# Tutorial

## – Biocurve Analyze

In this tutorial, we will guide you through the process of analyzing sample data using Biocurve Analyzer. To begin, please install the application by following the instructions available on our GitHub page. The app features a navigation bar at the top, which includes three main tabs to assist users in completing the analysis step-by-step (see Figure 1). Each main tab consists of two panels: the side panel for input and the main panel for output.

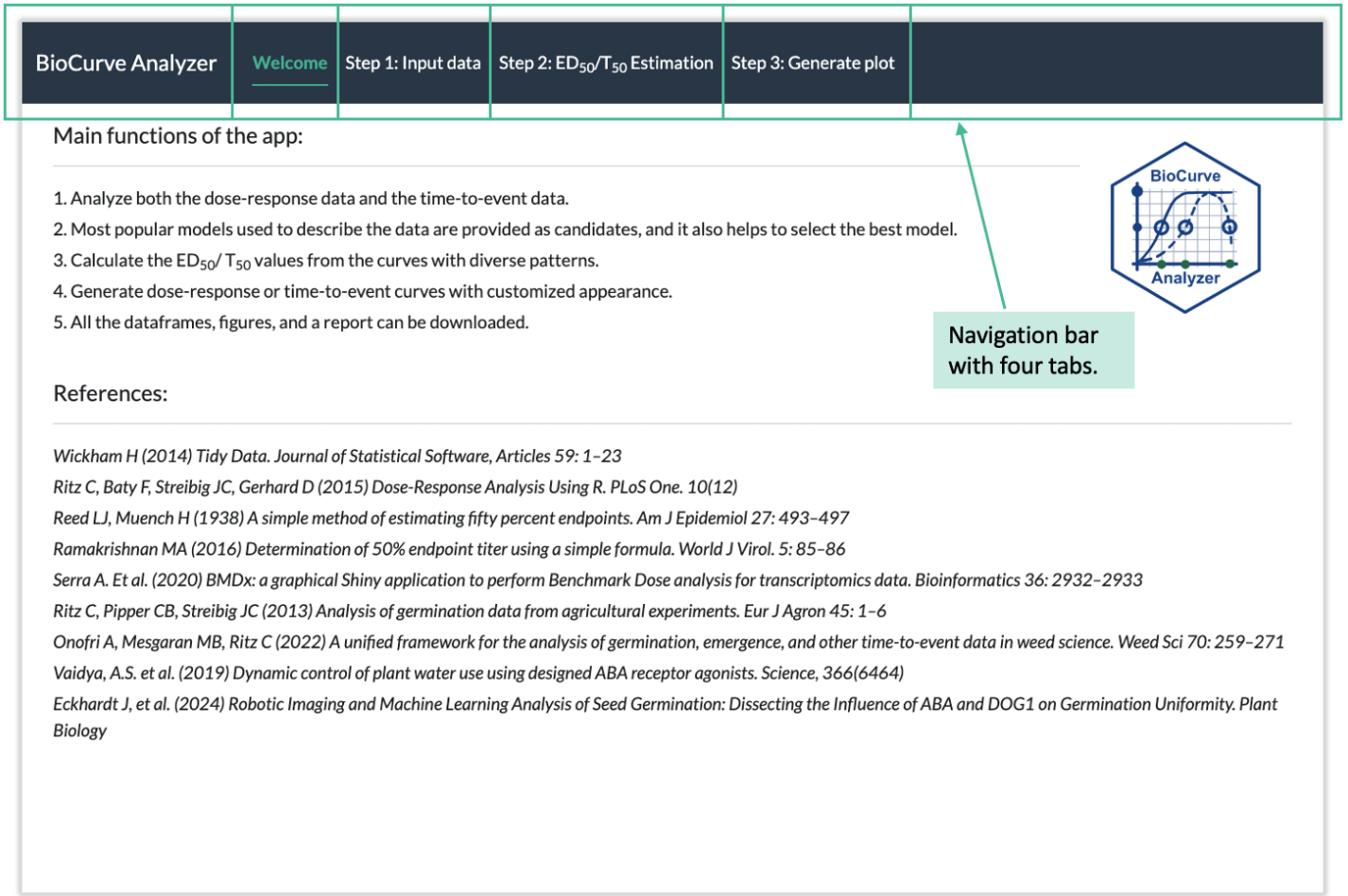


**Figure 1 Welcome Tab**

## Step1: Input Data

In this tab, users must first select the data type: either dose-response or time-to-event. Following that, the raw data needs to be submitted in a tidy format, with the columns arranged in a specified order. Users have three options to upload their data, as adapted from a previously published application [1]. Each data type requires specific information, and the necessary columns, along with their order, can be found by clicking the question mark next to the input data options. Additionally, users can download sample data to familiarize themselves with the required input format. To illustrate the functionality of this shiny app, we analyzed two datasets from our previously published papers [2–4]. Once the data is prepared, click the “Go” button to display it as a table and a simple line plot on the main panel (Figure 2).

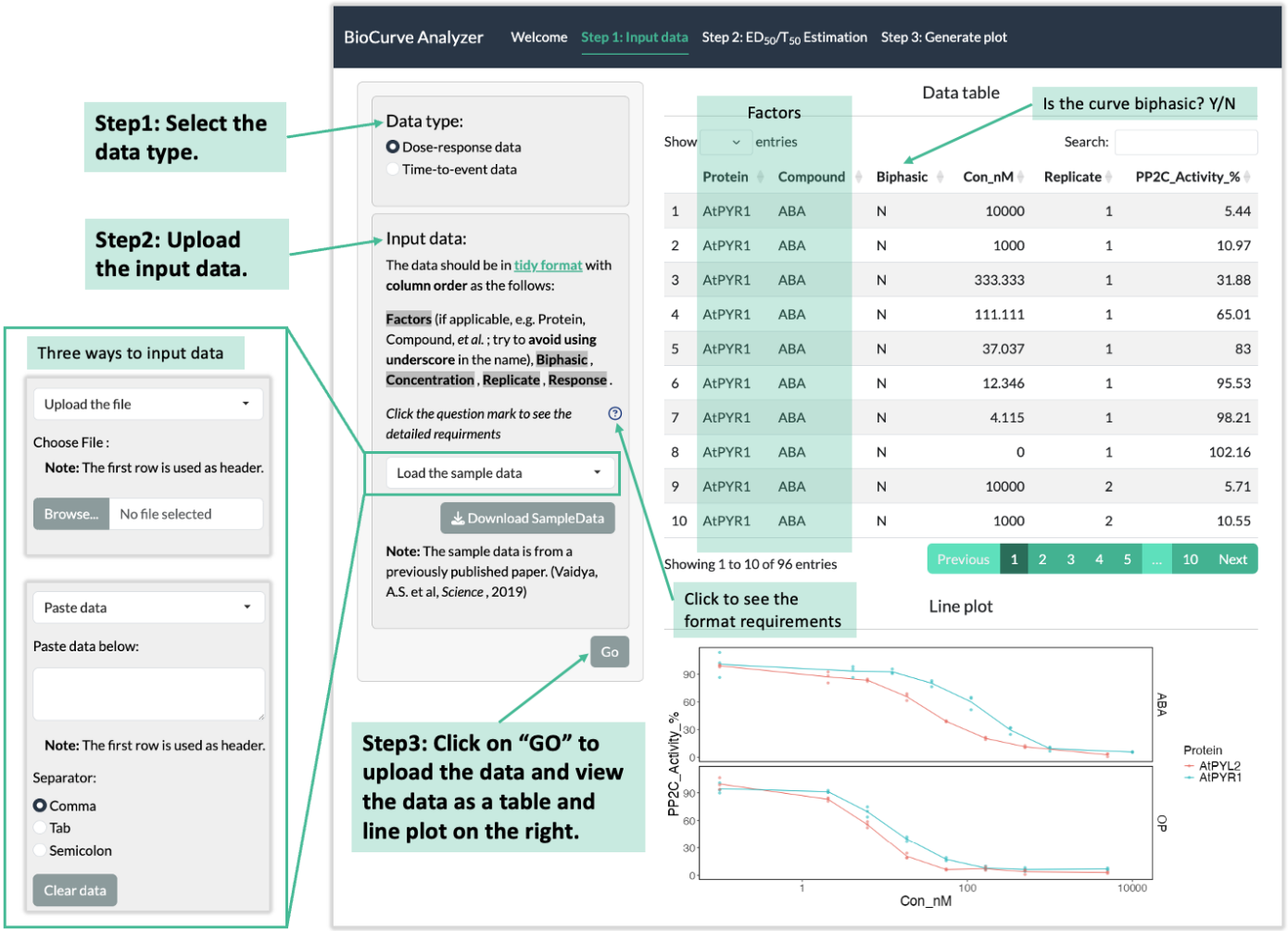


**Figure 2 Input Data Tab**

(Demonstrated by using dose-response data as the input data type)

## Step2: $ED_{50}/T_{50}$ Estimation

In this tab, users can estimate the $ED_{50}/T_{50}$ from their data by employing the best-fit model (see Figure 3). For both dose-response and time-to-event data, users should select a list of candidate models and determine the appropriate criterion for identifying the best-fit model. Additionally, users can specify constraints for the upper and lower limits of each model to better suit their data.

For dose-response data, there are several considerations to address before calculating the $ED_{50}$ values. The application enables users to estimate both relative and absolute $ED_{50}$ values, with three established methods available for value estimation. Furthermore, users can evaluate the models using the selected statistical tests. The final step involves specifying the minimum dose for plotting, as the concentration must be log-transformed. This step is optional, with the default method used being the one from the *drc* package. The differences between models, types of $ED_{50}$, three $ED_{50}$ estimation methods, and model assessment methods are available for the users by clicking on the questions mark at the top right corner.

## Step3: Generate plot

In this step, users can generate the final figure using default formatting settings in one tab, while also having the option to customize the figure by modifying labels, legends, fonts, and line colors (see Figure 4).

First, users should define the layout, especially if multiple variables are involved in their experiments. They can choose how to present their data, either by displaying all replicates as a dot plot or by plotting the mean value of each group with the corresponding standard deviation (SD) represented as error bars.

If some of the data do not conform to any of the models chosen in the previous step, there is an additional option available. Users can either return to the previous step to adjust the candidate model list or proceed to plot the data as needed. The application allows for either a simple line plot that connects the mean values or fitting the data to the Loess model for visualization. In such cases, a message box will alert users about the extra step required when creating the figure. The results of both options are displayed in Figure 4, making this function particularly useful for handling messy data or inactive chemicals in experiments.

Once all selections are made, users can click on “Generate plot” to view the plot in the main panel. They can choose whether to display the $ED_{50}/T_{50}$ on the plot and have further customization options available. The figures, data frames, and a comprehensive report can be downloaded from the app. Additional details and interpretations of the report are provided in Figure 5.

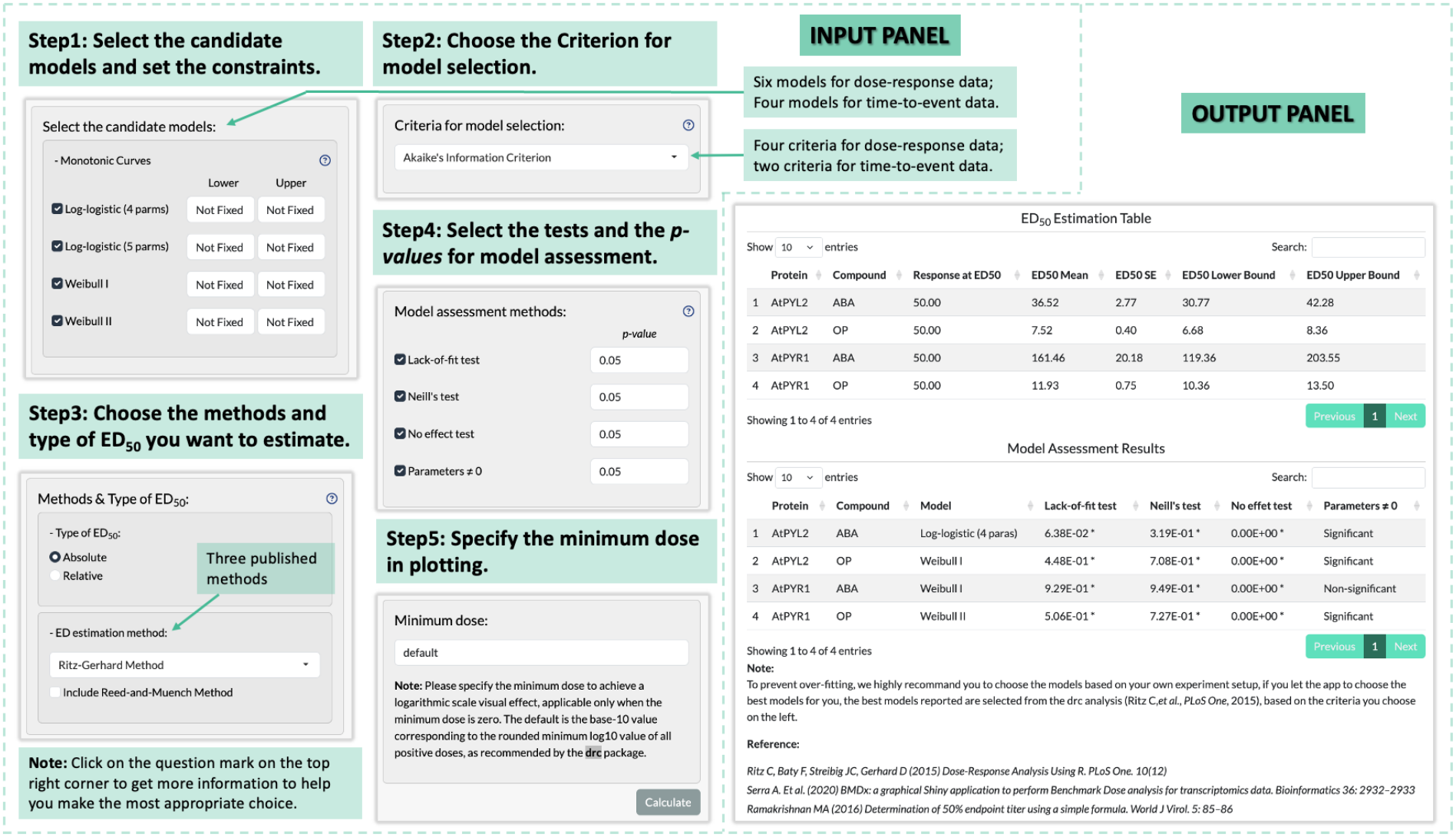


**Figure 3 ED50 Estimation Tab**

(Demonstrated by using dose-response data as the input data type)

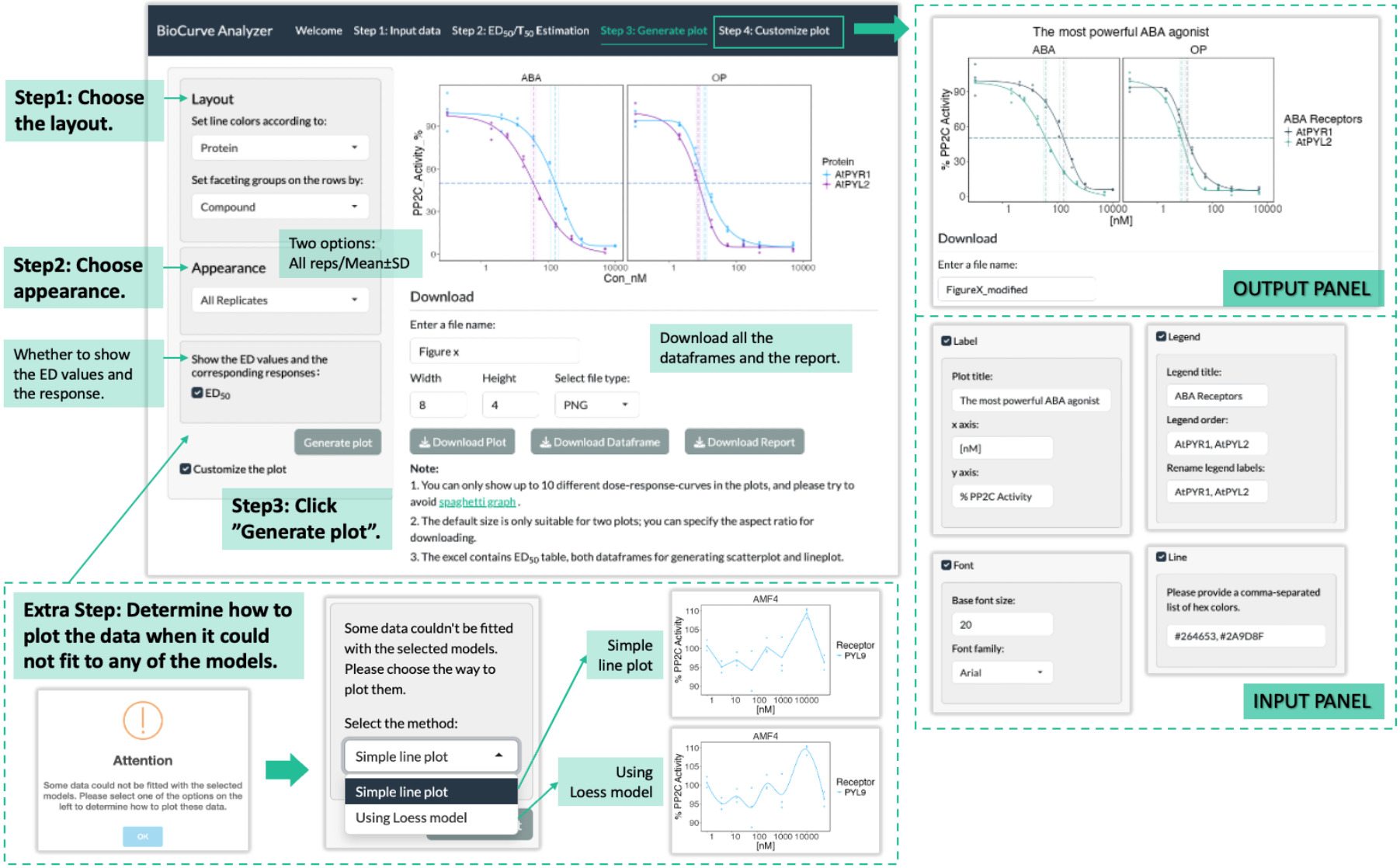


**Figure 4 Plot generation**

(Demonstrated by using dose-response data as the input)

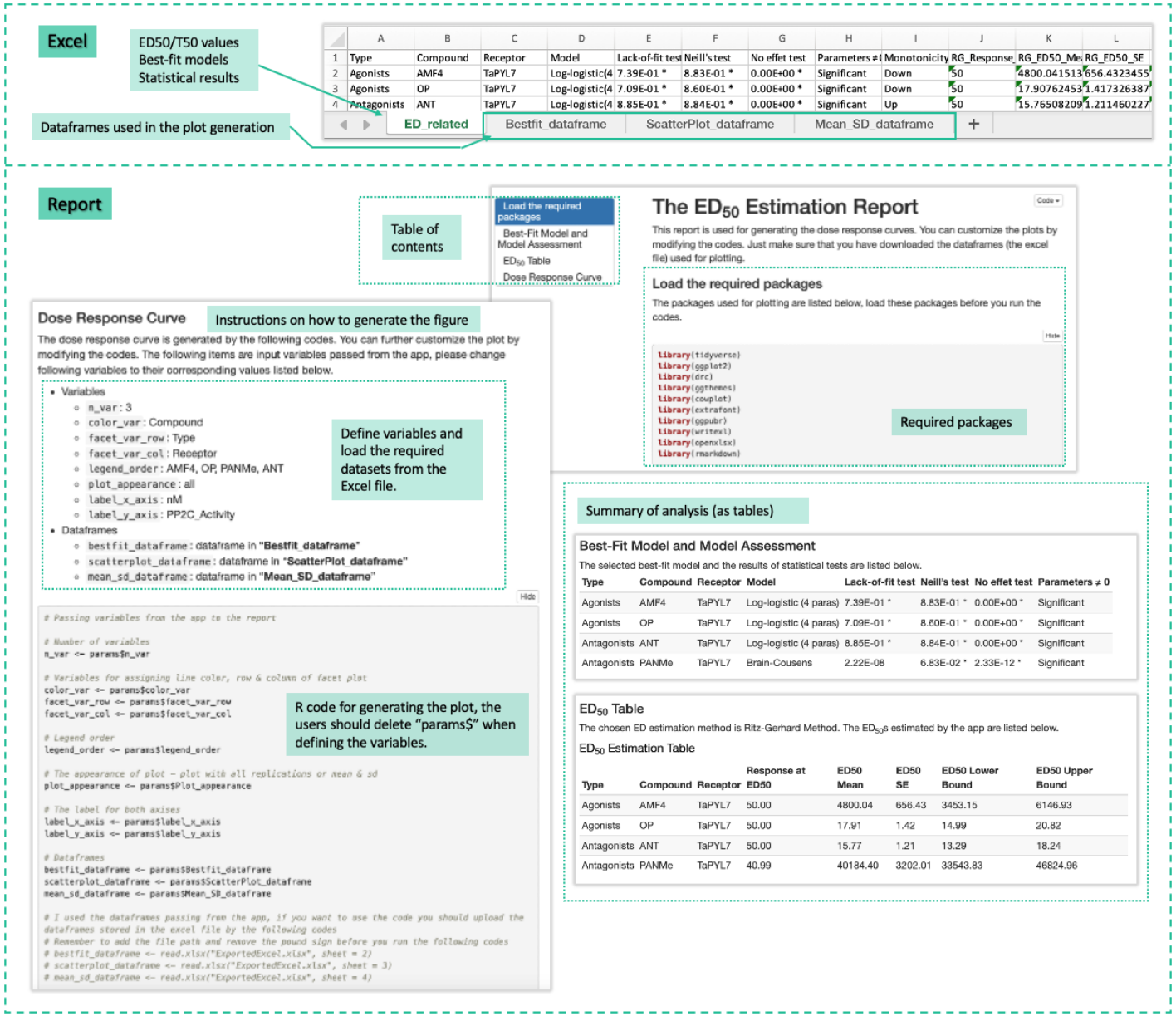


**Figure 5 Example of output files**

(Demonstrated by using dose-response data as the input data type)

## References:

1. Spitzer M, Wildenhain J, Rappsilber J, Tyers M. BoxPlotR: a web tool for generation of box plots. Nat Methods. 2014;11:121–2.

2. Vaidya AS, Helander JDM, Peterson FC, Elzinga D, Dejonghe W, Kaundal A, et al. Dynamic control of plant water use using designed ABA receptor agonists. Science. 2019;366:eaaw8848.

3. Vaidya AS, Peterson FC, Eckhardt J, Xing Z, Park S-Y, Dejonghe W, et al. Click-to-lead design of a picomolar ABA receptor antagonist with potent activity in vivo. Proc Natl Acad Sci U S A [Internet]. 2021;118. Available from: http://dx.doi.org/10.1073/pnas.2108281118

4. Eckhardt J, Xing Z, Subramanian V, Vaidya A, Cutler S. Robotic Imaging and Machine Learning Analysis of Seed Germination: Dissecting the Influence of ABA and DOG1 on Germination Uniformity [Internet]. Plant Biology. bioRxiv; 2024. Available from: https://www.biorxiv.org/content/10.1101/2024.05.10.593629v1.full.pdf