July 2025

geneXplain® platform 7.6 release

New method:

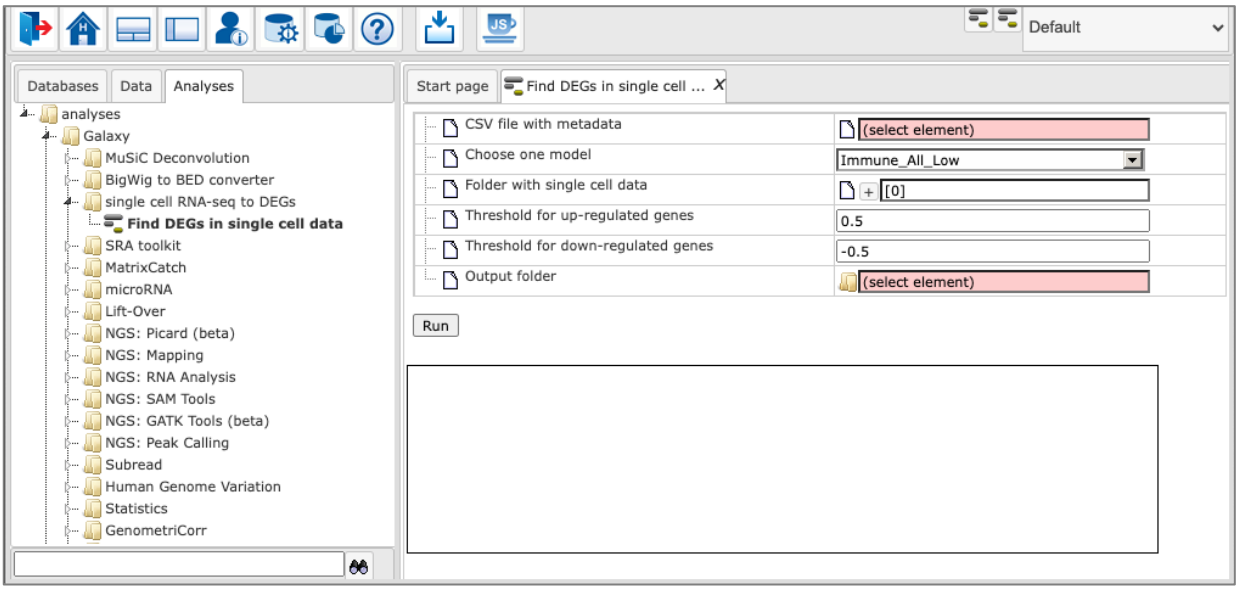
This release of the geneXplain platform introduces ability to process single cell RNA-seq data (scRNA-seq) and identify the differentially expressed genes (DEGs) using the newly developed method "Find DEGs in single cell data".

You will find the new method for DEGs identification from scRNA-seq under the: Analyses → Galaxy → Single cell RNA-seq to DEGs → Find DEGs in single cell data

This new tool identifies differentially expressed genes in single-cell RNA-seq data by comparing experimental conditions. It uses robust pseudobulk analysis and Wilcoxon test methods to accurately detect DEGs across different cell types, ensuring reliable biological insights.

Inputs

Tool input mask overview:



As input for this method a metadata file and a folder with single-cell count data should be provided. A cell-typing model is mandatory if cell types are not provided in the metadata file. **All files should be uploaded as generic files.**

- **Metadata File (CSV)** Sample metadata should be provided in the format of a CSV file. The **obligatory columns** include:
    - **Sample identifiers**.
    - An experimental group column named `group` or `condition` with the values **'control'** or **'experiment'**.
    - An optional `cell_type` column for annotations. If missing, the tool will perform automatic cell typing.

Example visualization of the input metadata file:

metadata

NAME	sample	cell_type	sex	group
ATTCACTGTAACAGGC-1_1	C51ctr	Epithelial cells	female	control
TAACTTCCAACCACGC-1_1	C51ctr	Myeloid	female	control
TTGGGTACACGACAAG-1_1	C51ctr	Epithelial cells	female	control
AGGCCACAGAGTCACG-1_1	C51ctr	Epithelial cells	female	control
CACTGAAGTCGAAGCA-1_1	C51ctr	Epithelial cells	female	control
ACTGATGTCTGCACCT-1_1	C51ctr	Epithelial cells	female	control
TTACCGCCACTCAGAT-1_1	C51ctr	Epithelial cells	female	control
TTGGTTTTCCTAGCTC-1_1	C51ctr	Myeloid	female	control
TGGGAAGTCAGTGATC-1_1	C51ctr	Epithelial cells	female	control
CCACGAGTCTCTTAAC-1_1	C51ctr	Fibroblasts	female	control

- **Folder with Single-cell RNA Sequencing Data** The tool accepts two formats:
    - **10X Genomics MTX Format**: Requires three files per sample, sharing a common prefix. `barcodes.tsv.gz` `features.tsv.gz` `matrix.mtx.gz`
    - **CSV Format**: A matrix with genes as rows and cells as columns (or vice-versa). The first row/column must contain identifiers.
- **Thresholds for up/down regulated genes** A floating pointer number which is used for filtering up and down regulated genes
- **Model for Cell-Typing** This is a required input **only if** your metadata file lacks a `cell_type` column. Select a pre-trained **CellTypist** model to automatically annotate cell types.

Workflow

The tool follows a sequential workflow from data loading to analysis.

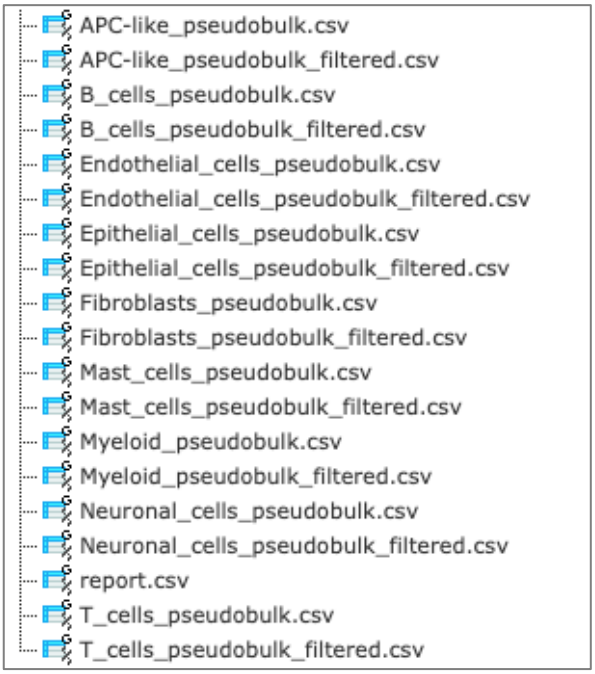
- **Data Loading**: Reads the input single-cell data (MTX or CSV) and metadata file.
- **Data Preparation**: A quality control pipeline filters low-quality genes and cells, calculates QC metrics (mitochondrial/ribosomal percentages), performs doublet detection with **Scrublet**, normalizes counts, and log-transforms the data.
- **Cell Typing (Optional)**: If the `cell_type` column is missing, the tool uses the selected **CellTypist**model to predict cell types.
- **Pseudobulk Aggregation**: Gene counts are aggregated for all cells belonging to the same sample and cell type, creating **pseudobulk profiles**.
- **Differential Expression**: For each cell type, the tool performs DEG analysis.
    - **Primary Method**: Uses **PyDESeq2** on pseudobulk profiles to compare 'experiment' vs 'control'.
    - **Fallback Method**: If `PyDESeq2` fails (e.g., due to low sample counts), it defaults to **Scanpy's** `rank_genes_groups` (Wilcoxon test) on the original single-cell data.
- **Output Generation**: The final DEG results are saved into separate CSV files for each cell type. The tool also generates a filtered csv file and a **report.csv** file which contains found cell types, number of cells corresponding to each type, and the type of analysis which was used.

Outputs

The tool returns a single **folder** containing the analysis results.

Inside the zip file there is one **CSV file per cell type**. The filenames indicate the cell type and the analysis method used (e.g., `T_cells_pseudobulk.csv` or `B_cells_rank_groups.csv`). The tool also generates a filtered csv file and a **report.csv** file which contains found cell types, number of cells corresponding to each type, and the type of analysis which was used.

Example output visualization:



Each CSV file includes:

- Gene names
- Log2 fold changes
- P-values
- Adjusted p-values

Database updates:

HumanPSD™ is updated to version 2025.1 (July 2025).

TRANSFAC® is updated to version 2025.1 (July 2025).

TRANSPATH® is updated to version 2025.1 (July 2025).

Please note that with the new release the analysis results might vary from the previous ones.