## 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 -Xmx10G -jar mzidentml-lib-1.6.10-SNAPSHOT.jar ProteoAnnotator -inputGFF **PATH\_TO\_ProteoAnnotator\_test\_files**\canonical\_gene\_model\ToxoDB-10.0\_TgondiiME49.gff -spectrum\_files **PATH\_TO\_ProteoAnnotator\_test\_files**\mgf\Orbitap-1DE\combined.mgf -outputFolder **PATH\_TO\_ProteoAnnotator\_test\_files**\output\_results -inputPredicted "**PATH\_TO\_ProteoAnnotator\_test\_files**\non\_canonical\_gene\_model\augustus\augustus.gff;**PATH\_TO\_ProteoAnnotator\_test\_files**\non\_canonical\_gene\_model\augustus\augustus.aa##**PATH\_TO\_ProteoAnnotator\_test\_files**\non\_canonical\_gene\_model\glimmer\Toxo-Glimmer-ME49.gff" -prefix orbitap-1DE\_toxo10\_ -compress false -searchParameters "**PATH\_TO\_ProteoAnnotator\_test\_files**\search.txt" -peptideThreshValue 0.01 -proteinThreshValue 0.01 –enableMsgf true

**IMPORTANT**: Replace **PATH\_TO\_ProteoAnnotator\_test\_files** with the actual path to the unzipped ProteoAnnotator\_test\_files folder.

### Example (change it to match your folder structures):

java -Xmx10G -jar mzidentml-lib-1.6.10-SNAPSHOT.jar ProteoAnnotator -inputGFF C:\ProteoAnnotator\_test\_files\canonical\_gene\_model\ToxoDB-10.0\_TgondiiME49.gff -spectrum\_files C:\ProteoAnnotator\_test\_files\mgf\Orbitap-1DE\combined.mgf -outputFolder C:\ProteoAnnotator\_test\_files\output\_results -inputPredicted "C:\ProteoAnnotator\_test\_files\non\_canonical\_gene\_model\augustus\augustus.gff;C:\ProteoAnnotator\_test\_files\non\_canonical\_gene\_model\augustus\augustus.aa##C:\ProteoAnnotator\_test\_files\non\_canonical\_gene\_model\glimmer\Toxo-Glimmer-ME49.gff" -prefix orbitap-1DE\_toxo10\_ -compress false -searchParameters "C:\ProteoAnnotator\_test\_files\search.txt" -peptideThreshValue 0.01 -proteinThreshValue 0.01 –enableMsgf true

 In this example the "**PATH\_TO\_ProteoAnnotator\_test\_files**" is "C:\ProteoAnnotator\_test\_files"

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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 file (only one input file currently supported, later builds will incorporate multiple files to be merged). |
| -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+ |