Using Seqmonk, import all total RNA-seq BAM files as RNA-seq data. Create a merged file for all primary culture systems (Cortex, Hippocampus, Striatum) and treatment groups (vehicle or KCl).

* Cortex, 6 files (3 vehicle, 3 KCl)
* Hippocampus, 6 files (3 vehicle, 3 KCl)
* Striatum, 7 files (3 vehicle, 4 KCl)
* Import the following annotation files:
  + MACS2 ATAC-seq peaks from **Step 5** - AllRegions\_ATACPeaks.bed
  + RefSeq curated gene annotations - RefSeq\_Curated.bed
  + UCSC gene annotations - UCSC\_RefSeq.bed
  + Ensemble gene annotations - Rn6\_v95\_gtf\_genes.txt
  + Contiguously transcribed regions over 1kb – Contigs.txt

1. Import All BAM files
   * Do not remove duplicate reads
   * Do not treat as HiC data
   * Min mapping quality - 20
   * Import primary alignments only
   * Treat as RNA-Seq data
   * Data Type – Paired end
   * Pair Distance Cutoff (bp) – n/a
2. Merge all files to make a single group track consisting of Veh and KCl samples from each regions using: Edit 🡪 Groups
3. Make probes around regions of open chromatin (ROCs)
4. Data 🡪 Define Probes 🡪 Feature Probe Generator
5. Features to design around 🡪 AllRegions\_ATACPeaks.bed
6. Split into subfeatures 🡪 No
7. Remove Exact duplicates 🡪 Yes
8. Ignore feature strand information 🡪 No
9. Make Probes 🡪 Over Feature +/-500 bp
10. Identifies **191,857** probes 🡪 Name “ROCs”
11. Add ROCs to annotation track (Right click probe list, select “Convert to annotation track”)
12. Filter ROCs that were are 1kbp of Refseq curated genes (Filtering 🡪 Filter by features)
    * 1. Features to design around – RefSeq\_Curated.bed
      2. Split into subfeatures – No
      3. Make Probes- Over feature +/-1000bp
      4. Select probes which are – overlapping
      5. Distance cutoff (bp) – n/a
      6. Use features on strand – Any
      7. Identifies 68,856 ROCs that are overlapping or within 1kb of RefSeq genes
13. Filter ROCs that were within 1kbp of UCSC genes (Filtering 🡪 Filter by features)
    * 1. Features to design around – UCSC\_RefSeq.bed
      2. Split into subfeatures – No
      3. Make Probes- Over feature +/-1000bp
      4. Select probes which are – overlapping
      5. Distance cutoff (bp) – n/a
      6. Use features on strand – Any
      7. Identifies 71,461 ROCs that are overlapping or within 1kb of UCSC genes
14. Filter ROCs that within 1kbp of Ensemble genes (Filtering 🡪 Filter by features)
    * 1. Features to design around – Rn6\_v95\_gtf\_genes.txt
      2. Split into subfeatures – No
      3. Make Probes- Over feature +/-1000bp
      4. Select probes which are – Overlapping
      5. Distance cutoff (bp) – n/a
      6. Use features on strand – Any
      7. Identifies 76,382 ROCs overlapping or within 1kb of Ensemble genes
15. Filtered ROCs that overlap Contiguously transcribed regions (Filtering 🡪 Filter by features)
    * 1. Features to design around – Contigs.txt
      2. Split into subfeatures – No
      3. Make Probes- Over feature +/-1000bp
      4. Select probes which are – Overlapping
      5. Distance cutoff (bp) – n/a
      6. Use features on strand – Any
      7. Identifies 57366 ROCs overlapping or within 1kb of Contiguously transcribed regions
16. Repeat filtering for miRNA, misc\_RNA, rRNA, snoRNA, snRNA, tRNA (Filtering 🡪 Filter by features)
    * 1. Features to design around – pick from above annotations provided by Seqmonk Rn6 genome assembly
      2. Split into subfeatures – No
      3. Make Probes- Over feature From +/- 0bp
      4. Select probes which are – Overlapping
      5. Distance cutoff (bp) – n/a
      6. Use features on strand – Any
      7. 839 ROCs overlapping various ncRNA genes
17. Filtering 🡪 intersect multiple lists…
    1. Include ROCs, exclude all ROCs overlapping features from sections 4-8 of Step 6
    2. Intersection leaves **100,767** “Intergenic ROCs” or iROCs
    3. Convert to annotation track (Right click probe list, select “Convert to annotation track”)

**Steps 7-8.** Using Seqmonk, quantify total RNA-seq transcription from iROCs. Then, filter for iROCs that are bidirectionally transcribed.

1. Design probes around iROCs (Data 🡪 Define Probes 🡪 Feature Probe Generator)
   1. Features to design around 🡪 iROCs
   2. Split into subfeatures 🡪 No
   3. Remove Exact duplicates 🡪 Checked
   4. Ignore feature strand information 🡪 Unchecked
   5. Make Probes 🡪 Over Feature from -0 to +0 bp
2. Quantify all sequenced transcripts (Probe quantitation 🡪 Difference Quantitation)
   * 1. Calculate Forward only as a percentage of All reads
     2. Min count = 0
     3. Ignore duplicates – unchecked
3. Filter unidirectionally transcribed iROCs (Filtering 🡪 Filter on Values)
   1. Merged RNA-seq group value must be between 5 and 95%
   2. This excludes probes with unidirectional transcription from either strand (likely unannotated genes or exons)
   3. Identifies **28,492** “Transcriptionally active putative enhancers” – TAPEs
   4. Convert to annotation track Right click probe list, select “Convert to annotation track”
4. Make probes defining TAPEs (Define probes 🡪 Feature Probe Generator)
   1. Features to design around 🡪 “TAPEs”
   2. Split into subfeatures 🡪 No
   3. Remove exact duplicates 🡪 Checked
   4. Ignore strand feature information 🡪 Unchecked
   5. Make probes 🡪 Over feature -0 to +0 bp
5. Quantify TAPE transcription (CPKM) (Data 🡪 Read count quantitation)
6. Count reads on strand 🡪 All reads
7. Correct for total read count 🡪 Checked
8. Correct to what? 🡪 per million reads
9. Count total only in probes? 🡪 Unchecked
10. Correct for probe length? 🡪 Checked
11. Log transform count 🡪 Unchecked
12. Count duplicate reads only once 🡪 Unchecked
13. Filtering 🡪 Probe Values filter
14. RNA-seq group value must be between 0.05 and 10000
15. Identifies 1916 highly expressed TAPEs
16. Create report for TAPEs
17. Reports 🡪 Annotated Probe Report
18. Annotate with overlapping Rn6\_v95\_gtf\_genes.txt
19. Annotation distance cutoff – 1Mbp
20. Include – unannotated probes
21. Include – data for currently visible stores