Oct 03, 2024

Systemic AAV administration through retro-orbital injection

DOI
dx.doi.org/10.17504/protocols.io.36wgqnw73gk5/v1

Gerard Michael Coughlin[1]

[1]California Institute of Technology

Gerard Michael Coughlin
California Institute of Technology

DOI: dx.doi.org/10.17504/protocols.io.36wgqnw73gk5/v1

Protocol Citation: Gerard Michael Coughlin 2024. Systemic AAV administration through retro-orbital injection. **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgqnw73gk5/v1

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

Created: October 02, 2024

Last Modified: October 03, 2024

Protocol Integer ID: 108966

Keywords: Adeno-associated viruses, Retro-orbital injection, Viral vectors

Funders Acknowledgement:
Aligning Science Across Parkinson's
Grant ID: ASAP-020495

Abstract

Adeno-associated viruses (AAVs) are popular tools for gene delivery to animal models. Natural and engineered AAVs can even achieve widespread transduction of target organs through systemic delivery. Fast (<3 minute per mouse) and reproducible transduction through systemic AAV delivery can be achieved with retro-orbital injection. This protocol describes how to systemically deliver AAVs via the retro-orbital sinus.

Safety warnings

AAVs are biohazardous materials and must be handled according to governmental and institutional regulations. Experiments involving AAVs were performed using biosafety level 2 practices as required by the California Institute of Technology and the US Centers for Disease Control and Prevention.

rAAVs, although replication-incompetent, are potent gene-delivery vehicles and must be handled according to governmental and institutional regulations. The safety of packaged transgenes (e.g., oncogenic genes) should be carefully considered. Perform all procedures in a certified biosafety cabinet and clean AAV-contaminated equipment, surfaces, and labware with fresh 10% (vol/vol) bleach.

Isoflurane is a halogenated anesthetic gas associated with adverse health outcomes in humans and must be handled according to governmental and institutional regulations. To reduce the risk of occupational exposure during rodent anesthesia, waste gas was collected in a biosafety cabinet using a charcoal scavenging system as approved by the California Institute of Technology.

Ethics statement

Animal husbandry and all procedures involving animals were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and approved by the Institutional Animal Care and Use Committee (IACUC) and by the Office of Laboratory Animal Resources at the California Institute of Technology.

Training

1 Though quick and less technically challenging than other injection routes, retro-orbital injections can still take practice to become proficient. Practicing by injecting adeno-associated viruses, especially at large doses, can be costly and time consuming. 1h

Injection of dyes can provide cheaper and faster paradigm for practicing retro-orbital injections, with more immediate feedback than needing to wait for AAV expression.

Injection of 50 µL of 1 mg/mL fluorophore-conjugated tomato lectin dye (e.g. https://vectorlabs.com/products/dylight-649-lycopersicon-esculentum-tomato) will label vasculature. 01:00:00 following injection, euthanize, perfuse (30 mL of heparinized 1x PBS, followed by 30 mL of 4% PFA, in 1x PBS), then collect and post-fix target organs. Process tissue with appropriate techniques (slicing, tissue clearing, etc.). Clear vascular labeling should be evident under fluorescence microscopy.

Systemic AAV administration through retro-orbital injection

2 Before beginning, ensure that all AAVs are prepared and recently (< 1 month) titered. If injecting multiple AAVs (either into the same animals, or animals in separate conditions), we recommend that all AAVs are titered in the same qPCR or ddPCR assay.

Make a plan for diluting AAVs in the injection buffer, considering number of animals per group and total dose per animal. Plan to make slightly more than necessary to ensure that each animal gets the full volume (e.g. 10% extra, but appropriate surplus will depend on number of animals to inject). To reduce variability in injected dose due to error when measuring volume using a syringe, we recommend larger volume injections (up to 120 µL)

Note

Example: you want to inject a 1e12 vg per animal into 3 animals, with a volume per animal of 100 µL. Your AAV virus was titered at 4.23e13 vg/mL. To ensure that all animals receive the full dose, you plan to prepare 10% extra.

For 1 animal, you need 1e12 vg in 100 µL
For 3 animals, you need 3e12 vg in 300 µL
To provide 10% surplus, you need 3.3e12 vg in 330 µL

To calculate volume of AAV stock to use in dilution, divide the desired amount by the titer:

3.3e12 vg / 4.23e13 vg/mL = 3.3e12 vg / 42.3e12 vg/mL = 0.078 mL = 78 µL

Fill to 330 µL by adding 252 µL of sterile saline.

3 Dilute AAVs in the injection buffer. We use sterile saline to dilute AAVs for injection. Briefly vortex AAV dilutions, then briefly centrifuge to collect at bottom of tube.

Note

Dilute AAVs on the day of injection, ideally immediately before injecting. If necessary to wait, keep dilutions on ice or at 4 °C until injection. Allow to warm to room temperature before injection, to minimize discomfort to animals.

AAVs can adsorb to the surface of plastic tubes. Thus, some AAVs will be lost due to adsorption, and the percent loss will be greater with lower concentrations. Thus, we don't recommend prolonged storage of AAV suspensions, especially those at low titer.

4 Prepare work area. Set up isoflurane anaesthesia system inside the biosafety cabinet (BSC), with an induction box. Ensure that you have easily access to biohazard sharps disposal. Place a small stack of paper towels near the front of the BSC.

5 Transfer mouse to induction chamber and anaesthetize with 5% isoflurane in 1L/min oxygen.

6 While animal is being anaesthetized, load a 31g insulin syringe with the virus. Draw up virus into the syringe, then gently eject virus back into the tube to expel air bubbles. Repeat this until no bubbles remain in the barrel of the syringe. Draw up virus to desired volume and place the syringe in a safe, sterile, and accessible place until needed.

7 Once the mouse's breathing rate is about 1 breath per second, turn off isoflurane and flush chamber. Transfer anaesthetized mouse to stack of paper towels, with nose pointing towards the experimenters dominant hand and ventral side facing the experimenter. The eye to inject will be facing upwards.

Using the index finger and thumb of the non-dominant hand, draw back skin around the eye. This will cause the eye to protrude slightly from the socket. Using the dominant hand, insert the needle bevel down at a 30°-45° angle into the medial canthus and through the conjunctival membrane. The needle should be positioned behind the eye, in the retro-orbital sinus. Slowly inject the virus into the sinus, then remove the needle by pulling in the opposite direction as it was inserted.

Note

Avoid touching the surface of the eye with the needle.

Retro-orbital injection should not result in bleeding and no liquid should leak out following injection. If either of these occur (bleeding or loss of viral dose due to leak), the animal may still be transduced, but just at a lower MOI. Take note of injection quality for all animals, and consider these when analyzing results later.

8 Apply 1-2 drops of 0.5% ophthalmic proparacaine to the corneal surface for local analgesia. Transfer animal to a clean cage and monitor until the animal is recovered.

Note

Once the animal has recovered from isoflurane anaesthesia, there should be no obvious behavioural changes due to the injection.

Protocol references

Challis, R.C. et al. Systemic AAV vectors for widespread and targeted gene delivery in rodents. *Nat. Protoc.* **14**, 379–414 (2019).