## ProteoAnnotator user guide

1. Download the latest version from: <http://www.proteoannotator.org/?q=installation>
2. Extract the zip file. Example, extract ProteoAnnotator-1.0.zip
3. Download the test files from: <http://www.proteoannotator.org/?q=installation>
4. Extract the zip file. Example, extract the ProteoAnnotator\_test\_files.zip
5. Change the directory to the extracted ProteoAnnotator-1.0.zip.
6. Run the following command:

java -Djava.util.Arrays.useLegacyMergeSort=true -jar E:\t\mzidlib-1.7\mzidlib-1.7.jar ProteoAnnotator2 -inputGFF E:\t\Homo\_sapiens.GRCh38.77.gff3 -inputFasta E:\t\Homo\_sapiens.GRCh38.pep.all.fa -spectrum\_files "E:\t\PXD000548\_sample\_input" -outputFolder E:\t\withmsgf1 -prefix PXD000548\_withmsgf\_ -compress false -searchParameters E:\t\search\_PXD000652.txt -peptideThreshValue 0.01 -proteinThreshValue 0.01 -inputPredicted "E:\t\1.gff3;E:\t\rp.fasta" -enableMsgf true

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| --- | --- | --- |
| Parameter | optional/ mandatory | Explanation |
| -prefix | Optional | A prefix to be attached to the output file names |
| -inputGFF\_A | Mandatory | The canonical GFF file |
| -inputFasta\_A | Optional if the canonical GFF contains the FASTA | The protein database |
| -outputFolder | Mandatory | The output folder for the analysis |
| -spectrum\_files | Mandatory | The MGF folder |
| -searchParameters | Mandatory | The search parameters file to be used for the search. |
| -inputPredicted | Optional | The non-canonical gene models, these are a set of GFF/FASTA files. The GFF and FASTA are separated by ';' and the pairs are separated by '##' |
| - peptideThreshValue | Mandatory | Peptide FDR Threshold value |
| - proteinThreshValue | Mandatory | Protein FDR Threshold value |
| -enableMsgf | Mandatory | Enable/Disable MSGF+ |

## ProteoAnnotator 2 stage search

In order to run two stage search:

1. Run 1st stage search as normal
2. To run 2nd stage: Re-run the same command as 1st stage with additional parameter -**enableTwoStageSearch 1**