

**CRISPR new project**

Before starting a CRISPR project, please provide the following info:

1. Please specify your gene of interest and the cell line to be transfected.

2. What is the expected phenotype of knocking-out the gene of interest? Please base your answer on previous studies, gene ontology (GO), gene cards, etc.

3. What can you tell on the DNA sequence (motives, repeats, SNPs, % GC), RNA seq (splice variants), number of exons, pseudo genes?

4. Please perform PCR based mycoplasma test to ensure that the culture is mycoplasma free and attach the gel image.

5. What is the cells' karyotype and number of nuclei per cell? Please note that if the cell's karyotype is unknown, it might be abnormal and thus affect the knockout efficiency.

6. Can your cell line form colony from a single cell? Please perform dilution experiment to ensure.

7. Please specify the transfection conditions of your cell line (DNA concentration, cells' density, which reagent, electroporator's parameters) and what is the transfection efficiency in percentages. Note, that you will be required to provide us medium and transfection reagent.

8. Please contact addgene to purchase pSpCas9(BB)-2A-GFP (PX458) plasmid. You will also need to sign MTA.

9. Please be aware that the regular workflow includes working with single sgRNA. Working with several sgRNA is possible upon demand.