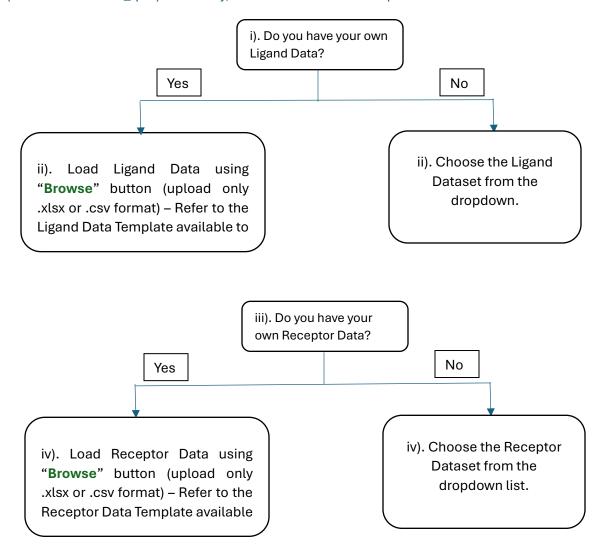# Analysis Tab

Access the application using the link https://sensoryomics.shinyapps.io/Interactome/

Step 1: Upload Ligand/Receptor Data and Generate Interactome Results

Note: Please refer to the templates if you are uploading your own data. Ensure that the gene symbols are in the first column for both the ligand and receptor data files. Each column after the first should contain gene expression data for a cell/tissue type, with the header of the column labeled as "cell unit" (ex. TRPA1neurons_tpm). Currently, the maximum allowed input file size is 5MB.

```
                    ┌─────────────────────────┐
                    │ i). Do you have your own│
                    │ Ligand Data?            │
                    └─────────────────────────┘
        ┌──────┐                          ┌──────┐
        │ Yes  │                          │  No  │
        └──────┘                          └──────┘
```

ii). Load Ligand Data using "**Browse**" button (upload only .xlsx or .csv format) – Refer to the Ligand Data Template available to

ii). Choose the Ligand Dataset from the dropdown.

```
                    ┌─────────────────────────┐
                    │ iii). Do you have your  │
                    │ own Receptor Data?      │
                    └─────────────────────────┘
        ┌──────┐                          ┌──────┐
        │ Yes  │                          │  No  │
        └──────┘                          └──────┘
```

iv). Load Receptor Data using "**Browse**" button (upload only .xlsx or .csv format) – Refer to the Receptor Data Template available

iv). Choose the Receptor Dataset from the dropdown list.

V.   Click the "**Generate Interactome Data**" button to run the analysis. Download the results using the '**Download excel**' button. This will be a large table containing possible interactions between all cell types in the ligand input file and all cell types in the receptor input file.

Step 2: Cell type selection for specific interactomes

- Specific cell types from the ligand dataset can be selected for interactome analysis using the "Select Cell Type" drop-down menu. From the chosen cell type, interactions with all the receptor cell types can be generated.

Step 3: Column selection for further steps (By default, all columns are auto populated)
Remove undesired columns from the auto populated columns. Only selected columns will be present in the output excel file for the specific interactome.

Step 4: Filtering the interactome data.

- Choose the column, condition, and value to filter: To select for the interactions that are most relevant from the list of all possible interactions, the user may filter out interactions based on up to 3 criteria. For each criteria, select a column with numerical values, enter the filtering threshold value, and select whether rows should be removed if they are greater than or less than the value in the given column.
- If you do not want to filter anything, don't select any of the columns, condition, and value prompts. Just click on the '**Filter data for above conditions**' button to run, and you will have the data ready for ranking.

Note: You can change the filter conditions any number of times and filter again using the **'Filter data for above conditions'** button, until you are satisfied with the number of records you want.

Step 5: Ranking the filtered data.

- You can choose a maximum of three columns with numerical values for ranking. If you are ranking only one or two columns, choose from only the first second dropdown buttons, and click on the '**Rank data based on above columns**' button.

Note: You can change the ranking conditions any number of times and rank again using the '**Rank data based on above columns**' button.

Step 6: Text file preparation for SankeyMATIC.

- Select the number of top Interactions you want from the ranked data (maximum allowed is 100 right now).
- Click on '**Prepare Data for Sankeymatics**' button to create data in the required format for the SankeyMATIC web application. The text files can be used to generate Sankey plots in the "**Explore Tab**" or at https://www.sankeymatic.com/build/.

Note: Steps 2-6 may be repeated multiple times to generate different interactomes from the same overall data (output of Step 1). For example, for an analysis with multiple immune cell types in the ligand data and DRG sensory neuron types in the receptor data, Steps 2-6 can be run once to generate the macrophage – DRG interactome, once again to generate the T cell – DRG interactome, and furthermore for interactomes between each immune cell type and the DRG neurons.

Explore Tab

Upload the Step 6 results of the "**Analysis tab**" into the input box in the "**Explore tab**", you will get the interactions Sankey plot generated according to the data. Utilize the plot parameters to control the interaction width, height, and other plot parameters.