P3DB Training Materials version 1.0

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Website:

http://p3db.org/PPPworkshop14.php

This manual can be downloaded from the above link.

0. Preparation

0.1 Go to http://p3db.org

0.2(optional) Create your account. Please "sign up" your own account from the top right button on our front page.

0.3(optional) Log in

0.4(optional) Download "sample upload data", which will be useful in 4.2 from http://p3db.org/PPPworkshop14.php "Document download->sample upload data download[zip]" and unzip it.

1. Search

1.1 Search a protein and browse the information

- ❖ You will know how to search a protein by its accession number and browse the data among protein page, phosphosite page, peptide page and spectra plot.
- ➢ Step 1. Go to the menu "search->protein", choose "phosphoprotein search", type "**AT5G64130.1**" in "protein accession number", leave the default or blank for anything else, and click "search".
- ➢ Step 2. Click the links in the "protein description" column, and the protein page will show up.
- ➢ Step 3. Click "protein chart". The chart can be zoomed in/out and saved after it is shown.
- ➢ Step 4. Back to the protein page at step 2. Change the "reference" to "Meyer et al.(2012)", click the red marked amino acid in "sequence" or click the "detail" button in the table of "phosphosites", and the phosphosite page will be shown.
- ➢ Step 5. Click the "detail" button in the "NR peptide", and the peptide page will be shown.
- ➢ Step 6. Scroll the page down to view the "spectra" information. Click the link in the "spectrum" column, and the spectrum will be shown on the next page.
- ➢ Step 7. Go back to the peptide page in Step 5. Click the "detail" button on the top of the page, and the protein page will be back. You can browse more information there.

1.2 Search a protein by other methods

❖ You can search a protein not only by its name or accession number, but also by its peptide sequence through Blast.

➢ Step 1. Go to menu "Search->protein", and click on phosphopeptide search; type in "**YFDSADWA**" in "amino acid sequence", and you can get all the proteins having that peptide cross all the plant species that we have.

➢ Step 2. Go to menu "Search->protein", and click on "phosphoprotein BLAST". Click on the "example" button, change the "E-value threshold" to "0.001", and click "BLAST".

➢ Step 3. Click on the "E-value" in the results table, twice, and the results will be sorted by the minimal E-value to the maximum.

➢ Step 4. Go to menu "Search->protein", and click on "phosphopeptide BLAST". Click on the "example" button, and click "BLAST". The protein sequence will be blasted against the peptide data and the results will be returned.

1.3 Search protein-protein interaction (PPI)

❖ You can search the PPI data from a protein list and play with the network.

➢ Step 1. Go to menu "Search->PPI", click on "example" and then "search".

➢ Step 2. The PPI data will be listed in the table. Click on "Draw PPI network".

➢ Step 3. On the top part of network page, select "maximize the connectivity" and click on "search", and the green nodes will connect the isolated parts of the network.

➢ Step 4. Change the layout to "circle" or anything else, and click on "apply", the network layout will be changed.

➢ Step 5. Click on any links, and you can see the interaction information and data source links in the table above the network graph.

➢ Step 6. Click on any nodes, and then click "expand neighbors". The white nodes representing the neighborhood will be shown. Click on "hide neighbors" and they will be hidden.

1.4 Search kinase and phosphatase

❖ You can search kinase by its name.

➢ Step 1. Go to menu "Search->Kinase", type in "MAP_Kinase" in the kinase key word text box, and click "search".

➢ Step 2. The result can be researched in the text box under each column name. Type in "3g" in the protein id column, and the data will be filtered.

➢ Step 3. Type in "MAP_kinase_kinase_5" to further filter the results. "_" is used to get the full match.

1.5 Search by Gene Ontology terms

❖ You can search the proteins related to any gene ontology term.

➢ Step 1. Go to menu "Search->Ontology", type in "phosphorylation" in the description text box, and click "search".

➢ Step 2. In the results list, click "Tree View" beside the first term "GO:0000160".

➢ Step 3. You can see the ontology term structure, and related proteins and term information.

> Step 4. In the ontology tree structure, click the parental term on the top "GO:0007165".
> Step 5. Scroll down to find the protein related to "signal transduction" in the box of "related protein list", and you can click the link if the protein is in P3DB and go to the protein page.

1.6 Search by domain name, ID, networks

❖ You can search by protein domains.
> Step 1. Go to menu "Search->domain", in the top panel "domain search", type in the "PHD zinc finger", click "search".
> Step 2. In the result page, if the protein ID is shown in red, you can click it and you will be directed to the protein page.
> Step 3. Go to menu "Search->domain", in the bottom panel "domain network search", type in the "PF00481", click "search".
> Step 4. Beside the network graph, uncheck "rice" and "soybean", and leave "Arabidopsis".
> Step 5. Click the link between "PP2C" and "FHA" in the network graph, and you will see the information shown below.
> Step 6. Click the related proteins in the bottom right panel.

2. Browse the data

2.1 Browse the dataset

❖ You can browse the datasets by publication list or organisms.
> Step 1. Go to menu "Browse->datasets".
> Step 2. Click the column name "Glycine max" twice, and you can sort the data sources by the number of phosphoproteins.
> Step 3. Click the number, and you can see all the proteins.
> Step 4. Go to menu "Browse->organism", and you can see all the species in P3DB.
> Step 5. Click the number, and you can see all the proteins.

2.2 Browse kinase

❖ You can browse kinase architecture and the phosphatase architecture in the same way.
> Step 1. Go to menu "Tool->kinase".
> Step 2. Click on the top "expand all".
> Step 3. Click on "1.1.1".

3. Tools

3.1 PPI network

❖ Already shown in 1.3.

3.2 Kic Assay

> Go to menu "Browse->kicassay".

3.3 Ontology

❖ You can browse the whole structure of ontology.
➢ Already shown in 1.5.

3.4 Prediction of phosphosites

❖ You can predict the phosphosites using plant-specific models.
➢ Step 1.Go to menu "Tool->Prediction"
➢ Step 2.Click on "example" and click "search".
➢ Step 3. Scroll the bar for changing the specificity.

3.5 Domain network

❖ You can search, browse, and save domain networks.
➢ Already shown in 1.6.

3.6 Kinase-substrate network

❖ You can search within the kinase-substrate network.
➢ Step 1. Go to "tool->kinase substrate network"
➢ Step 2. Click "example" and click on "search".
➢ Step 3. Scroll down and check the blue "phosphatase" and blue "phosphatase to substrate" to overlay the phosphatase data.
➢ Step 4. Click one node in the network graph, and click on the button "search by selected".
➢ Step 5. Click on the button bottom left to the network graph to save the graph.

4. Data

4.1 Data download

❖ You can download any public data sets.
➢ Step 1.Go to "Data->download"
➢ Step 2. Change organism to "Arabidopsis", change experimental study to "Salvato 2013", and click on "download".

4.2 Data Upload

❖ You can submit the data and create your own data page.
❖ Make sure you have already logged in and have the sample upload data ready from 0.4
➢ Step 1. Go to "Data->Submit".
➢ Step 2. Click on the "PTM SITE" circle.
➢ Step 3. Open "information.txt" from your sample data set.
➢ Step 4. Type "Solanum tuberosum" in the organism box and click when the right organism '4113' popped up.

- Step 5. Select the modification type. Filter by typing in "phosphor" and scroll down, and then check "phosphoserine, phosphothreonine, phosphotyrosine".
- Step 6. Type in the rest of the information according to "information.txt" file. Click "submit".
- Step 7. Select "share within group", and click on "create group".
- Step 8. Type in "temp group" in the "name", and leave the "size" blank. Click on "create".
- Step 9. Go to "share within group" again. Choose the "temp group" you just created. Click "submit".
- Step 10. Click "select files", and select the "peptides12714601.txt" from your sample data set. Click "upload". When it is done, click "define columns".
- Step 11. This is to define each column. Select "Protein ID" "peptide" "m/z" "Charge" from the dropdown list accordingly. Notice that the columns "First row" is showing the title row in the sample file, and "second row" is showing the sample data from your file, which helps you to identify the meaning of each column for the sample data. Choose "uniprot" as the ID type, and then click "submit".
- Step 12. Click "select files", and choose "Solanum_tuberosum.fasta" from your sample data set. Click "upload". When it is done, click "view".
- Step 13. Rename your website as "my temp data site", and click "submit".
- Step 14. Click the link that is created by the workflow.
- Step 15. You may view the data accordingly as in P3DB main site.
- Step 16. Go to http://p3db.org and click on your name on the right up corner, go to your user account.
- Step 17. Click "my website" on the left menu list.
- Step 18. You may view data, update information.
- Step 19. Click circle in the "delete" column to make the dataset selected, and then click "delete" button.

5. Help

5.1 Submit your questions from menu "Information->Help".

5.2 Submit your suggestion from menu "Information->contribute", and you may either suggest us to include the new data set available online, or any of your own data.

5.3 You may leave feedback in either 5.1 or 5.2.

5.4 More questions about this manual, please contact Qiuming Yao: qywt5@mail.mizzou.edu