**Chapter 8 Study Questions**

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

With partial solutions

1. The chapter compares editing manuscripts with editing genomes. Like all analogies, this one has strengths and limitations.
   1. Compare how the “search” function is carried out for genome editing using TALENs, ZFNs, and CRISPR-Cas9.
   2. For manuscript editing, having a “search” string that is too short or that allows mismatches (that is, that uses wild-card characters) can result in edits at locations other than or in addition to the desired one. In genome editing, these are known as off-target effects. In either case, off-target effects can be reduced or eliminated by using a longer or more specific search string. Is using a long search string feasible with these genome editing methods or not? What, if any, are the limitations of a longer search string for genome editing?
   3. The “Edit” function is the least discussed step in genome editing, and is largely dependent on the natural processes of the cell. Suppose that you wanted to improve this editing function to be able to specify what type of edits are obtained. What cellular process or processes might be fruitful avenues to explore?

1. Although TALENs and ZFNs have been largely supplanted by CRISPR-Cas9 as a preferred method for genome editing, both methods are still in use. A paper by Liu et al. in 2018 (G3 2018 8:3221-3230) used TALEN to make targeted mutations in the *Fascin2* gene in mice. The paper is open access and can be consulted in answering these questions.
   1. In one sentence, what is the goal of this set of experiments?
   2. Using information in the chapter and the target sequence in Figure 1B, design the TALE domain amino acid sequence necessary for TAL1 and TAL2. Only the RVD needs to be specified since the other amino acids in the TALE domain are constant.
   3. How was the TALEN introduced into the mice?
   4. How were the TALEN-induced mutations repaired, and how do you know this?
   5. The authors do not tell us why they chose to make these mutations using TALENs rather than using CRISPR, although it could be that the experiments were begun before CRISPR was in widespread use. Based on the sequence of the first exon as shown in Figure 1C, design the sgRNA that would be needed to carry out the same experiment using CRISPR-Cas9.
2. Many hundreds of experiments using CRISPR-Cas9 to create a targeted gene knockout have been done. Experiments by Xu et al (G3 2018 8: 2833-2840) studied the PAX4 gene in rabbits. The paper is open access and can be consulted in answering these questions.
   1. In one sentence, what was the rationale for carrying out these experiments in rabbits rather than in mice?
   2. How were the sgRNAs designed?
   3. Explain why two different sgRNAs were used.
   4. How were the sgRNAs and Cas9 introduced into the rabbit embryos?
   5. What was the procedure to look for possible off-target mutations? Can you think of any limitations of this procedure that might have missed some off-target mutations?
   6. One concern with many genome editing experiments is that the animals are chimeras of both edited and unedited cells. These experiments do not directly test for the possibility of chimerism in the rabbits. Why do you think this was not done?
   7. Based on Table 1, which step in the overall process was the least efficient?
   8. Based on the results in Figure 1E, how were these double-stranded breaks repaired?