# *In Vivo Delivery of Lenti-Cre or Adeno-Cre into Mice Using Intranasal Instillation*

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# In Vivo Delivery of Lenti-Cre or Adeno-Cre into Mice Using Intranasal Instillation

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## Abstract

Lung cancer remains the leading cause of cancer deaths among both men and women, with a lower rate of survival than both breast and prostate cancer. Development of the Cre/lox system and improved mouse models have allowed researchers to gain a better understanding of human disease, including lung cancer. Through the viral delivery of Cre, gene function in adult mice can be precisely studied at a specific developmental stage or in a specific cell/tissue type of choice. This protocol describes how to produce adenovirus-Cre precipitate. Using this adeno-Cre (or lentivirus-Cre), Cre can be expressed in mouse lungs. The virus is delivered by intranasal instillation.

## MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPE: Please see the end of this article for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

### Reagents

Adenovirus-Cre (University of Iowa, Gene Transfer Vector Core)

The University of Iowa sends virus at a high titer of $10^{12}$ particles/mL which is equal to $10^{10}$ PFU/mL. It is important to minimize freeze/thawing as the virus titer drops ten-fold with each freeze/thaw cycle. Upon arrival thaw the virus on ice, aliquot it, and store them at −80°C.

Alternatively, lentivirus-Cre, prepared in **Producing and Concentrating Lenti-Cre for Mouse Infections** (Gierut et al. 2014), can be used.



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Avertin (20 mg/mL) <R>

Avertin is our laboratory's preference, but other anesthetics may be used according to investigator preference.

$CaCl_2$ (2 M, prepared in $H_2O$)

Floxed mice, $p53^{fl}$ (Jackson Laboratory 008462; strain B6.129P2-*Trp53*$^{tm1Brn}$/J)

Minimal essential medium (MEM)

### Equipment

Biosafety hood (BL2)

Microcentrifuge tubes, polypropylene

Needles (30 gauge, ½ inch)

Protein gel pipette loading tips

Tuberculin syringes (1 mL)

Warming pad (Kent Scientific DCT-25)

## METHOD

### Preparation of Adenovirus-Cre Precipitate

The volume of virus prepared is determined by the number of mice that will be infected. Each mouse is infected with 62.5 μL of the virus mixture.

**1** Thaw the adenovirus on ice. In a polypropylene microcentrifuge tube, add 60 μL of MEM and 2.5 μL of adeno-Cre virus to obtain $5 \times 10^6$ PFU per dose. Mix by lightly flicking the tube.

**2** Slowly add 0.3 μL of 2 M $CaCl_2$ to the mixture to obtain a final concentration of 10 mM $CaCl_2$. Mix by lightly flicking the tube. Incubate for 20 min at room temperature to allow a calcium phosphate precipitate to form.

The adenovirus precipitate mixture should be used within 1 h of preparation. Adeno-Cre delivery requires ~5 min per mouse. Eight mice are normally infected at a time, so the virus is used within 1 h of preparation. If many mice will be infected, precipitates can be prepared sequentially.

### Intranasal Instillation of Virus into Mice

**3** Anesthetize the mice via intraperitoneal injection with 20 mg/mL avertin (use 0.4 mg/g body weight for females and 0.45 mg/g body weight for males). Check that mice are fully anesthetized by ensuring that they lack a toe reflex.

All animal procedures should be done in accordance with protocols approved by your Institutional Animal Care and Use Committee.

See Troubleshooting

4 In the biosafety hood, hold the mouse in your hand so that the ventral side is facing up. Tilt the mouse so that its head is positioned above its feet. Hold the protein gel loading tip directly over the opening of one nostril and slowly dispense the virus, in a dropwise fashion, until the entire 62.5 μL has been inhaled.

See Troubleshooting

5 Place the mouse on a warming pad to recover in the biosafety hood. Monitor the health of the mouse before returning it to its cage.

See Troubleshooting

## TROUBLESHOOTING

*Problem (Step 3):* The mouse is not falling asleep.

*Solution:* Administer more avertin. Increase the amount of avertin 50–100 μL at a time so not to harm the mouse.

*Problem (Step 3):* The mouse is over anesthetized and breathing very slowly and irregularly.

*Solution*: Do not administer virus to the mouse until breathing has become regular. Before viral delivery make sure that the mouse lacks the toe reflex.

*Problem (Step 4):* The mouse is coughing and ejecting virus from the nostril.

*Solution*: Too much virus was placed on the nostril before being inhaled. Let the coughing subside and let the animal recover. Using the pipette, collect the virus that is resting on the nostril for reuse. In a dropwise manner add the virus to the nostril.

*Problem (Step 5)*: The mouse does not recover well following anesthesia.

*Solution*: The mouse may be either too hot or too cold. If the mouse is too cold, increase the warming pad temperature and monitor until the mouse is awake. If the animal is too hot, turn off the warming pad and let the mouse recover.

## RECIPE

### Avertin (20 mg/mL)

1. To prepare a 1.6 g/mL avertin stock solution, add 15.5 mL of 2-methyl-2-butanol (tert-amyl alcohol) to 25 g of avertin (2-2-2 tribromoethanol). Seal the bottle and protect the solution from light. Stir the mixture overnight at room temperature to dissolve.

   Discard the solution if it turns yellow. The stock solution is stable at room temperature for ~1 yr.

2. To prepare an avertin working solution at 20 mg/mL, dilute the avertin stock solution in phosphate-buffered saline (PBS). Protect the solution from light, and stir

overnight at room temperature. Sterilize the working solution by passing through a 0.22-μm filter. Aliquot and store at 4°C in the dark for up to ~4 mo.

## References

Gierut JJ, Jacks TE, Haigis KM. Producing and concentrating lenti-Cre for mouse infections. Cold Spring Harb Protoc. 2014