PEPTIDE DEPOT

SOFTWARE USER MANUAL

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Introduction to Peptide Depot

What is Peptide Depot

Peptide Depot is a custom FileMaker inteface that provides a central location for phosphoproteomic peptide data management and analysis. Peptide Depot organizes peptide records by experiments and collections and provides an interface to easily browse through experiments. This software also offers an interface to manage and analyze comparisons between datasets such as timecourses or wild-type/mutant comparisons. Once viewing an experiment or comparison, many tools such as spectra viewers, protein network maps, and condensed timecourse data heatmaps can greatly enhance phosphoproteomic analysis.

What You'll Learn

This manual will show you how to quickly set up Peptide Depot in your lab and will guide you through all the functionalities available in different layouts of the software.

Setting Up Peptide Depot in Your Lab

1. Install FileMaker server 9 or higher on your computer .
   (If you don't have a copy, you need to purchase it from FileMaker corp.)

2. Unzip the downloaded file to any folder.

3. After installation of FileMaker server, copy all database files (.fp7) in the FileMaker folder to FileMaker server's data folder (PC Default: c:\program files\FileMaker server\data\).

4. Open FileMaker admin console and enable those files.

5. To verify FileMaker server and copied files, open FileMaker pro 9 or higher (if you don't have a copy, you need to purchase it from FileMaker corp.) and in open remote menu, connect to your FileMaker server's address and select to open 'peptide depot.'

6. You shall be asked for username and password. Use admin and default password: brownuniv
   (Since this username and password is used to open all related databases, you may change password for all databases if you change one).

7. if server is OK, you shall be able to open the database and explore data in FileMaker.

8. Go to the installer folder, install MySQL and mysql_connector_odbc.

9. Two types of MySQL database are provided in zip file under folder "MySQL DB": in sql format and folder dump. Both contains three sample data set. For sql, you can execute in terminal via command: mysql -h hostname -u username -p < ***your_path_to_the_sql***.sql; For the folder type, copy it to mysql's data folder and restart mysql server, you may receive errors but it should work.

10. You will also need to setup a system DSN for mysql database. Go to administrator tools and open "data sources (ODBC)" for management.

11. Go to system DSN panel and click Add New. Choose "MySQL ODBC 3.51 driver" (3.51.14) as your driver.

12. Configure DSN according to your mysql server info. Choose 'sequest' as the database

13. You are almost all set! Open 'peptide depot' in FileMaker and go to menu "file->manage->external data sources."

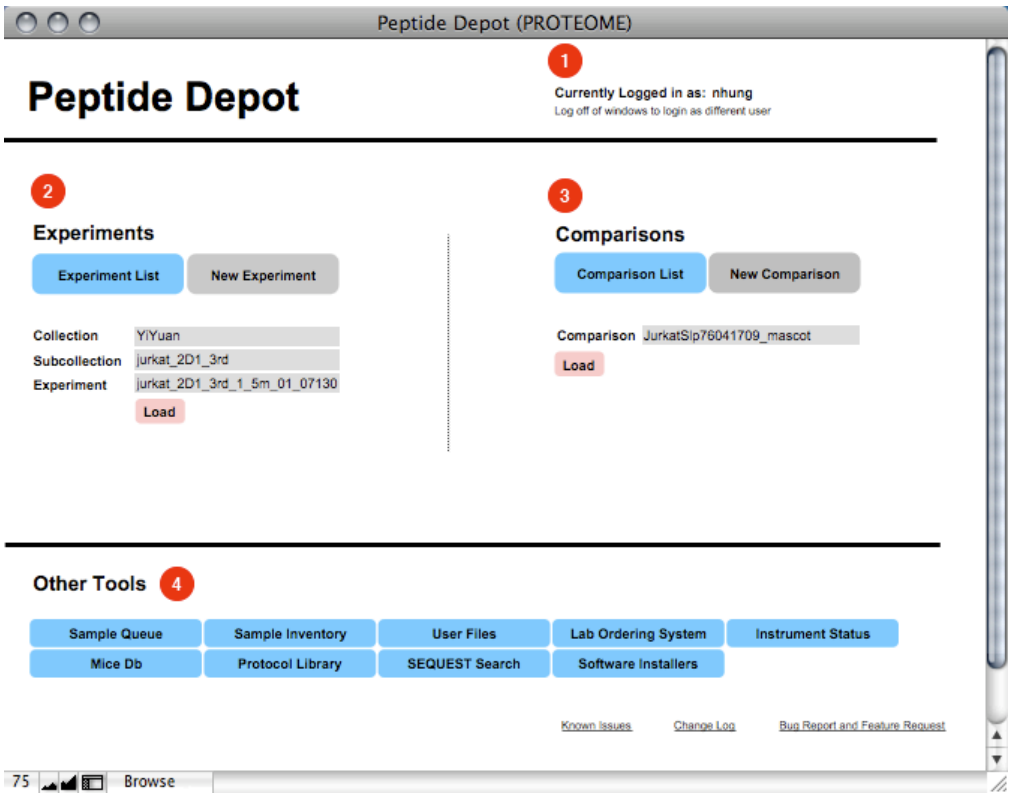
14. Select "Peptide_Depot_SQL" and type in username as password (this is optional. if password is not set, FileMaker will ask you password every time you login to this database)

15. Now your FileMaker and MySQL database should be all linked up. Try it out and tell us what you think!

Navigating Peptide Depot

Peptide Depot Home Page

This page is this first page you will see when you open up peptide depot. It acts as a portal to other views in the database. From here, you can access all experiments and experiment comparisons in the database. As analysis of experiments and comparisons is the main functionality of this software, this is probably what the homepage will be used for most of the time. However, buttons at the bottom will lead you to other features of the software.

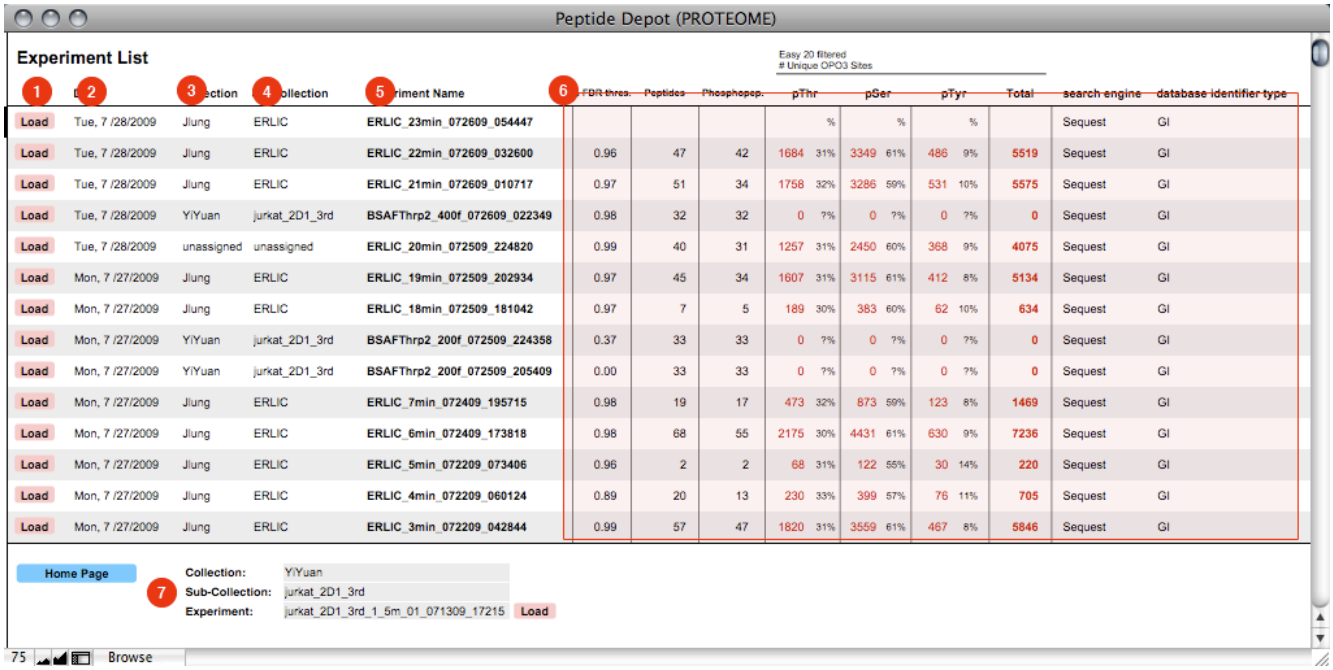


Category	Tool	Description
Experiments	Experiment List	*Shows a list of all experiments with summaries*
	New Experiment	*Switches to sample queue view to make new experiments*
	Experiment Loader	*Load an experiment directly by selecting an experiment from a drop down menu*
Comparisons	Comparison List	*Shows a list of all comparisons with summaries*
	New Comparison	*Allows user to make a new comparison*

	Comparison Loader	*Load a comparison directly*
Other Tools	Sample Queue	
	Mice DB	
	Sample Inventory	*Tells you where things are.*
	Protocol Library	
	User Files	
	SEQUEST Search	
	Lab Ordering System	*Doesn't work outside of Salomon Lab*
	Software Installer	*Doesn't work outside of Salomon Lab*
Misc.	User	*Shows currently logged in user*
	Known Issues	
	Change Log	
	Bug Report and Feature Request	

Experiment List

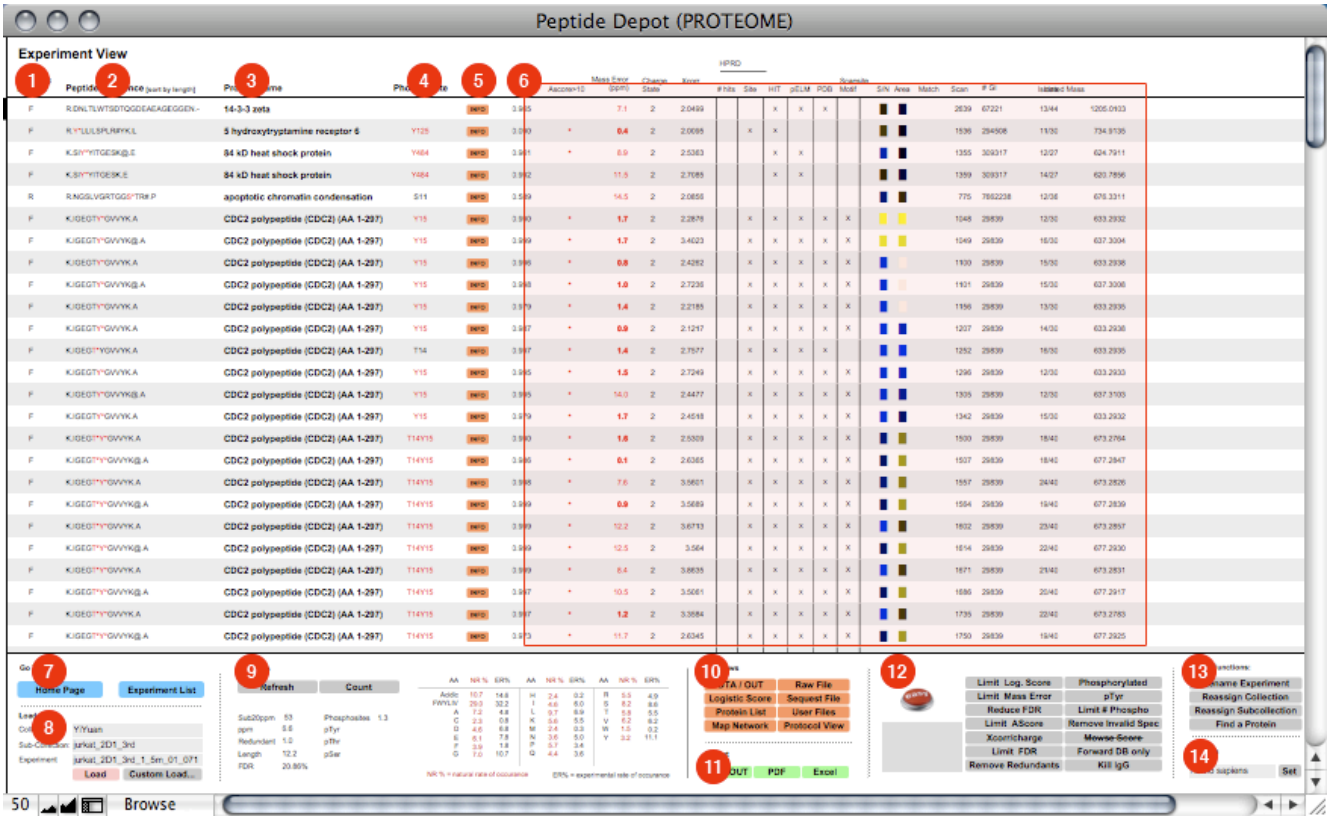
This view lists all of your experiments and relevant summary information about each experiment. To provide a more realistic picture of your experiment, the summaries only take into account proteins that survive a 1% FDR threshold. In addition, you can arrange experiments by name, date, or collection by clicking on their respective labels at the top.



Experiment View

This layout offers a view of all the peptides found in a experiment. In addition to the peptide itself, the software automatically determines a best-fit protein for each peptide as shown in the protein name – the associated protein can easily be changed by clicking on the protein name. Details and statistics on each peptide are also displayed.

Filtering options are available to narrow down peptides based on statistical confidence and protein relevance. In addition to the main experiment view, there are a variety of subviews available that provide either a more technical or detailed view of each peptide or additional information on the experiment. For example, to learn more about a particular peptide, you can click on the info button next to that peptide, which will show you existing online knowledge on that particular protein. When the experiment is ready for publication, multiple export options are offered.



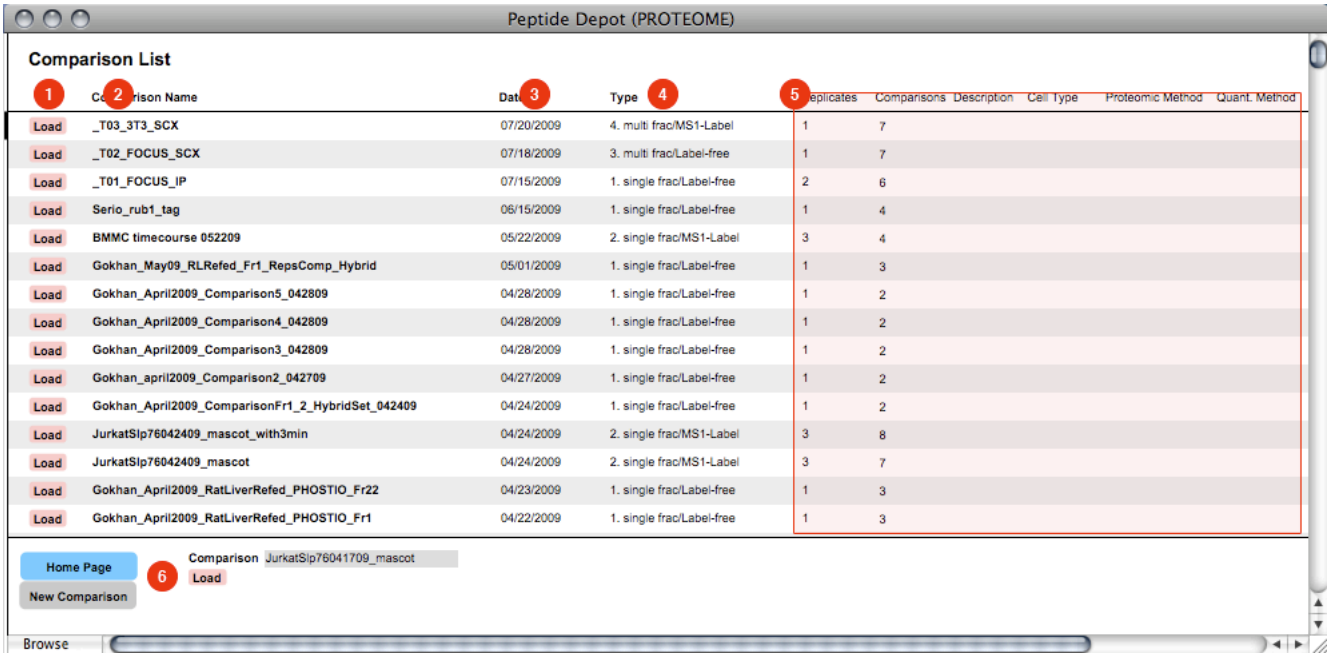
Category	Tool	Description
Main Table	Decoy DB Direction	*Describes whether peptide is a forward or reverse hit in decoy database.*
	Peptide Sequence	*Displays the peptide sequence of the peptide record. Phosphorylated sites are shown in red.* ***Click to view the peptide spectra.***
	Phosphosite	*Displays the phosphorylated amino acid(s) in the peptide. Phosphosites in red denote pTyr containing sites.*

	Protein Name	*Displays the chosen protein name. Hover over to choose other protein names.*
	Additional Names	*Shows other possible protein names for the given peptide.*
	Info Button	*Click to show online database information on the current peptide record. (e.g., HRPRD, NCBI, OMIM, Pubmed, Wikipedia)*
Go To	Home Page	*Brings you to the home page*
	Exp. List	*Shows a list of all experiments with summaries*
	Collection	*Use the drop-down menu to pick a collection of experiments*
	Sub-Collection	*Use the drop-down menu to pick a subcollection of experiments*
	Experiment	*Use the drop-down menu to pick an experiment*
	Load	*Loads the selected experiment*
	Custom Load…	*Use this to load records from more than one experiment. In order for this to work, records need to be from experiments with a matching unique keyword in their names.*
Subviews	DTA/OUT	*DTA = raw MS/MS Spectra file. OUT = database search output (mascot or sequest)*
	Logistic Score	*Probility score indicates the validity of peptide sequence identifications*
	Protein List	*Shows all proteins captured by the dataset*
	Map Network	*Move button to Comparison View*
	User Files	*Opens the user folder.*
	Protocols	*This view allows the user to view and edit protocols used to create this dataset.*
Export	DTA/OUT	*Exports the DTA/OUT*
	PDF	*Exports a PDF of all the records including heatmaps*
	Excel	*Exports all numerical and metadata as an excel database*
	Raw File	*Opens the raw data file for the entire experiment*
	Sequest File	*Opens a Zip file containing all database search output*
Filters	Autofilter	*Combination of most commonly used filters (limit mass error, remove redundant, kill IgG, etc)*
	Limit Log. Score	
	Limit Mass Error	
	Reduce FDR	*Many filters (remove unnamed proteins, Ig's) that help reduce the FDR*
	Limit Ascore	
	Xcorr/charge	*Custom Value*
	Limit FDR	
	Redundinator	*Removes all redundant peptides.*
	Phosphorylated	*Filters out all records with unphosphorylated peptides.*
	pTyr	*Filters out all records without phosphotyrosine.*
	Limit # Phospho	*Limits no. phosphorylation sites per peptide*
	Remove Invalid Spectra	*Removes any manually invalidated spectra.*
	Mowse Score	*Custom value.*
	Forward DB only	*Removes all records that are a hit against reverse sequences in the decoy database.*

	Kill IgG	*Removes any immunoglobulin.*
Statistics	Calculate	*Calculates and refreshses the statstics for the dataset*
	Sub20ppm	*Numbers peptides with mass error < 20ppm*
	Avg. pSites/peptide	
	ppm	*Average ppm in current dataset*
	Redundant	*Number of redundant peptides*
	Length	*Average length*
	FDR	*Average FDR*
	Natural/Experimental Rate Table	*Displays the difference between the natural rate of occurance of an amino acid and the experimental rate*
Other Functions	Rename Experiment	
	Reassign Collection	
	Reassign Subcollection	
	Find a Protein	
Species	Set Species	*Assigns current dataset to a particular species. Used for protein interaction.*

Comparison List

This view lists all of your comparisons and relevant summary information about each experiment. You can arrange experiments by name, date, or type by clicking on their respective labels at the top.



Comparison View

This view is use to do any analysis on two-state comparisons. Each peptide record displays its name, phosphorylated sites, phosphoproteomic data in heatmaps display, a free-for-all notes section, and the peptide sequence. Rollover the heatmaps for more detailed information on each datapoint. Also, clicking on the heatmap squares allows you to look at or validate the corresponding spectra. The comparison view offers various statistical and biological filtering tools to help clean up the data set. Peptides can be manual categorized into groups for further organization. When the data set is set for publication, several export options including pdf export for publication and raw data in excel format are available.

Category	Tool	Description
Main Table	Grouping Check Box	*Use the check box to select proteins for a new group.*
	Protein Name	*Displays the default (usually HPRD) or manually choosen protein name for the peptide record. Hover over to view other names. Click to manually edit the name.*
	Phosphosites	*Displays the phosphorylated amino acid(s) in the peptide. An asterisk defines sites that are known in the linked HPRD database. Click to manually edit the phosphorylation site.*
	Label-Free Heatmaps	*Displays Label-Free data in heatmap form. (Wild-Type data by default). Hover over to see more detailed information. Go to Reading Heatmaps section for more information.*
	SILAC Heatmaps	*Displays SILAC ratio data in heatmap form. Hover over to see more detailed information. Go to Reading Heatmaps section for more information.*
	Comments/References	*A free for all area to insert your own notes or references for each peptide. Click to edit.*
	Peptide Sequence	*Displays the peptide sequence of the peptide record. Phosphorylated sites are shown in red. Click to view the peptide spectra.*

Go To	Home Page	*Brings you to the home page.*
	Comparison List	*Shows a list of all comparisons with summaries.*
	Experiment Load	*Loads the selected experiment. Use the drop-down menu to pick an experiment.*
	Group Load	*Loads a specific group in the experiment. Use the drop-down menu to pick a group.*
Basic Functions	Rebuild Cache	*Rebuilds the heatmap cache. Use when heatmaps parameters have changed. (Caution: this script may take up to a couple of hours to complete)*
	Find a Peptide	*Search for a peptide by using keywords.*
	Make New Group	*Creates a new group from the selected peptides (use the check box to select).*
	Calculate Log Score Dist.	
Export	PDF	*Exports the records in PDF format*
	Excel	*Exports all comparison data in an excel table*
	Spectra by Counter	*Exports spectra in*
	Spectra by Name	
	Build ProteinNetVis	
	Run ProteinNetVis	
	Current DTA/OUT Set	
	All Raw Files	
SILAC Graph	SILAC Graph	
Filters	Applied Filters	*This area displays all currently applied filters*
	Reduce FDR	*Custom value.*
	Limit AScore	*Custom value.*
	Limit Logistic Score	*Custom value*
	Set Min. Timepoints	*Filters out any peptide records that have below a certain amount of timepoints. (Number of timepoints in manually set after clicking the button)*
	Show pTyr containing	*Filters out any peptide records that do not contain a phospho-tyrosine.*
Color Change Legends	LF	*Displays the heatmap color key for current label-free heatmap data.*
	SILAC	*Displays the heatmap color key for current SILAC heatmap data.*
	%CV	*Displays the color key for coefficient of variation percentage of each timepoint.*
Statistics	Proteins	*Displays number of proteins represented by the current dataset.*
	Sites	*Displays the number of phosophorylation sites represented by the current dataset.*
	% pTyr	*Displays the percentage of phosphorylation sites that are phospho-tyrosine sites represented by the current dataset.*
	pY	*Displays the number of phosopho-tyrosine sites represented by the current dataset.*

	pT	*Displays the number of phosopho-threonine sites represented by the current dataset.*
	pS	*Displays the number of phosopho-serine sites represented by the current dataset.*
	Count	*Counts and refreshes the statistics based on the current data set.*