**Type VI Secretion Systems Suite - User Manual**

**1. Overview**

The **Type VI Secretion Systems (T6SS) Suite** is a comprehensive bioinformatics tool designed to facilitate the analysis of Type VI Secretion Systems in bacterial genomes. This application is inspired by the work of Amy J. G. Anderson et al. (2023) and offers a range of functionalities including prediction tools, graphical interfaces for sequence analysis, and tools for phylogenetic tree construction. The suite integrates several well-known bioinformatics tools into a unified interface, making it easier for researchers to conduct their analyses.

**2. Key Features**

The app provides the following key features:

* **DeepSecE Prediction**: Predicts secretion systems using a deep learning-based framework.
* **SecRet6 Prediction**: Identifies Type VI Secretion Systems using BLASTp or HMMsearch.
* **cblaster GUI**: A graphical interface for the cblaster tool, enabling remote search and visualization of homologous gene clusters.
* **FastTree**: Constructs phylogenetic trees from sequence alignments.
* **DeepTMHMM**: Predicts transmembrane helices in protein sequences.
* **HMMER**: Tools for building and searching profile hidden Markov models.

**3. Installation Instructions**

**Accessing the App**

To access the **Type VI Secretion Systems Suite**, please ensure that you have R and the necessary packages installed. The app utilizes several Conda environments and external tools, which must be installed and configured correctly. Specific installation instructions for each tool can be found in their respective sections within the app or by following the provided links.

**Dependencies**

The app requires the following R packages:

* shiny
* shinyjs
* shinythemes
* DT

**System Requirements**

* **Operating System**: Windows 10/11, macOS, or Linux.
* **RAM**: At least 8 GB is recommended. For Windows users using WSL, 16G RAM is needed for DeepSecE
* **Software**: R, Conda, WSL (for Windows users), Ubuntu (for WSL), and various bioinformatics tools.

**Setting Up WSL and Ubuntu (For Windows Users)**

Some of the tools integrated into the app—namely **DeepSecE**, **SecRet6**, and **HMMER**—require a Unix-like environment. Windows users must set up the Windows Subsystem for Linux (WSL) and install Ubuntu to use these tools.

**Step 1: Install WSL**

1. **Enable WSL**:

Open PowerShell as Administrator.

Run the following command:

*wsl --install*

This command installs WSL and the default Linux distribution, typically Ubuntu. If you want a specific distribution, you can specify it like this:

*wsl --install -d Ubuntu*

Restart your computer if prompted.

1. **Verify Installation**:

After your computer restarts, open a new terminal window (Command Prompt, PowerShell, or Windows Terminal) and type:

*wsl --list --verbose*

You should see a list with Ubuntu installed and running.

**Step 2: Set Up Ubuntu**

1. **Launch Ubuntu**:

Open the Ubuntu application installed via WSL. This will prompt you to create a new user account and password for Ubuntu.

1. **Update and Upgrade**:

Once inside the Ubuntu terminal, update the package lists and upgrade the existing packages by running:

*sudo apt update*

*sudo apt upgrade*

1. **Install Basic Utilities**:

Install essential tools like build-essential and wget:

*sudo apt install build-essential wget*

Additionally, external tools like DeepSecE, SecRet6, cblaster, FastTree, DeepTMHMM, and HMMER need to be installed on your system. Please follow the instructions provided in each tool's respective section for installation.

**4. User Interface**

**Home Tab**

The **Home** tab provides an overview of the application, including background information on Type VI Secretion Systems and an example workflow overview. This tab serves as the entry point to the app, offering users guidance on how to navigate and utilize the various tools available.

**Tool-Specific Tabs**

Each tool integrated into the suite has its own tab:

* **DeepSecE Prediction**: Upload your FASTA files and specify parameters such as the Conda environment and model location. The tool will run predictions and display results.
* **SecRet6 Prediction**: Upload your genomic data in FASTA or GBK format, specify the necessary parameters, and run predictions using either BLASTp or HMMsearch.
* **Run cblaster GUI**: Configure your Python or Conda environment to run cblaster, and use the graphical interface for sequence analysis.
* **FastTree**: Upload alignment files and select the sequence and model types to construct phylogenetic trees.
* **DeepTMHMM**: Predict transmembrane helices in protein sequences by uploading your FASTA files and running the tool.
* **HMMER**: Build and search hidden Markov models by specifying your working directory and necessary files.

**5. Instructions for Use**

**IMPORTANT!! – It is recommended you are in your HOME directory for the separate tool installations as you must specify this directory within the app to make use of the tools**

**Using DeepSecE Prediction**

1. **Set Conda Environment**: Specify the Conda environment where DeepSecE is installed.
2. **Upload FASTA File**: Use the file input to upload your protein sequences in FASTA format.
3. **Specify Model Location**: Provide the path to the DeepSecE model file.
4. **(Optional) Select no cuda**: Runs prediction using device CPU
5. **(Optional) Select Save attention**: Add to save sequence attention of secreted protein
6. **Run Prediction**: Click on "Run prediction" to start the analysis. The results will be displayed in a table format.

**Using SecRet6 Prediction**

1. **Select Conda Environment**: Specify the Conda environment for SecRet6.
2. **Upload Input File**: Choose your genomic data file in GBK or FASTA format.
3. **Set Parameters**: Configure options such as E-value threshold, identity percentage, and maximum interval between T6SS components.
4. **Run Prediction**: Click on "Run prediction" to analyze the data. View the results in the output section.

**Running cblaster GUI**

1. **Configure Python/Conda Environment**: Set the path to your Python executable or specify a Conda environment.
2. **Launch cblaster GUI**: Start the cblaster graphical interface for sequence analysis.
3. **Analyze Sequences**: Use the interface to run searches and visualize homologous gene clusters.

**Constructing Phylogenetic Trees with FastTree**

1. **Upload Alignment File**: Choose your sequence alignment file in FASTA format.
2. **Select Sequence and Model Type**: Specify whether your sequences are nucleotide or protein-based and choose a model type.
3. **Run FastTree**: Click "Run FastTree" to generate the phylogenetic tree. The tree can be displayed directly in the app.

**Predicting Transmembrane Helices with DeepTMHMM**

1. **Set Conda Environment**: Provide the Conda environment for DeepTMHMM.
2. **Upload FASTA File**: Upload the protein sequence file for analysis.
3. **Run Prediction**: Click "Run prediction" to start the analysis. Results will be displayed on the same page.

**Using HMMER for Profile HMMs**

1. **Set Working Directory**: Specify the directory containing the necessary files.
2. **Run hmmbuild or hmmsearch**: Use the interface to build hidden Markov models or search sequences.
3. **View Results**: Output files and results will be displayed within the app.

**6. Common Scenarios**

**Example Workflow**

A typical use case involves starting with the **DeepSecE Prediction** to identify secretion systems, followed by **SecRet6 Prediction** for a more detailed analysis. Researchers can then use **cblaster GUI** for sequence comparison, and/or finally, **FastTree** to construct a phylogenetic tree of the sequences. The tools can be used in whatever order you prefer or are needed for your analysis. (*More tools will be added in the future).*

**7. Troubleshooting**

* **Issue**: The app does not recognize the specified Conda environment.
  + **Solution**: Ensure the environment is correctly installed and activated in your system. Verify that the paths are correct.
* **Issue**: Uploaded files are not being processed.
  + **Solution**: Check that the file format is correct and matches the expected input (e.g., FASTA, GBK).
* **Issue:** Incorrect File Path
  + **Solution:** Read the tooltips carefully for each tool, and ensure the FULL path is specified for that tool. Backslashes and forward slashes are handled by the app, there is no reason to change these.

**8. Tutorial**

**Getting Started**

**This tutorial will guide you through the basic workflow of using the Type VI Secretion Systems Suite. We will cover how to navigate the interface, upload data, and run predictions.**

**9. FAQs**

**Q:** Can the app be used on Windows? **A:** Yes, but certain tools require installation within the Windows Subsystem for Linux (WSL).

**Q:** How can I get support? **A:** For technical support, please refer to the contact information provided in the "Citations and User Manual" tab.

**9. Contact Information**

For further assistance, feedback, or reporting issues, please contact:

* **Developer:** Padhraig McNicholl
* **Email:** pmcnicholl06@qub.ac.uk