Characterization of small RNAs from Extracellular Vesicles

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INTRODUCTION & OVERVIEW

- The discovery of ribonucleic acid (RNA) inside extracellular vesicles (EV) has raised significant interest in the biological function and biomarker potential of small RNAs, miRNAs in particular.
- This project represents a first-pass attempt to use sequencing to compare EV small RNAs from vesicles and non-vesicles from the same individuals.

DATA

- Sequencing data was generated by Illumina HiSeq2000 and aligned to the reference using miRDeep2 software package.
- The data contained 42 matched subjects with sequenced vesicle and non-vesicle (flowthrough) material. These samples also had recorded background characteristics and cognitive measures, in particular diagnosis, Age, Sex, APOE genotype, MMSE and DRS score.

CLUSTERING: METHODS & RESULTS

- Consensus matrices were generated with PAM and hierarchical clustering, using 1 - Spearman correlation distance on log transformed RLE normalized counts with 80 percent sample resampling.

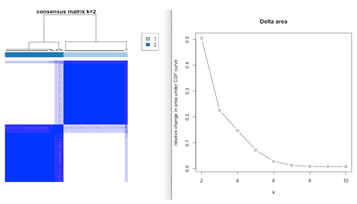


Figure 7: Consensus Matrix plot with k=2 and the relative change in area under the CDF for k from 1 to 10.

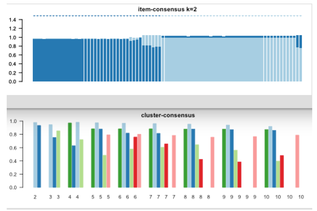


Figure 8: Item Consensus for k=2 and Cluster Consensus for k from 1 to 10.

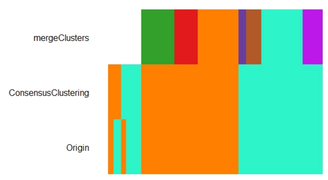


Figure 9: Comparison of Consensus Clustering and Combine Many method of clusterExperiment.

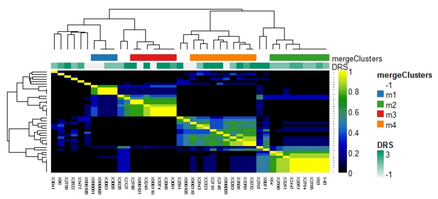


Figure 10: clusterExperiment heatmap within vesicle dataset with diagnosis as group.

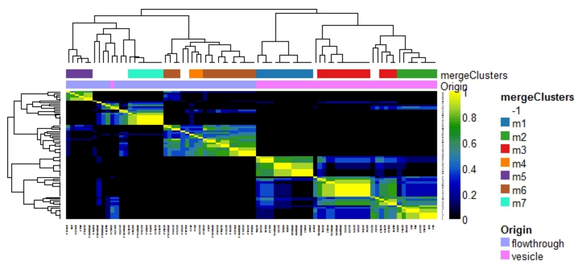


Figure 11: clusterExperiment heatmap with flowthrough vs. vesicle as groups.

EDA AND NORMALIZATION: METHODS & RESULTS

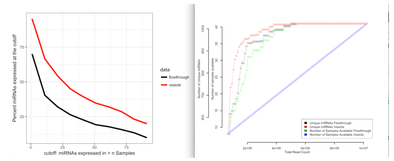


Figure 1: First graph depicts the percent of miRNAs expressed at a certain percent of samples in both flowthrough and vesicle datasets. Graph on the right shows the number of unique miRNAs and samples with at least 1 count for different total read sums.

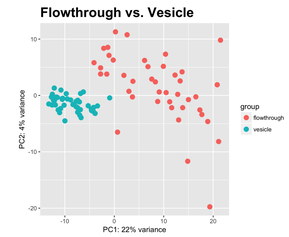


Figure 2: First two principal components depict differences between Flowthrough and Vesicle samples

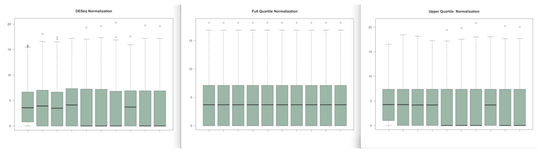


Figure 3: Boxplots for 10 randomly selected samples using 3 different normalization methods: DESeq, Full Quartile and Upper Quartile.

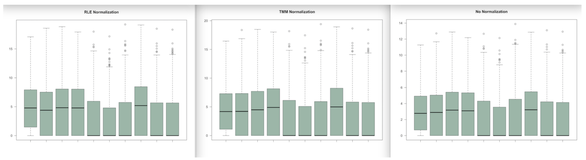


Figure 4: Boxplots for 10 randomly selected samples using 2 different normalization methods: RLE and TMM, as well as no normalization.

DIFFERENTIAL EXPRESSION: METHODS & RESULTS

Figure 5: estim. fold change

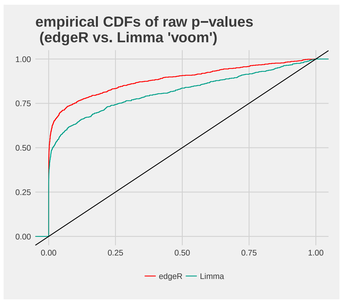
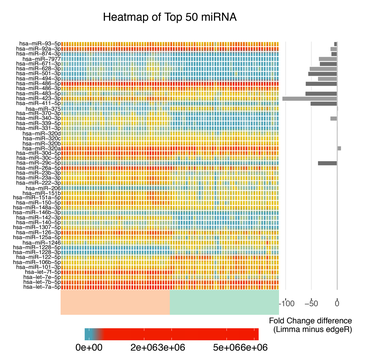


Figure 6: raw p-values



- There is some obvious discord between the set of differentially expressed genes.
- "Limma voom" controls the false positive rate more strictly than does "edgeR".

PRINCIPAL REFERENCES

[1] Daniel Enderle, Alexandra Spiel, Christine M Coticchia, Emily Berghoff, Romy Mueller, Martin Schlumpberger, Markus Sprenger-Haussels, Jonathan M Shaffer, Eric Lader, Johan Skog, et al. Characterization of RNA from exosomes and other extracellular vesicles isolated by a novel spin column-based method. *PloS one*, 10(8), 2015.

[2] Kevin P. McCormick, Matthew R. Willmann, and Blake C. Meyers. Experimental design, preprocessing, normalization and differential expression analysis of small rna sequencing experiments. *Silence*, 2(1):2, 2011.

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