Transient transfection and luciferase assay

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Introduction

Transient transfection into mammalian cells is a convenient way to over express and obtain protein expression.

Reagents

Penn/Streptmycin

Gibco-OPTI-MEM (Reduced serum Medium) (Gibco-Invitrogen).

FuGENE® 6 transfection Reagent (Roche Applied Science, Indianapolis, IN)

DNA: QIAGEN mini or maxi prep grade. Solution in TE is compatible. Basically, no need to linearize DNA.

Procedure

Culture conditions

1. One day before the transfection experiment, adjust the cell concentration, and plate the cells.
2. Plating 2-5x10^6 cells in a 60-mm culture dish in 3 ml of medium overnight will achieve the desired density of 60-80% confluency.

Transfection of experimental cells

1. The following protocol is for 60 cm dish (culture medium: 5 ml). If you use 6-well or 12-well plates, total volume of the medium should be 3ml and 1.5ml, respectively, and decrease the amount of each reagent accordingly.
2. Plate cells the night before to give 60-80% confluence at the day of transfection. The efficiency will decrease if reached 100% confluence. Less than 50% confluence may be OK but the amount of protein expressed will be low because of the small numbers of cells.
3. Prior to the transfection, change to medium. Volume should be 5 ml per dish.
4. Label the small sterile tube and pipet 97µl of serum-free medium and add 3µl of FuGENE 6 directly into the tubes with out allowing contact with the walls of the plastic tubes.
5. Add 2µg experimental DNA into the sterile tube, tap the tube or vortex for one second to mix the contents.
6. Incubate the transfection reagent:DNA complex for minimum of 15 min at room temperature.

7. When you are ready, add this mixture directly to the cells drop wise through the medium. Make sure you evenly sprinkle the droplet over the entire area. There is no need to remove and replace with fresh medium.
8. Incubate for 36-48 h. Harvest cells, or culture supernatant. As long as the cells are alive, they produce proteins.

Preparation of Mammalian Cell Lysate for Luciferase assay

1. Remove growth medium from cultured cells.
2. Rinse cells in 1x PBS. Do not dislodge cells. Remove as much of the final wash as possible.
3. Dispense a minimal volume of 1x lysis reagent (RLB; Promega) into each culture vessel (e.g., 200-400µl/60mm culture dish).
4. For culture dishes, scrape attached cells from the dish, and transfer the cells and solution to a micro centrifuge tube.
5. Pellet debris by brief centrifugation, and transfer the supernatant to a new tube.
6. Mix 20µl of cell lysate with 100µl of Luciferase Assay Reagent and measure the light produced using LUMAT LB9501 (Berthold, TN).

Troubleshooting

Because of differences in transfection efficiencies, an expression plasmid pCMV β-galactosidase will be used to co-transfect as a transfection efficiency control, and luciferease activities will be normalized based on β-galactosidase activity with the chemiluminescent β-gal reporter gene assay kit (Promega).

References

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