Intranasal instillation with Adenovirus
Kim Mercer 2/19/04, modified by C. Kim

Infect mice at 6 weeks of age.

You need:

Virus on ice (thawing virus that has been stored in –80°C is best done on regular ice).
MEM (Sigma catalog #M4655)
2M $CaCl_2$
syringes & needles for avertin
Avertin
Protein tips
Tubes to prepare virus in (50mL conical)
Filter tips to prepare virus (prepare in procedure room)
pipetmen

1. Make up Virus (total volume 62.8ul per mouse) to allow formation of Ad:CaP_i co-precipitates
   Mix MEM and Virus for a total of 62.5ul (2.5 x10^7 PFU dose per mouse)
   Then add 0.3ul $CaCl_2$, mix, and allow to stand for 20 mins*.

   Example: virus from Iowa is 4 x 10^{10} PFU/mL
   To give each mouse 2.5 x10^7 PFU, use 0.6 uL of virus + 61.9 uL MEM + $CaCl_2$

2. Anesthetize mice. Check that they are out by pinching feet. If mice are not fully under they will cough up the virus.

3. Using protein gel loading tip give 62.5ul to mice at nostril. Do not put the tip inside the nostril. Pipette slowly but steadily by placing a drop on the nose of the mouse held flat in your hand. Be sure the mouse is indeed inhaling the drop that is forming. If not stop expelling and wait until it inhales what is there.

4. Mouse will start to cough following the infection. Also, avertin depresses the body temperature and breathing rate. If you notice a mouse that is breathing really slowly or shivering I find that if you fill a large glove with hot water and place the mouse on the glove it often helps it to survive. Monitor their health before returning to the cage.

5. Perform this in the hood in the animal procedure room. Wear gloves, other standard protection, cages will be labeled with the reagent used and investigator will perform first cage change.

Notes:

*Original Jacks Lab protocol administered virus as follows.
Mix 121.9ul MEM and 2.5ul virus. Then add 0.6ul $CaCl_2$.
Viral mixture was given in 2 doses of 62.5ul, second dose being delivered after the coughing stopped. Modification above was made to enable a single administration of virus rather than two.

*The University of Iowa facility sends virus at a titer of 10^{12} particles/mL which is usually equal to 10^{10} PFU/mL. We order it aliquoted to avoid a thaw/freeze which could reduce titer. **Make sure you find out the exact PFU concentration in the lot we have in the freezer before you use it—it can vary by 2-10 fold with each batch.**

Ordering info. can be obtained from the following people:
The tech doing the ordering is:
Kate Ramsey
Research Assistant I
University of Iowa
Gene Transfer Vector Core
221 EMRB
Iowa City, IA 52242
phone: (319) 335-6726
fax: (319) 353-5572
kate-ramsey@uiowa.edu

The director of the facility:
Beverly L. Davidson, Ph.D.
Roy J. Carver Assoc Prof Internal Medicine
Assoc Prof in Neurology and Physiology & Biophysics
Director, Gene Transfer Vector Core
200 EMRB
University of Iowa College of Medicine
Iowa City, IA 52242
(319) 353-5511
beverly-davidson@uiowa.edu

Once we receive virus form the University of Iowa, it is aliquoted in 25ul volumes in and stored @ –80ºC. We should test all new batches of virus with 3TZ cells to make sure the Cre is active.

*When infecting with calcium phosphate precipitation, the size of the particle continues to increase with longer times of incubation and eventually gets to be a size that interferes with infections (usually 1 hour of incubation).
We normally infect 8 mice at a time so the virus is not sitting around for longer than an hour.