Midterm 1, Answer Key Oct. 26, 2009

Honor Pledge: I have neither given nor received any unauthorized help on this exam:

Name Printed: _____

Signature:_____

1a. (2 pts) Imagine you have two pure breeding plants of the same species. One has round leaves, red flowers and hairy stems and the other has long thin leaves, purple flowers and hairy stems. If you cross them, the F1 has long thin leaves, purple flowers and hairy stems.

Which allele is dominant for leaf shape?

Thin

Which allele is dominant for flower color

Purple

1b. (2 pts) You cross 2 F1s and get the following numbers of progeny. How many loci with different segregating alleles can be measured.

Two

18 thin, purple, hairy, 6 thin, red, hairy, 6 round, purple, hairy: 2 round, red, hairy

Are the loci linked or segregating independently?

Unlinked

2a. (1 pt) What physical structure is required for transfer of the F plasmid between *E. coli* bacteria?. Where are the genes for that structure located, plasmid or chromosome? **The pilus. On the F plasmid**

2b. (2 pts) Why did we have to include a streptomycin resistance gene in the recipient strain in our Hfr conjugation experiment?
The donor had wild type alleles for the genes that were auxotrophic in the recipient but could not grow on Streptomycin. Only recombinants could

2c. (2 pts) Hfr strains of *E. coli* have an F plasmid integrated into the bacterial chromosome. The F plasmid replicates with the bacterial chromosome instead of

replicating on its own, separate from the chromosome. Because of the integrated F plasmid, Hfr strains are capable of mating with F- *E. coli.*
What sequences on the F plasmid allow it to become integrated into the bacterial chromosome?

Insertion sequences or transposable elements common between the plasmid and the bacterial chromosome.

3. In the paper we discussed in the lecture, Lederberg and Tatum demonstrated that *E. coli* can exchange genetic information.

a. (2 pts) How did Lederberg prove that he found genetic recombination between strains and not simply back mutation. It took two separate experiments.

He measured the reverse mutation frequency for one trait. He then used multiple mutations to test if recombination was occurring. The frequency of exchange was too high to be explained by back mutations.

c. (2 pts) What extra experiment did Lederberg do to ensure that transformation was not responsible for the genetic exchange that he saw?

He cultured each mutant strain with medium from the opposite mutant culture and found no recombinants. Louise you can give half points for the U tube experiment

4. You are working with wild asters in the mountains of Virginia. You have found two plants in nature that both have white flowers. You bring them back to your home greenhouse. You find that if you self fertilize either plant, all the progeny plants are white. You also cross each plant to a plant with purple flowers. All the F1 from this cross have purple flowers. When you cross the two original white flowered plants together, the F1 progeny from your crosses all have purple flowers..

a. (2 pts) Are the alleles for white flowers in your two plants recessive or dominant?

Recessive

Each one has a recessive mutation in a gene for anthocyanin.

b. (2 pts) Why do the F1 progeny from the two different white plants you collected in nature have purple flowers?

The mutations complement. Each parent plant is homozygous for the functional allele at one locus and homozygous for the recessive loss of function allele at another locus.

c. (2 pts) You self fertilize some of the F1 plants and grow the F2 plants. No matter which parental cross you made, when you self fertilize a purple F2 plant, you get some plants that have purple flowers and the rest of the plants have white flowers. The ratio is 9 purple flowering plants to 7 white plants. What genotypes give purple flowers and what genotypes give white flowers?

Purple is P1-;P2-;
white is p1p1;P2-, P1-;p2p2, or p1p1;p2p2

5. (6 pts). If you see the following results on a yeast complementation test for adenine requiring mutants. Which individuals have allelic mutations? G means growth on minimal medium and NG means no growth. Are there any strains with mutation in more than one gene required for adenine synthesis?

Growth on minimal medium:

	Alpha-type mutants					
A-type mutants	Wild type control	Mutant 1	Mutant2	mutant3	mutant4	mutant5
Wild type control	G	G	G	G	G	G
Mutant 1	G	NG	NG	G	G	G
Mutant 2	G	NG	NG	NG	G	G
Mutant3	G	G	NG	NG	G	G
Mutant4	G	G	G	G	NG	G
Mutant 5	G	G	G	G	G	NG

1 and 2 are allelic, 2 is a double mutant, 3 and 2 are allelic

b. (1 pt) What did we learn from crossing all the mutants to a wild type strain? Why is this important to establishing the validity of the complementation test described here?

We learn that all the mutations are recessive to wild type. The complementation test cannot be done with dominant mutants.

6a. (1 pt) When yeast is exposed to UV light what are the most common chemical changes in the DNA that lead to mutations?

Thymidine dimers

b. (1 pt) Why did we use a strain of yeast that was defective in excision repair for our mutation lab?

Excision repair will correct UV light induced mutations. Using the mutant allowed us to expose the yeast to less UV light but it will not interfere with photoreactivation because that is an independent process.

c. (1 pt) What process that does require light can repair these mutations?

Photoreactivation (if you can't remember the word, describe the process)

7. (2 pts) In our lab exercise with mice, we had two alleles of the *C* locus, albino and wild-type. What information told us that the albino allele was epistatic to alleles at the Black (*B*) and Agouti (*A*) loci?

Segregants that were *cc* were white no matter what alleles were at *A* or *B*.

8. Mutations in more than one gene can affect the eye color of Drosophila. Imagine you have 4 true breeding inbred lines of mutant flies. All of them were isolated in different mutagenesis experiments so there is no chance one line is a descendent of the other. All the lines have white eyes instead of brick red eyes (wild type). You make the following intercrosses:

1x2 results in flies with brick red eyes
1X3 results in white eyes
1X4 results in white eyes
2X3 results in brick red eyes
2X4 results in brick red eyes
3X4 results in white eyes

a. (2 pts) How many different genes for eye colors are mutant in your four lines?

Two because some crosses of 2 X anything else result in complementation, While all the other crosses are not complementing.

b. (2 pts) Which lines have mutations in the same genes?

1, 3 and 4 all have mutations in the same gene.

9. Answer the following questions to design a screen to identify mutations in genes required for RNAi. (I am just looking for a simple screening plan. You do not need to include extra mutations such as *egl* that make the screening process easier to do).

a. (1 pt) How would you induce mutations in unknown genes in C. elegans? Assume you cannot predict what kinds of genes will be important for RNAi mechanisms.

Chemical mutagenesis

b. (1 pt) What genotype of worms would you mutagenize so that you will be able to monitor the loss of ability to support RNAi?

wild type (if anyone introduces the *egl* mutant that should be a bonus point)

c. (3 pts) After letting mutagenized worms self fertilize for two generations, you collect M2 progeny from each original parent. In this generation, some of the adult worms will be homozygous for mutations induced by the mutagenesis in step b. How would you treat the M2 worms to test them for the ability to support RNAi?

Feed with *E. coli* expressing dsRNA against any visible trait.

d. (1 pt) What phenotype will you expect with worms that have lost the ability to support RNAi?

they will not take on phenotypes of mutants deficient in gene for which dsRNA is introduced.

e. (1 pt) How will you be sure that your method to induce RNAi is working when you do your experiment ie. what control experiment will you do to make sure the mutants you select are not caused by failure of the RNAi mechanism?

Control will be to feed some worms that have not been mutagenized with *E. coli* making dsRNA at the same time and under the same conditions as you do for the mutagenized worms.

f. (1 pt) How can you check your individual mutant candidates for loss of RNAi mechanism after you have chosen them using the original screen?

Inject with ds RNA to a different clearly visible phenotype like unc. See if they become unc or are resistant.

g. (2 pts) Name two proteins (or protein complexes) known to be required specifically for RNAi that you would expect to find in your screen.

RITS
RdRp
Argonaut
Dicer
Risc choose any two

10. Dr Yeh described temperature sensitive mutations in kineticore function in meiosis of yeast. The screening procedure is more complex to get temperature sensitive mutations but in some cases it is worth the trouble.

a. (2 pts) What kinds of genes can best be identified using screens for temperature sensitive mutations Instead of screens for complete loss of function mutations?

Essential genes Genes where organism cannot survive loss of function

b. (2 pts) What kind of changes to the DNA sequence do you predict would most often lead to temperature sensitive mutations?

Changes in protein sequence, point mutations in protein coding sequences.