ChimeraRNA transfection protocol

Part I

1. Seeding 293T cells at 10^5 cells/well (24 well plate).
2. Cells were washed with PBS once. Add 0.5 ml fresh DMEM without serum into cells.
3. Add 1 ul Lipofectamine 2000 into 30 ul DMEM without serum, mixing well and wait for 5 min at room temperature.
4. Add 1 ug of expression plasmid with 25 ul corresponding chimeraRNA (2 uM solution) in 30 ul DMEM without serum and mixing well.
5. Add Lipofectamine 2000 mixture from step 3 to the mixture from step 4. Mix well at room temperature for 20 min.
6. Add mixture from step 5 to cells at 24 well plate. Incubating at 37℃ incubator for 4 hrs.
7. Exchange the medium with 0.5 ml fresh complete DMEM to each well of 24 well plate and incubate at 37℃ incubator for overnight.
8. Exchange the medium with 0.5 ml fresh complete DMEM per well the next day and incubate at 37℃ incubator for overnight.
9. Total cells were collected at 48 hrs after transfection for Western blotting.

Note:

This protocol is for overexpression and knock-down at the same time. If one wants to knock-down the endogenous one, it may take 72-96 hrs.

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Part II

Material

All the plastic wear should be RNase-free, including the micro-pipette tip and the micro tube. To avoid RNase contamination, please do not touch plasticware even before autoclave sterilization procedure. Please keep the experimental bench as clean as possible.

Protocol

1. Optimization the transfection condition

Generally, transfection optimization could be achieved by analyzing lamin A/C and GAPDH of the transfected endogenous gene with western blot. In contrast, the optimization is performed by a superior quantitative measure, reporter gene assay, in our system, allowing the transfected clones steadily secrete the reporter gene, such as GFP and luciferase, in the cell culture medium. The optimized transfection condition is used in a Chimera RNAi experiment.

Higher transfection efficiency is usually achieved by using more transfection reagent, which usually increases the cytotoxicity. Therefore, the concentration of the transfected reagent requires an appropriate adjustment to avoid the cytotoxicity.

In order to mimic the physiological concentration of miRNA, at least 5nM of Chimera RNAi is used. The ideal condition would involve the use of 1 nM with 90% reporter activity suppression.

We transfect the firefly luciferase with pcDNA3 vector into HeLa, SK-OV-3 and SiHa cells; the G418 treatment is used to separate the clones that reveal the luciferase chronically.

The Chimera RNAi for firefly luciferase has been designed and synthesized. Chimera RNAi is introduced under the conditions that are specifically designed for each clone derived from those transfected cells.

2. Cell culture

Before the transfection step, it should be assured that target cells are in exponential phase. The cells are thawed freshly at the room temperature before each experiment and the cell cultures are kept for 1-2 weeks to reach a stabilized condition. We recommend the use of ATCC cell lines to avoid differences among laboratories. Please make sure that the CO_2 incubator is installed horizontally to avoid bias results.

A. One day before the transfection procedure, please perform cell passage and keep the cells in log phase.
B. On the day transfection, the cell culture should be 30% confluent.
C. The cells are seeded with a calculated density and the medium contains 10% FCS without antibiotic. Please shake the cell culture gently and assure even cell distribution; special attention must be paid when the experiment is performed in 96 well plates, because cells tend to distribute themselves near the on the wall.

The table below is a reference for the cell density when a 6-well plate is used:

Cell line	Cell density per well
SiHa cell	1～2 x 10^5 cell/well
SK-OV-3 cell	0.5～1 x 10^5 cell/well
HeLa cell	0.5～1 x 10^5 cell/well

Note:

- ✓ *The cell density seeded is proportional to the culture plate.*
- ✓ *The Chimera RNAi transfection efficiency increases with a limited range of increasing cell density, since cytotoxicity could also increase by the increasing transfection reagent used.*
- ✓ *PBS wash and medium replacement for the transfected cells on the day of transfection are unnecessary.*

3. Chimera RNAi transfection (transfection at the density of 5 nM or less)

Lipofectamine 2000 (LP2000, Invitrogen) is used as the transfection reagent, and the optimized condition for Chimera RNAi transfection should be determined.

The Chimera RNAi is thawed (make aliquots of Chimera RNAi 10μL/tube with the final concentration 50μM) and freezed at -20°C. Opti-MEM I nutrient medium (Invitrogen) is used to dilute the Chimera RNAi to make the working concentration of 10μM.

Obtain 1μL of the diluted 10μM working solution, mix it with 50 μL the Opti-MEM I nutrient medium in a microtube and vortex gently.

A. Preparation of Lipofectamine 2000 solution

For SiHa and SK-OV-3 cells

Add 1.6μL LP2000 reagent to 48.4 μL Opti-MEM I nutrient medium, vortex the mixture for a few seconds and leave the mixture at the room temperature for 5-10 minutes.

Note: As for SiHa and SK-OV-3 cells, the transfection concentration needs to be higher than 25 nM, or the transfection efficiency will be decreased due to the LP2000 cytotoxicity.

For HeLa cells

Add 0.4-0.5 μL LP2000 reagent to 49.5-49.6 μL Opti-MEM I nutrient medium,,vortex the mixture for a few seconds and leave the mixture at the room temperature for 5-10 minutes.

Note: As for HeLa cells, the transfection concentration needs to be higher than 5 nM, or the transfection efficiency will be decreased due to the LP2000 cytotoxicity.

B. Add LP2000 dilution solution to Chimera RNAi solution and vortex for several seconds. Add 100μL of the mixture to 2 mL of the medium to make the final concentration of 5 nM.

*A low concentration transfection of Chimera RNAi, LP2000 needs to be diluted into 1nM.

A. Preparation of Lipofectamine 2000 solution

For SiHa cell and SK-OV-3 cell

Add LP2000 1.6 μL into Opti-MEM I medium till 100μL, vortex for a few seconds. After the dilution, place the mixture in room temperature for 5-10 minutes.

For HeLa cell

Add LP2000 0.4-0.5 μL into Opti-MEM I medium till 100μL, vortex for a few seconds. After the dilution, place the mixture in room temperature for 5-10 minutes.

B. Add 20 μL Chimera RNAi solution to 80 μL LP2000 dilution solution in a microtube and vortex for a few seconds. Place the mixture at the room temperature for 15-30 minutes. Add 1μL of Chimera RNAi-LP2000 mixture to 100μL culture medium. The amount of LP2000 reagent used is proportional to the concentration of Chimera RNAi, not the amount of the culture medium.
C. 48-72 hours after the transfection procedure, the analysis can be performed.

Note: After the transfection, medium replacement is not required.

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