Oct 08, 2022

Viral-mediated short-hairpin RNA knockdown

DOI
https://dx.doi.org/10.17504/protocols.io.q26g7y819gwz/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.q26g7y819gwz/v1

Protocol Citation: Chuyu Chen, Loukia Parisiadou 2022. Viral-mediated short-hairpin RNA knockdown. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g7y819gwz/v1

Protocol status: Working
We use this protocol and it's working

Created: October 08, 2022

Last Modified: May 31, 2024

Protocol Integer ID: 71031

Keywords: ASAPCRN, hairpin rna knockdown stereotaxic injection of viral vector, hairpin rna knockdown, viral vector, gene knockdown experiment, u6 vector for aav1 packaging, aav vector, using aav, vector biolab, aav1 packaging, stereotaxic injection, rna, gene, striatum of mice, u6 vector, mice

Funders Acknowledgements:
Aligning Science Across Parkinson's through the Michael J. Fox Foundation for Parkinson's Research (MJFF)
Grant ID: ASAP-020600

Abstract

Stereotaxic injection of viral vectors for gene knockdown experiments : The AAV vectors for knocking down the gene of interest were custom-made with Vector Biolabs using AAV-GFP-U6 vector for AAV1 packaging (Cat no, 7040, Vector Biolabs). They were then unilaterally injected into the striatum of mice as follows.

Just before the procedure

1 The following preparatory steps are followed:

1.1 Weigh the animals

1.2 Calibrate the David Kopf frame based on the manufacturer's instructions.

1.3 Clean the Hamilton Syringe with 70% ethanol and distilled water.

1.4 Shave the fur off the top of the animal's skull.

Viral injections

2 Anesthetize the animal with 3-4% Isoflurane. Monitor closely the animal until it reaches anesthesia (typically 5–10 min) and does not respond to a light pinch to the hind paw or tail.

3 Place the animal in the stereotaxic instrument by carefully placing one ear bar in the ear canal, securing the bar, and holding the animal in place as the other ear bar is placed and secured.

4 Secure the mouth in the incisor adapter of the stereotaxic instrument. It is critical to ensure that the airway is not blocked.

5 Cover the anesthetized animal's eyes with sterile ocular lubricant to keep them moist during the surgery.

6 Clean the skin's surface with alcohol and Iodine prep wipes.

7 Make a midline incision to the skin's surface's skull with small surgical scissors or a scalpel.

8 Clean the skull with cotton swabs.

9 Identify the coordinates relative to bregma:
- four sites in the striatum
- anterior-posterior 0.8 mm,
- mediolateral 2.4 mm
- dorsoventral −2.8 and −3.6 mm
- anterior-posterior 0.2 mm
- mediolateral 1.8 mm,
- dorsoventral −2.8 and −3.6 mm relative to bregma

10 Load the viral solution with Hamilton

11 Find bregma with the syringe needle and zero everything in the stereotactic frame

12 Determine your coordinates. Deliver virus by 100 nl/min speed (500-700 nl of AAV1-GFP-U6-shPKARIIβ or AAV1-GFP-U6-sh control viruses (5×10^12 genome copies/ml)

13 After injections, wait 10 minutes to allow the virus to diffuse

14 Remove the syringe very carefully and close the surgical sutures

15 Make a subcutaneous injection of Meloxicam right after the surgery and up to 48 hours.

16 Move the animal to the cage on the heat pad and monitor carefully its behavior.

17 Perform behavioral and biochemical experiments 4 weeks post operation.