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User Guide for SILVER

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1 INTRODUCTION

SILVER is an efficient tool for quantification of the stable isotope labeling mass spectrometry data with novel methods for quality control of quantification implemented at spectrum, peptide and protein levels respectively. Several new confidence filters and indices are applied to improve the accuracy of quantification results. Additionally, SILVER provides user-friendly interfaces for parameter setting, quantitative analysis, results visualization and manual validation as well as some useful statistics analyses. The core of SILVER was developed on the platform of Microsoft Visual C++ 2005 in Windows system. SILVER can be run directly on any Windows system (Microsoft Windows 7/Vista/2003/XP/2000, 64 bit version or 32 bit version). And the interfaces of SILVER was implemented in Java.

SILVER is freely available under the GNU General Public License v3.0 at http://bioinfo.hupo.org.cn/silver or https://sourceforge.net/projects/silver-bprc/

2 RUN SILVER

Before running SILVER, the JDK 7 or higher is required. Users can download it from: http://www.oracle.com/technetwork/java/javase/downloads/index.html. And for processing Thermo raw files, some computers need to install Xcalibur v2.2. Thus we highly recommend the public format mzXML files for quantification.

After installing the JDK, double click "SILVER.jar" then the interfaces of SILVER for parameter setting is shown in Fig. 1. A configuration file including input data and quantification parameters is necessary for SILVER to start quantification. Here, SILVER can provide two ways for making a configuration file.

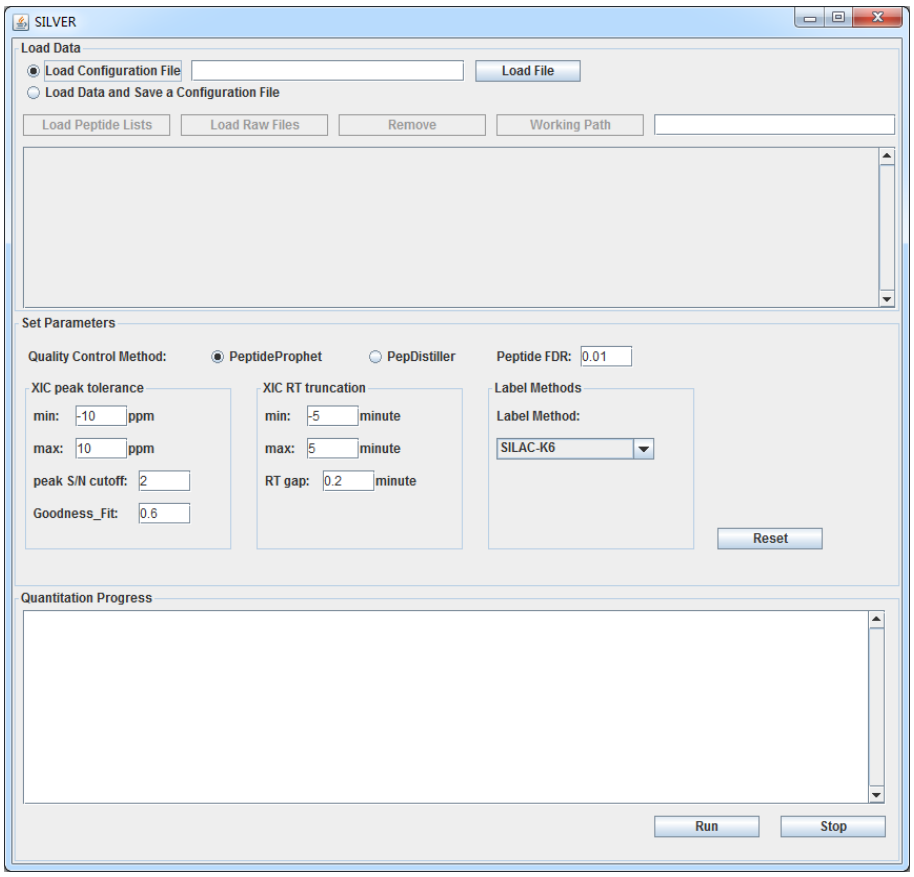

Figure 1: The interface for quantification

2.1 Loading an existing configuration file

The contents of the configuration file can be found in http://bioinfo.hupo.org.cn/silver.html. A user can write down his own configuration file using text editors such as Microsoft Word. If the user have an existing configuration file (*.config), then he can directly load this configuration file and start to run SILVER (Fig. 2).

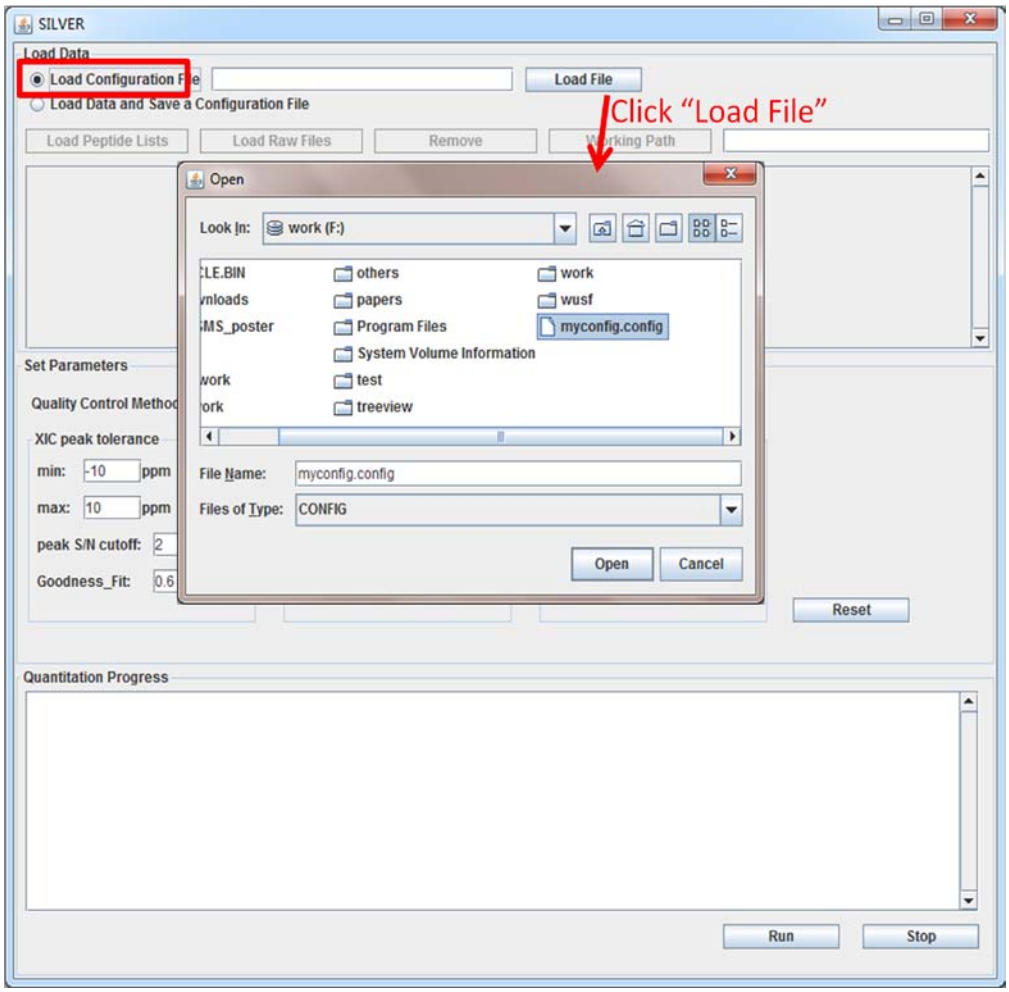

Figure 2: Load configuration file manually

2.2 Setting parameter and generating a new configuration file

The user can set parameters according to the interface in Fig. 3; then SILVER can generate a new configuration file and start to run.

Firstly, the mass spectrometry (MS) data and the high confident identification results are required to load into SILVER by clicking the buttons of "Load Raw Files" and "Load Peplist Files" (Fig. 3). Multiple files are allowed to choose at one time. The thermo raw files and public format mzXML files are supported by SILVER for now. Other format MS data can be processed in SILVER by converting the data format to mzXML. On the other hand, SILVER can take PeptideProphet's interact.pep.xml file (TPP version 4.5.2 and later versions) (Keller, et al., 2002) or PepDistiller's peplist file

(Li, et al., 2012) as input files. The "remove" button are used in case that some wrong files are loaded by mistake. Additionally, PepDistiller is developed in our lab for quality control and freely available at: http://www.bprc.ac.cn/PepDistiller/.

Secondly, click the "Working path" button and choose a file folder as the working path where the final results of SILVER will be saved. Then, the new configuration file will be generated in the working path you choose named by the system time (Silver-YY-MM-DD-HH-MM-SS.config). And quantification results of SILVER will be stored there.

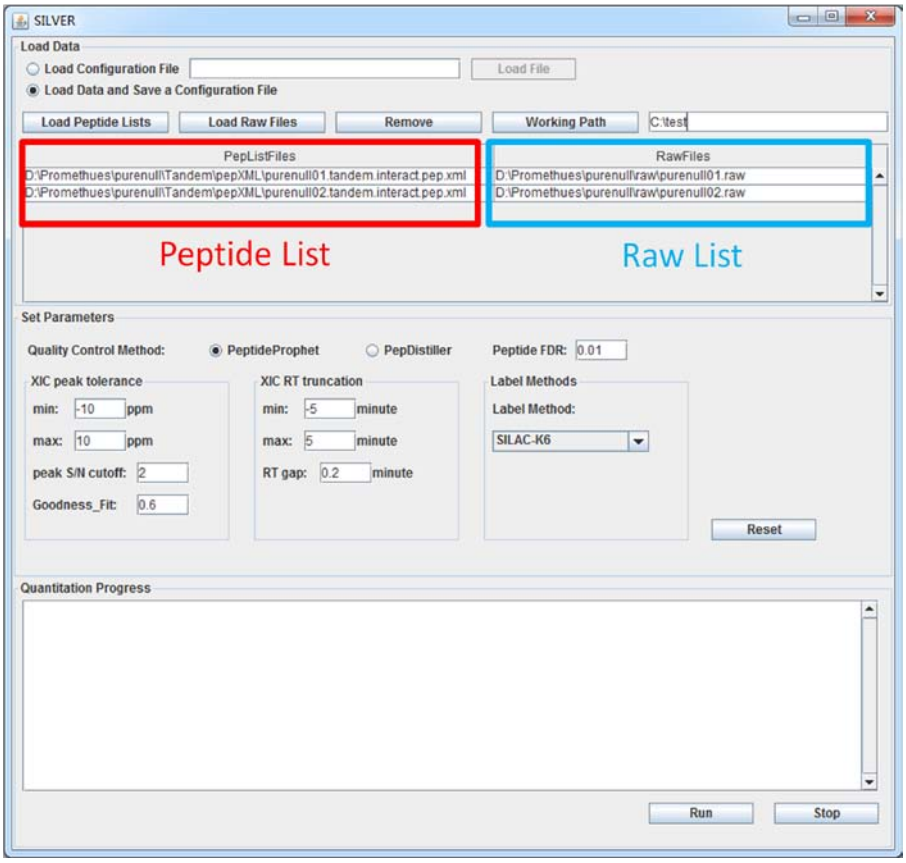

Figure 3: Set parameters and make a new configuration file

Thirdly, some necessary quantification parameters are required to be filled manually. Besides, the default settings are recommended for general quantification. The corresponding parameters are listed in Table 1.

Table 1. Annotations of parameters in SILVER

Parameters	Annotations
Working_path	the path to store the results of SILVER
Quality_Control_Method	PepDistiller and Peptideprophet are supported by SILVER for now.
Peptide_FDR	The FDR at peptide level, 0.01 is recommended.
Peak_ppm_min	The peptide m/z tolerance in ppm used during XIC construction, -10 is recommended.
Peak_ppm_max	The peptide m/z tolerance in ppm used during XIC construction, 10 is recommended.
SN_cutoff	The isotope signal-to-noise ratio cutoff during XIC construction, 2 is recommended.
Goodness_Fit	The goodness of the match between the observed and theoretical isotopic distribution, 0.6 is recommended.
RT_min	The peptide retention time minimum (in minutes) during XIC construction, -5 is recommended.
RT_max	The peptide retention time maximum (in minutes) during XIC construction, 5 is recommended.
RT_gap	The peptide retention time gap (in minutes) during XIC construction, 0.2 is recommended.
Label_Method	The label method in the experiment. SILAC-K6, SILAC-K6R6, SILAC-K8R10, 18O, 15N are supported in SILVER for now.

Finally, click "run" and the new configuration file are saved. Then SILVER starts to quantify the data listed in the configuration file. When the quantification is done, a pop-up message box comes up with words "SILVER done" and the quantification summary will be shown in the interfaces (Fig. 4).

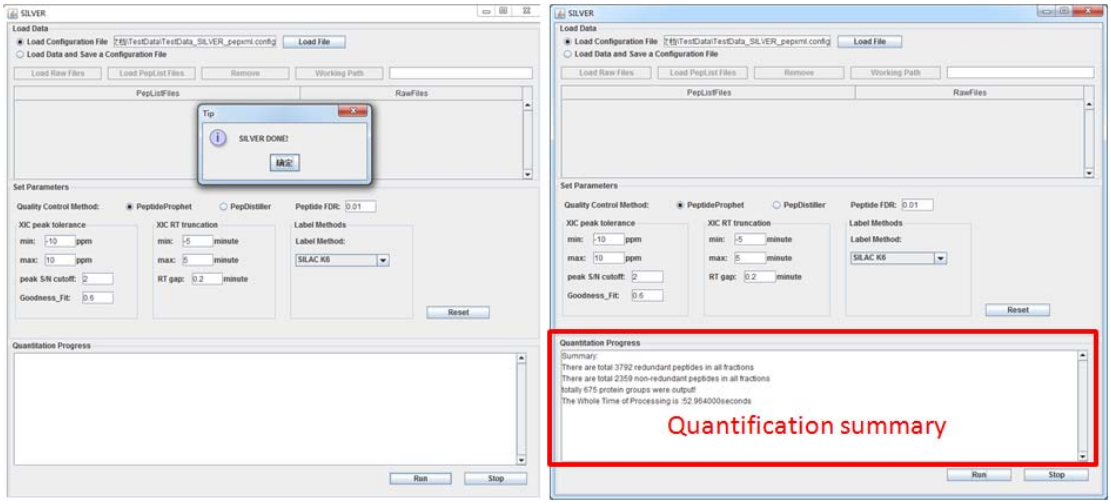

Figure 4: The quantification summary interface of SILVER

3 OUTPUT FILE FORMATS

Once the quantification is done, SILVER generates two folders in the working path which is set in the configuration file. One is "XIC_folder" where the retention time and intensity of every PSM's XIC are stored. The other folder is "quant_results" where the quantification results of SILVER are kept. The results of SILVER include four files (Table 2).

Table 2. Annotations of SILVER quantification results

File name	Annotations
allquant.log	General summary of the whole quantification
allquant.rPep.txt	List of redundant peptide (rPep) quantification results
allquant.nrPep.txt	List of non-redundant peptide (nrPep) quantification results
allquant.pro.txt	List of protein quantification results

Click the "SILVER-view.jar" and choose the working path in a pop-up message box (Fig. 5), then the interface for results visualization will be shown (Note: the working path is just the same path in the config file which means the parent directory

of both the "XIC_folder" and "quant_results" folders).

The protein list and the corresponding peptide list as well as their spectra list are shown in the three tables (Fig. 6). These flexible table can be sorted and permuted according to the labels in the first row.

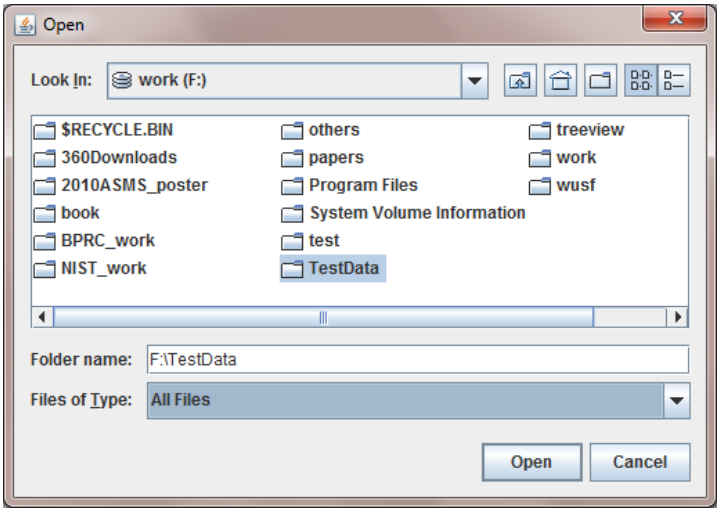

Figure 5: the message box for choosing the working path

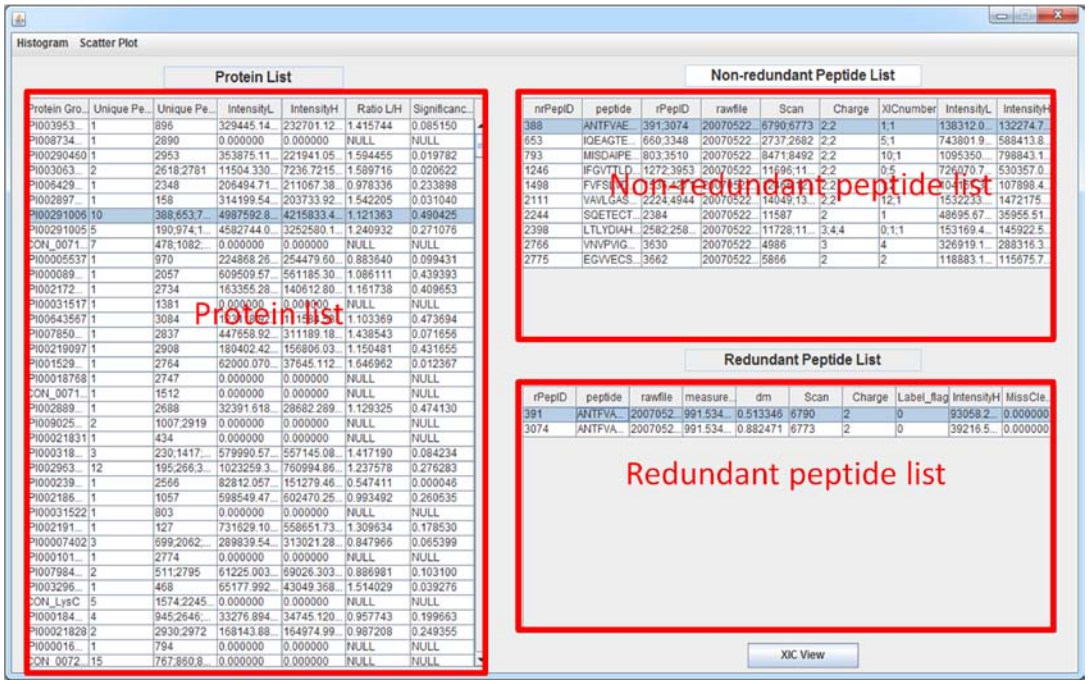

Figure 6: interface of SILVER-view for results visualization

4 RESULTS VISUALIZATION

Unlike common quantification tools which only give out the quantification results, SILVER could provide users a friendly interface to show the protein list and its corresponding peptides and spectra (Fig. 6). If users would like to search the peptides of a protein, all he need is just to double-click the protein in the protein list. So does in the nrPep list.

Some necessary statistic analyses such as the histogram and scattergram of the protein or peptide quantification distribution by clicking the menu "histogram" and "scattergram" (Fig. 7). What's more, in order to validate the quantification results, SILVER could also view the un-labeled and labeled XIC of each PSM (Fig. 8) by clicking the button of "XIC view". The data visualization especially the XIC plot could be helpful for further validation and analysis of specific proteins. In addition, the significance test algorithm(Cox and Mann, 2008) for differential expressed proteins mentioned in MaxQuant was implemented in SILVER.

Note: all the figures generated by SILVER-view are able to be zoomed in or out by clicking and dragging the mouse.

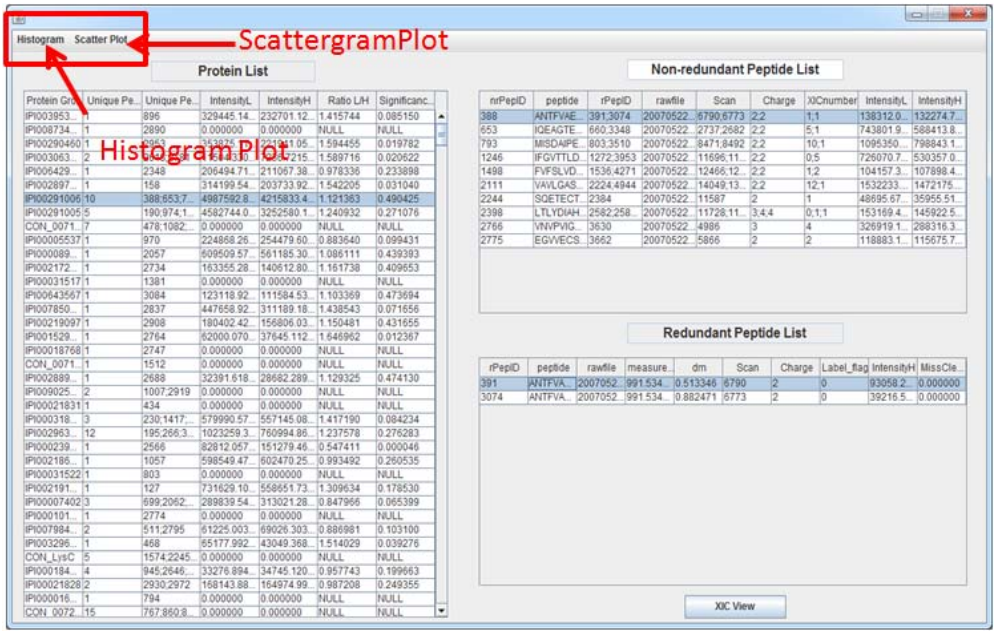

Figure 7: the histogram and the scatter gram analyses

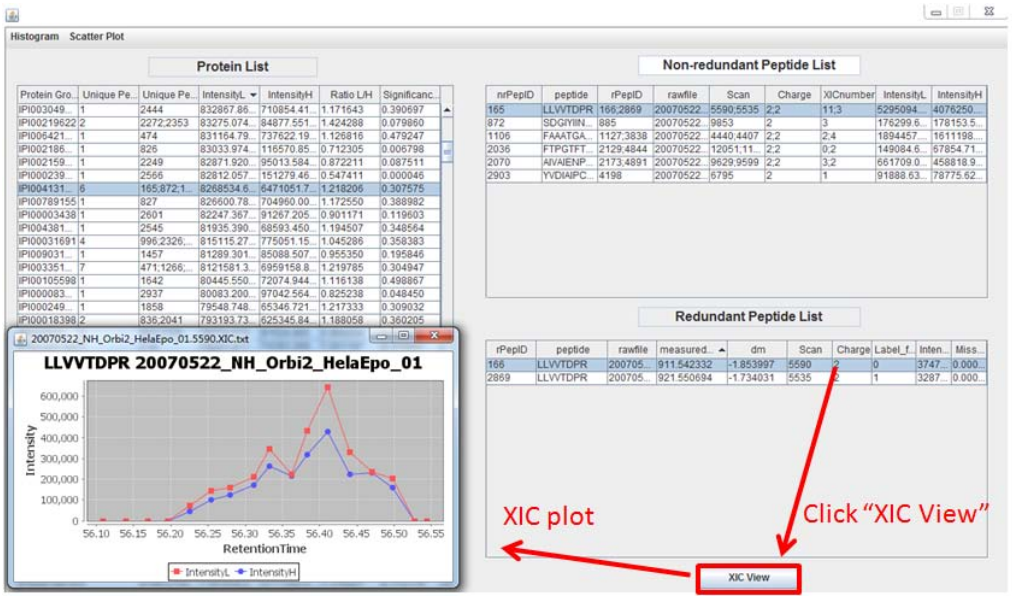

Figure 8: XIC view of each peptide

5 REFERENCES

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Keller, A., *et al.* (2002) Empirical statistical model to estimate the accuracy of peptide identifications made by MS/MS and database search, *Anal Chem*, **74**, 5383-5392.

Li, N., *et al.* (2012) PepDistiller: A quality control tool to improve the sensitivity and accuracy of peptide identifications in shotgun proteomics, *Proteomics*.