Planning Mamallian Transfections

Introduction

You have built all of your constructs, yay! It is now time to put them into mamallian cells

Materials

- Spreadsheet
- Midiprepped DNA
- Knowledge of the Cell line you are working with
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Procedure

How to Plan a Mammalian Transfection:

1. Have all required parts of your system midi prepped, so you have enough DNA to transfect
2. If your cells are adherent, 24 hours before transfection, seed your 5x10^4 cells into a 24-well plate.
3. Decide how much of each type DNA, in ng, is needed in each cell. (This value may need to determined experimentally).
4. Make sure you have an appropriate constitutively expressed color to act as a marker for how much plasmid made it into the cell.
5. Ensure you have proper negative controls for each variable you are testing.
6. Take the maximum amount of DNA needed in anyone of your cells, and set that as the amount of DNA for every cell. (This value should be between 1000-1500 ng of DNA).
7. For any cells that do not have the target amount of DNA, supplement the with junk DNA. (We used a P-Donor plasmid as junk in our experiment.)
8. Calculate the amount of transfection reagent you will need. This value is different across cell lines and reagent type. For our experiments, we used Viafect, at a 1:3 Viafect:DNA ratio.
9. Dilute your midi prep DNA to an appropriate concentration. (Usually between 10-150 ng/uL)
10. Determine the volume of DNA you will need to achieve the correct mass of DNA in ng.
11. Add media to bring the total volume of transfection mix up to 50 uL. (Make sure to include to volume of Viafect).

12. Once you have a plan for each well, run the transfection protocol

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