Count Splitting Controls For Type 1 Error in Differential Testing After Tree Merging of Gene Isoforms

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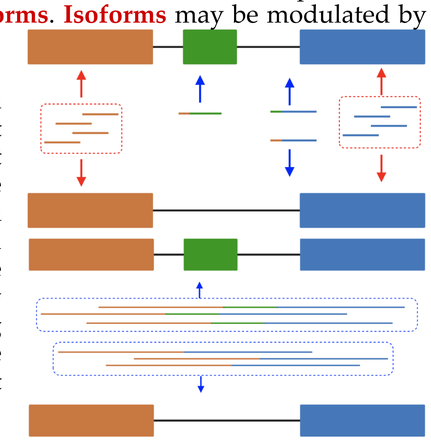
Background

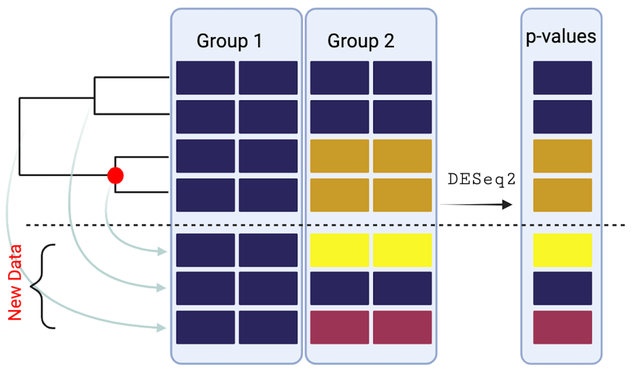
One **Gene** may be translated into *mRNA* and then spliced into multiple transcripts named **isoforms**. **Isoforms** may be modulated by splicing factors within the cell.[1]



RNAseq takes a snapshot of a cell's gene expression profile at the time of sequencing. Short read sequencing, 100-250 base pairs, required estimating which **isoform** is present since the read may be contained within one *exon*. **Long read sequencing** may span multiple *exons*, providing more confidence on the transcript **isoform** detected at the expense of read depth.

Goal: Develop an isoform-grouping method to facilitate isoform-level **differential expression (DE)** analysis using long-read sequencing data while controlling for False Positives for differential expression.

DE Testing of Inner Nodes



A gene with N isoforms implies $N-1$ inner nodes for its associated tree. These inner nodes are the sum of the leaves of a sample. Once we have our extended data, we perform `DESeq2`[2] and evaluate the resulting **pvalues** with our tree Climbing algorithm.

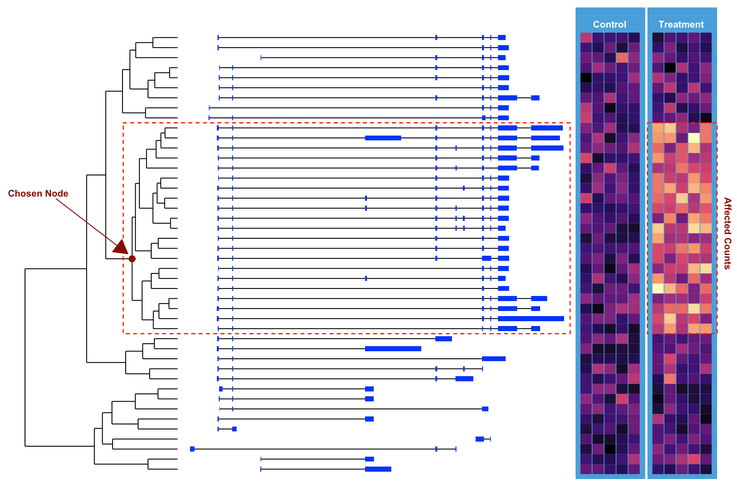
Cluster Tree Generation Method

To Generate Hierarchical clusters, we generated a **similarity metric** based on the similarities between transcripts as opposed to using data dependent counts. Let G represent a set of isoforms of size g. given any indexes $i,\ j \leq g$, define G_i and G_j as isoforms i and j from G such that they represent sets of exons of size N and M respectively. For any two i and j, we can define the similarity as:

$$S_{ij}(G_i, G_j) = \frac{2\sum_n^N \sum_m^M J(G_{i_n}, G_{j_m})}{N+M} \qquad J(G_{i_n}, G_{j_m}) = \frac{G_{i_n} \cap G_{j_m}}{G_{i_n} \cup G_{j_m}}$$

Simulation Methods

Choose an inner node within the tree and shift the mean of all leaves for a particular group by some delta. We can evaluate our Tree climbing algorithm based on how well it accurately chooses the known perturbed nodes.

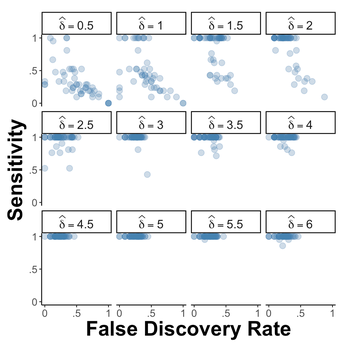


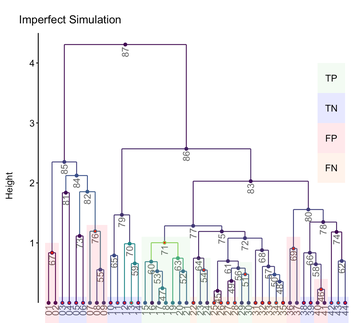
Let $\mu_{ij} = \mu_0 + \delta_{ij}$, $\mu_0 = 10$ and $\delta_{ij} = 0$ for i, j in the control group, and $\delta_{ij} = \hat{\delta}$ for i, j in the affected group. Each entry, X_{ij}, is sampled as follows.

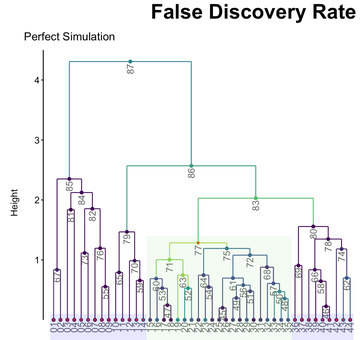
$$X_{ij} \sim \text{Nbinom}(\mu_{ij},\ \alpha = 100)$$

Simulation Sensitivity

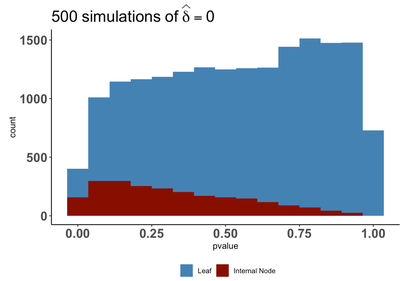
We conducted 50 simulations per $\hat{\delta} \in \{0.5, 1, 1.5, \ldots, 6\}$ and evaluated how often our tree climbing algorithm correctly merged the known perturbed data. Merged nodes with $\delta_{ij} = 0$ are **False Positives** and unmerged nodes of with $\delta_{ij} = \hat{\delta}$ are considered **False Negatives** in the tree climbing context.







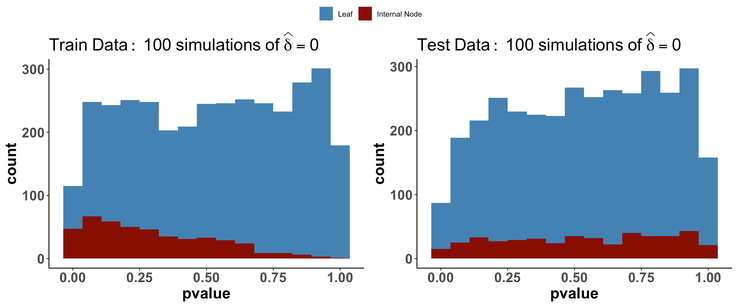
Count Splitting[3]



While at higher $\hat{\delta}$ shifts in our simulations are able to identify the correct inner node, but we also need to consider the case of the **null hypothesis**. To assess the distribution of pvalues under the assumption that there is no difference in the μ_{ij}, we ran simulations with $\hat{\delta} = 0$.

There is an **enrichment of low pvalues** among the **inner nodes** in the **null hypothesis simulations**. To correct this we can apply **count splitting**. The count splitting method is formulated for experiments that use the same data for feature selection as they use for analysis.[3]

$$X_{ij} \sim \text{Nbinom}(\mu_{ij}, \alpha = 100)$$
$$X_{ij}^{\text{train}} \sim \text{Bin}(X_{ij}, \theta = 0.5)$$
$$X_{ij}^{\text{test}} = X_{ij} - X_{ij}^{\text{train}}$$



Conclusions

1. Simulations of a single gene of 44 isoforms can reliably detect the correct node with $\log_2$ fold change of 0.5 between groups.
2. Utilization of count splitting controls for type 1 error under the null hypothesis.

Future Directions

1. Improve speed of data merging step.
2. Apply tree climbing and count splitting methods on real data sets.

References

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