Native Reference Construction Methods

* Tissue dissociation
* Library Prep
* Sequence -> FASTQs
* FASTQs -> cutadapt -> STARsolo
* velocyto matrix conversion
* rank (on Gene, not GeneFull) nUMI of all barcodes
* Evaluate kneeplot, extract all barcodes of rank > 30K (can be slightly different than 30,000 total)
* Observe data distributions
* QC plots
* Titrate filtration for each sample
* Filter according to titrated thresholds
* Observe all filtered data together to compare
* Integrate x4 native sample by chemistry (not by sample)
* Annotate cell class, clear multiplet clusters, low-info clusters, and RBCs
* Split cell classes
* Using the previously calculated integration slot, re-embed and cluster and identify low info clusters and multiplet clusters. Document. Remove.
* Perform initial annotation of the remaining cells (not to be used for publication, as they were made with the whole-tissue integrated slot which included some now-removed cells. This first annotation is just for reference in house)
* Save these objects, their markers, and their metadata.
* Merge the x4 now-cleaned class objects back together
* Split by chemistry
* Re-integrate by chemistry
* Scale and Regress on rat cell cycle genes
* Embed, visualize, cluster, changing resolution to make sure that previously tagged cell cycle cells all at least fall within a single cell class
* Class using this new clustering (Integrated res 1.4)
* save metadata
* break out each cell class individually
* Scale each cell class individually on integrated slot (without re-integrating) and scaling on rat cell-cycle genes present within the integration
* Cluster and embed
* Annotate cell types and save metadata
* Map this metadata back to the final integrated object (‘CellType\_Final’)