**MGCB 31400**

**AND**

**BIOS 21236**

**Genetic Analysis of Model Organisms**

**Fall 2015**

**Problem Set #1**

**Due Wednesday, October 14th in class**

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 the appropriate TA for your question, as indicated below, to set up a meeting time. Office Hours can also be found on Chalk.

**Question 1 (7 points)**

You are a botanist and have isolated two true-breeding stocks of the same species of flower, one with red petals on a short stem, and the other with white petals on a tall stem. Scientific curiosity takes hold, and you decide to cross the two lines of flowers and then self the resulting F1 generation to obtain the F2 stock. The offspring counts are listed below for each phenotypic class in order of the number of offspring:

Red Petals & Tall Stem: 55

Pink Petals & Tall Stem: 35

Red Petals & Short Stem: 17

Pink Petals & Short Stem: 13

White Petals & Tall Stem: 6

White Petals & Short Stem: 2

Total 128

1. Based on the observations above, how many genes control flower color? How many genes control stem height? What was the color and genotype for all the F1 flowers? What must the genotypes of the parental stocks of flowers have been? **(4 points)**
2. List all possible genotypes expected to produce true-breeding stocks of pink, short stemmed flowers. **(1 point)**

C) If we self a random pink/tall flower from the F2 generation, what is the probability of no segregation of the phenotype among the progeny (i.e. all progeny produced by the parent you selected will be identical to the parent-i.e. what fraction of pink/tall flower plants will be true breeding)? **(2 points)**

**Question 2 (8 points)**

In a summer rotation, you isolated three T4 bacteriophage mutants (t1 – t3). All mutants form tiny plaques when spotted on the B strain of *E. coli,* but form normal plaques in the K-12 strain of *E. coli*. As a control, you showed that only wild-type plaques formed when *E. coli* B were co-infected with wild-type and mutant phage at high m.o.i. To characterize your mutant phage, you mixed the mutant phage in pair-wise combinations and co-infected *E. coli* B at high m.o.i. You allowed plaques to form and scored their appearance (“+” indicates wild type plaques and “-” indicates tiny plaques). The scored phenotypes are as follows:

|  |  |  |  |
| --- | --- | --- | --- |
|  | t1 | t2 | t3 |
| t1 | - | - | + |
| t2 |  | - | - |
| t3 |  |  | - |

1. What type of test is this? What do you conclude from this test? **(2 points)**

To further characterize your mutant phage, you again mixed the mutant phage in pair-wise combinations, but then co-infected *E. coli* K-12 at high m.o.i. and allowed plaques to form. You used the phage from the lysed cells to infect both K-12 and B strains at low m.o.i. The ratio (# of wild type plaques on B strain)/(# of wild type plaques on K-12) is given below:

|  |  |  |  |
| --- | --- | --- | --- |
|  | t1 | t2 | t3 |
| t1 | 10-7 | 0.002 | 0.020 |
| t2 |  | <10-10 | 10-7 |
| t3 |  |  | 10-7 |

1. What type of test is this? Calculate the map distances between the mutations if possible. What kinds of mutations are t1, t2, and t3 (point or deletion)? Draw a map of the gene(s) involved, indicating the positions of the mutations in relation to each other. Label any map distances between the mutations that you were able to determine. **(6 points)**

**Question 3 (15 points)**

Your lab is trying to learn about the biosynthesis of uracil in *Saccharomyces cerevisiae*. A busy postdoctoral researcher gives you a strain of diploid yeast that is heterozygous for a mutation in a gene called ura100 (allele designation *ura100-1*)and asks you to replica plate colonies of the mutant to media containing and lacking uracil. He also asks you to sporulate the diploid, pull tetrads and test the resulting haploid ascosporal colonies, again by replica plating to medium either containing or lacking uracil. After replica plating you incubate the plates at either 20 or 37oC. The results are summarized in the table below.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | 20oC  Uracil in the media | 20oC  Media lacks uracil | 37oC  Uracil in the media | 37oC  Media lacks uracil |
| Diploid | grows normally | grows normally | grows normally | grows normally |
| Haploid | grows normally | 2 dead : 2 normal | grows normally | grows normally |

1. What type of mutation does this strain have? (Be concise and specific). **(1 points)**
2. Briefly describe how you would perform a suppressor screen for mutations that restore the ability of a *ura100-1* (or *ura100-1/ura100-1*) strain to grow at 20oC. Be sure to include the selection conditions (temperature, media conditions) and whether you are using haploid *MATa,* haploid *MATα*, or diploid yeast for every step. What is the advantage of this type of mutant search compared to standard searches for loss of function mutations? **(4 points)**
3. You find suppressors of your *ura100* mutation. How can you determine if they are dominant or recessive? How can you determine if the mutations are located within or outside of the *URA100* gene? (Hint: mutations in *URA100* should be in the same complementation group as *ura100*) **(6 points)**

1. You find that four of your suppressor mutations cause otherwise wild-typeyeast to grow very slowly at both 20oC and 37oC. Three of the mutations (m1, m2, and m3) are recessive, but one (M4) is dominant. Briefly describe how you would test if the mutations were in the same gene. Be sure to indicate the ploidy and mating type of each strain that you use. **(4 points)**

**Question 4 (12 points)**

You are studying genes involved in mercury sensitivity in yeast. WT yeast do not grow in 0.05% mercury (MryS phenotype). You have conducted a genetic screen and have found two mutant strains of yeast, *mry1* and *mry2* that seem to be MryR; these strains will grow in media containing up to 0.05% mercury. You also notice that both of these mutations segregate 2 MryS : 2 MryR when crossed to a WT yeast strain.

You begin to map your *mry* mutations by crossing the *mry1* mutant strain to a well-characterized *trp1* mutant strain, sporulating the diploid, and dissecting tetrads. (NOTE: *trp1* mutation is tightly linked to the centromere)

1. For each class of tetrad listed below, list the predicted mercury and Trp phenotypes of the 4 spores. **(3 points)**

Parental Ditype:

Non-Parental Ditype:

Tetratype:

1. In addition to the cross described above, you perform two more crosses, sporulate the diploids, and analyze tetrads from each. The three crosses are:

Cross 1*: MATa mry1 X MATα trp1*

Cross 2: *MATa mry2 X MATα trp1*

Cross 3: *MATa mry1 X MATα mry 2*

|  |  |
| --- | --- |
| **Cross 1** |  |
| **No. Tetrads** | **Tetrad phenotype** |
| 46 | 2 MryR Trp+ : 2 MryS Trp- |
| 46 | 2 MryS Trp+ : 2 MryR Trp- |
| 8 | 1 MryS Trp+ : 1 MryS Trp- : 1 MryR Trp+ : 1 MryR Trp- |

|  |  |
| --- | --- |
| **Cross 2** |  |
| **No. Tetrads** | **Tetrad phenotype** |
| 40 | 2 MryR Trp+ : 2 MryS Trp- |
| 40 | 2 MryS Trp+ : 2 MryR Trp- |
| 20 | 1 MryS Trp+ : 1 MryS Trp- : 1 MryR Trp+ : 1 MryR Trp- |

|  |  |
| --- | --- |
| **Cross 3** |  |
| **No. Tetrads** | **Tetrad Phenotype** |
| 82 | 0 MryS : 4 MryR |
| 2 | 2 MryS : 2 MryR |
| 16 | 1 MryS : 3 MryR |

Given the tetrad data above, draw a genetic map showing the position of *mry1*, *mry2* and *trp1* genes, their centromeres and any relevant distances. **(5 points)**

1. Consider the tetratype class of tetrads observed in the *mry1* x *trp1* cross above. Diagram how this class of tetrad may have arisen by recombination during meiosis I (4 strand stage). Include in your diagram any necessary crossovers; label all alleles. **(2 points)**

D) Consider the non-parental ditype class of tetrads observed in the *mer1* x *mer2* cross above. Diagram how this class of tetrad may have arisen by recombination during meiosis I (4 strand stage). Include in your diagram any necessary crossovers; label all alleles. **(2 points)**

**Question 5 (9 points)**

Upon graduating from the University of Chicago you have been placed in rotation in the graduate institution of your choice, but in your first rotation, you are working under Professor Bob whose organism is the blobfish. Professor Bob recently discovered a recessive single mutation in blobfish that causes it to appear to smile. He called the gene *smi*. He has also noticed that the gene appears to be linked to recessive mutations *fin* and *blk,* which make the blobfish finless and black respectively (wild-type has fins and is pink/gray). To map *smi,* you cross a smiling male to a black, finless female.

1. Describe the phenotype and genotype of the progeny. **(1 point)**
2. With males of what genotype should you cross your F1 females in order to perform a backcross? **(1 point)**

The results of the backcross were as follows:

fin, blk, smi 33

+, blk, smi 82

+, +, smi 390

fin, +, smi 3

+, +, + 32

fin, +, + 90

fin, blk , + 369

+, blk, + 1

total 1000

1. Find the recombination frequency between each pair of markers. Draw an appropriate map of the chromosome, describing the relative location of the markers and including the map distance between each marker. **(5 points)**
2. You may have noticed the number of double crossovers seems disproportionately low. Please name and describe the phenomenon that is responsible for this observation. Is it possible there are more than 4 double crossovers? **(2 points)**

**Question 6 (10 points)**

Draw 4 diagrams of respectively: (1) metaphase and (2) anaphase of meiosis I, and (3) metaphase and (4) anaphase of meiosis II. Make sure to label and explain ALL structural features of the meiotic chromosomes that allow for reductional segregation in meiosis I and sister chromatids to separate in meiosis II. Include at least 2 pairs of chromosomes or sister chromatids.

**Question 7 (8 points)**

CRISPR/Cas9, ZFN, and TALENs systems are all capable of introducing targeted mutations by causing double strand breaks at specific genetic locations. However, CRISPR/Cas9 is used far more commonly to edit genomes than either of the other two technologies. What is unique about the molecular mechanism of this system, and what practical benefits does this provide for researchers? **(3 Points)**

What is the major problem with genome editing, and what efforts have been made to improve the CRISPR/Cas9 system? **(3 Points)**

How have the CRISPR/Cas9 and ZFN systems been modified for use in applications other than genome editing, or what modifications can you envision that would enable them to be used in other types of experiments? (Hint: it involves modifying the nuclease domain) **(2 Points)**

**Question 8 (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.