**Chapter 7 Study Questions**

*Genetic Analysis: Genes, Genomes, and Networks in Eukaryotes*

1. The experimental approaches described in this chapter for yeast and mice have been termed “reverse genetics”.
	1. What is meant by this term, and how does it compare to the genetic approaches discussed in Chapter 4?
	2. Is this distinction still useful for experimental analysis? Why or why not?

1. Experimental methods that yield gene replacements and to carry out genome editing in yeast have been done successfully for more than 30 years. Initial attempts to carry out gene replacements in Drosophila and *C. elegans*, and to some extent, Arabidopsis, were often based on the methods used in yeast; however, these were not very successful. What were some aspects of the biology of these organisms, unappreciated at the time, that allowed these methods to succeed in yeast but not in other organisms?

1. The *TUB2* gene and its genetic interactions will be extensively discussed in Chapter 10. The procedure used to make a *tub2* mutant is depicted in Figure 7.7, Panel B; this overall strategy is typical of what has been used for many targeted gene replacements in yeast.
	1. Discuss the methods used in this strategy in detail.
	2. While a similar strategy was used for making targeted *tub1* and *tub3* mutations, what additional complication had to be considered, based on the information in this chapter?
	3. The *tub2* mutation depicted in Figure 7.7 B is a null allele. How might a hypomorphic allele of a gene be made by targeted gene replacement?
2. Most investigators who want to produce a “knockout” mouse rely on a local specialized facility that carries out the procedure rather than doing this in their own labs. Thus, although most mouse geneticists might not knock out a gene themselves, it is important to follow the steps of how this is done. Using Figures 7-10, 7-11, and 7-12, describe in detail the procedure to make a knockout mutant mouse. Be sure to explain each part of Figure 7.11 and its role.
3. Using Figure 7.11 as your template, draw other vectors that have the neoR and the HSV-tk genes in other locations with respect to the coding region of the gene and each other. In each case, diagram the expected outcome in ES cells with these other vectors.
4. Most knockout mutant mice are now made using the Cre-*lox* recombination system rather than the vector shown in Figure 7.11.
	1. What are some of the advantages of using Cre-*lox* to make targeted mutations?
	2. What are some of the limitations, and how can these be overcome?
	3. The diagram in Figure 7.15 shows the *lox* sites in direct orientation, which deletes a region of the gene. Invert one of the lox sites so that they are now in reverse orientation with respect to each other (in other words, so the arrows are pointing towards each other.) What type of change will this make to the gene? What are some experimental questions in which this reverse orientation might be useful?

1. These questions are based on a study by Guenther et al 2014 Nature Genetics 46: 748-752, which can be consulted for more information. However, the questions do not require any information directly from that paper. A genome-wide association study in humans found that variants in the regulatory region of the *Kit Ligand (KITLG)* gene are associated with blond hair in some Northern Europeans. In order to investigate if variants in this region are directly causative for blond hair, the investigators made transgenic mice and compared their fur color to those littermates without the mutation. *KITLG* is an essential gene, so simply knocking out the gene in all cells is lethal and the effects on hair color would not be able to be determined. For the purpose of these questions, two experimental questions needed to be addressed.
	1. Is the KITLG gene expressed in developing hair follicles? Based on some known mouse mutations and the blond hair studies in humans, the regulatory region thought to be responsible for expression in hair follicles is confined to be a 17 kb region upstream of the gene. Outline an experimental strategy using a reporter gene such as lacZ in transgenic mice to define a portion of this 17 kb region needed for hair follicle expression.
	2. Is a sequence change in the regulatory region directly responsible for the color variation? This strategy from Part A first defined a region of 6.7 kb and then a smaller region of 1.5 kb within that which is responsible for hair follicle expression. Single base changes between the dark-haired and light-haired mouse within this 1.5 kb region were identified, and these changes to the 1.5 kb region can be made in vitro. Outline a strategy to replace this 1.5 kb region from a dark-haired mouse line with the corresponding sequence engineered in vitro to be the putative blond-hair sequence.
	3. KITLG is expressed widely in developing mice. Imagine that you want to knock out the expression of the gene only in hair follicles and not in other tissues. Outline a strategy that might allow you to carry out that experiment. Assume that any mouse strain or experimental reagent that you might need is available to you.