Step-by-step Guide to Perform Automated Differential Expression Analysis using FlexStatv1 Pipeline

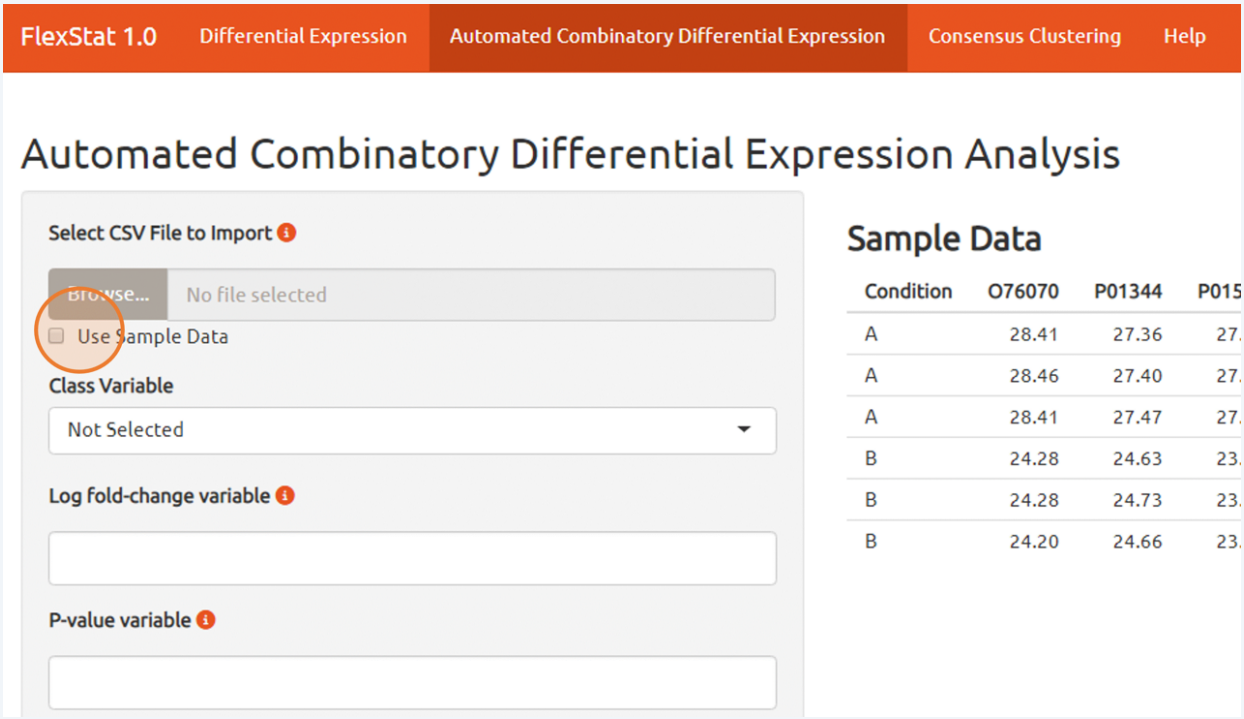
This feature facilitates combinatory differential expression analysis for datasets with more than two classes/conditions.

It systematically generates all possible pairwise comparisons, combines multiple classes/conditions, and presents detailed results for the differential expression analysis.

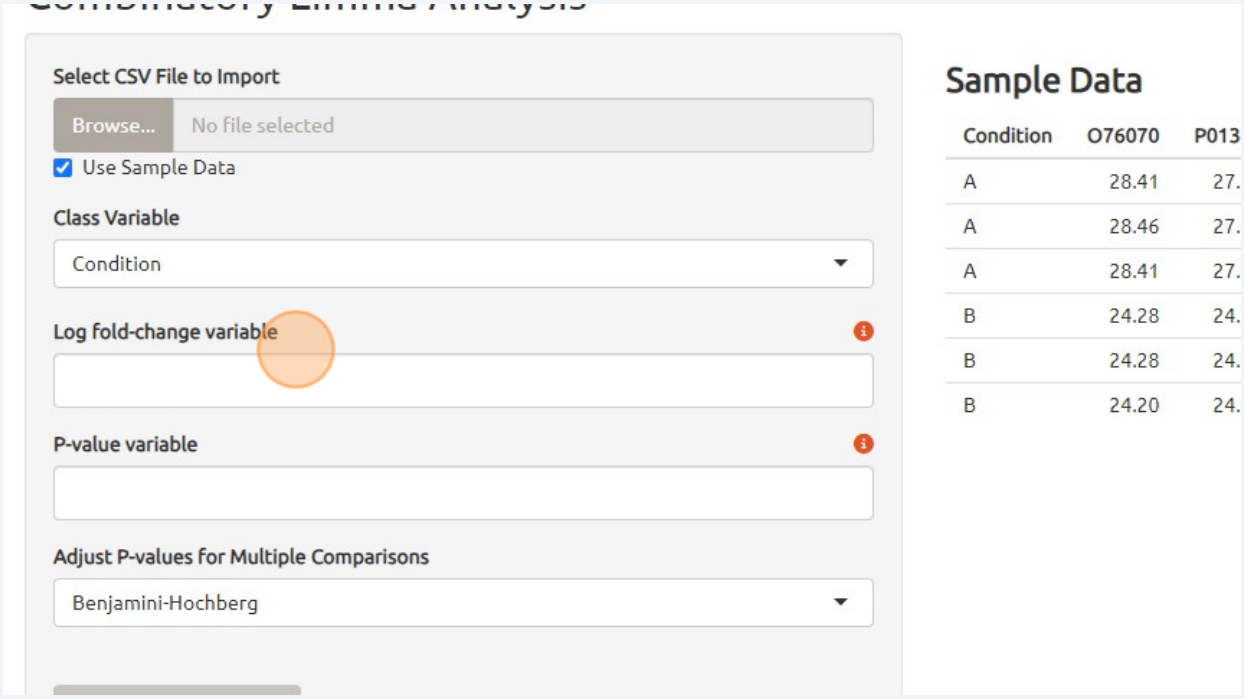
1 Navigate to https://jglab.shinyapps.io/flexstatv1-pipeline-only/

2 Go to the "Automated Combinatory Differential Expression" tab.

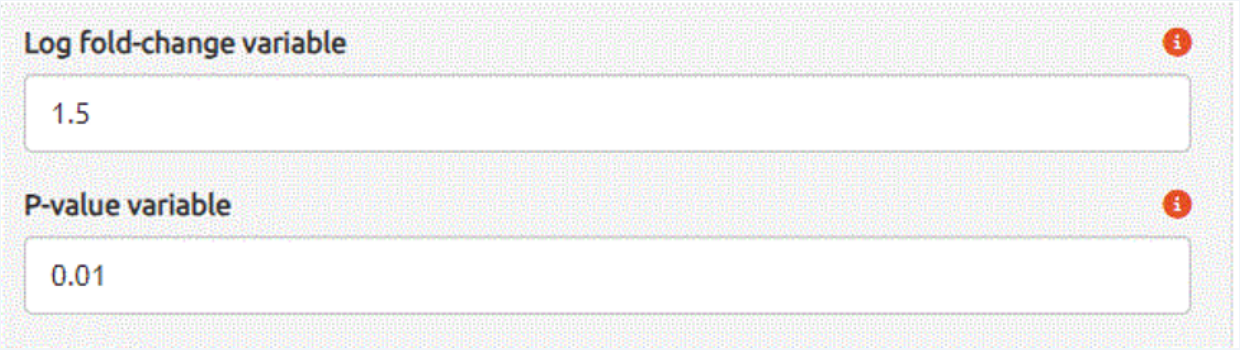
3 Click "Use Sample Data"



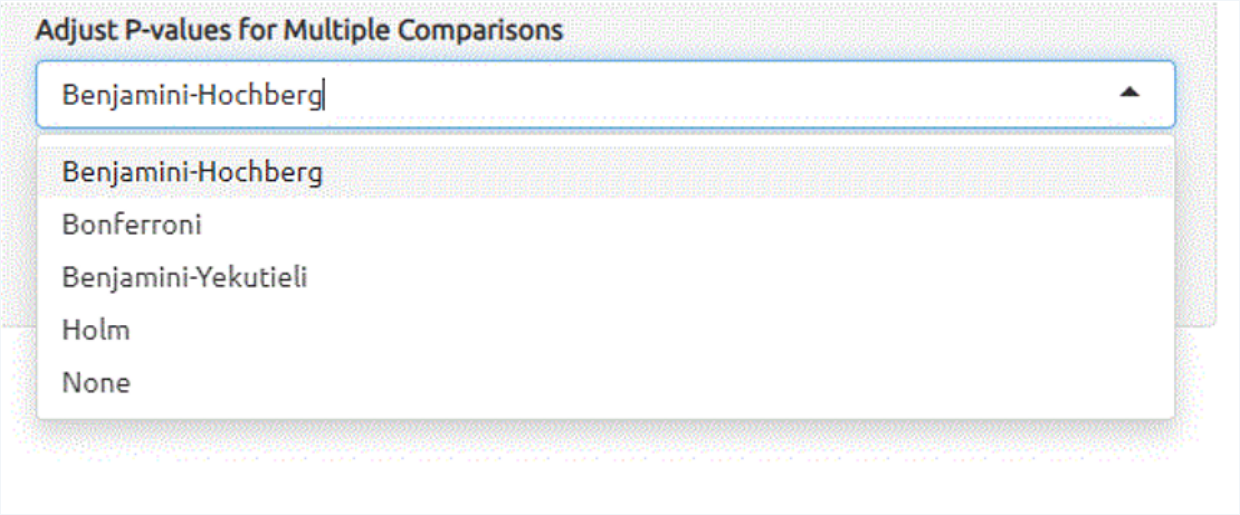
4 Click "Condition" as the Class variable and select correction for multiple comparisons.



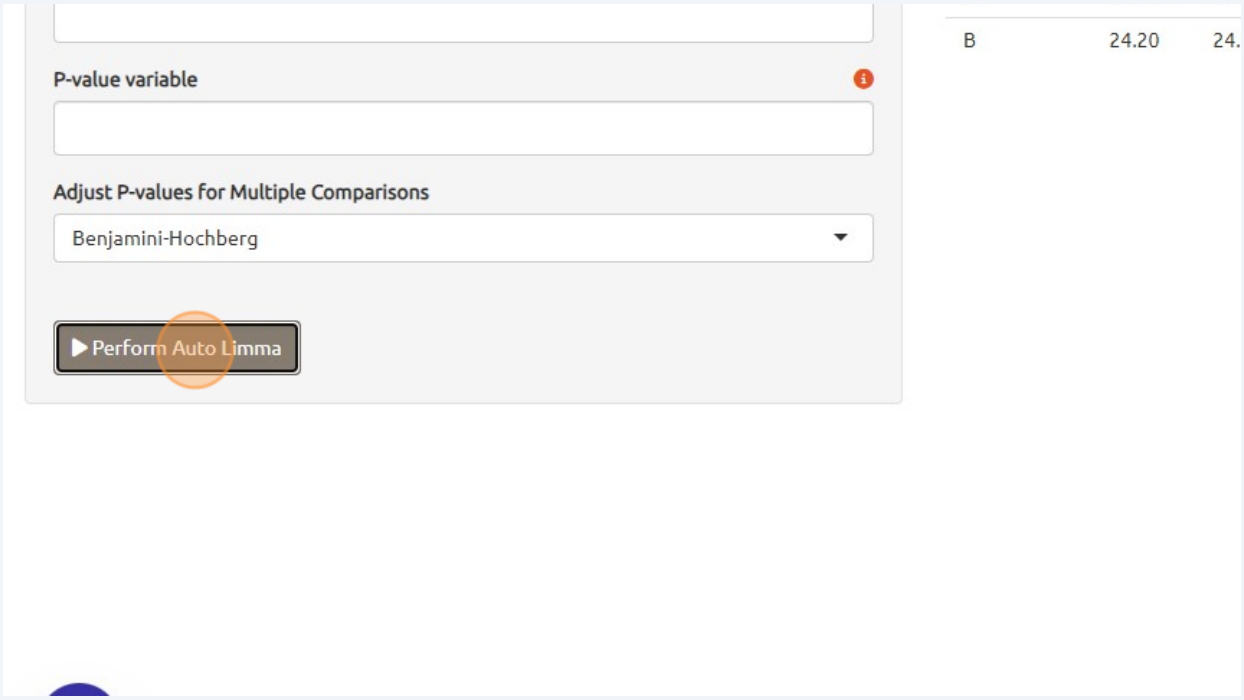
5 **[Optional]** Change log fold change and p-value cutoffs based on the research question at hand.



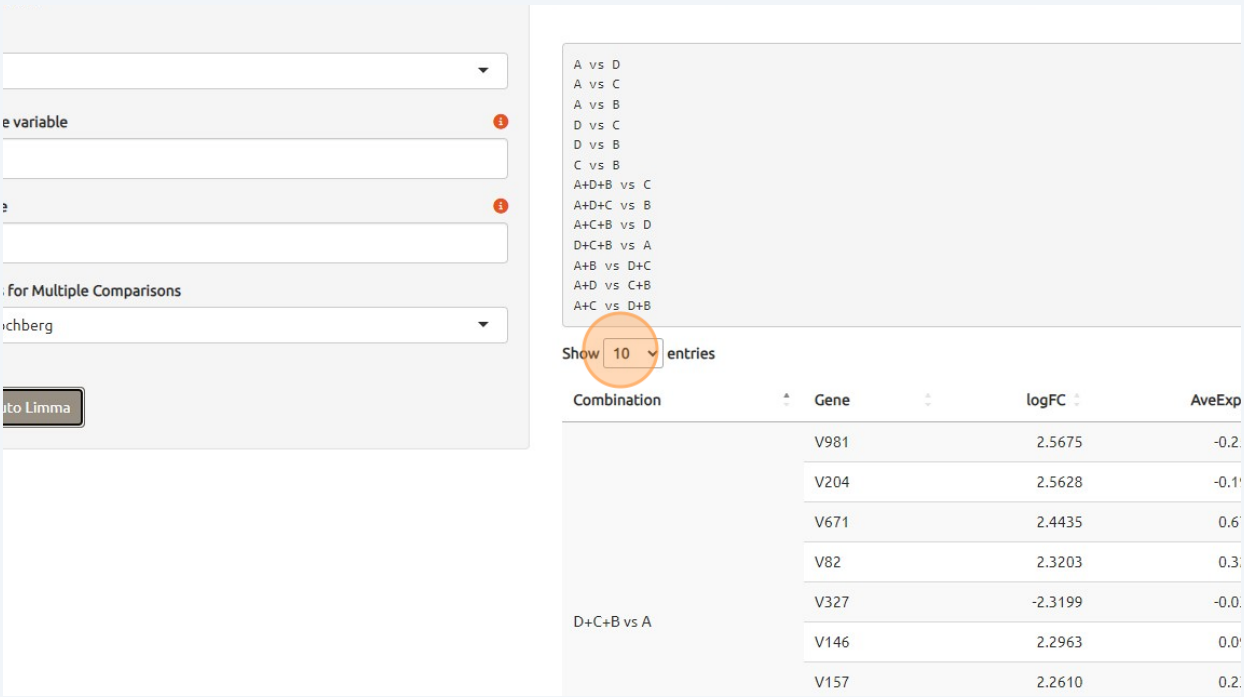
6 **[Optional]** Change the correction procedure for multiple comparison tests based on the research question at hand.



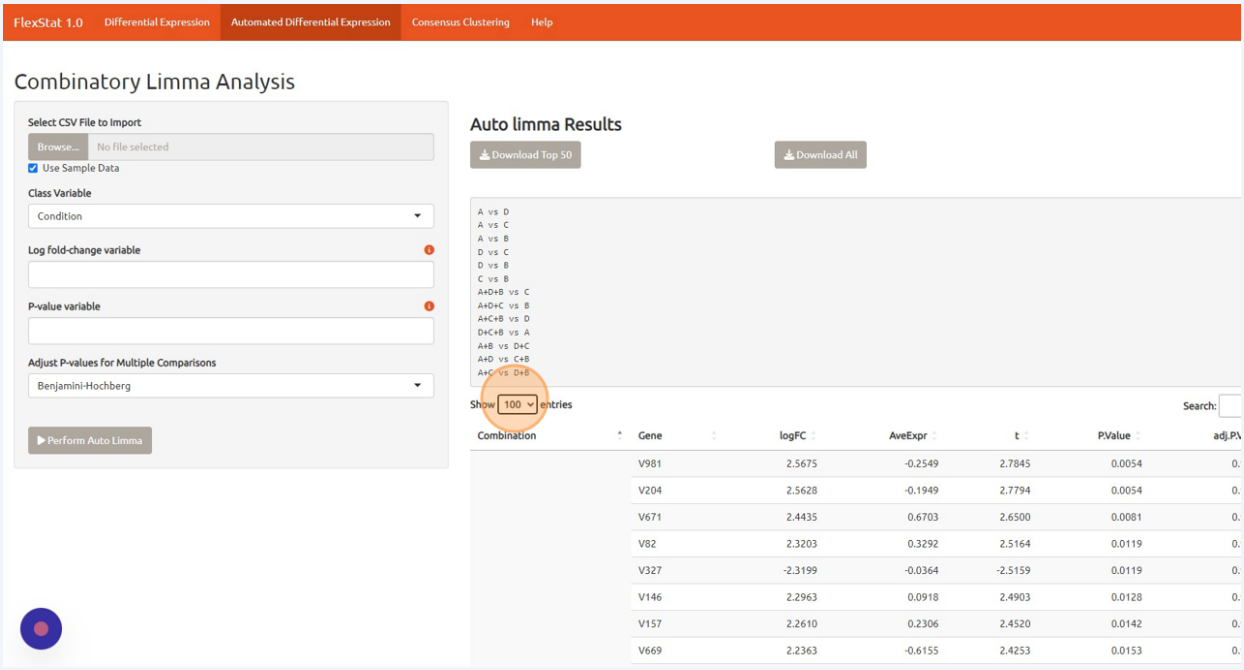
7 Click "Perform Auto Limma"



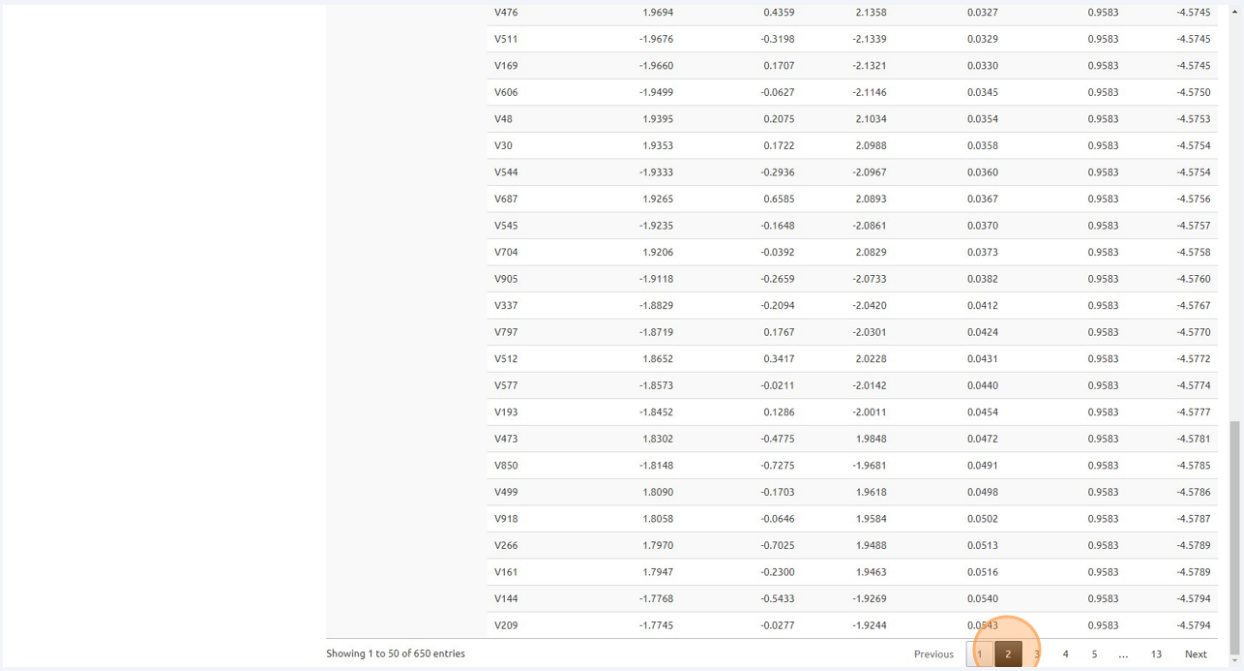
8 This will result in calculating all possible combinations ofthe classes.



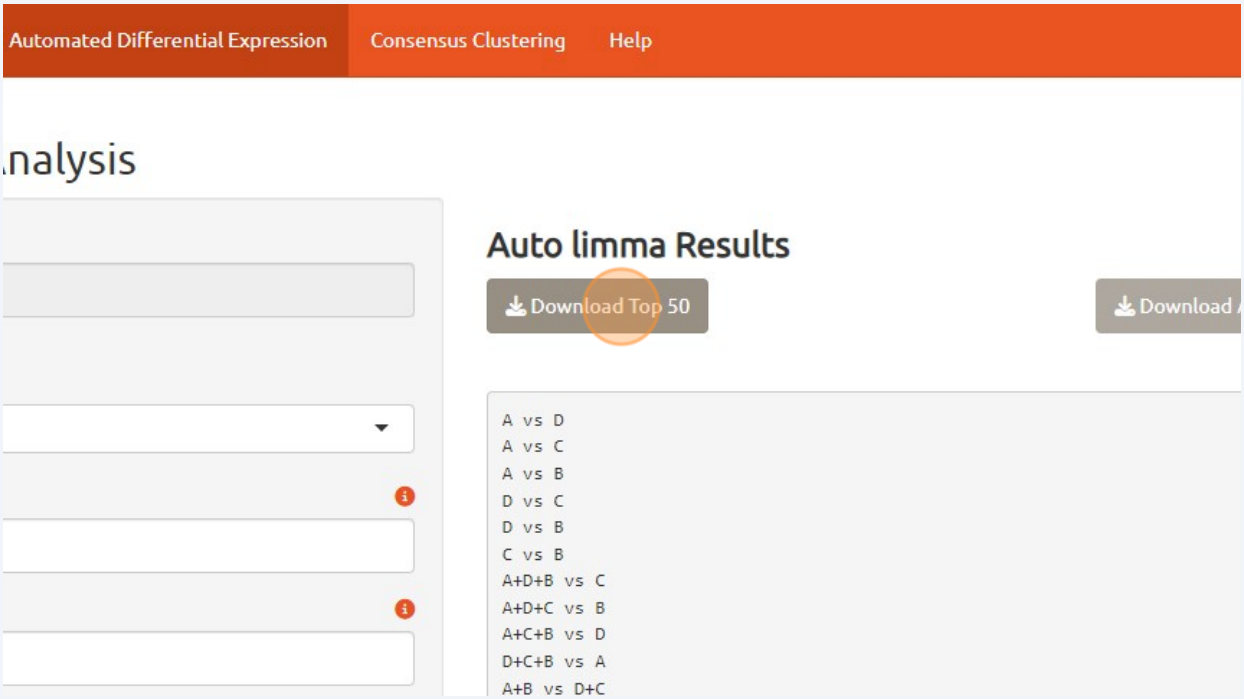
9 This will result in producing a table of differential expression results for each combination.



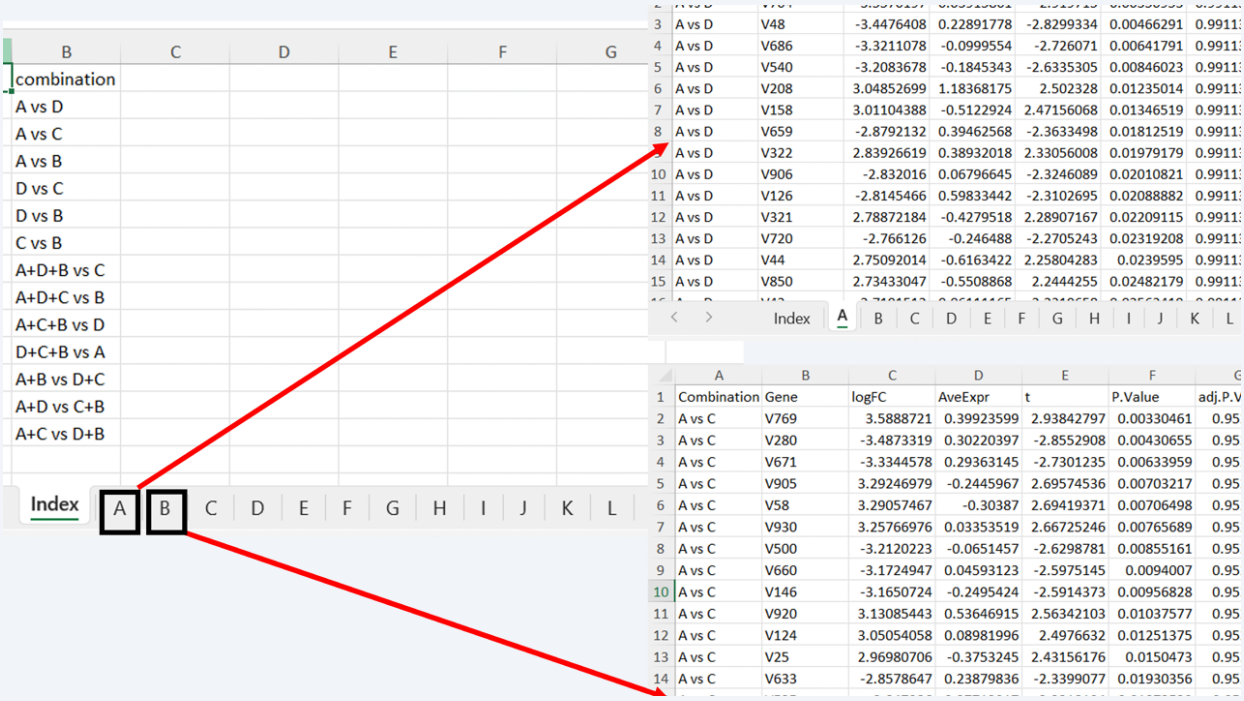
10 Navigate using page numbers to explore the results.



11 Click "Download Top 50" to download top 50 genes/proteins from each combination



12 Downloaded excel files will be as follows. The first sheet will be an index explaining the subsequent sheets representing different combinations.



13 Click "Download All" to download all results.