1. Running Proteinarium:

    - Create the Gene Set File 1. This must be a text file with one line per sample. Each line has the format <Sample Identifier> = <HGNC Symbol 1>, <HGNC Symbol 2>, ..., <HGNC Symbol n> for a sample with n seed genes.

    - (Optional) If you wish to analyze a dichotomous phenotype, a second gene set file can be created for Gene Set 2. This file must be formatted the same way as done for Gene Set 1.

    - In the GUI, select File > New analysis

    - Choose the data files for upload. If you are only analyzing one group or a single sample, leave the Geneset File 2 file field empty.

    - Enter a Project Name into the field "Project Name". If left blank, the program will create the following project name based on the input file "SIM"

    - Before running Proteinarium, confirm or make changes to the program's configurable parameters. A table with all possible parameters, their definitions and their default setting is provided if Help > Available Configurations is selected. Select File > Settings to view and change parameters. If changes are made click "Apply Changes" before exiting then you may re-run analysis with the changed settings by clicking File > New Analysis or the "Run Proteinarium" button in the Home screen.

    - For Advance Settings Select File > Settings and click on the "Advance Settings" button. Once changed click on "Set Settings" and the "Apply Changes" buttons.

    - Once files are uploaded and parameters are set, click "Run Proteinarium"

    - If more than one sample is contained within the input file (s), the dendrogam will be displayed. All output files generated by Proteinarium will be saved to the folder specified by the *outputDirectory* configuration option. By default, this goes to a folder called output in the same directory as the Proteinarium.jar file.
        - **<projectName>_ClusterAnalyses.csv**: cluster analysis files
        - **<projectName>_Dendrogram.png**: dendrogram image
        - **<projectName>_Dendrogram.txt**: representation of the dendrogram in Newick tree format

2. Viewing Clusters:

- When Proteinarium is done running, the GUI will navigate to a new window in which you can input the Cluster/Sample ID for which you are interesting in viewing or obtaining more info

- Enter sample ID or branch number (ex: *C12*) in the space provided.

- Then select either "View Cluster" or "Get Cluster Information".
- If "View Cluster" is selected, the corresponding the output files will be available in the \<outputDirectory\>/<cluster or sample ID\> folder. And the following files are generated:
    - **<cluster or sample ID>_Dendrogram.txt**
    - For each of the five possible output networks--Group 1, Group 2, [Group 1 + Group 2], [Group 1 - Group 2], [Group 2 - Group 1], three files are generated to summarize that network. For example:
        1. **<cluster or sample ID>_Group1_GeneSet.txt**: list of genes in the network and information about which input set they originated from (i.e. from Group 1, Group 2, or imputed from the interactome) and on how many samples the gene was found
        2. **<cluster or sample ID>_Group1_Interactions.txt**: network interaction matrix
        3. **<cluster or sample ID>_Group1.png**: image of the network

- If "Get Cluster Information" is selected, the following information will be displayed:
    - *Average Distance (Height)*
    - *Bootstrapping Confidence*
    - *Total Number of Samples*
    - *Number in Group 1* (number of samples)
    - *Number in Group 2* (number of samples)
    - *p-value (Fisher Exact test for Group 1 and Group 2)*
    - *Group 1 and Group 2 Clustering Coefficient*
    - *Group 1 Clustering Coefficient*
    - *Group 2 Clustering Coefficient*
    - *Group 1 minus Group 2 Clustering Coefficient*
    - *Group 2 minus Group 1 Clustering Coefficient*
    - *Group 1 Patients* (Sample IDs of the individuals)
    - *Group 2 Patients* (Sample IDs of the individuals)

3. Change the Parameter Configuration and Re-run Proteinarium:

    - Select File > Home to return to main screen for running Proteinarium

- Select File > Settings
- Change desired parameter values
- Click Apply Changes
- Click “Run Proteinarium” to re-run Proteinarium with new configuration