**PROCEDURE**

Here we respectively introduce the functions and parameter configuration of the nine criteria that belong to classic computational criteria and functional criteria.

**In Classic Computational Criteria**

Click the ‘CLASSIC’ button.

**Precision**

**Function of the criteria**

We assume that the normal tissue near the tumor can approximately represent the normal state of the tumor tissue. Therefore, we use the paired tumor tissue and the normal tissue near the tumor as the gold standard to evaluate the performance of the DEAs. Proteome abundance datasets containing paired matched tumor tissues and adjacent normal tissues were used to evaluate the DEAs (RankComp v1/v2, PenDA, Peng method, Quantile, T-test and Wilcoxon signed-rank test).

**The meaning of the parameters**

There are seven methods in the method list. RankComp represents RankComp v1/v2, and the other options also each represent a method.

**How to get sample results**

1) Go to the ‘Precision’ tab.

2) Go to the ‘parameters’ tab.

3) Follow the prompts to upload the corresponding five files.

4) Choose the methods you want.

5) Click the ‘CONFIRM’ button.

6) The chart after running will appear on the right.

**Type One Error**

**Function of the criteria**

Since there were no real tumor DEPs between samples of normal tissues, null data was constructed from normal tissue samples to evaluate the ability to control Type one error of algorithm. The individualized DEAs in IDEPA were used to analyze the null dataset to predict the individualized DEPs. In RankComp v1/v2, T-test, and Wilcoxon, the false discovery rate (FDR) was set to 0.05. Next, calculate the false-positive rate of the DEAs in each sample of each null dataset.

**The meaning of the parameters**

1) There are five methods in the method list. RankComp represents RankComp v1/v2,and the other options also each represent a method.

2) The nd\_sample\_size means null data with sample size.

3) The log\_label\_list, na\_label\_list and data\_label\_list are lists of the corresponding labels. It should be noted that the number of list elements entered in each parameter must be the same as the number of files entered, otherwise it will cause an error.

**How to get sample results**

1) Go to the ‘Type One Error’ tab.

2) Go to the ‘parameters’ tab.

3) Follow the prompts to upload the corresponding three files.

4) Choose the methods you want.

5) Modify the four parameters according to your needs.

6) Click the ‘CONFIRM’ button.

7) The chart after running will appear on the right.

**Stable pairs**

**Function of the criteria**

We used REOA package (https://github.com/pathint/reoa) to identify significantly stable REOs of protein pairs in a type of normal tissue samples accumulated from different laboratories.

**The meaning of the parameters**

1) If label\_specific\_protein is true, the output consistent pair and reversed pair must contain a specific protein and this specific protein is in the specific\_protein\_path file. If label\_specific\_protein is false, it is not required.

2) The sp threshold refers to the threshold of the number of stable pairs, reversal pairs, and consistent pairs.

3) The n\_visual refers to how many consistent pairs and reversal pairs will be provided for the user to choose.

**How to get sample results**

1) Go to the ‘Stable Pairs’ tab.

2) Go to the ‘parameters’ tab.

3) Follow the prompts to upload the corresponding four files.

4) Modify the four parameters according to your needs.

5) Click the ‘CONFIRM’ button.

6) The chart after running will appear on the right.

**Parameter Influence**

**Function of the criteria**

In individual-level DEAs, normal tissue samples provide an essential reference for difference analysis of tumor tissue samples. Generally, the stability of the reference group increases as the number of normal tissue samples increases. Also, as the number of proteins increases, the number of differential proteins also increases. We use real protein abundance data to analyze the influence of normal tissue sample size on the

performance of the DEAs.

**The meaning of the parameters**

1) There are five methods in the method list. RankComp represents RankComp v1/v2,and the other options also each represent a method.

2) The sample size label option indicates whether to evaluate the sample size.

3) The n protein label indicates whether to evaluate the amount of protein.

4) The sample size list is the number gradient of sample size.

5) The n protein list means the number of proteins.

**How to get sample results**

1) Go to the ‘Parameter Influence’ tab.

2) Go to the ‘parameters’ tab.

3) Follow the prompts to upload the corresponding four files.

4) Choose the methods you want.

5) Modify the four parameters according to your needs.

6) Click the ‘CONFIRM’ button.

7) The chart after running will appear on the right.

**Robustness Individual**

**Function of the criteria**

The robustness evaluation of the difference expression algorithm focuses on the robustness of the method when it was applied to the same tumor tissue sample but the normal tissue sample was different. When there were real difference proteins in tumor tissue samples, no matter what normal tissue sample was used as the reference group, the real difference proteins should be able to be detected. Therefore, the difference expression results obtained from different reference groups for the same tumor tissue sample should be highly consistent. When the result consistency was not high, it means that the algorithm was obviously affected by the reference group, and the robustness of algorithm was low.

The DEAs was used to obtain the individual-level DEAs results of tumor samples in lung-Xu under these two reference groups. Based on the two difference expression results, the consistency score was calculated to compare the individual-level robustness of different algorithms on the lung dataset. Similarly, the datasets gastric-Ni and gastric-Ge were also used to compare the individual-level robustness of different DEAs on the gastric dataset.

**The meaning of the parameters**

1) There are four methods in the method list. RankComp represents RankComp v1/v2,and the other options also each represent a method.

2) The meaning of other parameters is the same as in Robustness Group.

**How to get sample results**

1) Go to the ‘Robustness Individual’ tab.

2) Other steps are the same as in Robustness Group.

**Robustness Group**

**Function of the criteria**

To compare the group-level robustness of DEAs under different datasets in the same organization, seven DEAs used to analyze lung-Xu and lung-Gillette (gastric-Ni and gastric-Ge). Then the individualized difference protein results were obtained. Through the binomial distribution test, the difference protein result at the group level can be obtained from the difference protein result at the individual level.

**The meaning of the parameters**

1) There are five methods in the method list. RankComp represents RankComp v1/v2, and the other options also each represent a method.

2) Robustness is the same algorithm that processes two kinds of data. The first three parameters represent the preprocessing parameters of the two pieces of data.

3) The group threshold is the threshold of the binomial distribution test P-value in the process of obtaining the differential expression results at the individual level to obtain the differential expression results at the group level.

4) The n\_cc refers to the length of the consistency curve.

**How to get sample results**

1) Go to the ‘Robustness Group’ tab.

2) Go to the ‘parameters’ tab.

3) Follow the prompts to upload the corresponding six files.

4) Choose the methods you want.

5) Modify the five parameters according to your needs.

6) Click the ‘CONFIRM’ button.

7) The chart after running will appear on the right.

**Similarity**

**Function of the criteria**

To compare the similarities between differential expression algorithms (RankComp v1/v2, PenDA Pro, T-test, Wilcoxon), we use five real datasets (lung-Xu, lung-Gillette, gastric-Ge, gastric-Ni, liver-Gao) to calculate the consistency between different differential expression algorithms. For each sample in each dataset, we calculate the consistency curve of the top 150 proteins in each pair of algorithms. Average the consistency curve of all samples in the same dataset to obtain the area under the consistency curve of each dataset. Similar to the robustness of the comparison algorithm, the consistency score was used as an indicator to compare the similarity between different algorithms.

**The meaning of the parameters**

1) There are four methods in the method list. RankComp represents RankComp v1/v2,and the other options also each represent a method. It should be noted that the four methods must be selected, otherwise it will cause errors.

2) The n\_cc refers to the length of the consistency curve.

**How to get sample results**

1) Go to the ‘Similarity’ tab.

2) Go to the ‘parameters’ tab.

3) Follow the prompts to upload the corresponding three files.

4) Modify the parameter according to your needs.

5) Click the ‘CONFIRM’ button.

6) The chart after running will appear on the right.

**In Functional Criteria**

Click the ‘FUNCTIONAL’ button.

**Pathway Enrichment**

**Function of the criteria**

The DEPs at the population level were often used as input for subsequent pathway enrichment analysis. DEPs at the individual level contains more information and can also be used for pathway enrichment analysis to determine significant pathways. First, the individual-level differential expression analysis algorithm was used to obtain the individual-level differentially expressed proteins. Then the binomial distribution test was used to obtain the group-level DEPs for subsequent pathway enrichment analysis. Finally, compare the number and the significance of pathways obtained by different individual-level DEAs.

**The meaning of the parameters**

1) There are three methods in the method list. RankComp represents RankComp v1/v2,and the other options also each represent a method.

2) The group threshold is the threshold of the binomial distribution test P-value in the process of obtaining the differential expression results at the individual level to obtain the differential expression results at the group level.

3) The qvalue thres is the qvalue thres paths from small to large among all the pathways obtained by a certain differential expression algorithm.

**How to get sample results**

1) Go to the ‘Pathway Enrichment’ tab.

2) Go to the ‘parameters’ tab.

3) Follow the prompts to upload the corresponding three files.

4) Choose the methods you want.

5) Modify the two parameters according to your needs.

6) Click the ‘CONFIRM’ button.

7) The chart after running will appear on the right.

**Survival Analysis**

**Function of the criteria**

To compare the ability of different DEAs to find prognostic proteins, we performed the following steps. First, the DEAs was used to obtain the DEPs at the individual level. Then we selected proteins whose differential expression ratios were within a specific range as candidate prognostic proteomes. For each protein in the candidate prognostic protein group, we divide patients into two groups according to whether the protein was a DEPs. Use the univariate Cox proportional hazard regression model with 5% FDR control, we obtained the prognostic proteins found in different datasets through different algorithms and obtain their survival curves. Finally, compare the number and significance of survival-related proteins obtained by different individual-level DEAs.

**The meaning of the parameters**

1) There are three methods in the method list. RankComp represents RankComp v1/v2,and the other options also each represent a method.

2) The function of survival threshold is as follows: When the differential expression ratio of a certain protein in all samples is within the range of (survival threshold[0],survival threshold[1]), the protein will be used in subsequent survival analysis.

**How to get sample results**

1) Go to the ‘Survival Analysis’ tab.

2) Go to the ‘parameters’ tab.

3) Follow the prompts to upload the corresponding four files.

4) Choose the methods you want.

5) Modify the parameter according to your needs.

6) Click the ‘CONFIRM’ button.

7) The chart after running will appear on the right.