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DISECTION OF GIL063

GIL063 is the result of a cross between MP56 and JYL337:

homozygous mutant for: trp1, his3, leu2, ade2

heterozygous mutant for: CAN1/can1, HMLα/hmlαΔ::LEU2, BAR1/bar1Δ::ADE2, URA3/ura3

DATE	SPORE	-ura	-ade ~~-arg+CAN~~	-arg+CAN ~~-ade~~	-leu	+TESTER a	+TESTER α	MATING TYPE
2/11	1-A	+	−	−	−	+	−	α
2/11	1-B	−	+	+	+	−	+	a
2/11	1-C	+	−	−	−	+	−	α
2/11	1-D	−	+	+	+	−	+	a
2/11	2-A	+	−	+	−	−	+	a
	2-B	−	+	−	+	+	−	α
	2-C	−	−	+	−	+	−	α
	2-D	+	+	−	+	−	+	a
	3-A	+	+	+	−	+	−	α
	3-B	−	−	−	−	+	−	α
	3-C	−	+	+	+	−	+	a
	3-D	−	−	−	+	−	+	a
	4-A	−	+	+	−	−	+	a
	4-B	−	+	+	+	+	−	α
	4-C	+	−	−	+	+	−	α
	4-D	+	−	−	−	−	+	a
	5-A	+	+	−	+	−	+	a
	5-B	+	+	+	+	−	+	a
	6-A	−	−	+	+	+	−	α
2/13	7-A	−	+	+	−	−	+	a
	7-B	+	−	−	+	+	−	α
	7-C	−	+	−	+	−	+	a
	7-D	+	−	+	−	+	−	α
	8-A	−	−	+	−	+	−	α
	8-B	+	+	−	+	−	+	a
	8-C	+	+	−	−	−	+	a
	8-D	−	−	+	+	+	−	α
	9-A	−	+	+	+	+	−	α
	9-B	+	−	+	−	−	+	a
	9-C	−	−	−	−	−	+	a
	9-D	+	+	−	+	+	−	α

#1

DATE	SPORE ~~name~~	-ura	-ade ~~-arg+CAN~~	-arg+CAN ~~-ade~~	-leu	+TESTER a	+TESTER α	MATING TYPE
2/13	10-A	-	+	+	+	-	-	
	10-B	+	-	-	+	-	+	a
	10-C	+	+	-	-	-	+	a
	10-D	-	-	+	-	+	-	α
	11-A	-	+	+	+	+	-	α
	11-B	+	-	-	-	-	+	a
	11-C	-	+	-	-	-	+	a
	11-D	+	-	+	+	+	-	α
	12-A	-	-	-	-	+	-	α
	12-B	-	+	+	+	-	+	a
	12-C	+	+	+	+	-	+	a
	12-D	+	-	-	-	+	-	α
	13-A	-	+	-	+	+	-	α
	13-B	+	-	+	+	+	-	α
	13-C	+	-	-	-	-	+	a
	13-D	-	+	+	-	-	+	a
	14-A	-	+	+	-	-	+	a
	14-B	+	-	-	-	-	+	a
	14-C	-	+	-	+	+	-	α
	14-D	+	-	+	+	+	-	α
	15-A	-	+	+	-	+	-	α
	15-B	+	-	-	+	-	+	a
	15-C	+	-	-	+	-	+	a
	15-D	-	+	+	-	+	-	α
	16-A	+	+	-	+	-	+	a
	16-B	-	-	+	-	+	-	α
	16-C	+	-	-	-	-	+	a
	16-D	-	+	+	+	+	-	α
	17-A	-	-	-	+	+	-	α
	17-B	+	+	+	-	-	+	a
	17-C	+	+	-	+	-	+	a
	18-A	+	-	+	-	+	-	α
	18-B	-	+	+	+	+	-	α
	18-C	+	-	-	+	-	+	a
	19-A	-	+	-	-	-	+	a
	19-B	+	-	-	+	+	-	α
	19-C	-	+	+	+	-	+	a
	20-A	-	+	+	+	+	-	α
	20-B	+	-	+	-	-	+	a

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~~CROSS~~

- Cross spore 9-D with GIL087 (from freezer)
- Pick 10 zygotes → 8 survived
- Sporulate zygote #1
- Disect ~~18~~ 17 tetrads
  - tetrads #17 & #18 had only 3 spores survive.

DATE DISECTED	SPORE	-leu	-ade	-arg+CAN	+TESTER a	+TESTER α	MATING TYPE
2/21	1-A	−	−	−	−	+	a
	1-B	+	−	+	+	−	α
	1-C	−	+	+	−	+	a
	1-D	+	+	−	+	−	α
	2-A	+	−	+	−	+	a
	2-B	−	+	+	−	+	a
	2-C	−	+	−	+	−	α
	2-D	+	−	−	+	−	α
	3-A	+	+	+	−	+	a
	3-B	−	+	−	−	+	a
	3-C	+	−	−	+	−	α
	3-D	−	−	+	+	−	α
	4-A	−	+	+	−	+	a
	4-B	+	−	−	+	−	α
	4-C	−	−	−	−	+	a
	4-D	+	+	+	+	−	α
	5-A	+	−	+	+	−	α
	5-B	−	+	−	−	+	a
	5-C	+	+	−	+	−	α
	5-D	−	−	+	−	+	a
	6-A	−	+	+	−	+	a
	6-B	+	−	−	+	−	α
	6-C	+	+	−	−	+	a
	6-D	−	−	+	+	−	α
	7-A	+	−	+	+	−	α
	7-B	+	−	+	+	−	α
	7-C	−	+	−	−	+	a
	7-D	−	+	−	−	+	a
	8-A	+	−	+	+	−	α
	8-B	−	+	−	−	+	a
	8-C	−	−	−	+	−	α
	8-D	−	+	+	−	+	a

DATE DISECTED	SPORE	-leu	-ade	-arg+CAN	+TESTER a	+TESTER α	MATING TYPE
2/21	9-A	+	+	+	+	−	α
	9-B	−	−	+	−	+	a
	9-C	+	+	+	−	+	a
	9-D	−	−	−	+	−	α
	10-A	−	+	+	−	+	a
	10-B	+	−	+	+	−	α
	10-C	−	+	−	−	+	a
	10-D	+	−	−	+	−	α
	11-A	+	−	−	−	+	a
	11-B	−	+	+	+	−	α
	11-C	−	−	−	−	+	a
	11-D	+	+	+	+	−	α
	12-A	−	+	−	+	−	α
	12-B	+	−	−	−	+	a
	12-C	+	+	+	+	−	α
	12-D	−	−	+	−	+	a
	13-A	−	−	+	+	−	α
	13-B	+	+	−	−	+	a
	13-C	−	+	−	−	+	a
	13-D	+	−	+	+	−	α
	14-A	−	+	+	+	−	α
	14-B	−	+	−	+	−	α
	14-C	+	−	−	−	+	a
	14-D	+	−	+	−	+	a
	15-A	−	+	−	−	+	a
	15-B	+	−	−	+	−	α
	15-C	+	−	+	+	−	α
	15-D	−	+	+	−	+	a
	16-A	+	+	−	−	+	a
	16-B	−	−	−	−	+	a
	16-C	−	+	+	+	−	α
	17-A	+	+	+	−	+	a
	17-B	+	−	−	+	−	α
	17-C	−	−	+	+	−	α

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SUMMARY

parental

GIL087 URA3, leu2, trp1, can1, ade2, his3, MATa

~~GIL088~~ GIL063 spore (9D, pg 17)

9-D (GIL102) URA3, leu2, trp1, CAN1, ade2, his3, bar1Δ::ADE2, hml⍺Δ::LEU2, MAT⍺

1D ⍺
5C ⍺
6C — CAN1, bar1Δ, hml⍺Δ
13B
16A

2D ⍺
3C ⍺
4B ⍺
6B ⍺
10D ⍺ — CAN1, BAR1, hml⍺Δ
11A
12B
14C
15B ⍺
17B ⍺

3B
5B
7C
7D
8B
10C — CAN1, bar1Δ, HML⍺

[redacted]

12A ⍺
13C
14B ⍺
15A

1A
4C
8C ⍺ — CAN1, BAR1, HML⍺
9D ⍺
11C
16B

2× SC-ura

Yesterday I started o/N cultures of [redacted] strains. Today I will set up 100λ fluor. assays.

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Coulter Counts

As usual, sonicate 2 min. Make 3 dilutions of 10λ into 20 mL

Bkg	140	368	252						
GLC087 1	44912	46064	46450						
" 2	52484	52604	52216						
" 3	52094	52498	52164						
GLL102 1	59716	59428	59294						
" 2	59082	58796	57968						
" 3	58760	58602	58890						
3B 1	63812	64128	64260						
" 2	60612	60548	59530						
" 3	62322	62734	62490						
5B 1	56412	56342	55938						
" 2	52458	≠51230	51610						
" 3	53426	53312	53118						
7C 1	56082	57316	56166						
" 2	56360	56766	57130						
" 3	56376	55800	55986						
7D 1	66452	66314	66520						
" 2	63368	63240	63788						
" 3	65230	64166	65036						
8B 1	62560	63520	63688						
" 2	65792	65898	65504						
" 3	63342	59838	59548						
10 C 1	48610	47698	# 47584						
" 2	48372	47836	47832						
" 3	53104	49058	49428	48804					
13C 1	53910	53714	53594						
" 2	53148	52880	52878						
" 3	51294	50980	49970						
15A 1	66062	66962	67006						
" 2	56094	55138	56104						
" 3	66854	66072	65820						
Post Bkg	1350	810	518						

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5FOA PLATE COUNTS

GIL087	1	2	3	4	5	6	7	8	9
1	42	0	0	0	0	0	0	0	0
2	11	0	0	0	13	2	0	0	0
3	0	0	11	0	0	0	0	0	3
4	0	0	1	0	38	0	0	0	0
5	0	0	0	0	63	0	17	0	0
6	0	0	0	1	0	0	0	0	0
7 ·	3	x 3	0	0	10	2	0	0	0
8 ·	15	35	0	0	0	0	2	0	0

GIL102	1	2	3	4	5	6	7	8	9
1	7	50	7	3	9	0	9	3	0
2	0	1	3	17	9	0	3	9	1
3	1	7	5	5	1	5	9	6	0
4	10	12	7	16	14	0	18	0	5
5	0	6	9	9	8	53	8	4	6
6 ·	1	2	3	7	4	6	70	4	4
7 ·	18	10	21	5	5	4	1	~80	11
8 ·	2	1	3	0	3	20	11	1	2

3B	1	2	3	4	5	6	7	8	9
1	3	11	0	2	0	0	11	0	0
2 ·	0	0	2	24	0	0	16	0	0
3 ·	0	0	6	0	0	0	9	0	2
4 ·	0	0	0	0	0	0	0	1	0
5	0	1	0	0	4	1	30	0	43
6	4	0	16	13	1	0	7	0	34
7	4	0	0	0	0	1	0	0	0
8	0	131	0	0	1	0	9	0	0

5B	1	2	3	4	5	6	7	8	9
1	0	0	1	5	0	1	0	39	2
2	0	22	3	3	1	6	2	0	10
3	10	7	30	0	0	0	0	1	5
4	1	2	13	0	19	0	0	4	1
5	4	0	0	0	2	5	1	11	0
6 ·	5	5	8	0	19	0	2	0	1
7 ·	3	21	1	0	3	0	18	3	0
8 ·	0	4	0	0	0	0	0	3	3

7C	1	2	3	4	5	6	7	8	9
1	4	0	0	12	25	2	0	2	0
2	2	5	0	2	0	41	5	1	5
3	6	1	15	0	0	0	1	9	0
4	4	5	2	3	17	5	2	0	25
5	6	0	5	1	3	0	0	0	3
6 ·	0	~200	13	1	2	0	2	7	1
7 ·	1	0	0	14	15	1	0	1	2
8 ·	1	6	3	0	17	3	1	2	4

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7D	1	2	3	4	5	6	7	8	9
1	1	9	4	5	0	6	88	2	0
2	8	16	5	161	8	2	0	2	4
3	2	J(~1000)	0	0	25	2	53	10	1
4	0	0	2	2	2	5	2	7	0
5	5	0	0	1	2	10	35	10	27
6 ·	0	2	6	1	0	4	0	0	10
7 ·	1	0	4	0	1	3	2	1	2
8 ·	1	7	1	5	2	1	4	4	4

8B	1	2	3	4	5	6	7	8	9
1	0	0	0	14	19	3	0	0	0
2	0	5	0	0	0	0	0	1	0
3	7	19	0	4	0	0	1	17	1
4	3	0	0	52	0	0	7	7	0
5	5	0	0	7	1	46	0	0	11
6 ·	0	5	0	0	0	6	0	1	0
7 ·	0	0	0	11	6	5	0	0	1
8 ·	0	0	0	0	0	1	0	0	2

10C	1	2	3	4	5	6	7	8	9
1	1	10	0	10	0	1	0	10	3
2	0	0	0	0	0	0	5	0	0
3	1	0	0	0	0	1	0	0	0
4	2	11	4	0	5	10	0	21	0
5	3	0	4	23	2	0	0	10	0
6 ·	23 0	2	0	0	4	0	0	0	0
7 ·	2	4	1	0	0	0	6	0	0
8 ·	0	0	0	0	0	5	0	0	10

13C	1	2	3	4	5	6	7	8	9
1	0	15	0	1	37	3	0	5	5
2	5	1	0	1	12	1	1	0	0
3	2	0	5	2	0	0	0	2	0
4	12	0	2	6	J(~500)	2	19	10	7
5	57	7	4	0	0	8	7	17	8
6 ·	2	3	1	0	1	0	1	1	0
7 ·	0	1	1	0	5	15	24	0	2
8 ·	1	4	60	0	0	2	49	27	6

15A	1	2	3	4	5	6	7	8	9
1	0	5	9	2	4	0	4	0	0
2	0	0	0	0	0	0	0	0	25
3	4	0	0	0	0	0	3	0	0
4	9	0	9	0	0	0	2	0	0
5	8	0	0	17	4	0	0	31	2
6 ·	0	2	0	14	0	1	0	0	9
7 ·	8 0	0	0	10	0	0	0	0	0
8 ·	0	0	0	1	0	0	0	0	0

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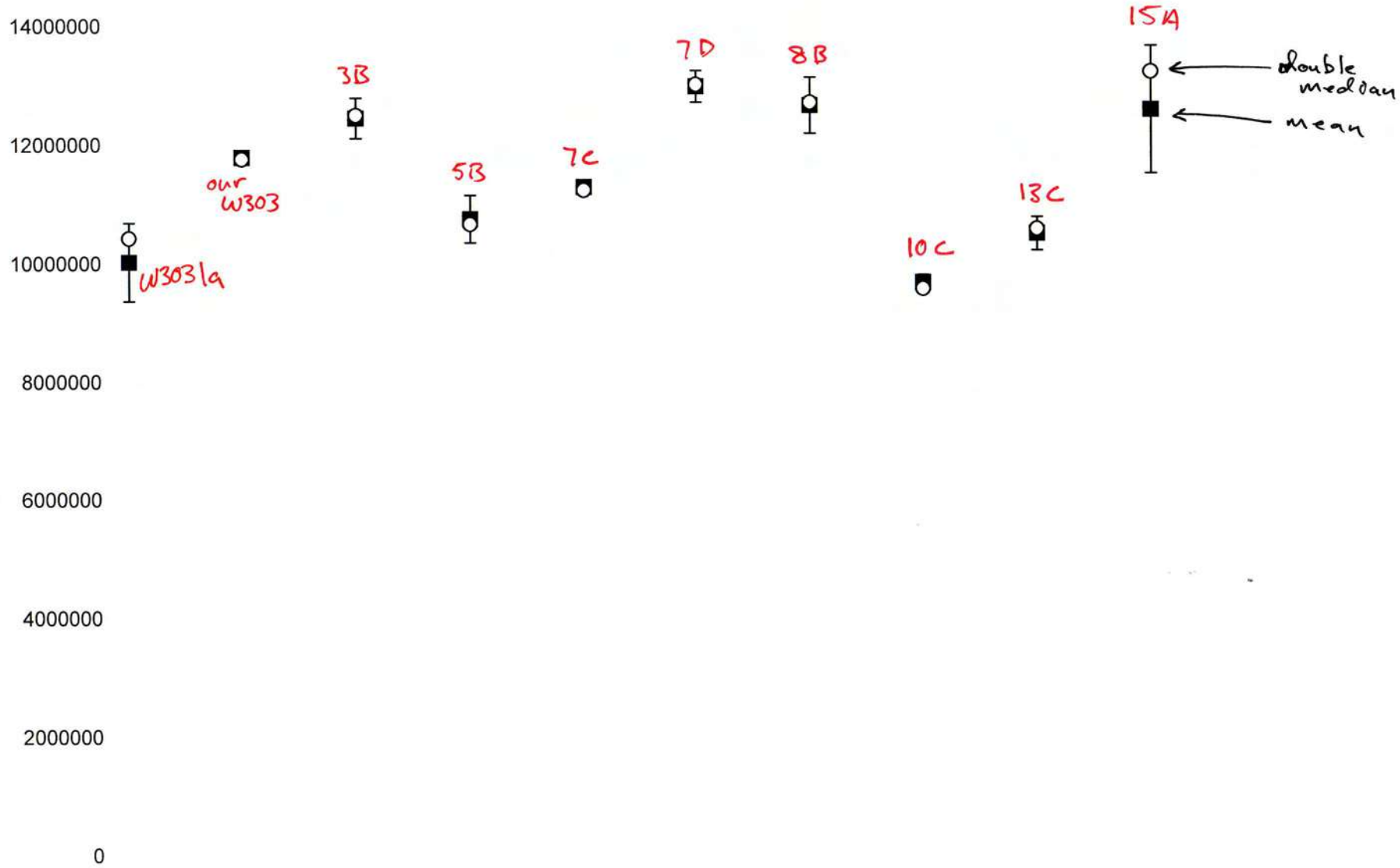
Freeze down spore 15A → now known as GIL104


14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
N
W303 1a
our W303
3B
5B
7C
7D
8B
10C
13C
15A
double median
mean

	W3031a									
strain	GIL087	GIL102	3B	5B	7C	7D	8B	10C	13C	15A
m	0.32	2.25	0.53	1.00	1.26	1.46	0.59	0.56	1.27	0.42
N	10432800	11759200	12498000	10662400	11233200	13007200	12704000	9567200	10576000	13214400
u	3.1	19.1	4.2	9.4	11.2	11.2	4.6	5.8	12.0	3.2
	42	7	3	0	4	1	0	1	0	0
	11	0	0	0	2	8	0	0	5	0
	0	1	0	10	6	2	7	1	2	4
	0	10	0	1	4	0	3	2	12	9
	0	0	0	4	6	5	5	3	57	8
	0	1	4	5	0	0	0	0	2	0
	3	18	4	3	1	1	0	2	0	0
	15	2	0	0	1	1	0	0	1	0
	0	50	11	0	0	9	0	10	15	5
	0	1	0	22	5	16	5	0	1	0
	0	17	0	7	1	1000	19	0	0	0
	0	12	0	2	5	0	0	11	0	0
	0	6	1	0	0	0	0	0	7	0
	0	2	0	5	200	2	5	2	3	2
	3	10	0	21	0	0	0	4	1	0
	35	1	131	4	6	7	0	0	4	0
	0	7	0	1	0	4	0	0	0	9
	0	3	2	3	0	5	0	0	0	0
	11	5	6	30	15	0	0	0	5	0
	1	7	0	13	2	2	0	4	2	9
	0	9	0	0	5	0	0	4	4	0
	0	3	16	3	13	6	0	0	1	0
	0	21	0	1	0	4	0	1	1	0
	0	1	0	0	3	1	0	0	60	0
	0	3	2	5	12	5	14	10	1	2
	0	17	24	3	2	161	0	0	1	0
	0	5	0	0	0	0	4	0	2	0
	0	16	0	0	3	2	52	0	6	0
	0	9	0	0	1	1	7	23	0	17
	1	7	13	0	1	1	0	0	0	14
	0	5	0	0	14	0	11	0	0	10
	0	0	0	0	0	1	0	0	0	1
	0	9	0	0	25	0	19	0	37	4
	13	9	0	1	0	8	0	0	12	0
	0	1	0	0	0	25	0	0	0	0

④ 12-9-4

⑪ 3-2-5-8

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Coulter Counts

Bkg	110	174	96
GIL087 1	63964	64380	64852
2	61064	59980	60924
3	64414	63258	64518
12A 1	73608	74450	72466
2	75852	73426	72662
3	72796	72164	71364
14B 1	72116	71228	70534
2	68906	67748	67162
3	71768	72812	70234
Post Bkg	2104	672	282

4/27

MOTIVATION: Previous work I have done suggested that our domesticated W303 carried several mutator loci. Therefore I wanted to backcross a spore from the GIL087/GIL102 cross to GIL087. Here I am performing fluctuation assays on the two Mat a spores with the correct phenotype (pg 5)

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5FOA PLATE COUNTS

GIL087

	1	2	3	4	5	6	7	8	9
1	0	0	0	6	8	5	0	3	0
2	0	~500	0	4	0	0	0	0	0
3	0	0	22	41	0	11	0	1	27
4	0	1	0	0	0	1	0	0	0
5	0	0	11	0	0	2	0	0	24
6	0	0	0	3	21	0	0	18	4
7	8	0	7	0	0	0	28	4	9
8	2	0	0	~~0~~ 4	~~4~~ 5	0	0	0	0

12A

	1	2	3	4	5	6	7	8	9
1	2	9	0	4	1	15	10	0	0
2	24	2	38	0	0	6	~~14~~ J(500)	14	1
3	4	4	0	1	1	2	1	23	0
4	13	4	0	15	~~21~~ 0	~~21~~ 31	~~12~~ 21	12	2
5	0	2	5	0	0	7	13	0	4
6	J(1000)	1	5	0	1	2	3	1	19
7	8	1	0	3	9	1	0	1	20
8	1	6	8	0	4	2	2	3	0

14B

	1	2	3	4	5	6	7	8	9
1	2	9	1	0	0	41	1	4	0
2	2	2	2	0	0	0	3	0	1
3	0	~25	0	3	12	7	17	30	5
4	37	11	15	1	2	0	1	0	0
5	6	1	0	1	0	1	4	11	0
6	J(1000)	2	22	3	6	3	0	17	3
7	5	1	0	13	1	1	3	4	0
8	5	1	32	0	0	0	1	15	3

4/4

Both 12A and 14B appear to have a high mutation rate; therefore, I will not backcross them to GIL087 as I had originally planned. ~~Instead~~ Instead I will use spore 15A ~~as~~ for the rest of the experiments.

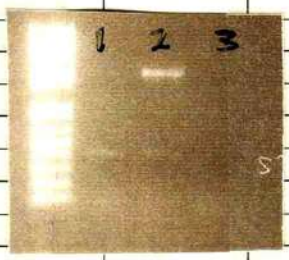
3/28

I want to sequence a large number of SFOA^R ura3 mutants as well as Can^R can1 mutants. In order to do this I first need to get a good primer pair for PCRing the CAN1 locus and get good internal primers for sequencing. Hopefully I can get the whole locus with 2x coverage using 4 primers.

- I pulled three random gDNA preps from the fridge GIL066, GIL076, and one from the Δ collection

- PCR using primers CAN1ext/intF1, CAN1extR1
  - use the URA3 program (except):
    anneal ~~45°~~ 49°
    extension ~~2 min~~ 2:30

1) GIL066
2) GIL076
3) Δ collection
} temp.



20522285
V.INDJEIAN
22032800 2/3/2006
CAN1int/extF1
5'-AAA AAA GGC ATA GCA ATG AC-3'
Tm= 49.3 °C , MW= 6,176.1
7.2OD$_{260}$ 33.40nmol 0.21mg

20522286
V.INDJEIAN
22032801 2/3/2006
CAN1extR1
5'-GTG TAT GAC TTA TGA GGG TG-3'
Tm= 49.9 °C , MW= 6,243.1
6.2OD$_{260}$ 30.80nmol 0.19mg

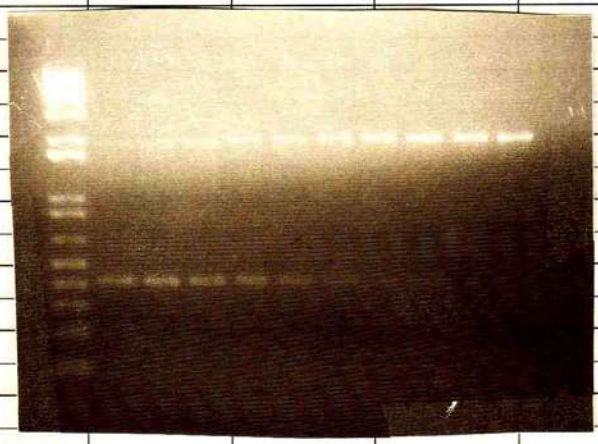
20522287
V.INDJEIAN
22032802 2/3/2006
CAN1intF1
5'-AAG TTT TAG CCA TTA TCG GG-3'
Tm= 50.2 °C , MW= 6,147.0
6.6OD$_{260}$ 33.50nmol 0.21mg

20522284
V.INDJEIAN
22032799 2/3/2006
CAN1intR1
5'-GGG AGA AGT AGA AAC GTA GG-3'
Tm= 51.9 °C , MW= 6,304.2
5.7OD$_{260}$ 25.90nmol 0.16mg

The rxn only worked on the one template (GIL076)

- Submit for sequencing.
- As it turns out there is a second possible product (~400bp) that can be amplified using these primers.
- The sequencing reads for the internal primers look good; however the outside primer reads are sloppy (2 temps) for the 1st ~400bp then clear up.
- I need to optimize the PCR.

ANNEALING TEMP:



- Purify the band 2 3 submit for sequencing.

4/4

Again when the sequencing came back the int primers looked fine but the ext. primers look like crap → perhaps they both bind internally??

Redesign the primers:

21735003 IDT
V.INDJEIAN
23248028 3/29/2006
CAN1extF2
5'- TCT TCA GAC TTC TTA ACT CC -3'
Tm= 48.6 °C , MW= 5,977.9
5.8 OD_{260} 32.50 nmol 0.20 mg

21735008 IDT
V.INDJEIAN
23248033 3/29/2006
CAN1extR2
5'- ATA GTA AGC TCA TTG ATC CC -3'
Tm= 48.9 °C , MW= 6,076.0
5.8 OD_{260} 29.60 nmol 0.18 mg

Typical Taq PCR

Anneal: 50°
Extension: 2:30

LANE

	1	CAN1 from spore 15A with F2/R2	
other expts	2	MSH6 SNP1	(MR4)
	3	MSH6 SNP2	(MR4)
	4	FAB1::KanMX deletion cassette	(MR 3)

PREP CAN1 PCR → submit for sequencing:

PRIMERS:
- CAN1extF2
- CAN1extR2
- CAN1extIntF1
- CAN1intR1

I suspect that CAN1extF2 might give a poor read since it is outside of the polyA tract. I will try to use CAN1extIntF1 – this will test the idea that it binds internally.

4/8

SEQUENCING ANALYSIS

- As expected CANextF2 gave a poor read.
- The others look good; however, there are still regions of 1× coverage and a region of No coverage near the start codon.

→ because of this I will must use 6 primers to sequence CAN1

6
4/11

Yesterday I resubmitted the sequencing of CAN1 using primers:

- CAN1ext/intF1
- CAN1extR2
- CAN1 intF2
- CAN1 intF3
- CAN1 intR2
- CAN1 intR3

- The sequences look good — 2-3× coverage of the gene except for 1× coverage of the region near the start codon.

4/11

DETERMINE MUTATION RATE OF ALL 4 SPORES OF THE TETRAD FROM WHICH GIL104 WAS DERIVED.

Counter	Counts								
Bkg	154	92	168						
15A 1	71356	71632	71290						
2	71010	69988	69556						
3	69470	69588	70230						
15B 1	61328	60342	60550						
2	62116	62480	62314						
3	60680	59958	60992						
15C 1	63566	63094	~~62968~~	62968					
2	63396	61866	62256						
3	62848	62484	61596						
15D 1	66374	67048	66960						
2	64222	63848	64138						
3	67570	66202	66220						
Post Bkg	1350	1220	848						

suggesting that our W303 carries 1 mutator allele

4/18

On pg 13 it looks like the mutation rates fall into two classes; however, previous work had suggested that ~~W303~~ our domesticated W303 carried several mutator loci.

I wanted to determine how the mutator locus (loci?) segregated in the tetrad from which GIL104 was derived.

The 2:2 segregation of mutation rate on the following page shows that even if there are multiple mutator alleles in ~~the~~ our W303 ~~the~~ those mutations are not present in GIL104

The ~~m~~ values for m (and thus μ) are higher here because I waited 7 days to count the plates instead of the usual 5 — I should figure out Z-scores for this expt.

4/17

COUNT 5FOA PLATES

15A (G1L104)	1	2	3	4	5	6	7	8	9
1	0	0	1	10	0	0	77	2	0
2	13	0	0	0	0	3	0	8	0
3	0	3	0	5	0	0	0	4	2
4	0	0	0	1	6	15	18	8	18
5	0	0	0	13	0	53	1	0	0
6	0	7	0	1	1	2	J(~1000)	2	0
7	5	0	1	0	5	9	0	4	1
8	3	13	5	2	3	0	2	19	1

15B	1	2	3	4	5	6	7	8	9
1	3	5	10	3	1	0	0	11	1
2	22	1	3	1	3	5	1	8	1
3	3	1	1	10	0	29	2	5	3
4	13	5	7	8	9	0	8	1	3
5	0	57	1	23	1	15	29	4	0
6	2	11	0	4	1	11	3	0	2
7	5	3	0	4	3	5	13	5	2
8	5	12	1	6	92	4	0	8	13

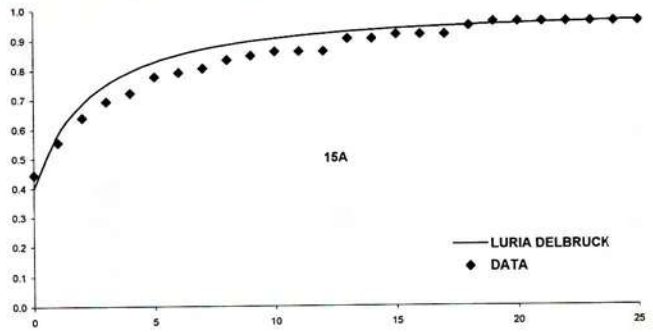
15C	1	2	3	4	5	6	7	8	9
1	0	10	7	17	3	6	2	0	6
2	J(~5000)	2	2	5	0	~200	1	57	4
3	2	19	0	0	9	4	120	2	7
4	0	10	2	2	6	9	7	6	3
5	3	13	0	9	38	1	8	1	47
6	23	18	6	5	1	1	5	8	6
7	3	24	0	1	2	4	0	13	3
8	2	0	2	5	0	2	3	1	9

15D	1	2	3	4	5	6	7	8	9
1	0	1	0	26	0	0	11	0	0
2	0	0	0	0	7	1	8	2	35
3	2	0	20	0	0	0	0	1	0
4	0	2	3	3	1	0	0	0	22
5	0	0	0	0	1	0	23	3	7
6	8	0	0	0	2	0	0	0	1
7	0	0	2	4	0	0	0	7	1
8	0	0	0	0	1	4	0	16	J(~200)

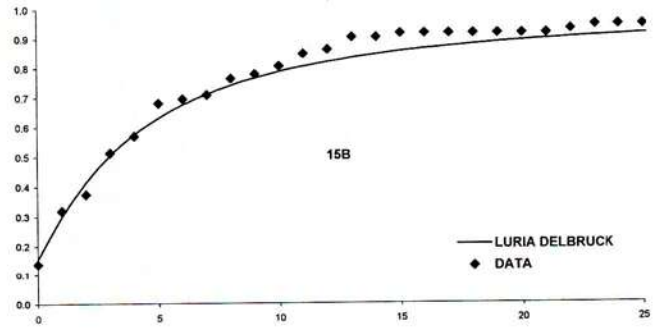
spore	15A	15B	15C	15D
m	0.9174	1.8662	1.9553	0.6118
N	13397600	12136000	12496800	13244000
u (x 10^8)	6.847	15.377	15.646	4.619

4/18

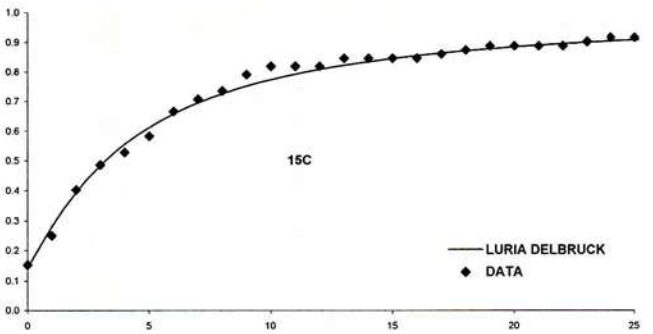
QUALITY OF FLUCTUATION DATA FROM PAGE 22



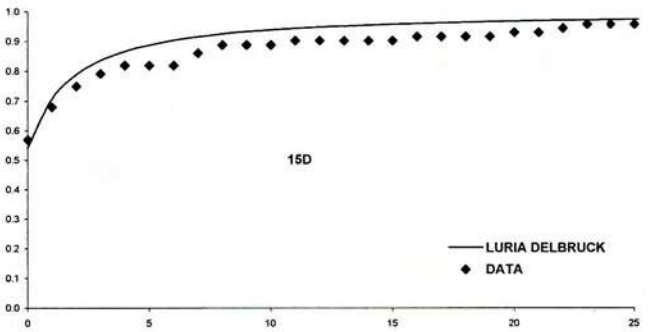
Z = 0.7394



Z = -0.1794



Z = -1.8118



Z = 0.9441

4/12

MAKING αF plates:

- ✓ 2L H_2O
- ✓ 60g Dextrose
- ✓ 60g Peptone
- ✓ 30g Yeast Extract
- ✓ 60g Bacto agar

— Stir —

- ✓ 1L H_2O

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

- ✓ 30 mL Ade + Trp (2 mg/mL each)

— AFTER COOL:

- ✓ 3 mL αF (10 mg/mL)

— POUR —

4/13

MAKING 10× CAN PLATES

- ✓ 2 L H_2O
- ✓ 60 g Dextrose
- ✓ 21 g YNB (w/o amino 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/L)
- ✓ 30 mL Canavanine (60 mg/mL)
- —— Pour ——————

Make 30 mL of Canavanine (60 mg/mL)

- Weigh out 1.8 g of Canavanine
- Put in 50 mL falcon tube
- Add 30 mL ddH_2O
- Vortex

4/12

THE PLAN

Soon I will be able to start the big experiment. Below is an outline of the plan

PART I: FLUCTUATION ASSAYS

DAY 1	Streak GIL104 from frozen stock
DAY 2	
DAY 3	Pick 10 indepent colonies → inoculate 3mL 2×SC-ura
DAY 4	For each culture, start fluct. assays for 5FOA^R, Can^R, & αF^R / put filters on αF plates
DAY 5	Plate αF ~~ent~~ plates / put filters on 5FOA & Can plates
DAY 6	Plate 5FOA & Can plates / Coulter count cultures for αF
DAY 7	Coulter count cultures for 5FOA & Can
DAY 8	Count αF plates
.	
.	
.	
DAY ~12	Count 5FOA & Can plates

PART II: gDNA preps:

- For both the 5FOA & the Can plates, pick 240 resistant colonies and inoculate 1 mL of YPD + ADE
- Take 100 λ of each culture to freeze down in 96-well plates
- make gDNA preps

PART III: Sequencing

- PCR URA3 & CAN1 loci respectively using primers URA3extF / URA3extR and CAN1extF2 / CAN1extR2
- PCR cleanup spin columns
- submit for sequencing

URA3

(1) URA3 ext F3
(2) URA3 ext R
(3) URA3 int F2
(4) URA3 int R2

CAN1

(1) CAN1 ext/int F1
(2) CAN1 ext R2
(3) CAN1 int F2
(4) CAN1 int F3
(5) CAN1 int R2
(6) CAN1 int R3

4/12

What needs to be done before I begin?

- Make 10× Can plates
- Test the αF and Can plates
- Test 5FOA plates
- Make ⅓ Culture media
- Determine appropriate culture conditions such that the zero class is in the range ~~20-5~~ 10-50% My expectation is as follows:

→ For $5FOA^R$: 200λ cultures in 2×SC

- For Can^R: 100λ cultures in 2×SC

- For αF^R: 10λ cultures in 2×SC (0.2% Glucose)

4/18

Yesterday I streaked GIL104 from the -80 freezer onto duplicate YPD plates.

4/19

Pick 10 colonies off of one of the YPD plates and inoculate 3mL 2×SC-ura (filtered)

↳ labeled A → J

4/18

PLATE TESTING / PRACTICE RUN

- On ~~Sunday~~ Friday (4/15) I started two fluctuation assays (100 λ and 200 λ into 2- 5C)
- On Sunday (4/16) I plated them onto SFOA (200 λ) and ~~Gr~~ 10x Canavanine (100 λ)
- ~~Yesterday (Sunday, 4/17) I s~~
- On Sunday (4/16) I started a fluct assay using 10 λ cultures ~~an o/n culture of GIL104~~
- Yesterday I plated the cultures onto αF plates (10 λ cultures + 90 λ H_2O)

4/19

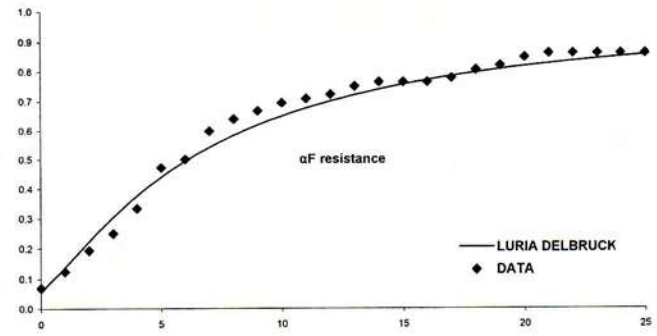
COUNT PLATES

αF^R

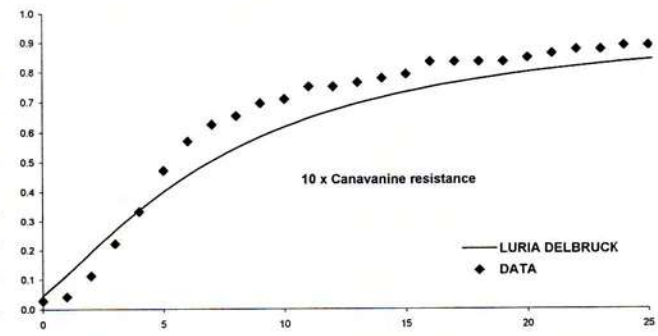
	1	2	3	4	5	6	7	8	9
1	10	32	4	4	7	12	5	4	19
2	21	13	5	3	~250	5	1	60	3
3	1	3	5	0	1	7	26	6	7
4	8	7	8	7	17	4	0	4	5
5	18	20	13	5	5	2	9	11	2
6	65	2	~230	33	5	1	10	5	7
7	18	64	0	~250	5	14	2	52	7
8	8	2	9	0	0	3	20	4	6

$10 \times Can^R$

	1	2	3	4	5	6	7	8	9
1	6(3)	16(0)	1(3)	6(2)	13(8)	5(1)	31(1)	5(1)	3(2)
2	20(0)	9(2)	40(4)	16(0)	4(2)	3(2)	4(1)	11(0)	21(0)
3	6(1)	10(1)	2(0)	5(0)	5(0)	4(0)	4(1)	4(1)	5(3)
4	14(2)	24(0)	5(0)	6(0)	6(1)	6(0)	9(5)	29(4)	22(0)
5	16(0)	5(1)	~200(0)	2(1)	9(2)	5(1)	2(0)	8(0)	56(1)
6	11(0)	2(1)	5(1)	11(1)	6(0)	5(0)	0(15)	3(0)	3(1)
7	7(1)	87(0)	3(8)	7(0)	68(0)	8(1)	0(1)	4(0)	4(02)
8	3(1)	7(1)	34(3)	7(2)	4(1)	2(0)	3(0)	3(0)	15(0)



$Z = -0.8570$



$Z = 1.2229$

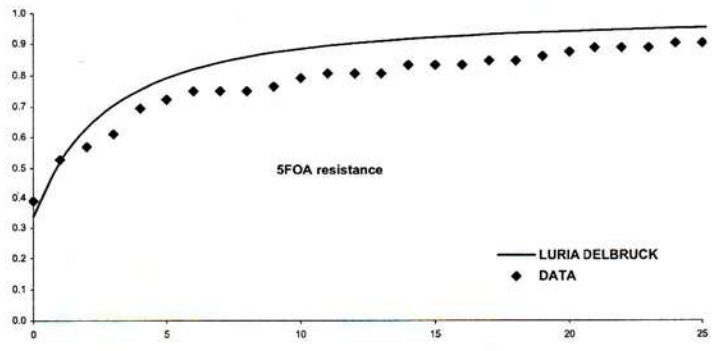
4/21

DISCUSSION OF PRACTICE RUN

- The counts from the 5FF plates look good but perhaps the m is too high. I will have to think about changing the media
- It looked like just about every spot was contaminated with some yellow bacteria. Some spots had a lot more contamination than others leading me to think that perhaps the contaminant was coming from one of the tips — but that doesn't seem to be the case. Regardless I will need to be more careful when plating the 5FF plates.
- I was still able to count the yeast colonies despite the contamination.
- The 10x Can plates are behaving more like 1x. There were more colonies than I expected and the cumulative plot on of the data on pg. 28 shows characteristics of post-plating growth.
- I will need to remake the plates tomorrow.

4/22 COUNT 5FOA PLATES

5FOA	1	2	3	4	5	6
1	3	0	0	0	0	68
2	0	0	0	68	0	6
3	0	14	4	0	0	1
4	4	38	2	10	5	24
5	0	9	1	0	5	4
6	1	0	0	20	0	162
7	1	0	1	19	0	0
8	0	1	21	17	0	0
9	32	0	0	0	1	11
10	0	6	3	10	1	3
11	73	14(4)	4	1	4	2
12	0(1)	54	4	2	1	0



4/22 REMAKE -arg+10x Canavanine PLATES

Today I am remaking the ~~10x~~ -arg+10x Canavanine plates according to the protocol on page 26.

I think the problem with the plates was either ~~not enough~~ that the canavanine was bad or (more likely) I added the drug to the media while it ~~was~~ was too hot

This time I have ordered new canavanine and I will be sure to let the media cool before adding the drug.

→ According to page 86 in the MR3 notebook, I ~~waited~~ should wait ~1h after taking ~~out~~ the flasks from the autoclave before adding the drug.

NOTES:

I waited ~1.5h following removal from the autoclave until I added the drug. I began pouring the plates shortly thereafter.

4/22

PLATE TESTING CONTINUED

- The ~~%~~ P_0 class from the αF^R practice run (p 30) was a little small (less than .1). I would like to redo the αF^R fluctuation assay using 2-fold diluted media (1x SC w/ only 0.1% Glucose)
  ↑ still 10λ cultures

- → start o/N culture ~~using~~ in 2x SC-ura using culture ~~"A"~~ single colony from one of the GIL104 YPD plates in the fridge.

- In order to test the -arg + 10x Can plates I am making today I will start a 100λ fluct assay using culture "A" from rollerdrum (see pg. 28)

4/23

- Start fluctuation assay to retest the αF plates: 10λ cultures in 1x SC (only 0.1% Glucose)
  ↳ 3:00 pm

4/24

Plate the fluctuation assays to test αF and the new -arg+10x Canavanine plates

TECAN PROGRAMS:

~~fluct~~
for αF: "fluctPlating_petri9well_10μl_060227"

for -arg+10x Canavanine: "fluctPlating_petri9well_060227"

- before I started plating I washed the tips by ~~pip~~ aspirating & dispensing 400λ of 70% EtOH 5 times using program "ethanol_wash_060424"
- plating between 11:30 - 12:30
- plates put in 30° incubator @ 6:00pm

4/26

Count Plates from second practice run

I probably let the αF plates grow too much.

αF	1	2	3	4	5	6	7	8	9
1	1(0)	13(0)	0(3)	4(1)	0(0)	2(0)	1(0)	3(~1000)	1(3)
2	2(3)	3(2)	6(~1000)	1(0)	2(1)	0(2)	3(0)	3(0)	6(1)
3	2(0)	4(0)	11(~1000)	12(1)	5(1)	2(0)	11(0)	14(1)	5(0)
4	1(0)	2(0)	4(0)	2(0)	12(2)	5(0)	11(0)	2(0)	2(0)
5	10(0)	7(1)	8(0)	0(0)	5(0)	4(1)	2(0)	7(1)	5(0)
6	1(0)	6(0)	2(0)	9(0)	8(0)	9(1)	2(2)	11(0)	13(0)
7	4(0)	13(0)	14(1)	9(1)	5(0)	2(3)	5(0)	0(0)	8(0)
8	4(0)	1(0)	4(3)	0(0)	0(1)	4(0)	5(0)	15(0)	15(0)

-avg+10×Canavan	1	2	3	4	5	6	7	8	9
1	9(1)	3(0)	176(0)	46(0)	17(0)	0(1)	2(1)	99(0)	2(0)
2	~225	5(0)	5(0)	32(1)	7(0)	0(0)	8(1)	6(0)	20(0)
3	2(0)	3(0)	30(0)	3(0)	11(2)	7(1)	2(0)	9(0)	12(0)
4	1(0)	15(2)	6(0)	2(0)	6(0)	1(0)	7(1)	10(0)	6(0)
5	4(0)	2(0)	0(1)	2(2)	6(0)	1(0)	3(2)	6(7)	6(0)
6	0(2)	80(0)	2(3)	13(0)	7(0)	39(0)	7(0)	34(0)	4(3)
7	10(0)	10(0)	17(0)	4(0)	3(1)	4(2)	1(0)	4(0)	2(0)
8	4(1)	11(0)	4(0)	3(1)	5(0)	14(0)	2(0)	4(0)	9(0)

4/24

THE REAL EXPERIMENT — TAKE II

- On Saturday (4/22) I streaked GIL104 from the -80 freezer onto duplicate YPD plates labeled "GIL104-3" and "GIL104-4" to distinguish them from the plates from 4/18 (pg 28)
- Today I picked 10 single colonies from plate GIL104-3 and inoculated 3 mL of filtered 2×SC-ura → labeled K→T

4/25

Today I need to start the fluctuation assays:

For SFOA^R: 200λ 2×SC

For $^{10\times}$Can^R: 100λ 2×SC

For αF^R: 10λ 1×SC (only 0.1% Glucose)

} all media filtered

I

- Dilute each o/N culture 1:10 in H_2O (100λ culture into 900λ H_2O)
- Inoculate 40 mL of 2×SC w/ 40λ of diluted cultures
- Using the multichannel pipettor, set up fluctuation assays for SFOA^R & Can^R
- I will make a second plate of "K" to be plated on 1× Can.

Put ~~in~~ 96-well plates in 30°C room ~12:45

- Put tubes K-T back in rollerdrum
- Dilute each o/N culture 1:100 by ~~serial~~ 2 1:10 dilutions as above.
- Inoculate 2 mL of 1×SC (only 0.1% Glucose) with 20λ of 1:100 dilution.
- Using multichannel set up fluctuation assays for αF^R

Put 96-well plates in 30°C room ~3:00

4/26

PLATING OF PLATES

NOTES

- Started ~ 1:30
- During the run "K" I noticed several spot fusions; therefore, I dropped the amount of water added to ~~at~~ only 80λ → I renamed the program "fluctPlating_petri9well_16μL_060426"
  ↳ I will have to remember this when calculating N.
- Some of the plates seem a little warped — if I notice this I will press down ~~at~~ on the 96-well plate so that the tip is not forced against the bottom of the well.
- Program run time: ~ 13m
- I washed the tips w/ 70% ethanol before I began using the program "ethanol_wash_060424"
- I still noticed several more spot fusions, but the rate of spot fusion is acceptable
- The ~~spots~~ plates with writing on the top "GREG-OF" seem to have bigger spots — more fusion. Since these were ~~on~~ the top plate in stacks of 20, they may be more dry.
- I accidentally plated plate "Q" on "P" plates and into the "P" tube
  ↳ I will need to remember to switch the labels later.
- I changed the water plate halfway through — after "O"
- Finished Plating @ 4:15 → Plates left out o/n to dry

~~4/18~~ 4/27

NOTES CONTINUED

- Today when I went to get the plates I noticed there was a lot of contamination — not the yellow bacteria I saw earlier (pg. 31) but it looked more like a mold. Hopefully I will be able to count the plates tomorrow and will be able to count around the contaminant.
- In the future I should add a drug to these plates (G418?, amp?); also I should use synthetic plates — not YPD — YPD seems to get contaminated more easily and also, the spots formed from spot-plating are bigger (this could also be due to the fact that these spots were mostly water however) and more difficult to see. These plates are expensive enough as it is — I may as well make sure that they work correctly. — Question — do Mata cells arrest as effectively on synthetic? Probably Not.

~~4/26~~ 4/27

PLATING CANVANING PLATES

NOTES

- Started ~ 10:30 am
- Last night I put filters on the Can & SFOA plates
- In order to improve plating efficiency I moved the plates on the tecan workspace — the plates were plated using this new program, "FunctPlating_petri9well_060427
- I have been adjusting the Zmax on the 96 well plate and the Z-dispense on the 9 well petri dishes throughout the run — there is still some liquid left in the wells of the 96-well plate after aspiration (I would say, at worst, 5 µl). This is not ideal but is acceptable.
- Prior to the run I washed the tips w/ ethanol as before.
- I noticed that the orbital shaker was not at 1050 rpm which is where I usually use it — it was around 950-1000 rpm. I don't know if this could contribute to the appearance of cells left behind in the wells
- Before I started plating (when I renamed the workspace) I edited the liquid classes, "water_slow" and "water_fast" to remove the leading and trailing air gaps — I noticed that the air gaps are added when the tips are at Z-travel and ~~are~~ are unnecessary. Getting rid of the air gaps should speed up the program.
- Program run time: ~ 8m 30s
- Finished ~ 12:30

5/2

- Plates were put in the coldroom Saturday night (4/29) and counted on Sunday (4/30).

4/27

PLATING SFOA PLATES

NOTES

- Started ~6:00 pm
- I started plating after the plates were on the shaker for ~10m
  ↳ it does not take long to get the cells in suspension.
- ~~I~~ I noticed a ~~slight~~ gradient of cells left in the wells → a few in column 1 ~~but~~ with a little more in each subsequent column.
  ↳ After "K" I put the 10λ leading & trailing overlaps back in the liquid classes (see comment on pg. 37) but this didn't seem to make a difference.
- Run time: ~10m 20s
- I washed the tips again w/ EtOH prior to the run.
- Finish ~8:30 pm

4/28

I accidentally left the pooled cultures (to be counted today) out at RT o/N — I don't think that this should make any difference.

4/27

Coulter Count of F cultures

- I am guessing I should add 100λ of culture to 10mL solution
  - ↳ this came from MRI Notebook (p535)
- For each sample → ~~sonic~~ vortex → sonicate 2m → Count on triplicate

		Bkg	22	122	54					
K	1		34924	35266	·35244					
	2		35708	·35654	35160					
	3		·36434	36508	35956					
L	1		41084	·40260	39540					
	2		·40108	40450	39540					
	3		·41838	42044	41516					
M	1		·39708	39820	39430					
	2		39020	·39732	39802					
	3		40432	·40132	37784					
N	1		41674	·40874	40850					
	2		42936	·41072	40766					
	3		41634	40500	·40746					
O	1		40318	40488	·40398					
	2		41506	·41284	40766					
	3		41956	40974	·41172					
P	1		42880	·42038	41546					
	2		·42038	42336	41702					
	3		42656	42368	·42430					
Q	1		44984	44078	·44862					
	2		44310	·44224	43508					
	3		44252	43846	·44042					
R	1		40630	42490	·42336					
	2		41516	·41202	41006					
	3		42130	·41874	41694					
S	1		42568	·42434	42046					
	2		·42822	42984	41992					
	3		43112	42660	·42710					
T	1		45300	45198	43550	43586	·44038			
	2		45096	·45070	44660					
	3		·45598	45756	45136					
Post Bkg			1486	364						

| = rinse & flush

After Post Bkg I counted a new sample of "K": | 34990 34658 34666

and "T": | 43362 42370 43794

~~4/28~~ 4/28

COULTER COUNT RANAVANINE CULTURES

vortex →
· Sonicate 2m → 10λ into 20mL solution

Bkg		206	72	82
K(iv)	1	64866	·65376	66306
	2	64284	65386	·64418
	3	64852	·64730	64314
K	1	69430	·68974	68856
	2	68394	67784	·67952
	3	69710	67404	·67856
L	1	72000	·71912	71546
	2	69714	·69420	69242
	3	70600	69724	·70200
M	1	60032	·59400	59348
	2	59014	59566	·59154
	3	59666	·59548	58534
N	1	·70588	69784	71014
	2	71070	72056	·71682
	3	71228	·71060	70884
O	1	68714	·67696	67616
	2	64132	64330	·64280
	3	·65870	66162	65306
mid Bkg		2238	1488	1356
P	1	66338	·66494	67372
	2	·67156	67308	66920
	3	64988	65244	·65018
Q	1	·73338	72264	73662
	2	71484	·71626	72158
	3	·72404	72202	72412
R	1	66696	·66632	66232
	2	·65334	64930	65398
	3	67678	66696	·67336
S	1	72982	71764	·72962
	2	72402	·72328	71768
	3	71570	·71472	70888
T	1	60444	59712	·60182
	2	61286	61942	·61792
	3	62036	61788	·61884
Post Bkg		3018	3042	2478

4/28

COULTER COUNT SFOA CULTURES

• Vortex → sonicate 2m → 10λ into 20 mL solution

Bkg		30	48	30			42	104	60	
K	1	43232	·44140	44738	K	1	·49932	49616	50572	
	2	45914	45364	·45686		2	51506	50864	·51374	
	3	44870	·44688	44452		3	51216	50124	·50576	
L	1	·37034	37442	36736	L	1	57732	·56586	56290	
	2	·37312	37120	37496		2	56366	55982	·56258	
	3	37546	·37466	37050		3	55338	·55486	55520	
M	1	42746	42068	·42456	M	1	·51696	52552	51548	
	2	42914	·42650	42110		2	51236	50780	·51194	
	3	·43620	43696	42930		3	50286	·49384	48678	
N	1	~~39998~~	·39518	38674	N	1	~~48678~~	39142	37878	·38600
	2	39148	·38946	38748		2	32122	·31780	31202	
	3	·39162	39210	38404		3	·31904	31874	32252	
O	1	44110	·44020	43790	O	1	52184	·52920	53270	
	2	·44272	43922	44556		2	50600	·49888	49792	
	3	·44350	44878	44296		3	·50170	59856	50290	
P	1	·50660	50128	50732	P	1	55212	·54962	53594	
	2	49942	·49502	49206		2	53906	54636	·54166	
	3	49608	·49262	48770		3	~~44096~~	54096	53644	·53960
Mid Bkg		1250	540	400						
Q	1	50218	·50218	51194	Q	1	·55890	56292	55596	
	2	51316	·51376	51966		2	·54990	54562	55424	
	3	51662	·51744	51878		3	55726	·55682	55488	
R	1	·41888	41450	42362	R	1	·52366	52562	52212	
	2	·41826	42056	41596		2	52100	·52080	51978	
	3	·41320	41594	40848		3	·52000	51590	52170	
S	1	40522	40278	·40296	S	1	·48242	48300	48090	
	2	·40138	40348	39668		2	49348	48384	·48884	
	3	39602	·39788	39902		3	49284	·48840	48790	
T	1	39380	37836	·38040	T	1	46222	·46640	46646	
	2	·37868	37914	37754		2	50264	·50916	52840	
	3	·38140	38194	37988		3	50478	49808	·49922	
Post Bkg		1794	1280	1226	N	4	50712	·50566	50268	
						5	·48418	47918	48458	
						6	48908	·49502	49860	
							1524	634	502	

4/29 RECOUNT SFOA CULTURES

4/29

NOTES ON COULTER COUNTING

Counting of αF cultures

- It looks like the counts were increasing with each sample — I wonder if the background played a role in this?
  ↳ Notice that twice when I rinsed and flushed the next count was lower.

Counting of 5FOA cultures

- Because of the way I set up the fluctuation assays I expected the cell densities for the 5FOA and Canavanine cultures to be the same. I was surprised to see that the 200λ cultures saturated at a significantly lower density to the point that some of the strains had almost as many total cells in the 100λ cultures as in the 200λ.

  ↳ After I finished counting on Friday (4/28) I looked at the cells from the canavanine cultures "K" and "L" and 5FOA cultures "K" and "L" to see if there really were significantly ~~more cells in the 100~~ higher densities of cells in the 100λ cultures. → it looked like the was indeed a greater cell density in the 100λ cultures, but there were still a number of cell clumps in the 200λ cultures — it could be that 2 min of sonication is not sufficient to break up cell clumps in the pooled 200λ cultures (4.5 mL total). To test this I removed 2 mL from the ~~"K"~~ "L" 5FOA tube and resonicated both "L" tubes for 2 min and recounted:

Bkg	706	710	734
*Can L 1	73008	74644	74314
2	73482	73782	74430
3	74096	73998	73748
5FOA L 1	55454	55652	55298
2	54978	54044	54356
3	56292	55896	55824
Post Bkg	2494	1696	1830

thus shows that increased sonication increases counts for 200λ cultures but not 100λ.

4/29

NOTES ON COULTER COUNTING CONT.

- From now on I should look at the strains under the 'scope before I coulter count them
- Today I vortexed the SFOA samples → removed 2 mL → resonicated and recounted. → the results are on p 41
  - ↳ before I recounted I looked at "M" and "P" under the 'scope.
    - ↳ All counts increased except "N" – I looked at this strain under the microscope and it looked well sonicated and looked to be about the same conc. as "M" – therefore I recounted this strain (bottom of p 41 ~~upt~~ "N4-N6".) Perhaps I did not vortex the strain well enough therefore the samples I removed were too dilute.

4/28 αF PLATE COUNTS

K

	1	2	3	4	5	6	7	8	9
1	0(0)	9(0)	J(500)	5(0)	2(1)	3(1)	: 1(0)	3(0)	· 2(0)
2	2(0)	9(1)	2(2)	14(0)	3(0)	4(0)	2(0)	· 15(1)	4(0)
3	: 10(0)	10(0)	5(0)	: 4(1)	1(0)	0(0)	5(1)	1(0)	2(0)
4	4(1)	0(1)	1(1)	13(2)	10(1)	1(1)	4(0)	2(0)	8(0)
5	9(0)	2(1)	12(3)	0(0)	2(1)	1(0)	3(0)	8(0)	4(2)
6	4(0)	· 3(0)	4(0)	0(0)	4(1)	1(0)	2(0)	1(0)	· 7(0)
7	9(1)	6(0)	5(0)	1(0)	: 4(0)	: 2(0)	13(2)	13(0)	7(0)
8	1(0)	6(0)	2(2)	2(0)	1(0)	4(0)	3(1)	2(1)	9(0)

L

	1	2	3	4	5	6	7	8	9
1	1(0)	17(1)	21(1)	3(0)	1(0)	: 1(0)	: 2(0)	29(0)	· 9(0)
2	· 1(0)	1(0)	8(0)	4(2)	6(1)	2(1)	0(0)	4(1)	4(0)
3	2(1)	5(1)	4(1)	: 7(1)	3(0)	6(0)	6(0)	4(2)	2(1)
4	13(3)	6(1)	8(0)	: 6(0)	: 0(0)	1(1)	12(0)	1(1)	0(4)
5	: 2(0)	4(0)	6(1)	6(0)	47(0)	3(0)	: 0(0)	3(0)	7(1)
6	5(0)	8(0)	4(0)	: 1(0)	· 1(0)	5(0)	1(0)	0(0)	4(0)
7	1(0)	3(0)	6(0)	2(0)	· 33(0)	: 11(0)	1(1)	: 14(2)	6(0)
8	8(0)	1(2)	0(0)	8(2)	4(0)	2(0)	5(4)	2(2)	: 5(0)

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

M

	1	2	3	4	5	6	7	8	9
1	10(0)	1(0)	7(0)	9(2)	2(3)	4(0)	0(0)	6(1)	5(1)
2	4(0)	J(200)	1(1)	4(0)	· 2(0)	5(0)	9(0)	18(0)	1(0)
3	16(0)	1(3)	: 1(0)	4(0)	10(1)	1(0)	19(1)	24(0)	2(0)
4	3(0)	1(1)	3(0)	4(2)	3(2)	0(2)	1(0)	3(1)	: 1(0)
5	: 18(0)	13(1)	8(2)	5(1)	2(0)	2(0)	4(1)	13(1)	3(2)
6	6(0)	· 3(0)	X 3(1)	: 5(0)	· 6(0)	6(0)	8(0)	3(1)	2(7)
7	8(1)	: 3(4)	0(0)	1(1)	· 1(1)	7(1)	11(0)	8(2)	5(0)
8	9(2)	6(0)	32(0)	5(0)	10(1)	4(0)	15(0)	0(1)	3(0)

N

	1	2	3	4	5	6	7	8	9
1	3(0)	5(2)	3(0)	2(0)	6(0)	2(1)	16(1)	9(0)	6(0)
2	6(2)	: 6(1)	: 8(0)	· 1(0)	· 4(1)	2(0)	: 4(0)	: 3(0)	4(1)
3	5(1)	6(2)	69(0)	3(1)	6(0)	3(1)	2(3)	5(3)	2(0)
4	3(6)	6(0)	5(2)	16(0)	0(2)	: 5(0)	0(0)	· 4(1)	0(0)
5	34(0)	2(0)	21(2)	4(0)	2(0)	6(1)	· 10(0)	12(0)	: 0(0)
6	3(3)	3(0)	22(0)	5(0)	4(0)	1(0)	7(0)	11(1)	5(0)
7	: 3(0)	3(2)	84(0)	: 11(0)	3(1)	: 4(0)	: 0(0)	5(0)	: 4(2)
8	30(2)	5(1)	· 6(3)	14(0)	: 2(0)	~~: 3(1) 4(0)~~ 5(1)	~~4(0)~~ 5(0)	5(1)	11(0)

O

	1	2	3	4	5	6	7	8	9
1	4(0)	6(0)	2(0)	: 2(2)	6(5)	17(1)	: 4(1)	1(3)	2(1)
2	6(0)	: 3(0)	2(0)	4(10)	: 2(0)	: 1(0)	10(1)	3(~100)	2(1)
3	83(0)	~200(0)	4(2)	25(1)	4(1)	1(0)	3(1)	! 10(0)	6(0)
4	6(0)	5(0)	3(2)	6(1)	5(0)	2(0)	2(0)	1(1)	11(0)
5	2(1)	2(7)	9(0)	2(2)	2(1)	2(4)	9(0)	18(1)	3(0)
6	2(0)	: 4(0)	2(1)	2(2)	: 3(0)	2(0)	9(0)	2(0)	5(0)
7	10(0)	5(0)	1(11)	· 0(0)	2(0)	2(1)	2(1)	0(1)	3(0)
8	2(1)	22(1)	1(0)	· 2(0)	3(0)	4(0)	5(0)	3(0)	5(1)

· - contamination - probably did not effect counts
: - " - may have effected counts
! - " - " "
· : ! - probably effected counts

Q

	1	2	3	4	5	6	7	8	9
1	1(0)	5(0)	5(0)	2(0)	16(0)	13(0)	2(1)	0(1)	8(2)
2	6(0)	6(0)	18(1)	8(0)	2(0)	3(0)	2(0)	1(0)	2(0)
3	2(0)	3(1)	3(0)	6(0)	:1(5)	2(2)	2(0)	:2(0)	12(1)
4	1(1)	:15(0)	2(2)	10(4)	2(0)	3(0)	0(0)	2(0)	5(0)
5	2(2)	4(0)	2(3)	3(0)	2(0)	9(6)	66(0)	2(0)	7(0)
6	2(0)	4(2)	3(1)	·9(1)	4(0)	:0(0)	4(0)	26(0)	5(0)
7	10(0)	3(2)	14(1)	14(0)	5(3)	5(0)	2(0)	57(3)	J(~200)
8	6(2)	:2(0)	12(0)	5(1)	:5(0)	5(1)	10(0)	:4(1)	7(1)

4/28

4/29

P

	1	2	3	4	5	6	7	8	9
1	:3(0)	28(0)	2(0)	:3(0)	:23(0)	2(1)	:1(0)	:9(8)	1(1)
2	4(1)	·1(3)	:100(2)	0(0)	:4(0)	·28(0)	5(0)	:7(0)	:2(0)
3	2(1)	2(0)	4(3)	3(1)	3(3)	3(1)	·6(0)	0(3)	:0(0)
4	2(0)	1(0)	5(1)	2(0)	5(1)	5(0)	2(0)	0(0)	2(2)
5	:20(0)	2(0)	2(2)	1(0)	:6(8)	·4(2)	:3(1)	:8(2)	5(0)
6	~200(0)	4(2)	4(1)	2(0)	4(0)	:21(2)	7(2)	0(0)	9(0)
7	13(2)	·1(0)	3(0)	2(1)	~~8(0)~~5(0)	3(2)	8(0)	8(0)	:14(0)
8	2(~50)	5(0)	3(0)	:3(0)	19(0)	4(0)	26(0)	4(5)	10(0)

R

	1	2	3	4	5	6	7	8	9
1	3(4)	4(5)	2(1)	4(1)	·4(0)	4(2)	11(1)	1(1)	3(0)
2	·1(0)	1(1)	5(0)	:3(1)	5(1)	4(0)	4(0)	·5(1)	8(0)
3	15(2)	0(0)	·3(0)	3(0)	2(0)	·15(4)	1(1)	3(0)	5(0)
4	9(0)	13(1)	10(1)	2(2)	2(0)	~~6(1)~~ 3(1)	6(1)	14(2)	8(0)
5	4(1)	2(2)	3(1)	13(0)	5(3)	3(1)	4(0)	5(0)	:2(0)
6	3(3)	:~~9(0)~~ 10(0)	~~~200~~ ~500	5(0)	3(2)	·3(2)	2(1)	4(2)	3(0)
7	:3(0)	5(1)	2(0)	:0(0)	2(1)	28(5)	·3(2)	19(0)	5(8)
8	7(0)	:2(0)	1(2)	~150(0)	13(2)	3(2)	1(1)	26(2)	2(0)

S

	1	2	3	4	5	6	7	8	9
1	:1(0)	:0(0)	12(~50)	:5(0)	·1(0)	8(0)	1(2)	59(0)	4(2)
2	2(0)	2(1)	7(1)	2(0)	4(0)	31(0)	6(7)	·~200	3(0)
3	5(0)	3(0)	63(2)	3(1)	10(1)	·3(2)	1(1)	7(3)	1(0)
4	6(1)	2(0)	3(0)	2(0)	2(0)	3(0)	6(0)	3(2)	:2(0)
5	5(3)	6(0)	4(3)	3(1)	10(0)	23(0)	14(24)	10(1)	6(1)
6	4(1)	17(0)	·3(4)	7(0)	:8(1)	:1(0)	2(1)	2(0)	5(2)
7	2(0)	1(0)	:6(0)	:3(4)	5(1)	1(1)	·10(1)	3(2)	2(0)
8	·2(0)	5(0)	8(2)	3(1)	3(0)	8(0)	4(1)	6(1)	4(2)

T

	1	2	3	4	5	6	7	8	9
1	20(0)	·0(0)	1(7)	2(3)	·3(1)	9(1)	3(0)	1(2)	5(1)
2	:4(1)	·1(0)	:16(0)	8(0)	2(0)	9(0)	:1(1)	·11(0)	1(1)
3	·7(0)	1(2)	·2(0)	7(0)	3(20)	3(2)	4(0)	:3(0)	:4(2)
4	1(0)	3(0)	·J(~200)	6(2)	:9(1)	3(1)	1(2)	:2(0)	16(2)
5	·16(1)	2(1)	8(3)	5(2)	2(0)	5(2)	·3(0)	4(0)	5(0)
6	3(2)	4(5)	4(0)	·1(0)	2(0)	6(3)	7(0)	3(0)	5(4)
7	1(1)	·14(0)	10(2)	:2(0)	:0(0)	J(~200)	14(0)	0(0)	15(0)
8	:6(15)	:10(0)	8(3)	2(1)	15(0)	:7(0)	9(5)	:3(0)	·2(0)

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

4/30 COUNT CANAUANINE PLATES

K(1x)

	1	2	3	4	5	6	7	8	9
1	2(0)	10(1)	12(2)	3(2)	0(0)	17(0)	8(5)	12(0)	15(1)
2	6(0)	15(2)	9(1)	33(2)	25(0)	4(0)	14(3)	8(0)	5(0)
3	8(1)	9(1)	5(0)	4(4)	3(2)	~~26(5)~~ 17(4)	~~5(5)~~ 26(5)	~~14(1)~~ 5(5)	14(1)
4	21(1)	3(0)	16(1)	24(3)	7(1)	6(2)	17(3)	4(2)	7(4)
5	56(1)	11(2)	2(0)	8(1)	1(0)	9(4)	1(0)	11(1)	5(0)
6	90(5)	3(3)	7(1)	10(1)	3(2)	3(2)	2(0)	11(1)	6(1)
7	58(0)	5(0)	5(2)	4(3)	4(1)	28(3)	8(1)	12(4)	8(1)
8	8(0)	20(6)	5(5)	11(0)	7(1)	9(4)	6(1)	10(2)	2(3)

K

	1	2	3	4	5	6	7	8	9
1	10(0)	6(1)	0(0)	13(4)	31(0)	3(0)	14(0)	1(0)	2(0)
2	5(1)	~~35(0)~~ 7(0)	35(0)	10(2)	1(0)	9(0)	3(1)	6(0)	1(1)
3	0(0)	12(0)	1(0)	34(0)	39(1)	77(1)	6(0)	21(0)	7(0)
4	0(1)	8(1)	3(1)	2(0)	5(1)	28(0)	4(0)	32(0)	12(1)
5	5(0)	4(0)	8(0)	2(0)	20(0)	8(0)	14(0)	6(1)	7(1)
6	1(2)	6(0)	4(0)	10(0)	5(0)	3(0)	0(0)	4(0)	29(0)
7	9(0)	4(0)	136	15(0)	16(1)	2(2)	3(18)	18(0)	3(0)
8	6(0)	5(0)	8(0)	4(0)	2(0)	7(1)	13(0)	5(0)	4(0)

L

	1	2	3	4	5	6	7	8	9
1	4(0)	142(0)	5(0)	2(0)	6(4)	19(0)	8(0)	1(1)	5(0)
2	0(0)	4(1)	9(0)	7(1)	0(0)	10(0)	20(0)	3(0)	2(0)
3	2(0)	3(2)	3(8)	2(0)	8(1)	3(0)	10(0)	1(0)	10(2)
4	3(0)	7(0)	3(0)	2(0)	4(0)	~250	3(1)	8(0)	12(0)
5	2(0)	3(0)	13(3)	49(0)	5(2)	6(1)	9(0)	3(0)	7(2)
6	6(1)	31(0)	4(0)	1(0)	1(1)	11(0)	0(1)	18(0)	3(0)
7	14(0)	1(0)	28(0)	1(0)	15(0)	6(0)	4(0)	5(4)	11(0)
8	10(3)	3(0)	6(0)	2(1)	4(0)	4(1)	~~6(0)~~ 4(1)	~~12(0)~~ 6(0)	12(0)

M

	1	2	3	4	5	6	7	8	9
1	12(1)	2(0)	21(2)	25(0)	6(1)	4(1)	2(0)	2(0)	9(0)
2	13(1)	9(0)	10(0)	39(3)	4(0)	4(0)	6(0)	4(2)	12(0)
3	6(0)	9(0)	4(1)	4(0)	9(0)	0(1)	4(2)	0(0)	2(0)
4	4(4)	40(0)	3(0)	2(0)	7(1)	4(5)	9(0)	7(0)	3(1)
5	3(0)	3(0)	6(0)	0(2)	4(0)	5(0)	24(0)	6(0)	7(0)
6	3(1)	2(1)	9(1)	0(0)	3(0)	12(1)	13(0)	16(0)	3(0)
7	107(0)	5(0)	1(0)	12(0)	2(7)	~~7(4)~~ 5(0)	7(1)	121(0)	7(2)
8	3(1)	16(1)	4(4)	20(0)	9(0)	1(0)	1(1)	1(0)	8(0)

N

	1	2	3	4	5	6	7	8	9
1	92(1)	1(0)	5(0)	11(0)	2(3)	4(0)	10(0)	11(0)	4(1)
2	7(0)	8(1)	3(1)	1(0)	6(0)	25(0)	2(0)	31(0)	8(0)
3	0(22)	3(0)	7(0)	3(1)	8(1)	4(0)	1(0)	3(0)	18(0)
4	4(0)	1(0)	36(2)	12(0)	4(0)	45(0)	6(1)	4(0)	86(0)
5	5(0)	4(0)	4(0)	9(0)	3(0)	3(0)	1(0)	5(0)	4(1)
6	5(1)	33(0)	1(0)	5(1)	4(0)	4(0)	15(0)	20(0)	4(0)
7	17(1)	13(0)	3(0)	58(0)	3(2)	20(1)	6(1)	7(5)	4(1)
8	3(0)	8(0)	2(0)	0(0)	6(0)	12(0)	6(0)	5(0)	0(0)

O

	1	2	3	4	5	6	7	8	9
1	14(0)	42(0)	1(0)	36(8)	4(0)	7(0)	2(1)	8(0)	6(1)
2	5(0)	1(1)	9(4)	17(1)	3(0)	15(0)	2(0)	3(0)	2(0)
3	28(2)	4(5)	2(0)	5(0)	3(0)	2(0)	4(0)	16(2)	2(17)
4	4(0)	10(0)	3(1)	4(4)	6(0)	5(0)	3(3)	9(0)	49(19)
5	5(0)	17(0)	19(0)	6(0)	16(0)	5(0)	14(0)	13(1)	5(0)
6	3(0)	29(0)	37(0)	5(1)	3(1)	~150	7(0)	3(0)	11(0)
7	7(0)	2(0)	0(0)	1(1)	2(1)	0(1)	1(0)	2(0)	11(0)
8	5(1)	1(1)	5(0)	8(1)	1(0)	23(0)	9(0)	11(0)	16(0)

P

	1	2	3	4	5	6	7	8	9
1	6(1)	5(2)	6(0)	13(0)	9(1)	4(0)	2(0)	4(0)	7(0)
2	8(0)	5(0)	8(1)	51(0)	29(2)	4(0)	1(0)	3(0)	6(0)
3	0(1)	1(0)	3(0)	0(0)	24(0)	1(0)	14(0)	4(0)	40(0)
4	18(0)	5(0)	4(0)	2(0)	1(2)	7(0)	4(0)	1(0)	5(1)
5	11(0)	4(0)	9(2)	8(2)	2(6)	8(0)	30(1)	51(0)	4(1)
6	23(0)	3(1)	2(0)	41(1)	2(0)	0(0)	18(0)	2(0)	10(0)
7	4(0)	17(0)	15(0)	0(3)	2(0)	4(0)	17(1)	20(0)	5(0)
8	2(0)	0(7)	19(1)	7(0)	11(2)	4(0)	2(0)	3(0)	13(0)

Q

	1	2	3	4	5	6	7	8	9
1	24(0)	0(0)	5(1)	3(0)	0(1)	1(3)	0(0)	2(0)	6(1)
2	5(0)	16(0)	20(6)	56(0)	32(8)	0(0)	3(0)	14(1)	49(0)
3	8(0)	3(0)	3(0)	1(0)	8(1)	8(1)	1(0)	5(0)	13(0)
4	4(0)	3(1)	10(59)	10(1)	1(2)	2(0)	25(0)	2(0)	2(1)
5	1(4)	60(2)	7(1)	9(0)	6(1)	13(0)	7(2)	4(0)	6(1)
6	0(0)	3(0)	10(0)	2(0)	5(0)	8(1)	20(6)	62(0)	8(13)
7	3(0)	5(0)	9(0)	6(0)	1(1)	24(0)	2(1)	12(0)	10(0)
8	0(0)	8(1)	13(0)	8(0)	14(0)	7(0)	6(2)	5(0)	2(0)

	1	2	3	4	5	6	7	8	9
1	5(0)	5(0)	26(7)	5(0)	5(1)	4(0)	2(0)	1(0)	2(0)
2	6(0)	4(1)	0(0)	7(1)	7(1)	5(0)	59(1)	59(1) 23(1)	23(1)
3									
4									
5									
6									
7									
8									

R

	1	2	3	4	5	6	7	8	9
1	5(0)	5(0)	26(7)	5(0)	5(1)	4(0)	2(0)	1(0)	2(0)
2	6(0)	4(0)	0(0)	7(1)	7(1)	13(0)	5(0)	59(1)	23(1)
3	13(0)	11(0)	8(0)	10(0)	7(1)	1(0)	3(0)	14(2)	2(0)
4	22(1)	4(0)	2(1)	0(0)	5(0)	9(0)	19(0)	2(0)	5(1)
5	9(0)	1(0)	2(2)	5(0)	11(0)	19(4)	~200	14(0)	7(0)
6	2(0)	1(1)	0(0)	6(0)	3(0)	~250	26(3)	66(1)	1(1)
7	4(3)	1(0)	3(0)	14(0)	23(34)	15(5)	6(2)	14(0)	9(0)
8	3(0)	2(0)	8(0)	13(0)	5(0)	0(0)	~200	5(0)	25(2)

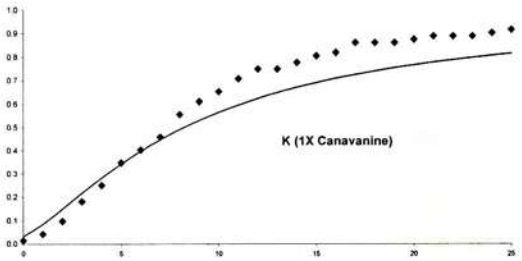
S

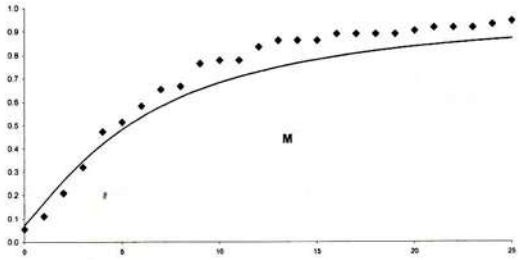
	1	2	3	4	5	6	7	8	9
1	~~2(0)~~ 3(0)	~~1(0)~~ 4(0)	8(0)	6(0)	3(0)	0(1)	8(0)	5(9)	15(0)
2	3(0)	7(1)	2(0)	0(4)	3(0)	3(0)	6(1)	25(0)	8(1)
3	11(0)	12(0)	6(0)	2(0)	3(0)	11(1)	4(1)	8(14)	5(0)
4	0(2)	0(2)	5(0)	9(0)	10(3)	6(3)	6(9)	12(0)	~500
5	15(0)	0(0)	3(0)	2(0)	7(34)	19(1)	~200	2(0)	5(0)
6	5(0)	11(0)	~~2(0) 49(0)~~	~~49(0) 1(0)~~	~~1(0) 4(0)~~	~~4(0) 16(0)~~	16(0)	~~6(1~~ 11(0)	6(0)
7	11(0)	4(0)	2(0)	1(0)	1(1)	18(1)	13(1)	5(0)	1(0)
8	~400	11(1)	5(0)	25(0)	5(1)	4(2)	2(4)	1(1)	94(0)

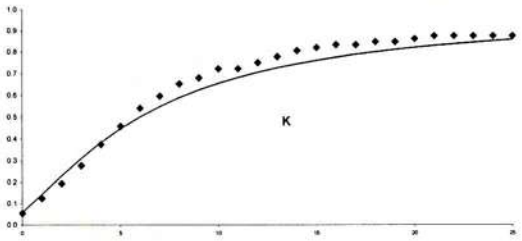
T

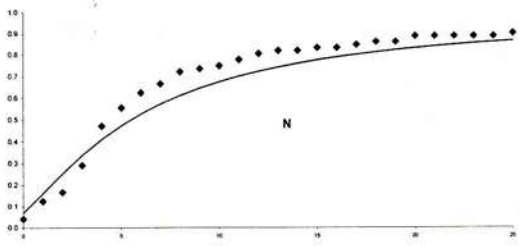
	1	2	3	4	5	6	7	8	9
1	2(0)	1(0)	13(0)	2(1)	3(1)	1(1)	1(1)	6(0)	0(1)
2	1(2)	4(1)	7(0)	2(0)	9(0)	4(0)	32(0)	12(0)	15(3)
3	1(0)	0(1)	16(0)	4(0)	8(0)	3(1)	3(1)	0(2)	2(0)
4	8(0)	10(1)	4(0)	0(0)	7(1)	9(0)	6(1)	43(0)	~500
5	3(0)	9(0)	8(0)	14(0)	5(0)	14(0)	5(0)	8(0)	16(0)
6	5(0)	11(0)	10(1)	11(0)	10(1)	3(0)	16(0)	3(1)	8(0)
7	7(2)	6(0)	8(0)	1(0)	2(0)	12(0)	3(2)	5(0)	4(0)
8	3(0)	3(0)	10(0)	3(0)	0(0)	22(0)	6(1)	6(1)	9(2)

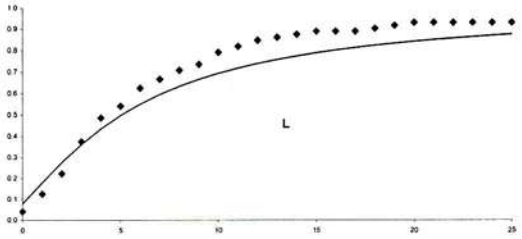
5/2 QUALITY OF DATA FOR CANAVANINE PLATES

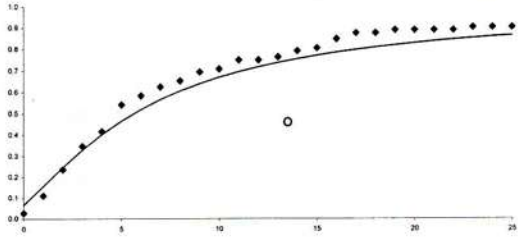


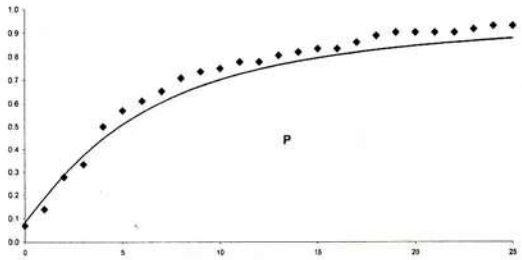


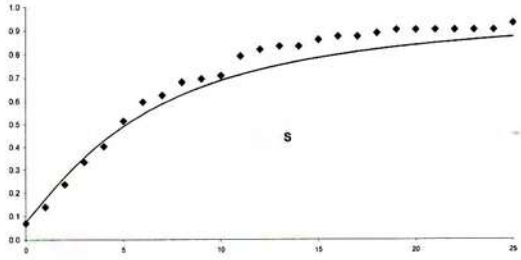


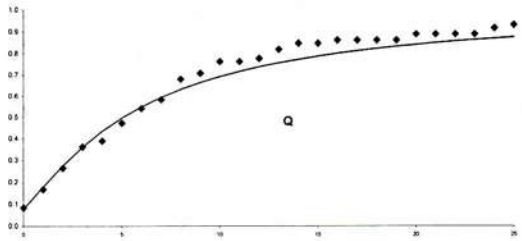


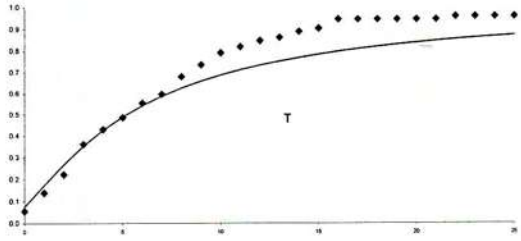


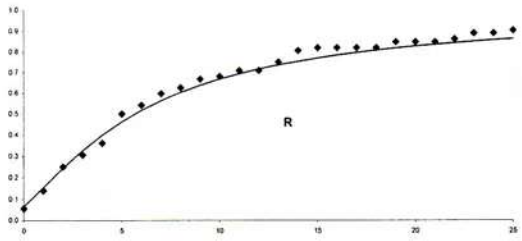


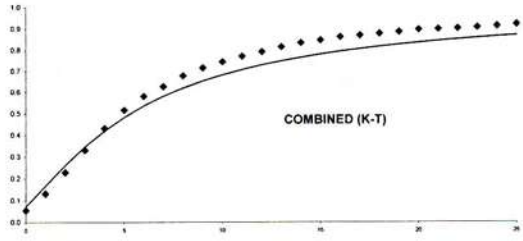




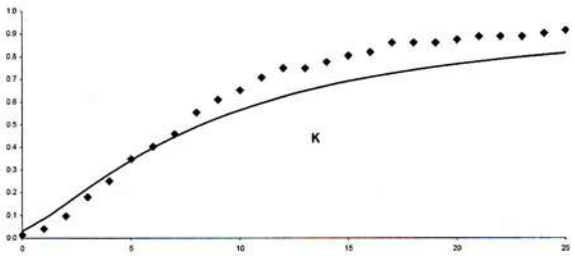


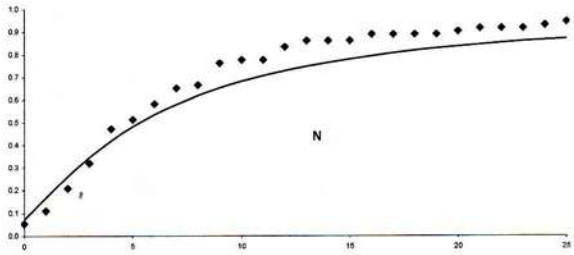


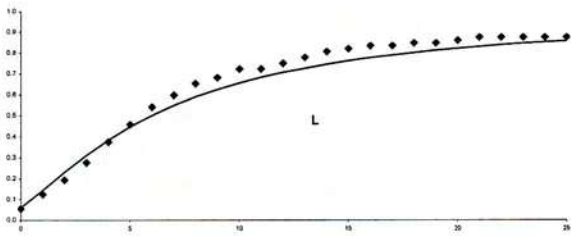


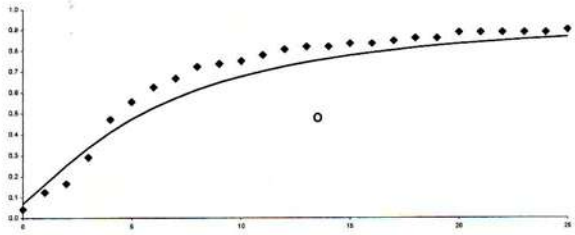


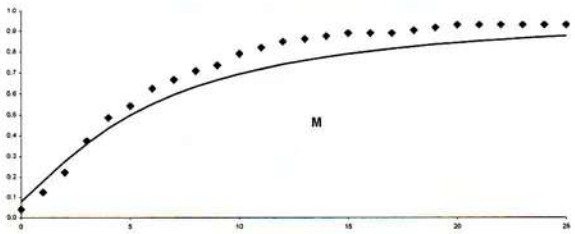
5/2 QUALITY OF DATA FOR αF PLATES

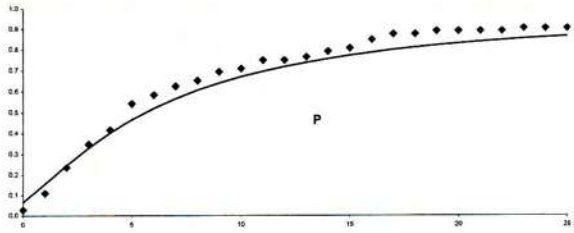


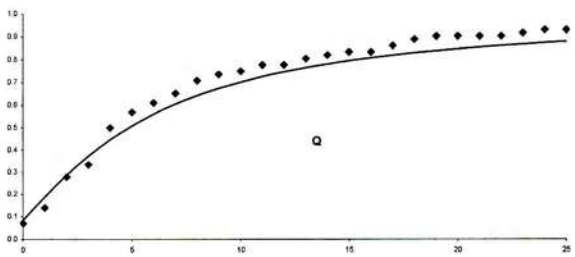


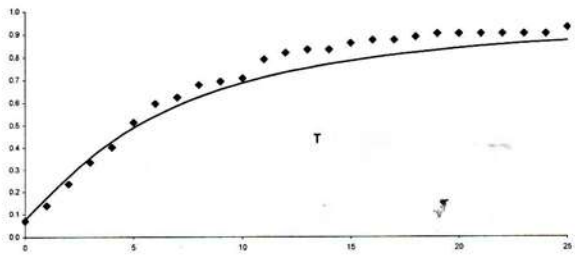


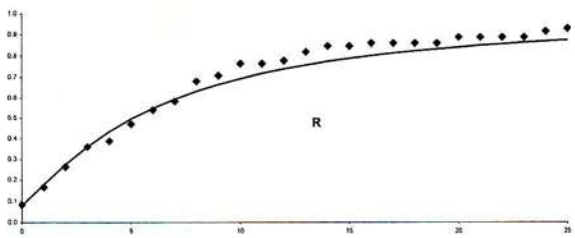


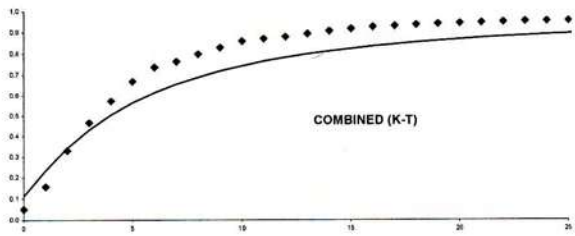


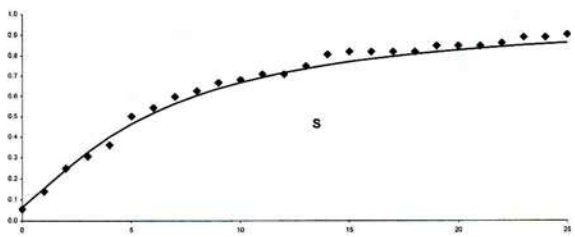












5/1 RECOUNTING SELECT CANAVANINE FLUCT. ASSAYS

K(1x)

	1	2	3	4	5	6	7	8	9
1	5(2)	12(3)	14(3)	5(5)	1(6)	18(1)	17(4)	14(3)	19(0)
2	9(5)	18(3)	11(1)	36(2)	27(2)	7(3)	18(1)	11(3)	5(0)
3	11(2)	9(2)	8(3)	9(4)	7(6)	25(5)	28(4)	12(5)	17(1)
4	25(4)	6(2)	19(2)	31(1)	11(3)	9(5)	24(2)	7(1)	13(6)
5	56(7)	16(4)	3(3)	13(1)	2(2)	17(2)	3(2)	12(3)	9(2)
6	89(6)	9(3)	10(4)	13(4)	7(3)	6(2)	5(5)	14(3)	13(0)
7	63(2)	7(0)	10(1)	8(4)	6(3)	31(2)	12(2)	19(6)	11(4)
8	10(1)	31(8)	10(8)	15(5)	12(2)	13(6)	9(2)	15(3)	7(4)

~~K~~M

	1	2	3	4	5	6	7	8	9
1	~~10(2)~~ 13(1)	2(2)	22(7)	24(1)	7(1)	5(29)	2(7)	2(2)	10(1)
2	15(2)	10(1)	10(3)	43(1)	5(0)	4(2)	6(0)	7(3)	13(2)
3	7(0)	9(4)	7(0)	4(2)	9(3)	1(2)	5(1)	1(3)	2(4)
4	8(3)	39(4)	4(1)	2(0)	8(1)	9(3)	9(0)	7(1)	6(1)
5	3(0)	3(1)	6(4)	2(2)	4(0)	7(2)	24(0)	6(3)	8(2)
6	4(4)	3(1)	9(0)	0(3)	3(0)	13(7)	14(0)	16(0)	3(1)
7	103(0)	4(3)	1(1)	12(3)	10(2)	6(3)	7(6)	117(2)	9(2)
8	4(0)	16(3)	8(0)	20(1)	9(1)	1(0)	2(1)	1(0)	9(1)

~~N~~

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

~~T~~

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

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

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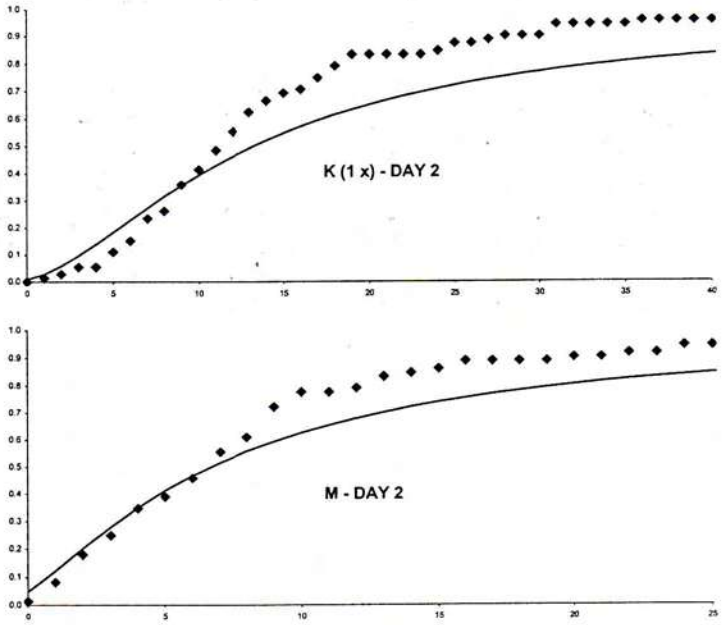
4									
5									
6									
7									
8									

3/2 QUALITY OF DATA FOR RECOUNTED FLUCT. ASSAYS



5/3

COUNTING SFOA PLATES

K

	1	2	3	4	5	6
1	2	9	1	13	6	20
2	3(5)	1	22	40	2	2
3	0	4	0	1	1	0
4	36	1	1	0	0	2
5	12	8	13	1	3	0
6	0	19	7(1)	0	1	13(1)
7	1	3	0	12	3	19
8	48	7	2	1	8	2
9	3	6	0	15	3	0
10	0	0	0	2	9	0
11	1	0	0	4	8	1
12	0	2	0	4	1	0

L

	1	2	3	4	5	6
1	0	0	0	6	1	0
2	0	1	1	1(1)	0	0
3	1	7	0(1)	1	17	2
4	13	0	0	0	~~45~~ 7	45
5	4	0	8	2	0	0
6	6	0	11	14	0	1
7	172	6	0	0	0	10
8	5	0	8	1	7	27
9	1	0	0	0	4	8
10	9	2	0	1(2)	19	0
11	10	3	14	13	4	0
12	0	9	7	0	12	0

M

	1	2	3	4	5	6
1	0	8	5	62	0	~200
2	0	1	1	25	1	5
3	0	0	7	1	24	0
4	6	1	10	0	0	15(1)
5	1	3(2)	0	7	0	11
6	2(1)	2	2(1)	0	10	9
7	14	16	1	8	0	9
8	0	3	1	55	266	0
9	0	8(1)	26	1	1	2
10	1	6(1)	1	5	1(1)	3
11	~~156(1) +~~	~~6(~~ 2	0	0	3	9
12	2	4	3(1)	20	0	7

N

	1	2	3	4	5	6
1	2	0	0	0	0	1
2	~~18~~ 0	18	3	0	12(9)	2
3	2	2	0	1	3	0
4	0	0	0	19	0	0
5	9	1	6	5(1)	6	1
6	5	0	0(3)	1	2	0
7	39	1	0	20	4	21
8	4	1	15	5	0	0
9	0	0	0	28	1	1
10	1	1	5	6	2	5
11	2	1	0	1(1)	18	2
12	0	1	0	1	9	0

O

	1	2	3	4	5	6
1	1	10(1)	0	1	1	0
2	~~1 6 (3)~~	6(3)	8	0	0	0
3	2	2	0	0	6	2
4	6	0	0	0	0	11
5	0	0	0	45	0	22
6	13	1	0	0	10	26
7	10	0	3	1	37	0
8	1	1	1	0	45	0
9	14	0	1	1	3	0
10	0	~175	3(1)	0	5	0
11	0	1	2	0	0	5
12	0	0	89	2	2	28

P

	1	2	3	4	5	6
1	5(1)	1	0	0	0	49
2	3	16(11)	1	8	0	7
3	25	19	15	0	4	0
4	0	9	14	1	6	0
5	12	22	3	0(1)	0	88
6	0	6	0	13	4	3
7	~~13~~ 100	~~100~~ 0	8	3(1)	21(21)	22
8	27	7	20(7)	0	8	0
9	0	3	11	8	1	0
10	0	14	0	2	0	3
11	3	12	157	17	0	0
12	1(1)	0	6(1)	0	0	21

Q

	1	2	3	4	5	6
1	1	0	22	127	1(1)	1
2	3	0	0	0	4	1
3	5	2(2)	2	20(1)	0	12
4	2	0	0	0	0	3
5	44	0	7	0	~200	10
6	0	2	1(1)	0(2)	0	4
7	4	5	11	9	4	12
8	6	0	0	1(1)	1	1
9	2	0	0	0	0	1
10	9	0	8	0	0	0
11	16	0	1	3	2	3
12	2	6	0	0	2	35

R

	1	2	3	4	5	6
1	0	1	0	12(1)	0(3)	0
2	1	2	0(1)	0	8	9(1)
3	9	2	0	0	0	0
4	0(5)	4	6(1)	2	13	0
5	2	2(1)	~150 E	0	7	3(1)
6	5	1	1	2	0	12
7	6	9	0	5	0	0
8	5(1)	0	0	3	3	2
9	2	0	0	4(2)	7	4(4)
10	1	3	6(1)	0	17	0
11	0	6	23	2	0	0
12	0(1)	0	0	5(3)	2	7(5)

S

	1	2	3	4	5	6
1	0	2(1)	6(4)	17(1)	14	0
2	0(1)	~150 E	1	1	1	3
3	2	4	7	0	12	87
4	4(1)	28	1	7(1)	2(1)	2
5	18	8	1	5(1)	0	0
6	0	48	31(4)	~500	54	10
7	0(1)	0	0	0	27(2)	4(2)
8	84	5	1	2	0	0
9	9	3	1	0	2	8
10	0(1)	4	0	2	1	0
11	0	0	11	4	0	0
12	2	10(1)	1	9	7	1

T

	1	2	3	4	5	6
1	0	12	0	0	0	18
2	0	1	0	0	0	0
3	5(3)	0	2(1)	0	0	3
4	0	4	0	0	4	5
5	0	0	0	0	0	0
6	69(1)	0(2)	9	83	3	0
7	6	2	3(3)	0	5	1
8	1	4	2	0	0	0
9	0	5(1)	0	0	0	9
10	2	0(1)	1	0	0	0
11	2	0	16	0	0	5
12	0	1	12	0	0	0

	1	2	3	4	5	6
1						
2						
3						
4						
5						
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7						
8						
9						
10						
11						
12						

	1	2	3	4	5	6
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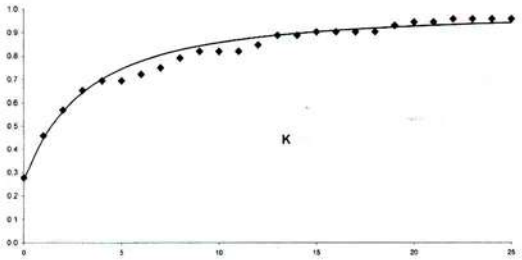
12						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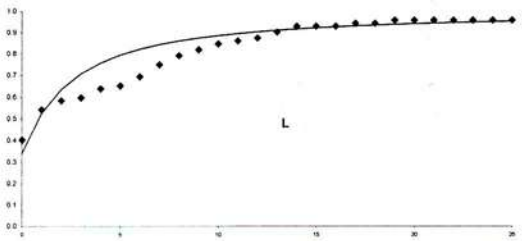
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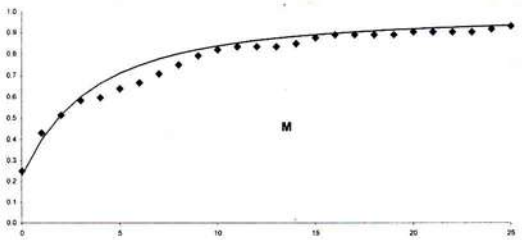
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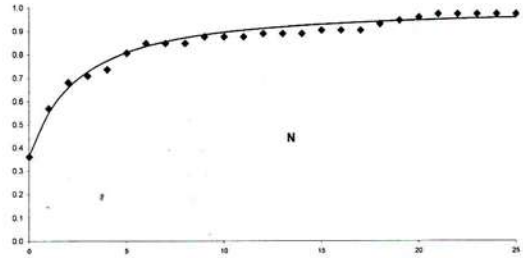
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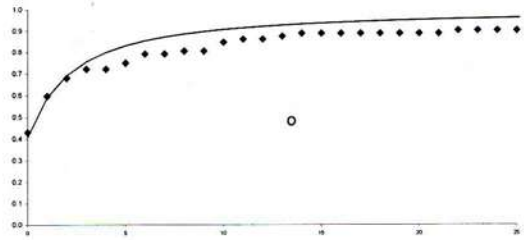
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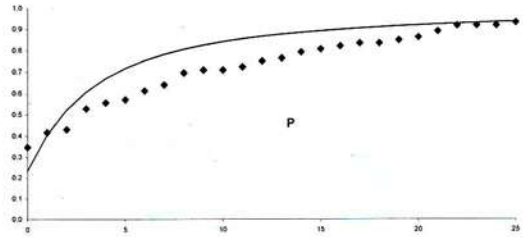
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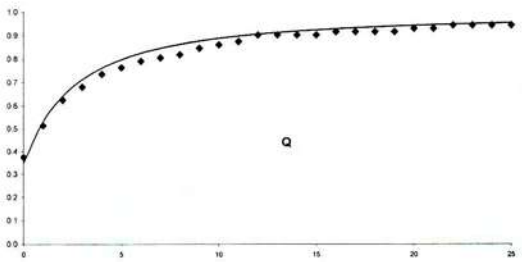
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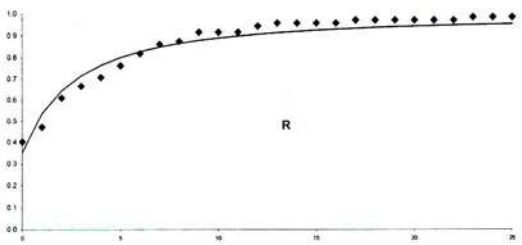
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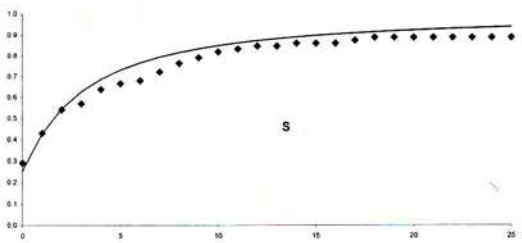
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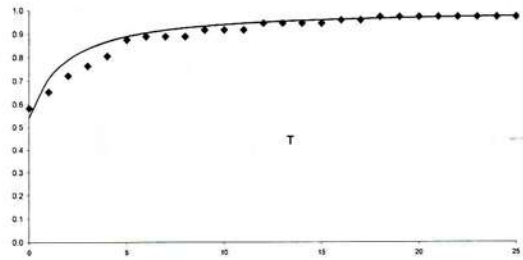
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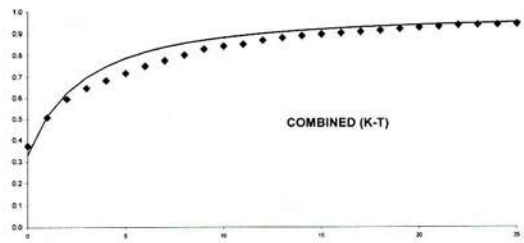

COMBINED (K-T)

4/29

NOTES ON PLATE COUNTING

CONTAMINATION OF αF PLATES

I noticed the day after plating that there was a white contaminant on many of the αF plates (see p. 36)

- Bacteria not confined to spots
- Swarms – moves better w/in spots (more surface tension)
- Not on plates left @ RT w/o filters, but on plates where filters were put on but not removed the bacteria are confluent – and the odor is gagging.
  → Therefore the white contaminant is from the filters – I did use a new box of filters but I don't remember when I opened them
  → perhaps in the future I will autoclave the filters (if possible)
- For the most part I could count around the contamination but it made counting more difficult. I marked which spots were contaminated and how I believe it effected my ability to count colonies.
- Many yeast colonies were visible poking through a bkg of white contaminating bacteria – to see if these were really yeast I picked one and looked at it under the microscope and sure enough it was a yeast colony – surprisingly I didn't see any bacteria come up when I picked the colony.
- There were two other contaminants which didn't effect counts because they were not in any spot – 1 occurrence at each
  – The "yellow" bacteria which I saw before (see p 31) were on plate P4
  – A very "glossy" colony was on T4
- All bacterial contaminants were rods

YEAST		∅ ~5 μm
"Yellow"		~1-3 μm
"White"		~10-15 μm – many connected head-to-tail
"Glossy"		~5 μm – single rods ~~about~~ t length about equal to the diameter of a yeast cell.

5/2

NOTES ON PLATE COUNTING CONT.

RECOUNTING CANAVANINE PLATES (p. 51)

- All of the canavanine plates were counted on Sunday (4/30); however, I recounted two sets (K 1x & M) on Monday (5/1). I noticed on sunday that there was much more colony size variability on the 1x Can plate than on the others — I expected the number of new *positively scored colonies to be greater on the 1x plates. I recounted the K 1x ~~pt~~ set and I chose to recount "M" because I had already picked colonies off of K and L plates.

THRESHOLD COUNTING

When I suspect that post plating growth is occurring on my plates I will use threshold counting when counting my fluct. assays.

PLATES	THRESHOLD	
αF	3 mm at	6x
Canavanine (4/30)	bigger than the #"6" @	10x
Canavanine (5/1)	"	@ 7x
5FOA (5/3)	"	@ 6x

5/4

COUNTING 5FOA PLATES

- As expected counting the 5FOA plates was easy because there were fewer colonies per spot and most colonies were large and uniformly sized. I
- I did use threshold counting for these plates (see above)
- There were two cases where I saw edge effects — where a gradient of colony size exists such that larger colonies are on the outside and small colonies in the center — this is presumably due to depletion ~~on~~ of uracil.

↳ these spots are marked with an "E" (5FOA^R-R-5-3 & 5FOA^R-S-2-2)

5/3

NOTES ON PLATE COUNTING CONT.

- There are ~~two~~ three explanations for small colonies:

(1) The mutations occurred post plating

(2) The mutants carry an additional mutation which results in loss of fitness

(3) They represent ~~a~~ mutations which allow weak growth on the selective media

3/6 SUMMARY OF FLUCTUATION ASSAYS

m

	alphaF	10 x Can	1 x Can (D2)	5FOA
K	1.97	2.82	4.80	1.31
L	2.00	2.54	--	1.07
M	2.24	2.63	--	1.47
N	2.42	2.68	--	1.01
O	2.07	2.72	--	0.90
P	2.30	2.48	--	1.45
Q	2.13	2.55	--	1.06
R	2.28	2.74	--	1.04
S	2.31	2.58	--	1.37
T	2.23	2.59	--	0.61
AVERAGE	2.20	2.63	--	1.13
COMBINED K-T (median)	2.19	2.63	--	1.11

Cells per culture

	alphaF	10 x Can	1 x Can	5FOA
K	3.57E+05	1.36E+07	1.29E+07	2.02E+07
L	3.62E+05	1.40E+07	--	2.25E+07
M	3.57E+05	1.19E+07	--	2.05E+07
N	3.68E+05	1.42E+07	--	1.98E+07
O	3.71E+05	1.32E+07	--	2.01E+07
P	3.78E+05	1.33E+07	--	2.17E+07
Q	3.98E+05	1.45E+07	--	2.23E+07
R	3.77E+05	1.33E+07	--	2.08E+07
S	3.84E+05	1.45E+07	--	1.95E+07
T	4.06E+05	1.24E+07	--	2.00E+07
AVERAGE	3.76E+05	1.35E+07	--	2.07E+07
COMBINED K-T (median)	3.74E+05	1.35E+07	--	2.04E+07

Mutation rate

	alphaF	10 x Can	1 x Can	5FOA
K	5.51E-06	2.08E-07	3.71E-07	6.49E-08
L	5.51E-06	1.81E-07	--	4.77E-08
M	6.28E-06	2.21E-07	--	7.19E-08
N	6.58E-06	1.88E-07	--	5.08E-08
O	5.60E-06	2.06E-07	--	4.48E-08
P	6.07E-06	1.87E-07	--	6.70E-08
Q	5.35E-06	1.76E-07	--	4.74E-08
R	6.05E-06	2.05E-07	--	5.01E-08
S	6.00E-06	1.79E-07	--	7.03E-08
T	5.50E-06	2.09E-07	--	3.05E-08
AVERAGE	5.85E-06	1.96E-07	--	5.45E-08
COMBINED K-T (median)	5.86E-06	1.95E-07	--	5.43E-08

αF^R

CULTURE	Z-score	# Greater
K	2.27	100
L	1.76	438
M	1.64	544
N	2.16	138
O	2.40	63
P	1.82	348
Q	1.30	1075
R	2.19	138
S	2.21	125
T	1.86	330
K-T	4.66	0

Can^R

K	-0.05	5105
L	1.44	838
M	1.35	975
N	1.02	1643
O	0.39	3492
P	0.38	3573
Q	0.14	4386
R	-0.61	7156
S	0.68	2537
T	1.88	313
K-T	3.39	0
K 1 x	1.85	351
K 1 x (2)		
M (2)		

5FOA^R

K	-0.60	7137
L	1.60	561
M	0.09	4624
N	-1.17	8759
O	1.23	1189
P	2.40	49
Q	-0.72	7581
R	0.09	4662
S	0.38	3557
T	0.58	2894
K-T	3.15	1

5/27

αF

	Z-score	# Greater
K	2.2592	101.0000
L	1.7380	430.0000
M	1.6113	582.0000
N	2.1715	116.0000
O	2.3748	69.0000
P	1.8207	339.0000
Q	1.3250	953.0000
R	2.1981	130.0000
S	2.2422	96.0000
T	1.8834	304.0000

5/1

PICKING CAN^R COLONIES FOR SEQUENCING

Number	Srain	Plate	Spot	Notes
500	K	1	1	
501	K	1	2	
502	K	1	4	
503	K	2	1	
504	K	2	2	
505	K	2	3	
506	K	3	2	
507	K	3	3	
508	K	3	4	
509	K	4	2	
510	K	4	3	
511	K	4	4	
512	K	5	1	
513	K	5	2	
514	K	5	3	
515	K	6	1	
516	K	6	2	
517	K	6	3	
518	K	7	1	
519	K	7	2	
520	K	7	3	
521	K	8	1	
522	K	8	2	
523	K	8	3	
524	L	1	1	
525	L	1	2	
526	L	1	3	
527	L	2	2	
528	L	2	3	
529	L	2	4	
530	L	3	1	
531	L	3	2	
532	L	3	3	
533	L	4	1	
534	L	4	2	
535	L	4	3	
536	L	5	1	
537	L	5	2	
538	L	5	3	
539	L	6	1	
540	L	6	2	
541	L	6	3	
542	L	7	1	
543	L	7	2	
544	L	7	3	
545	L	8	1	
546	L	8	2	
547	L	8	3	
548	M	1	1	
549	M	1	2	
550	M	1	3	
551	M	2	1	
552	M	2	2	
553	M	2	3	

5/2

5/2

554	M	3	1	
555	M	3	2	
556	M	3	3	
557	M	4	1	
558	M	4	2	
559	M	4	3	
560	M	5	1	
561	M	5	2	
562	M	5	3	
563	M	6	1	
564	M	6	2	
565	M	6	3	
566	M	7	1	edge
567	M	7	2	
568	M	7	3	
569	M	8	1	
570	M	8	2	
571	M	8	3	
572	N	1	1	edge
573	N	1	2	
574	N	1	3	
575	N	2	1	
576	N	2	2	
577	N	2	3	
578	N	3	2	
579	N	3	3	
580	N	3	4	
581	N	4	1	
582	N	4	2	
583	N	4	3	
584	N	5	1	
585	N	5	2	
586	N	5	3	
587	N	6	1	
588	N	6	2	
589	N	6	3	
590	N	7	1	
591	N	7	2	
592	N	7	3	
593	N	8	1	
594	N	8	2	
595	N	8	3	
596	O	1	1	
597	O	1	2	
598	O	1	3	
599	O	2	1	
600	O	2	2	
601	O	2	3	
602	O	3	1	
603	O	3	2	
604	O	3	3	
605	O	4	1	
606	O	4	2	
607	O	4	3	
608	O	5	1	

5/2

609	O	5	2	
610	O	5	3	edge
611	O	6	1	
612	O	6	2	edge
613	O	6	3	
614	O	7	1	
615	O	7	2	
616	O	7	4	
617	O	8	1	
618	O	8	2	
619	O	8	3	
620	P	1	1	
621	P	1	2	
622	P	1	3	
623	P	2	1	
624	P	2	2	
625	P	2	3	
626	P	3	1 2	
627	P	3	2 3	
628	P	3	5	
629	P	4	1	
630	P	4	2	
631	P	4	3	
632	P	5	1	
633	P	5	2	
634	P	5	3	
635	P	6	1	
636	P	6	2	
637	P	6	3	
638	P	7	1	
639	P	7	2	
640	P	7	3	
641	P	8	1	
642	P	8	3	
643	P	8	4	
644	Q	1	1	
645	Q	1	3	
646	Q	1	4	
647	Q	2	1	
648	Q	2	2	
649	Q	2	3	
650	Q	3	1	
651	Q	3	2	
652	Q	3	3	
653	Q	4	1	
654	Q	4	2	
655	Q	4	3	edge
656	Q	5	1	
657	Q	5	2	edge
658	Q	5	3	
659	Q	6	2	
660	Q	6	3	
661	Q	6	4	
662	Q	7	1	
663	Q	7	2	

5/2

664	Q	7	3	
665	Q	8	2	
666	Q	8	3	
667	Q	8	4	
668	R	1	1	edge
669	R	1	2	
670	R	1	3	edge
671	R	2	1	
672	R	2	2	
673	R	2	4	
674	R	3	1	
675	R	3	2	
676	R	3	3	
677	R	4	1	
678	R	4	2	
679	R	4	#3	
680	R	5	1	
681	R	5	2	
682	R	5	3	
683	R	6	1	
684	R	6	2	
685	R	6	4	
686	R	7	1	
687	R	7	2	
688	R	7	3	
689	R	8	1	
690	R	8	2	
691	R	8	3	
692	S	1	1	
693	S	1	2	
694	S	1	3	
695	S	2	1	
696	S	2	2	
697	S	2	3	
698	S	3	1	
699	S	3	2	
700	S	3	3	
701	S	4	3	
702	S	4	4	
703	S	4	5	
704	S	5	1	
705	S	5	3	
706	S	5	4	
707	S	6	1	
708	S	6	2	
709	S	6	3	
710	S	7	1	
711	S	7	2	
712	S	7	3	
713	S	8	2	
714	S	8	3	
715	S	8	4	
716	T	1	1	
717	T	1	2	
718	T	1	3	

5/2

719	T	2	1	
720	T	2	2	
721	T	2	3	
722	T	3	1	
723	T	3	3	
724	T	3	4	
725	T	4	1	
726	T	4	2	
727	T	4	3	
728	T	5	1	
729	T	5	2	
730	T	5	3	
731	T	6	1	
732	T	6	2	
733	T	6	3	
734	T	7	1	
735	T	7	2	
736	T	7	3	
737	T	8	1	
738	T	8	2	
739	T	8	3	

5/4 PICKING ~~EOLA~~ SFOA^R COLONIES FOR SEQUENCING

Number	Srain	Plate	Spot	Notes
801	K	1	1	
802	K	1	2	
803	K	2	1	
804	K	2	2	
805	K	3	2	
806	K	3	4	
807	K	4	1	edge
808	K	4	2	
809	K	5	1	
810	K	5	2	
811	K	6	2	
812	K	6	3	
813	K	7	1	
814	K	7	2	
815	K	8	1	
816	K	8	2	
817	K	9	1	
818	K	9	2	
819	K	10	4	
820	K	10	5	
821	K	11	1	
822	K	11	4	
823	K	12	2	
824	K	12	4	
825	L	1	4	
826	L	1	5	
827	L	2	2	
828	L	2	3	
829	L	3	1	
830	L	3	2	
831	L	4	1	
832	L	4	5	
833	L	5	1	
834	L	5	3	dilute
835	L	6	1	
836	L	6	3	edge
837	L	7	1	edge
838	L	7	2	
839	L	8	1	
840	L	8	3	
841	L	9	1	
842	L	9	5	
843	L	10	1	dilute
844	L	10	2	
845	L	11	1	
846	L	11	2	
847	L	12	2	
848	L	12	3	
849	M	1	2	
850	M	1	3	
851	M	2	2	
852	M	2	3	
853	M	3	3	
854	M	3	4	

855	M	4	1	
856	M	4	2	
857	M	5	1	
858	M	5	2	dilute
859	M	6	1	
860	M	6	2	
861	M	7	1	
862	M	7	2	
863	M	8	2	
864	M	8	3	
865	M	9	2	
866	M	9	3	
867	M	10	1	
868	M	10	2	
869	M	11	1	edge
870	M	11	2	
871	M	12	2	
872	M	12	3	
873	N	1	1	
874	N	1	6	
875	N	2	2	
876	N	2	3	
877	N	3	1	
878	N	3	2	
879	N	4	4	
880	N	~~4~~ 5	1	
881	N	5	2	
882	N	5	3	
883	N	6	1	
884	N	6	4	
885	N	7	1	
886	N	7	2	
887	N	8	1	
888	N	8	2	
889	N	9	4	edge
890	N	9	5	
891	N	10	1	
892	N	10	2	
893	N	11	1	
894	N	11	2	
895	N	12	2	
896	N	12	4	
897	O	1	1	
898	O	1	2	edge
899	O	2	1	
900	O	2	2	
901	O	3	1	
902	O	3	2	
903	O	4	1	
904	O	4	6	
905	O	5	4	edge
906	O	5	6	edge
907	O	6	1	
908	O	6	2	s
909	O	7	1	

910	O	7	3	
911	O	8	1	
912	O	8	2	
913	O	9	1	
914	O	9	3	s
915	O	10	2	edge
916	O	10	3	s
917	O	11	2	
918	O	11	3	
919	O	12	3	edge
920	O	12	4	
921	P	1	1	
922	P	1	2	
923	P	2	1	s dilute
924	P	2	2	
925	P	3	1	
926	P	3	2	
927	P	4	2	
928	P	4	3	edge
929	P	5	1	s
930	P	5	2	
931	P	6	2	dilute
932	P	6	4	
933	P	7	1	edge
934	P	7	3	small
935	P	8	1	edge
936	P	8	2	dilute
937	P	9	2	small
938	P	9	3	small
939	P	10	2	
940	P	10	4	
941	P	11	1	
942	P	11	2	
943	P	12	1	
944	P	12	3	
945	Q	1	1	
946	Q	1	3	
947	Q	2	1	
948	Q	2	5	
949	Q	3	1	
950	Q	3	2	
951	Q	4	1	small
952	Q	4	6	
953	Q	5	1	edge
954	Q	5	3	small
955	Q	6	2	
956	Q	6	3	small dilute
957	Q	7	1	
958	Q	7	2	
959	Q	8	1	
960	Q	8	4	small dilute
961	Q	9	1	
962	Q	9	6	
963	Q	10	1	
964	Q	10	3	small

965	Q	11	1	
966	Q	11	3	
967	Q	12	1	
968	Q	12	2	
969	R	1	2	small
970	R	1	4	edge
971	R	2	1	small
972	R	2	2	
973	R	3	1	
974	R	3	2	
975	R	4	2	small
976	R	4	3	small
977	R	5	1	small
978	R	5	2	
979	R	6	1	small
980	R	6	2	small
981	R	7	1	
982	R	7	2	
983	R	8	1	small
984	R	8	4	
985	R	9	1	
986	R	9	4	
987	R	10	1 2	~~small~~
988	R	10	2 3	
989	R	11	2	
990	R	11	3	edge
991	R	12	4	
992	R	12	5	
993	S	1	2	
994	S	1	3	small
995	S	2	2	edge
996	S	2	3	
997	S	3	1	
998	S	3	2	
999	S	4	1	small dilute
1000	S	4	2	edge
1001	S	5	1	edge
1002	S	5	2	
1003	S	6	2	edge
1004	S	6	3	edge
1005	S	7	5	edge
1006	S	7	6	small dilute
1007	S	8	1	edge
1008	S	8	2	
1009	S	9	1	
1010	S	9	2	small
1011	S	10	2	
1012	S	10	4	
1013	S	11	3	small dilute
1014	S	11	4	small
1015	S	12	1	
1016	S	12	2	
1017	T	1	2	edge
1018	T	1	6	
1019	T	2	2	

1020	T	~~23~~	1	
1021	T	3	3	small
1022	T	3	6	
1023	T	4	2	
1024	T	4	5	
1025	T	~~84~~	6	
1026	T	~~86~~	1	edge
1027	T	6	3	
1028	T	6	4	edge
1029	T	7	1	
1030	T	7	2	
1031	T	8	1	small
1032	T	8	2	
1033	T	9	2	
1034	T	9	6	
1035	T	10	1	small dirty
1036	T	10	3	.
1037	T	11	1	
1038	T	11	3	edge
1039	T	12	2	
1040	T	12	3	

5/4

NOTES ON COLONY PICKING

- I know from past work that bkg cells from a SFOA plate will not regrow when inoculated into YPD. I do not know that this is true for canavanine.
- On Monday (5/1) I picked ~~canav~~ Can^R colonies off of K and L plates (see p. 61) and inoculated 1 mL of YPD. I also took two large streaks of bkg cells and inoculated 1 mL YPD.
  - ↳ on tuesday morning all of tubes inoculated with Can^R colonies had saturated — by tuesday evening I could see growth in one of the bkg tubes — by wednesday morning both bkg tubes had saturated.
  - ↳ Therefore I should purify the strains on Canavanine plates before making gDNA preps.
- I left the tubes in the rollerdrum until today.
- On tuesday (5/2) I picked colonies off ~~the~~ M-T plates and inoculated 1 mL YPD — left for 2 days in rollerdrums.
- Today I froze down 100 λ of each of the 240 Can^R strains (numbered 500-739) in 3 96-well plates.
  - ↳ prior to freezing I pin ~~transferred to~~ each plate to another YPD liquid plate and to a 10× canavanine agar plate — from this plate I will pick 8 streaks to gDNA prep.
- I was careful not to pick any colonies that were below my size threshold and thus were not counted in my fluctuation analysis.
  - ↳ this is important because these may represent a class of resistance mutations that allow weak growth on the selective media.
  - ↳ if I want to know what these mutations are I should sequence small colonies of resistant mutants from where I had a jackpot below the size threshold (see Can^R 0-3-9 p 47 and $5FOA^R$-P-7-5 p 54).
- Today I will inoculate 240 1mL YPD tubes with $5FOA^R$ colonies
- I always ~~tried~~ tried to pick the colony closest to the center of the spot — where that was too difficult I picked one from the edge and indicated that in the notes.

5/5

NOTES ON COLONY PICKING CONT.

- Today I put 100λ of each 5FOA^R mutant strain into 3 · 96-well plates (strains numbered 801 - 1040) containing 43λ 50% Glycerol
  - pin transferred to 5FOA
  - pin transferred to 100λ YPD+ADE
  - froze down original plates.
- Some cultures were still dilute and may not have pin transferred ↳ I indicated those strains by writing "dilute" on pgs 66-70.
- Put Canavanine plates (from yesterday) and Can^R mutant cultures in fridge.

5/15

- Despite the note that I left in the 30° room, no one put my plates in the fridge — that includes my 3 96-well plates and my 5 5FOA plates.
  - ↳ all of the ~~spots~~ wells in the 96-well plates had pellets
  - ↳ two of the ~~420 spots~~ 240 spots on 5FOA showed no growth (see p. 74)
  - 37 spots showed evidence of additional mutations (see picture on p 73)
  - a few spots showed weak growth but that was probably because there were too few cells in the 96-well plates when I pin transferred them to the 5FOA plates.
- Today I pin transferred from the ~~30~~ 96-well plates to 5FOA and to ~~drop~~ another copy plate (100λ YPD+ADE) labelled ~~"COPY"~~ "COPY 2".

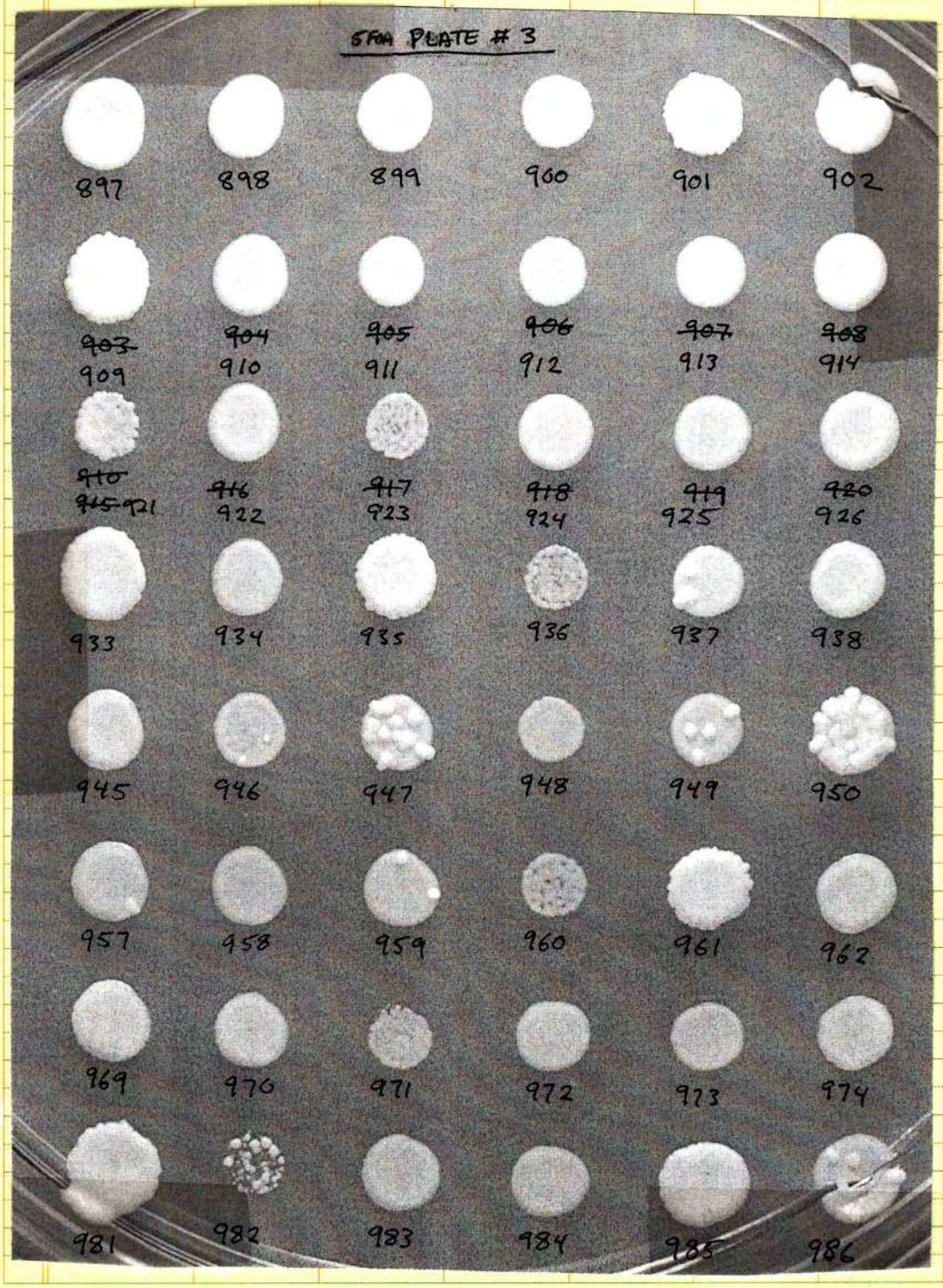
5/23

- A I left the 5FOA BKg tubes in the roller drum while I was on vacation — when I returned there was growth in the tubes — this indicates that 5FOA does not kill cells but cells take ~ 1 wk. to recover from 5FOA.

5/15

ANALYSIS OF 5FOA PLATES THAT WERE LEFT IN 30° ROOM

These papillations seen on the 5FOA plate could be due to two types of mutation:
(1) mutations which allow more rapid growth on 5FOA (general growth)
(2) mutations which increase resistance to 5FOA → because of this I need to be careful not to pick one of the papills since they may contain multiple ~~5FOA muts~~ URA3 mutations



ANALYSIS OF 5FOA PLATES LEFT IN 30° ROOM CONT.

Population

815
819
820
825
828
831
833
843
845
850
858 (could just be weak growth)
861
875
879
887
905
928
932
937
944
946
947
949
957
959
986
987
988
995
1000
1015
1017
1018
1019
1023
1037
1040

No Growth

999
1013

5/15

STARTING CULTURES FOR gDNA preps

- Today I will inoculate 1.25 mL of YPD+ADE with Can^R mutants ~~500-~~ from plates 1-3
  → that corresponds to: 500-595; 596-601, 608-613, 620-625, 632-637, 644-649, 656-661, 668-673, 680-685

5/18

NOTES ON gDNA preps:

- PROTOCOL:

(1) Pick cells off 10x Can plate (p 71) and inoculate 1.25 mL YPD+ADE; grow O/N

(2) spin down in microfuge tube; asp sup

(3) Add: 200 λ LYSIS buffer + 175 λ Phenol/Chloroform (for the first set (500-547) I used 200 λ P/C)
+ glass beads

(4) Vortex to resuspend → vortex in multi for 10 min

(5) Spin 10 min.

(6) Remove 125 λ sup → into 250 λ EtOH (first set (500-547) I did 150 into 300)

(7) -20 for at least 30 min.

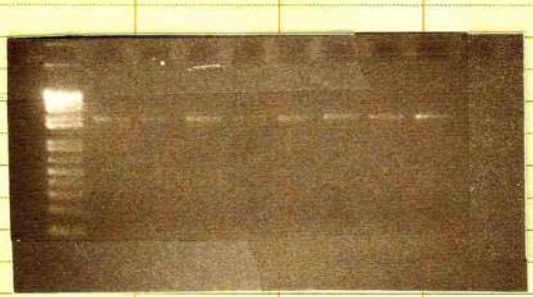
(8) spin 15 min @ 4°; ~~rem~~ asp EtOH → speedvac (~~rd 5~~ 20m)

(9) Add 50 λ TE → put in heating block (~20m)

- 632-637 - initially I ~~did~~ didn't add P/C
656-661 - added 2x P/C

5/23

I PCR tested the gDNA preps (including 632 & 656 see note above)



5/23

GENOMIC PREPS : NOTES

Over the weekend I (5/20 & 5/21) I finished the rest of the gDNA preps. ~~Not~~ For the most part I followed the protocol on pg. 75 — Notes and exceptions are below:

- In order to get pure SFOA^R colonies for sequencing, I took my "Copy 2" plate (pg 72) vortexed it on the plate shaker and pin transferred to SFOA and -URA — the results are shown on page 77.
  - 32 strains showed growth on -URA
    - of these
      - 24 showed full growth on SFOA
      - 5 showed weak growth on SFOA
      - 3 showed no growth on SFOA
    - two possibilities
      (1) mixed cultures → I can rep. plate to distinguish between these two
      (2) pure cultures w/ a mutation conferring SFOA^R/uracil prototrophs

- For the 3 that showed no growth (982, 999, & 1013) on SFOA I went back to the fluct^n plates and picked an additional colony from the same spots — I call these 982-B, 999-B, and 1013-B — these were streaked onto SFOA.
  - these strains grow very slowly on SFOA: could be cause the sat in the fridge for several weeks — after 2-3 days of growth only bkg comming up
  - they do look like real SFOA^R but they are comming on slowly — 999-B is the slowest of the three.

- On saturday (5/20) when I was starting the o/N cultures for gDNA preps I inoculated 982-B, 999-B, & 1013-B from the restreak SFOA plates (took a patch where it had grown in)
- 999-B did not grow up — I picked a patch off the SFOA restreak plate and resus. in 500 µl H_2O to start the gDNA prep
- 999-B and 982-B gDNA preps were resus. in only 25 µl TE
- gDNA preps I should verify by PCR:
  - Restreaks: 982-B, 999-B, & 1013-B
  - Small yeast pellets: 840, 883, 893
  - Small DNA pellets: 890

5FOA^ mutant number	GROWTH 5FOA	-ura
801	++	-
802	++	-
803	++	-
804	++	++
805	++	-
806	++	-
807	++	-
808	++	-
809	++	-
810	++	-
811	++	-
812	++	-
813	++	-
814	++	-
815	++	++
816	++	-
817	++	-
818	++	-
819	++	-
820	++	-
821	++	-
822	++	-
823	++	-
824	++	-
825	++	++
826	++	-
827	++	-
828	++	-
829	++	-
830	++	-
831	++	-
832	++	-
833	++	++
834	++	-
835	++	-
836	++	-
837	++	-
838	++	-
839	++	-
840	++	++
841	++	-
842	++	-
843	++	++
844	++	-
845	++	-
846	++	-
847	++	-
848	++	-
849	++	++
850	++	-
851	++	-
852	++	-
853	++	-
854	++	-
855	++	-
856	++	-
857	++	-
858	++	-
859	++	-
860	++	-
861	++	-
862	++	++
863	++	-
864	++	-
865	++	-
866	++	-
867	++	-
868	++	-
869	++	-
870	++	-
871	++	-
872	++	-
873	++	-
874	++	-
875	++	-
876	++	-
877	++	-
878	++	-
879	++	-
880	++	-
881	++	-
882	++	-
883	+	++
884	++	-
885	++	-
886	++	-
887	++	-
888	++	-
889	++	-
890	++	-
891	++	++
892	++	-
893	++	++
894	++	-
895	++	-
896	++	-
897	++	-
898	++	-
899	++	-
900	++	-
901	++	++
902	++	-
903	++	-
904	++	-
905	++	-
906	++	-
907	++	++
908	++	-
909	++	++
910	++	-
911	++	-
912	++	-
913	++	-
914	++	-
915	++	-
916	++	-
917	++	-
918	++	-
919	++	-
920	++	-
921	+	+
922	++	-
923	++	-
924	++	-
925	++	-
926	++	-
927	+	+
928	++	-
929	++	-
930	++	-
931	++	-
932	++	-
933	++	++
934	++	-
935	++	-
936	++	++
937	++	-
938	++	-
939	++	++
940	++	-
941	++	-
942	++	-
943	++	-
944	++	-
945	++	-
946	++	-
947	++	-
948	+	+
949	++	-
950	++	-
951	++	-
952	++	-
953	++	-
954	++	-
955	++	-
956	++	-
957	++	-
958	++	-
959	++	-
960	++	-
961	++	-
962	++	-
963	++	++
964	++	++
965	++	-
966	++	-
967	++	-
968	++	++
969	++	-
970	++	-
971	++	++
972	++	-
973	++	-
974	++	-
975	++	-
976	++	-
977	++	-
978	++	-
979	++	-
980	++	-
981	++	++
982	-	+
983	++	-
984	++	-
985	++	-
986	++	-
987	++	-
988	++	-
989	++	-
990	++	-
991	+	++
992	++	-
993	++	-
994	++	-
995	++	-
996	++	-
997	++	-
998	++	++
999	-	-
1000	++	-
1001	++	-
1002	++	-
1003	++	-
1004	++	-
1005	++	-
1006	++	-
1007	++	-
1008	++	-
1009	++	-
1010	++	-
1011	++	-
1012	++	-
1013	-	-
1014	++	-
1015	++	++
1016	++	++
1017	++	-
1018	++	-
1019	++	-
1020	++	-
1021	++	-
1022	++	-
1023	++	-
1024	++	-
1025	++	-
1026	++	-
1027	++	-
1028	++	-
1029	++	-
1030	++	-
1031	++	-
1032	++	-
1033	++	-
1034	++	-
1035	++	-
1036	++	-
1037	++	-
1038	++	-
1039	++	-
1040	++	-

5/23

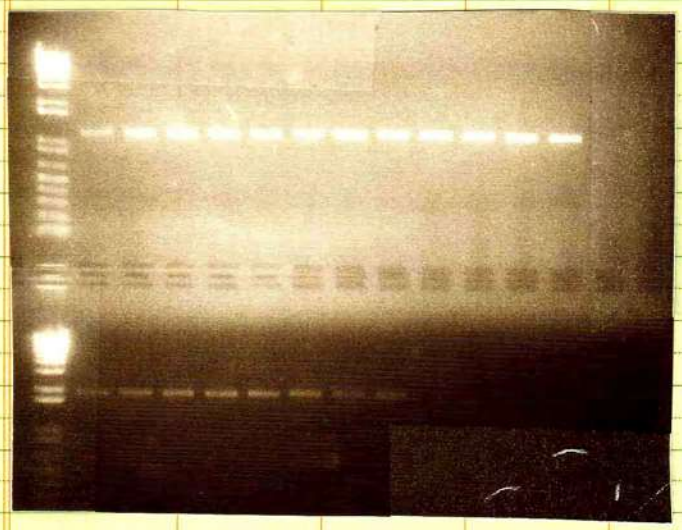
SEQENCNG

TEMPLATE

- PCR on the following qDNA preps: → URA3: 801 - 812, CAN1: 500 - 507

50 λ PCR → elute w/ 100 λ H_2O

run 10 λ



PRIMERS:

URA3: URA3 extF & URA3 extR

CAN1: CAN1extF2 & CAN1extR2 (see p 18)

PROGRAM:

URA3: Anneal: 47° Extension: 2 m — run time ~ 2h 29m

CAN1: Anneal: 50° Extension: 2 m 30 s (see p 18) — run time ~2h 40m

→ add 10 λ per sequencing rxn

PRIMER

CAN1 ext/int F1	$\frac{8\ \text{pmol}}{33.4\ \text{pmol}/\lambda} = 0.24 \simeq .3$	→ 4.5 λ into 295.5 λ H_2O
CAN1 ext R2	$\frac{8}{29.6} = 0.27 \simeq .3$	
CAN1 int F2	$\frac{8}{26.6} = 0.30 \simeq .3$	
CAN1 int F3	$\frac{8}{30.3} = 0.26 \simeq .3$	
CAN1 int R2	$\frac{8}{31.2} = 0.26 \simeq .3$	
CAN1 int R3	$\frac{8}{31.0} = 0.26 \simeq .3$	
URA3 ext F3	$\frac{8}{27.5} = 0.29 \simeq .3$	
URA3 ext R	$\frac{8}{18.5} = 0.43 \simeq .45$	→ 6.75 λ into 293.25 λ H_2O
URA3 int F2	$\frac{8}{30.0} = 0.27 \simeq .3$	
URA3 int R2	$\frac{8}{26.5} = 0.30 \simeq .3$	

→ add 20 λ per sequencing rxn.

- I submitted the sequences to the Dana-Farber/Harvard Cancer Center – they are cheaper than biopolymers

5/25

PCR PURIFICATIONS

- Yesterday I started the PCR purifications using the Qiagen kit → PCRs were eluted in 100 λ H_2O.
- Purified samples put in -20°C freezer until I am ready to set up sequencing.
- 500-507 were done on 5/23 (p. 79). Yesterday I did 508-657.

CHANI

5/25

URA3 sequencing results

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CLUSTAL W (1.83) multiple sequence alignment

URA3            ATGTCGAAAGCTACATATAAGGAACGTGCTGCTACTCATCCTAGTCCTGTTGCTGCCAAG 60
801             ATGTCGAAAGCTACATATAAGGAACGTGCTGCTACTCATCCTAGTCCTGTTGCTGCCAAG 60
802             ATGTCGAAAGCTACATATAAGGAACGTGCTGCTACTCATCCTAGTCCTGTTGCTGCCAAG 60
80              ATGTCGAAAGCTACATATAAGGAACGTGCTGCTACTCATCCTAGTCCTGTTGCTGCCAAG 60
804             ATGTCGAAAGCTACATATAAGGAACGTGCTGCTACTCATCCTAGTCCTGTTGCTGCCAAG 60
805             ATGTCGAAAGCTACATATAAGGAACGTGCTGCTACTCATCCTAGTCCTGTTGCTGCCAAG 60
806             ATGTCGAAAGCTACATATAAGGAACGTGCTGCTACTCATCCTAGTCCTGTTGCTGCCAAG 60
807             ATGTCGAAAGCTACATATAAGGAACGTGCTGCTACTCATCCTAGTCCTGTTGCTGCCAAG 60
808             ATGTCGAAAGCTACATATAAGGAACGTGCTGCTACTCATCCTAGTCCTGTTGCTGCCAAG 60
809             ATGTCGAAAGCTACATATAAGGAACGTGCTGCTACTCATCCTAGTCCTGTTGCTGCCAAG 60
810             ATGTCGAAAGCTACATATAAGGAACGTGCTGCTACTCATCCTAGTCCTGTTGCTGCCAAG 60
811             ATGTCGAAAGCTACATATAAGGAACGTGCTGCTACTCATCCTAGTCCTGTTGCTGCCAAG 60
812             ATGTCGAAAGCTACATATAAGGAACGTGCTGCTACTCATCCTAGTCCTGTTGCTGCCAAG 60
                ************************************************************

URA3            CTATTTAATATCATGCACGAAAAGCAAACAAACTTGTGTGCTTCATTGGATGTTCGTACC 120
801             CTATTTAATATCATGCACGAAAAGCAAACAAACTTGTGTGCTTCATTGGATGTTCGTACC 120
802             CTATTTAATATCATGCACGAAAAGCAAACAAACTTGTGTGCTTCATTGGATGTTCGTACC 120
803             CTATTTAATATCATGCACGAAAAGCAAACAAACTTGTGTGCTTCATTGGATGTTCGTACC 120
804_wt          CTATTTAATATCATGCACGAAAAGCAAACAAACTTGTGTGCTTCATTGGATGTTCGTACC 120
805             CTATTTAATATCATGCACGAAAAGCAAACAAACTTGTGTGCTTCATTGGATGTTCGTACC 120
806             CTATTTAATATCATGCACGAAAAGCAAACAAACTTGTGTGCTTCATTGGATGTTCGTACC 120
807             CTATTTAATATCATGCACGAAAAGCAAACAAACTTGTGTGCTTCATTGGATGTTCGTACC 120
808             CTATTTAATATCATGCACGAAAAGCAAACAAACTTGTGTGCTTCATTGGATGTTCGTACC 120
809             CTATTTAATATCATGCACGAAAAGCAAACAAACTTGTGTGCTTCATTGGATGTTCGTACC 120
810             CTATTTAATATCATGCACGAAAAGCAAACAAACTTGTGTGCTTCATTGGATGTTCGTACC 120
811             CTATTTAATATCATGCACGAAAAGCAAACAAACTTGTGTGCTTCATTGGATGTTCGTACC 120
812             CTATTTAATATCATGCACGAAAAGCAAACAAACTTGTGTGCTTCATTGGATGTTCGTACC 120
                ************************************************************

URA3            ACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGTTTACTAAAAACA 180
801             ACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGTTTACTAAAAACA 180
802             ACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGTTTACTAAAAACCA 180
803             ACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGTTTACTAAAAACA 180
804_wt          ACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGTTTACTAAAAACA 180
805             ACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGTTTACTAAAAACA 180
806             ACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGTTTACTAAAAACA 180
807             ACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGTTTACTAAAAACA 180
808             ACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGTTTACTAAAAACA 180
809             ACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGTTTACTAAAAACA 180
810             ACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGATCCCAAAATTTGTTTACTAAAAACA 180
811             ACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGTTTACTAAAAACA 180
812             ACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGTTTACTAAAAACA 180
                ********************************** ********************** **

URA3            CATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTA 240
801             CATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTA 240
80              CATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTA 240
803             CATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTA 240
804_wt          CATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTA 240
805             CATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTA 240
806             CATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTA 240
807             CATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTA 240
808             CATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTA 240
809             TATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTA 240
810             CATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTA 240
811             CATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTA 240
812             CATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTA 240
                ************************************************************

URA3            TCCGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACA 300
801             TCCGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACA 300
802             TCCGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACA 300
803             TCCGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACA 300
804_wt          TCCGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACA 300
805             TCCGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACA 300
806             TCCGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACA 300
807             TCCGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACA 300
808             TCCGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACA 300
809             TCCGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACA 300
810             TCCGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACA 300
811             TCCGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACA 300
812             TCCGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACA 300
                ************************************************************

URA3            GTCAAATTGCAGTACTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAAT 360
801             GTCAAATTGCAGTACTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAAT 360
802             GTCAAATTGCAGTACTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAAT 360
803             GTCAAATTGCAGTACTCTGCGGGTGTATACAGAATAGCAGAAGGGGCAGACATTACGAAT 360
804_wt          GTCAAATTGCAGTACTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAAT 360
805             GTCAAATTGCAGTACTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAAT 360
806             GTCAAATTGCAGTACTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAAT 360
807             GTCAAATTGCAGTACTCTGCGGGTGTATACAGAATAGAAGAATGGGCAGACATTACGAAT 360
808             GTCAAATTGCAGTACTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAAT 360
809             GTCAAATTGCAGTACTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAAT 360
810             GTCAAATTGCAGTACTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAAT 360
811             GTCAAATTGTAGTACTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAAT 360
812             GTCAAATTGCAGTACTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAAT 360
                ********* ************************** **** ****************

                GCACACGGTGTGGTGGGCCCAGGTATTGTTAGCGGTTTGAAGCAGGCGGCGGAAGAAGTA 420
                GCACACGGTGTGGTGGGCCCAGGTATTGTTAGCGGTTTGAAGCAGGCGGCGGAAGAAGTA 420
802             GCACACGGTGTGGTGGGCCCAGGTATTGTTAGCGGTTTGAAGCAGGCGGCGGAAGAAGTA 420
803             GCACACGGTGTGGTGGGCCCAGGTATTGTTAGCGGTTTGAAGCAGGCGGCGGAAGAAGTA 420
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ACA → CCA (802)
T → P

GGT → GAT (810, 80)
G → D

CAT → TAT (809)
H → Y

TGG → GGG (803)
W → G

GCA → GAA (807, 121)
A → E

CAG → TAG (811, 46, 103)
Q → STOP

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804_wt          GCACACGGTGTGGTGGGCCCAGGTATTGTTAGCGGTTTGAAGCAGGCGGCGGAAGAAGTA 420
805             GCACACGGTGTGGTGGGCCCAGGTATTGTTAGCGGTTTGAAGCAGGCGGCGGAAGAAGTA 420
806             GCACACGGTGTGGTGGGCCCAGGTATTGTTAGCGGTTTGAAGCAGGCGGCGGAAGAAGTA 420
807             GCACACGGTGTGGTGGGCCCAGGTATTGTTAGCGGTTTGAAGCAGGCGGCGGAAGAAGTA 420
808             GCACACGGTGTGGTGGGCCCAGGTATTGTTAGCGGTTTGAAGCAGGCGGCGGAAGAAGTA 420
809             GCACACGGTGTGGTGGGCCCAGGTATTGTTAGCGGTTTGAAGCAGGCGGCGGAAGAAGTA 420
810             GCACACGGTGTGGTGGGCCCAGGTATTGTTAGCGGTTTGAAGCAGGCGGCGGAAGAAGTA 420
811             GCACACGGTGTGGTGGGCCCAGGTATTGTTAGCGGTTTGAAGCAGGCGGCGGAAGAAGTA 420
812             GCACACGGTGTGGTGGGCCCAGGTATTGTTAGCGGTTTGAAGCAGGCGGCGGAAGAAGTA 420
                ************************************************************

URA3            ACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATTGTCATGCAAGGGCTCCCTATCT 480
801             ACATAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATTGTCATGCAAGGGCTCCCTATCT 480
802             ACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATTGTCATGCAAGGGCTCCCTATCT 480
803             ACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATTGTCATGCAAGGGCTCCCTATCT 480
804_wt          ACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATTGTCATGCAAGGGCTCCCTATCT 480
805             ACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATTGTCATGCAAGGGCTCCCTATCT 480
806             ACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATTGTCATGCAAGGGCTCCCTATCT 480
807             ACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATTGTCATGCAAGGGCTCCCTATCT 480
808             ACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATTGTCATGCAAGGGCTCCCTATCT 480
809             ACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATTGTCATGCAAGGGCTCCCTATCT 480
810             ACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATTGTCATGCAAGGGCTCCCTATCT 480
811             ACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATTGTCATGCAAGGGCTCCCTATCT 480
812             ACAAAGGAACCTAGAGGCCTTTTGATGTTAGCATAATTGTCATGCAAGGGCTCCCTATCT 480
                *** ***************************** ********** ***************

URA3            ACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTTATC 540
801             ACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTTATC 540
802             ACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTTATC 540
803             ACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTTATC 540
804_wt          ACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTTATC 540
805             ACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTTATC 540
806             ACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTTATC 540
807             ACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTTATC 540
808             ACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTTATC 540
809             ACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTTATC 540
810             ACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTTATC 540
811             ACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTTATC 540
812             ACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTTATC 540
                ************************************************************

URA3            GGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATG 600
801             GGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATG 600
802             GGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATG 600
803             GGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATG 600
804_wt          GGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATG 600
805             GGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATG 600
806             GGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATG 600
807             GGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATG 600
808             GGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATG 600
809             GGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATG 600
810             GGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATG 600
811             GGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATG 600
812             GGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATG 600
                ************************************************************

URA3            ACACCCGGTGTGGGTTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTG 660
801             ACACCCGGTGTGGGTTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTG 660
802             ACACCCGGTGTGGGTTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTG 660
803             ACACCCGGTGTGGGTTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTG 660
804_wt          ACACCCGGTGTGGGTTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTG 660
805             ACACCCGGTGTGGGTTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTG 660
806             ACACCCGGTGTGGGTT-AGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTG 659
807             ACACCCGGTGTGGGTTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTG 660
808             ACACACGGTGTGGGTTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTG 660
809             ACACCCGGTGTGGGTTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTG 660
810             ACACCCGGTGTGGGTTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTG 660
811             ACACCCGGTGTGGGTTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTG 660
812             ACACCCGGTGTGGGTTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTG 660
                **** *********** ********************************************

URA3            GATGATGTGGTCTCTACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAG 720
801             GATGATGTGGTCTCTACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAG 720
802             GATGATGTGGTCTCTACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAG 720
803             GATGATGTGGTCTCTACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAG 720
804_wt          GATGATGTGGTCTCTACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAG 720
805             GATGATGTGGTCTCTACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAG 720
806             GATGATGTGGTCTCTACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAG 719
807             GATGATGTGGTCTCTACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAG 720
808             GATGATGTGGTCTCTACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAG 720
809             GATGATGTGGTCTCTACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAG 720
810             GATGATGTGGTCTCTACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAG 720
811             GATGATGTGGTCTCTACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAG 720
812             GATGATGTGGTCTCTACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAG 720
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URA3            GGAAGGGATGCTAAGGTAGAGGGTGAACGTTACAGAAAAGCAGGCTGGGAAGCATATTTG 780
801             GGAAGGGATGCTAAGGTAGAGGGTGAACGTTACAGAAAAGCAGGCTGGGAAGCATATTTG 780
802             GGAAGGGATGCTAAGGTAGAGGGTGAACGTTACAGAAAAGCAGGCTGGGAAGCATATTTG 780
803             GGAAGGGATGCTAAGGTAGAGGGTGAACGTTACAGAAAAGCAGGCTGGGAAGCATATTTG 780
804_wt          GGAAGGGATGCTAAGGTAGAGGGTGAACGTTACAGAAAAGCAGGCTGGGAAGCATATTTG 780
805             GGAAGGGATGCTAAGGTAGAGGGTGAACGTTACAGAAAAGCAGGCTGGGAAGCATATTTG 780
806             GGAAGGGATGCTAAGGTAGAGGGTGAACGTTACAGAAAAGCAGGCTGGGAAGCATATTTG 779
807             GGAAGGGATGCTAAGGTAGAGGGTGAACGTTACAGAAAAGCAGGCTGGGAAGCATATTTG 780
808             GGAAGGGATGCTAAGGTAGAGGGTGAACGTTACAGAAAAGCAGGCTGGGAAGCATATTTG 780
809             GGAAGGGATGCTAAGGTAGAGGGTGAACGTTACAGAAAAGCAGGCTGGGAAGCATATTTG 780
810             GGAAGGGATGCTAAGGTAGAGGGTGAACGTTACAGAAAAGCAGGCTGGGAAGCATATTTG 780
```

AAG → TAG (801)
K STOP

TGC → TGA (805, 30)
C STOP

GAA → TAA (812)
E STOP

-T (806) @ 617

CCC → CAC (808, 40)
P H

82

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811             GGAAGGGATGCTAAGGTAGAGGGTGAACGTTACAGAAAAGCAGGCTGGGAAGCATATTTG 780
812             GGAAGGGATGCTAAGGTAGAGGGTGAACGTTACAGAAAAGCAGGCTGGGAAGCATATTTG 780
                ************************************************************

URA3            AGAAGATGCGGCCAGCAAAACTAA 804
801             AGAAGATGCGGCCAGCAAAACTAA 804
802             AGAAGATGCGGCCAGCAAAACTAA 804
803             AGAAGATGCGGCCAGCAAAACTAA 804
804_wt          AGAAGATGCGGCCAGCAAAACTAA 804
805             AGAAGATGCGGCCAGCAAAACTAA 804
806             AGAAGATGCGGCCAGCAAAACTAA 803
807             AGAAGATGCGGCCAGCAAAACTAA 804
808             AGAAGATGCGGCCAGCAAAACTAA 804
809             AGAAGATGCGGCCAGCAAAACTAA 804
810             AGAAGATGCGGCCAGCAAAACTAA 804
811             AGAAGATGCGGCCAGCAAAACTAA 804
812             AGAAGATGCGGCCAGCAAAACTAA 804
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5/25

CAN1 Sequencing Results

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CLUSTAL W (1.83) multiple sequence alignment

CAN1            ATGACAAATTCAAAAGAAGACGCCGACATAGAGGAGAAGCATATGTACAATGAGCCGGTC 60
500             ATGACAAATTCAAAAGAAGACGCCGACATAGAGGAGAAGCATATGTACAATGAGCCGGTC 60
501             ATGACAAATTCAAAAGAAGACGCCGACATAGAGGAGAAGCATATGTACAATGAGCCGGTC 60
50              ATGACAAATTCAAAAGAAGACGCCGACATAGAGGAGAAGCATATGTACAATGAGCCGGTC 60
50              ATGACAAATTCAAAAGAAGACGCCGACATAGAGGAGAAGCATATGTACAATGAGCCGGTC 60
504             ATGACAAATTCAAAAGAAGACGCCGACATAGAGGAGAAGCATATGTACAATGAGCCGGTC 60
505             ATGACAAATTCAAAAGAAGACGCCGACATAGAGGAGAAGCATATGTACAATGAGCCGGTC 60
506             ATGACAAATTCAAAAGAAGACGCCGACATAGAGGAGAAGCATATGTACAATGAGCCGGTC 60
507             ATGACAAATTCAAAAGAAGACGCCGACATAGAGGAGAAGCATATGTACAATGAGCCGGTC 60
                ************************************************************

CAN1            ACAACCCTCTTTCACGACGTTGAAGCTTCACAAACACACCACAGACGTGGGTCAATACCA 120
500             ACAACCCTCTTTCACGACGTTGAAGCTTCACAAACACACCACAGACGTGGGTCAATACCA 120
501             ACAACCCTCTTTCACGACGTTGAAGCTTCACAAACACACCACAGACGTGGGTCAATACCA 120
502             ACAACCCTCTTTCACGACGTTGAAGCTTCACAAACACACCACAGACGTGGGTCAATACCA 120
503             ACAACCCTCTTTCACGACGTTGAAGCTTCACAAACACACCACAGACGTGGGTCAATACCA 120
504             ACAACCCTCTTTCACGACGTTGAAGCTTCACAAACACACCACAGACGTGGGTCAATACCA 120
505             ACAACCCTCTTTCACGACGTTGAAGCTTCACAAACACACCACAGACGTGGGTCAATACCA 120
506             ACAACCCTCTTTCACGACGTTGAAGCTTCACAAACACACCACAGACGTGGGTCAATACCA 120
507             ACAACCCTCTTTCACGACGTTGAAGCTTCACAAACACACCACAGACGTGGGTCAATACCA 120
                ************************************************************

CAN1            TTGAAAGATGAGAAAAGTAAAGAATTGTATCCATTGCGCTCTTTCCCGACGAGAGTAAAT 180
500             TTGAAAGATGAGAAAAGTAAAGAATTGTATCCATTGCGCTCTTTCCCGACGAGAGTAAAT 180
501             TTGAAAGATGAGAAAAGTAAAGAATTGTATCCATTGCGCTCTTTCCCGACGAGAGTAAAT 180
502             TTGAAAGATGAGAAAAGTAAAGAATTGTATCCATTGCGCTCTTTCCCGACGAGAGTAAAT 180
503             TTGAAAGATGAGAAAAGTAAAGAATTGTATCCATTGCGCTCTTTCCCGACGAGAGTAAAT 180
504             TTGAAAGATGAGAAAAGTAAAGAATTGTATCCATTGCGCTCTTTCCCGACGAGAGTAAAT 180
505             TTGAAAGATGAGAAAAGTAAAGAATTGTATCCATTGCGCTCTTTCCCGACGAGAGTAAAT 180
506             TTGAAAGATGAGAAAAGTAAAGAATTGTATCCATTGCGCTCTTTCCCGACGAGAGTAAAT 180
507             TTGAAAGATGAGAAAAGTAAAGAATTGTATCCATTGCGCTCTTTCCCGACGAGAGTAAAT 180
                ************************************************************

CAN1            GGCGAGGATACGTTCTCTATGGAGGATGGCATAGGTGATGAAGATGAAGGAGAAGTACAG 240
500             GGCGAGGATACGTTCTCTATGGAGGATGGCATAGGTGATGAAGATGAAGGAGAAGTACAG 240
501             GGCGAGGATACGTTCTCTATGGAGGATGGCATAGGTGATGAAGATGAAGGAGAAGTACAG 240
502             GGCGAGGATACGTTCTCTATGGAGGATGGCATAGGTGATGAAGATGAAGGAGAAGTACAG 240
503             GGCGAGGATACGTTCTCTATGGAGGATGGCATAGGTGATGAAGATGAAGGAGAAGTACAG 240
504             GGCGAGGATACGTTCTCTATGGAGGATGGCATAGGTGATGAAGATGAAGGAGAAGTACAG 240
505             GGCGAGGATACGTTCTCTATGGAGGATGGCATAGGTGATGAAGATGAAGGAGAAGTACAG 240
506             GGCGAGGATACGTTCTCTATGGAGGATGGCATAGGTGATGAAGATGAAGGAGAAGTACAG 240
507             GGCGAGGATACGTTCTCTATGGAGGATGGCATAGGTGATGAAGATGAAGGAGAAGTACAG 240
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CAN1            AACGCTGAAGTGAAGAGAGAGCTTAAGCAAAGACATATTGGTATGATTGCCCTTGGTGGT 300
500             AACGCTGAAGTGAAGAGAGAGCTTAAGCAAAGACATATTGGTATGATTGCCCTTGGTGGT 300
501             AACGCTGAAGTGAAGAGAGAGCTTAAGCAAAGACATATTGGTATGATTGCCCTTGGTGGT 300
50              AACGCTGAAGTGAAGAGAGAGCTTAAGCAAAGACATATTGGTATGATTGCCCTTGGTGGT 300
50              AACGCTGAAGTGAAGAGAGAGCTTAAGCAAAGACATATTGGTATGATTGCCCTTGGTGGT 300
504             AACGCTGAAGTGAAGAGAGAGCTTAAGCAAAGACATATTGGTATGATTGCCCTTGGTGGT 300
505             AACGCTGAAGTGAAGAGAGAGCTTAAGCAAAGACATATTGGTATGATTGCCCTTGGTGGT 300
506             AACGCTGAAGTGAAGAGAGAGCTTAAGCAAAGACATATTGGTATGATTGCCCTTGGTGGT 300
507             AACGCTGAAGTGAAGAGAGAGCTTAAGCAAAGACATATTGGTATGATTGCCCTTGGTGGT 300
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CAN1            ACTATTGGTACAGGTCTTTTCATTGGTTTATCCACACCTCTGACCAACGCCGGCCCAGTG 360
500             ACTATTGGTACAGGTCTTTTCATTGGTTTATCCACACCTCTGACCAACGCCGGCCCAGTG 360
501             ACTATTGGTACAGGTCTTTTCATTGGTTTATCCACACCTCTGACCAACGCCGGCCCAGTG 360
502             ACTATTGGTACAGGTCTTTTCATTGGTTTATCCACACCTCTGACCAACGCCGGCCCAGTG 360
503             ACTATTGGTACAGGTCTTTTCATTGGTTTATCCACACCTCTGACCAACGCCGGCCCAGTG 360
504             ACTATTGGTACAGGTCTTTTCATTGGTTTATCCACACCTCTGACCAACGCCGGCCCAGTG 360
505             ACTATTGGTACAGGTCTTTTCATTGGTTTATCCACACCTCTGACCAACGCCGGCCCAGTG 360
506             ACTATTGGTACAGGTCTTTTCATTGGTTTATCCACACCTCTGACCAACGCCGGCCCAGTG 360
507             ACTATTGGTACAGGTCTTTTCATTGGTTTATCCACACCTCTGACCAACGCCGGCCCAGTG 360
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CAN1            GGCGCTCTTATATCATATTTATTTATGGGTTCTTTGGCATATTCTGTCACGCAGTCCTTG 420
500             GGCGCTCTTATATCATATTTATTTATGGGTTCTTTGGCATATTCTGTCACGCAGTCCTTG 420
501             GGCGCTCTTATATCATATTTATTTATGGGTTCTTTGGCATATTCTGTCACGCAGTCCTTG 420
502             GGCGCTCTTATATCATATTTATTTATGGGTTCTTTGGCATATTCTGTCACGCAGTCCTTG 420
503             GGCGCTCTTATATCATATTTATTTATGGGTTCTTTGGCATATTCTGTCACGCAGTCCTTG 420
504             GGCGCTCTTATATCATATTTATTTATGGGTTCTTTGGCATATTCTGTCACGCAGTCCTTG 420
505             GGCGCTCTTATATCATATTTATTTATGGGTTCTTTGGCATATTCTGTCACGCAGTCCTTG 420
506             GGCGCTCTTATATCATATTTATTTATGGGTTCTTTGGCATATTCTGTCACGCAGTCCTTG 420
507             GGCGCTCTTATATCATATTTATTTATGGGTTCTTTGGCATATTCTGTCACGCAGTCCTTG 420
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CAN1            GGTGAAATGGCTACATTCATCCCTGTTACATCCTCTTTCACAGTGTTCTCACAAAGATTC 480
500             GGTGAAATGGCTACATTCATCCCTGTTACATCCTCTTTCACAGTGTTCTCACAAAGATTC 480
501             GGTGAAATGGCTACATTCATCCCTGTTACATCCTCTTTCACAGTGTTCTCACAAAGATTC 480
502             GGTGAAATGGCTACATTCATCCCTGTTACATCCTCTTTCACAGTGTTCTCACAAAGATTC 480
503             GGTGAAATGGCTACATTCATCCCTGTTACATCCTCTTTCACAGTGTTCTCACAAAGATTC 480
504             GGTGAAATGGCTACATTCATCCCTGTTACATCCTCTTTCACAGTGTTCTCACAAAGATTC 480
505             GGTGAAATGGCTACATTCATCCCTGTTACATCCTCTTTCACAGTGTTCTCACAAAGATTC 480
506             GGTGAAATGGCTACATTCATCCCTGTTACATCCTCTTTCACAGTGTTCTCACAAAGATTC 480
507             GGTGAAATGGCTACATTCATCCCTGTTACATCCTCTTTCACAGTGTTCTCACAAAGATTC 480
                ************************************************************

CAN1            CTTTCTCCAGCATTTGGTGCGGCCAATGGTTACATGTATTGGTTTTCTTGGGCAATCACT 540
500             CTTTCTCCAGCATTTGGTGCGGCCAATGGTTACATGTATTGGTTTTCTTGGGCAATCACT 540
50              CTTTCTCCAGCATTTGGTGCGGCCAATGGTTACATGTATTGGTTTTCTTGGGCAATCACT 540
50              CTTTCTCCAGCATTTGGTGCGGCCAATGGTTACATGTATTGGTTTTCTTGGGCAATCACT 540
503             CTTTCTCCAGCATTTGGTGCGGCCAATGGTTACATGTATTGGTTTTCTTGGGCAATCACT 540
504             CTTTCTCCAGCATTTGGTGCGGCCAATGGTTACATGTATTGGTTTTCTTGGGCAATCACT 540
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505         CTTTCTCCAGCATTTGGTGCGGCCAATGGTTACATGTATTGGTTTTCTTGGGCAATCACT 540
506         CTTTCTCCAGCATTTGGTGCGGCCAATGGTTACATGTATTGGTTTTCTTGGGCAATCACT 540
507         CTTTCTCCAGCATTTGGTGCGGCCAATGGTTACATGTATTGGTTTTCTTGGGCAATCACT 540
            ************************************************************

CAN1        TTTGCCCTGGAACTTAGTGTAGTTGGCCAAGTCATTCAATTTTGGACGTACAAAGTTCCA 600
500         TTTGCCCTGGAACTTAGTGTAGTTGGCTAAGTCATTCAATTTTGGACGTACAAAGTTCCA 600
501         TTTGCCCTGGAACTTAGTGTAGTTGGCCAAGTCATTCAATTTTGGACGTACAAAGTTCCA 600
502         TTTGCCCTGGAACTTAGTGTAGTTGGCCAAGTCATTCAATTTTGGACGTACAAAGTTCCA 600
503         TTTGCCCTGGAACTTAGTGTAGTTGGCCAAGTCATTCAATTTTGGACGTACAAAGTTCCA 600
504         TTTGCCCTGGAACTTAGTGTAGTTGGCCAAGTCATTCAATTTTGGACGTACAAAGTTCCA 600
505         TTTGCCCTGGAACTTAGTGTAGTTGGCCAAGTCATTCAATTTTGGACGTACAAAGTTCCA 600
506         TTTGCCCTGGAACTTAGTGTAGTTGGCCAAGTCATTCAATTTTGGACGTACAAAGTTCCA 600
507         TTTGCCCTGGAACTTAGTGTAGTTGGCCAAGTCATTCAATTTTGGACGTACAAAGTTCCA 600
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CAN1        CTGGCGGCATGGATTAGTATTTTTTGGGTAATTATCACAATAATGAACTTGTTCCCTGTC 660
500         CTGGCGGCATGGATTAGTATTTTTTGGGTAATTATCACAATAATGAACTTGTTCCCTGTC 660
501         CTGGCGGCATGGATTAGTATTTTTTGGGTAATTATCACAATAATGAACTTGTTCCCTGTC 660
502         CTGGCGGCATGGATTAGTATTTTTTGGGTAATTATCACAATAATGAACTTGTTCCCTGTC 660
503         -TGGCGGCATGGATTAGTATTTTTTGGGTAATTATCACAATAATGAACTTGTTCCCTGTC 659
504         CTGGCGGCATGGATTAGTATTTTTTGGGTAATTATCACAATAATGAACTTGTTCCCTGTC 660
505         CTGGCGGCATGGATTAGTATTTTTTGGGTAATTATCACAATAATGAACTTGTTCCCTGTC 660
506         CTGGCGGCATGGATTAGTATTTTTTGGGTAATTATCACAATAATGAACTTGTTCCCTGTC 660
507         CTGGCGGCATGGATTAGTATTTTTTGGGTAATTATCACAATAATGAACTTGTTCCCTGTC 660
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CAN1        AAATATTACGGTGAATTCGAGTTCTGGGTCGCTTCCATCAAAGTTTTAGCCATTATCGGG 720
500         AAATATTACGGTGAATTCGAGTTCTGGGTCGCTTCCATCAAAGTTTTAGCCATTATCGGG 720
501         AAATATTACGGTGAATTCGAGTTCTGGGTCGCTTCCATCAAAGTTTTAGCCATTATCGGG 720
502         AAATATTACGGTGAATTCGAGTTCTGGGTCGCTTCCATCAAAGTTTTAGCCATTATCGGG 720
503         AAATATTACGGTGAATTCGAGTTCTGGGTCGCTTCCATCAAAGTTTTAGCCATTATCGGG 719
504         AAATATTACGGTGAATTCGAGTTCTGGGTCGCTTCCATCAAAGTTTTAGCCATTATCGGG 720
505         AAATATTACGGTGAATTCGAGTTCTGGGTCGCTTCCATCAAAGTTTTAGCCATTATCGGG 720
506         AAATATTACGGTGAATTCGAGTTCTGGGTCGCTTCCATCAAAGTTTTAGCCATTATCGGG 720
507         AAATATTACGGTGAATTCGAGTTCTGGGTCGCTTCCATCAAAGTTTTAGCCATTATCGGG 720
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CAN1        TTTCTAATATACTGTTTTTGTATGGTTTGTGGTGCTGGGGTTACCGGCCCAGTTGGATTC 780
500         TTTCTAATATACTGTTTTTGTATGGTTTGTGGTGCTGGGGTTACCGGCCCAGTTGGATTC 780
501         TTTCTAATATACTGTTTTTGTATGGTTTGTGGTGCTGGGGTTACCGGCCCAGTTGGATTC 780
502         TTTCTAATATACTGTTTTTGTATGGTTTGTGGTGCTGGGGTTACCGGCCCAGTTGGATTC 780
503         TTTCTAATATACTGTTTTTGTATGGTTTGTGGTGCTGGGGTTACCGGCCCAGTTGGATTC 779
504         TTTCTAATATACTGTTTTTGTATGGTTTGTGGTGCTGGGGTTACCGGCCCAGTTGGATTC 780
505         TTTCTAATATACTGTTTTTGTATGGTTTGTGGTGCTGGGGTTACCGGCCCAGTTGGATTC 780
506         TTTCTAATATACTGTTTTTGTATGGTTTGTGGTGCTGGGGTTACCGGCCCAGTTGGATTC 780
507         TTTCTAATATACTGTTTTTGTATGGTTTGTGGTGCTGGGGTTACCGGCCCAGTTGGATTC 780
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CAN1        CGTTATTGGAGAAACCCAGGTGCCTGGGGTCCAGGTATAATATCTAAGGATAAAAACGAA 840
500         CGTTATTGGAGAAACCCAGGTGCCTGGGGTCCAGGTATAATATCTAAGGATAAAAACGAA 840
501         CGTTATTGGAGAAACCCAGGTGCCTGGGGTCCAGGTATAATATCTAAGGATAAAAACGAA 840
502         CGTTATTGGAGAAACCCAGGTGCCTGGGGTCCAGGTATAATATCTAAGGATAAAAACGAA 840
503         CGTTATTGGAGAAACCCAGGTGCCTGGGGTCCAGGTATAATATCTAAGGATAAAAACGAA 839
504         CGTTATTGGAGAAACCCAGGTGCCTGGGGTCCAGGTATAATATCTAAGGATAAAAACGAA 840
505         CGTTATTGGAGAAACCCAGGTGCCTGGGGTCCAGGTATAATATCTAAGGATAAAAACGAA 840
506         CGTTATTGGAGAAACCCAGGTGCCTGGGGTCCAGGTATAATATCTAAGGATAAAAACGAA 840
507         CGTTATTGGAGAAACCCAGGTGCCTGGGGTCCAGGTATAATATCTAAGGATAAAAACGAA 840
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CAN1        GGGAGGTTCTTAGGTTGGGTTTCCTCTTTGATTAACGCTGCCTTCACATTTCAAGGTACT 900
500         GGGAGGTTCTTAGGTTGGGTTTCCTCTTTGATTAACGCTGCCTTCACATTTCAAGGTACT 900
501         GGGAGGTTCTTAGGTTGGGTTTCCTCTTTGATTAACGCTGCCTTCACATTTTAAGGTACT 900
502         GGGAGGTTCTTAGGTTGGGTTTCCTCTTTGATTAACGCTGCCTTCACATTTCAAGGTACT 900
503         GGGAGGTTCTTAGGTTGGGTTTCCTCTTTGATTAACGCTGCCTTCACATTTCAAGGTACT 899
504         GGGAGGTTCTTAGGTTGGGTTTCCTCTTTGATTAACGCTGCCTTCACATTTCAAGGTACT 900
505         GGGAGGTTCTTAGGTTGGGTTTCCTCTTTGATTAACGCTGCCTTCACATTTCAAGGTACT 900
506         GGGAGGTTCTTAGGTTGGGTTTCCTCTTTGATTAACGCTGCCTTCACATTTCAAGGTACT 900
507         GGGAGGTTCTTAGGTTGGGTTTCCTCTTTGATTAACGCTGCCTTCACATTTCAAGGTACT 900
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CAN1        GAACTAGTTGGTATCACTGCTGGTGAAGCTGCAAACCCCAGAAAATCCGTTCCAAGAGCC 960
500         GAACTAGTTGGTATCACTGCTGGTGAAGCTGCAAACCCCAGAAAATCCGTTCCAAGAGCC 960
501         GAACTAGTTGGTATCACTGCTGGTGAAGCTGCAAACCCCAGAAAATCCGTTCCAAGAGCC 960
502         GAACTAGTTGGTATCACTGCTGGTGAAGCTGCAAACCCCAGAAAATCCGTTCCAAGAGCC 960
503         GAACTAGTTGGTATCACTGCTGGTGAAGCTGCAAACCCCAGAAAATCCGTTCCAAGAGCC 959
504         GAACTAGTTGGTATCACTGCTGGTGAAGCTGCAAACCCCAGAAAATCCGTTCCAAGAGCC 960
505         GAACTAGTTGGTATCACTGCTGGTGAAGCTGCAAACCCCAGAAAATCCGTTCCAAGAGCC 960
506         GAACTAGTTGGTATCACTGCTGGTGAAGCTGCAAACCCCAGAAAATCCGTTCCAAGAGCC 960
507         TAACTAGTTGGTATCACTGCTGGTGAAGCTGCAAACCCCAGAAAATCCGTTCCAAGAGCC 960
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CAN1        ATCAAAAAAGTTGTTTTCCGTATCTTAACCTTCTACATTGGCTCTCTATTATTCATTGGA 1020
500         ATCAAAAAAGTTGTTTTCCGTATCTTAACCTTCTACATTGGCTCTCTATTATTCATTGGA 1020
501         ATCAAAAAAGTTGTTTTCCGTATCTTAACCTTCTACATTGGCTCTCTATTATTCATTGGA 1020
502         ATCAAAAAAGTTGTTTTCCGTATCTTAACCTTCTACATTGGCTCTCTATTATTCATTGGA 1020
503         ATCAAAAAAGTTGTTTTCCGTATCTTAACCTTCTACATTGGCTCTCTATTATTCATTGGA 1019
504         ATCAAAAAAGTTGTTTTCCGTATCTTAACCTTCTACATTGGCTCTCTATTATTCATTGGA 1020
505         ATCAAAAAAGTTGTTTTCCCTATCTTAACCTTCTACATTGGCTCTCTATTATTCATTGGA 1020
506         AT-AAAAAGGTTGTTTTCCGTATCTTAACCTTCTACATTGGCTCTCTATTATTCATTGGA 1019
507         ATCAAAAAAGTTGTTTTCCGTATCTTAACCTTCTACATTGGCTCTCTATTATTCATTGGA 1020
            ** ***** ********** ****************************************

CAN1        CTTTTAGTTCCATACAATGACCCTAAACTAACACAATCTACTTCCTACGTTTCTACTTCT 1080
500         CTTTTAGTTCCATACAATGACCCTAAACTAACACAATCTACTTCCTACGTTTCTACTTCT 1080
501         CTTTTAGTTCCATACAATGACCCTAAACTAACACAATCTACTTCCTACGTTTCTACTTCT 1080
502         CTTTTAGTTC-ATACAATGACCCTAAACTAACACAATCTACTTCCTACGTTTCTACTTCT 1079
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CAA → UAA (500)
Q STOP

−T (503)

CAA → UAA (501)
Q STOP

GAA → UAA (507)
E STOP

−C
AAA → AAG } (506)
K K

CGT → CCT (505)
R P

−C (502)

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503             CTTTTAGTTCCATACAATGACCCTAAACTAACACAATCTACTTCCTACGTTTCTACTTCT 1079
504             CTTTTAGTTCCATACAATGACCCTAAACTAACACAATCTACTTCCTACGTTTCTACTTCT 1080
505             CTTTTAGTTCCATACAATGACCCTAAACTAACACAATCTACTTCCTACGTTTCTACTTCT 1080
506             CTTTTAGTTCCATACAATGACCCTAAACTAACACAATCTACTTCCTACGTTTCTACTTCT 1079
507             CTTTTAGTTCCATACAATGACCCTAAACTAACACAATCTACTTCCTACGTTTCTACTTCT 1080
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CA              CCCTTTATTATTGCTATTGAGAACTCTGGTACAAAGGTTTTGCCACATATCTTCAACGCT 1140
500             CCCTTTATTATTGCTATTGAGAACTCTGGTACAAAGGTTTTGCCACATATCTTCAACGCT 1140
501             CCCTTTATTATTGCTATTGAGAACTCTGGTACAAAGGTTTTGCCACATATCTTCAACGCT 1140
502             CCCTTTATTATTGCTATTGAGAACTCTGGTACAAAGGTTTTGCCACATATCTTCAACGCT 1139
503             CCCTTTATTATTGCTATTGAGAACTCTGGTACAAAGGTTTTGCCACATATCTTCAACGCT 1139
504             CCCTTTATTATTGCTATTGAGAACTCTGGTACAAAGGTTTTGCCACATATCTTCAACGCT 1140
505             CCCTTTATTATTGCTATTGAGAACTCTGGTACAAAGGTTTTGCCACATATCTTCAACGCT 1140
506             CCCTTTATTATTGCTATTGAGAACTCTGGTACAAAGGTTTTGCCACATATCTTCAACGCT 1139
507             CCCTTTATTATTGCTATTGAGAACTCTGGTACAAAGGTTTTGCCACATATCTTCAACGCT 1140
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CAN1            GTTATCTTAACAACCATTATTTCTGCCGCAAATTCAAATATTTACGTTGGTTCCCGTATT 1200
500             GTTATCTTAACAACCATTATTTCTGCCGCAAATTCAAATATTTACGTTGGTTCCCGTATT 1200
501             GTTATCTTAACAACCATTATTTCTGCCGCAAATTCAAATATTTACGTTGGTTCCCGTATT 1200
502             GTTATCTTAACAACCATTATTTCTGCCGCAAATTCAAATATTTACGTTGGTTCCCGTATT 1199
503             GTTATCTTAACAACCATTATTTCTGCCGCAAATTCAAATATTTACGTTGGTTCCCGTATT 1199
504             GTTATCTTAACAACCATTATTTCTGCCGCAAATTCAAATATTTACGTTGGTTCCCGTATT 1200
505             GTTATCTTAACAACCATTATTTCTGCCGCAAATTCAAATATTTACGTTGGTTCCCGTATT 1200
506             GTTATCTTAACAACCATTATTTCTGCCGCAAATTCAAATATTTACGTTGGTTCCCGTATT 1199
507             GTTATCTTAACAACCATTATTTCTGCCGCAAATTCAAATATTTACGTTGGTTCCCGTATT 1200
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CAN1            TTATTTGGTCTATCAAAGAACAAGTTGGCTCCTAAATTCCTGTCAAGGACCACCAAAGGT 1260
500             TTATTTGGTCTATCAAAGAACAAGTTGGCTCCTAAATTCCTGTCAAGGACCACCAAAGGT 1260
501             TTATTTGGTCTATCAAAGAACAAGTTGGCTCCTAAATTCCTGTCAAGGACCACCAAAGGT 1260
502             TTATTTGGTCTATCAAAGAACAAGTTGGCTCCTAAATTCCTGTCAAGGACCACCAAAGGT 1259
503             TTATTTGGTCTATCAAAGAACAAGTTGGCTCCTAAATTCCTGTCAAGGACCACCAAAGGT 1259
504             TTATTTGGTCTATCAAAGAACAAGTTGGCTCCTAAATTCCTGTCAAGGACCACCAAAGGT 1260
505             TTATTTGGTCTATCAAAGAACAAGTTGGCTCCTAAATTCCTGTCAAGGACCACCAAAGGT 1260
506             TTATTTGGTCTATCAAAGAACAAGTTGGCTCCTAAATTCCTGTCAAGGACCACCAAAGGT 1259
507             TTATTTGGTCTATCAAAGAACAAGTTGGCTCCTAAATTCCTGTCAAGGACCACCAAAGGT 1260
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CAN1            GGTGTTCCATACATTGCAGTTTTCGTTACTGCTGCATTTGGCGCTTTGGCTTACATGGAG 1320
500             GGTGTTCCATACATTGCAGTTTTCGTTACTGCTGCATTTGGCGCTTTGGCTTACATGGAG 1320
501             GGTGTTCCATACATTGCAGTTTTCGTTACTGCTGCATTTGGCGCTTTGGCTTACATGGAG 1320
502             GGTGTTCCATACATTGCAGTTTTCGTTACTGCTGCATTTGGCGCTTTGGCTTACATGGAG 1319
503             GGTGTTCCATACATTGCAGTTTTCGTTACTGCTGCATTTGGCGCTTTGGCTTACATGGAG 1319
504             GGTGTTCCATACATTGCAGTTTTCGTTACTGCTGCATTTGGCGCTTTGGCTTACATGGAG 1320
505             GGTGTTCCATACATTGCAGTTTTCGTTACTGCTGCATTTGGCGCTTTGGCTTACATGGAG 1320
506             GGTGTTCCATACATTGCAGTTTTCGTTACTGCTGCATTTGGCGCTTTGGCTTACATGGAG 1319
507             GGTGTTCCATACATTGCAGTTTTCGTTACTGCTGCATTTGGCGCTTTGGCTTACATGGAG 1320
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CA              ACATCTACTGGTGGTGACAAAGTTTTCGAATGGCTATTAAATATCACTGGTGTTGCAGGC 1380
500             ACATCTACTGGTGGTGACAAAGTTTTCGAATGGCTATTAAATATCACTGGTGTTGCAGGC 1380
501             ACATCTACTGGTGGTGACAAAGTTTTCGAATGGCTATTAAATATCACTGGTGTTGCAGGC 1380
502             ACATCTACTGGTGGTGACAAAGTTTTCGAATGGCTATTAAATATCACTGGTGTTGCAGGC 1379
503             ACATCTACTGGTGGTGACAAAGTTTTCGAATGGCTATTAAATATCACTGGTGTTGCAGGC 1379
504             ACATCTACTGGTGGTGACAAAGTTTTCGAATGGCTATTAAATATCACTGGTGTTGCAGGC 1380
505             ACATCTACTGGTGGTGACAAAGTTTTCGAATGGCTATTAAATATCACTGGTGTTGCAGGC 1380
506             ACATCTACTGGTGGTGACAAAGTTTTCGAATGGCTATTAAATATCACTGGTGTTGCAGGC 1379
507             ACATCTACTGGTGGTGACAAAGTTTTCGAATGGCTATTAAATATCACTGGTGTTGCAGGC 1380
                ************************************************************

CAN1            TTTTTTGCATGGTTATTTATCTCAATCTCGCACATCAGATTTATGCAAGCTTTGAAATAC 1440
500             TTTTTTGCATGGTTATTTATCTCAATCTCGCACATCAGATTTATGCAAGCTTTGAAATAC 1440
501             TTTTTTGCATGGTTATTTATCTCAATCTCGCACATCAGATTTATGCAAGCTTTGAAATAC 1440
502             TTTTTTGCATGGTTATTTATCTCAATCTCGCACATCAGATTTATGCAAGCTTTGAAATAC 1439
503             TTTTTTGCATGGTTATTTATCTCAATCTCGCACATCAGATTTATGCAAGCTTTGAAATAC 1439
504             TTTTTTGCATGGTTATTTATCTCAATCTCGCACATCAGATTTATGCAAGCTTTGAAATAC 1440
505             TTTTTTGCATGGTTATTTATCTCAATCTCGCACATCAGATTTATGCAAGCTTTGAAATAC 1440
506             TTTTTTGCATGGTTATTTATCTCAATCTCGCACATCAGATTTATGCAAGCTTTGAAATAC 1439
507             TTTTTTGCATGGTTATTTATCTCAATCTCGCACATCAGATTTATGCAAGCTTTGAAATAC 1440
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CAN1            CGTGGCATCTCTCGTGACGAGTTACCATTTAAAGCTAAATTAATGCCCGGCTTGGCTTAT 1500
500             CGTGGCATCTCTCGTGACGAGTTACCATTTAAAGCTAAATTAATGCCCGGCTTGGCTTAT 1500
501             CGTGGCATCTCTCGTGACGAGTTACCATTTAAAGCTAAATTAATGCCCGGCTTGGCTTAT 1500
502             CGTGGCATCTCTCGTGACGAGTTACCATTTAAAGCTAAATTAATGCCCGGCTTGGCTTAT 1499
503             CGTGGCATCTCTCGTGACGAGTTACCATTTAAAGCTAAATTAATGCCCGGCTTGGCTTAT 1499
504             CGTGGCATCTCTCGTGACGAGTTACCATTTAAAGCTAAATTAATGCCCGGCTTGGCTTAT 1500
505             CGTGGCATCTCTCGTGACGAGTTACCATTTAAAGCTAAATTAATGCCCGGCTTGGCTTAT 1500
506             CGTGGCATCTCTCGTGACGAGTTACCATTTAAAGCTAAATTAATGCCCGGCTTGGCTTAT 1499
507             CGTGGCATCTCTCGTGACGAGTTACCATTTAAAGCTAAATTAATGCCCGGCTTGGCTTAT 1500
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CAN1            TATGCGGCCACATTTATGACGATCATTATCATTATTCAAGGTTTCACGGCTTTTGCACCA 1560
500             TATGCGGCCACATTTATGACGATCATTATCATTATTCAAGGTTTCACGGCTTTTGCACCA 1560
501             TATGCGGCCACATTTATGACGATCATTATCATTATTCAAGGTTTCACGGCTTTTGCACCA 1560
502             TATGCGGCCACATTTATGACGATCATTATCATTATTCAAGGTTTCACGGCTTTTGCACCA 1559
503             TATGCGGCCACATTTATGACGATCATTATCATTATTCAAGGTTTCACGGCTTTTGCACCA 1559
504             TATGCGGCCACATTTATGACGATCATTATCATTATTCAAGGTTTCACGGCTTTTGCAC-A 1559
505             TATGCGGCCACATTTATGACGATCATTATCATTATTCAAGGTTTCACGGCTTTTGCACCA 1560
506             TATGCGGCCACATTTATGACGATCATTATCATTATTCAAGGTTTCACGGCTTTTGCACCA 1559
507             TATGCGGCCACATTTATGACGATCATTATCATTATTCAAGGTTTCACGGCTTTTGCACCA 1560
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CAN1            AAATTCAATGGTGTTAGCTTTGCTGCCGCCTATATCTCTATTTTCCTGTTCTTAGCTGTT 1620
500             AAATTCAATGGTGTTAGCTTTGCTGCCGCCTATATCTCTATTTTCCTGTTCTTAGCTGTT 1620
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-C (504)

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501             AAATTCAATGGTGTTAGCTTTGCTGCCCGCCTATATCTCTATTTTCCTGTTCTTAGCTGTT 1620
502             AAATTCAATGGTGTTAGCTTTGCTGCCCGCCTATATCTCTATTTTCCTGTTCTTAGCTGTT 1619
503             AAATTCAATGGTGTTAGCTTTGCTGCCCGCCTATATCTCTATTTTCCTGTTCTTAGCTGTT 1619
504             AAATTCAATGGTGTTAGCTTTGCTGCCCGCCTATATCTCTATTTTCCTGTTCTTAGCTGTT 1619
505             AAATTCAATGGTGTTAGCTTTGCTGCCCGCCTATATCTCTATTTTCCTGTTCTTAGCTGTT 1620
506             AAATTCAATGGTGTTAGCTTTGCTGCCCGCCTATATCTCTATTTTCCTGTTCTTAGCTGTT 1619
507             AAATTCAATGGTGTTAGCTTTGCTGCCCGCCTATATCTCTATTTTCCTGTTCTTAGCTGTT 1620
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CAN1            TGGATCTTATTTCAATGCATATTCAGATGCAGATTTATTTGGAAGATTGGAGATGTCGAC 1680
500             TGGATCTTATTTCAATGCATATTCAGATGCAGATTTATTTGGAAGATTGGAGATGTCGAC 1680
501             TGGATCTTATTTCAATGCATATTCAGATGCAGATTTATTTGGAAGATTGGAGATGTCGAC 1680
502             TGGATCTTATTTCAATGCATATTCAGATGCAGATTTATTTGGAAGATTGGAGATGTCGAC 1679
503             TGGATCTTATTTCAATGCATATTCAGATGCAGATTTATTTGGAAGATTGGAGATGTCGAC 1679
504             TGGATCTTATTTCAATGCATATTCAGATGCAGATTTATTTGGAAGATTGGAGATGTCGAC 1679
505             TGGATCTTATTTCAATGCATATTCAGATGCAGATTTATTTGGAAGATTGGAGATGTCGAC 1680
506             TGGATCTTATTTCAATGCATATTCAGATGCAGATTTATTTGGAAGATTGGAGATGTCGAC 1679
507             TGGATCTTATTTCAATGCATATTCAGATGCAGATTTATTTGGAAGATTGGAGATGTCGAC 1680
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CAN1            ATCGATTCCGATAGAAGAGACATTGAGGCAATTGTATGGGAAGATCATGAACCAAAGACT 1740
500             ATCGATTCCGATAGAAGAGACATTGAGGCAATTGTATGGGAAGATCATGAACCAAAGACT 1740
501             ATCGATTCCGATAGAAGAGACATTGAGGCAATTGTATGGGAAGATCATGAACCAAAGACT 1740
502             ATCGATTCCGATAGAAGAGACATTGAGGCAATTGTATGGGAAGATCATGAACCAAAGACT 1739
503             ATCGATTCCGATAGAAGAGACATTGAGGCAATTGTATGGGAAGATCATGAACCAAAGACT 1739
504             ATCGATTCCGATAGAAGAGACATTGAGGCAATTGTATGGGAAGATCATGAACCAAAGACT 1739
505             ATCGATTCCGATAGAAGAGACATTGAGGCAATTGTATGGGAAGATCATGAACCAAAGACT 1740
506             ATCGATTCCGATAGAAGAGACATTGAGGCAATTGTATGGGAAGATCATGAACCAAAGACT 1739
507             ATCGATTCCGATAGAAGAGACATTGAGGCAATTGTATGGGAAGATCATGAACCAAAGACT 1740
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CAN1            TTTTGGGACAAATTTTGGAATGTTGTAGCATAG 1773
500             TTTTGGGACAAATTTTGGAATGTTGTAGCATAG 1773
501             TTTTGGGACAAATTTTGGAATGTTGTAGCATAG 1773
502             TTTTGGGACAAATTTTGGAATGTTGTAGCATAG 1772
503             TTTTGGGACAAATTTTGGAATGTTGTAGCATAG 1772
504             TTTTGGGACAAATTTTGGAATGTTGTAGCATAG 1772
505             TTTTGGGACAAATTTTGGAATGTTGTAGCATAG 1773
506             TTTTGGGACAAATTTTGGAATGTTGTAGCATAG 1772
507             TTTTGGGACAAATTTTGGAATGTTGTAGCATAG 1773
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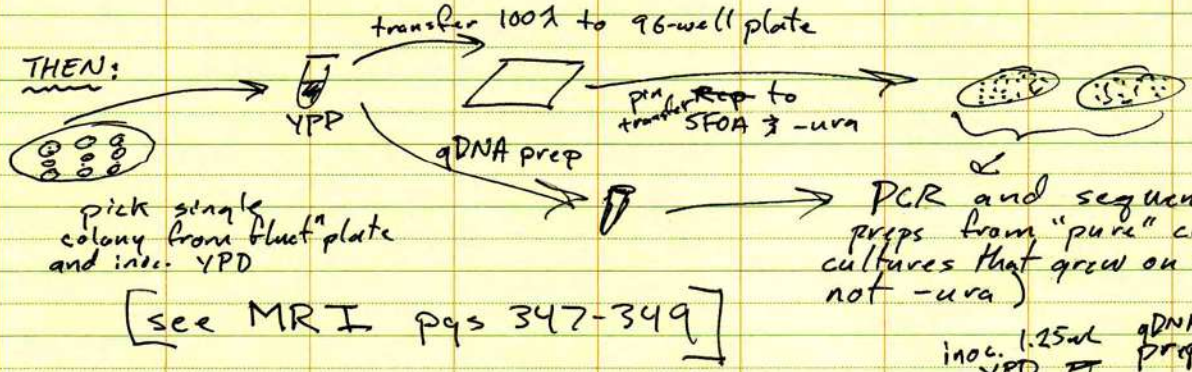
5/25

Analysis of sequencing results

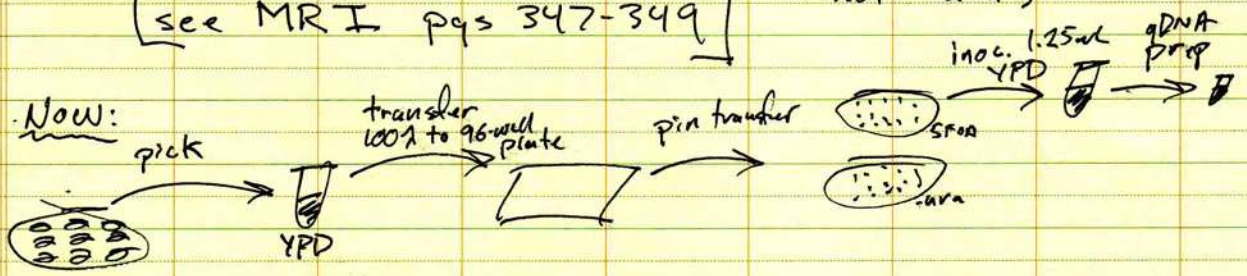
- The sequencing worked in that I got at least 2× coverage across each ura3 and can1 allele.
- One ura3 allele (804) ~~was~~ did not contain a coding mutation – nor a mutation in ~200bp upstream ± ~100 bp downstream.
  - ↳ in addition notice that 804 is a putative 5FOA^R uracil prototroph (p77).

From ~~my~~ my dataset of 144 5FOA^R ura3 alleles (see) I found only one WT URA3 allele leading me to claim that over 99% of 5FOA^R mutations are URA3 mutants. However I am starting to question that claim.

It will help to illustrate how I selected colonies to sequence then ~~and~~ (12/9/04) and now

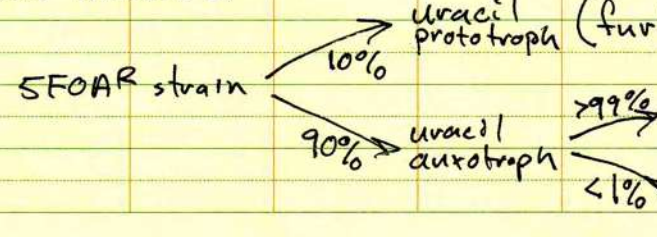


[see MRI pgs 347-349]



- When I first did the sequencing I was in effect selecting out the 5FOA^R uracil prototrophs, assuming that they were not real but were just "unpure" cultures.
- Looking at MRI pgs 347-349 I see that 25/237 strains showed growth on both 5FOA and -ura. This is the same proportion I see now: 24/240 (p 76377). Before I ignored them and did not sequence any — this time I will and I expect them to be WT URA3. ~~like a~~

NEW THOUGHTS:



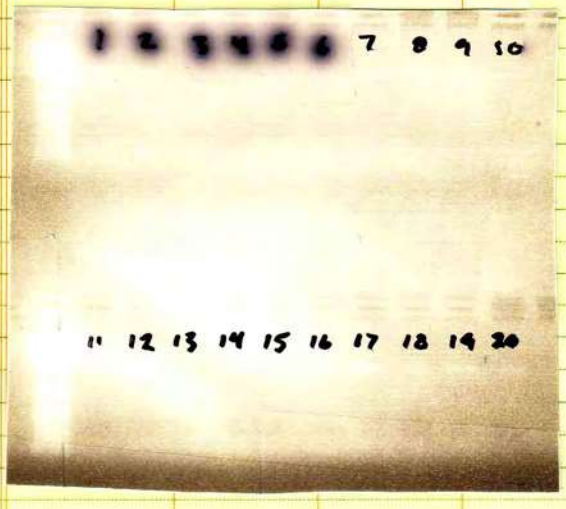
5/26

PCRs

- On tuesday (5/23) I set up the CAN1 PCR in a 96-well format.
  - 1 λ ~~protein~~ gDNA in each well
  - 49 λ of PCR mix in each well using multichannel
  - omitted template for 500-507 (already sequenced)
  - vortex well before PCR
  - aluminum plate seal
- On tuesday I put 1 λ gDNA temp. in 96-well plates for URA3 PCRs
  - spin down
  - store in -20
  - no temp added for 801-812
- On ~~Thursday~~ Wednesday (5/24) I added the PCR mix and ran the URA3 PCRs.
  - I noticed yesterday (5/25) that one well of the liquid had escaped,
    ↳ # 24 → I will redo #24 and the adjacent wells (23, 35, 36)
    ↳ reran these 4 on 5/26

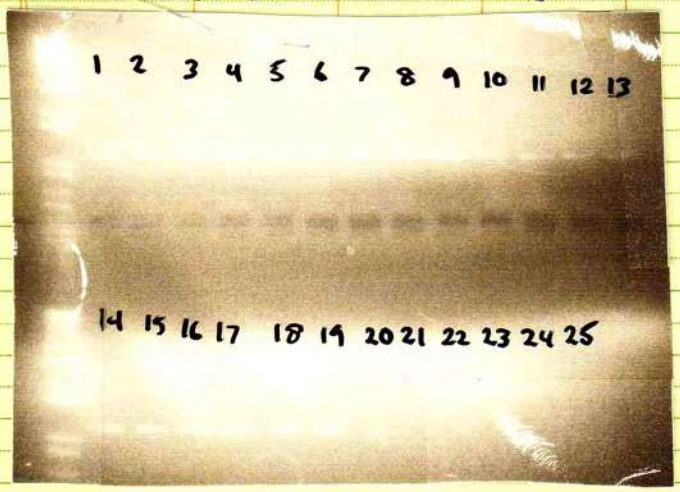
VERIFY CAN1 PCR

Run 2 λ from the last well of each row of the CAN1 plates



~~LANE#~~	STRAIN#	LANE#	STRAIN#
1	511	11	631
2	523	12	643
3	535	13	~~645~~ 655
4	547	14	~~657~~ 667
5	559	15	~~669~~ 679
6	571	16	~~681~~ 691
7	583	17	~~693~~ ~~693~~ 703
8	595	18	~~705~~ 715
9	607	19	~~717~~ 727
10	619	20	~~729~~ 739

5/27 VERIFY URA3 PCRS



LANE #	STRAIN#	LANE#	STRAIN#	
1	824	14	980	
2	836	15	992	
3	848	16	1004	
4	~~8~~860	17	1016	
5	872	18	1028	
6	884	19	1040	
7	896	20	807	there was no temp in these lanes
8	908	21	808	
9	920	22	809	
10	932	23	810	
11	944	24	811	
12	956	25	812	
13	968			

5/26

PCR PURIFICATIONS

- On Wed (5/24) I started the PCR purification ~~of th~~ using the Qiagen PCR kit → eluted in 100 λ H_2O
- Purified samples put in -20 freezer until I am ready to setup sequencing.
- On Wed I was able to prep 505-657 → run 5λ on gel:

← Bands are faint (only ran 5λ)

} here I ran 5 λ of PCR rxn from the rows w/o template (corresponding to 500-507) - this was to see if PCRs contaminate adjacent wells.

5/27

- Yesterday I ~~acc~~ prepped 658-687 - I accidentally eluted in ~~250~~ 500λ instead of 100λ - I am not sure if I should redo these or test them to see if they will sequence.

NUMBERING

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

PLATE #1

		1	2	3	4	5	6	7	8	9	10	11	12
								CAN1ext/intF1	CAN1extR2	CAN1intF2	CAN1intF3	CAN1intR2	CAN1intR3
URA3extF3	A	801	802	803	804	805	806	500	500	500	500	500	500
	B	807	808	809	810	811	812	501	501	501	501	501	501
URA3extR	C	801	802	803	804	805	806	502	502	502	502	502	502
	D	807	808	809	810	811	812	503	503	503	503	503	503
URA3intF2	E	801	802	803	804	805	806	504	504	504	504	504	504
	F	807	808	809	810	811	812	505	505	505	505	505	505
URA3intR2	G	801	802	803	804	805	806	506	506	506	506	506	506
	H	807	808	809	810	811	812	507	507	507	507	507	507

PLATE #2

		1	2	3	4	5	6	7	8	9	10	11	12
								CAN1ext/intF1	CAN1extR2	CAN1intF2	CAN1intF3	CAN1intR2	CAN1intR3
URA3extF3	A	813	814	815	816	817	818	508	508	508	508	508	508
	B	819	820	821	822	823	824	509	509	509	509	509	509
URA3extR	C	813	814	815	816	817	818	510	510	510	510	510	510
	D	819	820	821	822	823	824	511	511	511	511	511	511
URA3intF2	E	813	814	815	816	817	818	512	512	512	512	512	512
	F	819	820	821	822	823	824	513	513	513	513	513	513
URA3intR2	G	813	814	815	816	817	818	658	658	658	658	658	658
	H	819	820	821	822	823	824	659	659	659	659	659	659

PLATE #3

		1	2	3	4	5	6	7	8	9	10	11	12
URA3extF3	A	825	826	827	828	829	830	831	832	833	834	835	836
	B	837	838	839	840	841	842	843	844	845	846	847	848
URA3extR	C	825	826	827	828	829	830	831	832	833	834	835	836
	D	837	838	839	840	841	842	843	844	845	846	847	848
URA3intF2	E	825	826	827	828	829	830	831	832	833	834	835	836
	F	837	838	839	840	841	842	843	844	845	846	847	848
URA3intR2	G	825	826	827	828	829	830	831	832	833	834	835	836
	H	837	838	839	840	841	842	843	844	845	846	847	848

PLATE #4

		1	2	3	4	5	6	7	8	9	10	11	12
URA3extF3	A	849	850	851	852	853	854	855	856	857	858	859	860
	B	861	862	863	864	865	866	867	868	869	870	871	872
URA3extR	C	849	850	851	852	853	854	855	856	857	858	859	860
	D	861	862	863	864	865	866	867	868	869	870	871	872
URA3intF2	E	849	850	851	852	853	854	855	856	857	858	859	860
	F	861	862	863	864	865	866	867	868	869	870	871	872
URA3intR2	G	849	850	851	852	853	854	855	856	857	858	859	860
	H	861	862	863	864	865	866	867	868	869	870	871	872

PLATE #5

		1	2	3	4	5	6	7	8	9	10	11	12
URA3extF3	A	873	874	875	876	877	878	879	880	881	882	883	884
	B	885	886	887	888	889	890	891	892	893	894	895	896
URA3extR	C	873	874	875	876	877	878	879	880	881	882	883	884
	D	885	886	887	888	889	890	891	892	893	894	895	896
URA3intF2	E	873	874	875	876	877	878	879	880	881	882	883	884
	F	885	886	887	888	889	890	891	892	893	894	895	896
URA3intR2	G	873	874	875	876	877	878	879	880	881	882	883	884
	H	885	886	887	888	889	890	891	892	893	894	895	896

PLATE #6

		1	2	3	4	5	6	7	8	9	10	11	12
URA3extF3	A	897	898	899	900	901	902	903	904	905	906	907	908
	B	909	910	911	912	913	914	915	916	917	918	919	920
URA3extR	C	897	898	899	900	901	902	903	904	905	906	907	908
	D	909	910	911	912	913	914	915	916	917	918	919	920
URA3intF2	E	897	898	899	900	901	902	903	904	905	906	907	908
	F	909	910	911	912	913	914	915	916	917	918	919	920
URA3intR2	G	897	898	899	900	901	902	903	904	905	906	907	908
	H	909	910	911	912	913	914	915	916	917	918	919	920

PLATE #7

		1	2	3	4	5	6	7	8	9	10	11	12
URA3extF3	A	921	922	923	924	925	926	927	928	929	930	931	932
	B	933	934	935	936	937	938	939	940	941	942	943	944
URA3extR	C	921	922	923	924	925	926	927	928	929	930	931	932
	D	933	934	935	936	937	938	939	940	941	942	943	944
URA3intF2	E	921	922	923	924	925	926	927	928	929	930	931	932
	F	933	934	935	936	937	938	939	940	941	942	943	944
URA3intR2	G	921	922	923	924	925	926	927	928	929	930	931	932
	H	933	934	935	936	937	938	939	940	941	942	943	944

PLATE #8

		1	2	3	4	5	6	7	8	9	10	11	12
URA3extF3	A	945	946	947	948	949	950	951	952	953	954	955	956
	B	957	958	959	960	961	962	963	964	965	966	967	968
URA3extR	C	945	946	947	948	949	950	951	952	953	954	955	956
	D	957	958	959	960	961	962	963	964	965	966	967	968
URA3intF2	E	945	946	947	948	949	950	951	952	953	954	955	956
	F	957	958	959	960	961	962	963	964	965	966	967	968
URA3intR2	G	945	946	947	948	949	950	951	952	953	954	955	956
	H	957	958	959	960	961	962	963	964	965	966	967	968

PLATE #9

		1	2	3	4	5	6	7	8	9	10	11	12
URA3extF3	A	969	970	971	972	973	974	975	976	977	978	979	980
	B	981	982	983	984	985	986	987	988	989	990	991	992
URA3extR	C	969	970	971	972	973	974	975	976	977	978	979	980
	D	981	982	983	984	985	986	987	988	989	990	991	992
URA3intF2	E	969	970	971	972	973	974	975	976	977	978	979	980
	F	981	982	983	984	985	986	987	988	989	990	991	992
URA3intR2	G	969	970	971	972	973	974	975	976	977	978	979	980
	H	981	982	983	984	985	986	987	988	989	990	991	992

PLATE #10

		1	2	3	4	5	6	7	8	9	10	11	12
URA3extF3	A	993	994	995	996	997	998	999	1000	1001	1002	1003	1004
	B	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016
URA3extR	C	993	994	995	996	997	998	999	1000	1001	1002	1003	1004
	D	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016
URA3intF2	E	993	994	995	996	997	998	999	1000	1001	1002	1003	1004
	F	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016
URA3intR2	G	993	994	995	996	997	998	999	1000	1001	1002	1003	1004
	H	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016

PLATE #11

		1	2	3	4	5	6	7	8	9	10	11	12
URA3extF3	A	1017	1018	1019	1020	1021	1022	1023	1024	1025	1026	1027	1028
	B	1029	1030	1031	1032	1033	1034	1035	1036	1037	1038	1039	1040
URA3extR	C	1017	1018	1019	1020	1021	1022	1023	1024	1025	1026	1027	1028
	D	1029	1030	1031	1032	1033	1034	1035	1036	1037	1038	1039	1040
URA3intF2	E	1017	1018	1019	1020	1021	1022	1023	1024	1025	1026	1027	1028
	F	1029	1030	1031	1032	1033	1034	1035	1036	1037	1038	1039	1040
URA3intR2	G	1017	1018	1019	1020	1021	1022	1023	1024	1025	1026	1027	1028
	H	1029	1030	1031	1032	1033	1034	1035	1036	1037	1038	1039	1040

1

PLATE #12

	1	2	3	4	5	6	7	8	9	10	11	12
	CAN1ext/intF1		CAN1extR2		CAN1intF2		CAN1intF3		CAN1intR2		CAN1intR3	
A	516	524	516	524	516	524	516	524	516	524	516	524
B	517	525	517	525	517	525	517	525	517	525	517	525
C	518	526	518	526	518	526	518	526	518	526	518	526
D	519	527	519	527	519	527	519	527	519	527	519	527
E	520	528	520	528	520	528	520	528	520	528	520	528
F	521	529	521	529	521	529	521	529	521	529	521	529
G	522	530	522	530	522	530	522	530	522	530	522	530
H	523	531	523	531	523	531	523	531	523	531	523	531

2

PLATE #13

	1	2	3	4	5	6	7	8	9	10	11	12
A	532	540	532	540	532	540	532	540	532	540	532	540
B	533	541	533	541	533	541	533	541	533	541	533	541
C	534	542	534	542	534	542	534	542	534	542	534	542
D	535	543	535	543	535	543	535	543	535	543	535	543
E	536	544	536	544	536	544	536	544	536	544	536	544
F	537	545	537	545	537	545	537	545	537	545	537	545
G	538	546	538	546	538	546	538	546	538	546	538	546
H	539	547	539	547	539	547	539	547	539	547	539	547

3

PLATE #14

	1	2	3	4	5	6	7	8	9	10	11	12
A	548	556	548	556	548	556	548	556	548	556	548	556
B	549	557	549	557	549	557	549	557	549	557	549	557
C	550	558	550	558	550	558	550	558	550	558	550	558
D	551	559	551	559	551	559	551	559	551	559	551	559
E	552	560	552	560	552	560	552	560	552	560	552	560
F	553	561	553	561	553	561	553	561	553	561	553	561
G	554	562	554	562	554	562	554	562	554	562	554	562
H	555	563	555	563	555	563	555	563	555	563	555	563

4

PLATE #15

	1	2	3	4	5	6	7	8	9	10	11	12
	CAN1ext/intF1		CAN1extR2		CAN1intF2		CAN1intF3		CAN1intR2		CAN1intR3	
A	564	572	564	572	564	572	564	572	564	572	564	572
B	565	573	565	573	565	573	565	573	565	573	565	573
C	566	574	566	574	566	574	566	574	566	574	566	574
D	567	575	567	575	567	575	567	575	567	575	567	575
E	568	576	568	576	568	576	568	576	568	576	568	576
F	569	577	569	577	569	577	569	577	569	577	569	577
G	570	578	570	578	570	578	570	578	570	578	570	578
H	571	579	571	579	571	579	571	579	571	579	571	579

5

PLATE #16

	1	2	3	4	5	6	7	8	9	10	11	12
A	580	588	580	588	580	588	580	588	580	588	580	588
B	581	589	581	589	581	589	581	589	581	589	581	589
C	582	590	582	590	582	590	582	590	582	590	582	590
D	583	591	583	591	583	591	583	591	583	591	583	591
E	584	592	584	592	584	592	584	592	584	592	584	592
F	585	593	585	593	585	593	585	593	585	593	585	593
G	586	594	586	594	586	594	586	594	586	594	586	594
H	587	595	587	595	587	595	587	595	587	595	587	595

6

PLATE #17

	1	2	3	4	5	6	7	8	9	10	11	12
A	596	604	596	604	596	604	596	604	596	604	596	604
B	597	605	597	605	597	605	597	605	597	605	597	605
C	598	606	598	606	598	606	598	606	598	606	598	606
D	599	607	599	607	599	607	599	607	599	607	599	607
E	600	608	600	608	600	608	600	608	600	608	600	608
F	601	609	601	609	601	609	601	609	601	609	601	609
G	602	610	602	610	602	610	602	610	602	610	602	610
H	603	611	603	611	603	611	603	611	603	611	603	611

7

PLATE #18

	1 CAN1ext/intF1	2	3 CAN1extR2	4	5 CAN1intF2	6	7 CAN1intF3	8	9 CAN1intR2	10	11 CAN1intR3	12
A	612	620	612	620	612	620	612	620	612	620	612	620
B	613	621	613	621	613	621	613	621	613	621	613	621
C	614	622	614	622	614	622	614	622	614	622	614	622
D	615	623	615	623	615	623	615	623	615	623	615	623
E	616	624	616	624	616	624	616	624	616	624	616	624
F	617	625	617	625	617	625	617	625	617	625	617	625
G	618	626	618	626	618	626	618	626	618	626	618	626
H	619	627	619	627	619	627	619	627	619	627	619	627

8

PLATE #19

	1	2	3	4	5	6	7	8	9	10	11	12
A	628	636	628	636	628	636	628	636	628	636	628	636
B	629	637	629	637	629	637	629	637	629	637	629	637
C	630	638	630	638	630	638	630	638	630	638	630	638
D	631	639	631	639	631	639	631	639	631	639	631	639
E	632	640	632	640	632	640	632	640	632	640	632	640
F	633	641	633	641	633	641	633	641	633	641	633	641
G	634	642	634	642	634	642	634	642	634	642	634	642
H	635	643	635	643	635	643	635	643	635	643	635	643

9

PLATE #20

	1	2	3	4	5	6	7	8	9	10	11	12
A	644	652	644	652	644	652	644	652	644	652	644	652
B	645	653	645	653	645	653	645	653	645	653	645	653
C	646	654	646	654	646	654	646	654	646	654	646	654
D	647	655	647	655	647	655	647	655	647	655	647	655
E	648	656	648	656	648	656	648	656	648	656	648	656
F	649	657	649	657	649	657	649	657	649	657	649	657
G	650	514	650	514	650	514	650	514	650	514	650	514
H	651	515	651	515	651	515	651	515	651	515	651	515

10

PLATE #21

	1 CAN1ext/intF1	2	3 CAN1extR2	4	5 CAN1intF2	6	7 CAN1intF3	8	9 CAN1intR2	10	11 CAN1intR3	12
A	660	668	660	668	660	668	660	668	660	668	660	668
B	661	669	661	669	661	669	661	669	661	669	661	669
C	662	670	662	670	662	670	662	670	662	670	662	670
D	663	671	663	671	663	671	663	671	663	671	663	671
E	664	672	664	672	664	672	664	672	664	672	664	672
F	665	673	665	673	665	673	665	673	665	673	665	673
G	666	674	666	674	666	674	666	674	666	674	666	674
H	667	675	667	675	667	675	667	675	667	675	667	675

11

PLATE #22

	1	2	3	4	5	6	7	8	9	10	11	12
A	676	684	676	684	676	684	676	684	676	684	676	684
B	677	685	677	685	677	685	677	685	677	685	677	685
C	678	686	678	686	678	686	678	686	678	686	678	686
D	679	687	679	687	679	687	679	687	679	687	679	687
E	680	688	680	688	680	688	680	688	680	688	680	688
F	681	689	681	689	681	689	681	689	681	689	681	689
G	682	690	682	690	682	690	682	690	682	690	682	690
H	683	691	683	691	683	691	683	691	683	691	683	691

12

PLATE #23

	1	2	3	4	5	6	7	8	9	10	11	12
A	692	700	692	700	692	700	692	700	692	700	692	700
B	693	701	693	701	693	701	693	701	693	701	693	701
C	694	702	694	702	694	702	694	702	694	702	694	702
D	695	703	695	703	695	703	695	703	695	703	695	703
E	696	704	696	704	696	704	696	704	696	704	696	704
F	697	705	697	705	697	705	697	705	697	705	697	705
G	698	706	698	706	698	706	698	706	698	706	698	706
H	699	707	699	707	699	707	699	707	699	707	699	707

13

PLATE #24

	1	2	3	4	5	6	7	8	9	10	11	12
	CAN1ext/intF1		CAN1extR2		CAN1intF2		CAN1intF3		CAN1intR2		CAN1intR3	
A	708	716	708	716	708	716	708	716	708	716	708	716
B	709	717	709	717	709	717	709	717	709	717	709	717
C	710	718	710	718	710	718	710	718	710	718	710	718
D	711	719	711	719	711	719	711	719	711	719	711	719
E	712	720	712	720	712	720	712	720	712	720	712	720
F	713	721	713	721	713	721	713	721	713	721	713	721
G	714	722	714	722	714	722	714	722	714	722	714	722
H	715	723	715	723	715	723	715	723	715	723	715	723

14

PLATE #25

	1	2	3	4	5	6	7	8	9	10	11	12
A	724	732	724	732	724	732	724	732	724	732	724	732
B	725	733	725	733	725	733	725	733	725	733	725	733
C	726	734	726	734	726	734	726	734	726	734	726	734
D	727	735	727	735	727	735	727	735	727	735	727	735
E	728	736	728	736	728	736	728	736	728	736	728	736
F	729	737	729	737	729	737	729	737	729	737	729	737
G	730	738	730	738	730	738	730	738	730	738	730	738
H	731	739	731	739	731	739	731	739	731	739	731	739

6/19

SEQUENCING OF FUR1 ALLELES FROM URA3 WT STRAINS

- except 982, 999, & 1013
- 1024 is only uracil auxotroph.

6/26

PREPAIRING TO SELECT FOR SUPRESSORS

- Ann created a URA3Δ strain by transforming GIL104 with the ura3Δ cassette amplified from BY4741.
- She sequenced the Δ and found that the region between the HindIII site (218 bp upstream from ATG) and the XmaI site (75 bp downstream from TAA) is deleted

CLONING URA3 INTO THE MCS OF pRS414 & pRS415

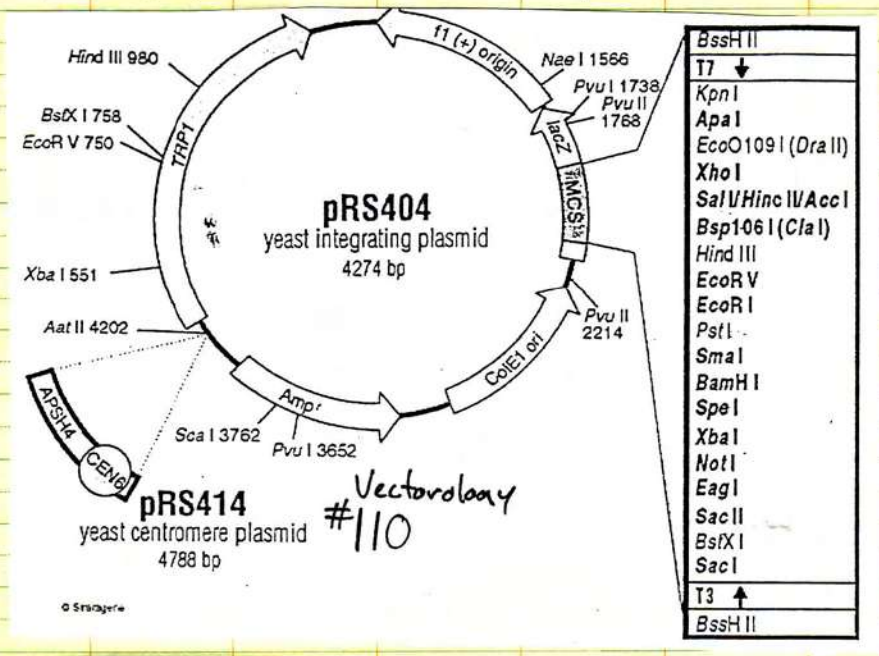
- Amplify WT URA3 from gDNA preps 804 & 815 using:

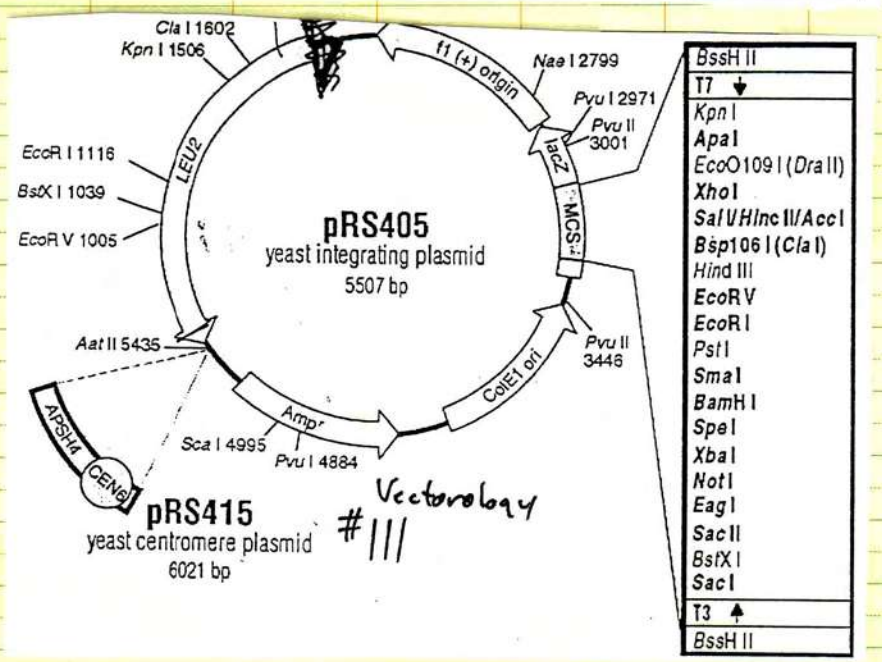
23377653 IDT
V.INDJEIAN
25005335 6/16/2006
URA3extF5_XhoI
5'- CAA TTA ATA CTC GAG AAG AAG AGT ATT GAG AAG
Tm= 57.9 °C , MW= 10,925.2
8.2 OD_{260} 21.90 nmol 0.24 mg

23377654 IDT
V.INDJEIAN
25005336 6/16/2006
URA3extR5_SpeI
5'- CAT AAT TAG ACT AGT ATA CGC CAG TAC ACC TTA
Tm= 57.4 °C , MW= 10,658.0
11.2 OD_{260} 32.30 nmol 0.34 mg

PCR:

Anneal: 54°
Extension: 2 m

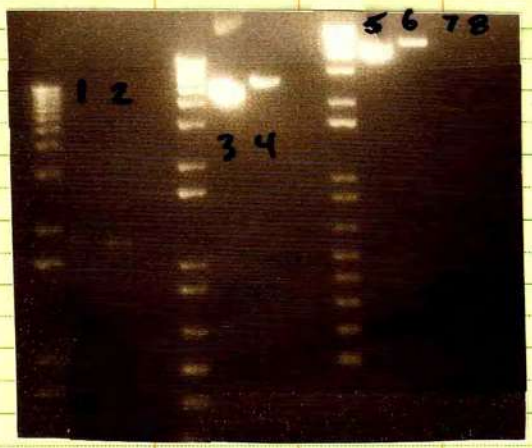




- Cut Purified PCRs and Plasmids w/ XhoI and SpeI.

Vectors		PCRs	
40.5	H_2O	43.5 λ	PCR
3 λ	Vector	5 λ	NEB 2
5 λ	NEB 2	.5 λ	BSA
.5 λ	BSA	.5 λ	SpeI
.5 λ	SpeI	.5 λ	XhoI
.5 λ	XhoI	50	
50			

- After digestion → heat kill enzymes → CIP vectors.
- Spin column purification for PCRs
- Gel purify vectors
- Ligate → transform Chemically comp. cells { 110 + 804, 110 + 815, 111 + 804, 111 + 815



LANE

1	PCR on 804
2	PCR on 815
3	110 double digested
4	111 double digested
5	110 gel purified
6	111 gel purified
7	PCR of 804 purified
8	PCR of 815 purified

~~6/25~~ 6/26

TRANSFORMATION RESULTS

- Pick 4 colonies off of each* plate and 2 off each control plate
  - ↳ miniprep → digest w/ BssHII

379.5 λ H_2O
55 λ 10× BssHII Buff.
5.5 λ BssHII
↳ 20 λ + 5 λ miniprep

Possible products

~~Expected Bands~~

double cut 110:	4615
double cut 111:	5848
insert:	1970
MCS:	173

LANE	
1	110 + 804
2	
3	
4	
5	110 + 815
6	
7	
8	
9	~~empty~~ 110
10	
11	111 + 804
12	
13	
14	
15	111 + 815
16	
17	
18	
19	111
20