



**QUEEN'S
UNIVERSITY
BELFAST**

TYPE VI SECRETION SYSTEMS SUITE - USER MANUAL

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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.

2. **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.

2. **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
```

3. **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.

Citations tab

Contains necessary links to each paper from which the tool was developed.

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. Typically, MAFFT is used before FastTree as it aligns biological 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. Tutorial

Getting Started

This tutorial will guide you through the basic workflow of using the Type VI Secretion Systems Suite. We will cover briefly how to navigate the interface and run an example prediction using the SecRet6 tool and DeepSecE.

Navigating the Home Tab

- **Explore the Home Tab:** The Home tab provides an overview of the application, including background information and an example workflow. Familiarize yourself with the content to understand the purpose of the app. Check out the Citations tab also for links to the papers from which each tool was developed, plus the paper that designed the original pipeline from which the selection of these tools came.

Type VI Secretion Systems Suite

[Home](#)
[DeepSecE Prediction](#)
[SecRet6 Prediction](#)
[Run cblaster GUI](#)
[FastTree](#)
[DeepTMHMM](#)
[HMMER](#)
[Citations and User Manual](#)

Welcome to Type VI Secretion Systems Suite

This application provides various bioinformatics tools for the analysis of Type VI Secretion Systems (T6SS) in bacterial genomes. The tools selected were chosen based on a pre-determined pipeline, created by Amy J. G. Anderson et al (2023), entitled 'Distribution and diversity of type VI secretion system clusters in *Enterobacter bugandensis* and *Enterobacter cloacae*'.

What This App Does

Type VI Secretion Systems Suite offers several features for bioinformatics analysis, including:

- DeepSecE Prediction: A tool for predicting secretion systems.
- SecRet6 Prediction: Predicts Type VI Secretion Systems (T6SS) using BLASTp or HMMsearch.
- Run cblaster GUI: A graphical interface for the cblaster tool.
- FastTree: Constructs phylogenetic trees from sequence alignments.
- DeepTMHMM: Predicts transmembrane helices in protein sequences.
- HMMER: Tools for building and searching profile hidden Markov models.
- More to follow ...

Below is an example workflow overview outlining where some of these tools were used, and how for *E. bugandensis*

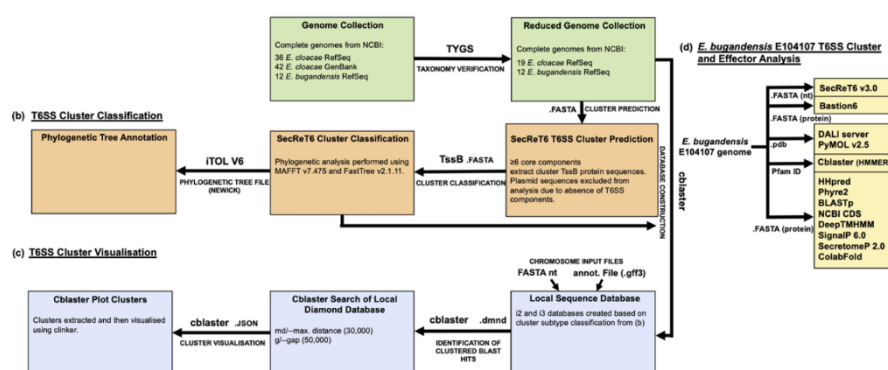


Figure 1. Workflow overview of T6SS analysis in *E. bugandensis* showing what tools were used and when. Taken from Amy J. G. Anderson et al (2023).

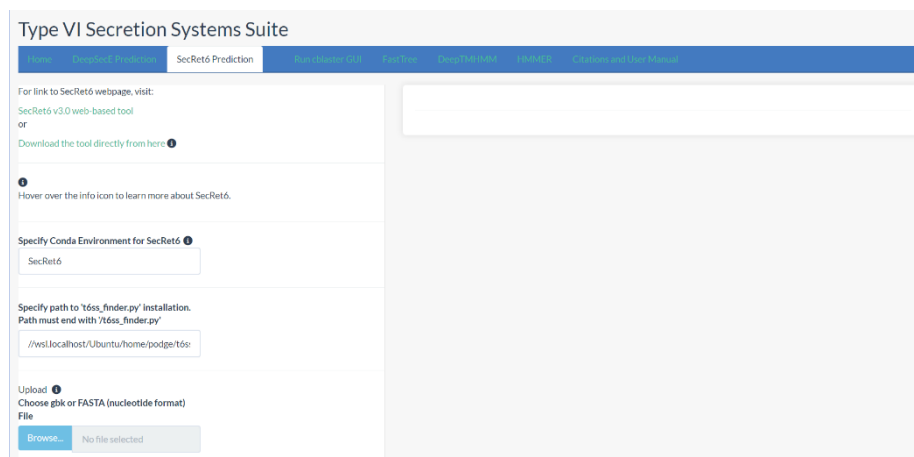
Underlying Biology

Type VI Secretion Systems (T6SS) are complex molecular machines used by many Gram-negative bacteria to transport proteins across their cell envelope. T6SS can inject toxic effector proteins into both eukaryotic and prokaryotic cells, facilitating bacterial competition and interactions with host organisms. Thirteen essential parts in the T6SS assembly model are needed for secretion. A baseplate complex (TssE/G/K) encircling a VgrG-PAAR (proline-alanine-alanine-arginine) spike can dock after the membrane complex (TssJ/L/M) secures the T6SS to the bacterial membrane. The inner tube, which is made of polymerized Hcp and is tipped by the VgrG protein, is then propelled towards the target cell by contraction of the sheath-like structure, delivering toxin effectors (see figure below). Finally, to recycle sheath components, the AAA + ClpV ATPase is drawn to the contracted sheath. Sheath contraction correlates with target cell lysis, so as with bacteriophages, the contraction of the T6SS sheath-like structure should result in the delivery of the effector in a single step.

Example tool usage 1. SecRet6

Below is a step-by-step example of how to run a SecRet6 prediction. Each tool is unique so be sure to read the information guiding you on how to use them.

1. **Navigate to the SecRet6 Tab:** Click on the "SecRet6 Prediction" tab at the top of the app interface.



Type VI Secretion Systems Suite

For link to SecRet6 webpage, visit:
SecRet6 v3.0 web-based tool
or
Download the tool directly from here

Hover over the info icon to learn more about SecRet6.

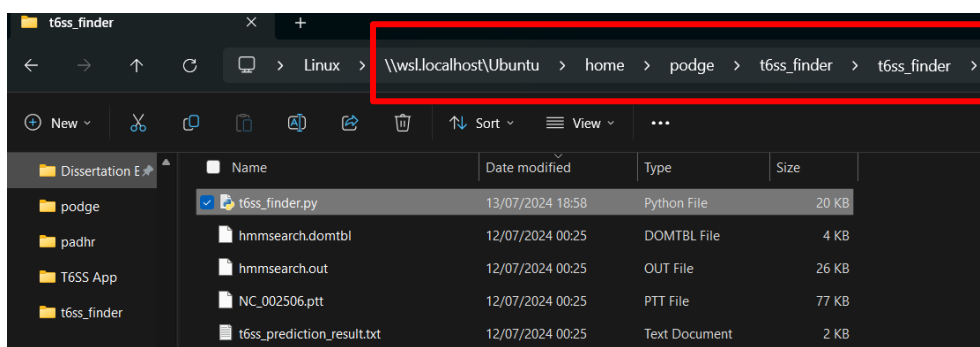
Specify Conda Environment for SecRet6
SecRet6

Specify path to 't6ss_finder.py' installation.
Path must end with '/t6ss_finder.py'

\\wsl.localhost\Ubuntu\home\podge\t6ss

Upload
Choose gbmk or FASTA (nucleotide format)
File
Browse... No file selected

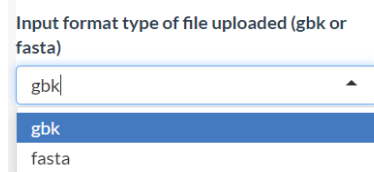
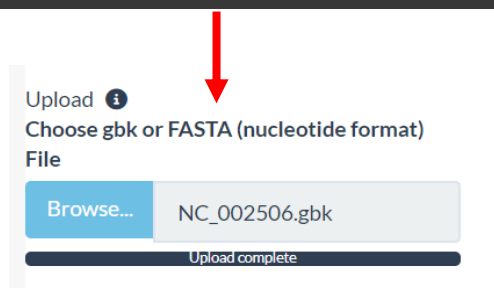
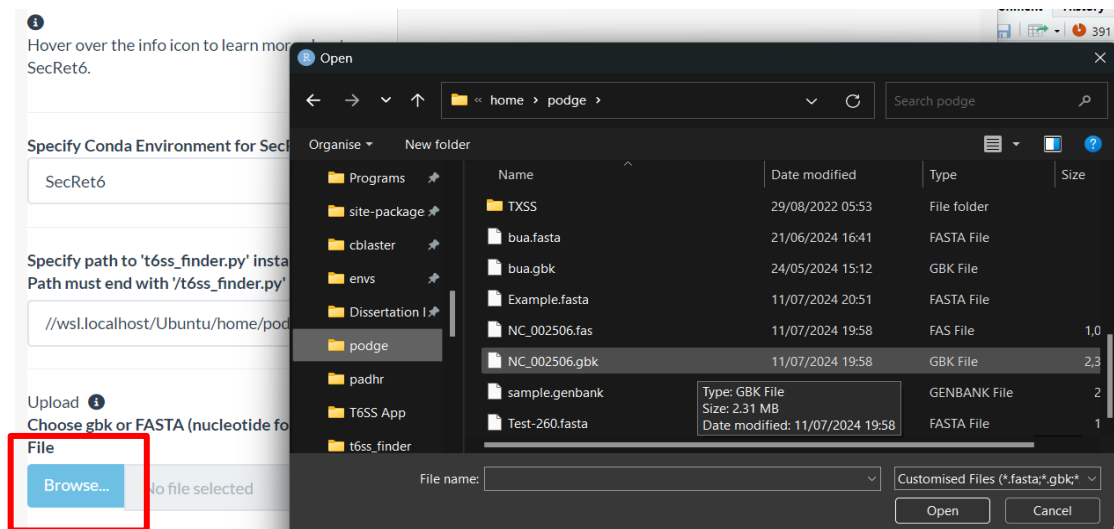
2. **Input Your File Path to Your SecRet6 Installation:** The file path must end with t6ss_finder.py. This will have to be manually inserted.



Specify path to 't6ss_finder.py' installation.
Path must end with '/t6ss_finder.py'

ge/t6ss_finder/t6ss_finder/t6ss_finder.py

- Upload Your Genomic Data:** Choose your file in GBK or FASTA format using the "Choose gbk or FASTA File" button.



4. **Set Prediction Parameters:** Configure options like the tool used for prediction, E-value threshold, identity percentage, and maximum interval between T6SS components.

Select the tool you wish to use for prediction
(blastp or hmmsearch)

blastp ▲

blastp

hmmsearch

Specify E-value threshold

0.001

Specify the percent identity for BLASTp
search against T6SS component proteins

30

Specify the maximum interval (bp) between
two co-localized T6SS components

15000

Specify the minimum number of T6SS
component proteins for each T6SS region

3

5. **Run the Prediction:** Click on "Run prediction" to analyze the data after specifying where you want the results to be saved (preferably the home directory). The results will be displayed in the output section after clicking the "Show Prediction Result".

Specify output directory to save results ⓘ

/home/podge

▶ Run prediction from Conda

📄 Show Prediction Result

For link to SecRet6 webpage, visit:
[SecRet6 v3.0 web-based tool](#)
 or
[Download the tool directly from here](#) 1

1
 Hover over the info icon to learn more about SecRet6.

Specify Conda Environment for SecRet6 1

Specify path to 't6ss_finder.py' installation.
 Path must end with '/t6ss_finder.py'

Upload 1
 Choose gbk or FASTA (nucleotide format)
 File

Input format type of file uploaded (gbk or fasta)

Script Contents:

```
[ -f $HOME/anaconda3/etc/profile.d/conda.sh ] && source $HOME/anaconda3/etc/profile.d/conda.sh || source
```

Activated Conda Environment: SecRet6
 Preparing for the input...
 Running BLASTp...
 Parsing BLASTp results for T6SS prediction...
 Writing T6SS prediction results to file...
 Prediction is done! Output saved to /home/podge

#Region1	Coordinates	Strand	Length	PID	Locus_tag	Product	T6CP_ID	Identities (%)	E-value	Bitscore
19781..20299	+	172	NP_232418.1	VCA0017	hcp protein	T6CP015517	98.256	3.46e-134		
20427..22511	+	694	NP_232419.1	VCA0018	vgrG protein	T6CP011000	70.376	0.0		
116846..117352	+	168	NP_232508.1	VCA0107	hypothetical protein	T6CP016002	100.0			
117393..118871	+	492	NP_232509.1	VCA0108	hypothetical protein	T6CP016003	100.0			
118874..119311	+	145	NP_232510.1	VCA0109	hypothetical protein	T6CP016004	100.0			
119317..121086	+	589	NP_232511.1	VCA0110	hypothetical protein	T6CP016005	100.0			
121090..122066	+	338	NP_232512.1	VCA0111	hypothetical protein	T6CP014868	100.0			
123559..124035	+	158	NP_232514.1	VCA0113	hypothetical protein	T6CP016008	99.367			
124042..125376	+	444	NP_232515.1	VCA0114	hypothetical protein	T6CP016009	100.0			
125379..126152	+	257	NP_232516.1	VCA0115	hypothetical protein	T6CP014865	100.0			
126178..128787	+	869	NP_232517.1	VCA0116	clpB protein	T6CP014864	99.885	0.0		
131045..132454	+	469	NP_232520.1	VCA0119	hypothetical protein	T6CP014863	100.0			
132470..136015	+	1181	NP_232521.1	VCA0120	IcmF-like protein	T6CP015990	100.0			
136063..137328	+	421	NP_232522.1	VCA0121	hypothetical protein	T6CP014268	100.0			
137581..140634	+	1017	NP_232524.1	VCA0123	vgrG protein	T6CP014269	100.0	0.0		
596382..597005	-	207	NP_233048.1	VCA0659	protein F-like protein	T6CP011752	38.095			
706534..708192	-	552	NP_233149.1	VCA0763	hypothetical protein	T6CP016176	32.323			
815881..817278	+	465	NP_233250.1	VCA0864	methyl-accepting chemotaxis protein	T6CP00345				

Here, the prediction was completed successfully and the results from that prediction were displayed in the UI. The full command that was run is shown at the top, followed by the console output from the WSL terminal.

Example tool usage 2. DeepSecE

Below is a step-by-step example of how to run a DeepSecE prediction. Each tool is unique so be sure to read the information guiding you on how to use them.

1. **Navigate to the DeepSecE Tab:** Click on the "SecRet6 Prediction" tab at the top of the app interface.

Type VI Secretion Systems Suite

Tools: **DeepSecE Prediction** | SecRet6 Prediction | Run Calculator CUB | FastTree | DeepTSS-001 | H0-002 | User Manual | Citations

For installation instructions, visit: [DeepSecE Installation](#) 1

Required

Specify Conda Environment for DeepSecE 1

Specify local Directory to DeepSecE Installation

Upload
 Choose FASTA File
 No file selected

Model Location Directory

2. **Input the conda environment name for prediction, followed by the file path to where you installed DeepSecE:** It is advised that you are in your home directory for installation. Please make sure to place the “predict.py” script into this directory also.

Type VI Secretion Systems Suite

Home DeepSecE Prediction Secretion Prediction Run Cluster C-IT FastTree DeepTSS-Web TSS-Web User Manual Citations

For installation instructions, visit: [DeepSecE Installation](#)

Required

Specify Conda Environment for DeepSecE

DeepSecE

Specify local Directory to DeepSecE Installation

/home/podge

Upload

Choose FASTA File

Browse... No file selected

Model Location Directory

/home/podge/.cache/torch/hub/checkpoi

3. **Upload Your FASTA File:** Use the "Choose FASTA File" button to upload your sequence data.

Required

Specify Conda Environment for DeepSecE

DeepSecE

Specify local Directory to DeepSecE Installation

/home/podge

Upload

Choose FASTA File

Browse... No file selected

Model Location Directory

/home/podge/.cache/torch/hub/checkpoi

Open

File explorer showing the contents of the /home/podge directory. The file Test-260.fasta is selected.

Name	Date modified	Type	Size
ncbi_dataset (1)	10/08/2024 14:16	File folder	
runs	10/06/2024 17:05	File folder	
t6ss_finder	13/07/2024 19:02	File folder	
t6ss_prediction	13/07/2024 19:00	File folder	
TXSS	29/08/2022 05:53	File folder	
bua.fasta	21/06/2024 16:41	FASTA File	
Example.fasta	11/07/2024 20:51	FASTA File	
Test-260.fasta	20/06/2024 15:01	FASTA File	

File name: Test-260.fasta

FASTA File (*.fasta)

Open Cancel

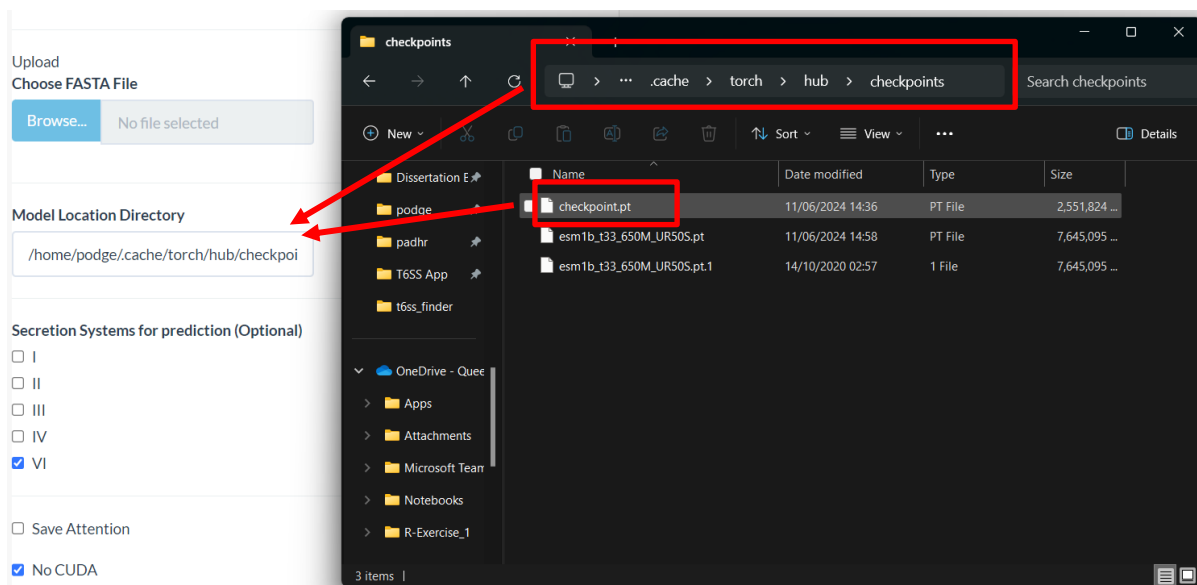
Upload

Choose FASTA File

Browse... Test-260.fasta

Upload complete

4. **Specify Model Path:** The model weights can be downloaded from the GitHub and placed into the same directory as where DeepSecE was installed.



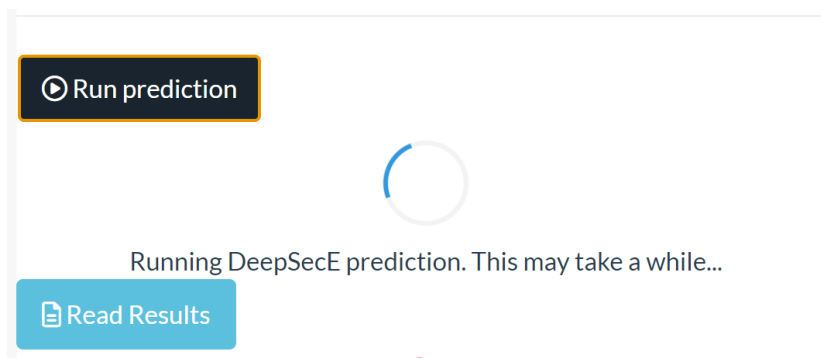
5. **Configure Parameters:** Set the necessary parameters such as the option to run from a CPU, save attention, or the secretion systems you may also want. These are optional, so for this test example, no secretion systems were selected, along with “No CUDA” to run the prediction with the device's CPU.

Secretion Systems for prediction (Optional)

☐ I
☐ II
☐ III
☐ IV
☒ VI

☐ Save Attention
☒ No CUDA

6. **Run the Prediction:** Click on the "Run prediction" button to start the analysis. You will see a loading spinner indicating that the process is running.



For installation instructions, visit: [DeepSecE Installation](#)

Required

Specify Conda Environment for DeepSecE

DeepSecE

Specify local Directory to DeepSecE Installation

/home/podge

Upload

Choose FASTA File

Browse... Test-260.fasta

Upload complete

Model Location Directory

/home/podge/cache/torch/hub/checkpoi

☐ Save Attention

☒ No CUDA

Run prediction Read Results

```
[1] "Activated conda environment: DeepSecE"
/home/podge/miniconda3/envs/DeepSecE/lib/python3.9/site-packages/numpy/_core/getli
0%| 0/260 [00:00<?, ?it/s]
0%| 1/260 [00:22<1:37:32, 22.60s/it]
1%| 2/260 [00:24<44:33, 10.36s/it]
1%| 3/260 [00:28<32:06, 7.50s/it]
2%| 4/260 [00:32<26:30, 6.21s/it]
2%| 5/260 [00:49<43:03, 10.13s/it]
2%| 6/260 [01:05<50:46, 11.99s/it]
3%| 7/260 [01:15<47:37, 11.30s/it]
3%| 8/260 [01:18<37:05, 8.83s/it]
3%| 9/260 [01:20<27:21, 6.54s/it]
4%| 10/260 [01:26<26:09, 6.28s/it]
4%| 11/260 [01:28<21:30, 5.18s/it]
5%| 12/260 [01:33<21:29, 5.20s/it]
5%| 13/260 [01:38<20:50, 5.06s/it]
5%| 14/260 [01:45<22:46, 5.55s/it]
6%| 15/260 [01:48<19:35, 4.80s/it]
6%| 16/260 [02:05<34:31, 8.49s/it]
7%| 17/260 [02:12<32:18, 7.98s/it]
7%| 18/260 [02:23<36:29, 9.05s/it]
7%| 19/260 [02:27<29:39, 7.38s/it]
8%| 20/260 [02:30<24:53, 6.22s/it]
8%| 21/260 [02:38<26:47, 6.72s/it]
8%| 22/260 [02:39<19:54, 5.02s/it]
9%| 23/260 [02:43<18:20, 4.64s/it]
9%| 24/260 [02:46<16:34, 4.21s/it]
10%| 25/260 [02:58<25:50, 6.60s/it]
10%| 26/260 [03:02<22:23, 5.74s/it]
10%| 27/260 [03:11<25:34, 6.59s/it]
11%| 28/260 [03:15<23:17, 6.03s/it]
11%| 29/260 [03:45<50:51, 13.21s/it]
12%| 30/260 [03:59<51:17, 13.38s/it]
12%| 31/260 [04:04<41:32, 10.88s/it]
12%| 32/260 [04:06<30:54, 8.13s/it]
13%| 33/260 [04:12<27:48, 7.35s/it]
13%| 34/260 [04:18<27:16, 7.24s/it]
```

7. **View Results:** After the prediction is complete, the results will be displayed in a table format below after clicking “Read Results”. You can scroll through the results to analyze the predicted secretion systems, and sort by ascending/descending order for whatever you are interested in. These results are also automatically saved to the directory you installed DeepSecE into.

Show entries Search:

	name	system	score	NonSecreted.prob	T1SE.prob	T2SE.prob
114	T6SE-9	VI	0.7369	0.2508	0.0032	0.0033
113	T6SE-8	VI	0.9968	0.0018	0.001	0.0001
112	T6SE-7	VI	0.9852	0.0002	0.0045	0.0003
111	T6SE-6	VI	0.9992	0	0.0005	0.0001
110	T6SE-5	VI	0.993	0.0023	0.0027	0.0004
109	T6SE-4	VI	0.996	0.0003	0.0032	0.0001
125	T6SE-20	III	0.9736	0.0036	0.0009	0.0007
108	T6SE-2	VI	0.9994	0.0001	0.0002	0
124	T6SE-19	III	0.9797	0.0025	0.0049	0.0043
123	T6SE-18	VI	0.9888	0.0066	0.002	0.0006

Showing 1 to 10 of 125 entries

Previous 1 2 3 4 5 ... 13 Next

In this test result, the table was sorted in descending order for the “T6SE.prob” column.

ob	T1SE.prob	T2SE.prob	T3SE.prob	T4SE.prob	T6SE.prob	length
0001	0.0002	0	0.0003	0	0.9994	165
0	0.0005	0.0001	0.0001	0	0.9992	176
0003	0.0001	0.0001	0.0007	0	0.9988	172
0005	0.0004	0.0001	0.0002	0.0001	0.9988	547
0002	0.0006	0.0003	0.0001	0.0002	0.997	392
0018	0.001	0.0001	0.0002	0	0.9968	1019
0015	0.0009	0.0003	0.0004	0.0004	0.9965	386
0003	0.0032	0.0001	0.0003	0.0001	0.996	669
0027	0.0008	0.0002	0.0027	0.0001	0.9934	485
0023	0.0027	0.0004	0.0004	0.0013	0.993	188

4 5 ... 13 Next

8. 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. Ensure the correct Anaconda navigator is installed and the conda environment is created in the correct place i.e. for tools that require WSL, Anaconda navigator for Linux must be installed and the env must be created within WSL.
- **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.

9. FAQs

Q: Can the app be used on all Operating Systems? **A:** Yes, but for Windows users, 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 or the contact information section below.

Q: Can I run this app on my laptop? **A:** Yes, you can, a minimum 8G RAM laptop can run most of these tools, however, Windows users must have a minimum of 16G RAM to run a DeepSecE prediction as this tool requires large computational resources and Windows uses more memory to run WSL.

10. Contact Information

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

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