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Lentivirus production and transduction

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Abstract

Lentivirus production and transduction

1. Lentiviruses containing shRNA targeting vectors were produced to test the knockdown efficiency of shRNA constructs in cultured primary astrocytes or to bulk transduce neurons with shRNA and GFP.

2. To produce lentivirus, HEK293T cells were transfected with a pLKO.1 shRNA Puro targeting plasmid (for astrocyte transduction), an envelope plasmid (VSVG), and a packaging plasmid (dR8.91) using X-tremeGENE (Roche).

3. One day after transfection, the media was replaced with AGM (for astrocyte transduction), and media containing lentivirus was collected on days 2 and 3 post-transfection.

4. To assess the knockdown efficiency of shRNAs in astrocytes, rat primary astrocytes at DIV 7 were plated in 6-well dishes in 2 ml of AGM.

5. On DIV 8, 1 ml of AGM was removed, and 500 µl of fresh AGM was added along with 500 µl of lentivirus-containing media and 1 µg/ml polybrene.

6. Cultured astrocytes were treated with puromycin (1 µg/ml) from DIV 10-15 to select for transduced cells.

7. Cultured astrocytes were lysed at DIV 15 for protein extraction and Western blot analysis.