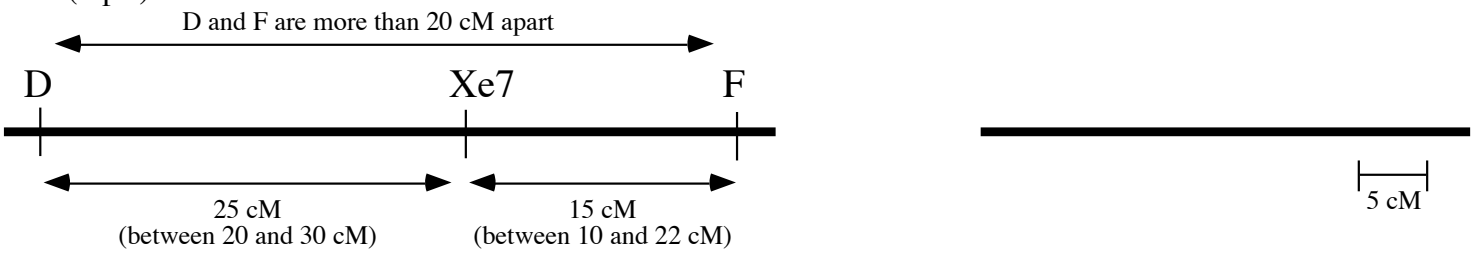
Final Exam Key, GENOME 371 Autumn 2005 Berg/Brewer

1A. (4 pts)
d. sex-linked recessive
Define notation: Derma = X^d; normal = X^D
I-1 $X^D X^d$
II-3 X^d Y
III-8 $X^D X^d$
IV-4 $X^D X^?$

1B. (4 pts)
b. autosomal recessive
Define notation: Freckles = f; no freckles = F
I-1 Ff
II-3 F?
III-8 Ff
IV-4 F?

1C. (5 pts)



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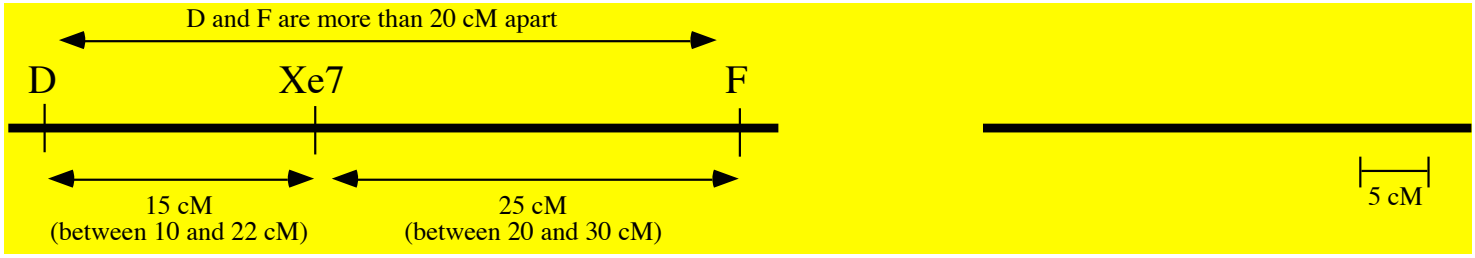


1D. (3 pts) Pedigree analysis reveals that Freckles is autosomal and Derma is sex-linked, yet the marker Xe7 is linked to both. This conflicting information can be resolved by realizing that Freckles resides on the pseudosutosomal region of the X and Y, while Derma is in the X-specific portion of the X. Freckles therefore behaves as if it were an autosomal gene—showing no differences in males and females, while Derma shows the classic signs of being X-linked and not having an allele on the Y. (Most people decided that Xe7 was a bad marker—that is, repeated in the genome. Repeated molecular markers are not used in mapping so this answer was not an entirely satisfactory answer.)

2A. (4 pts) Based on the maps of the wild type and orange alleles, the orange allele appears to be a deletion mutation. It could have occurred through improper recombination (or crossing-over) between misaligned retrotransposons on homologues or on sister chromatids, or by intrachromosomal recombination between the two retrotransposons on the same chromatid.

2B. (4 pts) To determine if the orange allele is dominant or recessive we need to test the phenotype of a heterozygous cell and ask whether that cell makes orange pigment or black pigment. Since the Orange locus is on the X chromosome and is subject to random X-chromosome inactivation, no cell in the heterozygous cat is actually expressing both alleles at the same time.

2C. (8 pts) There were four possible correct solutions to these linked questions. Here is only one of the correct options.

i. Will you inject DNA into a male or a female zygote? Male

ii. What is the genotype (O or o) of the zygote you will inject? $X^{O}Y$

iii. Which DNA would you inject? The o allele

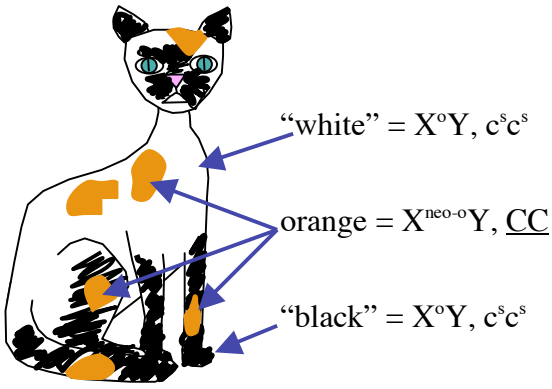
iv. What will be the genotype of the zygote after a successful injection? $X^{O}Y$; o inserted somewhere else. (In this male it could be on the X or on an autosome or even on the Y.)

v. What will you expect to find if the orange allele is a LOF mutation? The cat would be black.

2D. i. (5 pts) The thymidine kinase (tk) gene needs to be added to the "neo-o" construct at either the right or left end of the construct. TK is an important second selectable marker because cells with a random integration of the construct will most likely retain the tk gene and convert gancyclovir to a toxin. Cells with a homologous recombination between the neo-o/tk construct and the O locus on the X chromosome will delete the tk gene and thus survive in media with both neomycin and gancyclovir.

2D. ii. (3 pts) $X^{neo\text{-}o}Y$, CC

2D. iii and iv. (6 pts) Bungo chose a siamese cat because the siamese allele of the C gene is temperature sensitive and would only show significant pigmentation at the extremities (face, ears, tail and feet). (A complete loss of function mutation of the C gene in cats is extremely rare and not available for the experiment.) Since the ES cells are CC they will be able to show their pigment wherever the cells end up in the dermis of the cat—even in the warm parts of the body. If the neo-o allele produced orange pigment, then the resulting cat would be a Siamese cat with black extremities and orange spots at random locations in both the warm body and the cool extremities. (If the neo-o allele produced black pigment, Bungo would have seen the black patches in the warm body parts.)

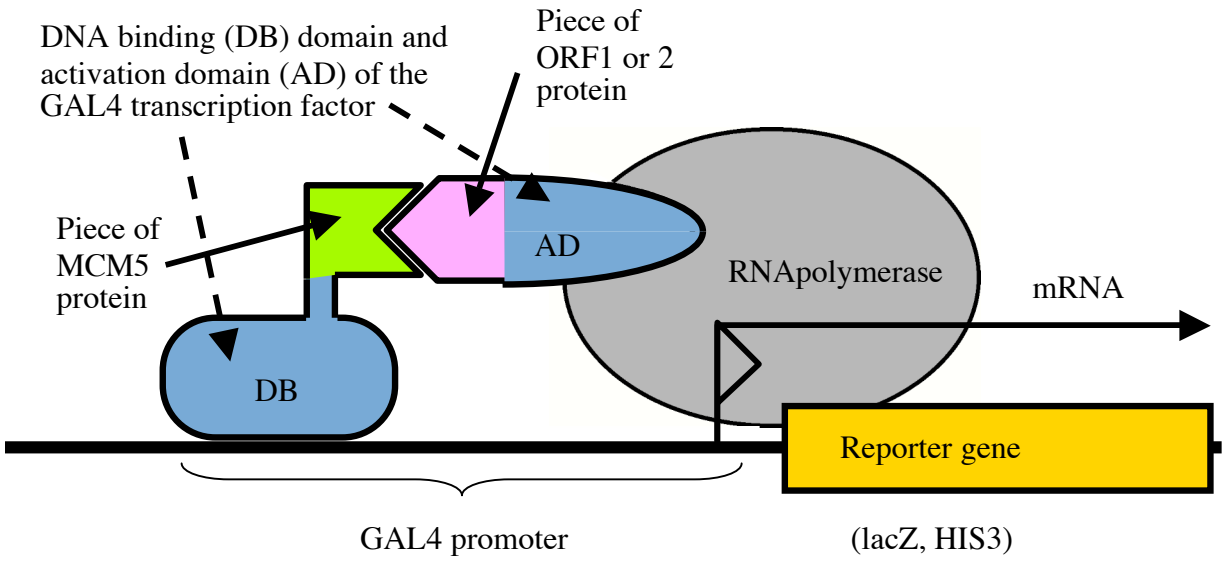


3A. (2 pts) To establish linkage groups of polymorphic (microsatellite) markers and to determine their order.

3B. (4 pts) Isolate DNA from each of the radiation hybrid cell lines.
Do PCR analysis on the DNA from each cell line using primers for microsatellite markers.

3C. (4 pts) Make a library of the cat genome containing random small fragments.
Do colony hybridization of a cat library using simple repeated sequences as a probe.
Sequence the inserts to find unique flanking DNA.

4A. (4 pts)



4B. (8 pts)

spore	MCM genotype	ORF genotype
A	$mcm5^{ts}$	orf1::URA3
B	$mcm5^{ts}$	ORF1
C	MCM5	orf1::URA3
D	MCM5	ORF1

spore	MCM genotype	ORF genotype
A	MCM5	ORF1
B	MCM5	orf1::URA3
C	$mcm5^{ts}$	ORF1
D	$mcm5^{ts}$	orf1::URA3

spore	MCM genotype	ORF genotype
A	MCM5	ORF2
B	MCM5	orf2::URA3
C	$mcm5^{ts}$	ORF2
D	$mcm5^{ts}$	orf2::URA3

spore	MCM genotype	ORF genotype
A	$mcm5^{ts}$	orf2::URA3
B	$mcm5^{ts}$	ORF2
C	MCM5	ORF2
D	MCM5	orf2::URA3

4C. (2 pts) There is evidence from the double mutant that ORF2 and MCM5 interact. (The two mutations are synthetically lethal.) There is no evidence from the double mutant that ORF1 and MCM5 interact.

5A. (4 pts) Starting with a collection of P-element mutagenized flies, they could be released into a container that contains two vials—one vial with a narcotic and a control vial with water (or other neutral compound). The vials have a one-way door, so that the flies can't leave after their initial choice of vial. At the end of some period of time, collect the flies that have chosen the narcotic vial and re-test them to be sure that they truly prefer the narcotic and didn't just make a random choice. If they pass this test numerous times, they would be considered as narcotic-seeking mutants. This example is a selection since the vial with the narcotics selected the desired flies. The assay could also be done on larvae on agar plates. It is also possible to do the mutant hunting as a screen, testing each individual mutagenized P-element stock, one at a time.

5B. (7 pts)
Step 1: Isolate DNA from the mutant fly.
2: Digest completely with EcoRI.
3: Dilute the DNA and incubate with ligase to circularize the plasmid present in the transposon.
4: Transform E. coli and plate cells on ampicillin plates.
5: Pick colonies that grew and isolate their plasmid DNA, which contains part of the P element, including ori and amp^R, as well as flanking DNA from one side of the transposon.
6: Using P-element sequence as a primer, sequence the flanking fly genomic DNA.
7: Perform a DNA BLAST to find the sequence in the fly genome database.
Partial credit was given when students added an extra step of colony hybridization using the *P* element as a probe. Less credit was given if the method described positional cloning from flanking markers. Pedigree analysis, LOD analysis, and radiation hybrid panels are not necessary with flies!

5C. (4 pts) Transposon mutant: A P-element insertion (perhaps in the promoter region) that created a gain-of-function mutation of this gene so the fly has a greater sensitivity to narcotics. (Recall the CDC6 Ty mutation in Quiz Section 8).

Knock-out mutant: A P-element insertion into the coding portion of the gene that causes a complete loss of function, so the fly loses its sense of smell for narcotics. (Recall the Ty insertion into ADE6 in Quiz section 8.)

6A. (7 pts) There is a problem with mitosis or segregation in cells that contain this double-CEN plasmid. In some cells the two centromeres may be tugged to opposite poles. Since this minichromosome remains at the metaphase plate even after cohesin is degraded, cells may delay the completion of cell division. The delay in mitosis would slow down the rate that cells can divide, thus producing smaller colonies. A second possibility is that this cell rips the plasmid into two pieces and continues with mitosis. If the plasmid gets broken, only one of the two cells might get a plasmid and the loser fails to leave descendants on the –uracil plates so the colony enlarges more slowly than cells with the single-CEN plasmid.

6B. (3 pts) Hypothesis: Deletion, damage or LOF mutation at one of the copies of CEN3.

(5 pts) Evidence: The new plasmid is smaller than the original plasmid. In particular, the small EcoRI fragment containing the CEN3 on the right of the plasmid is reduced from 2 kb to 1 kb. All or part of this copy of CEN3 was deleted.