

Step-by-step Guide to Perform Automated Differential Expression Analysis using FlexStatv1 Pipeline



This feature facilitates combinatory differential expression analysis for datasets with more than two classes/conditions.

It systematically generates all possible pairwise comparisons, combines multiple classes/conditions, and presents detailed results for the differential expression analysis.

1

Navigate to <https://jglab.shinyapps.io/flexstatv1-pipeline-only/>

2

Go to the "Automated Combinatory Differential Expression" tab.

3 Click "Use Sample Data"

FlexStat 1.0 Differential Expression Automated Combinatory Differential Expression Consensus Clustering Help

Automated Combinatory Differential Expression Analysis

Select CSV File to Import ⓘ

Browse... No file selected

☒ Use Sample Data

Class Variable

Not Selected

Log fold-change variable ⓘ

P-value variable ⓘ

Sample Data

Condition	O76070	P01344	P015
A	28.41	27.36	27.
A	28.46	27.40	27.
A	28.41	27.47	27.
B	24.28	24.63	23.
B	24.28	24.73	23.
B	24.20	24.66	23.

4 Click "Condition" as the Class variable and select correction for multiple comparisons.

Select CSV File to Import

Browse... No file selected

☒ Use Sample Data

Class Variable

Condition

Log fold-change variable ⓘ

P-value variable ⓘ

Adjust P-values for Multiple Comparisons

Benjamini-Hochberg

Sample Data

Condition	O76070	P013
A	28.41	27.
A	28.46	27.
A	28.41	27.
B	24.28	24.
B	24.28	24.
B	24.20	24.

5

[Optional] Change log fold change and p-value cutoffs based on the research question at hand.

Log fold-change variable

1.5

P-value variable

0.01

6

[Optional] Change the correction procedure for multiple comparison tests based on the research question at hand.

Adjust P-values for Multiple Comparisons

Benjamini-Hochberg

Benjamini-Hochberg

Bonferroni

Benjamini-Yekutieli

Holm

None

7 Click "Perform Auto Limma"

P-value variable i

Adjust P-values for Multiple Comparisons

Benjamini-Hochberg ▼

▶ Perform Auto Limma

B	24.20	24.
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8 This will result in calculating all possible combinations of the classes.

e variable i

for Multiple Comparisons

chberg ▼

Auto Limma

A vs D
A vs C
A vs B
D vs C
D vs B
C vs B
A+D+B vs C
A+D+C vs B
A+C+B vs D
D+C+B vs A
A+B vs D+C
A+D vs C+B
A+C vs D+B

Show **10** entries

Combination	Gene	logFC	AveExp
	V981	2.5675	-0.2
	V204	2.5628	-0.1
	V671	2.4435	0.6
	V82	2.3203	0.3
	V327	-2.3199	-0.0
D+C+B vs A	V146	2.2963	0.0
	V157	2.2610	0.2

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This will result in producing a table of differential expression results for each combination.

FlexStat 1.0 Differential Expression Automated Differential Expression Consensus Clustering Help

Combinatory Limma Analysis

Select CSV File to Import
 Browse... No file selected
☒ Use Sample Data

Class Variable
 Condition

Log fold-change variable

P-value variable

Adjust P-values for Multiple Comparisons
 Benjamini-Hochberg

Perform Auto Limma

Auto limma Results

Download Top 50 Download All

A vs D
 A vs C
 A vs B
 D vs C
 D vs B
 C vs B
 A+D+B vs C
 A+D+C vs B
 A+C+B vs D
 D+C+B vs A
 A+B vs D+C
 A+D vs C+B
 A+C vs D+B

Show 100 entries

Combination	Gene	logFC	AveExpr	t	PValue	adj.PV
V981		2.5675	-0.2549	2.7845	0.0054	0.
V204		2.5628	-0.1949	2.7794	0.0054	0.
V671		2.4435	0.6703	2.6500	0.0081	0.
V82		2.3203	0.3292	2.5164	0.0119	0.
V327		-2.3199	-0.0364	-2.5159	0.0119	0.
V146		2.2963	0.0918	2.4903	0.0128	0.
V157		2.2610	0.2306	2.4520	0.0142	0.
V669		2.2363	-0.6155	2.4253	0.0153	0.

10

Navigate using page numbers to explore the results.

V476	1.9694	0.4359	2.1358	0.0327	0.9583	-4.5745
V511	-1.9676	-0.3198	-2.1339	0.0329	0.9583	-4.5745
V169	-1.9660	0.1707	-2.1321	0.0330	0.9583	-4.5745
V606	-1.9499	-0.0627	-2.1146	0.0345	0.9583	-4.5750
V48	1.9395	0.2075	2.1034	0.0354	0.9583	-4.5753
V30	1.9353	0.1722	2.0988	0.0358	0.9583	-4.5754
V544	-1.9333	-0.2936	-2.0967	0.0360	0.9583	-4.5754
V687	1.9265	0.6585	2.0893	0.0367	0.9583	-4.5756
V545	-1.9235	-0.1648	-2.0861	0.0370	0.9583	-4.5757
V704	1.9206	-0.0392	2.0829	0.0373	0.9583	-4.5758
V905	-1.9118	-0.2659	-2.0733	0.0382	0.9583	-4.5760
V337	-1.8829	-0.2094	-2.0420	0.0412	0.9583	-4.5767
V797	-1.8719	0.1767	-2.0301	0.0424	0.9583	-4.5770
V512	1.8652	0.3417	2.0228	0.0431	0.9583	-4.5772
V577	-1.8573	-0.0211	-2.0142	0.0440	0.9583	-4.5774
V193	-1.8452	0.1286	-2.0011	0.0454	0.9583	-4.5777
V473	1.8302	-0.4775	1.9848	0.0472	0.9583	-4.5781
V850	-1.8148	-0.7275	-1.9681	0.0491	0.9583	-4.5785
V499	1.8090	-0.1703	1.9618	0.0498	0.9583	-4.5786
V918	1.8058	-0.0646	1.9584	0.0502	0.9583	-4.5787
V266	1.7970	-0.7025	1.9488	0.0513	0.9583	-4.5789
V161	1.7947	-0.2300	1.9463	0.0516	0.9583	-4.5789
V144	-1.7768	-0.5433	-1.9269	0.0540	0.9583	-4.5794
V209	-1.7745	-0.0277	-1.9244	0.0543	0.9583	-4.5794

Showing 1 to 50 of 650 entries

Previous 1 2 3 4 5 ... 13 Next

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Click "Download Top 50" to download top 50 genes/proteins from each combination

Automated Differential Expression Consensus Clustering Help

Analysis

Auto limma Results

[Download Top 50](#) [Download](#)

A vs D
 A vs C
 A vs B
 D vs C
 D vs B
 C vs B
 A+D+B vs C
 A+D+C vs B
 A+C+B vs D
 D+C+B vs A
 A+B vs D+C

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Downloaded excel files will be as follows. The first sheet will be an index explaining the subsequent sheets representing different combinations.

combination						
A vs D						
A vs C						
A vs B						
D vs C						
D vs B						
C vs B						
A+D+B vs C						
A+D+C vs B						
A+C+B vs D						
D+C+B vs A						
A+B vs D+C						
A+D vs C+B						
A+C vs D+B						

Index	A	B	C	D	E	F	G	H	I	J	K	L
1	Combination	Gene	logFC	AveExpr	t	P.Value	adj.P.V					
2	A vs C	V769	3.5888721	0.39923599	2.93842797	0.00330461	0.95					
3	A vs C	V280	-3.4873319	0.30220397	-2.8552908	0.00430655	0.95					
4	A vs C	V671	-3.3344578	0.29363145	-2.7301235	0.00633959	0.95					
5	A vs C	V905	3.29246979	-0.2445967	2.69574536	0.00703217	0.95					
6	A vs C	V58	3.29057467	-0.30387	2.69419371	0.00706498	0.95					
7	A vs C	V930	3.25766976	0.03353519	2.66725246	0.00765689	0.95					
8	A vs C	V500	-3.2120223	-0.0651457	-2.6298781	0.00855161	0.95					
9	A vs C	V660	-3.1724947	0.04593123	-2.5975145	0.0094007	0.95					
10	A vs C	V146	-3.1650724	-0.2495424	-2.5914373	0.00956828	0.95					
11	A vs C	V920	3.13085443	0.53646915	2.56342103	0.01037577	0.95					
12	A vs C	V124	3.05054058	0.08981996	2.4976632	0.01251375	0.95					
13	A vs C	V25	2.96980706	-0.3753245	2.43156176	0.0150473	0.95					
14	A vs C	V633	-2.8578647	0.23879836	-2.3399077	0.01930356	0.95					

13 Click "Download All" to download all results.

