Dharmacon siRNA Transfection of Hela Cells

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[Abstract] Small Interfering RNA (siRNA) is a class of double-stranded RNAs of 20-25 nucleotides that play important roles in many biological processes (Hamilton and Baulcombe, 1999). siRNAs act by "neutralizing" the mRNA of the target protein, facilitating degradation of the mRNA and hence altering the biological effect of the protein (reviewed in Hannon and Rossi, 2004). siRNAs may also change the intracellular levels of regulatory RNAs. Use of siRNAs for manipulating the expression of genes of interest in biological research is commonly referred to as RNA interference or knockdown technique (Elbashir *et al*., 2001). Synthetic siRNAs are an emerging tool that are now widely used in these studies. A variety of algorithms are employed by different companies for the design of siRNA products, which differ in efficacy, specificity and cost among other criteria. An example protocol of siRNA knockdown is explained here using the siGENOME SMARTpool reagents from Dharmacon.

Materials and Reagents

1. Human cervix epithelial carcinoma cell line Hela (ATCC, catalog number: CCL-2™)
2. Eagle's Minimum Essential Medium (ATCC, catalog number: 30-2003™)
3. Fetal bovine serum (FBS) (ATCC, catalog number: 30-2020™)
4. 5x siRNA buffer (GE Healthcare Dharmacon, catalog number: B-002000-UB-100)
5. DharmaFECT 1 siRNA Transfection Reagent (GE Healthcare Dharmacon, catalog number: T-2001-01)
6. siGENOME SMARTpool reagents (GE Healthcare Dharmacon)
7. Glyceral-dehyde-3-phosphate dehydrogenase (GAPD) (GE Healthcare Dharmacon, catalog number: D-001140-01)
8. Non-targeting siRNA control pool (GE Healthcare Dharmacon, catalog number: D-001206-13)

Equipment

1. 12-well polystyrene tissue culture plate (BD Biosciences, Falcon®, catalog number: 353043)

2. Cell culture incubator: 37 °C and 5% CO_2

Procedure

1. Carry Hela cells in Eagle's Minimum Essential Medium with 10% FBS.
2. Trypsinize, count cells and reseed cells 12-16 h before knockdown (See Note 1).
3. Resuspend siRNA in 1x siRNA buffer to reach a final concentration of 5 μM.
4. Add 5 μl of the 5 μM siRNA to 95 μl of serum-free medium in a low-adhesion tube 1, mix by gently tapping the tube or pipetting up and down.
5. Add 0.5~5 μl DharmaFECT 1 reagent (see Note 2) to 99 μl of serum-free medium in a separate tube 2, mix by gently tapping the tube or pipetting up and down (see Note 3).
6. The two tubes in steps 4-5 are incubated at room temperature for 5 min.
7. Add the content of tube 1 from step 4 to tube 2 from step 5 (siRNA into DharmaFECT), mix gently by pipetting up and down, and incubate at room temperature for an additional 20 min.
8. Add 800 μl of complete medium to the resulting mixture of step 7 (final siRNA concentration is 25 nM).
9. Remove culture medium from the 12-well tissue culture plate. Add the medium mixture of step 8 (total of 1 ml) to each well (see Note 4).
10. Grow Hela cells for additional 24-48 h before mRNA analysis, or >48 h for protein analysis.
11. Cytotoxicity should always be carefully monitored throughout the knockdown process. Experimental conditions should always be determined empirically.
12. Each experiment should have “control groups” including Non-treated cells, Positive control siRNA (*e.g.,* GAPD), negative control siRNA (*e.g.,* “Non-targeting”). Perform experiments in triplicates as a minimum.

Notes

1. Optimal cell seeding density for each cell type should always be determined empirically. For HeLa cells, the cell density should reach ~50% confluence at the beginning of the knockdown procedure.
2. 1 μl of DharmaFECT reagent was found to yield good knockdown results.
3. Use different DharmaFECT Transfection reagent for different cell lines. Check www.dharmacon.com for details.

4. For steps 8-9: Alternatively, one can also replenish the original medium in the well with 800 µl of fresh complete medium, followed by evenly "dropping" the 200 µl reagent mixture of step 7 into each well.

Acknowledgments

This protocol was developed in the Department of Immunology, Scripps Research Institute, La Jolla, CA, USA and adapted from Elbashir *et al.* (2001), Hamilton and Baulcombe (1999) and Hannon and Rossi (2004). The work was funded by NIH grants CA079871 and CA114059, and Tobacco-Related Disease, Research Program of the University of California, 15RT-0104 to Dr. Jiing-Dwan Lee [see Chen *et al*. (2009)].

References

1. Chen, Y., Lu, B., Yang, Q., Fearns, C., Yates, J. R., 3rd and Lee, J. D. (2009). Combined integrin phosphoproteomic analyses and small interfering RNA--based functional screening identify key regulators for cancer cell adhesion and migration. *Cancer Res* 69(8): 3713-3720.
2. Elbashir, S. M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K. and Tuschl, T. (2001). Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 411(6836): 494-498.
3. Hamilton, A. J., Baulcombe, D. C. (1999). A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science* 286(5441): 950-952.
4. Hanno, G. J. and Rossi, J. J. (2004). Unlocking the potential of the human genome with RNA interference. *Nature* 431(7006): 371-378.