PROTOCOL EXCHANGE | *COMMUNITY CONTRIBUTED*

High-efficiency transfection of small RNAs and plasmid DNA in human cells

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Introduction

miRNAs are small non-coding RNA involved in many different biological processes. Their overexpression in cultured cells is an essential tool to study their function.
Here we describe an optimized protocol for transient transfection of miRNAs and siRNAs in combination with plasmid DNA. In this experimental set-up, plasmid DNA is a luciferase reporter that allows quantitative measurements of the biological effect of the overexpressed microRNA on the transcription from a given natural or synthetic promoter. In our paper, this plasmid allowed to read the strength of the TGF-beta pathway activation (pCAGA12-lux).
siRNAs are well known reagents commonly used for gene knock-down; in our experimental set-up siRNA designed against the putative target of an miRNA was used as an experimental positive control and was therefore tested under the same conditions.

Subject terms: Cell culture Tissue culture Cell biology Developmental biology Genetic modification

Keywords: transfection microRNA luciferase

Reagents

Lipofectamine2000 (Invitrogen)
Lipofectamine RNAiMAX (Invitrogen)
Synthetic double-stranded RNAs (siRNAs or mature miRNAs) (Invitrogen)
OPTI-MEM (Reduced serum Medium) (Invitrogen)
24-wells plate (Falcon)
plasmid DNA (in sterile water)
Ligand of the pathway of interest (i.e. TGF-beta as recombinant protein from R&D)

Procedure

Day1:
1) Plate cells at ~25% confluency in 24-wells plates, using standard growth medium.

Day2:

2) Check cells confluency (it shoud be ~50%) and replace the medium with 500µl of growth medium without antibiotics.

3) Mix 600 ng of siRNA in 50ul of Opti-MEM (amounts and volumes used for a single well)

4) Mix 1 ul of RNAiMAX in 50 µl of Opti-MEM

5) Combine the 2 solutions, mix gently and incubate 10 minutes at room temperature

6) Add the solution uniformly to the cells

7) After 4-6 hours replace medium with standard growth medium

8) Incubate cells for 48 hours under standard conditions.

Day4:

9) Replace the medium with 500ul of growth medium without antibiotics

10) Mix 600ng of miRNA, 80ng of reporter plasmid and 40ng of pCS2-lacZ plasmid in 50ul of Opti-MEM (amounts and volumes used for a single well; pCS2-lacZ is used as normalizer for luciferase assays)

11) Mix 1ul of Lipofectamine2000 in 50µl of Opti-MEM

12) Combine the 2 solutions, mix gently and incubate 10 minutes at room temperature

13) After 4-6 hours replace medium with standard growth medium

Day5:

14) Replace the medium with 500µl of growth medium containing 0.1% serum and incubate for 8 hours

15) Dilute the protein ligand in 0.1% serum medium and add the solution to the cells

16) Incubate cells under standard conditions for 2-16 hours (according to the ligand activity)

17) Replace medium with 200µl per well of luciferase lysis buffer

18) Perform a standar luciferase assay

Timing

5 days

Critical Steps

In order to find the right concentration of extracellular ligand (i.e. giving an unambiguous reporter induction in treated cells, and thus a solid bioassay) we suggest to perform a titration experiment, using doses from 0. 1 ng/ml to 100 ng/ml

Troubleshooting

To maximize transfection efficiency, avoid cell clumping. For some epithelial cells, it may be advantageous to plate cells in collagen I coated dishes to facilitate spreading and even cell seeding

References

Martello et al., (2007) Nature (in press)

Associated Publications

This protocol is related to the following articles:

- MicroRNA control of Nodal signalling
  Graziano Martello, Luca Zacchigna, Masafumi Inui, Marco Montagner, Maddalena Adorno, Anant Mamidi, Leonardo Morsut, Sandra Soligo, Uyen Tran, Sirio Dupont, Michelangelo Cordenonsi, Oliver Wessely, and Stefano Piccolo

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Competing financial interests

The authors declare no competing financial interests.

Readers' Comments

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