
WEoN Documentation

Network Biology Lab

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The Fly Tool for Weighted Epigenomic Network (Fly T-WEoN) is a [Cytoscape](#) app that incorporates a filtering method to determine context-specific gene regulatory networks (GRNs). The method employs diverse data to filter out regulatory connections between genes. The tool uses histone modifications through Chromatin Immunoprecipitation followed by DNA sequencing (ChIP-seq), gene expression through RNA-seq, and chromatin accessibility through DNase-seq data to perform analysis of a GRN. A series of heuristic filters removes experimentally determined and computational inferred regulations (such as Transcription factors and regulatory RNAs) from a GRN considered as a Reference Network (e.g. a GRN with all known connections, regardless the developmental stage or cell type). The corresponding context-specific GRN considers weighted information, and the generated network can be further analyzed within the [Cytoscape](#) software.

Please write directly to us ([Leandro Murgas](#), [Alberto JM Martin](#)) or post an issue at the [Github repository](#) if you encounter any problem.

CHAPTER 1

Installation

First, be aware that Fly T-WEoN runs a Perl script that filter out regulations unlikely occurring from a Reference GRN. Please follow specific instructions for your platform to install the Perl interpreter [here](#).

Requeriments

- Java: Instructions to install Java depends on your operating system. Windows and MacOS users should download Java 8 from [here](#) and follow the installer instructions. For Unix users, Java could be installed from the repository packages *openjdk-8-jdk* and *openjdk-8-jre* (e.g. *apt-get install openjdk-8-jdk openjdk-8-jre*).
- Cytoscape: Download the Cytoscape software from [here](#). The webpage will automatically determine your operating system and prompt a download button.
- Perl: Similarly to Java, Windows users should install a Perl interpreter. Please download from [here](#) and follow the instructions. For MacOS and Unix operating systems, Perl can be already installed; if not, the user can install it from the repository.

Getting Fly T-WEoN

Fly T-WEoN is freely available to download from **Figshare**. Fly T-WEoN can be used in several operating systems (Linux, Windows and macOS) and you need to download a specific version (in zip format) of the tool for your operating system (OS).

- Fly T-WEoN for Linux [download here](#)
- Fly T-WEoN for Windows [download here](#)
- Fly T-WEoN for Mac [download here](#)

Once you are in the download page, you can get the tool by clicking on the **download button** as is shown in the following image.



Browse

Search on figshare...

C

Installation

With the corresponding zip file for your OS, you need to uncompress it. After completion, you have now a sub-directory called *WEoN_FlyT*, which correspond to the scripts executed by WEoN FlyT and the *WEoN_FlyT.jar* file corresponding to the Cytoscape app. The jar file contains a graphical user interface (GUI) that allows to Cytoscape execute the filtering process encoded in Fly T-WEoN.

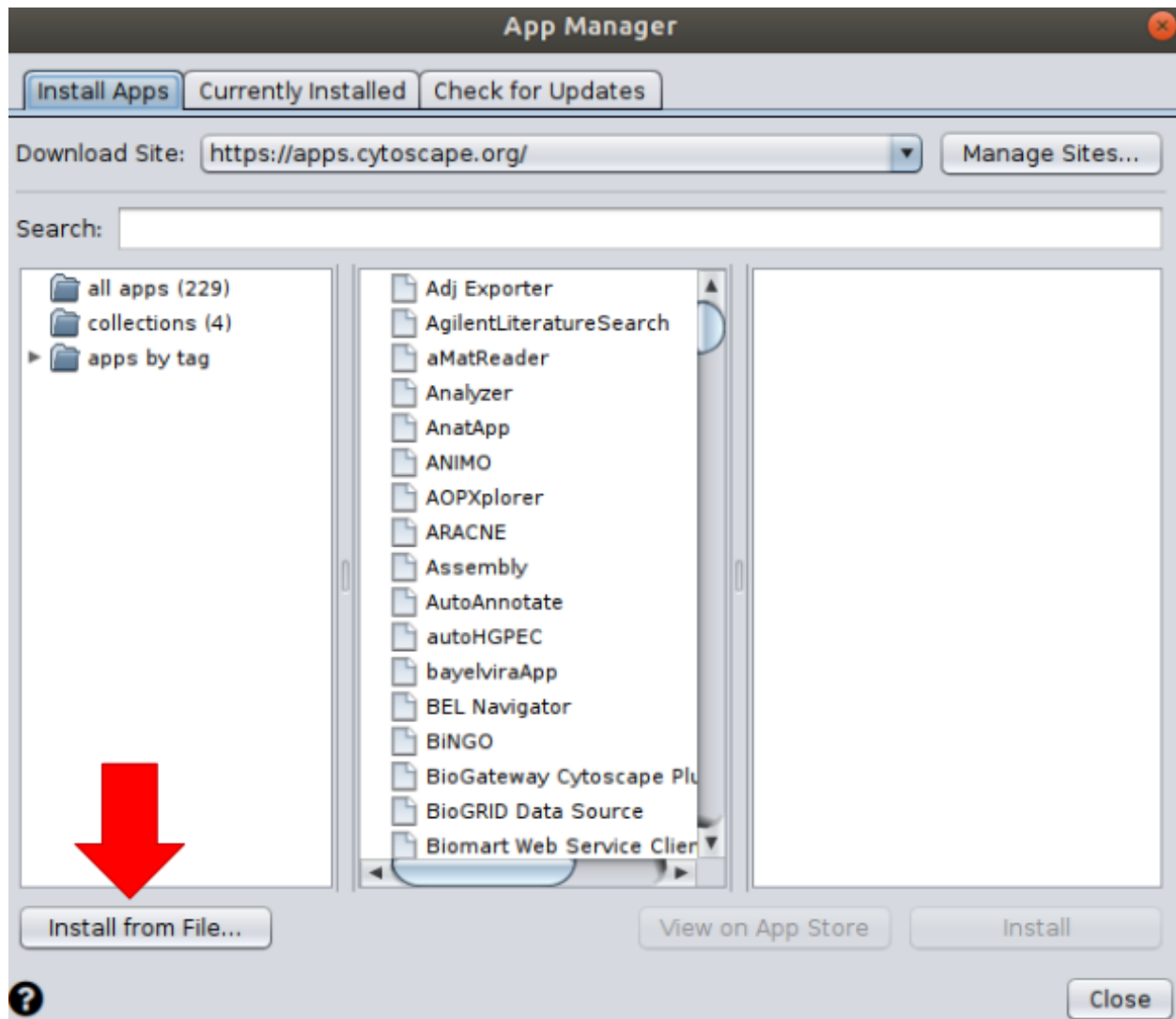
In order to install the scripts and the GUI, you need to do the following steps for the main folder and for the cytoscape app:

1. **WEoN_FlyT directory:** Fly T- - Linux users: copy the folder to */home/your_username*

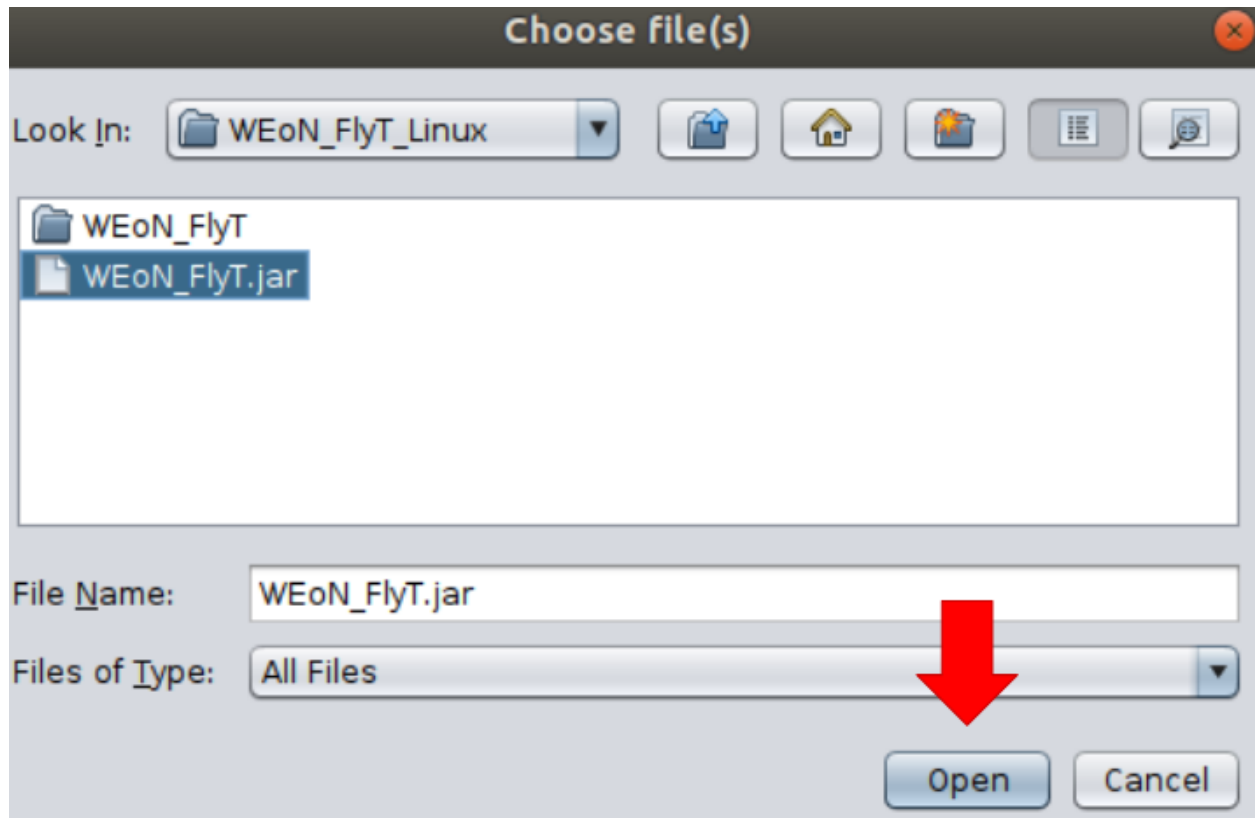
- Windows users: copy the folder to *C:*
- MacOS users: copy the folder to */Users/your_username*

Note: Additional instructions for Windows users: In some Windows operating systems, copying WEoN FlyT folder could require for admin permissions. For this case, we included an **installation.bat** file, which automatically will ask for these permissions and then will generate a copy of the folder in the *C:* directory.

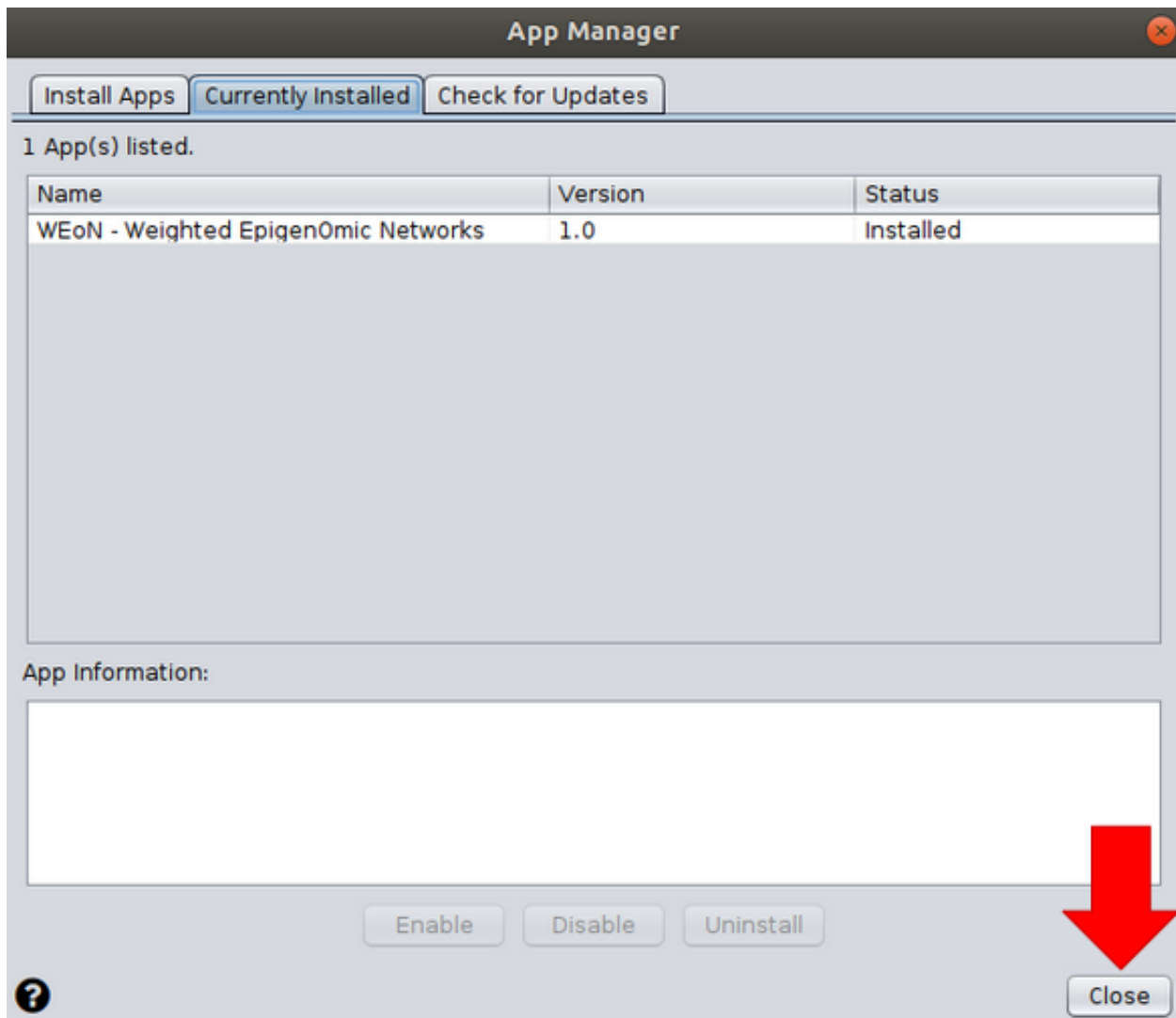
2. **WEoN_FlyT.jar file:** In order to install the GUI, you need to open Cytoscape and then navigate to *Apps menu* >> *App Manager* and click on *Install from File*, as it is indicated in the following image.



Clicking on the *Install from file* button will open a new window where you need to navigate to the directory containing the jar file and select it as shown in the next image.



After selecting the jar file, the resulting process will be similar to the shown in the next image, and the installation process is finalized by clicking on the *close* button.



Now you only need to go to the *Apps menu*, click on *Fly T-WEoN - Fly Tool for Weighted Epigenomic Network* to start to use the tool.

Note: Need Help? If you run into any problems with installation, please leave an issue in the official [Github repository](#).

Instructions to use Fly T-WEoN

This document contains the main instructions in order to use the **Fly T-WEoN (Fly Tool for Weighted Epigenetic Networks)** Cytoscape app.

1. **Open the Fly T-WEoN app**

Within Cytoscape, go to Apps >> Fly T-WEoN - Fly Tool for Weighted Epigenomic Network. This will display the user interface as shown in the figure.

2. **Interface** The Fly T-WEoN interface is an input screen where the user complete required and optional data. In the backend, Fly T-WEoN executes Perl scripts, therefore parsing correctly the data to arguments and call orderly the scripts to filter out unlikely occurring gene regulations from a reference gene regulatory network.

Fly T-WEoN - Fly Tool for Weighted Epigenomic Networks

Help

Required

Network Definition Distance: 1500nt

Expression File Select File
No File Selected

Result Folder Select Folder
No Folder Selected

Optional

DNase File Select File
No File Selected

DNase Score 0

Histone Mark Path Files Select Files

Methylation File Select File
No File Selected

Methylation Score 0

Run Fly T-WEoN

Note: There is a known issue occurring in MacOS. Users has reported that the *Network Definition* selector is not shown. However, the selector is still there; you only need to click on in this *blank space* and the menu will be

displayed to select a network definition.

- **Required data**

In order to use Fly T-WEoN, you need to provide at least three required data as listed as follow:

1. **Network Definition:** This field corresponds to the reference GRN to which apply filters to generate the weighted GRN. We provide three references networks, which were constructed within a different cut-off of 1500, 2000, and 5000 nucleotides: if a Transcription Factor Binding Site or other regulatory element is contained in the cut-off upstream the Transcription Start Site of *Drosophila melanogaster* genes, then the GRN defines a connection between gene and regulator.
2. **Expression File:** Fly T-WEoN employs RNA-seq data to filter out absent transcription factors and miRNAs from the Reference GRN. The RNA-seq data must be provided formatted in a two-columns file separated by tabulations. The first column is the gene name while the second is the expression of the corresponding gene. Expression could be reported as read counts, reads per kilobase million (RPKM) or fragments per kilobase million (FPKM). Data must be a single experiment or the average value of the experimental replication.
3. **Result folder:** A folder where results will be placed.

- **Optional data**

If you have data from DNase (or ATAC-seq), Histone modifications and/or methylation experiments, you can use it to complement RNA-seq data and get a more specific Gene Regulation Network.

So, for the `DNase file` and `Methylation file` fields, you need to provide four-columns files separated by tabulations. Each column correspond, in order, to the chromosome, the initial coordinate, the ending coordinate, and the score for the mapped feature. Both files has an associated `Score` field used as a threshold value to dismiss lower score values from the original data. Default is zero, which means that all mapped features in the DNase and Methylation files will be used in the filtering process.

Respect to the `Histone Mark Path Files` button, once you click it a new window will appear as shown in the next image, so in this new window you can select files for the corresponding post-translational modification of histone tails. Each data must be a three-columns files separated by tabulations, where each column correspond to the chromosome, the initial coordinate, and the ending coordinate of the mapped feature.

Histone Marks
✕

Histone marks: all modifications shown below are non mandatory, WeON will run using each of the marks for which you loaded a file.

H3K27me3	<input type="button" value="Select File"/>	No File Selected
H3K27ac	<input type="button" value="Select File"/>	No File Selected
H3K36me2	<input type="button" value="Select File"/>	No File Selected
H3K4me	<input type="button" value="Select File"/>	No File Selected
H3K4me2	<input type="button" value="Select File"/>	No File Selected
H3K4me3	<input type="button" value="Select File"/>	No File Selected
H3K79me2	<input type="button" value="Select File"/>	No File Selected
H3K9ac	<input type="button" value="Select File"/>	No File Selected
H3K9me2	<input type="button" value="Select File"/>	No File Selected
H3K9me3	<input type="button" value="Select File"/>	No File Selected
H3S10ph	<input type="button" value="Select File"/>	No File Selected
H4K16ac	<input type="button" value="Select File"/>	No File Selected
H4K20me3	<input type="button" value="Select File"/>	No File Selected

3. Execute filtering and visualization of results

After all data is selected, please click on Run Fly T-WEoN. The weighted GRN will be loaded in Cytoscape with the Create View button to display the time and/or tissue specific GRN.

Note: It is important to remark that the filtering process could take a long time depending on your machine hardware and the number of files and information on them provided to Fly T-WEoN. Please be patient.

4. Details of the Output Files

A set of files are created after executing Fly T-WEoN, which are located in the folder selected as output. These files are described as follow:

- *output_filter_PTMs.txt*: A file describing information about chromosome, id of the coding gene for the transcription factor (TF), starting coordinate of the transcription factor binding site (TFBS), ending coordinate of the TFBS, name of the post-translational modification (PTM) of histone tails (histone marks), starting coordinate of the PTM, ending coordinate of the PTM, open or closed chromatin state (represented as + or -) and place where the PTM was found (on the promotor region, P, or on the gene, G)

- *output_connect.txt*: A file describing information about PTMs. In the first column is the connection in the Gene Regulatory Network (GRN) and the second has info about PTM and the number of times that PTM was found and affecting the connection.
- *output_connection_analysis.txt*: A file describing all PTMs affecting the binding of a TF.
- *output_regulator_express.tsv*: A file describing connections from expressed regulators to genes.
- *output_regulator_no_express.tsv*: A file describing connections from non-expressed regulators to genes.
- *output_mirna_express.tsv*: A file describing connections from expressed miRNAs to genes.
- *output_mirna_no_express.tsv*: A file describing connections from non-expressed miRNAs to genes.
- *out_TFBS_network.tsv*: A copy of the reference GRN used as input.
- *specific_GRN.tsv*: A file that merge *output_regulator_express.tsv* and *output_mirna_express.tsv* results.
- *scored_GRN.tsv*: Same as *specific_GRN.tsv*, except for this file include information about the calculated score of the connection. This score is based on a set of filters (based on Expression, DNase, histone marks, and Methylation) indicating the evidence for the regulatory interaction. Importantly to note is that the scale for the evidence score go from one to five (only if all four types of experiments were provided by the user).

Note: If you close Cytoscape and need to visualize again the weighted GRN, please import the **specific_GRN.tsv** or **scored_GRN.tsv** into Cytoscape.

Note: Feel free to contact directly through the [Github repository](#) or to Dr. Alberto Martin's [e-mail](#).

CHAPTER 3

Indices and tables

- `genindex`
- `modindex`
- `search`