Question 1. In class, we discussed extensively three classical experiments in bacterial genetics that proved bacteria to *have* genetics, to be able to mate, and to transfer genetic information unidirectionally.

Sal Luria and Max Delbrück used resistance to infection by phage as a model system to study whether or not bacteria exhibit the phenomenon of heredity in the conventional sense of the word. The famous "fluctuation test" Luria performed answered this question in the affirmative. An important aspect of his success was the use of a "gain-of-signal assay." What was the assay and *what specific aspect of the assay* was most important in the case of this *specific* experiment? (10 points)

Joshua Lederberg proved that bacteria mate. He did so by mixing two bacterial strains auxotrophic for different nutrients and demonstrating that bacteria emerge that carry mixed genotypes. He proposed the term "conjugation" when he demonstrated the requirement for a direct contact between bacteria (they would not "mate" through a filter). As you know, this term is a misnomer – *E. coli* don't actually perform bidirectional exchange of genetic information the way *Paramecium* does. What was the origin of this mistake? Why didn't Lederberg discover unidirectional transfer? (10 points)

William Hayes made elegant use of streptomycin in his proof that genetic transfer in *E. coli* is directional. The slide shown in lecture has a mistake, specifically, in the part highlighted in ***bold-face italic.*** Identify the mistake, please, and write out the correct version of this sentence. (10 points)

Cross #1:
Strain A (Str^R, B-, M-) × Strain B (**Str^S**, L-, T-)
Result: streptomycin completely inhibits prototroph formation (i.e., appearance of B+,M+,L+,T+ bacteria) if added before conjugation is complete.
Cross #2:
Strain A (**Str^S**, B-, M-) × Strain B (Str^R, L-, T-)
Result: streptomycin has no effect whatsoever. You can add it all you want, ***at any time,*** and prototrophs will still form!!

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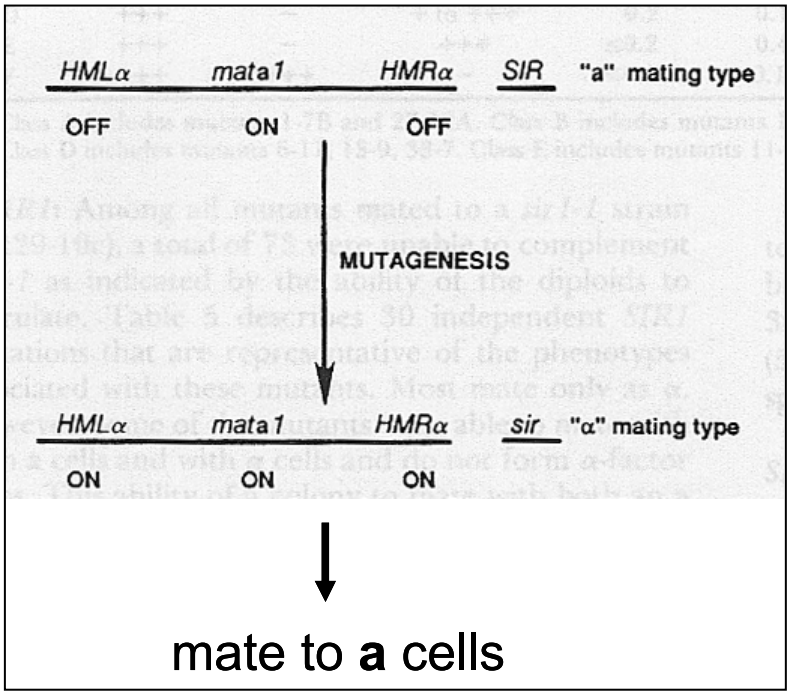
Question 2. The diagram on the right describes the outline of a screen done by Jasper Rine to identify genes responsible for the epigenetic silencing of mating type loci in budding yeast. As you can tell, Prof. Rine deliberately engineered a yeast strain for that purpose. Why wasn't this screen done in wild-type yeast? (10 points)



As you can see in the schematic, Prof. Rine's assay for a yeast cell carrying the desired mutation was mating it to a different yeast cell, to yield a diploid. This is a problem, is it not? We are trying to find a cell with a mutation, and instead we get a diploid yeast cell carrying one wild-type and one mutant copy. What two distinct solutions to this problem do yeast genetics and microbiology in general offer? (10 points)

1. _____

2. _____

We are accustomed to mutations being "100% penetrant" – in other words, every cell of a given genotype always has the expected phenotype. This is not the case for some mutant alleles of *SIR* genes – pools of cells of apparently the same genotype have a "mixed" phenotype (some cells retain silencing over the mating type loci, and some cells lose it). Provide an explanation for this phenomenon – be sure to compare this strange "incomplete penetrance" to a fundamentally similar process we discussed in class in the context of a different model system. Name that other process and explain the biological connection between these two (10 points)

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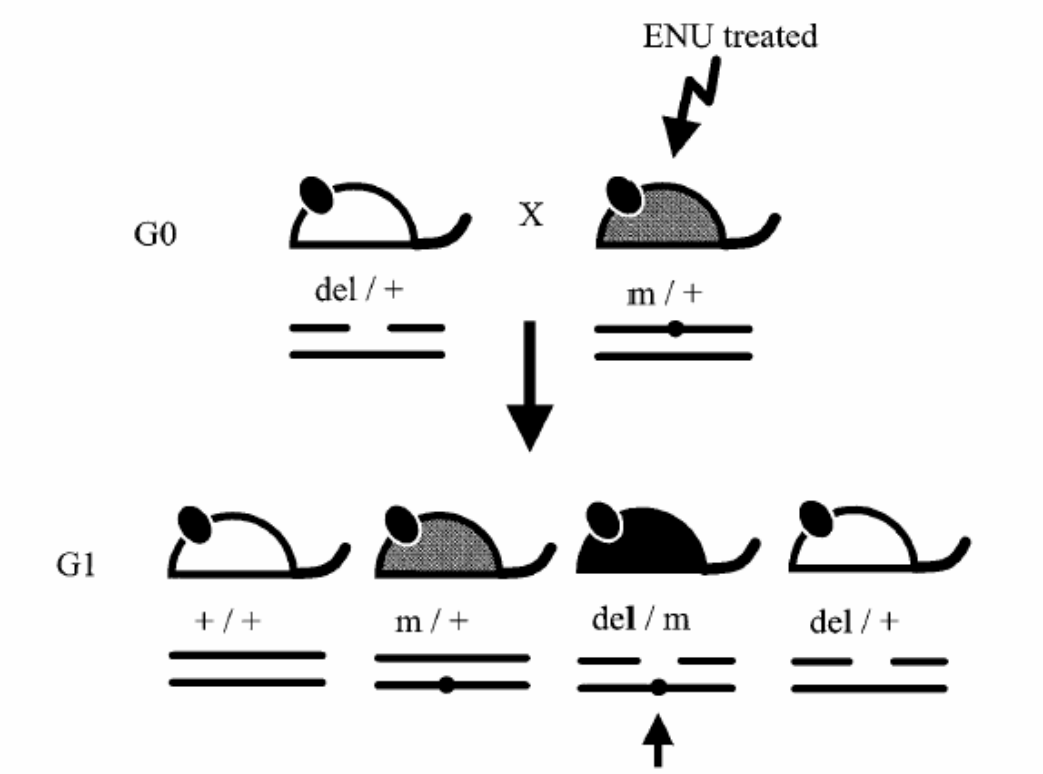
Question 3. The schematic below describes the outline of a forward genetic screen "over a deletion" for recessive mutations in the mouse.



To borrow a phrase from Denzel Washington's character in the movie *Philadelphia,* "explain this to me like I was a 4 year old."

What explains the need to screen for recessive mutations over a deletion, as opposed to in wild-type mice? (10 points)

The mouse identified with the arrow – when you do this screen, how exactly do you pick it out from the rest of the litter? How do you identify the animal that has a recessive mutation in the region spanned by the deletion? (10 points)

One cannot do such screens for every region in the mouse genome – not because one does not want to, but because the required mice cannot be obtained. The difficulty lies not in mutagenising the males and getting ENU to act, but rather in obtaining females carrying the relevant deletion. Why do you think that is? (10 points)

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Question 4. We talked a great deal about the utility of "mouse models of human disease," yet the fact remains that the mouse, however close to humans in its genetic makeup and physiology, cannot be used to model all human disease accurately. Cardiovascular disease, for example, is commonly studied in the pig and in the rabbit – animals with both heart and peripheral vasculature more reminiscent of their human counterparts.

Let us focus on the rabbit for this question. It is an animal whose physiology is very well understood, but genetic tools are, to a first approximation, entirely lacking. **Nothing is known about this animal's genetic makeup** (note: this is hyperbole for exam purposes). Assume you have the ability to trap rabbits in the wild – however many you wish. Describe two experiments designed to prove to reviewers on a federal funding panel that rabbits follow Mendel's laws. Use a "numbered list" format for both answers. (10 pts)

Expt. 1:	*Expt. 2*

Now that you have shown that rabbits engage in Mendelian genetics, describe what needs to be done in order to be able to do a forward genetic screen in the rabbit to identify loci involved in early-onset cardiac pathology (do not describe the screen itself, just what needs to be done to allow one to happen). Note that your goal is to make the rabbit as genetically tractable a system as the mouse is. For each step, state what you would do in the "What?" column, write a brief explication in the "What does that mean exactly?" column and a brief justification in the "Why" column. Think about how to state the correct answer succinctly – it must fit in the space below! (20 points)

What?	*What does this entail?*	*Why do this?*

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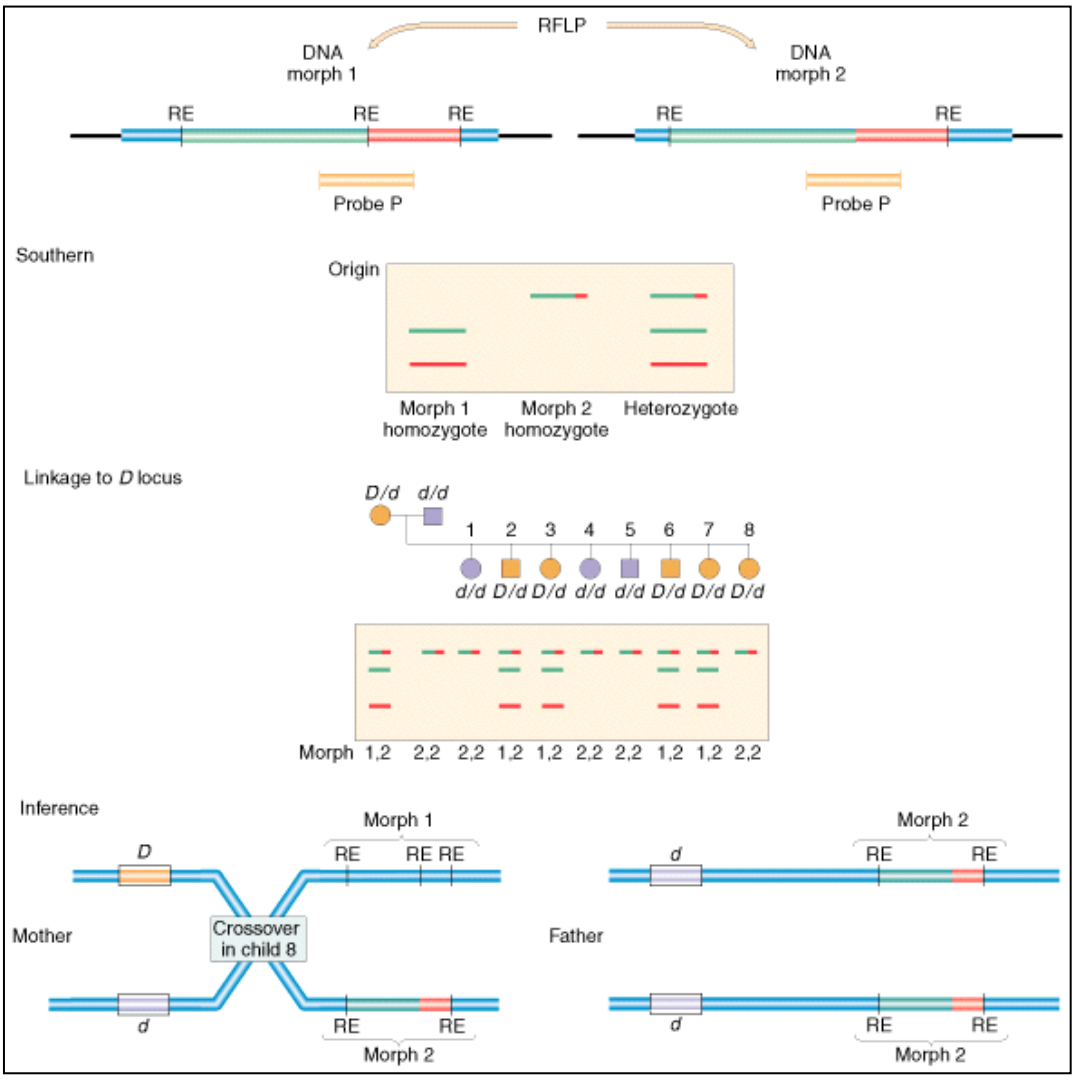
Question 5. As promised, here is a set of questions about "mapping by linkage" as shown in the figure below.



As seen above, there appear to be two "morphs" here differing in the presence of an "RE." Why is the existence of "morphs" – i.e., two different "allelic" forms of the same DNA stretch – so critically important for purposes of such analysis? Suggestion: in your answer, talk briefly about what would happen if the "morphs" were the same (5 points)

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Very strictly speaking, there is an error in this figure – specifically, in the boxed statement "crossover in child 8." There was, of course, no crossover **in** child 8. It seems the artist ran out of space to properly describe the actual event. Please remedy this error: assuming you have more space, write one full, proper English sentence that could go into that box and would accurately describe what actually happened in the case of child 8 (5 points).

As shown in this figure, a key component of "mapping by linkage" is the analysis of human pedigrees (e.g., multiple individuals in particular families). Why don't geneticists study random unrelated individuals when trying to identify loci that contribute to human disease? What explains this "focus on the family *per se*"? (5 points)

(a follow-up question to the previous one) While human geneticists cannot be picky and have to work with whatever subjects they can find, under most circumstances, they prefer to work with families where parents have more than one child (as shown in the figure). Why is that? (5 points)

The data in the figure have an important implication for this research project: the researchers still have a considerable amount of such "mapping by linkage" analysis to do before they can identify the locus containing the gene, mutations in which cause condition D. It is exceedingly likely they will have to analyze more families and look at more marker loci. What specifically about these data leads one to this conclusion? (10 points)