**Embryonic Mouse Dissection / Dissociation**

**Day 1:**

1. Plate \_\_\_\_ large coverslip or \_\_\_\_ small coverslip 6 well plates

**Day 2:**

**Plate Preparation:**

1. Placed coverslip plates under ultraviolet light for 30 minutes (with lids off).
2. Weighed out \_\_\_\_\_\_ mg Poly-L-Ornithine [sigma, P-3655, lot \_\_\_\_\_\_\_\_\_\_\_\_] (1 mg per 2ml Borate buffer) and dissolved in \_\_\_\_\_ml Borate Buffer made \_\_\_\_\_\_\_\_\_\_\_ by \_\_\_\_\_\_\_.
3. Filter sterilized PLO / Borate Buffer and added 2mL to each well and allow to coat for > 1hr (lids on).
	1. Incubated from \_\_\_\_\_\_ to \_\_\_\_\_\_\_\_.
4. Following incubation in PLO, removed media from each well and washed 2 times with tissue culture water.
5. After 2nd wash, remove water and allow plated to dry (lids off) for at least 1 hour.
	1. Drying time from \_\_\_\_\_\_\_\_ to \_\_\_\_\_\_\_.
6. Once plates were completely dry, add 1.5mL of Plating Media made \_\_\_\_\_\_\_\_\_ by \_\_\_\_\_\_\_\_ to each well and place in incubator until dissociation is complete.

**Animal Preparation:**

1. Retrieve E18 pregnant moms from the mouse colony.
2. Anesthetize via an i.p. injection of \_\_\_\_\_ mL of FatalPlus (Nembutol).
	1. Record use in log.
3. Remove E18 mouse pups.
	1. There were \_\_\_\_\_\_\_ pups.
4. Prepare tubes labeled by number based on the amount of pups.
	1. ­­­Numbered \_\_\_\_\_\_\_ 1.5mL microcentrifuge tubes
	2. Numbered \_\_\_\_\_\_\_ 15mL conical tubes containing 10mL of Hibernate Media
5. From each pup collect:
	1. Tail sample for PCR analysis and add to microcentrifuge tube
	2. Remove head and make initial cut towards the front of the skull but do not remove brain from skull… Place opened skull into 15mL conical tube containing the 10mL of Hibernate Media and place tubes at 4oC overnight.

**PCR Analysis to Determine Genotype for pooling of tissue:**

1. Remove Red Extract N-Amp Tissue PCR kit components from -20oC and allow to thaw at room temperature (Note: thawing bottles A and B can take up to 1.5 hrs).
	1. Extraction Solution
	2. Neutralizing Solution
	3. Tissue Prep Solution (Take this out immediately before use – liquid @

-20oC)

* 1. Red mix
1. Using filter-tip pipettes add the following to each microtube containing tails:
	1. 100μL of Extraction Solution (Note: Always add this first)
	2. 25μL of Tissue Prep Solution
		1. Be sure all tail tissue is completely immersed in solutions!
		2. Vortex quickly
		3. Incubate at room temperature for at least 10 minutes
			1. So, start timer as soon as first tube is filled…
2. Once incubation is complete, boil tail samples at 95oC for 5 minutes to stop digestion.
3. After boiling is complete, add 100μL of Neutralization Solution to each tube
	1. Total volume = ~225μL
	2. After vortexing again, this sample can then be used directly in the PCR reaction…
4. Prepare a master mix for PCR reactions as follows:
	1. Will need per tube:
		1. 10μL of Red mix
		2. 1μL of 10μM Neo13 primer (Forward)
		3. 1μL of 10μM Neo14 primer (Reverse)
		4. 1μL of 10μM CHIP2F primer (Forward)
		5. 1μL of 10μM CHIP2R primer (Reverse)
		6. 2μL of ddH20
			1. When calculating for master mix, include 3 extra tubes for a WT, Het and KO control sample.
			2. Vortex master mix after all components are included.
5. To each PCR tube add:
	1. 16μL of Master Mix
	2. 4μL of tail sample
6. Place PCR tubes into thermal cycler and run the “CHIP&NEO” program.

**Day 3:**

**PCR Gel and Analysis:**

1. Prepare a 2% agarose gel
	1. 2g agarose in 100mL 1xTAE Buffer
	2. Allow 20 – 30 minutes for gel to set
2. Prepare 1Kb Plus DNA Ladder as usual
3. Add all 10μL of each PCR sample directly to each lane
	1. May be less than 10uL, but add all remaining product
4. Run at 150 Volts for 35 minutes
5. Take picture of gel
6. Group animal tissue according to genotype

**Dissection Preparation:**

1. Thaw \_\_\_\_\_\_\_\_ aliquots of 0.025% Trypsin [sigma, T9935, lot \_\_\_\_\_\_\_\_\_\_\_\_] at room temperature.
	1. You will need 1 tube per E18 mouse pup
2. Remove brains from 4oC
3. Dump off Hibernate Media and place all WT heads in a 35mm dish containing 7mL of HBSS.
	1. Repeat for Het and KO mice
4. Remove brains from skull, remove meninges and dissect forebrain one genotype at a time.
	1. In between genotypes, clean dissecting tools!
5. Once all forebrains are dissected, pipette the appropriate amount of trypsin (again, 1mL per brain) into a 35mm dish.
6. Transfer forebrains to the trypsin dish and allow tissue to digest for 20 minutes at room temperature.
7. During digestion:
	1. Prepare 2 x 35mm dishes containing 5mL of HBSS (per genotype) for washing post-trypsinization.
	2. Prepare plating media
		1. This media should contain 1X NS21 and 1X N2 and 10μM BME.
		2. I usually prepare 100mL.
	3. Prepare trypan blue tubes – one for each genotype (450μL/tube).
	4. Label one 15mL conical tube per genotype and add 10mL of plating media.
	5. Label one 50mL conical tube per genotype.
	6. Remove plating media from PLO coated plates.
8. Once digestion in trypsin is complete, transfer tissue via plugged Pasteur pipette into HBSS dishes for washing.
	1. Use a new pipette for each genotype!
	2. Repeat for second wash in HBSS.
9. After washing is complete transfer brains to the appropriate 15mL conical tube containing 10mL plating media.
10. Triturate tissue in plating media with Pasteur pipette (~21 times).
11. After all genotypes have been triturated, place a 40 micron cell strainer into the 50mL conical tubes and filter the supernatants.
12. Add 50μL of each genotypes filtered cell suspension to the trypan blue and count three 4X4 squares on the hemocytometer.
	1. Record average count (Total Count / 3) for each genotype:
		1. WT =
		2. Het =
		3. KO =
	2. Sample calculation for determining plating density:
		1. \_\_\_\_\_\_ (live cells) x 10 x 10,000 ÷ 700,000 (or desired plating density) = \_\_\_\_\_\_\_ (dilution factor).
		2. \_\_\_\_\_\_(mL needed for plating) ÷ \_\_\_\_\_\_(above calculated dilution factor) = \_\_\_\_\_\_\_ (mL of stock cells needed).
		3. So added \_\_\_\_\_\_mL stock cells to \_\_\_\_\_\_\_mL plating media
	3. WT = \_\_\_\_\_\_\_\_\_\_ mL of cell suspension and \_\_\_\_\_\_\_\_\_mL plating Media
	4. Het = \_\_\_\_\_\_\_\_\_\_ mL of cell suspension and \_\_\_\_\_\_\_\_\_mL plating Media
	5. KO = \_\_\_\_\_\_\_\_\_\_ mL of cell suspension and \_\_\_\_\_\_\_\_\_mL plating Media
13. Plate cells and place in incubator.

**NOTES:**

We plate our mouse cells at 700,000 cells per well (double what we usually do our rat neurons b/c they do not survive as well…

The day after dissection we do a 100% media change to fresh plating media to get rid of any nasty floaters…

In addition, for the mouse cultures, we add NS21 to the plating media (2% final) as well.