

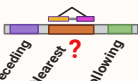
1. Data processing	Analysis	Feature			
		Gene	Exon	Jxn	Tx
	<p>Normalization</p> <p>Density</p> <p>$\log_2(\text{CPM} + 0.5)$</p>	Log-normalize raw counts to $\log_2(\text{CPM} + 0.5)$ using the TMM method to compute normalization factors		$\log_2(\text{CPM} + 0.5)$ with TMMwsp method to compute normalization factors	Log-transform TPM to $\log_2(\text{TPM} + 0.5)$
	<p>Feature filtering</p>	Keep features that have a minimum number of counts in at least n samples with <code>filterByExpr()</code> from <i>edgeR</i>			Filter txs based on a mean expression cutoff defined with <code>expression_cutoff()</code> from <i>jaffelab</i>
	<p>Sample separation</p>	Separate samples by tissue and age: <ul style="list-style-type: none"> Brain vs Blood Adults vs Pups 			

2. Exploratory Data Analysis	Quality Control Analysis	Evaluate and filter samples by QC metrics: <ul style="list-style-type: none"> Compare QC metrics of the samples in the different biological and technical sample groups Filter poor-quality samples based on their number of detected genes, library sizes and percentages of ribosomal/mitochondrial read counts <ul style="list-style-type: none"> Examine QC metrics of samples retained and removed after filtering 			
	Explore sample level effects	<ul style="list-style-type: none"> Principal Component Analysis (PCA) <ul style="list-style-type: none"> Identify major sources of feature expression variation in samples Manual sample filtering Explore gene expression variation in control and exposed samples Multidimensional scaling analysis (MDS) 			
	Explore gene level effects	Compute per-gene expression variance explained by explanatory variables to guide variable selection for DEA models	Not performed at these levels		

3. Differential Expression Analysis	Analysis	Feature			
		Gene	Exon	Tx	Jxn
	<p>Modeling</p> <p>$-\log_{10}(\text{FDR})$</p> <p>$\log_2\text{FC (Expt vs Ctrl)}$</p>	Perform DEA at this level for all brain and blood samples		Perform DEA for pup brain samples only	
	<p>Comparisons</p> <p>t-stats in 2nd group</p> <p>t-stats in 1st group</p> <p>DE</p> <p>52 Up nic, 21 Down smo, 9 overlap</p>	Compare signal for gene DE in: <ul style="list-style-type: none"> Smoking vs Nicotine Pups vs Adult Brain vs Blood Human¹ vs Mouse 		Compare signals for DE by smoking and nicotine exposures in txs/exons vs that of their genes	Not performed at this level
		Compare mean expression of genes with and without DE features			
		Quantify and compare up and downregulated DE features (and their genes) in the two conditions, and the overlap between DEGs and DE features' genes. Compare mouse DEGs vs mouse homologs of human genes associated with TUD ²			

4. Functional Enrichment Analysis	<p>Gene</p> <p>lognorm counts</p> <p>Ctrl Expt Nicotine</p>	For the DEGs, DE txs' genes and for genes with DE exons and txs in pups obtain their enriched: <ul style="list-style-type: none"> Biological processes (BP) Molecular functions (MF) Cellular components (CC) Pathways 	Not performed at this level
	<p>Prior disease</p> <p>Parkinson disease</p> <p>Down nic Up smo DEGs/DE features' genes</p>	Visualize the expression levels of genes involved in processes of interest in control and exposed samples	

5. DGE visualization	<p>Samples</p> <p>Ctrl Expt</p> <p>Genes</p>	Visualize expression patterns and clustering of DEGs	Not performed at these levels

6. Novel junction gene annotation	<div><p>Novel DE jxn</p></div>	Not performed at these levels	<div>Find the<ul style="list-style-type: none">• nearest• following• precedinggenes of fully novel DE jxs without assigned gene</div>