Protocol: Tissue Culture siRNA Transfection

Use this protocol to transfect cells in tissue culture with siRNA. This protocol describes the transfection of 3T3 cells in 6-well dishes using the IDT siRNA TriFECTa kit. The experiment can be scaled according to the manufacturer's directions.

Required Materials
Confluent culture of cells ready for passage
6-well dishes (tissue culture treated)
Lipofectamine RNAiMAX (Thermo Fisher)
OptiMEM media (no FBS no penicillin/streptomycin)
OptiMEM media + 5% FBS (no penicillin/streptomycin)
siRNA (including negative control)

IMPORTANT NOTES:

- IDT siRNAs are resuspended in 20μl nuclease-free water for a final stock concentration of 100μM. Dilute the stock siRNAs to 10μM (or other concentration depending on empirical evidence) in supplied duplex buffer to create a working stock. *Do this work under RNase-free conditions!!*
- We perform a 'reverse transfection' as described in the Lipofectamine RNAiMAX protocol. This involves plating the transfection reagent first, then adding the cells.
- One 100% confluent 10cm dish of 3T3 cells is enough for one 6-well plate. Scale up accordingly.
- 500,000 cells per well (6-well plate) results in approximately 80% confluency.
- **Controls are important!** Make sure to plan for the proper negative control, and positive control if applicable.

Part 1. Harvest Cells

1. Wash and trypsinize one 100% confluent 10cm dish of 3T3 cells per 6-well plate. Collect the cells in half the volume (5ml per plate) of OptiMEM +5% FBS. Multiple 10cm dishes can be combined into a single conical tube.
2. Perform a hemocytometer count. Dilute cells to **222,222 cells/ml** in Optimem +5% FBS (no penicillin/streptomycin). Set the diluted cells aside.

Part 2. Create Lipofectamine Transfection Reagent

1. Determine the number of transfection reactions needed (number of wells). The volumes below are for a single well of a 6-well dish. Complete the table below in your lab notebook.

Per siRNA experiment:

Reagent	***Volume per Rxn***	***Number of Rxns***	***Total volume***
OptiMEM (no FPS no P/S)	150μl		
Lipofectamine RNAiMAX	9μl		

Reagent	***Volume per Rxn***	***Number of Rxns***	***Total volume***
OptiMEM (no FPS no P/S)	150μl		
siRNA (desired μM concentration)	x μl (depending on final conc of siRNA)		

2. Using RNase-free microcentrifuge tubes, label one tube ***L*** (for lipofectamine) and the other ***R*** (for RNA). Add the calculated volume of OptiMEM and RNAiMAX Lipofectamine to the ***L*** tube. Add the calculated volume of OptiMEM and siRNA to the ***R*** tube.
3. Add the entire contents of the ***R*** tube to the ***L*** tube. Incubate at room temperature for 5 minutes.

Part 3. Reverse Transfection

1. Add 250µl of transfection reagent to each well of the 6-well dish. Make sure this pools in the center of each well.
2. Add 2.25ml of 3T3 cells from **Part 1** (diluted to 222,222 cells/ml in OptiMEM) directly to the transfection reagent in each well.
3. Allow transfection to occur for 24 hours.