Intratracheal delivery of lentivirus/cells in mice

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

The intranasal and intratracheal infection techniques described in this protocol are not restricted to tumor-initiating studies. Investigators may theoretically probe the role of any Cre/LoxP-controlled genes in various cells of the lung using this protocol to deliver Cre. Alternatively, the intranasal or intratracheal delivery methods can be used in applications other than the viral delivery of Cre recombinase. The viral delivery systems may be adapted to express cDNAs or shRNAs in cells of the lung by infection with lentiviruses. While either the intranasal or intratracheal techniques can be utilized for the aforementioned studies, only intratracheal intubation is recommended for the orthotopic transplantation of lung tumor cells or lung tumor cell lines to the alveolar space of the lungs.

While we initially delivered Cre to the lungs of anesthetized mice using intranasal (IN) instillation of the virus, we now prefer to deliver the virus to the lungs by intratracheal (IT) intubation. 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. However, intratracheal intubation requires additional equipment and practice to perform it correctly and in a timely manner. Therefore, it may be easier to begin with the IN delivery method to assess the tumor model and practice the IT method while continuing to breed animals for future experiments.

Age of Mice

Our laboratory has utilized mice between 6 and 12 weeks of age for tumor initiation by IN or IT delivery of viruses expressing Cre. 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 50–125 µl per mouse, but we recommend using a total volume of 75 µl per mouse.

Materials

- Mice between 6 and 12 weeks of age
- Avertin
  2-2-2 Tribromoethanol (Avertin, Sigma Aldrich T48402)

2-methyl-2-butanol (Tert-amyl alcohol, Sigma Aldrich 152463)

- Phosphate Buffered Saline (PBS)
- Minimal Essential Media (MEM, Sigma, M4655)
- 2M $CaCl_2$ (the one used for transfection)
- Bleach
- Lentivirus-Cre (Three plasmid transfection system of CMV-VSV-G (Addgene plasmid 8454), Δ8.2 (gag/pol). The virus need to be concentrated and titered prior to use.

Equipment

- Needles (26G gauge, ½ inch, Becton Dickinson, cat. no. 305111)
- Syringes (1 ml, Becton Dickinson, cat. no. 309602)
- Flat forceps
- IV catheters (24G x ¾ inch, Terumo SR-OX2419CA, order from Santa Cruz website)
- Intubation platform (Steve Boukedes, moc.liamg@snoitnevnibal)
- Fiber-Lite Illuminator
- Heat lamp (or latex gloves filled with warm water)

Procedure

1. Avertin Administration TIMING 5 min per mouse. For IT experiment, I will inject Avertin one mouse at a time.
Anesthetize mice via intra-peritoneal injection of room temperature 20 mg ml^{-1} avertin (use 0.4 mg g^{-1} body weight for females and 0.45 mg $^{-1}$ body weight for males). Confirm the mice are fully anesthetized by ensuring that they lack a toe reflex.

CRITICAL STEP Administering the correct amount of avertin is crucial to successfully delivering the virus.

2. Intratracheal infection method TIMING 1–5 min per mouse

The procedure described below has to be done in a Biosafety hood !

A. Place mouse on the platform so that it is hanging from its top front teeth on the bar.

B. Push the mouse towards the bar so that the chest is vertical underneath the bar (perpendicular to the platform).

C. Direct the Fiber-Lite Illuminator to shine on the mouse's chest, in between the front legs.

D. Prepare the IV catheter for the infection procedure. To ensure that the needle does not become exposed and impale the mouse, hold the square part of the needle with one's thumb and index finger, and using one's middle finger, push the catheter over the end of the needle completely and continue to hold the catheter in place during the infection protocol.

E. Using the Exel Safelet IV catheter, open the mouth and gently pull out the tongue with the flat forceps.

F. Locate the opening of the trachea by peering into the mouth and looking for the white light emitted from the trachea (Fig. 1e).

G. While holding the IV catheter vertically, position the catheter over the white light emitted from the opening of the 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.

H. While stabilizing the IV catheter with one hand, remove the needle from the mouth.

I. CRITICAL STEP Prior to removing the needle, the mouse cannot breathe through the catheter. Once the Exel Safelet IV catheter has been inserted into the trachea, promptly remove the needle to allow the mouse to breathe through the catheter.

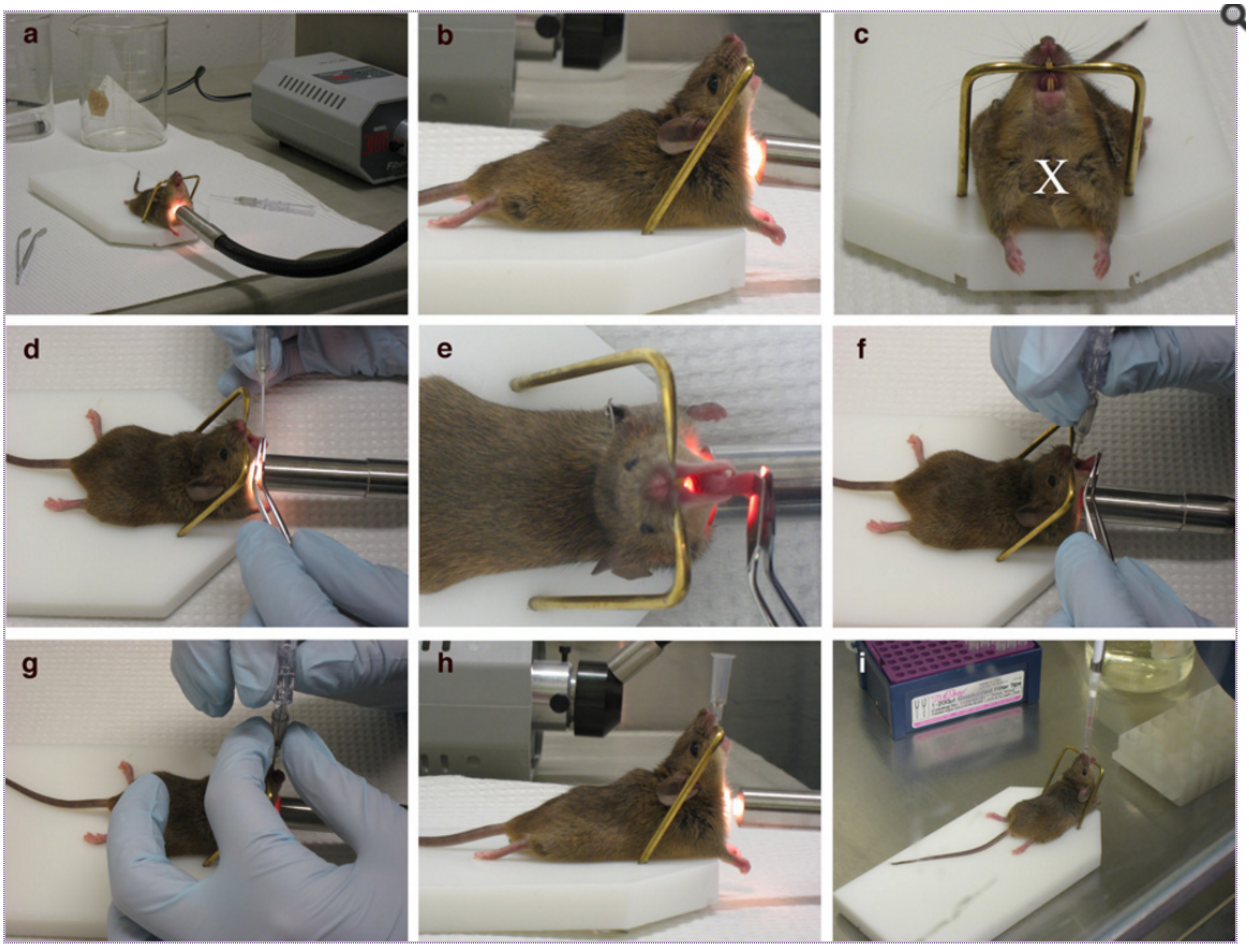
J. The proper placement of the catheter in the trachea can be confirmed by visualizing the white light shining through the opening of the catheter in the mouth.

K. Move the platform, mouse, and catheter into the biosafety hood.

L. Pipette the virus directly into the opening of the catheter to ensure the entire volume is inhaled.

M. If the catheter is correctly inserted into the trachea, the mouse will begin inhaling the virus immediately. 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.

3. Animal Recovery TIMING 10–15 min Place the mouse under a heat lamp or on a latex glove filled with warm water to recover in the biosafety hood.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