Comparison of Label Free Quantification Tools

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Introduction

With the development of high resolution mass spectrometry and liquid chromatographic (LC) separation techniques in recent years, the label-free quantitative method has greatly improved its sensitivity and accuracy. However with the increasing scale and complexity of quantitative proteomics studies, the subsequent data analysis becomes more and more challenging. Many computational tools and work flows have been developed to meet this challenge. In this poster, we compare a newly developed tool called PEAKS Q with the popular free software package MaxQuant [1]. The evaluation will focus on two aspects: the quantification linearity test and the sensitivity test for proteins.

Methods

We designed two different experiments to test the performance of two software packages. First experiment focused on testing the quantification linearity of the tools. It used a series of samples which contain the same set of proteins with different abundance across the samples. Then the correlation between the detected intensity and protein abundance is compared for two packages. Second experiment tried to test the ability to pick out proteins with significant change from a huge set of unchanged background proteins. A series of UPS-1 protein samples of different abundance were spiked in the same yeast lysate. We then compared the number of UPS-1 protein among the top 50 significant proteins reported by the two packages. PEAKS 7 and MaxQuant 1.4.1.2 software package are used for testing.

UPS2 Data [2]. In this experiment, UPS2 proteomic dynamic range standard set manufactured by Sigma-Aldrich was analyzed at 11 specified amounts ranging from 10 fmol/μL to 1500 fmol/μL by Thermo's LTQ Orbitrap mass spectrometer. Each of UPS2 samples was analyzed three to seven times. In total 38 LC-MS/MS runs were generated. Protein identification was then performed by PEAKS DB [3] and Andromeda search engine in MaxQuant. 38 LC-MS/MS runs were searched against fasta database with 48 UPS protein with the original contaminant database included in the MaxQuant package. The quantification were then performed by PEAKS Q and MaxQuant.

CPTAC Study Data. This data set is from "Clinical Proteomic Tumor Analysis Consortium" study 6 [4]. The samples were analyzed by four Thermo LTQ and four orbitrap instruments. Only four orbitrap data (OrbiO@65, OrbiP@65, OrbiW@56, Orbi@86) were used in our analysis. The samples were prepared like this: a yeast lysate was spiked with a mixture of 48 human proteins (Sigma-Aldrich UPS1) at five levels: 0.25, 0.74, 2.2, 6.7, and 20 fmol/μL, each 3-fold higher than the last. Each sample was then analyzed three times by each instrument.

From low level to high level, all 15 runs for each instrument were grouped into 5 groups: A, B, C, D, E, each contained triplicate runs for one sample. PEAKS DB and Andromeda were used to do protein identification. PEAKS Q and MaxQuant were used to do quantification.

Result

Quantification Linearity Test. The measured intensity of proteins should have linear response to its real abundance in the sample. The first measurement for the linearity is Pearson's r value. The second measurement evaluate the signal response under each abundance level for all protein. Since different proteins cannot be compared at the intensity level, for each protein the reported intensity values are normalized to their sum. Let $(I_1, I_2, \ldots, I_k)$ be the reported intensity values for the protein. The normalized values are calculated as $(\frac{I_1}{S}, \frac{I_2}{S}, \ldots, \frac{I_k}{S})$. $S = \sum_{k=1}^{n} I_k$. Each normalized value is then the percentage of the abundance in the current run to the total abundance from all the runs. These percentage values are then comparable between different proteins. The median values of percentage under each abundance level are used as the second measurement for the evaluation of linearity. The UPS2 data set has the 48 UPS2 protein loaded with 11 specified abundance. PEAKS DB quantified 23 UPS2 proteins and MaxQuant quantified 22 UPS2 proteins. The results for UPS2 data are shown in Figure 1.

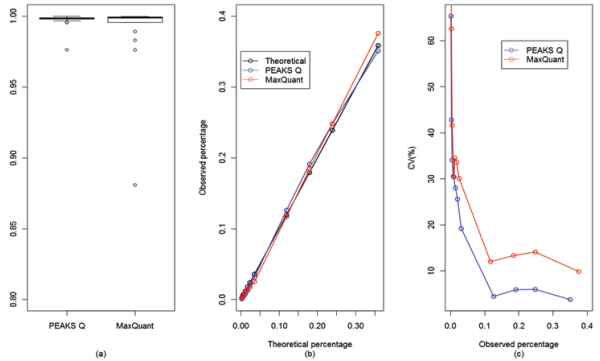

Figure 1. UPS2 data linearity test. (a) Boxplot for Pearson's r value. (b) Observed percentage compared to the theoretical percentage. (c) CV under different abundance levels.

Sensitivity and Accuracy of Protein Quantification. The ultimate goal of label free quantification is to find those proteins whose expression level changes across different biological samples among the background of many constant proteins. CPTAC study 6 provides a comprehensive data set to evaluate the efficiency of different scoring systems to find those significantly changed proteins. 48 UPS1 human proteins are spiked in a yeast lysate. From group A to group E, each with 3-fold change of human proteins compared to the group before it. Then all the human proteins are treated as the proteins with significant change. All the yeast proteins are treated as the constant background. Total 3 different scoring systems are evaluated: PEAKS Q significance, MaxQuant intensity value combined with t-test and MaxQuant intensity value combined with ANOVA test. The results of sensitivity and accuracy test are shown in Figure 2.

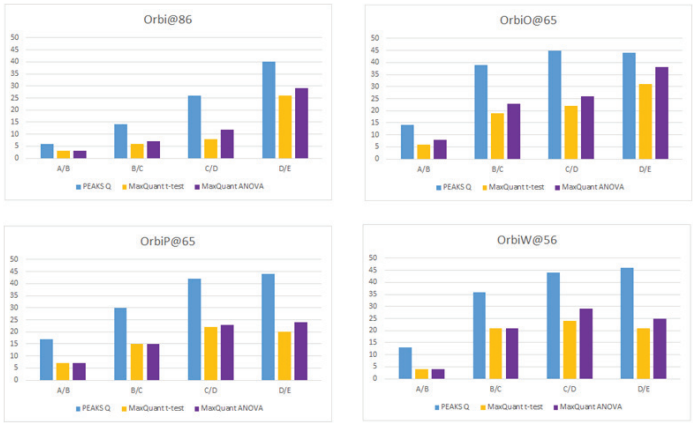

Figure 2. Gold standard for sensitivity evaluation.

Three scoring systems are evaluated on four sets of data. Each set of data has 15 runs divided into 5 groups: A, B, C, D, E. Evaluation is done in four pairs A/B, B/C, C/D, D/E. The numbers shown here are the number of human proteins in the top 50 most significant proteins under each scoring system.

Conclusion

In the sense of the accuracy of extracting intensity signals, both MaxQuant and PEAKS Q perform very well on the testing data sets. PEAKS Q might have slight advantage in smaller variances. But as to the sensitivity of quantification, with the novel design for significance calculation, PEAKS Q achieved great improvement in finding those significantly changed proteins.

References

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