**UMass SCGE Protocol for MCV1 reporter mice- protocol 3**

Mice injections:

* Take the RNP aliquot from the freezer and keep on ice until ready to use.

Let the RNP to warm at room temperature before injection (this can be done by loading the syringe first, while preparing the mice for the surgery).

* The mice are anesthetized with Fentanyl/Midazolam/Dexmedetomidine (0.1/5.0/0.25 mg/kg) via IP injection at a volume of 0.1 mL/10 g body mass.
* IS injection coordinates are: ML +/- 2.0 mm

AP +1.0 mm

DV -3.0 mm

* Speed of injections is: 500 nL/min (to a 2 uL total injection) and wait 2 min post injection. Perform injections on both sides of the brain.
* Administer the following reversal agents & analgesics with injection volumes of 0.1 mL/10 g body mass:
  + - Flumazenil/Atipamezole 0.5/5 mg/kg via IP injection (anesthetic reversal agents).
    - Buprenorphine 0.3 mg/kg via subcutaneous injection (analgesics).
* The mice are sacrificed 14 days post injection. (Our in-house tests thus far have gone for 7 days post-injection, but 14 days should be fine too.)

Mice dissections:

* Inject the mice with Fatal-Plus (Vortech Pharmaceuticals) dilution (0.1 ml/10 g body mass dose) via IP injection
  + Fatal-Plus stock solution is diluted 1:10 in 0.9% sterile saline
* Perfuse the mice with PBS.
* Collect the brains
* Soaked in 30 % sucrose + PBS, for 48 hours (until the brains sunk).
* OCT-embedded in cold 2-methylbutane and then stored at -80C until sectioned.
* Slides stored at -80C.

Immunofluorescence protocol for anti-Spycas9:

1. Fix slides in 10% formalin for 15 min.
2. Wash slides 3x in PBS.
3. Antigen retrieval by steaming in a citrate buffer (0.294% sodium citrate, 0.05% Tween 20 in DI water, pH 6.0):
   1. Preheated hybridization oven to 99.9C
   2. Heated in slide container in microwave for ~3 mins until boiling
   3. Incubated slides in buffer inside hybridization oven for 15 mins
   4. Cooled on ice for 10 mins
4. Rinse sections in PBS and block (5% goat serum, 0.2% Triton X-100 in PBS) for min 4 hours at 4C.
5. Incubate with primary Abx (Rabbit anti-SpyCas9 1:400) in blocking solution O/N at 4C.
6. Wash in PBS.
7. Incubate with secondary Abx (Donkey anti-Rabbit-Alexa Fluor 488 1:500) for 2 hr RT in blocking solution.
8. Wash 3x with PBS.
9. Mount with Prolong Gold Antifade Reagent with DAPI.