**BIO380: Cancer Biology**

**Homework Assignment #6: CRISPR-CAS9**

**50pt, Due Wednesday March 1**

Suppose you’d like to cure lung cancers caused by EGFR mutations, now that we’ve characterized the mutations involved. You select a patient with lung cancer, sequence their *egfr* gene and find a single mutation in exon 21 that encodes L858R (recall, this mutation *responds* to gefitinib).

**A.** Watch the Bozeman Science video explaining CRISPR: <https://www.youtube.com/watch?v=MnYppmstxIs>

Answer the following questions about the video:

1. Write a paragraph that includes the following terms: spacer, hairpin, CAS genes, immune system, helicases, nucleases, crRNA, tracrRNA and CAS9. (4pt)
2. How can a bacterial Cas protein be made to cut a gene of your choosing? (2pt)
3. How can a bacterial Cas protein be made to insert a gene? (2pt)
4. How is the CRISPR-Cas9 system similar to restriction digest? How is it different/better? (4pt)
5. The National Institutes of Health does not yet fund CRIPSR research involving human embryos. What are some ethical implications that arise when CRISPR technology is applied to humans? (2pt)

**B.** Read through the AddGene website explaining how to design a CRISPR experiment: <https://www.addgene.org/crispr/guide/>

Plan your CRISPR experiment, following the AddGene guide.

1. What is your desired genetic manipulation? (1pt)
2. What expression system would you use? (1pt)
3. What is your target gene? What specific part of the gene would you target? What specifically would you do to the gene? (5pt)
4. What is a PAM sequence and why are they important to identify when selecting your gRNA? (2pt)
5. Why are gRNA oligos inserted into plasmids? (2pt)
6. What delivery method would you select (hint, you will be using cells from a patient)? (1pt)
7. How will you know your gene has been delivered? (4pt)
8. Summarize your experiment in a paragraph or draw a picture. (4pt)

**C.** Read the short “Perspective” article on EGFR and CRISPR and answer the following questions. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4734839/pdf/EMMM-8-083.pdf>

1. How did the proposed approach differ from yours? (4pt)
2. What potential complications did the paper bring up that you did not consider when planning your experiment? (4pt)

**D.** Reflect upon your research.

1. Based on previous knowledge of CRISPR and what you know now, would you go ahead and perform your proposed experiment? If not, what research needs to be performed first? (4pt)
2. Do you think CRISPR research on human genes should be funded? Animal genes? Plant genes? Why or why not? (4pt)