**Figure 4:** Goals are to Identify genes enriched in OXT neurons and generate a molecular profile for OXT neurons.

Experiment: 3 WT Input vs. 3 WT IP samples

Data sets: Samples sequenced by Ovation RNAseq System (Ovation) on HiSeq

Samples sequenced by SoLo on MiSeq

Note: RNA was from same samples/tissue. Half used for each workflow.

Questions for analyzing the datasets:

1. Should we just focus on enriched genes and not present data for de-enriched genes? Should we present de-enriched genes in a separate supplementary table so that they are published if people are interested?
2. Which data set is best to use for this figure? It seems like probably the Ovation data set since this was high quality and done on the HiSeq. I also still have cDNA from these samples so I can go back and verify genes (de-enriched *Agrp* and *Cartpt* as well as other candidates of interest). If we choose the Ovation data set, should we also do a supplementary figure comparing Ovation vs. SoLo. Do we do this with just the IPs or also include the inputs? We will need something because in Figure 5 we switch to SoLo...so we will probably get asked to validate how closely SoLo and Ovation match. Also might be good data for Lieber to have internally to choose best workflows.
3. How do we filter out “noise” (i.e. transcripts that just stick to the beads during IP or transcripts that are on ribosomes in all cell types and aren’t really specific to OXT neurons…also are these 2 separate normalizations?). There are some available datasets for thinking about this:

* Shigeoka et al. 2016 (GSE79352). They use Ribotag in retinal cells.
* Nectow et al. 2017 (GSE89737). They use GFPL10a in MCH neurons in the LH.
* Our CST Input vs. IP Ribotag data set (Clonetech low input kit by Wei)

See mock up figure for ideas for the main figure.

Supplemental figures (review questions or further analysis?)

* Comparing our data to other TRAP data for validation. Again, no one has done Ribotag or GFPL10a in OXT neurons yet…but there is some single-cell RNAseq data from Chen et al. 2017 (GSE87544). Or is there anything else we should have on our radar for “validating.”
* Confirming genes of interest with qPCR or RNAscope. Some papers also use Allen Brain Atlas.
* GO analysis to identify some pathways enriched in OXT neurons.
* Since we also have the capacity to look at junctions and splicing…could we do something with these data?
* Some people include the entire excel sheet of DEGs…others make tables of top 75 DEG or whatever number they choose. Some focus on most significant, some the biggest fold change genes.
* We may get asked if there are differences between the RiboTag mouse and the AAV Ribotag. Should we compare the WT IP SoLo Ribotag mouse vs. WT IP SoLo AAV Ribotag to show that they are comparable. Caveat here is that the mouse probably has PVN and SON neurons whereas the AAV Ribotag only has PVN. But we could also discuss this as an interesting point if there are differences that maybe PVN and SON OXT neurons are different. But we may get asked, so we should think about this a little bit.
* Are there any other controls that we are missing?
* Should we do something with CSEA tool from Dougherty?