

User's manual

RiceProteomeDB

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INTRODUCTION

1.1 Web application Overview

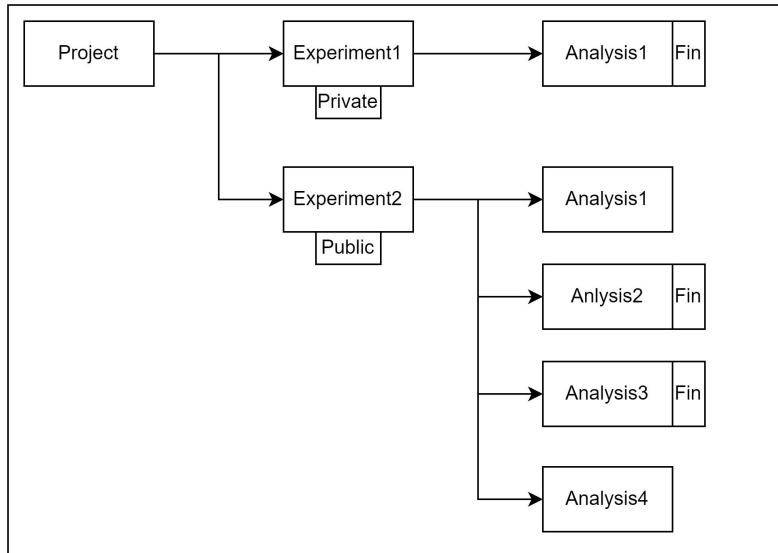


Figure 1. Overview of when users use RiceProteomeDB

- Users can create projects, upload experiments within those projects, and perform multiple analyses on the experiments. Among these, they can change the property of an experiment from 'Private' to 'Public' (Figure 1).
- At this point, users not involved in the project can access finished analyses of public experiments within the 'Experiment (Public)' section (Figure 2).

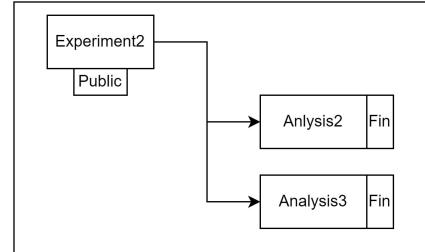


Figure 2. Cases of Users Not Participating in the Project

1.2 Organization Manual

The user manual is structured into sections for Account, Management, Ongoing Analysis, and Finish Analysis

1. **Account Section:** This section provides information on account and login.
2. **Management:** Within this section, users will find comprehensive information about project creation, participation, experiment uploads, and the addition of analyses.
3. **Ongoing Analysis:** The Ongoing Analysis section guides users through sequential DEP, GO, and NETWORK analyses. It also explains how to use the 'Protein Basket' feature, allowing users to add proteins of interest during ongoing analysis.
4. **Finish Analysis:** This section outlines the finalization of the analysis process, including accessing completed analyses and downloading associated files.

In addition, detailed terminology and explanations can be found in the GitHub Wiki section for reference.

1.3 Sample Prepare

Part to be used as value in "proteinGroups" file

LFQ intensity control rep1	LFQ intensity control rep2	LFQ intensity control rep3	LFQ intensity flg22 rep1	LFQ intensity flg22 rep2	LFQ intensity flg22 rep3	LFQ intensity MSP1 rep1	LFQ intensity MSP1 rep2	LFQ intensity MSP1 rep3
----------------------------	----------------------------	----------------------------	--------------------------	--------------------------	--------------------------	-------------------------	-------------------------	-------------------------



experimentname	samplename	condition	replicate
LFQ intensity control rep1	control_1	control	1
LFQ intensity control rep2	control_1	control	2
LFQ intensity control rep3	control_1	control	3
LFQ intensity flg22 rep1	flg22_1	flg22	1
LFQ intensity flg22 rep2	flg22_1	flg22	2
LFQ intensity flg22 rep3	flg22_1	flg22	3
LFQ intensity MSP1 rep1	MSP1_1	msp1	1
LFQ intensity MSP1 rep2	MSP1_1	msp1	2
LFQ intensity MSP1 rep3	MSP1_1	msp1	3

- Write down the experiment name, sample name, condition, and replicate compared to the protein groups above.

START

2.1 Account

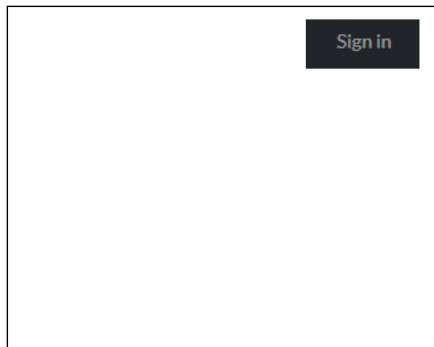
A screenshot of a sign-in dialog box. The title bar says "sign-in". Inside, there are two input fields: the top one is labeled "Manager_1" and the bottom one is labeled "yourpassword". Below the fields is a dark grey "sign-in" button. At the bottom left, there is a link "please sign up".

Figure 3.

Figure 4

- Access <http://riceproteome.plantprofile.net/>
- From here on, use the summarized screen to guide the explanation. Locate the 'Sign in' button (Figure 3).
- If it's your first visit, click the 'please sign up' button, enter an ID and password, and log in. In this manual, I have created 'Manager_1' to explain RiceProteomeDB (Figure 4).

2.2.1 Management(Create Project)

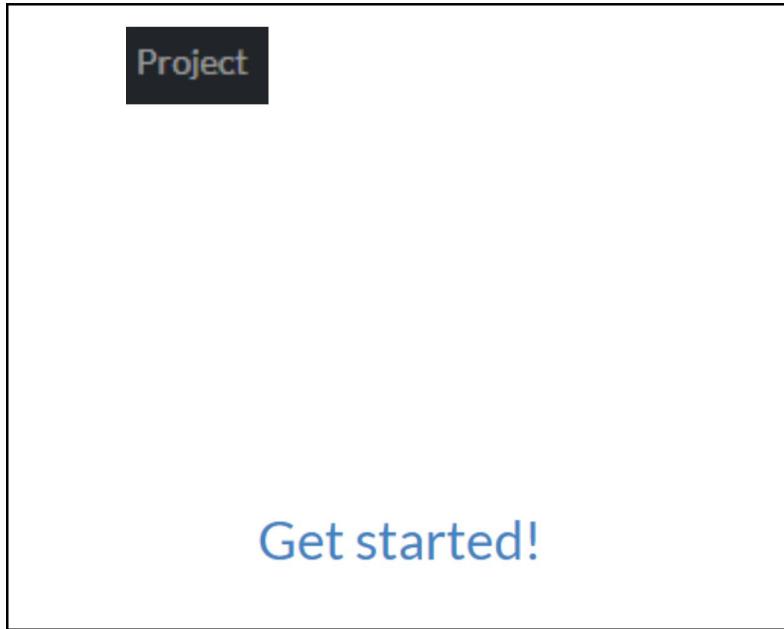


Figure 5

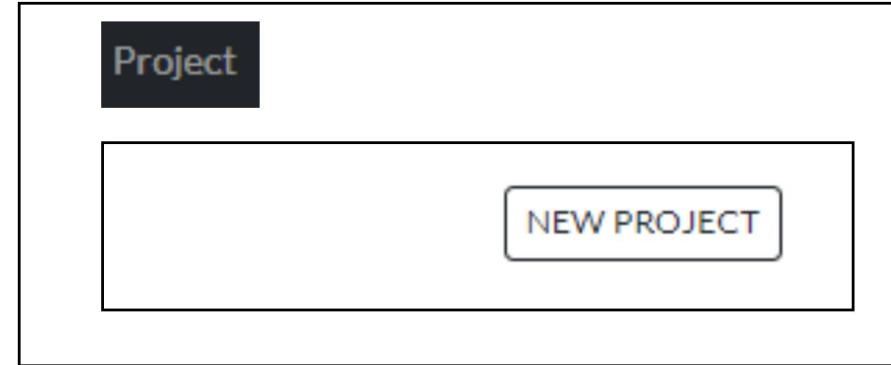


Figure 6

2.2.1 Management(Create Project)

New project

Project Name

Enter information that can describe the project (name, start date, end date, project purpose) and click the submit button.

Start Date

End Date

Project Description



Submit

Figure 7

Project				
Project Name	Administer	...	Experiment manage	Settings
Manager_project	manager_1	...	<u>select this project</u>	
NEW PROJECT				

Figure 8

2.2.1 Management(Create Project)

- Find the 'Project' button(Figure 5).
- There is an empty space with a 'New PROJECT' button(Figure 6).
- Under 'New project', enter information including 'Project Name' as 'Manager_project'(Figure 7).
- As a result, you can see a table displaying project information including 'Project Name' as 'Manager_project', 'Administrator' as 'manager_1' an option to proceed to experiment management labeled 'select this project,' and the ability to add participants labeled 'Settings symbol'(Figure 8).

2.2.1.1

Management(Join Project)

Edit project
ProjectName:Manager_project

Project User management

submissionA1212 ×

Period in Manager_project
10/01/2023-11/30/2023

Experiments in Manager_project

Add Experiment?

Upload link

Project Description

Create a project to explain in the manual

Submit x

- You can add users to 'Manager_project' by clicking the 'Settings symbol.'
- As an example, I added 'submissionA1212.'
- Similar to the project creator 'manager_1,' 'submissionA1212' can upload experiments to the project(Figure 9).

Figure 9

2.2.2 Management(Upload Experiments)

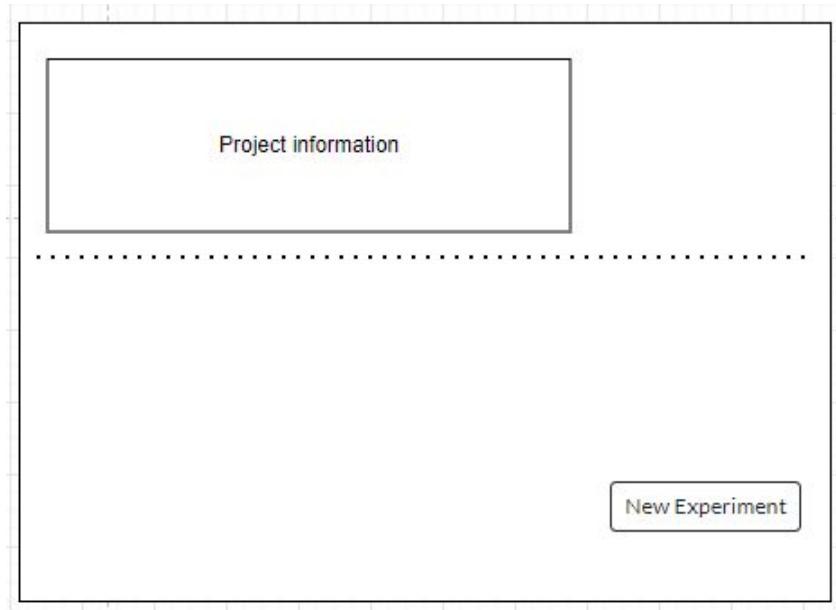


Figure 10

The screenshot displays a modal dialog box titled "Upload Experiment". It contains several input fields and options:

- Project Name:** Manager_project
- Experiment Name:** Manager_project_upload_1
- Label Information:** A radio button group where LFQ is selected. There is also a link "what is TMT/LFQ?".
- Filter row:** A radio button group where NA.omit is selected. There is also a link "what is filter row?".
- Write Experiment Information:** A section for "proteingroups(txt VERSION)". It includes a file selection button "파일 선택" and a preview area showing "1. proteinGro...Label-free.txt".
- Experiment Description:** A text area with placeholder text "Upload experiment for manual explanation." and a red circled number "1" in the bottom right corner.

Figure 11

2.2.2 Management(Upload Experiments)

- When you select 'select this project' in 'Manager_project,' you can easily review the 'Project Information' section and the experiments added to the project. However, for first-time visitors, only the 'New Experiment' button is visible(Figure 10).
- I uploaded 'Proteingroups quantified by maxquant for label-free data' to demonstrate in the manual. The 'Experiment Name' was set as 'Manager_project_upload_1,' the 'Label Information' was 'LFQ,' and the 'Filter Row' was selected as 'NA.omit.' I provided an experiment description as 'upload experiment for manual explanation'(Figure 11).
- Add some sample information and additional descriptions at
https://github.com/dongu7610/Riceproteome/wiki/RiceProteome_Manual-additional-explanation

2.2.2 Management(Upload Experiments)

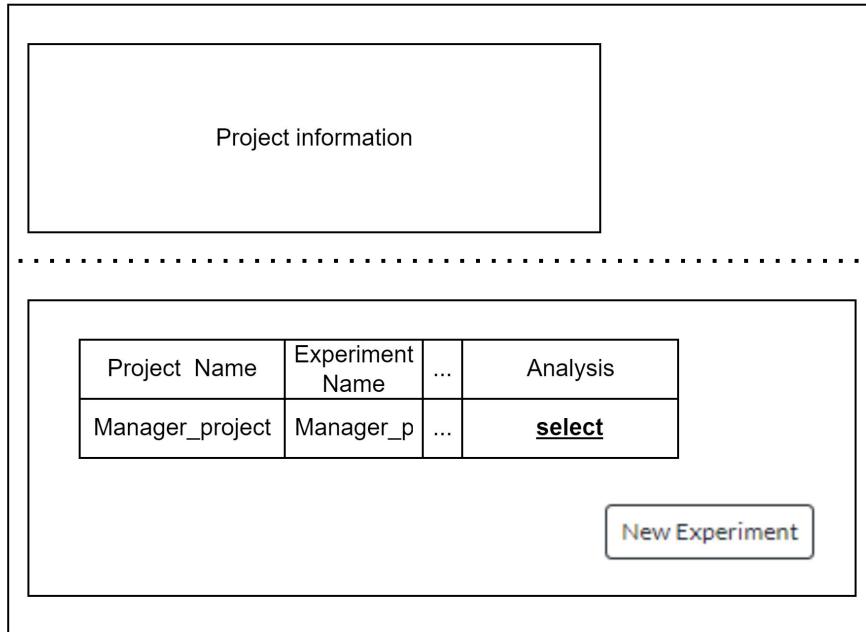


Figure 12

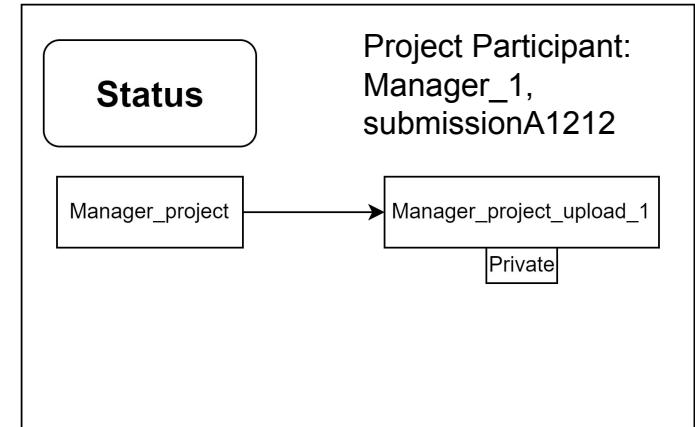


Figure 13

2.2.2 Management(Upload Experiments)

- Comparing to Figure 10, an entry labeled 'Experiment Name' as 'Manager_project_upload_1' has been added, which can be accessed for analysis via 'select'(Figure 12).
- To summarize the situation, the 'manager_1' account created a project named 'Manager_project,' involving the 'submissionA1212' account in the project. Additionally, 'Manager_project_upload_1' was uploaded for analysis. It is in a private state, thus not visible to other users(Figure 13).

2.2.3 Management(Add Analysis)

Experiment information

Property :	Private	Change to Public
Experiment Name :	Manager_project_upload_1	
Condition :	LFQ intensity MSP1 ,LFQ intensity flq22 ,LFQ intensity control	
...		
.....		
Finished Analysis		
.....		
Ongoing Analysis		

[New Analysis](#)

Figure 14

Add Analysis

Analysis Name

Write the analysis name and description.

Analysis Description

Analysis test for manual explanation.



[Submit](#) [X](#)

Figure 15

2.2.3 Management(Add Analysis)

- When accessing 'Manager_project_upload_1' via the 'select button,' it displays the 'Experiment Information,' including 'Experiment Name' and 'Condition.' Additionally, it features the 'Finished Analysis' section for completed analyses and the 'Ongoing Analysis' section for analyses in progress. However, for first-time visitors, only 'New Analysis' is visible(Figure 14).
- There is also a section where you can change the 'Private' to 'Public'.
- I created an 'analysis branch' where analysis can be conducted. In this manual, I selected the 'New Analysis' button and then entered 'Analysis1' for the 'Analysis Name' and 'Analysis test for manual explanation' for the 'Analysis Description'(Figure 15).

2.2.3 Management(Add Analysis)

Experiment information		
Property	:	Private Change to Public
Experiment Name	:	Manager_project_upload_1
Condition	:	LFQ intensity MSP1 ,LFQ intensity flq22 ,LFQ intensity control
...		
.....		
Finished Analysis		
.....		
Ongoing Analysis		
AnalysisName	...	Analysis
Analysis1	...	<u>select</u>

Figure 16

- Based on the input in 'Figure 15,' an entry 'Analysis1' appears under 'Ongoing Analysis,' accessible through the 'select button'(Figure 16).
- Once 'Analysis1' is completed, another analysis can be performed by using the 'New Analysis' button

2.3 Ongoing Analysis

Manager_project_upload_1_An1;Analysis1
— Analysis information: Analysis test for manual explanation,

Your experiment's condition

LFQ intensity MSP1
LFQ intensity flg22
LFQ intensity control
— Experiment Options LFQ;NA.omit

?

Number of Compare Group

Apply

Figure 17

- When initiating the analysis via the 'select' button it displays the Analysis information along with Your experiment's condition. In this context, the experiment's conditions are LFQ intensity MSP1, LFQ intensity flg22, and LFQ intensity control
- Here, set the 'Number of Compare Groups' to 2 and click 'Apply' button(Figure 17).



Figure 18

- 'compare 1' and 'compare 2,' and a dropdown menu appears.
- Enter 'control vs msp1' and 'control vs flg22' for each compare group(Figure 18).
- T-test analysis for each group.

2.3.1 Ongoing Analysis(Analysis DEP)

The screenshot shows a software interface with two main sections. The top section, titled 'Experiment information', contains fields for 'Property' (set to 'Private'), 'Experiment Name' ('Manager_project_upload_1'), 'Condition' ('LFQ intensity MSP1 ,LFQ intensity flq22 ,LFQ intensity control'), and an ellipsis (...). Below this is a horizontal dotted line. The bottom section, titled 'Ongoing Analysis', contains a table with two rows. The first row has columns for 'AnalysisName' ('...'), '...', and 'Analysis'. The second row has columns for 'Analysis1' ('...'), '...', and 'goDEP'. There is another horizontal dotted line below this table.

AnalysisName	...	Analysis
Analysis1	...	<u>goDEP</u>

Figure 19

- After entering, wait for a moment; the 'select' will change to 'goDEP'(Figure 19).

2.3.1 Ongoing Analysis(Analysis DEP)

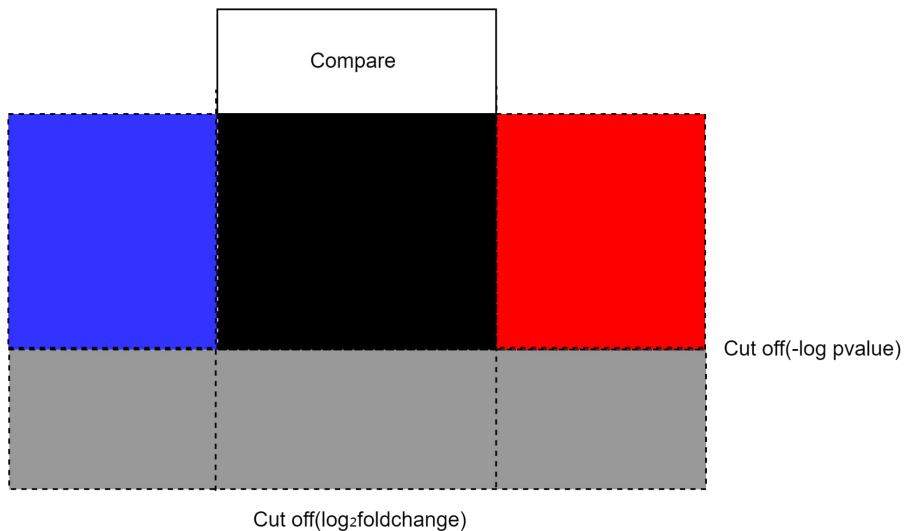


Figure 19

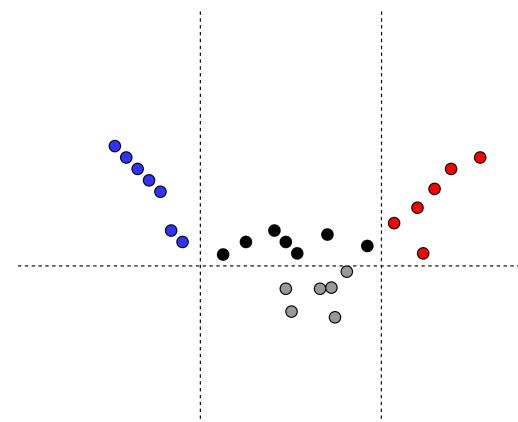


Figure 20

2.3.1 Ongoing Analysis(Analysis DEP)

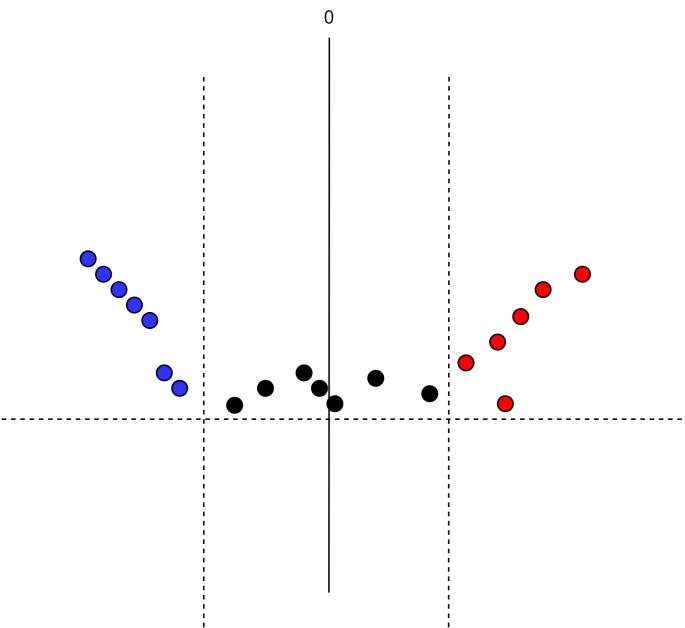


Figure 21

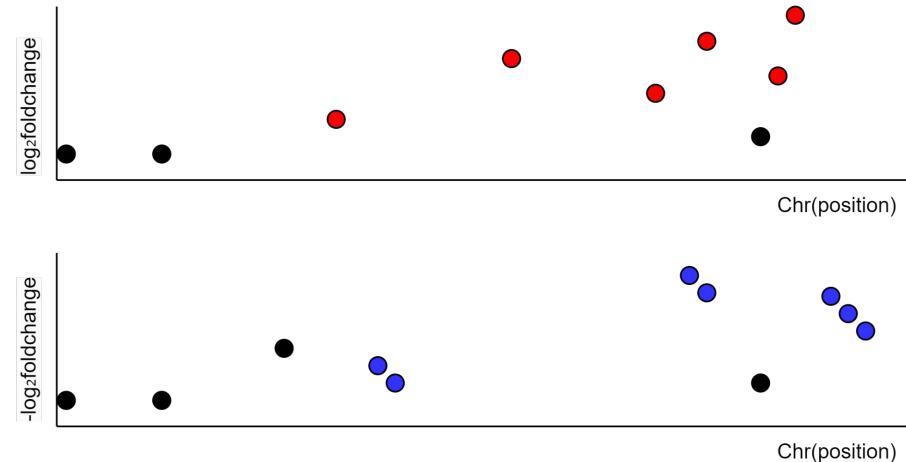


Figure 22

2.3.1 Ongoing Analysis(Analysis DEP)

- For the Volcano plot the x-axis represents 'log₂fold change,' and the y-axis represents '-log p-value.' The default 'cut-off' values are set at log₂fold change '-1.5, 1.5' and -log p-value 3. In this plot, the gray area signifies proteins eliminated based on p-value. The blue area represents down-regulated proteins in comparison, the black area designates similar proteins, and the red area signifies up-regulated proteins(Figure 19).
- Simply put, it can be graphed as follows(Figure 20).
- The Manhattan plot displays the log₂fold change values of proteins that have passed through the p-value, alongside their chromosome positions(Figure 21,Figure 22).

2.3.1 Ongoing Analysis(Analysis DEP)

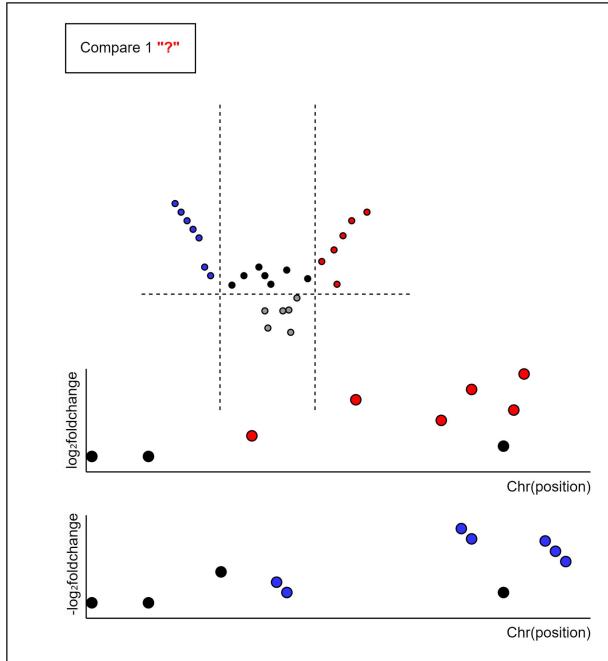


Figure 23

- In the manual, two comparisons, namely, compare1 and compare2, are prepared. A total of two volcano plots and four Manhattan plots are generated for each comparison(Figure 23).
- The red question mark indicates that clicking it allows you to verify which conditions were used.
- “compare 1(Control vs MSP1)”

2.3.1 Ongoing Analysis(Analysis DEP)

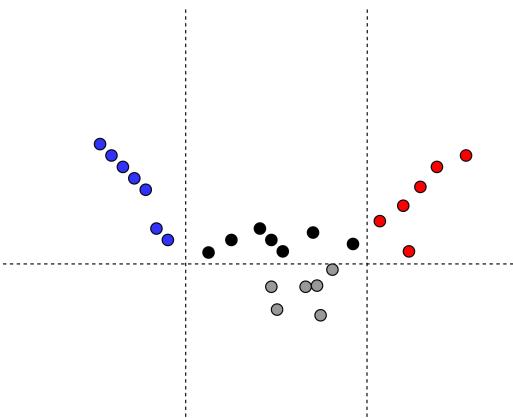


Figure 24

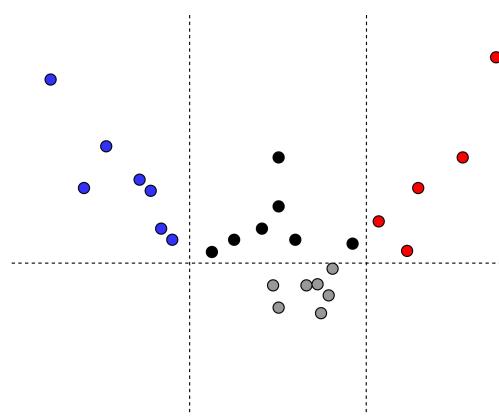


Figure 25

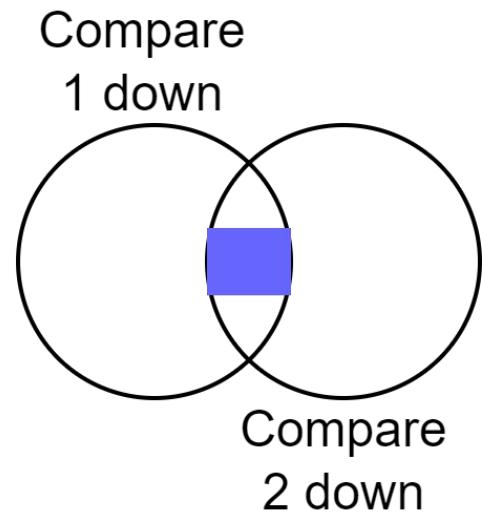


Figure 26

2.3.1 Ongoing Analysis(Analysis DEP)

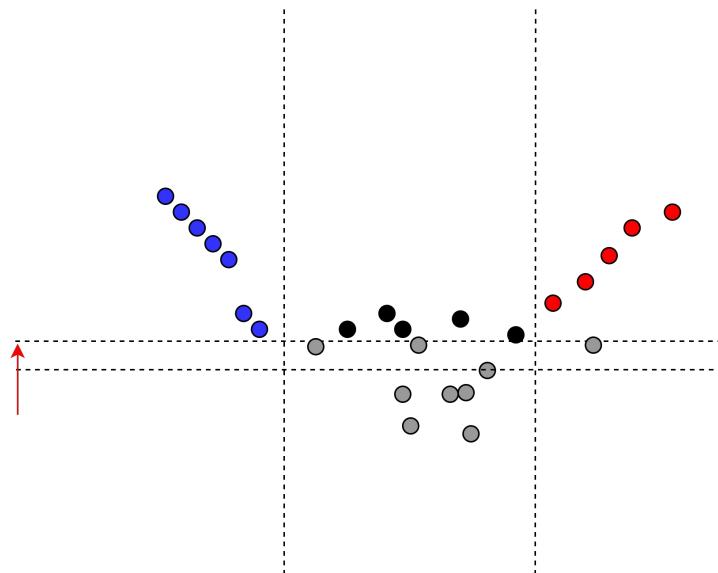


Figure 27

Change cutoff value?

log₂foldchange value

1.5

-log p value

3

Submit

Figure 28

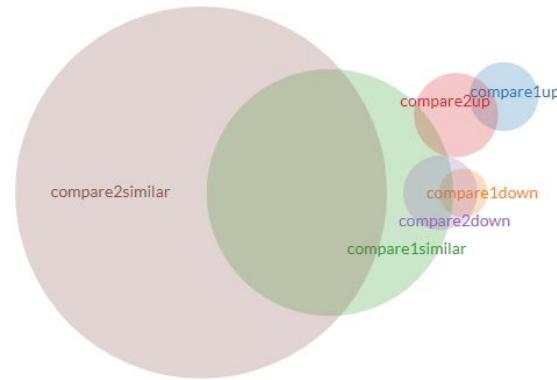
2.3.1 Ongoing Analysis(Analysis DEP)

- To prepare for network analysis, a Venn diagram is drawn for proteins that pass the cutoff p-value.
- For instance, there might be a different protein composition between compare 1 down and compare 2 down, but there could be an overlapping area(Figure 24,25).
- This overlap could be labeled as compare 1 down, compare 2 down for further analysis(Figure 26).
- You can tighten the cutoff to perform a more stringent analysis. By adjusting the p-value, represented by the red arrow, more proteins will be filtered out(Figure 27,28).

2.3.1 Ongoing Analysis(Analysis DEP)

VennDiagram

[Export svg\(Venn\)](#)



- This is the actual Venn diagram on the page. By clicking between compare1up and compare2up, it selects four proteins found in this region, and a Manhattan plot is drawn(Figure 29).

Figure 29

2.3.2 Ongoing Analysis(Analysis GO)

GO analysis ?

[go to GOEA](#)

GOEA

counts protein 623 (-log p value >3)

LOC_Os01g08410.1,LOC_Os01g59600.2,LOC_Os03g44

Analysis information

Manager_project_upload_1_Anlysis1

foldchange and p value

3.1.5

[Submit](#) [x](#)

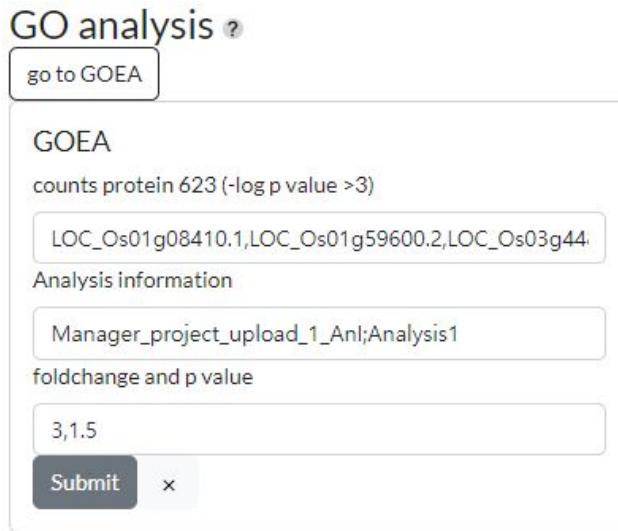


Figure 30

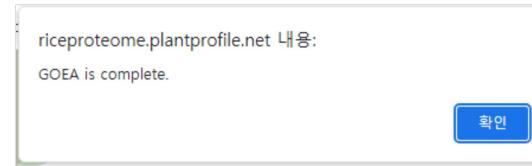


Figure 31

GO analysis ?

[go to GOEA](#)

wait a few seconds

[pvalue=3,fc=1.5,counts=623](#) [goGOEA](#)

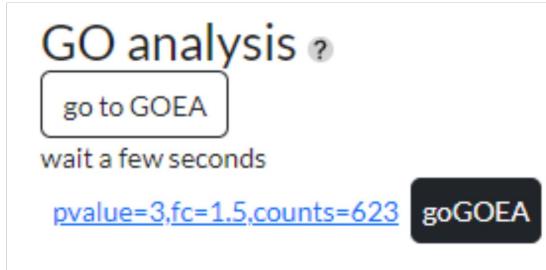


Figure 32

2.3.2 Ongoing Analysis(Analysis GO)

- I conduct GO analysis using these proteins that have passed the p-value threshold(Figure 30).
- After entering the details and waiting a few minutes, a completion window appears(Figure 31).
- Then, links and buttons for further progress are displayed(Figure 32).

2.3.2 Ongoing Analysis(Analysis GO)

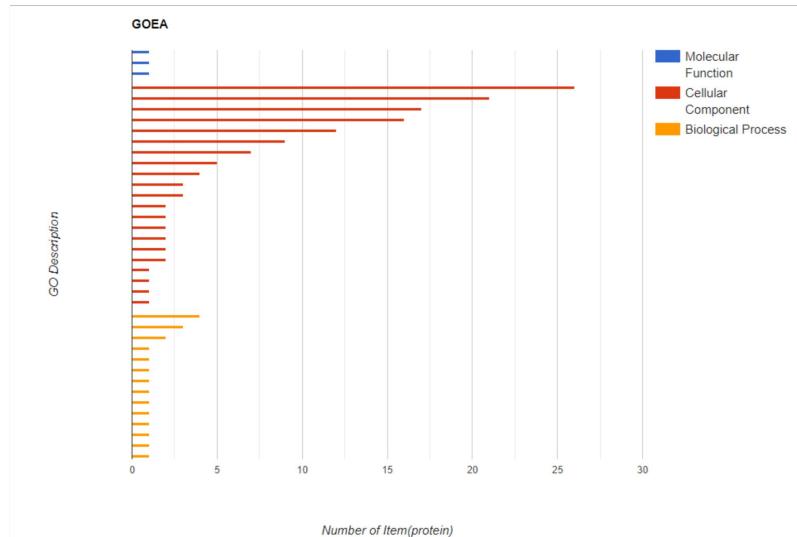


Figure 33

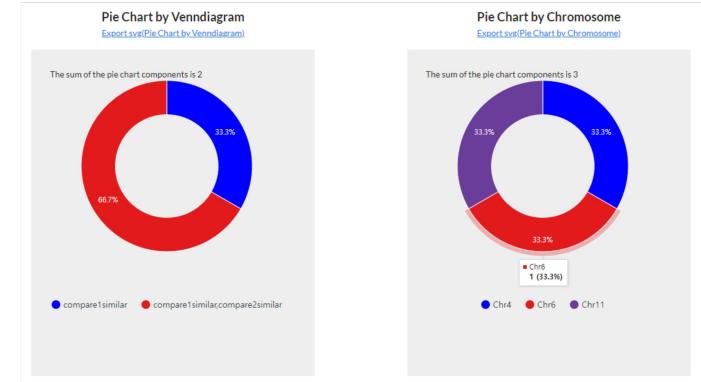


Figure 34

2.3.2 Ongoing Analysis(Analysis GO)

GO Table
You can click ProteinName in the table.

GOID	GO_fdr	GO_pv	ProteinName	Chrinfo	ProteinDescription	Vennarea
GO:0005777	1	0.021659326456192018	LOC_Os04g53210.3	Chr4	LOC_Os04g53210.3 protein(hydroxycacid oxidase 1, putative, expressed)	compare1similar, compare2similar
GO:0005777	1	0.021659326456192018	LOC_Os11g39220.2	Chr11	LOC_Os11g39220.2 protein(acyl-coenzyme A oxidase, putative, expressed)	compare1similar, compare2similar
GO:0005777	1	0.021659326456192018	LOC_Os06g14240.1	Chr6	LOC_Os06g14240.1 protein(hsp20/alpha crystallin family protein, putative, expressed)	compare1similar

Rows per page: 20 ▾

First | Prev | 1 | Next | Last

Figure 35

- This is the output from the Python library 'goatools.' From the results, I created a bar chart for three major categories(Figure 33).
- Among these, if you click the bar related to GO:0005777 (peroxisome), it displays a corresponding pie chart and table. In the Pie Chart by Venn diagram, the proteins connect with our experimental data. On the other hand, the Pie Chart by Chromosome connects protein information with their location data(Figure 34).
- The table provides information about the proteins associated with the selected GOID (GO:0005777)(Figure 35).

2.3.3 Ongoing Analysis(Protein basket)

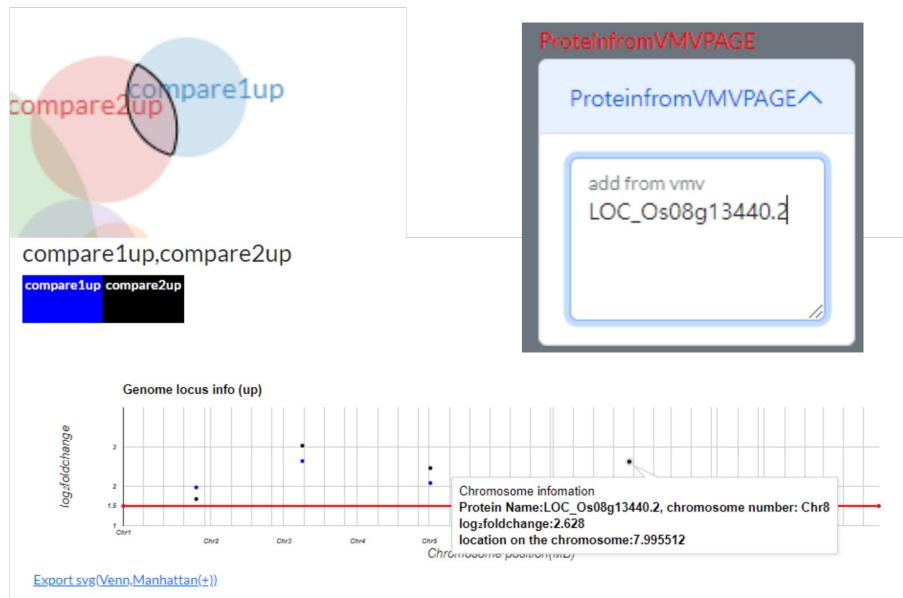


Figure 36



Figure 37

2.3.3 Ongoing Analysis(Protein basket)

- In RiceProteomeDB, you can add proteins you are interested in to the Protein Basket.
- For example, you can add the protein 'LOC_Os08g13440.2' among the proteins between 'compare1up' and 'compare2up' to the 'ProteinfromVMVPAGE'(Figure 36).
- Additionally, on the page performing the Analysis GO, by selecting an entry in the 'ProteinName' column from the Table that appears when clicking 'GO:0005777 (peroxisome),' you can add it to 'ProteinfromGO'(Figure 35,37).
- You can also utilize the list of proteins you already know by pasting it in bulk.
- I have added three proteins related to peroxisomes (LOC_Os04g53210.3, LOC_Os11g39220.2, LOC_Os06g14240.1) to ProteinfromGO in this manual.
- By clicking 'Find nodes' with the three proteins in the Protein Basket, you can perform Network Analysis!

2.3.4 Ongoing Analysis(Analysis Network)

Network Analysis?

Find nodes

'Group A from Protein Basket,Group B from Protein Basket' uses the protein added to the Protein Basket during the analysis process. Proteins selected from Group A draw a NETWORK (Group A; node, edge) as a two-step neighborhood.

Select the protein you want to know about the relationship with Group A drawn with Source from Protein Basket in Group B to get Network (Result).

VMV,GOEA,Bulk is represented by the colors red (V), yellow (G), and blue (B). If the selected protein possesses both VMV and GOEA properties, it is indicated by the color orange (VG).

Group A from Protein Basket

Search Group A

- LOC_Os04g53210.3
- LOC_Os11g39220.2
- LOC_Os06g14240.1

Submit

Figure 38

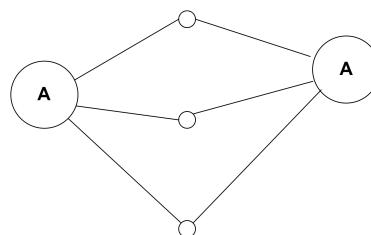


Figure 39

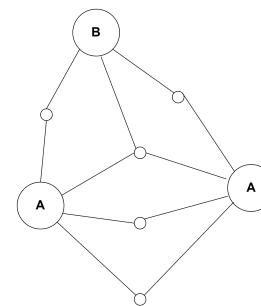


Figure 40

2.3.4 Ongoing Analysis(Analysis Network)

Group A from Protein Basket

✓ LOC_Os04g53210.3(G) ✗ ✓ LOC_Os11g39220.2(G) ✗

Group B from Protein Basket(optional)

✓ LOC_Os06g14240.1(G) ✗

Submit

Figure 41

- Perform Network Analysis using the added proteins. The three proteins added from ProteinfromGO will be displayed(Figure 38).
- When choosing the nodes for the network, you select whether to draw the network as added nodes to Group A(Figure 39), or consider the options from Group B as well(Figure 40).
- In this manual, the analysis was conducted considering the Group B option(Figure 41).

2.3.4 Ongoing Analysis(Analysis Network)

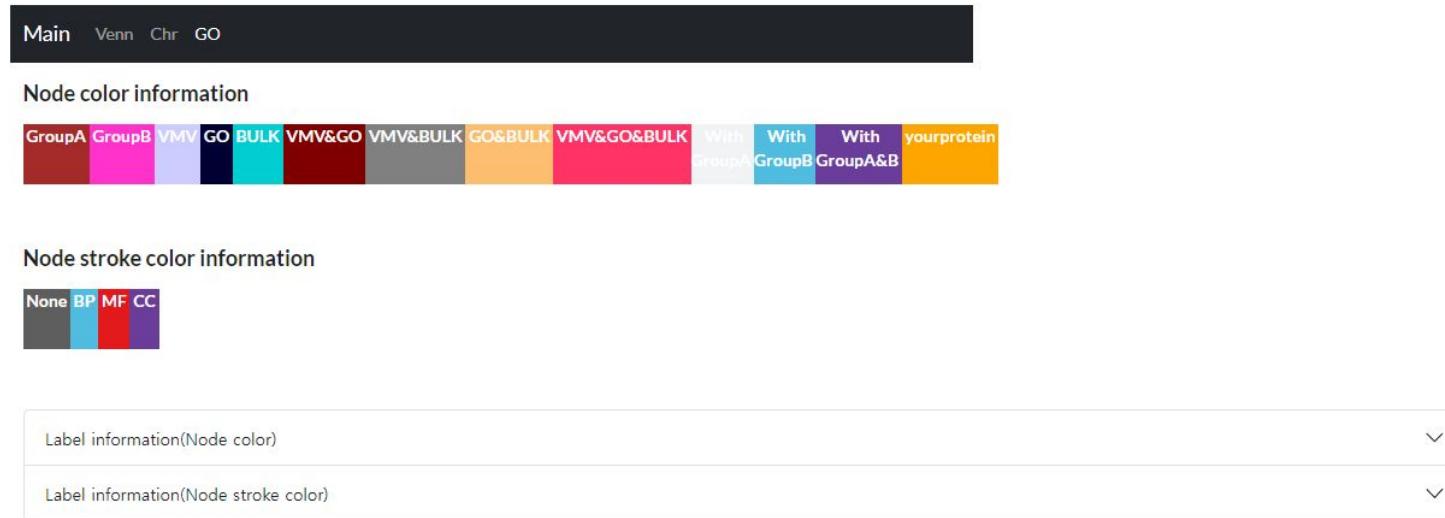


Figure 42

2.3.4 Ongoing Analysis(Analysis Network)

- From the results of the network analysis, there exists a Navigation bar comprising 'Main', 'Venn', 'Chr', 'GO' along with Node color and Node stroke color options.
- 'Main' represents the Normal Network. 'Venn' signifies the network with added experimental data.'Chr' provides Chromosome information, and 'GO' includes the BP, CC, MF categories.
- By expanding 'Label information' below (Node color, Node stroke color), you can easily review the simple Label information(Figure 42).

2.3.4 Ongoing Analysis(Analysis Network)

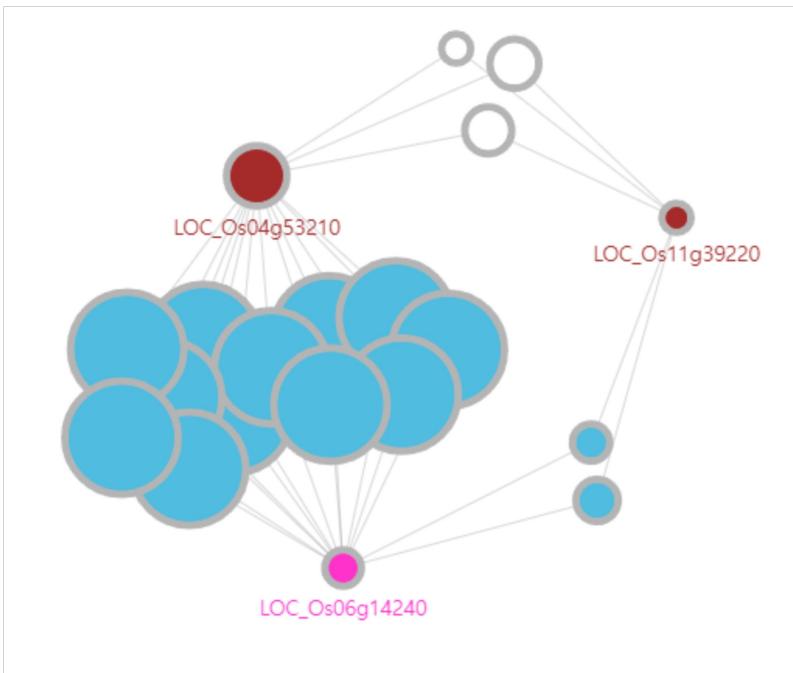


Figure 43(Main)

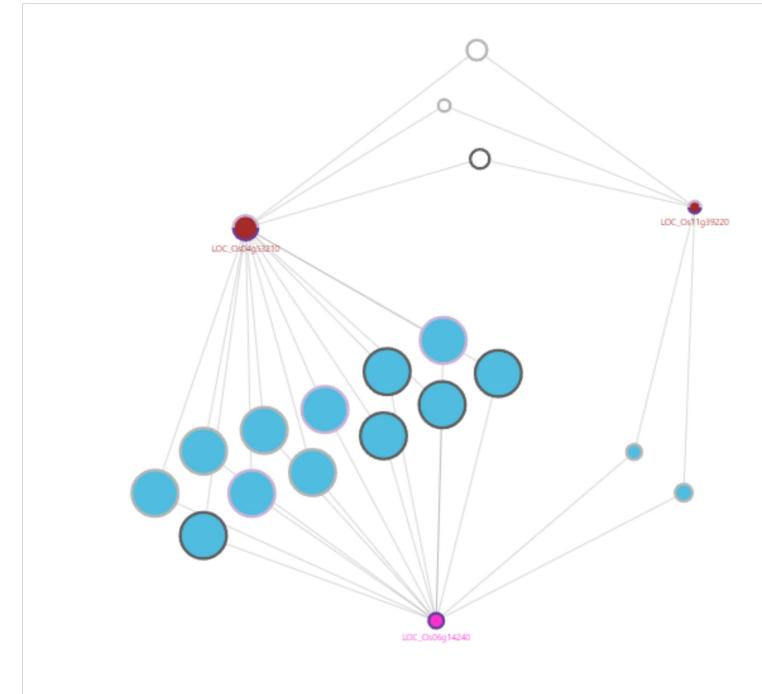


Figure 44(Venn)

2.3.4 Ongoing Analysis(Analysis Network)

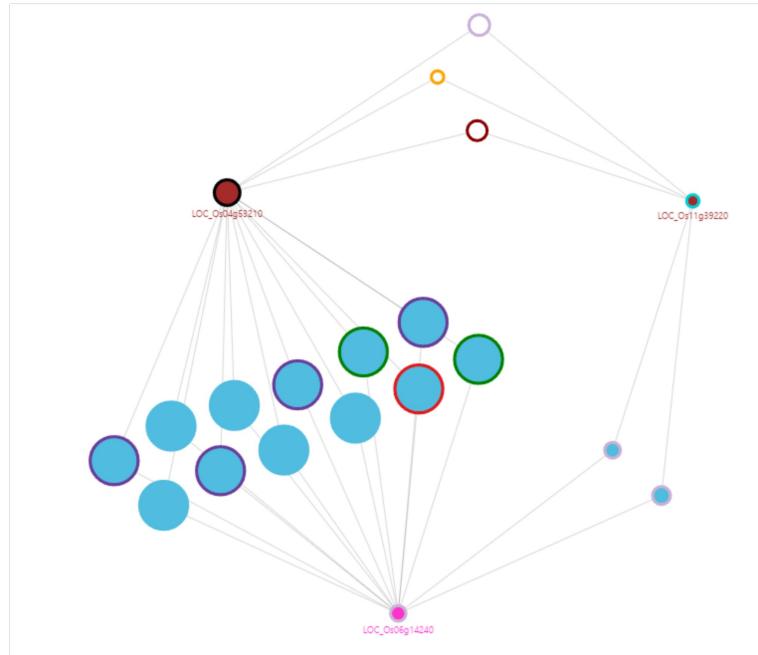


Figure 45(Chr)

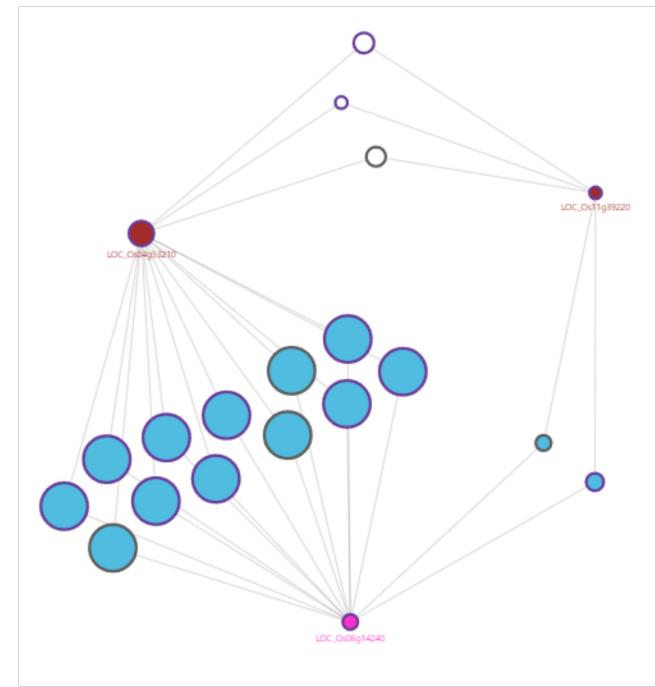


Figure 46(GO)

2.3.4 Ongoing Analysis(Analysis NETWORK)

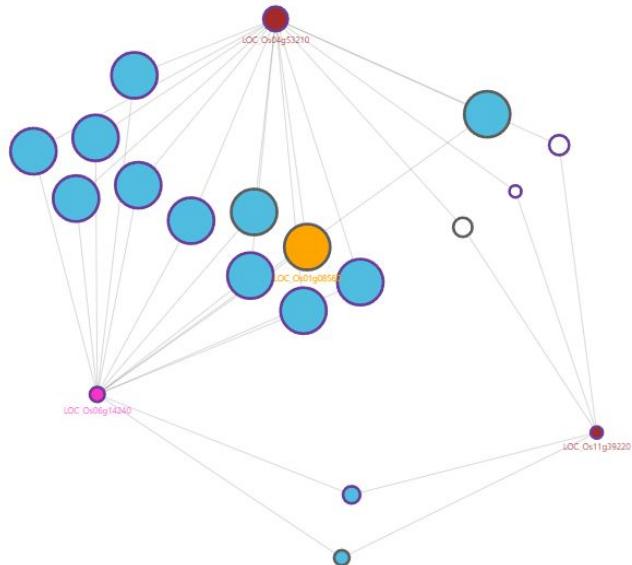


Figure 47

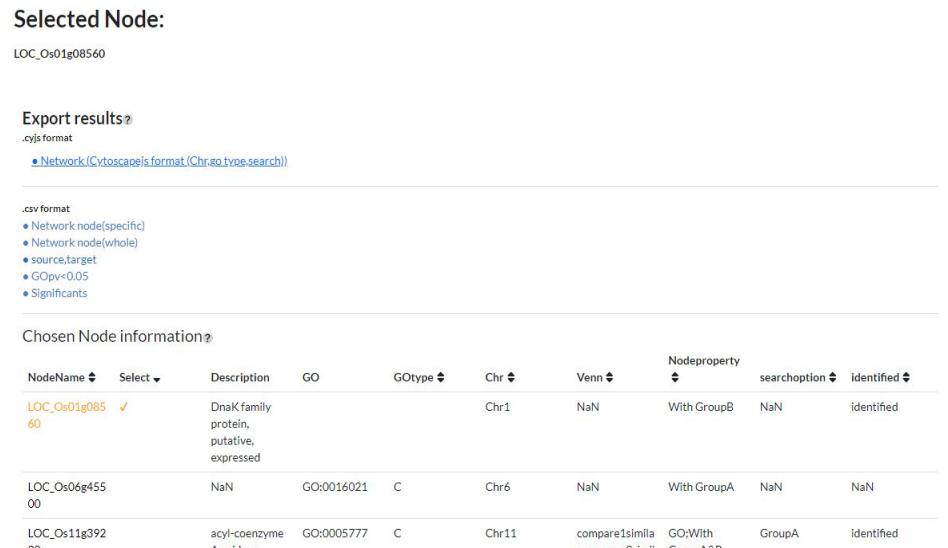


Figure 48

2.3.4 Ongoing Analysis(Analysis NETWORK)

- The Network in 'Figure(43-46)' represents individual examinations of 'Main,' 'Venn,' 'Chr,' 'GO.' You can observe the change in Node Stroke colors.
- When a researcher discovers an interesting protein while examining, a left-click selection marks it in orange color.
- The selected protein is then added to the 'Selected Node.'
- In this manual, I chose LOC_Os01g08560(Figure 47) and was able to gather basic protein information from the row marked with a check in the Table(Figure 48).

2.4 Finish Analysis

Are you sure you want to finalize?

[save the Analysis](#)

Figure 49

Experiment information		
Property	:	Private Change to Public
Experiment Name	:	Manager_project_upload_1
Condition	:	LFQ intensity MSP1 ,LFQ intensity flq22 ,LFQ intensity control
...		

Finished Analysis		
AnalysisName	...	Analysis
Analysis1	...	goDEP

Ongoing Analysis		
		New Analysis

Figure 50

2.4 Finish Analysis

- When finishing the analysis, click on 'Save the Analysis' at the bottom(Figure 49).
- This saves the analysis, and 'Analysis1,' the analysis performed in this manual, is added to the 'Finished Analysis' section. Since one analysis is completed, you can proceed to add a new analysis(Figure 50).

CONTACT

- Further details not covered in this manual are explained on the GitHub Wiki page:
https://github.com/dongu7610/Riceproteome/wiki/RiceProteome_Manual-additional-explanation For any additional queries or information not addressed in this manual, please refer to the GitHub Wiki.
- For any inquiries or additional information not covered in this manual, please contact us via email at dongu7610@naver.com