Fluctuation tests

To determine chromosome loss, recombination and mutation rates we perform fluctuation tests using the median method (Lea and Coulson J. Genet. **49:** 264-284 (1948)).

Haploid strains are used to determine recombination and mutation rates and diploids are used for chromosome loss rates. For both types fresh colonies should be used.

For diploids, we cross MATa and MATα strains, pull zygotes on YPD, and grow at 30C for 3 days (diploids can be kept at 4C for about 2 days but no longer). Some diploid strains, including the W303 background, will sporulate on YPD after several days and this will complicate chromosome loss and recombination rates which rely on the appearance of recessive markers. The basics of the genome instability assay in diploids, which identifies chromosome loss and recombination events that reveal the recessive *can-100* mutation and canavanine resistance, is shown below.

Events leading to canavanine-resistant diploids
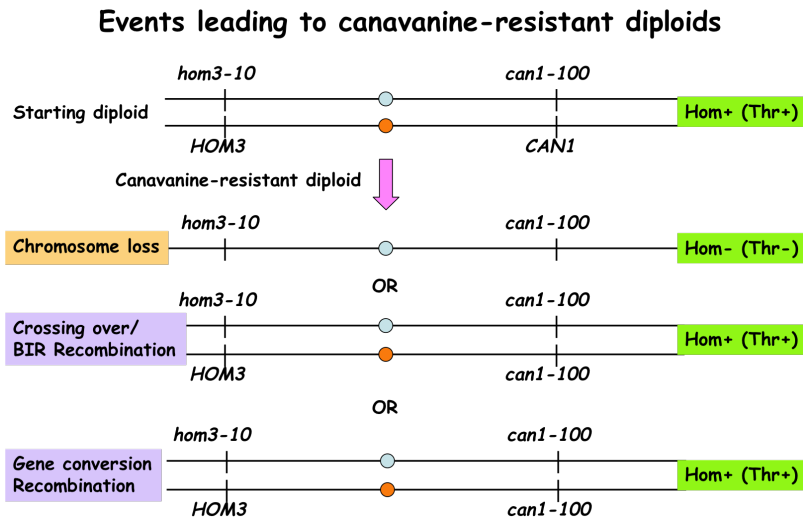
For each test 9 colonies are used.

1. Pick 9 colonies each from the nonselective growth plate disperse in I ml water in an eppendorf tube.

2. Make 10-fold serial dilutions for each colony, up to the 10^4 dilution.

3. Spread 25ul of the 10^4 dilution for each diploid on SC agar media, to estimate total number of viable cells, and 25ul of 10^0 and 10^1 dilutions on SC-arginine + canavanine (60ug/ml) agar plate, for events that reveal the recessive *can1-100* marker. Each plate is divided into quarters and 25ul of each dilution is spread on one quadrant so that cultures from two diploids can be put on one plate.

4. Incubate plates at 30C. Colonies are counted on the third day.

5. Replica-plate the SC-arginine+canavanine plates to SC-threonine plates and incubate at 30C for one more day.
6. Count the number of colonies that grow on the SC-threonine plates.
7. We calculate the chromosome loss rate and recombination rate by Lea-Coulson median method. $Can^r\ Thr^-$ colonies come from chromosome loss events and $Can^r\ Thr^+$ colonies come from recombination events. The number of Can^r colonies and the total number of colonies for each diploid are entered into an Excel spreadsheet along with the dilution factor, and event frequencies are calculated (see the example in the figure below). From the median frequency a rate is calculated using the equations from the Lea and Coulson paper. To calculate recombination and chromosome loss rate use the numbers you get from SC-threonine plates to enter into the spreadsheet. An Excel template with the embedded equations is available for download.

Example:

	A	B	C	D	E	F	G	H	I	J
						hpr5 rad51 test2				
1			STRAIN: hpr5::TRP1 rad51::LEU2 1333-13Ca hom3-10+ x 1307-12Calpha ADE+CAN					Test #2		
2			DATE:	5/6/03						
3										
4										
5			insert data in these two col.			insert data in these two col.				
6	Colony	SC	Dilution	Total Cells/ml	SC-arg+can	Dilution	Recombinant Cells/ml	Frequency		
7	1	41	400000	16400000	37	400	14800	0.00090244		
8	2	47	400000	18800000	103	400	41200	0.00219149		
9	3	60	400000	24000000	44	400	17600	0.00073333		
10	4	40	400000	16000000	44	400	17600	0.0011		
11	5	55	400000	22000000	47	400	18800	0.00085455		
12	6	45	400000	18000000	76	400	30400	0.00168889		
13	7	39	400000	15600000	60	400	24000	0.00153846		
14	8	42	400000	16800000	66	400	26400	0.00157143		
15	9	71	400000	28400000	89	400	35600	0.00125352		
16										
17		Comment:	Many small colonies on SC+thr plates .							
18		Recombination rate=1.957918E-5								
19		Chromosome loss rate=7.213382E-5								
20		SC (Can + thr)/Can + thr =		0.213483146						
21						median=	0.00125352			
22						ro =	35600			
23										
24										
25							table ro		table m	
26							35529.8656		3751.83375	
27							35924.8421		3789.54031	
28									m= 3758.52915	
29										
30								Canr rate =	9.1713E-05	
31										
32										

For recombination and mutation rates in haploids we streak out strains for single colonies on YPD or the appropriate nonselective medium and grow for 3 days.

Use 9 single colonies for one test.
1. To determine the recombination rate we use the recombination system: *leu2-ecoRI::URA3::leu2-bstEII*. The strain is firstly grown on SC-uracil plates to ensure that the strain has the recombination reporter and then streaked on a YPD agar plate. Plate the same way as for chromosome loss, using SC plates to determine the total number of cells and SC-leucine-uracil

for gene conversion events (Leu$^+$ Ura$^+$) and SC+5-FOA (750um/ml) for pop-out or single strand annealing events (Ura$^-$).

2. Dilute each colony in 1ml of water and make serial dilutions as above.

3. Spread 25ul of the 10^4 dilution for each diploid on SC medium, to estimate total number of viable cells, and 25ul of 10^0 and 10^1 dilutions on SC-uracil-leucine plates, for gene conversion recombination events, and onto SC + 5-FOA plates for SSA pop-out events that are Ura$^-$. Each plate is divided into quarters and 25ul of each dilution is spread on one quadrant so that cultures from two diploids can be put on one plate.

4. After growth at 30C for 30C for three days, colonies are counted and entered into the Excel spread sheet template.

To determine mutation rates we use strains that are *ADE2 CAN1*. We plate appropriate dilutions onto SC-arginine+canavanine plates to select for Can^r segregants.