

Differential Expression Pipeline Overview

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Joe Troy

Mouse Resident Experiment Recap

- ***Resident*** males nested with females for at least one week prior to the experiment.
- **Treatment Scenario:** female is removed and ***intruder*** male is introduced to the ***resident*** male for 5 minutes.
- **Control Scenario:** female is removed and paper cup is introduced to the ***resident*** male for 5 minutes.
- Brain region tissue (Amygdala, Hypothalamus, and Frontal Cortex) was collected from treatment and control animals at 30, 60 and 120 minutes after intruder or paper cup is removed.

Arrangement of Samples

	30 min	60 min	120 min
Amygdala (A)	T* = 5 C* = 5	T = 5 C = 5	T = 5 C = 5
Frontal Cortex (FC)	T = 5 C = 5	T = 5 C = 5	T = 6 C = 6
Hypothalamus (H)	T = 5 C = 3	T = 5 C = 5	T = 5 C = 5

***Count of Treatment (T) and Control (C) samples**

Mouse Differential Expression Design

- For each brain region and time-point, what is the gene DE between the control and treated samples?
- For treated samples within a brain region, what is the gene DE between 30 and 60 minutes, 30 and 120 minutes, and 60 and 120 minutes?

Data Analysis Pipelines

- Both edgeR and Cufflinks pipelines are used for differential expression analysis
- edgeR pipeline
 - Provides raw transcript counts conveniently
- Cufflinks pipeline
 - Provides isoform DE reporting
- For either pipeline, DAVID can be used for gene ontology analysis

TopHat (v 2.0.8) / Bowtie (v 2.1.0)

* align short reads to genome - one mismatch & unique



htseq-count (v 0.6.1)

* raw transcript counts by gene

Cufflinks (v 2.1.1)

* Transcript assembly & abundance



edgeR

* gene DE reporting
* outlier analysis & MDS plots
* dispersion analysis

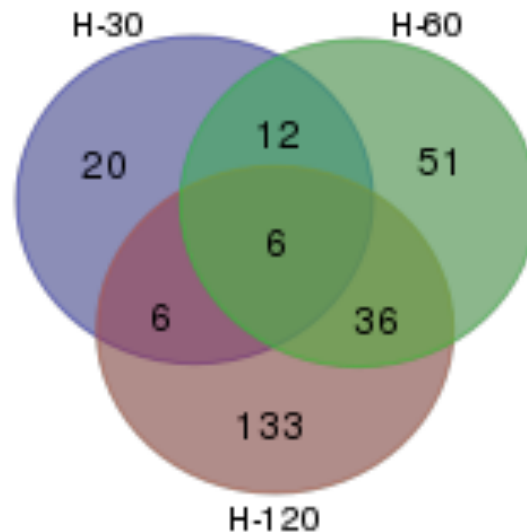
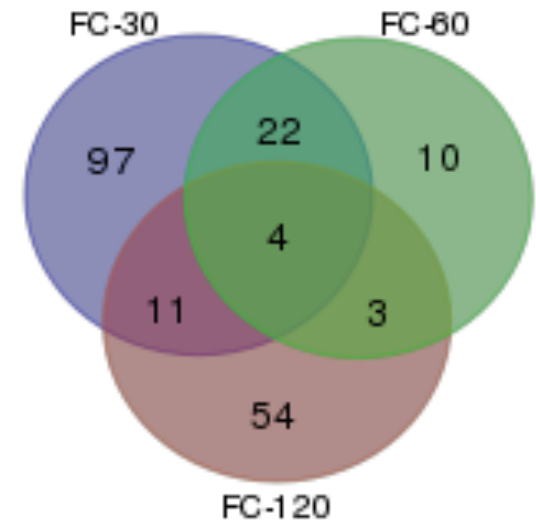
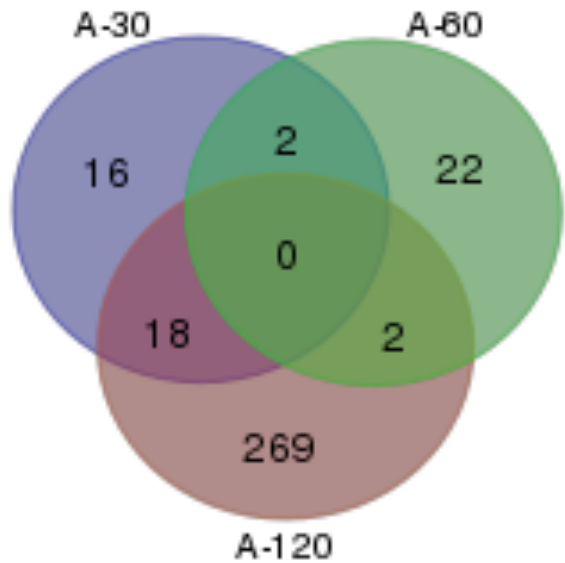
Cuffdiff (v 2.1.1)

* gene DE reporting
* isoform DE reporting



DAVID
Gene Ontology Analysis

Gene DE overview for control vs. treatment at each brain region/time-point



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Sample of BVC scatter chart – A 120

