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.

Selection--only the trp$^+$ cells will grow.

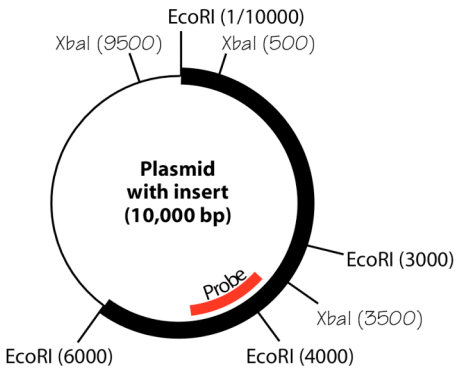
1b. Suggest two distinct hypotheses to explain how this trp$^+$ colony arose:

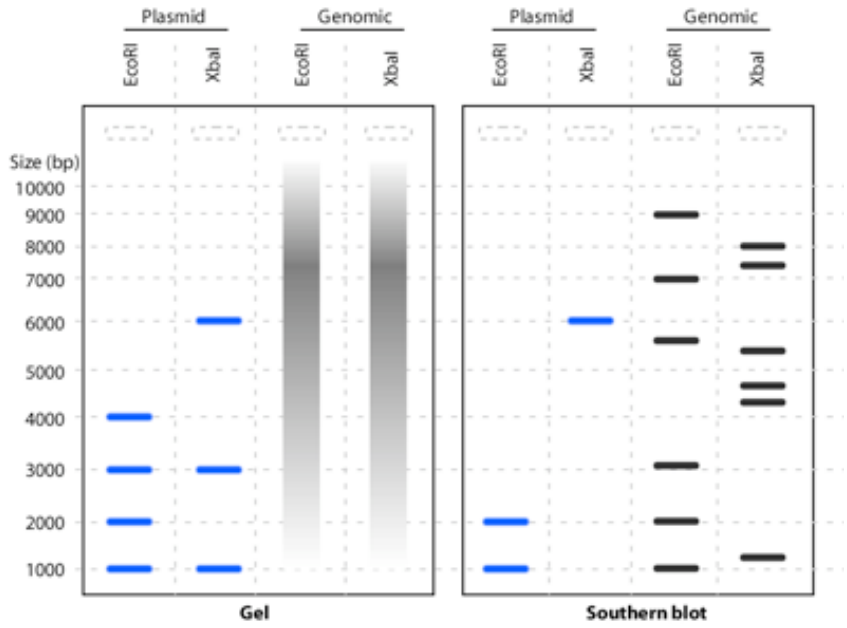
(i) A trp1 mutant cell took up a plasmid containing the wild type TRP1 gene, which allowed that cell to multiply and form a colony

(ii) The trp1 mutant allele in the host strain's genome spontaneously reverted to TRP1 (wild type)

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?

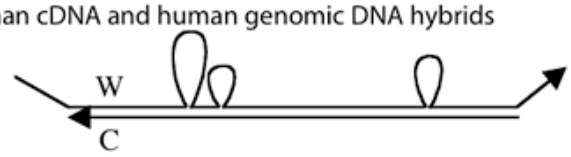
Repeated DNA present in the probe sequence allows the probe to recognize several different targets in the genome. Otherwise, we'd expect to see just one genomic DNA fragment hybridizing in the XbaI digest and two fragments in the EcoRI digest.

The reason why the 6 kb XbaI band doesn't show up in the Southern blot of genomic DNA is because the genomic DNA is from cells that do NOT have plasmid. On the plasmid, the fragment is part yeast (3500-6000) and part vector (6000-9500). The vector portion won't be present in the genomic DNA, so the size of the hybridizing genomic fragment will depend on how far away the next XbaI site is. In the EcoRI digest, the two fragments in the plasmid that the probe would recognize are both entirely genomic DNA, and do not contain vector DNA. We expect that the genomic DNA therefore would also contain the same spacing of EcoRI sites, and therefore show the same pattern of hybridization.

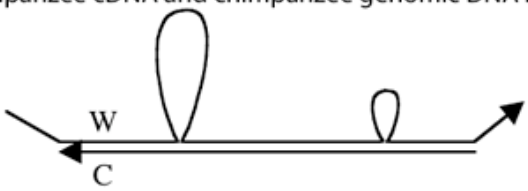
2. A human cDNA from a brain library was used as a probe to identify a genomic clone for this gene. The cDNA for this gene is 2 kb, the genomic clone for this gene is 4 kb. The human cDNA clone was also used to isolate clones from chimpanzee--both genomic and cDNA clones.

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.

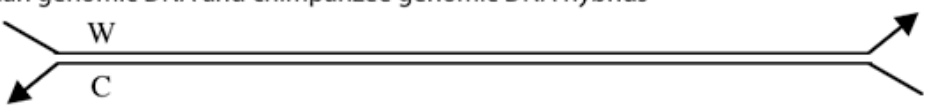
i. Human cDNA and human genomic DNA hybrids



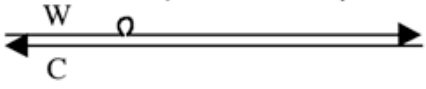
ii. Chimpanzee cDNA and chimpanzee genomic DNA hybrids



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)?

watson

2b. Based on figure i, how many exons __4 exons_____ and introns ___3 introns _____ are there in the human gene?

2c. Based on figure ii, how many exons _3 exons_____ and introns ___2 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?

The human and chimpanzee genes are homologous along most of their lengths, but the flanking DNA 5' and 3' differ.

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)?

Crick

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?

human exon 2

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?

To find type B mutants-mutagenize and plate with high levels of valine. To find type A mutants, grow in the presence of low levels of valine, replica plate, and screen for colonies that cannot grow in the absence of valine.

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

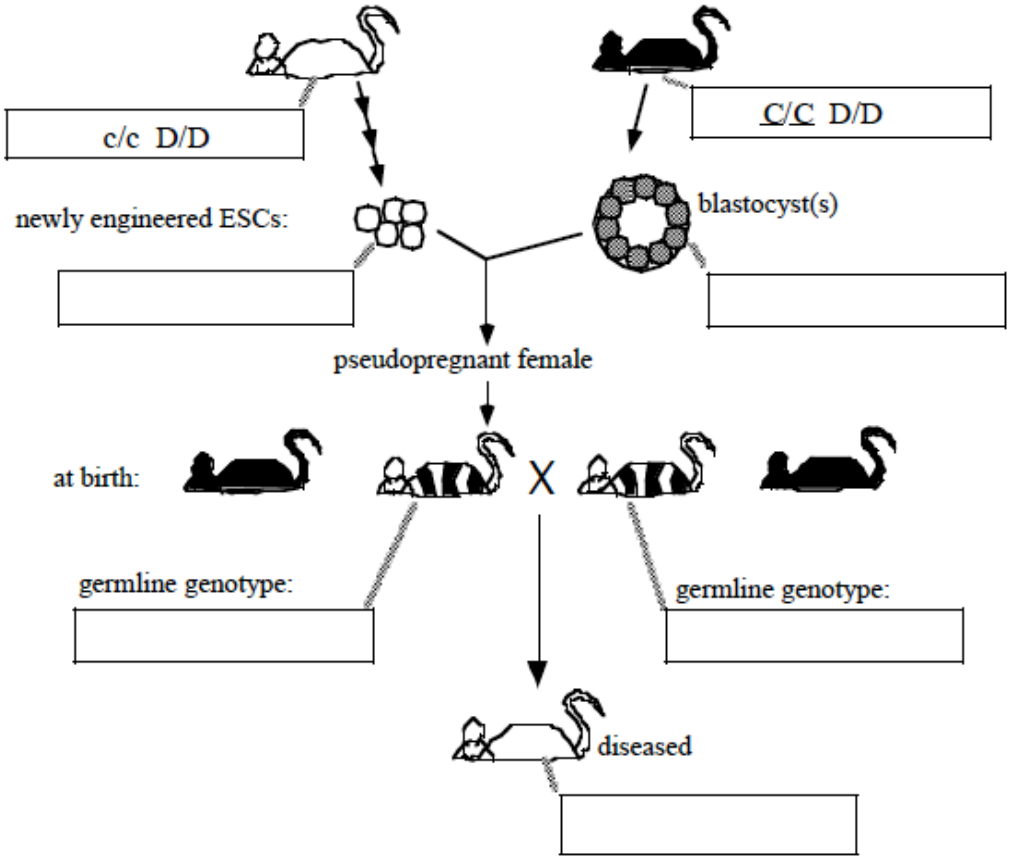
Begin with a type A mutant and transform it with a library constructed from the *E. coli* genome. Screen for colonies that are capable of growth in the absence of valine.

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)?

B1 class mutants are recessive to wild type and you could do a similar complementation cloning screen as in b. Transform in a wild-type E. coli library, selecting for transformants using a resistance gene that is also found on the plasmid. Then grow on plates lacking or with low levels of lysine, replicate plating onto plates with high levels of valine. Look for colonies that fail to grow when in high concentrations—these contain your mutants of interest. Since B2 is dominant to wild type, a similar yet slightly different approach could be used. Generate a library from B2 mutants and introduce into wild type cells. Grow these on plates with high valine concentrations—only those with the B2 fragment present will be able to grow.

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**.

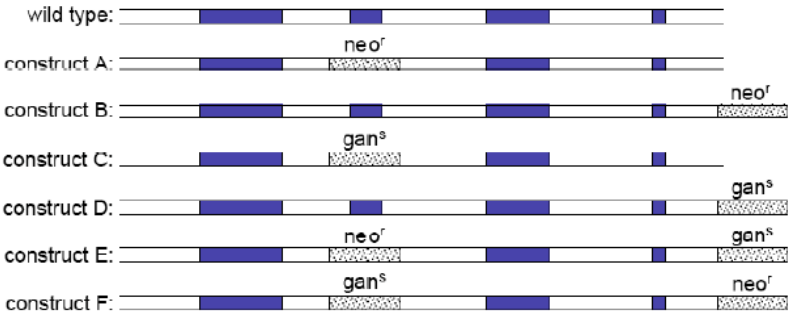


Newly engineered ESCs – c/c d/D
Blastocyst(s) – C/C D/D
Both germline genotypes – c/c d/D
Diseased – c/c d/d

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.

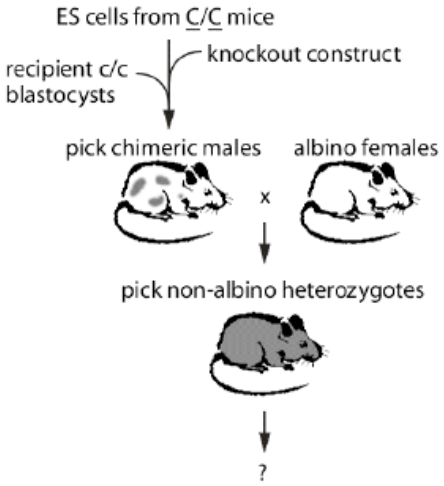
5a. Which of the above versions of the gene would you use to create a knockout of the mouse DIA1 gene (*dia1*^{KO})? Briefly describe how you would select for cells that have a knockout, and why your strategy would work?

ES cells would be transformed with Construct E and selection would be performed in media containing neomycin and gancylovir. Cells that survive would have had to undergo a homologous recombination event in which the normal DIA1 gene had been replaced with a construct bearing the neor gene, but lacking the gans gene. This selection strategy would eliminate cells in which an intact construct E had inserted randomly in the genome, as such cells would be sensitive to gancyclovir.

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?

Construct B would be used to transform ES cells and selection would be performed in media containing just neomycin. This would select for cells that had acquired the entire construct at a random location in the genome. Cells that undergo a homologous recombination event would lose the neor gene and would be sensitive to neomycin.

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}	1	Black; diabetic	Black; diabetic	Black
C/C; *DIA1/dia1*^{KO}	2	Black; diabetic	Black	Black
C/C; *DIA1/DIA1*	1	Black	Black	Black
C/c; *dia1*^{KO}/*dia1*^{KO}	2	Black; diabetic	Black; diabetic	Black
C/c; *DIA1/dia1*^{KO}	4	Black; diabetic	Black	Black
C/c; *DIA1/DIA1*	2	Black	Black	Black
c/c; *dia1*^{KO}/*dia1*^{KO}	1	White; diabetic	White; diabetic	White
c/c; *DIA1/dia1*^{KO}	2	White; diabetic	White	White
c/c; *DIA1/DIA1*	1	White	White	White