*~Supplementary notes~*

# NAguideR: performing and prioritizing missing value imputations for consistent bottom-up proteomic analyses

Shisheng Wang[1], Wenxue Li[2], Liqiang Hu[1], Jingqiu Cheng[1], Hao Yang[1,*] and Yansheng Liu[2,3,*]

[1] West China-Washington Mitochondria and Metabolism Research Center; Key Lab of Transplant Engineering and Immunology, MOH, West China Hospital, Sichuan University, Chengdu, 610041, China

[2] Yale Cancer Biology Institute, Yale University, West Haven, CT, 06516, USA

[3] Department of Pharmacology, Yale University School of Medicine, New Haven, CT, 06520, USA

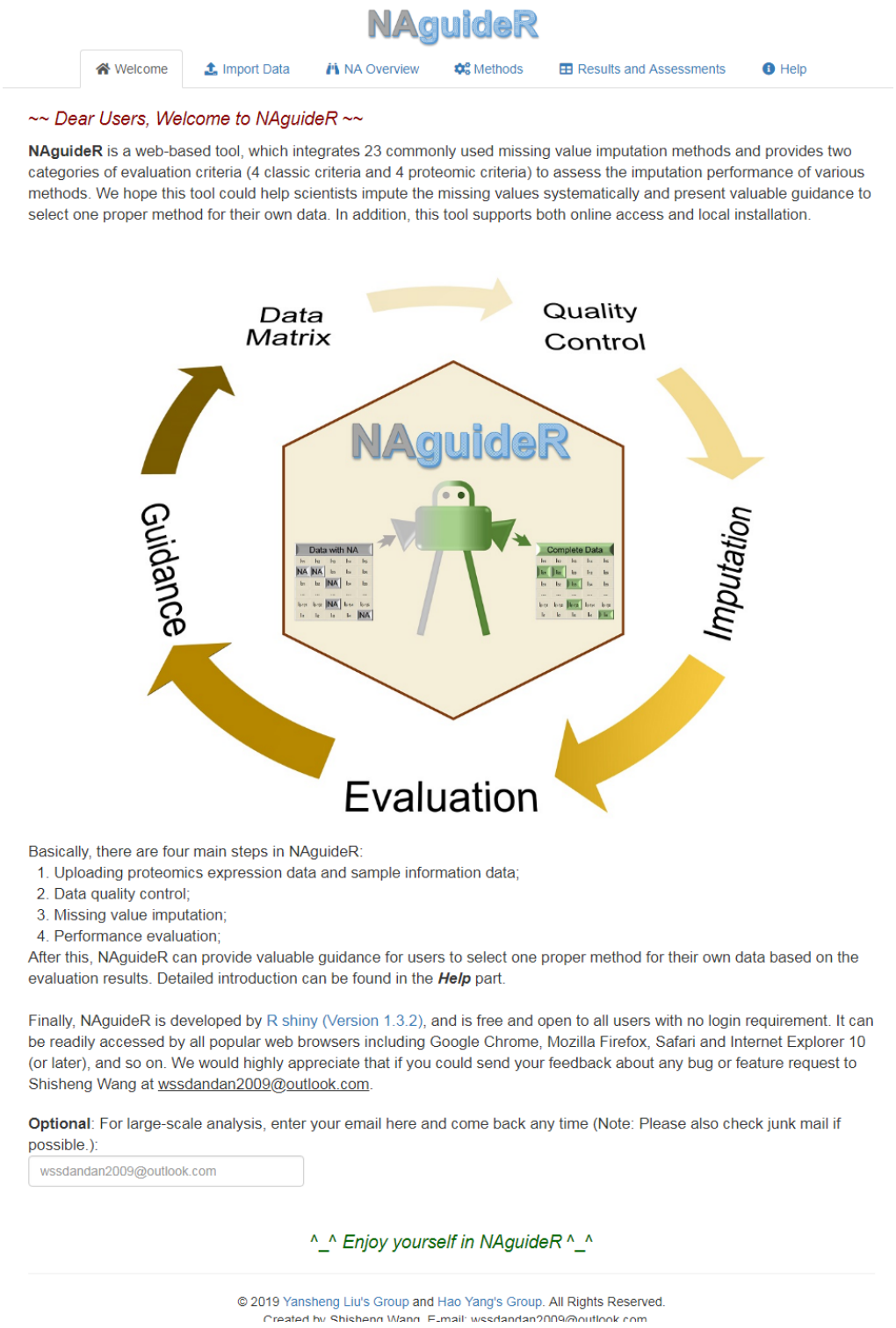
**Corresponding Author**

*Email address: yanghao@scu.edu.cn; yansheng.liu@yale.edu.

## Supplementary notes

NAguideR integrates up to 23 commonly used missing value imputation methods (described in Table S1) and provides two categories of evaluation criteria (four classic computational criteria and four empirical proteomics criteria) to assess the imputation performance of various methods. Here we present the detailed introduction and operation of NAguideR, by which users can follow to analyze their own data freely and conveniently.

Users can visit this site: http://www.omicsolution.org/wukong/NAguideR. Then the website homepage can be shown like this:

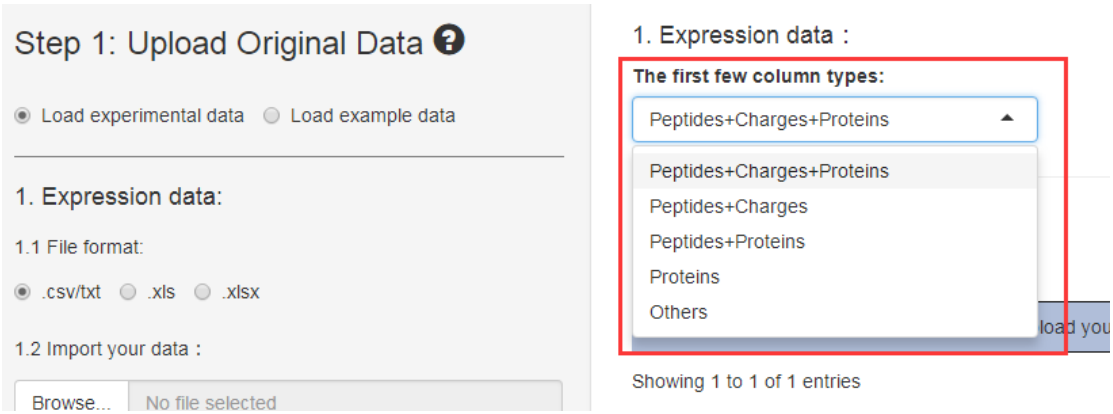


## 1. Data Preparation

NAguideR supports four basic file formats (.csv, .txt, .xlsx, .xls). Before analysis, users should prepare two required data: (i) Proteomics expression table for quantification and (ii) Sample information. The data required here could be readily generated based on results of several popular tools such as MaxQuant (1), PEAKS (2), Spectronaut (3), DIA-NN (4), OpenSWATH (5), and so on. The users then can upload the two data into NAguideR with right formats respectively and start subsequent analysis.

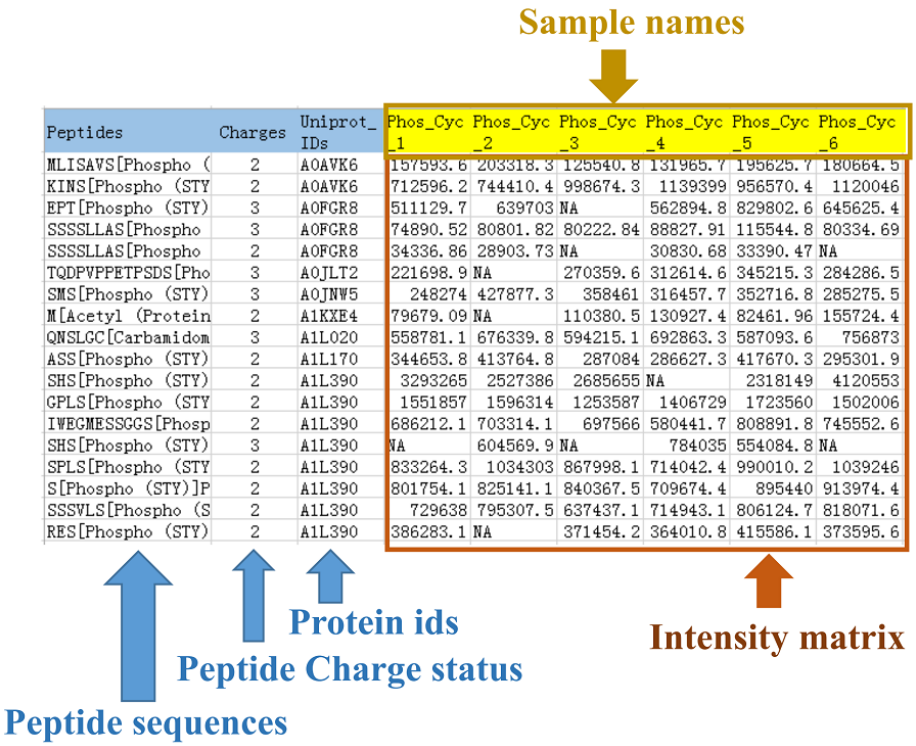
### 1.1 Expression data

There are currently four types of proteomics expression data supported in NAguideR (i.e., 'Peptides+Charges+Proteins', 'Peptides+Charges', 'Peptides+Proteins', 'Proteins'), among which the main differences are the first few columns. In addition, users may upload other kinds of omics data (i.e. Genomics, Metabolomics), they can just choose the fifth type ('Others'). Please note, the fifth type cannot generate the results based on those proteomic criteria.



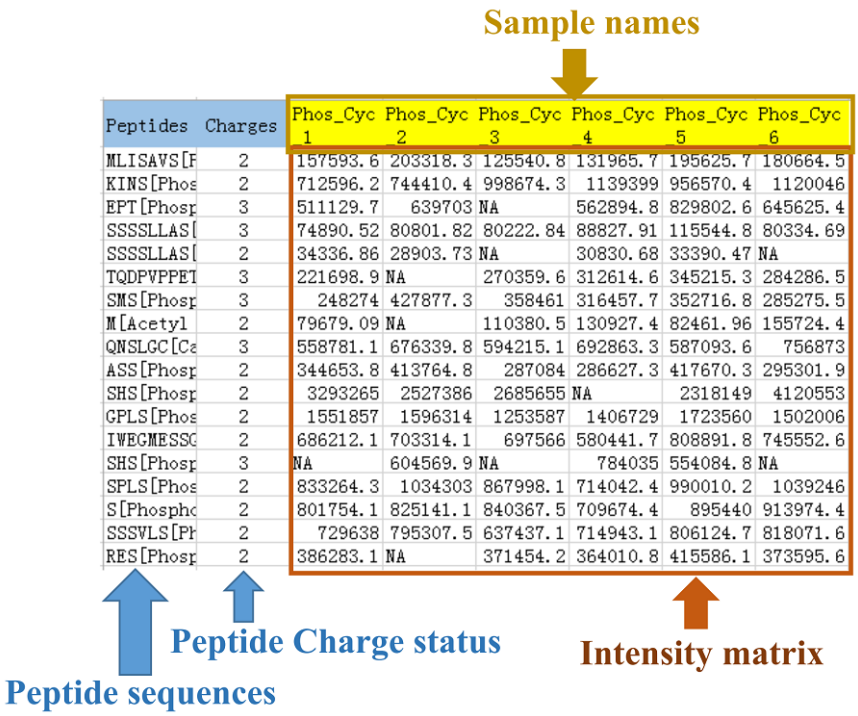

*1.1.1 Expression data with peptide sequences, peptide charge states, and protein ids*

In this situation, peptide sequences, peptide charge states, and protein ids are sequentially provided in the first three columns of input file. Peptide sequences in the first column can be peptides with any post-translational modification (PTM, written in any routine format) or stripped peptides (without PTM). The second column is peptide charge status. The protein ids in the third column should be UniProt ids. From the fourth column, peptides/proteins expression intensity or signal abundance in every sample should be listed. The data structure is shown as below:



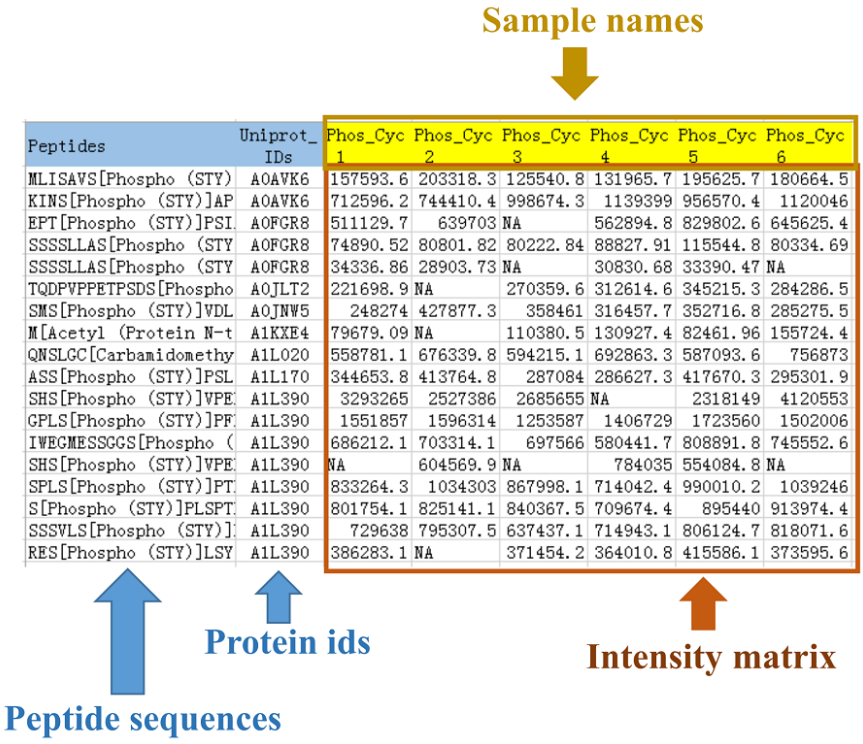

*1.1.2 Expression data with peptide sequences and peptide charge states*

Similar to the above situation, peptide sequences and peptide charge status are sequentially provided in the first two columns of input file. Peptide sequences in the first column can be peptides with post-translational modification (PTM, written in any routine format) or stripped peptides (without PTM). The second column is peptide charge states. From the third column, peptides/proteins expression intensity or signal abundance in every sample should be listed. The data structure is shown as below:



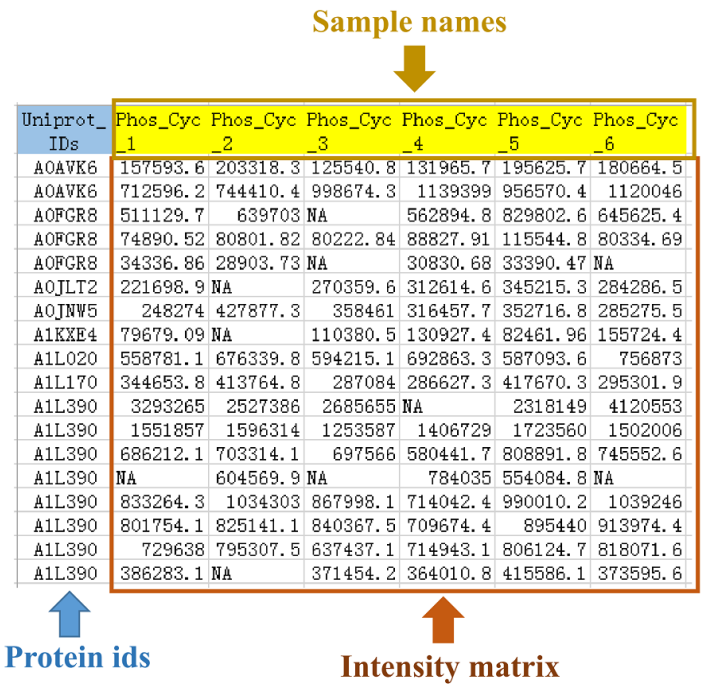
*1.1.3 Expression data with peptide sequences, and protein ids*

Under this circumstance, peptide sequences, and protein ids are sequentially provided in the first two columns of input file. Peptide sequences in the first column can be peptides with post-translational modification (PTM, written in any routine format) or stripped peptides (without PTM). The protein ids in the second column should be UniProt ids. From the third column, peptides/proteins expression intensity or signal abundance in every sample should be listed. The data structure is shown as below:



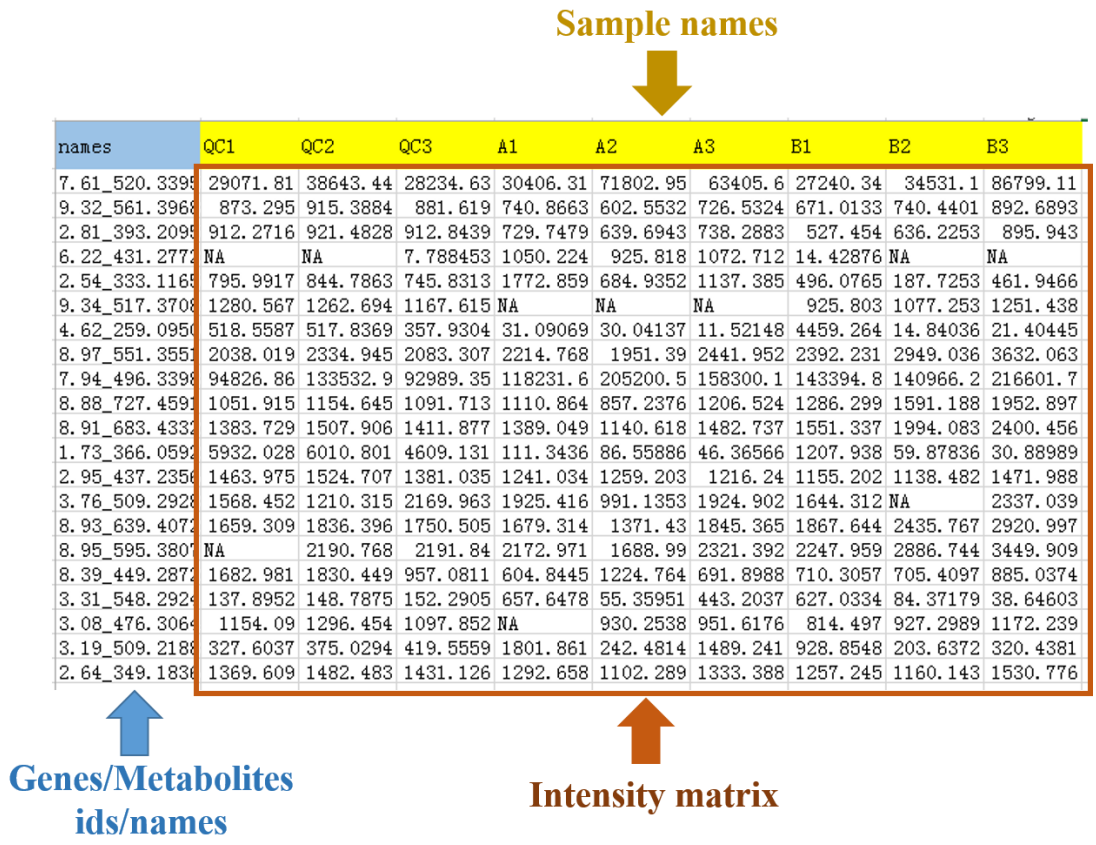

*1.1.4 Expression data with protein ids*

In this situation, protein ids are provided in the first two columns of input file. The protein ids here should be UniProt ids. From the second column, peptides/proteins expression intensity or signal abundance in every sample should be listed. The data structure is shown as below:



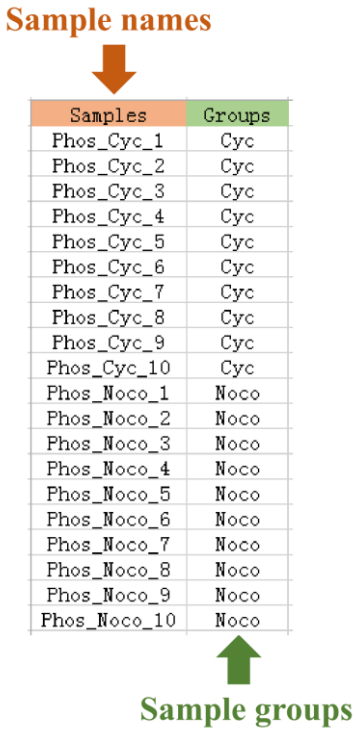

*1.1.5 Other kinds of omics data*

If users want to use NAguideR for other omics data (i.e. genomics, metabolomics), gene/metabolite ids/names should be provided in the first columns of input file. From the second column, genes/metabolites expression intensity or signal abundance in every sample should be listed. The data structure may be shown as below:



### 1.2 Samples information data

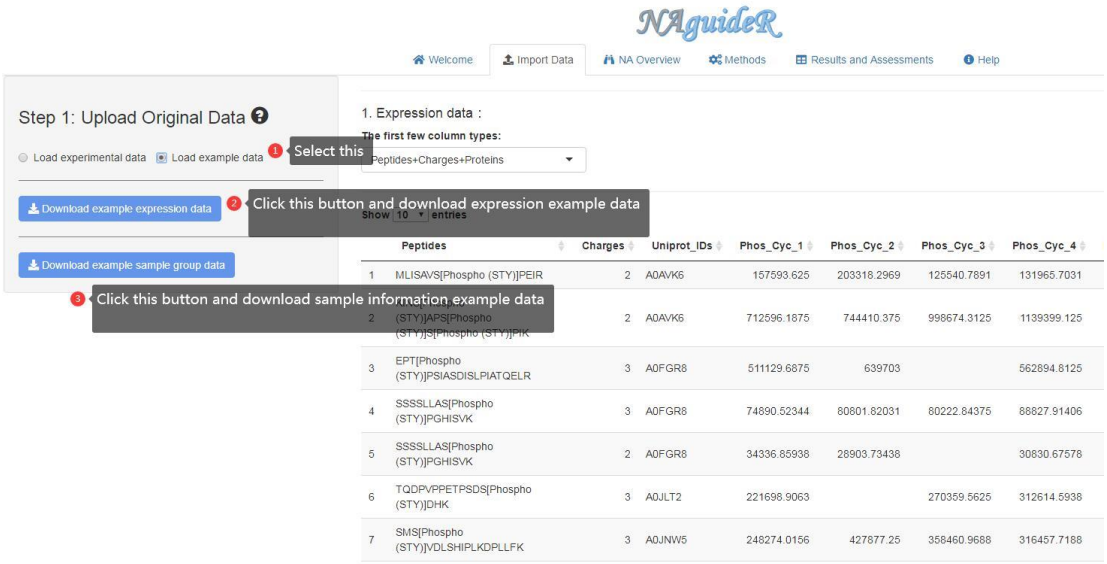
Sample information here means that users should provide sample group identity information. This information could e.g., enable filtration strategy for different group respectively in a later step (see below). The sample names are in the first column and their orders are same as those in the expression data. Group information is in the second column. The data structure is shown as below:




| Samples | Groups |
|---|---|
| Phos_Cyc_1 | Cyc |
| Phos_Cyc_2 | Cyc |
| Phos_Cyc_3 | Cyc |
| Phos_Cyc_4 | Cyc |
| Phos_Cyc_5 | Cyc |
| Phos_Cyc_6 | Cyc |
| Phos_Cyc_7 | Cyc |
| Phos_Cyc_8 | Cyc |
| Phos_Cyc_9 | Cyc |
| Phos_Cyc_10 | Cyc |
| Phos_Noco_1 | Noco |
| Phos_Noco_2 | Noco |
| Phos_Noco_3 | Noco |
| Phos_Noco_4 | Noco |
| Phos_Noco_5 | Noco |
| Phos_Noco_6 | Noco |
| Phos_Noco_7 | Noco |
| Phos_Noco_8 | Noco |
| Phos_Noco_9 | Noco |
| Phos_Noco_10 | Noco |

### 1.3 Download example datasets

If users want to download the example datasets to their own computer and check the data format locally, they can download them from here:




First, select “Load example data” and the example data will be shown on the right panel interactively. Users can visually observe what the data looks like.

Second, users can download the example data (expression data and sample information data) by clicking the corresponding button. The data are saved as .csv format and users can open them in other software, such as Excel.

## 2. Import data.

This is the first step, in which users should upload data here or load the example data to learn the data formats. By default, we use the example data to show each result of every step.

*2.1 Uploading data.* When users prepare their data (expression and sample information data set), they can upload these data from here:


There are two main panels: first, *parameters panel*, users can adjust parameters here; second, *results panel*, many results after users set the parameters will be shown here and users can also download these results.

In the *parameters panel* of “Import Data”, there are two choices for users:

*a. Load experimental data*. When users choose this option, they can upload their own data here. Users should select the right format based on their data and then click “Browse” button to import the data;

*First row as column names*: this means whether the first row is column names. If true, you should choose this parameter.

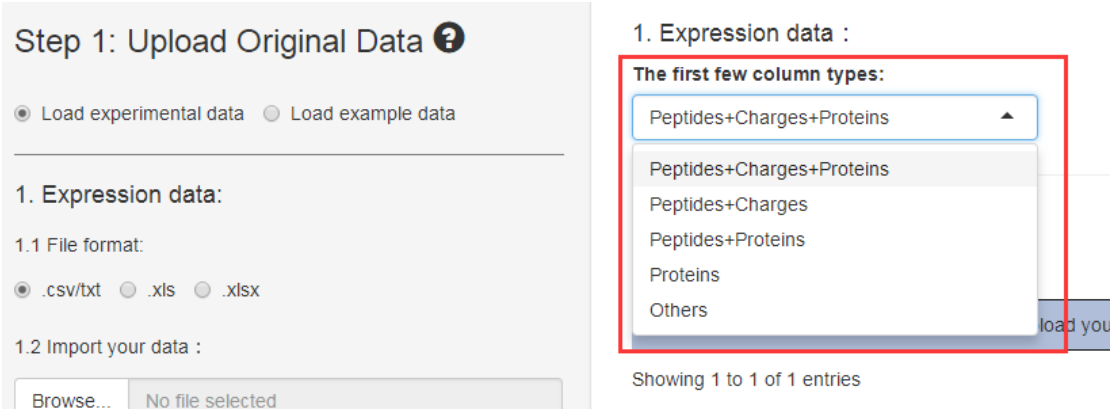
*First column as row names*: this means whether the first column is row names. If true, you should choose this parameter.

*b. Load example data*. As described in part 1.3, users can choose this option and download the example data to check them locally.

In the *results panel* of “Import Data”, if users don’t upload their data, here will show “NAguideR detects that you did not upload your data. Please upload the expression data (or sample information data), or load the example data to check first” to warn users.

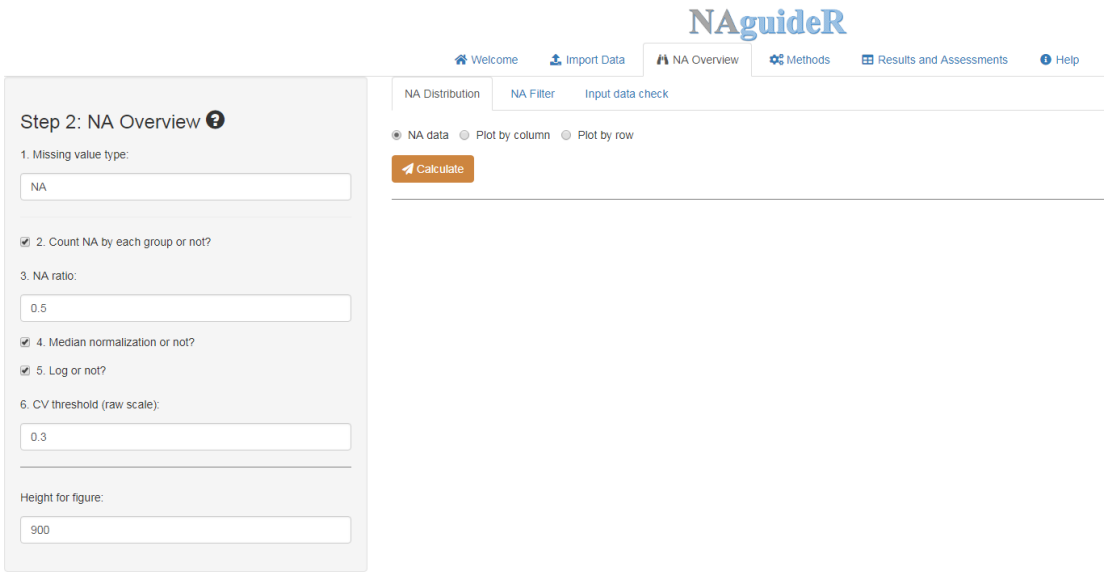
Before uploading expression data, users should also recognize which type their data belongs to and choose the right parameter by adjusting the “*The first few column types*”. The instruction of the

column types can be found above (*Data Preparation* part).

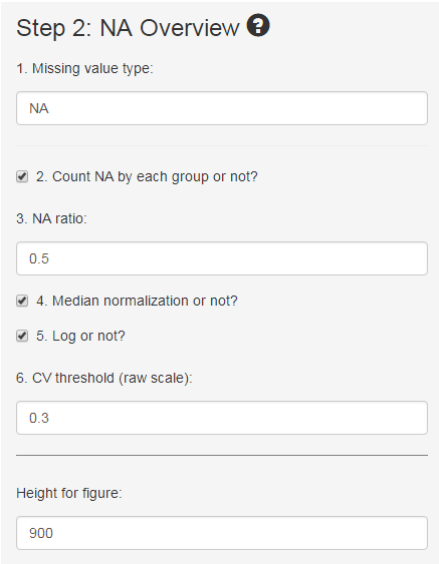


## 3. NA Overview

Users can check the missing value situation of their own data and filter those data with a high proportion of missing value in this step. Note, “NA” is short for Not Available, which means missing value here (see below).




### 3.1 Parameters




*1. Missing value type*: what the missing values look like in the expression data, for example, Spectronaut (6,7) software usually export “Filtered” as missing values, so users should change this parameter to “Filtered” if their data contain “Filtered”. NAguideR will recognize these characters and replace them with NAs. Any other characters indicating a missing value can be similarly defined.

*2. Count NA by each group or not*: if true, NAguideR will count the number of missing values in each group and calculate the NA ratio. Otherwise, it calculates the NA ratio across all groups, for example, as below:

| A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Peptides | Charges | Uniprot_IDs | Phos_Cyc_1 | Phos_Cyc_2 | Phos_Cyc_3 | Phos_Cyc_4 | Phos_Cyc_5 | Phos_Cyc_6 | Phos_Cyc_7 | Phos_Cyc_8 | Phos_Cyc_9 | Phos_Cyc_10 | Phos_Noc_1 | Phos_Noc_2 | Phos_Noc_3 | Phos_Noc_4 | Phos_Noc_5 | Phos_Noc_6 | Phos_Noc_7 | Phos_Noc_8 | Phos_Noc_9 | Phos_Noc_10 |
| M1Acetyl (Protein N-ter | 2 | A1KXE4 | 79679.09 | NA | 110380.5 | 130927.4 | 82461.96 | 155724.4 | 113495.3 | 136404.3 | 56171.31 | 98299.7 | NA | NA | 151027.6 | NA | 210179.9 | 182829.7 | 151424.3 | NA | NA | 181321.2 |

There are 2 groups (10 biological replicates in each group) here, if users select this parameter, NAguideR will calculate 2 NA ratios for this peptide (first group: 1/10=0.1, second group: 5/10=0.5), otherwise, only one NA ratio: 6/20=0.3.

*3. NA ratio*: the threshold of NA ratio. Those peptides/proteins with NA ratio above this threshold will be removed.

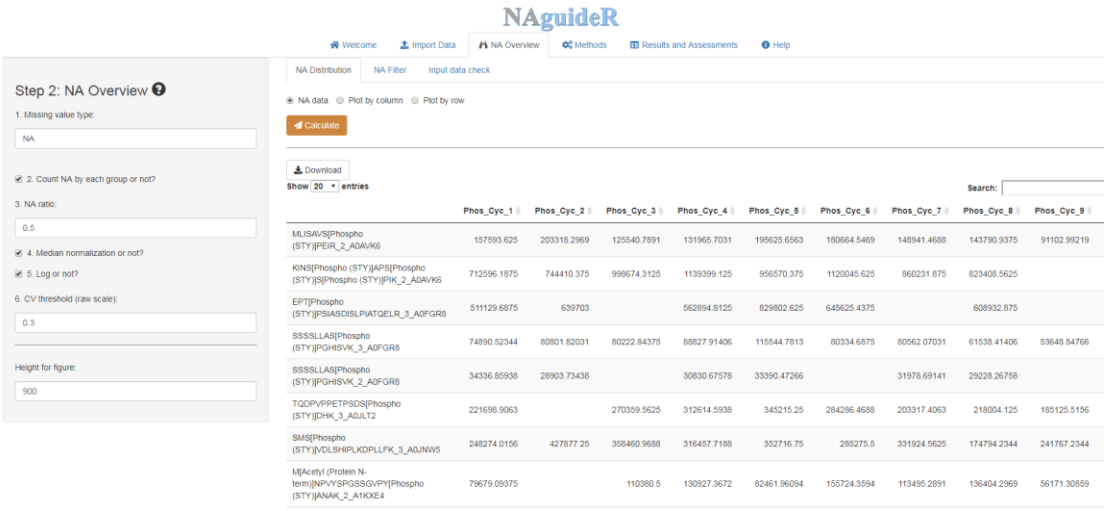
*4. Median normalization or not*: if true, NAguideR will process median normalization for original data. (Note, NAguideR was not designed to perform sophisticated normalization analysis. Any normalized datasets with NA can be accepted for analysis).

*5. Log or not*: if true, the data will be transformed to the logarithmic scale with base 2.

*6. CV threshold (raw scale)*: the threshold of coefficient of variation. Those peptides/proteins with NA ratio above this threshold will be removed. “raw scale” here means the CV of each peptide/protein is calculate using the data before logarithm transformation.

*7. Height for figure*: users can adjust the height of figures by changing this parameter.

If users set these parameters well, then click “calculate” button, the results will appear on the right panel.

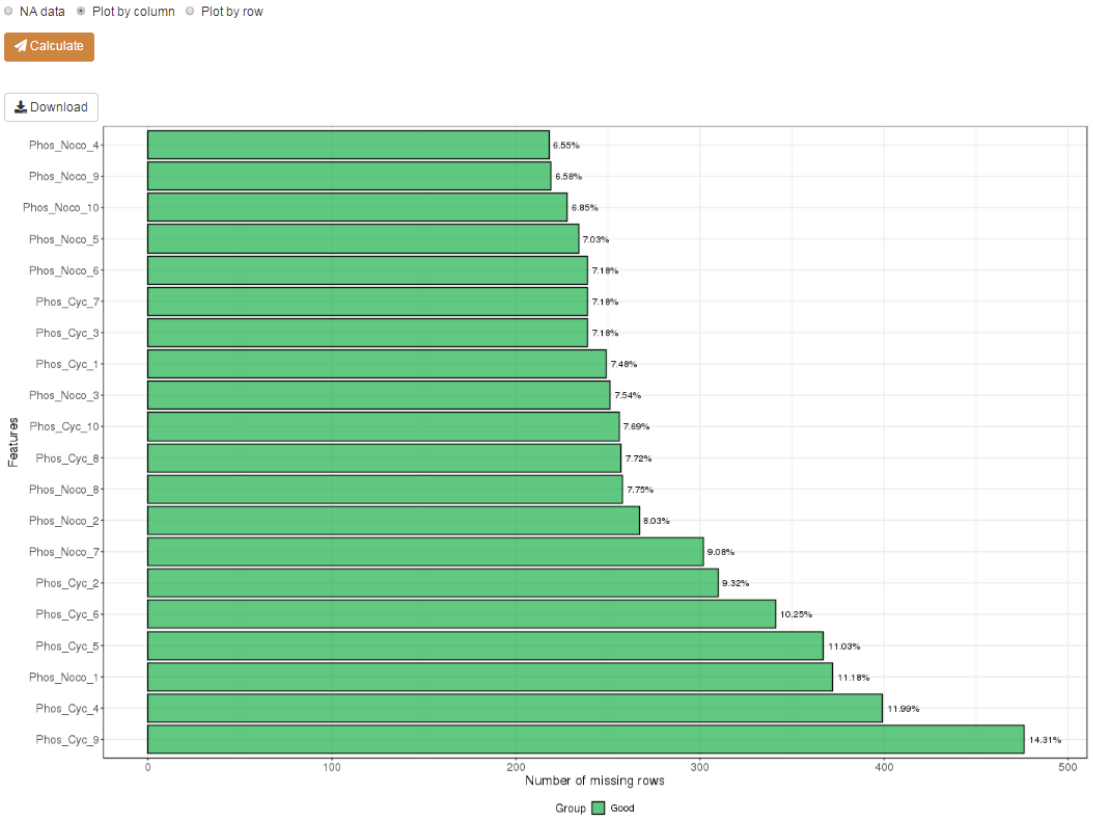



### 3.2 results of NA overview

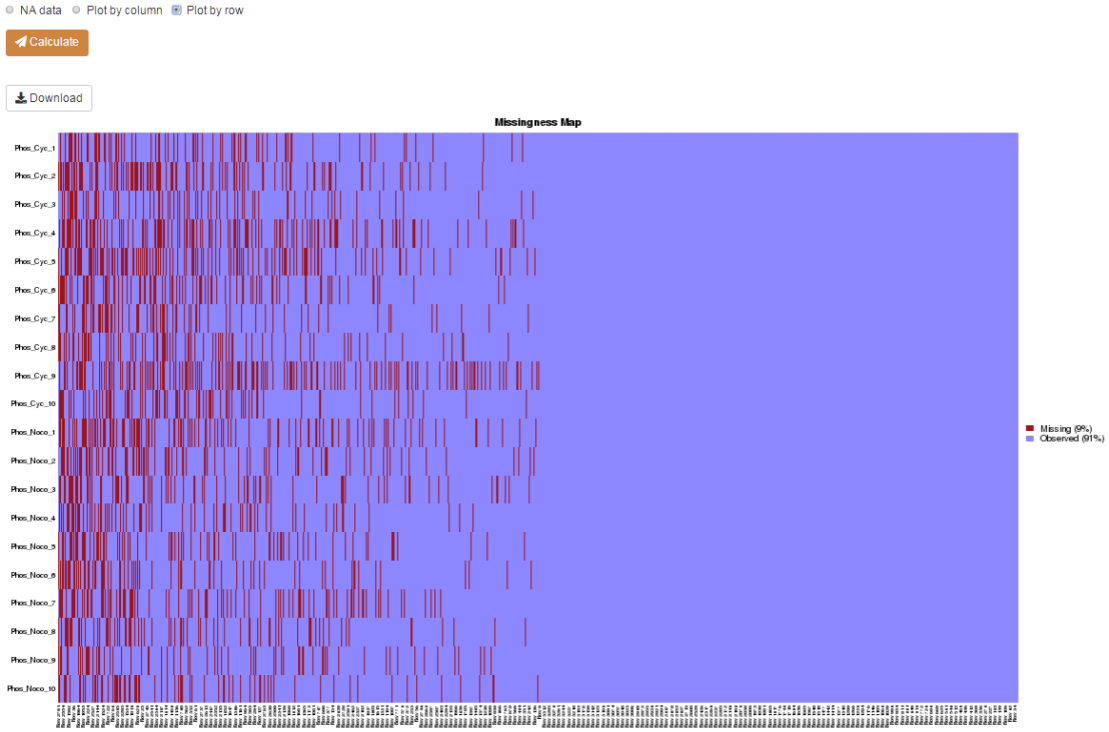
*a. NA Distribution*. This part contains three sub-parts:

*a.1 NA data*. Here shows the result where the “Missing value type” defined by “NA” will be shown with a blank cell and users can click “Download” button to download this result to their own computer:

*a.2 Plot by column*. Here shows the result of the NA distribution of every sample.

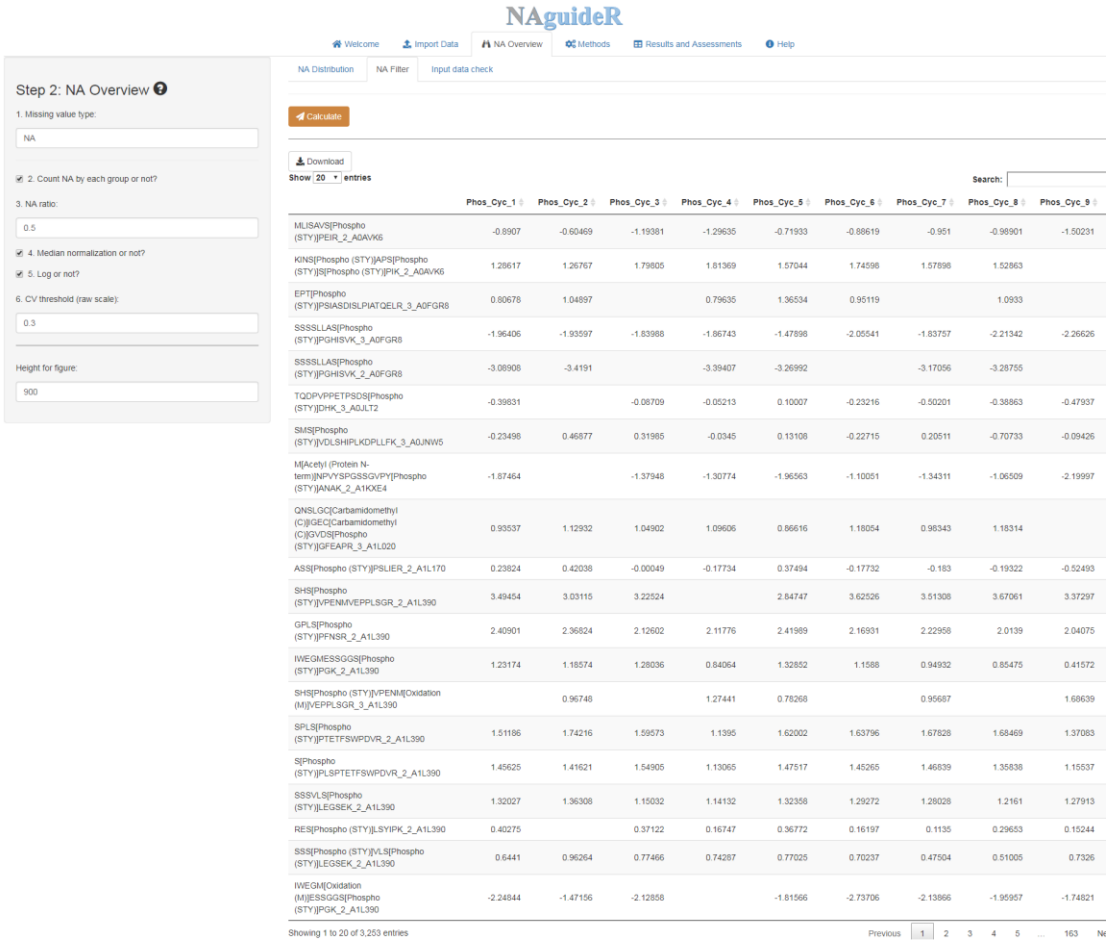



*a.2 Plot by row*. Here shows the result of the NA distribution of every peptide/protein.

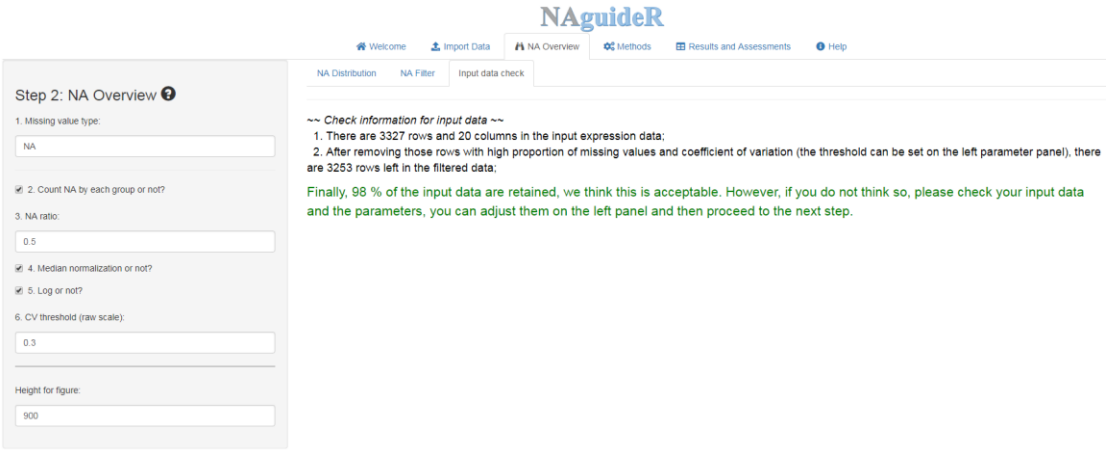



*b. NA filter*. This part will show the filtered result. That means, on the basis of the preset parameters

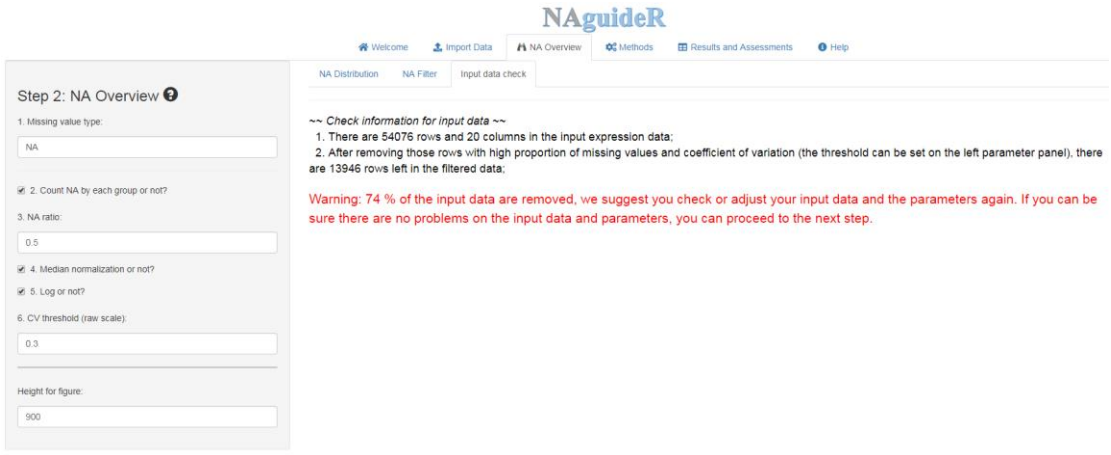
(i.e. NA ratio, CV threshold), those objects (peptides/proteins/genes/metabolites) without meeting these requirements would be removed.




*c. Input data check*. This part will show the checking information as a summary note for input data. By default, if there still remain more than half (>50%) objects in the filtered data, NAguideR will think it is acceptable, and will give users a message like below:

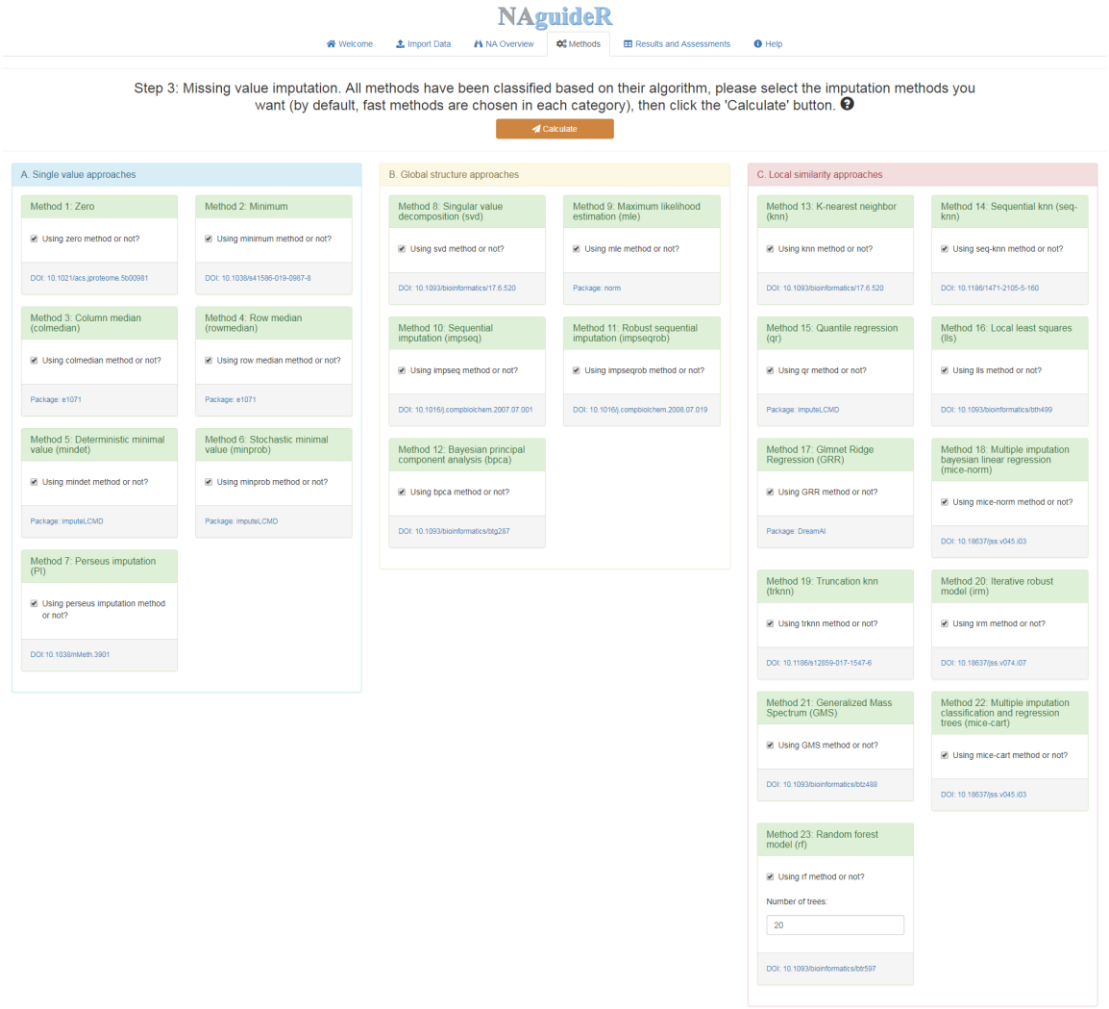



Otherwise, NAguideR will give some warnings to users, which means users should pay more attention to their own data and those preset parameters. It is recommended that the users should then make sure that there are no problems before they can proceed to the next step:

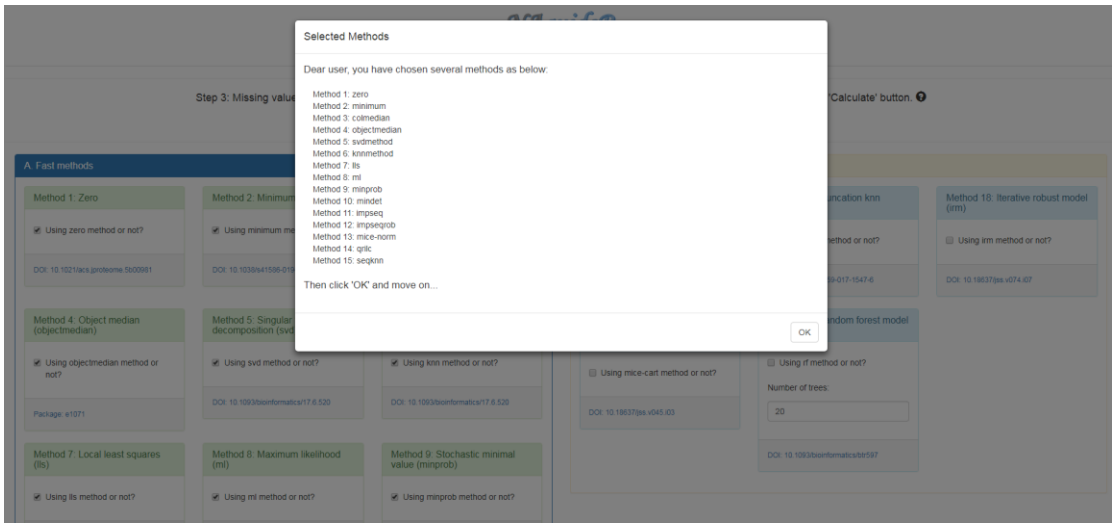

NAguideR
Welcome
Import Data
NA Overview
Methods
Results and Assessments
Help
Step 2: NA Overview
1. Missing value type:
NA
2. Count NA by each group or not?
3. NA ratio:
0.5
4. Median normalization or not?
5. Log or not?
6. CV threshold (raw scale):
0.3
Height for figure:
900
NA Distribution
NA Filter
Input data check
~~ Check information for input data ~~
1. There are 54076 rows and 20 columns in the input expression data;
2. After removing those rows with high proportion of missing values and coefficient of variation (the threshold can be set on the left parameter panel), there are 13946 rows left in the filtered data;
Warning: 74 % of the input data are removed, we suggest you check or adjust your input data and the parameters again. If you can be sure there are no problems on the input data and parameters, you can proceed to the next step.

## 4. Methods

In this step, users can select any of 23 missing value imputation methods that are currently supported. All methods have been classified into three categories based on their algorithm (Single value approaches, global structure approaches and local similarity approaches). In order to control the running time, we set these fast methods (17 methods) chosen by default. If users choose those slow methods (6 methods), that means the running time will be longer. If users want to try these slow methods, they just need to select the corresponding methods. The detailed information about each method can be found in Table S1. In addition, we also provide the reference for every method just blow each option on the web:

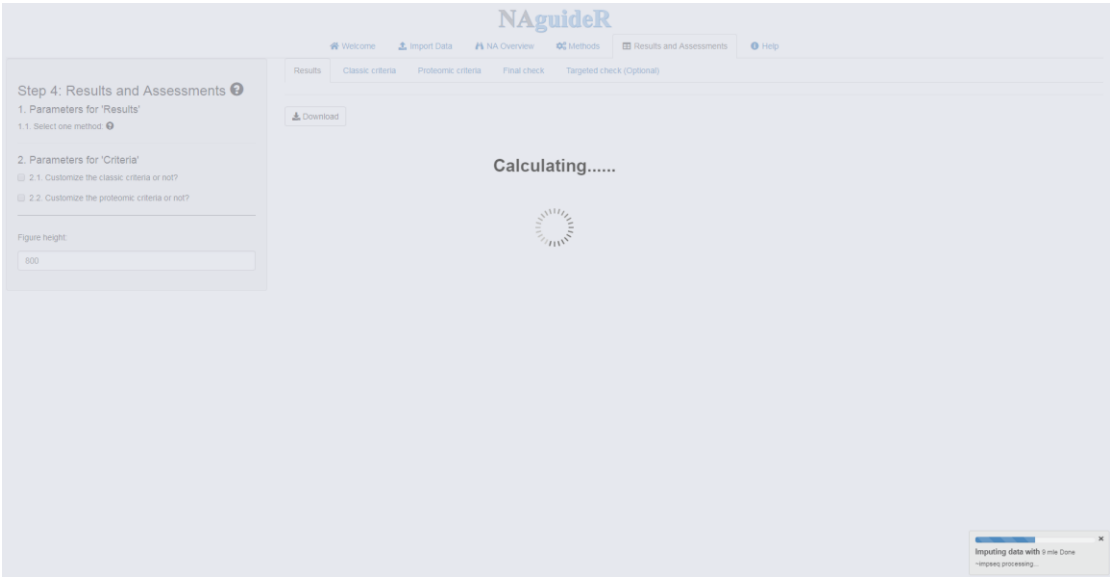



After selecting suitable methods, users need to click 'Calculate' button, and a popup window will be jumped out to show the selected methods, then click 'OK' button and continue:

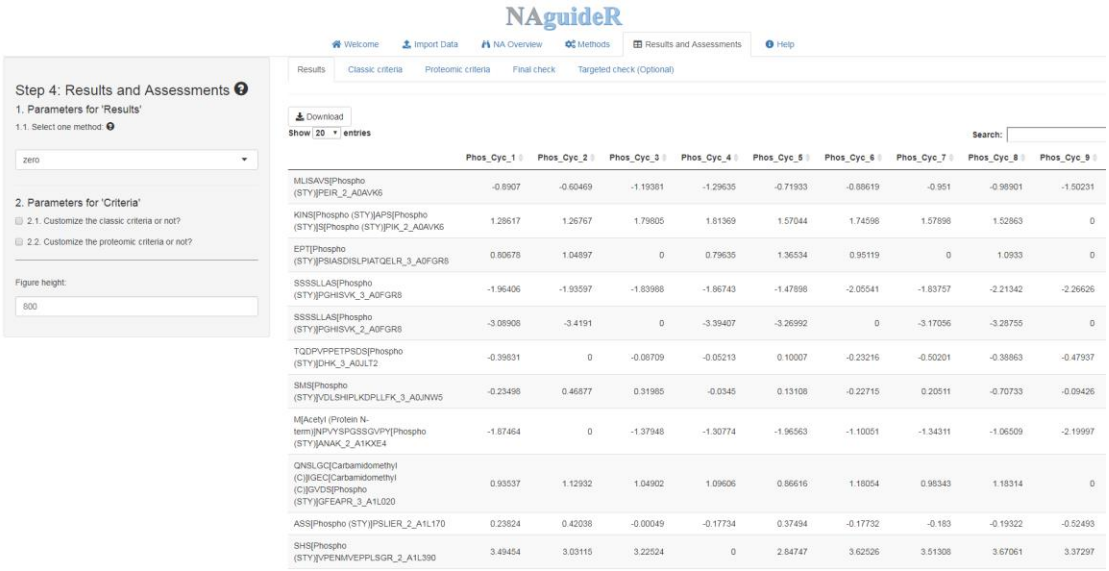

Selected Methods
Dear user, you have chosen several methods as below:
Method 1: zero
Method 2: minimum
Method 3: colmedian
Method 4: objectmedian
Method 5: svdmethod
Method 6: knnmethod
Method 7: lls
Method 8: ml
Method 9: minprob
Method 10: mindet
Method 11: impseq
Method 12: impseqrob
Method 13: mice-norm
Method 14: qrilc
Method 15: seqknn
Then click 'OK' and move on...
OK
Step 3: Missing value
'Calculate' button.
A. Fast methods
Method 1: Zero
Using zero method or not?
Method 4: Object median (objectmedian)
Using objectmedian method or not?
Method 7: Local least squares (lls)
Using lls method or not?
Method 8: Maximum likelihood (ml)
Using ml method or not?
Method 9: Stochastic minimal value (minprob)
Using minprob method or not?
Using mice-cart method or not?
Using rf method or not?
Number of trees:
20
Method 18: Iterative robust model (irm)
Using irm method or not?

## 5. Results and Assessments

This step will process missing value imputation and performance evaluation of every method that users select in “Methods” step. Click “Results and Assessments”, NAguideR will start to impute these missing value items, a process bar will appear in the bottom right corner to tell users where it goes:

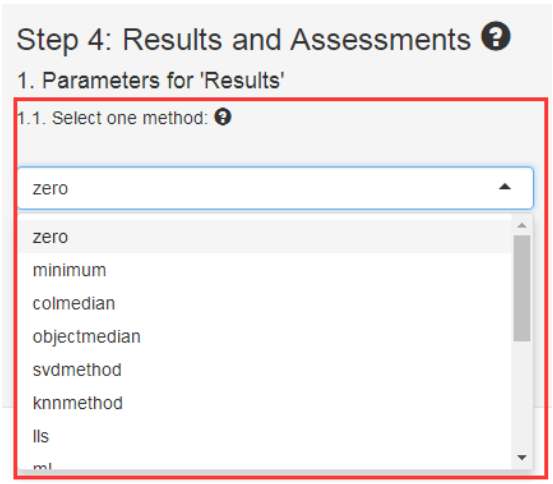



The result from every imputation method will be shown on the “Results” panel:

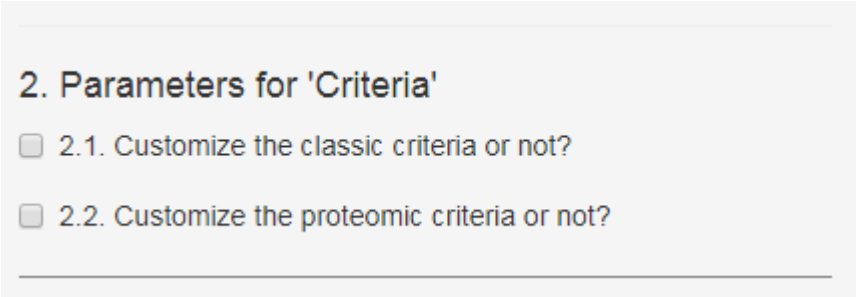



| | Phos_Cyc_1 | Phos_Cyc_2 | Phos_Cyc_3 | Phos_Cyc_4 | Phos_Cyc_5 | Phos_Cyc_6 | Phos_Cyc_7 | Phos_Cyc_8 | Phos_Cyc_9 |
|---|---|---|---|---|---|---|---|---|---|
| MLISAVS[Phospho (STY)]PEIR_2_A0AVK6 | -0.8907 | -0.60469 | -1.19381 | -1.29635 | -0.71933 | -0.88619 | -0.951 | -0.98901 | -1.50231 |
| KINS[Phospho (STY)]APS[Phospho (STY)]S[Phospho (STY)]PIK_2_A0AVK6 | 1.28617 | 1.26767 | 1.79805 | 1.81369 | 1.57044 | 1.74598 | 1.57898 | 1.52863 | 0 |
| EPT[Phospho (STY)]PSIASDISLPIATQELR_3_A0FGR8 | 0.80678 | 1.04897 | 0 | 0.79635 | 1.36534 | 0.95119 | 0 | 1.0933 | 0 |
| SSSSLLAS[Phospho (STY)]PGHISVK_3_A0FGR8 | -1.96406 | -1.93597 | -1.83988 | -1.86743 | -1.47898 | -2.05541 | -1.83757 | -2.21342 | -2.26626 |
| SSSSLLAS[Phospho (STY)]PGHISVK_2_A0FGR8 | -3.08908 | -3.4191 | 0 | -3.39407 | -3.26992 | 0 | -3.17056 | -3.28755 | 0 |
| TQDPVPPETPSDS[Phospho (STY)]DHK_3_A0JLT2 | -0.39831 | 0 | -0.08709 | -0.05213 | 0.10007 | -0.23216 | -0.50201 | -0.38863 | -0.47937 |
| SMS[Phospho (STY)]VDLSHIPLKDPLLFK_3_A0JNW5 | -0.23498 | 0.46877 | 0.31985 | -0.0345 | 0.13108 | -0.22715 | 0.20511 | -0.70733 | -0.09426 |
| M[Acetyl (Protein N-term)]NPVYSPGSSGVPY[Phospho (STY)]ANAK_2_A1KXE4 | -1.87464 | 0 | -1.37948 | -1.30774 | -1.96563 | -1.10051 | -1.34311 | -1.06509 | -2.19997 |
| QNSLGC[Carbamidomethyl (C)]IGEC[Carbamidomethyl (C)]GVDS[Phospho (STY)]GFEAPR_3_A1L020 | 0.93537 | 1.12932 | 1.04902 | 1.09606 | 0.86616 | 1.18054 | 0.98343 | 1.18314 | 0 |
| ASS[Phospho (STY)]PSLIER_2_A1L170 | 0.23824 | 0.42038 | -0.00049 | -0.17734 | 0.37494 | -0.17732 | -0.183 | -0.19322 | -0.52493 |
| SHS[Phospho (STY)]VPENMVEPPLSGR_2_A1L390 | 3.49454 | 3.03116 | 3.22524 | 0 | 2.84747 | 3.62526 | 3.51308 | 3.67061 | 3.37297 |

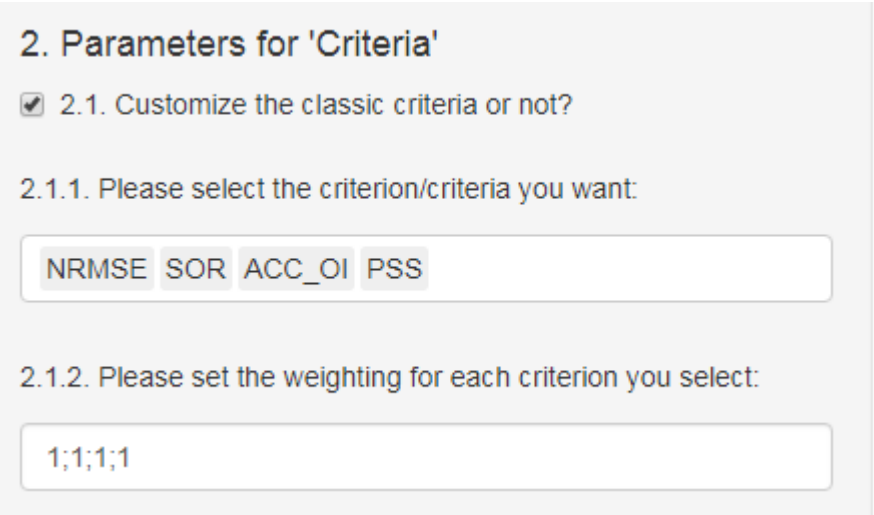
*a. Parameters for ‘Results’*. Herein users can change the parameter “*Select one method*” on the left panel to check relative result, for example, if users select “zero”, it will show the result derived from zero method:



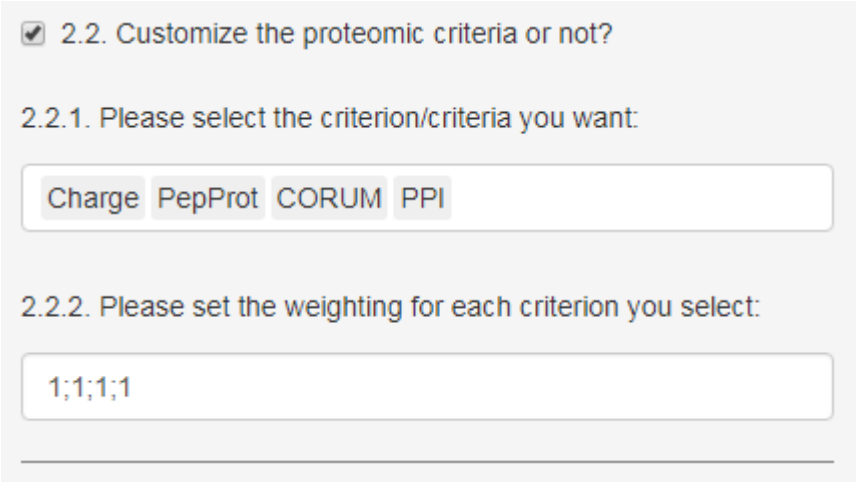

b. *Parameters for 'Criteria'*. Users can customize the criteria and relative weighting for specific experimental designs and aims. By default, these parameters are not selected and all criteria weights are equal.



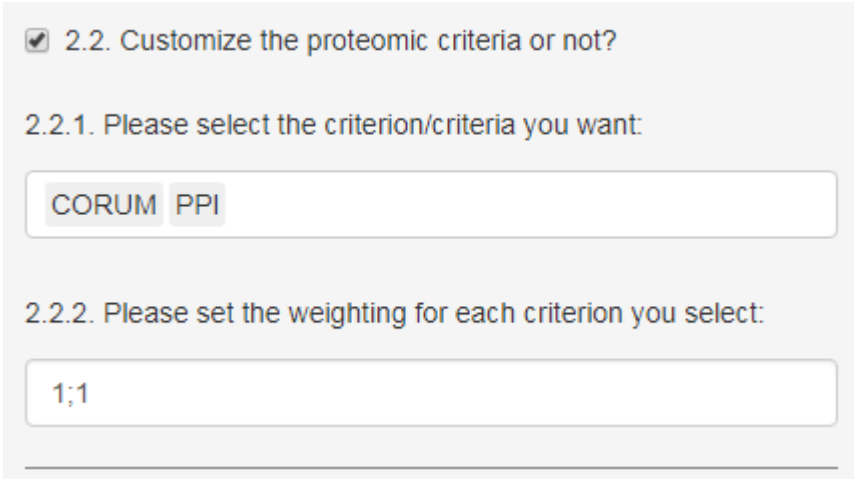

*b.1 Customize the classic criteria or not*? If true, users can set the classic criteria and relative weight they want, by default, four classic criteria (NRMSE, SOR, ACC_OI, PSS) are chosen and their weights are equal. Please note, the number of criteria and weights should be equal, for example, if users select 'NRMS', 'SOR', and 'PSS', the weights parameter should be type in '1;1;1', which are separated by semicolons, and in this situation, the three criteria weights are all 0.333 (1/3). If users think 'NRMS' should has a higher weight and type in '3;1;1', this means the weight of 'NRMS' is 0.6 (3/5), 'SOR' and 'PSS' is 0.2 (1/5), respectively:



b.2 *Customize the proteomic criteria or not*? If true, users can set the proteomic criteria and relative weight they want, by default, four proteomic criteria (Charge, PepProt, CORUM and PPI) are chosen and their weights are equal. Please also note, the number of criteria and weights should be equal and other descriptions are similar to those for classic criteria as above. Note, the b.1 and b.2 options enable users to customize the criteria and set relative weightings for those specific experimental designs (e.g., a mixture of protein standards being measured in which no in-vivo protein complex formation or interactions expected).




Especially for type 'Proteins' dataset (see part 1 above), Charge and PepProt criteria cannot be used (As there are no information about charges and peptides in the data), so users should change the parameters like this if they decide to customize the proteomic criteria:




Next, click "Classic criteria" and "Calculate" button. NAguideR will assess every method under the four classic criteria:


The tables and figures are provided here under the four classic criteria.

1. This table shows the comprehensive ranks of every imputation method. By default, all criteria weights are equal, if users change their weights, and the comprehensive ranks would also change correspondingly based on the new criteria and weights;

2-5, the tables show the scores of every imputation method based on 'Normalized root mean squared Error (NRMSE)', 'NRMSE-based sum of ranks (SOR)', 'Procrustes sum of squared errors (PSS)', and 'Average correlation coefficient between original value and imputed value (ACC_OI)', respectively;

6. Figures here show the normalized scores of every imputation method under the four classic criteria. 'Normalized Values' here means that every score is divided by the corresponding max value.

1. Comprehensive ranks under classic criteria:

Download

Show 20 entries Search:

| | Methods | NRMSE_Rank | SOR_Rank | ACC_OI_Rank | PSS_Rank | Rank_Mean |
|---|---|---|---|---|---|---|
| Method 2 | impseq | 1 | 1 | 1 | 1 | 1 |
| Method 3 | impseqrob | 2 | 2 | 2 | 2 | 2 |
| Method 13 | seqknn | 4 | 3 | 3 | 6 | 4 |
| Method 10 | ml | 3 | 6 | 5 | 3 | 4.25 |
| Method 4 | knnmethod | 5 | 4 | 4 | 5 | 4.5 |
| Method 5 | lls | 6 | 5 | 6 | 4 | 5.25 |
| Method 6 | mice-norm | 7 | 7 | 7 | 7 | 7 |
| Method 11 | objectmedian | 8 | 8 | 8 | 8 | 8 |
| Method 12 | qrilc | 9 | 10 | 9 | 11 | 9.75 |
| Method 14 | svdmethod | 10 | 9 | 10 | 12 | 10.25 |
| Method 1 | colmedian | 11 | 12 | 11 | 10 | 11 |
| Method 15 | zero | 12 | 11 | 12 | 9 | 11 |
| Method 7 | mindet | 13 | 13 | 13 | 13 | 13 |
| Method 9 | minprob | 14 | 14 | 14 | 14 | 14 |
| Method 8 | minimum | 15 | 15 | 15 | 15 | 15 |

Showing 1 to 15 of 15 entries Previous 1 Next

2. Normalized root mean squared Error (NRMSE):

Download

Show 20 entries Search:

| | Methods | NRMSE |
|---|---|---|
| Method 11 | impseq | 0.07796 |
| Method 12 | impseqrob | 0.07814 |
| Method 8 | ml | 0.10625 |
| Method 15 | seqknn | 0.11049 |
| Method 6 | knnmethod | 0.11513 |
| Method 7 | lls | 0.1237 |
| Method 13 | mice-norm | 0.16857 |
| Method 4 | objectmedian | 0.5063 |
| Method 14 | qrilc | 0.8632 |
| Method 5 | svdmethod | 0.93162 |
| Method 3 | colmedian | 1.00393 |
| Method 1 | zero | 1.08355 |
| Method 10 | mindet | 2.2209 |
| Method 9 | minprob | 2.25375 |
| Method 2 | minimum | 3.28021 |

Showing 1 to 15 of 15 entries Previous 1 Next

3. NRMSE-based sum of ranks (SOR):

Download

Show 20 entries Search:

| | Methods | SOR |
|---|---|---|
| Method 11 | impseq | 2122 |
| Method 12 | impseqrob | 2142 |
| Method 15 | seqknn | 3536 |
| Method 6 | knnmethod | 3625 |
| Method 7 | lls | 3676 |
| Method 8 | ml | 3696 |
| Method 13 | mice-norm | 4296 |
| Method 4 | objectmedian | 6526 |
| Method 5 | svdmethod | 8030 |
| Method 14 | qrilc | 8110 |
| Method 1 | zero | 8406 |
| Method 3 | colmedian | 8418 |
| Method 10 | mindet | 10135 |
| Method 9 | minprob | 10313 |
| Method 2 | minimum | 11769 |

Showing 1 to 15 of 15 entries Previous 1 Next

4. Procrustes sum of squared errors (PSS):

Download

Show 20 entries Search:

| | Methods | PSS |
|---|---|---|
| Method 11 | impseq | 0.00048 |
| Method 12 | impseqrob | 0.00051 |
| Method 8 | ml | 0.00064 |
| Method 7 | lls | 0.00094 |
| Method 6 | knnmethod | 0.00109 |
| Method 15 | seqknn | 0.00129 |
| Method 13 | mice-norm | 0.00556 |
| Method 4 | objectmedian | 0.02591 |
| Method 1 | zero | 0.05313 |
| Method 3 | colmedian | 0.05389 |
| Method 14 | qrilc | 0.05468 |
| Method 5 | svdmethod | 0.06779 |
| Method 10 | mindet | 0.10707 |
| Method 9 | minprob | 0.10904 |
| Method 2 | minimum | 0.13141 |

Showing 1 to 15 of 15 entries Previous 1 Next

5. Average correlation coefficient between original value and imputated value (ACC_OI):

Download

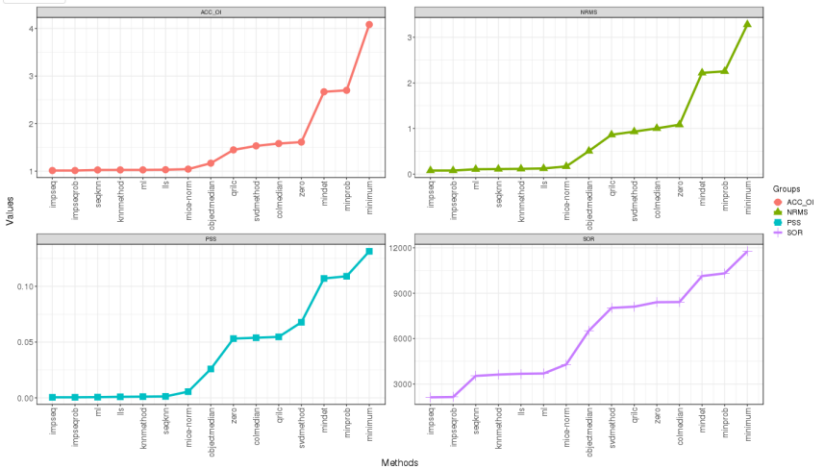
Show 20 entries Search:

| | Methods | Cor_mean |
|---|---|---|
| Method 11 | impseq | 0.98755 |
| Method 12 | impseqrob | 0.98748 |
| Method 15 | seqknn | 0.9757 |
| Method 6 | knnmethod | 0.975 |
| Method 8 | ml | 0.97447 |
| Method 7 | lls | 0.97116 |
| Method 13 | mice-norm | 0.95947 |
| Method 4 | objectmedian | 0.8567 |
| Method 14 | qrilc | 0.69105 |
| Method 5 | svdmethod | 0.653 |
| Method 3 | colmedian | 0.63258 |
| Method 1 | zero | 0.62062 |
| Method 10 | mindet | 0.37464 |
| Method 9 | minprob | 0.37038 |
| Method 2 | minimum | 0.24487 |

Showing 1 to 15 of 15 entries Previous 1 Next

6. Figures:

Download



Then click “Proteomic criteria” and “Calculate” button. NAguideR will assess every imputation method under the four proteomic criteria:


The tables and figures are provided here under the four proteomic criteria.

1. This table shows the comprehensive ranks of every imputation method. By default, all criteria weights are equal, if users change their weights, and the comprehensive ranks would also change correspondingly based on the new criteria and weights;

2-5, the tables show the scores of every imputation method based on 'Average correlation coefficient between peptides with different charges (ACC_Charge)', 'Average correlation coefficient between peptides in a same protein (ACC_PepProt)', 'Average correlation coefficient between protein complexes (ACC_CORUM)', 'Average correlation coefficient between protein complexes (ACC_PPI)', respectively;

6. Figures here show the correlation coefficient distribution of the original values and the imputed values from every imputation method under the four proteomic criteria. Figures will be instantly updated for a particular NA method that can be specified in “*1.1 Select one method*” parameter under Step 4 (left panel). The figure example below shows the results of method “zero”.

1. Comprehensive ranks under proteomic criteria:

Download

Show 20 entries Search:

| | Methods | Charge_Rank | PepProt_Rank | CORUM_Rank | PPI_Rank | Rank_Mean |
|---|---|---|---|---|---|---|
| Method 4 | knnmethod | 2 | 1 | 1 | 2 | 1.5 |
| Method 13 | seqknn | 1 | 2 | 4 | 1 | 2 |
| Method 2 | impseq | 3 | 3 | 2 | 3 | 2.75 |
| Method 3 | impseqrob | 4 | 4 | 3 | 4 | 3.75 |
| Method 5 | lls | 5 | 5 | 6 | 5 | 5.25 |
| Method 10 | mi | 6 | 6 | 5 | 6 | 5.75 |
| Method 6 | mice-norm | 7 | 7 | 7 | 7 | 7 |
| Method 11 | objectmedian | 8 | 8 | 8 | 8 | 8 |
| Method 12 | qrilc | 9 | 9 | 9 | 11 | 9.5 |
| Method 14 | svdmethod | 10 | 10 | 10 | 9 | 9.75 |
| Method 1 | colmedian | 11 | 11 | 12 | 10 | 11 |
| Method 15 | zero | 12 | 12 | 11 | 12 | 11.75 |
| Method 7 | mindet | 13 | 13 | 13 | 13 | 13 |
| Method 9 | minprob | 14 | 14 | 14 | 14 | 14 |
| Method 8 | minimum | 15 | 15 | 15 | 15 | 15 |

Showing 1 to 15 of 15 entries Previous 1 Next

2. Average correlation coefficient between peptides with different charges (ACC_Charge):

Download

Show 20 entries Search:

| | Methods | ACC_Charge |
|---|---|---|
| Method 15 | seqknn | 0.84803 |
| Method 6 | knnmethod | 0.84666 |
| Method 11 | impseq | 0.84525 |
| Method 12 | impseqrob | 0.84508 |
| Method 7 | lls | 0.84018 |
| Method 8 | mi | 0.83723 |
| Method 13 | mice-norm | 0.82996 |
| Method 4 | objectmedian | 0.73897 |
| Method 14 | qrilc | 0.62586 |
| Method 5 | svdmethod | 0.60933 |
| Method 3 | colmedian | 0.59157 |
| Method 1 | zero | 0.58832 |
| Method 10 | mindet | 0.43458 |
| Method 9 | minprob | 0.42645 |
| Method 2 | minimum | 0.35983 |

Showing 1 to 15 of 15 entries Previous 1 Next

3. Average correlation coefficient between peptides in a same protein (ACC_PepProt):

Download

Show 20 entries Search:

| | Methods | ACC_peppro |
|---|---|---|
| Method 6 | knnmethod | 0.54888 |
| Method 15 | seqknn | 0.54877 |
| Method 11 | impseq | 0.54602 |
| Method 12 | impseqrob | 0.54588 |
| Method 7 | lls | 0.54151 |
| Method 8 | mi | 0.54064 |
| Method 13 | mice-norm | 0.53333 |
| Method 4 | objectmedian | 0.47951 |
| Method 14 | qrilc | 0.40258 |
| Method 5 | svdmethod | 0.38689 |
| Method 3 | colmedian | 0.37715 |
| Method 1 | zero | 0.37693 |
| Method 10 | mindet | 0.27806 |
| Method 9 | minprob | 0.27274 |
| Method 2 | minimum | 0.22728 |

Showing 1 to 15 of 15 entries Previous 1 Next

4. Average correlation coefficient between protein complexes (ACC_CORUM):

Download

Show 20 entries Search:

| | Methods | ACC_CORUM |
|---|---|---|
| Method 6 | knnmethod | 0.30498 |
| Method 11 | impseq | 0.30475 |
| Method 12 | impseqrob | 0.30471 |
| Method 15 | seqknn | 0.30459 |
| Method 8 | mi | 0.29933 |
| Method 7 | lls | 0.29666 |
| Method 13 | mice-norm | 0.29583 |
| Method 4 | objectmedian | 0.2465 |
| Method 14 | qrilc | 0.21802 |
| Method 5 | svdmethod | 0.19725 |
| Method 1 | zero | 0.19269 |
| Method 3 | colmedian | 0.18941 |
| Method 10 | mindet | 0.15264 |
| Method 9 | minprob | 0.15054 |
| Method 2 | minimum | 0.127 |

Showing 1 to 15 of 15 entries Previous 1 Next

5. Average correlation coefficient between protein complexes (ACC_PPI):

Download

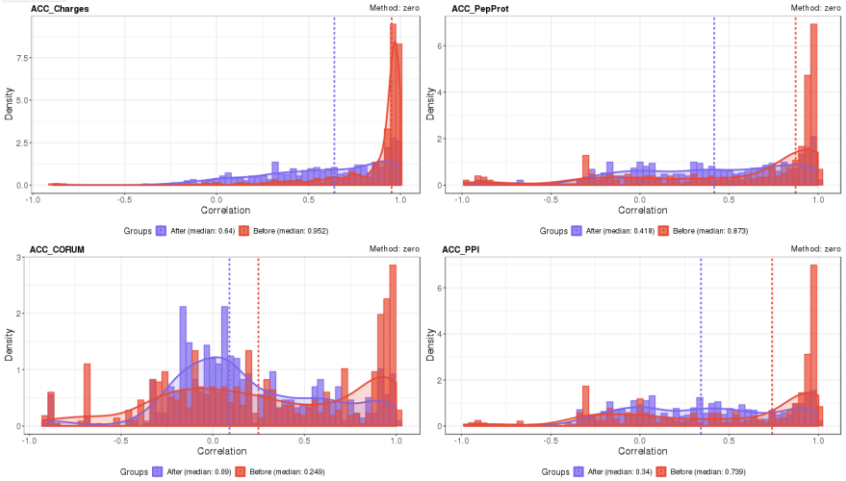
Show 20 entries Search:

| | Methods | ACC_PPI |
|---|---|---|
| Method 15 | seqknn | 0.48217 |
| Method 6 | knnmethod | 0.48201 |
| Method 11 | impseq | 0.48111 |
| Method 12 | impseqrob | 0.48108 |
| Method 7 | lls | 0.4779 |
| Method 8 | mi | 0.47428 |
| Method 13 | mice-norm | 0.46884 |
| Method 4 | objectmedian | 0.41256 |
| Method 5 | svdmethod | 0.35871 |
| Method 3 | colmedian | 0.34504 |
| Method 14 | qrilc | 0.33687 |
| Method 1 | zero | 0.33539 |
| Method 10 | mindet | 0.22936 |
| Method 9 | minprob | 0.22714 |
| Method 2 | minimum | 0.18582 |

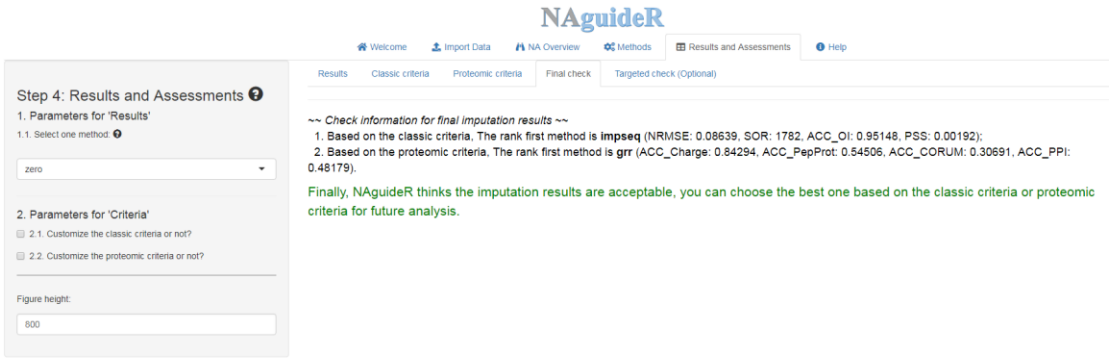
Showing 1 to 15 of 15 entries Previous 1 Next

6. Figures:

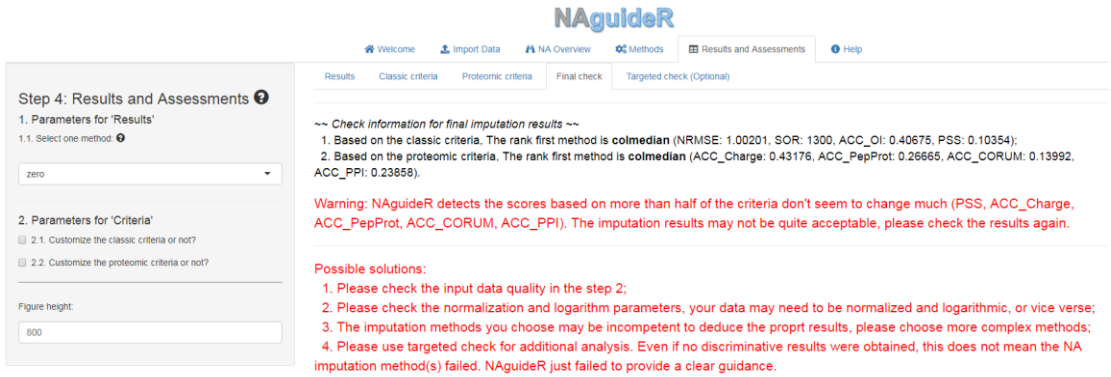
Download



Next, click ‘Final check’ for checking final imputation results as a summary note. NAguideR will re-check those scores based on every criterion. If everything is acceptable (see below), NAguideR will show a message like:




Here NAguideR performs a simple check to report if there is any big difference among these imputation methods under more than half of the criteria (by default, NAguideR check the fold change between the maximum score and the minimum score for each criterion, if the fold change is below 2, a fact suggesting that no big difference under the corresponding criterion, i.e., that NAguideR cannot provide a significantly discriminant guidance on NA method selection), NAguideR will give some warnings and possible solutions for users to review/re-calculate these imputation results:



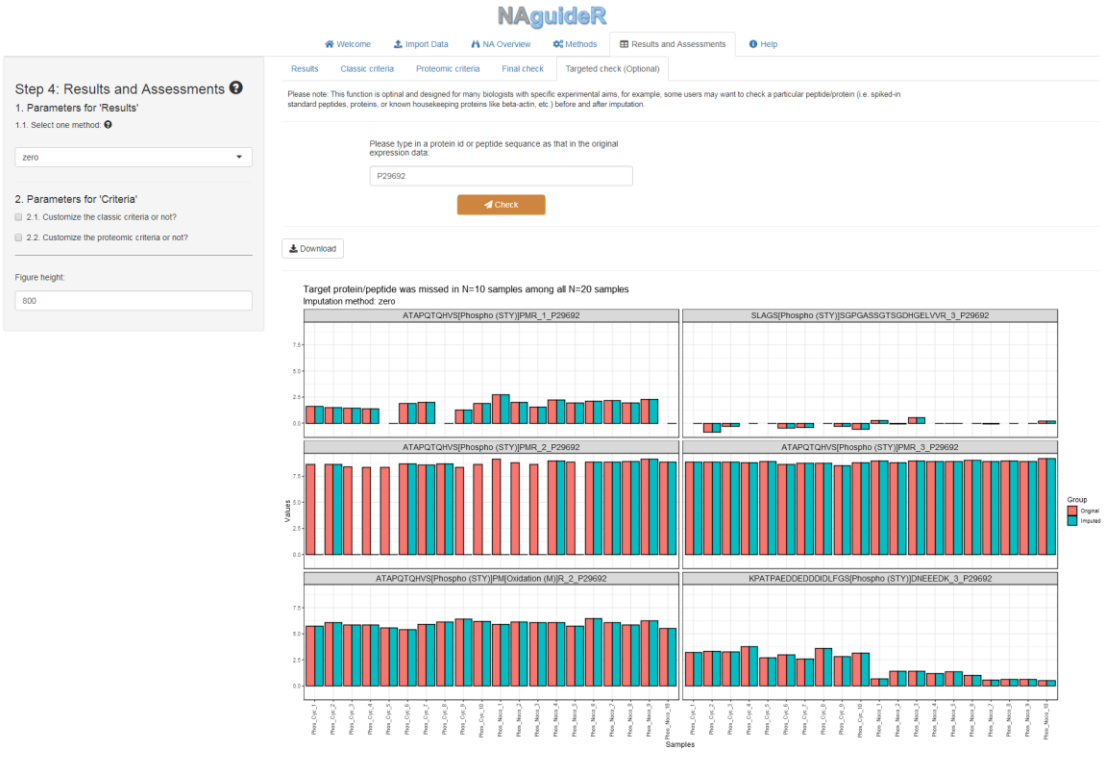

Last but not least, NaguideR implements one optional function, ‘Targeted check’, which is designed for many biologists with specific experimental aims. For example, this feature conveniently allows users to directly visualize the results of a particular peptide or protein item (i.e., spiked-in standard peptides, proteins, or known housekeeping proteins like beta-actin, etc.). Therefore, by following their experimental design, they can type in the peptide sequence or protein id in the text area and click the ‘Check’ button.

Then, NAguideR will locate this peptide or protein id in the input and resultant matrix (if the id was not listed in the user’s input, it will give a message, “Target protein/peptide not found. Please make sure the item is included in the input table”, example 1 as below). Then, NAguideR will show the results before and after imputation by using bar plots and provide a note “Target protein/peptide was missed in N=X samples among all N=Y samples” (example 2 as below). This plot should help the users to inspect results following their particular experimental design. If the target protein/peptide is quantified without the need of NA imputation, NAguideR will still display the bar plots and provide a

note, “Target protein/peptide was not missed in any sample” (example 3 as below).

Example 1 (Target protein/peptide not found. Please make sure the item is included in the input table):


Example 2 (Target protein/peptide was missed in N=10 samples among all N=20 samples):




Example 3 (Target protein/peptide was not missed in any sample):

NAguideR

Welcome | Import Data | NA Overview | Methods | Results and Assessments | Help

## Step 4: Results and Assessments

### 1. Parameters for 'Results'

1.1. Select one method:

zero

### 2. Parameters for 'Criteria'

2.1. Customize the classic criteria or not?

2.2. Customize the proteomic criteria or not?

Figure height:

600

Results | Classic criteria | Proteomic criteria | Final check | Targeted check (Optional)

Please note: This function is optinal and designed for many biologists with specific experimental aims, for example, some users may want to check a particular peptide/protein (i.e. spiked-in standard peptides, proteins, or known housekeeping proteins like beta-actin, etc.) before and after imputation.

Please type in a protein id or peptide sequance as that in the original expression data:

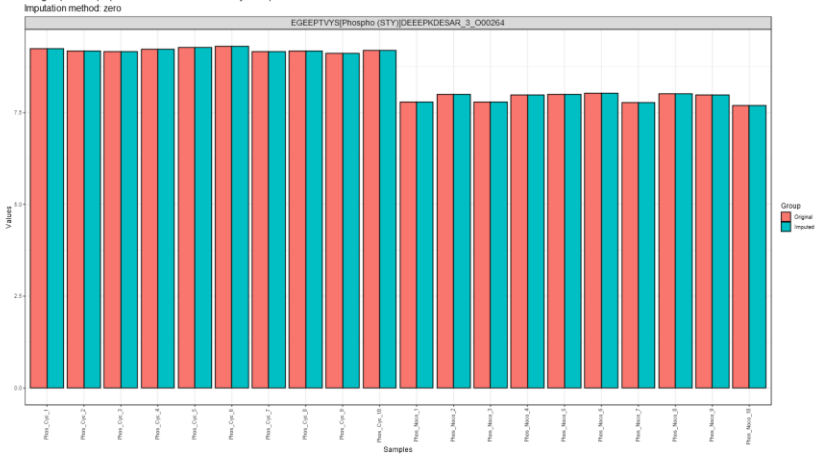
O00264

Check

Download

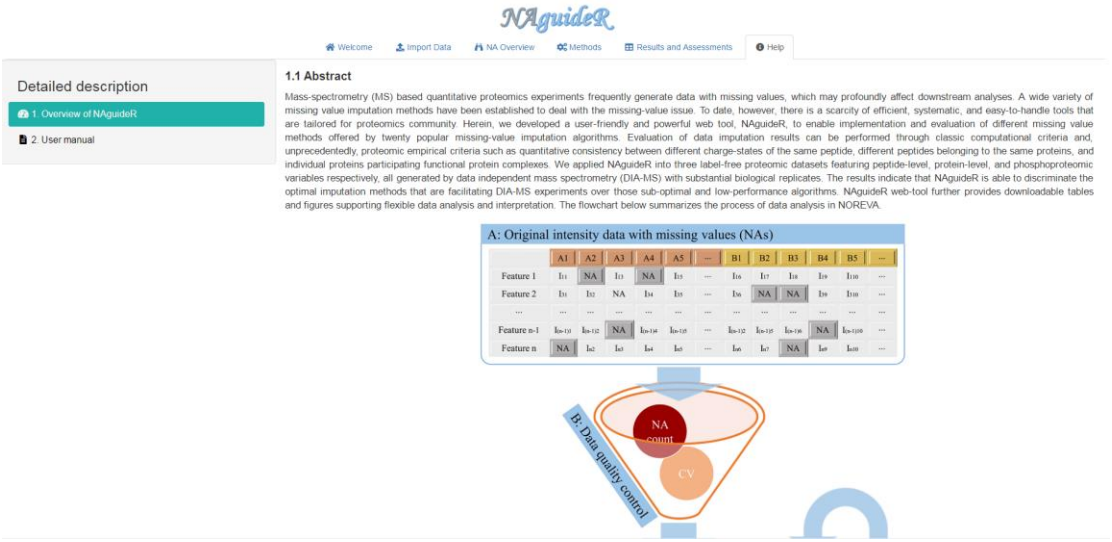
**Target protein/peptide was not missed in any sample.**

Imputation method: zero



## 6. Help

This part provides brief introductions and operation manual about NAguideR for users to quickly learn this tool and start to use this tool.





| Peptides | Charges | Uniprot_IDs | Phos_Cyc_1 | Phos_Cyc_2 | Phos_Cyc_3 | Phos_Cyc_4 | Phos_Cyc_5 | Phos_Cyc_6 |
|---|---|---|---|---|---|---|---|---|
| MLISAVS[Phospho ( | 2 | A0AVK6 | 157593.6 | 203318.3 | 125540.8 | 131965.7 | 195625.7 | 180664.5 |
| KINS[Phospho (STY | 2 | A0AVK6 | 712596.2 | 744410.4 | 998674.3 | 1139399 | 956570.4 | 1120046 |
| EPT[Phospho (STY) | 3 | A0FGR8 | 511129.7 | 639703 | NA | 562894.8 | 829802.6 | 645625.4 |
| SSSSLLAS[Phospho | 3 | A0FGR8 | 74890.52 | 80801.82 | 80222.84 | 88827.91 | 115544.8 | 80334.69 |
| SSSSLLAS[Phospho | 2 | A0FGR8 | 34336.86 | 28903.73 | NA | 30830.68 | 33390.47 | NA |
| TQDPYPFETPEDS[Pho | 3 | A0JLT2 | 221698.9 | NA | 270359.6 | 312614.6 | 345215.3 | 284286.5 |
| SMS[Phospho (STY) | 3 | A0JNW5 | 248274 | 427877.3 | 358461 | 316457.7 | 352716.8 | 285275.5 |
| M[Acetyl (Protein | 2 | A1XKE4 | 79679.09 | NA | 110380.5 | 130927.4 | 82461.96 | 155724.4 |
| QNSLGC[Carbamidom | 3 | A1L020 | 558781.1 | 676339.8 | 594215.1 | 692863.3 | 587093.6 | 756873 |
| ASS[Phospho (STY) | 2 | A1L170 | 344653.8 | 413764.8 | 287084 | 286627.3 | 417670.3 | 295301.9 |
| SHS[Phospho (STY) | 2 | A1L390 | 3293265 | 2527386 | 2685655 | NA | 2318149 | 4120553 |
| GPLS[Phospho (STY | 2 | A1L390 | 1551857 | 1596314 | 1253587 | 1406729 | 1723560 | 1502006 |
| IWEGMESSQGS[Phosp | 2 | A1L390 | 686212.1 | 703314.1 | 697566 | 580441.7 | 808891.8 | 745552.6 |
| SHS[Phospho (STY) | 3 | A1L390 | NA | 604569.9 | NA | 784035 | 554084.8 | NA |
| SPLS[Phospho (STY | 2 | A1L390 | 833264.3 | 1034303 | 867998.1 | 714042.4 | 990010.2 | 1039246 |
| S[Phospho (STY)]P | 2 | A1L390 | 801754.1 | 825141.1 | 840367.5 | 709674.4 | 895440 | 913974.4 |
| SSSVLS[Phospho (S | 2 | A1L390 | 729638 | 795307.5 | 637437.1 | 714943.1 | 806124.7 | 818071.6 |
| RES[Phospho (STY) | 2 | A1L390 | 386283.1 | NA | 371454.2 | 364010.8 | 415586.1 | 373595.6 |

## 7. How to run this tool locally?

*NAguideR* is an open source software for non-commercial use and all codes can be obtained on our GitHub: https://github.com/wangshisheng/NAguideR. If users want to run *NAguideR* on their own computer, they should operate as below:

7.1 As this tool was developed with R, you may:

a) Install R. You can download R from here: https://www.r-project.org/.

b) Install RStudio. (Recommendatory but not necessary). You can download RStudio from here: https://www.rstudio.com/.

c) Check packages. After installing R and RStudio, you should check whether you have installed these packages (shiny, shinyBS, shinyjs, shinyWidgets, DT, gdata, ggplot2, ggsci, openxlsx, data.table, DT, raster, Metrics, vegan, tidyverse, ggExtra, cowplot, Amelia, e1071, impute, SeqKnn, pcaMethods, norm, imputeLCMD, VIM, rrcovNA, mice, missForest, GMSimpute, DreamAI). You may run the codes below to check them:

```
if(!require(pacman)) install.packages("pacman")
pacman::p_load(shiny, shinyBS, shinyjs, shinyWidgets, DT, gdata, ggplot2, ggsci, openxlsx, data.table, DT, raster, Metrics, vegan, tidyverse, ggExtra, cowplot, Amelia, e1071, impute, SeqKnn, pcaMethods, norm, imputeLCMD, VIM, rrcovNA, mice, missForest, GMSimpute, DreamAI)
```

Please note, you may find the SeqKnn package (https://github.com/cran/SeqKnn) cannot be installed rightly as it has not been updated for a long time. If so, please download this package from here: https://github.com/wangshisheng/NAguideR/blob/master/SeqKnn_1.0.1.tar.gz. Then you can install this separate package locally:

```
setwd('path') #path is where the two packages are.
install.packages("SeqKnn_1.0.1.tar.gz", repos = NULL,type="source")
```

d) Run this tool locally

```
if(!require(NAguideR)) devtools::install_github("wangshisheng/NAguideR")
library(NAguideR)
NAguideR_app()
```

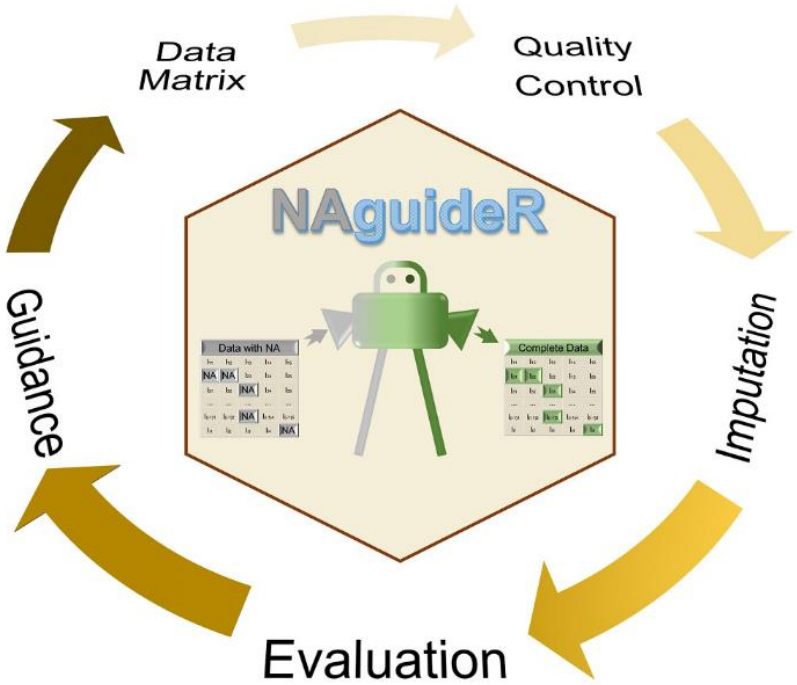
Then NAguideR will be started as below, and the detailed operation about NAguideR can be found in the Supplementary Notes part 1-6:

# NAguideR

Welcome | Import Data | NA Overview | Methods | Results and Assessments | Help

*~~ Dear Users, Welcome to NAguideR ~~*

**NAguideR** is a web-based tool, which integrates 23 commonly used missing value imputation methods and provides two categories of evaluation criteria (4 classic criteria and 4 proteomic criteria) to assess the imputation performance of various methods. We hope this tool could help scientists impute the missing values systematically and present valuable guidance to select one proper method for their own data. In addition, this tool supports both online access and local installation.




Basically, there are four main steps in NAguideR:

1. Uploading proteomics expression data and sample information data;
2. Data quality control;
3. Missing value imputation;
4. Performance evaluation;

After this, NAguideR can provide valuable guidance for users to select one proper method for their own data based on the evaluation results. Detailed introduction can be found in the ***Help*** part.

Finally, NAguideR is developed by R shiny (Version 1.3.2), and is free and open to all users with no login requirement. It can be readily accessed by all popular web browsers including Google Chrome, Mozilla Firefox, Safari and Internet Explorer 10 (or later), and so on. We would highly appreciate that if you could send your feedback about any bug or feature request to Shisheng Wang at wsslearning@omicsolution.com.

*^_^ Enjoy yourself in NAguideR ^_^*


Created by Shisheng Wang. E-mail: wsslearning@omicsolution.com.

## III. Reference


1. Tyanova, S., Temu, T. and Cox, J. (2016) The MaxQuant computational platform for mass spectrometry-based shotgun proteomics. *Nat Protoc*, **11**, 2301-2319.
2. Ma, B., Zhang, K., Hendrie, C., Liang, C., Li, M., Doherty-Kirby, A. and Lajoie, G. (2003) PEAKS: powerful software for peptide de novo sequencing by tandem mass spectrometry. *Rapid communications in mass spectrometry : RCM*, **17**, 2337-2342.
3. Bruderer, R., Bernhardt, O.M., Gandhi, T., Miladinović, S.M., Cheng, L.-Y., Messner, S., Ehrenberger, T., Zanotelli, V., Butscheid, Y. and Escher, C. (2015) Extending the limits of quantitative proteome profiling with data-independent acquisition and application to acetaminophen-treated three-dimensional liver microtissues. *Mol Cell Proteomics*, **14**, 1400-1410.
4. Demichev, V., Messner, C.B., Vernardis, S.I., Lilley, K.S. and Ralser, M. (2020) DIA-NN: neural networks and interference correction enable deep proteome coverage in high throughput. *Nature methods*, **17**, 41-44.
5. Rost, H.L., Rosenberger, G., Navarro, P., Gillet, L., Miladinovic, S.M., Schubert, O.T., Wolski, W., Collins, B.C., Malmstrom, J., Malmstrom, L. *et al.* (2014) OpenSWATH enables automated, targeted analysis of data-independent acquisition MS data. *Nature biotechnology*, **32**, 219-223.
6. Bruderer, R., Bernhardt, O.M., Gandhi, T., Miladinovic, S.M., Cheng, L.Y., Messner, S., Ehrenberger, T., Zanotelli, V., Butscheid, Y., Escher, C. *et al.* (2015) Extending the limits of quantitative proteome profiling with data-independent acquisition and application to acetaminophen-treated three-dimensional liver microtissues. *Molecular & cellular proteomics : MCP*, **14**, 1400-1410.
7. Bruderer, R., Bernhardt, O.M., Gandhi, T., Xuan, Y., Sondermann, J., Schmidt, M., Gomez-Varela, D. and Reiter, L. (2017) Optimization of Experimental Parameters in Data-Independent Mass Spectrometry Significantly Increases Depth and Reproducibility of Results. *Molecular & cellular proteomics : MCP*, **16**, 2296-2309.