

Reading:

Minimal PAM specificity of a highly similar SpCas9 ortholog.

Chatterjee P, Jakimo N, Jacobson JM.

Sci Adv. 2018 Oct 24;4(10):eaau0766. doi: 10.1126/sciadv.aau0766. eCollection

2018 Oct. PMID: 30397647

These questions will be discussed in class. Please hand in written answers on 11/19 for a total of 5 "class discussion" points per question.

1. Describe how ScCas9 was identified. 4-5 sentences.
2. Why was ScCas9 initially suspected to have an altered PAM specificity? 4-5 sentences.
3. Be able to diagram the PAM-SCANR system on the board and explain how it works. Do not be scared to check this paper:
<https://www.ncbi.nlm.nih.gov/pubmed/27041224>
4-5 sentences.
4. What are the structural differences between ScCas9 and SpCas9 that determine their differences in specificity? Be able to describe an experiment and outcome from the paper that is the basis for this conclusion. 6-8 sentences.
5. What is the experiment that led to results in Figures 3A and 3B? How does the experiment address the PAM specificity of ScCas9? Does the experiment agree with the results in Figure 2A? Which specific results are not in agreement? 6-8 sentences.
6. Describe one assay that was used to assess off-target activity of ScCas9. How did its off-target activity compare with that of SpCas9? 6-8 sentences.
7. How does SPAMALOT help to define the PAM specificity of Cas9 proteins? 6-8 sentences.