

Virus injection

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 Works for me

[dx.doi.org/10.17504/protocols.io.bctxiwpm](https://doi.org/10.17504/protocols.io.bctxiwpm)



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ABSTRACT

This protocol is used to inject viral vectors expressing proteins such as ion channels, transcription factors, enzymes, receptors, fluorescent proteins or other non-toxic, non-hazardous payloads. The viral vectors include AAV, Adenovirus, modified RV, Lentivirus, Modified Herpes Simplex Virus, and Pseudorabies Virus.

The protocol is often performed in conjunction with the headbar implantation protocol ([dx.doi.org/10.17504/protocols.io.bcrsiv6e](https://doi.org/10.17504/protocols.io.bcrsiv6e)). Please refer to the headbar implantation protocol for more details.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

This procedure was originally developed in: Petreanu, L., T. Mao, S. M. Sternson, and K. Svoboda. "The Subcellular Organization of Neocortical Excitatory Connections." *Nature* 457, no. 7233 (January 18, 2009): 1142–45.

GUIDELINES

Standard sterile procedures for surgeries apply.

MATERIALS

NAME	CATALOG #	VENDOR
Petri Dish	LI-PD01100	P212121
Microcentrifuge		
Micropipettes (2-20 ul, 50-250ul, 100-1000ul)		
1 ml syringes (or U-100 Insulin Syringe)	329461	BD Biosciences
Anesthetic (Isoflurane)	NC9259743	Fisher Scientific
ethanol		
Betadine solution	AJ159701	VWR International Ltd
Stereomicroscope	GC01650	Microscope Depot
Gloves	PK20782	Ansell
Heating Pad	TP22B	Gaymar
Clippers Oster MiniMax trimmer	07-842-4245	Patterson Veterinary
Ketoprofen	07-803-7389	Patterson Veterinary
Buprenorphine (Buprenex)	191.26890.3	Midwest Veterinary Supply
Ice Bucket	M16807-1104	
Applicators, Applicator stick; Length: 6 in.; Cotton-tipped	23400101	Thermo Fisher
Shandon™ Disposable Scalpel No. 10, Sterile, Individually Wrapped, 5.75 (14.6cm)	3120032	Thermo Fisher

NAME	CATALOG #	VENDOR
Shandon™ Iris Scissors, Probe/Point, Angular, Premium, 4.5 in. (11.4cm)	71906	Thermo Fisher
Dumont Forceps (Cover Slip Forceps)	11251-33	Fine Science Tools
Glass Pipettes		Drummond Scientific
Virus		
Light Source		
Stereotax		
Drill		
Eye lubricant		
Dental Cement		
Cortex buffer		
Marcaine		
Gelfoam		
Krazy glue		
Volumetric microinjection system		

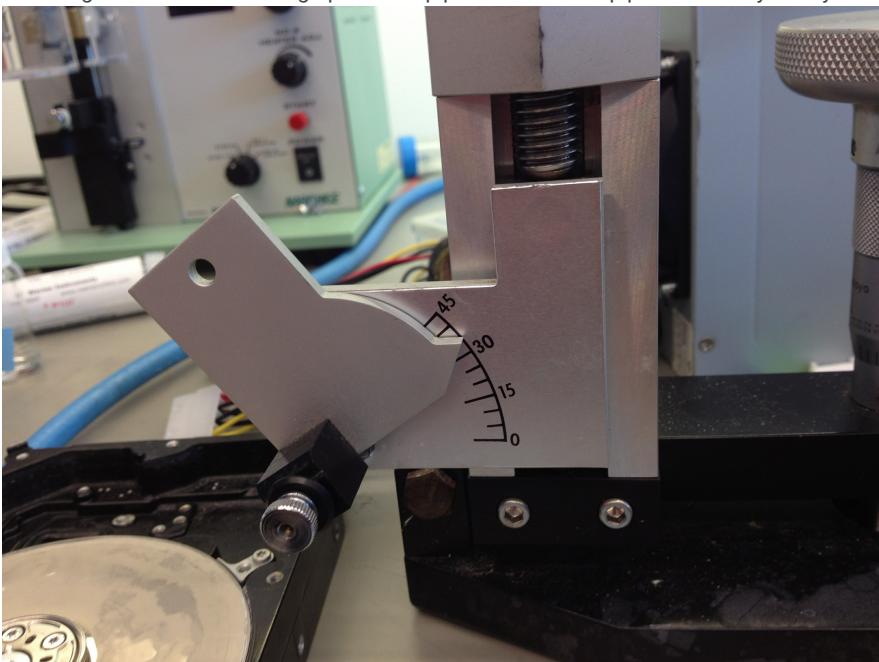
BEFORE STARTING

Please refer to the headbar implantation protocol for more details on the surgical setup (dx.doi.org/10.17504/protocols.io.bcrsiv6e).

Pipette preparation

- 1 Using thin-walled Drummond glass, pull symmetrically on the P-2000 (Sutter) or equivalent.
- 2 Under visual inspection (e.g. microforge or microscope) inspect the tip and carefully break the tip by hitting it against a solid surface. Aim for approximately 20µm tip diameter.

- 3 Mount pipette on a beveler at a 35° angle or less to horizontal, with the broken end roughly flush with the platter. Wet a kimwipe with 70% EtOH. Start the hard drive. Maintain a wet drive surface by holding the wet wipe softly above the platter. The EtOH prevents glass dust from kicking up into the pipette. Lower the pipette and very briefly touch the spinning surface and return.



Beveler on top of a harddrive.

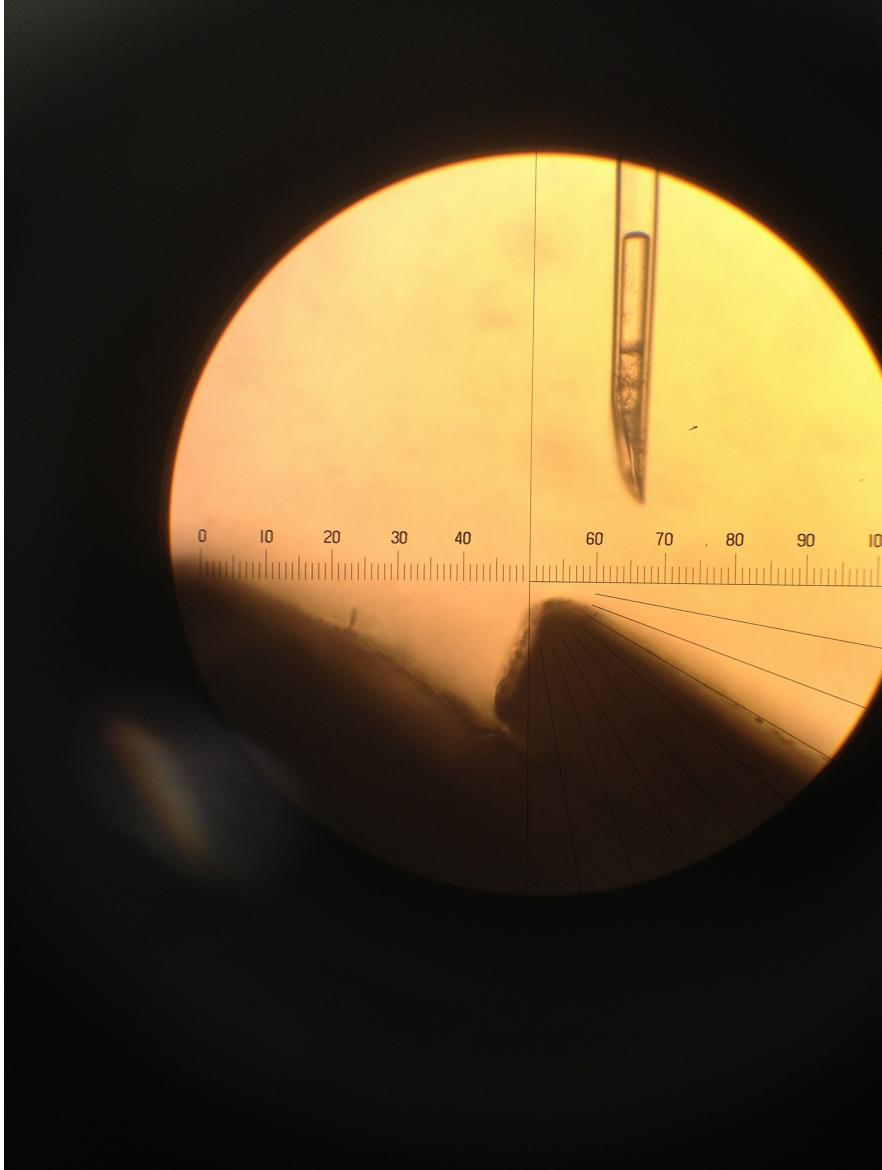


EtOH going inside through the tip of the pipette during sharpening usually indicates a successfully sharpened tip.

- 4 Inspect the pipette. Try to blow dust out of the inside and off the tip with a canned air.

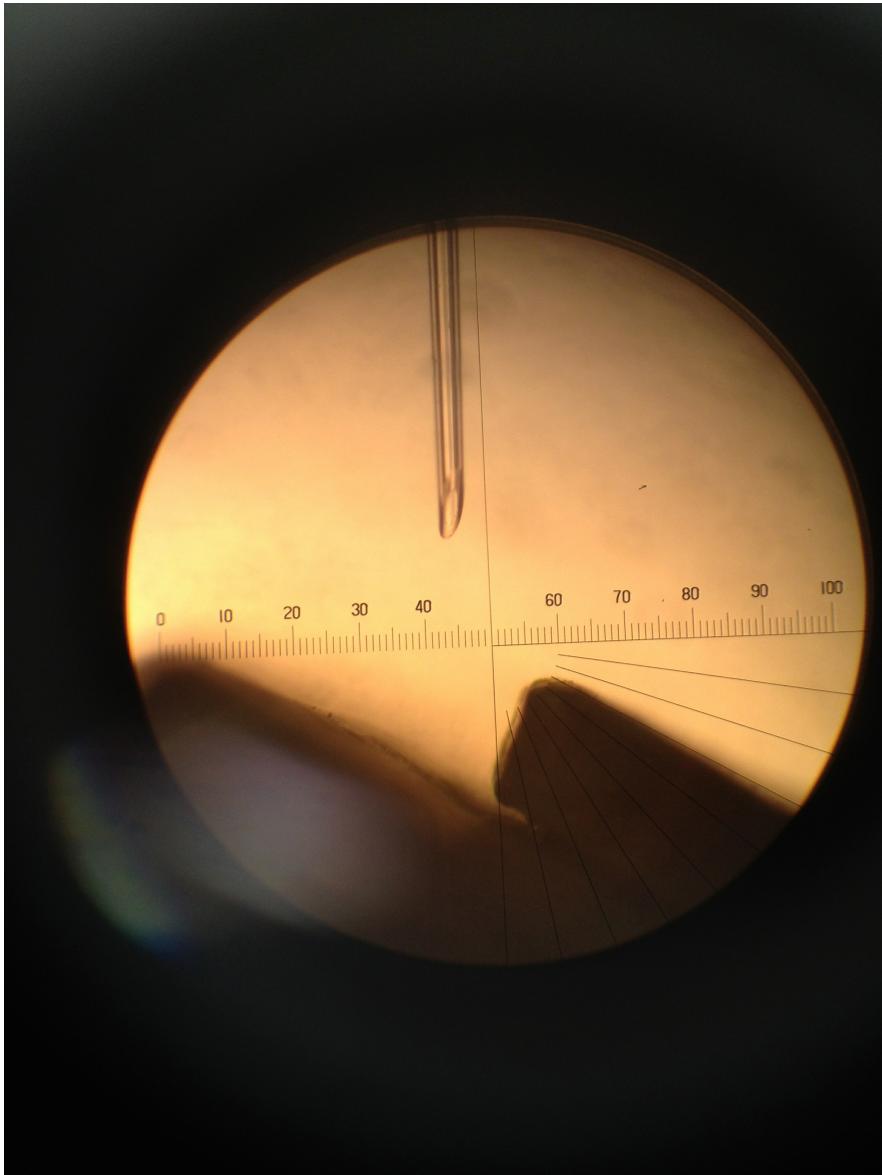


If you did a bad job it looks dusty like this.



Pipette with dust.

If you did a good job it looks clean and sharp like this.



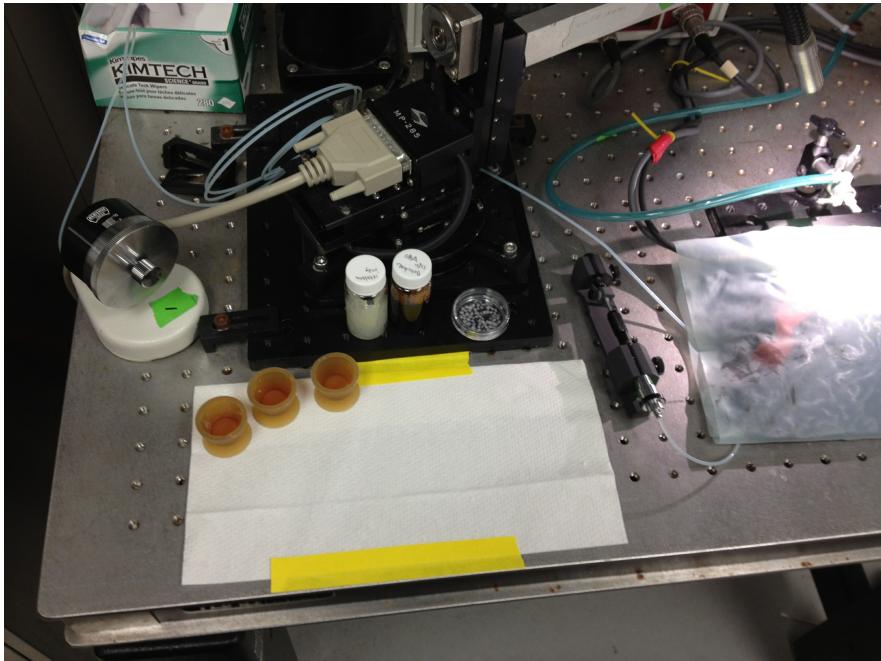
Clean and sharpened pipette.

5 Bevel at least 3 pipettes and arrange by quality. Use the best one first, with the others as backup (if you break / clog the first).

Injection setup

6 Thaw virus aliquot ($2\mu\text{L}$) on ice.

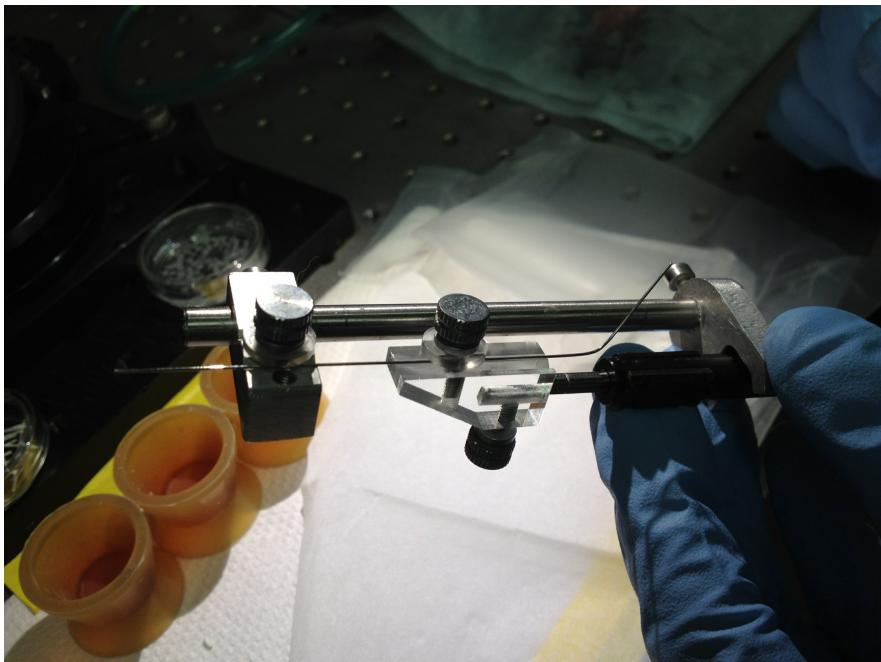
- 7 Sterilize the workspace and prepare tools. See headbar protocol for more details.



Workspace setup with the injection system, micromanipulator, and gel foam.

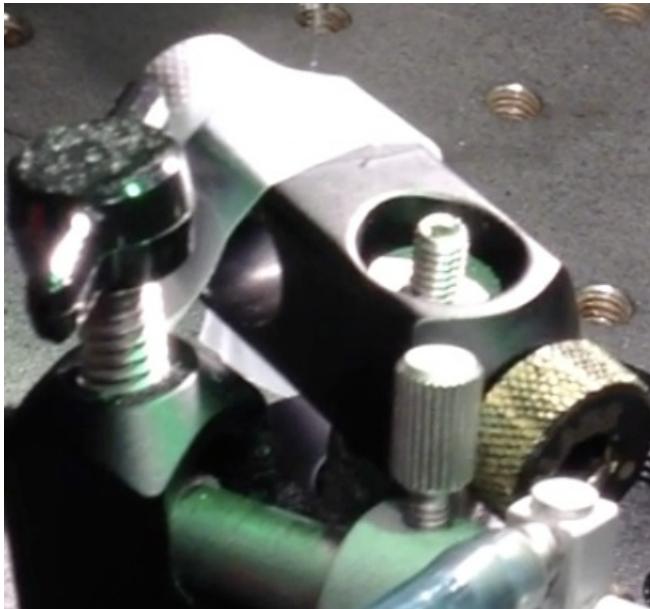
- 8 Cut gel foam into 1mm cubes and soak in cortex buffer

- 9 Adjust the alignment of the pipette holder and the plunger holder. The two attachment channels should be straight and co-linear.



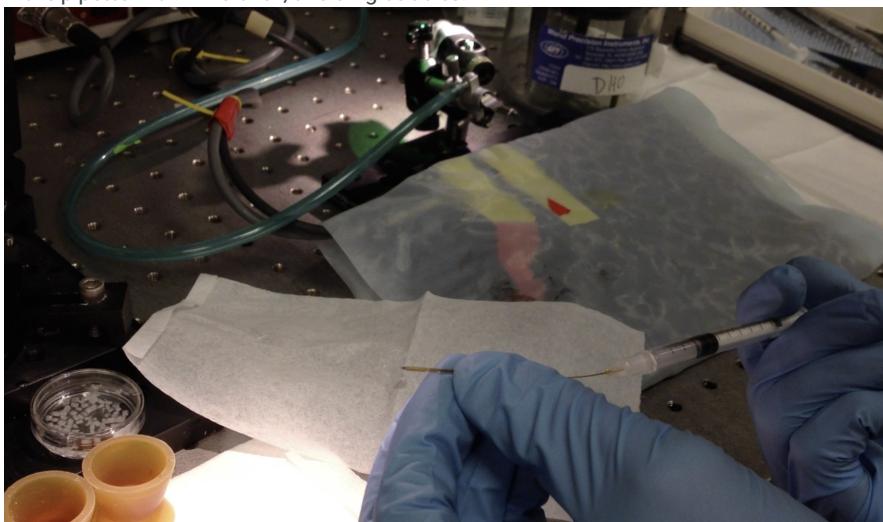
The pipette holder and the plunger in the plunger holder are aligned.

- 10 Place a tiny platform adjacent to the mouse head holder and wrap with parafilm for virus loading.



Parafilm for virus loading from the tip

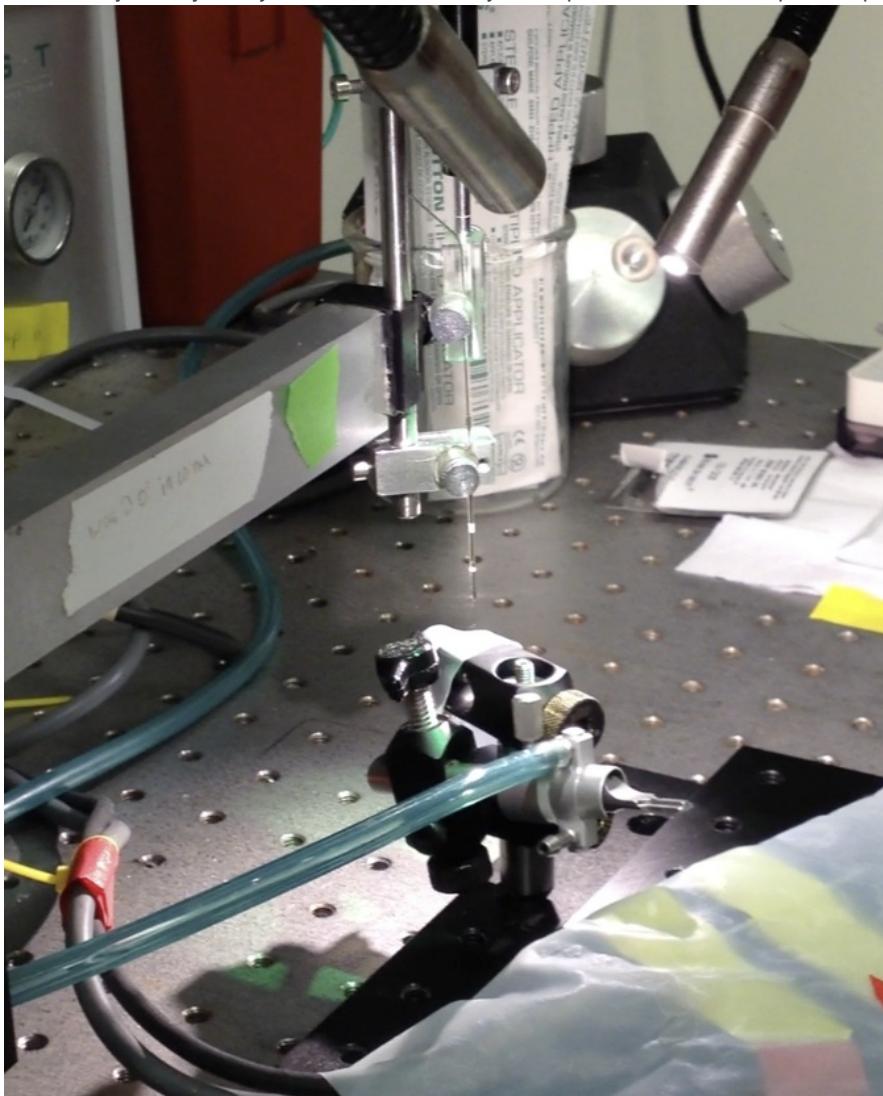
- 11 Fill the pipette with mineral oil, avoiding bubbles.



Back filling the pipette with mineral oil.

- 12 Mount the pipette in the pipette holder, and insert the plunger into the back end of the pipette. Turn the knob of Narishige injection system clockwise to eject and counter-clockwise to suck.

- 13 Mount the injector, eject any bubbles, then carefully suck up the virus from a drop on the parafilm.

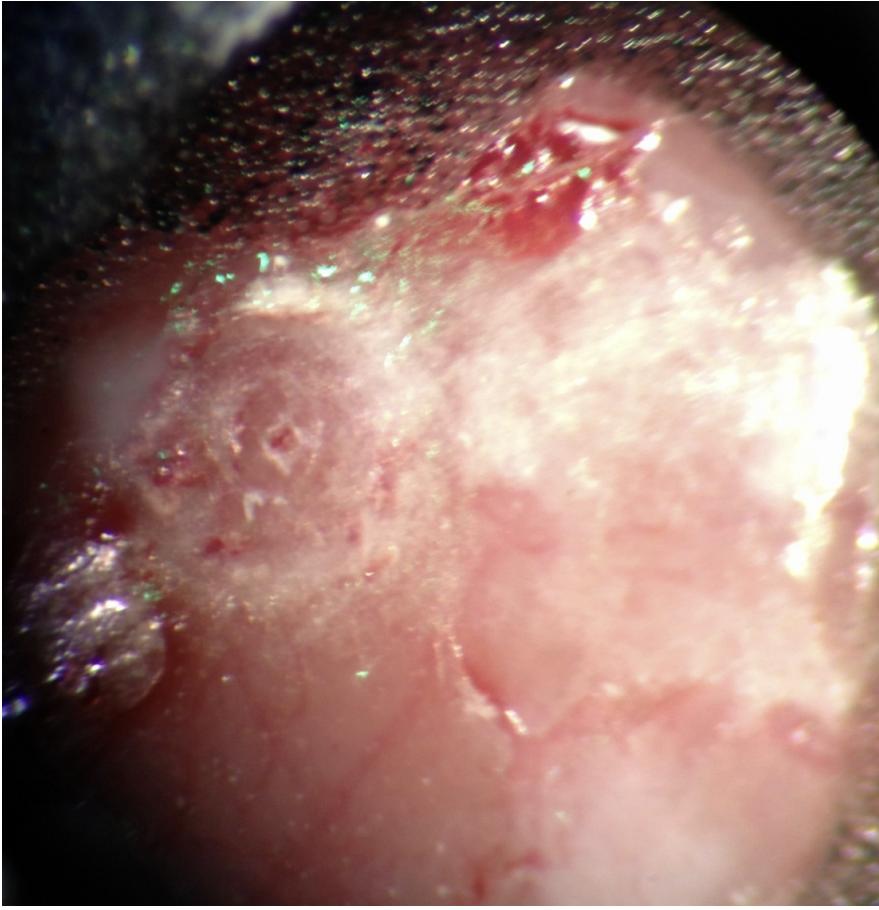


Loading the virus by suction from the tip.

Surgery and injection

- 14 Wet the skull with cortex buffer to visualize the vasculature.

- 15 Confirm the target, dry the skull and drill a small hole directly above the target. Look carefully for cracking or peeling of the bone, this indicates you are at or very near the dura, and can stop drilling.



Drill a small hole.

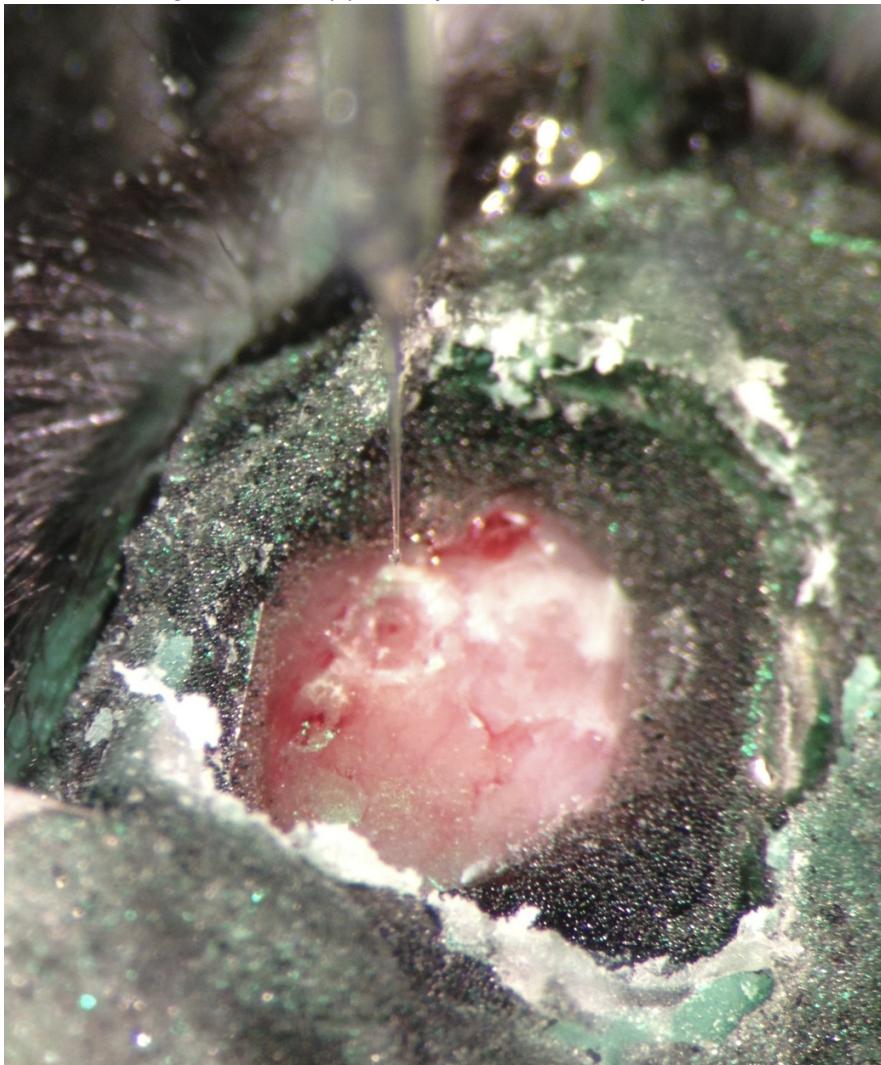
- 16 Re-wet the area and zoom in.

- 17 Position the pipette directly above the craniotomy. Watch out to not hit the hydraulics on the injector with the scope support.



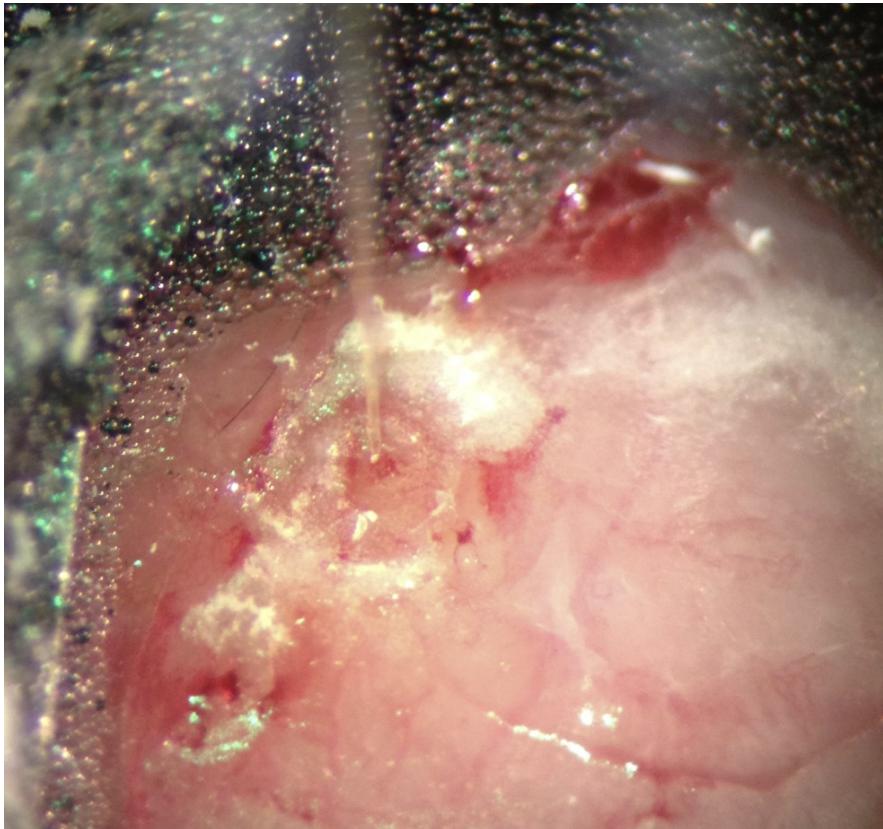
Injection system above the mouse.

- 18 Eject a small volume of virus to remove any bubbles and wick away the drop using a saline soaked piece of gel foam. Keep the lighting at a fair distance from the pipette, at moderate brightness, to reduce volume expansion from heating. Once the lighting is set, do not change it so that the pipette stays in a thermal steady state.



Pipette above the target with a drop of virus suspension at the tip.

- 19 Position the needle just onto the surface of the craniotomy. Zero the manipulator.



Manipulator above the craniotomy.

- 20 Lower the pipette to the desired depth at 2 $\mu\text{m}/\text{s}$, and inject the desired amount (typically 10-100 nL) of virus at 0.5 nL/s.
- 21 After each injection, leave the pipette in tissue for **00:20:00**. Maintain a wet surface on the craniotomy to prevent tissue sticking to the pipette.
- 22 Slowly retract the pipette out of the brain at 5-10 $\mu\text{m}/\text{s}$. After the pipette is removed from the brain, there should be no bleeding at the injection site.
- 23 Test the pipette for clogs by ejecting a small volume.
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- If it comes out easily, the injection probably worked. If nothing comes out, don't despair, it might have gotten clogged on the way out. If clogged, then repeat the injection with a new pipette.
- 24 Wipe the pipette with wet gel foam, then eject another tiny drop to seal the end. If you thinned a large area of skull, coat the thinned area with a small volume of Krazy glue. Fill well with Kwik-Cast and cover with dental acrylic.
- 25 Dispose of virus handling parts in a labeled sealed 50mL conical.

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