GENOME 371, Problem Set 6

1. *S. pombe* is a distant relative of baker's yeast (which you used in quiz section). Wild type *S. pombe* can grow on plates lacking tryptophan (-trp plates). A mutant has been isolated that cannot grow on –trp plates.

A genomic DNA library was made from wild type *S. pombe* cells. Mutant trp^- *S. pombe* were transformed with this library of plasmids and many millions of these cells were plated on a –trp plate. One colony grew on the –trp plate.

1a. Was this a *selection* or a *screen* for trp^+ cells? Explain.

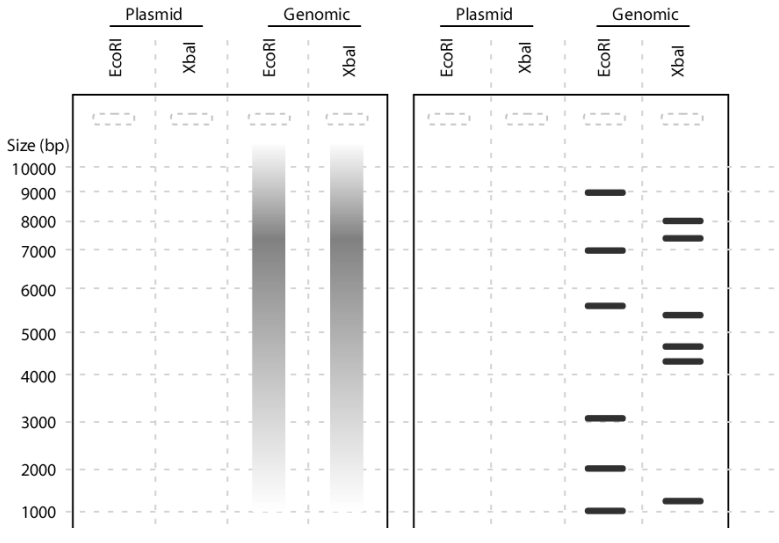
1b. Suggest two distinct hypotheses to explain how this trp^+ colony arose:

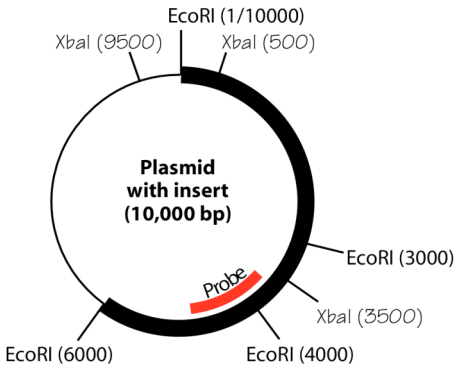
(i)

(ii)

1c. A plasmid that was recovered from the trp^+ colony is shown below. (The thick black line represents the genomic DNA insert; numbers in parentheses indicate distance in bp from position 1.) Purified plasmid (containing the insert) was cut to completion in separate reactions with (i) EcoRI; and (ii) Xba I. *S. pombe* genomic DNA (from cells without the plasmid) was also cut in parallel reactions, and all samples were analyzed by gel electrophoresis and Southern blotting.

On the picture below, mark the bands you would predict for the **plasmid** samples on the gel, and on the Southern blot after probing with the indicated probe.



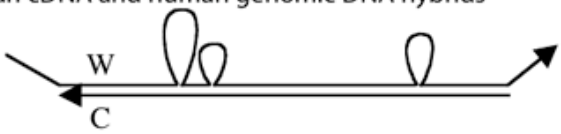


1d. Explain the Southern blot result you see for the **genomic** DNA samples. Why is it that one of the two digests shares fragments between the plasmid and genomic DNA southern blot, but not the other digest?

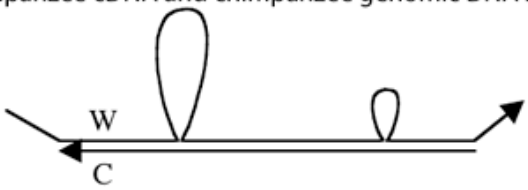
2. A human cDNA from a brain library was used as a probe to identify a genomic clone for this gene. The cDNA for this gene is 2 kb, the genomic clone for this gene is 4 kb. The human cDNA clone was also used to isolate clones from chimpanzee--both genomic and cDNA clones.

Starting with purified double-stranded DNA inserts from both the cDNA and genomic DNA clones, hybridization experiments were carried out. The hybrid DNA molecules were examined in the electron microscope (EM) where individual DNA duplexes were photographed. The drawings below show cartoons of what was seen in the EM.

i. Human cDNA and human genomic DNA hybrids

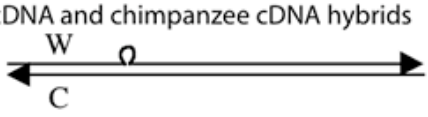


ii. Chimpanzee cDNA and chimpanzee genomic DNA hybrids



iii. Human genomic DNA and chimpanzee genomic DNA hybrids

iv. Human cDNA and chimpanzee cDNA hybrids



2a. In figure i, which strand is from the genomic clone (W or C)?

2b. Based on figure i, how many exons _____ and introns _____ are there in the human gene?

2c. Based on figure ii, how many exons _____ and introns _____ are there in the chimpanzee gene?

2d. Based on figure iii, what conclusions can you make about the human and chimp genes and why are the ends of the genomic clones not hybridizing?

2e. Based on all of the data from figures i-iv, which strand in figure iv is from the chimpanzee cDNA clone (W or C)?

2f. Using the combined set of data from all four figures, what specific DNA is present in the small loop in the watson strand in figure iv?

3. Wild type E. coli cells can grow in the absence of valine, or in the presence of low levels of valine, but NOT in the presence of high levels of this amino acid (presumably because high intracellular levels of valine are toxic). Genetic studies of valine metabolism in E. coli have revealed two sorts of conditional mutant. One type of mutant (mutant A) can grow in the presence of low levels of valine, but not in its absence. The other type of mutant (mutant B) can grow in the presence of both low and high levels of valine. Type B mutants can also grow in the absence of this amino acid. You wish to clone the gene corresponding to these two mutant categories and hypothesize that class A mutants are defective in valine synthesis (the mutations are recessive to wild type), and that the B class mutants are defective in a pump responsible for importing valine from the media. However, after thinking about this, you realize that the mutations responsible for

the B class could be of two sorts, which you call B1 and B2. The B1 sort might simply be defective in pumping valine (recessive to wild type), whereas the B2 sort might reverse the flow of the valine pump (pumping it out of the cell; this sort of mutation might well be dominant to wild type). With this information in mind, please answer the following questions:

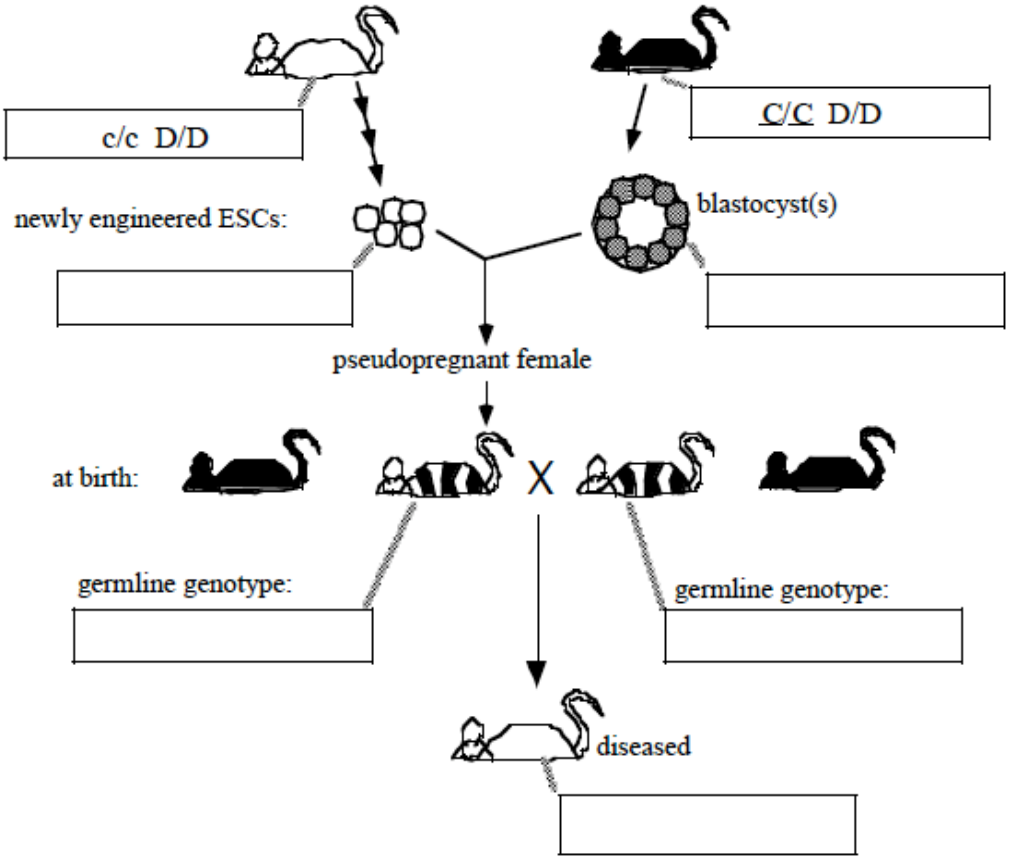
3a. How do you suppose the genetic screens were carried out to identify the mutants falling into the A and B categories?

3b. How would clone the gene corresponding to mutant category A?

3c. How would you clone the gene corresponding to mutant category B (be sure to address both possible explanations for this mutant type; assume that the B2 mutants ARE dominant to wild type)?

4. To make a mouse "model" for a **recessive** inherited human disease, embryonic stem cells (ESCs) made by mating two **fully homozygous** white (albino) non-disease mice are used. One of the normal alleles (**D**) of the mouse "disease" gene is replaced by an inactivated allele (**d**). The modified ESCs are then injected into mouse blastocysts made by mating two **fully homozygous** black nondisease mice. Black coat color is conferred by an allele (**C**) that is dominant to the allele causing albinism (**c**). The resulting embryos are implanted in a pseudopregnant female (this is a fancy term for a surrogate mother) and allowed to develop. (See the drawings below).

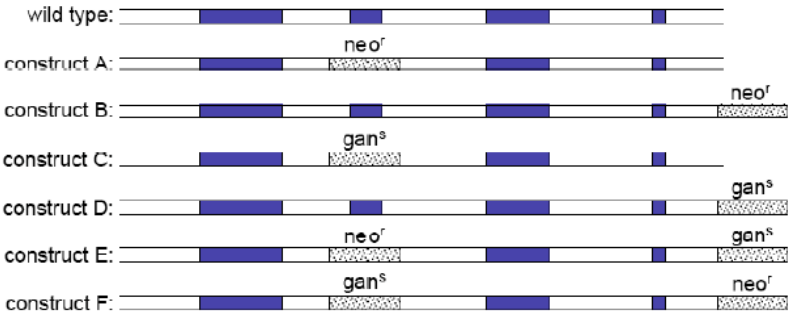
Some of the mice from these embryos are coat color chimeras. Two of these black/white mice are mated to each other. One offspring is found that has a completely white (albino) coat and shows the disease trait! (See the drawings below)**.** In each of the boxes in the drawing below, write in the genotypes **for the coat color gene** and **for the disease gene**.



5. You have identified a diabetic mutant strain of mice. Your studies indicate that mice that are heterozygous or homozygous for this mutation both have an identical diabetic phenotype. From these findings, you propose the following three models to explain the effect of this mutation:

Model 1: The mutation is haploinsufficient.
Model 2: The mutation acts in a dominant-negative fashion.
Model 3: The mutation acts through a gain-of-function mechanism.

To distinguish your three models, you map and clone the gene responsible for this genetic form of diabetes, which you name *DIA1,* and then proceed to generate constructs that will allow you to study this gene further in mice. The following linear DNA fragments, all of which contain portions of the mouse *DIA1* gene (exons are darkly shaded) and other DNA sequences that maybe important in generating your desired mouse strains are available for this analysis:



neo^R: confers resistance to the drug neomycin

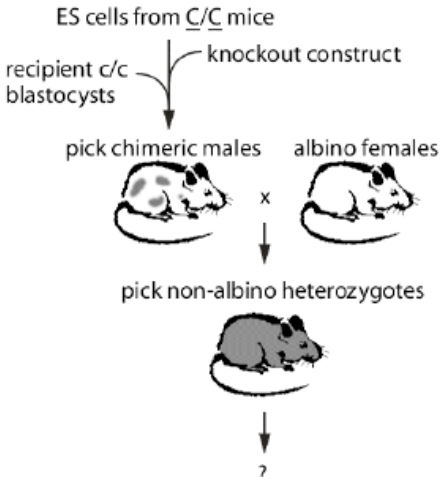
gan^S: confers sensitivity to the drug gancyclovir

You obtain homozygous C/C ; *DIA1*/*DIA1* embryonic stem cells (ES cells; note that the tyrosinase genotype of the ES cells is the opposite of what was discussed in class) and now proceed to create two different genotypes of mice. Assume for this question that you have the appropriate culture medium and also have access to neomycin and gancyclovir.

5a. Which of the above versions of the gene would you use to create a knockout of the mouse DIA1 gene (*dia1*^{KO})? Briefly describe how you would select for cells that have a knockout, and why your strategy would work?

5b. Which of the above versions of the gene would you use to create a mouse with three wild type copies of the mouse *DIA1* gene (i.e., one extra copy)? Briefly describe how you would select for cells that have a third copy of the gene, and why your strategy would work?

You follow standard procedures to make mice that are heterozygous (C/c; *DIA1*/*dia1*^{KO}) as described in the figure at right. You now proceed to cross these mice to one another to create homozygous knockout animals.



5c. What progeny would you expect (in terms

of coat color and diabetes phenotype), and in what proportions given the three genetic models that you are considering? If a particular model does not predict a diabetes phenotype for the knockout animals, you may assume that the knockout animals behave like wild type mice.

Genotype	**Expected ratios**	**Phenotype (MODEL 1) haploinsufficient**	**Phenotype (MODEL 2) dominant negative**	**Phenotype (MODEL 3) Gain-of-function**
C/C; *dia1^{KO}/dia1^{KO}*				
C/C; *DIA1/dia1^{KO}*				
C/C; *DIA1/DIA1*				
C/c; *dia1^{KO}/dia1^{KO}*				
C/c; *DIA1/dia1^{KO}*				
C/c; *DIA1/DIA1*				
c/c; *dia1^{KO}/dia1^{KO}*				
c/c; *DIA1/dia1^{KO}*				
c/c; *DIA1/DIA1*				