

**Biology 423L Laboratory in Genetics, Final Exam Key,
Dec. 14, 2009**

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

Name Printed: _____

Signature: _____

1. If you use X-rays to make mutations in yeast instead of UV as we did:

a. (2 pts) What kind of mutations are commonly made with X-rays that are not commonly caused by UV light?

Deletions and gross recombinations caused by breakage of the DNA backbone.

b. (2 pts) Could photoreactivation correct these kind of mutations?
Explain why or why not.

Photoreactivation repairs a thymidine dimer. X-rays do not generate thymidine dimers.

2. (2 pts) What two features of yeast make it a good model organism to identify mutations in biochemical pathways relevant to multicellular organisms.

Yeast is a eukaryote but it grows as a haploid. Mutants can be selected in the haploid phase. I will accept growing as a haploid for mutation and diploid for complementation as a correct answer.

3. In which of the following cases can you use RNAi to induce a mutation and in which would it be better to use a chemical mutagen? Explain your choice.

a. (4 pts) You want to identify a group of genes that is essential for development of the gonads of *C. elegans*. Assume that you cannot predict what the protein sequences might be from similarity to proteins from other organisms.

Use chemical mutagens because you do not know what gene sequences you expect to find.

b. (4 pts) You have cloned a gene from *C. elegans* that has strong homology to a transcription factor that affects wing development in *Drosophila* and you want to determine the function of the gene in *C. elegans*.

Use RNAi to block expression of genes homologous to your gene of interest.

4. An Hfr strain is used to map three genes (a b and c) in an interrupted mating experiment. The cross is *Hfr/a+b+c+rif-* X *F-/a-b-c-rifR* (*rif-* is sensitive to the antibiotic

rifampicin and *rifR* is resistant to rifampicin). The *a+* gene is required for synthesis of nutrient A, the *b+* gene for nutrient B and the *c+* gene for nutrient C. The minus alleles are auxotrophs for these nutrients. The cross is initiated at time = 0 and at various times the mating mixture is plated on three types of medium. Each plate contains minimal medium plus rifampicin plus the nutrients indicated in the following table.

| | Time of interruption | | | |
|-------------------|----------------------|--------|--------|--------|
| Supplements added | 5 min | 10 min | 15 min | 20 min |
| Nutrients A and B | 0 | 0 | 4 | 21 |
| Nutrients B and C | 0 | 5 | 23 | 40 |
| Nutrients A and C | 4 | 25 | 60 | 82 |

a. (6 pts) Based on these data determine the approximate location on the chromosome of the *a*, *b* and *c* genes relative to one another and the F factor. Show your work.

Grows on AB rif: has taken up C+ 1 pt for getting this part right

Grows on BC rif has taken up A+

Grows on AC rif has taken up B+

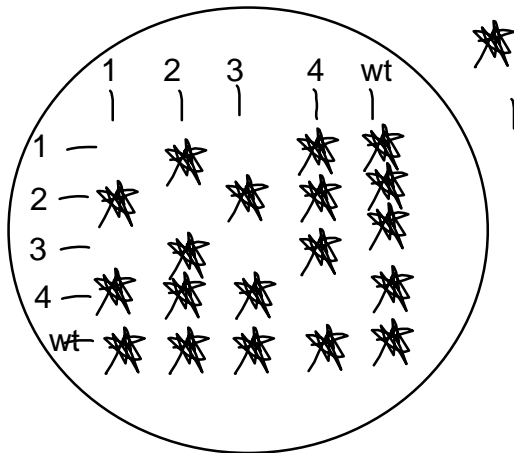
First is taken up B+, next is taken up A+ final is taken up C+ 1 pt for correct order.

Order is oriT, B, A, C

b. (2 pts) What is the purpose of the rifampicin in this experiment?

To select for recombinants on minimal media. Only the recipient parent which has the auxotrophic mutations is rifampicin resistant.

5. You have done a mutant screen taking UV-irradiated yeast and used replica plating to identify mutants that require histidine to grow. You have 4 mutants and you do a complementation test to see which mutants have mutations in the same genes. The results of streaking the mated colonies on a minimal medium plate (lacking histidine) are illustrated below. There is a wild type control.



Line indicates parental streak

Squiggle indicates grown colony after mating

a. (4 pts) Which mutants have a defect in the same genes?

1 and 3

b. (2 pts) Are any of the mutations dominant to wild type? **No**

6. (2 pts) Agouti brown looks like agouti black. Explain the phenotype of agouti and why agouti brown looks like agouti black. Explain why the dominant or wild type allele of Agouti can be considered epistatic to black or brown alleles at the B locus in mice.

Agouti makes color get laid down on the mouse hairs in stripes. The mice look grey because it is not easy to see if the stripes are brown pigment or black pigment. The B locus controls the color of the stripes. Therefore if the mouse has the dominant agouti allele, it will be difficult to tell if it has the black or the brown allele at B.

7. The inheritance of coat color in a group of cocker spaniels is controlled by two genes. The coat colors and genotypes are as follows:

| | |
|-------------|---------|
| Black | $A- B-$ |
| Light brown | $aaB-$ |
| Red | $A-bb$ |
| Yellow | $aa bb$ |

a. (2 pts) Are the relationships between between recessive alleles of A epistatic to any alleles of B? Explain your answer.

aa is not epistatic because you can distinguish which alleles are at the B locus in an aa genotype. aaB- is different from aabb.

b. (2 pts) What does this mean in terms of the number of independent biochemical pathways leading to coat color in dogs?

This means there are two different coat color pathways, one involves the product of gene *A* and the other involves the product of gene *B*. The final coat color is the combination of the effects of the two pathways.

8. (2 pts) *C. elegans* have two sexual types. What are they?

Male and hermaphrodite

9. *Drosophila* females of wild type appearance but heterozygous for alleles at three autosomal genes are mated with males having the recessive alleles for all three traits. The recessive alleles are not necessarily all on the same chromosome in the females. The recessive traits are: glassy eyes, coal-colored bodies and striped thoraxes. 1,000 progeny are distributed into the following progeny classes:

Wild type: 144
 Striped thorax: 81
 Coal body 236
 Glassy eye 43
 Coal body, striped thorax 41
 Glassy eye, coal body 78
 Glassy eye, striped thorax 237
 Glassy eye, coal body, striped thorax 140

a. (2 pts) What is the arrangement of alleles on the two homologous chromosomes in the heterozygous parental females?

Glassy eye, striped thorax, wild type body and wild type eye, wild type thorax and coal body.

b. (2 pts) Which gene is in the middle of the three on the chromosome?

Striped thorax

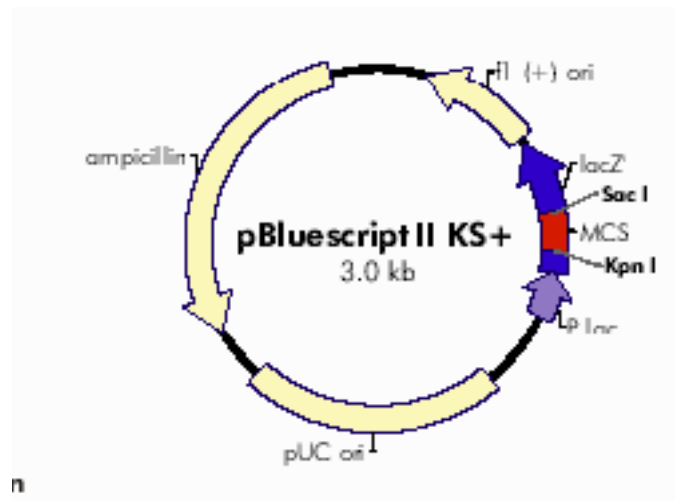
c. (6 pts) Estimate the map distance between the three genes.

Glassy eye to striped thorax $(78 + 81 + 43 + 41)/1000 = 24.3$ cM

Striped thorax to coal body $(140 + 144 + 43 + 41)/1000 = 36.8$ cM

Total is 61.1 cM

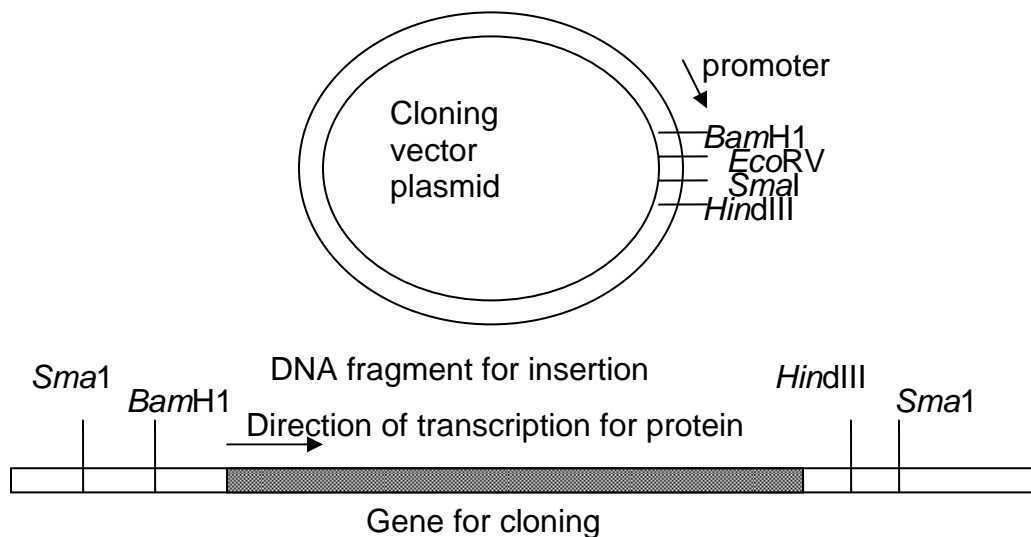
10. (2 pts) If we cloned a DNA fragment into the MCS region of the plasmid illustrated below, and then transformed *E. coli* with the resulting insert bearing plasmid and grew them on ampicillin and X-gal, would the colonies be blue or white? Explain.



The colonies will be white.

Insertion of DNA in the MCS separates the lacZ promoter from the coding sequence. Therefore the LacZ protein (Beta galactosidase) is not made. As a result, the colonies do not secrete the protein which can cleave X-gal converting it from a colorless compound to a blue chemical.

11. You want to clone a gene into a cloning vector. The vector and a DNA fragment that includes the gene to be cloned are illustrated below. The vector has a multiple cloning site that can be cut with the enzymes shown on the illustration for insertion of foreign DNA. The entire gene to be cloned is contained in the stretch of DNA illustrated as a shaded rectangle on a linear DNA region below. The gene can be excised from its current location for cloning using the restriction sites illustrated on the diagram.



Restriction sites and positions of DNA strand cutting for

SmaI 5'... CCC[▼]GGG... 3'
3'... GGG[▲]CCC... 5'

BamHI 5'... GGATCC... 3'
3'... CCTAGG... 5'

HindIII 5'... A[▼]AGCTT... 3'
3'... TTCGA[▲]A... 5'

EcoRV 5'... GAT[▼]ATC... 3'
3'... CTA[▲]TAG... 5'

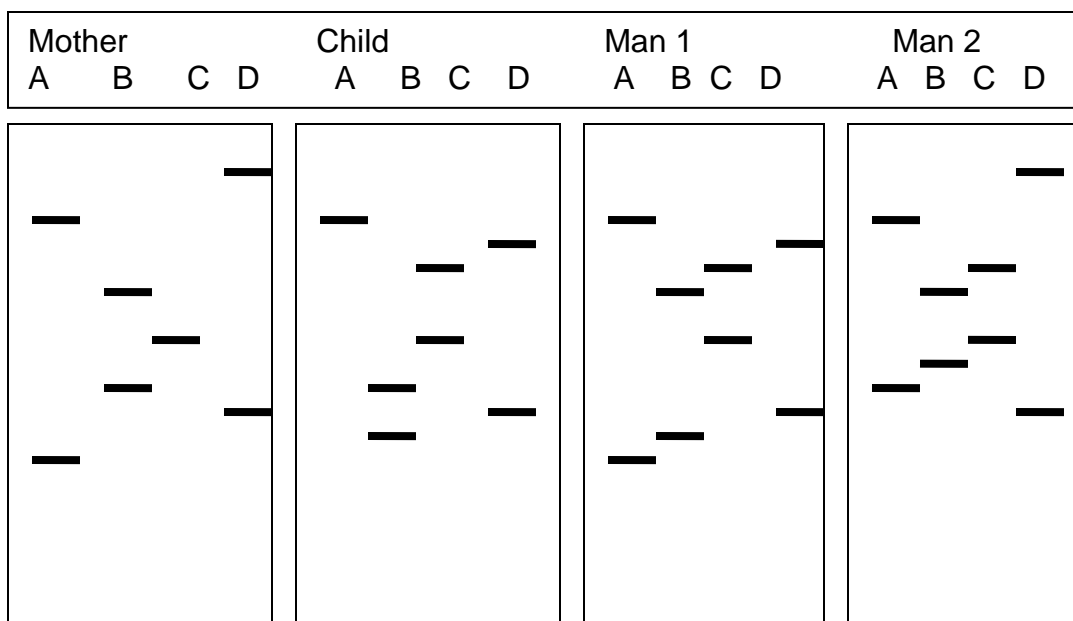
a. (4 pts) Which enzymes would you use to cut the fragment and the vector to be sure the gene was cloned into the vector so that the protein could be expressed from the promoter on the vector. Explain your choice.

***Bam*HI and *Hind*III will cut the insert and the vector in the correct places. Because they are different enzymes that leave distinct single stranded overhangs, the fragment will only ligate in the correct orientation.**

b. (2 pts) How would you cut the fragment so that you could insert the gene into the *Eco*RV site of the vector?

You could cut the fragment out with *Sma*I. Because it is a blunt end cutter and *Eco*RV is also a blunt end cutter the blunt *Sma*I cut fragment could be ligated into the *Eco*RV site.

12. You are working in a forensics lab on a paternity case. You have done PCR reactions to test each individual involved, mother child and two potential fathers. You have tested 4 diagnostic loci A B C and D.



a. (4 pts) Which man do you suggest should be paying child support?

Man 1

b. (4 pts) State all the loci that are informative for your decision?

B and D

13. (4 pts) List two major advantages of PCR based markers for forensic studies over methods such as Southern Blot techniques?

You can use very small amounts of DNA

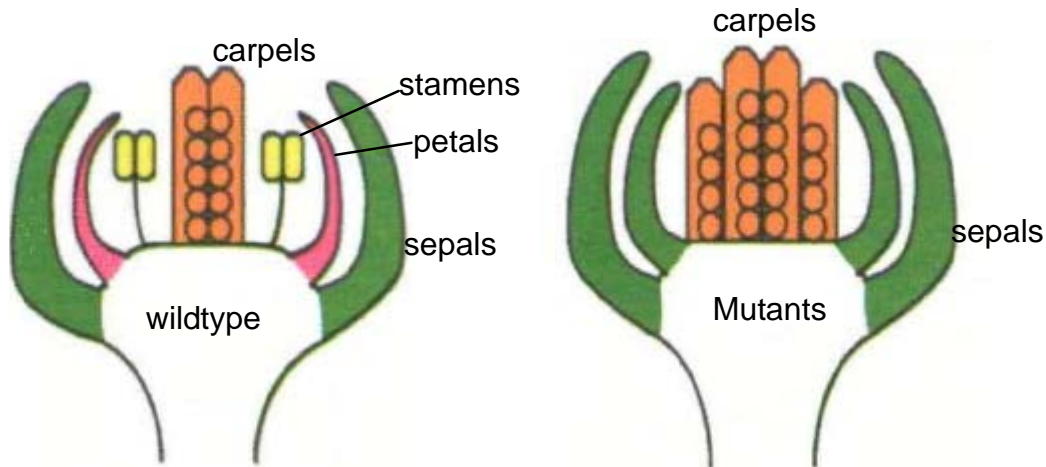
The PCR technique is accomplished more quickly

You don't need radioactivity for the PCR technique

14. (4 pts) DNA markers are often designed around sites of DNA repeats of about 10-100 base pairs. Why are these VNTR sites or minisatellites highly variable.

Minisatellites can expand or contract if the sequences pair incorrectly in meiosis.

15. (8 pts) John Bowman studied the genetics of floral organ identity for his doctoral thesis. After screening thousands of M2 plants derived from mutagenized seeds, he found 10 mutants that made sepals and carpels but had no petals or stamens as illustrated below.



In order to do an allelism test, he should cross two of the mutants with the same phenotype together. He could not do that because they do not have stamens and do not produce pollen. Outline a protocol that would allow him to test two different mutants with the no stamens, no petals phenotype to see if the mutations are in the same genes and state the expected outcomes of the experiment if the mutants had a defect in the same gene or in two different genes.

Cross each back to wild type get independent heterozygotes.

Cross the two distinct F1s together. Offspring will be 3:1 if same gene.

Offspring will be wild type if two genes.

F1s will be $aA BB$ and $AABb$. Crossing together gives $1aABB$: $2 AaBb$: $1AABb$ all wild type.

16. The father of the family depicted below has a dominant trait for tasting the chemical PTC. His wife cannot taste the chemical so she has two recessive alleles at that locus. The illustrated with filled-in boxes or filled-in circles can taste PTC. You have investigated the inheritance of two randomly chosen DNA markers in this family. In each case, the mother is homozygous for her alleles at the marker in question, making it easier to follow the inheritance of the father's alleles.

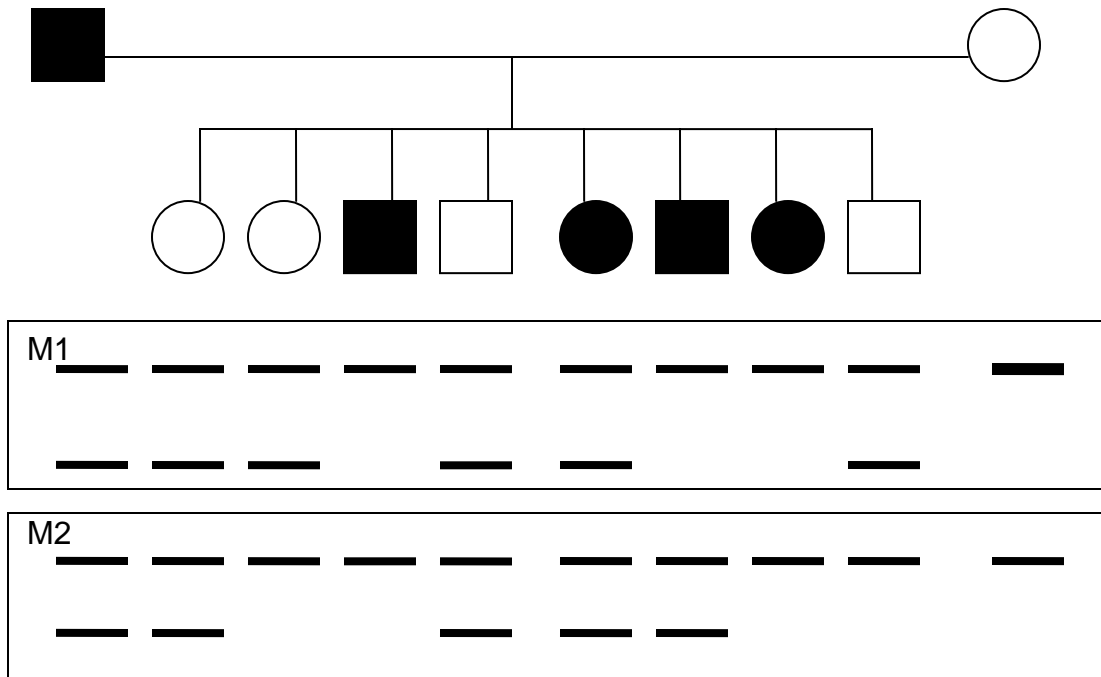
a. (6 pts) Which of the markers is linked to the gene for tasting PTC? And which is unlinked

M1 has one recombination event between the trait and the marker (II 5)

M2 is unlinked, half the affected children and half the wild type children have each marker.

b. (2 pts) Can the DNA sequence of the linked marker be part of the gene for PTC. Explain your answer.

No because there is a recombination event



17. Dr Duronio presented you with a lecture on his research in *Drosophila* and how it relates to cancer.

a. (2 pts) what part of the life cycle of *Drosophila* is he focusing on?

Embryo development

b. (2 pts) How does this part of the *Drosophila* life cycle provide an unusual tool to study cancer?

The *Drosophila* embryo goes through very rapid cell division. This makes it a good model to identify components of DNA replication and cell cycle control.

18. Explain the following two differences between positional cloning of a gene as we discussed for cystic fibrosis and genome wide association mapping.

a. (2 pts) One difference is represented in the words genome wide.

Markers covering the entire genome are examined at once, in positional cloning a small area is examined carefully.

b. (2 pts) the other difference is represented in the words association mapping.

We look for linkage of a marker to phenotypes in an entire population, not just in families.

c. (2 pts) Why does information about human haplotypes (ie regions of DNA that are never broken apart by recombination) make genome wide association mapping easier?

Fewer polymorphisms can be used to sample the entire genome because only one per haplotype is needed.

19. You have used map positional cloning to identify a candidate gene responsible for a mutation in a model organism like Arabidopsis plants. If you find a point mutation in the alleles of the candidate gene in your homozygous mutant, you still cannot be sure that you have found the gene of interest.

a. (2 pts) Why would a point mutation not necessarily mean you have found the affected gene?

A point mutation may not alter function of protein. First it may not change the amino acid sequence. Even if it does change the amino acid sequence, some substitutions still leave the protein functional.

b. (2 pts) What would you still need to do to prove this was the gene of interest?

Transform mutant to complement mutation or sequence another recessive mutant allele.