**EPdigger: an automatic dig tool of Epitope Peptide** 

# guideline

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## 1 Software Introduction

This software uses a mathematical and computational modeling method to automatically mine epitope peptides.

# 2 Operating Environment

#### 2.1 Hardware Environment

Ordinary PC (normal complete configuration is sufficient)

#### 2.2 Software Environment

Windows 7/8/10 platform

#### 3 Software Installation and Uninstallation

The software uses a no-installation mode, you can simply unzip the software package. To uninstall, just delete the software directory.

#### 4 Instructions for Use

#### 4.1 Opening and Closing the Software

To open the software: Double-click on the EPdigger.exe in the software directory to run and open the software.

To close the software: Click on the "x" close button in the upper right corner of the main interface.

#### 4.2 Main Interface of the Software

This software is an epitope peptide automatic mining software, and its main interface is shown in Figure 1 below.

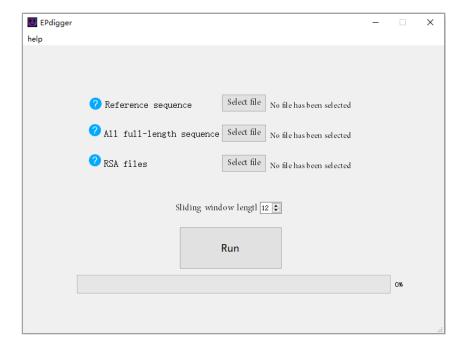


Figure 1

#### 4.3 Data Preparation

To predict epitope peptides using EPdigger, you need to prepare three input files. The first file, Reference sequence, contains only one antigen protein sequence for epitope prediction and is saved in fasta format. The second file, all full-length sequences, contains more than 2 protein sequences that are highly homologous to the Reference sequence. This is used for conservative analysis of the predicted candidate epitope peptides and is also saved in fasta format. The last file, RSA file, is used to analyze the Relative Solvent Accessibility (RSA) of the predicted epitope peptides.

#### 1) Data Acquisition:

**Reference Sequence**: Save the target protein sequence (which you wish to use for peptide epitope prediction) in fasta format.

All Full-Length Sequences: Use the reference sequence as a query sequence, perform a Blastp search on the NCBI website, obtain all protein sequences that are homologous to the reference sequence and of equal length, and save them in fasta format. There should be no less than two sequences.

Relative Solvent Accessibility: Use the SABLE website (http://sable.cchmc.org/) as shown in Figure 2. At the location marked 1, upload the reference sequence, at the location marked 2, fill in your email. SABLE will send the analysis results to the user-specified email. Copy the data in red box from the "Relative solvent accessibility prediction (real values)" part of the analysis results in your email as shown in Figure 3, save it as a txt format file.

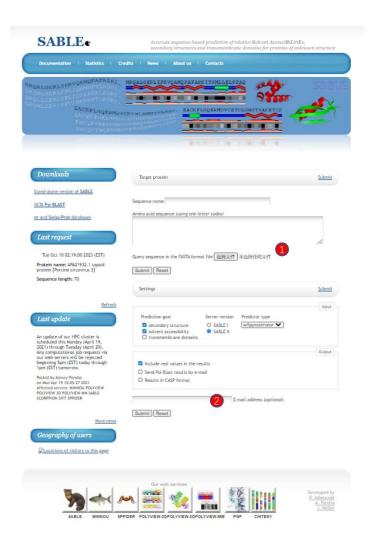


Figure 2

```
SECTION_SA_PERCENT
Relative solvent accessibility prediction (real values)
0 -> fully Buried
100 -> fully Exposed
```

```
M R H R A I F R R P R P R R R R R H R R R Y V R
 47 47 30 31 21 12 13 31 32 27 44 35 40 39 29 27 29 18 18 17 36 44 31 19 44
> 26
 R K L F I R R P T A G T Y Y T K K Y S T M N V I S
 30 26 13 9 11 22 22 23 47 30 41 16 33 10 32 16 30 27 41 37 46 40 24 29 44
 V G T P Q N N K P W H A N H F I T R L N E W E T A
 20 46 35 43 52 55 35 32 33 17 14 8 17 15 5 12 5 27 8 44 39 14 42 35 38
 I S F E Y Y K I L K M K V T L S P V I S P A Q Q T
 12 20 8 23 15 6 25 6 31 25 5 17 8 20 8 16 10 16 26 24 32 15 23 38 28
 K T M F G H T A I D L D G A W T T N T W L Q D D P
 49 46 26 23 14 16 10 6 8 11 24 30 41 54 17 33 45 37 36 51 25 44 34 28 19
 Y A E S S T R K V M T S K K K H S R Y F T P K P I
 18 23 45 15 31 35 21 32 26 11 38 38 51 45 33 8 36 18 22 11 32 17 37 14 24
 LAGTTSAHPGQSLFFFSRPTPWLNT
 8 43 36 30 43 45 26 22 33 35 22 22 24 15 17 37 32 36 26 34 20 8 8 30 18
> 176
 Y D P T V Q W G A L L W S I Y V P E K T G M T D F
 31 50 47 26 17 25 12 8 7 8 7 8 8 13 29 18 25 49 61 43 47 24 17 40 19
                                                214
 YGTKEVWIRYKSVL
 12 16 12 29 11 4 24 8 32 7 31 41 13 13
```

END\_SECTION

Figure 3

2) Example: Examples of three input files are placed in the "example" folder in the software directory, as shown in Figure 4.

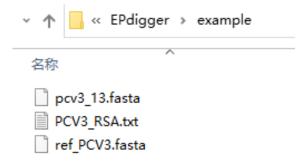


Figure 4

#### 4.4 Data Input

For the first line "Reference sequence", click the Browse button to select and upload the protein sequence file of the reference sequence. However, if there is an error in the file, a popup window will appear to remind you, as shown in Figure 3 below.

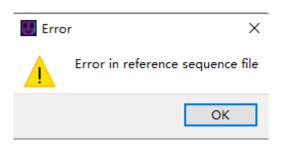


Figure 5

For the second line "All full-length sequence", click the Browse button to select and upload all full-length protein sequence files that are the same length as the reference sequence (at least two sequences), which should not contain unknown amino acids. However, if there is an error in the file, a pop-up window will appear to remind you, as shown in Figure 4 below.

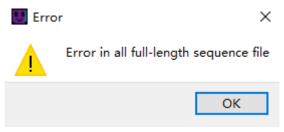


Figure 6

For the third line "RSA file", click the Browse button to select and upload the relative solvent accessibility file of the reference sequence. However, if there is an error in the file, a pop-up window will appear to remind you, as shown in Figure 5 below.

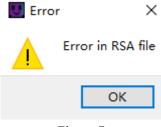


Figure 7

# 4.5 Sliding window length

You can adjust the length of the sliding window through the button, as shown in Figure 6 below.

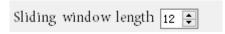


Figure 8

#### 4.6 Run

After the data input and window length selection are completed, click the Run button and the software will run. During the software operation, the progress bar will run synchronously. When the program is finished, a result pop-up window will appear, as shown in Figure 6 below.



Figure 9

At the same time, the output result file will also be saved in the "result" folder under the software directory, and the file name is "result.xlsx".

### 5 Technical Support

If you encounter any problems during the use of the system, you can describe the problems in the form of an email and send it to our mailbox. We will do our best to solve all your problems. If you have any suggestions, we are also very willing to discuss them together.