**MGCB 31400**

**AND**

**BIOS 21236**

**Genetic Analysis of Model Organisms**

**Fall 2015**

**Problem Set #1**

**Due Wednesday, October 12th in class**

**We will not accept late problem sets.**

Please answer each question in the space provided using LEGIBLE writing. If you need additional space, please use the back of the same sheet ONLY. If you would prefer to type up your answers, the assignment with be available on the Chalk site the day it is distributed. Please print SINGLE-SIDED however.

**Please write your full name on the top of each page to assist grading.**

If you have questions or concerns regarding this problem set please email Caroline at coldstonemoore@uchicago.edu

**Question #1 (11 points)**

Your lab is interested in the genetic control of flower color. You cross two true-breeding lines that have yellow flowers, self the resulting F1s, and score flower color in the F2 plants. F2 plants exhibit yellow, orange, and red flowers. You observe the following numbers of F2 progeny:

Yellow flowers: 454

Orange flowers: 684

Red flowers: 75

A. How many genes control flower color? What are the possible genotypes for each of the flower colors? What was the color and genotype for all the F1 flowers? What must have been the genotypes of the two parental yellow flower strains? (5 points)

B. If one orange plant from the F2 generation is chosen at random and selfed, what is the probability that there will be no segregation of the phenotype among its progeny (i.e. all progeny will be the same color)? (2 points)

C. If one yellow plant from the F2 generation is chosen at random and selfed, what is the probability that there will be no segregation of the phenotype among its progeny? (2 points)

D. If one red flower from the F2 generation is chosen at random and selfed, what is the probability that there will be no segregation of the phenotype among its progeny? (2 points)

**Question #2 (8 points)**

You wish to do a genome-wide screen for mutants defective in assembly of mitotic spindles. You plan to do a primary screen for temperature sensitive mutants that arrest with large buds at the restrictive temperature, followed by a secondary screen to look for abnormal tubulin staining patterns at the restrictive temperature. You consider the following options for mutagenesis prior to beginning your screen.

1. Use of the yeast haploid knockout collection from GE Darmacon.

2. Treatment of cells with ethyl-methane sulfonate (EMS)

3. Treatment of cells with X-rays.

4. Treatment of cells with UV light.

5. PCR mutagenesis.

A. Why did you decide to look for temperature sensitive mutants? (1 point)

B. What subset of these 5 possible approaches makes sense given the goals of your project? Your answer should include the type(s) of mutation you are attempting to generate (nonsense, missense etc.) and the reasoning behind this. For methods you think are unsuitable to the goal, explain why this is the case. (7 points)

**Question #3 (8 points)**

You find a recessive mutation *rol* in *Drosophila* *melanogaster* that causes flies to roll over when you shine green light on them. Rough mapping shows that *rol* is linked to two recessive markers: *tremble* (*tre*) and *black body* (*blb*). You cross rolling (*rol/rol*) females with a trembling, black-bodied male to produce an F1 generation.

A. You want to map the position of *rol* with respect to *tre* and *blb*. What type of cross will you use? Males of what genotype should be crossed to your F1 females? (2 points)

B. You count the following numbers of F2 progeny from your cross in A:

|  |  |
| --- | --- |
| wild type | 92 |
| rolling | 305 |
| trembling | 85 |
| black body | 22 |
| rolling, trembling | 17 |
| rolling, black body | 80 |
| trembling, black body | 313 |
| rolling, trembling, black body | 86 |
| Total | 1000 |

Calculate the recombination frequency for each pair of markers. Draw a map of the chromosome, indicating the relative positions of the markers and the map distance between them. (6 points)

**Question #4 (12 points)**

You are studying genes involved in amino acid synthesis in *S. cerevisiae*.You obtain several haploid strains that cannot grow in the absence of different individual amino acids. These four strains are *leu2, trp1, ura3* and *lys1*.You note that each of these mutations segregates 2:2 when crossed with wild type yeast.

To map these mutations, you begin by crossing the *leu2* mutant strain with the *ura3* mutant strain, sporulating the diploid, and dissecting tetrads.

A. For each class of tetrad listed below, give the predicted Leu and Ura phenotypes of the four spores. (3 points)

Parental Ditype:

Non-Parental Ditype:

Tetratype:

You go on to perform two more crosses, sporulate the diploids and dissect tetrads. The three crosses are:

Cross 1: *MATa* *leu2 x MATα ura3*

Cross 2: *MATa leu2 x MATα trp1*

Cross 3: *MATa lys1 x MATα trp1*

You obtain the following results:

|  |  |
| --- | --- |
| Cross 1 |  |
| No. of Tetrads | Tetrad phenotype |
| 94 | 2 Leu- Ura+ : 2 Leu+ Ura- |
| 1 | 2 Leu- Ura- : 2 Leu+ Ura+ |
| 5 | 1 Leu- Ura+ : 1 2 Leu+ Ura- : 1 Leu- Ura- : 1 Leu+ Ura+ |

|  |  |
| --- | --- |
| Cross 2 |  |
| No. of Tetrads | Tetrad phenotype |
| 100 | 2 Leu- Trp+ : 2 Leu+ Trp- |
| 0 | 2 Leu- Trp- : 2 Leu+ Trp+ |
| 0 | 1 Leu- Trp+ : 1 Leu+ Trp+ : 1 Leu- Trp- : 1 Leu+ Trp- |

|  |  |
| --- | --- |
| Cross 3 |  |
| No. of Tetrads | Tetrad phenotype |
| 40 | 2 Lys- Trp+ : 2 Lys+ Trp- |
| 40 | 2 Lys- Trp- : 2 Lys+ Trp+ |
| 20 | 1 Lys- Trp+ : 1 Lys+ Trp+ : 1 Lys- Trp- : 1 Lys+ Trp- |

B. Given the data above, calculate distances between each pair of markers and draw a genetic map. Indicate if markers are centromere linked. (IMPORTANT NOTE: *trp1* is known to be very tightly centromere linked.) (5 points)

C. Consider the tetratype class of tetrads in the *lys1* x *trp1.* Diagram how this class of tetrad may have arisen by recombination during meiosis I (4 strand stage). Include in your diagram any crossovers and label all alleles. (2 points)

D. Consider the non-parental ditype class of tetrads in the *leu1* x *ura3* cross. Diagram how this class may have arisen by recombination during meiosis I. Include any crossovers and label all alleles. (2 points)

**Question #5 (9 points)**

You isolated three T4 phage mutants (m1 – m3) that give minute plaques in *E. coli* K strains, but have no effect on plaque size in *E. coli* B strains.

You mixed the phage strains pairwise and infect *E. coli* K strain at high m.o.i. The phenotypes of the resulting plaques is scored as below (“+” indicates wildtype phenotype and “-“ indicates minute plaques):

|  |  |  |  |
| --- | --- | --- | --- |
|  | m1 | m2 | m3 |
| m1 | - | + | - |
| m2 | + | - | - |
| m3 | - | - | - |

A. What type of test is this? What conclusions can you draw about these mutations? (3 points)

You then mixed the phage stocks pairwise and infect *E. coli* B strain at high m.o.i, then titrated the lysate on both K and B strains at low m.o.i. The ratio of (# wild type plaques on K strain)/(# wild type plaques on B strain) is listed.

|  |  |  |  |
| --- | --- | --- | --- |
|  | m1 | m2 | m3 |
| m1 | 1x10-7 | 0.015 | 2x10-7 |
| m2 |  | 3x10-7 | 0.003 |
| m3 |  |  | 0 |

B. What type of test is this? What kinds of mutations are m1, m2, and m3? Calculate the distance between these mutations and draw a map of M genes and the positions of three mutations in relation to one another, labeling the distance between them. (6 points)

**Question #6 (6 points)**

You have access to all the materials you need to manipulate DNA in the lab. You also have the supplies required to transform and culture budding yeast. Give a brief (4-6 sentence) description of how you would use the single step gene “transplacement” along with standard yeast genetic crosses to create a tetraploid strain of yeast.

**Question #7 (15 points)**

*leu1-15* is a cold sensitive mutant in *Saccharomyces cerevisiae*. At 20°C, a *leu1-15* strain requires Leu+ media to grow. The mutant cells arrest in medium lacking Leu. (Note: when answering, please indicate whether you are using diploid or haploid yeast, the yeast mating type, and the growth media and other relevant conditions. Assume that you have all mutant combinations in both mating types.)

A. Design a suppressor screen for *SLE* genes (suppressor of *leu1-15*). How would you perform the selection? What are the temperature and media conditions? (3 points)

B. 1) How do you know if the suppressor mutations are dominant or recessive? 2) How can you determine if the mutations are likely to be located within the *LEU1* gene? (6 points)

C. Assume you find three recessive suppressor mutations of *leu1-15* that you provisionally designate *sle1, sle2, sle3*. How would you perform a complementation test to determine if these mutations lie in the same or different complementation groups? (3 points)

D. You are disappointed to discover that *sle1, sle2,* and *sle3* mutants have no phenotype of their own in a wild-type background. How would you clone these genes corresponding to each complementation group? (3 points)

**Question #8 (6 points)**

In three sentences or less, explain why CRISPR/Cas9 has become the predominant method for targeting deletion and “knock-in” mutations, as compared to the zinc-finger nuclease and TALEN methods.