Tutorial on "Cancer Genes Analysis Across Multiple Cancer Types via Topological Classification of scRNA-seq Data"

1. Open the Repository
Go to the following GitHub link:
https://github.com/Sudarshan-Gogoi/Topological_Classification

2. Download the Required Data Files and Code
Download the following files from the repository:

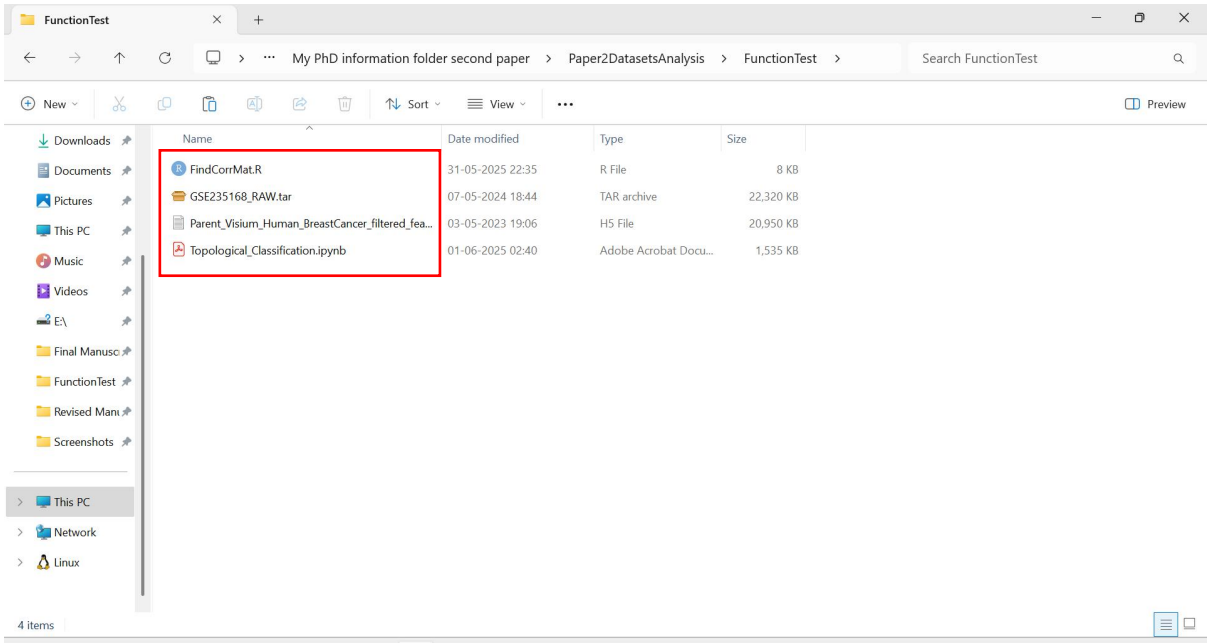
- **Example Data Files** (choose one depending on your dataset):

✧ `GSE235168_RAW.tar`
or

✧ `Parent_Visium_Human_BreastCancer_filtered_feature_bc_matrix.h5`

- **Code Files**:

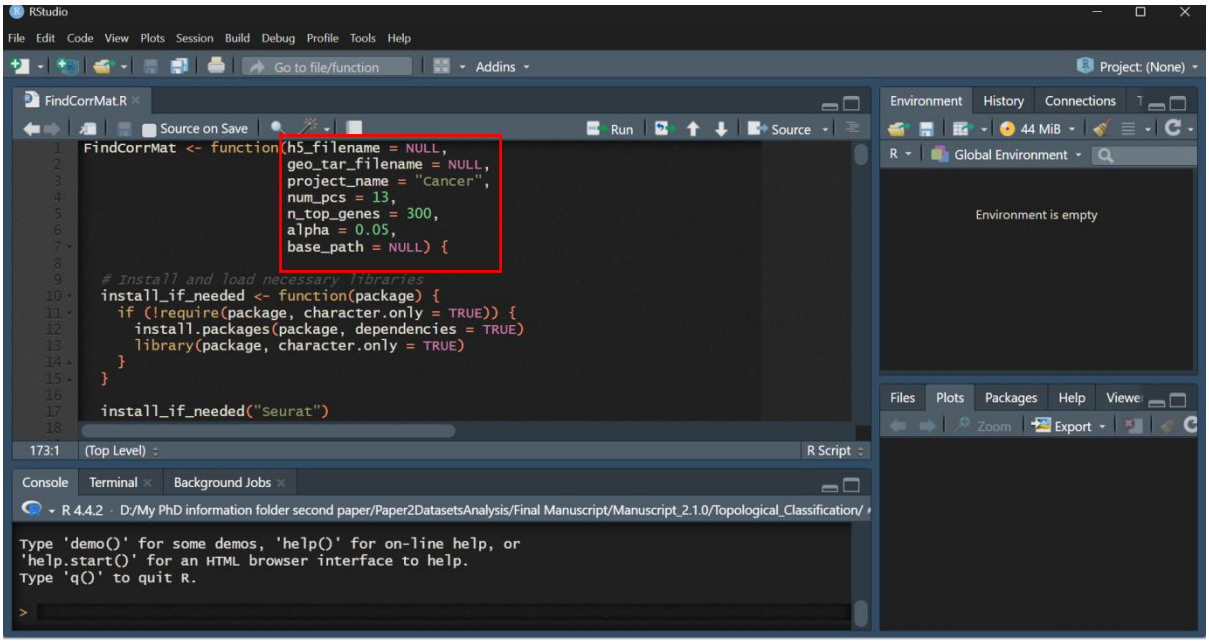
✧ `FindCorrMat.R`
✧ `Topological_Classification.ipynb`



3. Open the `FindCorrMat.R` Script in RStudio
Launch RStudio (version: RStudio-2024.09.1-39) and open the file `FindCorrMat.R`.

4. Set the Input Parameters for the Function:
The function accepts the following input parameters. You can use the default values provided for the example dataset, or adjust them based on your specific dataset as described below:

- `num_pcs`: Number of principal components to use.
  Default: 13
  To determine the optimal value for your dataset, run the function and examine the **Elbow Plot**. The "elbow point" in the plot typically indicates the best value to use for `num_pcs`.
- `n_top_genes`: Number of top genes to select per principal component.
  Default: 300
- `alpha`: Significance threshold for correlation analysis.
  Default: 0.05



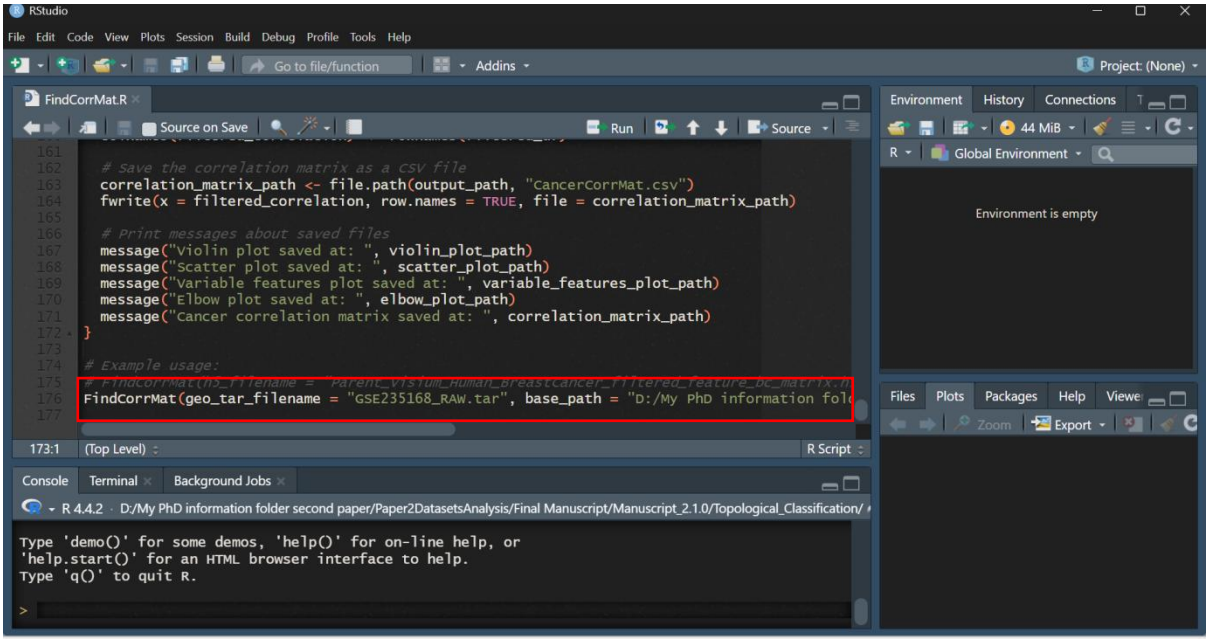
5. Set the Required Input Paths:
Before running the code, provide the following input parameters:

- `geo_tar_filename`: Specify the name of the dataset file you are using. Choose one of the following:

✧ `GSE235168_RAW.tar`
or

✧ `Parent_Visium_Human_BreastCancer_filtered_feature_bc_matrix.h5`

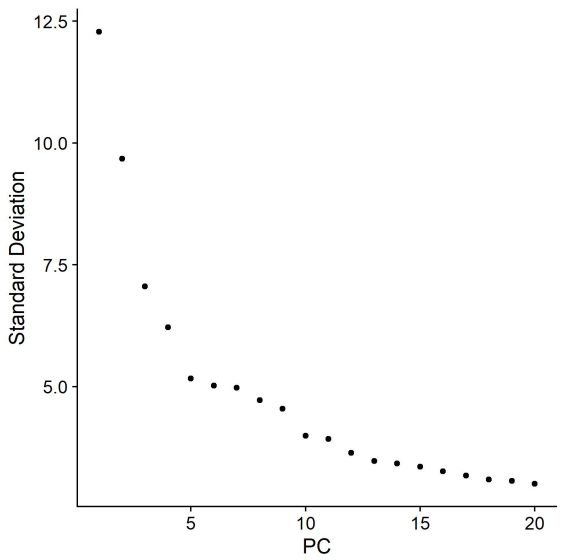
- `base_path`: Define the directory path where all your data files and code are located.
  Tip: Use the same `base_path` throughout all scripts and notebooks to simplify the workflow and avoid file path errors.



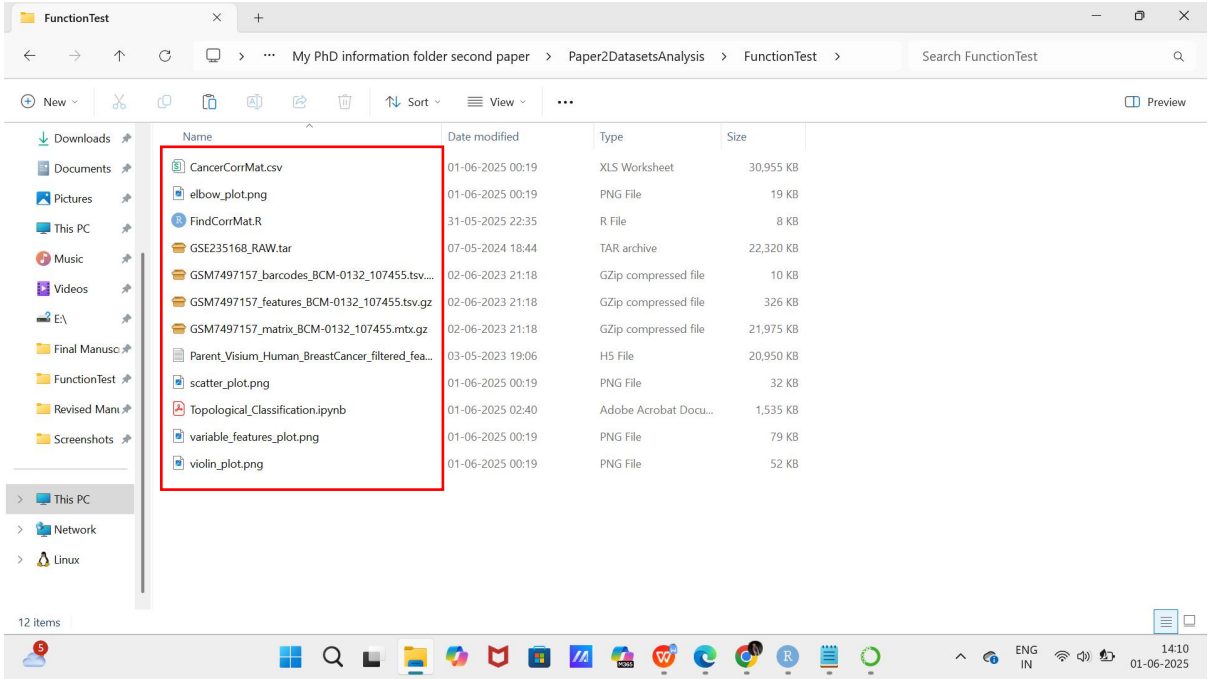
6. Output Files (Saved in `base_path`)

After running the code, the following output files will be generated and saved in the specified `base_path` directory:

- `violin_plot.png` – Quality control plots showing metrics such as gene counts, feature counts, etc.
- `scatter_plot.png` – Scatter plot of selected features for visual assessment.
- `variable_features_plot.png` – Plot displaying the most variable genes across the dataset.
- `elbow_plot.png` – PCA elbow plot used to determine the optimal number of principal components (`num_pcs`).
- `CancerCorrMat.csv` – Final correlation matrix of top genes used for downstream topological classification.



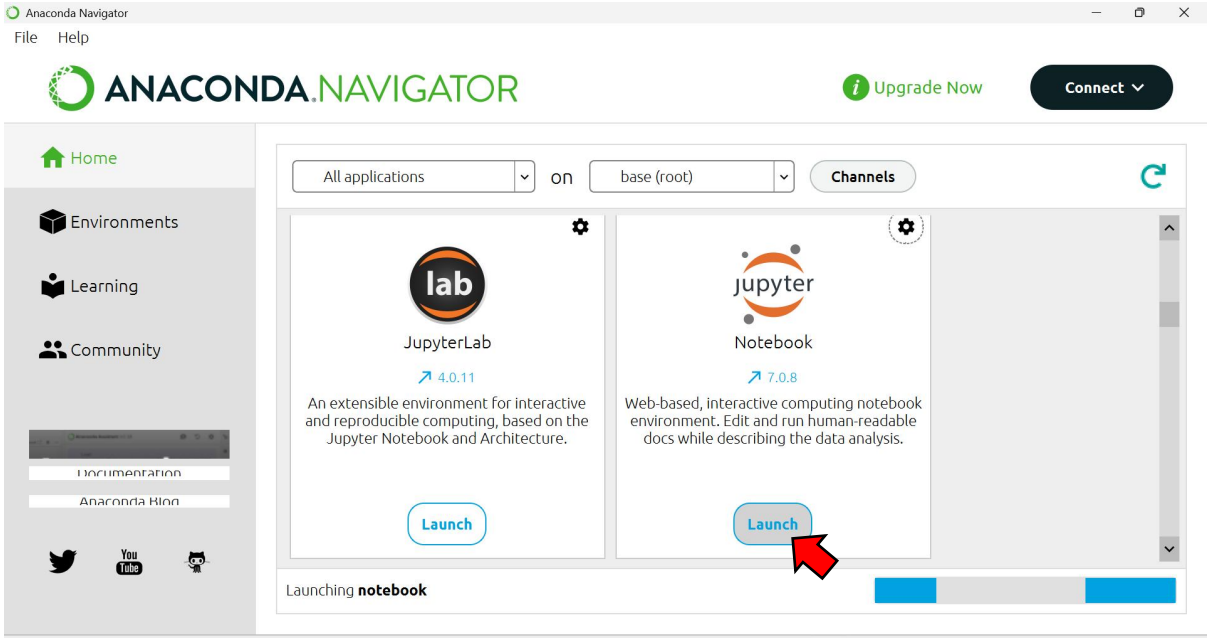
Elbow Plot



7. Launch Jupyter Notebook via Anaconda Navigator

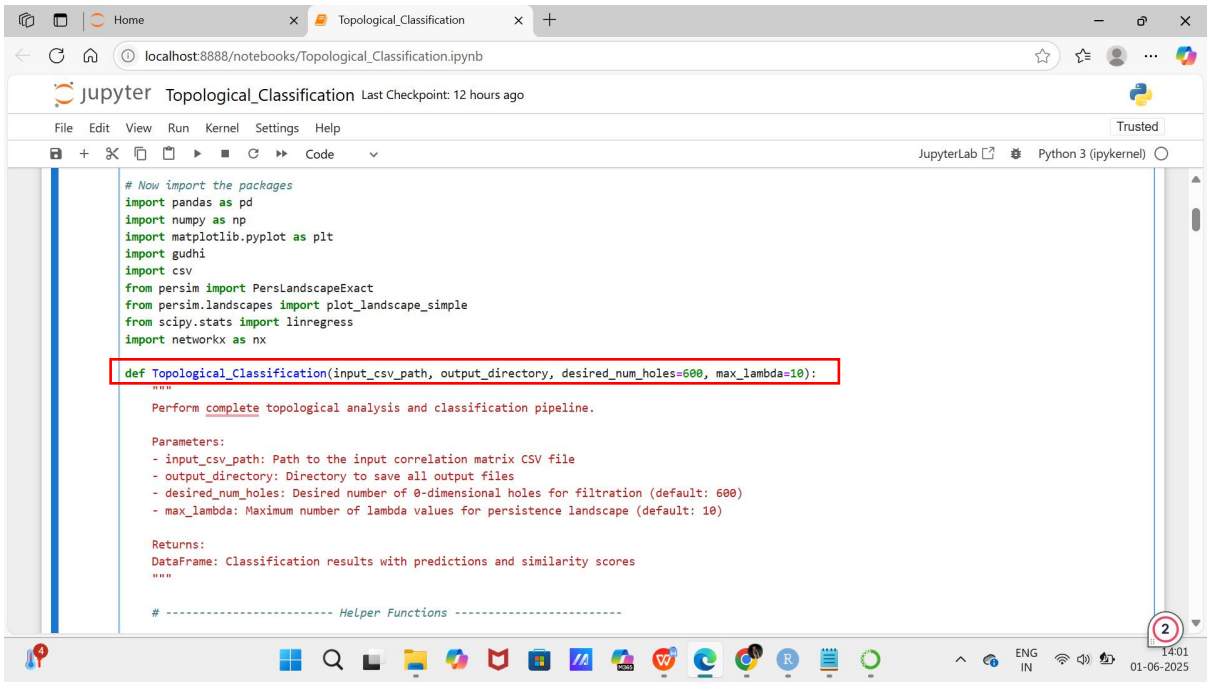
- **Open Anaconda Navigator**
  Start Anaconda Navigator from your system's applications or start menu.
- **Launch Jupyter Notebook (Version 7.0.8)**

✧ In the Anaconda Navigator interface, locate **Jupyter Notebook**.
✧ Click the **Launch** button beneath it.
✧ This will open Jupyter Notebook in your default web browser.



8. Load the Analysis Notebook

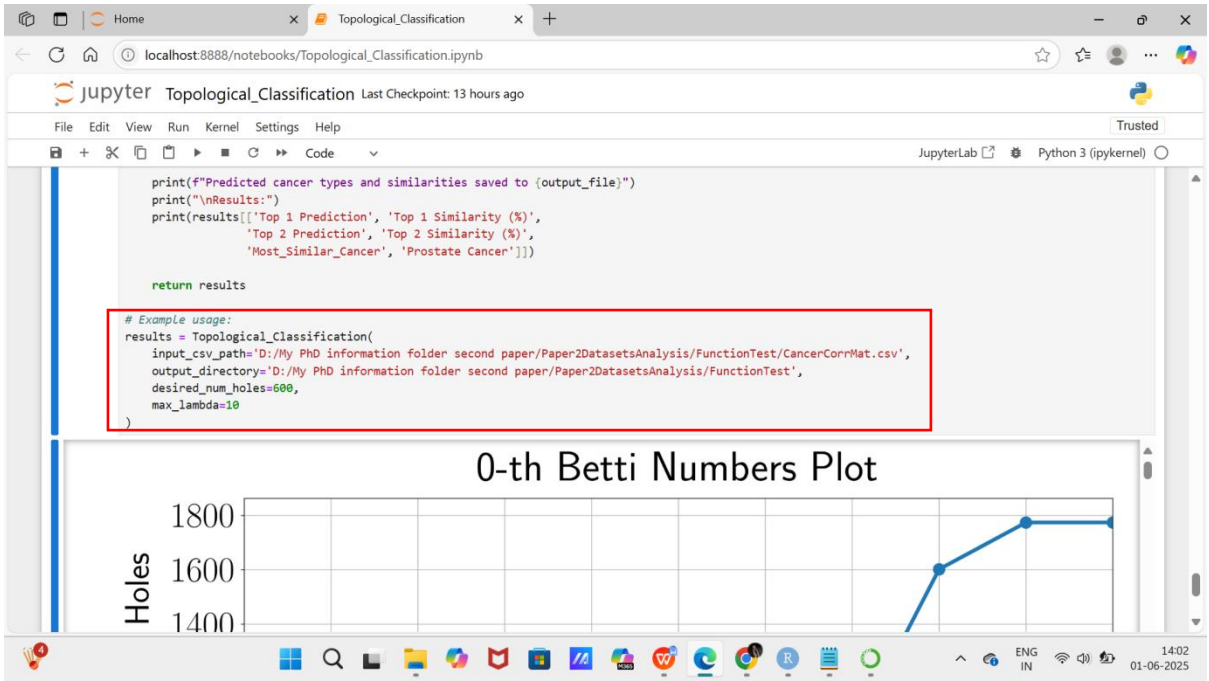
- Once Jupyter Notebook is open in your browser, navigate to the directory where you saved the project files (i.e., the `base_path`).
- Click on `Topological_Classification.ipynb` to open the notebook.



9. Set the Input Parameters in the Notebook

Before running the cells in `Topological_Classification.ipynb`, set the following input parameters:

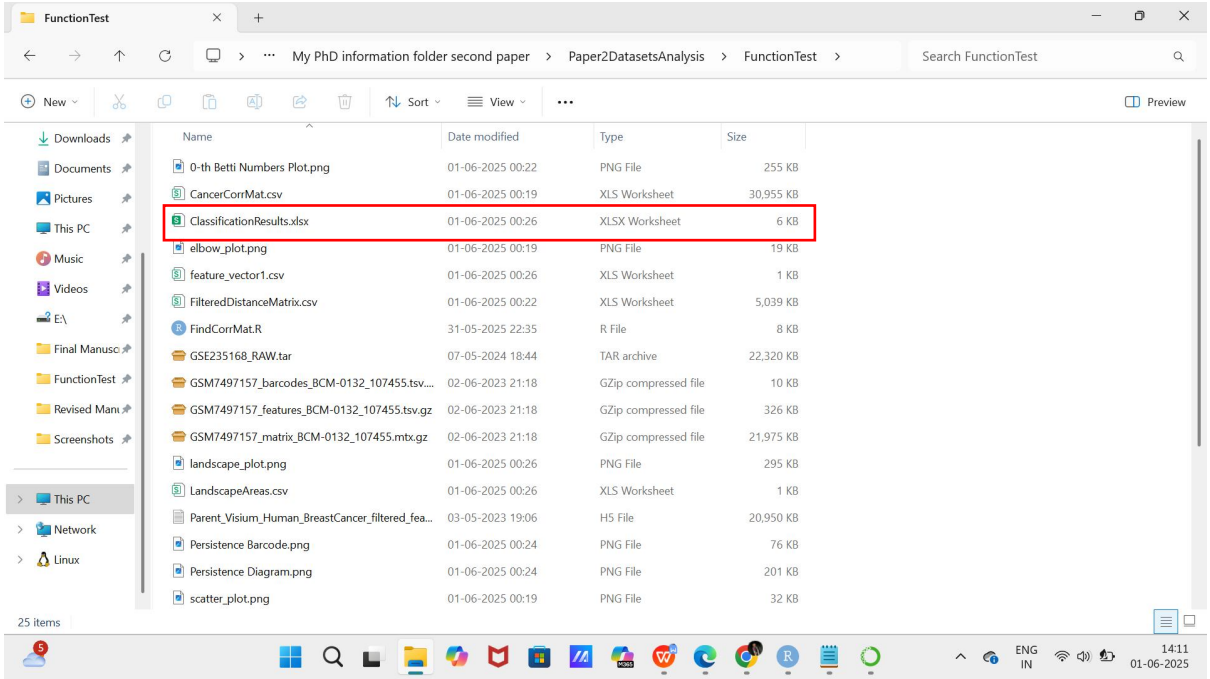
- `input_csv_path`:
  Path to the input correlation matrix CSV file generated by the `FindCorrMat` function.
- `output_directory`:
  Directory where all output files will be saved. This is required.
- `desired_num_holes` *(optional)*:
  Desired number of 0-dimensional topological features (holes) to retain.
  Default: 600
- `max_lambda` *(optional)*:
  Maximum lambda value used for computing the persistence landscape.
  Default: 10



10. Output Files (Saved in `output_directory`)

After running the notebook, the following output files will be generated and saved in the specified `output_directory`:

- `0-th Betti Numbers Plot.png` – Visualizes the number of 0-dimensional topological features (connected components or "holes").
- `FilteredDistanceMatrix.csv` – Filtered version of the correlation matrix used for topological analysis.
- `SignificantGenes.csv` – List of genes identified as significant based on topological filtering.
- `Persistence Diagram.png` – Persistence diagram showing birth and death of topological features.
- `Persistence Barcode.png` – Barcode plot representing the lifespan of topological features.
- `simplicial_complex_plot.png` – Network visualization of the simplicial complex structure.
- `simplicial_complex_data.csv` – Contains network statistics derived from the simplicial complex.
- `feature_vector1.csv` – Topological features extracted for classification.
- `landscape_plot.png` – Plot of the persistence landscape for the selected features.
- `LandscapeAreas.csv` – Numerical values of landscape areas used for trend and classification analysis.
- `TrendlinePlot.png` – Visual trendline of landscape areas.
- `TrendlineDetails.csv` – Statistical details of the trendline analysis.
- `ClassificationResults.xlsx` – Final classification results based on extracted topological features.



11. Interpretation of Significant Genes

The **significant genes** identified in `SignificantGenes.csv` are considered **primary significant genes**. These are initially selected based on the topological analysis of the correlation matrix.

To further refine these results, a **comparative filtering step** is performed:

- Run the same analysis pipeline on other cancer datasets.
- Compare the significant genes across datasets.
- Use **classification performance metrics** to identify genes that are consistently significant and contribute meaningfully to accurate classification.