Punching and extraction strategy note – Won Lee

[excerpted from my own pilot experiment notes]

**Pilot 5 - Sample collection date: 08/22/2019**

**Punching strategy used:**

- Make sure to put sufficient OCT around tissue or the tissue will crack like a cracker

- Place slide glasses in the cryostat in advance (this will make the tissue not stick to well, but if the glasses are kept outside the tissue melts as I press it against. Gore lab just place the glasses outside of the cryostat but they also use bigger punching tool for rat brains so I am not sure). Use finger warmth to make the tissue slightly melted but not too much if the slice is not sticking at all.

- Collected tissue in 1.5ml RNase-free tube, labeled then placed in the cryostat in advance

- Cool down the freezer box in dry ice in advance

- Put the punched tissue (in the tube) straight into dry ice.

- Once punching is done, add lysis buffer under the hood (really quickly), vortex 30 sec

🡪 This is actually very important (yet super annoying once tubes pile up…) step. Make sure the tissue is thoroughly lysed, not just sticking to the tube wall or floating around. If fails, the RNA will degrade during the storage

- Incubate additional 5 minutes at RT

- I froze in dry ice for 45 min (imitating freeze-thaw cycle)

**Pilot 5 – Extraction date: 08/22/19; used KingFisher**

**Extraction strategy used:**

- Thaw in RT while preparing mixes and plates

- Transfer lysates to Deep Well plate under the hood

- Cover plates while shaking

- Used A3-D4 (12 wells) on the Deep Well plate

- Add bead binding mix very carefully, straight into liquid in each well, do not use repeater – Avoid any splash or drops remaining on the wall of each well

🡪 I think this made difference! NO contamination at all

- Prepped wash solution plates (Plate 2,3,5,6) before first shake – while samples are thawing

- Prepped Elution plate during the second shake and DNase I plate during the last shake

- While shaking plates, start KingFisher and start inserting plates that are ready and close the lid

- DNase I extra processing step is at 0:19 (remaining time); approximately 30 min after the initial start (0:48 min as remaining time at the initial start)

- Elution volume: 30ul NFW

**Next experiment:**

* Using yellow punch (0.74mm). The red (0.91mm) is too big, can’t do precise punches. Rather stick to smaller but do multiple punches
* Do full punches for each brain regions (15 regions), punch more than 1 for each left/right if the ROI is big enough
* I wonder if I could decrease elution volume to 24-25ul?
* Punch tissue, lyse, then store in -80C for the weekend then extract on Sunday
* Forget Trizol it doesn’t work – stick to mirVana kit
* Make sure I have enough reagents for 12 extractions before start…!