Supporting Manual for:

NAguideR: performing and prioritizing missing value imputations for data independent acquisition 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.

Table 1. Description of 20 missing value imputation methods.

Class	Abbreviation	Manipulation Method	Remark	Function	Package/References
Fast	zero	zero	Replaces the missing values by 0.	0	base (1)
	minimum	minimum	Replaces the missing values by the smallest non-missing value in the data.	min	base (2)
	colmedian	Column median	Replaces the missing values by the median of non-missing value in each column.	impute	e1071 (3)
	rowmedian	Row median	Replaces the missing values by the median of non-missing value in each row.	impute	e1071 (3)
	SVD	Singular value decomposition imputation	Initializes all missing elements with zero then estimate them as a linear combination of the k most signifcant eigen-variables iteratively until reaches certain convergence threshold.	svdPca	pcaMethods (4)
	KNN	K Nearest Neighbors imputation	K-nearest neighbors in the space of peptides/proteins to impute missing expression values.	impute.knn	impute (4)
	Seq-KNN	Sequential K-nearest neighbor	Imputes the missing values sequentially from the peptide/protein having least missing values based on KNN method, and uses the imputed values for the later imputation.	SeqKNN	SeqKnn (5)
	LLS	Local least squares imputation	K variables (peptides/ proteins) are selected by Pearson, spearman or Kendall correlation coefficients. Then missing values are imputed by a linear combination of the k selected variables. The optimal combination is found by LLS regression.	llsImpute	pcaMethods (6)
	QR	Quantile regression imputation of left-censored data	A missing data imputation method that performs the imputation of left-censored missing data using random draws from a truncated	impute.QRILC	imputeLCMD (7)

			distribution with parameters estimated using quantile regression.		
	MLE	Imputation based on maximum likelihood estimation	Maximum likelihood-based imputation method using the EM algorithm.	prelim.norm, em.norm, imp.norm	norm (8)
	Mindet	Deterministic minimum imputation	Perform the imputation of left-censored missing data using a deterministic minimal value approach. Considering an expression data with n samples and p features, for each sample, the missing entries are replaced with a minimal value observed in that sample. The minimal value observed is estimated as being the q-th quantile of the observed values in that sample.	impute.MinDet	imputeLCMD (9)
	Minprob	Probabilistic minimum imputation	Performs the imputation of left-censored missing data by random draws from a Gaussian distribution centred to a minimal value. Considering an expression data matrix with n samples and p features, for each sample, the mean value of the Gaussian distribution is set to a minimal observed value in that sample. The minimal value observed is estimated as being the q-th quantile of the observed values in that sample. The standard deviation is estimated as the median of the feature standard deviations.	impute.MinProb	imputeLCMD (9)
	Impseq	Sequential imputation of missing values	Estimates sequentially the missing values in an incomplete observation by minimizing the determinant of the covariance of the augmented data matrix. Then the observation is added to the complete data matrix and	impSeq	rrcovNA (10)

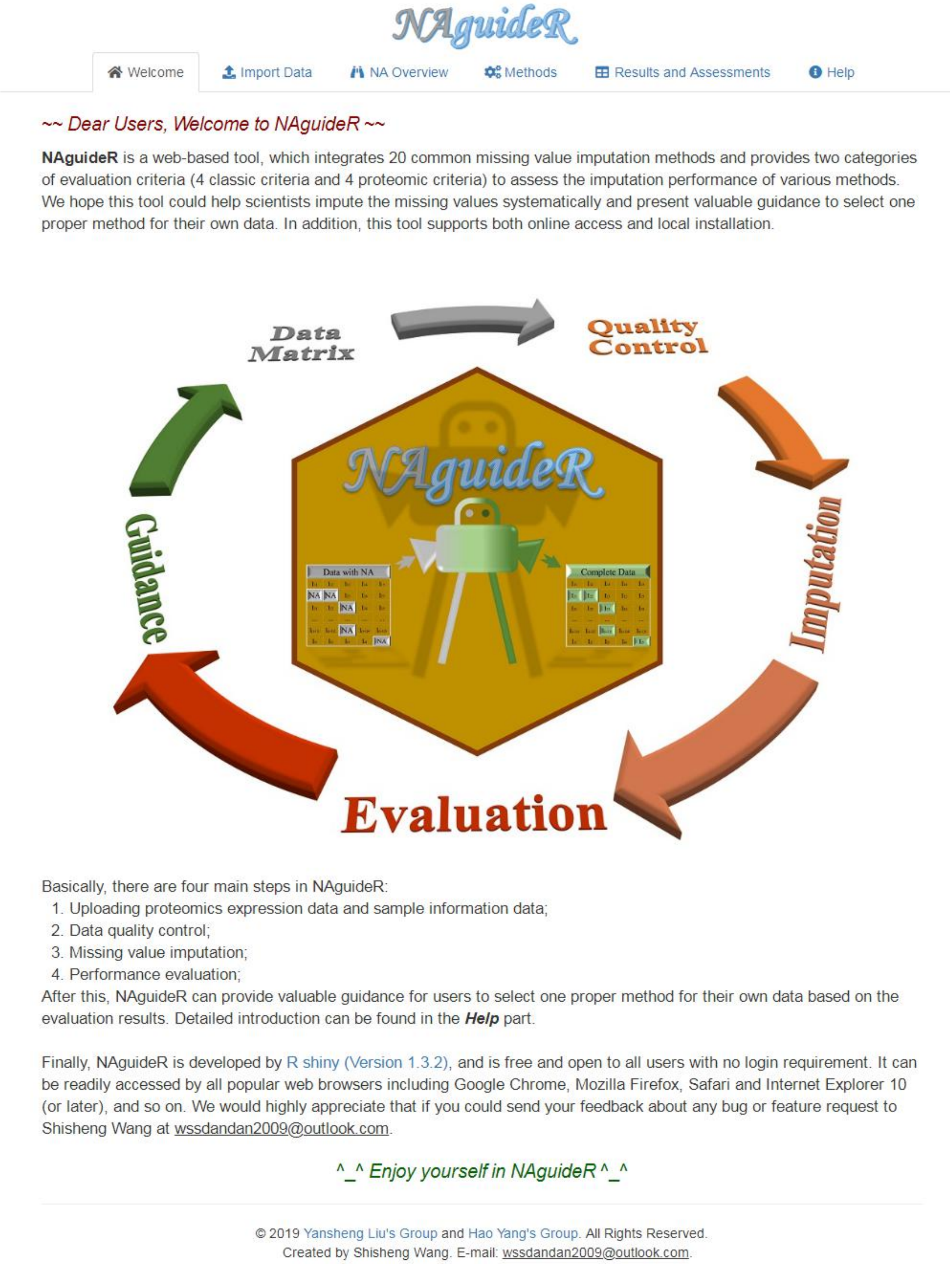
			the algorithm continues with the next observation with missing values.		
	Impseqrob	Robust sequential imputation of missing values	Similar to Impseq, but improved by plugging in robust estimators of location and scatter.	impSeqRob	rrcovNA (11)
	Mice-norm	Multivariate Imputation by Chained Equations-Bayesian linear regression	Generates multiple imputations for incomplete multivariate data by Gibbs sampling. Missing data can occur anywhere in the data. The algorithm imputes an incomplete column (the target column) by generating 'plausible' synthetic values given other columns in the data. Each incomplete column must act as a target column, and has its own specific set of predictors. The default set of predictors for a given target consists of all other columns in the data. For predictors that are incomplete themselves, the most recently generated imputations are used to complete the predictors prior to imputation of the target column. The imputation method depends on Bayesian linear regression.	mice (method='norm')	mice (12)
Slow	BPCA	Bayesian PCA missing value estimation	An iterative method using a Bayesian model to handle missing values.	bpca	pcaMethods (13)
	trKNN	Truncation k-nearest neighbors imputation	Applies a Newton-Raphson (NR) optimization to estimate the truncated mean and standard deviation. Then, Pearson correlation was calculated based on standardized data followed by correlation-based kNN imputation.	sim_trKNN_wrapper	Imput_funcs.R (14)
	IRM	Iterative	In each step of the iteration, one	irmi	VIM (15)

		robust model-based imputation	variable is used as a response variable and the remaining variables serve as the regressors.		
	Mice-cart	Multivariate Imputation by Chained Equations-classification and regression trees	Generates multiple imputations for incomplete multivariate data by Gibbs sampling. Missing data can occur anywhere in the data. The algorithm imputes an incomplete column (the target column) by generating 'plausible' synthetic values given other columns in the data. Each incomplete column must act as a target column, and has its own specific set of predictors. The default set of predictors for a given target consists of all other columns in the data. For predictors that are incomplete themselves, the most recently generated imputations are used to complete the predictors prior to imputation of the target column. The imputation method depends on classification and regression trees.	mice (method=’cart’)	mice (12)
	RF	Random forest	Imputes missing values particularly in the case of mixed-type data based on a random forest. It can be used to impute continuous and/or categorical data including complex interactions and nonlinear relations. It yields an out-of-bag (OOB) imputation error estimate.	missForest	missForest (16)

Supplementary notes

NAguideR integrates up to 20 common missing value imputation methods (described in Table S1) and provides two categories of evaluation criteria (four classic computational criteria and four common knowledge-based proteomics criteria) to assess the imputation performance of various methods. Here we present the detailed introduction and operation of NAguideR, users can follow this manuscript 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:



~~ Dear Users, Welcome to NAguideR ~~

NAguideR is a web-based tool, which integrates 20 common 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 wssdandan2009@outlook.com.

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

Created by Shisheng Wang. E-mail: wssdandan2009@outlook.com.

1. Data Preparation

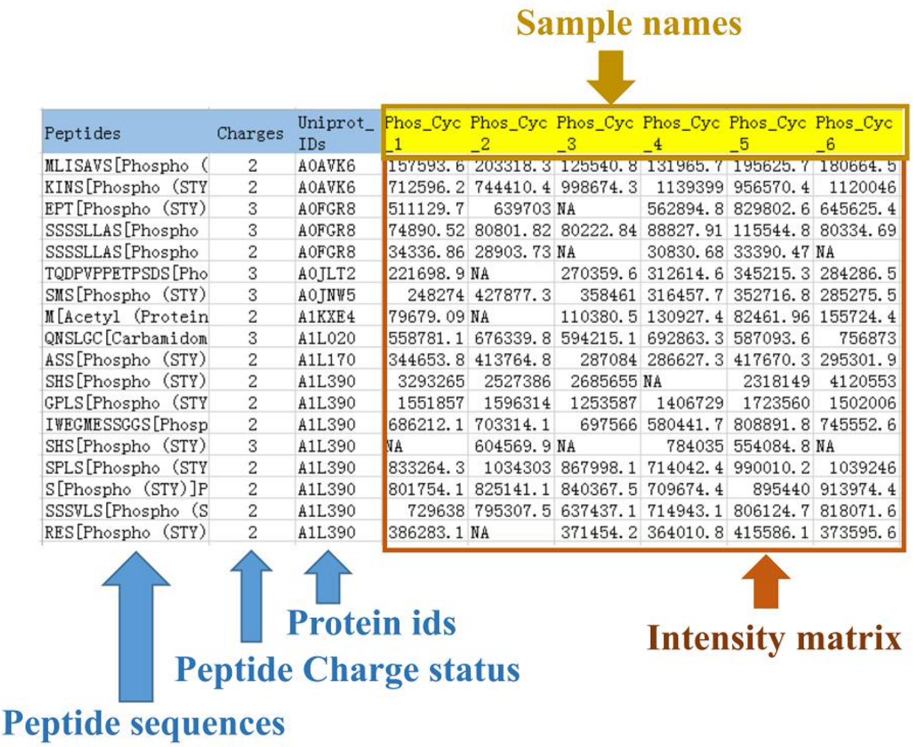
NAguideR supports four basic file formats (.csv, .txt, .xlsx, .xls). Before analysis, users should prepare two required data: (1) Proteomics expression data and (2) Sample information data. The data required here could be readily generated based on results of several popular tools such as MaxQuant, PEAKS,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

1.1 Expression data

There are four types of proteomics expression data supported in NAguideR, among which the main differences are the first few columns.

1.1.1 Expression data with peptide sequences, peptide charge status, and protein ids

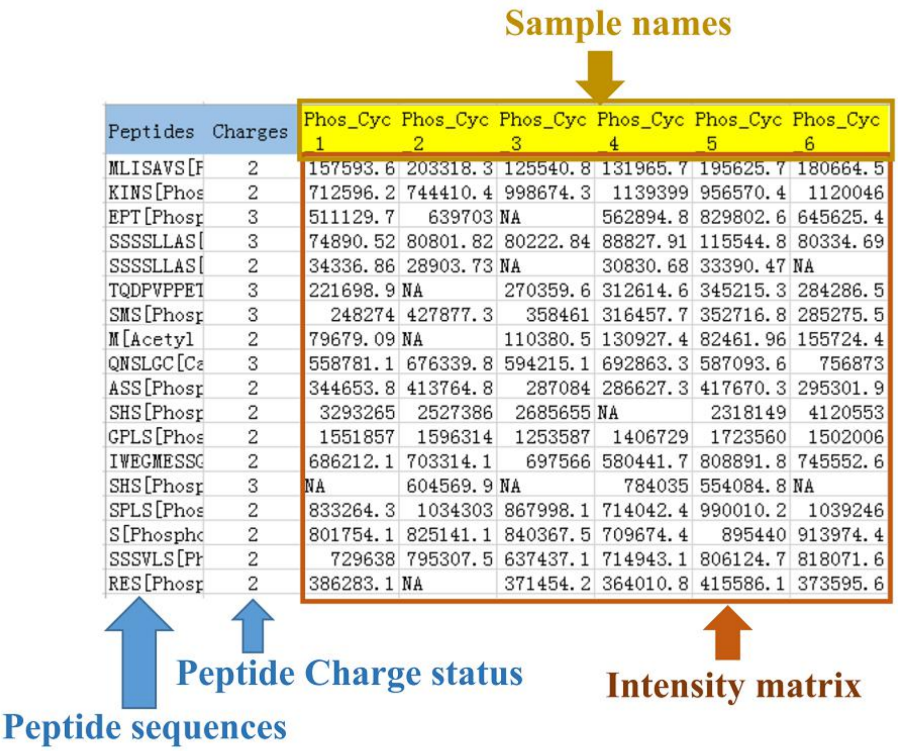
In this situation, peptide sequences, peptide charge status, and protein ids are sequentially provided in the first three columns of input file. Peptide sequences in the first column can be peptides with post-translational modification (PTM) or stripped peptides (without PTM). The second column is peptide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



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
TQDPVPPETPSDS[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	A1KXE4	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
IWEGMESSGGS[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

1.1.2 Expression data with peptide sequences and peptide charge status

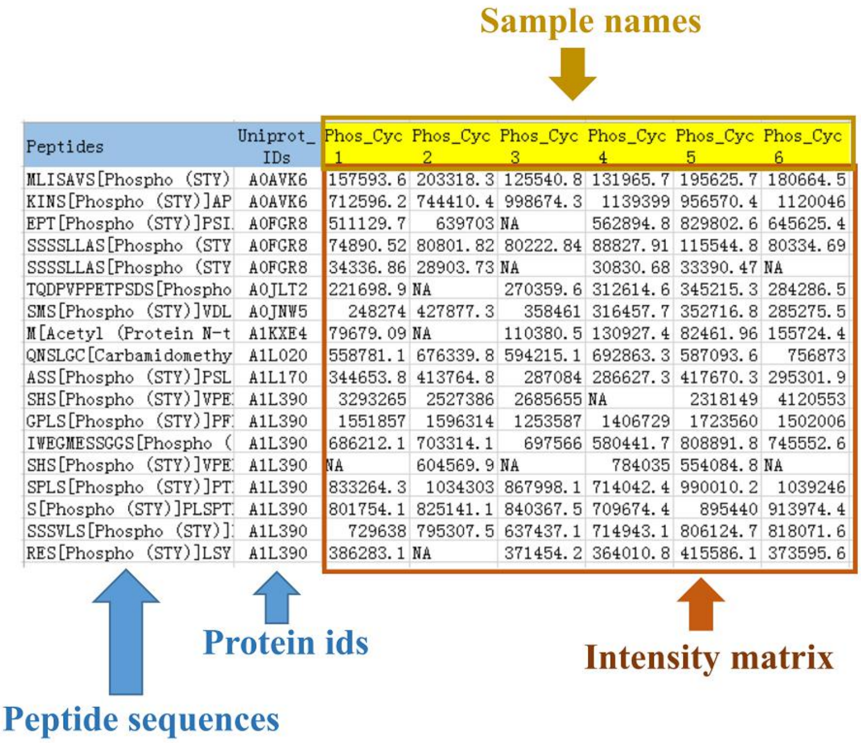
Similar to the above situation, peptide sequences and peptide charge status are sequentially provided in the first two columns of input file. Peptide sequences in the first column can be peptides with post-translational modification (PTM) or stripped peptides (without PTM). The second column is peptide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



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

1.1.3 Expression data with peptide sequences, and protein ids

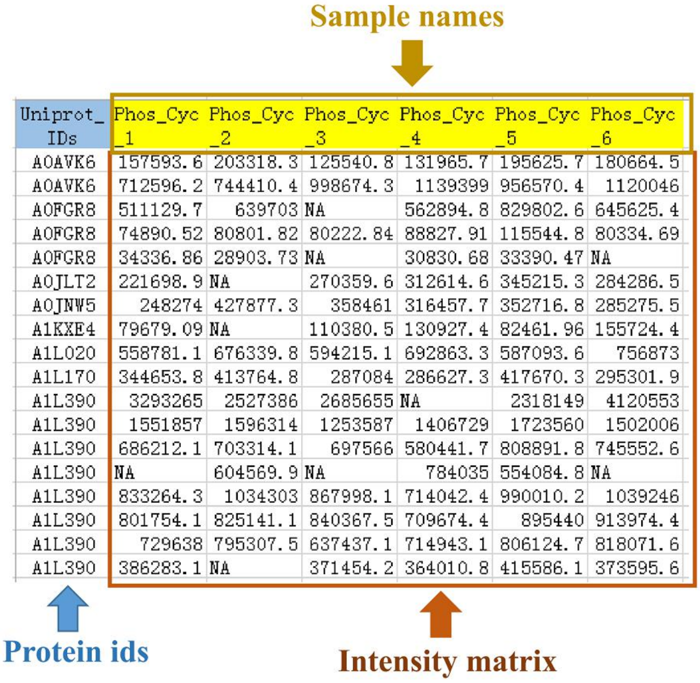
Under this circumstance, peptide sequences, and protein ids are sequentially provided in the first two columns of input file. Peptide sequences in the first column can be peptides with post-translational modification (PTM) or stripped peptides (without PTM). The protein ids in the second column should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



Peptides	Uniprot_IDs	Phos_Cyc_1	Phos_Cyc_2	Phos_Cyc_3	Phos_Cyc_4	Phos_Cyc_5	Phos_Cyc_6
MLISAVS[Phospho (STY)	A0AVK6	157593.6	203318.3	125540.8	131965.7	195625.7	180664.5
KINS[Phospho (STY)]AP	A0AVK6	712596.2	744410.4	998674.3	1139399	956570.4	1120046
EPT[Phospho (STY)]PSI	A0FGR8	511129.7	639703	NA	562894.8	829802.6	645625.4
SSSSLLAS[Phospho (STY	A0FGR8	74890.52	80801.82	80222.84	88827.91	115544.8	80334.69
SSSSLLAS[Phospho (STY	A0FGR8	34336.86	28903.73	NA	30830.68	33390.47	NA
TQDPVPPETPSDS[Phospho	A0JLT2	221698.9	NA	270359.6	312614.6	345215.3	284286.5
SMS[Phospho (STY)]VDL	A0JNW5	248274	427877.3	358461	316457.7	352716.8	285275.5
M[Acetyl (Protein N-t	A1KXE4	79679.09	NA	110380.5	130927.4	82461.96	155724.4
QNSLGC[Carbamidomethy	A1L020	558781.1	676339.8	594215.1	692863.3	587093.6	756873
ASS[Phospho (STY)]PSL	A1L170	344653.8	413764.8	287084	286627.3	417670.3	295301.9
SHS[Phospho (STY)]VPE	A1L390	3293265	2527386	2685655	NA	2318149	4120553
GPLS[Phospho (STY)]PF	A1L390	1551857	1596314	1253587	1406729	1723560	1502006
IWEGMESSGGS[Phospho (	A1L390	686212.1	703314.1	697566	580441.7	808891.8	745552.6
SHS[Phospho (STY)]VPE	A1L390	NA	604569.9	NA	784035	554084.8	NA
SPLS[Phospho (STY)]PT	A1L390	833264.3	1034303	867998.1	714042.4	990010.2	1039246
S[Phospho (STY)]PLSPT	A1L390	801754.1	825141.1	840367.5	709674.4	895440	913974.4
SSSVLS[Phospho (STY)]	A1L390	729638	795307.5	637437.1	714943.1	806124.7	818071.6
RES[Phospho (STY)]LSY	A1L390	386283.1	NA	371454.2	364010.8	415586.1	373595.6

1.1.4 Expression data with protein ids

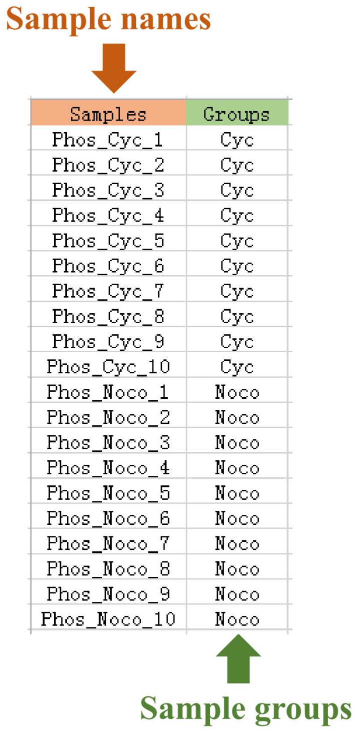
In this situation, protein ids are provided in the first two columns of input file. The protein ids here should be UniProt ids. From the second column on, they are peptides/proteins expression intensity in every sample. The data structure is shown as below:



Uniprot_IDs	Phos_Cyc_1	Phos_Cyc_2	Phos_Cyc_3	Phos_Cyc_4	Phos_Cyc_5	Phos_Cyc_6
A0AVK6	157593.6	203318.3	125540.8	131965.7	195625.7	180664.5
A0AVK6	712596.2	744410.4	998674.3	1139399	956570.4	1120046
A0FGR8	511129.7	639703	NA	562894.8	829802.6	645625.4
A0FGR8	74890.52	80801.82	80222.84	88827.91	115544.8	80334.69
A0FGR8	34336.86	28903.73	NA	30830.68	33390.47	NA
A0JLT2	221698.9	NA	270359.6	312614.6	345215.3	284286.5
A0JNW5	248274	427877.3	358461	316457.7	352716.8	285275.5
A1KXE4	79679.09	NA	110380.5	130927.4	82461.96	155724.4
A1L020	558781.1	676339.8	594215.1	692863.3	587093.6	756873
A1L170	344653.8	413764.8	287084	286627.3	417670.3	295301.9
A1L390	3293265	2527386	2685655	NA	2318149	4120553
A1L390	1551857	1596314	1253587	1406729	1723560	1502006
A1L390	686212.1	703314.1	697566	580441.7	808891.8	745552.6
A1L390	NA	604569.9	NA	784035	554084.8	NA
A1L390	833264.3	1034303	867998.1	714042.4	990010.2	1039246
A1L390	801754.1	825141.1	840367.5	709674.4	895440	913974.4
A1L390	729638	795307.5	637437.1	714943.1	806124.7	818071.6
A1L390	386283.1	NA	371454.2	364010.8	415586.1	373595.6

1.2 Samples information data

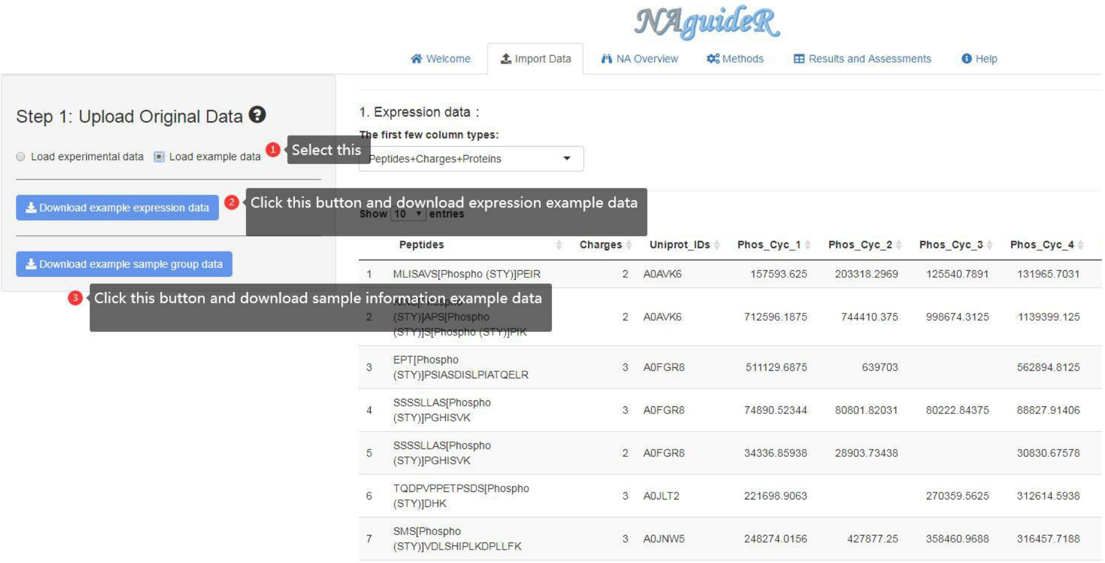
Sample information here means users should identify sample group information. The sample names are in the first column and their orders are same as those in the expression data. Group information is in the second column. The data structure is shown as below:



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:



Step 1: Upload Original Data

○ Load experimental data ● Load example data

1 Select this

2 Click this button and download expression example data

3 Click this button and download sample information example data

1. Expression data :

The first few column types: Peptides+Charges+Proteins

Show 10 entries

	Peptides	Charges	Uniprot_IDs	Phos_Cyc_1	Phos_Cyc_2	Phos_Cyc_3	Phos_Cyc_4
1	MLISAVS[Phospho (STY)]PEIR	2	A0AVK6	157593.625	203318.2969	125540.7891	131965.7031
2	[illegible] (STY)]APS[Phospho (STY)]S[Phospho (STY)]PIK	2	A0AVK6	712596.1875	744410.375	998674.3125	1139399.125
3	EPT[Phospho (STY)]PSIASDISLPIATQELR	3	A0FGR8	511129.6875	639703		562894.8125
4	SSSSLLAS[Phospho (STY)]PGHISVK	3	A0FGR8	74890.52344	80801.82031	80222.84375	88827.91406
5	SSSSLLAS[Phospho (STY)]PGHISVK	2	A0FGR8	34336.85938	28903.73438		30830.67578
6	TQDPVPPETPSDS[Phospho (STY)]DHK	3	A0JLT2	221698.9063		270359.5625	312614.5938
7	SMS[Phospho (STY)]VDLSHIPLKDPLLFK	3	A0JNW5	248274.0156	427877.25	358460.9688	316457.7188

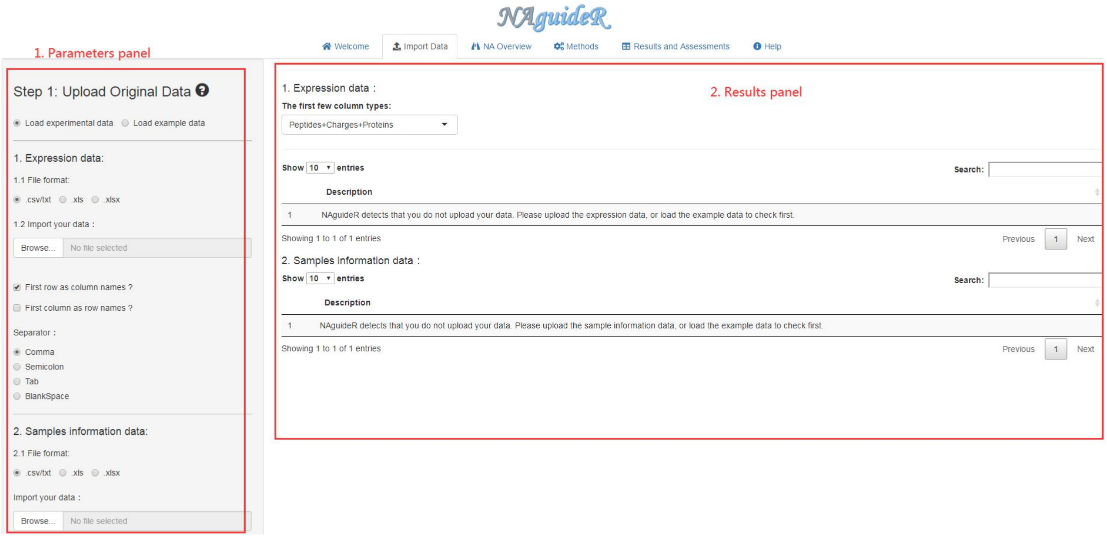
First, select "Load example data" and the example data will be shown on the right panel interactively. Users can visually observe what the data looks like.

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

2. Import data.

This is the first step, 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:



Step 1: Upload Original Data

● Load experimental data ○ Load example data

1. Expression data:

1.1 File format:

● .csv/txt ○ .xls ○ .xlsx

1.2 Import your data :

Browse... No file selected

☑ First row as column names ?

☐ First column as row names ?

Separator :

● Comma
○ Semicolon
○ Tab
○ BlankSpace

2. Samples information data:

2.1 File format:

● .csv/txt ○ .xls ○ .xlsx

Import your data :

Browse... No file selected

1. Expression data :

The first few column types: Peptides+Charges+Proteins

Show 10 entries Search:

	Description
1	NAguideR detects that you do not upload your data. Please upload the expression data, or load the example data to check first.

Showing 1 to 1 of 1 entries Previous 1 Next

2. Samples information data :

Show 10 entries Search:

	Description
1	NAguideR detects that you do not upload your data. Please upload the sample information data, or load the example data to check first.

Showing 1 to 1 of 1 entries Previous 1 Next

There are two main panels: first, *parameters panel*, users can adjust some 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 from here. Users should select the right format based on their own data and then click "Browse" button to import the data;

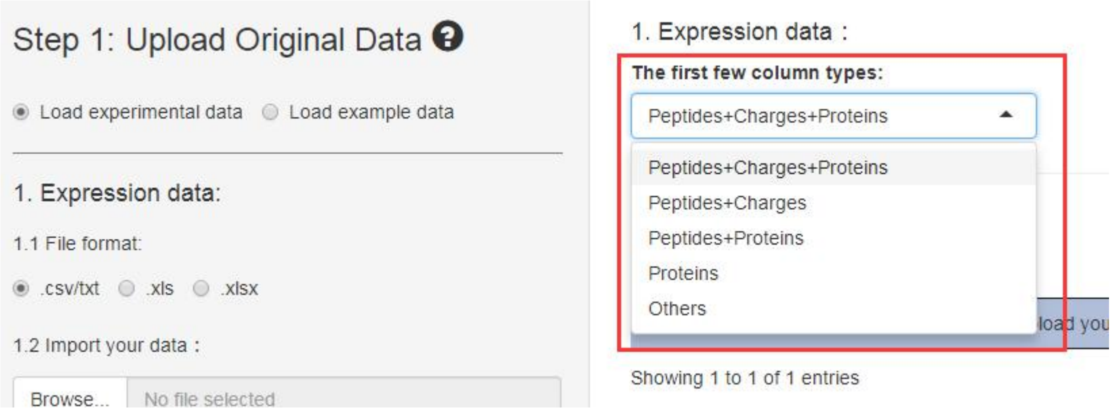
First row as column names: this means whether the first row is column names. If true, you should choose this parameter.

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 do 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 (part 1).



Step 1: Upload Original Data

● Load experimental data ○ Load example data

1. Expression data:

1.1 File format:

● .csv/txt ○ .xls ○ .xlsx

1.2 Import your data：

Browse... No file selected

1. Expression data：

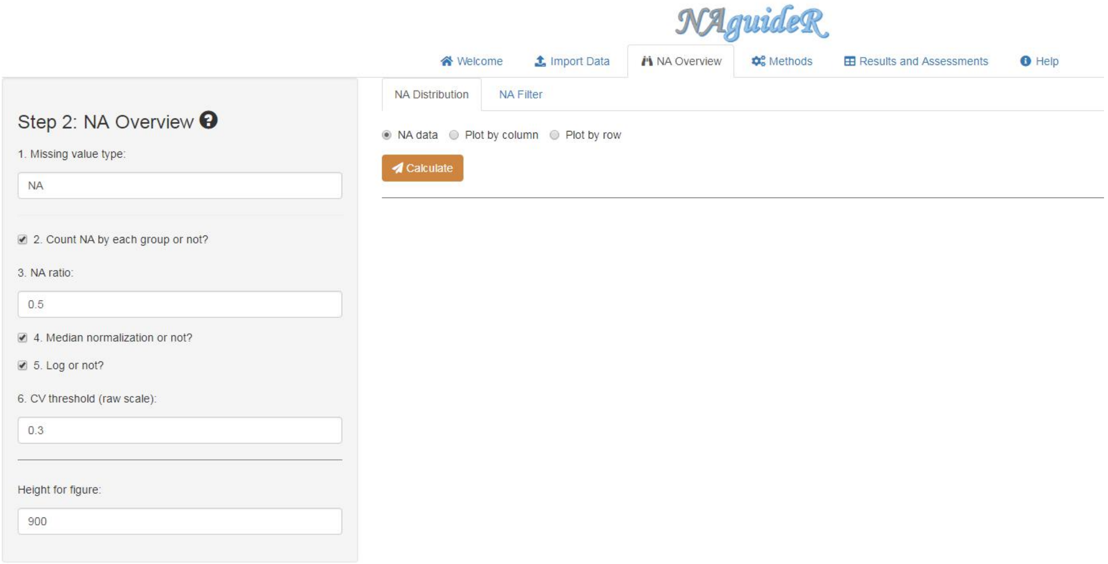
The first few column types:

Peptides+Charges+Proteins

Showing 1 to 1 of 1 entries

3. NA Overview

Users can check the missing value situation of their own data and filter those data with high proportion of missing value in this step. NA is short for Not Available, which means missing value here.



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?

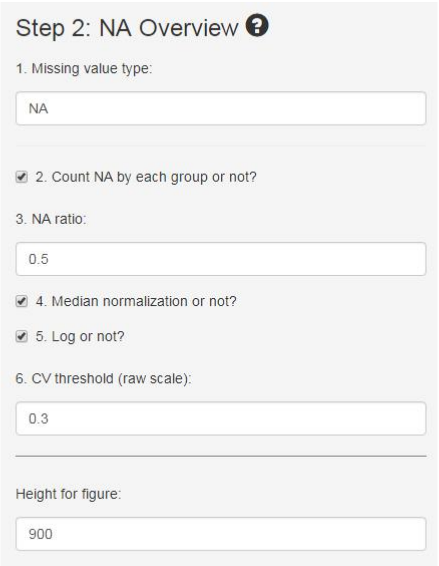
☑ 5. Log or not?

6. CV threshold (raw scale): 0.3

Height for figure: 900

● NA data ○ Plot by column ○ Plot by row

3.1 Parameters



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?

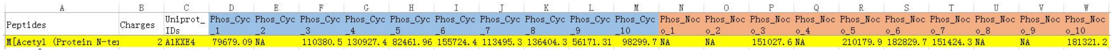
☑ 5. Log or not?

6. CV threshold (raw scale): 0.3

Height for figure: 900

1. Missing value type: what the missing values look like in the expression data, for example, Spectronaut (17,18) 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.

2. Count NA by each group or not: if true, NAguideR will count the number of missing value by each group and calculate the NA ratio, otherwise, calculate 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_Noco_1	Phos_Noco_2	Phos_Noco_3	Phos_Noco_4	Phos_Noco_5	Phos_Noco_6	Phos_Noco_7	Phos_Noco_8	Phos_Noco_9	Phos_Noco_10
M[Acetyl (Protein N-te	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.

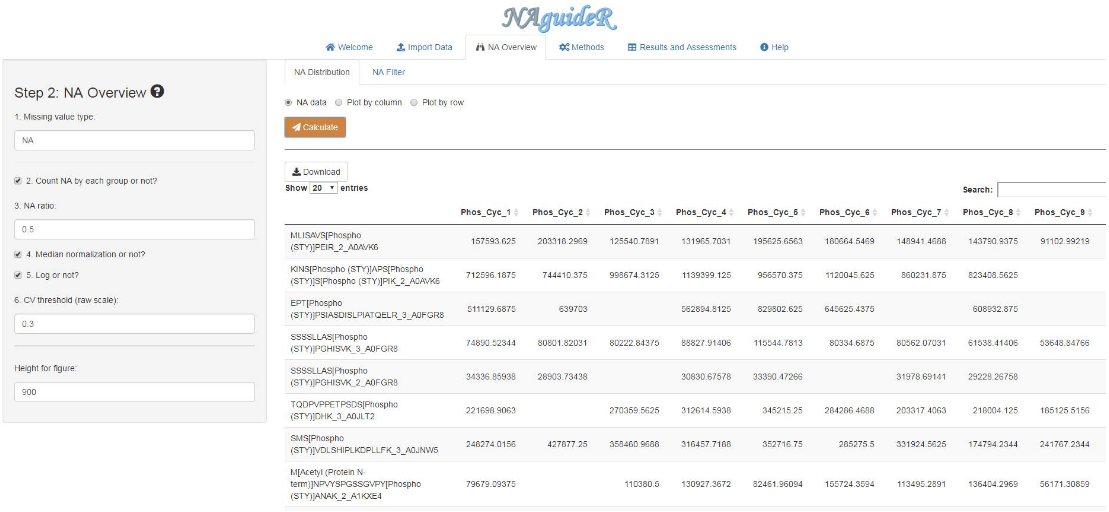
4. Median normalization or not: if true, NAguideR will process median normalization for original data.

5. Log or not: if true, the data will be logarithmic 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.



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 data ○ Plot by column ○ Plot by row

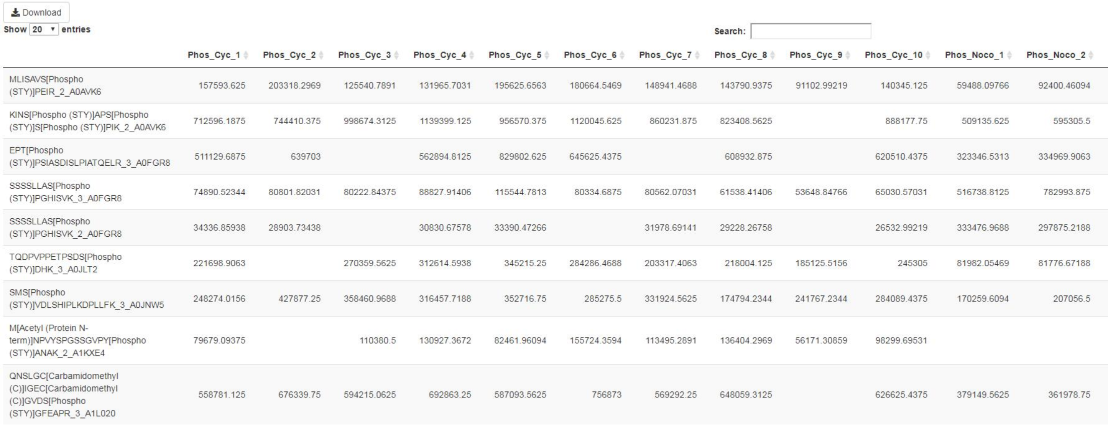
Show 20 entries

	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	157593.625	203318.2969	125540.7891	131965.7031	195625.6563	180664.5469	148941.4688	143790.9375	91102.99219
KINS[Phospho (STY)]APS[Phospho (STY)]S[Phospho (STY)]PIK_2_A0AVK6	712596.1875	744410.375	998674.3125	1139399.125	956570.375	1120045.625	860231.875	823408.5625	
EPT[Phospho (STY)]PSIASDISLPIATQELR_3_A0FGR8	511129.6875	639703		562894.8125	829802.625	645625.4375		608932.875	
SSSSLLAS[Phospho (STY)]PGHISVK_3_A0FGR8	74890.52344	80801.82031	80222.84375	88827.91406	115544.7813	80334.6875	80562.07031	61538.41406	53648.84766
SSSSLLAS[Phospho (STY)]PGHISVK_2_A0FGR8	34336.85938	28903.73438		30830.67578	33390.47266		31978.69141	29228.26758	
TQDPVPPETPSDS[Phospho (STY)]DHK_3_A0JLT2	221698.9063		270359.5625	312614.5938	345215.25	284286.4688	203317.4063	218004.125	185125.5156
SMS[Phospho (STY)]VDLSHIPLKDPLLFK_3_A0JNW5	248274.0156	427877.25	358460.9688	316457.7188	352716.75	285275.5	331924.5625	174794.2344	241767.2344
M[Acetyl (Protein N-term)]NPVYSPGSSGVPY[Phospho (STY)]ANAK_2_A1KXE4	79679.09375		110380.5	130927.3672	82461.96094	155724.3594	113495.2891	136404.2969	56171.30859

3.2 results

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

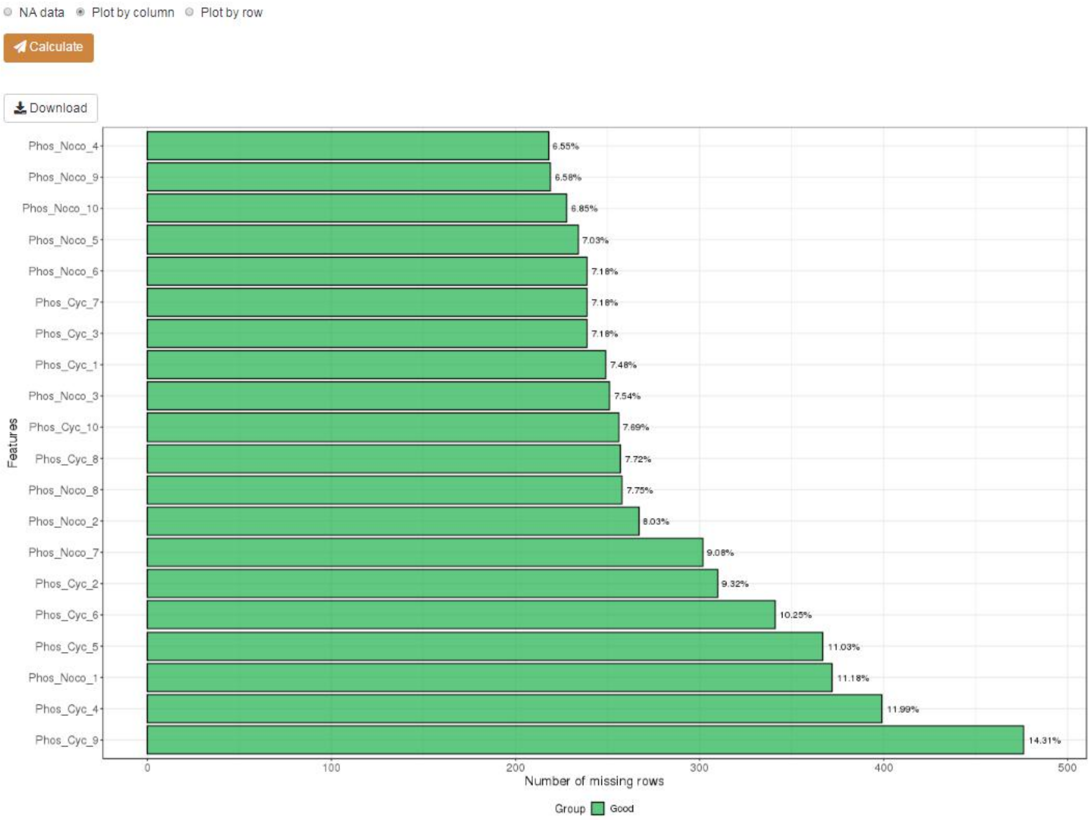
a.1 NA data. Here shows the result where the "Missing value type" will be replaced with NA and users can click "Download" button to download this result to their own computer:



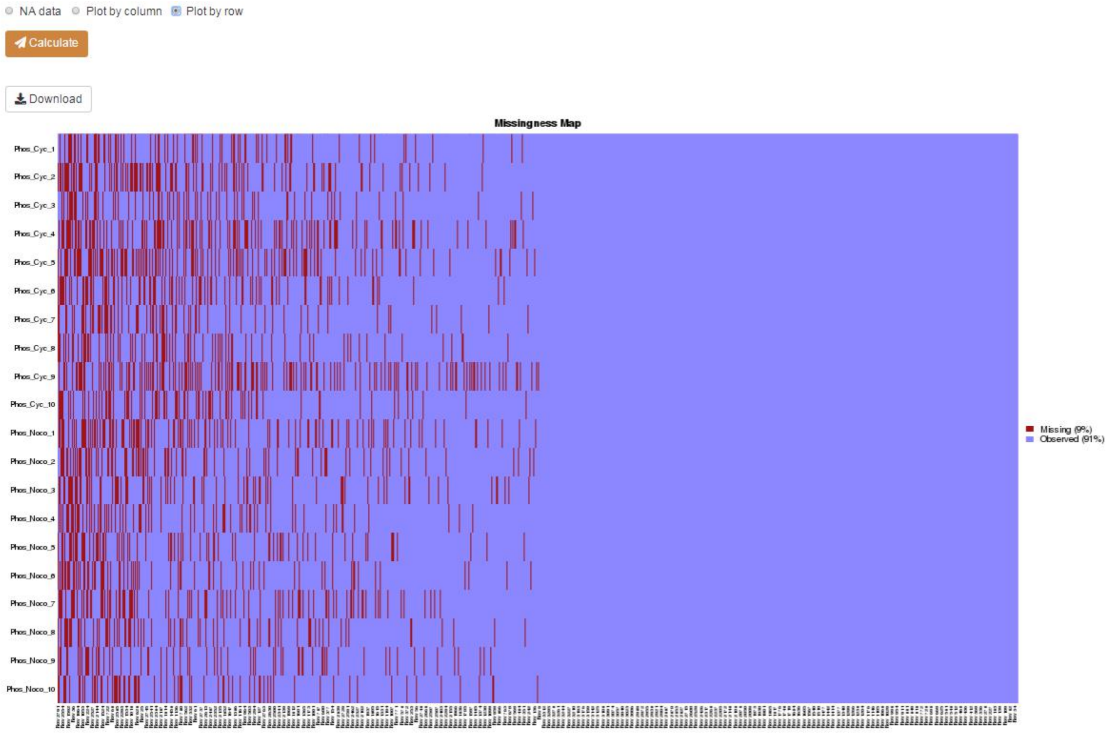
Show 20 entries

	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_Noco_1	Phos_Noco_2
MLISAVS[Phospho (STY)]PEIR_2_A0AVK6	157593.625	203318.2969	125540.7891	131965.7031	195625.6563	180664.5469	148941.4688	143790.9375	91102.99219	140345.125	59488.09766	92400.46094
KINS[Phospho (STY)]APS[Phospho (STY)]S[Phospho (STY)]PIK_2_A0AVK6	712596.1875	744410.375	998674.3125	1139399.125	956570.375	1120045.625	860231.875	823408.5625		888177.75	509135.625	595305.5
EPT[Phospho (STY)]PSIASDISLPIATQELR_3_A0FGR8	511129.6875	639703		562894.8125	829802.625	645625.4375		608932.875		620510.4375	323346.5313	334969.9063
SSSSLLAS[Phospho (STY)]PGHISVK_3_A0FGR8	74890.52344	80801.82031	80222.84375	88827.91406	115544.7813	80334.6875	80562.07031	61538.41406	53648.84766	65030.57031	516738.8125	782993.875
SSSSLLAS[Phospho (STY)]PGHISVK_2_A0FGR8	34336.85938	28903.73438		30830.67578	33390.47266		31978.69141	29228.26758		26532.99219	333476.9688	297875.2188
TQDPVPPETPSDS[Phospho (STY)]DHK_3_A0JLT2	221698.9063		270359.5625	312614.5938	345215.25	284286.4688	203317.4063	218004.125	185125.5156	245305	81982.05469	81776.67188
SMS[Phospho (STY)]VDLSHIPLKDPLLFK_3_A0JNW5	248274.0156	427877.25	358460.9688	316457.7188	352716.75	285275.5	331924.5625	174794.2344	241767.2344	284089.4375	170259.6094	207056.5
M[Acetyl (Protein N-term)]NPVYSPGSSGVPY[Phospho (STY)]ANAK_2_A1KXE4	79679.09375		110380.5	130927.3672	82461.96094	155724.3594	113495.2891	136404.2969	56171.30859	98299.69531		
QNSLGC[Carbamidomethyl (C)]IGEC[Carbamidomethyl (C)]GVDS[Phospho (STY)]GFEAPR_3_A1L020	558781.125	676339.75	594215.0625	692863.25	587093.5625	756873	569292.25	648059.3125		626625.4375	379149.5625	361978.75

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



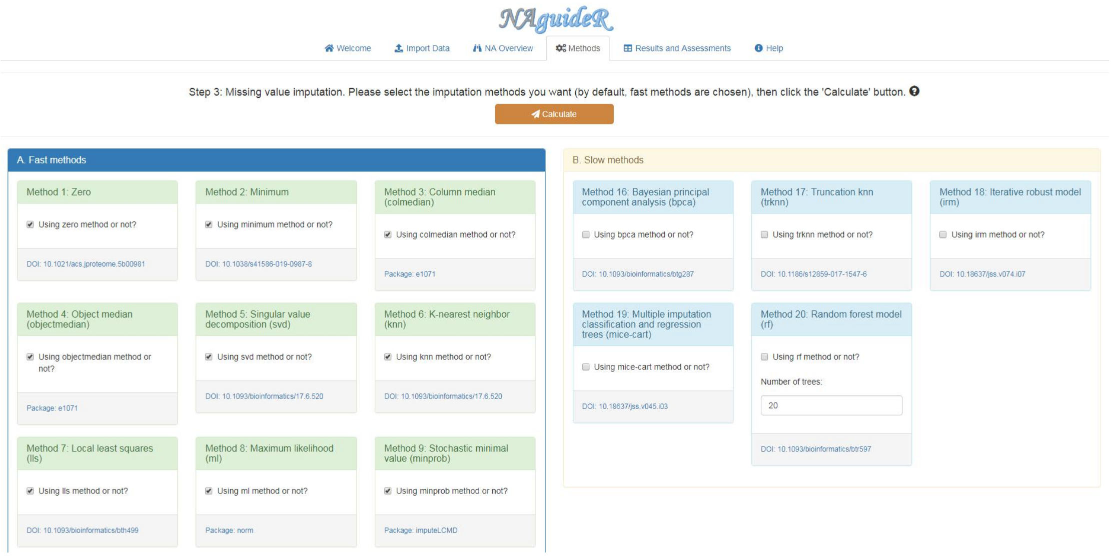
a.2 Plot by row. Here shows the result of the NA distribution of every peptide/protein.



b. NA filter. This part will show the filtered result.

4. Methods

In this step, users can choose or cancel any missing value imputation method. With regard to the running time, we set these fast methods (left part, 15 methods) chosen by default. If users choose those slow methods (right part, 5 methods), that means the running time will be longer. By default, the fast methods are selected. If users want to try these slow methods, they just select the corresponding methods. The detailed information about each method can be found in Table S2. In addition, we also provide the reference for every method just blow each option on the web:



Step 3: Missing value imputation. Please select the imputation methods you want (by default, fast methods are chosen), then click the 'Calculate' button.

A. Fast methods

Method 1: Zero
☑ Using zero method or not?
DOI: 10.1021/acs.jproteome.5b00981

Method 2: Minimum
☑ Using minimum method or not?
DOI: 10.1038/s41586-019-0987-8

Method 3: Column median (colmedian)
☑ Using colmedian method or not?
Package: e1071

Method 4: Object median (objectmedian)
☑ Using objectmedian method or not?
Package: e1071

Method 5: Singular value decomposition (svd)
☑ Using svd method or not?
DOI: 10.1093/bioinformatics/17.6.520

Method 6: K-nearest neighbor (knn)
☑ Using knn method or not?
DOI: 10.1093/bioinformatics/17.6.520

Method 7: Local least squares (lls)
☑ Using lls method or not?
DOI: 10.1093/bioinformatics/bth499

Method 8: Maximum likelihood (ml)
☑ Using ml method or not?
Package: norm

Method 9: Stochastic minimal value (minprob)
☑ Using minprob method or not?
Package: imputeLCMD

B. Slow methods

Method 16: Bayesian principal component analysis (bpca)
□ Using bpca method or not?
DOI: 10.1093/bioinformatics/btg287

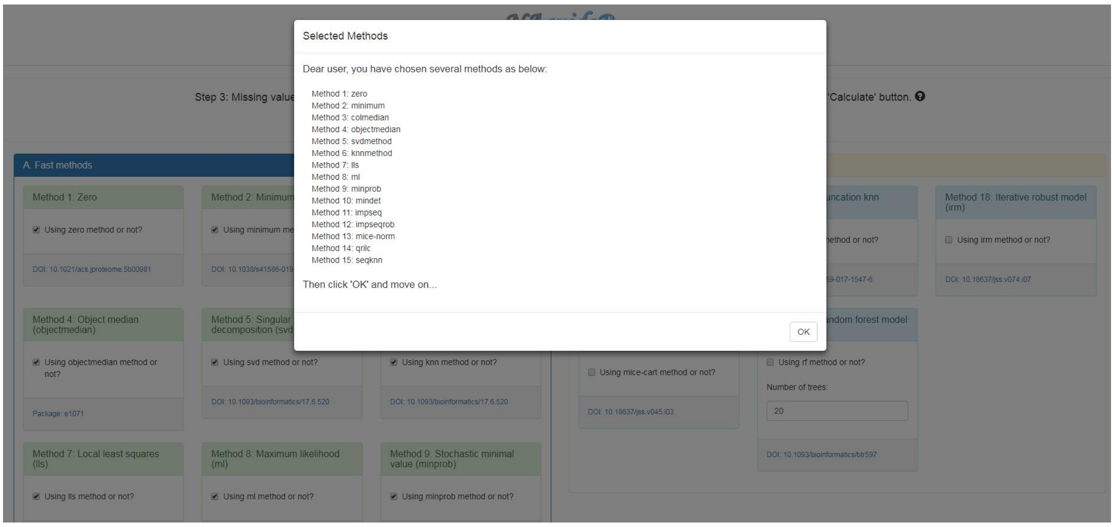
Method 17: Truncation knn (trknn)
□ Using trknn method or not?
DOI: 10.1186/s12859-017-1547-6

Method 18: Iterative robust model (irm)
□ Using irm method or not?
DOI: 10.18637/jss.v074.i07

Method 19: Multiple imputation classification and regression trees (mice-cart)
□ Using mice-cart method or not?
DOI: 10.18637/jss.v045.i03

Method 20: Random forest model (rf)
□ Using rf method or not?
Number of trees: 20
DOI: 10.1093/bioinformatics/btr597

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

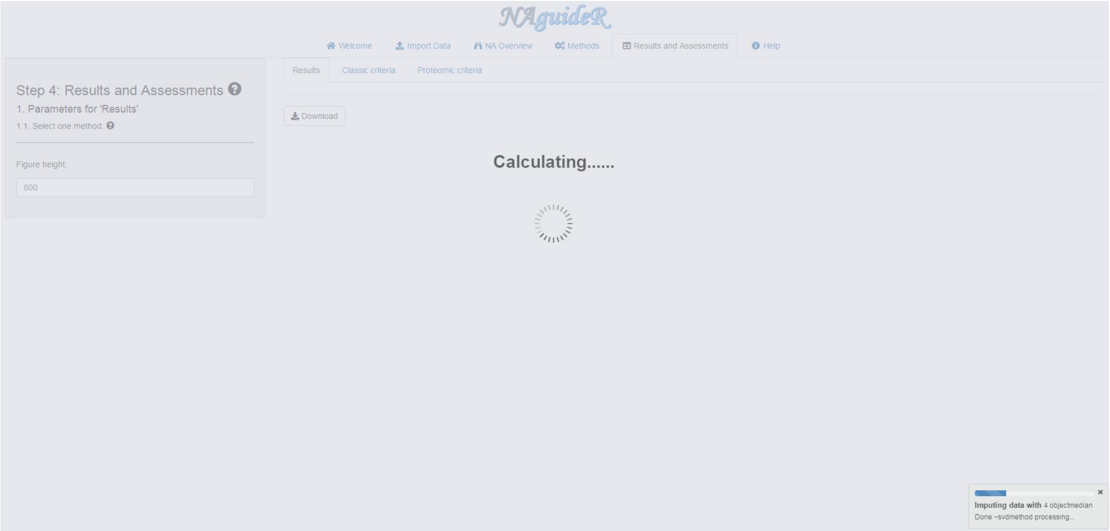
Dear user, you have chosen several methods as below:

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...

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, a process bar will appear in the bottom right corner to tell users where it goes:



Step 4: Results and Assessments

1. Parameters for 'Results'

1.1. Select one method:

Figure height:

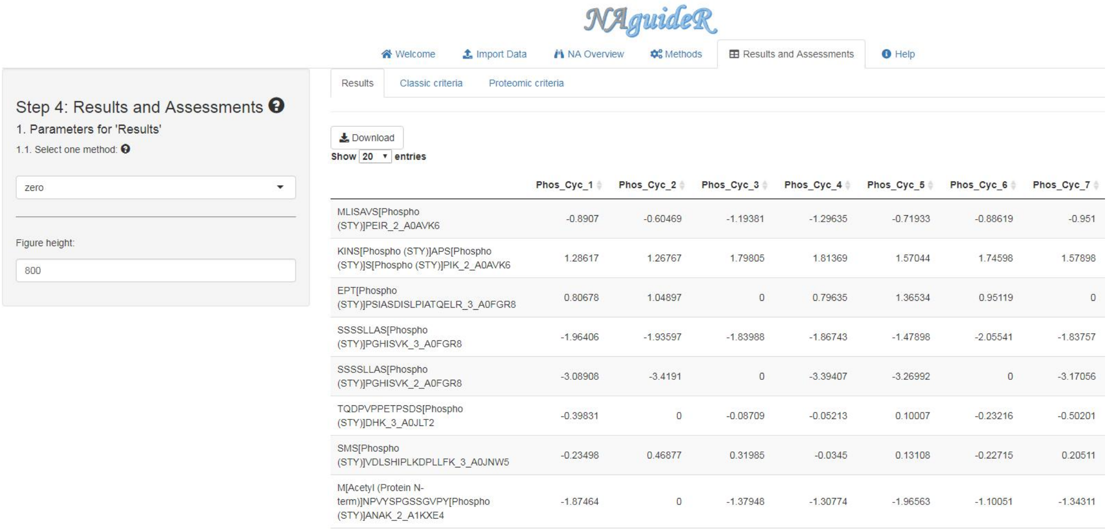
800

Calculating......

Imputing data with 4 objectmedian

Done ~svdmethod processing...

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



Step 4: Results and Assessments

1. Parameters for 'Results'

1.1. Select one method:

zero

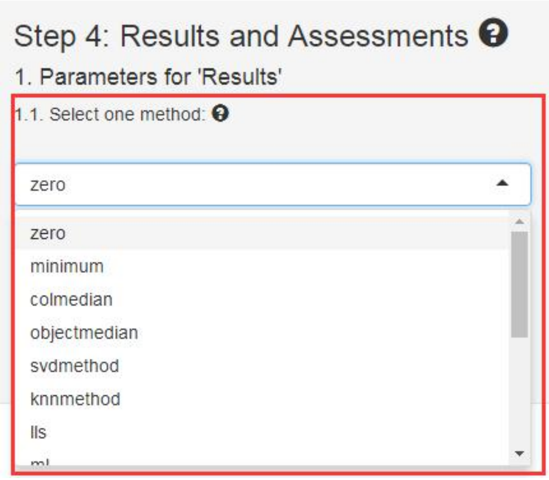
Figure height:

800

Show 20 entries

	Phos_Cyc_1	Phos_Cyc_2	Phos_Cyc_3	Phos_Cyc_4	Phos_Cyc_5	Phos_Cyc_6	Phos_Cyc_7
MLISAVS[Phospho (STY)]PEIR_2_A0AVK6	-0.8907	-0.60469	-1.19381	-1.29635	-0.71933	-0.88619	-0.951
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
EPT[Phospho (STY)]PSIASDISLPIATQELR_3_A0FGR8	0.80678	1.04897	0	0.79635	1.36534	0.95119	0
SSSSLLAS[Phospho (STY)]PGHISVK_3_A0FGR8	-1.96406	-1.93597	-1.83988	-1.86743	-1.47898	-2.05541	-1.83757
SSSSLLAS[Phospho (STY)]PGHISVK_2_A0FGR8	-3.08908	-3.4191	0	-3.39407	-3.26992	0	-3.17056
TQDPVPPETPSDS[Phospho (STY)]DHK_3_A0JLT2	-0.39831	0	-0.08709	-0.05213	0.10007	-0.23216	-0.50201
SMS[Phospho (STY)]VDLSHIPLKDPLLFK_3_A0JNW5	-0.23498	0.46877	0.31985	-0.0345	0.13108	-0.22715	0.20511
M[Acetyl (Protein N-term)]NPVYSPGSSGVPY[Phospho (STY)]ANAK_2_A1KXE4	-1.87464	0	-1.37948	-1.30774	-1.96563	-1.10051	-1.34311

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:



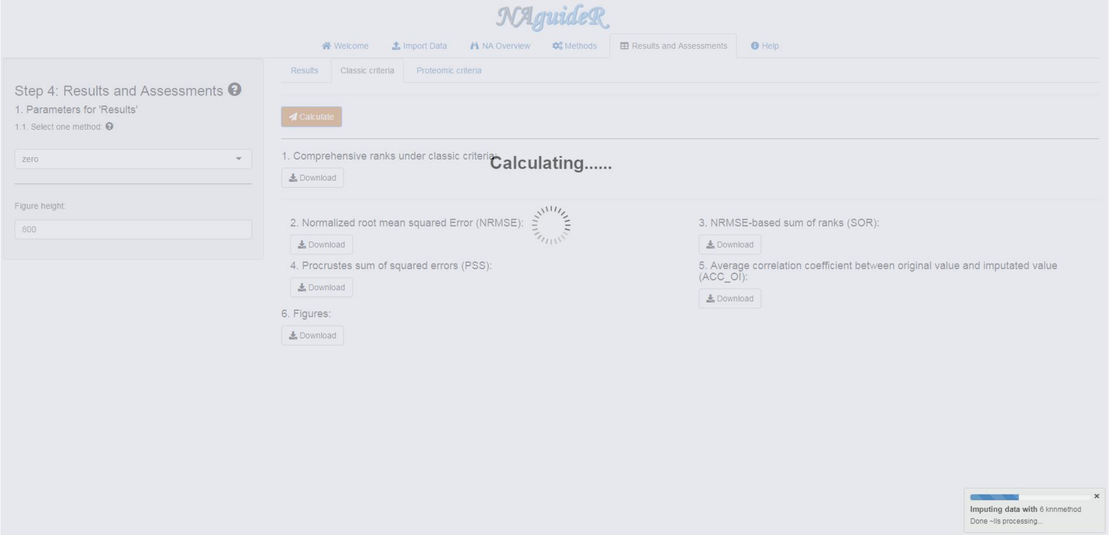
Step 4: Results and Assessments

1. Parameters for 'Results'

1.1. Select one method:

zero

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



Step 4: Results and Assessments

1. Parameters for 'Results'

1.1. Select one method:

Figure height:

1. Comprehensive ranks under classic criteria:

Calculating......

2. Normalized root mean squared Error (NRMSE):

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

4. Procrustes sum of squared errors (PSS):

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

6. Figures:

Imputing data with 6 knnmethod

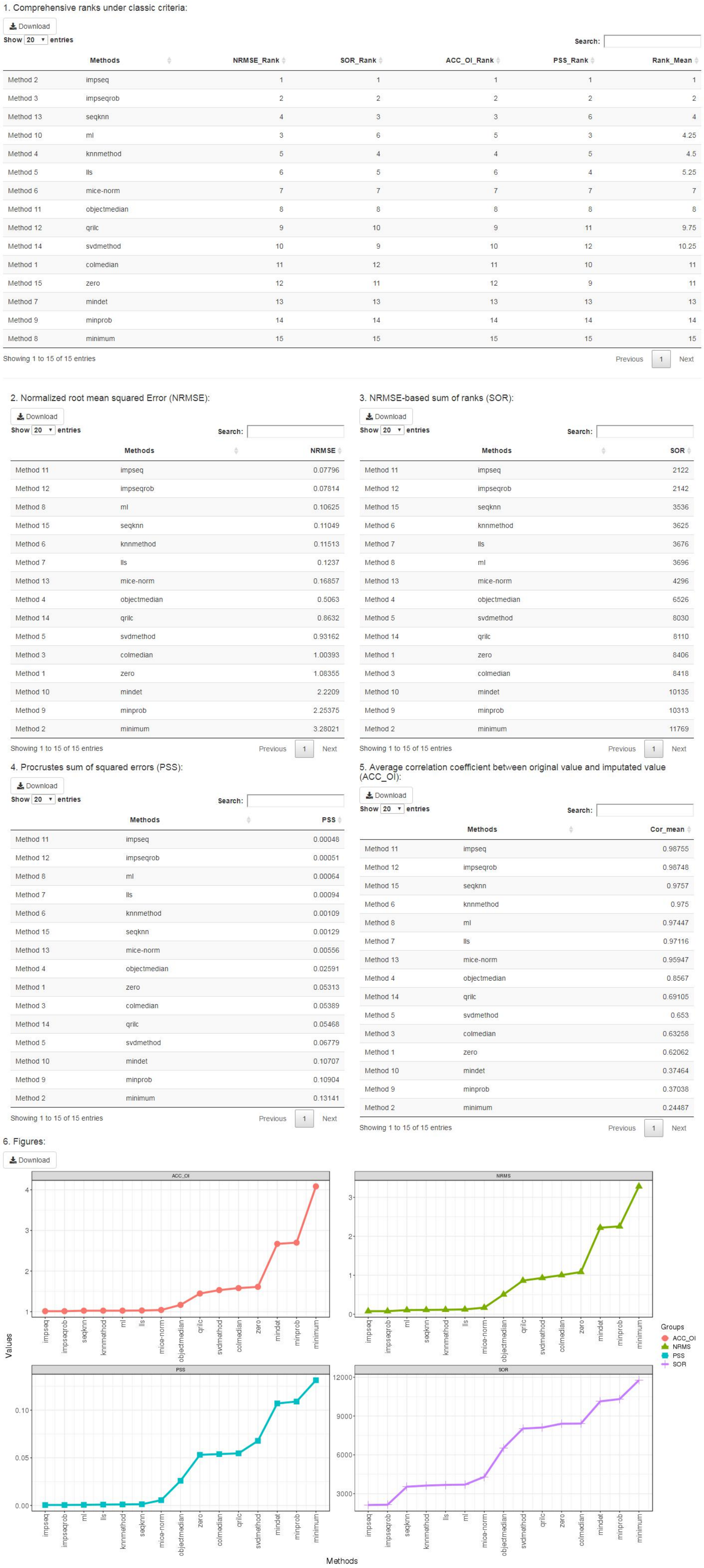
Done ~lls processing...

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

1. This table shows the comprehensive ranks of every imputation method;

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 imputated value (ACC_OI)', respectively;

6. Figures here show the normalized scores of every imputation method under the four classic criteria. 'Normalized Values' here means every score divides by 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

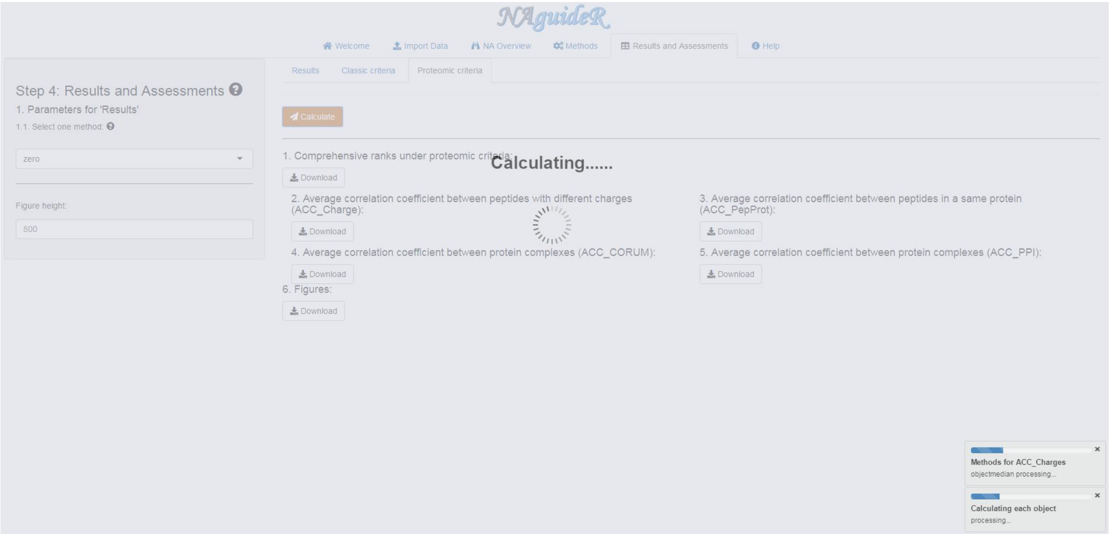
Showing 1 to 15 of 15 entries

Previous 1 Next

6. Figures:

Download

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



Step 4: Results and Assessments

1. Parameters for 'Results'

1.1. Select one method: zero

Figure height: 800

1. Comprehensive ranks under proteomic criteria:

Calculating......

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

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

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

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

6. Figures:

Methods for ACC_Charges
objectmedian processing...

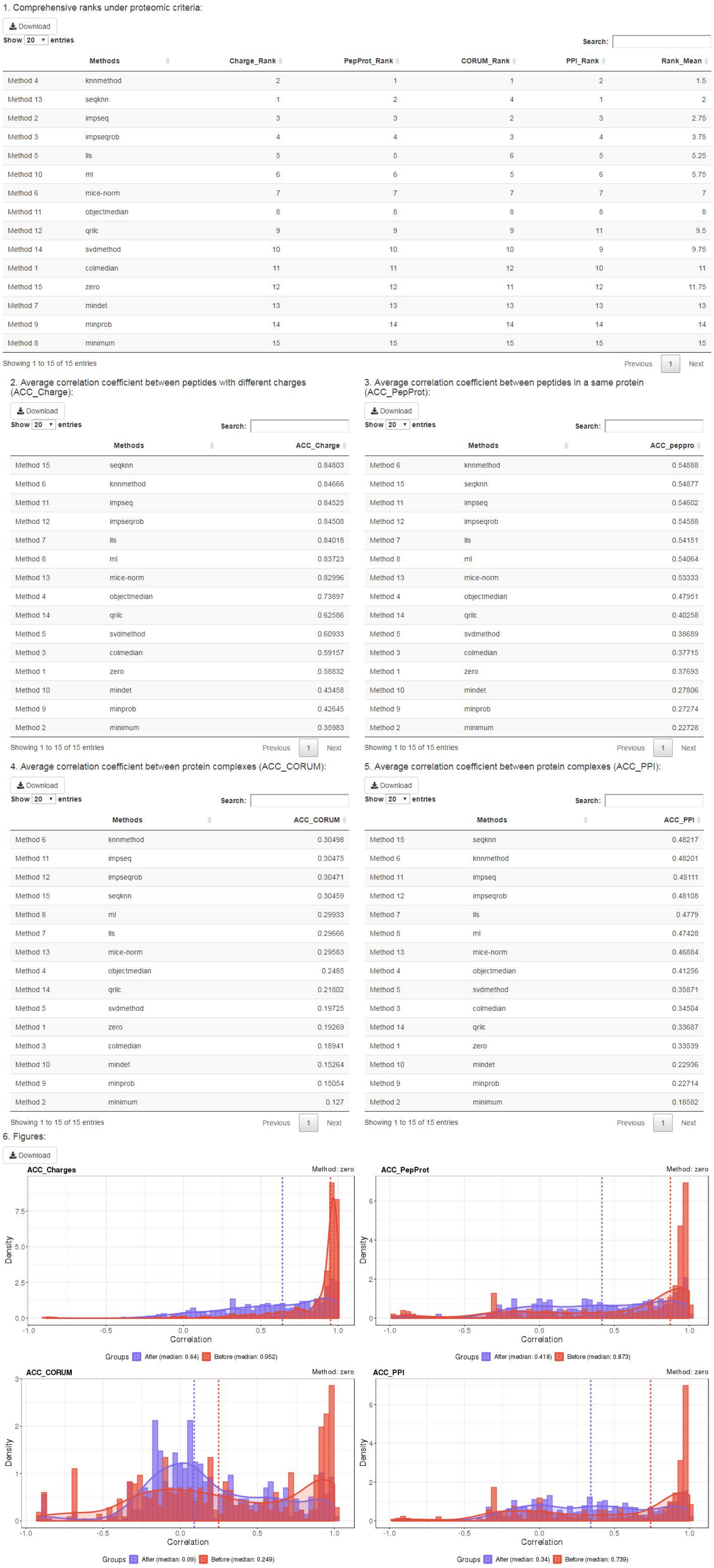
Calculating each object
processing...

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

1. This table shows the comprehensive ranks of every imputation method;

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.



1. Comprehensive ranks under proteomic criteria:

	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	ml	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

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

	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	ml	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

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

	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	ml	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

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

	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	ml	0.29933
Method 7	lls	0.29666
Method 13	mice-norm	0.29583
Method 4	objectmedian	0.2485
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

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

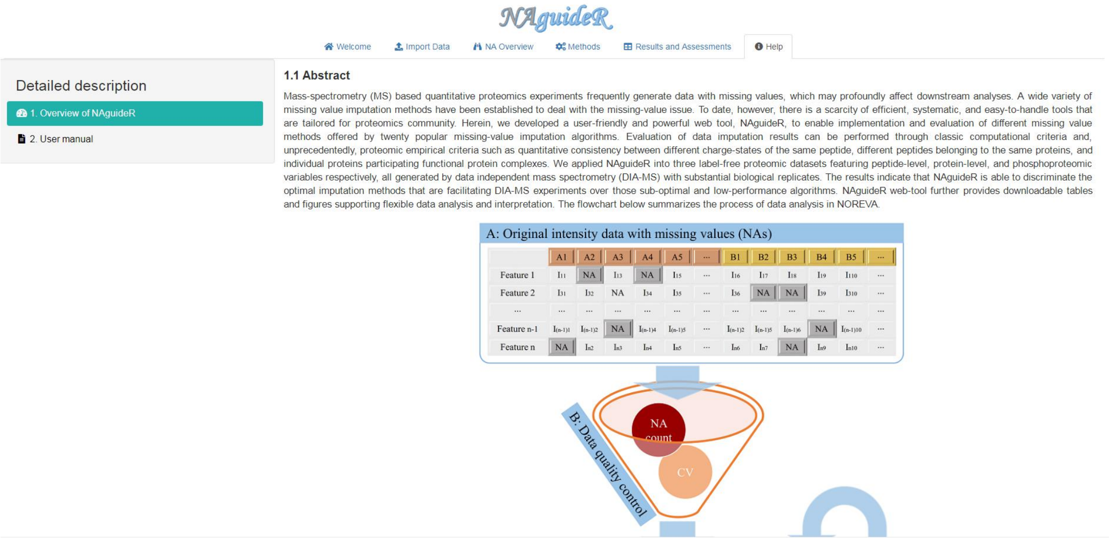
	Methods	ACC_PPI
Method 15	seqknn	0.48217
Method 6	knnmethod	0.48201
Method 11	impseq	0.48111
Method 12	impseqrob	0.48108
Method 7	lls	0.4779
Method 8	ml	0.47428
Method 13	mice-norm	0.46884
Method 4	objectmedian	0.41256
Method 5	svdmethod	0.35871
Method 3	colmedian	0.34504
Method 14	qrilc	0.33687
Method 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

6. Figures:

6. Help

This part provides some introduction and operation manual about NAguideR, so that users can quickly learn what this tool is and how to use this tool.

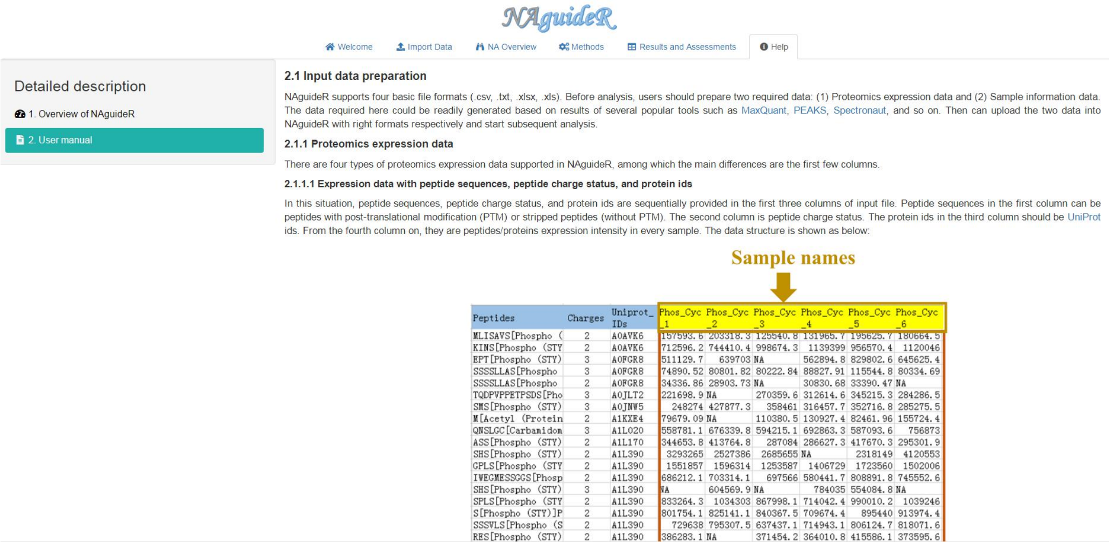


1.1 Abstract

Mass-spectrometry (MS) based quantitative proteomics experiments frequently generate data with missing values, which may profoundly affect downstream analyses. A wide variety of missing value imputation methods have been established to deal with the missing-value issue. To date, however, there is a scarcity of efficient, systematic, and easy-to-handle tools that are tailored for proteomics community. Herein, we developed a user-friendly and powerful web tool, NAguideR, to enable implementation and evaluation of different missing value methods offered by twenty popular missing-value imputation algorithms. Evaluation of data imputation results can be performed through classic computational criteria and, unprecedentedly, proteomic empirical criteria such as quantitative consistency between different charge-states of the same peptide, different peptides belonging to the same proteins, and individual proteins participating functional protein complexes. We applied NAguideR into three label-free proteomic datasets featuring peptide-level, protein-level, and phosphoproteomic variables respectively, all generated by data independent mass spectrometry (DIA-MS) with substantial biological replicates. The results indicate that NAguideR is able to discriminate the optimal imputation methods that are facilitating DIA-MS experiments over those sub-optimal and low-performance algorithms. NAguideR web-tool further provides downloadable tables and figures supporting flexible data analysis and interpretation. The flowchart below summarizes the process of data analysis in NOREVA.

A: Original intensity data with missing values (NAs)

	A1	A2	A3	A4	A5	···	B1	B2	B3	B4	B5	···
Feature 1	I_{11}	NA	I_{13}	NA	I_{15}	···	I_{16}	I_{17}	I_{18}	I_{19}	I_{110}	···
Feature 2	I_{31}	I_{32}	NA	I_{34}	I_{35}	···	I_{36}	NA	NA	I_{39}	I_{310}	···
···	···	···	···	···	···	···	···	···	···	···	···	···
Feature n-1	$I_{(n-1)1}$	$I_{(n-1)2}$	NA	$I_{(n-1)4}$	$I_{(n-1)5}$	···	$I_{(n-1)2}$	$I_{(n-1)5}$	$I_{(n-1)6}$	NA	$I_{(n-1)10}$	···
Feature n	NA	I_{n2}	I_{n3}	I_{n4}	I_{n5}	···	I_{n6}	I_{n7}	NA	I_{n9}	I_{n10}	···



2.1 Input data preparation

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

2.1.1 Proteomics expression data

There are four types of proteomics expression data supported in NAguideR, among which the main differences are the first few columns.

2.1.1.1 Expression data with peptide sequences, peptide charge status, and protein ids

In this situation, peptide sequences, peptide charge status, and protein ids are sequentially provided in the first three columns of input file. Peptide sequences in the first column can be peptides with post-translational modification (PTM) 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 on, they are peptides/proteins expression intensity in every sample. The data structure is shown as below:

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
TQDPVPPETPSDS[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	A1KXE4	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
IWEGMESSGGS[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). 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)
```

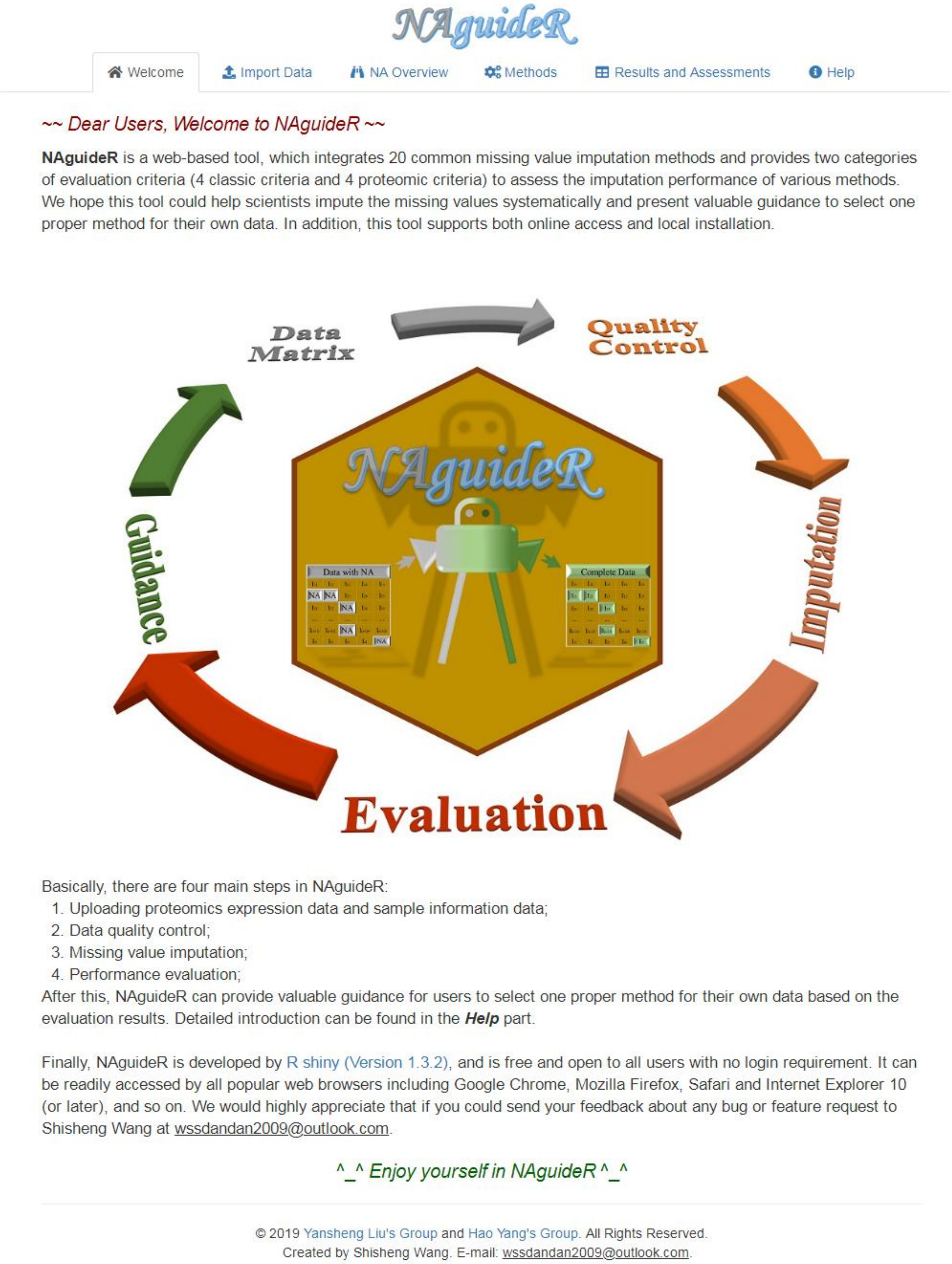
Please note, you may find the SeqKnn package (https://github.com/cran/SeqKnn) can not be installed rightly as it has not been updated for a long time. If so, please download this package from here: https://github.com/wangshisheng/NAguideR/blob/master/SeqKnn_1.0.1.tar.gz. Then you can install this package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



~~ Dear Users, Welcome to NAguideR ~~

NAguideR is a web-based tool, which integrates 20 common 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 wssdandan2009@outlook.com.

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

III. Reference

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