

PROCEDURE

Notice

The web version of PhosMap is for quick start of visualization only due to the low-level hardware of R shiny server. It is single-threaded and we recommend users to analyze small data sets using the demo server. For larger datasets, upgraded hardware is necessary according to the possible computational cost of the data. We recommend users to use the local docker version of PhosMap.

Introduction of example data

Here, we reanalysis (phospho)proteomic profilings of WiDr colorectal cancer cells harbouring the BRAF(V600E) mutation after treatment using vemurafenibin a time course of 0, 2, 6, 24, and 48 hour^[1]. The raw files were deposited in ProteomeXchange Consortium(PXD007740). The raw data were processed in Firmiana, a one-stop proteomic cloud platform^[2], to obtain quantitative peptide and protein files. You can download example data in <https://github.com/liuzan-info/PhosMap/tree/master/examplefile/mascot> and <https://github.com/liuzan-info/PhosMap/tree/master/examplefile/maxquant>.

Preprocessing for Maxquant data

Import MaxQuant data

How to import your MaxQuant data

1. Go to the 'Import data' tab.
2. Choose 'Maxquant' to start with data from Maxquant.
3. Click 'Browse' to upload phosphoproteomics experimental design file in .txt format, and phospho (STY)Sites.txt. Proteomics experimental design file is optional.
4. Uploaded data will be shown in the 'Data Overview' secondary tab.
5. You can also choose 'load exmaple data' to use exmaple files.

| | Experiment_Code | Group | Description |
|---|-----------------|-------|-----------------------|
| 1 | Exp027015 | 0 | ctr_0h_R2_IMAC_1.raw |
| 2 | Exp027016 | 0 | ctr_0h_R2_IMAC_2.raw |
| 3 | Exp027017 | 0 | ctr_0h_R2_IMAC_3.raw |
| 4 | Exp027031 | 6 | PLX_6h_R2_IMAC_1.raw |
| 5 | Exp027032 | 6 | PLX_6h_R2_IMAC_2.raw |
| 6 | Exp027033 | 6 | PLX_6h_R2_IMAC_3.raw |
| 7 | Exp027046 | 48 | PLX_48h_R2_IMAC_1.raw |
| 8 | Exp027047 | 48 | PLX_48h_R2_IMAC_2.raw |
| 9 | Exp027048 | 48 | PLX_48h_R2_IMAC_3.raw |

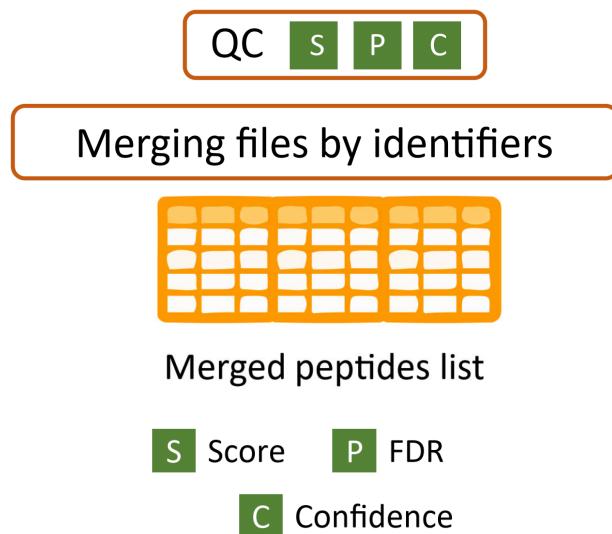
Showing 1 to 9 of 9 entries

Previous 1 Next

Quality control and merging

Function

Generate merged phosphoproteomics data frame based on peptides files.



How to get analysis results

1. Go to the 'Preprocessing' tab.
2. Modify the parameters in Step1 according to your needs.

3. Click the running button in Step1 and the file will appear on the right.

The screenshot shows the PhosMap Preprocessing interface. It consists of three main sections: Step1: Quality Control, Step2: Normalization & Imputation & Filtering, and Step3: Normalization based on proteomics data. Step3 is currently active. In Step3, there is a checkbox 'With proteomics data' which is checked. Below it are parameters for 'Proteomics data preprocessing parameters': 'intensity type' set to iBAQ, 'minimum unique peptide' set to 1, and 'minimum detection frequency' set to 1. There are also dropdowns for 'normalization method' (set to global), 'imputation method' (set to minimum/10), and 'control label' (set to 0). On the right side, under 'QC result:', there is a table titled 'QC result:' showing 10 entries. The columns are ID, Exp027015, Exp027016, Exp027017, Exp027031, and Exp027032. The table lists various peptides with their corresponding values across the experiments. At the bottom of the table, it says 'Showing 1 to 10 of 4,862 entries' and provides navigation links for previous, next, and page numbers (1, 2, 3, 4, 5, ..., 487).

| ID | Exp027015 | Exp027016 | Exp027017 | Exp027031 | Exp027032 |
|-----------------------|----------------|-----------|-----------|-----------|-----------|
| A0AVK6_E2F8_S102 | E2F8_S102 | 0 | 15484000 | 0 | 0 |
| A0AVK6_E2F8_S71 | E2F8_S71 | 0 | 0 | 0 | 3934000 |
| A0FGR8_ESYT2_S743 | ESYT2_S743 | 0 | 0 | 0 | 0 |
| A0JLT2_MED19_S226 | MED19_S226 | 0 | 96244000 | 0 | 78867000 |
| A0JNW5_UHRF1BP1L_S989 | UHRF1BP1L_S989 | 24650000 | 43735000 | 33424000 | 84046000 |
| A0MZ66_KIAA1598_S506 | KIAA1598_S506 | 36034000 | 24778000 | 27576000 | 23404000 |
| A0MZ66_KIAA1598_S494 | KIAA1598_S494 | 0 | 28114000 | 21070000 | 0 |
| A1KXE4_FAM168B_S6 | FAM168B_S6 | 8743300 | 0 | 0 | 7431400 |
| A1L170_C1orf226_S223 | C1orf226_S223 | 0 | 0 | 11539000 | 13014000 |
| A1L390_PLEKHG3_S76 | PLEKHG3_S76 | 31566000 | 30739000 | 31883000 | 21461000 |
| | | | | | 23385000 |

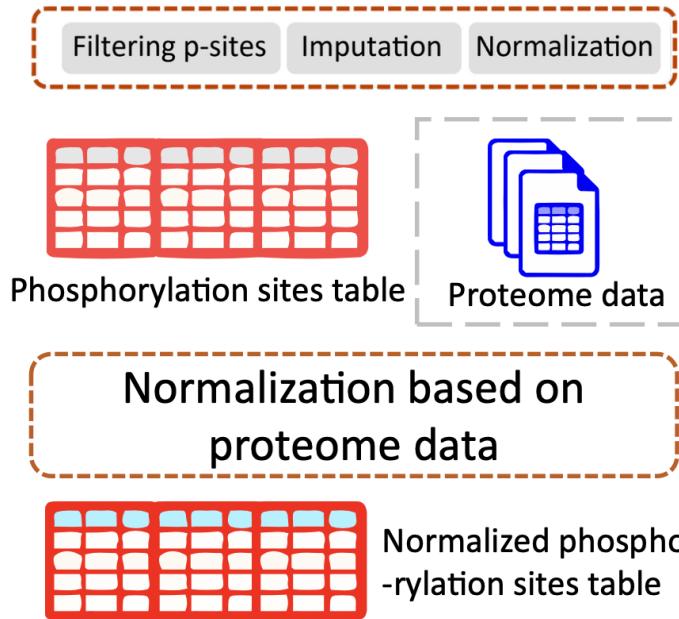
Interpretation of analysis results

We performed quality control for identified phosphopeptides using PhosMap, those phosphopeptides that met 1% FDR at peptide level and had ion score greater than 20 and the highest confidence probabilities of p-sites computed by Mascot, were kept. We merged phosphopeptides list with quantitative value from all experiments to generate a matrix for analysis.

Data normalization

Function

PhosMap provides two kinds of normalizations, a total sum scaling normalization and normalizing phosphoproteomics data based on proteomics data.



How to get analysis results

1. Go to the 'Preprocessing' tab.
2. Modify the parameters according to your needs.
3. Click the running button in the Step2 and the normalized data of p-sites based on a total sum scaling will appear on the right.
4. Click the running button in Step3 and normalized data of p-sites based on proteomics data will appear on the right.

Preprocessing

Step1: Quality Control

- minimum score: 40
- minimum localization probability: 0.75
- minimum detection frequency: 1

Step2: Normalization & Imputation & Filtering

- normalization method: global
- impute globally
- imputation method: minimum/10
- top: 100

Step3: Normalization based on proteomics data

With proteomics data

Proteomics data preprocessing parameters

- intensity type: iBAQ
- minimum unique peptide: 1
- minimum detection frequency: 1
- normalization method: global
- imputation method: minimum/10
- control label: 0

Phosphorylation data frame:

| upsID | Sequence | Exp027015 | Exp027016 | Exp027017 |
|------------------------|-------------------------|--------------------|------------------|--------------------|
| 1 P09651_HNRNPA1_S6 | SEsPKEPEQLRK | 806.618091022792 | 2998.8334004027 | 1111.03246252 |
| 2 P08238_HSP90AB1_S226 | EKEIlsDDEAEEEK | 278.047800130604 | 115.498146334136 | 282.4869745970 |
| 3 P35579_MYH9_S1943 | GAGDGsDEEVDGKADGAEAKPAE | 1953.88447950694 | 2300.79755266096 | 1817.772424792 |
| 4 Q9UHD8_SEPT9_S82 | HVDLsQRSPK | 0.0399062994401786 | 11.579644197159 | 0.0399062994401786 |
| 5 P19338_NCL_S67 | KVVVsPTK | 186.438898802424 | 278.545579522404 | 620.3806900931 |
| 6 Q9UHD8_SEPT9_S85 | HVDLsQRSPK | 773.387502005132 | 2022.61281463548 | 798.942023482 |
| 7 P27824_CANX_S583 | AEEDEILNRsPRNR | 1322.41059475714 | 932.414428041142 | 1296.909157804 |
| 8 Q01518_CAP1_T307 | PFSAPKPGQsPSPK | 14.5065193340688 | 1249.64221603336 | 1889.073452038 |
| 9 Q15019_SEPT2_S218 | IYHLPDAEsDEDDEFK | 1198.66488669975 | 561.252864309285 | 714.5303476555 |
| 10 P02545_LMNA_S392 | LSPsPTSQR | 1237.68881410961 | 1539.68661125762 | 1287.551194862 |

Showing 1 to 10 of 4,862 entries

Result Step 1 Step 2 Step 3

[Go to analysis tools](#)

Preprocessing

Step1: Quality Control

- minimum score: 40
- minimum localization probability: 0.75
- minimum detection frequency: 1

Step2: Normalization & Imputation & Filtering

- normalization method: global
- impute globally
- imputation method: minimum/10
- top: 100

Step3: Normalization based on proteomics data

With proteomics data

Proteomics data preprocessing parameters

- intensity type: iBAQ
- minimum unique peptide: 1
- minimum detection frequency: 1
- normalization method: global
- imputation method: minimum/10
- control label: 0

Phosphorylation data frame:

| Sequence | Exp027015 | Exp027016 | Exp027017 |
|-------------------------|-------------------|-------------------|-------------------|
| SEsPKEPEQLRK | 0.492501712729907 | 1.82935858424327 | 0.6781397030268 |
| EKEIlsDDEAEEEK | 1.23284655936821 | 0.514694395775893 | 1.25245904485 |
| GAGDGsDEEVDGKADGAEAKPAE | 0.96530283949402 | 1.13660512261286 | 0.8980920378930 |
| HVDLsQRSPK | 0.212812721625771 | 2.57437455874846 | 0.212812721625771 |
| KVVVsPTK | 0.516661790292904 | 0.770547205021484 | 1.712791004686 |
| HVDLsQRSPK | 0.645691977914281 | 1.68730843076805 | 0.6669959313176 |
| AEEDEILNRsPRNR | 1.11688570299748 | 0.787750403223905 | 1.095363893778 |
| PFSAPKPGQsPSPK | 0.014738997408384 | 1.18873971011006 | 1.7965212901494 |
| IYHLPDAEsDEDDEFK | 1.45270234401247 | 0.680845178487121 | 0.8664524775004 |
| LSPsPTSQR | 0.913503804086326 | 1.13622006129149 | 0.950276134622 |

Showing 1 to 10 of 4,862 entries

Proteomics data frame:

| Symbol | Exp026982 | Exp026983 | Exp026995 | Exp026996 | Exp026997 |
|---------|-------------------|-------------------|------------------|------------------|-----------|
| 4 RBM47 | 7.92400365808776 | 7.25563751717897 | 8.6101214704357 | 8.56304505356731 | 11.678994 |
| 5 UBA6 | 11.6340311498454 | 8.63246776857612 | 10.0785527153132 | 12.1772669538328 | 9.0558354 |
| 6 ESYT2 | 0.697671483550616 | 0.701310509897537 | 1.12387693059222 | 1.36932355653506 | 0.6385380 |

All Done

You can now download the 'Phosphorylation data frame' for further analysis.

OK

Result Step 1 Step 2 Step 3

[Go to analysis tools](#)

Preprocessing for Firmiana data

Import Firmiana data

How to import your Firmiana data

1. Go to the 'Import data' tab.
2. Choose 'Firmiana' to start with data from Firmiana.
3. Click 'Browse' to upload phosphoproteomics experimental design file in .txt format.
4. Zip your Mascot xml files and Phosphoproteomics peptide files, and then upload. The folder tree is shown below. File names of Mascot xml files and Phosphoproteomics peptide files must be consistent with 'Experiment_Code' of phosphoproteomics experimental design file.

```
.           └── mascot_xml
                  ├── Exp027012
                  │   └── Exp027012_F1_R1.xml
                  ├── Exp027020
                  │   └── Exp027020_F1_R1.xml
                  ├── Exp027028
                  │   └── Exp027028_F1_R1.xml
                  ├── Exp027036
                  │   └── Exp027036_F1_R1.xml
                  └── Exp027044
                      └── Exp027044_F1_R1.xml
            └── phosphorylation_peptide_txt
                  ├── Exp027012_peptide.txt
                  ├── Exp027020_peptide.txt
                  ├── Exp027028_peptide.txt
                  ├── Exp027036_peptide.txt
                  └── Exp027044_peptide.txt
> phosphorylation_exp_design_info_csv
      Experiment_Code Group          Description
      1             Exp027012    0  ctr_0h_R1_IMAC_1.raw
      9             Exp027020    2  PLX_2h_R1_IMAC_1.raw
     17             Exp027028    6  PLX_6h_R1_IMAC_1.raw
     25             Exp027036   24  PLX_24h_R1_IMAC_1.raw
     33             Exp027044   48  PLX_48h_R1_IMAC_2.raw
```

6. Proteomics data is optional. Click 'Browse' to upload proteomics experimental design file in .txt format. Zip your Profiling_gene_txt and upload. The folder tree is shown below. File names of Profiling_gene_txt must be consistent with 'Experiment_Code' of proteomics experimental design file.

```
Profiling_gene_txt
  ├── Exp026921_gene.txt
  ├── Exp026986_gene.txt
  ├── Exp026993_gene.txt
  ├── Exp026999_gene.txt
  └── Exp027006_gene.txt
> profiling_exp_design_info
      Experiment_Code Group          Description
      1             Exp026921    0  ctr_0h_R1_injection_2.raw
      2             Exp026986    2  PLX_2h_R1_injection_1.raw
      3             Exp026993    6  PLX_6h_R1_injection_1.raw
      4             Exp026999   24  PLX_24h_R1_injection_1.raw
      5             Exp027006   48  PLX_48h_R1_injection_3.raw
```

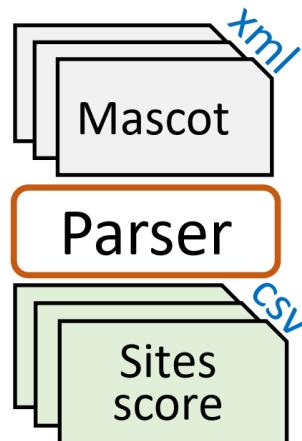
7. Uploaded data will be shown in the 'Data Overview' secondary tab.
8. You can also choose 'load example data' to use example files.

| Experiment_Code | Group | Description |
|-----------------|-------|-----------------------|
| 1 Exp027015 | 0 | ctr_0h_R2_IMAC_1.raw |
| 2 Exp027016 | 0 | ctr_0h_R2_IMAC_2.raw |
| 3 Exp027017 | 0 | ctr_0h_R2_IMAC_3.raw |
| 4 Exp027031 | 6 | PLX_0h_R2_IMAC_1.raw |
| 5 Exp027032 | 6 | PLX_0h_R2_IMAC_2.raw |
| 6 Exp027033 | 6 | PLX_6h_R2_IMAC_3.raw |
| 7 Exp027046 | 48 | PLX_48h_R2_IMAC_1.raw |
| 8 Exp027047 | 48 | PLX_48h_R2_IMAC_2.raw |
| 9 Exp027048 | 48 | PLX_48h_R2_IMAC_3.raw |

Parser

Function

If you start with .xml files from mascot results, you can run this button to parser them to sites score files, based on which .csv files of phosphorylation sites with confidence score will be generated.



How to get analysis results

1. Go to the 'Preprocessing' tab.
2. Click the running button in Step1 and the file will appear on the right.

The screenshot shows the 'Preprocessing' interface with several tabs at the top: Home, Import Data, Preprocessing (selected), Analysis, Tutorial, FAQ, Download, and Help. The main area is divided into four sections:

- Step1: Parser**: no parameter
- Step2: Quality Control & Merging**: minimum score: 20, minimum FDR: 0.01
- Step3: Mapping**: species: human, id type: RefSeq_Protein_GI, fasta type: refseq
- Step4: Filtering & Normalization & Imputation**: minimum detection frequency: 1, normalization method: global, impute globally checked, imputation method: minimum/10, top: 100

A progress bar at the top indicates Step1 is running. On the right, a table lists peptide identification files with psites scores for 'Exp027015_psites_score'. The table includes columns for pep_seq and pep_var_mod_conf, showing 10 entries from 1 to 10. The first entry is ITLPVDFTADKFDENAA.

Quality control and merging

Function

Generate merged phosphoproteomics data frame based on peptides files.

QC S P C

Merging files by identifiers



Merged peptides list

S Score P FDR

C Confidence

How to get analysis results

1. Go to the 'Preprocessing' tab.
2. Modify the parameters according to your needs.
3. Click the running button in Step2 and the file will appear on the right.

The screenshot shows the PhosMap software interface with the 'Preprocessing' tab selected. The interface is divided into several sections:

- Step1: Parser**: No parameter.
- Step2: Quality Control & Merging**: minimum score: 20, minimum FDR: 0.01.
- Step3: Mapping**: species: human, id type: RefSeq_Protein_GI, fasta type: refseq.
- Step4: Filtering & Normalization & Imputation**: minimum detection frequency: 1, normalization method: global, impute globally checked, imputation method: minimum/10, top: 100.
- Step5: Normalization based on proteomics data**: With proteomics data checked, US cutoff: 1, minimum detection frequency: 1.
- Result**: Peptide data frame through phosphorylation sites quality control. A table shows 10 entries of peptide sequences and their phosphorylation sites across four experiments (Exp027015, Exp027016, Exp027017, Exp027031). The table includes columns for ID_of_seq_gi_site, sequence, and experimental values.

Interpretation of analysis results

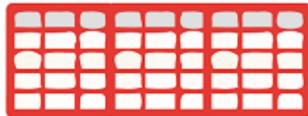
We performed quality control for identified phosphopeptides using PhosMap, those phosphopeptides that met 1% FDR at peptide level and had ion score greater than 20 and the highest confidence probabilities of p-sites computed by Mascot, were kept. We merged phosphopeptides list with quantitative value from all experiments to generate a matrix for analysis.

Mapping p-sites to protein

Function

Mapping protein gi number to gene symbol and outputting expression profile matrix with gene symbol. Constructing the data frame with unique phosphorylation site for each protein sequence.

Mapping p-sites to protein



Raw phosphorylation sites table

How to get analysis results

1. Go to the 'Preprocessing' tab.
2. Modify the parameters according to your needs.
3. Click the running button in Step3 and the file will appear on the right.

The screenshot shows the PhosMap software interface. On the left, there are three main sections: Step1: Parser (no parameter), Step2: Quality Control & Merging (minimum score: 20, minimum FDR: 0.01), and Step3: Mapping (species: human, id type: RefSeq_Protein_GI, fasta type: refseq). Step4: Filtering & Normalization & Imputation includes settings for minimum detection frequency (1), normalization method (global), impute globally (checked), imputation method (minimum/10), and top (100). Step5: Normalization based on proteomics data includes a checkbox for 'With proteomics data' and parameters for US cutoff (1) and minimum detection frequency (1). On the right, a table titled 'Data frame mapped ID to Gene Symbol:' shows 10 entries of phosphorylation sites. The columns include AA_in_protein, AA_in_peptide, Sequence, ID, and Modification. The table lists various protein IDs (e.g., gi|39930517_s161, gi|88900495_s50, gi|110681708_s483) with their corresponding peptide sequences and phosphorylation sites.

| AA_in_protein | AA_in_peptide | Sequence | ID | Modification |
|-------------------|---------------|----------|-------------------------------------|--------------------------|
| gi 39930517_s161 | s161 | s11 | AAAAAATAPPsPGPAQPGPR | gi 39930517 Phosph (11) |
| gi 88900495_s50 | s50 | s13 | AAAGEEETAAAGsPGRK | gi 88900495 Phosph (13) |
| gi 110681708_s483 | s483 | s14 | AAALQALQAOAPTsPPPPPPPLK | gi 110681708 Phosph (14) |
| gi 4758248_s281 | s281 | s5 | AAAlSsLSTLASPK | gi 4758248 Phosph (5) |
| gi 4758248_s283 | s283 | s7 | AAALSLsTLASPK | gi 4758248 Phosph (7) |
| gi 4758248_s287 | s287 | s11 | AAALSLSTLASPK | gi 4758248 Phosph (11) |
| gi 4758248_t284 | t284 | t8 | AAALSLsTLASPK | gi 4758248 Phosph (8) |
| gi 4758248_t294 | t294 | t5 | GGSGIAGTEPSDIIPRL | gi 4758248 Phosph (6) |
| gi 13491174_s104 | s104 | s5 | LSGLsFK | gi 13491174 Phosph (5) |
| gi 13491174_s22 | s22 | s15 s12 | APRGDVTAEAAAGAsPAK GDVTAEEAAGAsPAK | gi 13491174 Phosph (15) |

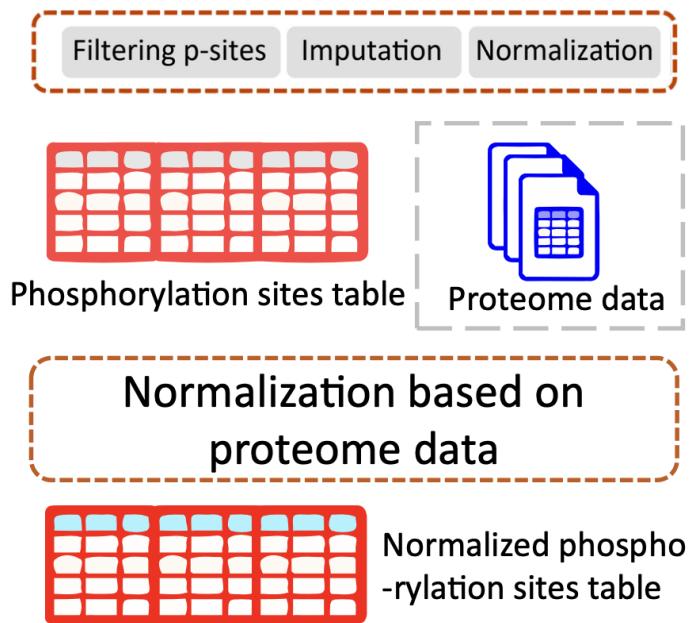
Interpretation of analysis results

Combining the phosphopeptides sequence, modification position, attached protein ID and the built-in human protein reference database of PhosMap, all p-sites were mapped to the corresponding protein sequence and represented by unique p-sites identifier (upsID) that consisted of a protein GI number/accession, gene symbol and location of the p-site in the protein sequence. In addition, the matched proteome data with phosphoproteome were collected at each time point in Ressa, et al. study. Finally, 3,649 unique p-sites were obtained and their quantitative values were normalized by matched protein profiling data using PhosMap.

Data normalization

Function

PhosMap provides two kinds of normalizations, a total sum scaling normalization and normalizing phosphoproteomics data based on proteomics data.



How to get analysis results

1. Go to the 'Preprocessing' tab.
2. Modify the parameters according to your needs.
3. Click the running button in the Step4 and the normalized data of p-sites based on a total sum scaling will appear on the right.
4. Click the running button in Step5 and normalized data of p-sites based on proteomics data will appear on the right.

127.0.0.1

Home Import Data Preprocessing Analysis Tutorial FAQ Download

Preprocessing

Step1: Parser
no parameter

Step2: Quality Control & Merging
minimum score: 20
minimum FDR: 0.01

Step3: Mapping
species: human
id type: RefSeq_Protein_GI
fasta type: refseq

Step4: Filtering & Normalization & Imputation
minimum detection frequency: 1
normalization method: global
impute globally
imputation method: minimum/10
top: 100

This step also filters modification types, remaining only 'S/T/Y'

Step5: Normalization based on proteomics data
 With proteomics data
Proteomics data preprocessing parameters
US cutoff: 1
minimum detection frequency: 1

Phosphorylation data frame:
Show 10 entries
Search:
upsID Sequence
1 g|55956788_NCL_S67 KVVVsPTK||KVVVsPTK||VVVsPTK
2 g|156523970_AHSG_S138 CDSSPDsAEDVR||CDSSPDsAEDVRK
3 g|56118310_NUCKS1_S214 EKTPSPKEEDEEPesPPEK||TPSPKEEDEEPesPPEK
4 g|56118310_NUCKS1_S181 ATVtPsPVK||ATVtPsPVK
5 g|24234699_KRT19_S35 FGPGVAFRAPsHNGGSGGR
6 g|45359848_G3BP2_T227 STtPPPAEPVSLPQEPPPKPR
7 g|4503423_DUT_S11 PCSEETPAIsPSK
8 g|61743954_AHNAK_S5731 GGVTGsPEASISGSK||GGVTGsPEASISGsK||GGVTGsPEASISGSKGDLK||GGVTGsPEASISGsKGDLK
9 g|61743954_AHNAK_S5841 GHYEVGTgsDDETGK||GHYEVGTgsDDETGKLOQSGGVSLASK||SKGHYEVGTgsDDETGK
10 g|506327594_CARHSP1_S52 TfSAtVR||TRTFsAtVR

Showing 1 to 10 of 2,090 entries Previous 1 2 3 4 5 ... 209 Next

127.0.0.1

Home Import Data Preprocessing Analysis Tutorial FAQ Download

Preprocessing

Step1: Parser
no parameter

Step2: Quality Control & Merging
minimum score: 20
minimum FDR: 0.01

Step3: Mapping
species: human
id type: RefSeq_Protein_GI
fasta type: refseq

Step4: Filtering & Normalization & Imputation
minimum detection frequency: 1
normalization method: global
impute globally
imputation method: minimum/10
top: 100

This step also filters modification types, remaining only 'S/T/Y'

Step5: Normalization based on proteomics data
 With proteomics data
Proteomics data preprocessing parameters
US cutoff: 1
minimum detection frequency: 1

All Done
You can now download the 'Phosphorylation data frame' for further analysis.

OK

Phosphorylation data frame:
Show 10 entries
Search:
Sequence
1 KVVVsPTK||KVVVsPTK||VVVsPTK
2 CDSSPDsAEDVR||CDSSPDsAEDVRK
3 EKTPSPKEEDEEPesPPEK||TPSPKEEDEEPesPPEK
4 ATVtPsPVK||ATVtPsPVK
5 FGPGVAFRAPsHNGGSGGR
6 STtPPPAEPVSLPQEPPPKPR
7 PCSEETPAIsPSK
8 GGVTGsPEASISGSK||GGVTGsPEASISGsK||GGVTGsPEASISGSKGDLK||GGVTGsPEASISGsKGDLK
9 GHYEVGTgsDDETGK||GHYEVGTgsDDETGKLOQSGGVSLASK||SKGHYEVGTgsDDETGK
10 TfSAtVR||TRTFsAtVR

Showing 1 to 10 of 2,090 entries Previous 1 2 3 4 5 ... 209 Next

Proteomics data frame:
Show 10 entries
Search:
Symbol Exp026982 Exp026983 Exp026995 Exp026996
1 AAAS 1.14544335883623 0.000164691226643703 0.886230524292994 0.177342286346101 0.00016469
2 AAC8 0.616028373603419 1.29427243522537 0.61757036413145 2.92073094008856 0.65814
3 AAGAB 1.59913959060195 1.19412666610487 0.000164691226643703 1.05454385172275 0.00016469

Analysis and visualization

PhosMap incorporated six analysis modules: dimension reduction analysis, differential expression analysis, time course analysis, kinase activity prediction, phosphorylation motif enrichment analysis and survival analysis.

Upload data

Function

In this step, you can upload your preprocessed data to PhosMap, such as the phosphorylation dataframe. If you have not preprocessed your data, you must preprocess it with PhosMap (go to the 'Preprocessing' tab) or do it yourself.

How to upload your data

1. Go to the 'Analysis Data Upload' under 'Analysis' tab.
2. Choose 'example data' or follow the prompts to upload your own corresponding three files.

The screenshot shows the PhosMap web application interface. On the left, there's a sidebar titled 'Analysis Data Upload' with three sections: 'Experimental design file', 'Phosphorylation data frame', and 'Clinical data file [optional]'. Each section has a 'Browse...' button, a file name, and an 'Upload complete' button. The 'Clinical data file [optional]' section also includes 'Download Template' and 'View Upload Instructions' links. On the right, there's a 'Data Overview' section with a table titled '4. Clinical data file :'. The table has columns 'PatientID', 'status', and 'time'. It lists 10 entries from 1 to 10, with values like Exp027012, 0, 1290. A search bar and pagination controls (Previous, Next) are at the bottom of the table.

| PatientID | status | time |
|-----------|--------|------|
| 1 | 0 | 1290 |
| 2 | 0 | 1187 |
| 3 | 1 | 1106 |
| 4 | 1 | 1264 |
| 5 | 1 | 948 |
| 6 | 0 | 1401 |
| 7 | 1 | 961 |
| 8 | 0 | 1867 |
| 9 | 1 | 986 |
| 10 | 0 | 1593 |

Dimension reduction analysis

Function

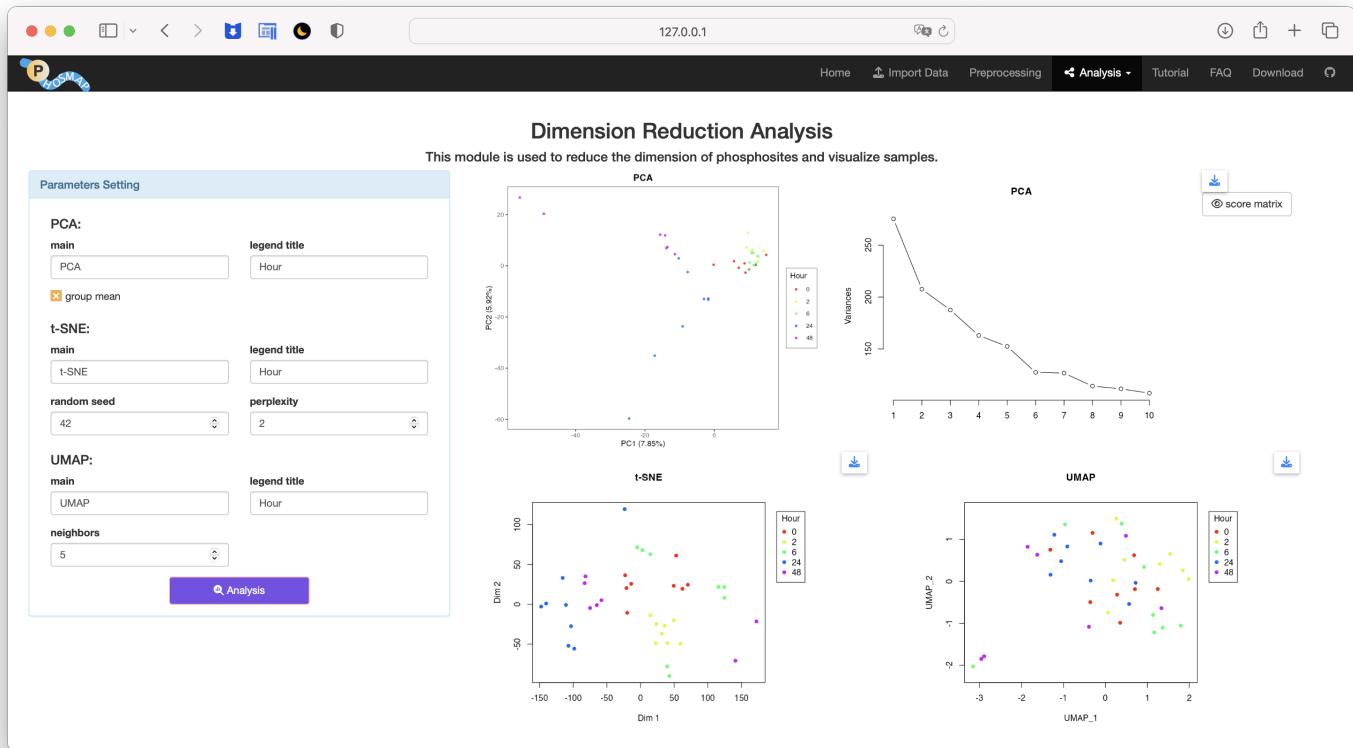
In PhosMap, Dimension reduction analysis methods allowed for PCA, t-SNE and UMAP.

The meaning of the parameters

1. 'Title' refers to the main title of the plot.
2. 'Legend title' refers to the title of the legend in the plot.
3. 'Random seed' is a parameter for t-SNE that sets the seed for the random number generator. This can be used to ensure reproducibility of results.
4. 'Perplexity' is a numerical value for t-SNE, with a default value of 2. It balances the focus between preserving the local and global structure of the data.
5. 'Neighbors' is a parameter for UMAP that refers to the size of the local neighborhood (in terms of the number of neighboring sample points) used for manifold approximation. Larger values result in more global views of the manifold, while smaller values result in more local data being preserved.

How to get analysis results

1. Go to the 'Dimension reduction analysis' under 'Analysis' tab.
2. Modify the parameters according to your needs.
3. Click the 'Analysis' button.
4. The PCA, t-SNE and UMAP plot after running will appear on the right.
5. Click the download button to download the plot file.



Interpretation of analysis results

To extract an overview of the effect of the different time course treatments, we performed PCA analysis in the downstream analysis module of PhosMap. We could see that phosphorylation expression profiles of colorectal cancer cells after longer (24h and 48h) vemurafenib treatment were quite different from those after short treatment (2h and 6h). In addition, it shows that principal component 1 (PC1), with 31.77%, is superior to 20% from original literature and demonstrates phosphorylation expression profile normalized by matched proteomics data has an advantage over representing the variation over time in the BRAFi-treated samples.

Differential expression analysis

Function

In PhosMap, differential expression analysis methods allowed for limma, SAM and ANOVA Data analysis.

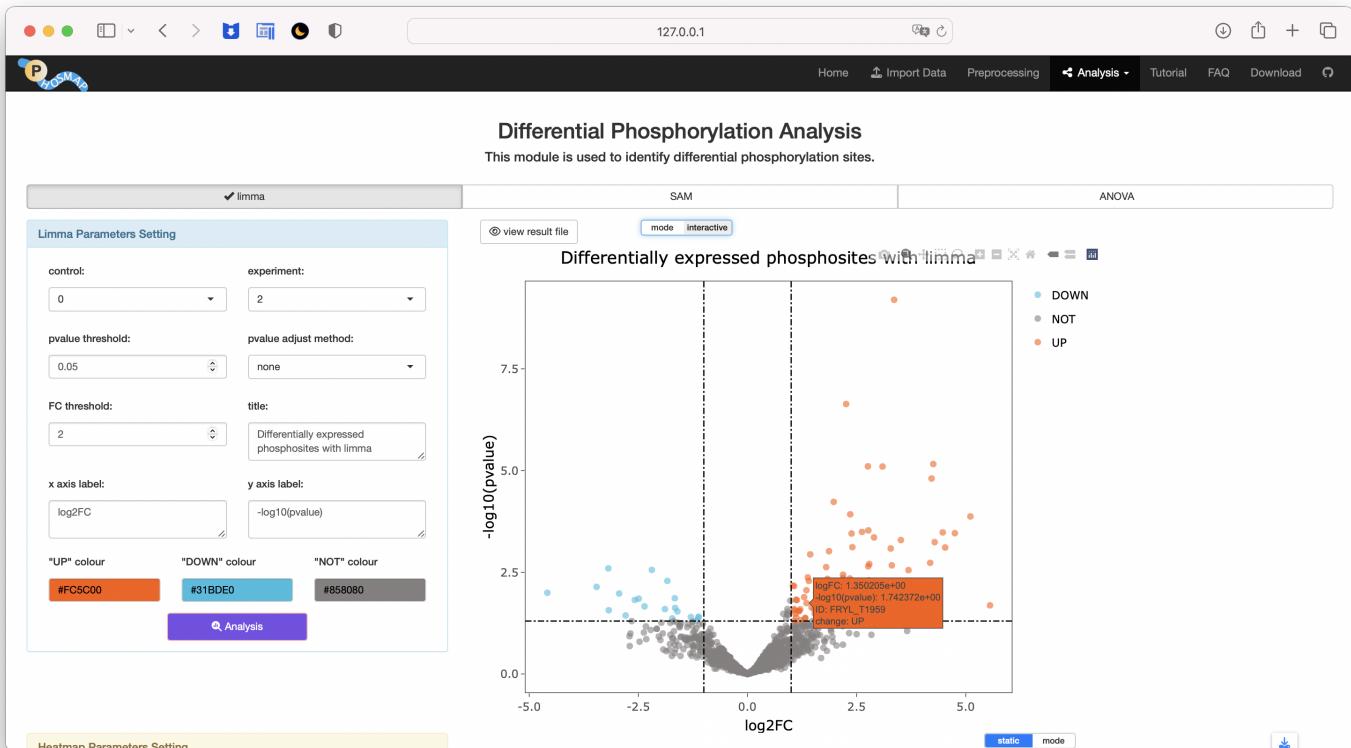
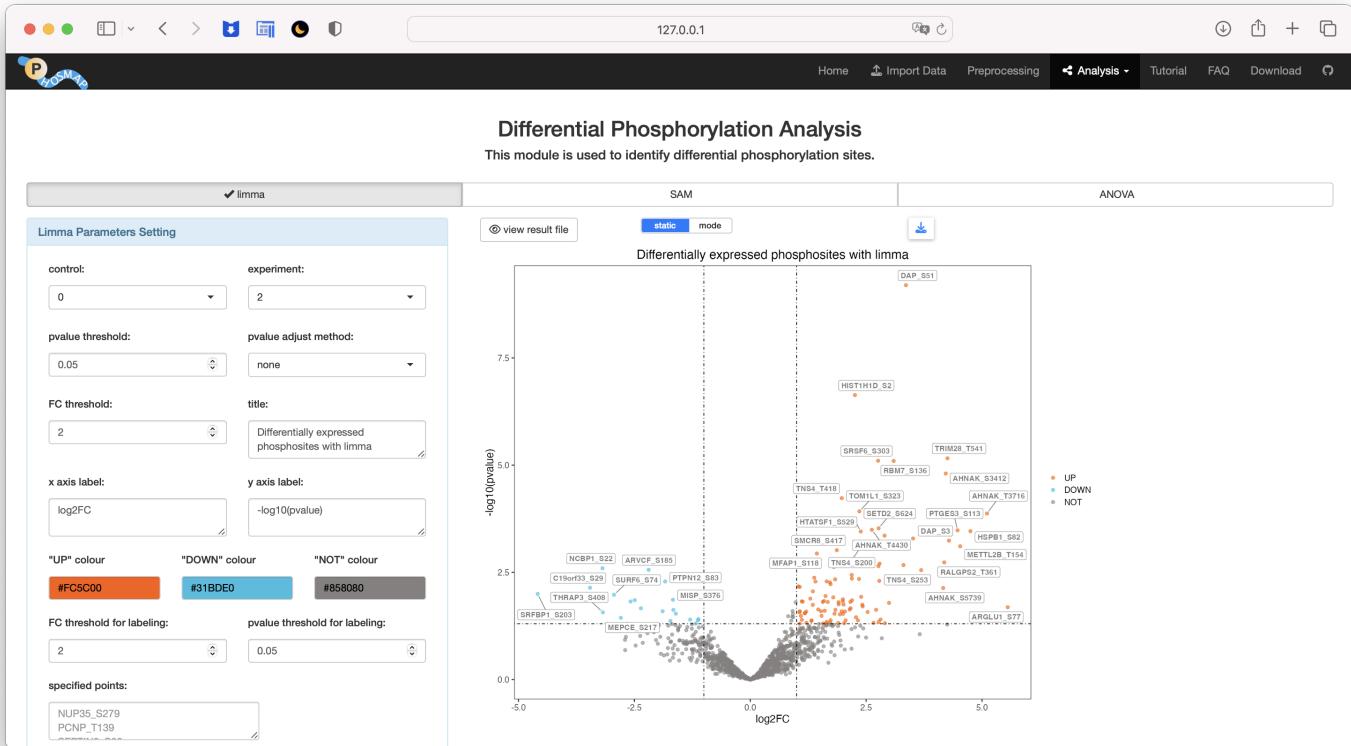
The meaning of the parameters

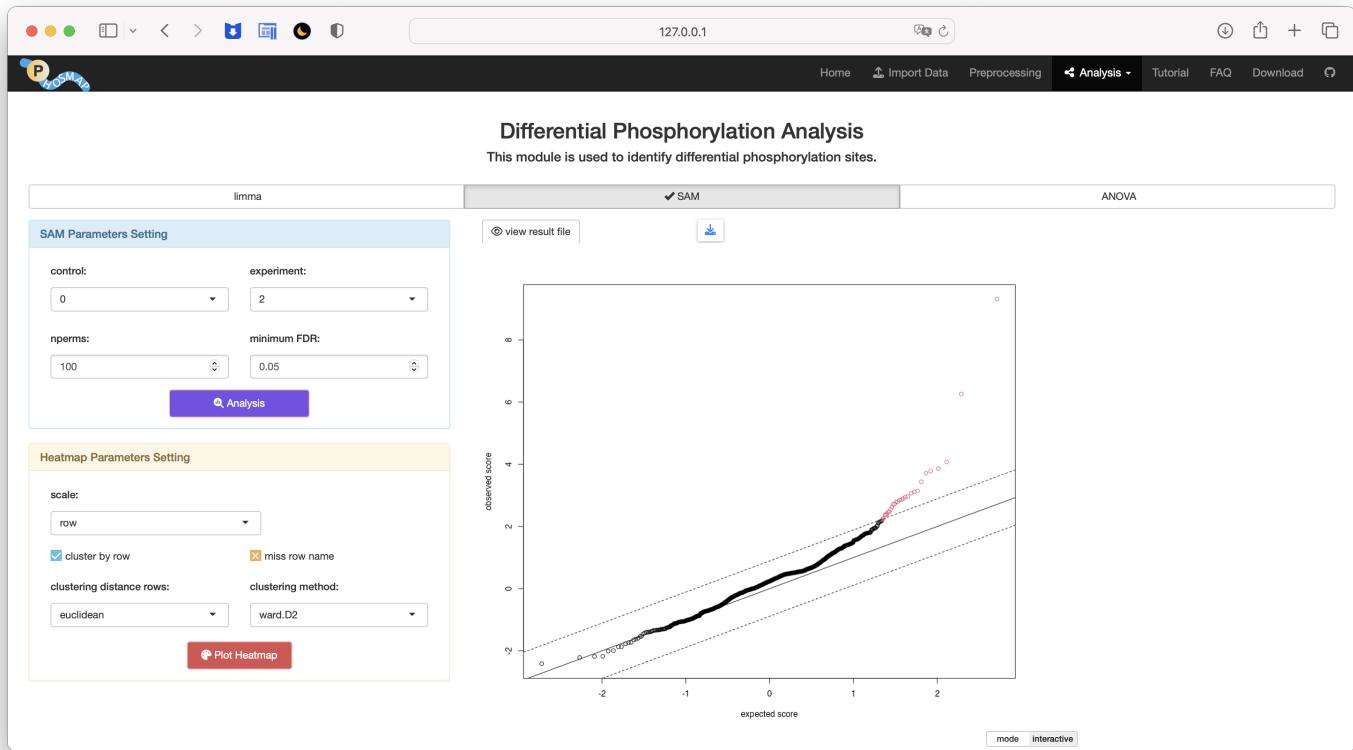
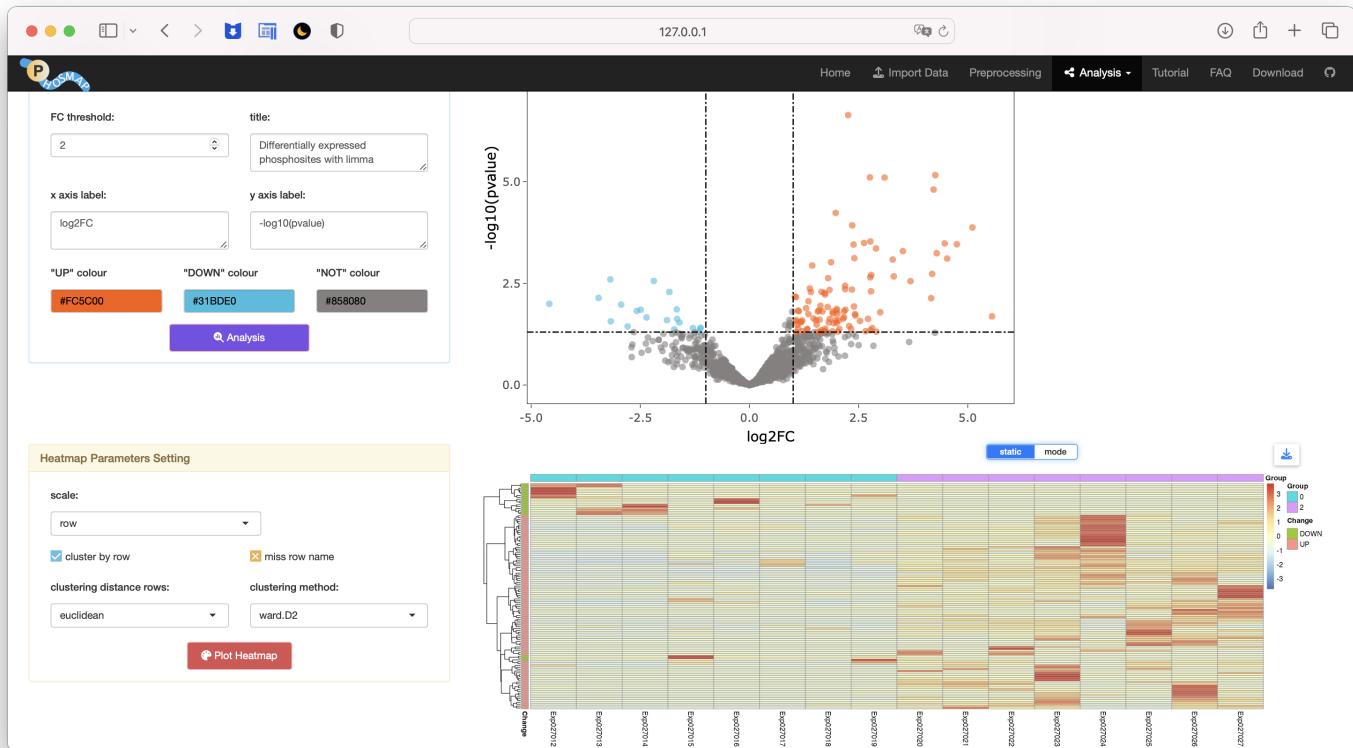
1. 'Control' refers to the control group in the experiment.
2. 'Experiment' refers to the experimental group in the experiment.
3. 'P-value threshold' is the threshold for determining statistical significance based on the p-value.

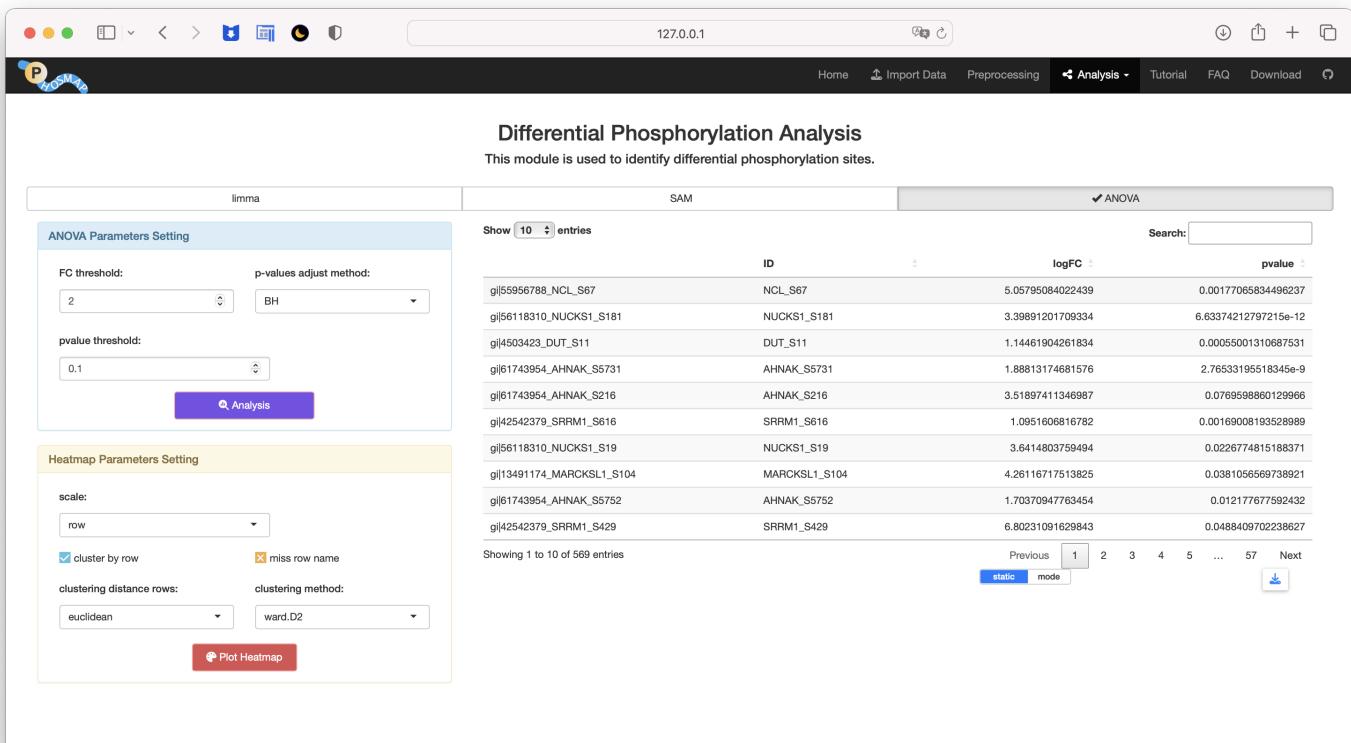
4. 'P-value adjust method' is the method used to adjust p-values for multiple comparisons.
5. 'FC threshold' is the fold change threshold for determining significant changes in phosphorylation levels.
6. 'nperms' is a parameter for the SAM method that specifies the number of permutations to perform.
7. 'Minimum FDR' is the minimum false discovery rate threshold for determining statistical significance.
8. 'Clustering distance rows' is a parameter for heatmap generation that specifies the distance metric used for clustering rows.
9. 'Clustering method' is a parameter for heatmap generation that specifies the clustering method used to cluster rows and columns.

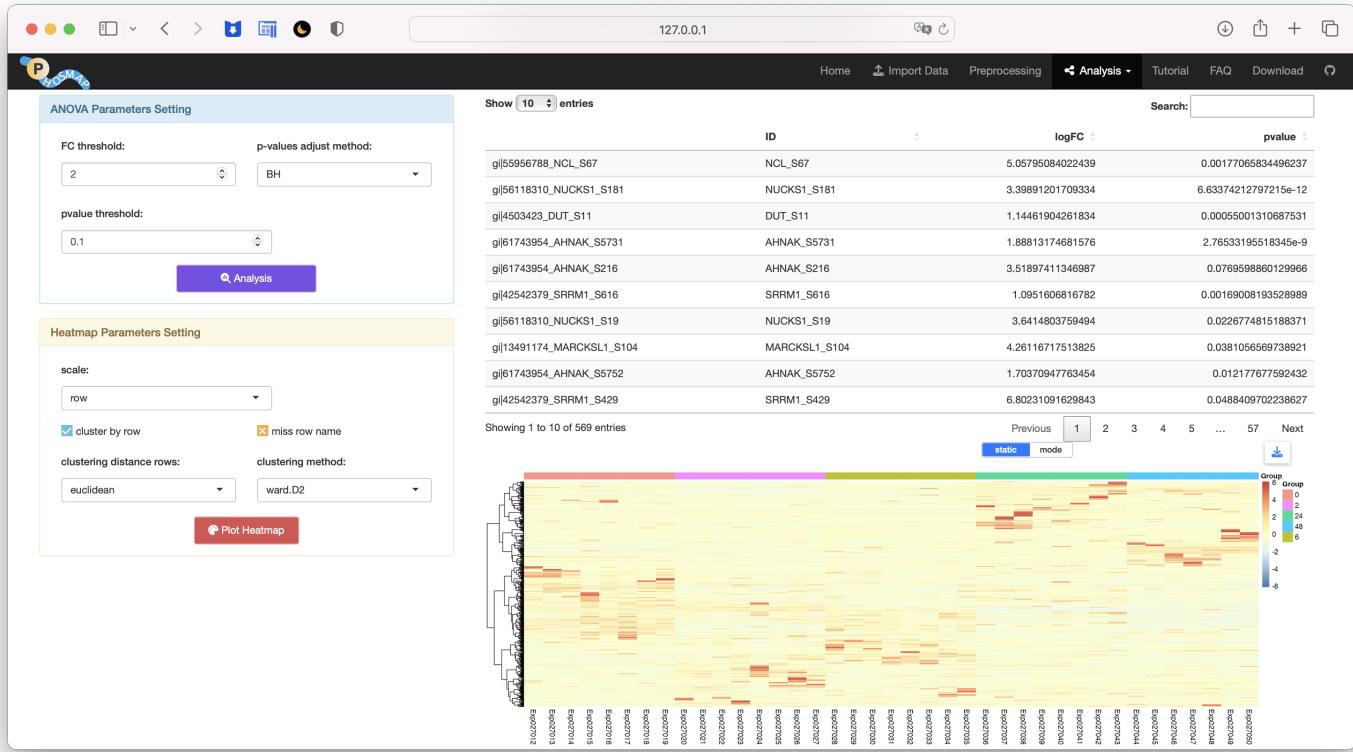
How to get analysis results

1. Go to the 'Differential Expression Analysis' under 'Analysis' tab.
2. Go to the 'limma', 'SAM' or 'ANOVA' secondary tab.
3. Choose Control and Experiment used for differential Expression Analysis.
4. Choose 'Interactive mode' and click the 'Analysis' button. The interactive plot after running will appear on the right.
5. Choose 'Static mode' and click the 'Analysis' button. The static plot after running will appear on the right.
6. Click 'Plot Heatmap' button. The heatmap will appear in the pop-up window.
7. Click the download button to download the plot file.









Interpretation of analysis results

In order to show differential expression analysis between two experimental conditions. We use the limma method integrated into differential expression analysis module of PhosMap to identify 128 significant differently expressed p-sites (DEPs) between the samples with BRAFi-treated for two hours and control samples (P value < 0.05 and fold change > 2). 139 p-sites were up-regulated in the BRAFi-treated samples. The most disparate difference is observed in DAP_S51, whose phosphoserine is related to the MTOR pathway. 99 p-sites were down-regulated in the BRAFi-treated samples.

For the multiple experimental conditions, we leveraged the embedded ANOVA analysis of PhosMap and identified 548 DEPs among the five time points (P value < 0.1 and fold change > 2).

Time Course Analysis

Function

Fuzzy clustering was applied to time course analysis for discovering patterns associated with time points in PhosMap. The corresponding line chart combined with membership for each cluster was also drawn.

The meaning of the parameters

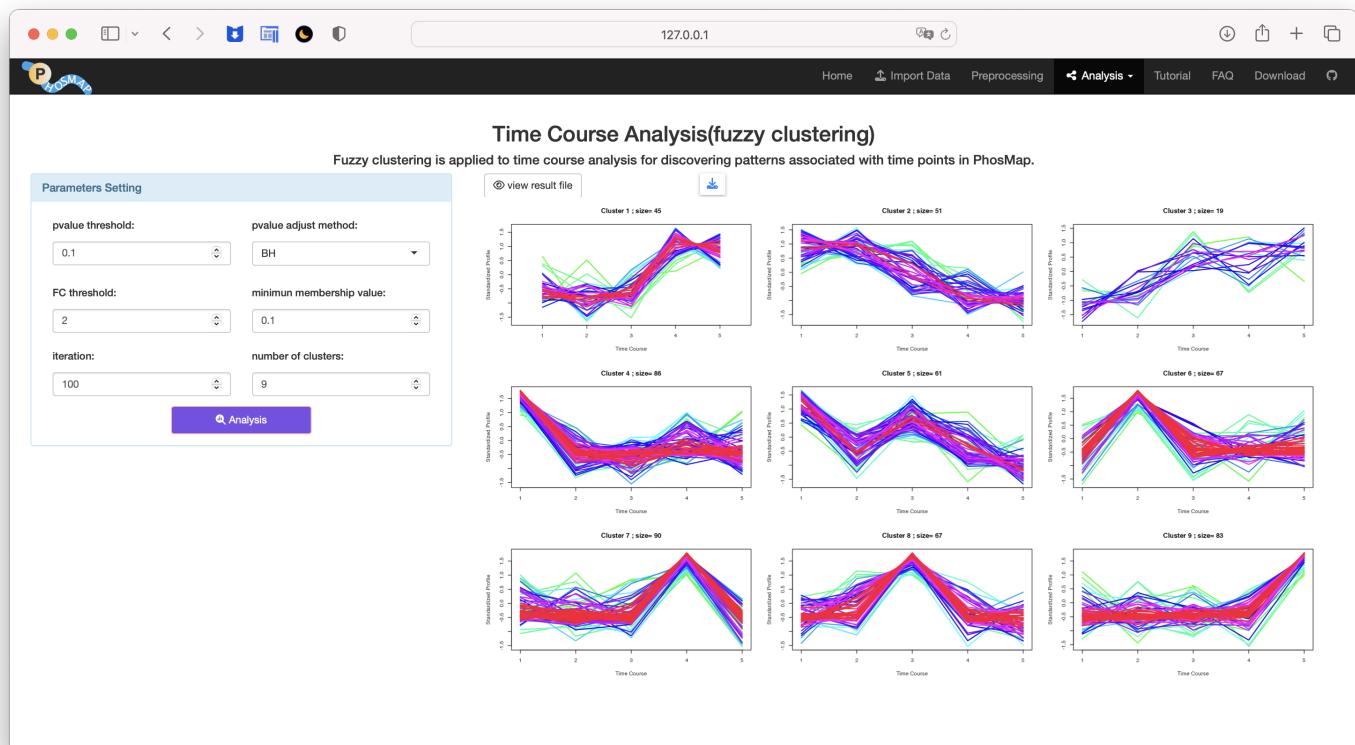
1. ‘Minimum membership value’ is a threshold for determining the minimum membership value for a data point to be included in a cluster.
2. ‘Iteration’ is the number of iterations to perform in the clustering algorithm.
3. ‘Number of clusters’ is the number of clusters to generate in the clustering algorithm.

How to get analysis results

1. Go to the ‘Time course Analysis (fuzzy clustering)’ under ‘Analysis’ tab.
2. Modify the parameters according to your needs.
3. Click the ‘Analysis’ button. The plot after running will appear on the right.
4. Click the download button to download the plot file.

Interpretation of analysis results

These 548 DEPs were used as inputs in the time course analysis module of PhosMap, then 9 strong expression patterns were generated. Two major clusters show significant downregulation at the phosphoproteomics signalling level upon BRAFi treatment in line with the original literature. Cluster 1 responds within 2 hours, an early treatment response. Cluster 2 responds within 24 hours, a late treatment response.



Kinase activity prediction (KSEA)

Function

In PhosMap, KSEA was used to predict kinase activity.

The meaning of the parameters

1. 'Control' refers to the control group in the experiment.
2. 'Experiment' refers to the experimental group in the experiment.
3. 'Species' refers to the species of the organism being studied.
4. 'Scale' is a parameter for scaling the data before generating the heatmap.
5. 'Clustering distance rows' is a parameter for heatmap generation that specifies the distance metric used for clustering rows.
6. 'Clustering method' is a parameter for heatmap generation that specifies the clustering method used to cluster rows and columns.

How to get analysis results

1. Go to the 'Kinase-Substrate Enrichment Analysis' under 'Analysis' tab.
2. Select 'Multiple groups' or 'Two groups' according to the number of groups of your data.
3. Click the first 'Analysis' button. If 'Multiple groups' is selected, after running, the plot will appear on the right. Click 'view result' to view and download the kinase prediction time course result. If 'Two groups' is selected, only the phosphorylation dataframe will appear on the right.
4. Select a cluster if 'Multiple groups' is selected. Click the second 'Analysis' button. After running, the heatmap will appear on the right.
5. Click the download button to download the plot file.

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Kinase-Substrate Enrichment Analysis

This module is used to predict kinase activity.

Result Step 1 Step 2

Clustering Parameters Setting [Step 1]

✓ Multiple groups Two groups

pvalue threshold: 0.1 pvalue adjust method: BH

FC threshold: 2 minimum membership value: 0.1

Iteration: 100 number of clusters: 9

KSEA Parameters Setting [Step 2]

select a cluster: 1 species: human

scale: none clustering distance rows: euclidean

clustering method: ward.D2 title: Kinase-Substrate Enrichment Analysis of Cluster 1

Cluster 1 : size= 45 Cluster 2 : size= 51 Cluster 3 : size= 19

Cluster 4 : size= 86 Cluster 5 : size= 61 Cluster 6 : size= 67

Cluster 7 : size= 90 Cluster 8 : size= 67 Cluster 9 : size= 83

Standardized Profile Time Course

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Kinase-Substrate Enrichment Analysis

This module is used to predict kinase activity.

Result Step 1 Step 2

Clustering Parameters Setting [Step 1]

✓ Multiple groups Two groups

pvalue threshold: 0.1 pvalue adjust method: BH

FC threshold: 2 minimum membership value: 0.1

Iteration: 100 number of clusters: 9

KSEA Parameters Setting [Step 2]

select a cluster: 1 species: human

scale: none clustering distance rows: euclidean

clustering method: ward.D2 title: Kinase-Substrate Enrichment Analysis of Cluster 1

Kinase-Substrate Enrichment Analysis of Cluster 1

Show 10 entries Search:

| group | CDK5 | MTOR | CDK2 | CDK1 | AKT1 | SRPK2 | HIPK1 | MARK1 |
|-------|------|------|------|------|------|-------|-------|-------|
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PhosMap

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Kinase-Substrate Enrichment Analysis

This module is used to predict kinase activity.

Result Step 1 Step 2

Show 10 entries Search:

| ID | Exp027012 | Exp027013 | Exp027014 | Exp027015 | Exp027016 | Exp027017 | Exp027018 | Exp027019 |
|---------------------------|---------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| gi 134152708_ARGLU1_S77 | ARGLU1_S77 | 0.001223459 | 1.907614887 | 2.105814833 | 0.001223459 | 0.001223459 | 2.524966705 | 1.456709738 |
| gi 103471995_SRFBP1_S203 | SRFBP1_S203 | 0.693174887 | 0.001999739 | 0.001999739 | 6.381098837 | 0.001999739 | 0.470005869 | 0.44772145 |
| gi 157649073_CAP1_S308 | CAP1_S308 | 2.82446145 | 2.836969402 | 1.083492138 | 0.000935231 | 0.000935231 | 0.000935231 | 0.89337952 |
| gi 56549640_SEPTIN2_S218 | SEPTIN2_S218 | 1.472866354 | 2.36419485 | 1.950855675 | 0.28779756 | 0.489017824 | 0.414760843 | 0.587088319 |
| gi 10835067_SSB_S366 | SSB_S366 | 1.600235315 | 1.13598565 | 1.116441327 | 0.001298198 | 0.896975825 | 0.001298198 | 1.94535899 |
| gi 61743954_AHNAK_S135 | AHNAK_S135 | 0.001920669 | 0.001920669 | 0.001920669 | 4.053823919 | 0.001920669 | 3.934652066 | 0.001920666 |
| gi 13491174_MARCKSL1_S104 | MARCKSL1_S104 | 0.833577284 | 0.988007631 | 0.912591979 | 1.197880732 | 0.718407402 | 1.390739206 | 1.369987996 |
| gi 42542379_SRRM1_T220 | SRRM1_T220 | 6.881830552 | 0.159738493 | 0.159738493 | 0.159738493 | 0.159738493 | 0.159738493 | 0.159738493 |
| gi 23308579_PTGES3_S113 | PTGES3_S113 | 0.039680827 | 0.304635154 | 0.039680827 | 0.039680827 | 0.651020846 | 0.696630111 | 2.005772064 |
| gi 118572613_SRRM2_T1177 | SRRM2_T1177 | 0.045546703 | 0.045546703 | 0.045546703 | 6.995319693 | 0.731400087 | 0.045546703 | 0.045546703 |

Showing 1 to 10 of 409 entries Previous 1 2 3 4 5 ... 41 Next

Analysis

PhosMap

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Kinase-Substrate Enrichment Analysis

This module is used to predict kinase activity.

Result Step 1 Step 2

mode interactive

Kinase-Substrate Enrichment Analysis

Search:

| Exp027012 | Exp027013 | Exp027014 | Exp027015 | Exp027016 | Exp027017 | Exp027018 |
|-----------|--------------------|-------------------|-------------------|--------------------|-------------------|-------------------|
| CSNK2A2 | 0.752026733638193 | 1.46852108295774 | 0.869666231504994 | -1.74472749489669 | -2.15490195998574 | -1.79588001734408 |
| DYRK4 | 1.1611509026274 | -2.04575749056068 | -1.76955107862173 | -1.79588001734408 | 0.943095148663527 | -1.74472749489669 |
| CDK7 | -0.294136287716081 | -1.72124639904717 | -2.22184874961636 | -0.503070351926785 | -0.34008379993015 | -1.06048074738138 |
| PAK4 | -1.17392519729917 | -1.11350927482752 | -1.16749108729376 | 1.14266750536873 | -1.30908391997148 | 0.88605647693163 |
| GSK3A | -1.19382002601611 | -1.13667713987954 | -1.1249387366083 | 1.07058107428571 | -1.3010299966398 | 0.954677021213343 |
| MAPKAPK5 | 0.75448733218585 | 0.742321425130815 | -0.27818938478453 | -0.876148359032914 | 2 | -0.91721462968355 |

Analysis

Interpretation of analysis results

Afterwards, the substrates from the two clusters are imported into the KSEA module of PhosMap to infer kinase activities. The results indicate that CDK1/2, MAPK1/3 and AKT1 are suppressed during

BRAFi treatment.

Motif enrichment analysis

Function

PhosMap allowed for performing MEA on user defined phosphopeptides lists to provide clues for finding candidate kinases that are not present in the database.

The meaning of parameters

1. 'Fasta type' refers to the type of fasta file used as input for the analysis.
2. 'Selected row number for plotting motif logo' is the number of rows to be selected for generating the motif logo plot.
3. 'Matched seqs threshold' is the threshold for determining the minimum number of matched sequences required for a motif to be considered significant.
4. 'Scale' is a parameter for scaling the data before generating the heatmap.
5. 'Distance metric' is a parameter for heatmap generation that specifies the distance metric used for clustering rows.
6. 'Clustering method' is a parameter for heatmap generation that specifies the clustering method used to cluster rows and columns.

How to get analysis results

1. Go to the 'Motif Enrichment Analysis' under 'Analysis' tab.
2. Modify the parameters according to your needs.
3. Click the 'Analysis' button.
4. The foreground dataframe mapped to motifs is shown on the right after running.
5. Select row number for plotting logo.
6. Click the first 'Plot' button, and the logo will appear on the right.
7. Modify the parameters below.
8. Click the second 'Plot' button.
9. The heatmap will appear on the right.
10. Click the download button to download the plot file.

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Home Import Data Preprocessing Analysis Tutorial FAQ Download Motif-Kinase Relation

Motif Enrichment Analysis

This module is used to find and visualize enriched motifs.

Parameters Setting

species: human
fasta type: refseq
pvalue threshold: 0.01

Motif Selection

selected row number for plotting motif logo: 1

Heatmap Parameters Setting

Assign quantitative values of peptides to their motif
matched seqs threshold: 50

Motif enrichment analysis result:

| motif | score | foreground_matches | foreground_size | background_matches | background_size | fold_increase |
|----------------|------------------|--------------------|-----------------|--------------------|-----------------|------------------|
|SP.K.K. | 34.0470844488047 | 25 | 2920 | 254 | 868625 | 29.2789680185525 |
| A...RS.SP.... | 35.8342606069076 | 9 | 2895 | 45 | 868371 | 59.9910880829015 |
|SPSK.... | 32.7445608653088 | 29 | 2886 | 304 | 868326 | 28.7019162381004 |
| ...RA.SPS.... | 32.6013002890178 | 7 | 2857 | 32 | 868022 | 66.4612574378719 |
| ...T.QSP.... | 31.3575151281482 | 5 | 2850 | 10 | 867990 | 152.278947368421 |
| .KY...SP..P.. | 32.8402738146705 | 5 | 2845 | 10 | 867980 | 152.544815465729 |
| ...R.SPPP.... | 32.0349538755237 | 15 | 2840 | 99 | 867970 | 46.3065514297909 |
|S.SPV.R.. | 31.8576048646771 | 7 | 2825 | 25 | 867871 | 86.0190725663717 |
| ...R..SPT.... | 26.6461515471161 | 15 | 2818 | 216 | 867846 | 21.3864738585285 |
| .RK....SP.... | 27.2951660844078 | 14 | 2803 | 162 | 867630 | 26.7500429434072 |
| ...D...SP....K | 26.720126704985 | 11 | 2789 | 124 | 867468 | 27.5915404989648 |
| ...AP.SP..K.. | 28.2641728543008 | 6 | 2778 | 28 | 867344 | 66.9040419623573 |
| ...VS.SP....K | 29.8281846942537 | 5 | 2772 | 14 | 867316 | 111.744485673057 |
| .KK....SP.... | 25.5857789899066 | 13 | 2767 | 169 | 867302 | 24.1111450890996 |
|SPK.... | 22.7741198377676 | 79 | 2754 | 2447 | 867133 | 10.1651759494456 |

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Motif logo

selected row number for plotting motif logo: 1

Parameters Setting

species: human
fasta type: refseq
pvalue threshold: 0.01

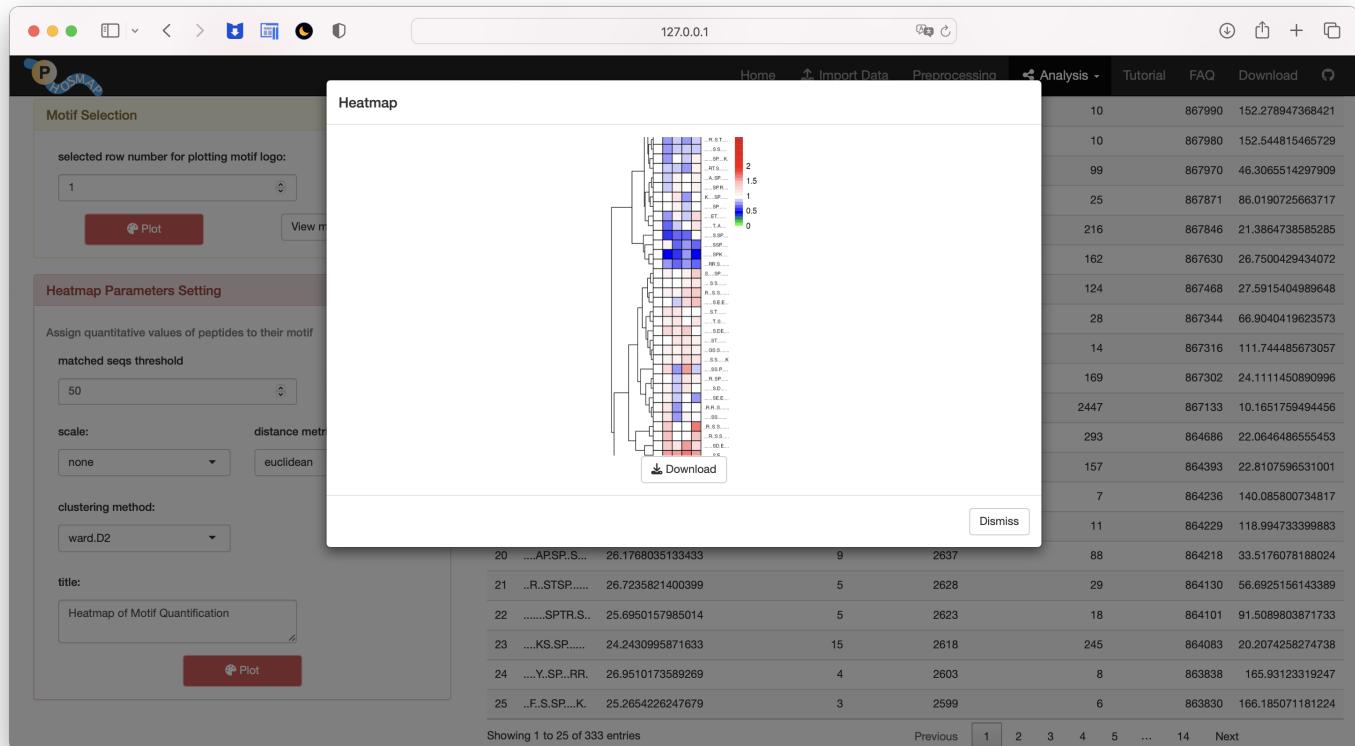
Motif Selection

Heatmap Parameters Setting

Assign quantitative values of peptides to their motif
matched seqs threshold: 50

The motif logo visualization shows the sequence "SPK" at position 11. The logo is composed of vertical bars of different heights and colors (green, blue, black, purple, red) representing the frequency of each amino acid (Polar, Basic, Hydrophobic, Neutral, Acidic) at each position. Below the logo, there is a legend for "chemistry" and a "Download" button.

| motif | score | foreground_matches | foreground_size | background_matches | background_size | fold_increase |
|----------------|------------------|--------------------|-----------------|--------------------|-----------------|------------------|
| ...R..SPT.... | 26.6461515471161 | 15 | 2818 | 216 | 867846 | 21.3864738585285 |
| .RK....SP.... | 27.2951660844078 | 14 | 2803 | 162 | 867630 | 26.7500429434072 |
| ...D...SP....K | 26.720126704985 | 11 | 2789 | 124 | 867468 | 27.5915404989648 |
| ...AP.SP..K.. | 28.2641728543008 | 6 | 2778 | 28 | 867344 | 66.9040419623573 |
| ...VS.SP....K | 29.8281846942537 | 5 | 2772 | 14 | 867316 | 111.744485673057 |
| .KK....SP.... | 25.5857789899066 | 13 | 2767 | 169 | 867302 | 24.1111450890996 |
|SPK.... | 22.7741198377676 | 79 | 2754 | 2447 | 867133 | 10.1651759494456 |



Interpretation of analysis results

The 3,649 identified phosphor-peptides as foreground sequences are used for MEA of PhosMap and the results further strengthen the evidence of CDK and MAPK pathway deactivation in BRAF mutant CRC cells in response to BRAFi treatment.

Survival analysis

Function

This module is used to identify phosphorylation sites or kinases associated with clinical outcomes of patients. Using kinases or phosphorylation locations files and patients' survival information as input matrices, coxph function from survival R package was used to calculate the hazard ratio (HR) and P-value.

How to get analysis results

1. Go to the 'Survival Analysis' under 'Analysis' tab.
2. Modify the parameters according to your needs.
3. Click the 'Analysis' button.
4. The summary dataframe list will appear on the right.
5. Click the 'Plot' button.

6. The plot after running will appear on the right.
7. Click the download button to download the plot file.

Survival Analysis

This module is used to identify phosphorylation sites or kinases associated with clinical outcomes of patients.

Parameters Setting

| | |
|-----------------------|-------------------|
| pvalue adjust method: | pvalue threshold: |
| BH | 0.01 |

Feature Selection

Select a statistically significant Psite:
g|110556636_TNKS1BP1_S872

or input any Psite:
g|56118310_NUCKS1_S181

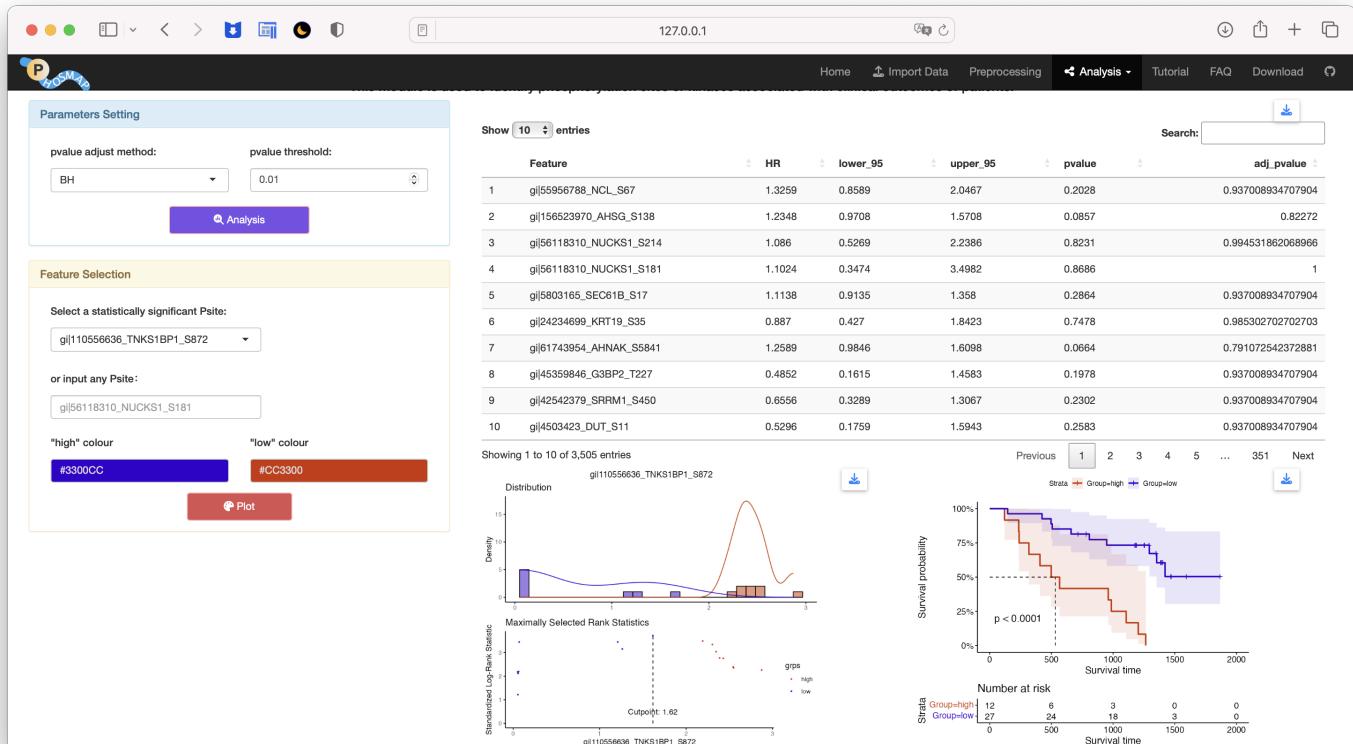
"high" colour: #3300CC "low" colour: #CC3300

Show 10 entries

| Feature | HR | lower_95 | upper_95 | pvalue | adj_pvalue |
|------------------------|--------|----------|----------|--------|-------------------|
| g 55956788_NCL_S67 | 1.3259 | 0.8589 | 2.0467 | 0.2028 | 0.937008934707904 |
| g 156523970_AHSQ_S138 | 1.2348 | 0.9708 | 1.5708 | 0.0857 | 0.82272 |
| g 56118310_NUCKS1_S214 | 1.086 | 0.5269 | 2.2386 | 0.8231 | 0.994531862068966 |
| g 56118310_NUCKS1_S181 | 1.1024 | 0.3474 | 3.4982 | 0.8686 | 1 |
| g 5803165_SEC61B_S17 | 1.1138 | 0.9135 | 1.358 | 0.2864 | 0.937008934707904 |
| g 24234699_KRT19_S35 | 0.887 | 0.427 | 1.8423 | 0.7478 | 0.985302702702703 |
| g 61743954_AHNAK_S5841 | 1.2589 | 0.9846 | 1.6098 | 0.0664 | 0.791072542372881 |
| g 4559846_G3BP2_T227 | 0.4852 | 0.1615 | 1.4583 | 0.1978 | 0.937008934707904 |
| g 42542379_SRMM1_S450 | 0.6556 | 0.3289 | 1.3067 | 0.2302 | 0.937008934707904 |
| g 4503423_DUT_S11 | 0.5296 | 0.1759 | 1.5943 | 0.2583 | 0.937008934707904 |

Showing 1 to 10 of 3,505 entries

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References

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