

XGBMut1.0

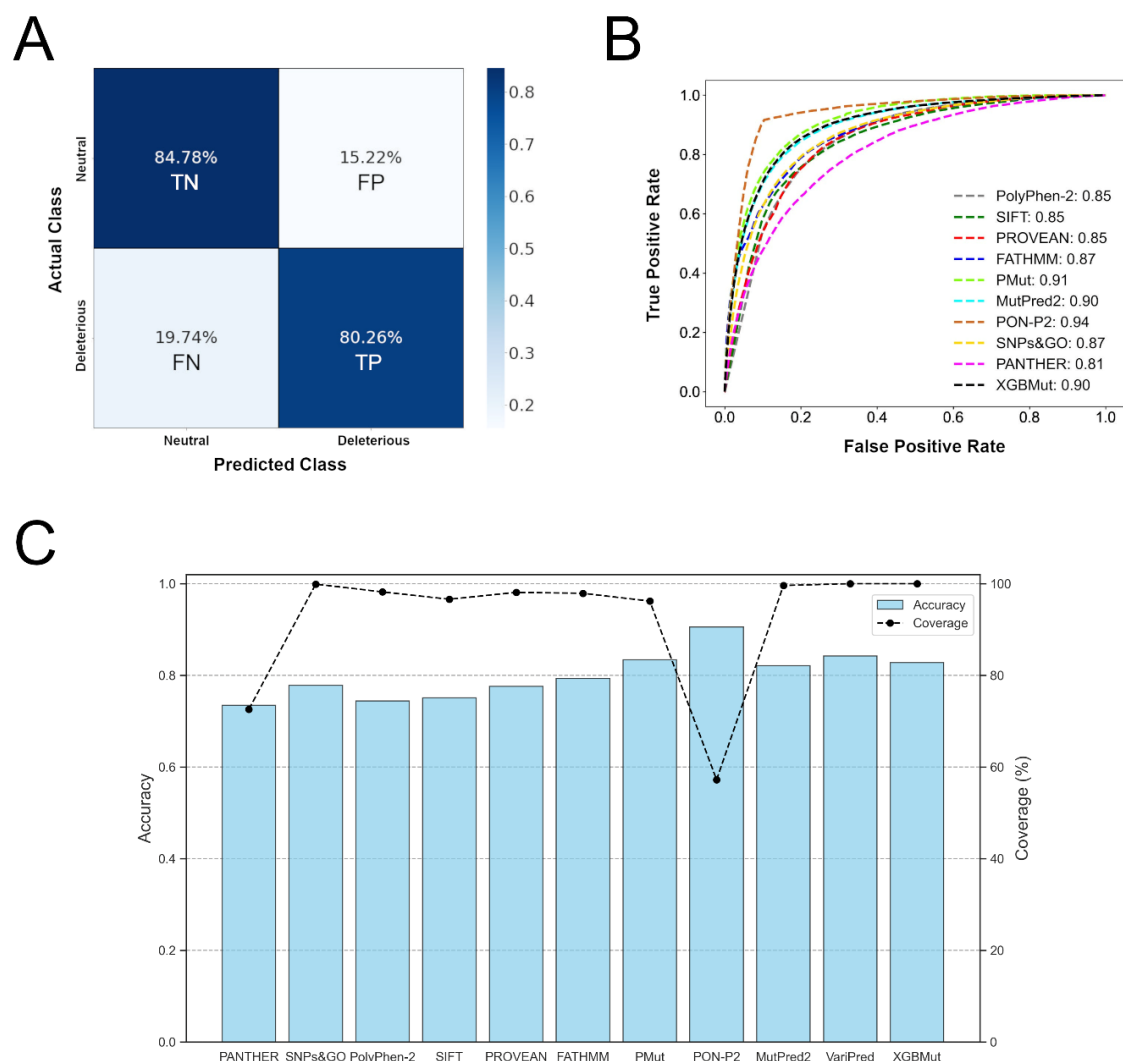
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## 1. PRESENTATION

XGBMut1.0 is a machine learning method with integrated software that uses multiple genetic and molecular data to predict whether a missense mutation is neutral or deleterious together with its probability of being deleterious for the protein's function. XGBMut was trained on a balanced dataset derived from ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) containing 86.336 labeled mutations and validated using a non-redundant dataset derived from HumSavar (<https://www.uniprot.org/docs/humsavar>), which contained 69.887 labeled mutations. XGBMut is an extreme gradient boosting model presenting roughly 83% accuracy and 0.9 ROC-AUC in the validation set. This outcome is within the performance range of ten functional prediction algorithms when analyzing the same validation set, including PANTHER (<http://www.pantherdb.org/tools/>), SNPs&GO (<https://snps-and-go.biocomp.unibo.it/snps-and-go/>), PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), SIFT (<https://sift.bii.a-star.edu.sg/>), PROVEAN (<https://www.jcvi.org/research/provean>), FATHMM (<http://fathmm.biocompute.org.uk/>), PMut (<http://mmb.irbbarcelona.org/PMut/>), PON-P2 (<http://structure.bmc.lu.se/PON-P2/>), and MutPred2 (<http://mutpred2.mutdb.org/>), VariPred (<https://github.com/wlin16/VariPred>) (Figure1).

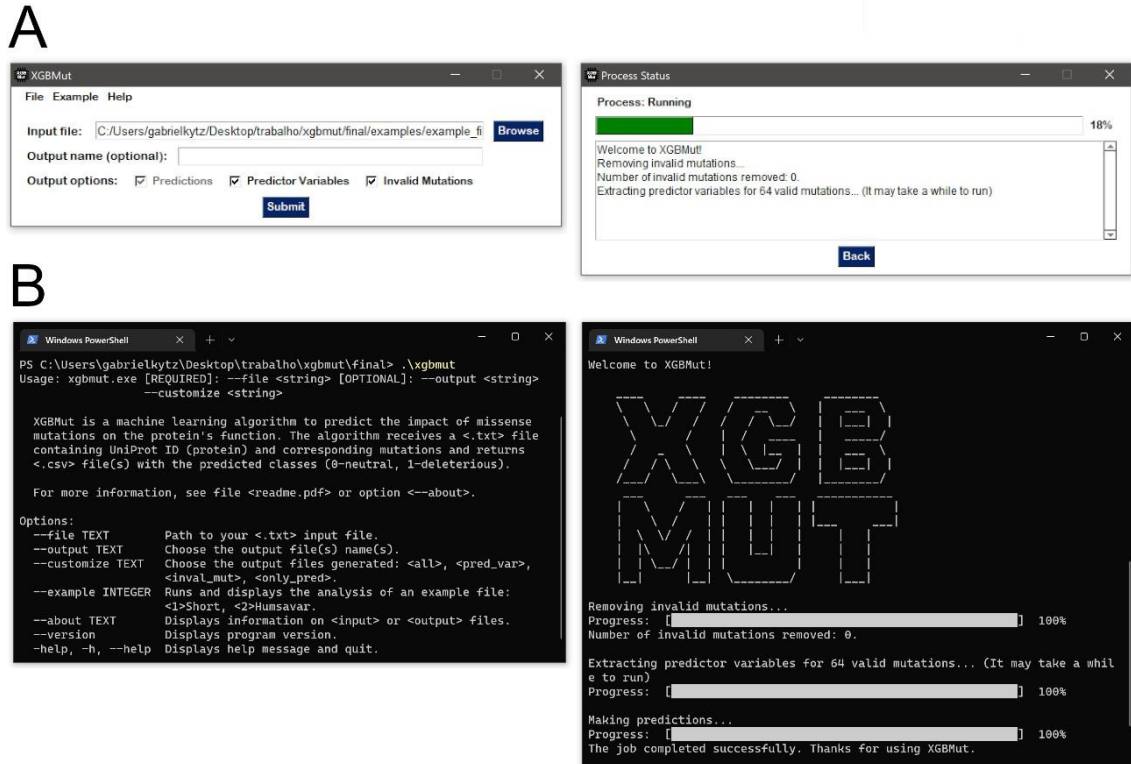
The software has two user interface options: a graphical user interface (GUI) and a command line interface (CLI), which are compatible with **Windows 10** and **Linux/Ubuntu 20.04** or higher and run within an executable file with no need for a Python interpreter or modules installed. The graphical interface allows the use of XGBMut in an intuitive and user-friendly way, facilitating its use by third parties, including professionals from other areas, such as medical doctors or wet-lab researchers who are not familiar with command line tools. XGBMut was developed in Python using the *Pandas* library for data cleaning and transformation (<https://pandas.pydata.org/>), in addition to the *xgboost* library (<https://xgboost.readthedocs.io/>) for creating and deploying the predictive model. PySimpleGui library was used to develop the GUI (<https://www.pysimplegui.org/>), while *click* library was used to develop the CLI (<https://click.palletsprojects.com/>). PyInstaller was used to compile and generates the executable file (<https://pyinstaller.org/>).



**Figure 1. Performance metrics computed for XGBMut in the test set derived from HumsaVar database.** (A) Confusion matrix computed for XGBMut. (B) AUC-ROC calculated for XGBMut and well-established methods. AUC-ROC curves comparing XGBMut with other established methods. VariPred was excluded as it directly outputs the class, without providing probabilities. (C) Accuracy and mutation coverage on the test set for the compared algorithms.

The software can be divided into two main parts: an initial menu where the input file is loaded and the customization options selected, as well as a loading menu containing information about the algorithm's progress (Figure 2). In addition to the standard analyzes, XGBMut allows output customizations, so that the user can choose the file name and which files will be generated. To guide the user experience, two usage examples are available within the software options. A help menu containing essential information about XGBMut and the required file formats was also provided. The maximum RAM usage by the software was estimated at 3GB in the tests performed, which enables its use in most computers currently available. XGBMut does not require installation, as

the executable file already includes the Python interpreter, and the necessary modules compiled in a single file. The software is available at the link: <https://github.com/gabrielktyz2/XGBMut/>.



**Figure2. XGBMut interface is available in both Windows and Linux platforms. (A) Command line interface (CLI). (B) Graphical User Interface.**

XGBMut requires as the input a **.txt** file with two columns separated by a tab, containing: the UniProt ID of the affected protein, and the corresponding amino acid substitution under the one-letter code notation. Two input file examples are provided in the `<examples>` folder, *i.e.* **example\_file.txt** and **humsavar\_example.txt**. The algorithm initially checks for and removes invalid mutations and protein IDs, which can be written into **invalid\_mutations.csv** (optional). Then, XGBMut search for the corresponding predictor variables in the databases files, which can be saved into **predictor\_variables.csv** (optional). At last, the algorithm predicts whether the mutation is neutral (0) or deleterious (1), together with its probability of being deleterious, which are finally written into **output.csv**. See **usage options** below.

XGBMut was developed by Gabriel Pereira, PhD, as a ready-to-use executable file that supports both CLI and GUI interfaces, requiring no installation. Note that for the software to function properly, the executable file must remain within the downloaded folder.

## **2. COMMAND LINE INTERFACE (CLI)**

### **2.0. Installation**

XGBMut is a ready-to-use executable file that supports both CLI and GUI interfaces, requiring no installation. For proper functionality, the executable must remain in the downloaded folder, the databases must be kept in the designated database folder, and the model must be within its respective folder.

### **2.1. Usage**

xgbmut.exe [REQUIRED]: --file <string> [OPTIONAL]: --output <string> --customize <string>

### **2.2. Options**

--file TEXT	Path to your <.txt> input file.
--output TEXT	Choose the output file(s) name(s).
--customize TEXT	Choose the output files generated: <all>, <pred_var>, <inval_mut>, <only_pred>.
--example INTEGER	Runs and displays the analysis of an example file: <1>Short, <2>Humsavar.
--about TEXT	Displays information on <input> or <output> files.
--version	Displays program version.
-help, -h, --help	Displays help message and quit.

### **2.3. Input File**

The input file must be a <.txt> file separated by a tab containing Uniprot ID and mutation, one mutation per row, as follows:

<Uniprot ID><tab><Mutation>

The mutation must be written in one letter code for amino acid:

<wild-type amino acid><position><mutated amino acid>

***e.g., example.txt:***

P04217	H52R
P04217	H395R
Q9NQ94	V555M
Q9NQ94	A558S
P01023	R704H
P01023	C972Y

Additional example inputs are provided in the <examples> folder.

## 2.4. Output File

XGBMut generates up to three <.csv> files as output:

### 1) *predictions.csv*

A 4 columns file containing the UniProt ID, mutation, predicted class (0-neutral, 1-deleterious), and probability of being deleterious (always generated).

### 2) *predictor\_variables.csv*

A 27 columns file containing the UniProt ID, mutation, and 25 predictor variables needed to run the predictive model.

### 3) *invalid\_mutations.csv*

A 2 columns file containing the UniProt ID and mutation for all invalid mutations submitted to XGBMut.

The outputs can be customized by choosing <--output> and <--customize> options.

--output <string>: choose output file(s) name(s).

--customize <string>: choose output file(s) generated:

<all>: predictions.csv, predictor\_variables.csv, invalid\_mutations.csv.

<pred\_var>: predictions.csv, predictor\_variables.csv.

<inval\_mut>: predictions.csv, invalid\_mutations.csv.

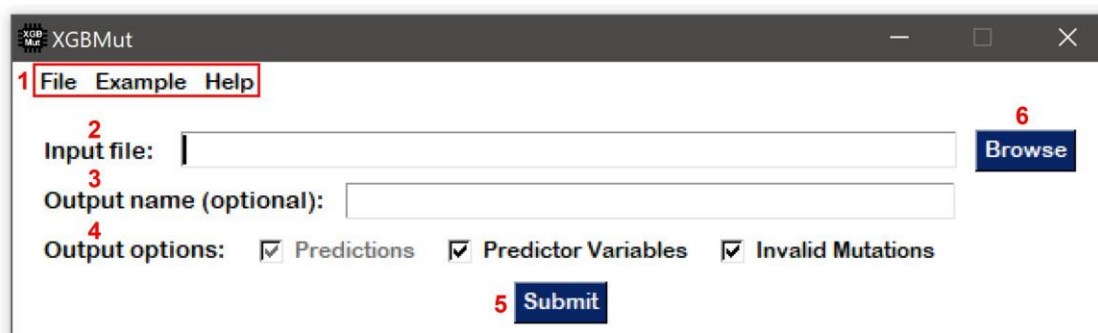
<only\_pred>: predictions.csv.

### 3. GRAPHICAL USER INTERFACE (GUI)

#### 3.0. Installation

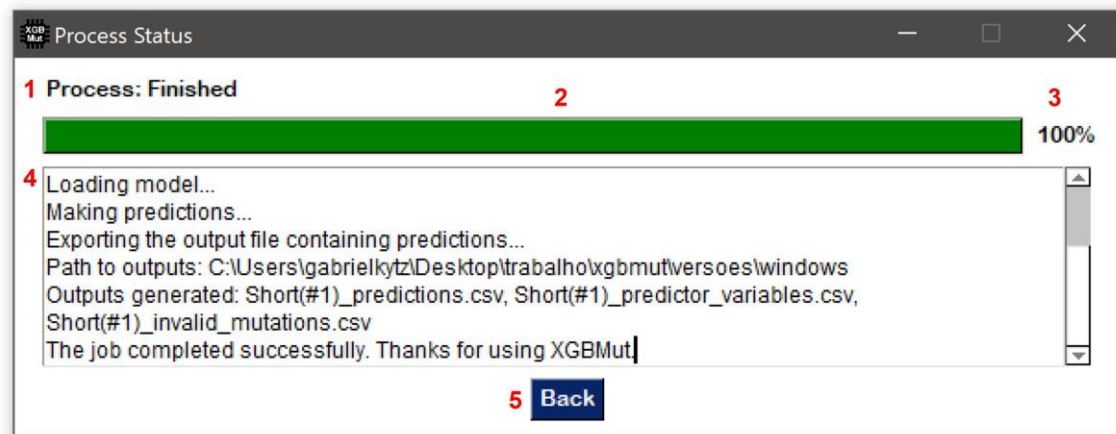
XGBMut is a ready-to-use executable file that supports both CLI and GUI interfaces, requiring no installation. For proper functionality, the executable must remain in the downloaded folder, the databases must be kept in the designated database folder, and the model must be within its respective folder.

#### 3.1. Initial Menu



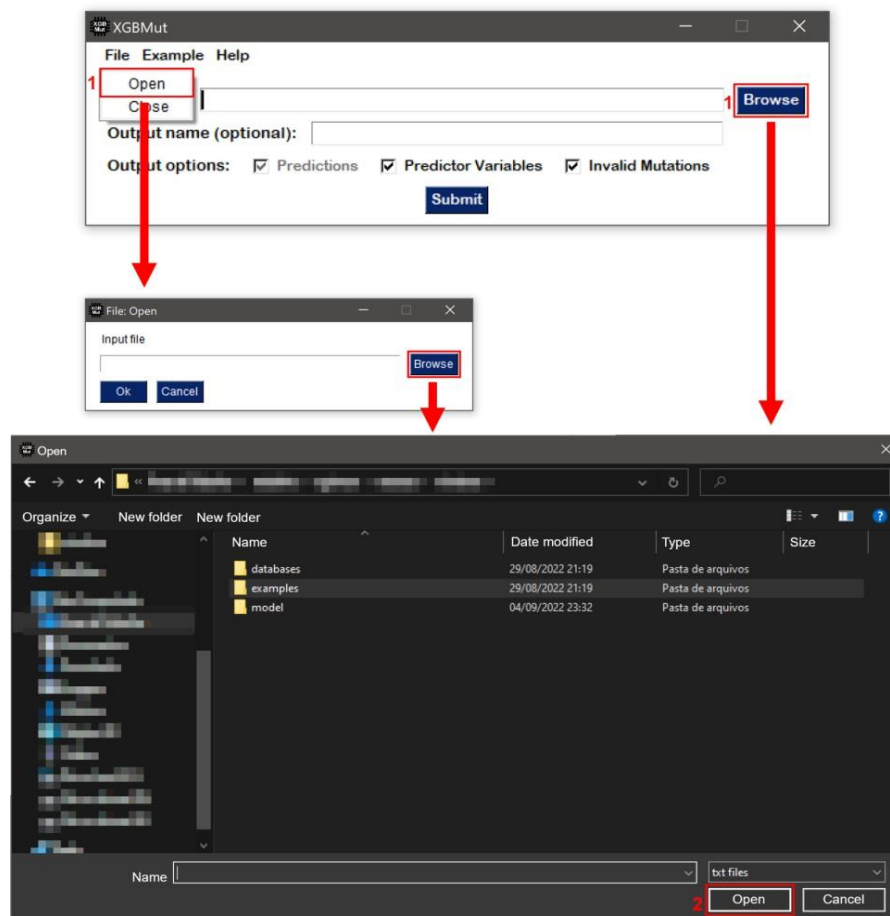
- 1) *Menu*: contains File, Example, and Help options;
- 2) *Input file*: write or select Browse button (6) to choose the path to input file;
- 3) *Output name*: write the output file name (optional);
- 4) *Output options*: customize the output files generated by checking the boxes.
- 5) *Submit button*: submit the analysis and open the Progress window.
- 6) *Browse button*: open a popup window to choose the path to the input file.

### 3.2. Progress Menu



- 1) *Process*: progress status information;
- 2) *Loading bar*: visual indicator of the analysis progress;
- 3) *Progress*: percentage of the analysis progress;
- 4) *Output console*: information about the analysis.
- 5) *Back menu*: returns to the Initial window.

### 3.3. Loading File





- 1) *Open menu/Browse*: find and load the input file from your computer.
- 2) *Loading button*: upload the selected file from your computer.

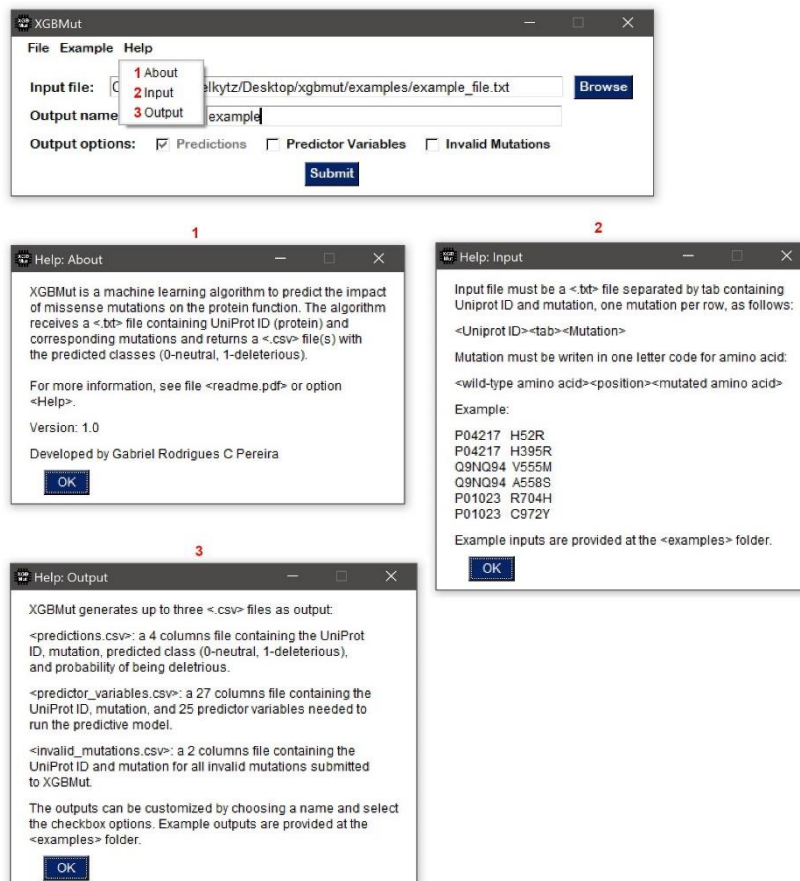
### 3.4. Running Analysis

The screenshot shows the XGBMut application window with the following fields and buttons:

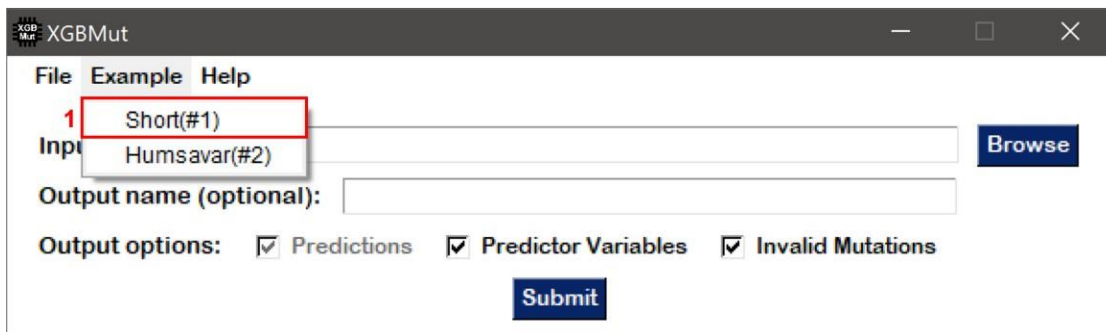
- Input file:** A text box containing the path `C:/Users/gabrielkytz/Desktop/xgbmut/examples/example_file.txt`. A red number '1' is above the text box, and a blue 'Browse' button is to its right.
- Output name (optional):** A text box containing the word 'example'. A red number '2' is above the text box.
- Output options:** Three checkboxes: 'Predictions' (checked), 'Predictor Variables' (unchecked), and 'Invalid Mutations' (unchecked). A red number '3' is above the 'Predictions' checkbox.
- Submit:** A blue button with a red border. A red number '4' is to its left.

- 1) Write the path to the input file (mandatory);
- 2) Choose an output name (optional);
- 3) Customize the output files generated (optional)
- 4) Submit the analysis (mandatory);

### 3.5. Help options



### 3.6. Running Examples



XGBMut

File Example Help

1 Short(#1)  
Humsavar(#2)

Input file:  Browse

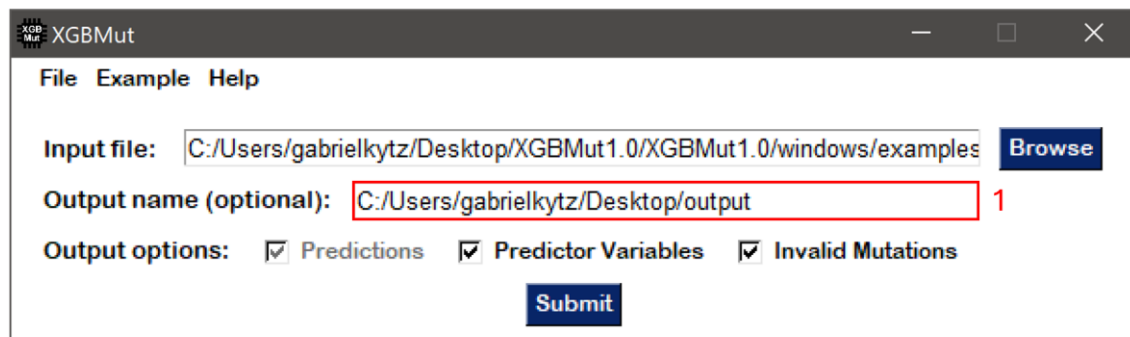
Output name (optional):

Output options: ☒ Predictions ☒ Predictor Variables ☒ Invalid Mutations

Submit

1) Choose an example option: *Short(#1)* or *Humsavar(#2)*.

### 3.7. Output Selection



XGBMut

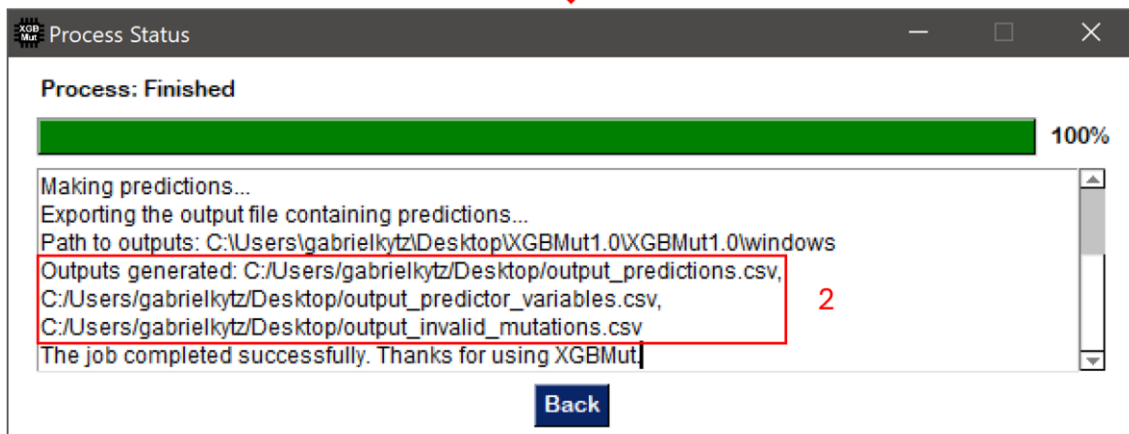
File Example Help

Input file:  Browse

Output name (optional):  1

Output options: ☒ Predictions ☒ Predictor Variables ☒ Invalid Mutations

Submit



Process Status

Process: Finished

100%

Making predictions...  
Exporting the output file containing predictions...  
Path to outputs: C:\Users\gabrielkytz\Desktop\XGBMut1.0\XGBMut1.0\windows  
Outputs generated: C:\Users\gabrielkytz\Desktop\output\_predictions.csv,  
C:\Users\gabrielkytz\Desktop\output\_predictor\_variables.csv,  
C:\Users\gabrielkytz\Desktop\output\_invalid\_mutations.csv 2  
The job completed successfully. Thanks for using XGBMut

Back

- 1) Choose the path to the output files.
- 2) Output information.

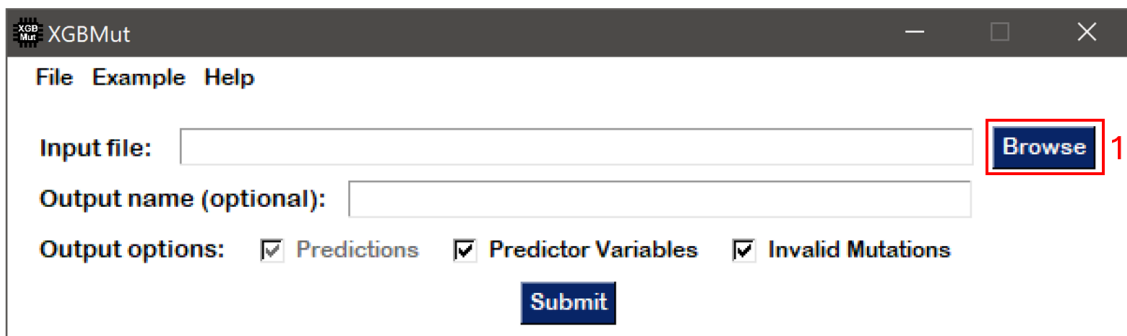
### 3.8. Tutorial

- I) Assess the Uniprot page related to human frataxin protein (<https://www.uniprot.org/uniprotkb/Q16595/entry>);
- II) Obtain the variants associated with Friedreich's Ataxia to be used as the tutorial input;

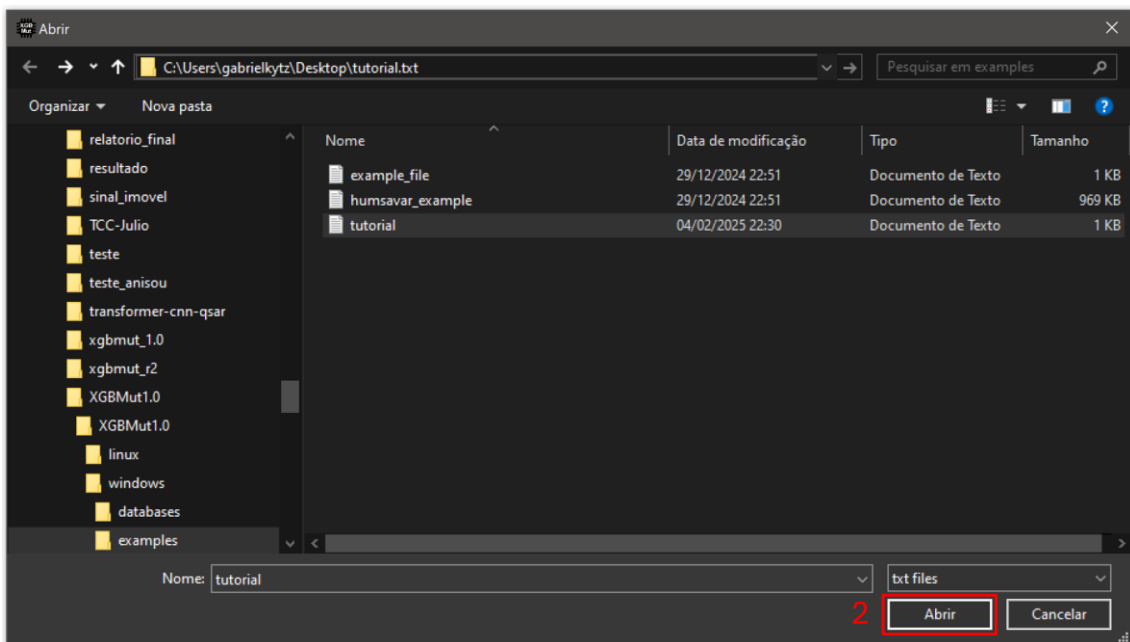
***e.g., tutorial.txt:***

Q16595	L106S
Q16595	D122Y
Q16595	G130V
Q16595	I154F
Q16595	W155R
Q16595	R165C
Q16595	L182F
Q16595	L198R

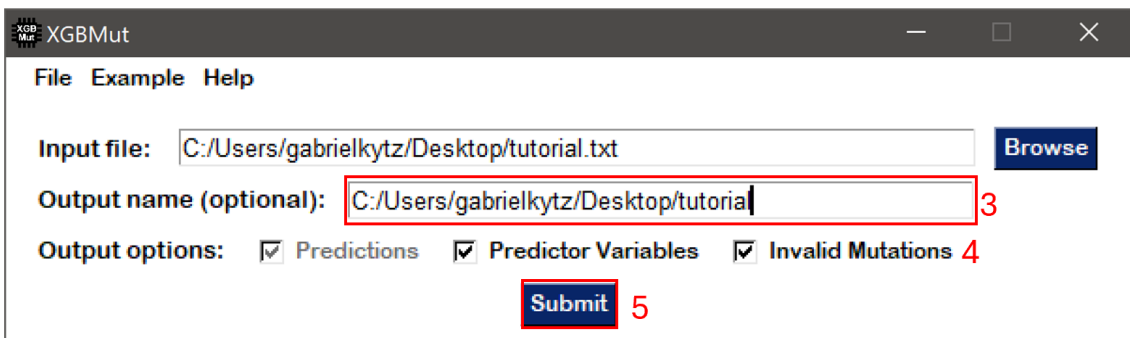
- III) Run XGBMut;



- 1) Browse the input file.



2) Select and load the input file.



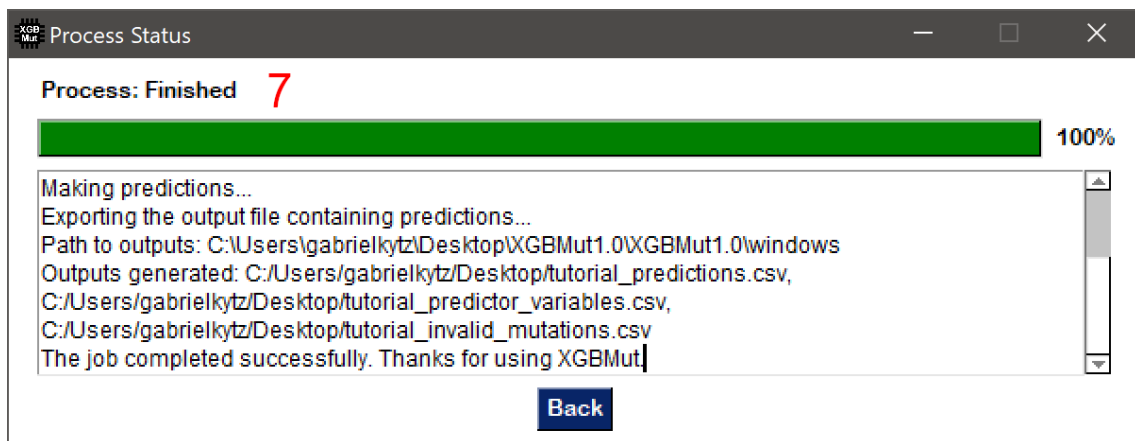
3) Define the output path.

4) Define the output options (set to default).

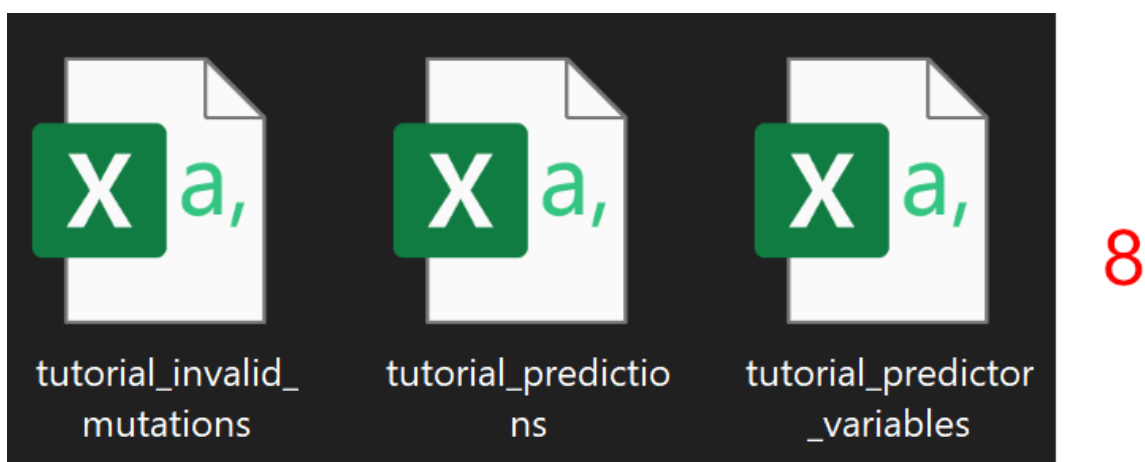
5) Submit the job.



6) Wait for the job to run.



7) Process finished.



8) Check the results.

	A	B
1	ACC,AA_change	
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		

	A	B	C	D
1	ACC,AA_change,Predicted_Class,Probability			
2	Q16595,L106S,1,0.9463191			
3	Q16595,D122Y,1,0.83736426			
4	Q16595,G130V,1,0.85918033			
5	Q16595,I154F,1,0.8533724			
6	Q16595,W155R,1,0.9870625			
7	Q16595,R165C,1,0.72111994			
8	Q16595,L182F,0,0.4916693			
9	Q16595,L198R,1,0.63907915			

9

tutorial\_predictions.csv

tutorial\_invalid\_mutations.csv

	A	B	C	D	E	F	G	H	I	J	K	L
1	ACC,AA_change,MMseq2_conservation_level,MMseq2_conservation_score,SCRIBERscore,VSL2_score,Average_conservation,pssm_score,											
2	Q16595,L106S,3,0.2,3,0.075,0.43,4.3142857143,-2,1,29,6,27,1,0,0,0,0,0,0,-2,0,0,0,0,211,-26.0,-77.7,-4.6											
3	Q16595,D122Y,5,0.2,65,0.129,0.33,4.3142857143,-2,1,29,6,27,1,0,0,0,0,0,0,-3,0,1,0,0,293,48.0,82.5,2.2											
4	Q16595,G130V,4,0.2,54,0.084,0.24,4.3142857143,-2,1,29,6,27,1,0,0,0,0,0,0,-3,0,0,0,0,494,42.0,79.9,4.6											
5	Q16595,I154F,6,0.2,68,0.1,0.5,4.3142857143,-1,1,29,6,27,1,0,0,0,0,0,0,0,0,0,218,34.0,23.2,-1.7											
6	Q16595,W155R,9,0.4,34,0.127,0.54,4.3142857143,-2,1,29,6,27,1,0,0,0,0,0,0,-3,0,1,0,0,235,-30.0,-54.4,-3.6											
7	Q16595,R165C,7,0.2,94,0.22,0.58,4.3142857143,1,1,29,6,27,1,0,0,0,0,0,0,-3,0,1,0,1,1369,-53.0,-64.9,7.0											
8	Q16595,L182F,3,0.2,29,0.115,0.33,4.3142857143,-2,1,29,6,27,1,0,0,0,0,0,0,0,0,0,765,34.0,23.2,-1.0											
9	Q16595,L198R,1,0.1,53,0.114,0.54,4.3142857143,-1,0,29,6,27,1,0,0,0,0,0,0,-2,0,1,0,0,266,43.0,6.7,-8.3											

tutorial\_predictor\_variables.csv

9) Open the output files using Google Sheets, Microsoft Excel, or Notepad.

#### 4. SOFTWARE UPDATE POLICY

The authors are committed to providing regular updates to the software to ensure its compatibility with current operating systems and relevant packages. Updates will include bug fixes and performance improvements. Whenever necessary, deprecated dependencies will be replaced, and the software will be tested to maintain optimal functionality. Major updates will be detailed in the documentation.