

Aug 06, 2020

## **③** Database Preparation and Search for Protein Identification

DOI

dx.doi.org/10.17504/protocols.io.bjdwki7e

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**Document Citation:** avinash.kale 2020. Database Preparation and Search for Protein Identification. **protocols.io** <a href="https://dx.doi.org/10.17504/protocols.io.bjdwki7e">https://dx.doi.org/10.17504/protocols.io.bjdwki7e</a>

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Created: August 06, 2020



Last Modified: August 06, 2020

**Document Integer ID: 40086** 

**Keywords:** search, identification

## Troubleshooting



## **Database Preparation and Search for Protein Identification**

The mass-spec data were searched against the customized mosquito larvicidal toxin database which included well established larvicidal toxins like Cry1 to Cry73, Bin-A/B, VIP-1/2/3/4, and Mtx-1/2/3. (28). The default search parameters used for the analysis are as follows: (a) trypsin as the proteolytic enzyme with up to one missed cleavage; (b) peptide mass error tolerance of 20ppm; (c) fragment mass error tolerance of 0.1 Da; (d) carb-amidomethylation of cysteine as fixed modification (e) oxidation of methionine as a variable modification. A false discovery rate of 1% was applied while identifying the peptide-spectrum matches. Two criteria used to report a positive find, 1) reported protein/toxin should have least two numbers of peptides in amino acid sequence; and 2) the length of each of the identified peptide sequences using LC-MS has a minimum of 10 amino acids.