**UMass SCGE Protocol for mTmG reporter mice- protocol 1**

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 10% formalin.
* Collect the brains
* Fix in 10% formalin for 18 hours at 4oC.
* Transfer the brains into 1XPBS pH 7.4
* Section the paraffin embedded brains at 10 µm and mount on slides.
* Stain slices using IHC for GFP.

IHC Protocol for FFPE tissue:

Dewax Slides:

* Wear nitrile gloves when handling xylene.
* Use Tissue Tek II Rack for dewaxing. Check baths to be sure all xylene and ethanol baths are filled to the line.
* Slides should not be allowed to dry.

Inside chemical hood:

1. Place slides in white dunking apparatus, spaced apart to avoid slides sticking together.
2. Immerse slides in Xylene 3 times (once per bath) for 5 min each.
3. Immerse slides in 100% ethanol 3 times (once per bath) for 5 min.
4. Immerse 95% ethanol 1 time for 5 min.
5. Immerse 70% ethanol 1 time for 5 min.
6. Wash slides 3X in PBS for 5 min using gentle agitation.

Place slides in black Simport slide chamber. Add sterile water or diH2O to perimeter of chamber to keep slides hydrated.

1. Use PAP pen to surround tissue with hydrophobic barrier.

Peroxidase Block:

1. Dilute 30% H2O2 to 3% H2O2 (1:10 dilution) with distilled water. Mix well and store at 4ºC for up to 3 months. Each slide holds about 500 µL.
2. Add 3% hydrogen peroxide to tissues and wait 3 min.
3. Wash 3 times for 5 min each with PBS on shaker.
4. Block with Serum (Rabbit) in the Anti-Rabbit Ig ImmPress kit.

Use a few drops of ready-to-use 2.5% horse serum to cover tissue and incubate for 2hr at room temperature.

Primary Antibody:

1. Discard the serum.
2. Prepare 200 µl anti-GFP antibody solution at 1:200 dilution for each slide in 2.5% horse serum from the ImmPress kit.
3. Add primary antibody.
4. Make sure the black tray has ample amount of water to work as humidified chamber.
5. Incubate slides overnight at 4°C.

Secondary Antibody for IHC:

1. Wash with PBST (Tween 0.1%) 3 times for 5 minutes each on shaker.
2. Add 2 drops of ImmPress Kit anti-rabbit secondary solution.
3. Let incubate for 30 minutes at room temperature in Simport slide chamber.

DAB Staining:

1. Wash with PBST (Tween 0.1%) 3 times for 5 min each on shaker.
2. Make 1X DBS from DAB Quanto kit.
3. Add 1 drop (30 µL) DAB chromagen into 1 mL DAB Quanto substrate.
4. One slide at a time, add DAB to cover tissue sample completely and incubate for appropriate 2-5 min. Re-stain with more DAB as necessary.
5. Quench in distilled water ASAP by dipping in dH2O; use a second tub for leaving in water for extended period of time.

Counterstaining (optional):

1. Immerse slides in hematoxylin to evenly stain tissue for ~3 secs.
2. Dunk in distilled water about 3 times ­until hematoxylin is only on the tissues
3. Immerse in bluing reagent for 30 secs. This turns hematoxylin from purple/violet to blue, leading to better contrast with DAB (brown).

Dehydration:

1. Immerse slides in 70% ethanol for 1 min.
2. Immerse slides in 95% ethanol for 1 min.
3. Immerse slides in 100% ethanol for 1 min.
4. Immerse slides in xylene for 1 min in glass jar.

Coverslip mounting:

1. Add 2-3 drops of Cryoseal near the tissue and lower cover glass slowly at an angle to avoid air bubbles. Make sure the slide is still wet with xylene to avoid water bubbles
2. Dry slide facing up on a paper towel for at least 5 min.
3. Slides are ready for imaging.