MFPaQ v4 documentation

1) Overview

MFPaQ (Mascot File Parsing and Quantification) is a software developed on the IPBS (Institut de Pharmacologie et de Biologie Structurale, Toulouse, France) proteomics platform and dedicated to parse, validate, and quantify proteomic data. It allows fast and user-friendly verification of Mascot result files, as well as data quantification using isotopic labeling methods (SILAC/ICAT) or label free approaches (spectral counting, MS signal comparison).

This web application provides a convenient interface to retrieve Mascot protein lists, sort them according to Mascot scoring or to user-defined criteria based on the number, the score and the rank of identified peptides, and to validate the results. The software extracts quantitative data from raw files obtained by nanoLC-MS/MS, calculates peptide ratios, and generates a non-redundant list of proteins identified in a multi-search experiment with their calculated averaged ratio.

Related publications:

1: Bouyssié D, Gonzalez de Peredo A, Mouton E, Albigot R, Roussel L, Ortega N, Cayrol C, Burlet-Schiltz O, Girard JP, Monsarrat B. Mascot file parsing and quantification (MFPaQ), a new software to parse, validate, and quantify proteomics data generated by ICAT and SILAC mass spectrometric analyses: application to the proteomics study of membrane proteins from primary human endothelial cells. Mol Cell Proteomics. 2007 Sep;6(9):1621-37.

2: Roux-Dalvai F, Gonzalez de Peredo A, Simó C, Guerrier L, Bouyssié D, Zanella A, Citterio A, Burlet-Schiltz O, Boschetti E, Righetti PG, Monsarrat B. Extensive analysis of the cytoplasmic proteome of human erythrocytes using the peptide ligand library technology and advanced mass spectrometry. Mol Cell Proteomics. 2008 Nov;7(11):2254-69.

3: Raymond AA, Gonzalez de Peredo A, Stella A, Ishida-Yamamoto A, Bouyssie D, Serre G, Monsarrat B, Simon M. Lamellar bodies of human epidermis: proteomics characterization by high throughput mass spectrometry and possible involvement of CLIP-170 in their trafficking/secretion. Mol Cell Proteomics. 2008 Nov;7(11):2151-75.

4: Stanislas T, Bouyssie D, Rossignol M, Vesa S, Fromentin J, Morel J, Pichereaux C, Monsarrat B, Simon-Plas F. Quantitative proteomics reveals a dynamic association of proteins to detergent-resistant membranes upon elicitor signaling in tobacco. Mol Cell Proteomics. 2009 Sep;8(9):2186-98.

5: Bousquet-Dubouch MP, Nguen S, Bouyssié D, Burlet-Schiltz O, French SW, Monsarrat B, Bardag-Gorce F. Chronic ethanol feeding affects proteasome-interacting proteins. Proteomics. 2009 Jul;9(13):3609-22.

6: Mouton-Barbosa E, Roux-Dalvai F, Bouyssié D, Berger F, Schmidt E, Righetti PG, Guerrier L, Boschetti E, Burlet-Schiltz O, Monsarrat B, Gonzalez de Peredo A. In depth exploration of cerebrospinal fluid by combining peptide ligand library treatment and label free protein quantification. Mol Cell Proteomics. 2010 Jan 21.

2) <u>Download and installation instructions</u>

2.1 Download
The application can be downloaded from http://sourceforge.net/projects/mfpaq

2.2 Prerequisites

MFPaQ is a web-based application that runs on a windows server. Its installation needs some other softwares/packages to be installed:
- IIS (Internet Information Services): this is a component of Windows that can be installed from the "add/remove programs" (configuration panel). Select "add/remove windows components", check "IIS", click "next" and validate (you may have to insert the Windows CD-ROM).
- Mascot server 2.1 or above
- Perl 5.8 or above.

On the client side, users must use Mozilla Firefox as the web-browser.

2.3 Installation

2.3.1 Execution of the automated-installer

The downloaded file is an executable that will help you through the installation process. When it is launched, it detects the presence of the needed software and copies the application files in the appropriate directory. It will show a message if a needed component is absent. The application will be installed in the web-server "root" directory ("C:\Inetput\wwwroot" by default).
MFPaQ is built on a system called PAIRS (Proteomic Analyses Integrating and Reporting System). The MFPaQ application directory has thus been named "pairs".
By default MFPaQ data are stored in the "data" sub-directory of the application root (this should be "C:\Inetput\wwwroot\pairs\data"). The installer will ask you if you want to use the default location or to define another one ("D:\mfpaq_data" for instance).

2.3.2 Windows configuration

Decimal symbol as to be the point character (see windows regional settings).
Important: the windows group "User" needs to have a total control on the "config" and "data" directories.

3) <u>Getting started</u>

The user interface is accessible via a web browser. Currently only Mozilla Firefox is supported. The application is available by typing in the address bar the name of the mascot web-server followed by '/pairs/www' (EX: **http://myserver/pairs/www** ; this assumes that Mascot is available at **http://myserver/mascot**).

The MFPaQ home page will be displayed and you are ready to use the application. To get started, follow theses steps:

1. Log-in using the admin account (user=admin password=admin). Create an empty project and then go in "Configuration => Administration". In the "Users" tab you can create new user accounts. Click on "New User" and fill the form to create a new one.

2. Log-in using the recently created user account. Create a new project and select it. Note: you can only select one project at a time (it is equivalent to a session). Once selected you can create and browse datasets for the corresponding project.

3. In order to parse Mascot dat files, you need first to set up the parsing criteria that you wish to use. Go in "Configuration => Parameters" and configure your profile by setting the filters based on Mascot criteria.
The MFP module will extract protein entries from Mascot files and rank them according to either the Mascot "Standard scoring" or "Mudpit scoring". Choose which type of scoring you wish to apply.
In order to facilitate manual validation, the software applies to the proteins of the list a two-colour code related to the filtering rules defined by the user under its configuration profile. Proteins that passed the "validation criteria" are displayed in green. They can be considered as confident hits that do not need further verification and will automatically be checked in the validation window. Proteins that meet the "exclusion criteria" are discarded and are not displayed in the list. All other proteins, which are considered as ambiguous identifications, appear in red and can be manually verified by the user.
The filtering rules used for the classification of a protein in green and red are based either on the protein score defined in Mascot (Click on "Filter according to protein scores"), or on multiple criteria related to the peptide matches assigned to this protein (Click on "Filter according to peptide scores"). In the first case, the software basically displays in green colour the "significant hits" list given in the Mascot (proteins with total scores higher than the significance threshold, which depends on the database size and is calculated by default with the probability for a match to occur at random with a probability of less than 5%). In the other case, if you choose to apply criteria based on the number, the rank, and the score of the peptide matches assigned to a protein, the proteins displayed in MFPaQ will still be ranked according to Mascot scoring, but only proteins matching this criteria will appear in green and will be automatically validated. Proteins that do not fulfill these criteria, while being in the significant list of Mascot, will appear in red.
You can also use computer defined criteria if you performed a decoy search (decoy option must be enabled in your Mascot search parameters). Currently this mode doesn't work at the data extraction step. This means that you will have to apply this automatic validation after the extraction process.
Once you have chosen which criteria you want to apply, click the "save" button.

4. You can now extract and validate your Mascot results. Go in "Experiment manager => Create a new experiment". An experiment corresponds to a set of Mascot result files. You can provide a name and a description for an experiment. You have to give the numbers of the mascot result files you want to include in the experiment.

5. Once created, the experiments are available through "Experiment manager => Experiment Browser". Here you can browse identification results and use some Macros and Exporters on your datasets.

Some help is directly available in the application (mainly at the bottom of the pages).

If you have some trouble regarding the installation process or the use of the program you are invited to contact the developer at mfpaq@ipbs.fr.