DNA Transfection into HEK Cells

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

- OptiMEM (Serum-free media)
- Viafect
- DNA from midipreps

Procedure

Phase I: Calculations

1. Select DNA to be transfected and determine the concentration through Nanodrop
2. Determine the volume of DNA required to have 0.5 ug in reaction mixture. [1000 ng = 1 ug]

   Ex. for a 521 ng/ul stock of DNA you would need approximately 1 uL. If the calculated volume you need is less than 0.5 uL (i.e. hard to pipette) you may want to dilute your DNA solution so that you can get an appropriate volume.
3. Determine the amount of Viafect to use from the following table:

Table1

	A	B	C	D	E	F	G	H
1	Volume of cells in media (per well)	Total volume transfection complex (per well)	Amount of DNA (per well)	Amt of Viafect Reagent for 1.5:1 Reagent:DNA	2:1	3:1	4:1	6:1
2	500 uL	50 uL	0.5 ug	0.75 uL	1 uL	1.5 uL	2 uL	3 uL

4. Calculate the amount of serum free DMEM media required to get a reaction volume of 50 uL

   Ex. if you are adding 1 uL of DNA and 3 uL of Viafect, you would want to add 46 uL of DMEM

Phase II: Reaction Mixture

5. This phase consists of mixing the reactants required to create the transfection complex. Do so for all ratios of reagent to DNA above - add media first, then reagent, then DNA.
6. Let the reaction mixture sit for 10-20 minutes

   Closer to 20 min is better. [Molly says 16 min]

Phase III: Transfection

7. Retrieve cells from incubator. Make sure to leave this step for last because you don't want to keep the cells at room temperature for too long.

8. Add entire 50 uL transfection complex to each well in the well plate and store your cells back in the incubator.

Phase IV: Checking for Fluorescence

9. Close off lights in room and turn on the microscope's light.

10. Apply the appropriate fluorescence filter.

11. Check for fluorescence

Protocol From BioCel:

1. Decide which DNA to add in each tube. DNA stocks are kept at 150 ng/ul, so 2 ul of each DNA will yield 300 ng of DNA. Depending on the group, you should be aiming to have 1800 or 2100 ng of DNA total in each tube.

12. DNA mass 1: 1800 ng

13. DNA total volume 1: 12 ul total (if all DNA at 150 ng/ul)

14. Viafect volume 1: 3 ul

15. Optimem volume 1: 35 ul

16. DNA mass 2: 2100 ng

17. DNA total volume 2: 14 ul total (if all DNA at 150 ng/ul)

18. Viafect volume 2: 4.67 ul

19. Optimem volume 2: 31.33 ul

20. Mix DNA in tube. Add enough junk DNA to bring it up to appropriate total volume

21. Add appropriate amount of viafect (1:4 ratio, viafect:DNA ratio)

22. Add enough optimem to bring total volume to 50 ul

23. Incubate for 12 minutes

24. Add total volume of tube to well.