Supporting Information for:

EnrichVisBox: A versatile and powerful web toolbox for visualizing complex functional enrichment results of omics data

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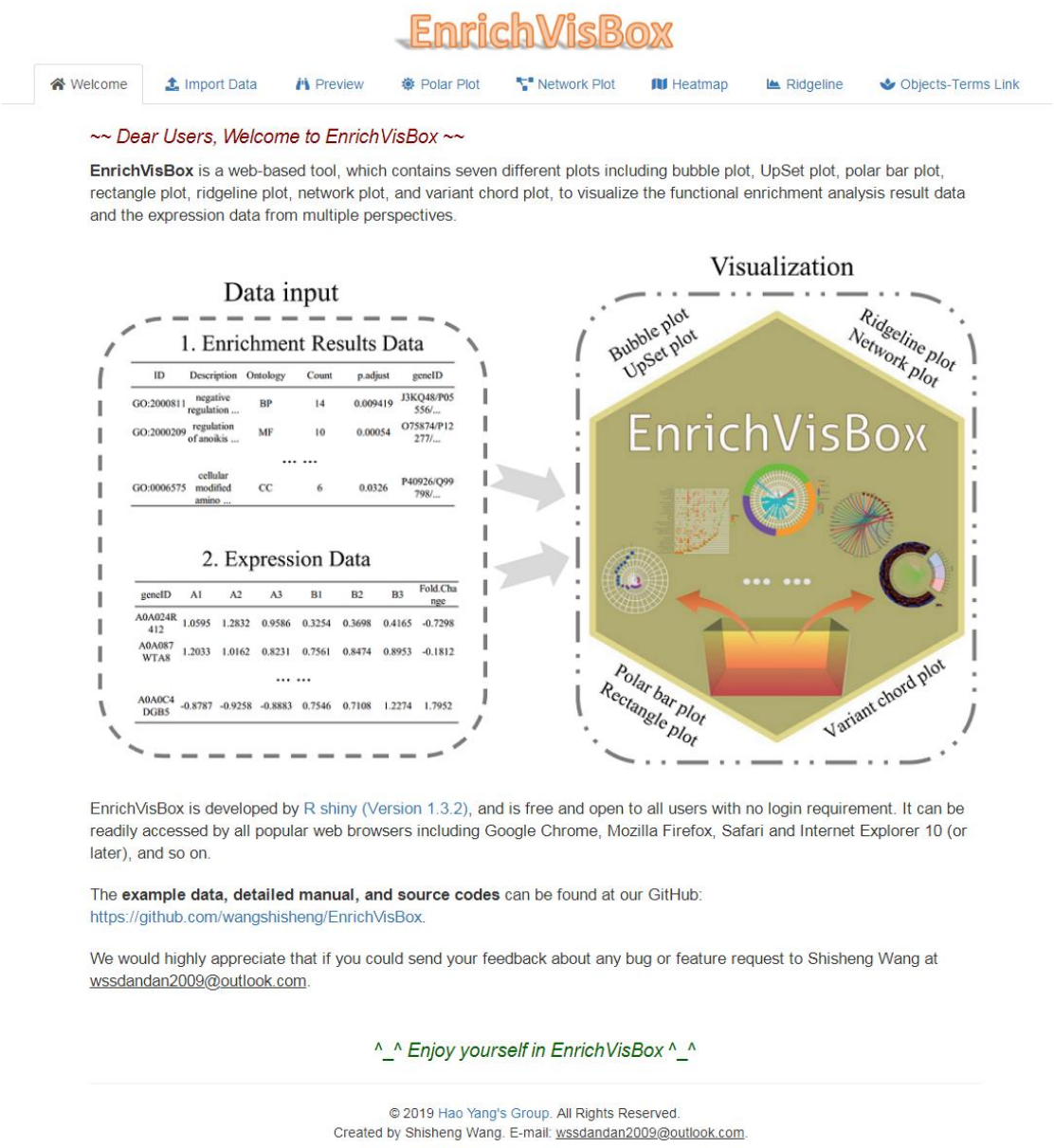
I. Supplementary Notes

EnrichVisBox, which is powerful and integrated software, can automatically visualize functional enrichment analysis results, such as gene ontology (GO) enrichment analysis results (Carbon et al. 2009) and KEGG pathway enrichment analysis results (Kanehisa and Goto 2000). Here, the detailed implementation and operation of EnrichVisBox are described. Users can follow this description to visualize their own data freely.

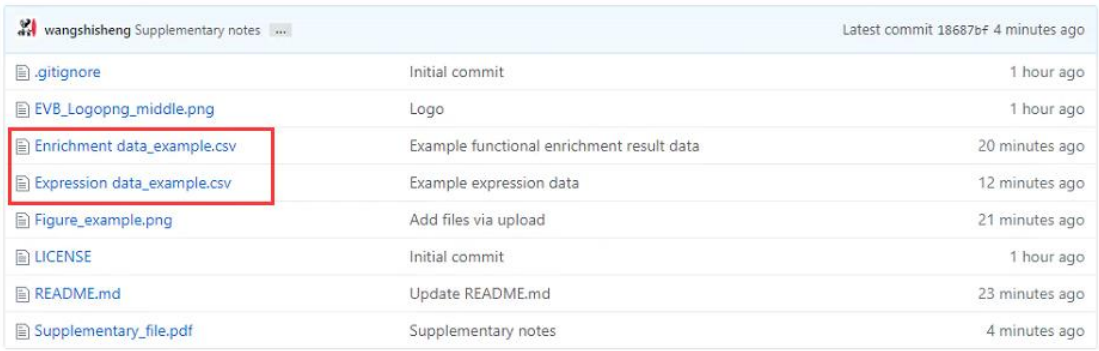
Users can open the site https://www.omicsolution.org/wukong/EnrichVisBox in their browsers. It is recommended that users use Chrome (downloaded from https://chrome.en.softonic.com).

The website homepage is as below:



1. Data preparation

Because EnrichVisBox aims mainly at visualizing functional enrichment analysis results, it is necessary to prepare two types of data here: object (genes/proteins/metabolites) expression data and enrichment analysis resulting data. If users cannot understand what the 'functional enrichment analysis' is, they can read this reference (Reimand et al. 2019) for further learning. Example data can be downloaded from https://github.com/wangshisheng/EnrichVisBox.



The 'Expression data_example.csv' file contains protein expression data, which are extracted from PXD008522 (Das et al. 2018). The 'Enrichment data_example.csv' file contains the enrichment analysis results of these proteins.

1.1 Objects (genes/proteins/metabolites) expression data

Different object data need different strategies to obtain the expression data (Brazma and Vilo 2000; Xia and Wishart 2010; Zhang et al. 2004), but the final formats are usually as shown below.

	A	B	C	D	E	F	G
1	a.	BT549_1	BT549_2	BT549_3	BT549DoxR_1	BT549DoxR_2	BT549DoxR_3
2	A0A024R412	1.059506	1.283269	0.214464	-0.822823392	-0.909236124	-0.82518021
3	A0A087WTA8	1.203369	1.016294	0.423007	-0.951003402	-0.796641649	-0.895024749
4	A0A0A0MRM8	-0.87877	-0.9258	-0.88837	0.754659969	0.710834499	1.227454102
5	A0A0A0MTH3	-0.90821	-0.94375	-0.8573	0.716695613	1.155494034	0.83707639
6	A0A0C4DGB5	-0.76534	-0.97416	-0.87332	1.331006196	0.87486418	0.406952287
7	B4DHE8	-0.7318	-0.92715	-0.92916	0.634944351	1.440765632	0.512403765
8	B7ZL14	0.444225	0.738294	1.414579	-0.794439954	-0.883569709	-0.919087885
9	C9J3D7	-0.85009	-0.96627	-0.89219	0.896350298	0.680054135	1.132144448
10	C9J6P4	1.11088	0.898826	0.702195	-0.828559798	-0.991580309	-0.891760745
11	C9JTE9	0.813344	1.100614	0.793451	-0.719331199	-1.038144629	-0.949933464
12	E7EV99	1.209935	0.820583	0.654708	-0.9219523	-1.003077083	-0.760196609
13	F5H345	-1.02479	-0.74981	-0.95088	0.977722465	0.865618721	0.882141359
14	J3KQ48	-0.96425	-0.91211	-0.7643	0.646871137	0.644714482	1.34907925
15	J3QRU1	1.145576	1.00803	0.525084	-0.887914161	-0.940037309	-0.850738713
16	O00151	-0.78797	-0.90148	-0.98002	0.801803834	1.264754783	0.602911702
17	O00592	1.015921	1.045755	0.649597	-0.881145621	-0.939818539	-0.890308402
18	O00625	-0.88022	-0.84651	-0.80439	1.286553014	0.157593249	1.086980006
19	O14907	0.853003	1.352031	0.400099	-0.765250921	-0.975289783	-0.864592167
20	O15075	0.429311	0.989474	1.226264	-0.859006902	-0.897560576	-0.888482192
21	O43852	0.299224	1.003515	1.288304	-0.883249245	-0.905318909	-0.802474524
22	O43854	-0.81727	-0.81507	-0.80942	0.074459052	0.863518832	1.503782181
23	O60701	1.213452	0.903693	0.561584	-0.905306442	-0.84545998	-0.927962439
24	O75874	1.053606	0.751383	0.909227	-0.836992278	-1.072967803	-0.804256174
25	O95340	1.329013	0.669268	0.658082	-0.897059668	-0.894562498	-0.864740307
26	O95671	1.49785	0.199451	0.798682	-0.82347645	-0.804478886	-0.86802776
27	P05161	1.014788	0.077106	1.384383	-0.851998474	-0.848894233	-0.775384158
28	P05556	0.953843	0.906458	0.873183	-0.863287075	-0.855203781	-1.014813431

a. b. c.

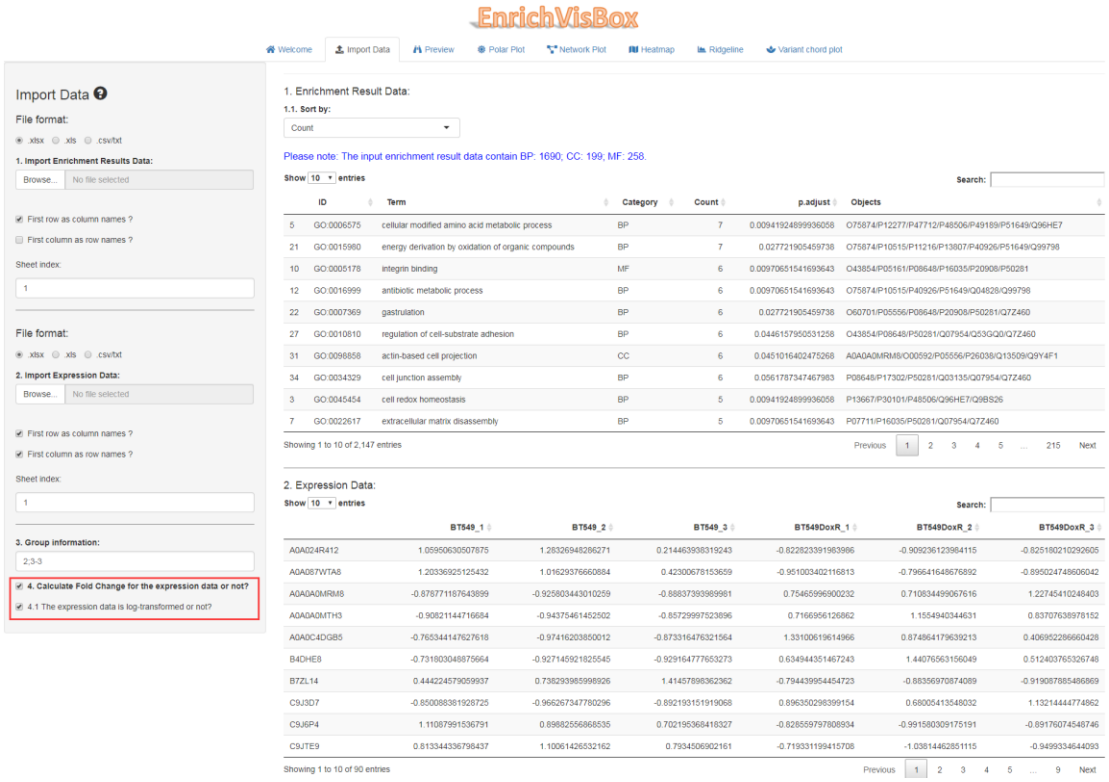
There are three main sections:

a. Column name: these are sample names. In the example data, there are two group samples (three biological replicates in each group).
b. Row names: these can be gene/protein/metabolite names or some kind of ID, for instance, the ones here are protein Uniprot IDs.
c. Expression values: these can be original measured values or normalized values, for example, the ones here are normalized peak intensities.

Here, in EnrichVisBox, users could include an additional column, fold change, the variable importance in projection (VIP) value derived from the PLSDA or OPLSDA model (Worley, B. and Powers, R. 2013), or other similar values. For example, the log2 fold change between two groups is added. Shown as below:

		BT549_1	BT549_2	BT549_3	BT549DoxR_1	BT549DoxR_2	BT549DoxR_3	Fold.Change
2	A0A024R412	1.059506	1.283269	0.214464	-0.822823392	-0.909236124	-0.82518021	-2.998247456
3	A0A087WTA8	1.203369	1.016294	0.423007	-0.951003402	-0.796641649	-0.895024749	-2.571503195
4	A0A0A0MRM8	-0.87877	-0.9258	-0.88837	0.754659969	0.710834499	1.227454102	4.822678072
5	A0A0A0MTH3	-0.90821	-0.94375	-0.8573	0.716695613	1.155494034	0.83707639	1.435108882
6	A0A0C4DGB5	-0.76534	-0.97416	-0.87332	1.331006196	0.87486418	0.406952287	1.575034541
7	B4DHE8	-0.7318	-0.92715	-0.92916	0.634944351	1.440765632	0.512403765	1.77553431
8	B7ZL14	0.444225	0.738294	1.414579	-0.794439954	-0.883569709	-0.919087885	-2.161046453
9	C9J3D7	-0.85009	-0.96627	-0.89219	0.896350298	0.680054135	1.132144448	4.156198372
10	C9J6P4	1.11088	0.898826	0.702195	-0.828559798	-0.991580309	-0.891760745	-1.179184593
11	C9JTE9	0.813344	1.100614	0.793451	-0.719331199	-1.038144629	-0.949933464	-1.458409586
12	E7EV99	1.209935	0.820583	0.654708	-0.9219523	-1.003077083	-0.760196609	-1.85472371
13	F5H345	-1.02479	-0.74981	-0.95088	0.977722465	0.865618721	0.882141359	1.401252417
14	J3KQ48	-0.96425	-0.91211	-0.7643	0.646871137	0.644714482	1.34907925	1.389542565
15	J3QRU1	1.145576	1.00803	0.525084	-0.887914161	-0.940037309	-0.850738713	-1.310422403
16	O00151	-0.78797	-0.90148	-0.98002	0.801803834	1.264754783	0.602911702	1.507742165
17	O00592	1.015921	1.045755	0.649597	-0.881145621	-0.939818539	-0.890308402	-2.68989838
18	O00625	-0.88022	-0.84651	-0.80439	1.286553014	0.157593249	1.086980006	2.550562206
19	O14907	0.853003	1.352031	0.400099	-0.765250921	-0.975289783	-0.864592167	-1.535320574
20	O15075	0.429311	0.989474	1.226264	-0.859006902	-0.897560576	-0.888482192	-6.177120425
21	O43852	0.299224	1.003515	1.288304	-0.883249245	-0.905318909	-0.802474524	-1.573375864
22	O43854	-0.81727	-0.81507	-0.80942	0.074459052	0.863518832	1.503782181	6.582184599
23	O60701	1.213452	0.903693	0.561584	-0.905306442	-0.84545998	-0.927962439	-1.616736253
24	O75874	1.053606	0.751383	0.909227	-0.836992278	-1.072967803	-0.804256174	-1.759064714
25	O95340	1.329013	0.669268	0.658082	-0.897059668	-0.894562498	-0.864740307	-2.61542742
26	O95671	1.49785	0.199451	0.798682	-0.82347645	-0.804478886	-0.86802776	-3.335527393
27	P05161	1.014788	0.077106	1.384383	-0.851998474	-0.848894233	-0.775384158	-2.611417709
28	P05556	0.953643	0.906458	0.873183	-0.863267075	-0.855203781	-1.014813431	-1.0718328

If users don't want to add the fold change by themselves, EnrichVisBox can calculate it for users after they select this parameter "4. Calculate Fold Change for the expression data or not?" below. The detailed description of this parameter can be found in the "Import data" part.



1.2 Enrichment analysis resulting data

After obtaining the object (gene/protein/metabolite) list, it is generally desirable to obtain the biological interpretation. A standard method of solving this problem is functional enrichment analysis, which summarizes the large object list as a smaller list of more easily interpretable biological functions based on some existing databases, such as the GO database(Carbon et al. 2009) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database(Kanehisa and Goto 2000). The final file format may be like the one below, which is an example result of GO enrichment analysis.

	ID	Description	ONTOLOGY	Count	p.adjust	geneID
2	GO:2000811	negative regulation o	BP	4	0.009419	J3KQ48/P05556/P08648/Q03135
3	GO:2000209	regulation of anoikis	BP	4	0.009419	J3KQ48/P05556/P08648/Q03135
4	GO:0045454	cell redox homeostasi	BP	5	0.009419	P13667/P30101/P48506/Q96HE7/Q9BS26
5	GO:0006099	tricarboxylic acid cy	BP	4	0.009419	O75874/P10515/P40926/Q99798
6	GO:0006575	cellular modified ami	BP	7	0.009419	O75874/P12277/P47712/P48506/P49189/P51649/Q96HE7
7	GO:0006101	citrate metabolic pro	BP	4	0.009707	O75874/P10515/P40926/Q99798
8	GO:0022617	extracellular matrix	BP	5	0.009707	P07711/P16035/P50281/Q07954/Q7Z460
9	GO:0043276	anoikis	BP	4	0.009707	J3KQ48/P05556/P08648/Q03135
10	GO:0033631	cell-cell adhesion me	BP	3	0.009707	O00592/P05556/P08648
11	GO:0005178	integrin binding	MF	6	0.009707	O43854/P05161/P08648/P16035/P20908/P50281
12	GO:0072350	tricarboxylic acid me	BP	4	0.009707	O75874/P10515/P40926/Q99798
13	GO:0016999	antibiotic metabolic	BP	6	0.009707	O75874/P10515/P40926/P51649/Q04828/Q99798
14	GO:0035313	wound healing, spread	BP	3	0.009761	P08648/P20908/Q7Z460
15	GO:1901888	regulation of cell ju	BP	5	0.023368	P17302/P50281/Q03135/Q07954/Q7Z460
16	GO:0016616	oxidoreductase activi	MF	5	0.024569	O60701/O75874/P40926/Q04828/Q53GQ0
17	GO:0001968	fibronectin binding	MF	3	0.026621	P05556/P07711/Q53GQ0
18	GO:0030175	filopodium	CC	5	0.026621	O00592/P05556/P26038/Q13509/Q9Y4F1

ID: GO ID;

Description: A statement about the function of objects;

ONTOLOGY: A formal representation of a body of knowledge within a given domain — the detailed information about ontology can be found here: http://geneontology.org/docs/ontology-documentation;

Count: Number of enriched objects (genes/proteins/metabolites);

p.adjust: Adjusted p values;
geneID: IDs/names of enriched objects (genes/proteins/metabolites).
It is recommended that users prepare their enrichment analysis resulting data with the same columns, but the content of each column can be different.

An example result of KEGG enrichment analysis can be like below:

ID	Description	ONTOLOGY	Count	p.adjust	geneID
hsa00020	Citrate cycle (TCA cycle	KEGG	5	0.000375	O75874/P10515/P40926/Q16822/Q99798
hsa00620	Pyruvate metabolism	KEGG	4	0.021521	P10515/P40926/P49189/Q16822
hsa01100	Metabolic pathways	KEGG	18	0.081561	O60701/O75874/O95340/P10515/P11216/P12277/P13
hsa05205	Proteoglycans in cancer	KEGG	6	0.081561	P05556/P07711/P08648/P26038/Q03135/Q14643
hsa00330	Arginine and proline met	KEGG	3	0.120031	P12277/P13674/P49189
hsa04922	Glucagon signaling pathw	KEGG	4	0.120031	P11216/P13807/Q14643/Q16822
hsa01210	2-Oxocarboxylic acid met	KEGG	2	0.143221	O75874/Q99798
hsa01200	Carbon metabolism	KEGG	4	0.164541	O75874/P10515/P40926/Q99798
hsa00010	Glycolysis / Gluconeogen	KEGG	3	0.173616	P10515/P49189/Q16822
hsa05412	Arrhythmogenic right ven	KEGG	3	0.173616	P05556/P08648/P17302
hsa00053	Ascorbate and aldarate m	KEGG	2	0.173616	O60701/P49189
hsa00650	Butanoate metabolism	KEGG	2	0.173616	P51649/P55809
hsa05100	Bacterial invasion of ep	KEGG	3	0.173616	P05556/P08648/Q03135
hsa00500	Starch and sucrose metab	KEGG	2	0.173616	P11216/P13807
hsa04512	ECM-receptor interaction	KEGG	3	0.173616	P05556/P08648/P12111
hsa04540	Gap junction	KEGG	3	0.173616	P17302/Q13509/Q14643
hsa00630	Glyoxylate and dicarboxy	KEGG	2	0.173616	P40926/Q99798
hsa03030	DNA replication	KEGG	2	0.186366	P25205/P33992
hsa04151	PI3K-Akt signaling pathw	KEGG	6	0.186366	P05556/P08648/P12111/P13807/P61952/Q16822
hsa04141	Protein processing in en	KEGG	4	0.191075	P13667/P19525/P30101/Q96HE7
hsa04912	GnRH signaling pathway	KEGG	3	0.192732	P47712/P50281/Q14643
hsa04145	Phagosome	KEGG	4	0.192732	P05556/P07711/P08648/Q13509
hsa04931	Insulin resistance	KEGG	3	0.192732	P11216/P13807/Q16822
hsa04216	Ferroptosis	KEGG	2	0.232831	P48506/Q15043

Please note, as KEGG doesn't have three namespaces as in GO, users could add one more column "ONTOLOGY" easily by themselves, in which all values could be "KEGG".

How to process functional enrichment analysis?

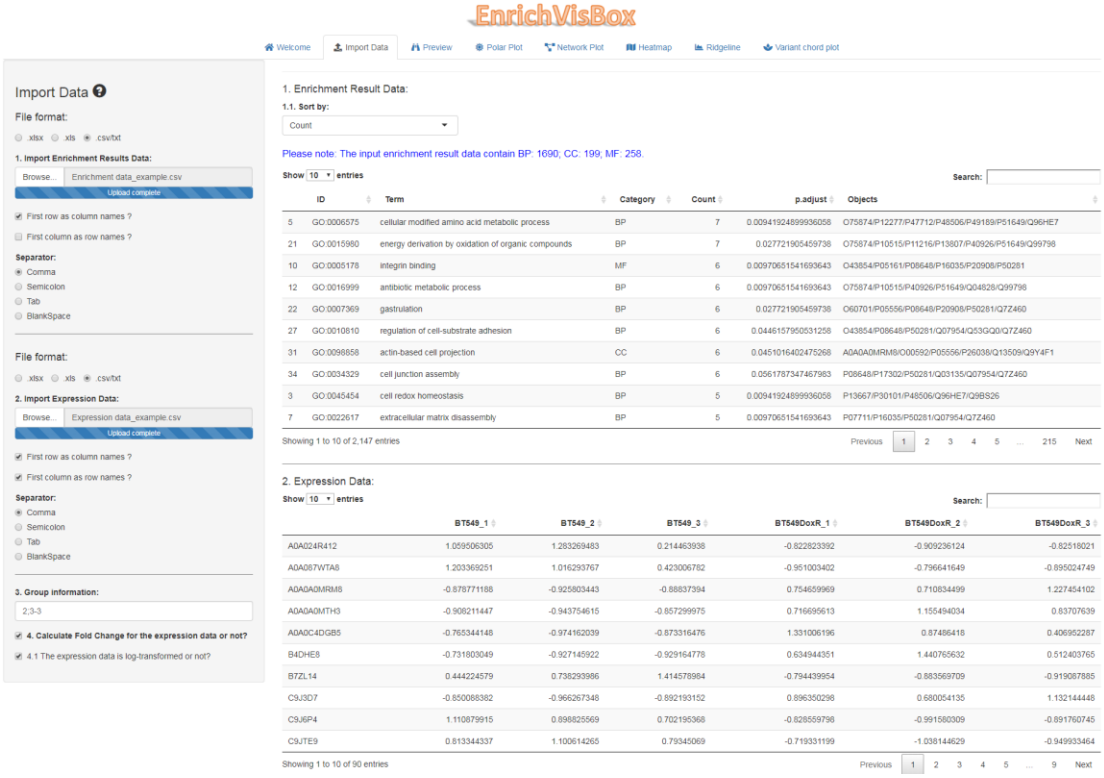
Some users, especially beginners, may have this question. Here, some published software tools are recommended.

Data type	Tool names	Links
Genomics	Enrichr(Chen et al. 2013)	https://amp.pharm.mssm.edu/Enrichr
	DAVID(Dennis et al. 2003)	https://david.ncifcrf.gov
Proteomics	MixProTool(Wang et al. 2018)	https://www.omicsolution.org/wukong/MixProTool
	FunRich(Pathan et al. 2015)	http://www.funrich.org
Metabolomics	MetaboAnalyst(Xia et al. 2009)	https://www.metaboanalyst.ca
	ConsensusPathDB(Kamburov et al. 2008)	http://consensuspathdb.org

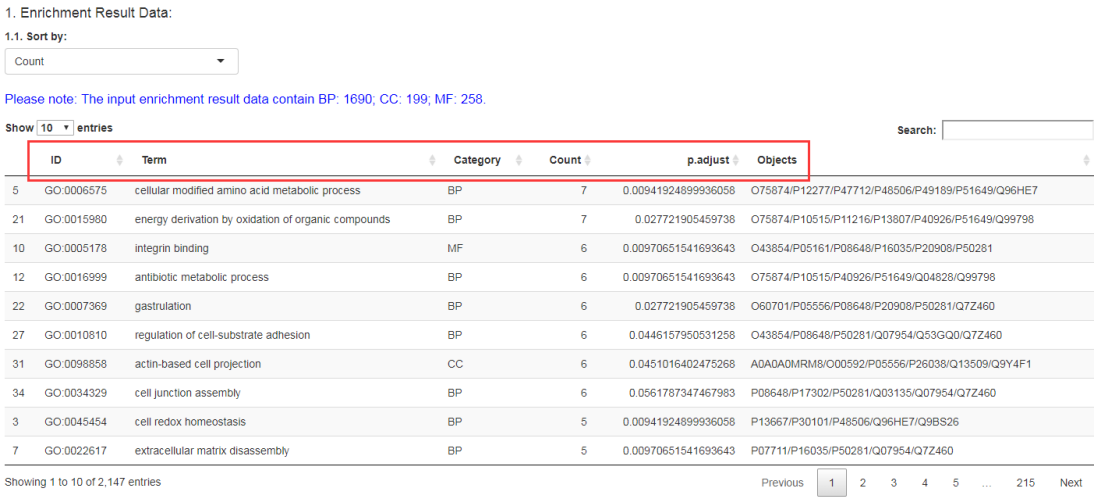
If users are familiar with the R language, they can choose to use some R packages, such as clusterProfiler**(Yu et al. 2012)**, and topGO**(Alexa and Rahnenfuhrer 2010)**. Whichever tool users choose to use, it is recommended that they assign their enrichment results as above.

2. Import data

When users prepare their data (expression data and enrichment analysis resulting data), they can click ‘Import Data’ and upload the data from here.



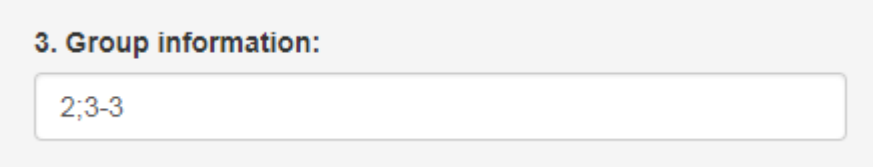
Users should choose the right file format based on their data (the example data have been saved as a .csv file, so.csv/.txt is chosen here) and then click the ‘Browse’ button to import the data. To facilitate subsequent analysis, the columns in the enrichment analysis resulting data (Section 1.2) were renamed as: *ID*, *Term*, *Category*, *Count*, *p.adjust*, and *Objects*.



Header: this means whether the first row is column names. If true, you should choose this parameter.
First column: this indicates whether the first column is row names. If true, you should choose this parameter.

Separator: this is the field separator character. Values on each line of the file are separated by this character.

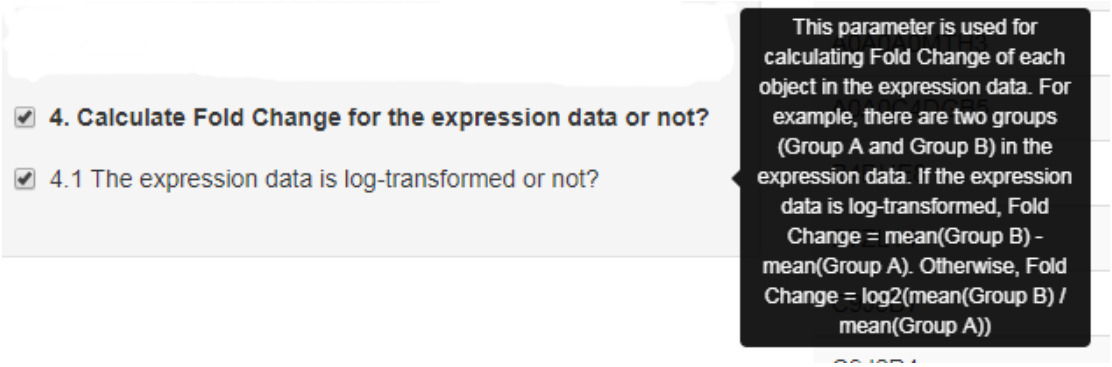
Here, one should also type in the ‘Group information’:



As described above, there are two group samples (three replicates in each group) in the example data, so here one should input ‘2;3-3’. Please note this format. One more example is given here for users to understand this parameter better.
If your data have five groups and six replicates in each group, you should input ‘5;6-6-6-6-6’.

Then, users should notice that whether their input expression data contain one more column (e.g. fold change). If they have calculated the fold change, just unselect this parameter below “4. Calculate Fold Change for the expression data or not?” And if not, users can select this parameter, and this tool will calculate fold change for users.

When calculating fold change, this tool considers two situations, which controlled by the parameter “4.1 The expression data is log-transformed or not?” For example, there are two groups (Group A and Group B) in the expression data. If the expression data is log-transformed, Fold Change = mean(Group B) - mean(Group A). Otherwise, Fold Change = log2(mean(Group B) / mean(Group A)).



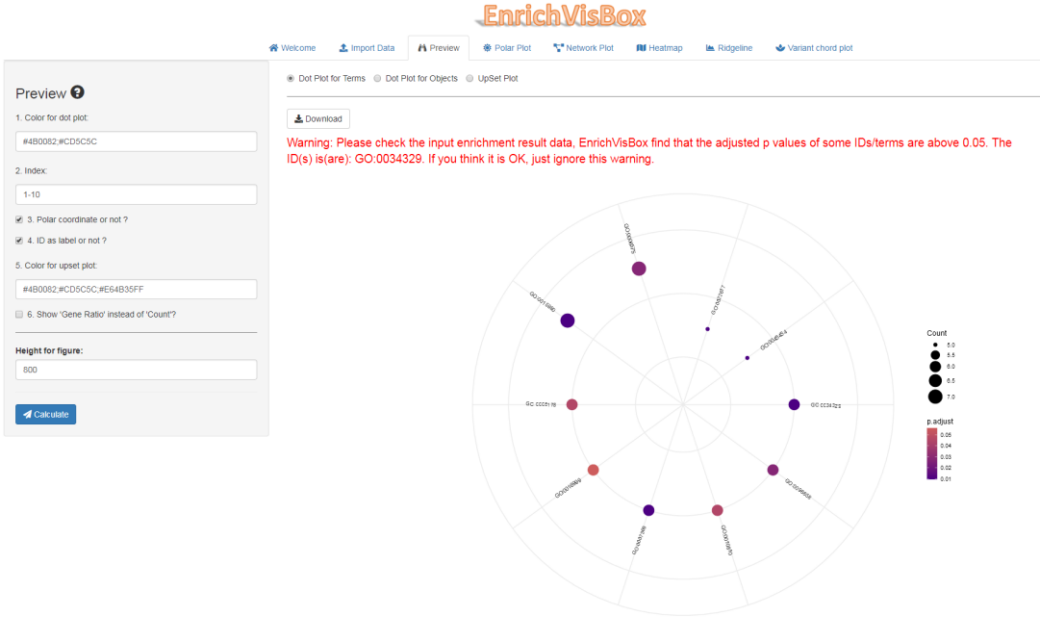
If the data have been uploaded successfully, the results will be shown simultaneously on the right panel.

3. Preview

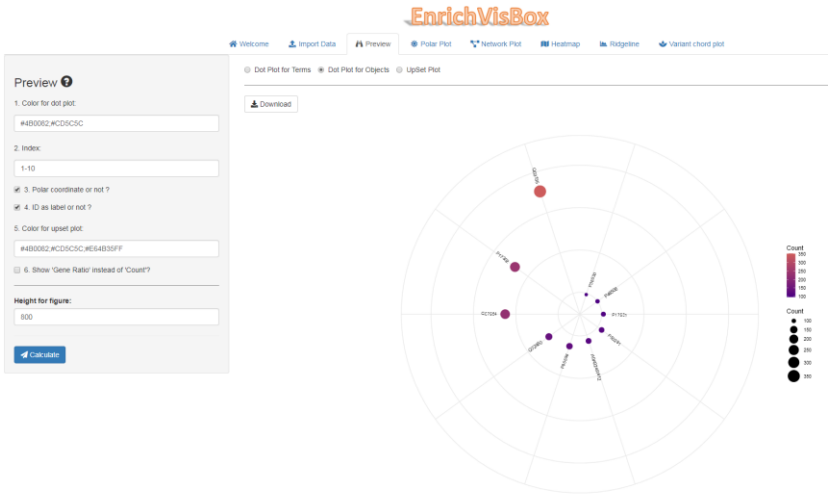
After importing data with the right format, users can click 'Preview', which means users can visualize some basic information about their data in this step. There are three parts in this module.

a. *Dot Plot for Terms*: to show the terms with bubbles. The count of one term is larger, the bubble size is bigger, and the colour of each bubble corresponds to the adjusted p value. This plot was implemented with geom_point and coord_polar functions in the ggplot2 package (Wickham 2016).

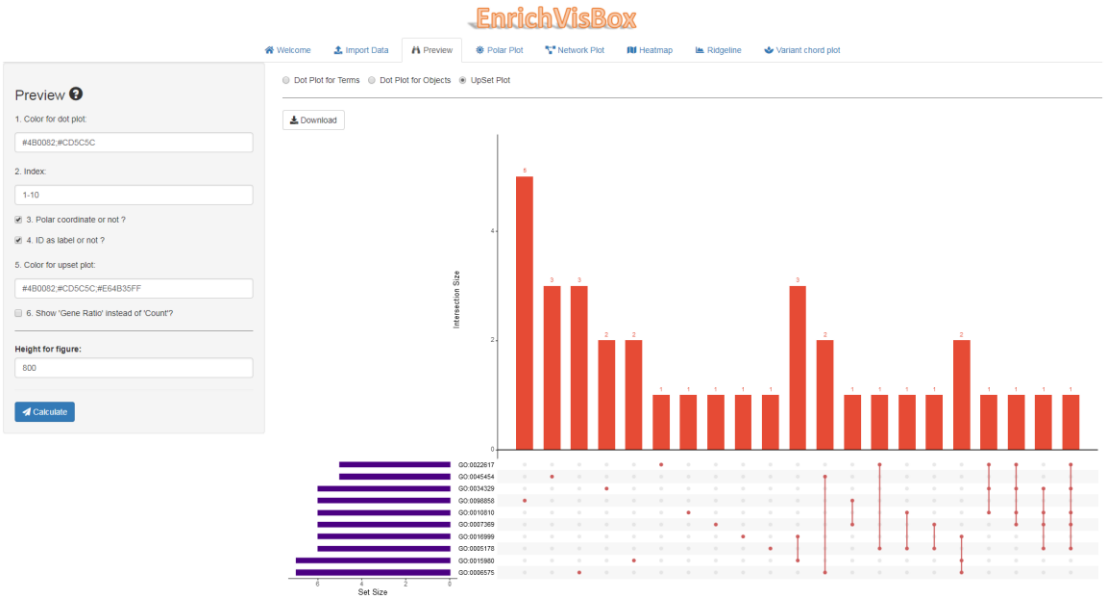
Here, this tool will check the adjusted p values, if it find any adjusted p-values above 0.05, it will give users a warning and tell users which IDs, as below:



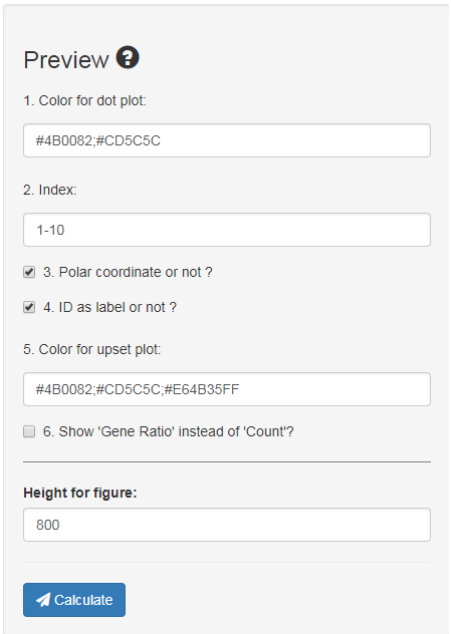
b. *Dot Plot for Objects*: to show the objects (genes/proteins/metabolites) with bubbles. Because most of objects may be overrepresented in more than one term, this part can count how many terms every object is assigned to. The number is larger, the bubble size is bigger, and the colour of each bubble here corresponds to the number. This plot was implemented with geom_ point and coord_polar functions in the ggplot2 package (Wickham 2016).



c. *UpSet Plot*: to show interactions among the terms. Because there are the same or different objects between each two terms, this part can show their overlap. This plot was implemented with the upset function in the UpSetR package (Conway et al. 2017).



Parameters in this module:



1. Color for dot plot: to change bubble colour. Users can type in two colour names with a semicolon. One color name can be a common English name (e.g. red, blue, yellow etc.), or a hex triplet (e.g. #FF0000, #0000FF, #FFFF00 etc.). The first one is for the lowest p.adjust, and the second one is for the highest p.adjust.

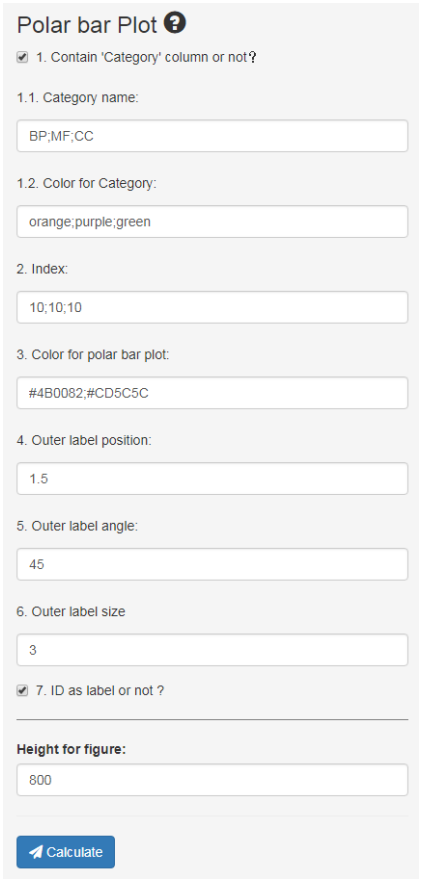
2. Index: which terms or objects will be plotted. The default '1-10' means the top 10 (1 to 10) terms or objects are shown. If users type in '10-21', it means it shows the 10th to 21st terms or objects (total of 12 terms or objects). If users input '1,10,21', this means it will show the 1st,

the 10th, and the 21st terms or objects (total three terms or objects).
3. Polar coordinate or not: if the dot plot is shown in a circle or not, the default is true, otherwise, the dot plot is shown in a normal rectangular coordinate system.
4. ID as label or not: the label in the dot plot is shown with IDs or Terms, the default is with IDs.
5. Color for upset plot: to change colours in the upset plot. Users can type in three colour names with two semicolons. One color name can be a common English name (e.g. red, blue, yellow etc.), or a hex triplet (e.g. #FF0000, #0000FF, #FFFF00 etc.). The first one is the colour of the set size bar plot, the second one is the colour of the intersection points, and the third one is the colour of the main bar plot.
6. Show 'Gene Ratio' instead of 'Count'?: If true, users should type in the total ID number below. And the total number can be obtained easily after they process the enrichment analysis. Then the count number will be replaced by gene ratio in the dot plot. If false, just ignore this parameter.
6.1. Please type in the total ID number: Please change this value based on your own data, the default value is used for the example data.
Height for figure: adjusting the height of the figure.

4. Polar plot

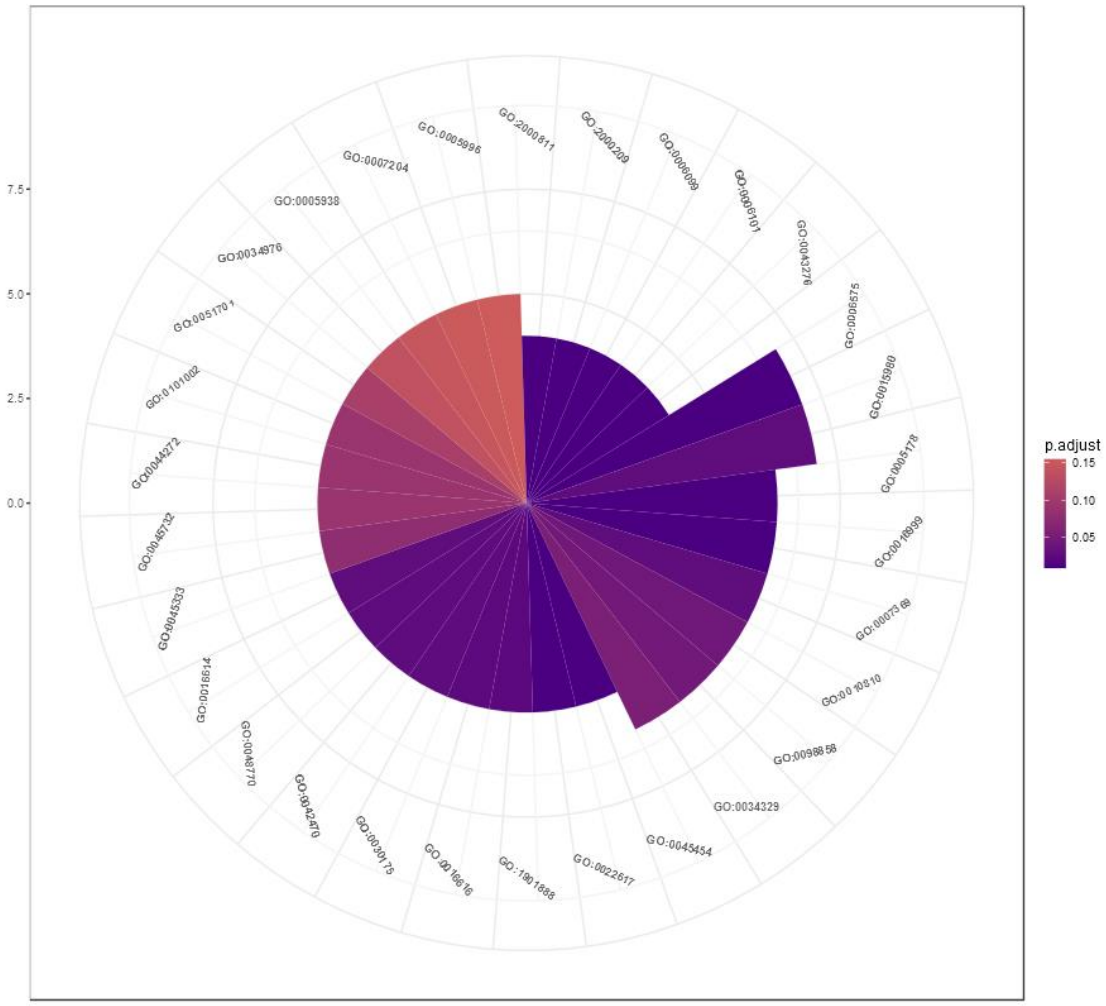
This module shows data with the polar bar plot, which involves in *ID (Term)*, *Category*, *Count*, and *p.adjust* columns. Different *Categories* show discrete colours in the outermost circle, and the bar colours are filled with continuous colours corresponding to adjusted p values. This plot was implemented with the geom_bar, geom_segment and coord_polar functions in the ggplot2 package (Wickham 2016).

Parameters in this module:



Contain 'Category' column or not: if there is a '*Category*' column in the enrichment analysis resulting data, users should select this parameter, and then input category names in the *Category name* parameter and colour names in the *Color for category* parameter. The number should be same, which means there are three categories, so there should be three colours here. For example, there are three namespaces in GO analysis results (e.g. BP, MF, CC), users can type in three names here ("BP;MF;CC"), then type in three colors next. In addition, users can also type in one namespace, e.g. BP, then this tool will show a figure just for BP.

Index: which terms will be plotted. If users select the *Contain 'Category' column or not* parameter, the index number should be the same as the category number and linked with semicolons. For instance, there are three categories in the example data, and, to show top 10 (1 to 10) terms in each category, '10;10;10' is input here. If users unselect the *Contain 'Category' column or not* parameter, the number here will sum up, which means the plot will show the top 30 ('10;10;10' is typed in here) terms in all the data, as below.



Color for polar bar plot: to change the bar colour. Users can type in two colour names with a semicolon. One color name can be a common English name (e.g. red, blue, yellow etc.), or a hex triplet (e.g. #FF0000, #0000FF, #FFFF00 etc.). The first one is for lowest p.adjust, and the second one is for highest p.adjust.

Outer label position: to change the position of outer labels.

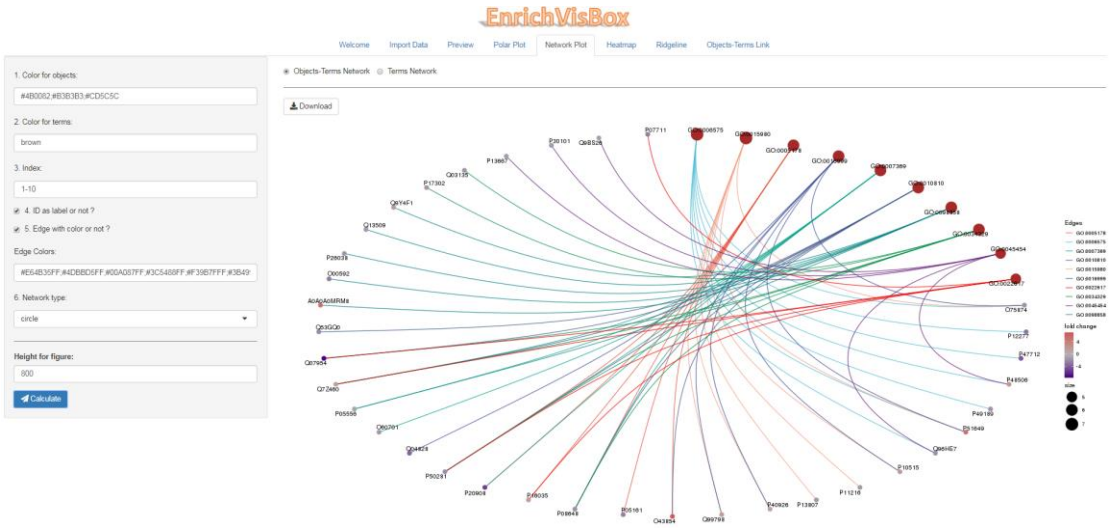
Outer label angle: to change the angle of outer labels.

Outer label size: to change the size of outer labels.
Height for figure: adjusting the height of the figure.

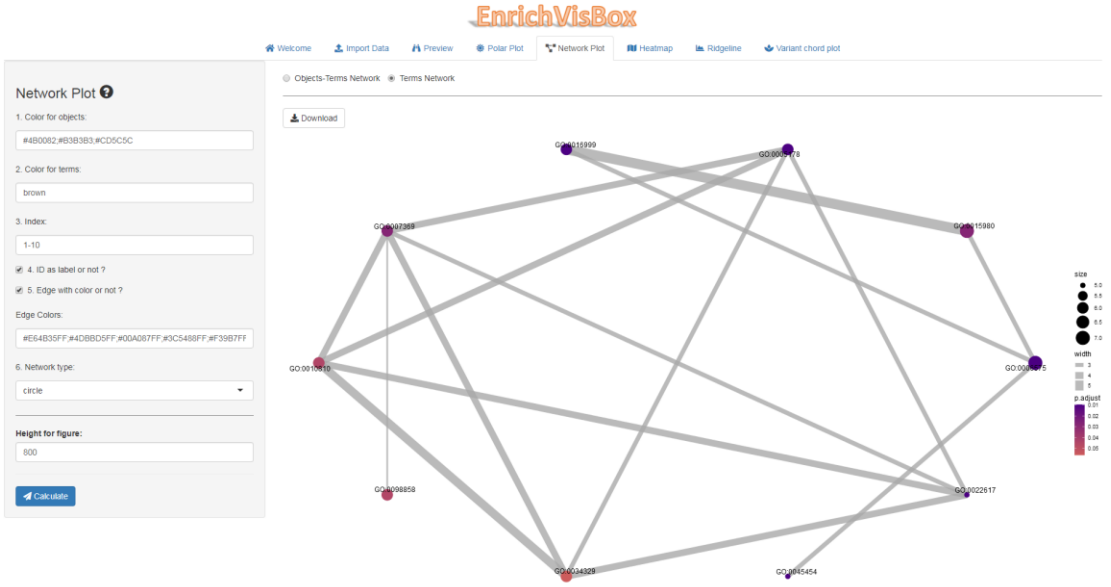
5. Network plot

There are two types in this module. The first one shows the interactions between each object and ID/term, and the second one shows the interactions between every two IDs/terms. This plot was implemented with the cnetplot and emapplot functions in the enrichplot package (Yu et al. 2012).

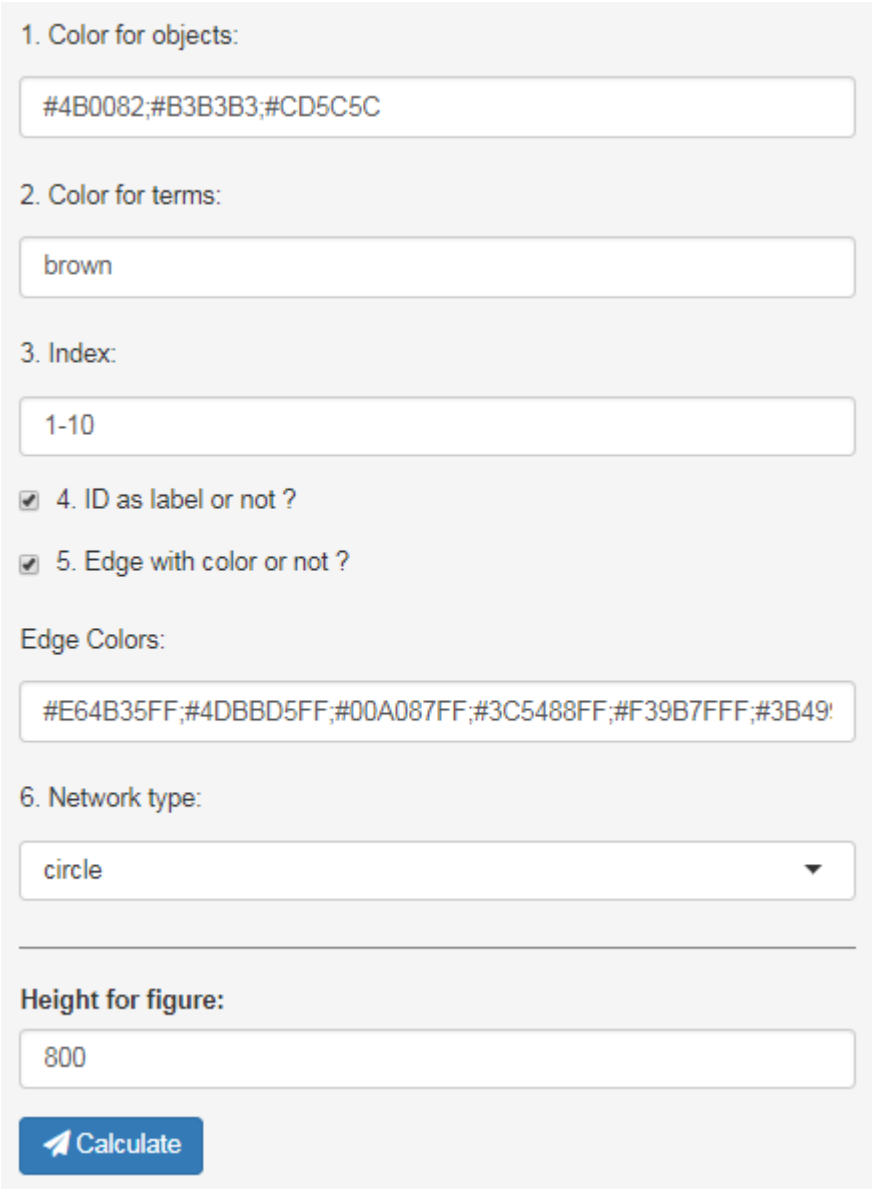
a. *Objects-Terms Network:* the objects are linked via coloured lines to their assigned IDs/terms. The line colours mean different IDs/terms, the colour of each bubble corresponds to fold change (log2 here), and, the larger the count of one term, the bigger the bubble size.



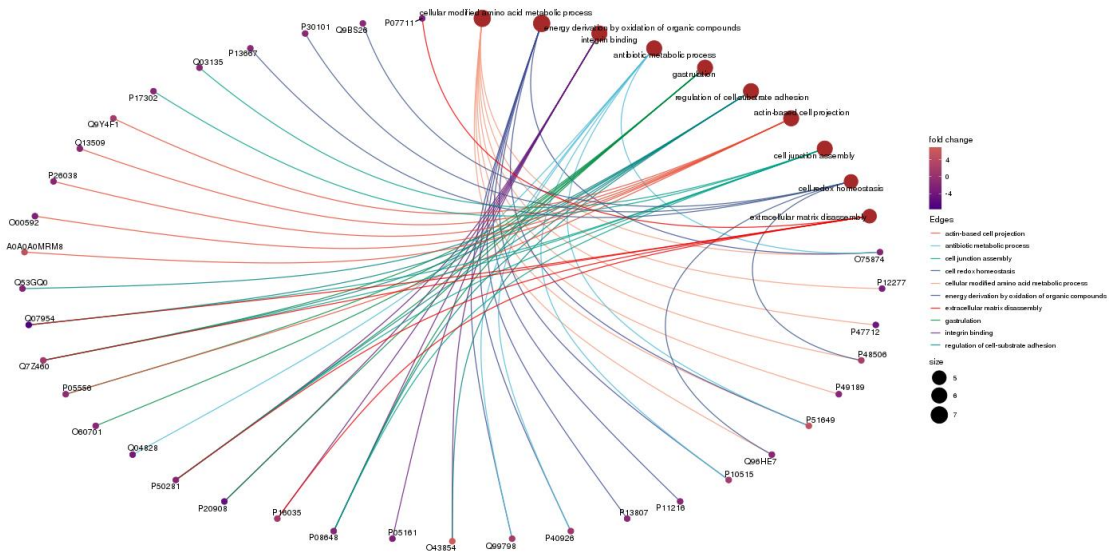
b. *Terms Network:* this plot shows each ID/term with edges linking overlapped objects between every two enriched IDs/terms. When the number of overlapped objects between two IDs/terms is larger, the line size is bigger. Colours of points correspond to adjusted p values.



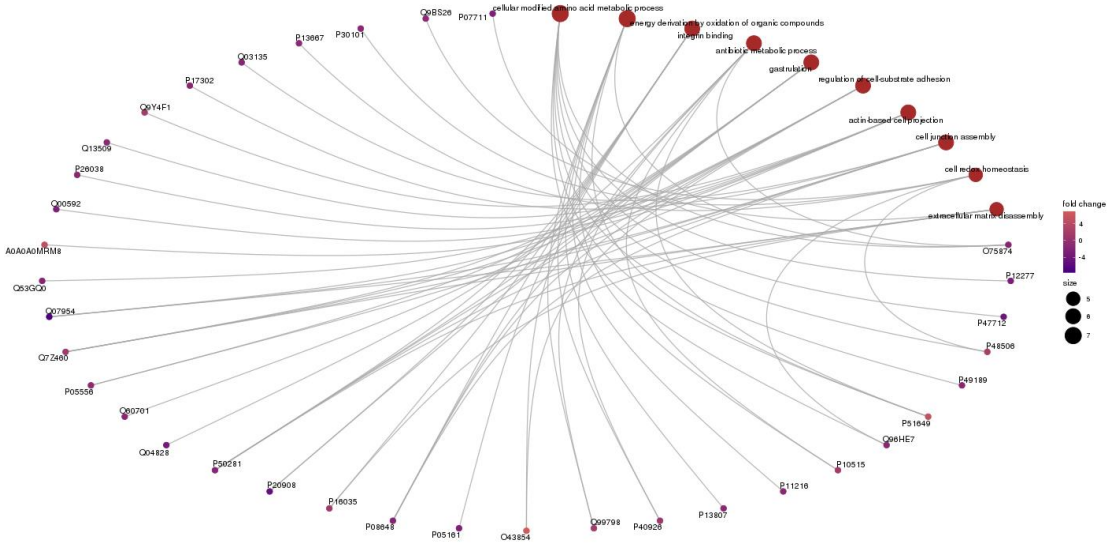
Parameters in this module:



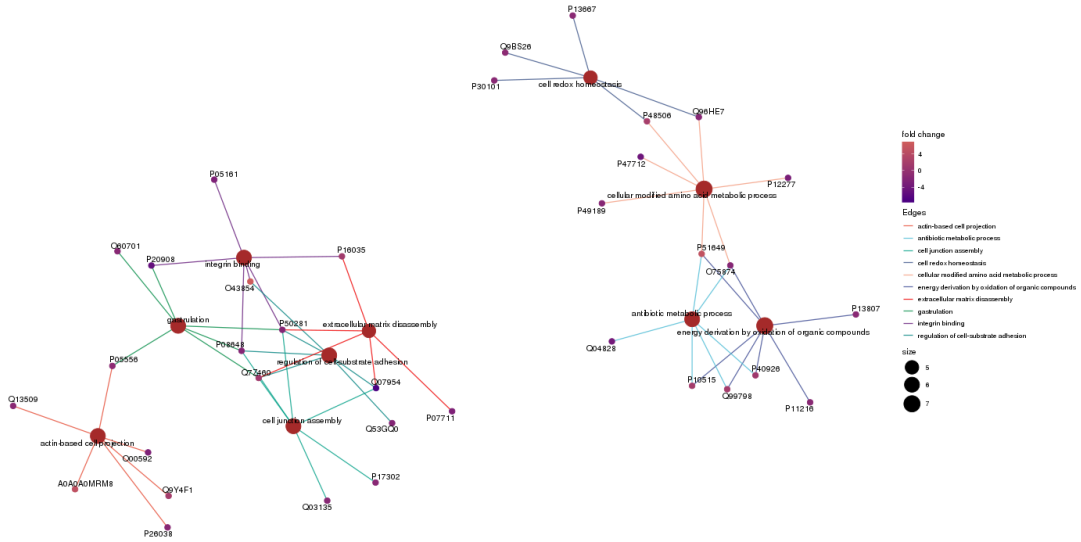
Color for objects: to change point (object) colour. Users can type in two or three colour names with a/two semicolon(s). One color name can be a common English name (e.g. red, blue, yellow etc.), or a hex triplet (e.g. #FF0000, #0000FF, #FFFF00 etc.). The first one is for the lowest fold change value (log2 here), and the last one is for the highest fold change value (log2 here).
Color for terms: to change point (ID/term) colour.
Index: which terms or objects will be plotted. The default '1-10' means the top 10 (1 to 10) terms or objects are shown. If users type in '10-21', it means it shows the 10th to 21st terms or objects (total of 12 terms or objects). If users input '1,10,21', this means it will show the 1st, 10th, and 21st terms or objects (total of three terms or objects).
ID as label or not: if selected, the IDs will be shown; otherwise, the terms will be shown, as below.



Edge with color or not: if selected, users should type in the same number of colours in the *Edge colors* parameter. For example, if '1-10' is input in the *index* parameter, here one should type in 10 colour names for each ID/term. If it is not selected, the line colours will become grey, as below.



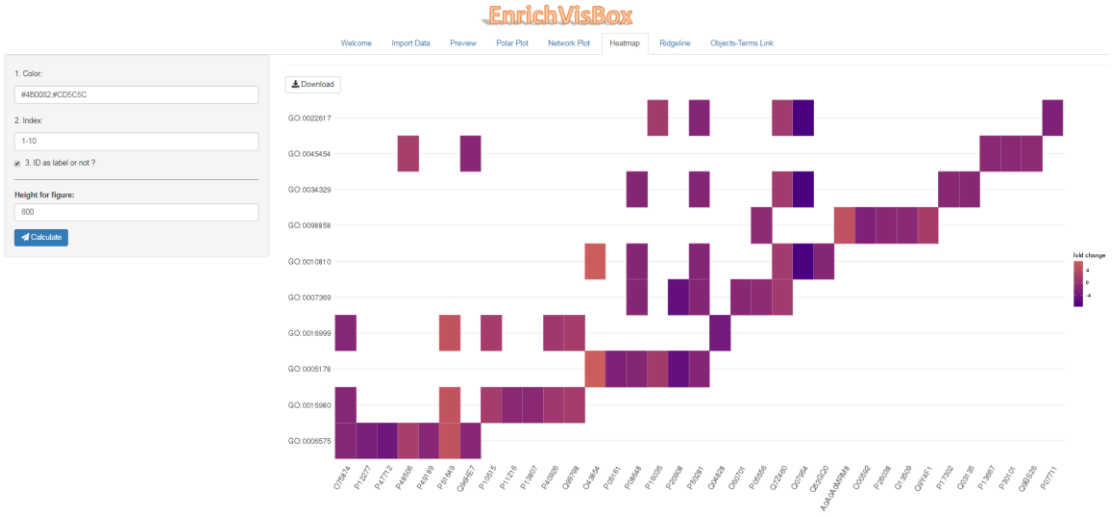
Network type: to determine how the vertices are placed on the plot. There are two types here: circle and kk. Circle means showing the plot with a circular layout, and kk means placing the vertices on the plane based on the Kamada–Kawai layout algorithm (Kamada and Kawai 1989).



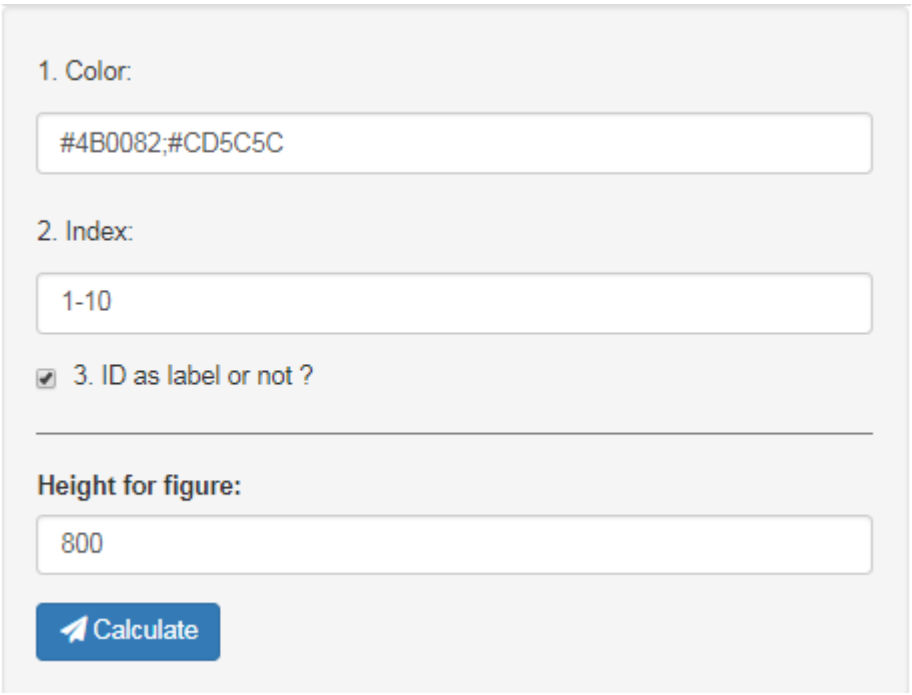
Height for figure: adjusting the height of the figure.

6. Heatmap

This module shows a heatmap-like plot. There is a coloured rectangle if one object is mapped in one ID/term, and the colour corresponds to the fold change (log2 here); otherwise, there is nothing in the position. This plot was implemented with the geom_tile function in ggplot2 package (Wickham 2016).



Parameters in this module:

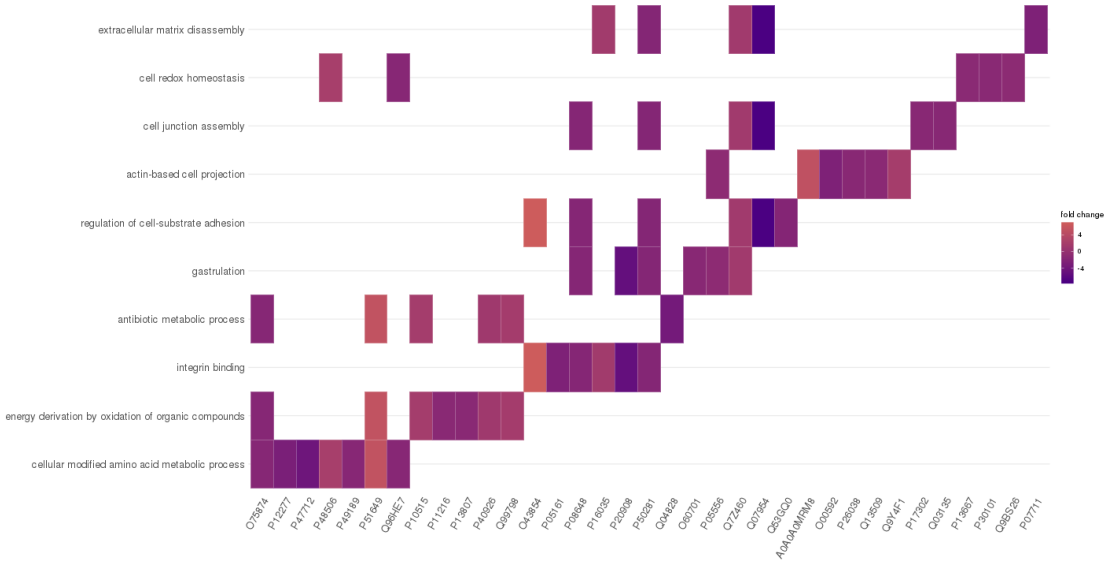


Color: to change rectangles colour. Users can type in two colour names with a semicolon. One color name can be a common English name (e.g. red, blue, yellow etc.), or a hex triplet (e.g. #FF0000, #0000FF, #FFFF00 etc.). The first one is for the lowest fold change value (log2 here), and the second one is for the highest fold change value (log2 here).

Index: which terms or objects will be plotted. The default '1-10' means the top 10 (1 to 10) terms or objects are shown. If users type in '10-21', it means it shows the 10th to 21st terms or objects (total of 12 terms or objects). If users input '1,10,21', this means it will show the 1st,

10th, and 21st terms or objects (total of three terms or objects).

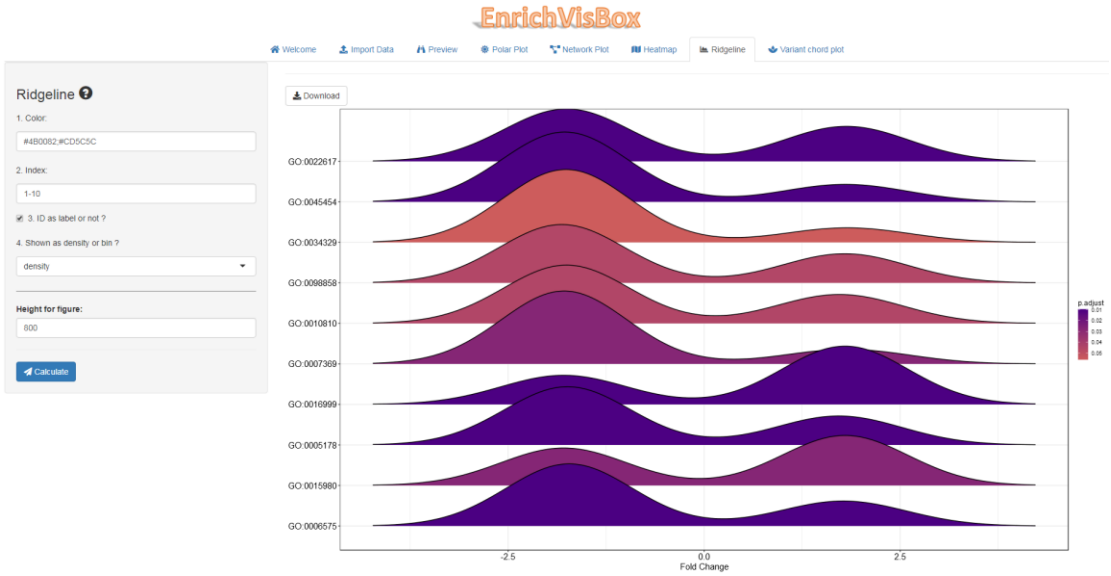
ID as label or not: if selected, the IDs will be shown; otherwise, the terms will be shown, as below.



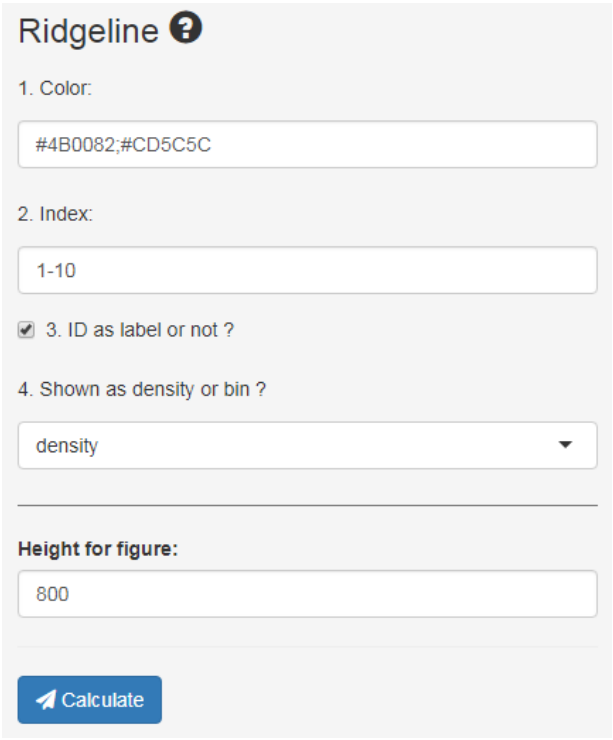
Height for figure: adjusting the height of the figure.

7. Ridgeline

This module shows the density distribution of all object fold changes (log2 here) across each ID/term, and colour corresponds to adjusted p values. This plot was implemented with the ridgeplot function in the enrichplot package(Yu et al. 2012).



Parameters in this module:

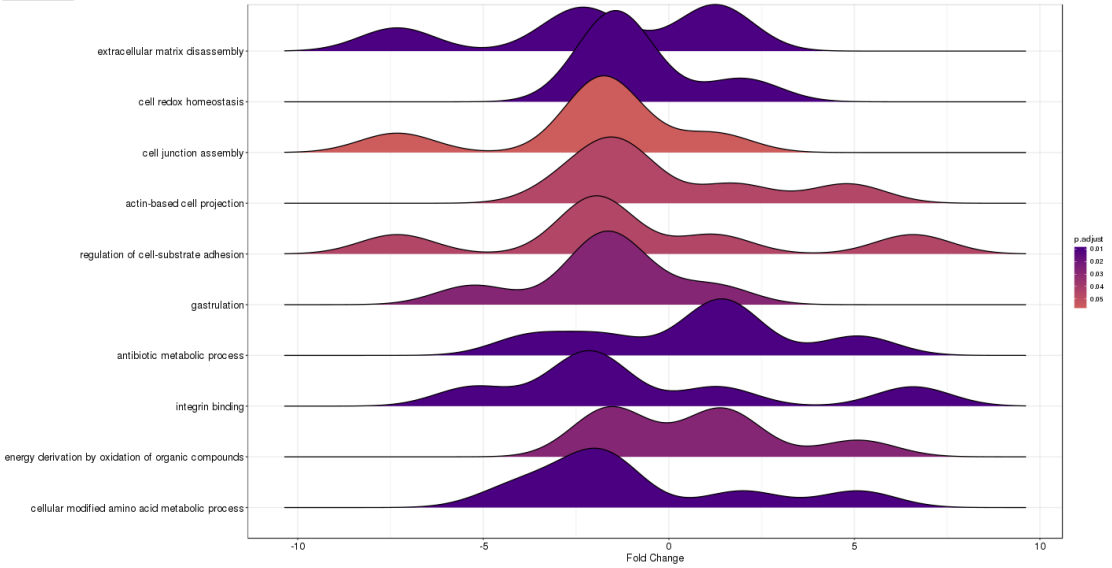


Color: to change the ridgeline colour. Users can type in two colour names with a semicolon. One color name can be a common English name (e.g. red, blue, yellow etc.), or a hex triplet (e.g. #FF0000, #0000FF, #FFFF00 etc.). The first one is for the lowest adjusted p value, and the second one is for the highest adjusted p value.

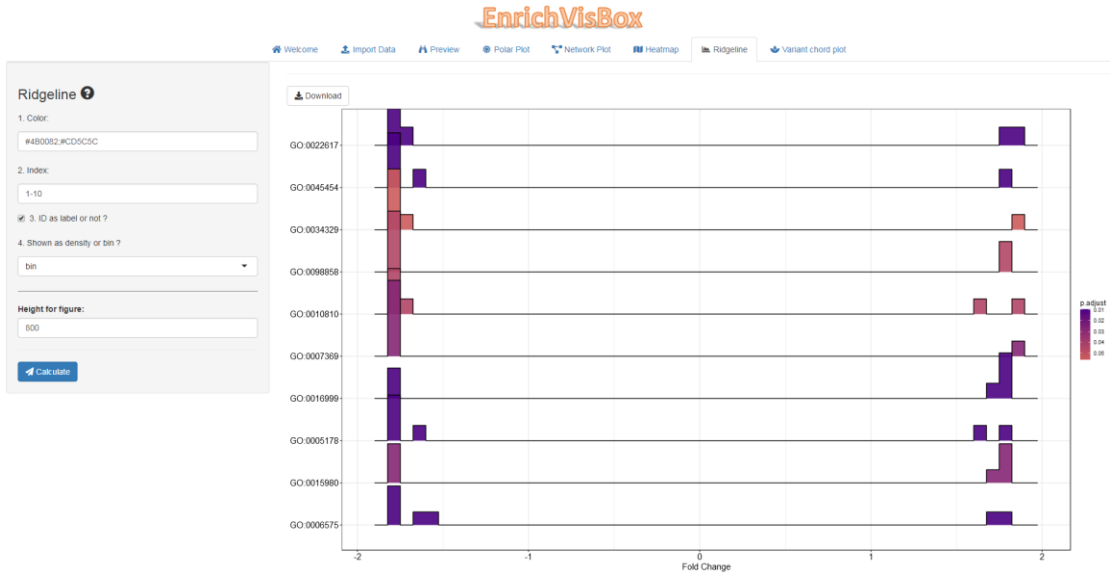
Index: which terms or objects will be plotted. The default '1-10' means the top 10 (1 to 10) terms or objects are shown. If users type in '10-21', it means it shows the 10th to 21st terms or

objects (total of 12 terms or objects). If users input '1,10,21', this means it will show the 1st, the 10th, and the 21st terms or objects (total of three terms or objects).

ID as label or not: if selected, the IDs will be shown; otherwise, the terms will be shown, as below.



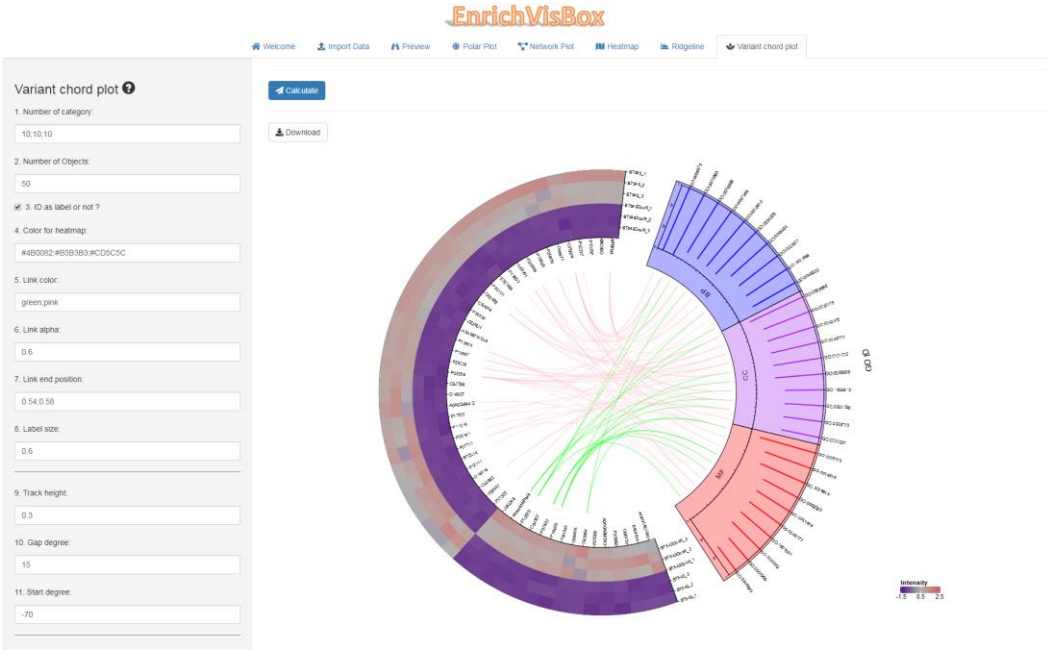
Shown as density or bin: "density" means the distributions are shown as densities; "bin" means the distributions are shown as bins. It is not quite proper to show the distribution as density when the number of objects is low, so users can choose "bin" to show the distribution. The bins are shown as below:



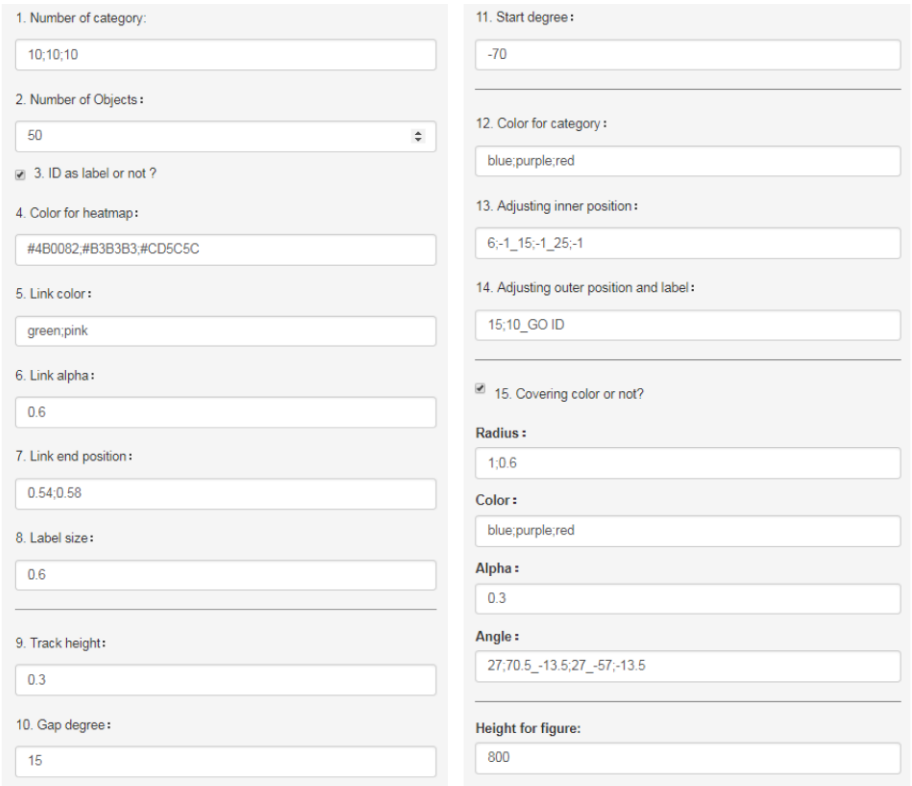
Height for figure: adjusting the height of the figure.

8. Variant chord plot

This plot can show the "Objects-Terms Link", which means it can present the enrichment result data and the expression data together. The left part is the intensity heatmap of proteins in every sample, and the right part is a bar plot of each ID/term; the two parts are linked via coloured lines (green means up-regulated, brown means down-regulated, and colours can be adjusted in the corresponding parameter). This plot was implemented with the circos.trackPlotRegion, circos.rect, circos.lines, and draw.sector functions in the circlize package (Gu et al. 2014).



Parameters in this module:



Warm tips:
Because this module is somewhat complicated, users need to be careful when analysing their own data, especially when adjusting these parameters.

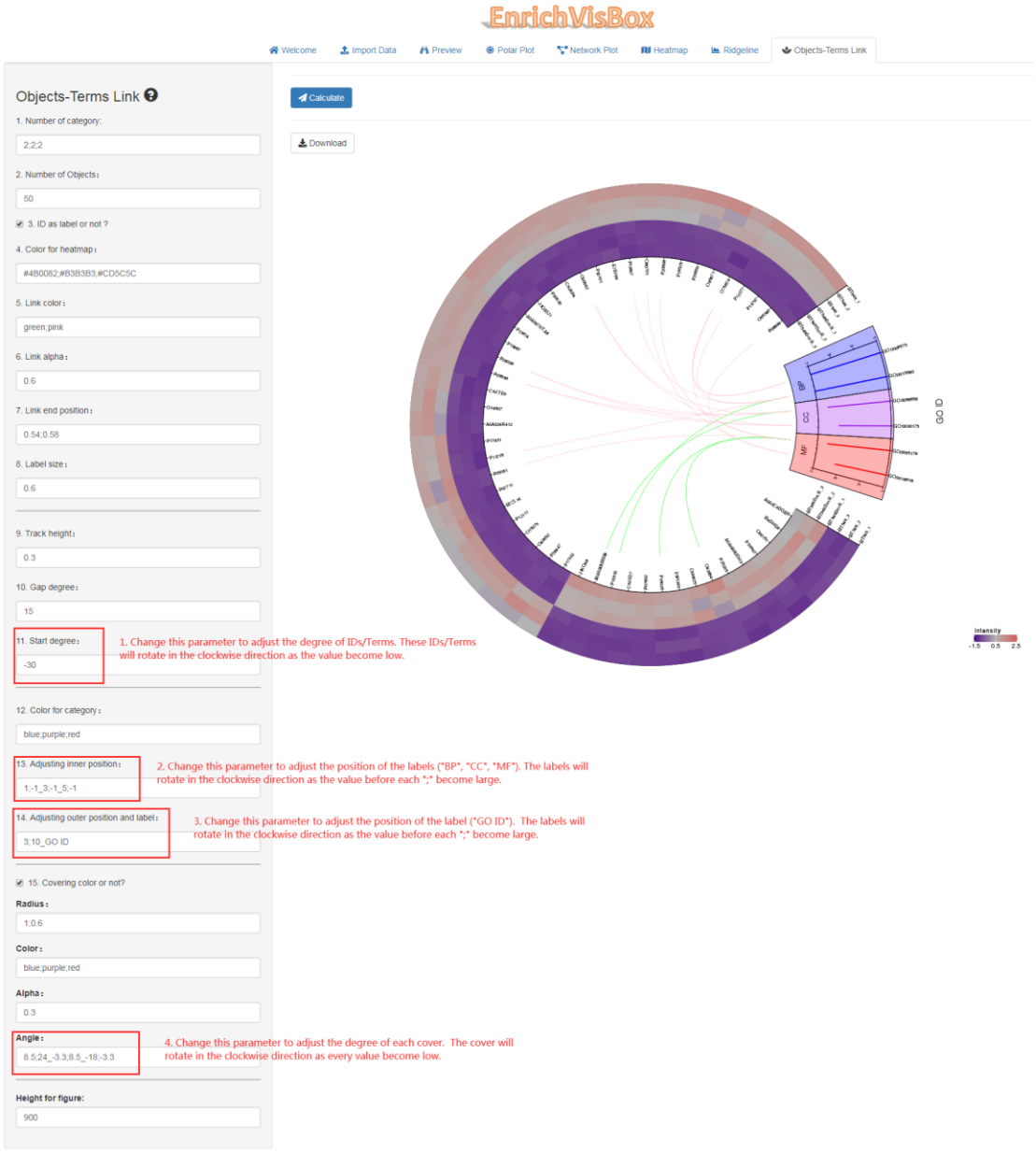
1. Number of category: which terms will be plotted. The index number should be same as the category number and linked with semicolons. For instance, there are three categories in the example data, and, to show the top 10 (1 to 10) terms in each category, one inputs '10;10;10' here.
2. Number of objects: how many objects in the expression data users want to show. For instance, if the user types in '50' here, it means the first 50 proteins in the expression data will be placed on the plot.
3. ID as label or not: if selected, the IDs will be shown; otherwise, the terms will be shown.
4. Color for heatmap: to change the heatmap colour, which corresponds to the intensity value in the expression data. Users should input three colour names here. The first one is for the lowest intensity value, the second one is for the middle intensity value, and the third one is for the highest intensity value.
5. Link color: to change the line colour. Users should type in two colour names with a semicolon. The first one indicates the negative fold change value (log2 here), and the second one corresponds to the positive fold change value (log2 here).
6. Link alpha: to change line colour transparency. The value should be in (0, 1).
7. Link end position: to change the line position to objects or IDs/terms. Users should type in two values here. The first one is for adjusting the line end distance to objects, and the second one is for adjusting the line end distance to IDs/terms. When the value is larger, the line end is further from the objects or IDs/terms.
8. Label size: to change the size of labels.
9. Track height: to change the height of tracks. It is the percentage according to the radius of the unit circle. The height includes the top and bottom cell paddings but not the margins.
10. Gap degree: to change the gap between two neighbour sectors.
11. Start degree: to change the starting degree from which the circle begins to draw.
12. Color for category: to change the category colour. The colour names and category number should be the same, for instance, in the example data, there are three categories, so there should be three colours here, which are linked with semicolons.
13. Adjusting inner position: to change the inner category title position. The position contains the values on the *x*-axis and *y*-axis linked with a semicolon. Every two positions are linked with an underline. For example, if '6;-1_15;-1_25;-1' is input here, it means that '6;-1' is for the first category ('BP') position ('6' is *x*-axis value, '-1' is *y*-axis value), '15;-1' is for the second category ('CC') position ('15' is *x*-axis value, '-1' is *y*-axis value), and '25;-1' is for the third category ('MF') position ('25' is *x*-axis value, '-1' is *y*-axis value).
14. Adjusting outer position and label: to change the outer title position and title text, they are connected with an underline. For example, if '15;10_GO ID' is input here, '15;10' is for the title position ('15' is *x*-axis value, '10' is *y*-axis value), and 'GO ID' is the title.
15. Covering color or not: whether to change the sector colours for every category. If selected, users will adjust the parameters, including *Radius* (radii for the outer arc and the inner arc in the sector, linked with a semicolon), *Color* (colours for sectors), *Alpha* (colour transparency), *Angle* (start and end degrees for every sector in a counterclockwise direction, linked with a semicolon, while the

angles between every two sectors are linked with an underline — for instance, if '27;70.5_-13.5;27_-57;-13.5' is input here, '27;70.5' is for the first sector ('27' is the start degree, '70.5' is the end degree), '-13.5;27' is for the second sector ('-13.5' is the start degree, '27' is the end degree), and '-57;-13.5' is for the third sector ('-57' is the start degree, '-13.5' is the end degree)).
16. Height for figure: adjusting the height of the figure.

If users set the correct parameters based on their own data, and then click the 'Calculate' button in each module, the figure will be placed on the right panel. They can also click the 'Download' button to save the results as a pdf file on their local computer.

To make users understand and utilize this plot better, we take two more examples here.

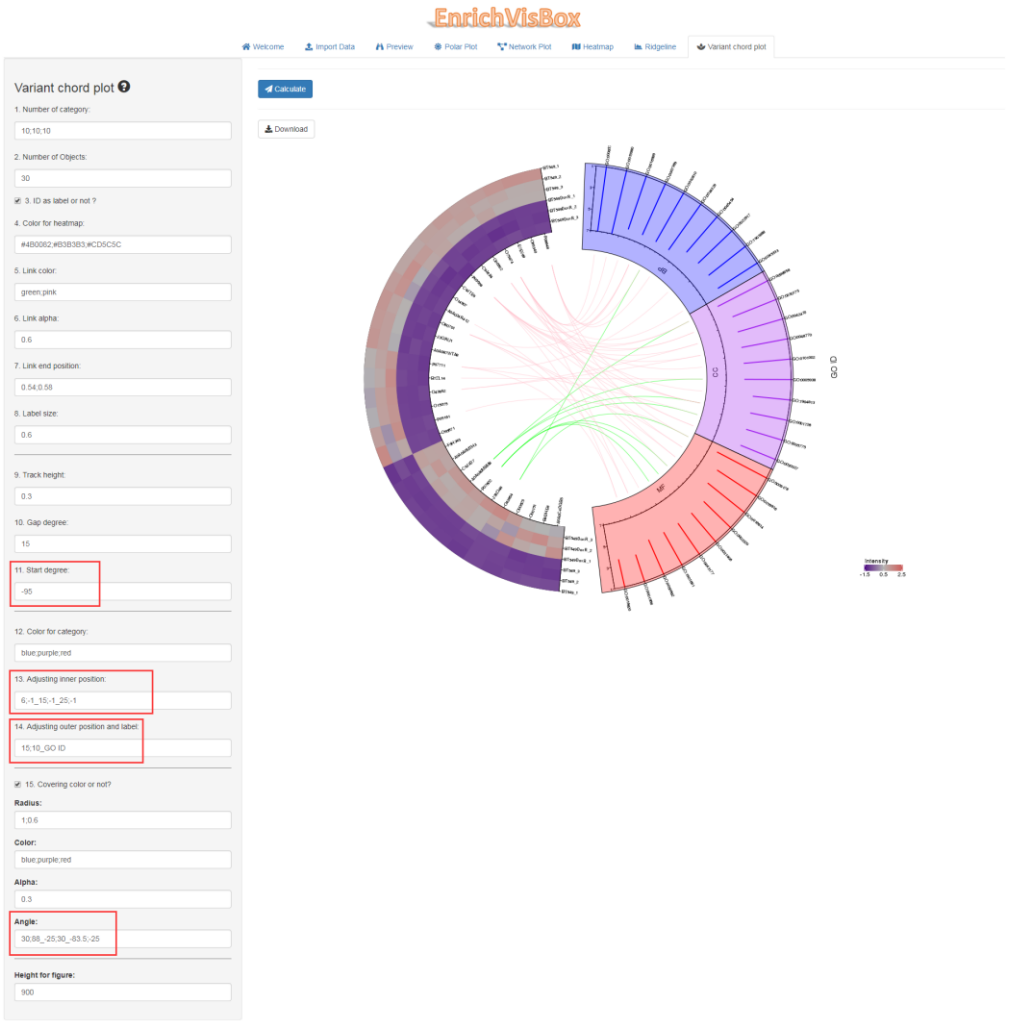
(1) For instance, if we change the number of categories to 2;2;2, which means that users only choose two terms from each category, the final figure is shown as below:



For this situation, users should notice change four parameters in the red box as above: *1. Change*

the "11. Start degree" parameter. Users should notice that when the number of IDs/terms become more or less (default number is 15;15;15), the angle of the IDs/terms panel will not be horizontal. So users can adjust this parameter and the IDs/terms panel will rotate in the clockwise direction as the value become low. If users never mind whether the IDs/terms panel is horizontal, just ignore this parameter and keep the default value. *2. Change the "13. Adjusting inner position" parameter*. When the position of the IDs/terms panel changes, the labels (e.g. "BP", "CC", "MF") will not be just in the front of it. The labels will rotate in the clockwise direction as the value before every ";" become large. 3. Change the "14. Adjusting outer position and label" parameter. As above, the label (e.g. GO ID) will not be just in the bottom of the IDs/terms panel. Users can adjust the value before ";". When the value become large, the label will rotate in the clockwise direction. 4. Change the "Angle" in the "15. Covering color or not?" parameter. As above, the cover will not be just on the IDs/terms panel. User should change this parameter if they want to cover different color on the IDs/terms panel. The cover will rotate in the clockwise direction as every value becomes low and users should also note that the cover width, which can be controlled by the difference between the values on both sides of ";".

(2) If we change the number of objects to 30, Users should also mainly adjust the four parameters mentioned above. The final figure is shown as below:



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