### **User Input Testing**

This document displays a number of example searches that constitute user input data in different formats.

- i) <u>User input AZ20.tsv</u>
- ii) User input AZ20.tsv, however missing the CV column
- iii) User input AZ20.tsv, however CV column is in a random location
- iv) User input AZ20.tsv, however the residue type and number does not contain any 'nones'
- v) <u>User input AZ20.tsv</u>, however the substrate name column has been placed as the final column
- vi) User input AZ20.tsv, however the substrate name column has been placed in a random position
- vii) User input AZ20.tsv, however the p values column is missing
- viii) User input AZ20.tsv, however the p values column is in a random column
- ix) User input AZ20.tsv, however the fold values column is missing
- x) User input AZ20.tsv, however the fold values column has been moved to a different column

**Test case scenario 1 = User input AZ20.tsv** 



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Upload data file for processing

Choose File az20.tsv

### Upload

Data analysis takes some time - Please wait

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Results for AZ20.tsv are as follows with no error messages displayed.

File az20 successfully analysed

### Phospho-proteomics Data Analysis Results

Processed Table	Volcano Plot	Kinase & Substrate Frequencies	Kinase activities	Summary Charts	
Download data					

**General overview:** This table corresponds to hits, whose corrected p-values meet an error rate threshold of <=0.05 and have a CV of <=25%. If the original upload table didn't include CV columns, then only the p-value threshold is applied.

Clickable entries: Try clicking entries in columns: Substrate/Isoform in DB (gene name), Phosphosite in DB (ID) and Kinase in DB (gene name). We guarantee a pleasant journey!

**Intensity columns:** Original intensity values were transformed by dividing each substrate/site intensity by the maximum intensity of the row (both conditions). This scaling allows the application of a heatmap to the cells, for visual clarification of intensity differences.

**Log2 fold changes:** Barplots are integrated into the column, that scale with the fold change values. *Note*: Cells that are fully coloured, denote hits detected in only one condition (see intensity columns).

Sorting: The Log2 fold changes are used, giving a gradated ordering of hits as you scroll through the table.

Number	Substrate (gene name)	Phospho site ID	Substrate/Isoform in DB (gene name)	-	Kinase in DB (gene name)	Fold control intensity over maximum	Fold condition intensity over maximum	Log2 fold change - condition over control	corrected p-value
1	SYNE2	S6361	SYNE2	56361	not in DB	0	1		0.00092
2	GTF3C1	S739	GTF3C1	5739	not in DB	0	1		0.0012
3	WWC3	T909	WWC3	T909	not in DB	0	1		0.0063
4	PLEC	S4626	PLEC	S4626	not in DB	0	1		0.0085
5	CTAGE5	S559	not in DB	not in DB	not in DB	0	1		0.0088
6	SNRNP70	S410	SNRNP70	S410	not in DB	0	1		0.0091
7	CC2D1A	S118	CC2D1A	S118	not in DB	0	1		0.011
8	PACS1	S430	PACS1	S430	not in DB	0	1		0.014
9	TMF1	S112	TMF1	S112	not in DB	0	1		0.014
10	TRPS1	S1041	TRPS1	S1041	not in DB	0	1		0.015
11	ZFHX3	S2230	ZFHX3	52230	not in DB	0	1		0.021
12	ZFHX3	S2226	ZFHX3	S2226	not in DB	0	1		0.021
13	FOXK1	T436	FOXK1	T436	not in DB	0.15	1	2.7	0.00048
14	FOXK1	S441	FOXK1	S441	not in DB	0.15	1	2.7	0.00048

Test case scenario 2 = User input AZ20.tsv, however missing the CV column

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# Online analysis for your phospho-proteomics

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A csv file of the analysed data is provided for download when the analysis is finished and individual data charts can be saved from the site.

Please see the documentation for information about how to format your datafile correctly and for further information about the analysis output results.

We hope you enjoy your PhosphoQuest.

Upload data file for processing

Choose File az2o\_no\_cvs.tsv



Data analysis takes some time - Please wait

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Results for AZ20.tsv without the CV column. Here, the data analysis is still performed, however if the original file does not include a CV column, then only the p-value threshold is applied towards the calculation.

File az2o\_no\_cvs successfully analysed

### Phospho-proteomics Data Analysis Results

Processed Table Volcano Plot Kinase & Substrate Frequencies Kinase activities Summary Charts

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**General overview:** This table corresponds to hits, whose corrected p-values meet an error rate threshold of <=0.05 and have a CV of <=25%. If the original upload table didn't include CV columns, then only the p-value threshold is applied.

Clickable entries: Try clicking entries in columns: Substrate/Isoform in DB (gene name), Phosphosite in DB (ID) and Kinase in DB (gene name). We guarantee a pleasant journey!

**Intensity columns:** Original intensity values were transformed by dividing each substrate/site intensity by the maximum intensity of the row (both conditions). This scaling allows the application of a heatmap to the cells, for visual clarification of intensity differences.

**Log2 fold changes:** Barplots are integrated into the column, that scale with the fold change values. *Note*: Cells that are fully coloured, denote hits detected in only one condition (see intensity columns).

Sorting: The Log2 fold changes are used, giving a gradated ordering of hits as you scroll through the table.

Number	Substrate (gene name)	Phospho site ID	Substrate/Isoform in DB (gene name)	-	Kinase in DB (gene name)	Fold control intensity over maximum	Fold condition intensity over maximum	Log2 fold change - condition over control	corrected p-value
1	SYNE2	S6361	SYNE2	56361	not in DB	0	1		0.00092
2	GTF3C1	S739	GTF3C1	S739	not in DB	0	1		0.0012
3	WWC3	T909	WWC3	T909	not in DB	0	1		0.0063
4	PLEC	S4626	PLEC	S4626	not in DB	0	1		0.0085
5	CTAGE5	S559	not in DB	not in DB	not in DB	0	1		0.0088
6	SNRNP70	S410	SNRNP70	S410	not in DB	0	1		0.0091
7	CC2D1A	S118	CC2D1A	S118	not in DB	0	1		0.011
8	PACS1	S430	PACS1	S430	not in DB	0	1		0.014
9	TMF1	S112	TMF1	S112	not in DB	0	1		0.014
10	TRPS1	S1041	TRPS1	S1041	not in DB	0	1		0.015
11	ZFHX3	S2230	ZFHX3	52230	not in DB	0	1		0.021
12	ZFHX3	S2226	ZFHX3	S2226	not in DB	0	1		0.021
13	VANGL1	S86	VANGL1	S86	not in DB	0	1		0.024
14	VANGL1	588	VANGL1	588	not in DB	0	1		0.024
15	RRP1B	S458	RRP1B	S458	not in DB	0	1		0.029

Test case scenario 3 = User input AZ20.tsv, however the CV column is in a random location



All analysis on this website is done on the fly, therefore please do not click back or other links (in same tab) unless you have finished looking through your results.

A csv file of the analysed data is provided for download when the analysis is finished and individual data charts can be saved from the site.

Please see the documentation for information about how to format your datafile correctly and for further information about the analysis output results.

We hope you enjoy your PhosphoQuest.

Upload data file for processing

Choose File | az20\_cvs\_asRandomColumn.tsv

### Upload

Data analysis takes some time - Please wait

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Results for AZ20.tsv with the CV columns in a different location. Here, the data analysis does not perform, as it expects the CV columns to be at the end fo the user data file.

Anomaly detected..PhosphoQuest will self-destruct in T minus 10 seconds...just kidding! Please check your fold change calculations, a discrepancy has been detected.

# Online analysis for your phospho-proteomics

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We hope you enjoy your PhosphoQuest.

Upload data file for processing

Choose File No file chosen



Data analysis takes some time - Please wait

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Test case scenario 4 = User input AZ20.tsv, however the residue type and number does not contain any 'nones'



All analysis on this website is done on the fly, therefore please do not click back or other links (in same tab) unless you have finished looking through your results.

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We hope you enjoy your PhosphoQuest.

Upload data file for processing

Choose File az2o\_no\_nones.tsv

Upload

Data analysis takes some time - Please wait

#### **Browse Database**

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Results for AZ20.tsv\_noNones show that the analysis functions as normal and the lack of 'nones' within the substrate column does not affect the analysis.

File az20 no nones successfully analysed

### Phospho-proteomics Data Analysis Results

Processed Table Volcano Plot Kinase & Substrate Frequencies Kinase activities Summary Charts

Download data

**General overview:** This table corresponds to hits, whose corrected p-values meet an error rate threshold of <=0.05 and have a CV of <=25%. If the original upload table didn't include CV columns, then only the p-value threshold is applied.

Clickable entries: Try clicking entries in columns: Substrate/Isoform in DB (gene name), Phosphosite in DB (ID) and Kinase in DB (gene name). We guarantee a pleasant journey!

**Intensity columns:** Original intensity values were transformed by dividing each substrate/site intensity by the maximum intensity of the row (both conditions). This scaling allows the application of a heatmap to the cells, for visual clarification of intensity differences.

**Log2 fold changes:** Barplots are integrated into the column, that scale with the fold change values. *Note*: Cells that are fully coloured, denote hits detected in only one condition (see intensity columns).

Sorting: The Log2 fold changes are used, giving a gradated ordering of hits as you scroll through the table.

Number	Substrate (gene name)	Phospho site ID	Substrate/Isoform in DB (gene name)	Phosphosite in DB (ID)	Kinase in DB (gene name)	Fold control intensity over maximum	Fold condition intensity over maximum	Log2 fold change - condition over control	corrected p-value
1	SYNE2	S6361	SYNE2	56361	not in DB	0	1		0.00092
2	GTF3C1	S739	GTF3C1	5739	not in DB	0	1		0.0012
3	WWC3	T909	WWC3	T909	not in DB	0	1		0.0063
4	PLEC	S4626	PLEC	S4626	not in DB	0	1		0.0085
5	CTAGE5	S559	not in DB	not in DB	not in DB	0	1		0.0088
6	SNRNP70	S410	SNRNP70	S410	not in DB	0	1		0.0091
7	CC2D1A	S118	CC2D1A	S118	not in DB	0	1		0.011
8	PACS1	S430	PACS1	S430	not in DB	0	1		0.014
9	TMF1	S112	TMF1	S112	not in DB	0	1		0.014
10	TRPS1	S1041	TRPS1	S1041	not in DB	0	1		0.015
11	ZFHX3	S2230	ZFHX3	52230	not in DB	0	1		0.021
12	ZFHX3	S2226	ZFHX3	S2226	not in DB	0	1		0.021
13	FOXK1	T436	FOXK1	T436	not in DB	0.15	1	2.7	0.00048

Test case scenario 5 = User input AZ20.tsv, however the substrate name column has been placed as the final column



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We hope you enjoy your PhosphoQuest.

Upload data file for processing

Choose File az2o\_substrateColumn\_asFinalColumn.tsv



Data analysis takes some time - Please wait

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Results for AZ20.tsv substrate Column as Final Column show that the current analysis requires the substrate name information to be as the first column of the user input file.

An error occurred please try again

could not convert string to float: '1A24\_HUMAN(S356)'

Sadly the dragons ate your analysis An error occurred



Please restart your PhosphoQuest. Try going back to analysis

Test case scenario 6 = User input AZ20.tsv, however the substrate name column has been placed in a random position



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Upload data file for processing

Choose File az2o\_substrateColumn\_asRandomColumn.tsv



Data analysis takes some time - Please wait

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Results for AZ20.tsv substrate Column as Random Column show that the current analysis requires the substrate name information to be as the first column of the user input file.

An error occurred please try again

could not convert string to float: '1A24\_HUMAN(S356)'

Sadly the dragons ate your analysis

An error occurred



Please restart your PhosphoQuest. Try going back to analysis

Test case scenario 7 = User input AZ20.tsv, however the p values column is missing



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Please see the documentation for information about how to format your datafile correctly and for further information about the analysis output results.

We hope you enjoy your PhosphoQuest.

Upload data file for processing

Choose File | az20\_missingPvalues.tsv



Data analysis takes some time - Please wait

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Results for AZ20.tsv\_missingP values show that the current analysis requires a P value column to be present for the analysis to be completed.

An error occurred please try again

single positional indexer is out-of-bounds

Sadly the dragons ate your analysis

An error occurred



Please restart your PhosphoQuest. Try going back to analysis

Test case scenario 8 = User input AZ20.tsv, however the p values column is in a random column



All analysis on this website is done on the fly, therefore please do not click back or other links (in same tab) unless you have finished looking through your results.

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Please see the documentation for information about how to format your datafile correctly and for further information about the analysis output results.

We hope you enjoy your PhosphoQuest.

Upload data file for processing

Choose File az2o\_Pvalues\_asRandomColumn.tsv



Data analysis takes some time - Please wait

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Results for AZ20.tsv\_missingPvalues show that the current analysis requires a P value column to be present for the analysis to be completed.

Anomaly detected..PhosphoQuest will self-destruct in T minus 10 seconds...just kidding! Please check your fold change calculations, a discrepancy has been detected.



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Upload data file for processing

Choose File No file chosen



Data analysis takes some time - Please wait

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Test case scenario 9 = User input AZ20.tsv, however the fold values column is missing



All analysis on this website is done on the fly, therefore please do not click back or other links (in same tab) unless you have finished looking through your results.

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Please see the documentation for information about how to format your datafile correctly and for further information about the analysis output results.

We hope you enjoy your PhosphoQuest.

Upload data file for processing

Choose File az20\_missingFoldvalues.tsv

### Upload

Data analysis takes some time - Please wait

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Results for AZ20.tsv\_missingFoldvalues show that the current analysis requires a Fold value column to be present for the analysis to be completed.

An error occurred please try again

single positional indexer is out-of-bounds

### Sadly the dragons ate your analysis An error occurred



Please restart your PhosphoQuest. Try going back to analysis

### Test case scenario 10 = User input AZ20.tsv, however the fold values column has been moved to a different column



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We hope you enjoy your PhosphoQuest.

Upload data file for processing

Choose File az20\_FoldValues\_asRandomColumn.tsv

Upload

Data analysis takes some time - Please wait

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Results for AZ20.tsvFoldValuesasRandomColumn show that the current analysis requires a Fold value column to be present in the expected column.

Anomaly detected..PhosphoQuest will self-destruct in T minus 10 seconds...just kidding! Please check your fold change calculations, a discrepancy has been detected.



### Online analysis for your phospho-proteomics

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A csy file of the analysed data is provided for download when the analysis is finished and individual data charts can be saved from the site.

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We hope you enjoy your PhosphoQuest.

Upload data file for processing

Choose File No file chosen



Data analysis takes some time - Please wait

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### Summary

In this short user app testing, we have tested a number of different scenarios in relation to different user input files:-

- User input AZ20.tsv
- User input AZ20.tsv, however missing the CV column
- User input AZ20.tsv, however the CV column is in a random location
- User input AZ20.tsv, however the residue type and number does not contain any 'nones'
- User input AZ20.tsv, however the substrate name column has been placed as the final column
- User input AZ20.tsv, however the substrate name column has been placed in a random position
- User input AZ20.tsv, however the p values column is missing
- User input AZ20.tsv, however the p values column is in a random column
- Test case scenario 9 = User input AZ20.tsv, however the fold values column is missing
- Test case scenario 10 = User input AZ20.tsv, however the fold values column has been moved to a different column

We have tried to graphically illustrate the results of such searches and show that in each of the different

scenarios, the website does not crash, but the errors are captured in different ways. These error messages denote that there is something wrong with the input file in terms of order or columns of required information are missing. The user input testing also shows how missing CV columns can be accounted and the analysis completes without taking CVs into consideration.