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| Stereotax: holder/measurer | Virus Surgery Tray | KX Boosters(25% of induction dose/0.4ml) – **0.5 ml syr.** |
| Stereotax: level arm | Dura pic (brown needle) | Pre-op: Meloxicam(0.05cc) –**0.5 ml syr.** |
| Stereotax: needle arm | EZ-Clip box or 4-0 Sutures | Pre-op: Lidocaine (0.2cc) –**0.5 ml syr.** |
| Pulled Pipette Tips | 50mL Bleach | Post-op: Ringers (1ml/hour of surgery/4ml)– **5 ml syr., Blue needle** |
| Virus Box | Silicon epoxy |
| Styrofoam box/dry ice | Nanoject | Biohazard Bag |
| Virus Construct | Micropipette p10 | Retractor (in cetylcide, gel bead sterilized) |
| Disposable scalpel head | Small drill bit |  |

**Preparation**

Pull micropipette tips:

* Program 89 in Turrigiano Puller.
* Do not use gloves, Do not break the filament, Do not change settings.
* once tips are pulled, mount needle in scope and adjust scope sight so that it’s parallel with mounted needle.
* Use scissors to cut needle where two units of measure thick (cut a bit ahead of two-unit mark, glass will likely fracture)
* Turn on beveller and lubricate with MiliQ water.
* Take cut needle mounted in attachment and \*\*\*lightly\*\*\* touch tip to spinning beveler at a roughly 45° angle, you should hear soft hiss.
* Check needle tip under the scope, should have an edge like a hypodermic needle.
* Take care to bevel at the same angle if more than one grind is required.
* Clean up and re-cover scope and beveler!

1. **Day before:** make sure all items are in stock and virus is available and put screws in cetylcide. Make sure to have scheduled nanoject and surgery room time on google calendar.
2. Get dry ice from SSC and virus from TGF.
3. Print animal surgery log
   * + ENTER THOMAS
4. Weigh animal, bring animal and cage to main lab room.
5. Turn ISO box on for at least 5 minutes by turning green button on box, and press induction button.
6. Fill syringe with KX induction dose (use doses on the chart above drawer).
   1. 1.0mL for 250g + 0.1mL/25g over -**3mL syringe, 2 mL blue needle**
   2. E.g. 350g animal = 1.4mL KX
7. Place animal in iso box, check toe pinch after minute to see if animal is down.
8. IP inject KX induction and place animal back in cage.
9. Fill syringe with bupivacaine (0.2mL local analgesic; Lidocaine); pull all post-ops so they can warm to RT.
10. Gather all items for surgery while animal is going down.
11. Check for toe-pinch to determine if animal is ready.
12. Shave animal’s head just behind ears and just before “eyebrows”
    1. Use tape to clean off fur.

**Craniotomy**

1. Place animal in stereotaxis and fix with earbars
2. Apply eye gel liberally (may need to repeat as surgery goes on)
3. Clean head with alternating alcohol/iodine/alcohol wipes
4. Inject bupivacaine into scalp along incision site; wait 3 minutes.
5. Use scalpel to make an incision down the midline - about 1 inch.
6. Use retractor to hold wound open.
   1. Can use petroleum jelly-covered hemostats in absence of retractor.
7. Use cotton swabs to remove tissue and clean/dry skull.
   1. only want skull, any tissue could get caught in drill.
8. Use level arm to level skull.
   1. line up with bregma and lambda; both points should touch their respective sutures at the same time.
   2. readjust nose clamp.
9. Attach needle (yellow) to bar mount to accurately obtain position of bregma.
   1. Record bregma coordinate from stereotaxis arms - A/P; M/L
   2. Go to GC **For GC:**
      1. **A/P: +1.4**
      2. **M/L:** Right: +**5.0**, Left: **-5.0**
   3. Use cauterizer to mark GC spot(s) on skull.
   4. Go to PC **For PC:**
      1. **A/P: -1.4**
      2. **M/L:** Right: +**5.5**, Left: **-5.5**
   5. Use cauterizer to mark PC spot(s) on skull.
10. Use drill and *slowly* bore hole into skull.
    1. Cautious not to go too fast.
    2. Make sure to create flat-surfaced bore.
       1. keep drill parallel to floor.
    3. Make donut hole; must be large enough to fit micropipette and fiber.
11. Use needle to slice opening in dura (create as little “damage” as possible)
12. Cover head/craniotomy while setting up nanoject
    1. alternatively set up nanojet while dura bleeding stops.

**Virus Injection**

1. Attach Nanoject-holder to stereotax.
   1. have circle for nanoject on top, above the sterotax arm.
2. Insert Nanoject in holder and tighten.
3. Plug in:
   1. Nanoject cord to “Head” and nanoject itself
   2. Nanoject box to wall
4. Touch pipette tip into Fast Green FCF (second Katz drawer).
5. Grab a weighboat and fill with PBS, take acetone canister with IOC parts and stretch parafilm over top.
6. use p10 to pipette 5 μL PBS and 5μL virus to stretched parafilm on canister.
7. touch pipette tip to PBS bubble and mix so that bubble is blue (this will make it easier to visualize injection of virus and verify micropipette injection).
8. Use one of the pulled pipette tips and backfill it *completely* with oil (in Nanoject box).
9. Attach pulled pipette tip to Nanoject (see nanoject SOP)
   1. Screw black piece up (to the right) to tighten pipette into place.
10. Turn on Nanoject box.
    1. Menu.
    2. Empty.
       1. Will stop by itself.
       2. Leaves about ½ - ¾ oil remaining.
11. Place parafilm over jar lid.
12. Spin down Virus in centrifuge (Katz drawer) – 2.5μl.
    1. Use p10 pipette and draw up virus.
    2. Pipette virus onto blue PBS bubble on parafilm and mix thoroughly.
13. Place jar beneath pulled pipette and click “Fill”.
14. Hit:
    1. Exit
    2. Program 01 – (JY):Nanoject 4 program 01: Vol: 15.2 ; Rate 008; cycles 015; time 008
    3. Hit Test.
       1. make sure liquid comes out.
    4. Mark virus on pipette with sharpie to verify fluid injected.
15. Measure D/V value where pipette at brain surface (record on surgery sheet).
    1. Move pipette up and hit:
       1. Test.
16. Reposition and higher to desired depth (2mm/per run).
    1. **For GC: take D/V value measured at brain surface and subtract 4.4**
       1. Wait 2-min to allow for tissue settling.
       2. Hit:
          1. Run
    2. **For PC: take D/V value measured at brain surface and subtract 7**
       1. Wait 2-min to allow for tissue settling.
       2. Hit:
          1. Run
17. Focus scope on the oil/virus merge-line to ensure that solution is expelled every time you hear a beep (infusion)
18. Once it finishes, wait 3 minutes minimum.
    1. Go set up virus cage.
19. Very slowly retract needle from brain (counter-clockwise on the stereotax knob).
20. Once needle is completely outside of brain, swivel the stereotax arm out of the way and press ‘test’ on console to ensure needle has no blockages and will expel liquid for second injection.
21. Repeat injection on contralateral side if necessary.

**Implant**

1. Drill 4 shallow holes for screws.
2. Screw in each screw using screwdriver and forceps.
   * 1. Just until stable in skull
3. Dye the ends of the fiber with Vybrant Dil.
4. Use multi-joined holder arm and alligator clip to grip fiber.
5. Straighten fiber so it will enter brain straight - look at distant straight items in background (cabinet lines, door) to help decide.
6. Position over craniotomy
   * 1. WRITE DOWN WHICH FIBER NUMBER IS ON WHAT SIDE OF THE HEAD (important for knowing calibration number).
7. Check point at which tip touches dura; record
8. **GC:** D/V = **- 4.4**
9. **PC:** D/V = **- 7**
10. Lower 1mm at a time.
11. Once at desired depth, Use glue/kicker to attach the fiber to a screw. Make sure it is secure.
12. clean out CSF and use silicone to fill in around the hole.
13. Release from alligator clip and repeat on other side.
14. Mix dental cement solutions and begin creating retaining walls around entire set-up, really liquidy base to adhere to skull.
    * 1. Build up until fibers are secure up to the permanent marker line that indicates where the ferrule connects.
15. Place dust caps back on fiber tops.

**Post-op care on animal**

1. Get white bedding (alpha dry) and place in clean cage + new enrichment.
2. Remove metal rack, place dry food in carafe in bottom of cage.
3. Provide filled water bottle (square).
4. Give animal Post-op shots.
   1. Rest of Ringer’s.
   2. 0.3mL Penicillin.
5. Clean head with betadine and then cover in bacitracin ointment.
6. Post-operative procedures 2 days following including medication (meloxicam), bacitracin application, weighing.
7. Daily after surgery for at least 1 week must weigh the animal and monitor for 85% pre-surgery weight maintenance.