## Corra v2.0 User's Guide



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### **BMC Bioinformatics**

Methodology article



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Corra: Computational framework and tools for LC-MS discovery and targeted mass spectrometry-based proteomics

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Corra is an open source software Licensed under the Apache License, Version 2.0 and it's source code , demo data and this guide can be downloaded at the <a href="http://tools.proteomecenter.org/Corra/corra.html">http://tools.proteomecenter.org/Corra/corra.html</a>.

This user guide is written by Micheleen Harris (<u>mharris@systemsbiology.org</u>) and Mi-Youn Brusniak (<u>mbrusniak@systemsbiology.org</u>)

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#### 1. Introduction

Corra is a single, user-friendly, informatic framework, that is simple to use and fully customizable, for the enabling of LC-MS-based quantitative proteomic workflows of any size, able to guide the user seamlessly from MS data generation, through data processing, visualization, and statistical analysis steps, to the identification of differentially abundant or expressed candidate features for prioritized targeted identification by subsequent MS/MS. In the first published version of Corra software with the paper was v 1.5 in 2008 and since then, there were more update in the pipeline. In detail, Corra v1.5 pipeline ended by generating target list from statistical analysis. Corra v2.0 added additional feature extracting alignment tools as well as customized target list generation and annotation step using target LS-MS run. This guide uses the yeast gene knock out example used in Corra paper to illustrate the step of using v2.0 extended pipeline steps.

#### 1.1 Login

Website: Ask administer in your institution which server the Corra is deployed to and ask Corra admin to add your account. For this guide, we will use guest account.

The URL should be something like the following.

http://corrademo.systemsbiology.net

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1.2 Once logged in, click "New" to create a new project and give it a name (here it is "CourseExample").



1.3 Choose the instrument type under drop down menu "MS Instrument"

1.4 Adding Data. Your data must be in mzXML format (if not, there are several converters from RAW data to mzXML, such as ReAdW and mzWiff). Click "Add" next to mzXML files to add mzXML formatted data to the project (required before you save the project). Select the mzXML files from the drop-down menu with which you want to run Corra (you can hold down the Shift key to select a group of files). Then press "Save" and reopen Project Setup by pressing "Edit."



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Check the files to label and use the dropdown menu to select the condition label

appropriate for this group of files.

1.5 Defining Conditions, Sample IDs, Replicates and Time points. Make sure you have clicked "Edit" to continue setting up the project.

Click on "Condition\_1" or "Condition\_2" to rename these labels. If you wish to add any more conditions, click "Add."

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Each group of files (e.g. replicates belonging to a particular biological group) should share the same "Sample ID." Assign a numerical ID by clicking on a number in the "Sample ID" column as shown left side.

Sample Information:

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Define replicates using the drop-down menu in the "MS Replicates" column. If you have more than 3 replicates increase the replicate count by clicking on the number next to "Max. Replicate Count." If you have more than one defined time point, add it by clicking the "Add" button next to "Time1" and rename by replacing "t\_1" or "t\_2" etc. Then specify them in the "Time Point" column using the drop-down menu.

#### Don't forget to Save your work!

Alternatively, you could setup the project by importing a "Sample Information File." This is useful if, say, you have a similar project with many mzXML files, as entering all of the setup information by hand could be a rather long process or you can use "Copy" project option which will create a new project with current project setup page. This "Copy" option can be used to analyze data using alternative Corra pipeline options.

2. Feature Picking

2.1 Click on "Feature Picking", then "Edit"

2.2 Program for feature picking

Select the desired program (e.g. SpecArray is used for TOF-MS data and SuperHirn /msBID for FT-MS/Orbitrap)



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For the Feature Picking step it might be useful to view the mzxml file(s) data. Using Pep3D, a .png (image) file can be created and viewed in a generic graphic viewer. For example, using the SuperHirn program, the elution window is set by default to begin at 12 and end at 87 minutes. Viewing the mzxml file in a program like Pep3D can help you decide if you wish to exclude (or include) parts of the experiment based on how the elution profile looks (Pep3D is a viewer of LC-MS or LC-MS/MS data in a general 2D "gel-like" format).

2.3 Set "Parameters" or import a parameter file

2.4 Click "Run Feature Picking"

**Note**: Text in **yellow** indicates a process that is currently running and text in **green** is a process which has completed successfully. Text in **red** indicates an error has occurred and Corra log files may be referenced for further information.

When Feature Picking is done, you can scroll down to view the resulting feature counts for each input file.

Note: The "FT peak detect MS1 intensity min threshold" could be increased in the case where there are too many features and/or you desire the subsequent runs to be faster (adjust the parameter and rerun Feature Picking).



Here is a picture of the result of the feature counting:

Note: These pictures can be downloaded as a .pdf file through the link below this graph.

#### 3. Alignment

3.1 Click on the next step, "Alignment." Click "Edit" to setup the Alignment parameters.

3.2 Select a program (this should correspond to the program selected during Feature Picking)

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#### 3.3 Parameters

Adjust parameters to meet the specifications of your analysis and then click "Run Alignment."

**Note:** It might be a good idea to start with a value of 5 for the MS1 retention time tolerance.

#### 3.4 Alignment Results



An APML (Annotated Putative Peptide Markup Language) file is created and maybe downloaded (by clicking the APML link in red) and viewed in an APML viewer comes with Corra (

# <u>http://sourceforge.net/projects/corra/files/Corra-APML/APMLv2.0.1/APMLv2.0.1.tgz/download</u>). This will help the user to view in a graphical way, the amount of aligned features. See next section for details about the APML Viewer.

#### 3.4.1 APML Viewer

Open an .apml file in the viewer to see the aligned features in a m/z vs. Tr plot.

Try this: In the "Plotting Tool Bar", go to "Selected Plot View" -> "Times Aligned View" and click on a point in the graph to get a dialog box which shows the aligned features for that point (in this case there are three features aligning):



Click on "Statistical Analysis" and "Edit." The program in use is a collection of R modules called CorraStatistics.R. Set the "B-Statistics Cutoff" (B = -[ log odds ratio]) or use the default of 2.2. Here we change it to 0. Usually, having a "N/A Replace Method" of "none" is satisfactory. The "N/A Replace Method" is to be used if you wish to fill in missing features with a value, either a minimum value or userdefined value. Use the drop-down menu to select the type of N/A Replace Method to use.

Select the comparisons to be calculated (red circle on figure below). Save the setup and the Statistics step will begin.

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#### 4.2 Results

The result of the statistical analysis is displayed in two plots, a volcano plot and hierarchical cluster (unsupervised) as shown below. The red dots are features which are found in some of the samples, but not all and have a Log Odds ratio greater than the value set in the "B-Statistics Cutoff" field (I set it to 0, here, but the default is 2.2). The blue dots represent features that were found in all of the samples with a Log Odds greater than the default or user defined limit. A Log Fold Change which is negative, indicates that a feature is more abundant in Condition 2 (here, WildType) whereas a positive Log Fold Change indicates that the feature is more abundant in Condition 1 (here, DeletionStrain).

#### The Volcano plot:

#### The Hierarchical cluster tree:



A tab delimited file (.tsv) is created for the aligned features and can be downloaded by clicking "Differentially Expressed Feature List (.tsv)" link below the Volcano Plot. The data contained in the .tsv file comes from an analysis using CorraStatistics.R (Bioconductor) as a backend. It is used as input in the following step, "Inclusion List Builder."

The "Differentially Expressed Feature List (.tsv)" file is shown below, opened in MS Excel. Note in addition to the data there is statistical information such as logFC (log fold change), p-value and B value.

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	15	904.0772	52	3	0			30.89987	31.00546	31.2237	27.81261	27.64044	27.71927	3.318903	15.46571	3.25E-06	0.004356	5.336085	- 1
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Go to the "Inclusion List Builder" section. (Note: Inclusion List Builder depends upon the Statistical Analysis step which must be completed successfully).

Click "Create Inclusion List" and give it a name.

Click "F" to add a filter and "Add Filter." Select a type of filter by clicking on the "+" sign (circled in blue in next figure) and using the drop down menu. To delete this filter click on the "-" sign (circled in orange).

A "#LC aligned" filter might be useful if you wish to focus on the number of features aligned across all samples a certain amount of times.

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Projects       Stratter Deknig         Feature Picking       Alignment         Statistical Analysis       Inclusion List Builder         Target Feature Annotation       Step 4:         Inclusion List Builder       Target Feature Annotation         Statistical Analysis       Inclusion List Builder         Target Feature Annotation       Step 4:         Inclusion List Builder       Target Feature Annotation         Statistical Analysis       Inclusion Lists:         Source Feature Vicking       Conceate Inclusion Lists:         Source Feature List:       Source Feature Strain & Wild Type         Statistical Analysis       Inclusion Lists:         Source Feature List:       Source Feature List:         Source Feature List:       Source Feature List:         Source Feature List:       Feature Annotation         I ListA       DeletionStrain & Wild Type         Conceate Inclusion List:       Interview         I ListA       DeletionStrain & Feature Source         Statistical Analysis       Interview         I ListA       DeletionStrain & Vild Type         Conceate Inclusion List:       Interview         I ListA       DeletionStrain & Vild Type         Conceate Inclusion ListA       Vild Type <t< th=""><th></th><th></th><th>DISCOVERY AND</th><th>TARGETED ANALY</th><th><b>*5#5</b></th><th></th><th></th><th></th><th>a/z</th><th></th><th>me (min)</th></t<>			DISCOVERY AND	TARGETED ANALY	<b>*5#5</b>				a/z		me (min)
CourseExample Date: 119/2009 Project: CourseExample Date: 119/2009 Project Setup  Feature Picking Alignment Statistical Analysis Enclusion List Builder Target Feature Annotation Step 4: Inclusion List Builder Inclusion List Builder Inclusion List Source Feature List: DefetionStrain & WidType I I List DefetionStrain & E S D  Project: CourseExample Date: 119/2009 Project Setup  Feature Statistical Analysis Inclusion List Builder Inclusion List Inclusion List Inclusion List Inclusion List Enclusion List I I List DefetionStrain & E S D  Project: CourseExample Date: 119/2009 IIIIII IIIII IIIII IIIII IIIII IIIIII IIII	Projects	Save Cance	el								
Step 4: Inclusion List Builder Taget Feature Annotation Taget Feature Annotation Taget Feature Annotation Taget Feature List: Source Feature List: Deletion/Strain & Wild/Type Create Inclusion List:  ApML About Correa Taget Taget Feature List Taget Feature List: Deletion/Strain & Wild/Type Teature List: Deletion/Strain & Wild/Teature List: Deleti	C CourseExample Feature Picking Alignment	Project: Course Date: 11/9/2009 Project Setup ■ F	Example	Alignment 🔳 S	tatistical Analys	is 🔳 Inclu	usion List	Builde	r 🔳 Targe	t Feature Ann	otation
Inclusion Lists:       Target Feature Annotation       Target Feature Annotation     Source     Feature & Total     Feature & min     max     min     min     max     min     max     min     max     min     min     max     min     min <td>Statistical Analysis</td> <td>Step 4:</td> <td></td> <td>Inclusion List</td> <td>Builder</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>	Statistical Analysis	Step 4:		Inclusion List	Builder						
Arget Feature Annotation     Source Feature List:     DeletionStrain & WidType x     Oranne Inclusion List:       # Name     Source     Total     Filtered     min     max     min     max     mean     intensity     <	Inclusion List Builder				Inclusion	Lists:					
APML. Corra v2.0	rarget Feature Annotation	Source Feature Li	st: DeletionStra	ain & WildType [	Create Inclu	usion List	1				
APML About Corra telp Corra v 2.0		# Name	Source	Total Features	Filtered Features	min m/z	max m/z	min RT	max RT	mean intensity	
APML About Corra Help Sorra v.2.0		1 ListA Wild	Туре								ESD
APML About Corra Help Corra v20											
About Corra Help Corra v.20	APML										
Help Corra v.2.0	About Corra										
	Help										
	Corra V.2.0										

A "Mean Intensity" filter might be useful when features with low intensities wish to be excluded (Note: mean intensity is the log<sub>2</sub> Intensity of a peak).



#	Name	Source	Total Features	Filtered Features	min m/z	max m/z	min RT	max RT	mean intensity			
1	ListA	DeletionStrain & WildType	699	100	327.01	1810.57	17.46	85.52	26.81	E	<u>s</u>	D
	AND Charge	Negate:     Filter Min: 3	•	Max: 6			Negate	:				
	AND	Negate:	+	Max: 4			Negate	-				
T	otal Feat Add Filt	ures: 699 Filtered Fea er Save Inclusion L	tures: 100 isti Downlo	ad List								

Hit "Save Inclusion List" and it will show how many total features and filtered features there are, plus some information such as min and max m/z (filters may be applied to limit these as well). Press "F" again to close the filter menu.

#	Name	Source	Total Feature	s Fe	iltered atures	min m/z	max m/z	min RT	max RT	mean intensity	
1	ListA	DeletionStrain & WildType	699	100		327.01	1810.57	17.46	85.52	26.81	<u>s</u>
5	Segment	Length	5.0		Segmen	t Overlap		2.5			
\$	Segment	Delay	0.1								
F	First Seg	ment Start	0.0		First Seg	gment End	I	14.0			
	/lin. Feat	ures per Segment	1		Max. Fe	atures per	Segment	250			
	Create \$	Segments View	/ Segment Sum	mary	Export to E	Excalibur					

Click the red "S" to modify the segment settings. Segments can be useful when using the Thermofinnagin machine as these can be programmed into a target run. They can

allow the machine to focus on certain parts of the run and not focus on others.

Segment Length is the "window" so to speak (minutes). The segment overlap is how many minutes one wishes to expand the window before and after the segment.

"First Segment Start" is usually just zero, but "First Segment End" is important as this first segment might capture parts of the run (usually at the beginning) where nothing very informative is happening. The "Min. Features per Segment" and "Max. Features per Segment" might be useful to play around with if there are too many features or too few.

Click "Create Segments."

To view the result of segmentation click on "View Segment Summary" and something like this should be displayed:

	ListA	WildType	699	100	327.01	1810.57	17.46	85.52	26.81	E	<u>s</u>			
S	egment	Length	5.0		Segment Overla	ар	2.5	2.5						
Segment Delay			0.1											
F	irst Segr	ment Start	0.0		First Segment I	End	14.0							
Min. Features per Segment			1		Max. Features	per Segme	nt 250							
10	) Featu	ires Begin Segri	ent End	Segment Seg	ment Size Ove	erlap Prev	ious C	verlap N	lext					
1.	0	0	14	14	2.5		2.	5						
2.	1	14	19	5	2.40	000009536	74316 2.	5						
3.	3	19	24	5	2.40	000009536	74316 2	5						
4.	4	24	29	5	2.400000953674316 2.5									
5.	6	29	34	5	2.400000953674316 2.5									
6.	10	34	39	5	2.40	2.400000953674316 2.5								
7.	9	39	44	5	2.400000953674316 2.5									
8.	10	44	49	5	2.40	000009536	74316 2.							
9.	8	49	54	5	2.40	000009536	74316 2.	5						
1(	). 11	54	59	5	2.40	000009536	74316 2.	5						
11	1. 5	59	64	5	2.40	000009536	74316 2							
12	2.9	64	69	5	2.40	74316 2.	4316 2.5							
13	3. 4	69	74	5	2.40	000009536	74316 2	5						
14	1.8	74	79	5	2.40	000009536	74316 2.	5						
15	5. 9	79	84	5	2.40	000009536	3674316 2.5							
16	5.3	84	89	5	2.40	000009536	74316 2.	5						
n	clusion L Segmer Create S	ist Name: ListA, N it: 11, Avg. Feature Segments Hide	lum. Segm es Per Seg e Segment	ents: 16, Num. ment: 6 Summary E	Features Covere xport to Excalib	d: 100, Min ur	Feature	s In Segr	nent: 0, Ma:	x Featu	ires			

In this case I only had 100 features to begin with so there are very few features in my segments so I might try to increase my segment length.

You may save list inclusion list by clicking "Export to Excalibur."

#### 6. Target Feature Annotation

This module is to be used after MS/MS identification of peptide fragments to add sequence (and other protein descriptions) annotations to the original sample spectra, beginning the process of identifying proteins of interest. These could be the focus of future DDA or SRM analyses.

Target Feature Annotation annotates the statistical analysis output data (volcano plot data) based on the m/z values in a *.pep.xls* input file (provided by user).

6.1 Add an input file *somefile*.pep.xls

Note: This input file can be created using a pepxml viewer to convert a *.pep.xml* file to *.pep.xls* (e.g. PepXML Viewer – part of the TPP, see tutorial at <a href="http://tools.proteomecenter.org/wiki/index.php?title=TPP\_Tutorial#PepXML\_Viewer">http://tools.proteomecenter.org/wiki/index.php?title=TPP\_Tutorial#PepXML\_Viewer</a>).

This is a screenshot from PepXMLViewer (uses PeptideProphet analysis):

S	ummary D	isplay Options Pic	ck C	olumns	Filtering Options Other Actions	[Hide options]		A			
se Ify P P P h h S S S S S S S S S S S S S S S	Summary       Display Options       Pick Columns       Filtering Options       Other Actions       [Hide options]         select the columns you want to display.       If you want to change the order that the columns are displayed, highlight the column and use the up / down buttons.       undisplayed columns:       columns to display.         undisplayed columns:       columns to display.       index       index										
Pa	Page 1 of 23										
1	1 FIRST 1 2 3 4 5 6 11 NEXT LAST										
4	MZRATIO	RETENTION_TIME_SEC	2	PROB	SPECTRUM	PEFTIDE	PROTEIN	PROTEIN_DESCR			
	MZRATIO 2 570.2882	RETENTION_TIME_SEC 1633.37	2 2	PROB	SPECTRUM OrbiDeletionStrain_1.00810.00810.2 <sup>St</sup>	peptide R.VHGEEDPTKK <sup>P</sup> A	PROTEIN YFL014W +1	PROTEIN_DESCR [YFL014W;UPSP:HSP12_YEAST;GENSCAN0000000			
	MZRATIO 2 570.2882 3 941.3402	RETENTION_TIME_SEC 1633.37 1638	2 2 2	PROB 0.9051 1.0000	SFECTROM OrbiDeletionStrain_1.00810.00810.2 <sup>ST</sup> OrbiDeletionStrain_1.00814.00814.2 <sup>ST</sup>	refide R.VHGEEDPIKK <sup>R</sup> F.QNEGHEC160.16QC160.16QC160.16GSC160.16K.B <sup>R</sup>	PROTEIN <u>YFL014W +1</u> <u>YHR053C +2</u>	PROTEIN_DESCR [YFL014W;UPSP:HSP12_YEAST;GENSCAN0000000 [YHR053C;YHR055C;UPSP:MTCU_YEAST;gij632184			
	MZRATIO 2 570.2882 3 941.3402 4 712.8362	RETENTION_TIME_SEC 1633.37 1638 1682.36	2 2 2 2	PROB 0.9051 1.0000 0.9885	SPECTROM           OrbiDeletionStrain_1.00810.00810.2 <sup>ST</sup> OrbiDeletionStrain_1.00814.00814.2 <sup>ST</sup> OrbiDeletionStrain_1.00852.00852.2 <sup>ST</sup>	PEPTIDE R. VHOEEDPIKK <sup>1</sup> / <sub>1</sub> F. OMEGRECIED. 16QC160.16GSC160.16K.N <sup>R</sup> K. FYEEDEKESGR.1 <sup>R</sup>	PROTEIN           YFL014W +1           YHR053C +2           YAL005C +5	PROTEIN_DESCR [YFL014W;UPSP:HSP12_YEAST;GENSCAN0000000 [YHR053C;YHR055C;UPSP:MTCU_YEAST;gil63218- [YAL005C;GENSCAN0000000437;GENEFINDER00			
	MZRATIO 2 570.2882 3 941.3402 4 712.8362 5 560.7489	RETENTION TIME SEC 1633.37 1638 1682.36 1687.16	2 2 2 2 2 2	PROB 0.9051 1.0000 0.9885 1.0000	SPECTROM           OrbiDeletionStrain_1.00810.00810.2 <sup>ST</sup> OrbiDeletionStrain_1.00814.00814.2 <sup>ST</sup> OrbiDeletionStrain_1.00852.00852.2 <sup>ST</sup> OrbiDeletionStrain_1.00856.00856.2 <sup>ST</sup>	PEPTIDE R. VHGEEDPIKK Å F. ONEGRECIED. 16QC160.16GSC160.16K.NÅ K. FREEDEKESGR. IÅ R. YAGEVSHDOK.HÅ	PROTEIN           YFL014W +1           YHR053C +2           YAL005C +5           YGR192C +6	PROTEIN_DESCR NFL014W;UPSP:HSP12_YEAST;GENSCAN0000000 NHR053C;YHR055C;UPSP:MTCU_YEAST;gil63218- [YAL005C;GENSCAN0000000437;GENEFINDER00 NGR192C;GENSCAN00000002700;GENEFINDER00			
	MZRATIO           2         570.2882           3         941.3402           4         712.8362           5         560.7489           6         618.2842	RETENTION_TIME_SEC 1633.37 1638 1682.36 1687.16 1723.13	2 2 2 2 2 2 2 2	PROB 0.9051 1.0000 0.9885 1.0000 0.9853	SECCENCM           OrbiDeletionStrain_1.00810.00810.2 <sup>ST</sup> OrbiDeletionStrain_1.00814.00814.2 <sup>ST</sup> OrbiDeletionStrain_1.00852.00852.2 <sup>ST</sup> OrbiDeletionStrain_1.00856.00856.2 <sup>ST</sup> OrbiDeletionStrain_1.00888.00888.2 <sup>ST</sup>	PEPTIDE R. VHGEEDPIKK R F. QNEGHECIeo.leQCieo.leQCieo.leGSCieo.leK.N <sup>R</sup> K. FREEDEKESQR.I <sup>R</sup> R. SRGESDDSLNR.L <sup>R</sup> R. SRGESDDSLNR.L <sup>R</sup>	PROTEIN           YFL014W +1           YHR053C +2           YAL005C +5           YGR192C +6           YJL136C +4	PROTEIN_DESCR VFL014W,UPSP:HSP12_YEAST;GENSCAN0000000 (VHR053C;VHR055C;UPSP:MTCU_YEAST;gl63218/ (VAL005C;GENSCAN0000000437;GENEFINDER00 (YGR192C;GENSCAN00000002700;GENEFINDER01 (VJL136C;UPSP:RS21B_YEAST;gl6322325[ref]NP_C			
	MZRATIO           2         570.2882           3         941.3402           4         712.8362           5         560.7489           6         618.2842           7         932.8249	RETENTION TIME_SEC 1633.37 1638 1682.36 1687.16 1723.13 1729.63	2 2 2 2 2 2 2 2 2 2 2	PROB           0.9051           1.0000           0.9885           1.0000           0.9853           1.0000	SPECTNM           OrbiDeletionStrain_1.00810.00810.2 <sup>ST</sup> OrbiDeletionStrain_1.00814.00814.2 <sup>ST</sup> OrbiDeletionStrain_1.00852.00852.2 <sup>ST</sup> OrbiDeletionStrain_1.00855.00856.2 <sup>ST</sup> OrbiDeletionStrain_1.00888.00888.2 <sup>ST</sup> OrbiDeletionStrain_1.00888.00888.2 <sup>ST</sup> OrbiDeletionStrain_1.00888.00888.2 <sup>ST</sup>	PEFTIDE R.VHGEEDPTKKÅ F.QNEGHEC160.16QC160.16GSC160.16K.NÅ K.YKEEDEKESQR.TÅ R.YAGEVSNDDK.HÅ R.SRGESDDSLIR.LÅ F.Q111.10NEGHEC160.16QC160.16QC160.16GSC160.16K.NÅ	PROTEIN           YFL014W +1           YHR053C +2           YAL005C +5           YGR192C +6           YJL136C +4           YHR053C +2	PROTEIN_DESCR (YFL014W;UPSP:HSP12_YEAST;GENSCAN0000000 (YHR053C;YHR055C;UPSP:MTCU_YEAST;gil632184 /YAL095C;GENSCAN0000000437;GENEFINDER00 (YGR192C;GENSCAN000000002700;GENEFINDER01 (YJL136C;UPSP:R8218_YEAST;gil6322325[refINP_C (YHR053C;YHR055C;UPSP:MTCU_YEAST;gil632184			
	MZRATIO           570.2882           941.3402           712.8362           50.60.7489           618.2842           932.8249           908.8651	RETENTION TIME SEC 1633.37 1638 1682.36 1687.16 1723.13 1729.63 1747.26	2 2 2 2 2 2 2 2 2 2 2 2 2	PROB 0.9051 1.0000 0.9885 1.0000 0.9853 1.0000 1.0000	BFECTREM           OrbiDeletionStrain_1.00810.00810.2 <sup>SF</sup> OrbiDeletionStrain_1.00814.00814.2 <sup>SF</sup> OrbiDeletionStrain_1.00852.00852.2 <sup>SF</sup> OrbiDeletionStrain_1.00888.00888.2 <sup>SF</sup> OrbiDeletionStrain_1.00888.00884.2 <sup>SF</sup> OrbiDeletionStrain_1.00894.00894.2 <sup>SF</sup> OrbiDeletionStrain_1.00994.00894.2 <sup>SF</sup>	R.VHOEEDFIKK <sup>[]</sup> F.QHEGHECieo.ieQCieo.ieQCieo.ieGSCieo.ieK.N <sup>[]</sup> K.FKEEDEKESQR.I <sup>[]</sup> R.YAGEVSHOCK.H <sup>[]</sup> R.SRGESDDSINR.L <sup>[]</sup> F.Q111.10HERCieo.ieQCieo.ieQCieo.ieGSCieo.ieK.N <sup>[]</sup> R.N110.0HESNYINNNOCHOGR.G <sup>[]</sup>	PROTEIN           YFL014W +1           YHR053C +2           YAL005C +5           YGR192C +6           YJL136C +4           YHR053C +2           YOR204W +1	Important         Description           INFL014W.UPSP:HSP12_YEAST.GENSCAN0000000         IVHR053C;YHR055C;UPSP.MTCU_YEAST.gil63218-           IVAL005C;GENSCAN00000000437;GENEFINDER00         IVGR192C;GENSCAN00000002700;GENEFINDER00           IVAL136C;UPSP.MS21B_YEAST.gil63218-         IVHR053C;YHR055C;UPSP.MTCU_YEAST.gil63218-           IVRC92C;YHR055C;UPSP.MTCU_YEAST.gil63218-         IVRC9204W;UPSP.DED1_YEAST.gil63218-			
	MZRATIO           570.2882           941.3402           712.8362           50.7489           618.2842           932.8249           908.8651           923.4267	RETENTION TIME SEC 1633.37 1638 1682.36 1687.16 1723.13 1729.63 1747.26 1753.73	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	PROB           0.9051           1.0000           0.9885           1.0000           0.9853           1.0000           1.0000           1.0000           1.0000	BFECTREM           OrbDeletionStrain_100810.00810.2 <sup>SF</sup> OrbDeletionStrain_1.00814.00814.2 <sup>SF</sup> OrbDeletionStrain_100855.00856.2 <sup>SF</sup> OrbDeletionStrain_100858.00888.2 <sup>SF</sup> OrbDeletionStrain_100854.0084.2 <sup>SF</sup> OrbDeletionStrain_100810.00910.2 <sup>SF</sup> OrbDeletionStrain_100910.00910.2 <sup>SF</sup>	$\label{eq:refine} \hline $$ $$ $$ $$ $$ $$ $$ $$ $$ $$ $$ $$ $$	PROTEIN           YFL014W +1           YHR053C +2           YAL005C +5           YGR192C +6           YJL136C +4           YHR053C +2           YOR204W +1           YML008C +1	PROTECH_DESCR           INFL014W:UPSP:HSP12_YEAST;GENSCAN0000000           [YHR053C;YHR055C;UPSP:MTCU_YEAST;gli632184           YAL005C;GENSCAN0000000437;GENEFINDER00           YGR192C;GENSCAN00000002700;GENEFINDER00           YML05C;YHR055C;UPSP:MTCU_YEAST;gli632184           YMR053C;YHR055C;UPSP:MTCU_YEAST;gli632184           YOR204W;UPSP:DED1_YEAST;GENSCAN0000000           YML008C;GENSCAN00000003886;GENEFINDER00			
	MZRATIO           570.2882           941.3402           712.8362           560.7489           618.2842           932.8249           908.8651           923.4267           934.3937	RETENTION TIME BEC 1633.37 1638 1682.36 1687.16 1723.13 1729.63 1747.26 1753.73 1781.41	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	PROB           0.9051           1.0000           0.9885           1.0000           0.9853           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000	BFECTRIM           OrbiDeletionStrain_100810.00810.2 <sup>ST</sup> OrbiDeletionStrain_100814.00814.2 <sup>ST</sup> OrbiDeletionStrain_100852.00852.2 <sup>ST</sup> OrbiDeletionStrain_100886.0888.2 <sup>ST</sup> OrbiDeletionStrain_100884.00884.2 <sup>ST</sup> OrbiDeletionStrain_100894.00894.2 <sup>ST</sup> OrbiDeletionStrain_100910.0910.2 <sup>ST</sup> OrbiDeletionStrain_100910.0910.2 <sup>ST</sup> OrbiDeletionStrain_100910.0912.2 <sup>ST</sup>	$\label{eq:refine} \\ \begin{array}{l} \textbf{R}. \textbf{yhgeedptikk}, - \boldsymbol{\tilde{N}} \\ \textbf{F}. \underline{O} \textbf{R} C \textbf{HEC} \textbf{1} \textbf{e} \underline{O} C \textbf{1} \textbf{e} \underline{O} ( \textbf{1} \textbf{e} \underline{O} \textbf{1} \textbf{e} \textbf{0} \textbf{1} \textbf{e} \underline{O} \textbf{I} \textbf{E} \textbf{I} \textbf$	PROTEIN YFL014W ±1 YHR053C ±2 YAL005C ±5 YGR192C ±6 YJL136C ±4 YHR053C ±2 YOR204W ±1 YML008C ±1 YML008C ±1	BROTEIN         DESCR.           [NFL014W;UPSP:HSP12_YEAST;GENSCAN0000000         [NHR053C;YHR055C;UPSP:MTCU_YEAST;gil3218-           [NAL005C;GENSCAN0000000437;GENEFINDER00         [NGR192C;GENSCAN00000002700;GENEFINDER00           [YG192C;GENSCAN00000002700;GENEFINDER00         [NHR053C;YHR055C;UPSP:MTCU_YEAST;gil6322325]refINP_C           [YMR053C;YHR055C;UPSP:MTCU_YEAST;gil63218-         [YOR204W;UPSP:DED1_YEAST;GENSCAN000000]           [YML008C;GENSCAN000000368;GENEFINDER00         [YML008C;GENSCAN000000386;GENEFINDER00]           [YML008C;GENSCAN000000368;GENEFINDER00]         [YML173W;GENSCAN000000368;GENEFINDER00]			
	MZRATIO           MZRATIO           2         570.2882           3         941.3402           4         712.8362           5         560.7489           6         618.2842           932.8249         908.8651           9         923.4267           9         934.3937           1         665.8027	RETENTION TIME SEC 1633.37 1638 1682.36 1687.16 1723.13 1729.63 1747.26 1753.73 1781.41 1783.73	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	PROB           0.9051           1.0000           0.9885           1.0000           0.9853           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000	BFECTRIM           OrbiDeletionStrain_1.00810.00810.2 <sup>ST</sup> OrbiDeletionStrain_1.00814.00814.2 <sup>ST</sup> OrbiDeletionStrain_1.00852.00852.2 <sup>ST</sup> OrbiDeletionStrain_1.00886.00886.2 <sup>ST</sup> OrbiDeletionStrain_1.00884.00884.2 <sup>ST</sup> OrbiDeletionStrain_1.00894.00884.2 <sup>ST</sup> OrbiDeletionStrain_1.00910.00910.2 <sup>ST</sup> OrbiDeletionStrain_1.00916.00916.2 <sup>ST</sup> OrbiDeletionStrain_1.00944.00842.2 <sup>ST</sup> OrbiDeletionStrain_1.00944.00942.2 <sup>ST</sup>	$\label{eq:refine} \hline $$ $$ $$ $$ $$ $$ $$ $$ $$ $$ $$ $$ $$	PROTEIN YFL014W/+1 YHR053C +2 YAL005C +5 YGR192C +6 YJL136C +4 YHR053C +2 YOR204W +1 YML008C +1 YML03C +1 YML7TW +3	PROTEIN_DESCR           (YFL014W;UPSP:HSP12_YEAST;GENSCAN0000000           (YHR055C;UPSP:MTCU_YEAST;gli632184           (YAL005C;GENSCAN0000000437;GENEFINDER00           (YGR192C;GENSCAN00000002700;GENEFINDER00           (YL136C;UPSP:RS218_YEAST;gli6322325)refINP_C           (YHR055C;UPSP:MTCU_YEAST;gli632184           (YAR055C;UPSP:MTCU_YEAST;gli632184           (YAR055C;UPSP:MTCU_YEAST;gli632184           (YOR204W;UPSP:DED1_YEAST;GENSCAN0000000           (YML008C;GENSCAN0000000388;gli6323826[refINP_C           (YML030;GENSCAN0000000388;gli6323826[refINP_C           (YDR177W;UPSP:UBC1_YEAST;gli6320382[refINP_C			
	MZRATIO           MZRATIO           2         570.2882           3         941.3402           4         712.8362           5         560.7489           6         618.2842           932.8249         908.8651           923.4267         923.4267           0         934.3937           1         665.8027           2         1127.4712	RETENTION TIME SEC 1633.37 1638 1682.36 1687.16 1723.13 1729.63 1747.26 1753.73 1781.41 1783.73 1785.91	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	PROB           0.9051           1.0000           0.9885           1.0000           0.9853           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000	SFECTRIM           OrbiDeletionStrain_1.00810.02%T           OrbiDeletionStrain_1.00814.00814.2%T           OrbiDeletionStrain_1.00852.00852.2%T           OrbiDeletionStrain_1.00886.00888.2%T           OrbiDeletionStrain_1.00884.00888.2%T           OrbiDeletionStrain_1.00894.00884.2%T           OrbiDeletionStrain_1.00910.00910.2%T           OrbiDeletionStrain_1.00910.00916.2%T           OrbiDeletionStrain_1.00944.00942.2%T           OrbiDeletionStrain_1.00944.00942.2%T           OrbiDeletionStrain_1.00946.00942.2%T           OrbiDeletionStrain_1.00946.00942.2%T           OrbiDeletionStrain_1.00946.00942.2%T	$\label{eq:refine} \hline $$ $$ $$ $$ $$ $$ $$ $$ $$ $$ $$ $$ $$	PROTEIN YFL014W +1 YFL014W +1 YHR0530; +2 YGR1920; +5 YGR1920; +5 YJL1360; +4 YHR0530; +2 YOR204W +1 YML0080; +1 YMR173W +3 YMR173W; +3	PROTEIN_DESCR           INFOTEIN_DESCR           INFL014W;UPSP:HSP12_YEAST,GENSCAN0000000           INFR053C;YHR055C;UPSP:MTCU_YEAST,gil632184           INAL005C;GENSCAN0000000437;GENEFINDER00           IYG192C;GENSCAN00000002700;GENEFINDER00           IVL136C;UPSP:RS21B_YEAST,gil6322325/refINP_C           IYHR053C;YHR055C;UPSP:MTCU_YEAST,gil632184           IYOR204W;UPSP:DED1_YEAST;GENSCAN0000003886;GENEFINDER00           IYML008C;GENSCAN00000003886;GENEFINDER00           IYML103W;GENSCAN00000003886;GENEFINDER00           IYMR173W;GENSCAN0000000386;gil6323826[refINP_C           IYMR173W;GENSCAN0000000386;gil6323826[refINP_C			
	MZRATIO           MZRATIO           570.2882           941.3402           712.8362           560.7489           618.2842           932.8249           908.8651           92.34267           93.34267           93.4267           1665.8027           1127.4712           877.3722	AFTENTION TIME SEC 1633.37 1638 1682.36 1687.16 1723.13 1729.63 1747.26 1753.73 1781.41 1783.73 1785.91 1792.63	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	PROB 0.9051 1.0000 0.9885 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000	BFECTREM           OrbiDeletionStrain_100810_00810_2 <sup>SF</sup> OrbiDeletionStrain_100852_00852_2 <sup>SF</sup> OrbiDeletionStrain_100852_00852_2 <sup>SF</sup> OrbiDeletionStrain_100888_00882_2 <sup>SF</sup> OrbiDeletionStrain_100888_00882_2 <sup>SF</sup> OrbiDeletionStrain_100910_00910_2 <sup>SF</sup> OrbiDeletionStrain_100910_00910_2 <sup>SF</sup> OrbiDeletionStrain_100910_00916_2 <sup>SF</sup> OrbiDeletionStrain_100916_00942_2 <sup>SF</sup> OrbiDeletionStrain_100944_00942_2 <sup>SF</sup>	$\label{eq:refine} \\ $$ R.VHOEEDPTKK, -$$ \\ $$ R.VHOEEDPTKK, -$$ \\ $$ R.VHOEEDPTKK, $$ $$ $$ $$ $$ $$ $$ $$ $$ $$ $$ $$ $$$	FROTE IN           YFL014W +1           YHR053C +2           YAL005C +5           YGR192C +6           YJL136C +4           YHR053C +2           YOR204W +1           YMR02C +1           YMR02C +1           YMR173W +3           YMR173W +3           YMR173W +3	Importative         Description           PROTEXTY_DESCH         PROTEXTY_DESCH           IVFL014/W.UPSP-HSP12_YEAST,GENSCAN0000000         IVFR053C;YHR055C;UPSP-MTCU_YEAST,gII632184           IVAL005C;GENSCAN0000000437;GENEFINDER00         IVGR192C;GENSCAN0000000437;GENEFINDER00           IVAL136C;UPSP-MS21B_YEAST,gII63232326]refl/P_C         IVFR053C;YHR055C;UPSP-MTCU_YEAST,GENSCAN0000000           IVAL006C;GENSCAN0000003866;GENEFINDER00         IVMR053C;YHR055C;UPSP-MTCU_YEAST,GENSCAN0000000           IVML008C;GENSCAN00000003866;GENEFINDER00         IVML008C;GENSCAN00000003866;GENEFINDER00           IVML008C;GENSCAN00000003866;GENEFINDER00         IVML008C;GENSCAN00000003866;GENEFINDER00           IVML008C;GENSCAN00000003866;GENEFINDER00         IVMR173W;GENSCAN00000003866;GENEFINDER00           IVMR173W;GENSCAN00000003866;GENEFINDER01         IVMR173W;GENSCAN00000003866;GENEFINDER01           IVMR173W;GENSCAN00000003866;GENEFINDER01         IVMR173W;GENSCAN00000003866;GENEFINDER01			
	MZBATIO           570.2882           941.3402           712.8362           550.7489           560.7489           932.8249           908.8651           903.8249           903.8249           1 665.8027           1 1665.8027           2 1127.4712           3 877.3722           4 948.9063	AFTENTION TIME BEC 1633.37 1638 1682.36 1687.16 1723.13 1729.63 1747.26 1753.73 1781.41 1783.73 1785.91 1792.63 1838.32	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	PROB           0.9051           1.0000           0.9885           1.0000           0.9853           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000           1.0000	B#ECTREM           OrbiDeletionStrain_100810_00810_2 <sup>SPI</sup> OrbiDeletionStrain_100852_00852_2 <sup>SPI</sup> OrbiDeletionStrain_100856_00856_2 <sup>SPI</sup> OrbiDeletionStrain_100856_00886_2 <sup>SPI</sup> OrbiDeletionStrain_100894_00842_S <sup>SPI</sup> OrbiDeletionStrain_100916_00916_2 <sup>SPI</sup> OrbiDeletionStrain_100916_00916_2 <sup>SPI</sup> OrbiDeletionStrain_100940_0942_2 <sup>SPI</sup> OrbiDeletionStrain_100946_0946_2 <sup>SPI</sup> OrbiDeletionStrain_100946_0946_2 <sup>SPI</sup> OrbiDeletionStrain_100946_0946_2 <sup>SPI</sup> OrbiDeletionStrain_100946_0946_2 <sup>SPI</sup> OrbiDeletionStrain_100946_0986_2 <sup>SPI</sup> OrbiDeletionStrain_100946_0986_2 <sup>SPI</sup> OrbiDeletionStrain_100940_0946_2 <sup>SPI</sup> OrbiDeletionStrain_100962_00952_2 <sup>SPI</sup> OrbiDeletionStrain_100990_00990_2 <sup>SPI</sup>	PEPTIDE R. VHGEEDPTIK, $-\frac{R}{2}$ F. QHEGHECieo. 1eQCieo. 1eQCieo. 1eGSCieo. 1eK, N <sup>R</sup> K. FREEDEKESQR. I <sup>R</sup> R. YAGEVSHOCK, H <sup>R</sup> R. SRESDDSINR, L <sup>R</sup> F. Q111. 10NEGHECieo. 1eQCieo. 1eGSCieo. 1eK, N <sup>R</sup> R. N111. 0eHESUNINNIGOVHGR, G <sup>R</sup> R. KPENAETPSQTSQEATQ <sup>R</sup> N. INNINGSONNIQGDVYTK, A <sup>R</sup> K. SLOPHONITAIR. I <sup>R</sup> F. ANSINGNINGOVYTK, A <sup>R</sup> K. DIEEGTNEASSQSSINK, N <sup>R</sup>	PROTEIN           YFL014W +1           YHR053C +2           YAL005C +5           YGR192C +6           YJL136C +4           YHR053C +2           YOR204W +1           YMR03C +1           YMR173W +3           YMR173W +3           YMR173W +3           YMR173W +3           YMR173W +3           YKR006C +2	Instruct Desce           INFORTED DESCH           INFL014W:UPSP:HSP12_YEAST,GENSCAN0000000           INFR053C;YHR055C;UPSP.MTCU_YEAST,gil632184           INAL005C;GENSCAN0000000437;GENEFINDER00           ING1392C;GENSCAN00000002700;GENEFINDER00           ING1392C;GENSCAN00000002700;GENEFINDER00           INL136C;UPSP.MS21B_YEAST,gil632325[refl/P_C           INR053C;YHR055C;UPSP.MTCU_YEAST,gil6323826[refl]           INR050C;GENSCAN00000003886;GENEFINDER00           INIL088C;GENSCAN00000003886;GENEFINDER00           INR173W;GENSCAN00000003886;gil6323826[refl]           IVIR173W;GENSCAN00000003886;gil6323826[refl]           IVIR173W;GENSCAN00000003886;gil6323826[refl]           IVIR173W;GENSCAN00000003886;gil6323826[refl]           IVIR173W;GENSCAN00000003886;gil6323826[refl]           IVIR173W;GENSCAN00000003886;gil6323826[refl]           IVIR173W;GENSCAN00000003886;gil6323826[refl]           IVIR173W;GENSCAN00000003886;gil6323826[refl]           IVIR173W;GENSCAN00000003886;gil6323826[refl]           IVIR173W;GENSCAN00000003886;gil632826[refl]           IVIR173W;GENSCAN00000003886;gil6323826[refl]			
111111111111111111111111111111111111111	MZmatto           2         570.2882           3         941.3402           4         712.8362           5         560.7489           3         618.2842           932.8249         932.8249           9         934.3937           1         665.8027           2         1127.4712           3         877.3722           4         948.9063           5         855.3297	RETENTION TIME BEC 1633.37 1638 1682.36 1687.16 1723.13 1729.63 1747.26 1753.73 1781.41 1783.73 1785.91 1792.63 1838.32 1876.3	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	PROB 0.9051 1.0000 0.9885 1.0000 0.9853 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000	BFECTREM           OrbDeletionStrain_100810_00810_2 <sup>SF</sup> OrbDeletionStrain_100852_00852_2 <sup>SF</sup> OrbDeletionStrain_100856_00856_2 <sup>SF</sup> OrbDeletionStrain_100884_00884_2 <sup>SF</sup> OrbDeletionStrain_100810_00916_2 <sup>SF</sup> OrbDeletionStrain_100916_00916_2 <sup>SF</sup> OrbDeletionStrain_100916_00916_2 <sup>SF</sup> OrbDeletionStrain_100916_00946_2 <sup>SF</sup> OrbDeletionStrain_100940_00942_2 <sup>SF</sup> OrbDeletionStrain_100940_00982_2 <sup>SF</sup> OrbDeletionStrain_100920_00982_2 <sup>SF</sup> OrbDeletionStrain_100920_00982_2 <sup>SF</sup> OrbDeletionStrain_100920_00982_2 <sup>SF</sup> OrbDeletionStrain_10090_00980_2 <sup>SF</sup>	PEPTIDE R. VHGEEDETKK. $-\frac{R}{2}$ F. QHEGHECieo. 1eQCieo. 1eQCieo. 1eGSCieo. 1eK. N <sup>R</sup> / <sub>2</sub> K. FKEEDEKESQR. I <sup>R</sup> / <sub>2</sub> R. YAGEVSHDDK. H <sup>R</sup> / <sub>2</sub> F. Qili. 10NEGHECieo. 1eQCieo. 1eQCieo. 1eGSCieo. 1eK. N <sup>R</sup> / <sub>2</sub> R. NILS. 0eSISSININININGOYHGGR. $G^{R}/_{2}$ R. KPENAETPSQTSQEATQ. $-\frac{R}{2}$ M. INNINGSININGOPYTK. A <sup>R</sup> / <sub>2</sub> K. SLEDENDMITAIR, I <sup>R</sup> / <sub>2</sub> F. ANSINONINGSININGOPYTK. A <sup>R</sup> / <sub>2</sub> M. INNINGSININGOPYTK. A <sup>R</sup> / <sub>2</sub> M. DIEECTHEASSQSSINK. N <sup>R</sup> / <sub>2</sub> N. DSYGSINIDOSYGSSIK. K <sup>R</sup> / <sub>2</sub>	PROTEIN           YFL014W +1           YHR053C +2           YAL005C +5           YGR192C +6           YJL138C +4           YHR053C +2           YOR204W +1           YMR098C +1           YMR173W +3           YDR177W +3           YMR173W +3           YMR173W +3           YMR173W +2	PROTECTAL DESCR.           INFL014W:UPSP:HSP12_YEAST;GENSCAN0000000           [YHR053C;YHR055C;UPSP:MTCU_YEAST;gli632184           YAL005C;GENSCAN0000000437;GENEFINDER00           YGR192C;GENSCAN00000002700;GENEFINDER00           YGR192C;GENSCAN00000002700;GENEFINDER00           YML055C;UPSP:MTCU_YEAST;gli632382184           YOR204W;UPSP:DED1_YEAST;GENSCAN0000000           YML008C;GENSCAN00000003686;GENEFINDER00           YML008C;GENSCAN00000003686;GENEFINDER00           YMR173W;GENSCAN00000003686;GENEFINDER00           YMR173W;GENSCAN00000003686;gli6323826[reft]			

The input interact.pep.xls file should have **at least** these headers (but you may add more, like the index and spectrum for instance):

#### assumed\_charge MZratio peptide retention\_time\_sec protein

In order to run "Target Feature Annotation" you must Add an .xls (e.g., interact.pep.xls) file which has all of the possible annotations that may be queried and added to your data (volcano.tsv file actually). See next figure for adding a xls file.

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Inclusion List Builder <u>Target Feature Annotation</u>	Exported xIs file from pepxml viewer must include the following four columns (order are ignored) along with other selected columns. assumed_charge MZratio peptide retention_time_sec protein											
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You may adjust the "m/z tolerance in ppm" which is set at a default to 25 ppm. Also, you may wish to adjust the" rt tolerance" in minutes. These are worth playing with if you do not get very many features annotated.

Then, hit "Run Target Features Annotation."

6.2 Results of Target Feature Annotation

Once Target Feature Annotation has run, you will have an annotated volcano.tsv file from section 4.



At this point, you may click "Download Target Feature Annotation TSV," (circled in red) a file which looks similar to the "Differentially Expressed Feature List (.tsv)" from section 4, but with additional information including peptide descriptions for some of the features. Below, the annotated *.tsv* file is shown opened in MS Excel.

You may also download the "IPI file TSV" which just contains just the features which are associated with IPI(s).

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2 554.7447	66.79		2	0		31.26033	31.2904	31.37812	27.33401	27.18726	27.49832	3.96975	18.79458	9.83E-07	0.004356	6.345008	3										
3 879.409	42.28		2	0		31.6695	31.69408	31.62893	28.20053	28.14507	28.12132	3.508527	17.7143	1.41E-06	0.004356	6.048911	L										
4 940.3353	41.45		2	0		24.61068	24.49723	24.46404	27.89854	27.95469	27.97635	-3.41921	-17.0427	1.79E-06	0.004356	5.850541	L										
5 649.2925	40.94		2	0		25.03555	25.5542	25.13524	28.44259	28.55321	28.66767	-3.31283	-13.8135	6.46E-06	0.004476	4.711708	3										
6 802.3516	48.38		2	0		27.70408	27.32301	27.68703	23.54547	22.86078	23.75026	4.185872	13.59143	7.13E-06	0.004476	4.620129	•										
7 598.7434	39.72		2	0		28.21089	28.53429	28.28021	24.77582	25.19627	25.24779	3.2685	13.44017	7.63E-06	0.004476	4.556633	3										
8 846.898	57.11		2	0		29.97933	30.01585	30.12589	27.13921	27.37376	27.3878	2.740098	13.05556	9.10E-06	0.004476	4.39093	3										
9 1036.862	45.2		2	0		26.24666	26.95686	26.28742	29.93871	29.93383	30.10834	-3.49665	-12.7938	1.03E-05	0.004476	4.274528	3										
10 742.8234	39.92		2	0		28.7278	28.64235	28.6594	24.56388	24.60421	23.50981	4.450547	12.78001	1.04E-05	0.004476	4.268328	3				Orbite day	40		v000070	Inconcerce.	4004 557	-
12 671 8002	49.09		2	0		24.0000	24.03953	24.70473	27.77355	27.4077	27.40551	2.00521	12.7440	1.000-05	0.004476	4.232442	2 1291.33	5215			UDDelet	10	0 11/14/12/14/	TOROUTC	[TOROUTC,	1251.337	
12 994 9099	45.00		2	0		20.21420	20.20033	20.00011	27 16799	26 70646	25.02072	2.0000003	12,40733	1.225-05	0.004476	4.120103	2										
14 613 7706	64 77		2	0		26 83977	26 53221	26.83004	24.09517	23 86489	23 69824	2 847909	12 29556	1.315-05	0.004476	4 044503	2										
15 931 8226	45.32		2	0		23 39631	23 5265	24 29432	27 49889	27 33224	27 53066	-3 71488	-12 2922	1.31E-05	0.004476	4 042912	,										
16 857.3401	43.7	-	2	0		27.78583	27,72179	28.03372	25.32124	25,21108	25,18465	2,608122	12,21926	1.36E-05	0.004497	4.008251											
17 620,2919	56.23		2	0		26.05889	26,19464	25,93825	28,48642	28,53196	28,71227	-2.51295	-11.8197	1.66E-05	0.004819	3.813784	1										
18 687.2952	53.69		2	0		27.48284	27.61566	27.3376	24.92539	24.55259	24.37431	2.861273	11.71512	1.75E-05	0.004819	3.761556	5										
19 638.726	39.32		2	0		29.56937	29.99119	29.73484	26.98445	26.99605	26.54	2.924968	11.67957	1.78E-05	0.004819	3.743676	5										
20 637.2892	35.75		2	0		31.57199	31.72433	31.53729	29.41148	29.37029	29.30156	2.25009	11.05644	2.47E-05	0.005183	3.419342	2										
21 1084.906	30.14		2	1		27.21314	26.97768	27.47043	22.34231	NA	23.01833	4.540095	13.49993	2.83E-05	0.005454	3.351947	7										
22 780.8486	43.11		2	0		32.48825	32.05767	32.5114	29.7922	29.56009	29.84541	2.619874	10.92593	2.66E-05	0.005333	3.348693	3 780.8475	9 2452.78	2		L OrbiWild	132	4 K.DNAEGO	YFL014W,	[YFL014W)	780.8479	0
23 677.2717	38.24		2	0		29.40865	29.40844	29.39685	26.26973	26.98771	26.43908	2.839139	10.83027	2.80E-05	0.005454	3.296287	7										
24 513.7358	49.55		2	0		28.38896	28.40148	28.5531	26.07774	25.52409	25.45001	2.763904	10.79579	2.85E-05	0.005454	3.277266	5										
25 511.2571	53.22		2	0		26.70707	26.59197	26.85218	24.41918	24.57384	24.50566	2.217516	10.62531	3.14E-05	0.005658	3.182181	L										
26 429.6794	35.52		2	0		29.60005	29.67084	29.08329	26.22143	26.68734	26.68023	2.921725	10.61105	3.16E-05	0.005658	3.174152	2										
27 806.35	48.11		2	1		26.83652	26.8165	26.99627	23.71145	NA	23.48487	3.284937	12.91708	3.54E-05	0.005776	3.151112	2										
28 686.8199	39.26		2	0		23.93155	23.96476	24.38345	26.91559	27.39246	26.73841	-2.92223	-10.5578	3.26E-05	0.005682	3.14405	5										
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#### 6.3 PIPE2

You may load the results of Targeted Feature Annotation into PIPE2 by clicking "Load Proteins to Pipe2." Alternatively, you can copy and paste your IPIs, ORFs or other feature identifiers into PIPE2 to map them to several other databases, providing additional information about these important features. The PIPE2 link is here:

http://db.systemsbiology.net:8070/PIPE2/

Note: you must have firegoose extension installed in your computer when using Mozilla Firefox browser (http://gaggle.systemsbiology.org/docs/geese/firegoose/install/).

Here is a screenshot of our yeast DeletionStrain and Wildtype data into PIPE2 after Target Feature Analysis (remember we found 52 aligned and annotated features).

This links to a tutorial of PIPE2:

http://db.systemsbiology.net:8070/PIPE2/PIPE2/docs/PIPE2\_tutorial.doc

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YAL038W	Entrez Gene ID	Gene Symbol	Description	
1 YBR118W	852415	TEF2	Tef2p	
2 YBR118W	852415	TEF2	Tef2p	
3 YBR181C	852479	RPS6B	Protein component of the small (40S) ribosomal subunit; identical to Rps6Ap and has similarity to rat S6 ribosomal prot	ein
4 YCL037C	850320	SRO9	Cytoplasmic RNA-binding protein that associates with translating ribosomes; involved in heme regulation of Hap1p as a component of the HMC complex, also involved in the organization of actin filaments; contains a La motif	
5 YDL110C	851448	TMA17	Protein of unknown function that associates with ribosomes	
6 YDL223C	851303	HBT1	Hbt1p	
7 YDL223C	851303	HBT1	Hbt1p	
8 YDR328C	851928	SKP1	Evolutionarily conserved kinetochore protein that is part of multiple protein complexes, including the SCF ubiquitin ligas complex, the CBF3 complex that binds centromeric DNA, and the RAVE complex that regulates assembly of the V-ATPase	e
9 YDR450W	852061	RPS18A	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps18Bp and has similarity to E. coli S13 a rat S18 ribosomal proteins	nd
10 YFL014W	850532	HSP12	Hsp12p	

#### 7. Trouble Shooting

Problem	Fix
The mzxml file format is invalid	try using indexmzXML.exe to correct errors
PIPE2 button does nothing in firefox browser	Install firegoose extension