Pre-Surgical Setup and Animal Restraint
1) Prepare surgical area by wiping the area down with 70% EtOH. Sterilize all tools that will be used during surgery.
2) Anesthetize the animal for surgery. Weigh animal and calculate approximate dose. I use a mixture of ketamine/xylazine at a dose of 100/10 mg/kg body weight given intraperitoneally for an adult animal.
3) While animal is beginning to go down, shave the animals skull and clean the skin with 70% EtOH before incision.
4) Place animal in stereotaxic apparatus that has a 37°C heat blanket under where the animal will be placed. Fix one ear bar in the apparatus and gently position the animal’s ear canal against the ear bar. Keeping the animal still, slide the other ear bar to the opposite ear canal to complete the fixation. (Apply only moderate pressure). NOTE: use non-rupture ear bars with a wide angle tip when fixing the animal.
5) Insert the incisor adapter in the animal’s mouth, gently lock the front teeth in the adapter, making sure not to break the teeth. Lower the nose clamp with moderate pressure. Make sure the animal’s tongue is out and it is able to breathe unobstructed.
6) Apply lubricant eye ointment (Vaseline) to prevent corneal drying.

Surgery
1) Make a midline incision with small surgical scissors or a scalpel across the shaved portion of the skull. Separate the subcutaneous and muscle tissue from the skull using small surgical hooks to keep the area open. (I bend a 22 gauge needle to make the surgical hooks)
2) With the skull exposed, gently clean bregma and lambda with a small bone scraper. Level the head of the animal measuring the z-coordinate of bregma and lambda, and adjust head height using the incisor adapter height adjustment screw. (* Keep the skull moist with PBS throughout the procedure).
3) Measure the position of the x and y coordinates of bregma and calculate (subtract) the coordinates of the target injection area.
4) Thin the skull over the target area using a hand-held drill by horizontally moving the spinning drill bit over the skull. Stop when the bone is very thin and the blood vessels begin to become visible. (*Do not drill through the bone). Using a needle tip, “flip up” a piece of the thinned bone and then remove the thinned skull with fine forceps. Keep the skull and expose dura moist with PBS.
5) Place the viral-filled micropipette into the holder of the stereotaxic arm and connect the tubing to the pipette.
6) Bring the tip of the micropipette to bregma and calculate the x,y and z coordinates that will be used for injection. First, bring micropipette to correct x and y coordinate and lower it until the pipette barely touches the expose dura. Dura in younger animals can easily be penetrated with a sharp-angled cut pipette tip. Older animals have a tougher dura, and making small incision in the dura will help micropipette penetration. (* Be
careful to not push the dura too hard while trying to penetrate it, if there is resistance, make a small incision in the dura with a new 27G needle tip.

7) After penetrating the dura, lower the pipette to the desired z-coordinate. I lower the pipette slowly to reduce tearing of the tissue. Once at the proper z-coordinate, lower pipette one more full turn and bring back to desired coordinate. Begin to inject at a slow rate, \(~75\text{nl/minute}\). The slower the rate the better, as too fast of an injection will cause spreading and damage of the injection site. Once the injection is finished, wait 2-3 minutes before withdrawing the pipette, to prevent reflux.

8) If the skull incision is large, cover the craniotomy with bone wax. Suture the skin and apply antibiotic ointment to the sutured area.

9) Inject the animal with sterile PBS (30ml/kg body weight) subcutaneously to prevent dehydration.

10) Keep the animal warm during recovery by placing the animals cage on a 37C heat blanket. Place moistened food pellets in the cage so the animal has easy access to food and hydration.

11) Depending on survival time after surgery, check the animals everyday, to make sure the animal is not in pain or exhibiting bizarre behaviors.