RNAi – dsRNA Transfection using FuGENE 6

Materials:
6-well plates or 3cm dishes
15ml conical tubes
sterile Eppendorf tubes
FuGENE 6 Transfection Reagent* (Roche, Cat # 1 814 443)

* FuGENE 6 is a multi-component lipid-based transfection reagent that complexes with and transports DNA into the cell during transfection.

A) Preparation of cells for transfection

Use cells that are healthy and proliferating well.

1) Tap the flask to loosen cells from the surface. Harvest cells by pipetting up and down 10X.
2) Set aside a small aliquot of cells for counting.
3) Collect remaining cells in a 15ml conical tube. Note the volume of cells.
4) Determine the number of cells in the conical tube.
5) Centrifuge cells in the conical tube at 1000rpm for 3-5min (setting 4 on centrifuge)
6) Aspirate supernatant using sterile Pasteur pipette
7) Resuspend to 10^6 cells/ml in S2 medium with 10% serum. Pipette up and down 10X.
8) For a 6-well plate, seed 2 million cells into each well by adding 2ml of cell suspension with a 2ml pipette.

B) Preparation of FuGENE-RNA complex

General notes:

-Do not allow the undiluted FuGENE reagent to come into contact with any plastic surface other than the pipette tip.
-Always use more FuGENE reagent (µl volume) than RNA (µg mass)
-A ratio of 3µl FuGENE to 1-2µg RNA works well in S2 cells
-3 uL of FuGENE with 2 uL of dsRNA at a concentration of 1ug/uL is ideal
-For co-transfection experiments, if the total amount of RNA exceeds 2µg, increase the amount of FuGENE proportionally.

1) Based on the table on the following page, determine the volume of each component to use. It may be necessary to dilute your dsRNA for accurate pipetting. If so, dilute dsRNA in DEPC-treated water or nuclease-free water. Do not use a volume of more than 15µl of dsRNA.

2) (Optional) To transfect multiple wells with the same set of components, determine the volume required to make a master mix. If you have n well, prepare a mix enough for n+0.5 wells.

3) Mix the following components in the tissue culture hood. Note that the order of addition is critical.

Step	Component	Amount	Note
I	Serum-Free Medium (SFM)	X µl to a total of 100 µl	SFM must be pipetted into the tube first
II	FuGENE 6 Transfection Reagent	3 µl	Add FuGENE directly into the medium. Tap gently to mix.
III	dsRNA	0-2µg	Use a total volume between 0-15µl.

9) Tap the Eppendorf tube gently to mix contents.
10) Incubate for 20 min at room temperature.
11) When you finish, check that the cap of the FuGENE bottle is closed tightly.

C) <u>Transfection of cells</u>

1) After 20 minute of incubation, gently tap the Eppendorf tube to mix contents.
2) Dropwise, add FuGENE:RNA mixture to cells.
3) Gently rock the plate to ensure even dispersal of Fugene:RNA mixture.
4) Return plate to the incubator.