTTACTC TTCGAAGACA
trans_3    TCCATGGAGG GCACAGTTAA GCCGCTAAAG GCATTATCCG CCAAGTACAA TTTTTTACTC TTCGAAGACA
Consensus  TCCATGGAGG GCACAGTTAA GCCGCTAAAG GCATTATCCG CCAAGTACAA TTTTTTACTC TTCGAAGACA

           841                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 910
trans_1    GAAAATTTGC TGACATTGGT AATACAGTCA AATTGCAGTA CTCTGCGGGT GTATACAGAA TAGCAGAATG
trans_2    GAAAATTTGC TGACATTGGT AATACAGTCA AATTGCAGTA CTCTGCGGGT GTATACAGAA TAGCAGAATG
trans_4    GAAAATTTGC TGACATTGGT AATACAGTCA AATTGCAGTA CTCTGCGGGT GTATACAGAA TAGCAGAATG
trans_3    GAAAATTTGC TGACATTGGT AATACAGTCA AATTGCAGTA CTCTGCGGGT GTATACAGAA TAGCAGAATG
Consensus  GAAAATTTGC TGACATTGGT AATACAGTCA AATTGCAGTA CTCTGCGGGT GTATACAGAA TAGCAGAATG

           911                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
trans_1    GGCAGACATT ACGAATGCAC ACGGTGTGGT GGGCCCAGGT ATTGTTAGCG GTTTGAAGCA GGCGGCGGAA
trans_2    GGCAGACATT ACGAATGCAC ACGGTGTGGT GGGCCCAGGT ATTGTTAGCG GTTTGAAGCA GGCGGCGGAA
trans_4    GGCAGACATT ACGAATGCAC ACGGTGTGGT GGGCCCAGGT ATTGTTAGCG GTTTGAAGCA GGCGGCGGAA
trans_3    GGCAGACATT ACGAATGCAC ACGGTGTGGT GGGCCCAGGT ATTGTTAGCG GTTTGAAGCA GGCGGCGGAA
Consensus  GGCAGACATT ACGAATGCAC ACGGTGTGGT GGGCCCAGGT ATTGTTAGCG GTTTGAAGCA GGCGGCGGAA

           981                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
trans_1    GAAGTAACAA AGGAACCTAG AGGCCTTTTG ATGTTAGCAG AATTGTCATG CAAGGGCTCC CTATCTACTG
trans_2    GAAGTAACAA AGGAACCTAG AGGCCTTTTG ATGTTAGCAG AATTGTCATG CAAGGGCTCC CTATCTACTG
trans_4    GAAGTAACAA AGGAACCTAG AGGCCTTTTG ATGTTAGCAG AATTGTCATG CAAGGGCTCC CTATCTACTG
trans_3    GAAGTGACAA AGGAACCTAG AGGCCTTTTG ATGTTAGCAG AATTGTCATG CAAGGGCTCC CTATCTACTG
Consensus  GAAGTaACAA AGGAACCTAG AGGCCTTTTG ATGTTAGCAG AATTGTCATG CAAGGGCTCC CTATCTACTG

           1051                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
trans_1    GAGAATATAC TAAGGGTACT GTTGACATTG CGAAGAGCGA CAAAGATTTT GTTATCGGCT TTATTGCTCA
trans_2    GAGAATATAC TAAGGGTACT GTTGACATTG CGAAGAGCGA CAAAGATTTT GTTATCGGCT TTATTGCTCA
trans_4    GAGAATATAC TAAGGGTACT GTTGACATTG CGAAAAGCGA CAAAGATTTT GTTATCGGCT TTATTGCTCA
trans_3    GAGAATATAC TAAGGGTACT GTTGACATTG CGAAGAGCGA CAAAGATTTT GTTATCGGCT TTATTGCTCA
Consensus  GAGAATATAC TAAGGGTACT GTTGACATTG CGAAgAGCGA CAAAGATTTT GTTATCGGCT TTATTGCTCA

           1121                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
trans_1    AAGAGACATG GGTGGAAGAG ATGAAGGTTA CGATTGGTTG ATTATGACAC CCGGTGTGGG TTTAGATGAC
trans_2    AAGAGACATG GGTGGAAGAG ATGAAGGTTA CGATTGGTTG ATTATGACAC CCGGTGTGGG TTTAGATGAC
trans_4    AAGAGACATG GGCGGAAGAG ATGAAGGTTA CGATTGGTTG ATTATGACAC CCGGTGTGGG TTTAGATGAC
trans_3    AAGAGACATG GGTGGAAGAG ATGAAGGTTA CGATTGGTTG ATTATGACAC CCGGTGTGGG TTTAGATGAC
Consensus  AAGAGACATG GGtGGAAGAG ATGAAGGTTA CGATTGGTTG ATTATGACAC CCGGTGTGGG TTTAGATGAC

           1191                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
trans_1    AAGGGAGACG CATTGGGTCA ACAGTATAGA ACCGTGGATG ATGTGGTCTC TACAGGATCT GACATTATTA
trans_2    AAGGGAGACG CATTGGGTCA ACAGTATAGA ACCGTGGATG ATGTGGTCTC TACAGGATCT GACATTATTA
trans_4    AAGGGAGACG CATTGGGTCA ACAGTATAGA ACCGTGGATG ATGTGGTCTC TACAGGATCT GACATTATTA
trans_3    AAGGGAGACG CATTGGGTCA ACAGTATAGA ACCGTGGATG ATGTGGTCTC TACAGGATCT GACATTATTA
Consensus  AAGGGAGACG CATTGGGTCA ACAGTATAGA ACCGTGGATG ATGTGGTCTC TACAGGATCT GACATTATTA
```

```
           1261                                                                                  1330
trans_1    TTGTTGGAAG AGGACTATTT GCAAAGGGAA GGGATGCTAA GGTAGAGGGT GAACGTTACA GAAAAGCAGG
trans_2    TTGTTGGAAG AGGACTATTT GCAAAGGGAA GGGATGCTAA GGTAGAGGGT GAACGTTACA GAAAAGCAGG
trans_4    TTGTTGGAAG AGGACTATTT GCAAAGGGAA GGGATGCTAA GGTAGAGGGT GAACGTTACA GAAAAGCAGG
trans_3    TTGTTGGAAG AGGACTATTT GCAAAGGGAA GGGATGCTAA GGTAGAGGGT GAACGTTACA GAAAAGCAGG
Consensus  TTGTTGGAAG AGGACTATTT GCAAAGGGAA GGGATGCTAA GGTAGAGGGT GAACGTTACA GAAAAGCAGG

           1331                                                                                  1400
trans_1    CTGGGAAGCA TATTTGAGAA GATGCGGCCA GCAAAACTAA AAAACTGTAT TATAAGTAAA TGCATGTATA
trans_2    CTGGGAAGCA TATTTGAGAA GATGCGGCCA GCAAAACTAA AAAACTGTAT TATAAGTAAA TGCATGTATA
trans_4    CTGGGAAGCA TATTTGAGAA GATGCGGCCA GCAAAACTAA AAAACTGTAT TATAAGTAAA TGCATGTATA
trans_3    CTGGGAAGCA TATTTGAGAA GATGCGGCCA GCAAAACTAA AAAACTGTAT TATAAGTAAA TGCATGTATA
Consensus  CTGGGAAGCA TATTTGAGAA GATGCGGCCA GCAAAACTAA AAAACTGTAT TATAAGTAAA TGCATGTATA
```

URA3 STOP

```
           1401                                                                                  1470
trans_1    CTAAACTCAC AAATTAGAGC TTCAATTTAA TTATATCAGT TATTACCCGG GAATCTCGGT CGTAATGATT
trans_2    CTAAACTCAC AAATTAGAGC TTCAATTTAA TTATATCAGT TATTACCCGG GAATCTCGGT CGTAATGATT
trans_4    CTAAACTCAC AAATTAGAGC TTCAATTTAA TTATATCAGT TATTACCCGG GAATCTCGGT CGTAATGATT
trans_3    CTAAACTCAC AAATTAGAGC TTCAATTTAA TTATATCAGT TATTACCCGG GAATCTCGGT CGTAATGATT
Consensus  CTAAACTCAC AAATTAGAGC TTCAATTTAA TTATATCAGT TATTACCCGG GAATCTCGGT CGTAATGATT

           1471                                                                                  1540
trans_1    TCTATAATGA GATATGAATA AATTGCAGTT TCATTTGATG CTCGATGAGT TTTTCTAATC AGTACTGACA
trans_2    TCTATAATGA GATATGAATA AATTGCAGTT TCATTTGATG CTCGATGAGT TTTTCTAATC AGTACTGACA
trans_4    TCTATAATGA GATATGAATA AATTGCAGTT TCATTTGATG CTCGATGAGT TTTTCTAATC AGTACTGACA
trans_3    TCTATAATGA GATATGAATA AATTGCAGTT TCATTTGATG CTCGATGAGT TTTTCTAATC AGTACTGACA
Consensus  TCTATAATGA GATATGAATA AATTGCAGTT TCATTTGATG CTCGATGAGT TTTTCTAATC AGTACTGACA
```

URA3extRintegration

```
           1541                                                                                  1610
trans_1    ATAAAAAGAT TCTTGTTTTC AAGAACTTGT CATTTGTATA GTTTTTTTAT ATTGTAGTTG TTCTATTTTA
trans_2    ATAAAAAGAT TCTTGTTTTC AAGAACTTGT CATTTGTATA GTTTTTTTAT ATTGTAGTTG TTCTATTTTA
trans_4    ATAAAAAGAT TCTTGTTTTC AAGAACTTGT CATTTGTATA GTTTTTTTAT ATTGTAGTTG TTCTATTTTA
trans_3    ATAAAAAGAT TCTTGTTTTC AAGAACTTGT CATTTGTATA GTTTTTTTAT ATTGTAGTTG TTCTATTTTA
Consensus  ATAAAAAGAT TCTTGTTTTC AAGAACTTGT CATTTGTATA GTTTTTTTAT ATTGTAGTTG TTCTATTTTA

           1611                                                                                  1680
trans_1    ATCAAATGTT AGCGTGATTT ATATTTTTTT TCGCCTCGAC ATCATCTGCC CAGATGCGAA GTTAAGTGCG
trans_2    ATCAAATGTT AGCGTGATTT ATATTTTTTT TCGCCTCGAC ATCATCTGCC CAGATGCGAA GTTAAGTGCG
trans_4    ATCAAATGTT AGCGTGATTT ATATTTTTTT TCGCCTCGAC ATCATCTGCC CAGATGCGAA GTTAAGTGCG
trans_3    ATCAAATGTT AGCGTGATTT ATATTTTTTT TCGCCTCGAC ATCATCTGCC CAGATGCGAA GTTAAGTGCG
Consensus  ATCAAATGTT AGCGTGATTT ATATTTTTTT TCGCCTCGAC ATCATCTGCC CAGATGCGAA GTTAAGTGCG

           1681                                                                                  1750
trans_1    CAGAAAGTAA TATCATGCGT CAATCGTATG TGAATGCTGG TCGCTATACT GCTGTCGATT CGATACTAAC
trans_2    CAGAAAGTAA TATCATGCGT CAATCGTATG TGAATGCTGG TCGCTATACT GCTGTCGATT CGATACTAAC
trans_4    CAGAAAGTAA TATCATGCGT CAATCGTATG TGAATGCTGG TCGCTATACT GCTGTCGATT CGATACTAAC
trans_3    CAGAAAGTAA TATCATGCGT CAATCGTATG TGAATGCTGG TCGCTATACT GCTGTCGATT CGATACTAAC
Consensus  CAGAAAGTAA TATCATGCGT CAATCGTATG TGAATGCTGG TCGCTATACT GCTGTCGATT CGATACTAAC

           1751                  1774
trans_1    GCCGCCATCC AGTGTCGAAA ACG
trans_2    GCCGCCATCC AGTGTCGAAA ACGA
trans_4    GCCGCCATCC AGT
trans_3    GCCGCCATCC AGT
Consensus  GCCGCCATCC AGT....... ....
```

Summary

transformant 1 — 1 mutation
transformant 2 — No mutations
transformant 3 — 2 mutations (1 insertion)
transformant 4 — 2 mutations, both in URA3 gene. both synonamous

the insertion mutation was in the primer

Summary

Transcription 1 — 1 mutation

Transcription 2 — NO MUTATIONS

Transcription 3 — 2 mutations (1 insertion)

Transcription 4 — 2 mutations, both in USA3 genes, both synonymous

the insertion mutation was in the primer

8/5

I let the restriction enz. digests from 8/3 to go for ~1.5 h, then put them in the fridge.

Yesterday I gel extracted the digested fragments.

Lane	
1	½ of pFA6 double digest
2	"
3	double digest of transf. PCR 1
4	" 2
5	" 3
6	" 4

Expected Sizes

pFA6:

3.9 - singly cut vector

2.4 - vector

1.5 - kanMX6

inserts:

1.9 - ℓ insert

Not a complete digestion of vector.

According to Lang, I should have CIP'd the vector before I gel extracted. Thus morning I added 4 λ of NEBuff 3, 5.5 λ H_2O and .5 λ CIP to the 30 λ of plasmid 1 (ext. from lane 1 above). Incubated 60 min @ 50° (According to Jun-Yi, CIP has nuclease activity at lower temps.)

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

8/5

LIGATION

Set up ligations w/ CIP & Non-CIP treated vector and insert #2 — the only one w/o a PCR induced mutation.

7 λ H_2O
2 λ 10× Ligase Buffer
5 λ Plasmid
5 λ insert #2
1 λ T4 ligase
20 λ

RT @ 1:45

TRANSFORMATION

I transformed 2 λ of each ligation or 2 λ H_2O as a control into Electrocompetent cells — however I had trouble with the samples arcing — perhaps too much salt in ligations

Try Thomas' chemically competent cells as well

8/6

No colonies on either H_2O plate

~100 colonies on electroporation, w/ CIP plate
~200 colonies on electroporation, No CIP plate

thousands of colonies on both w/ and w/o CIP chemically comp plates.

- pick 10 colonies from both CIP plates (less likely to be empty vector) and inoc. 2 mL 2×YT+amp

8/6

LANE

1 CIP treated vector
2 non-CIP treated vector
3 insert #2 after RE digest
4 insert #2 before RE digest

} ran 2λ of each

· from this gel you can see that I added a lot more vector than insert — I am concerned that I may have too much religated vector transformants.

· I should have done a vector only ligation so that I can estimate the vector-only bkg.

Yesterday I started an o/N culture of the yeast transformant #2. Today I made a permanent stock of that strain, now called GHL085.

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 692 10VG

8/7

MINIPREP PUTATIVE TRANSFORMANTS

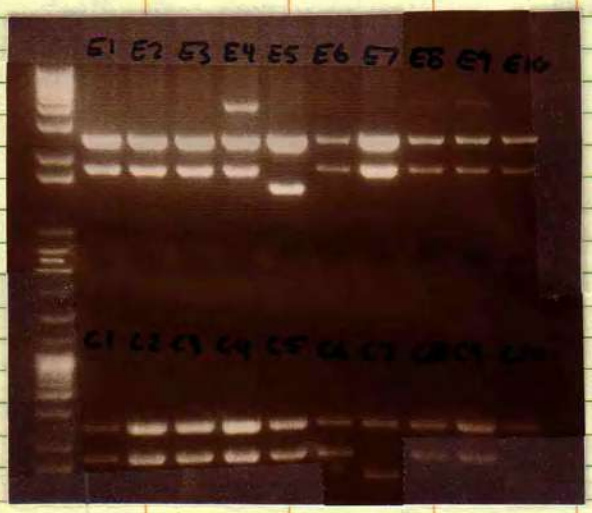
· miniprep the 20 transformants from yesterday
  - labeled E1-10 and C1-10 (for electroporation & chemical transformation, respectively)

RESTRICTION ENZYME DIGEST

737 λ	H_2O
110 λ	10×EcoRI Buffer
11 λ	100×BSA
11 λ	EcoRI
11 λ	BamHI

→ 20 λ mix + 5 λ miniprep

} 1.5 hours in 37° air incubator



Possible fragments:

Size (kb)

~~3.9 - uncut~~

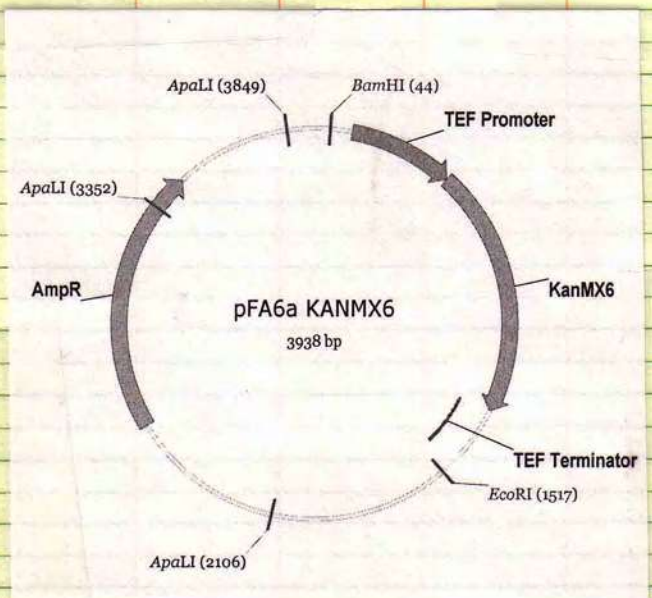
2.4 - vector

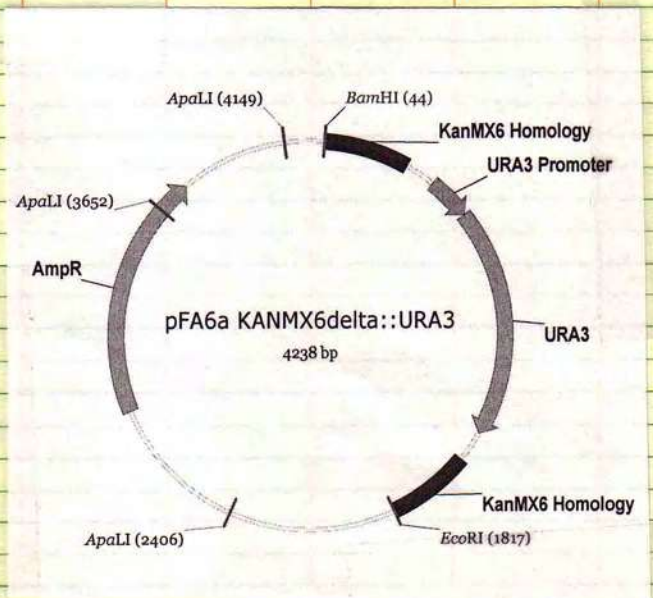
1.9 - KanMX6Δ::URA3

1.5 - KanMX6

3.9 - singly cut vector + KanMX6

4.3 - singly cut vector + KanMX6Δ::URA3





8/8

Yesterday when I set up the EcoRI/BamHI double digest, I also did a ApaLI digest. Since the U1 & ~~U1~~ D1 primer sites lie outside the E/B cut sites I cannot sequence off of the E/B d. digests.

748 λ	H_2O	1.5 h in 37° air incubator and o/N on benchtop
110 λ	NEBuffer 4	
11 λ	100×BSA	
11 λ	ApaLI	

→ 20 λ Mix + 5 λ miniprep

Pick 6 digests from E & C

Expected fragment sizes:

Size (kb)
2.5
1.2
.5

NOTES ON RE DIGESTS

Correct plasmid (pFA6a-KanMX6Δ::URA3): E1, E2, E3, E4, E6, E7, E8, E9, E10, C1, C2, C3, C4, C5, C6, C8, C9

Incorrect plasmid (pFA6a-KanMX6, religated singly cut vector): E5, C7, C10

There are clearly different intensities — may reflect either copy number or quality of minipreps or something else — genomic integration?

HIGH: E1, E2, E3, E4, E5, E7, C2, C3, C4, C5, C9

LOW: E6, E8, E9, E10, C1, C6, C7, C8, C10

E4 has a large amount of singly cut ~~vector~~ plasmid — perhaps this ~~plasmid a is a mixed~~ bacterial transformant contains multiple plasmids and/or this plasmid has a mutation in one of the RE sites.

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 692 10VG

8/8

Sequencing of Plasmid inserts

- Use PCR purification kit on E1, E2, E3, E7, C1, C2, C3, & C4
- Same primers as on page 14

STRIP #	Position 1 (Primer, Temp)	2	3	4	5	6	7	8
1	A, E1	A, E2	A, E3	A, E7	B, E1	B, E2	B, E3	B, E7
2	C, E1	C, E2	C, E3	C, E7	D, ~~E1~~ E2	D, ~~E2~~ E1	D, E3	D, E7
3	E, E1	E, E2	E, E3	E, E7	F, E1	F, E2	F, E3	F, E7
4	A, C1	A, C2	A, C3	A, C4	B, C1	B, C2	B, C3	B, C4
5	C, C1	C, C2	C, C3	C, C4	D, C1	D, C2	D, C3	D, C4
6	E, C1	E, C2	E, C3	E, C4	F, C1	F, C2	F, C3	F, C4

Sequencing Notes:

- I switched positions 5 & 6 in strip 2, as indicated above.
- The new Princeton separation columns seem to be a bit inconsistent. Several samples had less than 10 λ of liq, so I added H_2O to bring the vol. above 10 λ.

Sample (strip-Position)	Vol. added
1-8	8 λ
3-1	5 λ
4-2	10 λ
5-4	10 λ
5-8	10 λ

8/9 Sequencing Results

```
           1                                                                    70
EXPECTED   GATGTCCACG AGGTCTCTTA GTAGTAGTAG TAGTAGTACG TACGCTGCAG GTCGACGGAT CCCCGGGTTA
C2
E2
E3
C3
E1
E7
Consensus  .......... .......... .......... .......... .......... .......... ..........

           71                                                                  140
EXPECTED   ATTAAGGCGC GCCAGATCTG TTTAGCTTGC CTCGTCCCCG CCGGGTCACC CGGCCAGCGA CATGGAGGCC
C2
E2
E3
C3
E1
E7
Consensus  .......... .......... .......... .......... .......... .......... ..........

           141                                                                 210
EXPECTED   CAGAATACCC TCCTTGACAG TCTTGACGTG CGCAGCTCAG GGGCATGATG TGACTGTCGC CCGTACATTT
C2
E2
E3
C3
E1
E7
Consensus  .......... .......... .......... .......... .......... .......... ..........

           211                                                                 280
EXPECTED   AGCCCATACA TCCCCATGTA TAATCATTTG CATCCATACA TTTTGATGGC CGCACGGCGC GAAGCAAAAA
C2          GCCCATACA TCCCCATGTA TAATCATTTG CATCCATACA TTTTGATGGC CGCACGGCGC GAAGCAAAAA
E2              CATACA TCCCCATGTA TAATCATTTG CATCCATACA TTTTGATGGC CGCACGGCGC GAAGCAAAAA
E3
C3
E1                      ATGTA TAATCATTTG CATCCATACA TTTTGATGGC CGCACGGCGC GAAGCAAAAA
E7
Consensus  .......... .....atgta taatcatttg catccataca ttttgatggc cgcacggcgc gaagcaaaaa

           281                                                                 350
EXPECTED   TTACGGCTCC TCGCTGCAGA CCTGCGAGCA GGGAAACGCT CCCCTCACAG ACGCGTTGAA TTGTCCCCAC
C2         TTACGGCTCC TCGCTGCAGA CCTGCGAGCA GGGAAACGCT CCCCTCACAG ACGCGTTGAA TTGTCCCCAC
E2         TTACGGCTCC TCGCTGCAGA CCTGCGAGCA GGGAAACGCT CCCCTCACAG ACGCGTTGAA TTGTCCCCAC
E3                          A CCTGCGAGCA GGGAAACGCT CCCCTCACAG ACGCGTTGAA TTGTCCCCAC
C3
E1         TTACGGCTCC TCGCTGCAGA CCTGCGAGCA GGGAAACGCT CCCCTCACAG ACGCGTTGAA TTGTCCCCAC
E7
Consensus  ttacggctcc tcgctgcaga cctgcgagca gggaaacgct cccctcacag acgcgttgaa ttgtccccac

           351                                                                 420
EXPECTED   GCCGCGCATC AAAGAAGGTT AATGTGGCTG TGGTTTCAGG GTCCATAAAG CTTTTCAATT CATCTTTTTT
C2         GCCGCGCATC AAAGAAGGTT AATGTGGCTG TGGTTTCAGG GTCCATAAAG CTTTTCAATT CATCTTTTTT
E2         GCCGCGCATC AAAGAAGGTT AATGTGGCTG TGGTTTCAGG GTCCATAAAG CTTTTCAATT CATCTTTTTT
E3         GCCGCGCATC AAAGAAGGTT AATGTGGCTG TGGTTTCAGG GTCCATAAAG CTTTTCAATT CATCTTTTTT
C3                                          GGTTTCAGG GTCCATAAAG CTTTTCAATT CATCTTTTTT
E1         GCCGCGCATC AAAGAAGGTT AATGTGGCTG TGGTTTCAGG GTCCATAAAG CTTTTCAATT CATCTTTTTT
E7
nsensus    gccgcgcatc aaagaaggtt aatgtggctg tggtttcagg gtccataaag cttttcaatt catcttttt

           421                                                                 490
EXPECTED   TTTTTTATTC TTTTTTTTGA TTTCGGTTTC CTTGAAATTT TTTTGATTCG GTAATCTCCG AACAGAAGGA
C2         TTTTTTATTC TTTTTTT-GA TTTCGGTTTC CTTGAAATTT TTTTGATTCG GTAATCTCCG AACAGAAGGA
```

Sequencing Results

8/9

```
E2        TTTTTTATTC TTTTTTT-GA TTTCGGTTTC CTTGAAATTT TTTTGATTCG GTAATCTCCG AACAGAAGGA
E3        TTTTTTATTC TTTTTTT-GA TTTCGGTTTC CTTGAAATTT TTTTGATTCG GTAATCTCCG AACAGAAGGA
C3        TTTTTTATTC TTTTTTT-GA TTTCGGTTTC CTTGAAATTT TTTTGATTCG GTAATCTCCG AACAGAAGGA
E1        TTTTTTATTC TTTTTTT-GA TTTCGGTTTC CTTGAAATTT TTTTGATTCG GTAATCTCCG AACAGAAGGA
E7
Consensus tttttattc tttttt.ga tttcggtttc cttgaaattt ttttgattcg gtaatctccg aacagaagga

          491                                                                      560
EXPECTED  AGAACGAAGG AAGGAGCACA GACTTAGATT GGTATATATA CGCATATGTA GTGTTGAAGA AACATGAAAT
C2        AGAACGAAGG AAGGAGCACA GACTTAGATT GGTATATATA CGCATATGTA GTGTTGAAGA AACATGAAAT
E2        AGAACGAAGG AAGGAGCACA GACTTAGATT GGTATATATA CGCATATGTA GTGTTGAAGA AACATGAAAT
E3        AGAACGAAGG AAGGAGCACA GACTTAGATT GGTATATATA CGCATATGTA GTGTTGAAGA AACATGAAAT
C3        AGAACGAAGG AAGGAGCACA GACTTAGATT GGTATATATA CGCATATGTA GTGTTGAAGA AACATGAAAT
E1        AGAACGAAGG AAGGAGCACA GACTTAGATT GGTATATATA CGCATATGTA GTGTTGAAGA AACATGAAAT
E7                                       T GGTATATATA CGCATATGTA GTGTTGAAGA AACATGAAAT
Consensus agaacgaagg aaggagcaca gacttagatT GGTATATATA CGCATATGTA GTGTTGAAGA AACATGAAAT

          561                                                                      630
EXPECTED  TGCCCAGTAT TCTTAACCCA ACTGCACAGA ACAAAAACAT GCAGGAAACG AAGATAAATC ATGTCGAAAG
C2        TGCCCAGTAT TCTTAACCCA ACTGCACAGA ACAAAAACAT GCAGGAAACG AAGATAAATC ATGTCGAAAG
E2        TGCCCAGTAT TCTTAACCCA ACTGCACAGA ACAAAAACAT GCAGGAAACG AAGATAAATC ATGTCGAAAG
E3        TGCCCAGTAT TCTTAACCCA ACTGCACAGA ACAAAAACAT GCAGGAAACG AAGATAAATC ATGTCGAAAG
C3        TGCCCAGTAT TCTTAACCCA ACTGCACAGA ACAAAAACAT GCAGGAAACG AAGATAAATC ATGTCGAAAG
E1        TGCCCAGTAT TCTTAACCCA ACTGCACAGA ACAAAAACAT GCAGGAAACG AAGATAAATC ATGTCGAAAG
E7        TGCCCAGTAT TCTTAACCCA ACTGCACAGA ACAAAAACAT GCAGGAAACG AAGATAAATC ATGTCGAAAG
Consensus TGCCCAGTAT TCTTAACCCA ACTGCACAGA ACAAAAACAT GCAGGAAACG AAGATAAATC ATGTCGAAAG
```

URA3 START

```
          631                                                                      700
EXPECTED  CTACATATAA GGAACGTGCT GCTACTCATC CTAGTCCTGT TGCTGCCAAG CTATTTAATA TCATGCACGA
C2        CTACATATAA GGAACGTGCT GCTACTCATC CTAGTCCTGT TGCTGCCAAG CTATTTAATA TCATGCACGA
E2        CTACATATAA GGAACGTGCT GCTACTCATC CTAGTCCTGT TGCTGCCAAG CTATTTAATA TCATGCACGA
E3        CTACATATAA GGAACGTGCT GCTACCCATC CTAGTCCTGT TGCTGCCAAG CTATTTAATA TCATGCACGA
C3        CTACATATAA GGAACGTGCT GCTACTCATC CTAGTCCTGT TGCTGCCAAG CTATTTAATA TCATGCACGA
E1        CTACATATAA GGAACGTGCT GCTACTCATC CTAGTCCTGT TGCTGCCAAG CTATTTAATA TCATGCACGA
E7        CTACATATAA GGAACGTGCT GCTACTCATC CTAGTCCTGT TGCTGCCAAG CTATTTAATA TCATGCACGA
Consensus CTACATATAA GGAACGTGCT GCTACtCATC CTAGTCCTGT TGCTGCCAAG CTATTTAATA TCATGCACGA

          701                                                                      770
EXPECTED  AAAGCAAACA AACTTGTGTG CTTCATTGGA TGTTCGTACC ACCAAGGAAT TACTGGAGTT AGTTGAAGCA
C2        AAAGCAAACA AACTTGTGTG CTTCATTGGA TGTTCGTACC ACCAAGGAAT TACTGGAGTT AGTTGAAGCA
E2        AAAGCAAACA AACTTGTGTG CTTCATTGGA TGTTCGTACC ACCAAGGAAT TACTGGAGTT AGTTGAAGCA
E3        AAAGCAAACA AACTTGTGTG CTTCATTGGA TGTTCGTACC ACCAAGGAAT TACTGGAGTT AGTTGAAGCA
C3        AAAGCAAACA AACTTGTGTG CTTCATTGGA TGTTCGTACC ACCAAGGAAT TACTGGAGTT AGTTGAAGCA
E1        AAAGCAAACA AACTTGTGTG CTTCATTGGA TGTTCGTACC ACCAAGGAAT TACTGGAGTT AGTTGAAGCA
E7        AAAGCAAACA AACTTGTGTG CTTCATTGGA TGTTCGTACC ACCAAGGAAT TACTGGAGTT AGTTGAAGCA
Consensus AAAGCAAACA AACTTGTGTG CTTCATTGGA TGTTCGTACC ACCAAGGAAT TACTGGAGTT AGTTGAAGCA

          771                                                                      840
EXPECTED  TTAGGTCCCA AAATTTGTTT ACTAAAAACA CATGTGGATA TCTTGACTGA TTTTTCCATG GAGGGCACAG
C2        TTAGGTCCCA AAATTTGTTT ACTAAAAACA CATGTGGATA TCTTGACTGA TTTTTCCATG GAGGGCACAG
E2        TTAGGTCCCA AAATTTGTTT ACTAAAAACA CGTGTGGATA TCTTGACTGA TTTTTCCATG GAGGGCACAG
E3        TTAGGTCCCA AAATTTGTTT ACTAAAAACA CATGTGGATA TCTTGACTGA TTTTTCCATG GAGGGCACAG
C3        TTAGGTCCCA AAATTTGTTT ACTAAAAACA CATGTGGATA TCTTGACTGA TTTTTCCATG GAGGGCACAG
E1        TTAGGTCCCA AAATTTGTTT ACTAAAAACA CATGTGGATA TCTTGACTGA TTTTTCCATG GAGGGCACAG
E7        TTAGGTCCCA AAATTTGTTT ACTAAAAACA CATGTGGATA TCTTGACTGA TTTTTCCATG GAGGGCACAG
Consensus TTAGGTCCCA AAATTTGTTT ACTAAAAACA CaTGTGGATA TCTTGACTGA TTTTTCCATG GAGGGCACAG

          841                                                                      910
EXPECTED  TTAAGCCGCT AAAGGCATTA TCCGCCAAGT ACAATTTTTT ACTCTTCGAA GACAGAAAAT TTGCTGACAT
C2        TTAAGCCGCT AAAGGCATTA TCCGCCAAGT ACAATTTTTT ACTCTTCGAA GACAGAAAAT TTGCTGACAT
E2        TTAAGCCGCT AAAGGCATTA TCCGCCAAGT ACAATTTTTT ACTCTTCGAA GACAGAAAAT TTGCTGACAT
E3        TTAAGCCGCT AAAGGCATTA TCCGCCAAGT ACAATTTTTT ACTCTTCGAA GACAGAAAAT TTGCTGACAT
C3        TTAAGCCGCT AAAGGCATTA TCCGCCAAGT ACAATTTTTT ACTCTTCGAA GACAGAAAAT TTGCTGACAT
```

```
E1        TTAAGCCGCT AAAGGCATTA TCCGCCAAGT ACAATTTTTT ACTCTTCGAA GACAGAAAAT TTGCTGACAT
E7        TTAAGCCGCT AAAGGCATTA TCCGCCAAGT ACAATTTTTT ACTCTTCGAA GACAGAAAAT TTGCTGACAT
Consensus TTAAGCCGCT AAAGGCATTA TCCGCCAAGT ACAATTTTTT ACTCTTCGAA GACAGAAAAT TTGCTGACAT

          911                                                                      980
EXPECTED  TGGTAATACA GTCAAATTGC AGTACTCTGC GGGTGTATAC AGAATAGCAG AATGGGCAGA CATTACGAAT
C2        TGGTAATACA GTCAAATTGC AGTACTCTGC GGGTGTATAC AGAATAGCAG AATGGGCAGA CATTACGAAT
E2        TGGTAATACA GTCAAATTGC AGTACTCTGC GGGTGTATAC AGAATAGCAG AATGGGCAGA CATTACGAAT
E3        TGGTAATACA GTCAAATTGC AGTACTCTGC GGGTGTATAC AGAATAGCAG AATGGGCAGA CATTACGAAT
C3        TGGTAATACA GTCAAATTGC AGTACTCTGC GGGTGTATAC AGAATAGCAG AATGGGCAGA CATTACGAAT
E1        TGGTAATACA GTCAAATTGC AGTACTCTGC GGGTGTATAC AGAATAGCAG AATGGGCAGA CATTACGAAT
E7        TGGTAATACA GTCAAATTGC AGTACTCTGC GGGTGTATAC AGAATAGCAG AATGGGCAGA CATTACGAAT
Consensus TGGTAATACA GTCAAATTGC AGTACTCTGC GGGTGTATAC AGAATAGCAG AATGGGCAGA CATTACGAAT

          981                                                                     1050
EXPECTED  GCACACGGTG TGGTGGGCCC AGGTATTGTT AGCGGTTTGA AGCAGGCGGC GGAAGAAGTA ACAAAGGAAC
C2        GCACACGGTG TGGTGGGCCC AGGTATTGTT AGCGGTTTGA AGCAGGCGGC GGAAGAAGTA ACAAAGGAAC
E2        GCACACGGTG TGGTGGGCCC AGGTATTGTT AGCGGTTTGA AGCAGGCGGC GGAAGAAGTA ACAAAGGAAC
E3        GCACACGGTG TGGTGGGCCC AGGTATTGTT AGCGGTTTGA AGCAGGCGGC GGAAGAAGTA ACAAAGGAAC
C3        GCACACGGTG TGGTGGGCCC AGGTATTGTT AGCGGTTTGA AGCAGGCGGC GGAAGAAGTA ACAAAGGAAC
E1        GCACACGGTG TGGTGGGCCC AGGTATTGTT AGCGGTTTGA AGCAGGCGGC GGAAGAAGTA ACAAAGGAAC
E7        GCACACGGTG TGGTGGGCCC AGGTATTGTT AGCGGTTTGA AGCAGGCGGC GGAAGAAGTA ACAAAGGAAC
Consensus GCACACGGTG TGGTGGGCCC AGGTATTGTT AGCGGTTTGA AGCAGGCGGC GGAAGAAGTA ACAAAGGAAC

          1051                                                                    1120
EXPECTED  CTAGAGGCCT TTTGATGTTA GCAGAATTGT CATGCAAGGG CTCCCTATCT ACTGGAGAAT ATACTAAGGG
C2        CTAGAGGCCT TTTGATGTTA GCAGAATTGT CATGCAAGGG CTCCCTATCT ACTGGAGAAT ATACTAAGGG
E2        CTAGAGGCCT TTTGATGTTA GCAGAATTGT CATGCAAGGG CTCCCTATCT ACTGGAGAAT ATACTAAGGG
E3        CTAGAGGCCT TTTGATGTTA GCAGAATTGT CATGCAAGGG CTCCCTATCT ACTGGAGAAT ATACTAAGGG
C3        CTAGAGGCCT TTTGATGTTA GCAGAATTGT CATGCAAGGG CTCCCTATCT ACTGGAGAAT ATACTAAGGG
E1        CTAGAGGCCT TTTGATGTTA GCAGAATTGT CATGCAAGGG CTCCCTATCT ACTGGAGAAT ATACTAAGGG
E7        CTAGAGGCCT TTTGATGTTA GCAGAATTGT CATGCAAGGG CTCCCTATCT ACTGGAGAAT ATACTAAGGG
Consensus CTAGAGGCCT TTTGATGTTA GCAGAATTGT CATGCAAGGG CTCCCTATCT ACTGGAGAAT ATACTAAGGG

          1121                                                                    1190
EXPECTED  TACTGTTGAC ATTGCGAAGA GCGACAAAGA TTTTGTTATC GGCTTTATTG CTCAAAGAGA CATGGGTGGA
C2        TACTGTTGAC ATTGCGAAGA GCGACAAAGA TTTTGTTATC GGCTTTATTG CTCAAAGAGA CATGGGTGGA
E2        TACTGTTGAC ATTGCGAAGA GCGACAAAGA TTTTGTTATC GGCTTTATTG CTCAAAGAGA TATGGGTGGA
E3        TACTGTTGAC ATTGCGAAGA GCGACAAAGA TTTTGTTATC GGCTTTATTG CTCAAAGAGA CATGGGTGGA
C3        TACTGTTGAC ATTGCGAAGA GCGACAAAGA TTTTGTTATC GGCTTTATTG CTCAAAGAGA CATGGGTGGA
E1        TACTGTTGAC ATTGCGAAGA GCGACAAAGA TTTTGTTATC GGCTTTATTG CTCAAAGAGA CATGGGTGGA
E7        TACTGTTGAC ATTGCGAAGA GCGACAAAGA TTTTGTTATC GGCTTTATTG CTCAAAGAGA CATGGGTGGA
Consensus TACTGTTGAC ATTGCGAAGA GCGACAAAGA TTTTGTTATC GGCTTTATTG CTCAAAGAGA cATGGGTGGA

          1191                                                                    1260
EXPECTED  AGAGATGAAG GTTACGATTG GTTGATTATG ACACCCGGTG TGGGTTTAGA TGACAAGGGA GACGCATTGG
C2        AGAGATGAAG GTTACGATTG GTTGATTATG ACACCCGGTG TGGGTTTAGA TGACAAGGGA GACGCATTGG
E2        AGAGATGAAG GTTACGATTG GTTGATTATG ACACCCGGTG TGGGTTTAGA TGACAAGGGA GACGCATTGG
E3        AGAGATGAAG GTTACGATTG GTTGATTATG ACACCCGGTG TGGGTTTAGA TGACAAGGGG GACGCATTGG
C3        AGAGATGAAG GTTACGATTG GTTGATTATG ACACCCGGTG TGGGTTTAGA TGACAAGGGA GACGCATTGG
E1        AGAGATGAAG GTTACGATTG GTTGATTATG ACACCCGGTG TGGGTTTAGA TGACAAGGGA GACGCATTGG
E7        AGAGATGAAG GTTACGATTG GTTGATTATG ACACCCGGTG TGGGTTTAGA TGACAAGGGA GACGCATTGG
Consensus AGAGATGAAG GTTACGATTG GTTGATTATG ACACCCGGTG TGGGTTTAGA TGACAAGGGa GACGCATTGG

          1261                                                                    1330
EXPECTED  GTCAACAGTA TAGAACCGTG GATGATGTGG TCTCTACAGG ATCTGACATT ATTATTGTTG GAAGAGGACT
C2        GTCAACAGTA TAGAACCGTG GATGATGTGG TCTCTACAGG ATCTGACATT ATTATTGTTG GAAGAGGACT
E2        GTCAACAGTA TAGAACCGTG GATGATGTGG TCTCTACAGG ATCTGACATT ATTATTGTTG GAAGAGGACT
E3        GTCAACAGTA TAGAACCGTG GATGATGTGG TCTCTACAGG ATCTGACATT ATTATTGTTG GAAGAGGACT
C3        GTCAACAGTA TAGAACCGTG GATGATGTGG TCTCTACAGG ATCTGACATT ATTATTGTTG GAAGAGGACT
E1        GTCAACAGTA TAGAACCGTG GATGATGTGG TCTCTACAGG ATCTGACATT ATTATTGTTG GAAGAGGACT
E7        GTCAACAGTA TAGAACCGTG GATGATGTGG TCTCTACAGG ATCTGACATT ATTATTGTTG GAAGAGGACT
Consensus GTCAACAGTA TAGAACCGTG GATGATGTGG TCTCTACAGG ATCTGACATT ATTATTGTTG GAAGAGGACT
```

```
          1331                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
EXPECTED  ATTTGCAAAG GGAAGGGATG CTAAGGTAGA GGGTGAACGT TACAGAAAAG CAGGCTGGGA AGCATATTTG
C2        ATTTGCAAAG GGAAGGGATG CTAAGGTAGA GGGTGAACGT TACAGAAAAG CAGGCTGGGA AGCATATTTG
E2        ATTTGCAAAG GGAAGGGATG CTAAGGTAGA GGGTGAACGT TACAGAAAAG CAGGCTGGGA AGCATATTTG
E3        ATTTGCAAAG GGAAGGGATG CTAAGGTAGA GGGTGAACGT TACAGAAAAG CAGGCTGGGA AGCATATTTG
C3        ATTTGCAAAG GGAAGGGATG CTAAGGTAGA GGGTGAACGT TACAGAAAAG CAGGCTGGGA AGCATATTTG
E1        ATTTGCAAAG GGAAGGGATG CTAAGGTAGA GGGTGAACGT TACAGAAAAG CAGGCTGGGA AGCATATTTG
E7        ATTTGCAAAG GGAAGGGATG CTAAGGTAGA GGGTGAACGT TACAGAAAAG CAGGCTGGGA AGCATATTTG
Consensus ATTTGCAAAG GGAAGGGATG CTAAGGTAGA GGGTGAACGT TACAGAAAAG CAGGCTGGGA AGCATATTTG

          1401                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
EXPECTED  AGAAGATGCG GCCAGCAAAA CTAAAAAACT GTATTATAAG TAAATGCATG TATACTAAAC TCACAAATTA
C2        AGAAGATGCG GCCAGCAAAA CTAAAAAACT GTATTATAAG TAAATGCATG TATACTAAAC TCACAAATTA
E2        AGAAGATGCG GCCAGCAAAA CTAAAAAACT GTATTATAAG TAAATGCATG TATACTAAAC TCACAAATTA
E3        AGAAGATGCG GCCAGCAAAA CTAAAAAACT GTATTATAAG TAAATGCATG TATACTAAAC TCACAAATTA
C3        AGAAGATGCG GCCAGCAAAA CTAAAAAACT GTATTATAAG TAAATGCATG TATACTAAAC TCACAAATTA
E1        AGAAGATGCG GCCAGCAAAA CTAAAAAACT GTATTATAAG TAAATGCATG TATACTAAAC TCACAAATTA
E7        AGAAGATGCG GCCAGCAAAA CTAAAAAACT GTATTATAAG TAAATGCATG TATACTAAAC TCACAAATTA
Consensus AGAAGATGCG GCCAGCAAAA CTAAAAAACT GTATTATAAG TAAATGCATG TATACTAAAC TCACAAATTA
                                URA3 STOP
          1471                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
EXPECTED  GAGCTTCAAT TTAATTATAT CAGTTATTAC CCGGGAATCT CGGTCGTAAT GATTTCTATA ATGAGATATG
C2        GAGCTTCAAT TTAATTATAT CAGTTATTAC CCGGGAATCT CGGTCGTAAT GATTTCTATA ATGAGATATG
E2        GAGCTTCAAT TTAATTATAT CAGTTATTAC CCGGGAATCT CGGTCGTAAT GATTTCTATA ATGAGATATG
E3        GAGCTTCAAT TTAATTATAT CAGTTATTAC CCGGGAATCT CGGTCGTAAT GATTTCTATA ATGAGATATG
C3        GAGCTTCAAT TTAATTATAT CAGTTATTAC CCGGGAATCT CGGTCGTAAT GATTTCTATA ATGAGATATG
E1        GAGCTTCAAT TTAATTATAT CAGTTATTAC CCGGGAATCT CGGTCGTAAT GATTTCTATA ATGAGATATG
E7        GAGCTTCAAT TTAATTATAT CAGTTATTAC CCGGGAATCT CGGTCGTAAT GATTTCTATA ATGAGATATG
Consensus GAGCTTCAAT TTAATTATAT CAGTTATTAC CCGGGAATCT CGGTCGTAAT GATTTCTATA ATGAGATATG

          1541                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
EXPECTED  AATAAATTGC AGTTTCATTT GATGCTCGAT GAGTTTTTCT AATCAGTACT GACAATAAAA AGATTCTTGT
C2        AATAAATTGC AGTTTCATTT GATGCTCGAT GAGTTTTTCT AATCAGTACT GACAATAAAA AGATTCTTGT
E2        AATAAATTGC AGTTTCATTT GATGCTCGAT GAGTTTTTCT AATCAGTACT GACAATAAAA AGATTCTTGT
E3        AATAAATTGC AGTTTCATTT GATGCTCGAT GAGTTTTTCT AATCAGTACT GACAATAAAA AGATTCTTGT
C3        AATAAATTGC AGTTTCATTT GATGCTCGAT GAGTTTTTCT AATCAGTACT GACAATAAAA AGATTCTTGT
E1        AATAAATTGC AGTTTCATTT GATG
E7        AATAAATTGC AGTTTCATTT GATGCTCGAT GAGTTTTTCT AATCAGTACT GACAATAAAA AGATTCTTGT
Consensus AATAAATTGC AGTTTCATTT GATGctcgat gagtttttct aatcagtact gacaataaaa agattcttgt

          1611                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
EXPECTED  TTTCAAGAAC TTGTCATTTG TATAGTTTTT TTATATTGTA GTTGTTCTAT TTTAATCAAA TGTTAGCGTG
C2        TTTCAAGAAC TTGTCATTTG TATAGTTTTT TTATATTGTA GTTGTTCTAT TTTAATCAAA TGTTAGCGTG
E2        TTTCAAGAAC TTGTCATTTG TATAGTTTTT TTATATTGTA GTTGTTCTAT TTTAATCAAA TGTTAGCGTG
E3        TTTCAAGAAC TTGTCATTTG TATAGTTTTT TTATATTG
C3        TTTCAAGAAC TTGTCATTTG TATAGTTTTT TTATATTGTA GTTGTTCTAT TTTAATCAAA TGTTAGCGTG
E1
E7        TTTCAAGAAC TTGTCATTTG TATAGTTTTT T
Consensus tttcaagaac ttgtcatttg tatagttttt ttatattgta gttgttctat tttaatcaaa tgttagcgtg

          1681                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
EXPECTED  ATTTATATTT TTTTTCGCCT CGACATCATC TGCCCAGATG CGAAGTTAAG TGCGCAGAAA GTAATATCAT
C2        ATTTATATTT TTTTTCGCCT CGACATCATC TGCCCAGATG CGAAGTTAAG TGCGCAGAAA GTAATATCAT
E2        ATTTATATTT TTTTT
E3
C3        ATTTATATTT TTTTTCGCCT CGACATCATC TGCCCAGATG CGAAGTTAAG TGCGCAGAA
E1
E7
Consensus atttatattt ttttt..... .......... .......... .......... .......... ..........
```

8/10

SEQUENCING NOTES

- Yeast transformant #2 — which is the parent of all of the plasmids — has a -1 frameshift in the "unsequencable" poly-T tract.
  - I have never been able to sequence across this region, so I don't know wether this mutation exists in WT W303 URA3 or was introduced by PCR.
  - I ~~ten~~ now know that the difficulty in sequencing across this region is from PCR, not sequencing
- I tried to sequence using primers U1 & D1, but these sites are not present in the plasmid, so I only got good runs from the internal primers.
- C2, C3, & E1 contain no mutations
  - Out of the 3 good plasmids, I got the longest read from C2, so I will use this plasmid for my yeast transformations.

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 692 10VG

8/10

PREPARATION OF PLASMID FOR YEAST TRANSFORMATION

· Last night I grew up ~~150~~ 125 mL of 2×YT+AMP with the bacterial transformant C2.

Maxi-Prep plasmid → resuspend in 1 mL EB (Tris-Cl pH 8.5)

According to the kit protocol, the Maxi-column saturates with 500 μg of plasmid → ∴ plas. conc. ~ .5 μg/μl

RE digest

70 λ	H_2O
800 λ	Plasmid
100 λ	10×EcoRI Buffer
10 λ	100×BSA
10 λ	EcoRI
10 λ	BamHI
1000 λ	

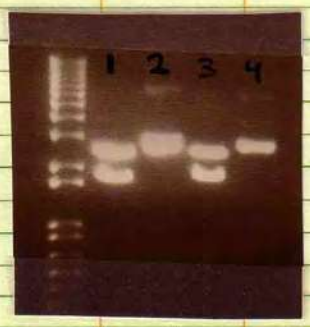
After 2.5 h

Lane

1) this digest after 2.5 h
2) this uncut plasmid
3) Miniprep plasmid C2 double digest (p22)
4) maxiprep plasmid C2 uncut

8/11

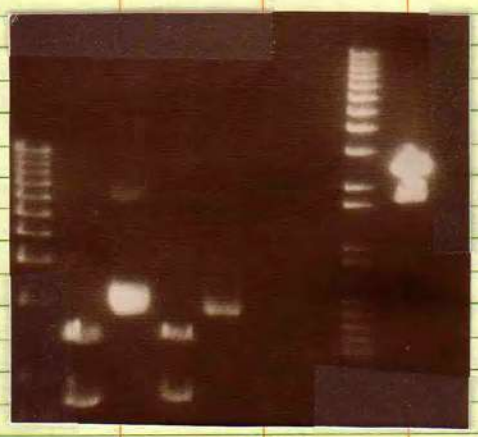
After 19.5 h – lanes the same as above



8/11

PREPARATION OF PLASMID FOR TRANS (CONT.)

- Phenol/Chloroform ~~ppt~~ extract digested DNA from digest
  ↳ 1 mL P/C per 1 mL digest
  remove ~ 800 λ
- add 100 λ 3M NaOAc
- add 1 mL isopropanol. incubate RT for ~3h
- spin 10 min 4° 14000
- wash with 70% EtOH
- spin 10 min. 4° 14000
- decant. → speed vac 10 min
- resuspend in 500 λ EB (Tris-Cl pH 8.5)



same as p. 30

after P/C EtOH ppt

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 692 10VG

8/10

TRANSFORMATION OF YEAST STRAINS

- Inoculate 12.5 mL YPD+ADE tubes with each of the 49 strains (p 1 & 5).
  - ~~Fo~~ Strains on YPD+G418 plates in fridge
  - ~~W~~ For most strains I picked a single colony. Ones where the colonies were too close together, I picked from the corner of a streak
  - For #27(LOC1), I picked a streak of cells since I know that this strain has a severe fitness defect.

8/11

- Strains were put in rollerdrum ~8:30pm last night.

- @ 9:30am

STRAIN	OD_{600}
13	0.82
27	0.35

- Dilute all strains ~1:20 (0.5mL into 10mL YPD+ADE)
  - Except: 15, 26, & 35 – dilute 1:10 (1 mL into 10)
  - 16, 27 – do not dilute at all

} 12:00

- @ 1:45

STRAIN	OD_{600}		
3	0.36		
13	0.27		
16	0.61	dilute 1:2 →	0.33
27	0.59	dilute 1:2 →	0.30
26	~~0.23~~ 0.30		
15	0.23		
~~13~~ 35	0.27		

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 692 10VG

@ 3:45

STRAIN	OD_{600}
10	0.73
24	0.66
31	0.69
42	0.70

start transformations — 4:15
finish ~ 11:00

2000 λ LiOAC mix
500 λ ssssDNA
500 λ plasmid
3000 λ

Bodo's Lithium Acetate Transformation

LiOAc mix

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

PEG mix

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

~~25 mL culture~~

1) Grow culture to OD 0.5-0.7 in YPD. (0.7) (~~not stationary~~)
→ 5 ~ for winner
2) Spin, transfer to Eppendorf tube and wash ~~twice~~ 3x with sterile water cold Spin 10 mL
↳ ~~setting 5 on centrifuge (in 50 mL falcon tube)~~ 5min @ ~~20,000~~ 2,000
3) Wash twice in 1 ml LiAc mix.
~~+20~~ ~~90λ~~ ~~40λ~~ 10 mL ~~20~~ 10λ
4) Resuspend in ~~360~~μl LiAc mix per ~~50~~ ml culture and ~~40~~μl carrier DNA (0°C).
~~30~~ 50λ
5) use 10 μl cells/plasmid transformation and ~~50~~μl for integrative transformation.
, sometimes preferable
6) Add thawed cells to DNA (maximally 2μl DNA per 10μl competent cells) and mix.
~~1λ per 10λ above~~ ~~(3λ for 30λ)~~ 10λ DNA + 50λ Cells
7) Add sixfold volume of 40% PEG, mix and incubate 30', ~~30°C (room temp if ts)~~. @ room temp
360λ
8) Add DMSO to final concentration of 10%. → helps cells survive temperature shock.
↳ not necessary 47 λ DMSO
9) Heat shock 15', ~~42°C~~ 37°C ~~if ts~~.
10) Sediment cells for 2-3 min at 2000rpm in Eppendorf centrifuge.
11) Resuspend in 200 μl YPD and plate out on selective medium.

Stock Sol'ns

45% PEG
1 M Tris (pH 7.5)
.5 M EDTA
1 M LiOAc

PEG mix: 100ml:
10ml 1M LiOAc
1ml 1M ~~Tris~~ pH 7.5
0.2ml 0.5M EDTA
88.8ml 45% PEG
+3.9 mL DMSO
to 30 ML PEG mix

Bodo's Lithium Acetate Transformation

LiOAc mix
100mM LiOAc
10mM Tris pH7.5
1mM EDTA

PEG mix
40%PEG
100mM LiOAc
10mM Tris pH 7.5
1mM EDTA

1) Grow culture to OD 0.5-0.7 in YPD.
2) Spin, transfer to Eppendorf tube and wash once with sterile water.
3) Washtwice in 1 ml LiAc mix.
4) Resuspend in 30ml LiAc mix per 50ml culture and 40ul carrier DNA (0°C).
5) use 10 ul cells/lasmid transformation and 50ul for integrative transformation.
6) Add thawed cells to DNA (maximally 2ul DNA per 10ul competent cells) and mix.
7) Add sixfold volume of 40% PEG, mix and incubate 30' 30°C (room temp ok).
8) Add DMSO to final concentration of 10%.
9) Heat shock 15' 42°C (37°C ok).
10) Sediment cells for 2-3 min at 2000rpm in Eppendorf centrifuge.
11) Resuspend in 200 ul YPD and plate out on selective medium.

Stock solus
45% PEG
1 M Tris (pH 7.5)
.5 M EDTA
1 M LiOAc

PEG mix: 100ml
10ml 1M LiOAc
1ml 1M Tris pH 7.5
0.2ml 0.5M EDTA
88.8ml 45% PEG

+3.9 ml DMSO
to 30 ml PEG mix

8/15

NOTES ON TRANSFORMATIONS:

- It looks like my transformation efficiency was very good; however, in some cases my plating efficiency was poor.
  - Next time I will use 3-5 mL cultures instead of 10 mL.
- For 33 of the transformants, I will ~~inoc~~ innoculate two tubes 3 mL of 2xSC-URA with ~~duplicate~~ colonies from the -URA transformation plate.
- For 16 of the transformants, ~~I will~~ – which had poor plating efficiency – I will take a streak from the plate and restreak twice onto 2xSC-ura.

  #'s 1, 2, 3, 10, 11, 13, 18, 19, 20, 21, 37, 38, 39, 40, 44, 45

⇒ Actually I will wait until tomorrow when I have enough plates.

- leave plates on benchtop

8/16 VERIFICATION OF TRANSFORMANTS

- For the 33 transformants (see above) streak duplicate URA+ colonies onto -ura
- For the 16 transformation plates (see above) streak a streak from the plate onto -ura

8/18

Pick single colony from each plate (labeled 1A, 2A, ..., 49A) and inoculate 2 mL YPD+ADE

8/19

gDNA preps on all 49 strains
- Strain #27 was given ~ an extra ~3h in vollerdrum.
- All strains ethanol ppt o/N

8/20

spin gDNA preps 15min 14000 4°. Aspirate EtOH. speed Vac resus in 50λ TE.

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 692 10VG

8/19

using the same cultures as I did for the gDNA preps, streak 5 λ of each culture onto YPD+2xG418. As ~~a~~ controls I included transformant #2 (pgs 13-19, also called GL-0) and YCaIΔ from Δ collection.

8/20

All strains appear to be $G418^S$ but (2-3) ~~except:~~

~~#1 -~~

• NOTE: All strains show a number of $G418^R$ colonies within the streak — even though this is 2xG418

Possible $G418^R$ strains

#1 shows a weak $G418^R$ phenotype — possibly a jackpot of $G418^R$ within culture.

#16 & #27 show a $G418^R$ phenotype — interestingly these are my reduced fitness lines

On 8/16 I restreaked 2 colonies from the transformation plates of #16 & #27. I will go back and pick a single colony from the other ~~strain~~ streak of both of these strains. I will ~~call~~ call these #16B and #27B — YPD+ADE liquid cultures.

~~8/21~~ 8/22

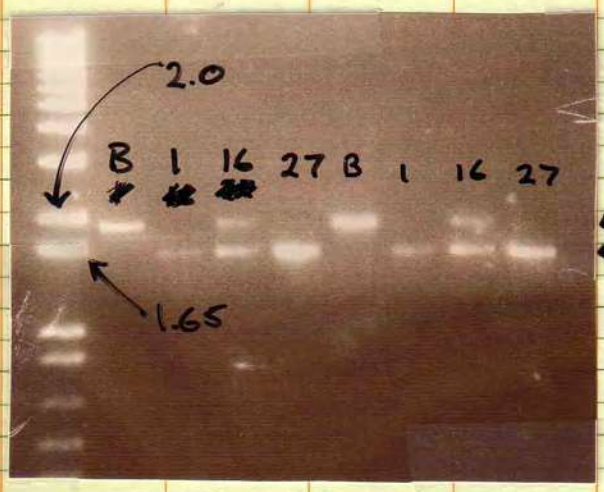
Last night I ran a PCR ~~using~~ on all 49 ~~strn~~ gDNA preps using primers URA3wl & URA3dl (p13).

I used a old program: differences from p13: 5 min hotstart &
2:00 extention &
52° annealing

I am not sure why I didn't just use the program on p13.

· PCRs didn't work:

· Redo PCRs on the questionable strains (1, 16, 17) (p 38) and transformant # 2 (pgs 13-17, labeled "B" below) using program on p13, new Taq and 2 diff Taq buffers.



B: positive control

CONCLUSIONS:

1 & 27 : incorrect integration

16 : multiple KanMX6 copies (amplified?)

see p7

PICK MORE TRANSFORMANTS TO SCREEN

Given the result above I will ~~do~~ need to screen more transformants.

· Pick ~~another~~ single colony from the restreaked #1 plate (opposite side of A) Label this 1B – YPD+ADE

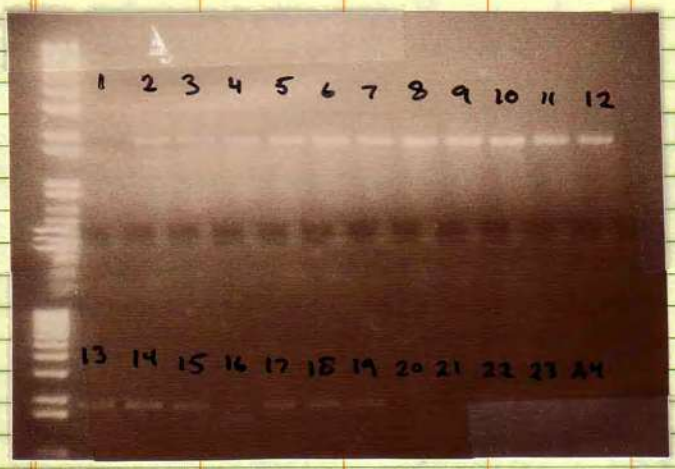
· Inoculate 3 mL of YPD+ADE with 10 λ of the cultures of #16B & 27B which I started on 8/20 (p38)

· Go back to transformation plate and restreak 6 more colonies from each 1, 16, & 27 onto -ura.

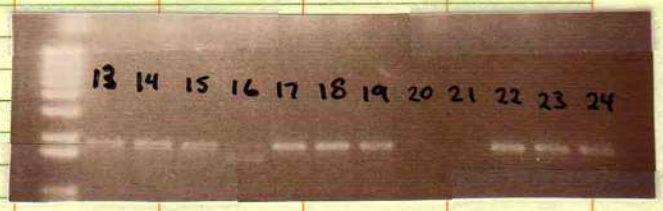
~~8/21~~ 8/22

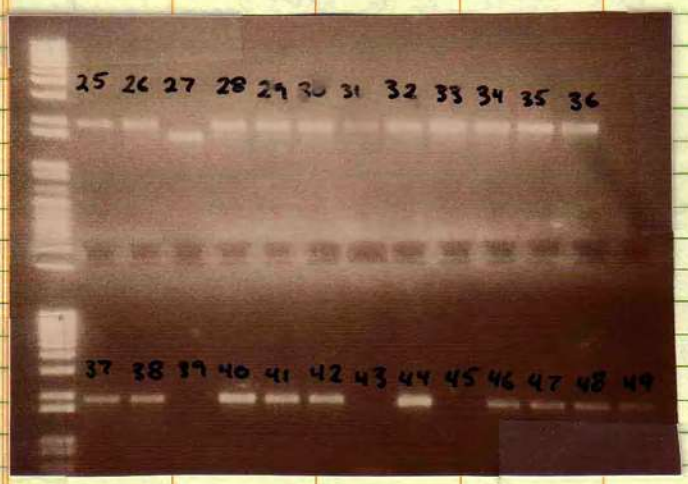
Redo PCRs - all 49.

~~8/22~~ 8/23



brighter





NOTES:

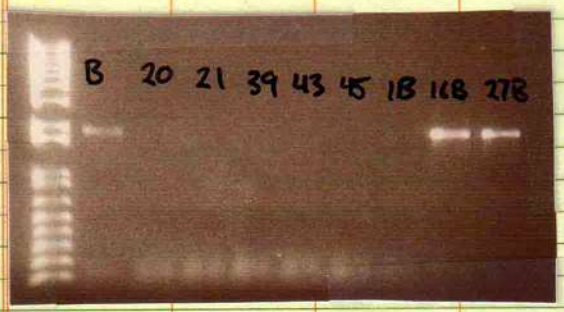
- 41 correct ~~of~~ URA3 transformants
- 6 - no product (including #1, which I know is wrong, p 59)
- 2 incorrect (#27 & 16)

8/23

I did gDNA preps on 1B, 16B, and 27B (p39). I was more careful w/ these – I added a 70% EtOH wash after the 95% ppt.

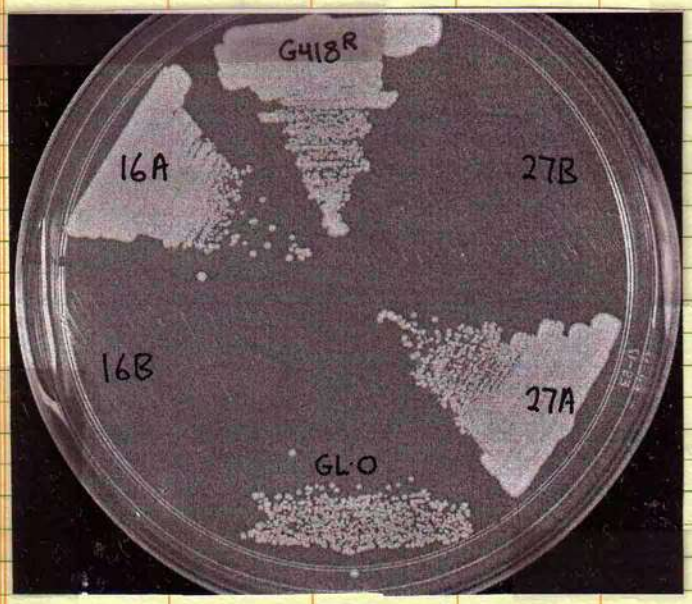
Rerun PCRs that failed earlier: 20, 21, 39, 43, 45. & In addition run PCRs for new gDNA preps.

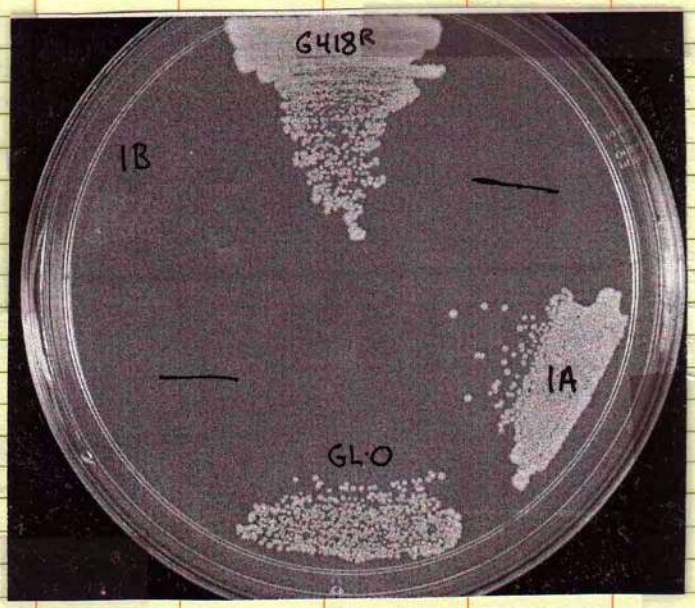
PCR – same as before ~~(p13)~~ (p13)



- Perhaps 16A (just labeled "16" on p39) is not annuplord like I thought, but a gDNA prep made from a mixed colony.
- It appears that 16B & 27B are correct.
- Streak ~~1B~~ 1A, 1B, 16A, 16B, 27A, & 27B on YPD+2xG418

8/25





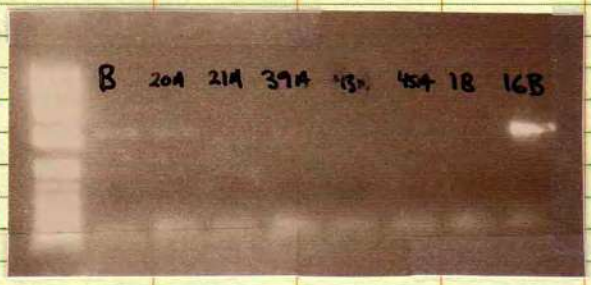
LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 692 10VG

8/25

Yesterday I made new gDNA preps for 20, 21, 39, 43, 45. I was more careful to get all of the phenol out by chloroform extracting afterwards.

I ran the PCR but nothing worked — even the positive control.

Overnight I reran the PCR ~~with~~ with 2 pos. controls — "B" and 16B.



It is faint but it appears that 20A has the correct band

In review:

~~[illegible]~~

- 44 correctly verified by PCR
- 5 cannot get a PCR product (tried multiple gDNA preps)
- all 49 are phenotypically correct (URA+, G418^S)

Since I am going away this weekend I put all of the correct PCRs in the −20° to purified and sequenced next week. I put all of the YPD cultures in my fridge, and all of the transformation/streaked plates in the coldroom.

8/31

Before I left I PCR purified GL-26 and submitted it for sequencing using primers:

A – F (see p 14),

~~G = KanInt~~

sequences Uptag

sequences downtag

G:

17791355
V.INDJEIAN
19057257 8/19/2005
KanMX6intR
5 AAT TCA ACG CGT CTG TGA GG -3
Tm= 55.6 °C , MW= 6,157.0
4.7OD$_{260}$ 24.03 nmol 0.15 mg

and

H:

17791356
V.INDJEIAN
19057258 8/19/2005
KanMX6intF
5 CAG TTT CAT TTG ATG CTC GAT G -3
Tm= 52.5 °C , MW= 6,731.4
5.4OD$_{260}$ 26.28 nmol 0.18 mg

Section 1

```
                    1         10        20        30        40        50        60        72
« glang_20050824...  (1)                   TAGCCAG-AGCACACGCAGCGTACGCTGCAGGTCGACGGATCCCCGGGTTA
» glang_20050824...  (1)                                                                      A
« glang_20050824...  (1)
» glang_20050824...  (1)
« glang_20050824...  (1)
» glang_20050824...  (1)
« glang_20050824...  (1)
» glang_20050824...  (1)
» KANMX_URA3_...     (1) GATGTCCACGAGGTCTCTTAGTAGT-AGTAGTAGTAGTA-CGTACGCTGCAGGTCGACGGATCCCCGGGTTA
        Contig 1     (1) GATGTCCACGAGGTCTCTTAGTAGYCAGTAGYASWMGYAGCGTACGCTGCAGGTCGACGGATCCCCGGGTTA
                                                 ++   +   + +++ + +
```

uptag

Section 2

```
                   73        80        90       100       110       120       130       144
« glang_20050824...  (51) ATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAACGACATGGAGGCCCA
» glang_20050824...  (1) ATNNAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAACGACATGGAGGCCCA
« glang_20050824...  (1)
» glang_20050824...  (1)
« glang_20050824...  (1)
» glang_20050824...  (1)
« glang_20050824...  (1)
» glang_20050824...  (1)
» KANMX_URA3_...     (72) ATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGACATGGAGGCCCA
        Contig 1     (72) ATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAACGACATGGAGGCCCA
                              . .                                                      +
```

Section 3

```
                  145       150       160       170       180       190       200       216
« glang_20050824...  (123) GAATACCCTCCTTGACAGTCTTGACGTGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGTACATTTAGCC
» glang_20050824...  (73) GAATACCCTCCTTGACAGTCTTGACGTGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGTACATTTAGCC
« glang_20050824...  (1)
» glang_20050824...  (1)
« glang_20050824...  (1)
» glang_20050824...  (1)
« glang_20050824...  (1)
» glang_20050824...  (1)
» KANMX_URA3_...     (144) GAATACCCTCCTTGACAGTCTTGACGTGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGTACATTTAGCC
        Contig 1     (144) GAATACCCTCCTTGACAGTCTTGACGTGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGTACATTTAGCC
```

Sequence of GL26

Section 4

```
                  217       230       240       250       260       270       288
« glang_20050824...  (195) CATACATCCCCATGTATAATCATTTGCATCCATACATTTTGANGGCCGCACGGCGCGAAGCAAAAATTACGG
» glang_20050824...  (145) CATACATCCCCATGTATAATCATTTGCATCCATACATTTTGATGGCCGCACGGCGCGAAGCAAAAATTACGG
« glang_20050824...  (1)
» glang_20050824...  (1)
« glang_20050824...  (1)
» glang_20050824...  (1)
« glang_20050824...  (1)
» glang_20050824...  (1)
» KANMX_URA3_...     (216) CATACATCCCCATGTATAATCATTTGCATCCATACATTTTGATGGCCGCACGGCGCGAAGCAAAAATTACGG
        Contig 1     (216) CATACATCCCCATGTATAATCATTTGCATCCATACATTTTGATGGCCGCACGGCGCGAAGCAAAAATTACGG
                                                                     .
```

Section 5

```
                  289       300       310       320       330       340       350       360
« glang_20050824...  (267) NNCC
» glang_20050824...  (217) CTCCTCGCTGCAGACCTGCGAGCAGGGAAACGCTCCCCTCACAGACGCGTTGAATTGTCCCCACGCCGCGCA
« glang_20050824...  (1)
» glang_20050824...  (1)
« glang_20050824...  (1)
» glang_20050824...  (1)
« glang_20050824...  (1)
» glang_20050824...  (1)
» KANMX_URA3_...     (288) CTCCTCGCTGCAGACCTGCGAGCAGGGAAACGCTCCCCTCACAGACGCGTTGAATTGTCCCCACGCCGCGCA
        Contig 1     (288) CTCCTCGCTGCAGACCTGCGAGCAGGGAAACGCTCCCCTCACAGACGCGTTGAATTGTCCCCACGCCGCGCA
                              . .
```

Section 6

```
                  361       370       380       390       400       410       420       432
« glang_20050824... (•270)
» glang_20050824...  (289) TCAAAGAAGGTTAATGTGGCTGTGGTTTCAGGGTCCATAAAGCTTTTC
« glang_20050824...  (1)
» glang_20050824...  (1)
« glang_20050824...  (1)
» glang_20050824...  (1)
« glang_20050824...  (1)
» glang_20050824...  (1)
» KANMX_URA3_...     (360) TCAAAGAAGGTTAATGTGGCTGTGGTTTCAGGGTCCATAAAGCTTTTCAATTCATCTTTTTTTTTTTTATTC
        Contig 1     (360) TCAAAGAAGGTTAATGTGGCTGTGGTTTCAGGGTCCATAAAGCTTTTCAATTCATCTTTTTTTTTTTTATTC
```

polyT region

Section 7

```
                    433       440       450       460       470       480       490       504
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824...   (1)         TTGATTTCGGTTTCCTTGAAATTTTTTTGATTCGGTAATCTCCGAACAGAAGGAAGAACGAAGGAA
» glang_20050824...   (1)
« glang_20050824...   (1)
» glang_20050824...   (1)
« glang_20050824...   (1)
» glang_20050824...   (1)
» KANMX_URA3_...  (432) TTTTTTTTGATTTCGGTTTCCTTGAAATTTTTTTGATTCGGTAATCTCCGAACAGAAGGAAGAACGAAGGAA
      Contig 1    (432) TTTTTTTTGATTTCGGTTTCCTTGAAATTTTTTTGATTCGGTAATCTCCGAACAGAAGGAAGAACGAAGGAA
```

Section 8

```
                    505       510       520       530       540       550       560       576
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824...  (66) GGAGCACAGACTTAGATTGGTATATATACGCATATGTAGTGTTGAAGAAACATGAAATTGCCCAGTATTCTT
» glang_20050824...   (1)             TTAGNNTGGTATATATACGCATATGTAGTGTTGAAGAAACATGAAATTGCCCAGTATTCTT
« glang_20050824...   (1)
» glang_20050824...   (1)
« glang_20050824...   (1)
» glang_20050824...   (1)
» KANMX_URA3_...  (504) GGAGCACAGACTTAGATTGGTATATATACGCATATGTAGTGTTGAAGAAACATGAAATTGCCCAGTATTCTT
      Contig 1    (504) GGAGCACAGACTTAGATTGGTATATATACGCATATGTAGTGTTGAAGAAACATGAAATTGCCCAGTATTCTT
```

Section 9

```
                    577       590       600       610       620       630       648
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824... (138) AACCCAACTGCACAGAACAAAAACATGCAGGAAACGAAGATAAATCATGTCGAAAGCTACATATAAGGAACG
» glang_20050824...  (61) AACCCAACTGCACAGAACAAAAACATGCAGGAAACGAAGATAAATCATGTCGAAAGCTACATATAAGGAACG
« glang_20050824...   (1)
» glang_20050824...   (1)
« glang_20050824...   (1)
» glang_20050824...   (1)
» KANMX_URA3_...  (576) AACCCAACTGCACAGAACAAAAACATGCAGGAAACGAAGATAAATCATGTCGAAAGCTACATATAAGGAACG
      Contig 1    (576) AACCCAACTGCACAGAACAAAAACATGCAGGAAACGAAGATAAATCATGTCGAAAGCTACATATAAGGAACG
```

URA3 START

Section 10

```
                    649       660       670       680       690       700       710       720
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824... (210) TGCTGCTACTCATCCTAGTCCTGTTGCTGCCAAGCTATTTAATATCATGCACGAAAAGCAAACAAACTTGTG
» glang_20050824... (133) TGCTGCTACTCATCCTAGTCCTGTTGCTGCCAAGCTATTTAATATCATGCACGAAAAGCAAACAAACTTGTG
« glang_20050824...   (1)
» glang_20050824...   (1)
« glang_20050824...   (1)
» glang_20050824...   (1)
» KANMX_URA3_...  (648) TGCTGCTACTCATCCTAGTCCTGTTGCTGCCAAGCTATTTAATATCATGCACGAAAAGCAAACAAACTTGTG
      Contig 1    (648) TGCTGCTACTCATCCTAGTCCTGTTGCTGCCAAGCTATTTAATATCATGCACGAAAAGCAAACAAACTTGTG
```

Section 11

```
                    721       730       740       750       760       770       780       792
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824... (282) TGCTTCATTGGATGTTCGTACCACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGTTT
» glang_20050824... (205) TGCTTCATTGGATGTTCGTACCACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGTTT
« glang_20050824...   (1)
» glang_20050824...   (1)
« glang_20050824...   (1)
» glang_20050824...   (1)
» KANMX_URA3_...  (720) TGCTTCATTGGATGTTCGTACCACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGTTT
      Contig 1    (720) TGCTTCATTGGATGTTCGTACCACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGTTT
```

Section 12

```
                    793       800       810       820       830       840       850       864
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824... (354) ACTAAAAACACATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTATC
» glang_20050824... (277) ACTAAAAACACATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTATC
« glang_20050824...   (1)         AACACATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTATC
» glang_20050824...   (1)
« glang_20050824...   (1)
» glang_20050824...   (1)
» KANMX_URA3_...  (792) ACTAAAAACACATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTATC
      Contig 1    (792) ACTAAAAACACATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTATC
```

Section 13

```
                         865     870       880       890       900       910       920       936
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824...  (426) CGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACAGTCAAATTGCAGTA
» glang_20050824...  (349) CGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACAGTCAAATTGCAGTA
« glang_20050824...   (66) CGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATNGGTAATACAGTCAAATTGCAGTA
» glang_20050824...    (1)
« glang_20050824...    (1)
» glang_20050824...    (1)
» KANMX_URA3_...  (864) CGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACAGTCAAATTGCAGTA
        Contig 1  (864) CGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACAGTCAAATTGCAGTA
```

Section 14

```
                         937       950       960       970       980       990      1008
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824...  (498) CTCTGCGGGTGTATACAGAATAGCAGAANGGGCAGACATTACGAANG
» glang_20050824...  (421) CTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAATGCACACGGTGTGGTGGGCCCAGGTAT
« glang_20050824...  (138) CTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAATGCACACGGTGTGGTGGGCCCAGGTAT
» glang_20050824...    (1)                                                                         T
« glang_20050824...    (1)
» glang_20050824...    (1)
» KANMX_URA3_...  (936) CTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAATGCACACGGTGTGGTGGGCCCAGGTAT
        Contig 1  (936) CTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAATGCACACGGTGTGGTGGGCCCAGGTAT
```

Section 15

```
                        1009      1020      1030      1040      1050      1060      1070      1080
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824... (•544)
» glang_20050824...  (493) TGTTAGCGGTTTGAAGCAGGCGGCGGAAGAAGTAACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATT
« glang_20050824...  (210) TGTTAGCGGTTTGAAGCAGGCGGCGGAAGAAGTAACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATT
» glang_20050824...    (1) TGTTAGCGGTTTGAAGCNGGCGGCGGAAGAAGTAACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATT
« glang_20050824...    (1)                                AAGTAACAAAGGANCCTAGAGGCCTTTTNATGTTAGCAGAATT
» glang_20050824...    (1)
» KANMX_URA3_... (1008) TGTTAGCGGTTTGAAGCAGGCGGCGGAAGAAGTAACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATT
        Contig 1 (1008) TGTTAGCGGTTTGAAGCAGGCGGCGGAAGAAGTAACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATT
```


Section 16

```
                        1081      1090      1100      1110      1120      1130      1140      1152
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824... (•544)
» glang_20050824...  (565) GTCATGCAAGGGCTCCCTATCTACTG
« glang_20050824...  (282) GTCATGCAAGGGCTCCCTATCTACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGA
» glang_20050824...   (73) GTCATGCAAGGGCTCCCTATCTACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGA
« glang_20050824...   (43) GTCATGCAAGGGCTCCCTATCTACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGA
» glang_20050824...    (1)
» KANMX_URA3_... (1080) GTCATGCAAGGGCTCCCTATCTACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGA
        Contig 1 (1080) GTCATGCAAGGGCTCCCTATCTACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGA
```

Section 17

```
                        1153      1160      1170      1180      1190      1200      1210      1224
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824... (•544)
» glang_20050824... (•590)
« glang_20050824...  (354) TTTTGTTATCGGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATGAC
» glang_20050824...  (145) TTTTGTTATCGGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATGAC
« glang_20050824...  (115) TTTTGTTATCGGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATGAC
» glang_20050824...    (1)
» KANMX_URA3_... (1152) TTTTGTTATCGGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATGAC
        Contig 1 (1152) TTTTGTTATCGGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATGAC
```

Section 18

```
                        1225      1230      1240      1250      1260      1270      1280      1296
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824... (•544)
» glang_20050824... (•590)
« glang_20050824...  (426) ACCCGGTGTGGGTTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTGGATGATGTGGTCTC
» glang_20050824...  (217) ACCCGGTGTGGGTTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTGGATGATGTGGTCTC
« glang_20050824...  (187) ACCCGGTGTGGGTTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTGGATGATGTGGTCTC
» glang_20050824...    (1)
» KANMX_URA3_... (1224) ACCCGGTGTGGGTTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTGGATGATGTGGTCTC
        Contig 1 (1224) ACCCGGTGTGGGTTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTGGATGATGTGGTCTC
```

Section 19

```
                                1297      1310      1320      1330      1340      1350      1368
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824... (•544)
» glang_20050824... (•590)
« glang_20050824... (498) TACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAGGGAAGGGATGCTAAGGTAGAGGGTGA
» glang_20050824... (289) TACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAGGGAAGGGATGCTAAGGTAGAGGGTGA
« glang_20050824... (259) TACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAGGGAAGGGATGCTAAGGTAGAGGGTGA
» glang_20050824... (1)
» KANMX_URA3_... (1296) TACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAGGGAAGGGATGCTAAGGTAGAGGGTGA
Contig 1 (1296) TACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAGGGAAGGGATGCTAAGGTAGAGGGTGA
```

Section 20

```
                                1369      1380      1390      1400      1410      1420      1430      1440
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824... (•544)
» glang_20050824... (•590)
« glang_20050824... (570) ACGTTACAGAAAAGCAGGCTGGGAAGCATATTTGAGAAGATGCGGCCAGCAAAACTAAAAAACTGTATTATA
» glang_20050824... (361) ACGTTACAGAAAAGCAGGCTGGGAAGCATATTTGAGAAGATGCGGCCAGCAAAACTAAAAAACTGTATTATA
« glang_20050824... (331) ACGTTACAGAAAAGCAGGCTGGGAAGCATATTTGAGAAGATGCGGCCAGCAAAACTAAAAAACTGTATTATA
» glang_20050824... (1)
» KANMX_URA3_... (1368) ACGTTACAGAAAAGCAGGCTGGGAAGCATATTTGAGAAGATGCGGCCAGCAAAACTAAAAAACTGTATTATA
Contig 1 (1368) ACGTTACAGAAAAGCAGGCTGGGAAGCATATTTGAGAAGATGCGGCCAGCAAAACTAAAAAACTGTATTATA
```

URA3 STOP

Section 21

```
                                1441      1450      1460      1470      1480      1490      1500      1512
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824... (•544)
» glang_20050824... (•590)
« glang_20050824... (642) AGTAAATGCATGTATACTNAACTCACAAATTAGAGCTTCAATTNNATTA
» glang_20050824... (433) AGTAAATGCATGTATACTAAACTCACAAATTAGAGCTTCAATTTAATTATATCAGTTATTACCCGGGAATCT
« glang_20050824... (403) AGTAAATGCATGTATACTAAACTCACAAATTAGAGCTTCAATTTAATTATATCAGTTATTACCCGGGAATCT
» glang_20050824... (1)
» KANMX_URA3_... (1440) AGTAAATGCATGTATACTAAACTCACAAATTAGAGCTTCAATTTAATTATATCAGTTATTACCCGGGAATCT
Contig 1 (1440) AGTAAATGCATGTATACTAAACTCACAAATTAGAGCTTCAATTTAATTATATCAGTTATTACCCGGGAATCT
```


Section 22

```
                                1513      1520      1530      1540      1550      1560      1570      1584
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824... (•544)
» glang_20050824... (•590)
« glang_20050824... (•690)
» glang_20050824... (505) CGGTCGTAATGATTT
« glang_20050824... (475) CGGTCGTAATGATTTCTATAATGAGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAA
» glang_20050824... (1)
» KANMX_URA3_... (1512) CGGTCGTAATGATTTCTATAATGAGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAA
Contig 1 (1512) CGGTCGTAATGATTTCTATAATGAGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAA
```

Section 23

```
                                1585      1590      1600      1610      1620      1630      1640      1656
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824... (•544)
» glang_20050824... (•590)
« glang_20050824... (•690)
» glang_20050824... (•519)
« glang_20050824... (547) TCAGTACTGACAATAAAAAGATTCTTGTTTTCAAGAACTTGTCATTTGTATAGTTTTTTTATATTGTAGTTG
» glang_20050824... (1)                                  AACTTGTNNTTTGTATAGTTTTTTTATATTGTAGTTG
» KANMX_URA3_... (1584) TCAGTACTGACAATAAAAAGATTCTTGTTTTCAAGAACTTGTCATTTGTATAGTTTTTTTATATTGTAGTTG
Contig 1 (1584) TCAGTACTGACAATAAAAAGATTCTTGTTTTCAAGAACTTGTCATTTGTATAGTTTTTTTATATTGTAGTTG
```

Section 24

```
                                1657      1670      1680      1690      1700      1710      1728
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824... (•544)
» glang_20050824... (•590)
« glang_20050824... (•690)
» glang_20050824... (•519)
« glang_20050824... (619) TTCTATTTTAATCAAATGTTAGCGTGATTTATATTTTTTTTCGCCTCGACATCATCTGCCCAGATGCGAAGT
» glang_20050824... (37) TTCTATTTTAATCAAATGTTAGCGTGATTTATATTTTTTTTCGCCTCGACATCATCTGCCCAGATGCGAAGT
» KANMX_URA3_... (1656) TTCTATTTTAATCAAATGTTAGCGTGATTTATATTTTTTTTCGCCTCGACATCATCTGCCCAGATGCGAAGT
Contig 1 (1656) TTCTATTTTAATCAAATGTTAGCGTGATTTATATTTTTTTTCGCCTCGACATCATCTGCCCAGATGCGAAGT
```

Section 25

```
                          1729      1740      1750      1760      1770      1780      1790      1800
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824... (•544)
» glang_20050824... (•590)
« glang_20050824... (•690)
» glang_20050824... (•519)
« glang_20050824...  (691) TAAGTGCGCAGAAAGTAATATCATGCGTCAATCGTATGTGAATGCTGGTCGCTATACTGCTGTCGATTCGAT
» glang_20050824...  (109) TAAGTGCGCAGAAAGTAATATCATGCGTCAATCGTATGTGAATGCTGGTCGCTATACTGCTGTCGATTCGAT
» KANMX_URA3_... (1728) TAAGTGCGCAGAAAGTAATATCATGCGTCAATCGTATGTGAATGCTGGTCGCTATACTGCTGTCGATTCGAT
        Contig 1 (1728) TAAGTGCGCAGAAAGTAATATCATGCGTCAATCGTATGTGAATGCTGGTCGCTATACTGCTGTCGATTCGAT
```

Section 26

```
                          1801      1810      1820      1830      1840      1850      1860      1872
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824... (•544)
» glang_20050824... (•590)
« glang_20050824... (•690)
» glang_20050824... (•519)
« glang_20050824...  (763) ACTAACGCCGCCANCCAG
» glang_20050824...  (181) ACTAACGCCGCCATCCAGTGTCGAAAACGAGCTCGAATTCATCGATCATCAATGGTC-TACCGT
» KANMX_URA3_... (1800) ACTAACGCCGCCATCCAGTTT--AAA-CGAGCTCGAATTCATCGATTAGTAGTAGTAGTA--GTAGTACTAC
        Contig 1 (1800) ACTAACGCCGCCATCCAGTKTCGAAAACGAGCTCGAATTCATCGATYAKYARTRGTMGTACCGTAGTACTAC
                                        .      + ++    +                      + ++  + +   ++   ++
```

downtag

Section 27

```
                          1873      1885
« glang_20050824... (•270)
» glang_20050824... (•336)
« glang_20050824... (•544)
» glang_20050824... (•590)
« glang_20050824... (•690)
» glang_20050824... (•519)
« glang_20050824... (•780)
» glang_20050824... (•244)
» KANMX_URA3_... (1872) GAGACCGACACCG
        Contig 1 (1872) GAGACCGACACCG
```

```
Section 1
                    1         10        20        30        40        51
« glang_20050...  (1)
« glang_20050...  (1) NGNNGNNNANGGNNNNNNNNNGNGNNGNNNNGNTCNANNNNNNNNNNNN
     Contig 1     (1) NGNNGNNNANGGNNNNNNNNNGNGNNGNNNNGNTCNANNNNNNNNNNNN
                      . .. ... .  ........... . .. .... .  . ............

Section 2
                   52        60        70        80        90       102
« glang_20050...  (1)
« glang_20050...  (51) NNNNNNNNNNNNNATNTATTNNANNNCNNANNTNANNNNNNNNNTNNTNNN
     Contig 1     (51) NNNNNNNNNNNNNATNTATTNNANNNCNNANNTNANNNNNNNNNTNNTNNN
                      .............  .  .. ... .. ... ......... .. ...

Section 3
                  103       110       120       130       140       153
« glang_20050...  (1)
« glang_20050...(102) NCCTGNNNNNNNNNNCNNNNNNNNNNNNNNNTNNNNNNNNNTGATGTCCA
     Contig 1 (102) NCCTGNNNNNNNNNNCNNNNNNNNNNNNNNNTNNNNNNNNNTGATGTCCA
                    .  ........... ................ .........

Section 4
                  154       160       170       180       190       204
« glang_20050...  (1)                TAGCCAGAGCACACGCAGCGTACGCTGCAGGTCGACGGA
« glang_20050...(153) CGAGGTCTCTTTTAGCCAGAGCACACGCAGCGTACGCTGCAGGTCGACGGA
     Contig 1 (153) CGAGGTCTCTTTTAGCCAGAGCACACGCAGCGTACGCTGCAGGTCGACGGA

Section 5
                  205       210       220       230       240       255
« glang_20050...(39) TCCCCGGGTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTCCCCG
« glang_20050...(204) TCCCCGGGTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTCCCCG
     Contig 1 (204) TCCCCGGGTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTCCCCG

Section 6
                  256       270       280       290       306
« glang_20050...(90) CCGGGTCACCCGGCCAACGACATGGAGGCCCAGAATACCCTCCTTGACAGT
« glang_20050...(255) CCGGGTCACCCGGCCAACGACATGGAGGCCCAGAATACCCTCCTTGACAGT
     Contig 1 (255) CCGGGTCACCCGGCCAACGACATGGAGGCCCAGAATACCCTCCTTGACAGT

Section 7
                  307       320       330       340       357
« glang_20050...(141) CTTGACGTGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGTACATTTAG
« glang_20050...(306) CTTGACGTGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGTACATTTAG
     Contig 1 (306) CTTGACGTGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGTACATTTAG

Section 8
                  358       370       380       390       408
« glang_20050...(192) CCCATACATCCCCATGTATAATCATTTGCATCCATACATTTTGANGGCCGC
« glang_20050...(357) CCCATACATCCCCATGTATAATCATTTGCATCCATACATTTTGANGGCCGC
     Contig 1 (357) CCCATACATCCCCATGTATAATCATTTGCATCCATACATTTTGANGGCCGC
```

Uptag

postnatal

Section 1

```
                    1         10        20        30        40        51
« glang_20050...  (1) NNNGNNGNNANNNCNNNNNTNNNNNNNNNNNNNNNNNNNNANNNNNNNNNN
« glang_20050...  (1)
   Contig 1       (1) NNNGNNGNNANNNCNNNNNTNNNNNNNNNNNNNNNNNNNNANNNNNNNNNN
                      ..... ..... ..... ..... ..... ..... ..... .....
```

Section 2

```
                   52        60        70        80        90       102
« glang_20050...  (51) NANNNTNNNNGGNNANCTAAATNNAAANANNNNNNNNNNNAANAAAGATTA
« glang_20050...  (1)
   Contig 1       (51) NANNNTNNNNGGNNANCTAAATNNAAANANNNNNNNNNNNAANAAAGATTA
                      .... .... .... ..   . ........... .
```

Section 3

```
                  103       110       120       130       140       153
« glang_20050... (102) ATTGNTAANCTTTATGNNNCCTNNANNNNCANNCNCNTTNNNNTNTNTNTN
« glang_20050...  (1)
   Contig 1      (102) ATTGNTAANCTTTATGNNNCCTNNANNNNCANNCNCNTTNNNNTNTNTNTN
                       .  .      ...  .. .... .. .  .  .... . . . .
```

Section 4

```
                  154       160       170       180       190       204
« glang_20050... (153) NNNNNNNNNNGNNNAATTNAANNTCGGTGTCGGTCTCGTAGTACGGTAGAC
« glang_20050...  (1)                                          ACGGTAGAC
   Contig 1      (153) NNNNNNNNNNGNNNAATTNAANNTCGGTGTCGGTCTCGTAGTACGGTAGAC
                       .......... ...     .  ..
```

Section 5

```
                  205       210       220       230       240       255
« glang_20050... (204) CATTGATGATCGATGAATTCGAGCTCGTTTTCGACACTGGATGGCGGCGTT
« glang_20050...  (9) CATTGATGATCGATGAATTCGAGCTCGTTTTCGACACTGGATGGCGGCGTT
   Contig 1      (204) CATTGATGATCGATGAATTCGAGCTCGTTTTCGACACTGGATGGCGGCGTT
```

possible EcoRI translamation-induced mutation

Section 6

```
                  256       270       280       290       306
« glang_20050... (255) AGTATCGAATCGACAGCAGTATAGCGACCAGCATTCACATACGATTGACGC
« glang_20050...  (60) AGTATCGAATCGACAGCAGTATAGCGACCAGCATTCACATACGATTGACGC
   Contig 1      (255) AGTATCGAATCGACAGCAGTATAGCGACCAGCATTCACATACGATTGACGC
```

Section 7

```
                  307       320       330       340       357
« glang_20050... (306) ATGATATTACTTTCTGCGCACTTAACTTCGCATCTGGGCAGATGATGTCGA
« glang_20050... (111) ATGATATTACTTTCTGCGCACTTAACTTCGCATCTGGGCAGATGATGTCGA
   Contig 1      (306) ATGATATTACTTTCTGCGCACTTAACTTCGCATCTGGGCAGATGATGTCGA
```

Section 8

```
                  358       370       380       390       408
« glang_20050... (357) GGCGAAAAAAAATATAAATCACGCTAACATTTGATTAAAATAGAACAACTA
« glang_20050... (162) GGCGAAAAAAAATATAAATCACGCTAACATTTGATTAAAATAGAACAACTA
   Contig 1      (357) GGCGAAAAAAAATATAAATCACGCTAACATTTGATTAAAATAGAACAACTA
```

downtag

Uptag

8/31

Sequencing notes:

GL·26

```
UPTAG primer
TATAACCTCCCTTGAATGGATGTCCACGAGGTCTCT  TTTAGCCAGAGCACACGCAG CGTACGCTGCAGGTCGAC

DOWNTAG primer
GGTTTATATATTATTTTACGGTGTCGGTCTCGTAG   TACGGTAGACCATTGCCGAG ATCGATGAATTCGAGCTCG

---------------------------------------------------

uptag - from deletion pages
TTTAGCCAGAGCACACGCAG

uptag - from sequence
TTTAGCCAGAGCACACGCAG

comparison
TTTAGCCAGAGCACACGCAG
TTTAGCCAGAGCACACGCAG

---------------------------------------------------

downtag - from deletion pages
TACGGTAGACCATTGCCGAG

downtag - from sequence
TACGGTAGACCATTGATGATCGATGAATT

comparison
TACGGTAGACCATTGCCGAG || ATCGATGAATTCGAGCTCG
TACGGTAGACCATTGATG   || ATCGATGAATTCGAGCTCG
```

- ~~uptag~~ uptag is correct (20/20)
- downtag is not quite correct (15/20)

  difference: CCGAG ⟶ ATG——

Note that the end of the transformed fragment lies close to the tag (EcoRI site, p50).

It is also interesting that the mutation looks like a tandem duplication:

ATTGATG ATCGATG

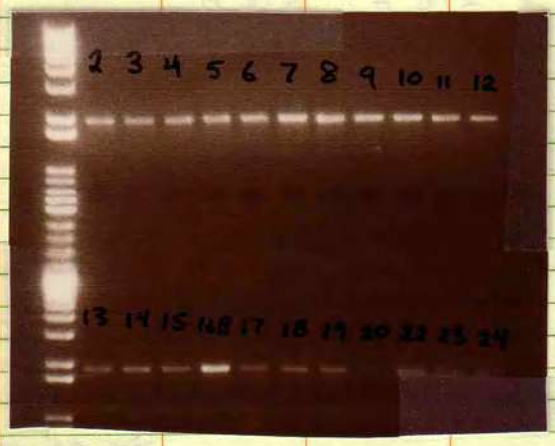
This could be a transformation-induced mutation

This could also explain why I cannot get transformants from some of my strains (p42) — perhaps ~~my primer~~ one of the primer sites was destroyed by trans. induced mutation. The primer sites are immediately distal to the tags.

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

8/31

Yesterday I PCR purified all of the cassettes from the PCR-verified strains. These were put in -20 on 8/25 (p 42).



100 λ PCR
↓ elute
30 λ EB

- There are two "16B"s — one from p41 & one from p42.
- 20 is by far the faintest band — I will need to use additional template during sequencing
- All are from "A" transformants (see p 37 8/18) except 16B and 27B (see p38 8/16)

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

8/31

SEQUENCING OF TRANSFORMANTS

Primers: A–H (p 42)

- Submit 2 strips to MCB: 16B A – 16B H & 27B A – 27B H
- Submit 3 plates to Biopolymers:

PLATE 1

row \ column	1	2	3	4	5	6	7	8	9	10	11	12
Primer A → A	2	3	4	5	6	7	8	9	10	11	12	15
Primer B → B	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
" C → C	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
" D → D	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
" E → E	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
" F → F	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
" G → G	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
H	20,A	20,B	20,C	20,D	20,E	20,F	20,G	23,A	23,B	23,C	23,D	23,E

Temp, Primer

~~for column H, use 27 of temp because of faint bands only~~

PLATE 2

A →	14	15	17	18	19	22	25	28	29	30	32	33
B →	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
C →	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
D →	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
E →	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
F →	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
G →	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
	23,F	23,G	24,A	24,B	24,C	24,D	24,E	24,F	24,G	31,A	31,B	31,C

PLATE 3

A →	34	35	36	37	38	40	41	42	44	46	47	48
B →	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
C →	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
D →	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
E →	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
F →	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
G →	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
	31,D	31,E	31,F	31,G	49,A	49,B	49,C	49,D	49,E	49,F	49,G	Tr2,G

Transformant #2 (lane #4, p19)
use 3 λ

SEQUENCING OF TRANSFORMANTS CONT.

for biopolymers:

- I want 8 pmol primer / 30 λ sample
- Sample vol = 30 λ

PRIMER	CONC. (pmol/λ)	VOL. PER SAMPLE		
A	32	$\frac{8}{32} = 0.25$	1203.75 λ H_2O + 11.25 λ Primer = 1215 λ (27×45)	
B	30.3	$\frac{8}{30.3} = 0.26$	1203.3 H_2O + 11.7 λ Primer	
C	27.5	$\frac{8}{27.5} = 0.29$	~~1201.95~~ 1202 H_2O	13 λ Primer
D	18.5	$\frac{8}{18.5} = 0.43$	1195.65	19.35
E	30	$\frac{8}{30} = 0.27$	1202.85	12.15
F	26.5	$\frac{8}{26.5} = 0.30$	1201.5	13.5
G	24	$\frac{8}{24} = 0.33$	1200.15	14.85

- Add 27 λ of primer mix to each well
- Add 3 λ of PCR product to each well

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

9/1

SEQUENCING RESULTS FOR GL·16 AND GL·27

- No mutations in the URA3 of either strain
- No mutations in the downtags of either strain or the uptag of GL·27
- Another mutation of the deletion/tandem duplication type (see p 51)
  - This one is more clear than the previous example (GL·26).

tagsGL16

UPTAG primer
CAGACTGTAATAGCCATGGATGTCCACGAGGTCTCT ATATAGCTCCCACATTGCAG CGTACGCTGCAGGTCGAC

DOWNTAG primer
CTGTCTCCATATGGGTTACGGTGTCGGTCTCGTAG ATGACACCCTCGTAACGGAG ATCGATGAATTCGAGCTCG

---

uptag - from deletion pages
ATATAGCTCCCACATTGCAG

uptag - from sequence
ATATAGCTCCCATATAGCTCC

comparison
ATATAGCTCCCACATTGCAG
ATATAGCTCCCATATAGCTCC

---

downtag - from deletion pages
ATGACACCCTCGTAACGGAG

downtag - from sequence
ATGACACCCTCGTAACGGAG

comparison
ATGACACCCTCGTAACGGAG
ATGACACCCTCGTAACGGAG

tagsGL27

UPTAG primer
TACAATTAAGGAAAGATGGATGTCCACGAGGTCTCT CGCTACATATACCTACAGAG CGTACGCTGCAGGTCGAC

DOWNTAG primer
GTTTAGTCAATCAAACTACGGTGTCGGTCTCGTAG CTCCGTTGAATCCCATTGAG ATCGATGAATTCGAGCTCG

---

uptag - from deletion pages
CGCTACATATACCTACAGAG

uptag - from sequence
CGCTACATATACCTACAGAG

comparison
CGCTACATATACCTACAGAG
CGCTACATATACCTACAGAG

---

downtag - from deletion pages
CTCCGTTGAATCCCATTGAG

downtag - from sequence
CTCCGTTGAATCCCATTGAG

comparison
CTCCGTTGAATCCCATTGAG
CTCCGTTGAATCCCATTGAG

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6992 10VG

9/2 ALIGNMENT OF ALL UPTAGS SEQUENCED THUS FAR

```
UPTAG primer
TACAATTAAGGAAAGATGGATGTCCACGAGGTCTCTxxxxxxxxxxxxxxxxxxxxxCGTACGCTGCAGGTCGAC

TCTCTxxxxxxxxxxxxxxxxxxxxxCGTACGCTGCAGGTCGAC --- from sequencing
TCTCTxxxxxxxxxxxxxxxxxxxxxCGTACGCTGCAGGTCGAC --- from deletion pages

>GL02
TCTCT     ACTGCCATACGAGGTAAGAG   CGTACGCTGCAGGTCGACGGATCCCCG
TCTCT     ACTGCCATACGAGGTAAGAG   CGTACGCTGCAGGTCGAC

>GL03
TCTCT     CTCCTAATGTACGCTAAGAG   CGTACGCTGCAGGTCGACGGATC
TCTCT     CTCCTAATGTACGCTAAGAG   CGTACGCTGCAGGTCGAC

>GL04
TCTCT     CCGGTAACTTCGTATAAGAG   CGTACGCTGCAGGTCGACG
TCTCT     CCGGTAACTTCGTATAAGAG   CGTACGCTGCAGGTCGAC

>GL05
TCTCT     AGCTAAGCCCGACGTTTCAG   CGTACGCTGCAGGTCGACGG
TCTCT     AGCTAAGCCCGACGTTTCAG   CGTACGCTGCAGGTCGAC

>GL06
TCTCT     CCTAACCGGAGATATTTCAG   CGTACGCTGCAGGTCGACGGAT
TCTCT     CCTAACCGGAGATATTTCAG   CGTACGCTGCAGGTCGAC

>GL07
TCTCT     CGACTAGCATGACATTTCAG   CGTACGCTGCAGGTCGACGGATC
TCTCT     CGACTAGCATGACATTTCAG   CGTACGCTGCAGGTCGAC

>GL08
TCTCT     ACCAGTATGTGGAAGCTCAG   CGTACGCTGCAGGTCGACG
TCTCT     ACCAGTATGTGGAAGTTCAG   CGTACGCTGCAGGTCGAC

>GL09
TCTCT     TAGACGATGCGCCATGTCAG   CGTACGCTGCAGG
TCTCT     TAGACGATGCGCCATGTCAG   CGTACGCTGCAGGTCGAC

>GL10
TCTCT     ACACCTACACGATTAGTCAG   CGTACGCTGCAGGTCGAC
TCTCT     ACACCTACACGATTAGTCAG   CGTACGCTGCAGGTCGAC

>GL11
TCTCT     G-ACCATTT-CTAAGCTCAG   CGTACGCTGCAGGTCGACGGATC
TCTCT     GGACCATTTACTAAGCTCAG   CGTACGCTGCAGGTCGAC

>GL12
NNTCT     TCAGAGGCGTAAGCACTCAG   CGTACGCTGCAGGTCGACGGA
TCTCT     TCAGAGGCGTAAGCACTCAG   CGTACGCTGCAGGTCGAC

>GL13
TNTNT     -GAGCCACGACCTATATCAG   CGTACGCTGCAGGTC
TCTCT     GGAGCCACGACCTATATCAG   CGTACGCTGCAGGTCGAC

>GL14
TCTCT     CCATCCAGCATAGTAATCAG   CGTACGCTGCAGGTCGAC
TCTCT     CCATCCAGCATAGTAATCAG   CGTACGCTGCAGGTCGAC

>GL15
TCTCT     CCTTAGTGTGAAGTTTGCAG   CGTACGCTGCAGGT
TCTCT     CCTTAGTGTGAAGTTTGCAG   CGTACGCTGCAGGTCGAC

>GL16
TCTCT     ATATAGCTCCCATATAGCTCC   CGTACGCTGCAGGTCGAC
TCTCT     ATATAGCTCCCACATTGCAG    CGTACGCTGCAGGTCGAC

>GL17
TCTCT     ACGACCGTCTAACTATGCAG   CGTACGCTGCAGGTCGACGGATCC
TCTCT     ACGACCGTCTAACTATGCAG   CGTACGCTGCAGGTCGAC

>GL18
TCTCT     ACAGACTCCCTAAGATGCAG   CGTACGCTGCAGGTCGACG
TCTCT     ACAGACTCCCTAAGATGCAG   CGTACGCTGCAGGTCGAC

>GL19
TCTCT     ATTCACGCCACATTGGGCAG   CGTACGCTGCAGGTCGAC
TCTCT     ATTCACGCCACATTGGGCAG   CGTACGCTGCAGGTCGAC

>GL20
TCTCT     CTATATGGCTAATGAGGCAG   CGTACGCTGCAGGT
TCTCT     CTATATGGCTAATGAGGCAG   CGTACGCTGCAGGTCGAC

>GL22
TCTCT     AGGCACTCACTCTAAGGCAG   CGTACGCTGCAGGTCGACG
TCTCT     AGGCACTCACTCTAAGGCAG   CGTACGCTGCAGGTCGAC

>GL23
TCTCT     ACAAGCGGATCTAATCGCAG   CGTACGCTGCAGGTCG
TCTCT     ACAAGCGGATCTAATCGCAG   CGTACGCTGCAGGTCGAC

>GL24
TCTCT     AGAGATCACTGACACCGCAG   CGTACGCTGCAGGTCGACGGATC
TCTCT     AGAGATCACTGACACCGCAG   CGTACGCTGCAGGTCGAC
```

All 45/45 correct uptag

2 uptags have significant mutations:

GL·16 & GL·31

also one on the downtag of GL·26 (see p51)

```
>GL25
TCTCT     GACACCTACTTAATACGCAG   CGTACGCTGCAGGTCGA
TCTCT     GACACCTACTTAATACGCAG   CGTACGCTGCAGGTCGAC

>GL26
TCTCT     TTTAGCCAGAGCACACGCAG   CGTACGCTGCAGGTCGA
TCTCT     TTTAGCCAGAGCACACGCAG   CGTACGCTGCAGGTCGA

>GL27
TCTCT     CGCTACATATACCTACAGAG   CGTACGCTGCAGGTCGA
TCTCT     CGCTACATATACCTACAGAG   CGTACGCTGCAGGTCGA

>GL28
TCTCT     CGATACTCCAGCAGTCAGA-   CGTACGCTGCAGGTCGACGGAT
TCTCT     CGATACTCCAGCAGTCAGAG   CGTACGCTGCAGGTCGAC

>GL29
TCTCT     AGGGTAACGTACATTCAGAG   CGTACGCTGCAGGTCGAC
TCTCT     AGGGTAACGTACATTCAGAG   CGTACGCTGCAGGTCGAC

>GL30
TCTCT     CATCAGGACCGTACAGAGAG   CGTACGCTGCAGGTCGACGGAT
TCTCT     CATCAGGACCGTACAGAGAG   CGTACGCTGCAGGTCGAC

>GL31
TCTCT     C-TCAGGCATTAAGTAGAGAG   CGTACGCTGCAGGTCGACGGA
TCTCT     CATCAGGACCGTAC AGAGAG   CGTACGCTGCAGGTCGAC

>GL32
NNTCT     TTNTTACACACCCAGGAGAG   CGTACGCTGCAGGTCGACGGANNC
TCTCT     TTCTTACACACCCAGGAGAG   CGTACGCTGCAGGTCGAC

>GL33
TCTCT     TAACTGGACCTAAGTGAGAG   CGTACGCTGCAGGTCG
TCTCT     TAACTGGACCTAAGTGAGAG   CGTACGCTGCAGGTCGAC

>GL34
TCTCT     ACCGTACAGCCATTTGAGAG   CGTACGCTGCAGGTCGACGGAT
TCTCT     ACCGTACAGCCATTTGAGAG   CGTACGCTGCAGGTCGAC

>GL35
TCTCT     CATTGCATGT--GGCTAGAG   CGTACGCTGCAGGTCGACGGATC
TCTCT     CATTGCATGTAAGGCTAGAG   CGTACGCTGCAGGTCGAC

>GL36
TCTCT     CCGTTAAGCCCATTGTAGAG   CGTACGCTGCA
TCTCT     CCGTTAAGCCCATTGTAGAG   CGTACGCTGCAGGTCGAC

>GL37
NCTCT     TAGTAGCCCATCCGAACGAG   CGTACGCTGCAGGTCGA
TCTCT     TAGTAGCCCATCCGAACGAG   CGTACGCTGCAGGTCGAC

>GL38
NCTCT     TGACACGATTACCATACGAG   CGTACGCTGCAGGTC
TCTCT     TGACACGATTACCATACGAG   CGTACGCTGCAGGTCGAC

>GL40
TNTCT     GACTACCAGGCACGTTGA-C   CGTACGCTGCAGGTCGACGG
TCTCT     GACTACCAGGCACGTTGATC   CGTACGCTGCAGGTCGAC

>GL41
TCTNT     GAGTAGTG-CCACCCATATC   CGTACGCTGCAGGTCGACG
TCTCT     GAGTAGTGACCACCCATATC   CGTACGCTGCAGGTCGAC

>GL42
TCTCT     GACCGTGCATAACTTCTATC   CGTACGC
TCTCT     GACCGTGCATAACTTCTATC   CGTACGCTGCAGGTCGA

>GL44
TCTCT     AGGGACCATAGACCATTATC   CGTACGCTGCA
TCTCT     AGGGACCATAGACCATTATC   CGTACGCTGCAGGTCGAC

>GL46
TCTCT     ATGACAAGAGGGTCGAACTC   CGTACGCTGCAGGTC
TCTCT     ATGACAAGAGGGTCGAACTC   CGTACGCTGCAGGTCGAC

>GL47
NNTNT     NANNTAACGCNCGCGAACTC   CGNACGCTGCANGNNGACGGA
TCTCT     GATCTAACGCACGGGAACTC   CGTACGCTGCAGGTCGAC

>GL48
TCTCT     AGAGGCTATCTCGTGA-CTC   CGTACGCTGCAGGT
TCTCT     AGAGGCCATCTCGTGAACTC   CGTACGCTGCAGGTCGAC

>GL49
NNTCT     CCNNCAGG-TA-TTTAACTC   CGNACGCTGCANGNNGACG
TCTCT     CCACCAGGGTAATTTAACTC   CGTACGCTGCAGGTCGAC

>GL00
TCTCT     CAGATGACTGAATGGCTCTC   CGTACGCTGCAGGTCGACGGATCCC
TCTCT     CAGATGACTGAATGGCTCTC   CGTACGCTGCAGGTCGAC
```

9/7

Last week I ordered ORF-specific primers for the 5 ORFs that I have not been able to get a PCR from

GL·01

17925101 IDT
V.INDJEIAN
19282987 8/31/2005
YFL063Wf
5 ACA GTT ACC CTC CAA TTA CC -3
Tm= 51.7 °C , MW= 5,981.0
5.8 OD_{260} 30.92 nmol 0.18 mg

17925098 IDT
V.INDJEIAN
19282986 8/31/2005
YFL063Wr
5 AGA GCA TAG TGG TAT GAC GG -3
Tm= 53.8 °C , MW= 6,246.1
5.9 OD_{260} 28.11 nmol 0.18 mg

GL·21

17925102 IDT
V.INDJEIAN
19282988 8/31/2005
YFL011Wf
5 GGA CAC AAG AAA TGT GTT GG -3
Tm= 51.9 °C , MW= 6,230.1
5.6 OD_{260} 27.01 nmol 0.17 mg

17925103 IDT
V.INDJEIAN
19282989 8/31/2005
YFL011Wr
5 GTG ACG CCA TTA ATA AAG GC -3
Tm= 51.9 °C , MW= 6,150.1
6.1 OD_{260} 30.26 nmol 0.19 mg

GL·39

17925097 IDT
V.INDJEIAN
19283065 8/31/2005
YFR030Wf
5 ATA TAA GCA ACA GAG GGC AG -3
Tm= 52.1 °C , MW= 6,208.1
5.8 OD_{260} 26.94 nmol 0.17 mg

17925106 IDT
V.INDJEIAN
19282992 8/31/2005
YFR030Wr
5 AGA AAT GTT CAA GAC AGC TTC -3
Tm= 50.6 °C , MW= 6,478.3
6.1 OD_{260} 28.11 nmol 0.18 mg

GL·43

17925104 IDT
V.INDJEIAN
19282990 8/31/2005
YFR043Cf
5 TTC GAA TTC TGA AAA GAG AGG -3
Tm= 50.1 °C , MW= 6,518.3
5.9 OD_{260} 27.10 nmol 0.18 mg

17925105 IDT
V.INDJEIAN
19282991 8/31/2005
YFR043Cr
5 TTT TAC TAG CAT ACT TCC CTG -3
Tm= 49.4 °C , MW= 6,322.2
5.2 OD_{260} 27.47 nmol 0.17 mg

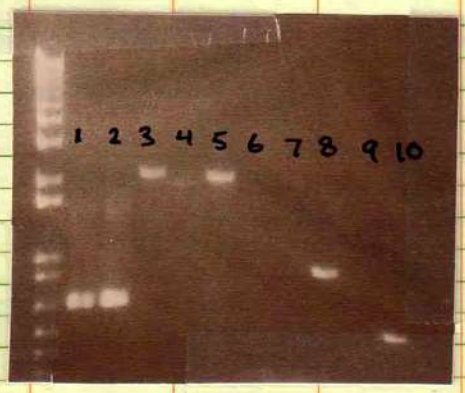
GL·45

17925100 IDT
V.INDJEIAN
19283067 8/31/2005
YFR049Wf
5 ACC ATT AAG GAG TAC TTC CC -3
Tm= 51.1 °C , MW= 6,061.0
5.8 OD_{260} 29.87 nmol 0.18 mg

17925099 IDT
V.INDJEIAN
19283066 8/31/2005
YFR049Wr
5 TCA TCA CTC TTC TCT CTT CC -3
Tm= 50.8 °C , MW= 5,904.9
5.6 OD_{260} 34.06 nmol 0.20 mg

EXPECTED FRAGMENT SIZES

TEMP ~~STRAIN~~	KanMX	KanMX Δ::URA3	incorrect strain
01	1.8	2.1	.65
21	1.8	2.1	1.9
39	1.8	2.1	3.3
43	1.8	2.1	.93
49	1.8	2.1	.56



LANE	PRIMER PAIR	TEMPLATE (p41-43)
1	GL·01	1B
2	"	B
3	GL·21	21A
4	"	B
5	GL·39	39A
6	"	B
7	GL·43	43A
8	"	B
9	GL·45	45A
10	"	B

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 692 10VG

9/8

- PCR prep lanes 3 and 5 from page 61
- ~~Submit~~ submit for sequencing

9/9

GL·21

```
   GL21 GATGTCCACGAGGTCTCTGATACACGAGTCACAGGCAG
     U1 GATGTCCACGAGGTCTCT
  UPTAG                    GATACACGAGTCACAGGCAG

   GL21  CACAGTACAAGAATATTTAGAAATTACGGTATATGAG
     D1  CGGTGTCGGTCTCGTAG
DOWNTAG                    CACTTAGGTGGAATATCGAG
```

GL·39

```
   GL39 GATGTCCACGAGGTCTCTTAACGGACTCTAATCCC-AGC
     U1 GATGTCCACGAGGTCTCT
  UPTAG                    TAACGGACTCTAATCCCGAGC

   GL39  CGGTGTCGG-CTCGCAGACGTATGACCCATTCGATAGA
     D1  CGGTGTCGGTCTCGTAG
DOWNTAG                    ACGCATGACCCATTCGATAGA
```

- no mutations in the URA3 for either strain
- mutations in both D1s → explains why U1/D1 PCRs didn't work (p40-42)
- mutations in both downtags
- no mutations in GL·21 uptag
- 1 mutation in GL·39 uptag

8/20

PRACTICE RUN

I started a practice run of what will be the big expt. using two strains:

(1) GIL051 - this is BY4741 transformed w/ W303 URA3 - hereafter in this expt called GL-51

(2) YEL020CΔ from the Δ collection transformed with W303 URA3 (transformant #2, pg 13-19) - hereafter called GL-0

Wednesday night (8/17) I started 2×SC-ura + ADE/TRP cultures of GL-0 & GL-51

Thursday (8/18) I started fluct^n tests of each strain

96 cultures in 100λ of YPD+ADE

→ half for URA3 fluct^n test, half for CAN1 test

Today I will plate them using the TECAN

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

8/21

COULTER COUNTS

Bkg	30			
GL.0 F1	170256	169732	170628	
" 2	127396	127598	127828	
" 3	137104	141414	134210	
Post Bkg				
Bkg				
GL.0 C1	134506	133232	134162	
" 2	141504	139920	140220	
" 3	108276	108804	108032	
Post Bkg				
Bkg				
GL.51 F1	120458	129152	136310	
" 2	150494	148016	147386	
" 3	124302	123044	121034	
Post Bkg				
Bkg				
GL.51 C1	155106	154962	154142	
" 2	112554	111810	113594	114682
" 3	~~114682~~	115798	115302	117594
Post Bkg	1448	1004	584	572

F = from 5FOA half of the 96 well plate

C = from the Carcinomma half of the plate

8/23

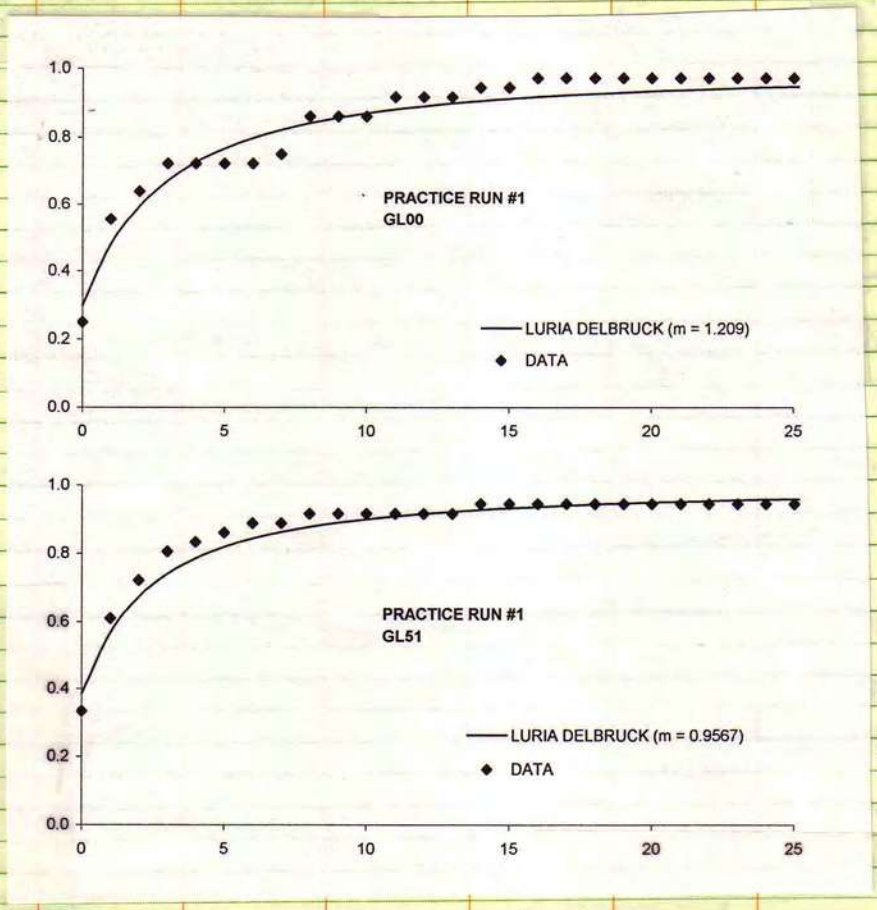
Counting CAN plates

GL·0

	1	2	3	4	5	6	7	8	9
1	11(1)	1(0)	1(0)	1(0)	8(0)	0(0)	11(0)	1(0)	0(0)
2	1(0)	1(1)	16(1)	1(0)	3(0)	0(0)	140	2(0)	8(0)
3	8(0)	0(0)	0(0)	1(0)	143	0(2)	1(0)	1(0)	0(0)
4	0(0)	8(0)	7(0)	3(0)	2(0)	2(1)	1(0)	3(1)	0(0)

GL·51

	1	2	3	4	5	6	7	8	9
1	5	3(0)	4(1)	1(0)	1(0)	0(0)	2(0)	1(0)	0(0)
2	0(0)	1(0)	0(0)	0(1)	8(0)	3(0)	14(0)	0(0)	2(2)
3	~~6(1)~~ 77	6(1)	1(0)	0(0)	1(0)	~~0(0)~~ 1(0)	0(0)	1(0)	2(0)
4	2(0)	0(0)	47(0)	3(0)	1(1)	1(1)	0(0)	0(0)	0(1)



Cumulative frequency

of Can^R cells per culture

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

9/1

I never finished my last practice run. The 5FOA plates did not turn out well.

- very few colonies on GL·0 plates
- lots of jackpots on GL·51 plates

PLATE		~0 colonies	thousands
51	1	3	6
51	2	2	7
51	3	1	8
51	4	1	8

} jackpot in starter culture?

→ but I used -ura media (unless I accidently put CSM instead of CSM-ura in media.

8/31 PRACTICE RUN #2

~~Start over~~

Make several adjustments and redo practice run

(1) Use 250λ cultures of media for 5FOA plates.

(2) Due to (#1) I will now only put 6 spots/plate ⟹ reprogram TECAN

(3) Try both 2xSC and YPD

GL·0 in 2xSC

GL·0 in YPD

GL·51 in 2xSC

GL·51 in YPD

(4) Filter all media

9/4

Coulter Counts

using 10 λ

Bkg 94

GL·0 sc 1	141188	140140	140668	
" 2	141106	141488	140716	
" 3	141822	141156	140178	
GL·51 sc 1	119484	119940	123136	120076
" 2	123708	123946	123368	
" 3	125136	125338	125256	
GL·0 YPD 1	161260	164350	155874	← partially obstructed
" 2	136054	135344	136138	
" 3				
"				
GL·51 YPD 1	125346	126888	126872	
" 2	129022	130498	130238	
" 3	109442	109096	109092	

using 5 λ

Bkg

GL·0 sc 1			
" 2			
" 3			
GL·51 sc 1			
" 2			
" 3			
GL·0 YPD 1	64990	65156	64348
" 2	67432	68092	67156
" 3	56404	57456	55828
GL·51 YPD 1	59574	58880	59566
" 2	63716	62604	63070
" 3	60792	58438	57928

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

9/5

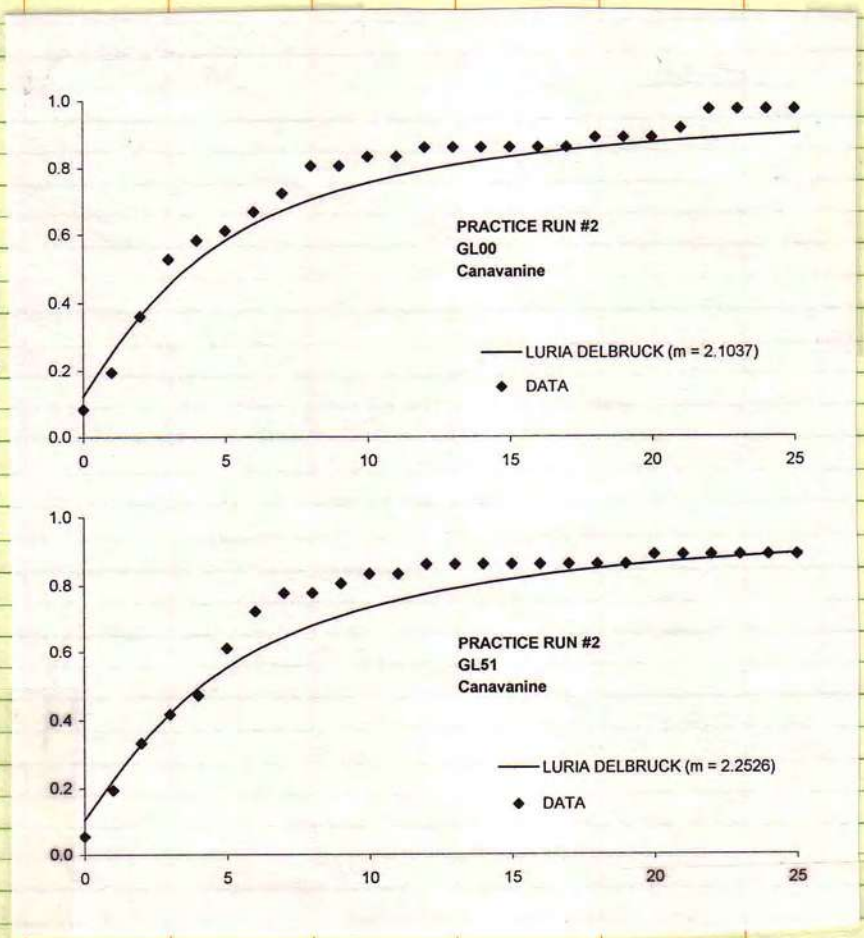
Count Canavanine plates

GL0 - SC

	1	2	3	4	5	6	7	8	9
1	6(3)	1(0)	18(1)	1(2)	~~7(2)~~ 8(1)	8(0)	1(0)	2(4)	8(0)
2	4(0)	35(0)	2(0)	1(1)	22(1)	21(0)	3(1)	0(1)	21(0)
3	2(1)	3(7)	3(1)	6(0)	2(0)	3(0)	5(1)	2(0)	7(0)
4	3(0)	3(0)	0(0)	7(1)	2(0)	4(0)	10(1)	0(0)	12(0)

GL51 - SC

	1	2	3	4	5	6	7	8	9
1	4(0)	51(0)	6(0)	9(0)	48(0)	5(0)	1(1)	3(0)	1(0)
2	2(0)	5(0)	10(1)	6(0)	0(1)	7(2)	34(2)	4(1)	6(4)
3	7(0)	6(0)	12(0)	20(0)	2(0)	1(0)	2(0)	35(1)	5(0)
4	3(1)	5(0)	1(0)	2(1)	3(0)	5(0)	2(0)	0(0)	1(0)



of Can^R cells per culture

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

9/10

It looks like I can actually count the 5FOA plates from last week.

GL·0 SC

	1	2	3	4	5	6
1	0	0	J(1000)	0	0	0
2	0	3	4	4	0	0
3	0	0	1	0	0	1
4	0	0	J(~60)	5	2	8
5	~~9~~ 32	9	0	1	0	0
6	0	0	0	2	2	4

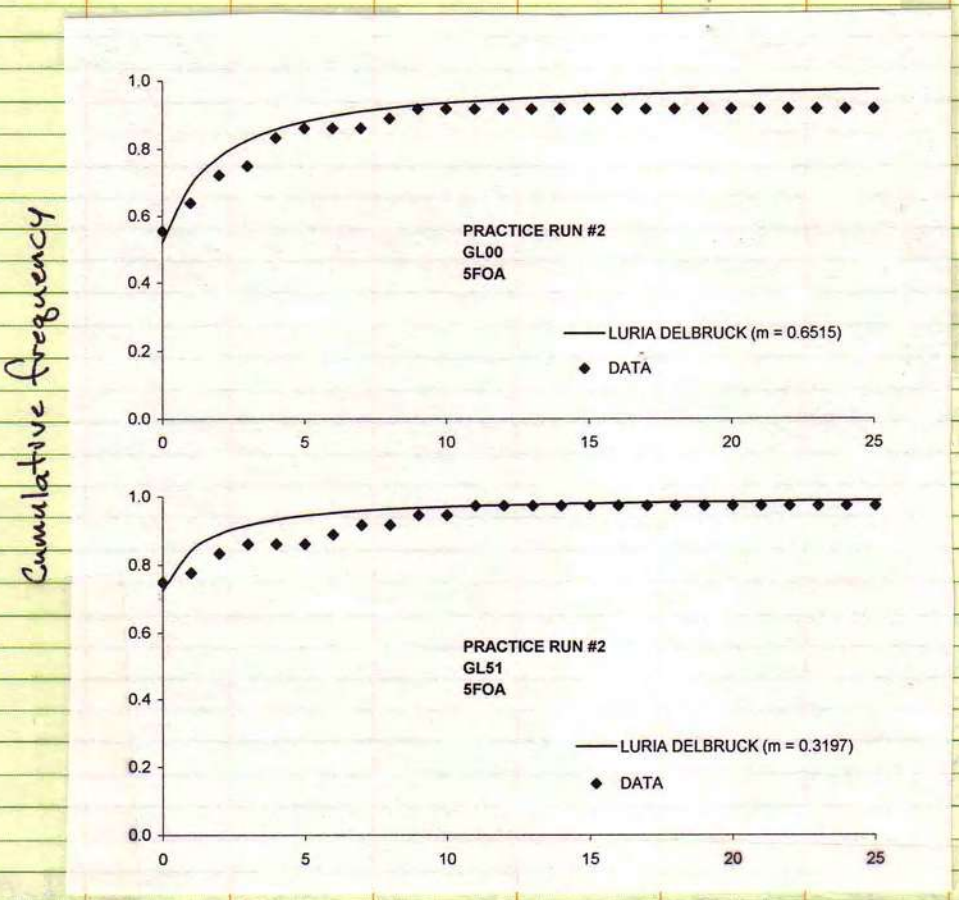
GL·51 SC

	1	2	3	4	5	6
1	3	0	0	0	1	11
2	0	0	0	0	0	2
3	0	0	0	0	0	0
4	0	0	0	6	0	0
5	9	2	0	0	0	0
6	0	0	28	7	0	0

Note: I was obvious on these plates the colonies grew better at the edge of the spots than in the middle — the was especially clear on the jackpots.

This suggests that the problem may be uracil depletion

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 692 10VG


Cumulative frequency
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
PRACTICE RUN #2
GL00
5FOA
LURIA DELBRUCK (m = 0.6515)
DATA
PRACTICE RUN #2
GL51
5FOA
LURIA DELBRUCK (m = 0.3197)
DATA
# of 5FOA^R cells per culture

9/7

NOTES FROM PRACTICE RUNS #1 & #2

Which nonselective media should I use? YPD or 2×SC?

- With YPD cultures fewer cells are left behind in the wells of the 96 well plates → perhaps YPD has higher surface tension. → perhaps I could get the same effect by adding a small amount of glycerol to the media
- YPD is easier to see → a benefit for YPD, but not a reason to choose it.
- YPD cultures supposedly saturate at a higher density although I don't see a significant difference in my coulter counts
- 2×SC cultures seem to clog the coulter counter less than YPD (even when both have been filtered).

What dilution should I do for the Coulter Counter?

According to Kevin (although I should look it up for myself) the coulter counter is most accurate in the 10,000 count range:

10 λ in 10 mL → 100,000 count range

5 λ in 10 mL → 50,000 range, but may be less accurate because of the error in pipetting 5 λ.

- Perhaps I should do 10 λ into 20 mL.

PLATE PROBLEMS:

Again my 5FOA plates do not look good. For PR#1 I used lab stock plates, for PR#2 I made my own plates with 1.5× the normal 5FOA conc. Both times I had too much background growth and few (less than expected) countable colonies.

These two problems (too much bkg growth and too few countable colonies) could be linked — overgrowth on the plate uses up all of the available uracil and prevents any true ura3 mutant from forming a colony

9/9

Possible solutions to the problem:

A) Bigger Spots

(1) drier plates (twice filtered?)

(2) add H_2O to cultures before plating

(3) reduce surface tension — but then I would leave more cells behind.

NOTE:

Average diameter of 100λ spot ~ 13 mm ⟶ ~~530~~ 66 mm^2

Average diameter of 250λ spot ~ 17 mm ⟶ ~~910~~ 114 mm^2

$\frac{250}{100} = 2.5 \times$ # of cells, only $\frac{\cancel{910}\ 114}{\cancel{530}\ 66} = 1.7\times$ area

*I want ~~~20.5 mm~~ ~20.5 mm diameter ~~circ~~ spots

B) Drug Concentration

It could be that my effective drug concentrations are too low. This could explain the bkg growth and the lack of positive colonies if the bkg uses up all of the uracil.

~~How could the dru~~

Compare the curves from PR1 & PR2 (ps 65 and 69). The ones from PR2 show characteristics of post-plating growth: small zero class and a poisson shoulder.

How could ~~I get~~ the drug concentration be too low?

- filters removed too much liquid
- I made the plates for PR2 and I recall adding the drug ~~to~~ right after the flasks came out of the autoclave — perhaps some of the drug degraded.

(1) smaller filters

(2) more drug

9/9

→ I could put only 4 spots/plate, but this would require 9 plates

c) Too much liq. added to plate (1.5 mL on 5FOA compared to 0.9 mL on ~~Canavanine~~)

This could cause a few problems:

(1) cells could divide before absorption into plate
- unlikely because these are in stationary phase and would need to go through lag
- I could arrest cells on the plate (for ~~the~~ example with αF). They would then need to wait for the αF to degrade before they could divide.

(2) added uracil could inhibit the action of 5FOA

2×SC: 40 μg/mL URACIL

5FOA plate: 10 μg/mL (0.25 mg/25 mL plate)

~~adding 10 μg/mL chan~~

adding 1.5 mL of 2×SC changes the uracil concentration in the plate from 10 μg/mL to 12.4 μg/mL – unlikely to be significant. Besides some uracil would be lost with the filter.

- I could always add less uracil to the 5FOA plates

(3) added nutrients from 2×SC could allow blq to grow
- I could switch to 1×SC.

(4) More liquid → more cells → greater depletion of uracil

(D) pH

5FOA plates need to be below pH 4.5 to work well. I used a pH strip to determine that both ~~an u~~ an unused and a used plate has a pH near 3.5, so this is not a cause of the problem.

LITHOGRAPHED IN U.S.A. ✦ ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 692 10VG

9/10

TROUBLESHOOTING THE PLATES

Yesterday I started 2×SC-ura cultures of GL·0, GL·51, GL·26, & JYL338.

Today I will start cultures ~~to test diffe~~ which will be used to test several of the possible reasons why the plates have not been looking good (p)

"small" = 8.26 cm diam
"large" = 9.0 cm diam
"smallest" = 7.5 cm diam

Plating Strategy:

5FOA – JUST GL·0

A 2 plates: 9 spots of 100λ cultures on 1×5FOA w/ small filters

B 2 plates: —— " —— w/ large filters

C 2 plates: —— " —— 1.5 × 5FOA w/ large filters

D 2 plates: —— " —— dry 1.5×5FOA ~~1×5FOA~~ w/ large filters

E 2 plates: —— " —— 1×5FOA w/ smallest filters

F 2 plates: 6 spots of 250λ cultures on 1.5×5FOA w/ large filters

G 2 plates: —— " —— 200 ~~250~~λ cultures + 50λ H_2O —— " ——

H 3 plates: 4 spots of 250λ cultures —— " ——

I 3 plates: —— " —— of 200λ cultures + 50λ H_2O —— " ——

5FOA – ALL 4 STRAINS

J 2 plates: 6 spots of 250λ cultures on 1× 5FOA w/ large filters

K 2 plates: —— " —— 200λ cultures + 50λ H_2O —— " ——

L 3 plates: 4 spots of 250λ cultures —— " ——

M 3 plates: —— " —— 200λ cultures + 50λ H_2O —— " ——

Canavanine – ALL 4 STRAINS

N 2 plates: 9 spots of 100λ cultures on old 10× Canavanine w/ large filters

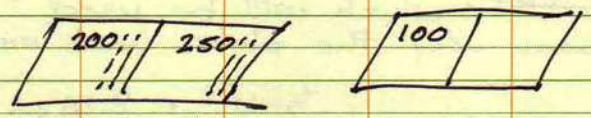
O 2 plates: —— " —— new —— " ——

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

9/10

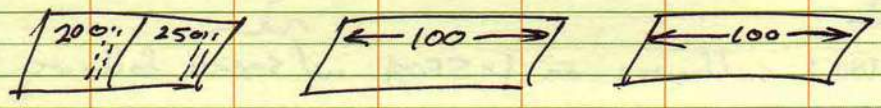
Set up the following 96-well plates:

All 4 strains:



6 plates

in addition, for GL-0



liquid:

GL-0 } 60 mL

GL-51
GL-26
J41338 } 30 mL

9/12

Plate A-O (p77) using the TECAN

- A-E, N, O using program "fluctPlating_9well"
- F-M using program "platingTest_050912"
- Allow plates to dry upright for at least 1.5h prior to putting them in 30° incubator

9/14

Count N & O plates

GL·0 (N)

	1	2	3	4	5	6	7	8	9
1	22(13)	2(0)	21(~60)	15(5)	6(1)	8(1)	5(0)	2(2)	27(0)
2	3(0)	4(0)	1(1)	11(0)	14(1)	0(3)	4(1)	7(0)	16(0)

GL·0 (O)

	1	2	3	4	5	6	7	8	9
1	2(0)	3(0)	0(1)	1(1)	0(0)	0(0)	0(0)	2(0)	0(0)
2	4(0)	6(0)	33(0)	1(0)	1(1)	4(0)	1(0)	0(0)	0(0)

GL·51 (N)

	1	2	3	4	5	6	7	8	9
1	8(0)	6(1)	2(0)	2(0)	4(2)	0(0)	21(0)	2(0)	1(1)
2	4(0)	1(0)	2(0)	4(0)	2(0)	13(0)	1(1)	6(0)	4(0)

GL·51 (O)

	1	2	3	4	5	6	7	8	9
1	3(0)	0(0)	1(0)	5(0)	0(0)	9(0)	5(1)	12(0)	0(0)
2	0(1)	3(1)	0(0)	0(1)	2(0)	2(0)	2(0)	7(2)	1(0)

GL·26 (N)

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

GL·26 (O)

~~JYL338 (N)~~

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

JYL338 (N)

	1	2	3	4	5	6	7	8	9
1	34(2)	15(7)	24(2)	19(6)	45(3)	6(12)	4(6)	16(1)	13(3)
2	2(1)	15(5)	15(5)	8(0)	2(2)	11(3)	21(4)	15(0)	32(1)

JYL338 (O)

	1	2	3	4	5	6	7	8	9
1	13(0)	7(1)	5(0)	4(1)	13(0)	5(0)	2(0)	5(0)	16(0)
2	16(0)	1(0)	6(3)	9(0)	4(1)	3(1)	6(0)	7(0)	6(0)

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

9/16

Recount N ≠ 0 plates – they sat on the benchtop for two days (since 9/14)

GL·0 (N)

	1	2	3	4	5	6	7	8	9
1	42(0)	7(3)	91(0)	23(0)	11(1)	10(3)	7(1)	5(2)	29(1)
2	4(2)	10(3)	3(0)	15(0)	16(1)	8(5)	11(3)	12(2)	23(12)

GL·0 (0)

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

GL·51 (N)

	1	2	3	4	5	6	7	8	9
1	12(1)	13(1)	5(5)	3(1)	8(1)	7(0)	34(15)	8(4)	5(0)
2	8(0)	5(3)	6(3)	7(2)	6(4)	16(0)	5(1)	7(1)	8(3)

GL·51 (0)

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

GL·26 (N)

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

GL·26 (0)

	1	2	3	4	5	6	7	8	9
1	2(0)	3(0)	1(0)	2(0)	17(0)	0(1)	0(0)	0(0)	9(0)
2	1(0)	5(0)	0(0)	1(0)	10(0)	5(1)	3(2)	1(0)	1(0)

JYL338 (N)

	1	2	3	4	5	6	7	8	9
1									
2									

JYL338 (0)

	1	2	3	4	5	6	7	8	9
1									
2									

9/16

Count K plates

GL·0

	1	2	3	4	5	6
1		0(0)			0(0)	
2			0(0)			0(0)

GL·51

	1	2	3	4	5	6
1	0(0)		0(0)	0(0)	0(0)	
2	0(0)		0(0)	0(0)	0(0)	

GL·26

	1	2	3	4	5	6
1					0(0)	0(0)
2			J	0(0)		0(0)

JYL338

	1	2	3	4	5	6
1				0(0)		
2						

I ~~of~~ only counted for what clearly looked like zeros and what did not. I will recount these plates in a few days.

9/19

Recount K plates

GL·0

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

GL·51

	1	2	3	4	5	6
1	0(0)	2(2)	0(0)	0(0)	0(0)	24(0)
2	1(0)	2(0)	0(0)	0(0)	0(0)	19(0)

GL·26

	1	2	3	4	5	6
1	10(5)	3(0)	37(1)	3(0)	0(0)	0(0)
2	0(0)	0(0)	J(~1000)	0(0)	1(0)	0(0)

JYL338

	1	2	3	4	5	6
1	3(0)	20(0)	45(0)	0(0)	96(0)	7(0)
2	51(0)	2(0)	8(0)	20(0)	10(0)	5(0)

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

9/13

PRACTICE RUN #3

Today I started o/N cultures for my third (and final?) practice run before I do the big expt.

~~(1) 3 mL~~ · GL·0 (5λ) into 3 mL 2×SC-ura (filtered)

· GL·51 (5λ) into 3 mL 2×SC-ura (filtered)

For this run I will:

(1) filter all media — this is especially helpful in the nonselective media because it keeps the Coulter count bkg low — but for consistence I will filter ~~everything I can~~ all liquids.

(2) dilute 10λ into 20 mL for Coulter counter

~~9/17~~ 9/16

On wednesday (9/14) I set up fluct^n tests for GL·0 and GL·51.

· dilute o/N SC-ura cultures 1:10 (100λ into 900λ) in filtered H_2O

· ~~dilute~~ 20λ of 1:10 dilution into 20 mL filtered 2×SC

· 200λ into columns 1-6 of 96 well plate

· 100λ into columns 7-12 of 96 well plate

· plate seal

· 30° ~ 3:00

9/16

· Plate GL·0 & GL·51 fluctuation tests using TECAN program "fluctPlating_9and6well_050916"

· let plates dry upright o/N.

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

9/17

MAKING PLATES

5FOA

2 L H_2O

60 g Dextrose

21 g YNB (w/o amino acids)

60 g Agar

3 g CSM-ura

— Stir —

1 L H_2O

— Autoclave — (P3, liquid 30 min exposure)

3 g 5FOA

30 mL Uracil (1 g/L)

15 mL Ade + Trp (2 mg/mL each)

— Pour —

Notes

- made ~~15L~~ 9L (3 flasks of 3L)
- each flask labeled uniquely (1, 2, or 3 black stripes on plates)
- Drug added ~45 min after liq came out of autoclave.
- Started pouring ~1h after liq came out of autoclave.
- flask #3 was put in 60° waterbath ~30 min as a precautionary measure

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 692 10VG

-arg + 10 × Canavanine

2 L H_2O

60 g Dextrose

21 g YNB (w/o ammo acids)

60 g Agar

3 g CSM-arg

— Stir ———— ⧖.

1 L H_2O

— Autoclave ———— (P3, liquid 30 min exposure)

30 mL Ade + Trp (2 mg/mL each)

15 mL Uracil (1 g/mL)

30 mL Canavanine (60 mg/mL)

— Pour ————

Notes

- made 6 L (2 flasks of 3 L)
- each batch labeled uniquely (1 or 2 red stripes on plates)
- drug added ~1h after liq came out of autoclave
- Started pouring ~~1.2h~~ ~1.25 h after liq came out of autoclave
- flask #2 put in 60° water bath ~15 min as a precautionary measure.

9/17

THE REAL THING — O/N STARTER CULTURES

- Pull cold YPD cultures out from fridge — all 50 strains, labeled GL:0 – GL:49
- Inoculate 5λ of each culture into 3 mL of filtered 2×SC-ura
- ~~30°~~ for my 2 strains of reduced fitness (GL:16 & GL:27) I will also inoculate 100λ of culture into 3mL of filtered 2×SC-ura
- 30°C rollerdrum — 10:45

9/18

SET UP FLUCTUATION TESTS

- For the two strains of reduced fitness (GL:16 & GL:27) I will use the cultures started from a 5λ inoculum — they seem to be of equal density as all other cultures.
- Dilute all cultures 1:10 in filtered H_2O (100λ into 900λ).
- Dilute all cultures 1:1000 in filtered 2×SC (20λ into 20 mL).
- 200λ into columns 1-6 of 96 well plate
- 100λ into columns 7-12 of 96 well plate
- plate seal
- 30°C incubator: 0,1,3-24 – 2:45
  2,25-36 – 4:45
  37-49 – 5:45

9/19

- Overdry the plates (300 5FOA & 200 10×CAN) by placing a Whatman filter on them (Grade 3, 90mm diameter) using the replicating block

9/20

- ~~Plate all~~

PLATING THE FLUCTUATION TESTS

- Remove filters from plates
- ~~Put 9~~ Vortex each 96-well plate for ~~at least prior t~~ ~1h @ 1200 rpm ~~or~~ on the Titramax orbital shaker prior to plating
- plate cultures using TECAN program: "fluctPlating_9and6well_~~090520~~ 050920"
- allow to dry upright o/N on benchtop
- put tubes for coulter counting in fridge.

NOTES ON PLATING

- Completed in 2 rounds:

  (1) plates 0-15, 17-25 11:30 - 5:00

  (2) plates 16, 26-49 7:00 - 1:00

- I am glad that I made sure to uniquely label each flask of media when I made the plates (p). ~~For~~ for each strain I used two plates from each batch ~~(5FOA 1,~~ (2× 5FOA1, 2×FOA 2, 2× 5FOA 3, 2× CAN 1, 2× CAN 2) and I kept them in the same order throughout.
- I noticed during the plating of GL.0 & GL.1 that the spots on the 5FOA 3 plates were larger than on the other two. ~~Therefore, a~~ Because of this I had several spot-fusion events on these plates. Therefore, after the plating of GL.1, I no longer added the 50λ of H_2O to the cultures prior to plating on the 5FOA3 plates.

9/21

COULTER COUNT CULTURES

Since I am not sure if I should use 10 or 20 mL of coulter soln. per sample I will try both options on sample GL·0.

	Bkg		208	46	74
20 mL	GL·0	1	68206	69020	70956
	"	2	68428	68348	68568
	"	3	67870	67070	67700
10 mL	GL·0	1	126580	128160	125542
	"	2	125272	124292	124882
	"	3	127084	126196	126812

3 counts of the same dilution

X1	X2	X3
Y1	Y2	Y3
Z1	Z2	Z3

3 independent dilutions

	Bkg		346	246	232
	GL·1	1	60178	60716	61126
		2	60044	60416	60128
		3	57906	57826	57908
	GL·2	1	67970	68070	66762
		2	68060	67164	67364
		3	69078	68138	68388
	GL·3	1	60614	59220	59210
		2	59302	58580	58608
		3	58820	57966	57618
	GL·4	1	57464	57548	57244
		2	57172	57396	56748
		3	58142	57124	57944
	GL·5	1	65038	64108	63236
		2	67566	66932	67164
		3	66660	66486	66524
	GL·6	1	64534	64448	64262
		2	61626	64596	64586 63956
		3	60464	60514	59430
	GL·7	1	65368	64018	64210
		2	64852	64848	65062
		3	63894	63738	62836
	GL·8	1	62450	62044	62274
		2	63904	64612	64002
		3	65774	64198	66000
	GL·9	1	72432	73218	70654
		2	77214	71962	72888 72314
		3	71236	71046	71084
	GL·10	1	67250	66586	66646
		2	60930	59638	60114
		3	67564	67774	67078

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 692 10VG

GL·11	1	77798	74482	72964	73838	
	2	73092	73394	73546		
	3	78804	75910	72160	72686	72064
GL·12	1	68570	71130	71496		
	2	65848	66020	66510		
	3	64754	65120	65236		
Post Bkg		2432	1426	1438		
Bkg		192	140	96		
GL·13	1	59356	59494	61068		
	2	60926	~~40~~ 60470	60238		
	3	63610	60662	60208	60412	
GL·14	1	54418	53670	52948		
	2	52476	51528	51168		
	3	56496	56020	56402		
GL·15	1	38094	38696	38958		
	2	40100	38986	39188		
	3	37678	37940	37180		
GL·16	1	56358	55582	56562		
	2	53666	52962	53114		
	3	54258	54326	55364		
GL·17	1	66320	65548	64168		
	2	68226	67652	68054		
	3	65476	65424	64848		
GL·18	1	62758	61650	61760		
	2	60856	60986	60774		
	3	62014	63198	62802		
GL·19	1	63872	63864	63392		
	2	66418	64044	64512		
	3	65570	64354	64860		
GL·20	1	66216	66042	66690		
	2	65542	65126	65514		
	3	66090	65660	65742		
GL·21	1	71532	72488	72234		
	2	69378	69254	69724		
	3	71168	71016	70196		
GL·22	1	75986	75450	75008		
	2	70986	71496	70460		
	3	71948	73096	72430		

GL·23	1	69272	68924	69160
	2	75410	74098	73740
	3	74386	74310	73972
GL·24	1	61180	66212	63198
	2	65218	65750	64806
	3	64214	64624	64056
Post Bkg		1942	758	638

9/22

Continue Coulter counts

Bkg		128	80	108
GL·25	1	62692	63382	63340
	2	61362	61496	61920
	3	61566	61996	60612
GL·26	1	55962	55040	54978
	2	56314	51588	47288
	3	54884	54780	54616
GL·27	1	55982	55170	54526
	2	54500	54620	53856
	3	57256	56246	57570
GL·28	1	59762	59238	58746
	2	58106	57130	58188
	3	60252	59358	58870
GL·29	1	61344	61062	60868
	2	61492	60700	61108
	3	62324	62446	62458
GL·30	1	60926	61472	61062
	2	61854	61214	61900
	3	62186	62164	63116
GL·31	1	73024	69010	70030
	2	67580	67028	67642
	3	69044	67540	68338
GL·32	1	65522	65332	65326
	2	64200	63220	64384
	3	65592	65280	65008
GL·33	1	57630	58270	57804
	2	57690	57930	57924
	3	61174	59958	58840

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

GL·34	1	65258	65126	64316	
	2	68680	63730	64236	
	3	64028	63614	63836	
GL·35	1	57458	56450	56412	
	2	57734	57556	57026	
	3	55826	56266	56938	
GL·36	1	61054	~~61144~~ 61104	59794	
	2	62034	61336	61562	
	3	60616	60682	60318	
Post Bkg		1906	1160	836	
	~~1~~				
	~~2~~				
Bkg		104	86	30	
GL·37	1	66880	66098	65864	
	2	65356	66438	65030	
	3	67194	65656	65518	
GL·38	1	65536	64504	64700	
	2	65356	65420	64992	
	3	66946	66856	66876	
GL·39	1	62774	61294	61976	
	2	62272	62740	62574	
~~GL·40~~	3	63060	61892	62640	
GL·40	1	68394	68472	67674	
	2	63834	64746	63906	
	3	66828	66532	66052	
GL·41	1	71746	70966	71018	
	2	71938	71354	71438	
	3	71418	71234	71476	
GL·42	1	70152	69924	70246	
	2	69418	69224	69688	
	3	69808	69592	69412	
GL·43	1	71986	71784	72494	
	2	70614	74658	74148	73796
	3	71024	71744	71782	
GL·44	1	53884	53770	53880	
	2	51920	52314	51996	
	3	53818	53650	53364	
GL·45	1	63044	62538	62100	
	2	63212	67698	63138	63404
	3	62938	62086	63006	

GL·46	1	62242	60836	61022
	2	61172	60782	61250
	3	60546	59688	60082
GL·47	1	67652	66722	67572
	2	61908	62578	62062
	3	68106	66912	66984
GL·48	1	67412	66512	66734
	2	66092	66282	65732
	3	66208	65974	66944
GL·49	~~1~~	~~66208~~	~~65974~~	~~66944~~
	1 2	73242	71892	72390
	2 3	70456	70330	70694
	3	70074	70692	70962
GL·13	1	66070	65966	66048
	2	62722	62150	62448
	3	61874	61332	62066
		2364	1386	1072

NOTES ON COULTER COUNTING

- While the cultures were being spot plated, the TECAN put 3 cultures from each column into a 5 ml Falcon tube. Once all of the cultures were plated the 50 tubes were put in the fridge.
- The counting was done in 4 sessions over two days. Sample GL·13 was processed on both days to make sure that the counts were not appreciably different between the days — GL·13 was therefore sonicated twice.
- Each sample:
  - remove from fridge
  - vortex
  - sonicate 2 min
  - vortex
  - 10 λ into 20 ml Coulter fluid (repeat twice)
- All of the coulter liquid was filtered through 0.45 μm filter
- | – rinse and flush
- after 3 counts, if one appears to be an outlier, I will recount the same dilution until I get 3 counts with good agreement.

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

9/23

COUNTS FROM CANAVANINE PLATES

GL·0

	1	2	3	4	5	6	7	8	9
1	5(0)	0(0)	1(0)	2(0)	1(0)	2(0)	2(0)	1(1)	0(0)
2	3(0)	0(0)	7(0)	0(0)	3(0)	0(0)	1(0)	3(0)	12(0)
3	3(0)	0(1)	0(1)	0(0)	3(0)	2(0)	3(0)	17(0)	5(0)
4	14(0)	2(1)	0(0)	2(0)	7(0)	3(0)	4(0)	0(0)	0(0)

GL·1

	1	2	3	4	5	6	7	8	9
1	2(0)	6(0)	13(0)	1(0)	1(0)	5(1)	6(0)	2(0)	17(0)
2	41(0)	6(0)	4(0)	1(0)	3(0)	60(0)	3(1)	4(0)	3(2)
3	0(0)	3(0)	0(1)	3(0)	5(0)	0(0)	1(0)	0(0)	1(0)
4	1(0)	3(0)	0(0)	3(0)	1(0)	9(0)	3(1)	2(0)	0(0)

GL·2

	1	2	3	4	5	6	7	8	9
1	2(0)	3(1)	4(0)	1(0)	2(0)	19(0)	0(0)	4(0)	3(0)
2	1(0)	3(0)	1(0)	0(0)	0(3)	0(0)	1(0)	27(0)	0(0)
3	0(0)	3(0)	25(1)	1(0)	6(0)	1(0)	3(0)	0(0)	2(0)
4	4(1)	0(0)	2(0)	4(0)	0(0)	0(0)	0(0)	3(0)	5(1)

GL·3

	1	2	3	4	5	6	7	8	9
1	0(0)	0(0)	1(0)	12(0)	2(0)	1(1)	12(0)	0(0)	4(2)
2	2(9)	3(0)	77(0)	91	0(0)	1(0)	1(0)	0(0)	3(0)
3	10(0)	1(2)	1(0)	6(0)	14(0)	0(2)	1(0)	29(0)	0(0)
4	0(0)	2(0)	2(0)	0(0)	0(1)	0(0)	2(0)	2(0)	1(0)

GL·4

	1	2	3	4	5	6	7	8	9
1	2(1)	3(0)	2(0)	0(0)	4(2)	2(1)	1(2)	1(0)	1(0)
2	5(1)	0(0)	4(0)	2(0)	1(0)	0(0)	0(0)	2(0)	1(0)
3	18(0)	1(0)	2(0)	4(0)	2(0)	3(0)	1(0)	0(0)	88(0)
4	0(0)	1(0)	1(0)	6(0)	5(0)	1(0)	0(0)	1(0)	2(0)

GL·5

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

GL·6

	1	2	3	4	5	6	7	8	9
1	0(1)	1(0)	10(0)	0(0)	2(0)	43(2)	1(0)	0(0)	1(1)
2	0(0)	1(0)	10(0)	1(0)	2(0)	4(2)	0(0)	28(0)	0(0)
3	0(0)	12(0)	3(0)	0(0)	22(0)	2(0)	1(0)	105(0)	3(0)
4	2(0)	3(3)	26(0)	25(0)	0(0)	1(1)	0(0)	1(0)	3(0)

GL·7

	1	2	3	4	5	6	7	8	9
1	6(0)	97	1(0)	0(0)	1(0)	5(1)	1(0)	1(1)	1(0)
2	7(0)	3(0)	1(1)	3(0)	1(0)	1(1)	2(0)	1(1)	0(0)
3	2(0)	2(0)	1(0)	2(0)	7(0)	2(0)	3(1)	1(1)	2(0)
4	2(1)	0(0)	0(1)	1(1)	1(1)	2(0)	4(0)	0(0)	2(0)

GL·8

	1	2	3	4	5	6	7	8	9
1	1(1)	13(1)	7(0)	0(1)	3(0)	16(1)	3(0)	2(0)	1(0)
2	12(0)	1(0)	0(0)	0(0)	0(0)	2(0)	1(0)	2(1)	4(1)
3	2(1)	0(1)	6(1)	0(0)	2(0)	1(0)	3(0)	2(1)	21(2)
4	10(0)	4(2)	0(0)	1(0)	0(0)	0(0)	5(0)	6(0)	4(0)

GL·9 9/23

	1	2	3	4	5	6	7	8	9
1	3(0)	9(0)	2(0)	4(0)	0(0)	3(0)	2(1)	10(0)	5(0)
2	0(1)	10(0)	31(1)	0(0)	2(0)	5(0)	6(0)	17(0)	0(0)
3	2(0)	20(0)	1(0)	0(0)	0(0)	6(0)	6(0)	0(0)	8(0)
4	1(0)	0(0)	28(0)	0(1)	18(0)	0(1)	1(0)	1(0)	49(1)

GL·10

	1	2	3	4	5	6	7	8	9
1	3(0)	3(0)	2(0)	2(0)	1(0)	0(0)	0(0)	6(0)	33(0)
2	21(1)	1(0)	0(0)	3(10)	0(0)	1(0)	2(2)	1(1)	14(0)
3	2(0)	0(1)	3(1)	0(0)	0(0)	0(0)	1(0)	1(1)	0(1)
4	1(0)	2(0)	0(0)	2(1)	1(0)	2(0)	11(1)	4(3)	0(0)

GL·12

	1	2	3	4	5	6	7	8	9
1	1(0)	118	6(0)	3(0)	1(0)	1(1)	3(0)	27(0)	8(0)
2	1(0)	1(1)	0(0)	2(1)	1(1)	3(0)	0(0)	4(0)	7(0)
3	5(0)	1(0)	0(0)	1(1)	3(0)	3(0)	2(0)	1(1)	2(0)
4	0(0)	3(0)	2(0)	0(0)	3(0)	0(0)	0(0)	2(1)	0(0)

GL·13

	1	2	3	4	5	6	7	8	9
1	4(0)	2(0)	18(0)	2(0)	8(1)	1(0)	2(75)	4(0)	0(1)
2	13(0)	23(2)	0(0)	2(0)	0(1)	1(0)	3(2)	0(0)	0(1)
3	0(1)	1(2)	26(0)	0(0)	0(1)	1(0)	0(1)	0(1)	2(0)
4	6(0)	0(1)	1(0)	1(2)	5(2)	0(0)	2(0)	0(0)	0(8)

GL·14

	1	2	3	4	5	6	7	8	9
1	38	1(0)	2(0)	14(0)	4(1)	17(0)	1(1)	4(0)	1(0)
2	22(1)	1(1)	4(0)	2(0)	0(1)	0(0)	16(0)	2(1)	2(0)
3	0(1)	0(19)	4(0)	0(3)	2(0)	1(0)	0(1)	34(0)	0(1)
4	5(0)	1(0)	8(0)	1(0)	0(0)	7(0)	3(1)	1(0)	0(1)

GL·15

	1	2	3	4	5	6	7	8	9
1	0(1)	2(0)	0(0)	0(0)	9(1)	4(0)	1(3)	0(1)	0(1)
2	1(1)	2(1)	2(0)	2(2)	1(0)	1(3)	3(0)	1(0)	0(0)
3	2(0)	1(0)	2(1)	1(0)	8(0)	1(0)	2(0)	0(24)	0(0)
4	0(0)	2(0)	0(0)	1(0)	1(0)	0(0)	0(0)	0(0)	2(0)

GL·17

	1	2	3	4	5	6	7	8	9
1	0(1)	0(0)	0(0)	16(4)	3(12)	11(0)	3(0)	0(0)	1(1)
2	4(0)	1(0)	0(0)	1(0)	4(0)	16(0)	0(0)	6(1)	1(1)
3	0(1)	3(25)	0(1)	0(0)	3(1)	3(0)	6(0)	103	4(1)
4	0(2)	2(0)	1(0)	0(1)	7(2)	2(2)	0(0)	1(2)	2(4)

GL·18

	1	2	3	4	5	6	7	8	9
1	10(0)	4(1)	4(0)	0(1)	36(2)	0(1)	2(0)	48(0)	1(0)
2	13(3)	0(5)	1(0)	0(1)	29(0)	0(0)	8(0)	1(0)	3(1)
3	9(0)	1(0)	0(0)	4(0)	0(0)	33(0)	0(0)	0(2)	2(0)
4	0(0)	0(0)	2(0)	13(0)	7(0)	2(0)	0(0)	0(0)	0(0)

GL·19

	1	2	3	4	5	6	7	8	9
1	0(0)	0(0)	0(0)	5(0)	0(0)	2(1)	44(0)	2(0)	0(0)
2	2(1)	0(0)	36(1)	19(0)	1(1)	0(0)	2(0)	27(0)	11(0)
3	3(0)	1(0)	0(0)	2(0)	3(0)	3(0)	0(8)	17(0)	3(0)
4	3(0)	0(0)	1(0)	8(0)	1(0)	1(0)	0(0)	6(18)	0(0)

9/23

GL·20

	1	2	3	4	5	6	7	8	9
1	7(6)	1(0)	3(2)	2(0)	1(0)	1(0)	0(0)	0(0)	5(0)
2	0(0)	19(0)	1(0)	1(0)	1(0)	3(1)	16(0)	4(0)	0(2)
3	0(3)	2(0)	2(0)	2(10)	1(1)	0(0)	0(0)	20(1)	0(0)
4	0(0)	2(1)	0(1)	8(23)	7(0)	0(1)	4(1)	0(0)	4(1)

GL·21

	1	2	3	4	5	6	7	8	9
1	0(0)	1(0)	1(0)	0(0)	3(0)	1(0)	0(0)	4(0)	2(0)
2	0(1)	0(0)	2(0)	2(0)	0(2)	4(0)	0(6)	25(0)	3(0)
3	2(0)	79(0)	2(1)	10(0)	15(0)	4(0)	5(0)	1(1)	4(0)
4	46(0)	4(0)	0(0)	2(0)	1(0)	2(1)	2(1)	1(0)	3(0)

GL·22

	1	2	3	4	5	6	7	8	9
1	2(1)	9(4)	7(0)	0(0)	31(2)	0(0)	2(0)	0(0)	0(0)
2	6(0)	3(0)	5(0)	0(0)	2(0)	0(0)	3(2)	1(2)	31(1)
3	0(1)	2(1)	3(0)	0(0)	30(0)	11(1)	0(0)	2(0)	3(0)
4	1(0)	1(0)	1(0)	10(0)	15(1)	0(1)	6(0)	0(1)	0(0)

GL·23

	1	2	3	4	5	6	7	8	9
1	2(0)	0(0)	2(0)	1(0)	1(0)	2(0)	0(0)	1(0)	0(0)
2	5(0)	0(0)	4(0)	1(1)	17(1)	43(2)	1(0)	0(1)	2(0)
3	6(0)	0(0)	0(0)	3(0)	0(0)	5(0)	0(0)	0(0)	0(0)
4	1(0)	3(0)	4(0)	0(0)	2(0)	0(0)	1(0)	2(1)	1(0)

GL·24

	1	2	3	4	5	6	7	8	9
1	0(0)	6(0)	4(0)	6(0)	1(0)	J(~1000)	1(0)	2(0)	1(0)
2	0(0)	3(0)	1(0)	68(1)	J(~1000)	1(0)	4(0)	0(0)	0(0)
3	5(0)	3(0)	0(0)	2(0)	3(0)	0(0)	2(0)	4(0)	1(0)
4	1(2)	1(0)	0(0)	0(0)	2(3)	0(0)	2(0)	30(0)	3(0)

GL·25

	1	2	3	4	5	6	7	8	9
1	3(2)	1(0)	9(0)	7(1)	7(0)	0(0)	0(1)	20(2)	5(0)
2	1(0)	16(0)	6(1)	3(0)	0(0)	2(0)	0(1)	15(1)	14(1)
3	1(0)	24(0)	0(0)	1(0)	0(0)	2(0)	2(0)	1(0)	6(1)
4	2(1)	49(0)	0(0)	1(0)	7(0)	2(0)	1(1)	0(0)	0(1)

9/23

9/24

GL·16

	1	2	3	4	5	6	7	8	9
1	1(2)	1(1)	2(1)	28(6)	21(3)	0(1)	2(0)	3(1)	3(2)
2	1(0)	0(0)	0(0)	0(0)	2(1)	11(0)	1(0)	4(0)	8(0)
3	2(0)	2(3)	2(2)	6(3)	1(0)	2(2)	0(0)	2(2)	7(2)
4	1(0)	3(1)	5(0)	4(0)	0(7)	1(1)	1(3)	1(6)	1(3)

GL·26

	1	2	3	4	5	6	7	8	9
1	1(0)	0(0)	0(0)	1(1)	1(1)	17(3)	0(0)	0(0)	3(0)
2	0(0)	0(0)	0(0)	2(0)	6(2)	0(1)	1(0)	0(0)	0(0)
3	0(1)	0(0)	1(0)	9(1)	0(0)	27(0)	0(0)	3(1)	0(0)
4	2(0)	3(0)	0(0)	1(0)	0(0)	10(1)	1(0)	0(0)	1(1)

GL·28

	1	2	3	4	5	6	7	8	9
1	1(0)	2(0)	0(0)	0(0)	6(1)	4(1)	1(0)	3(0)	0(0)
2	J(~300)	1(0)	1(1)	1(1)	4(0)	13(0)	13(0)	2(0)	6(2)
3	1(0)	4(1)	0(0)	2(0)	0(2)	0(0)	1(0)	2(0)	2(1)
4	0(0)	4(2)	1(0)	1(1)	1(1)	1(0)	4(0)	1(0)	0(0)

9/24

GL·29

	1	2	3	4	5	6	7	8	9
1	1(0)	0(3)	2(0)	21(1)	5(2)	2(2)	10(3)	0(0)	0(0)
2	2(0)	1(0)	1(0)	8(0)	0(0)	0(0)	0(1)	0(0)	16(1)
3	2(0)	0(1)	1(0)	3(0)	13(0)	1(0)	2(0)	6(0)	2(0)
4	57(14)	4(0)	5(0)	2(0)	0(0)	2(0)	1(0)	1(0)	4(0)

GL·30

	1	2	3	4	5	6	7	8	9
1	0(0)	0(0)	5(0)	0(0)	4(1)	1(0)	4(0)	2(0)	0(0)
2	0(0)	0(0)	2(0)	1(0)	0(0)	0(0)	10(0)	4(0)	8(0)
3	4(2)	0(0)	1(0)	0(0)	2(0)	1(0)	0(0)	2(0)	4(0)
4	0(0)	40(0)	19(0)	0(0)	1(0)	0(0)	2(0)	27(0)	5(0)

GL·31

	1	2	3	4	5	6	7	8	9
1	9(0)	24(0)	2(0)	0(0)	0(0)	4(0)	0(0)	0(0)	0(0)
2	0(0)	4(0)	5(~300)	1(2)	0(0)	1(0)	0(0)	5(0)	2(0)
3	0(0)	10(0)	0(0)	1(0)	0(0)	2(0)	1(0)	2(0)	5(0)
4	0(0)	1(0)	0(0)	2(0)	0(0)	1(0)	0(0)	3(0)	0(0)

GL·32

	1	2	3	4	5	6	7	8	9
1	1(0)	5(0)	5(0)	1(2)	4(3)	22(0)	0(1)	0(0)	2(1)
2	30(0)	0(1)	0(0)	1(0)	1(1)	1(0)	1(0)	12(0)	4(0)
3	2(0)	3(0)	0(0)	1(2)	1(1)	5(0)	2(0)	0(1)	0(0)
4	0(0)	7(0)	1(0)	0(0)	23(0)	5(0)	0(0)	1(0)	4(0)

GL·33

	1	2	3	4	5	6	7	8	9
1	2(0)	5(0)	0(0)	1(0)	0(0)	7(1)	10(0)	41(0)	0(0)
2	2(0)	2(5)	6(0)	0(0)	0(0)	5(0)	0(1)	2(0)	6(0)
3	14(0)	2(0)	0(0)	13(0)	0(0)	1(0)	10(0)	2(0)	14(0)
4	0(0)	0(0)	0(5)	0(0)	0(1)	2(0)(1)	0(0)	6(2)	2(0)

GL·34

	1	2	3	4	5	6	7	8	9
1	2(1)	1(0)	0(0)	0(0)	7(0)	6(1)	3(0)	14(0)	1(0)
2	5(0)	1(0)	27(2)	3(1)	6(1)	20(1)	0(0)	1(0)	6(0)
3	2(0)	2(1)	1(0)	0(0)	8(0)	67(0)	0(0)	3(0)	3(0)
4	1(0)	2(0)	5(6)	2(0)	0(0)	2(2)	11(6)	0(0)	4(0)

GL·35

	1	2	3	4	5	6	7	8	9
1	4(0)	1(0)	3(0)	0(0)	0(0)	0(0)	1(0)	9(0)	0(0)
2	0(0)	3(0)	0(0)	15(0)	0(0)	0(1)	2(0)	0(1)	4(0)
3	0(0)	2(0)	5(0)	12(0)	0(0)	5(1)	8(0)	2(1)	0(0)
4	0(0)	0(0)	10(0)	0(0)	2(1)	1(1)	5(0)	1(0)	1(0)

GL·36

	1	2	3	4	5	6	7	8	9
1	3(0)	3(0)	1(0)	0(1)	10(2)	0(1)	22(0)	0(1)	2(0)
2	4(0)	0(0)	1(3)	2(0)	1(1)	5(1)	5(17)	1(0)	0(0)
3	1(0)	2(0)	0(0)	2(0)	12(1)	3(0)	0(1)	8(0)	6(0)
4	2(2)	1(0)	0(0)	21(0)	4(0)	1(0)	1(0)	1(0)	1(0)

GL·37

	1	2	3	4	5	6	7	8	9
1	6(0)	2(0)	0(0)	0(1)	0(0)	11(1)	2(0)	2(0)	5(0)
2	1(0)	1(0)	0(0)	6(1)	12(0)	0(1)	8(0)	1(0)	6(0)
3	4(1)	50(0)	40(0)	0(0)	7(0)	0(0)	0(0)	2(0)	3(0)
4	2(0)	2(0)	0(0)	9(0)	1(1)	2(0)	3(0)	0(0)	4(7)

9/24

GL-38

	1	2	3	4	5	6	7	8	9
1	3(1)	1(2)	1(0)	0(0)	125(0)	1(1)	2(0)	1(0)	3(0)
2	0(0)	3(0)	~200	1(0)	3(0)	17(0)	0(1)	2(0)	0(0)
3	1(1)	2(0)	0(0)	0(12)	5(0)	1(3)	1(0)	1(0)	0(0)
4	1(1)	3(0)	2(1)	0(0)	0(0)	0(1)	0(0)	0(1)	1(0)

GL-39

	1	2	3	4	5	6	7	8	9
1	1(1)	1(1)	2(0)	14(0)	24(0)	3(0)	1(0)	4(0)	3(25)
2	7(0)	5(0)	24(0)	6(0)	0(0)	0(0)	~~1(1)~~ 2(0)	1(0)	15(0)
3	1(0)	6(0)	2(0)	2(1)	1(1)	8(7)	2(0)	4(0)	11(0)
4	12(1)	13(0)	1(0)	7(0)	0(0)	5(5)	9(0)	0(0)	1(0)

GL-40

	1	2	3	4	5	6	7	8	9
1	4(0)	1(0)	5(0)	39(0)	6(1)	39(0)	5(0)	2(0)	3(0)
2	12(0)	3(1)	9(0)	0(0)	4(2)	0(0)	1(0)	2(0)	2(1)
3	5(0)	6(0)	2(1)	0(1)	5(0)	4(0)	4(0)	2(0)	1(0)
4	0(0)	0(0)	0(1)	1(0)	0(2)	3(0)	0(0)	1(0)	9(0)

GL-41

	1	2	3	4	5	6	7	8	9
1	2(0)	18(2)	1(0)	2(0)	1(0)	4(0)	11(0)	0(0)	1(1)
2	0(0)	1(1)	2(0)	9(0)	4(1)	0(0)	0(0)	0(17)	7(0)
3	0(0)	39(1)	0(1)	6(0)	16(0)	1(0)	0(0)	19(3)	13(0)
4	0(1)	8(0)	0(1)	0(0)	0(0)	1(0)	11(0)	1(0)	1(0)

GL-42

	1	2	3	4	5	6	7	8	9
1	2(0)	0(0)	0(1)	17(0)	1(9)	2(1)	2(0)	3(0)	11(0)
2	1(2)	1(0)	1(1)	11(5)	0(0)	1(0)	1(0)	44(0)	5(2)
3	2(1)	3(0)	27(0)	2(0)	1(0)	7(0)	2(0)	2(0)	1(1)
4	4(1)	0(0)	3(0)	0(0)	11(2)	0(0)	0(1)	~~2(3)~~ 23(1)	6(0)

GL-43

	1	2	3	4	5	6	7	8	9
1	3(2)	96	3(0)	3(0)	1(0)	31(0)	2(0)	1(3)	2(2)
2	6(0)	6(0)	0(0)	2(0)	4(0)	0(1)	3(0)	5(1)	0(1)
3	4(0)	0(0)	7(0)	0(0)	3(0)	0(0)	156	19(1)	0(1)
4	2(0)	1(0)	1(0)	5(7)	10(0)	6(0)	6(0)	0(1)	9(0)

GL-44

	1	2	3	4	5	6	7	8	9
1	1(0)	14(0)	1(0)	4(0)	1(1)	0(0)	1(0)	1(0)	12(0)
2	0(0)	3(0)	1(0)	2(0)	3(1)	2(1)	0(2)	1(0)	2(0)
3	0(0)	4(0)	0(0)	5(0)	8(0)	1(0)	6(0)	0(0)	5(1)
4	0(2)	0(0)	0(0)	2(0)	0(1)	0(0)	2(1)	0(1)	4(1)

GL-45

	1	2	3	4	5	6	7	8	9
1	0(0)	33(2)	0(2)	0(2)	23(1)	0(0)	1(0)	5(0)	1(0)
2	1(0)	15(1)	14(0)	3(1)	0(1)	2(0)	2(1)	2(0)	3(1)
3	3(0)	1(0)	4(0)	0(0)	0(0)	2(0)	0(0)	5(2)	5(0)
4	2(1)	0(0)	4(0)	57	1(0)	1(0)	1(0)	1(0)	1(0)

GL-46

	1	2	3	4	5	6	7	8	9
1	0(1)	0(0)	14(0)	3(0)	13(0)	1(2)	12(0)	0(3)	4(0)
2	9(0)	3(1)	8(0)	1(0)	27(0)	3(1)	0(1)	21	0(2)
3	4(2)	0(0)	0(0)	1(0)	4(0)	36(0)	1(0)	63(1)	0(0)
4	1(0)	3(0)	8(0)	0(0)	10(0)	65(0)	0(0)	1(0)	2(1)

9/24

GL·47

	1	2	3	4	5	6	7	8	9
1	1(0)	48(0)	0(0)	3(1)	1(1)	9(0)	0(2)	2(0)	0(0)
2	5(0)	1(0)	0(1)	0(0)	15(0)	1(0)	7(0)	0(0)	6(0)
3	2(0)	3(0)	3(3)	0(0)	0(0)	8(0)	2(2)	15(0)	0(0)
4	1(1)	1(0)	25(0)	0(0)	1(0)	0(0)	6(0)	3(0)	0(0)

GL·48

	1	2	3	4	5	6	7	8	9
1	1(0)	0(0)	~200	11(15)	5(1)	1(0)	0(0)	4(0)	0(0)
2	0(0)	0(0)	1(0)	0(0)	7(0)	6(1)	0(0)	1(0)	2(0)
3	18(0)	1(1)	1(2)	1(0)	4(1)	0(0)	0(0)	4(0)	1(1)
4	0(0)	0(0)	1(0)	7(0)	3(0)	1(0)	4(0)	3(0)	3(0)

GL·49

	1	2	3	4	5	6	7	8	9
1	3(0)	1(0)	1(0)	5(0)	0(0)	15(2)	2(0)	1(0)	11(0)
2	0(1)	12(0)	0(0)	1(0)	1(0)	1(2)	0(0)	0(0)	0(1)
3	1(0)	39(1)	1(1)	1(1)	3(0)	5(0)	11(0)	9(0)	0(0)
4	2(1)	1(0)	4(0)	1(0)	4(1)	5(0)	5(0)	4(0)	5(0)

9/24

9/26

GL·11

	1	2	3	4	5	6	7	8	9
1	24(3)	37(1)	59(9)	50(2)	99(2)	J(~200)	24(2)	23(0)	68(2)
2	4(4)	40(1)	2(1)	J(~200)	76(1)	73(31)	29(1)	127(5)	36(11)
3	4(1)	61(15)	2(0)	7(2)	117	22(2)	57(1)	26(5)	~~66(5)~~ 5(2)
4	~~3~~66(5)	3(1)	15(1)	21(3)	45(9)	15(0)	25(0)	J(~100)	3(4)

GL·27

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

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

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

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

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

9/28

COUNTS FROM 5FOA PLATES

GL·0

	1	2	3	4	5	6
1	8(4)	0(0)	0(0)	2(1)	0(0)	26(21)
2	1(2)	1(1)	0(0)	1(2)	0(0)	0(4)
3	0(0)	4(1)	0(0)	0(0)	0(0)	0(1)
4	1(0)	12(4)	0(1)	2(0)	4(1)	0(0)
5	2(0)	2(0)	0(0)	0(0)	1(1)	0(0)
6	1(1)	11(1)	0(0)	2(0)	0(0)	1(0)

9/28

GL·1

	1	2	3	4	5	6
1	J(~10^4) E	J(~10^4) E	32(23) E	31(24) E	101(51) E	90(42) E
2	7(19)	15(24)	13(20)	153 E	61(12)	39(24)
3	64(13)	70(51)	36(11)	26(18)	49(37)	49(29)
4						
5						
6						

GL·2

	1	2	3	4	5	6
1	5(1)	17(4)	128 E	1(1)	9(3)	24(9)
2	7(1)	1(0)	2(0)	3(1)	15(5)	14(7)
3	0(2)	7(0)	6(7)	0(1)	0(0)	2(0)
4	1(0)	3(0)	0(2)	2(0)	3(0)	2(1)
5	15(6)	3(2)	2(0)	3(1)	2(0)	2(1)
6	10(2)	3(1)	J(~500) E	0(0)	6(4)	1(0)

9/29

GL·3

	1	2	3	4	5	6
1	4(2)	7(3)	3(1)	1(0)	51 E	5(2)
2	3(2)	0(3)	24(12)	2(4)	2(1)	5(2)
3	2(1)	13(14)	1(1)	4(2)	15(7)	6(0)
4	0(0)	3(0)	3(3)	0(0)	14(2)	2(0)
5	47(3)	4(0)	2(0)	1(0)	2(2)	20(1)
6	6(1)	4(0)	2(0)	0(2)	3(0)	14(13)

GL·4

	1	2	3	4	5	6
1	0(0)	0(0)	1(0)	0(0)	7(2)	5(1)
2	1(0)	14(8)	3(1)	1(0)	2(0)	7(5)
3	1(2)	11(6)	3(2)	0(0)	9(3)	2(0)
4	6(1)	0(0)	5(0)	0(2)	3(1)	6(0)
5	1(0)	82 E	0(0)	4(1)	23(9)	3(0)
6	2(1)	6(2)	4(2)	1(3)	6(0)	5(1)

GL·5

	1	2	3	4	5	6
1	1(2)	1(1)	~~11(3)~~ 1(0)	11(3)	0(0)	2(1)
2	1(0)	8(3)	4(2)	1(6)	4(4)	2(0)
3	~~5(3)~~ 3(1)	1(1)	5(3)	1(0)	1(1)	7(5)
4	1(0)	0(1)	3(0)	3(2)	2(3)	4(0)
5	0(1)	17(4)	3(1)	1(0)	9(9)	29(4)
6	3(1)	8(3)	1(0)	2(1)	1(6)	2(0)

9/28

GL·6

	1	2	3	4	5	6
1	2(0)	129 E	0(0)	30(17)	1(2)	6(2)
2	6(1)	2(0)	0(0)	2(0)	2(0)	19(19)
3	0(0)	2(0)	1(0)	2(0)	5(0)	0(0)
4	0(0)	10(5)	7(0)	13(2)	0(0)	0(3)
5	0(2)	102 E	1(1)	0(0)	14(2)	J(~1000) E
6	103 E	5(3)	1(1)	3(0)	9(2)	8(2)

9/28

GL·7 ~~GL·0~~

	1	2	3	4	5	6
1	18(9)	4(1)	3(2)	1(0)	4(1)	0(0)
2	0(0)	3(2)	32(3)	7(0)	1(2)	3(1)
3	12(0)	3(0)	2(2)	0(2)	7(1)	5(4)
4	11(5)	1(0)	0(1)	1(0)	53 E	19(4)
5	168 E	1(0)	1(0)	5(2)	10(5)	6(0)
6	1(0)	6(0)	20(7)	1(0)	3(0)	0(0)

GL·8

	1	2	3	4	5	6
1	3(0)	2(1)	2(2)	5(1)	2(0)	2(0)
2	17(2)	2(0)	~~7(2)~~ 0(0)	7(2)	1(0)	8(5)
3	1(0)	0(0)	1(1)	19(10)	3(2)	1(0)
4	4(3)	5(0)	5(2)	0(0)	15(3)	9(2)
5	0(0)	3(0)	2(2)	0(0)	J(~1000) E	1(1)
6	~~J(~1000) E~~ 6(6)	6(1)	0(0)	1(1)	1(0)	0(0)

9/28

GL·9

	1	2	3	4	5	6
1	4(15)	5(5)	0(1)	7(4)	1(0)	4(0)
2	1(2)	2(0)	0(1)	0(0)	1(0)	1(0)
3	0(0)	5(1)	4(4)	1(1)	2(1)	1(0)
4	11(3)	8(5)	11(12)	4(2)	6(4)	7(2)
5	8(0)	0(0)	12(2)	~~1(1)~~ 84 E	~~12(3)~~ 3(2)	2(1)
6	1(1)	12(3)	2(1)	3(1)	2(1)	2(0)

GL·10

	1	2	3	4	5	6
1	1(1)	1(1)	4(3)	0(0)	0(2)	8(19)
2	3(1)	7(0)	5(0)	1(0)	4(3)	0(0)
3	3(3)	2(1)	0(0)	9(22)	3(1)	1(2)
4	15(7)	J(~1000) E	1(0)	0(0)	2(0)	0(0)
5	2(1)	57 E	11(8)	3(2)	3(1)	0(0)
6	2(4)	1(2)	18(8)	6(1)	13(2)	12(1)

GL·11

	1	2	3	4	5	6
1	3(1)	J(~E5)	29(2)	J(~E5)	23(11)	J(~E5)
2	6(1)	67 E	14(4)	30(5)	J(~E5)	120 E
3	20(10)	J(~E5)	4(2)	11(1)	J(~E5)	32(11)
4	J(E5)	J(~E5)	J(~E5)	J(~E5)	J(~E5)	J(~E5)
5	18(6)	66(13)	J(~E5)	19(9)	26(4)	J(~E5)
6	89 E	31(7)	43(5)	J(~E5)	41(15)	J(~E5)

GL·12

	1	2	3	4	5	6
1	5(2)	2(0)	1(1)	1(1)	5(0)	2(0)
2	0(0)	5(2)	1(0)	9(5)	0(0)	0(0)
4~~3~~	2(0)	2(0)	8(0)	12(6)	4(0)	0(0)
5~~4~~	0(0)	13(0)	3(0)	0(0)	52 E	1(4)
6~~5~~	2(0)	5(1)	15(13)	1(0)	1(0)	0(0)
3~~6~~	3(0)	1(0)	1(0)	2(0)	0(0)	7(5)

9/29

GL·13

	1	2	3	4	5	6
1	0(0)	14(0)	2(0)	7(1)	0(0)	5(0)
2	4(0)	0(0)	115 E	1(0)	1(3)	55 E
3	0(0)	0(0)	22(14)	3(0)	1(0)	J(104)
4	1(1)	6(4)	1(0)	0(0)	0(0)	1(0)
5	6(2)	2(1)	3(0)	2(0)	J(~1000)	0(1)
6	2(0)	52(8)	0(1)	1(0)	5(1)	0(0)

9/29

GL·14

	1	2	3	4	5	6
1	0(0)	0(1)	1(1)	6(3)	1(0)	4(0)
2	2(0)	1(3)	0(0)	1(0)	1(2)	0(0)
3	1(0)	4(0)	0(0)	2(1)	0(5)	0(0)
4	15(3)	0(0)	24(5)	2(0)	7(1)	1(2)
5	8(2)	6(0)	J(~1000) E	2(1)	2(1)	13(2)
6	13(5)	8(0)	0(1)	J(~1000) E	0(0)	4(0)

GL·15

	1	2	3	4	5	6
1	3(0)	2(2)	5(0)	3(3)	3(0)	0(0)
2	J(~400) E	3(6)	0(0)	0(0)	1(0)	2(0)
3	26(7)	1(0)	0(0)	1(0)	0(0)	16(11)
4	0(2)	8(2)	15(16)	0(1)	1(0)	1(0)
5	0(0)	9(1)	2(1)	0(0)	0(1)	1(0)
6	1(0)	J(~500) E	1(0)	2(0)	4(2)	1(1)

GL·16

	1	2	3	4	5	6
1	0(0)	6(0)	2(0)	8(2)	24(5)	10(3)
2	1(0)	1(1)	0(0)	29(22)	6(4)	2(1)
3	3(1)	1(0)	0(0)	24(0)	0(0)	15(0)
4	2(1)	0(0)	3(0)	2(0)	9(3)	8(1)
5	2(0)	2(0)	6(1)	4(0)	3(1)	0(0)
6	7(1)	8(2)	1(0)	2(2)	2(1)	3(0)

GL·17

	1	2	3	4	5	6
1	0(0)	0(1)	0(1)	0(0)	~~0(0)~~ 1(1)	1(2)
2	0(0)	1(0)	0(0)	1(0)	2(2)	0(0)
3	1(0)	3(0)	0(0)	1(0)	0(0)	2(0)
4	0(0)	0(0)	1(0)	0(0)	3(1)	5(2)
5	2(0)	2(0)	0(0)	0(0)	0(0)	6(1)
6	0(0)	1(0)	0(0)	80 E	4(2)	3(1)

GL·18

	1	2	3	4	5	6
1	1(1)	1(0)	0(0)	5(1)	0(0)	2(1)
2	3(2)	5(6)	6(1)	J(~1000) E	1(1)	6(0)
3	0(0)	1(0)	1(1)	1(0)	2(0)	0(0)
4	0(0)	0(0)	1(0)	0(0)	0(0)	2(0)
5	3(0)	94 E	0(0)	1(0)	1(1)	1(0)
6	1(2)	0(0)	1(0)	5(0)	1(0)	0(0)

GL·19

	1	2	3	4	5	6
1	5(4)	2(0)	1(3)	0(0)	1(0)	0(0)
2	0(0)	0(0)	1(0)	0(0)	0(0)	0(0)
3	4(3)	4(2)	1(0)	5(0)	0(0)	0(0)
4	1(0)	3(2)	1(0)	0(0)	0(0)	0(0)
5	0(0)	0(0)	0(0)	2(0)	1(1)	0(1)
6	0(0)	4(2)	0(0)	3(0)	0(0)	0(0)

GL·20

	1	2	3	4	5	6
1	0(0)	1(0)	9(4)	1(0)	0(0)	0(0)
2	3(2)	20(1)	0(0)	1(0)	0(0)	0(0)
3	0(0)	J(~500) E	~~1(0)~~ 0(1)	2(0)	6(0)	0(0)
4	0(0)	0(0)	0(2)	1(2)	1(0)	0(0)
5	1(0)	0(0)	3(2)	0(0)	0(0)	0(0)
6	1(0)	0(0)	0(0)	2(0)	0(0)	0(0)

9/29

9/30

GL·21

	1	2	3	4	5	6
1	1(6)	4(1)	0(0)	0(0)	16(5)	7(2)
2	0(0)	5(2)	0(0)	0(0)	1(0)	0(1)
3	0(1)	1(1)	1(0)	1(0)	0(0)	3(0)
4	0(0)	0(0)	0(0)	0(1)	0(0)	94(55)
5	0(0)	0(0)	0(0)	2(0)	2(0)	0(0)
6	0(0)	0(0)	2(0)	0(2)	4(7)	0(0)

GL·22

	1	2	3	4	5	6
1	0(0)	0(0)	0(0)	1(1)	2(0)	0(0)
2	0(0)	0(0)	3(2)	0(1)	0(0)	0(0)
3	1(0)	0(0)	0(0)	0(0)	0(0)	~~0(0)~~ 1(1)
4	0(0)	18(4)	0(0)	1(0)	1(0)	37(7)
5	1(0)	1(0)	2(0)	1(0)	1(0)	2(0)
6	0(0)	0(0)	0(0)	0(0)	1(1)	0(0)

GL·23

	1	2	3	4	5	6
1	J(~10^4)	0(0)	0(0)	0(0)	0(0)	11(3)
2	0(0)	7(1)	0(0)	0(0)	14(9)	1(0)
3	0(0)	0(0)	1(0)	0(0)	0(0)	6(3)
4	1(4)	1(0)	0(0)	2(3)	0(0)	1(0)
5	0(0)	2(1)	0(0)	1(1)	10(6)	2(0)
6	0(0)	3(0)	1(2)	0(0)	2(2)	0(0)

GL·24

	1	2	3	4	5	6
1	0(0)	3(0)	0(0)	0(0)	0(0)	0(0)
2	0(0)	5(0)	0(0)	0(0)	0(0)	0(0)
3	1(0)	1(0)	0(0)	0(0)	0(2)	1(0)
4	2(0)	0(1)	0(0)	0(0)	1(0)	0(0)
5	0(0)	4(0)	0(0)	1(0)	0(0)	1(1)
6	0(0)	0(0)	0(0)	0(0)	1(0)	8(0)

GL·25

	1	2	3	4	5	6
1	1(2)	14(5)	2(0)	0(0)	0(0)	1(0)
2	0(0)	0(0)	42 E	0(0)	0(0)	0(0)
3	0(0)	1(1)	0(0)	0(0)	0(0)	1(0)
4	0(0)	0(0)	0(0)	2(0)	0(0)	1(0)
5	2(1)	3(0)	0(0)	0(0)	1(1)	0(0)
6	1(0)	3(0)	0(0)	0(0)	6(1)	J(~500) E

GL·26

	1	2	3	4	5	6
1	0(1)	1(0)	1(0)	1(1)	0(0)	3(0)
2	0(0)	7(2)	2(0)	0(0)	0(0)	3(0)
3	1(0)	1(0)	1(1)	1(0)	1(0)	0(0)
4	0(0)	0(2)	0(0)	0(0)	29 E	1(0)
5	4(0)	23(4)	0(0)	0(1)	4(2)	2(0)
6	12(1)	2(0)	0(0)	1(0)	3(0)	0(0)

GL·27

	1	2	3	4	5	6
1	0(1)	1(1)	0(0)	5(0)	0(0)	0(0)
2	0(0)	12(2)	1(0)	0(0)	0(0)	0(0)
3	13(7)	0(0)	0(0)	0(0)	0(0)	0(0)
4	2(0)	0(0)	0(0)	0(0)	0(0)	0(0)
5	0(0)	0(0)	3(1)	5(0)	0(0)	0(0)
6	1(0)	1(0)	1(1)	2(0)	0(0)	34(0)

GL·28

	1	2	3	4	5	6
1	1(0)	3(2)	2(1)	2(0)	1(1)	0(0)
2	0(0)	4(2)	1(0)	1(0)	4(0)	0(0)
3	0(0)	0(0)	0(0)	49	11(7)	1(0)
4	0(0)	2(0)	0(0)	15(6)	0(0)	0(0)
5	2(0)	4(0)	0(0)	1(0)	0(0)	14(6)
6	4(1)	0(0)	0(0)	0(0)	0(0)	0(0)

GL·29

	1	2	3	4	5	6
1	0(0)	9(1)	1(0)	1(0)	0(0)	1(0)
2	0(0)	0(0)	0(0)	0(0)	1(3)	1(0)
3	1(0)	0(0)	0(0)	0(0)	1(3)	6(1)
4	1(0)	0(0)	4(0)	0(0)	0(0)	0(0)
5	0(0)	0(1)	0(0)	0(0)	0(0)	2(1)
6	0(1)	0(0)	0(0)	0(0)	3(1)	3(0)

GL·30

	1	2	3	4	5	6
1	4(0)	8(2)	1(0)	0(0)	1(0)	0(0)
2	0(0)	0(0)	0(0)	0(0)	1(1)	1(2)
3	0(0)	2(1)	1(2)	2(0)	0(2)	1(0)
4	8(10)	0(0)	0(0)	1(0)	6(4)	2(2)
5	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
6	0(0)	1(1)	0(0)	2(0)	1(0)	1(0)

GL·31

	1	2	3	4	5	6
1	0(0)	0(0)	6(0)	2(0)	2(0)	1(0)
2	186 E	1(0)	3(0)	0(0)	6(0)	205(0)
3	21(0)	1(0)	12(0)	16(0)	0(0)	9(0)
4	3(0)	5(0)	5(0)	8(0)	3(0)	1(0)
5	14(0)	3(0)	5(0)	4(0)	7(0)	0(0)
6	18(0)	0(0)	0(0)	2(0)	6(0)	4(0)

GL·32

	1	2	3	4	5	6
1	0(0)	1(0)	1(0)	~~2(2)~~ 0(0)	~~0(0)~~ 2(2)	0(0)
2	~~0(0)~~ 13(5)	0(0)	1(0)	0(0)	0(0)	1(1)
3	1(0)	21(3)	4(1)	1(0)	1(0)	0(0)
4	3(1)	4(1)	0(0)	4(0)	2(0)	0(0)
5	4(10)	2(0)	0(0)	1(2)	0(0)	1(0)
6	2(3)	0(0)	0(0)	1(0)	0(0)	0(0)

GL·33

	1	2	3	4	5	6
1	0(0)	5(2)	1(0)	0(0)	0(0)	4(5)
2	1(0)	~~3(0)~~ 4(0)	0(3)	~~1(0)~~ 0(0)	0(0)	1(0)
3	0(0)	0(0)	1(0)	2(0)	~~26(10)~~ 0(0)	26(10)
4	0(0)	2(1)	10(12)	0(0)	6(3)	2(0)
5	1(0)	0(0)	0(0)	0(0)	13(6)	1(0)
6	2(1)	1(0)	J(~10^4)	0(0)	3(2)	0(0)

GL·34

	1	2	3	4	5	6
1	~~1(0)~~ 0(0)	2(2)	3(1)	1(0)	6(0)	0(0)
2	2(1)	0(0)	2(7)	0(0)	0(0)	0(0)
3	26(22)	1(0)	0(0)	1(0)	1(1)	0(2)
4	1(0)	2(2)	1(0)	0(0)	1(0)	0(0)
5	~175 E	2(0)	1(1)	0(0)	0(0)	2(0)
6	0(0)	1(1)	1(1)	0(0)	1(0)	1(0)

9/30

GL·35

	1	2	3	4	5	6
1	5(0)	0(0)	0(0)	1(0)	0(0)	1(0)
2	3(0)	15(0)	7(0)	20(0)	8(0)	4(0)
3	0(0)	0(0)	4(0)	1(0)	8(0)	13(0)
4	0(0)	12(0)	78(0)	3(0)	31(0)	14(0)
5	2(0)	21(0)	12(0)	0(0)	2(0)	12(0)
6	0(0)	27(0)	0(0)	0(0)	7(0)	5(0)

GL·36

	1	2	3	4	5	6
1	0(0)	0(0)	0(0)	2(0)	0(0)	~~0(0)~~ 0(0)
2	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
3	0(0)	2(0)	~~0(0)~~ 2(0)	0(0)	0(0)	0(0)
4	0(0)	2(0)	0(0)	3(0)	1(0)	0(0)
5	5(0)	2(1)	5(3)	2(0)	6(3)	0(0)
6	0(0)	2(0)	0(0)	0(0)	1(0)	0(0)

GL·37

	1	2	3	4	5	6
1	0(0)	2(1)	2(1)	0(0)	1(1)	0(0)
2	1(0)	3(0)	2(0)	1(0)	0(0)	0(0)
3	0(0)	1(0)	0(0)	0(0)	0(0)	1(0)
4	0(0)	2(1)	0(0)	0(0)	0(0)	44 E
5	0(0)	0(0)	0(0)	0(1)	0(1)	1(0)
6	2(0)	1(0)	0(0)	30 E	0(0)	1(0)

9/30

GL·38

	1	2	3	4	5	6
1	3(1)	11(2)	0(0)	0(0)	0(0)	0(0)
2	0(0)	4(0)	1(1)	0(0)	2(0)	0(0)
3	0(0)	0(0)	6(0)	0(0)	0(0)	0(0)
4	0(0)	5(0)	0(0)	1(0)	0(1)	0(0)
5	1(2)	0(0)	0(0)	0(0)	0(0)	1(0)
6	0(0)	0(0)	2(1)	0(0)	6(2)	4(1)

GL·39

	1	2	3	4	5	6
1	0(0)	0(0)	0(1)	3(7)	2(0)	9(7)
2	1(1)	0(0)	0(0)	5(26)	1(0)	2(0)
3	0(0)	0(0)	0(0)	5(3)	2(1)	0(0)
4	3(0)	3(0)	3(0)	0(1)	2(0)	3(0)
5	2(0)	J(~500) E	0(1)	32 E	4(3)	0(0)
6	1(0)	3(0)	7(1)	0(0)	0(0)	0(0)

10/1

GL·40

	1	2	3	4	5	6
1	~~2(1)~~ 9(1)	14(3)	2(1)	~~17(9)~~ 0(0)	2(1)	17(9)
2	0(1)	4(0)	J(~1000) E	5(1)	35(3)	0(0)
3	0(0)	0(1)	2(2)	26(5)	6(3)	30(11)
4	0(0)	0(2)	6(5)	7(1)	1(1)	0(0)
5	3(0)	8(5)	0(0)	3(0)	0(0)	14(0)
6	0(0)	2(0)	2(2)	9(1)	28(13)	0(0)

GL·41

	1	2	3	4	5	6
1	1(0)	2(0)	7(8)	0(0)	8(7)	2(1)
2	3(4)	3(2)	4(0)	2(2)	4(0)	12(6)
3	0(0)	1(0)	0(0)	0(0)	2(0)	2(0)
4	0(0)	2(0)	1(0)	4(0)	3(0)	26(1)
5	1(1)	11(5)	0(1)	5(1)	0(0)	1(0)
6	1(1)	10(4)	4(0)	4(0)	3(0)	0(0)

10/1

GL·42

	1	2	3	4	5	6
1	1(0)	0(0)	2(0)	1(1)	J(~500) E	6(8)
2	1(0)	7(1)	2(0)	0(0)	0(0)	5(3)
3	0(0)	7(12)	0(0)	2(0)	0(0)	1(0)
4	1(0)	1(1)	1(0)	4(2)	0(0)	0(0)
5	52(4)	J(~500) E	0(0)	2(0)	6(0)	0(1)
6	1(1)	9(5)	0(0)	4(0)	0(0)	6(1)

GL·43

	1	2	3	4	5	6
1	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
2	0(0)	0(0)	0(0)	0(0)	0(0)	1(0)
3	0(0)	0(0)	0(0)	0(0)	0(0)	1(0)
4	0(0)	0(0)	0(0)	4(1)	0(0)	0(0)
5	0(0)	0(0)	14(2)	1(0)	0(0)	0(0)
6	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

GL·44

	1	2	3	4	5	6
1	5(6)	15(4)	0(0)	7(4)	0(0)	0(0)
2	0(0)	4(3)	2(0)	0(0)	3(2)	10(2)
3	0(0)	7(2)	0(0)	1(0)	0(1)	2(1)
4	0(0)	0(0)	0(0)	0(0)	0(0)	2(1)
5	2(1)	5(0)	0(0)	0(0)	0(1)	12(1)
6	6(2)	27(10)	0(0)	5(0)	2(1)	4(0)

GL·45

	1	2	3	4	5	6
1	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
2	4(0)	0(0)	0(0)	0(0)	0(0)	5(0)
3	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
4	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
5	0(0)	1(0)	0(0)	0(0)	0(0)	0(0)
6	0(0)	1(0)	0(0)	0(0)	22(16)	2(0)

GL·46

	1	2	3	4	5	6
1	0(0)	10(3)	61 E	2(0)	35(15)	3(0)
2	2(0)	3(0)	1(0)	5(1)	1(0)	1(0)
3	0(0)	2(0)	1(0)	0(0)	0(0)	0(0)
4	9(7)	3(2)	1(0)	0(0)	5(0)	6(1)
5	16(22)	0(0)	0(0)	7(4)	0(0)	5(0)
6	17(6)	J(~250) E	37(10)	22(12)	3(0)	2(0)

GL·47

	1	2	3	4	5	6
1	0(0)	11(1)	1(0)	2(0)	0(0)	0(0)
2	0(0)	2(3)	2(0)	1(0)	1(0)	0(0)
3	0(0)	5(0)	3(0)	0(1)	1(0)	3(2)
4	6(0)	2(0)	20(4)	1(0)	0(0)	7(4)
5	1(0)	1(0)	1(0)	0(0)	0(0)	2(0)
6	0(0)	2(0)	0(0)	1(0)	1(0)	4(4)

GL·48

	1	2	3	4	5	6
1	4(0)	0(0)	0(0)	6(4)	1(0)	0(0)
2	0(0)	4(2)	1(0)	4(1)	2(0)	8(4)
3	1(0)	0(0)	15(2)	2(1)	3(0)	0(0)
4	0(0)	0(0)	2(0)	0(0)	6(2)	1(0)
5	2(0)	1(0)	0(0)	1(0)	0(0)	1(1)
6	0(0)	0(0)	0(0)	0(0)	7(1)	8(1)

10/1

GL·49

	1	2	3	4	5	6
1	0(0)	1(0)	2(1)	J(~500) E	1(1)	6(1)
2	26(0)	4(1)	0(0)	1(0)	0(0)	9(0)
3	1(2)	23(20)	2(3)	0(0)	0(0)	1(0)
4	1(4)	0(1)	0(0)	2(0)	3(0)	5(0)
5	1(0)	1(1)	0(0)	2(0)	0(0)	5(2)
6	1(0)	41(0)	0(0)	10(2)	0(0)	4(0)

	1	2	3	4	5	6
1						
2						
3						
4						
5						
6						

	1	2	3	4	5	6
1						
2						
3						
4						
5						
6						

	1	2	3	4	5	6
1						
2						
3						
4						
5						
6						

	1	2	3	4	5	6
1						
2						
3						
4						
5						
6						

	1	2	3	4	5	6
1						
2						
3						
4						
5						
6						

	1	2	3	4	5	6
1						
2						
3						
4						
5						
6						

10/4

NOTES ON PLATE COUNTING

- I used a threshold when counting the plates
  - I have always used a threshold when counting colonies on Canavanine plates because a lot of the small colonies are ones that grew post-plating.
  - With W303 I have never used a threshold when counting 5FOA plates because colonies were always clearly 5FOA^R or not, and I know that there is little (No?) growth post plating with W303.

  → At least one of these is not true w/ S288c. All of the spots (with the exception of 31 & 35 showed almost continuous variability ~~with~~ in colony size from pinpoint to large.

  Strains GL31 & GL35 did not show any variability in colony size on 5FOA. All of the colonies were large, easy to count, and clearly 5FOA^R.

  - Threshold size:

  for Canavanine plates: larger than the number "6" on the eyepiece (~2mm) at 6× magnification.

  for 5FOA plates: larger than the number "6" on the eyepiece (~2mm) at 10× magnification.

  - Notation:

  X (Y)

  above threshold — below threshold

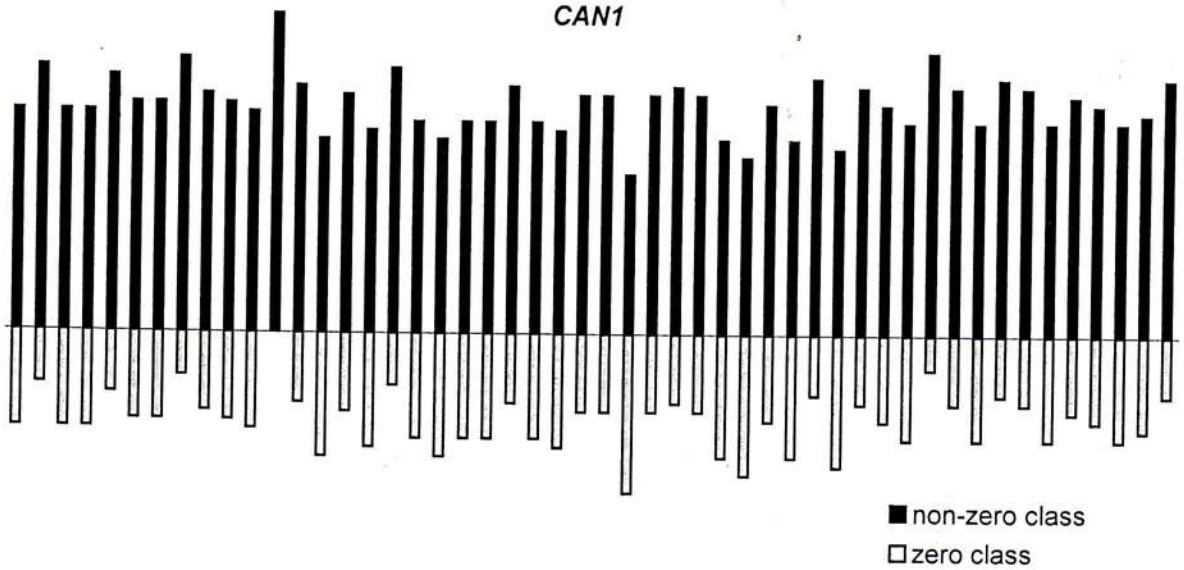
  In some cases I did not count the # of colonies below the threshold. Either I did not feel comfortable estimating or there were severe edge effects or a jackpot and the below threshold colonies were counted as real.

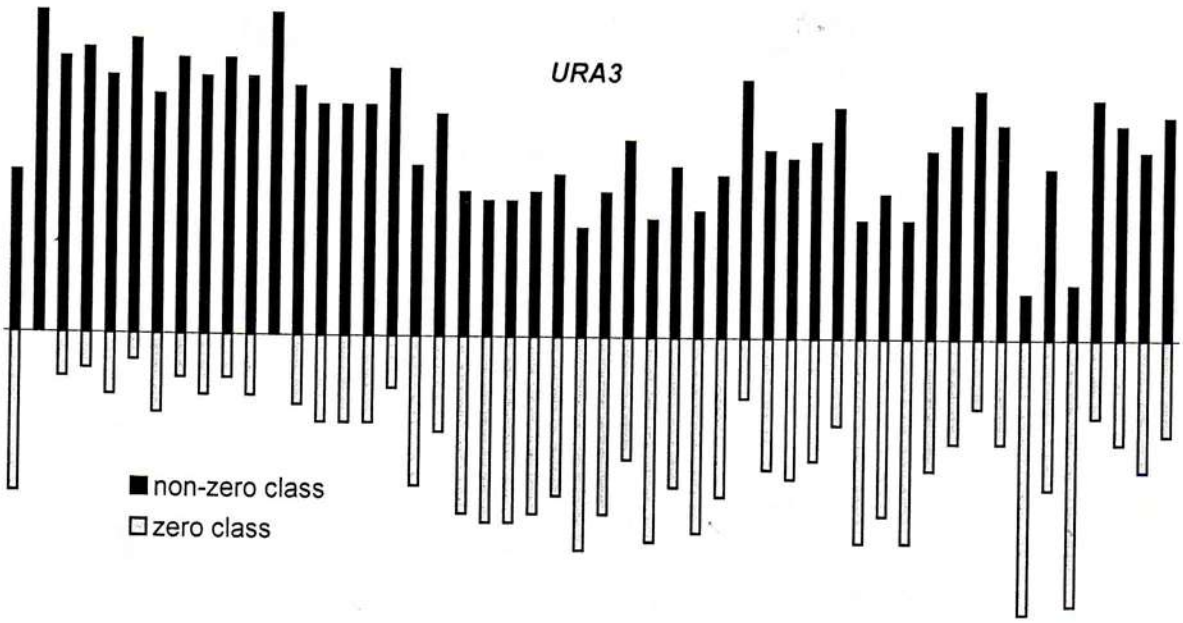
  E – edge effect where the size of the colonies is large on the outside of the spot and become increasingly smaller toward the center of the spot. In these cases I counted all of the colonies I could and pooled them into one value

  J – jackpot: far too many colonies to count – these were estimated

10/3

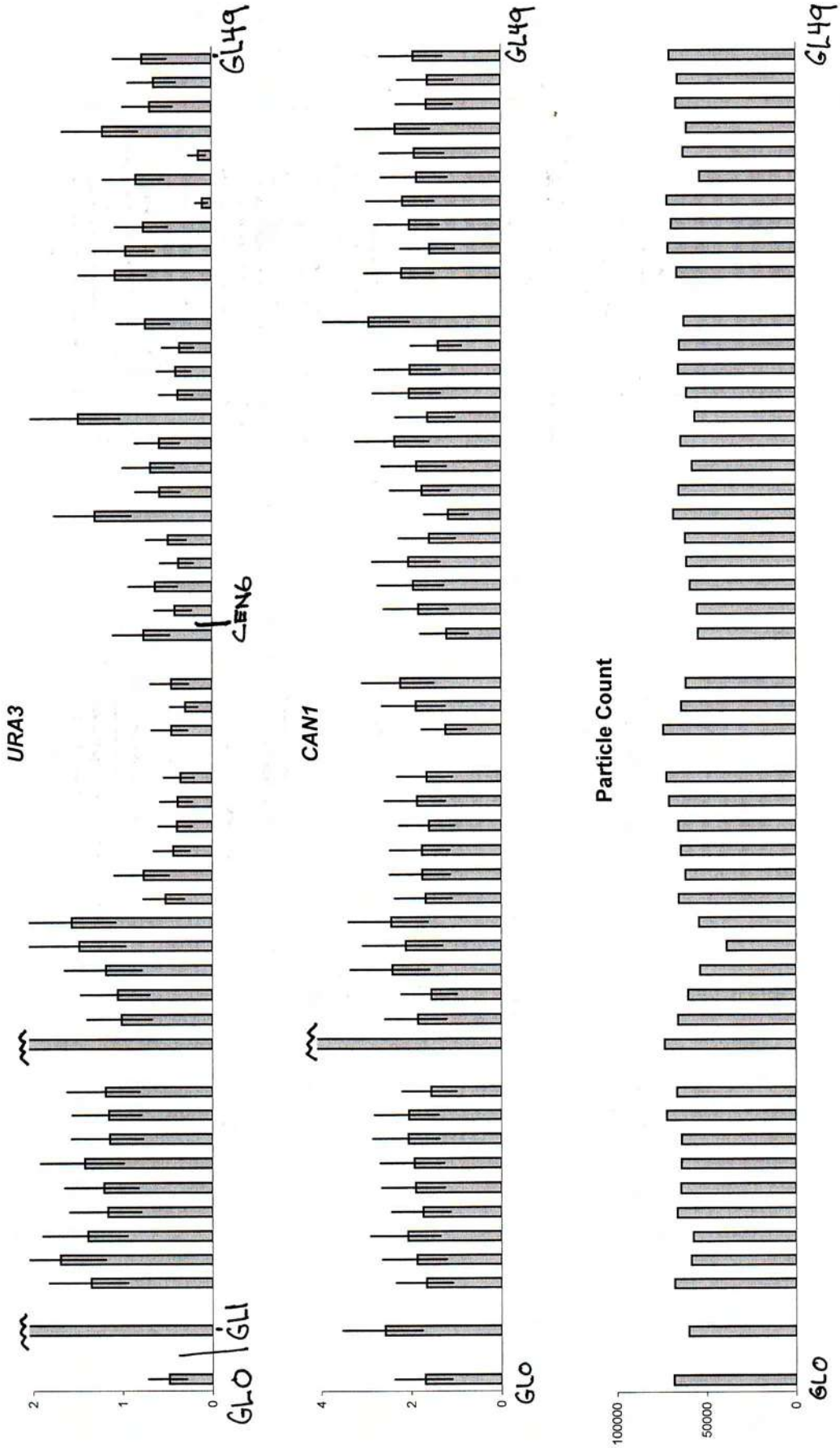
SUMMARY OF PLATE COUNTS





10/3

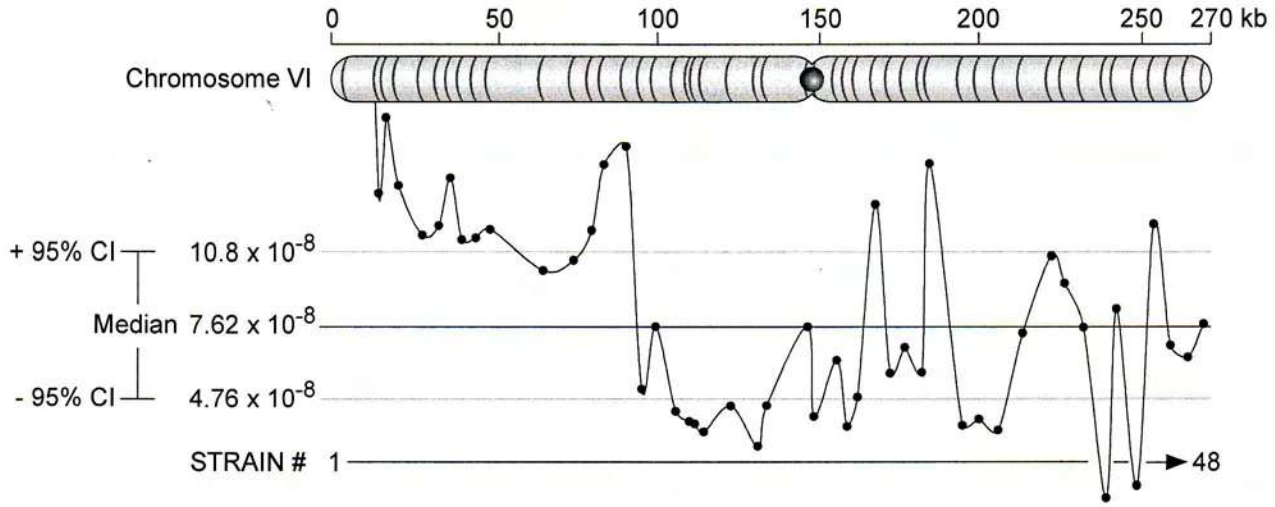
SUMMARY OF RESULTS



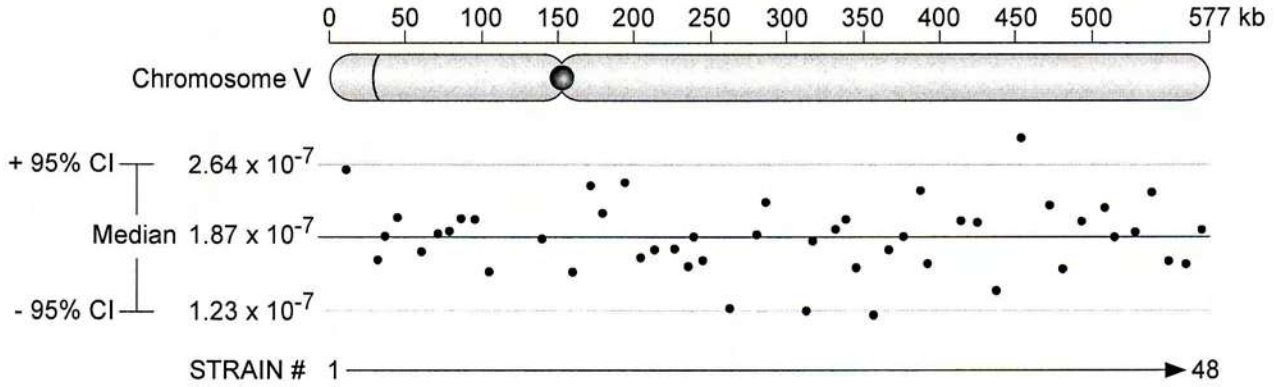
1/17/06 A fancy plot of the data – as presented at ISCB2005

Mutation rate varies across Chromosome VI

Mutation Rate at *URA3* - different position in each strain



Mutation Rate at *CAN1* - same position in each strain



11/28

The following work I have been doing over the last few weeks:

PURPOSE:

I noted earlier that strains GL31 and GL35 showed little variability in colony size on 5FOA (see p 109).

I would like to remake my URA3 collection in this background

Presumably these strains carry mutations which allow them to develop uniformly on 5FOA.

I will need to do two ~~things~~ things:

(1) Determine if it is the deletion of these ORFs themselves (YFR016C and YFR019W/FAB1) that give these strains this phenotype.

(2) Create a starter strain by returning the originally deleted ORF to one of the two strains.

STRAINS:

YFR019Δ & YFR016Δ — from Δ collection

auxotrophic for: ura, leu, his, met, G418R — Mat a

GIL052 - his3Δ, leu2Δ, lys2Δ Mat α

- Cross: YFR019Δ × GIL052, YFR016Δ × GIL052 } select diploids by streaking on -ura+G418
  → pick 2 diploids of each, labeled
  31-1, 31-2 } 16Δ×52
  35-1, 35-2 } 19Δ×52
- Sporulate using S288c protocol
- Dissect 5 tetrads of each

→ I put the dissection plates in the incubator last monday (11/21) and today I will patch the tetrads onto a YPD plate so that I can replica plate them tomorrow.

11/28

Notes of an tetrad dissections:

31-1

· I only picked 3 spores from tetrad # 5 - don't know what happened to the other one

31-2

· looks good

35-1

· Tetrads 3 & 4 show 2:2 seg. of fitness defects (3b, 3d, 4b, 4d - small colonies)
· Tetrad 5 shows 2:2 lethality (5c, 5d dead)

35-2

· 4b, 5a small colonies

11/30

RESULTS FROM TETRAD DISECTIONS

DIPLOID	TETRAD	SPORE	-ura	-met	G418	5FOA	Mat a	Mat α	Mating type
31-1	1	a	+ 1	−	−	−	+	−	α
		b	−	+	+	+	+	−	α
		c	+ 2	+	+	−	−	+	a
		d	−	−	−	+	−	+	a
	2	a	+ 3	+	+	−	+	−	α
		b	+ 4	−	+	−	−	+	a
		c	−	−	−	+	−	+	a
		d	−	+	−	+	+	−	α
	3	a	−	−	+	+	+	−	α
		b	−	+	−	+	−	+	a
		c	+ 5	+	+	−	+	−	α
		d	+ 6	−	−	−	−	+	a
	4	a	+ 7	−	+	−	−	+	a
		b	+ 8	+	+	−	+	−	α
		c	−	−	−	+	+	−	α
		d	−	+	−	+	−	+	a
	5	a	−	+	−	+	+	−	α
		b	−	−	−	+	+	−	α
		c	+ 9	+	+	−	−	+	a
		d							
31-2	1	a	−	+ 19	−	+	−	+	
		b	−	−	−	+	+	−	
		c	10 + 9	+ 20	+	−	+	−	
		d	11 + 10	−	+	−	−	+	
	2	a	12 + 11	+	+	−	+	−	
		b	13 + 12	−	+	−	−	+	
		c	−	−	−	+	−	+	
		d	−	+	−	+	+	−	
	3	a	−	−	+	+	+	−	
		b	−	+	+	+	−	+	
		c	14 + 13	+	−	−	−	+	
		d	15 + 14	−	−	−	+	−	
	4	a	16 + 15	−	+	−	+	−	
		b	−	−	−	+	+	−	
		c	17 + 16	+	+	−	−	+	
		d	−	+	−	+	−	+	
	5	a	18 + 17	+	−	−	+	−	
		b	19 + 18	−	−	−	−	+	
		c	−	+	+	+	−	+	
		d	−	−	+	+	+	−	

11/30

DIPLOID	TETRAD	SPORE	-ura	-met	G418	5FOA	Mat a	Mat α
35-1	1	a	20 + 19	+	+	−	+	−
		b	−	−	+	+	−	+
		c	21 + 20	−	−	−	+	−
		d	−	+	−	+	−	+
	2	a	22 + 21	+	+	−	+	−
		b	−	+	+	+	−	+
		c	23 + 22	+	−	−	+	−
		d	−	+	−	+	−	+
	3	a	24 + 23	+	−	−	−	+
		b	−	−	+	−	+	−
		c	25 + 24	+	−	−	−	+
		d	−	−	+	+	+	−
	4	a	−	+	+	+	+	−
		b	26 + 25	−	+	−	−	+
		c	27 + 26	−	−	−	+	−
		d	−	+	−	+	−	+
	5	a	−	+	−	+	−	+
		b	−	+	−	+	+	−
		c	←		DEAD		→	
		d	←				→	
35-2	1	a	28 + 27	−	+	−	+	−
		b	−	+	−	+	−	+
		c	−	−	−	+	−	+
		d	29 + 28	+	+	−	+	−
	2	a	30 + 29	−	−	−	+	−
		b	31 + 30	+	−	−	−	+
		c	−	+	+	+	+	−
		d	−	−	+	+	−	+
	3	a	32 + 31	+	−	−	+	−
		b	−	−	+	+	−	+
		c	33 + 32	+	−	−	+	−
		d	−	−	+	+	−	+
	4	a	34 + 33	−	+	−	+	−
		b	35 + 34	+	+	−	−	+
		c	−	−	−	+	−	+
		d	−	+	−	+	+	−
	5	a	36 + 35	−	+	−	−	+
		b	37 + 36	+	+	−	−	+
		c	−	−	−	+	+	−
		d	−	+	−	+	+	−

12/3

37 spores are URA3. Inoculate 1 mL YPD+ade with each of these.

also, inoculate 1 mL of YPD+ade w/ GIL051, GL31, & GL35

1/17

- I have not been keeping up with my notebook. Over the last few weeks I have been doing plating tests to determine the segregation pattern of the colony size variability (see pgs 109 & 115) phenotype; hereafter called CSV phenotype.
- The 1st expt. (analyzed on 1/12 pg 120): I grew 2 mL ~~in~~ of each strain in nonselective media – as it turns out it was a 50:50 mix of YPD and 2×SC+ADE. This was because I had 1 mL cold cultures of the strains in YPD to which I added 1 mL of 2×SC. I spot plated 9 100λ spots of each onto overdrived 5FOA.
- The 2nd expt (analyzed on 1/14 pg 120): I did this expt just like how I did the big fluct expt – 200 λ cultures in 96-well plates / grow 1st in 2×SC-ura / then 2×SC / add 50λ H_2O / spot 6 spots onto overdrived 5FOA.

Notes:

spore 26: slight fitness defect
spore 35: severe fitness defect
} observed during growth in liquid.

I noticed on plates that 22, 26, 35, & 36 looked slightly different:

- in expt 1 the spots were more diffuse
- in expt 2 the spots were smooth (not wavy like the others)

this was probably due to the fitness defects in these strains. I noticed fitness defects in log cultures of 26 & 35 – I must have missed defects in 22 & 36.

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

1/12 1/14

too few colonies to tell (usually 0-2)

	STRAIN	(CSV−) W303-like	(CSV+) S288c-like	Unclear	Between W303 & S288c-like
	1		X	X	
	2			X	X
	3			X	X
	4	X		X	
	5			X	X
	6			X X	
	7	X X			
	8			X	X
	9			X	X
31	10	X		X	
	11			X	X
	12	X		X	
	13			X	X
	14		X		X
	15		X X		
	16	X	X		
	17	X		X	
	18		X X		
	19		X	X	
	20	X X			
	21		X	X	
	22	X		X	
	23		X X		
	24		X X		
	25		X X		
	26	X X			
	27		X	X	
	28	X X			
	29	X X			
35	30		X	X	
	31		X X		
	32		X X		
	33		X X		
	34	X X			
	35	X X			
	36	X		X	
	37	X		X	
	GLC051		X X		
	GL31	X		X	
	GL35	X		X	

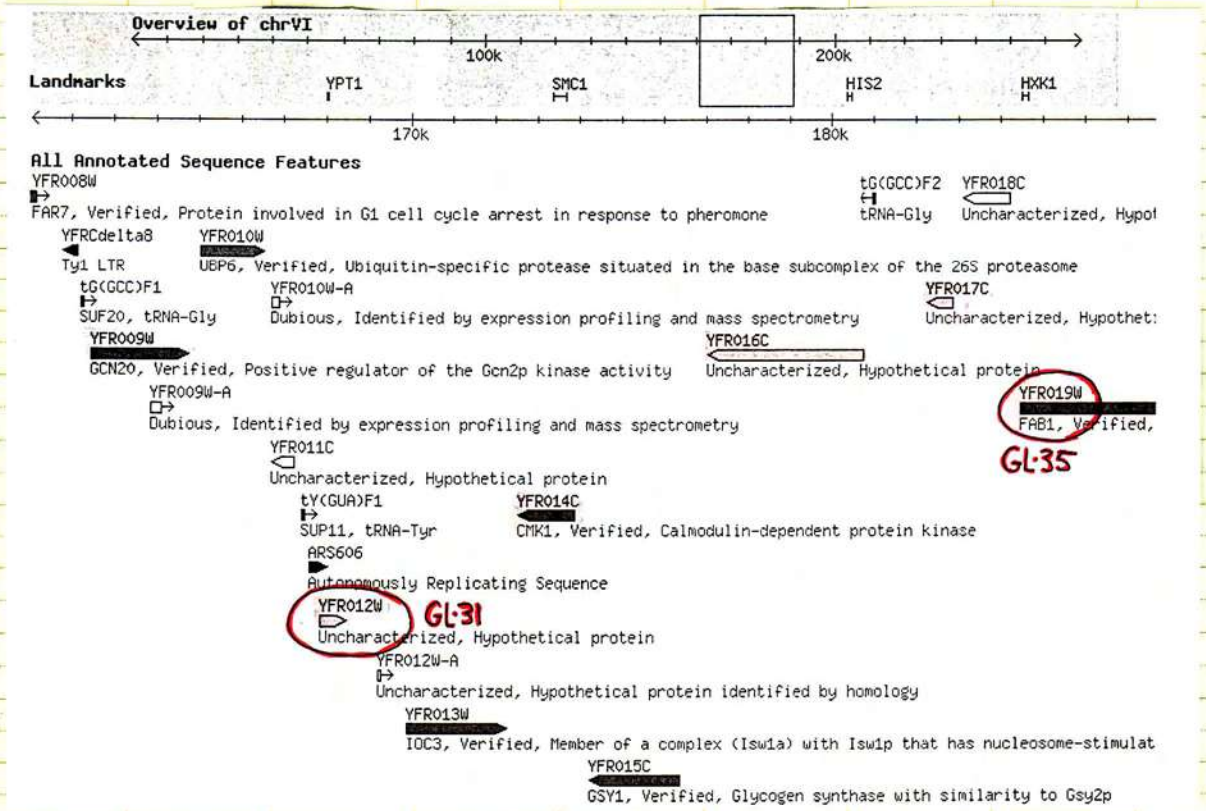
= KanMX

1/17

ANALYSIS OF SPORE PHENOTYPING

DIPLOIDS 31-1 & 31-2		DIPLOIDS 35-1 & 35-2	
CSV- KAN+	6	CSV- KAN+	9
CSV+ KAN-	5	CSV+ KAN-	9
CSV- KAN-	0	CSV- KAN-	0
CSV+ KAN+	0	CSV+ KAN+	0
UNKNOWN	8	UNKNOWN	0

In both strains, ~~this app~~ the ~~Ct~~ CSV phenotype appears to be linked to the KAN cassette



SEVERAL POSSIBILITIES:

(1) Deletion of either YFR012W or FAB1 results in CSV- ϕ.

(2) There exists a mutation(s) in the region which results in CSV- ϕ. Both of these strains carry this mutation(s) thus ~~are~~ the KAN cassettes are linked to the CSV- ϕ.

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

1/21

COMPLEMENTATION TEST

~~the~~
To test whether these two strains #31 & #35 carry ~~the~~ a mutation in the same gene or two diff genes I will perform a complementation test:

TEST CROSS: 31-1-4a met⁻/URA3⁺/KAN⁺/Mat a × 35-1-4a MET⁺/ura3⁻/KAN⁺/Mat α

POSITIVE CONTROL: 35-1-4a MET⁺/ura3⁻/KAN⁺/Mat α × 35-1-4b met⁻/URA3⁺/KAN⁺/Mat a

NEGATIVE CONTROL: 35-1-4c met⁻/URA3⁺/kan⁻/Mat α × 35-1-4d MET⁺/ura3⁻/kan⁻/Mat a

A positive result (complementation) in the test cross would indicate that either:

(1) the mutations are in diff genes, or
(2) at least one mutation is dominant.

To ~~the~~ test (2) I will perform the following crosses:

#31: 31-1-4a met⁻/URA3⁺/KAN⁺/Mat a × 31-1-[redacted] 5a MET⁺/ura3⁻/kan⁻/Mat α

#35: 35-1-4b met⁻/URA3⁺/KAN⁺/Mat a × 35-1-5b MET⁺/ura3⁻/kan⁻/Mat α

1/23

Streak all 5 crosses above onto -ura-met

2/13

RESULTS FROM THE COMPLEMENTATION TEST / DOMINANCE TEST

For each of the strains on page 122 (3 diploids from the complementation test and 2 from the dominance test) I grew o/N in -ura, diluted 1:10,000 in 2xSC and started 6×200λ and 9×100λ cultures.

- All of the positive control diploids from the complementation test have a fitness defect.
- All plates look like W303 — perhaps this test does not work in diploids, or ~~that~~ this phenotype in S288c is observed only for point mutations not for loss of the entire gene as is what happens in diploids

TRANSFORMATION TEST: MOVING CASSETTES INTO GL-35 BACKGROUND

To see if I can transfer some of my ORFΔ::KanMXΔ::URA3 cassettes into the bkg of ~~GL31 and~~ GL-35 I transformed 35-1-4a ~~an~~ (MET+/Mata) and 35-2-3b (met-/Mata) with PCR products from GL-21 and GL-39 using primers YFL011wf & YFL011wr and YFR030wf & YFR030wr, respectively (see pg. 61)

Like above I plated two plates per strain (6 200λ spots & and 9 100λ spots)

The 6 spot plate for 35-2-3b / GL-39 transformation has some small colonies, but I believe that they all look like W303 sized colonies on the other plates.

↳ therefore I can move my constructs into this bkg.

TRANSFORMATION TEST II: MOVING KANMXΔ CASSETTE FROM GL-31 TO GIL051

I wanted to move the KANMX cassette with 100 bp homology and 1kb homology from strains #31 and #35 (the parental strains from which GL-31 and GL-35 were derived by transformation) into GIL051 to see if I could transfer the colony morphology phenotype.

20301622 IDT
V.INDJEIAN
21586586 1/18/2006
GL31100F
5'- GAA ATA CAC TTT TAT GAT CTG TCC -3'
Tm= 49.7 °C , MW= 7,301.8
8.7OD260 37.60nmol 0.27mg

20301618 IDT
V.INDJEIAN
21586218 1/18/2006
GL311kbF
5'- CAC CCC CAT ATT TCT CGA AC -3'
Tm= 53.1 °C , MW= 5,916.9
5.9OD260 33.40nmol 0.20mg

20301619 IDT
V.INDJEIAN
21586219 1/18/2006
GL35100F
5'- TCT TGA CGA ACG CGA AAA CC -3'
Tm= 56.0 °C , MW= 6,095.0
6.5OD260 33.20nmol 0.20mg

20301617 IDT
V.INDJEIAN
21586217 1/18/2006
GL351kbF
5'- CGA ATT CTT GTC ATC GTG CC -3'
Tm= 54.2 °C , MW= 6,059.0
6.8OD260 37.70nmol 0.23mg

20301615 IDT
V.INDJEIAN
21586215 1/18/2006
GL31100R
5'- TAC ACA GAT TGG TCT GTG CC -3'
Tm= 54.7 °C , MW= 6,108.0
5.1OD260 27.10nmol 0.17mg

20301616 IDT
V.INDJEIAN
21586216 1/18/2006
GL311kbR
5'- GTC AAC GAT GTG GAA TAC GG -3'
Tm= 53.5 °C , MW= 6,206.1
5.9OD260 28.90nmol 0.18mg

20301620 IDT
V.INDJEIAN
21586584 1/18/2006
GL35100R
5'- CGT AAC ATT CAT TGA ACC ACC -3'
Tm= 52.0 °C , MW= 6,334.2
6.4OD260 31.90nmol 0.20mg

20301621 IDT
V.INDJEIAN
21586585 1/18/2006
GL351kbR
5'- GGA AAG GGA TTG ATT CAG AAC C -3'
Tm= 53.6 °C , MW= 6,832.5
7.5OD260 33.00nmol 0.23mg

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 8/9/21 6/92 10VG

2/13

- Unfortunately I was only able to get a PCR using the 1st set of primers.
- I was able to successfully transform GIL051 with this PCR product.
- Today I will set up fluct tests (as done previously, pg 123) to assay the phenotype of the transformants.

2/21

TRANSFORMANT	W303-like	S288c-like	Unclear
1			X
2	X	X	
3	X	X	
4		X	
5			X
~~X~~6			X
~~X~~7			X
~~X~~8			X
9		X	
10			X
11			X
12		X	
13			X
14			
15			
16			
17			
GIL051		X	
GL·31		X	
GL·35	X		

2/14/06

14

S288c v. YJM789				S288c v. RM11-1a			
ORF	Ka	Ks		ORF	Ka	Ks	
YEL021W	0	0		YEL021W	0.140416	0.007421	requires manual alignn
YFL063W	0.135545	0.019169	requires manual alig	YFL063W	0.123223	0.028754	requires manual alignn
YFL056C	0.006012	0	requires manual alig	YFL056C	0.032782	0.102381	requires manual alignn
YFL055W	0	0		YFL055W	0.002087	0.009182	
YFL054C	0	0		YFL054C	0.001333	0.009084	
YFL052W	0.000914	0.003297		YFL052W	0.000914	0.003297	
YFL050C	0.003292	0.007855		YFL050C	0.003127	0.008459	
YFL049W	0.000902	0.009298		YFL049W	0.003158	0.009298	requires manual alignn
YFL047W	0.001173	0.013636		YFL047W	0.000978	0.012879	
YFL044C	0.000467	0.006944		YFL044C	0.000467	0.006944	
YFL041W	0.000227	0.001668		YFL041W	0.000907	0.006672	
YFL036W	0.000209	0.004245		YFL036W	0.000313	0.005403	
YFL034W	0.000526	0.000484		YFL034W	0.000658	0	
YFL032W	0.083223	0.019417	requires manual alig	YFL032W	0.080581	0.019417	requires manual alignn
YFL027C	0.001688	0.007551		YFL027C	0.001406	0.00863	
YFL025C	0.000686	0.006546		YFL025C	0.000961	0.006042	
YFL023W	0.083377	0.025424	requires manual alig	YFL023W	0.08633	0.016243	requires manual alignn
YFL021W	0.000558	0.004921		YFL021W	0.132012	0.025591	requires manual alignn
YFL019C	0.058683	0	requires manual alig	YFL019C	0.064671	0.008811	requires manual alignn
YFL015C	0.113438	0.020649	requires manual alig	YFL015C	0.117801	0.00885	requires manual alignn
YFL012W	0.001876	0.007273		YFL012W	0	0	
YFL011W	0.001052	0.011607		YFL011W	0	0	
YFL010C	0.013414	0.002398	requires manual alig	YFL010C	0.013414	0	requires manual alignn
YFL007W	6.55E-05	0.000992	manually truncated	YFL007W	0.000655	0.003966	
YFL004W	0.000168	0.002626		YFL004W	0.000337	0.004596	manually truncated
YFL003C	0.00016	0.0024		YFL003C	0.000801	0.0024	
YFL001W	0.000316	0.001209		YFL001W	0.000949	0.004837	
YFR001W	0	0.005222		YFR001W	0	0.007833	
YFR006W	0.000778	0.002064		YFR006W	0.000778	0.007224	
YFR007W	0.001201	0.002903		YFR007W	0.001201	0.002903	
YFR009W	0.000187	0.003516		YFR009W	0.000187	0.004219	
YFR012W	0.039625	0.006834	requires manual alig	YFR012W	0.038184	0.009112	requires manual alignn
YFR014C	0.000629	0.007134		YFR014C	0.000943	0.007134	
YFR016C	0.087572	0.000439	requires manual alig	YFR016C	0.087686	0.000439	requires manual alignn
YFR017C	0	0		YFR017C	0	0.002639	
YFR019W	0.105406	0.000697	requires manual alig	YFR019W	0.105344	0.000697	requires manual alignn
YFR021W	0	0		YFR021W	0	0	
YFR023W	0	0.001813		YFR023W	0.000227	0.000907	
YFR026C	0.056619	0.006079	requires manual alig	YFR026C	0.056619	0.006079	requires manual alignn
YFR030W	0.000138	0.000962		YFR030W	0.000276	0.000962	
YFR032C	0	0		YFR032C	0	0	
YFR035C	0.03625	0.017021		YFR035C	0.0375	0.017021	
YFR039C	0.135408	0.012821	requires manual alig	YFR039C	0.135408	0.012821	requires manual alignn
YFR043C	0.002897	0.007212		YFR043C	0.002317	0.004808	
YFR045W	0.000999	0.003503		YFR045W	0.000999	0.007005	
YFR049W	0	0		YFR049W	0	0	
YFR053C	0.001163	0.012821		YFR053C	0.000873	0.001068	
YFR054C	0.002247	0.004975		YFR054C	0	0	
YFR055W	0.000425	0.005594	requires manual alig	YFR055W	0.00085	0	
YFR057W	0.123959	0.020906	requires manual alig	YFR057W	0.000925	0	

no contig.

15

2/14/06

1 Calculated in 500 bp window upstream and downstream of ORF
2 REFERENCE: Gerton JL et. al. Global mapping of meiotic recombination .... PNAS. 2000 Oct 10;97(21):11383-90.
3 REFERENCE: Raghuraman MK et. al. Replication dynamics of the yeast genome. Science. 2001 Oct 5;294(5540):115-21.

LOCUS	MUT RATE	%GC[1]	RECOMB RATE[2]	REP TIME[3]	Ks, S288c vs. YJM789	Ks, S288c vs. RM11-1a	
YEL021W	0.484670018	0.364	0.85	27.6938	0	0	
YFL063W	9.363690793	0.432	1.39	49.7317	0.040268456	0.045901639	
YFL056C	1.353391202	0.389	0.85	45.5572	0	0.104761905	
YFL055W	1.696952634	0.35	0.78	44.4609	0	0.00918197	*
YFL054C	1.388869553	0.332	0.84	43.6606	0	0.009084027	
YFL052W	1.165819854	0.377	0.83	43.9345	0.003296703	0.003296703	
YFL050C	1.208260924	0.341	0.87	42.6926	0.007854985	0.008459215	
YFL049W	1.424388724	0.349	0.86	42.4417	0.009298394	0.009306261	
YFL047W	1.145667323	0.345	0.81	42.9517	0.013636364	0.012878788	
YFL044C	1.153303648	0.361	0.98	43.6687	0.006944444	0.006944444	
YFL041W	1.191144255	0.329	0.93	43.9375	0.001668057	0.006672227	
YFL034W	1.011890336	0.296	0.82	36.2196	0.000484262	0	*
YFL032W	1.056247103	0.427	1.61	38.6249	0.005076142	0	
YFL027C	1.186696478	0.368	1.02	42.5949	0.007551241	0.008629989	
YFL025C	1.484908001	0.332	0.82	43.7822	0.006545821	0.006042296	
YFL023W	1.567573538	0.406	1.12	40.2538	0.008474576	0.002401922	
YFL021W	0.52152621	0.427	2.13	38.0255	0.00492126	0.00625	
YFL019C	0.764005829	0.367	1.095	36.763	0	0.004587156	*
YFL015C	0.433717758	0.396	1.38	32.7423	0.003021148	0.006024096	
YFL012W	0.394420614	0.314	1.22	29.2385	0.007272727	0	
YFL011W	0.385406106	0.322	1.4	27.1748	0.011607143	0	*
YFL010C	0.353168577	0.364	2.18	25.3505	0.002604167	0	
YFL007W	0.455342924	0.353	0.87	23.3455	0.000991572	0.003966287	
YFL004W	0.297676519	0.341	0.83	26.4715	0.002626395	0.004596192	
YFL003C	0.456339538	0.332	1.07	25.9189	0.00239952	0.00239952	
YFL001W	0.763234757	0.323	0.78	26.7957	0.00120919	0.004836759	
YFR001W	0.413358709	0.324	0.85	26.8613	0.005221932	0.007832898	
YFR006W	0.634052466	0.362	1.02	24.7213	0.002063983	0.007223942	
YFR007W	0.374173594	0.362	0.91	22.7203	0.002902758	0.002902758	
YFR009W	0.489378213	0.342	0.92	20.1885	0.003516174	0.004219409	
YFR012W	1.303667067	0.337	0.94	18.6297	0.00887574	0.01183432	
YFR014C	0.58325674	0.351	1.03	19.5332	0.007134364	0.007134364	

* k_s estimates differ $X \geq 2$

$$X = \frac{|Ks_{YJM} - Ks_{RM11}|}{(Ks_{YJM} + Ks_{RM11})/2}$$

YFR016C	0.683309164	0.427	0.91	23.4242	0.001154734	0.001154734	
YFR017C	0.586898312	0.432	1.56	25.1464	0	0.002638522	*
YFR019W	1.489105403	0.4	0.86	21.1616	0.00265252	0.00265252	
YFR021W	0.378435483	0.362	1.11	12.8011	0	0	
YFR023W	0.402705008	0.34	1.62	13.7177	0.001813237	0.000906618	
YFR026C	0.35965965	0.368	1.52	15.0252	0.008849558	0.008849558	
YFR030W	0.73888516	0.365	0.91	22.6155	0.000961538	0.000961538	
YFR032C	1.075873264	0.379	1.52	33.8172	0	0	
YFR035C	0.956131507	0.435	1.87	36.6154	0.017021277	0.017021277	
YFR039C	0.760863318	0.357	0.9	37.3668	0.001805054	0.001805054	
YFR043C	0.102936682	0.355	1.035	37.8963	0.007211538	0.004807692	
YFR045W	0.843429637	0.365	1.03	39.3621	0.003502627	0.007005254	
YFR049W	0.148876672	0.335	0.98	41.878	0	0	
YFR053C	1.215588831	0.335	1.23	42.5638	0.012820513	0.001068376	
YFR054C	0.691284486	0.366	0.88	45.3261	0.004975124	0	*
YFR055W	0.645843403	0.322	0.88	48.5842	0.005602241	0	
YFR057W	0.775971821	0.33	1.06	49.2339	NA	0	

no YJM789 sequence

	mut. rate	%GC	recomb rate	rep time	Ks YJM	Ks RM11	
vs mutation rate	1 0	0.1444 0.1588	-0.302 0.0188	0.5587 0	0.163 0.1319	0.4293 0.0011	
vs %GC		1 0	0.3798 0.0036	-0.0525 0.3639	0.3019 0.0014		← YJM/RM11 combined
vs recomb rate			1 0	-0.2377 0.0477	0.3919 0.0005		← YJM/RM11 combined, remove $X \geq 2$
vs rep time				1 0			

for each of the blocks above the top value is C and the bottom number is the p-value (p-value of "0" is actually $p < 0.0001$

NOTES FROM LAB MEETING (3/20/06) AND COMMITTEE MEETING (3/21/06)

- Normalized Z-values
- Don't use the term "mutational isochores"
- How much aneuploidy is in Δ collection? – email Jeff Boeke (Hopkins)
- Compare Ks (S288c v. YJM/RM11) to tREP genomewide
- calculate % GC with different window sizes
- I can explain the correlation btw Recomb rate & mut rate w/ the corr. btw. tREP & Recomb rate.
- Meel separation of function alleles which separate Vpt function & tREP – Rad6
  - → talk to Matt Michael
- ~~[illegible]~~
- Moving early origins to a late region sometimes works.
- Where do my outliers fall on the plot of μ_{SFOAR} vs. μ_{CANR}?
- S288c has bkg. growth on 5FOA which causes variable colony size
  - more drug on plates
  - deletion of FABI
  - backcross
  - nothing
- The outliers on my plot of Ks (~~WSO~~ S288c v. YJM) vs. Ks (S288c v. RM11) can be the result of 2 different things:
  - ~~If~~ Since S288c is a mut strain, these could ~~by~~ be regions ~~where~~ which come from different parental origins.

    or
  - These could be regions where the mutation rate has changed.

4/4

Transformation of GIL051 w/ FAB1Δ::KanMX6

I could not generate a PCR product using the primers GL35100F & R on pg. 123. I redesigned the primers hoping that this solves the problem:

21735005 IDT
V.INDJEIAN
23248030 3/29/2006
FAB1extF2
5'-AGA CCA ATT AGT CGC AAA GG-3'
Tm= 52.7 °C , MW= 6,159.1
5.6OD260 26.70nmol 0.17mg

21735007 IDT
V.INDJEIAN
23248032 3/29/2006
FAB1extR2
5'-TCC TCA GAA AAG CAA TTA GG-3'
Tm= 49.9 °C , MW= 6,134.1
5.7OD260 28.10nmol 0.17mg

- Use typical Taq protocol w/ annealing @ 50° and extention time of 2:30.
- Single product formed (see gel in MRS, p)
- PCR prep → transform into GIL051
- Today I need to rep. from YPD to YPD+G418

4/8

I still do not see any colonies on the YPD+G418 plate leading me to think that the transformation did not work.

The original PCR was not great so I decided to retry the PCR varying annealing temp. (48, 50, & 52) and using both the FAB1Δ::KanMX gDNA ~~as a tem~~ and the previous PCR product as template. — I also made ext. time to 3:00

Very faint product using gDNA in all 3 temps — good product in all 3 temps using PCR product as temp.

→ I pooled the 3 PCRs and prepped them into 60λ

Retry transformations: Choose 2 GIL051 cultures to transform → OD_{600} of .44, → OD_{600} of .71

4/11

Results of transformation

- Saturday (4/9) I replica plated the YPD plates to YPD+G418
- Today I see a number of colonies on both YPD+G418 plates (from the OD=0.44 and 0.71 transformations
- I also see a single ~~trans~~ colony on one of the YPD+G418 plates from 4/4
- Today I will restreak the putative transformants onto YPD+G418 labeled as follows:

~~From 4/4~~
~~From~~

From 4/4: 1.1
From OD=0.44: 2.1 – 2.8
From OD=0.71: 3.1 – 3.8
Bkg from the 0.71 YPD plate

4/12

- It looks like 1.1 & Bkg did not grow upon restreaking
- Inoculate 3mL YPD+ADE with a streak from the restreaked G418 plates (2.1-2.8, 3.1-3.8)
  ↳ tomorrow I will gDNA prep and PCR to confirm fab1 deletion.

4/18

Last week I gDNA prepped 2.1-2.8 and 3.1-3.8 and ran a PCR using primers FAB1extR3 & FAB1extF3:

21882925 IDT
V.INDJEIAN
23403629 4/5/2006
FAB1extF3
5'- CCT GAA ATA GCA CCA ACA GG -3'
Tm= 53.6 °C , MW= 6,104.0
5.2OD260 25.70nmol 0.16mg

21882923 IDT
V.INDJEIAN
23403627 4/5/2006
FAB1extR3
5'- AAG AGA TTC TGT CCC AGT GG -3'
Tm= 54.0 °C , MW= 6,157.0
5.0OD260 25.30nmol 0.16mg

Typical Taq PCR

Annealing 50°

Extension time 3m

PCR confirmation of FAB1 deletion in 2.4, 2.6, and 2.7

4/18

Fab1Δ::KanMX transformation continued : NOTES

Last Thursday (4/13) when I did the gDNA preps / PCRs, I also inoculated 3ml cultures of 2×SC-ura with all 16 ~~strains~~ putative transformants.

On Friday (4/14) I wanted to start fluct assays of the confirmed transformants however they had not yet saturated. The o/n cultures of 2.4, 2.5, 2.6, & 2.7 were still at low density whereas the other 12 had saturated.

On Sunday 2.4-2.7 were well saturated — I started ~~24~~ 16 100λ cultures of each (along with GL104, and GL051 as + & - controls respectively) to determine their plating phenotype.

Looking back at the restreaked G418 plates, I notice that the colonies from 2.4-2.7 look smaller. This could give me a way of screening transformants phenotypically rather than by PCR which would be nice especially since the transformation efficiency was so low.

QUESTIONS:

- I didn't notice a fitness defect previously in GL.35 — what is the source of the defect I see now? It could be that fab1Δ strains go through a longer lag (or slower log doubling time) but saturate at the same density.
- Is the colony morphology difference I see connected to this growth defect?

8/16 PREPARING TO TEST RELATIONSHIP BTW t_{REP} AND MUTATION RATE

Last week I presented this work at the Gordon Conference on Mutagenesis. Many people were intrigued by the idea that mutation rate may be correlated to replication timing. Tom Petes has done similar work integrating URA3 randomly across the genome, and he sees no correlation between mutation rate and t_{REP}. There are four explanations for this disagreement.

1) my data are ~~cr~~ crap

2) his data are crap

3) there is a systematic diff. in mut. rate between chromosomes, ∴ integrating URA3 randomly blurrs the relationship

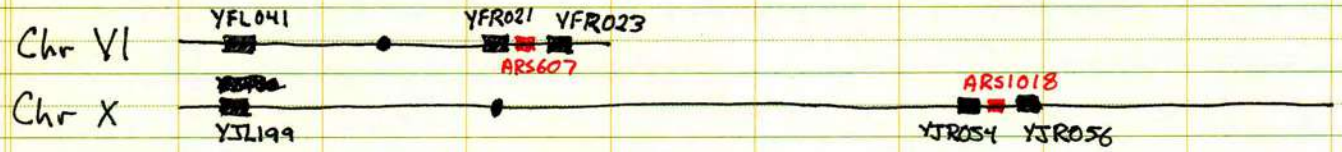
4) ~~it con~~ Tom's ~~cells~~ URA3 reporter contains a di-nucleotide ~~set~~ microsatellite. It could be that mutations at microsat. do not show this correlation

I am planning on doing an experiment to test wether the correlation I see between t_{REP} and μ is causal or coincidental.

The experimental setup is as follows:

- Take two strains that are in a low μ (early replicating) region because of their proximity to ARS607
- Delete ARS607 in these strains and look for an increase in mut. rate
- As controls, delete ARS607 in strains where URA3 is far away on the same chromosome and or on another chromosome.
- Repeat this for another location (ARS1018)

↳ The two most well characterized chromosomes with regard to rep. timing and origin location are VI & III. ChrX was used as a test chromosome for validation in two high-throughput papers – one looking at t_{REP} and one looking for origins – therefore ChrX is a good candidate for manipulation.



- I initially wanted to pick YJR055, but the delete appears to have a slight fitness defect.

STRAINS NEEDED

NAME	DISCRIPTION
GL·36	YFR021Δ::URA3
GL·37	YFR023Δ::URA3
GL·10	YFL041Δ::URA3
GL·51	YJL199Δ::URA3
GL·52	YJR055Δ::URA3
GL·53	YJR056Δ::URA3

GL·36$^{\Delta 607}$
GL·37$^{\Delta 607}$
GL·10$^{\Delta 607}$
GL·51$^{\Delta 607}$
} deleted for ARS607

GL·51$^{\Delta 1018}$
GL·52$^{\Delta 1018}$
GL·53$^{\Delta 1018}$
GL·10$^{\Delta 1018}$
} deleted for ARS1018

- I will disrupt the ARSs by replacing the ARS seq. with the Clonat resistance cassette — I picked NAT because it is a small cassette.

↳ This is not ideal because I will be inducing transcription in the region.

8/16

- On Sunday (8/13) I streaked ~~YJ~~ YJL199Δ::KanMX, YJR055Δ::KanMX, and YJR056Δ::KanMX from the deletion collection.
- Also on sunday I streaked GL-10, GL-36, & GL-37 to singles from my collection.
- Last night I started o/N cultures which I diluted back 1:400 this morning.
- Today I will ~~the~~ transform the strains with the KanMX cassette with my plasmid.

Preparation of plasmid.

- pGIL001 containing bacteria were streaked to singles
- two colonies used to inoc. 3 ml ½×YT+amp
- each 3ml culture miniprepped
- minipreps pooled and digested w/ EcoRI & BamHI

43.5 λ	Prep1	
43.5	Prep2	digest o/N
10	EcoRI Buffer	digest o/N
1	BSA	digest o/N
1	EcoRI	digest o/N
1	BamHI	digest o/N
100 λ	TOTAL	

- add 1 vol P/C; vortex; spin 5 min
- remove sup add 10 λ NaOAc (3M) and 200 λ EtOH; put in -20
- spin 10 min; asp sup; speed vac → resus in 40 λ H_2O

LANE #

1 Cut plasmid after EtOH before transformation
2, 3 } plasmid preps

- Transform strains L199Δ::KanMX, R054Δ::KanMX, & R056Δ::KanMX with purified plasmid. (ODs btw 0.53-0.56)

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

8/31

My transformations failed — bad PEG mix I think.

- Yesterday I redid the transformations using freshly made LiOAc & PEG Mix.
- Prepare plasmid same as before (p135).
- Transform:

L199Δ		KanMXΔ::URA3
R54Δ		"
R56Δ		"
GL·10	w/	607Δ
GL·10		~~1040Δ~~ 1018Δ
GL·36		607Δ
GL·36		~~1040Δ~~ 1018Δ
GL·37		607Δ
GL·37		~~1040Δ~~ 1018Δ

Sample ID wl 600.0

	Abs	
1	0.5686	L199
2	0.5824	R 54
3	0.6002	R 56
4	0.6861	GL·10
5	0.6616	GL·36
6	0.6897	GL·37
7		

- Today I replica plated the ars deletions to YPD+ Clonat

9/2

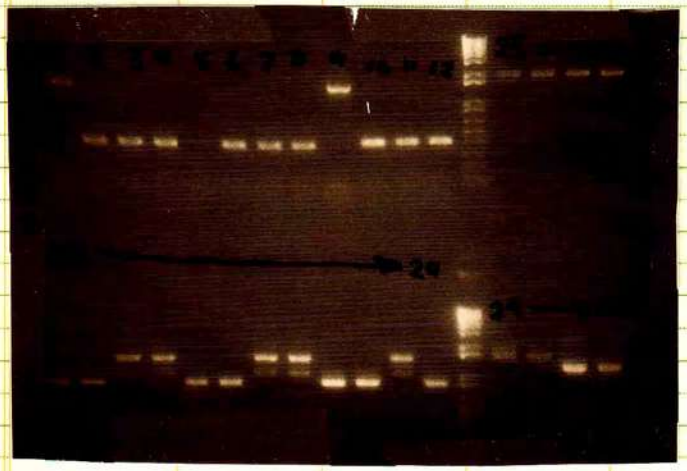
- Today I picked 2 colonies off of each transformed plate
  - URA3 plates → into SC-ura
  - others → into YPD
- Set up another PCR to amplify the Nat^R cassette so that I can knock out the ARSs in the strains with URA3 on ChrX.

9/5

- On Sunday (9/3) I did gDNA preps on the putative transformants
- Yesterday I set up PCRs to verify transformations

9/5

Verification of transformants



NOTES

- GL-36$^{\Delta 607}$A did not form a product with ARS607 primers. This strain is now URA−; therefore a more complicated rearrangement may have occurred using pringle homology ~~in the~~ left over from the ~~of~~ KanMX cassette.

LANE			
1	GL-10$^{\Delta 607}$	A	w/ primers ARS607extF1, ARS607extR1
2		B	
3	GL-10$^{\Delta 1018}$	A	
4		B	
5	GL-36$^{\Delta 607}$	A	
6		B	
7	GL-36$^{\Delta 1018}$	A	
8		B	
9	GL-37$^{\Delta 607}$	A	
10		B	
11	GL-37$^{\Delta 1018}$	A	
12		B	
13			w/ primers ARS1018extF1, ARS1018extR1
14			
15			
16			
17	same as 1-12		
18			
19			
20			
21			
22			
23			
24			
25	YJL199Δ::KanMXΔ::URA3	A	w/ primers URA3u1, URA3d1 (see p. 13)
26		B	
27	YJR54Δ::KanMXΔ::URA3	A	
28		B	
29	YJR56Δ::KanMXΔ::URA3	A	
30		B	
31	ARS607Δ::NatR		to transform lanes 25-30
32	ARS1018Δ::NatR		

24225974 IDT
V.INDJEIAN
25956939 8/14/2006
ARS607extF1
5'-GTT TTC AGT TTG AGG GAT CC-3'
Tm= 51.2 ºC , MW= 6,154.0
6.6OD260 34.80nmol 0.21mg

24225972 IDT
V.INDJEIAN
25956937 8/14/2006
ARS607extR1
5'-GTG TCG CAG TCC ATA GAA GG-3'
Tm= 55.2 ºC , MW= 6,182.1
5.5OD260 27.20nmol 0.17mg

24225973 IDT
V.INDJEIAN
25956938 8/14/2006
ARS1018extF1
5'-GTA CTC CAA AGA AGT CTG GG-3'
Tm= 52.4 ºC , MW= 6,166.1
5.5OD260 27.30nmol 0.17mg

24225975 IDT
V.INDJEIAN
25956940 8/14/2006
ARS1018extR1
5'-CCT CGA GGA TAT AGG AAT CC-3'
Tm= 51.1 ºC , MW= 6,126.0
6.5OD260 32.60nmol 0.20mg

SUMMARY

Correct:
- GL-10$^{\Delta 607}$ A
- GL-10$^{\Delta 1018}$ A
- GL-10$^{\Delta 1018}$ B
- GL-36$^{\Delta 1018}$ A
- GL-36$^{\Delta 1018}$ B
- GL-37$^{\Delta 607}$ A
- GL-37$^{\Delta 1018}$ A
- YJL199::URA3 A
- " B
- YJR54::URA3 A
- " B
- YJR56::URA3 B

Incorrect:
- GL-10$^{\Delta 607}$ B
- GL-36$^{\Delta 607}$ A
- GL-36$^{\Delta 607}$ B
- GL-37$^{\Delta 607}$ B
- GL-37$^{\Delta 1018}$ B
- YJR56::URA3 A

LITHOGRAPHED IN U.S.A. ∴ ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

9/5

gDNA preps an PCR on additional putative GL-36$^{\Delta 607}$ transformants

- Yesterday I picked 7 additional colonies off the GL-36$^{\Delta 607}$ plate and inoculated 3mL 2x SC-ura to ensure that the cassette did not integrate at the URA3 cassette.

↳ 5 of the 7 are URA+

↳ make gDNA preps → PCR using ARS607 primers

LANE

1	GL-36$^{\Delta 607}$	H
2	"	J
3	"	K
4	"	L
5	"	M
6	"	A
7	GL-36$^{\Delta 1018}$	A

PHENOTYPING THE STRAINS I WILL USE IN EXPT.

- Start o/N 3 mL cultures of the following strains

(1) GL-36
(2) GL-37
(3) GL-10
(4) GL-36 Δ607 – K (above)
(5) GL-37 Δ607 – A
(6) GL-10 Δ607 – A
(7) GL-36 Δ1018 – A
(8) GL-37 Δ1018 – A
(9) GL-10 Δ1018 – A

} pg. 137

9/6

Passage the 9 cultures

9/5

TRANSFORM YJL199Δ::URA3, YJR54Δ::URA3, & YJR56Δ::URA3 w/ ARS607Δ::Nat^R & ARS1018Δ::Nat^R

- PCR of ARS Δ cassettes
  - template: pAG25 (NatMx4) - vectorology #33 - use 1 λ of 1:10 dilution.
  - primers:

Sequence - ars1018_F2 — 250 nmole DNA oligo, 100 bases

5'- TTG GGT AAT AGT TGA CAT ACC GAT GTT CTG CTG GTA GCA TCG ATG AAC GAA AAT AGG TAA CGG CTT GCC GCT GAA GAT AGA CAT GGA GGC CCA GAA TAC C -3'

Sequence - ars1018_R2 — 250 nmole DNA oligo, 100 bases

5'- CAG CTT CCT CTA ACT TCG ATG ACA GTT CCT CGT ATC TTA TGT CAT CGT CTA ACA CCG TAT ATG ATA ATA TAT TGG TAG TGT CGA CAG CAG TAT AGC GAC C -3'

Sequence - ars607_F2 — 250 nmole DNA oligo, 100 bases

5'- CCA TTA GAG ACA GAG AAA CTA TTC ATT GTA CAT TCT CCA AAT GTG GTG ATA TAA ACA CTA CAT TCG CTA AAT TAC ATT AGA CAT GGA GGC CCA GAA TAC C -3'

Sequence - ars607_R2 — 250 nmole DNA oligo, 100 bases

5'- GAA TGT GAT TCT ATG CTT TCT AGT ACC TAC TGT GCC GAA TAA TGT GTA AGT CTC AAA ATT CTT TTC TTC CAA AGC ATA TCT CGA CAG CAG TAT AGC GAC C -3'

  - Cycle: 50° anneal
    2m extension
  - 100λ PCR → elute ~~resuspend~~ in 40λ H_2O
  - PCR products (lanes 31 & 32, pg 137) — this is the same as the transformation of GL10, GL36, & GL37 (pg 136-137)
- Grow o/n culture last night — this morning dilute back: 100λ into 10 mL YPD+ADE
-

Sample ID w1 600.0

Starting OD

	Abs	
1	0.5873	YJL199
2	0.7197	YJR54
3	0.6817	YJR56
4		

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

9/7

SEQUENCING OF CHR X PUTATIVE TRANSFORMANTS.

- On 9/5 I submitted lanes 25, 27, 30 (pg. 137) for sequencing using primers A–H (pg 14 & 42)
- YJR56Δ::URA3 B still has the in-tact KanMX cassette as I should have known from the size of the band on pg. 137
- Like Usual – no mutations in URA3 using plasmid transformation.
- Unfortunately from looking at the tags, the strains I thought were YJR054Δ and YJR056Δ are actually YJR031Δ and YJR009Δ — Dammit!

  ↳ the only correct strain is YJL199Δ::URA3 — I need to go back to the freezer — perhaps I will go to the original Δ collection.

```
YJL199

uptag - from deletion pages
GAAGGGAGATATTGAATGGATGTCCACGAGGTCTCT   ATGGCTGACCGATTCGCTCT   CGTACGCTGCAGGTCGAC

uptag - from sequence
                    CACCAGGTCTCT   ATGGCTGACCGATTCGT      ACTCTGCAGGTCGAC

comparison
ATGGCTGACCGATTCGCTCT
ATGGCTGACCGATTCGT---

downtag - from deletion pages
CTCCCTTATATATTGTTACGGTGTCGGTCTCGTAG   AACTGCTAGGCACTCGTGTG   ATCGATGAATTCGAGCTCG

downtag - from sequence
              TCGGTGTCGGGTCTCGTAG   AACTGCTAGGCACTCGTGTG   ATCGATGAATTCGAG
comparison
AACTGCTAGGCACTCGTGTG
AACTGCTAGGCACTCGTGTG

-----------------------------------------------------------------------

YJR054

uptag - from deletion pages
AACAGAAAATCAAAAATGGATGTCCACGAGGTCTCT   TATCCGCGCTTCATGCGTGT   CGTACGCTGCAGGTCGAC

uptag - from sequence
              TTGATGTCCACCGAGGTCTCT   ATGCACCGCAGAGCTTGTAG   CGTACGCTGCAGGTCGAC

comparison
TATCCGCGCTTCATGCGTGT
ATGCACCGCAGAGCTTGTAG - YJR031C

downtag - from deletion pages
GCTTTTGCCAGCCTATTACGGTGTCGGTCTCGTAG   ATTGTCAAGTGCTGCGAACG   ATCGATGAATTCGAGCTCG

downtag - from sequence

comparison

-----------------------------------------------------------------------

YJR056

uptag - from deletion pages
AGAATATATTCAATTATGGATGTCCACGAGGTCTCT   CCTCACATGAAAGTTAGCCA   CGTACGCTGCAGGTCGAC

uptag - from sequence
              TTGATGTCCACCAGGTCTCT   CCGCTTCATCAAGACTGTTACA   TACGCTGCAGGTCGAC

comparison
CCTCACATGAAAGTTAGCCA
CCGCTTCATCAAGACTGTTACA - YJR009C

downtag - from deletion pages
TCTTCCCGGGTGTTCTTACGGTGTCGGTCTCGTAG   CACTAGGCATGATGGTAACG   ATCGATGAATTCGAGCTCG

downtag - from sequence
              TCGGTGTCGGGTCTCGTAG   CAGCCGTGCATACGCAT      ATCGATGAATTCGAGCTCG

comparison
CACTAGGCATGATGGTAACG
CAGCCGTGCATACGCAT - YJR009C
```

STRAINS I WANTED:

	PLATE	POSITION
YJR054	40	B2
YJR056	40	B4

STRAINS I GOT:

	~~PLATE~~	~~POSITION~~
~~YJR031~~	~~[illegible]~~	~~[illegible]~~ G12
~~YJR009~~	~~[illegible]~~	

	PLATE	POSITION
YJR031	40	A3
YJR009	39	G12

9/6

TEST PLATING

- Yesterday, I started 12 200 λ cultures of the 9 strains (bottom of p 138) in 96-well plates

9/7

- Yesterday, Sara made 2 3-L batches of 5FOA (labeled 1 & 2)
- Today for each of the 9 strains I plated 6 200 λ spots onto an over-dried plate from each batch.
  ↳ allow to dry several hours.

9/12

- Yesterday I counted the colonies on the test plates — initially I was very excited because it was obvious that GL36$^{\Delta 607}$ and GL37$^{\Delta 607}$ had many more mutants than the other plates; however, I when I counted the colonies — every culture was a jackpot
  ↳ this is far too high of an elevation to be due to ARS deletion.

  I didn't realize initially but when I transformed in the Nat^R cassette I put direct repeats flanking URA3 — this is because the Δ collection was created using the pringle system and about 300 bp of pFA6 homology remains on either side of URA3 (pgs 7 & 22)

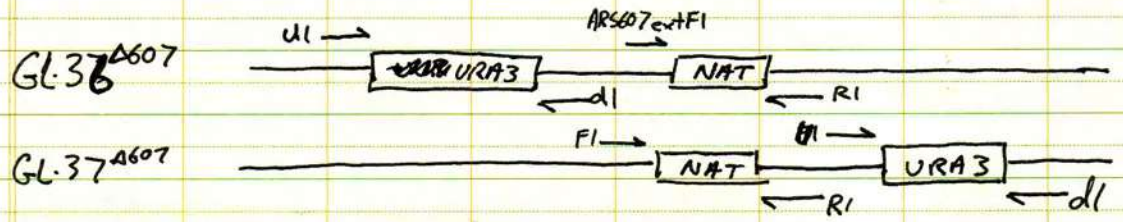
  ↳ also I got unlucky in that these were all on the same strand therefore the repeats were direct (not inverted)

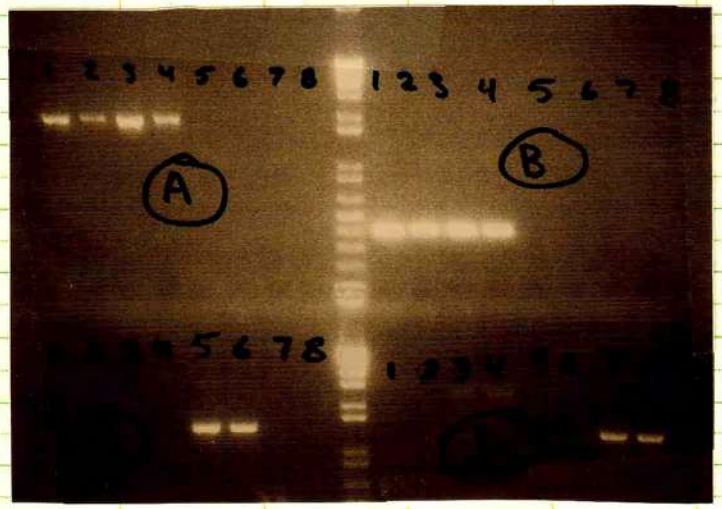
  ↳ although this may be lucky in that inverted repeats may elevate the mut. rate slightly and I may not have picked up on it until much later.

- Yesterday I started ~~gDNA~~ cultures of GL36, GL37, GL36$^{\Delta 607}$ & GL37$^{\Delta 607}$ to be gDNA prepped to determine if the mut. events I see in the Δ607 strains is the result of excision events.
- So I need to redo the ARS deletions using a diff. marker.
  ↳ I ordered primers to knock out ARS607 & ARS1018 with ~~his~~ HIS5 from the pRS vector system — also, just for the hell of it I reversed the orientation of the marker.

9/14

-ura PHENOTYPE IN GL36$^{\Delta 607}$ & GL-37$^{\Delta 607}$ IS DUE TO EXCISION OF URA3 via REC. BTW INV. REPEATS





LANE	SFOA^R mut of strain
1	GL-36
2	GL-36
3	GL-37
4	GL-37
5	GL-36 Δ607
6	GL-36 Δ607
7	GL-37 Δ607
8	GL-37 Δ607

PRIMERS

(A) ul / dl

(B) ARS607extF1 / ARS607extR1

(C) ul / ARS607extR1

(D) ARS607extF1 / dl

10/17/06

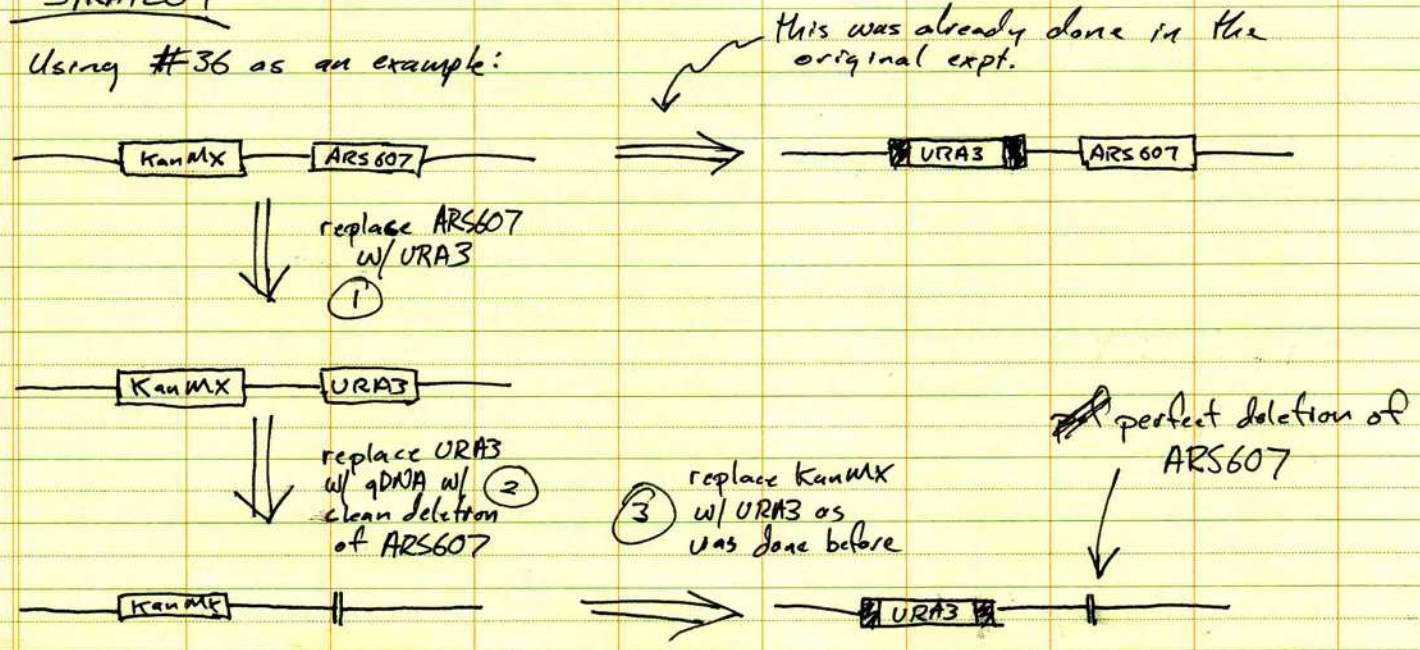
A DIFFERENT STRATEGY TO DELETE ARS607

After the problem with deleting ARSs using NAT^R (pg 142) I decided to make a clean deletion of the ARS.

Why? → No matter what I put in place of the ARS one could argue that what I put in place of the ARS (and not the absense of the ARS) is responsible for any elevation of mut. rate I might see

STRATEGY

Using #36 as an example:



The replacement of ARS607 w/ URA3 requires primers ARS607_F4 & ARS607_R4

Sequence - ars607_F4 — 100 nmole DNA oligo, 90 bases

5'- ACA GAG AAA CTA TTC ATT GTA CAT TCT CCA AAT GTG GTG ATA TAA ACA CTA CAT TCG CTA AAT TAC ATT AAT CAA AGA AGG TTA ATG TGG -3'

Sequence - ars607_R4 — 100 nmole DNA oligo, 90 bases

5'- CTA TGC TTT CTA GTA CCT ACT GTG CCG AAT AAT GTG TAA GTC TCA AAA TTC TTT TCT TCC AAA GCA TAT CTC ATT ATA GAA ATC ATT ACG -3'

- These two primers have 20 bp of homology to URA3 at the 3' end
  ↳ the highlighted regions are identical to primers URAextF & URAextR (see p 9)
- The primers contain 70 bp homology to the chromosomal region around ARS607

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

10/17/06

STRATEGY CONT...

The second transformation uses primers ARS607_F5 and ARS607_R5

Sequence - ars607_F5	100 nmole DNA oligo, 90 bases
5'- CAT TAG AGA CAG AGA AAC TAT TCA TTG TAC ATT CTC CAA ATG TGG TGA TAT AAA CAC TAC ATT CGC TAA ATT ACA TTA GAT ATG CTT TGG -3'	

Sequence - ars607_R5	100 nmole DNA oligo, 90 bases
5'- GCG AAT GTG ATT CTA TGC TTT CTA GTA CCT ACT GTG CCG AAT AAT GTG TAA GTC TCA AAA TTC TTT TCT TCC AAA GCA TAT CTA ATG TAA -3'	

- These primers contain 20 bp sequences at the 3' end that are the reverse-comp. of each other; therefore, they form a primer dimer and extend using each other as a template to form a ~~180~~ 180 bp dsDNA fragment that has ~90 bp homology to the region surrounding ARS607 but lacks the ARS sequence.

The third transformation will use pGIL001 to replace the KanMX cassette with URA3 as described earlier

---

FIRST ROUND OF TRANSFORMATIONS

- So far I have performed the first transformation (replacing ARS607 with URA3) twice.
- On the 1st attempt I did 2 100λ PCRs using ARS607_F4 & R4. For a template I used a genomic prep from GIL104 that I had in the fridge from another expt. I used standard PCR program I use with primers URA3extF & R (48°, 2m).
  ↳ the PCR was not that great, but I pcR purified & transformed anyway.
- Use standard transformation protocol where I use 10 mL starting culture, end in ~100λ and split into two 50λ transformations.
  ↳ transform
  - YFL041WΔ::KanMX (Δ10)
  - YFR021WΔ::KanMX (Δ36)
  - YFR023WΔ::KanMX (Δ37)

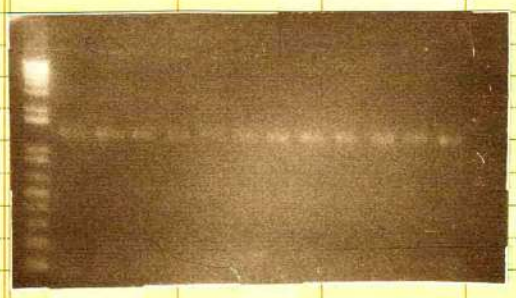
  } label trans. plates 10A, 10B, 36A, 36B, 37A, 37B
- Only 1 colony on all 6 plates (37A)

10/17/06

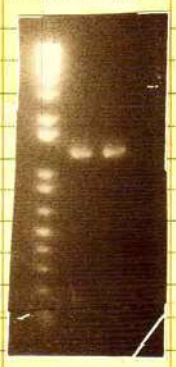
TRANSFORMATIONS CONT...

Since the PCR using ARS607-F4/R4 was inefficient I wanted to use the gradient to optimize the rxn.

→ I prepared ~~50~~ 12 50λ rxns but the gradient cycler was being used so I ran all 12 on another machine @ annealing T = 50°.



- not great but not terrible either.
- pool the 1st 6 and last 6 and PCR prep



use this to transform Δ10, Δ36, & Δ37 (10C-37D)

Results of transformation

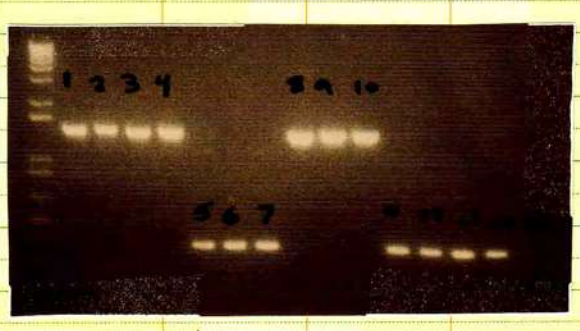
PLATE	#colonies
10C	
10D	
36C	
36D	
37C	
37D	

- I picked two colonies off of each plate and inoculated 3mL 2xSC-URA
  ↳ ~~you~~ grow O/N
- gDNA prep
- PCR using primers ARS607extF1 & ARS607extR1 (p137)

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

10/17/06

Results of transformation cont...



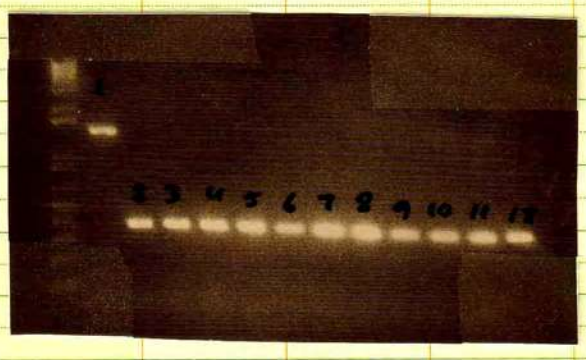
LANE	PUTATIVE TRANS. #	
1	10 C1	CORRECT
2	10 C2	
3	36 C1	
4	36 C2	
5	37 C1	
6	37 C2	
7	10 D1	
8	10 D2	
9	36 D1	
10	36 D2	
11	37 D1	
12	37 D2	
13	Δ10	controls (Δ37 was a poor gDNA prep)
14	Δ36	
15	Δ37	

- Pick the remaining putative transformants off of plates 37A, C, & D

  37A1, 37C3-10, 37D3-5 → inoculate 3 mL 2×SC-URA → grow O/N

10/19/06

Yesterday I did gDNA preps & PCRs on the putative transformants:



LANE	PUTATIVE TRANSFORMANT #	
1	37A 1	← correct
2	37C 3	
3	37C 4	
4	37C 5	
5	37C 6	
6	37C 7	
7	37C 8	
8	37C 9	
9	37C 10	
10	37D & 3	
11	37D & 4	
12	37D & 5	

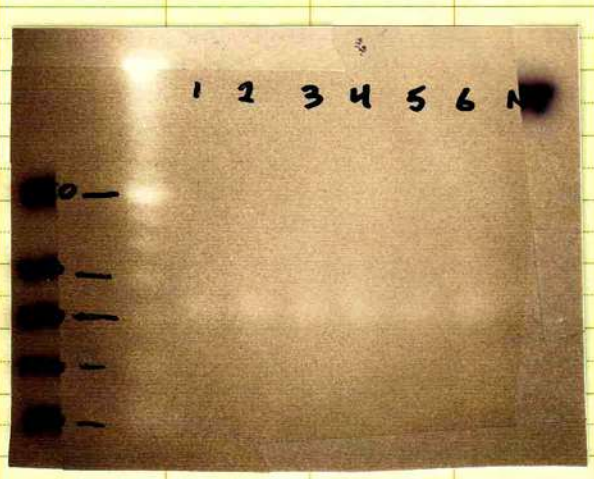
10/19/06

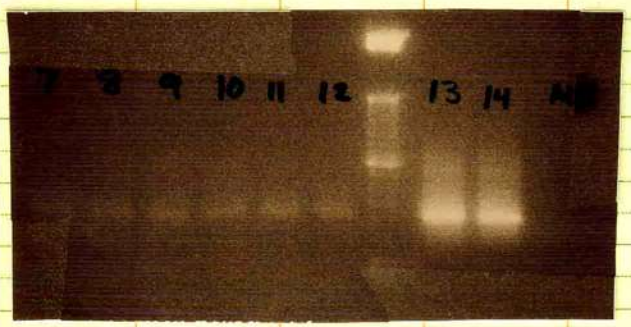
SECOND ROUND OF TRANSFORMATIONS: REPLACING URA3 w/ ARS607Δ CASSETTE

Preparation of cassette:

- Extend ars607_F5 and ars607_R5 (pg 144) → same as PCR rxn but no template.

↳ • two days ago I ran 6 100λ rxns plus a no-Taq control (1-6 below)
• today I ran 6 more 100λ rxns plus a no-dNTP control (7-12 below)
• today I pooled 1-6 & 7-12 and PCR purified them using a Qiagen spin column.





To pool & concentrate the rxns, I used 4 PCR Qiagen purification columns.

1-3 → elute w/ 30λ H_2O } pool
4-6 → "
7-9 → " } pool
10-12 → "

LANE	RXN
1	1
2	2
3	3
4	4
5	5
6	6
7	7
8	8
9	9
10	10
11	11
12	12
13	1-6 pooled & concentrated
14	7-12 pooled & concentrated
NT	No Taq
ND	No dNTPs

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

10/20/06

SECOND ROUND OF TRANSFORMATION CONT...

- Two days ago I started o/N cultures of 10C1, 10D2, 36C1, 36D2, & 37A1 in 2×SC-URA
- Yesterday morning I diluted the cultures back 1:50 and allowed them to grow ~6h before transforming (~~200~~ 250λ into 10mL)

	~~Abs~~ OD_{600}	
1	0.7831	10C1
2	0.7832	
3	0.8542	10D2
4	0.8556	
5	0.5805	36C1
6	0.5805	
7	0.6139	36C2
8	0.6143	
9	0.7953	37A1
10	0.7964	
11		

- Final LiOAc Mix suspension volume = 120λ
- Split into 3 50λ tubes
- transformations: A - 10λ 1-6 pooled
  B - 10λ 7-12 pooled
  C - 10λ H_2O

10/25/06

Verification of putative transformants

Two days ago I picked 48 total colonies off of the transformation plates

#s	Plate
1-5	10C1 - A
6-10	10C1 - B
11-15	10D2 - A
16-20	10D2 - B
21-25	36C1 - A
26-30	36C1 - B
31-35	36D2 - A
36-40	36D2 - B
~~[illegible]~~ 41	37A1 - A
42-43	37A1 - B
44	10C1 - C
45-46	10D2 - C
47	36C1 - C
48	36D2 - C

Yesterday I made gDNA preps of all 48 except 18, 19, 41, & 43 which ~~do grow very s~~ were too dilute.

PCR using primers ARS607extF1 and ARS607extR1 revealed that all of the $5FOA^R$ mutants were URA3 mutations. Tomorrow I will redo the transformations.

Modifications to protocol:

- Use 2×SC-Ura throughout to reduce bkg.
- Only 3 strains — 10C1, 36C1, & 37A1
- Larger culture — 50 mL
- Co-transform w/ pRS415 (LEU2) ~~to e~~ and select on -leu 5FOA — this will select for cells that were competent during the transformation.
  - I chose LEU2 since the Δ collection is deleted for this gene
  - I chose a CEN plasmid after talking to Soni about this transformation
    ↳ she said that I should be able to lose the CEN plasmid at a rate of 1 in 10-100 divisions — this can be done by replica plating.
- Add DMSO to ~~peg~~ PEG mix

10/25/06

DETAILS ON THE pRS415 PLASMID

- This is #111 in vectorology
- According to the nanodrop the conc. is 1.8 ng/λ
- Cut 1λ with EcoRI to verify that this plasmid is correct

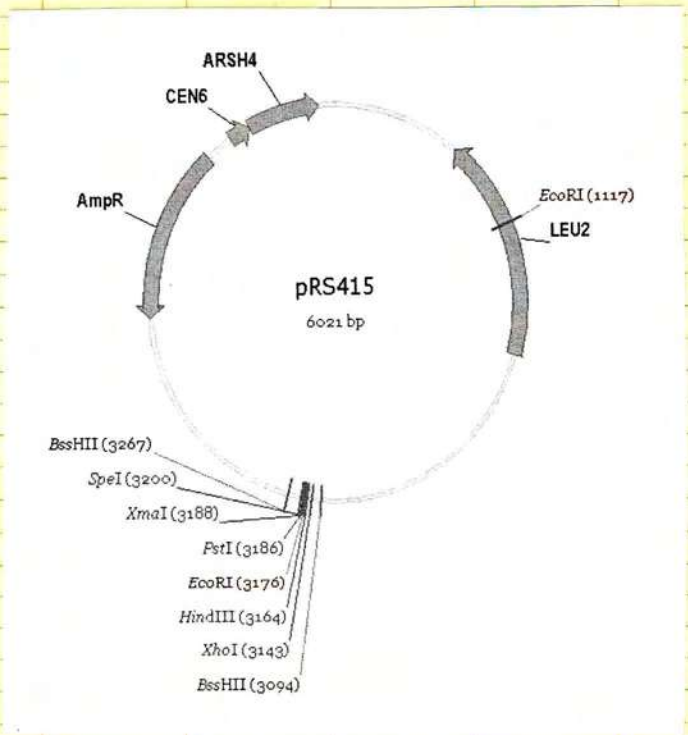
16.8 λ	H_2O
1 λ	vector #111
2 λ	NEB Buffer EcoRI
0.2 λ	EcoRI
20 λ	

Expected sizes

4.0 kb
2.0 kb

These bands seem a little high, but that is probably due to the buffer. If I purified this digest and reran it it would probably match up better.

ran this gel longer — looks fine.



10/26/06

- Dilute o/N cultures of 10C1, 36C1, & 37A1 1:100 (600λ into 60 mL) in 2×SC-ura — water bath shaker 9:20.

- 1:20, OD_{600}: 10 0.097
  36 0.074
  37 0.058

- 4:30 : 10 0.33 → dilute to 0.22 (remove 25 mL; add 25 mL 2×SC-Ura)
  36 0.24 → dilute to 0.22 (remove 10 mL; add 10 mL 2×SC-Ura)
  37 0.15

- 8:20 : 10 0.73
  36 0.73 ⟹ start transformations.
  37 0.53

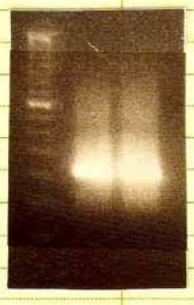
10/27/06

SECOND ROUND OF TRANSFORMATIONS – TAKE TWO

- Last night I transformed the strains 10C1, 36C1, & 37A1 with the product of ARS607_F5 & ARS607_R5 (pg. 144).

PREPARATION OF PLASMID

- Run 96 100 rxns → Prep using QIAGEN spin column
  - 12 columns
  - 8 rxns / column
  - elute 8 rxns into 30 mL EB
  - pool 1-6 & 7-12
  - run 2 λ



→ pool these two → this is a lot of DNA. If this transformation doesn't work I am not going to try it again.

this gives ~300 λ of this fragment.

TRANSFORMATIONS

	PCR	PLASMID	MEDIA	#'s 10	36	37
A	✓	✓	-leu 5FOA	8	8	7
B	✓	✓	5FOA	1	1	1
C	×	✓	-leu 5FOA	1	1	1
D	×	✓	-leu	1	1	1

NOTES:

- 1st spin 50 mL in 50 mL Falcon tube → ~~re~~ 10 m @ 2000 → resus in 10 mL H_2O → transfer to 15 mL Falcon tube → spin 5 m @ 2000 → resus in 1 mL H_2O → transfer to microfuge tube.

- Add DMSO to PEG: 14.4 mL PEG mix + 1880 λ DMSO → add 40 λ to each rxn
- Resus final pellet in 360 λ LiOAC + 40 mL ssDNA
- Added 15 λ plasmid to each trans. (before aliquoting)
- Aliquot ~~to~~ 50 λ into two microfuge tubes → (C & D above)
- to the remaining add 110 λ of prepped PCR
- aliquot 60 λ into as many tubes as possible (B and A above)

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

10/31/06

SECOND ROUND OF TRANSFORMATIONS - TAKE TWO CONT...

- It does not look like this ~~th~~ transformation worked.
  - No transformants on (A) plates
  - 2-5 on (B) ~~that~~ plates
  - Nothing on (C) plates
  - 200-500 on (D) plates
- ~~Just to~~ I expect the (B) plate colonies to be ura3 point mutants — just to be sure I yesterday I started o/n cultures for verification.
- Also, I put all of the plates back in the incubator — the real transformants may take a while to grow up.
  - → perhaps I should have plated onto YPD and replica plated onto 5FOA.

11/4/06

- All of the colonies I picked off of the B plates (2 each) are ura3 point mutants
- Nothing new growing up on the A plates

10/31/06

ANOTHER NEW STRATEGY TO DELETE ARS607.

- Since I accidentally created a situation where URA3 popped out due to recombination between ~~tande~~ direct repeats (pgs 141 & 142), I decided to do something similar in order to generate the ARS607 deletion.

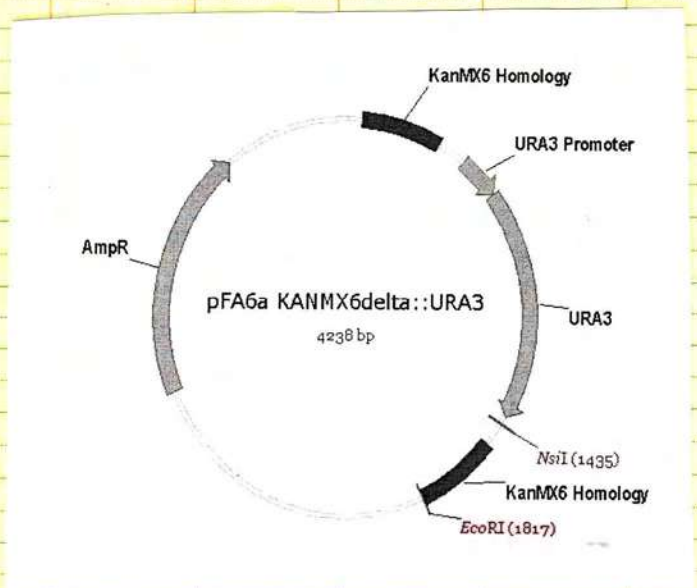
Here is the strategy:

(1) Clone the ARS607_F5/ARS607_R5 extension product (p 144) into the vector pGIL001 (the pFA6a-KanMX Δ::URA3 vector, pg 22)

- amplify ARS607_F5/R5 product with the following primers to introduce RE sites:

GL_ars607_F6_NsiI
AAGTAAATGCATCATTAGAGACAGAGAAAC

GL_ars607_R6_EcoRI
ATCGATGAATTCGCGAATGTGATTCTATGC



(2) Amplify off of this new plasmid using primers ARS607_F4 (p 143) and ARS607_R5 (p 144)

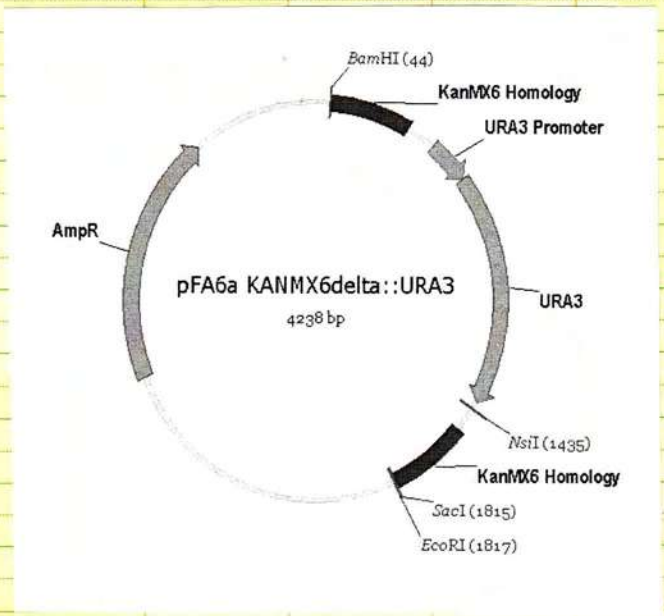
(3) Transform into Δ strains — select for URA3

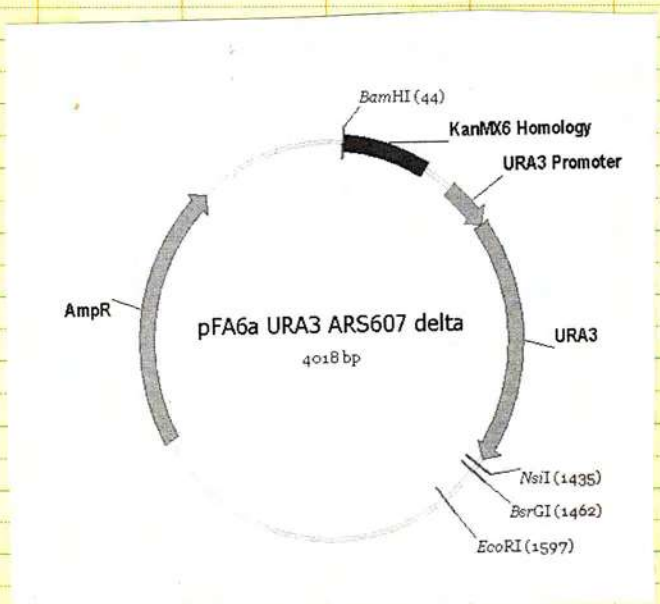
(4) Select for popout by selecting on 5FOA

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC. READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

11/4/06

CONSTRUCTION OF PLASMID pFA6 URA3-ARS607Δ





I made pFA6 URA3-ARS607Δ by cloning the ARS607F5/R5 fragment into GIL001 (pFA6 KanMX6Δ::URA3)

PREPARATION OF INSERT

- On Wednesday (11/1) I did a 100λ rxn with ARS607F5/R5 as usual (p 147)
- On Thursday → two 100λ rxns 100λ PCRs using the above as temp and using primers ARS607_F6_NsiI & ARS607_R6_EcoRI (p)
  - standard protocol w/ 50° Anneal & 1 min ext.
- Prep PCR w/ Qiagen Column → elute rxns in 30λ ddH_2O
- Cut w/ NsiI & EcoRI

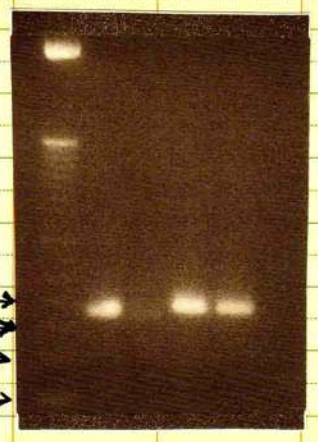
22 λ	PCR 1	Digest for ~4h
22 λ	PCR 2	
5 λ	NEB Buffer 3	
.5 λ	NsiI	
.5 λ	EcoRI	
50 λ		

11/4/06

PREPARATION OF INSERT CONT...

2% gel

2λ each



LANE	
1	Double cut insert
2	PCR using ARS607_F5 / ARS607_R5
3	PCR1 } using ARS607_F6_NsiI & ARS607_R6_EcoRI
4	PCR2 }

Purify Double digest using spin column → elute in 30λ ddH_2O

PREPARATION OF VECTOR

- On Wednesday (11/1) I started o/N bacterial cultures from two independent colonies carrying the plasmid GIL001
- On Thursday (11/2) → miniprep → elute 30λ ddH_2O
- Digest w/ NsiI & EcoRI (see pg 153 for RE sites)

24λ	H_2O
10λ	Miniprep #1
10λ	Miniprep #2
5λ	NEBuffer 3
.5λ	NsiI
.5λ	EcoRI
50λ	

Digest ~3h ; cip ~45 min (just add 1λ cip to digest)

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

11/4/06

PREPARATION OF VECTOR CONT...

LANE		Expected fragment sizes
1	Double digest	- 3856 bp - 382 bp
2	Miniprep #1	
3	Miniprep #2	

- Gel prep vector → elute in 30λ ddH$_2$O

LIGATION / TRANSFORMATION

Set up 3 ligations:

5λ insert & 5λ vector
8λ insert & 2λ vector
5λ vector & 5λ H$_2$O
} add 10λ 2× Quick ligase Buffer + 1λ Quick ligase

- incubate 10 min @ RT then use 5λ to transform competent cells
- I used two diff batches of cells (Thomas' & Grzegorz's)
- Labeled T5:5, T8:2, T–, G5:5, G8:2, G–

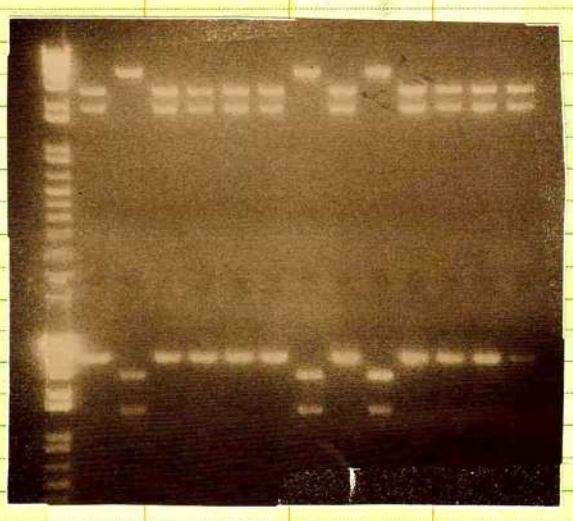
- On Friday I was a bit worried that the ligation/transformation didn't work – there were lots of colonies on the control (vector only) plates. For the "G" plates there were about the same number of colonies on all plates; for the "T" plates there were more colonies on the 5:5 & 8:2 plates than the control plates.

- I picked 5 colonies off of ~~the~~ the 5:5 and 8:2 plates and 2 off of the control plate → grow in 2 mL 2×YT+AMP
  ↳ labeled 5A-E, 8A-E, –A, B

11/4/06

- Today I did miniprepps on the 12 ~~strains~~ colonies I picked yesterday → elute 30λ ddH$_2$O
- Digest with either BamHI & SacI or BamHI & BsrGI

5λ	vector	NEBuffer 1 for SacI
2.5λ	NEBuffer	NEBuffer 2 for BsrGI
0.25	BSA	
0.25	BamHI	
0.25	RE #2 (SacI / BsrGI)	→ pg 154 shows how these enzymes cut the two vectors
16.75	H_2O	
25λ		



BamHI, SacI

BamHI, BsrGI

LANE	
1	5A
2	↓
3	
4	
5	5E
6	8A
7	↓
8	
9	
10	8E
11	-A
12	-B
13	GIL001

CORRECT
5B
8B
8D

EXPECTED FRAGMENT SIZES

- See pg 154 for RE sites
- Proper cloning of the ARS607Δ fragment into pGIL001 removes a SacI site and adds a BsrGI site.
- BamHI cuts both vectors once

FOR BAMHI & SACI

pGIL001 : 2467, 1771

Correct plasmid: 4018

FOR BAMHI & BSRGI

pGIL001 : 4238

Correct Vector: 2600, 1418

11/6/06

TESTING THE ROLE OF TRANSLESION SYNTHESIS

My current model for why late replicating regions have a higher mutation rate is that they are more likely to be subjected to translesion synthesis whereas lesions that are encountered early by the replication fork are more likely to be replicated by a template switching-like mechanism.

~~The~~ This would predict that disrupting TLS ~~in~~ would have a greater effect on late replicating regions than early.

To test this I want to knock out rev1 in two late replicating/high mutation rate strains and two early replicating/low mutation rate ~~a~~ strains.

Why rev1?

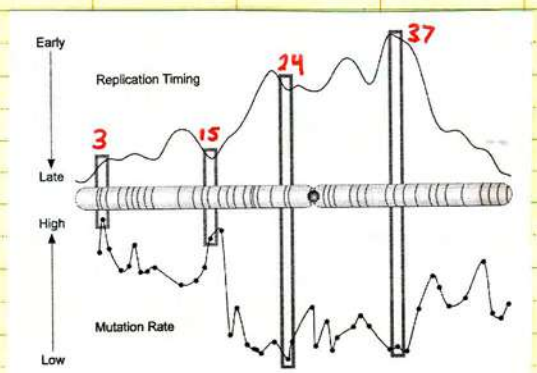
- 3 yeast TLS polymerases:

Pol η	Rad30
Pol ζ	Rev3/Rev7
	Rev1

- Rev3, 7, & 1 are essential for TLS
- Pol ζ may play roles other than TLS
- Rev1 not expressed until late S-phase – regulator of TLS

Which strains?

GL-3, GL-15 } late/high
GL-24, GL-37 } early/low



STRAIN CONSTRUCTION

- I streaked the above strains on YPD
- I streaked rev1Δ::KanMX from the deletion collection
  - rev1 is on ChrXV – ~~I~~ should I be concerned about introducing ~~mor~~ homology to the Pringle system into my strains?
- Picked 4 colonies off rev1 plate: 2 big colonies (B1, B2) and 2 small colonies (S1 & S2).

11/6/06

I was having trouble amplifying the rev1Δ cassete off of my gDNA preps and I was not able to get the WT REV1 gene off of good gDNA preps.

PRIMERS:

26433019 IDT
B.NEUGEBOREN
28138286 10/26/2006
GL-REV1extF1
5'-TCG CAT CAA CTT AAA CAT TGG -3'
Tm= 51.5 ºC , MW= 6,389.2
6.1OD260 30.00nmol 0.19m

26433018 IDT
B.NEUGEBOREN
28138285 10/26/2006
GL-REV1extR1
5'-GAG TCG GCC ATT CCA ATA CC-3'
Tm= 55.7 ºC , MW= 6,062.0
7.3OD260 38.20nmol 0.23m

I tried adjusting annealing etc. I was beginning to think that the strain was incorrect and that the WT was too big to be amplified by Taq.

I realized that the 3' end of REV1extF1 can make 5 bp dimer to REV1extR1

5'TCGCATCAACTTAAAC
ATTGG 3'
3'CCATAACCTTACCGGCTGAG 5'

To determine if I have the correct strain I tried a PCR using:

- REV1extF1 & REV1extR1
- KANMXintF1 & REV1extR1 (pg 42)
- U1 & D1 (pg 7)

LANE	gDNA	PRIMERS
1	B1	REV1extF1 & REV1extR1
2	B2	↓
3	S1	
4	S2	
5	B1	KANMXintF1 & REV1extR1
6	B2	↓
7	S1	
8	S2	
9	B1	U1/D1
10	B2	↓
11	S1	
12	S2	
13	- for another expt	

→ The strain is correct — the reason why the PCR didn't work is because of the primers

11/6/06

TRANSFORMATIONS

Yesterday I did two sets of transformations:

(1) Transform Δ10, Δ36, & Δ37 with URA3-ARS607Δ

(2) Transform GL-3, GL-15, GL-24, GL-37 with rev1Δ::KanMX6 cassette

- Prepare the URA3-ARS607Δ cassette by PCR w/ ARS607_F4 / ARS607_R5 using plasmid ~~XXX~~ 5B as a template (pg 158)

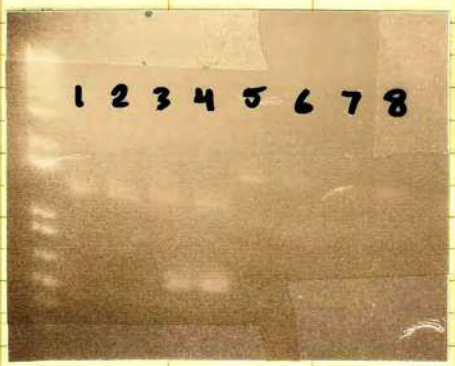
- Prepare the rev1Δ::KanMX6 cassette by two rounds of PCR
  (1) {Primers: U1 (pg 7) & REV1extR1 (pg 160) Template: rev1Δ (B1)
  {Primers: Rev1extF1 (pg 60) & D1 (pg 7) Template: rev1Δ (B1)

  (2) Purify and anneal/extend the two products above.
  - I put the purified products in the 100° block for ~5 min before extending
  - Program – 96° Denature for 1 min
  – 3 m extension

80 λ	PCRS
1 λ	dNTPs
1 λ	Taq
10 λ	Buffer
10 λ	H_2O
~100	

I diluted the strains above 1:40 in YPD+ADE and grew ~5h to an OD_{600} of:

Δ10	0.65
Δ36	0.57
Δ37	0.74
GL-3	0.89
GL-15	0.67
GL-24	0.79
GL-37	0.86



LANE	PRIMERS	TEMPLATE
1, 2	ARS607_F4 & ARS607_R5	5B cut w/ BamH1
3, 4	"	5B uncut
5, 6	REV1extF1 & D1	rev1Δ (B1)
7, 8	U1 & REV1extR1	"

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

11/6/06

TRANSFORMATIONS

Yesterday I did two sets of transformations:

(1) Transform Δ10, Δ36, & Δ37 with URA3-ARS607Δ

(2) Transform GL-3, GL-15, GL-24, GL-37 with rev1Δ::KanMX6 cassette

- Prepare the URA3-ARS607Δ cassette by PCR w/ ARS607_F4 / ARS607_R5 using plasmid ~~XXX~~ 5B as a template (pg 158)

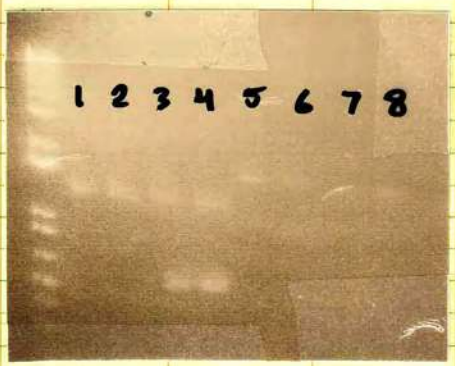
- Prepare the rev1Δ::KanMX6 cassette by two rounds of PCR
  (1) {Primers: U1 (pg 7) & REV1extR1 (pg 160) Template: rev1Δ (B1)
  {Primers: Rev1extF1 (pg 60) & D1 (pg 7) Template: rev1Δ (B1)

  (2) Purify and anneal/extend the two products above.
  - I put the purified products in the 100° block for ~5 min before extending
  - Program – 96° Denature for 1 min
  – 3 m extension

80 λ	PCRS
1 λ	dNTPs
1 λ	Taq
10 λ	Buffer
10 λ	H_2O
~100	

I diluted the strains above 1:40 in YPD+ADE and grew ~5h to an OD_{600} of:

Δ10	0.65
Δ36	0.57
Δ37	0.74
GL-3	0.89
GL-15	0.67
GL-24	0.79
GL-37	0.86



LANE	PRIMERS	TEMPLATE
1, 2	ARS607_F4 & ARS607_R5	5B cut w/ BamH1
3, 4	"	5B uncut
5, 6	REV1extF1 & D1	rev1Δ (B1)
7, 8	U1 & REV1extR1	"

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

11/14/06

ANOTHER ATTEMPT AT TRANSFORMATION

It is clear that I am having trouble with transformations.

Possible explanations:

(1) carrier DNA: I used a new tube of ssDNA (prepared by Grzegorz) on 10/27/06 (pg 151). I left it the 100° block for several days. I think most of the DNA degraded and a ppt was formed. On a gel this runs <100bp, and the solution is not as viscous as it ~~sho~~ usually is.

(2) LiOAc Mix: I prepared new LiOAc mix on 10/19/06 (pg. 147).

I have not had a successful transformation w/ either of these.

Yesterday I redid the transformations and I used new
- LiOAc mix
- PEG mix
- ssDNA (prepared by taking a new tube of Grzegorz's, thawing it in the fridge, vortexing for several min, heating in 100° block ~10 min, and immediately cooling in ice & water bath.)

PCR

- I did a total of 11 100λ PCRs of the URA3-ARS607Δ fragment (pg 154) and revIΔ::KanMX fragment

using primers
- REV1extF1 (pg 160)
- REV1extR2

template: revIΔ B1 (pg 159)

using primers:
- ~~ARS607GATA~~
- ARS607_F4 (pg 143)
- ARS607_R5 (pg 144)

template: plasmid 8:2D cut w/ BamHI/SacI (pg 158)

26782963 IDT
B.NEUGEBOREN 11/6/2006
28478825
GL-REV1extR2
5'-TCC ATT CAA GGA TTC AAA GG-3'
Tm= 50.2 °C , MW= 6,125.0
5.8 OD_{260} 28.80 nmol 0.18 mg

- Pool PCRs 1&2, 3-5, 6&7, 8&9, 10&11 → spin column → elute 30λ ddH_2O → pool 1-5, 6-11

LANE
1 ~~ARS607~~ URA3-ARS607Δ 1-5
2 URA3-ARS607Δ 6-11
3 revIΔ::KanMx 1-5
4 revIΔ::KanMx 6-11

11/14/06

TRANSFORMATIONS CONT...

- On ~~Saturday~~ Friday (11/10) I started 3 mL cultures in YPD of the following:

  GL·3
  GL·15
  GL·24
  GL·37
  Δ10
  Δ24
  Δ36
  Δ37

- On Sunday (11/12) I ~~start~~ diluted the saturated cultures 1:10^6 into 25 mL YPD+ADE/TRP
  - serially dilute to 10^{-3} → add 25 λ to 25 mL
  - put in water bath shaker ~3:00

- Yesterday (11/13) I started transformations ~4:30

STRAIN	OD_{600}
GL·3	0.70
GL·15	0.61
GL·24	0.60
GL·37	0.72
Δ10	0.32
Δ24	0.68
Δ36	0.41
Δ37	0.50

NOTES

- I pelleted the cells by spinning in 15 mL Falcon tube 2000 rpm, 5 min; decanting and adding the remaining ~~cells~~ culture and spinning again
- The cells were resuspended in 70 λ LiOAc mix, 10 λ ssDNA
- Aliquoted into ~60 λ → add 12 λ of DNA
  - pooled PCRs 1-5 labeled "A"
  - pooled PCRs 6-11 labeled "B"
- Add 420 λ PEG – vortex
- My only worry is that cells may be too concentrated although the OD is not as high as usual.
- Today I replica plated the YPD plates to YPD+G418

11/16/06

VERIFICATION OF PUTATIVE TRANSFORMANTS

· I picked three putative transformants off of the plates for each strain.

↳ for the colonies on -Ura, I innoculated 3mL of -Ura; for the G418 plates, I inoculated 3mL YPD (Although I should have done -Ura, see pg 162.

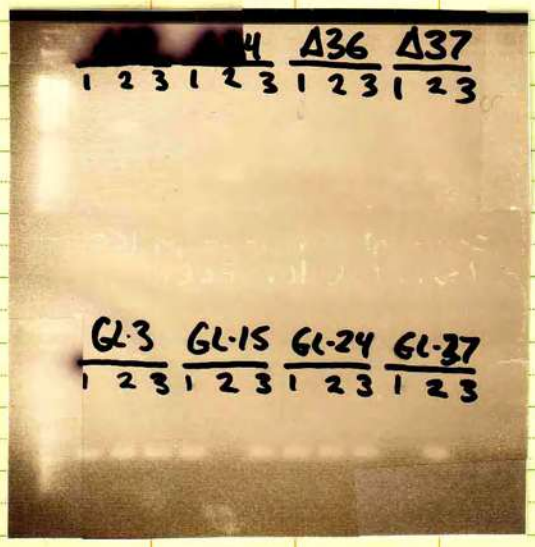
11/18/06

Yesterday I made gDNA preps of the cultures from 11/16. To verify the transformations by PCR I used the following primers:

ARS607extF1 & ARS607 extR1 for ARS deletions (pg 137)

KANMXintF1 & REV1extR2 (pg 163) for rev1 deletions (pg 160) →

these are not the best primers to use — I need to order a set of primers that are external to F1 & R2 and an internal primer for REV1



For ARS607 deletions:

Correct: Δ10.2, Δ36.1
Incorrect: Δ24.2, Δ37.1, Δ37.2, Δ37.3
Not Sure: Δ10.1, Δ10.3, Δ24.1, Δ24.3, Δ36.2, Δ36.3

- Yesterday I picked 8 more colonies off of the Δ24 & Δ37 plates and inoculated 3mL 2×SC-URA
- Today I will plate 200λ of the following cultures onto dupicate 5FOA plates: Δ10.2, Δ36.1, Δ24.1, Δ24.3, Δ37.1
- Also streak these same strains to single colonies on YPD. This is in case I find that there are untransformed 5FOA^R cells in my culture.
- These strains were labeled Δ10x-z Δ24x-z, etc.

11/19/06

For the rev1Δ

· I innoculated each of the putative rev1Δ strains into 2×SC-ura on 11/17.

↳ Only GL-37.1 did not grow indicating that the rev1Δ cassette replaced the kanMXΔ::URA3 cassette using the Pringle homology.

11/19/06

Today I am making gDNA preps of the additional 8 putative transformants from the Δ24 and Δ37 plates. → labeled Δ24P–W and Δ37P–W

PCR w/ ARS607extF1 & ARS607extR1

LANE	TEMP
1	Δ24 P
2	↓
3	↓
4	↓
5	↓
6	↓
7	↓
8	Δ24 W
9	Δ37 P
10	↓
11	↓
12	↓
13	↓
14	↓
15	↓
16	Δ37 W
17	Δ10 x
18	Δ10 Z
19	Δ24 x
20	Δ24 Z
21	Δ36 Y
22	Δ36 Z

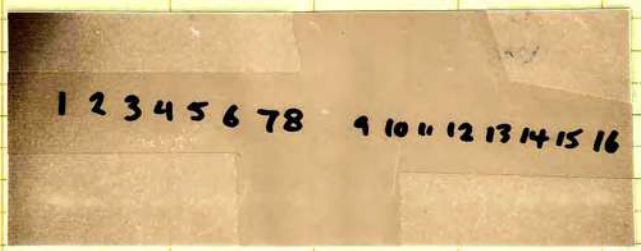
same gDNA preps as pg 165
↳ redrew the PCRs

11/28/06

SELECT FOR POPOUT OF URA3 REPORTER

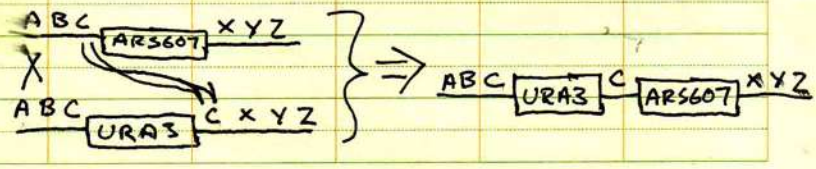
I took strains Δ10Y, Δ36x, Δ24Q, and Δ24S → grew O/N in 2x SC-Ura and plated 200λ onto duplicate 5FOA plates.

↳ I picked 4 colonies of each and grew them O/N in YPD → gDNA prep
↳ PCR w/ ARS607extF1/R1



11/30/06

I sequenced lanes 1, 5, and 9 on the left. To my surprise and dismay they are the WT ARS607. The band to the left is the size I would expect for WT ARS607 (410 bp compared to 300 bp for the ars607Δ). Why? I think that my integration happened in this way:

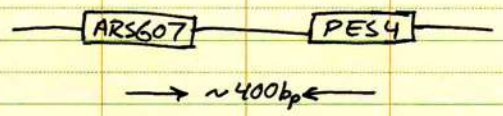


11/29/06

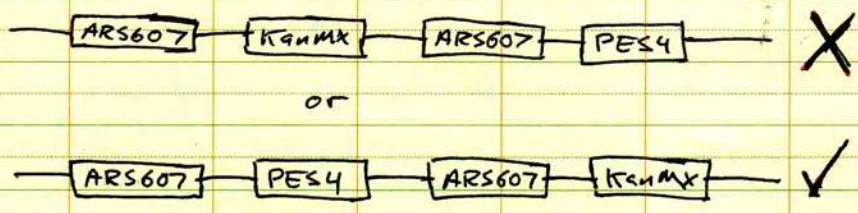
NOTES ON STRAIN CONSTRUCTION

I have not been able to transform the strain Δ37 (pes4Δ::KanMX6) with the ~~URA~~ ARS607Δ::URA3 cassette. I now think that this is because the strain Δ37 contains two ARS607s

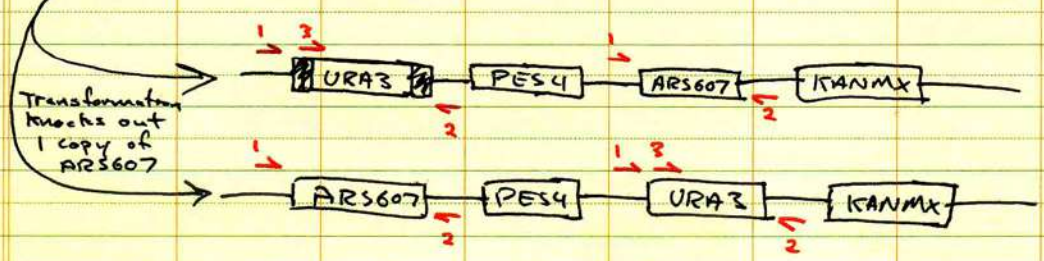
In a WT strain:



I think that the strain in the Δ collection had a tandem duplication, probably before strain construction so that the strain look like

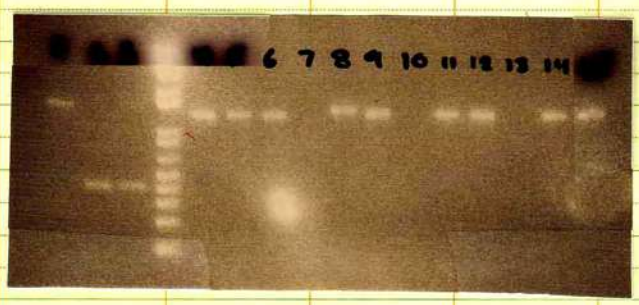


Of these two possibilities I think it would be the second since when I replace KanMX w/ URA3 the LOF rate is low.



PRIMERS

1) ARS607ext F1
2) ARS607ext R1
3) URA3ext F



LANE	TEMPLATE	PRIMERS
1	Δ10Y	ARS607extF1/ARS607extR1
2	Δ24Y	"
3	Δ10Y & Δ24Y	"
4	Δ37 X	URA3extF/ARS607extR1
5	" Y	
6	" Z	
7	" P	
8	" Q	
9	" R	
10	" S	
11	" T	
12	" U	
13	" V	
14	" W	
15	Δ10Y	

CONCLUSIONS

(1) When both templates are present, the smaller band (ARS607) is favored over the larger (ars607Δ::URA3)

(2) 8 of the 11 Δ37 strains contain the URA3 reporter in place of one ARS607 copy

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC. READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

11/29/06

NOTES ON STRAIN CONSTRUCTION CONTINUED

The notes on pg suggest that the strain I pulled from the Δ collection contains a duplicated region containing PES4 and ARS607

QUESTIONS

(1) Were the Δ strains screened negatively for the loss of a PCR product corresponding to the gene being deleted?

(2) The band in lane 8 (pg 167) seems larger than the rest. ~~Does this~~ ~~or indicate that~~
A way this might happen:
- Since the URA3 fragment has homology ~~to the~~ upstream of ARS607 on both ends it is possible to get an integrant that looks like

11/20/06

START OF FLUCTUATION ASSAYS

- Yesterday I started o/N cultures → 3mL 2×SC-Ura
  → Started from cold YPD cultures

GL·3
GL·15
GL·24
GL·37
GL·3 revlΔ
GL·15 revlΔ
GL·24 revlΔ
GL·37 revlΔ

11/22/06

ON Monday (11/20) I started the fluctuation assays (similar to pg 87)

- Dilute o/N cultures 1:10 in H_2O (100λ into 900λ)
  → 40λ of this dilution into 40 mL 2×SC → 200λ into each well of a 96-well plate
  100λ — " —

- All media was sterile filtered
- Plate sealed; incubated without shaking (~ 4:00 pm)

Today I am plating the fluctuation assays

The 200λ plates go onto 12 5FOA plates
The 100λ plates go onto 8 10×Canavanine plates

- Plating was done as before (pg 88) except the programs used were

"fluctPlating_6well_060427" for 5FOA plates

"fluctPlating_9well_060427" for 10×Canavanine plates

Notes on plating

- I autoclaved the Whatman filters before use to avoid contamination as was seen in another experiment (see notebook MR
- One batch of 10×Canavanine plates was quite dry – there were lots of splits in the agar – they should still work fine. These are plates 5-8 in each 10×Can fluct. assay
- The spots do not seem to spread out as far as before (smaller spots on both 5FOA and 10×Can) The plates still dry in ~20 min; therefore, I don't think that the plates are too wet.

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC. READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

11/28/06

NOTES ON THE STRAINS USED IN THE FLUCTUATION ASSAY

- I am getting ahead of myself as I often do. I have done the fluctuation assay before I verified that the strains are correctly deleted for rev1.

- It is possible that the rev1Δ::KanMX cassette integrated in place of the KanMXΔ::URA3 reporter instead of at REV1 — if this occurs, there should not be a PCR product using KANMX intF & REV1extR2 since R2 lies outside of the Pringle homology. These strains would also be Ura auxotrophs

↳ this appears to be the case for GL-37.1 (pg 165)

- GL-37.2 grew slowly; therefore, I used GL-37.3 as the rev1Δ strain in this expt (since it is phenotypically correct)

11/24/06

COULTER COUNTS – FOR 200λ CULTURES (5FOA PLATES)

- 10λ culture into 20 mL Solution
- Sonicate 2 min

Bkg		76	24	86
GL-3	1	69300	69954	69496
"	2	70822	70262	70806
"	3	70256	70322	70912
GL-15	1	42666	41994	41532
"	2	42108	42240	41498
"	3	41400	41432	41558
GL-24	1	59146	58884	59344
"	2	56870	56692	56602
"	3	59574	59480	59884
GL-37	1	66584	66286	67204
"	2	67316	66576	67804
"	3	67392	67326	68768
GL-3 rev1Δ	1	65940	66536	66470
"	2	66784	66138	66370
"	3	65584	65636	66450
GL-15 rev1Δ	1	42894	43870	43944
"	2	43440	42788	43558
"	3	43790	42918	43074
GL-24 rev1Δ	1	59488	59366	59696
"	2	59570	59124	59320
"	3	59586	58694	58370
GL-37 rev1Δ	1	75642	74162	75084
"	2	75088	74738	74900
"	3	73492	72268	73218
Post Bkg		1494	600	650

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC. READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

COULTER COUNTS - FOR 100λ CULTURES (10 × CAN PLATES)

Bkg	62	146	50
GL-3 1	103288	104238	104506
" 2	102804	103574	102582
" 3	103536	103022	102574
GL-15 1	92708	92858	92408
" 2	93972	92726	93538
" 3	92674	93082	92026
GL-24 1	89400	88208	88882
" 2	88318	86714	85888
" 3	91370	89584	89154
GL-37 1	98038	97064	97206
" 2	96496	97780	97660
" 3	97486	97726	96850
GL-3 revΔ 1	104986	104720	104386
" 2	105836	105502	106144
" 3	102306	103352	102548
GL-15 revΔ 1	93222	92496	92724
" 2	88846	89532	89086
" 3	91728	91928	91638
GL-24 revΔ 1	96648	96320	96444
" 2	96622	96294	96136
" 3	91944	93376	92012
GL-37 revΔ 1	104272	105134	104324
" 2	103556	104436	103254
" 3	101864	101060	103300
Post Bkg	1530	1030	560

11/29/06

COUNTS FROM 10x CANAVANINE PLATES

GL·3

	1	2	3	4	5	6	7	8	9
1	2	0	0	4(1)	4(1)	4	3	3	3(1)
2	0	2	1(1)	0	0	2	5	8	1
3	18	2	9	1	5	6(1)	27	0	0
4	25	1	42	2	2(3)	3(1)	2	3	0
5	0	0	0	1	0	0	10	0(37)	4
6	0	2	9	0	4	22	4(4)	1	4
7	1	1	5	9	0	3	12	1(2)	0(1)
8	3	2	3(1)	0	4(1)	1(2)	1	1	4

GL·15

	1	2	3	4	5	6	7	8	9
1	0(1)	4(1)	5(1)	5(1)	1	0	1	0	0
2	0	1	1	2	0	1	3	1	0
3	5	3	2(1)	0	6(2)	3	0(2)	0	1
4	5	0(1)	4	0	15(1)	1	4	0	1
5	0	2	2	2(1)	1	0	0(54)	0	0(1)
6	0	1	1	0	0	3	1	1(1)	0
7	1	1	0	1	7	0	2	1	2
8	3(1)	0	2	2	5	0	0	1	1

GL·24

	1	2	3	4	5	6	7	8	9
1	0	1	0	10(1)	4	1	1	3	2
2	0	0	2(1)	6	0	5	17	1	0
3	1	0	0	1	3	0	0	2	38
4	4	5	2	1	1	1	8	1	1
5	0	3	3	0	0	2	1	2(1)	7
6	0	0	0	0	2	7	1	0	1
7	0	0	3	1	0	13	25	2	0
8	0	5	1	5(1)	1	4	1	2	2

GL·37

	1	2	3	4	5	6	7	8	9
1	6	3	1(1)	6	4	J(~100)	10	4	2
2	6	3	0	4	2	10	1(1)	1	0
3	0	0	12	1	0	1(1)	2	43	13
4	11(1)	9	2	0	4	12	8	1	0
5	0	0	0(1)	2	0	1(3)	0	2(1)	9(1)
6	1	3	0	0	0	1(1)	0	0	4
7	0	1	7	5	0	6	3	4	1
8	4	0	1	7(1)	0	4(2)	1	2	0

GL·3 revlΔ

	1	2	3	4	5	6	7	8	9
1	0	6	0	7	0	1	3(1)	0	2
2	3	1	2	0	0	0	0	1	0
3	2	1	0	0	0	1(1)	3	5	1
4	1	~115	0	0	1	0	0	1	0(1)
5	0	12(1)	0	3	0	4	2	14	0
6	0	0	0(1)	1	3	3	0	2	5
7	28	0	2	11(1)	1	3(2)	1	0	0
8	9	2	0	0	4	4(1)	2	2	0

GL·15 revlΔ

	1	2	3	4	5	6	7	8	9
1	2	1	0	0	0	0(1)	2(1)	0	4
2	0	1	0	0	6(1)	0	1	0	1
3	18	12	1	0	2	0	0	1	0
4	1	0	3	7	2	2	1	5	0
5	5	2(1)	0	0	0	4	0	0	1
6	0	0	2	3	2	0	2(1)	0	2
7	2	0	2	0	0	0(1)	2	5	2
8	1	5	2	8	0	0	1	0	1

GL·24 revlΔ

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

GL·37 revlΔ

	1	2	3	4	5	6	7	8	9
1	8	0	2	0	31	0(1)	1(1)	5(2)	0
2	1	4	8(7)	4(1)	0	0(1)	2	2	2
3	2	1	3	0	0	5	1	10(1)	1
4	1	0	6	2	2	0(1)	0	5	28
5	3	6	6(1)	1	33	1	2(1)	0	25
6	2(2)	0	0	0	0	2	0(1)	7	1
7	3	1	1	2	0(1)	0	1	1	1
8	0	0	0	39	1(1)	2	3	41(8)	1

11/29/06

COUNTS FROM 5FOA PLATES

GL·3

	1	2	3	4	5	6
1	2(0)	2(0)	0(0)	2(0)	1(0)	4(3)
2	2(1)	1(0)	5(2)	3(2)	7(3)	8(2)
3	28 E	5(9)	0(0)	0(0)	1(0)	6(4)
4	2(0)	0(1)	0(0)	1(1)	0(1)	3(3)
5	0(0)	3(0)	0(0)	0(0)	1(0)	6(5)
6	2(0)	1(3)	1(0)	~125 E	2(1)	0(0)
7	5(0)	1(0)	3(4)	1(0)	0(1)	2(1)
8	9(5)	9(0)	0(0)	0(0)	0(0)	15(10)
9	~100 E	2(4)	4(5)	3(3)	4(1)	5(8)
10	1(0)	1(0)	2(1)	2(0)	2(0)	0(0)
11	1(2)	3(2)	3(2)	0(0)	0(0)	4(2)
12	40 E	2(0)	3(0)	3(1)	0(2)	~200 E

GL·15

	1	2	3	4	5	6
1	0(0)	0(0)	0(0)	0(0)	1(0)	0(0)
2	0(0)	0(0)	0(0)	2(0)	0(0)	0(0)
3	0(0)	5(1)	0(0)	0(0)	1(1)	2(1)
4	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
5	0(0)	0(0)	0(0)	6(4)	0(0)	0(0)
6	0(0)	0(0)	1(1)	0(0)	0(0)	0(0)
7	0(0)	0(0)	0(0)	0(0)	0(0)	2(0)
8	1(2)	2(1)	3(0)	0(0)	0(0)	2(0)
9	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
10	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
11	3(0)	~100 E	0(0)	1(0)	2(0)	1(0)
12	0(0)	0(0)	0(0)	0(0)	0(0)	1(0)

GL·24

	1	2	3	4	5	6
1	1(0)	0(1)	0(0)	13(0)	0(0)	0(0)
2	0(0)	1(0)	43 E	0(0)	0(0)	0(1)
3	0(0)	0(0)	0(0)	0(0)	0(0)	1(0)
4	1(0)	0(0)	0(0)	0(0)	0(0)	0(0)
5	0(0)	0(0)	0(0)	0(3)	0(0)	3(1)
6	10(0)	0(0)	0(0)	0(0)	1(0)	1(1)
7	2(0)	0(0)	0(0)	3(1)	0(0)	0(0)
8	1(0)	0(0)	0(0)	0(0)	1(0)	0(0)
9	0(0)	0(0)	0(0)	0(0)	0(0)	0(1)
10	1(0)	2(0)	1(1)	~30 E	2(0)	0(0)
11	0(0)	0(0)	0(0)	0(0)	0(0)	36 E
12	1(0)	0(0)	4(0)	2(0)	0(0)	0(0)

GL·37

	1	2	3	4	5	6
1	4(0)	0(0)	2(0)	0(0)	0(0)	0(0)
2	33 E	0(0)	2(0)	2(0)	4(0)	0(0)
3	0(0)	0(0)	0(0)	0(0)	4(8)	0(0)
4	0(0)	0(0)	1(1)	0(0)	0(0)	2(1)
5	1(0)	0(0)	0(0)	1(0)	0(0)	0(0)
6	0(0)	0(0)	0(0)	0(1)	1(1)	1(0)
7	1(0)	0(0)	0(0)	0(0)	0(1)	4(2)
8	3(1)	0(0)	0(0)	0(0)	0(0)	3(1)
9	2(2)	2(0)	1(0)	0(0)	J(~1000) E	0(0)
10	0(0)	0(0)	2(1)	0(0)	0(0)	1(0)
11	4(0)	0(0)	1(0)	0(0)	0(0)	1(0)
12	33 E	5(1)	6(8)	0(0)	0(0)	0(0)

GL·3 revΔ

	1	2	3	4	5	6
1	3(2)	0(0)	2(0)	~~0(0)~~ 2(0)	0(0)	2(0)
2	0(0)	0(0)	1(1)	31 E	0(0)	0(0)
3	0(0)	1(0)	7(0)	0(0)	0(0)	1(0)
4	0(0)	8(1)	1(0)	0(0)	42 E	1(0)
5	0(0)	0(0)	2(0)	0(0)	0(0)	2(0)
6	0(0)	0(0)	7(1)	0(0)	0(0)	0(0)
7	0(0)	0(0)	6(3)	0(0)	0(0)	1(0)
8	0(0)	3(1)	1(0)	0(0)	0(0)	2(0)
9	0(0)	0(0)	2(0)	0(0)	0(0)	10(5)
10	0(0)	0(0)	0(0)	0(0)	1(0)	0(0)
11	0(0)	1(2)	1(0)	0(0)	1(0)	0(0)
12	0(1)	2(0)	1(0)	0(0)	0(0)	0(0)

GL·15 revΔ

	1	2	3	4	5	6
1	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
2	0(0)	0(0)	1(0)	0(0)	2(0)	0(0)
3	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
4	0(0)	0(0)	0(0)	1(0)	0(0)	0(0)
5	0(0)	0(0)	0(0)	0(0)	0(1)	0(0)
6	0(0)	0(0)	0(0)	0(0)	2(0)	0(0)
7	1(1)	1(0)	0(0)	0(0)	0(0)	1(0)
8	0(0)	2(2)	1(0)	0(0)	1(1)	0(0)
9	0(0)	0(0)	0(0)	0(0)	0(0)	2(0)
10	0(1)	0(0)	0(1)	0(0)	0(0)	0(0)
11	0(0)	2(1)	0(0)	0(0)	0(0)	0(0)
12	43 E	0(0)	0(0)	0(0)	0(0)	0(0)

GL·24 revΔ

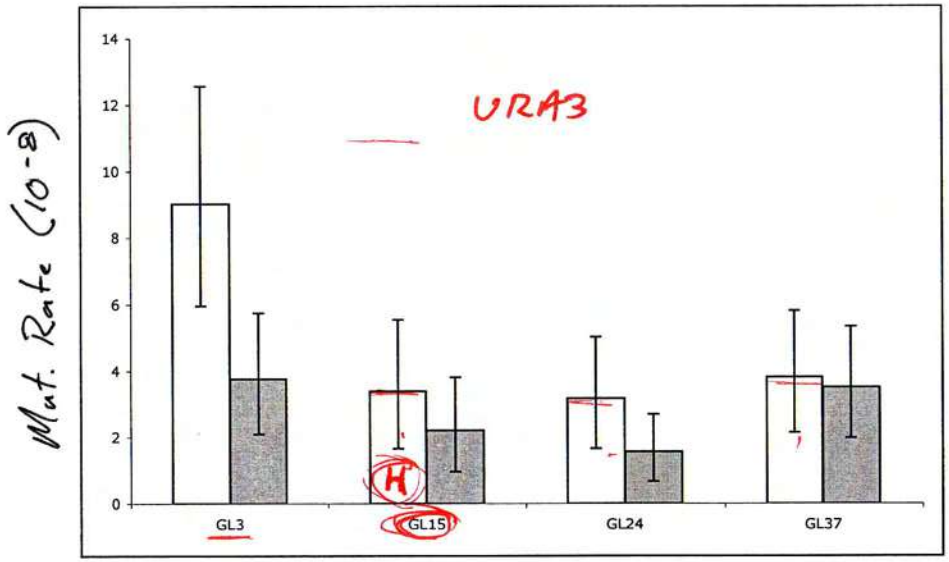
	1	2	3	4	5	6
1	5(0)	0(0)	1(0)	0(0)	0(0)	0(0)
2	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
3	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
4	0(1)	0(0)	0(0)	0(0)	0(0)	0(0)
5	0(0)	0(0)	0(0)	0(1)	0(0)	0(1)
6	2(1)	43 E	0(0)	0(0)	0(0)	0(0)
7	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
8	0(0)	0(0)	0(0)	1(0)	0(0)	0(0)
9	0(0)	1(0)	0(0)	0(0)	0(0)	0(0)
10	0(0)	0(0)	0(0)	~200 E	5(1)	0(0)
11	1(0)	41 E	0(0)	0(0)	4(0)	1(0)
12	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

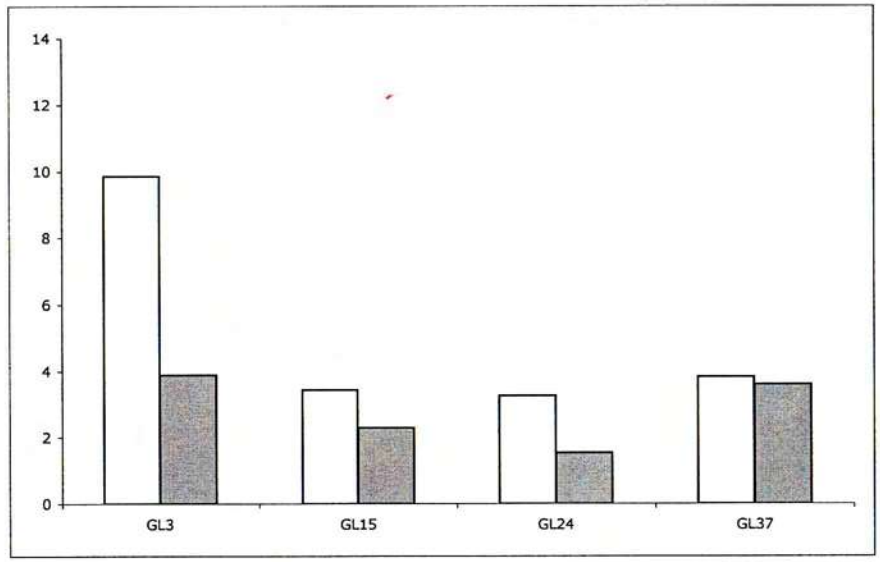
GL·37 revΔ

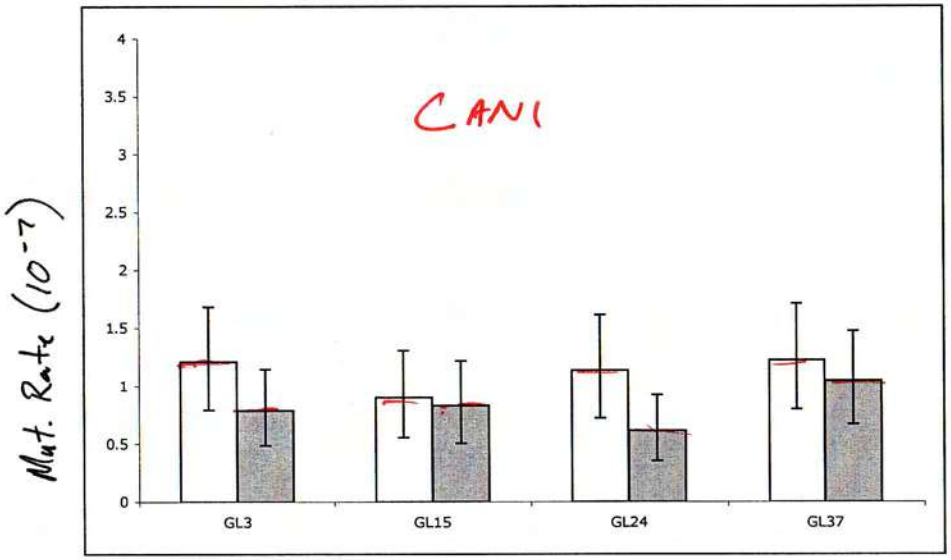
	1	2	3	4	5	6
1	0(0)	0(0)	0(0)	0(0)	0(0)	3(1)
2	10(0)	7(1)	0(0)	3(0)	0(0)	0(0)
3	0(0)	6(1)	5(6)	0(0)	0(0)	1(0)
4	1(0)	0(0)	0(0)	1(0)	0(0)	0(0)
5	1(0)	0(0)	3(2)	0(0)	0(0)	2(0)
6	0(0)	0(0)	0(0)	2(0)	4(0)	0(0)
7	3(3)	2(0)	0(0)	0(0)	1(0)	7(8)
8	0(0)	2(0)	0(1)	0(1)	3(0)	0(0)
9	0(0)	0(0)	0(0)	4(0)	1(1)	1(0)
10	0(0)	0(1)	0(0)	0(1)	0(0)	0(0)
11	0(0)	1(1)	1(0)	0(0)	2(0)	0(0)
12	1(0)	1(0)	0(0)	1(0)	0(1)	4(0)

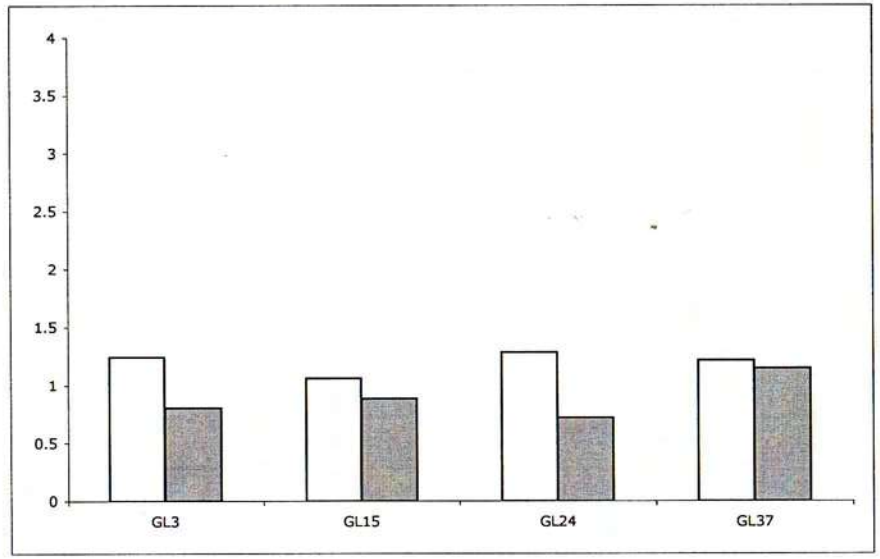
Maximum likelihood

Po

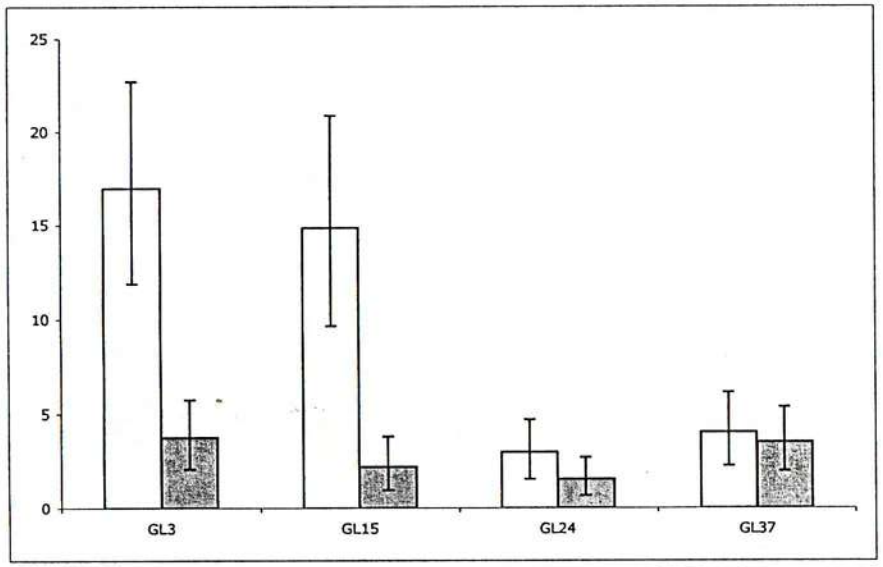


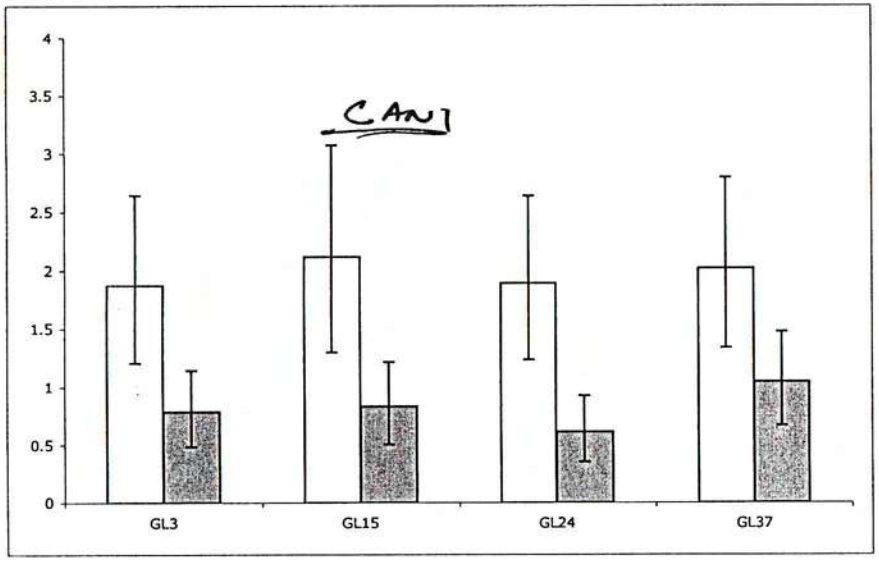






white bars – original expt.





2/20/07

MORE STRAIN CONSTRUCTION

I need to verify the strains I have made ~~and construct~~ for the rev1Δ experiment and construct strains for the ARS607Δ experiment.

- Streak bacteria containing the following plasmids onto LB-AMP:

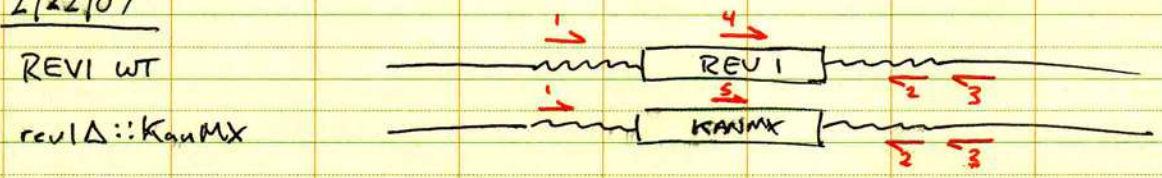
  pGIL001
  pGIL006
  pGIL007
  pGIL008

- Start o/N cultures GL3, GL15, GL24, GL37, & the rev1Δ versions.

2/21/07

- This morning I picked a single colony off of the LBamp plates and innocalated 2 mL 2×YT+AMP
- This afternoon I miniprepped the plasmids
- I made gDNA preps of the 8 yeast strains above

2/22/07

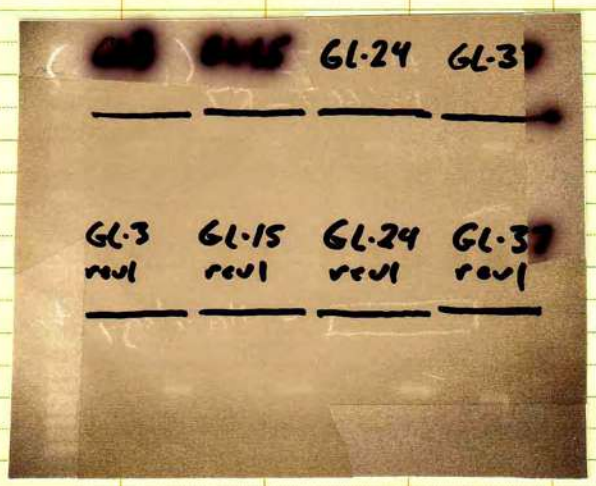


PRIMERS

(1) REV1extF1 } used for cloning
(2) REV1extR2 }
(3) REV1extR3
(4) REV1 int F1
(5) KANMX6 int F

For each of the eight strains above I did 3 PCRs:

LANE 1: REV1extF1 & REV1extR3
" 2: REV1intF1 & "
" 3: KANMX6intF & "



2/24/07

On thursday (2/22) I did 3 PCRs amplifying the revl Δ cassette so that I can redo the transformation of GL-37. → using primers REV1extF1 REV1extR2

Purify using Qiagen kit → elute in 30 λ H_2O

~~Also I minipropped~~

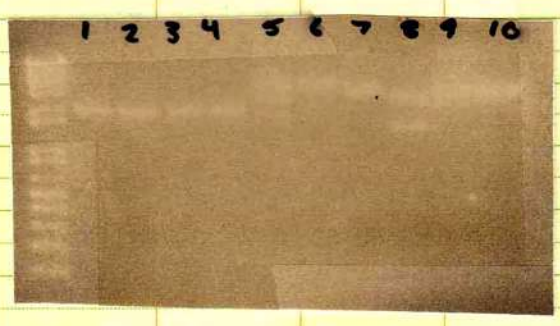
The minipreps I made on 2/21 (pg 179) did not contain plasmid.

↳ Possibly there was a problem with my Amp.

I thawed a fresh tube of Amp and regrew the strains and repeated the minipreps.

↳ Only the minipreps from pGIL001 & pGIL008 appear to have plasmid

↳ I digested to verify correct plasmids (see p. 158)



LANE		
1	purified revl Δ::KanMX PCR	(GL-3 revl Δ temp)
2	"	(GL-15 revl Δ temp)
3	"	(GL-24 revl Δ temp)
4	"	(pooled GL-3,15,24 see p. 179)
5	pGIL001	cut w/ BamHI/SacI
6	8	
7	1	cut w/ BamHI/BsrGI
8	8	
9	1	uncut
10	8	

partial digestions

PCR OF ARS607Δ:URA3 CASSETTE

Set up 6 100 λ PCRS ← (I did 10 rxns)

template: pGIL008 cut w/ BamHI/SacI (above)

primers: ARS607_F4 (p. 143)
ARS607_R7

Sequence - GL_ars607_R7 — 100 nmole DNA oligo, 90 bases

5'- TTC GGT ACG ACA CAA AAA CAA CTA TTG TGT CGC AGT CCA TAG AAG GAG CAA GAA AAA AGA ATG TCT TTA AAG GCG AAT GTG ATT CTA TGC -3'

[ ] - Homology to plasmid

2/25/07

TRANSFORMATION OF GL-37 WITH rcV1Δ::KANMX6 CASSETTE

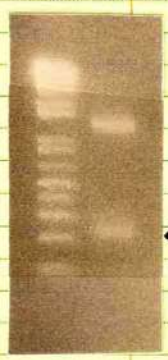
- Yesterday I transformed GL-37 with the 4 PCR products of the rcv1Δ::KanMX6 cassette (p 180)
- Start cultures @ 1:30 by diluting o/N culture
  1:10, 1:25, 1:50, & 1:100 in 10 mL.
- ~~5:30~~

CULTURE	OD_{600} @ 5:30		OD_{600} @ 6:30	
1:10	1.32			
1:25	0.81	← Dilute 1:2 →	0.67	} use these two
1:50	0.49	← →	0.66	
1:100	0.27			

- Pool both cultures → resus. in 2 × 140λ LiOAc Mix + 20λ carrier
  ↳ Aliquot into 4 50 mL tubes → add 10λ of the 4 PCR products
- Today I replica plated from YPD onto YPD + G418

TRANSFORMATION OF Δ10, Δ24, Δ36, & Δ37 W/ ARS607Δ::URA3 CASSETTE

- Pool all 10 PCRs (see p. 180) → purify, elute in 60λ H_2O



← CASSETTE

← PRIMER DIMER? (This was not a very efficient PCR)

- Start 1:25, 1:50 dilutions of o/Ns in 10 mL (11:30)
- OD_{600} @ 3:30

	1:50	1:25	
Δ10	.34	.58	} Transform the 1:25 dilutions → resus in 40λ LiOAc Mix + 15λ carrier + 15λ PCR
Δ24	.33	.57	
Δ36	.34	.59	
Δ37	.30	.54	

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC. READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

3/2/07

ANALYSIS OF revlΔ TRANSFORMATION

- I picked 3 ~~K~~ putative transformants off of each plate and inoculated 3 ml of 2xSC-URA — this is to make sure that the reporter did not integrate at the URA3 reporter using pringle homology.

↳ I have named the transformants

- GL-37 revlΔ _ 3A – C
- ~~GL~~ " _ 15A – C
- " _ 24A – C
- " _ PA – C

} the #s indicate which PCR product was used for transformation (see p. 180)

- 24A, 24B, PA, & PC did not grow in –URA
- qDNA prep the other 8 that grew in –URA
- PCR w/
  - ~~REVlextF1 &~~
  - REVlintF1 & REVlextF3 (R)
  - KANMX6intF & " (K)

3A	3B	3C	15A	15B
R K	R K	R K	R K	R K

15C	24A	PB	WT	revlΔ
R K	R K	R K	R K	R K

3/7/07

ANALYSIS OF THE ARS607Δ TRANSFORMATIONS

- After ~~a 5 days o~~ several days of growth on -URA I picked colonies.
- I know there is a problem with the Δ37 strain (see p 167) ~~so~~ but I included it in the transformation anyway
- There were hundreds of colonies on the Δ10, Δ24, & Δ36 plates
  ↳ only ~10 on Δ37
- Most colonies were small, but a few big ones.
- For each plate pick 1 large colony & 3 small ones
  ↳ labeled ex. Δ10 L1, Δ10 S2, Δ10 S3, & Δ10 S4
- Of the 16 putative transformants only 5 grew in -URA:
  Δ10L1, Δ10S2, Δ24L1, Δ24S2, & Δ36L1
- I took 200λ of each culture and plated on duplicate 5FOA plates (except Δ24 S2, which did not integrate in the right place)
- PCR using primers ARS607extF1/ARS607extR1

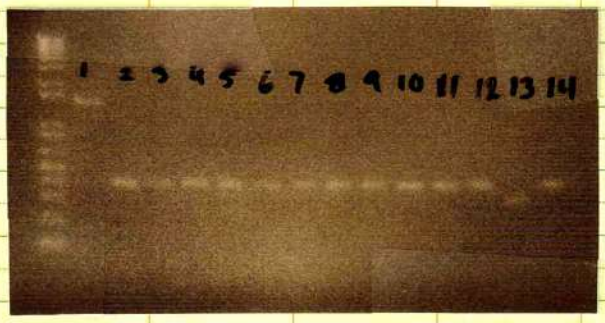
LANE	TEMPLATE
1	Δ10L1
2	Δ10S1
3	Δ24L1
4	Δ24S2
5	Δ36L1
6	WT ARS607

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC. READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

3/7/07

SELECTING FOR POPOUT OF URA3

- After several days growth on 5FOA I see ~100 colonies ~~on~~ for ~~At~~ Δ10L1, Δ10S2, Δ24L1 and only 10-20 on Δ36L1
- I picked 3 5FOA^R colonies from each strain and grew on YPD
  ↳ labeled ex. 10L1A, 10L1B, and 10L1C
- PCR w/ ARS607extF1/ARS607extR1



LANE	TEMPLATE
1	Δ10L1 (lane 1 p 183)
2	~~Δ10L1 A~~ WT ARS607 (lane 6, p183)
3	Δ10L1 ~~B~~ A
4	" ~~C~~ B
5	" C
6	Δ10S2 A
7	" B
8	" C
9	Δ24L1 A
10	" B
11	" C
12	Δ36L1 A
13	" B
14	" C

NOTES ON THE ARS607 STUFF

- It looks like I may finally been successful in deleting ARS607 in one strain.
- Initially when I saw some of the putative transformants on the -URA plates were growing slowly, I suspected that those would be the real ARS607 deletes and that the larger colonies were ones where the URA3 gene integrated next to the ARS – but since the ~~tang~~ smaller ones tended not to regrow on -URA that does not appear to be the case.
- I am confused by the fact that Δ36L1 A, B, & C are not identical. Δ36L1 was a single clone picked off of a -URA transformation plate and grown in -URA. Could I have picked two ~~etc~~ transformants adjacent to each other?
  ↳ Restreak Δ36L1 and Δ36L1 B to singles on YPD
- Even if ~~I~~ Δ36L1 B is the only ARS607Δ strain I can generate it should work for all of my experiments. ~~I~~ The KANMX6 cassette should be linked to the ARS deletion.

3/7/07

NOTES CONTINUED

- Streak BY4742 to singles on YPD from frozen stock
  ↳ I will use this strain to cross to Δ36L1B
  so that I can get the ARS deletion in a Mata strain.

3/8/07

Yesterday I sequenced PCR products

PCR Product

Page	Lane	Primer	Strain
184	12	ARS607ext F1	36L1A
"	"	ARS607ext R1	"
"	13	ARS607ext F1	36L1B
"	"	ARS607ext R1	"
183	1	URA3int F	10L1
"	"	ARS607ext R1	"
"	5	URA3int F	36L1
"	"	ARS607ext R	"

I got the sequences back today. Our new Vector NTI package sucks so I didn't analyze all of the sequences. But I did verify that strain 36L1B is a clean deletion of ARS607.

- I picked 4 single colonies of Δ36L1B off of the YPD plate I streaked out.
  Labeled Δ36L1Ba, Δ36L1Bb, Δ36L1Bc, & Δ36L1Bd (this notation is getting redundant)
  ↳ patch on YPD (for mating)
  and
  start culture of YPD (for gDNA preps to verify deletion)

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC. READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

3/9/07

MORE OF THE ~~ΔGCN~~ ΔARS607 STUFF

- Now that I successfully deleted ARS607 in one strain there are (2) ways I can delete it in other strains: (1) Mating or (2) PCR using the Δ36L1 strain as temp. I will discuss the second one first.

PCR

Using Δ36L1 as temp I can get as much homology as I want – I should design primers that will give ~500bp ~~on~~ homology.

PROBLEM: Δ36L1 is heterogeneous (see p. 184). Therefore I streaked Δ36L1 to singles.

- Today I picked 4 colonies and inc 2x SC-URA
  - I will select for loss of URA3 on 5FOA tomorrow.
  - Labeled Δ36L1 clone 1, Δ36L1 clone 2, Δ36L1 clone 3, & Δ36L1 clone 4

MATING

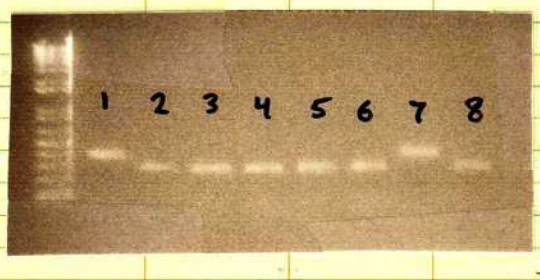
The KanMX cassette is 1kb from the deleted ARS607 – can be used as marker.

Δ36L1Ba, b, c, & d	×	BY4742
ura3Δ		ura3Δ
his3Δ		his3Δ
leu2Δ		leu2Δ
met15Δ		lys2Δ
KANMX6		MATα
MATa		

- Pick 4 zygotes from each cross – tomorrow I will streak the sat. mating patch on -lys-met.

3/9/07

· I made gDNA preps of Δ36LIB, a, b, c, & d
· PCR using ARS607extEI / ARS607extRI



LANE	TEMPLATE
1	LANE 12 p. 184 } just reloaded flow gels
2	LANE 13 p. 184 }
3	Δ36LIB a
4	" b
5	" c
6	" d
7	WT
8	Δ36LIB

It looks like Δ36LIB is a pure culture of ARS607Δ

3/10/07

TRANSFORMATION OF Δ36LIB w/ KanMX6Δ::URA3 CASSETTE

In order to generate the strain GL36ARS607Δ I need to transform Δ36LIB w/ pGIL001

Last night I picked 2 clones of pGIL001 and inoculated 3 mL 2-YT+AMP.
– labeled pGIL001-1 & pGIL001-2

Today I miniprepped the plasmids and set up digests

11.75 λ	H_2O
10 λ	plasmid
2.5 λ	10× EcoRI Buffer
.25 λ	100× BSA
.25 λ	EcoRI
.25 λ	BamHI
25 λ	TOTAL

after 3.5h

← single cut
← } double cut
←

LANE	PLASMID
1	pGIL001-1
2	pGIL001-2

PCR purification kit on RE digests.

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC. READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

TRANSFORMATION CONTINUED

Dilute o/N cultures of Δ36LIBa & Δ36LIBb 1:25 and 1:50 in YPD+ADE
↳ 10:40 — 10 ml

OD_{600}

	STRAIN CULTURE	3:30	4:20
1:50	Δ36LIB a	0.45	0.65
	Δ36LIB b	0.47	0.67
1:25	Δ36LIB a	0.79	—
	Δ36LIB b	0.79	—

} Start transformation
· transform each strain with both plasmid preps (p187)

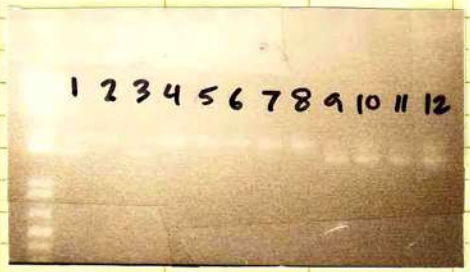
3/19

ANALYSIS OF TRANSFORMATION

I picked two ~~a~~ single ~~colony~~ colonies off of the plates which were transformed with pGL1001-1 (see p 187).

↳ labeled GL-36 ~~ΔA~~ ARS607Δ al-1, al-2, bl-1, & bl-2

PCR with U1/D1



LANE			
1	GL-36 ARS607Δ	al-1	
2	"	al-2	CORRECT
3	"	bl-1	
4	"	bl-2	
5	GL-603		
6	GL-2415		
7	GL-3624		
8	GL-37		
9	GL-603		
10	GL-2415	reul Δ	
11	GL-3624		
12	GL-37		

3/25/07

Phenotypically al-2 & bl-2 are correct (Ura+, G418S)
al-1 & bl-1 are incorrect (Ura+, G418R)

STRAINS GL-36 ARS607Δ al-2 and GL-36 ARS607Δ bl-2 are unable to sporulate

I will not be able to cross the ARS607Δ into the other strains. Both al-2 & bl-2 mate well but neither will sporulate. The gene deleted (ATG18) has been implicated in sporulation - but that was as a ~~heterozy~~ homozygous null (Numsmith). In that paper it still sporulated as a hetero. Also during the const. of the Δ collection it would have been sporulated as a hetero.

Could the ARSΔ be acting epistatically w/ the ATG18 mutation - unlikely but would be interesting

I should have crossed the ATG18Δ with WT ARS as control.

3/25/07

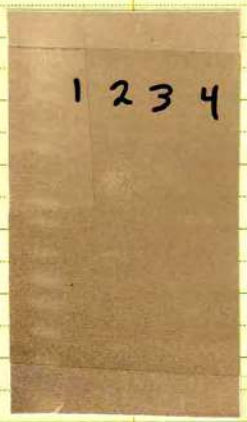
RE-TRANSFORM STRAINS W/ ARS607Δ::URA3 CASSETTE

- Now that I have a proper insertion of the URA3 popout cassette in ARS607 I can use this strain as a template and add more homology to both sides
- Primers ARS607_F2 & ARS607_R2 add 300 bp upstream and 500 bp downstream

29234304 IDT
B.NEUGEBOREN 3/19/2007
31108547
ARS607_F2
5'-CAT TTA CGC ACT CTA ACT GG-3'
Tm= 50.9 ºC , MW= 6,052.0
5.7 OD_{260} 30.70 nmol 0.19 mg

29234305 IDT
B.NEUGEBOREN 3/19/2007
31108548
ARS607_R2
5'-TTT TCG TGG TAG TCG TCT GG-3'
Tm= 54.5 ºC , MW= 6,161.0
5.2 OD_{260} 28.10 nmol 0.17 mg

- PCR Using these primers w/ Δ36L1-Clone1 (p191) as template



LANES 1-3: Δ36L1-Clone1

LANE 4: WT ARS607 as control

- POOL lanes 1-3 (3 100λ PCRs) and elute 100λ H_2O
- Transform the following

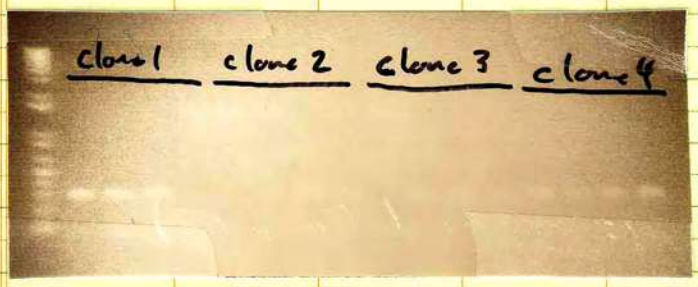
- Δ10
- Δ24
- Δ37
- BY4741
- BY4742

→ 5,Δ10 ; 5,Δ24; 6 Δ37 ; 4,41; 4,42

- Yesterday I picked putative transformants and inoc. - Uma

3/25/07

- I picked 4 clones off of the plate where I restreaked Δ36L1 (p. 184) → clone 1, clone 2, clone 3, & clone 4
- I grew each o/N in -Ura and plated 200λ onto 5FOA
- pick ~~the~~ 4 $5FOA^R$ ~~clo~~ colonies for each clone
  ↳ PCR w/ ARS607F / ARS607R



All of these bands are either the size of the ARS607Δ (URA3 pgpont) or the size of the URA3 (pt. mutation). None of the bands are the size of the ARS607 (400bp). Therefore, all 4 clones are correct.

FREEZEDOWN STRAINS

Yesterday I made perm. stocks of the following from o/N cultures

New Name	Culture
GIL 112	GL-3 rev1Δ
" 113	GL-15 rev1Δ
" 114	GL-24 rev1Δ
" 115	GL-37 rev1Δ (3A, p 182)
" 116	GL-36 ARS607Δ::URA3 clone 1
" 117	—— " —— clone 2
" 118	—— " —— clone 3
" 119	GL-36 ARS607Δ (a1-2, p 189)
" 120	GL-36 ARS607Δ (b1-2, p 189)

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

3/25/07

PREPARATION FOR FLUCTUATION ASSAYS

- Yesterday I made 3 L of 10× Can plates according to p. 86
- On Friday I started O/N YPD cultures of the following:

~~GL-3/16~~ GL-3
GL-15
GL-36
GL-37
GL-3
GL-15
GL-36 } rev1Δ
GL-37
GL-36
GL-36 ARS607Δ (a1-2)

- Yesterday ~~detect~~ Mor. - Ura
- Today I set up Fluct. assays as usual (see p 87)
  ↳ ~ 4:00

3/27/07

PLATING THE FLUCTUATION ASSAYS

- Used program "fluctPlating_9and6well_070327"
- The new plate seals I am using sucks - several have failed to form a seal and I a getting some loss of liq. from wells

3/28/07

COULTER COUNT CULTURES

- sonicate, vortex
- 10λ into 20mL of coulter solution

STRAIN

Bkg		130	92	56
GL-3	1	60732	60052	59950
"	2	60890	58702	58886
"	3	59678	59474	60048
GL-15	1	48346	48362	47938
"	2	48142	47674	48272
"	3	46480	46830	47266
GL-24	1	60590	60206	60516
"	2	58842	59440	58426
"	3	58774	58946	59056
GL-37	1	62866	62238	62670
"	2	63596	64370	63006
"	3	65992	64692	63676
GL-36	1	62822	63324	63094
"	2	62728	62412	63468
"	3	62514	61522	63008
GL-3 revlΔ	1	60364	60826	60894
"	2	60004	59174	59408
"	3	61076	60948	60814
GL-15 revlΔ	1	45278	45036	44570
"	2	44948	45508	45146
"	3	45278	44342	44764
GL-24 revlΔ	1	66832	67340	66590
"	2	66234	66854	65854
"	3	67536	66980	66754
GL-37 revlΔ	1	63534	63848	63262
"	2	63740	62972	63794
"	3	62484	62552	61952
GL-36 ABS6070	1	58702	59388	57836
"	2	57708	57528	58054
"	3	58088	58164	56782
Post Bkg		476	342	356

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG

COUNTING CANAVANINE PLATES 3/30/07

GL-3

	1	2	3	4	5	6	7	8	9
1	12(1)	20(1)	2(0)	2(0)	0(0)	~~2(0)~~ 1(1)	~~2(0)~~ 1(1)	3(0)	~200
2	0(0)	0(1)	0(1)	0(0)	1(2)	3(3)	6(0)	5(0)	1(0)
3	0(0)	0(0)	1(0)	0(0)	0(0)	0(7)	6(0)	1(0)	4(0)
4	0(0)	1(0)	1(0)	0(0)	3(1)	0(0)	0(0)	5(0)	54(0)

GL-15

	1	2	3	4	5	6	7	8	9
1	11(1)	9(0)	1(0)	0(0)	0(1)	10(0)	1(0)	0(0)	2(0)
2	0(0)	2(0)	0(1)	6(1)	1(0)	3(0)	1(1)	2(0)	5(1)
3	2(0)	2(0)	17(0)	5(0)	2(0)	5(1)	1(0)	1(0)	3(0)
4	0(1)	3(0)	1(0)	4(2)	10(0)	0(0)	26(0)	0(0)	0(1)

GL-24

	1	2	3	4	5	6	7	8	9
1	1(0)	1(0)	1(0)	3(0)	1(0)	0(0)	1(0)	1(0)	1(0)
2	10(0)	29(0)	0(0)	23(1)	5(0)	1(0)	~1000	0(0)	0(1)
3	0(0)	0(0)	4(1)	0(0)	1(0)	4(0)	1(0)	0(0)	2(0)
4	2(0)	1(1)	2(0)	2(0)	1(0)	0(1)	21(0)	2(0)	2(0)

GL-37

	1	2	3	4	5	6	7	8	9
1	1(0)	0(0)	1(0)	10(0)	6(0)	3(0)	2(0)	5(0)	3(0)
2	3(0)	1(1)	14(1)	1(0)	0(0)	0(0)	1(0)	3(0)	1(0)
3	1(0)	4(0)	1(0)	0(0)	3(0)	0(1)	0(1)	7(0)	3(0)
4	5(5)	4(4)	27(0)	113	1(0)	3(0)	1(1)	1(0)	1(1)

GL-36

	1	2	3	4	5	6	7	8	9
1	1(0)	4(0)	3(0)	2(0)	0(0)	6(0)	1(0)	0(0)	0(0)
2	25(0)	3(0)	4(0)	2(1)	0(0)	0(0)	2(0)	1(0)	0(0)
3	0(0)	3(2)	7(0)	0(0)	2(0)	1(0)	0(0)	1(1)	1(0)
4	1(0)	4(1)	2(0)	0(0)	3(0)	0(1)	1(0)	3(0)	2(0)

GL-3 revΔ

	1	2	3	4	5	6	7	8	9
1	0(0)	0(0)	0(0)	2(0)	16(0)	0(0)	0(0)	0(0)	0(0)
2	1(0)	0(1)	0(1)	1(0)	0(0)	4(0)	0(0)	2(0)	1(0)
3	1(0)	1(0)	10(0)	0(0)	0(0)	0(0)	1(0)	0(1)	0(0)
4	1(0)	20(0)	0(0)	0(0)	0(0)	2(0)	1(1)	1(0)	1(0)

GL-15 revΔ

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

GL-24 revΔ

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

GL-37 revΔ

	1	2	3	4	5	6	7	8	9
1	15(0)	0(0)	1(0)	0(0)	0(0)	0(0)	0(0)	2(0)	0(0)
2	0(0)	0(0)	1(0)	1(0)	1(0)	7(0)	7(0)	1(0)	1(0)
3	0(0)	1(0)	3(0)	0(0)	0(0)	0(0)	1(1)	0(0)	0(8)
4	0(0)	1(2)	0(0)	0(0)	0(0)	3(0)	0(0)	2(0)	0(0)

GL-36 ARS607Δ

	1	2	3	4	5	6	7	8	9
1	0(0)	3(2)	0(0)	0(0)	2(1)	4(0)	8(0)	0(1)	7(1)
2	2(0)	0(0)	0(0)	2(0)	1(0)	2(2)	0(1)	10(1)	0(0)
3	7(0)	0(0)	12(0)	0(0)	1(0)	4(0)	1(0)	1(0)	0(0)
4	0(0)	0(0)	0(0)	2(0)	1(0)	1(0)	9(0)	6(0)	0(0)

4/3/07

COUNT SFOA PLATES

GL·3

	1	2	3	4	5	6
1	3	68 E	4	10	113 E	1
2	4	6	18	1	0	1
3	3	1	15	6	6	1
4	8	2	28	2	11	17
5	1	4	2	J(105) E	1	6
6	81 E	3	0	0	1	0

GL·15

	1	2	3	4	5	6
1	1	9	0	0	1	0
2	0	0	3	0	6	0
3	0	0	2	0	0	1
4	0	0	15	0	0	6
5	0	15	0	2	0	0
6	3	0	8	0	12	1

GL·24

	1	2	3	4	5	6
1	49	2	0	0	2	0
2	7	0	0	0	5	2
3	0	0	0	0	25	2
4	0	0	0	1	0	0
5	3	0	0	0	0	0
6	5	0	0	0	0	1

GL·37

	1	2	3	4	5	6
1	0	7	0	1	0	0
2	0	0	0	0	0	1
3	0	3	0	0	0	0
4	27 E	0	0	1	0	0
5	0	12	2	2	0	0
6	1	1	0	~100 E	0	0

GL·36

	1	2	3	4	5	6
1	0	0	8	0	0	0
2	1	0	0	0	1	11
3	13	5	3	0	0	1
4	0	0	0	0	0	2
5	0	1	0	3	1	2
6	0	0	0	3	6	0

GL·3 revl Δ

	1	2	3	4	5	6
1	0	0	0	0	1	0
2	0	10	0	0	0	2
3	0	0	0	1	0	1
4	0	0	0	0	0	2
5	4	0	1	2	75 E	0
6	0	2	4	0	0	0

GL·15 revl Δ

	1	2	3	4	5	6
~~1~~	~~0~~	~~2~~	~~4~~	~~0~~	~~0~~	~~0~~
2	2	3	0	0	0	0
3	0	0	9	8	0	0
4	3	5	74 E	1	2	2
5	0	0	0	0	0	0
6	0	1	0	0	0	0
	0	0	0	0	0	0

GL·24
revl Δ

	1	2	3	4	5	6
1	0	0	0	0	0	9
2	0	0	0	14	2	3
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	5	0	1	0	8	0
6	0	0	1	0	0	1

GL·37
revl Δ

	1	2	3	4	5	6
1	0	0	4	0	0	0
2	2	2	0	0	0	0
3	0	0	0	0	47 E	4
4	1	0	1	0	1	1
5	3	0	0	0	0	3
6	1	0	0	0	0	1

GL·36
ARS607Δ

	1	2	3	4	5	6
1	3	0	1	2	0	2
2	0	0	0	0	20	0
3	3	13	0	0	0	10
4	0	1	0	2	11	0
5	5	19	0	0	0	2
6	0	0	0	7	0	3

	1	2	3	4	5	6
1						
2						
3						
4						
5						
6						

	1	2	3	4	5	6
1						
2						
3						
4						
5						
6						

	1	2	3	4	5	6
1						
2						
3						
4						
5						
6						

	1	2	3	4	5	6
1						
2						
3						
4						
5						
6						

7/11/07

Deletion of ARS607 does not result in the predicted elevation of mutation rate on GL36. One possibility is that the usually inefficient ARS608 compensates for the absense of ARS607.

Design primers to delete ARS608 — As a template I will use the deletion collection strain YFR030W — which partially overlaps w/ ARS608; however, I will design a new downstream primer which will extend the deleted region beyond the ACS.

ARS608 - 348 bp

GACTTGGATGCCGCAATTGAAGAATTAAAGGAAGCATCAAGATACATTTTAGAAGTCTACTAAATTAATATAGTAATAAAAACTAAATA
Overlap with YFR030W (MET10)

TCTATTTATTGAACCTGTCTTGAACATTTCTATTTTTTTTTTACTTTTAGTTTTCTTCTATGCGCAAGCTTTTCTATTGGCTGCCAAAT
ACS

AGAAAATTATTGAAATATGATTACATTACAATATTTATTTGTCTTATGAAAACTAACCATCACATTATACTAACTACGGAGGTACCATC
Overlap with YFR031C (SMC2)

TTGAAATCTCGTCCTGAAGACCCTATTAGCATTGGCAAACATACCTTCTTTAAGCGAGACTACAATGAACTGAGATCCTTT

Primers for deletion of ARS608

YFR030W_extF
GCTTATAGAAGCACGTGACC - 242 bp upstream

ARS608delta_R
ATAATTTTCTATTTGGCAGCCAATAGAAAAGCTTGCGCATAGAAGAAAACCGGTGTCGGTCTCGTAG
50 bp homology — **Primer D1 from deletion collection**

LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226 A-W Dataform® 87921 6/92 10VG