Functional analysis of RNA-seq data

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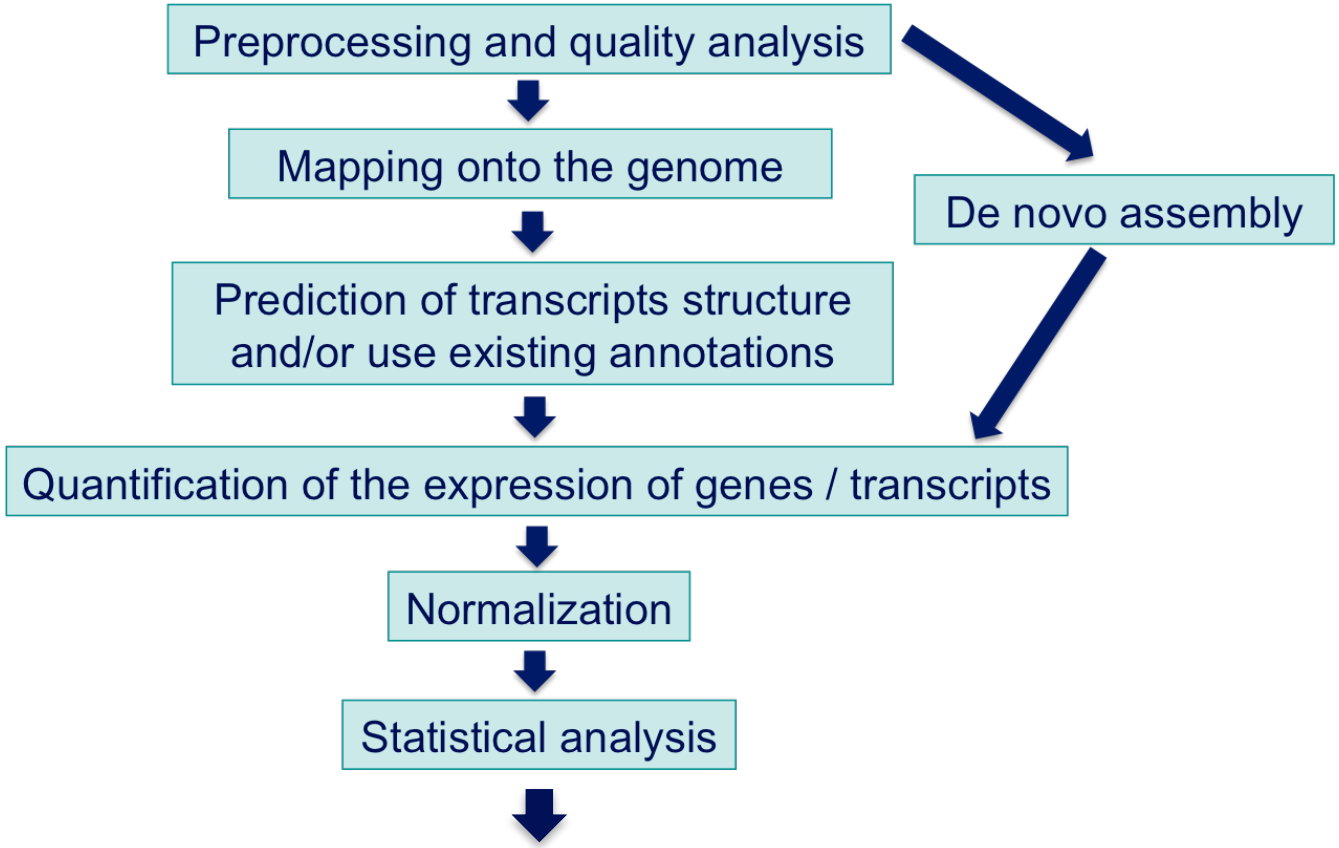
Analysis of RNA-seq data

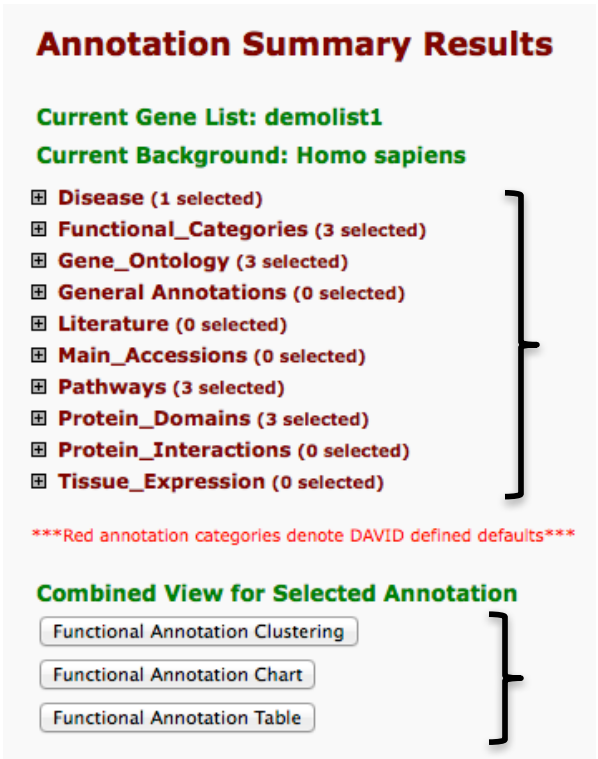


Functional enrichment analysis, pathway analysis, integration with other data, …

Functional analysis

- A lot of functional analysis tools available
  - Initially developed for microarray data
  - e.g. GO tools listed in
    https://omictools.com/search?q=gene+ontology
  - Methods specific to RNA-seq data
    - goseq (Young et al., Genome Biology 2010;11:R14)
    - SeqGSEA (Wang et al. BMC Bioinformatics 2013, 14(Sup5):S16)
    - GSAASeqSP (Xiong et al Scientific Reports 2014; 4:6347)
- DAVID will be used for this practical session because
  - Graphical interface & free software
- DAVID
  - **D**atabase for **A**nnotation, **V**isualization and **I**ntegrated **D**iscovery
  - https://david-d.ncifcrf.gov/
  - A very interested article describing how to use DAVID :
    Huang et al. Nature Protocols 2009;4(1):44-57.

DAVID



Different sources of annotation

- Disease (OMIM)
- Gene Ontology
- Pathways (KEGG, Biocarta)
- Protein Domains (InterPro, SMART)
- Protein Interaction (BIND)
- …

Different tools

- Functional Annotation Clustering
  - Cluster functionally similar terms associated with a gene list into groups
- Functional Annotation Chart
  - Identify enriched annotation terms associated with a gene list
- Functional Annotation Table
  - Query associated annotations for all genes from a list

Gene Ontology

- Defines concepts/classes used to describe gene function and relationships between these concepts
- Classifies functions along three aspects
  - Molecular function : molecular activities of gene products
  - Cellular component : where gene products are active
  - Biological process : pathways and larger processes made up of the activities of multiple gene products

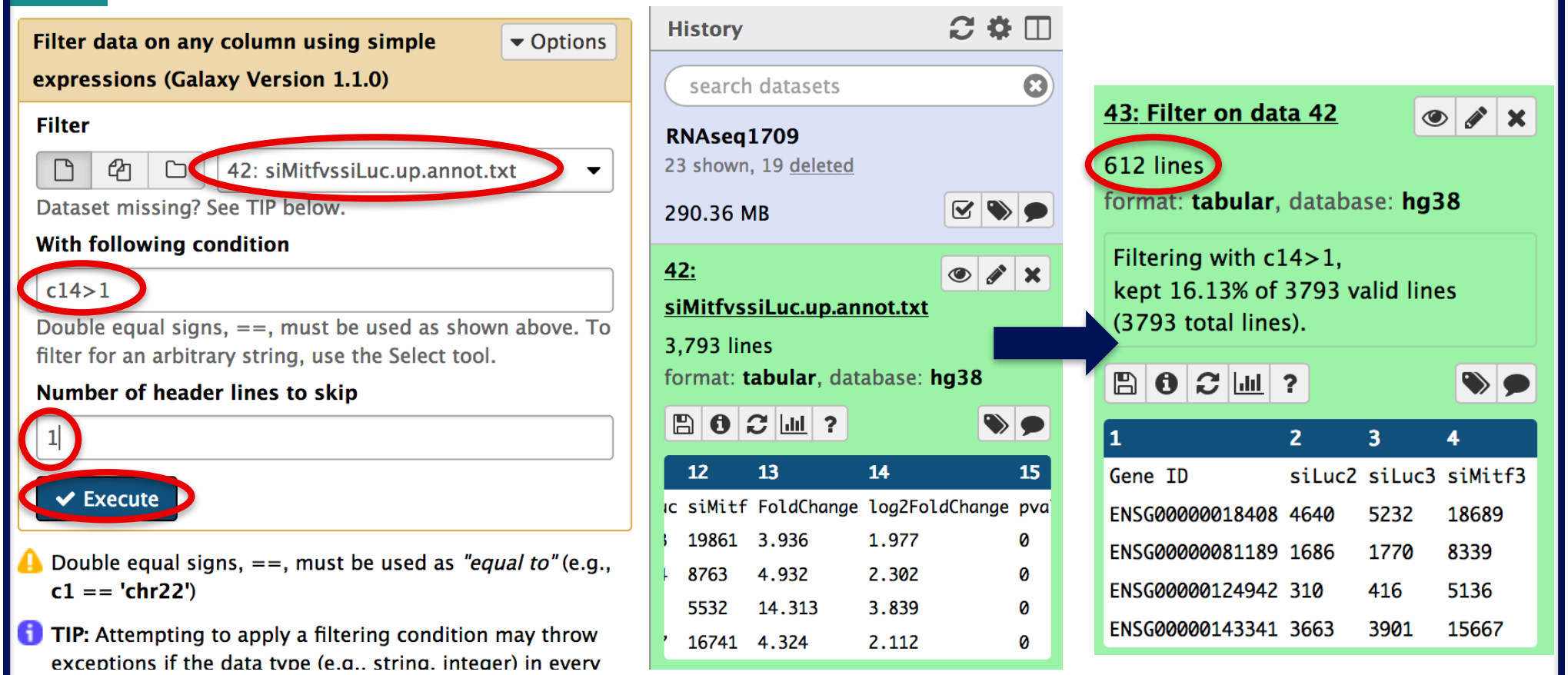
Exercise : functional analysis

- Use DAVID to perform functional analysis of genes significantly over-expressed in siMitf vs siLuc samples

1. Select over-expressed genes using the filter tool on GalaxEast
   - Proposed thresholds :
     Adjusted p-value < 0.05 and $\log_2$(FoldChange) > 1
2. Create a file with gene name for all these genes using the cut tool on GalaxEast
3. Analyse this gene list using DAVID

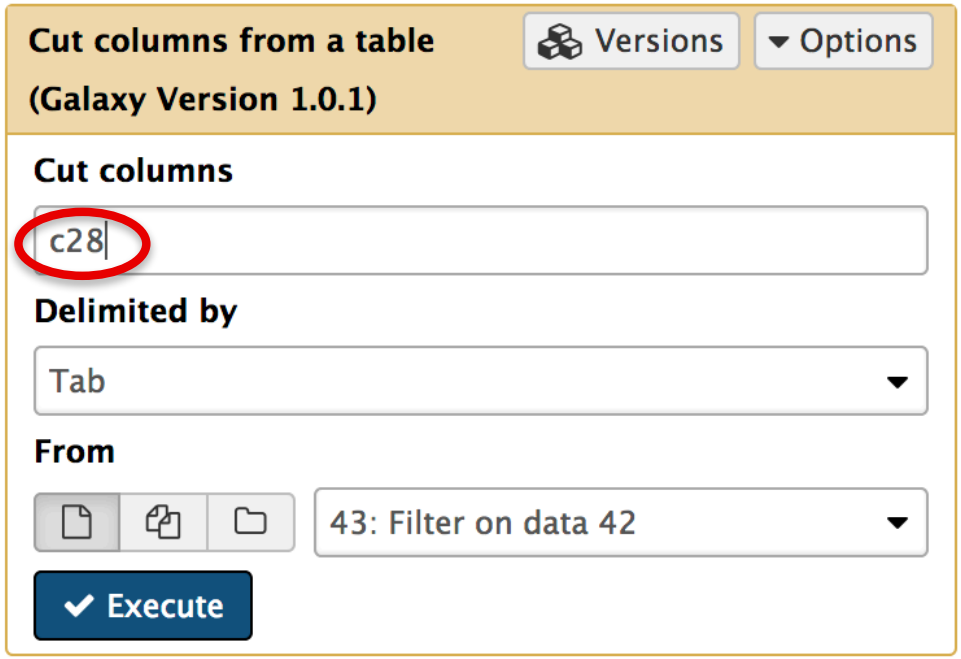
1. Select over-expressed genes

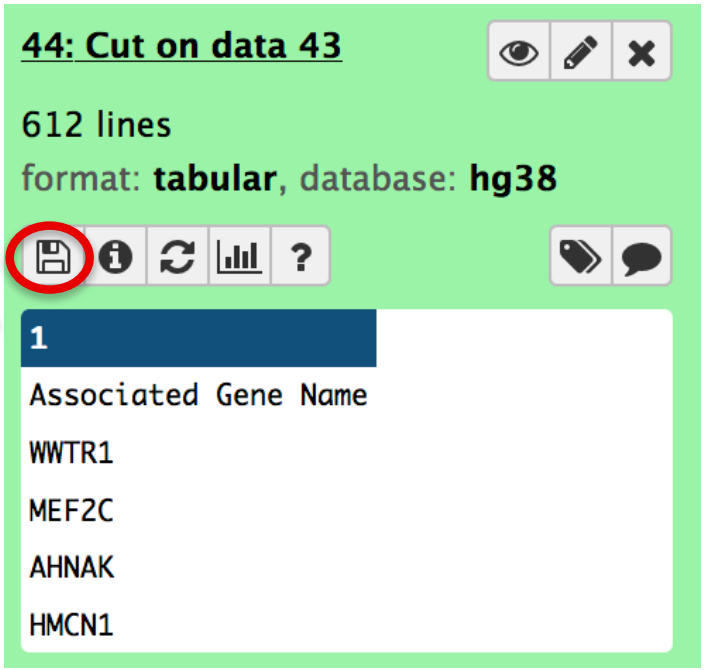
- Among significantly differentially expressed genes, select genes with $\log_2$(FoldChange) > 1



2. Create a list of gene names

- Select associated gene names in the previous table

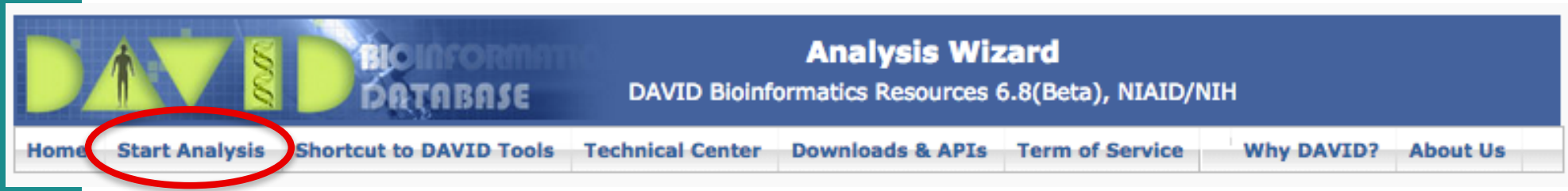




siMitfvssiLuc_upgenes_lfc1_padj005.txt file

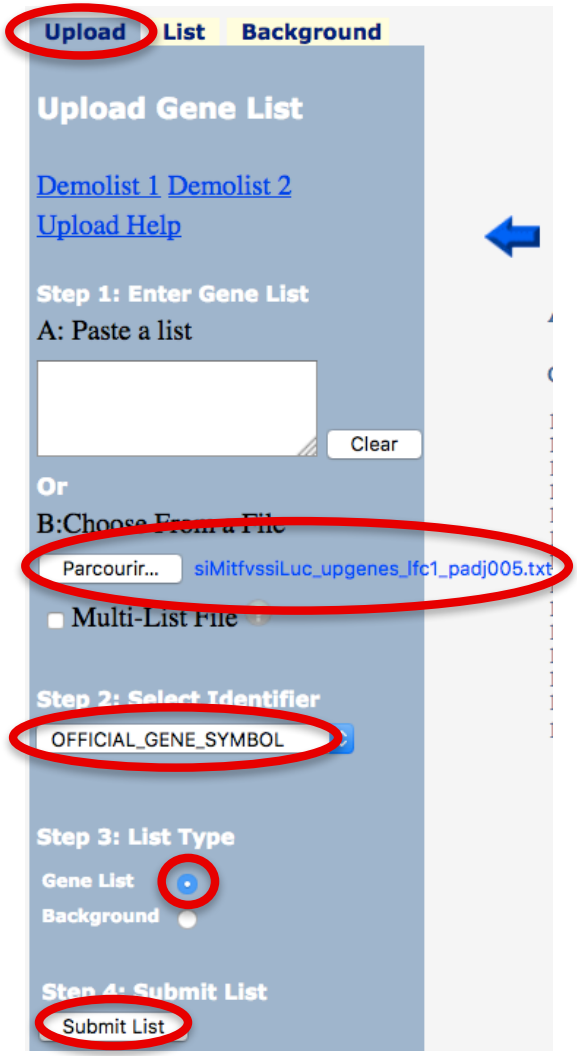
3. Analyse your gene list using DAVID

- Go to https://david-d.ncifcrf.gov
- Click on Start Analysis

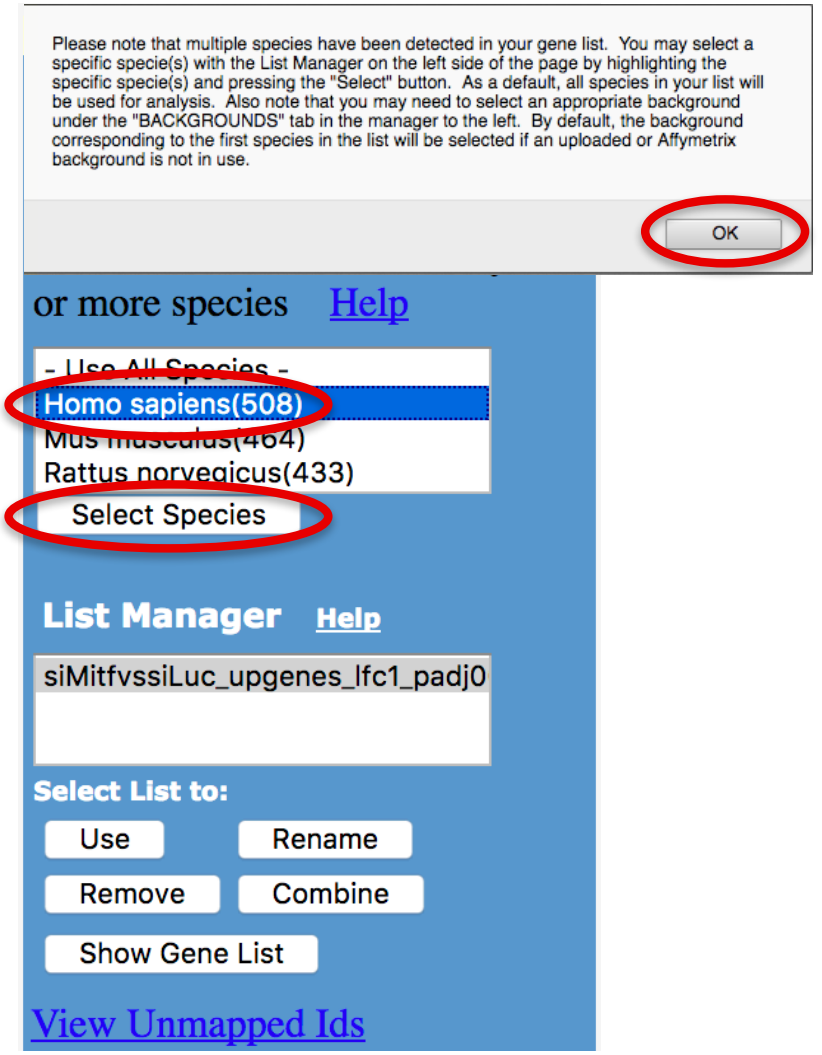


3. Start DAVID analysis

- Enter your gene list



- Select species



Exercise : functional analysis

1. What are the 10 most enriched functional annotation terms among annotations of the genes from your list ?

   How many genes are annotated with each of these terms ?

   Which genes are annotated with the most enriched term ?

2. As you see redundancy in previous results, it could be interesting to cluster functionally similar terms into groups.

   Look at the results of this clustering. What is the first identified cluster ?

   Click on  to visualize members of this cluster (genes and annotations).

3. *Claudin 15* gene is a member of this cluster.

   What are all associated annotations for this gene ?

   Among these annotations you will find the KEGG pathway "Cell adhesion molecules".
   Are other genes from your list member of this pathway ?