

Title: Intratracheal Delivery of AAV in Mice
Lab: Heaney SATC for Gao Validation
Submitted: 9/12/21

Intratracheal (IT) delivery of AAV in mice – Xue lab/Simon Liang

Adapt from Nat Protoc. 2009; 4(7): 1064–1072.

IT delivery provides the most direct and consistent method for the virus to reach the lungs. Reproducible delivery of the virus is critical because it directly affects the number of tumors generated in the mice.

Mice

Our laboratory has utilized Ai9 mice between 6 and 8 weeks of age for lung editing by IT delivery of viruses expressing sgRNA and spCas9. Mice of this age are old enough to recover from the anesthetic, the volume of virus administered to the lung, and the intubation of the trachea with the catheter.

Volume of virus

Mice can be infected with a volume ranging from 40–100 μ l per mouse, but we recommend using a total volume of 40 μ l per mouse.

Materials

- Mice
- Isoflurane
- AAV targeting loxP and expressing spCas9
- PBS

Equipment

- Flat forceps and IV catheter

<ul style="list-style-type: none">• Flat forceps (Roboz, cat. no. RS-8260)	<ul style="list-style-type: none">• Roboz	<ul style="list-style-type: none">• RS-8260
--	---	---

<ul style="list-style-type: none"> • Exel Safelet IV catheters (22 gauge, 1 inch, Fisher, cat. no. 14-841-20) 	<ul style="list-style-type: none"> • FISHER SCIENTIFIC COMPANY LLC 	<ul style="list-style-type: none"> • 14-841-20
--	---	---

- Endotracheal Intubation Kits (Kent scientific, ETI-MSE)
<https://www.kentscientific.com/products/endotracheal-intubation-kits/>

Procedure

1. Anesthetize mice via isoflurane
2. Place mouse on the platform so that it is hanging from its top front teeth on the platform.
3. Prepare the IV catheter for the infection procedure. 40ul of total AAV for each mouse.
4. Open the mouth and gently pull out the tongue with the flat forceps.
5. Locate the opening of the trachea by peering into the mouth, position the catheter to the opening trachea, and allow the catheter to slide into the trachea until the top of the catheter reaches the mouse's front teeth. There should be no resistance while inserting the catheter into the trachea.
6. Pipette the virus directly into the opening of the catheter to ensure the entire volume is inhaled.
7. Once the virus is no longer visible in the opening of the catheter, wait a few seconds for the entire volume to travel down the catheter before removing the catheter from the trachea and disposing of it in 50% bleach.
8. Place the mouse under a heat lamp to recover in the biosafety hood.

