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Lentivirus Production and Astrocyte Transduction

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Shiyi Wang[1]

[1]Duke University

ASAP Collaborative Rese…

Shiyi Wang
Duke University

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Abstract

Lentivirus Production and Astrocyte Transduction

1 1. **Materials Required**

1.1 - pLKO.1 shRNA Puro targeting plasmid (for astrocyte transduction)

1.2 - Envelope plasmid (VSVG)

1.3 - Packaging plasmid (dR8.91)

1.4 - HEK293T cells

1.5 - X-tremeGENE transfection reagent (Roche)

1.6 - Astrocyte growth media (AGM)

2 - Puromycin (for selection)

2.1 2. **Transfection of HEK293T Cells**

2.2 - Plate HEK293T cells in appropriate culture vessels (e.g., T75 flask) to achieve 70-80% confluence on the day of transfection.

2.3 - Prepare transfection mix per flask:

2.4 - Combine 2 µg pLKO.1 shRNA Puro plasmid, 1.5 µg VSVG plasmid, and 1.5 µg dR8.91 plasmid in Opti-MEM.

2.5 - Add X-tremeGENE transfection reagent according to the manufacturer’s instructions.

2.6 - Incubate transfection mix at room temperature for 20 minutes.

2.7 - Add transfection mix dropwise to cells and gently swirl flask to mix. Incubate at 37°C with 5% CO2.

3 3. **Collection of Lentivirus**

3.1 - Replace media with fresh AGM 24 hours post-transfection to enhance virus production.

3.2 - Collect lentivirus-containing media on days 2 and 3 post-transfection.

3.3 - Filter collected media through a 0.45 µm syringe filter to remove cell debris.

3.4 - Store lentivirus aliquots at -80°C for future use.

4 4. **Transduction of Rat Primary Astrocytes**

4.1 - **Day 7 In Vitro (DIV 7)**

4.2 - Plate rat primary astrocytes in 6-well dishes at a density suitable for transduction experiments (e.g., 2 ml AGM per well).

4.3 - **Day 8 In Vitro (DIV 8)**

4.4 - Remove 1 ml of AGM from each well and replace with 500 µl fresh AGM + 500 µl lentivirus-containing media.

4.5 - Add 1 µg/ml polybrene to enhance transduction efficiency.

4.6 - **Day 10-15 In Vitro (DIV 10-15)**

4.7 - Treat transduced astrocytes with puromycin (1 µg/ml) to select for cells expressing the shRNA construct.

4.8 - **Day 15 In Vitro (DIV 15)**

4.9 - Lysate astrocytes to extract proteins for Western blot analysis to assess knockdown efficiency.

5 Notes:

5.1 - Handle lentiviruses in a biosafety level 2 (BSL-2) laboratory following institutional guidelines.

5.2 - Perform all steps involving lentivirus production under sterile conditions to prevent contamination.

5.3 - Optimize lentiviral titration to achieve desired transduction efficiency in astrocyte cultures.

5.4 - Ensure proper disposal of materials used for lentivirus production according to institutional biohazard waste disposal protocols.