Arrays Analysis With GIANT

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Version January 2019

Files required to start an analysis (datasets in your history)

Expression
Need to Upload

- CEL files
- Or Normalized Expressions file (tabular format) ⚠

Study Design
Upload or
Create in Galaxy*

- Conditions File (tabular format) ⚠

*** Recommended to create in Galaxy to avoid mistakes in file (cf. Next Slide)**

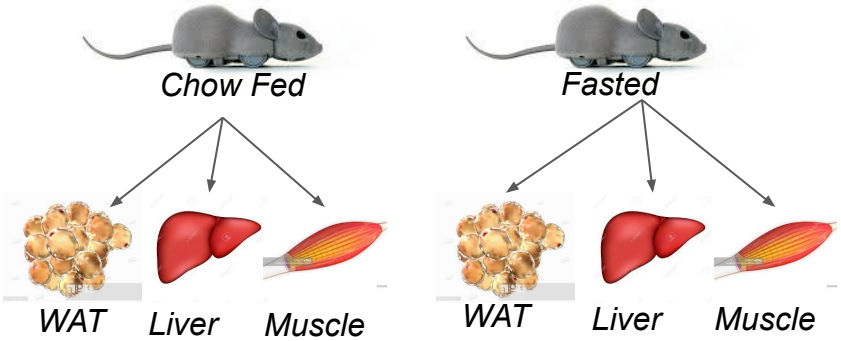
⚠ Precise the format when you upload your datasets

Example Dataset

Expression

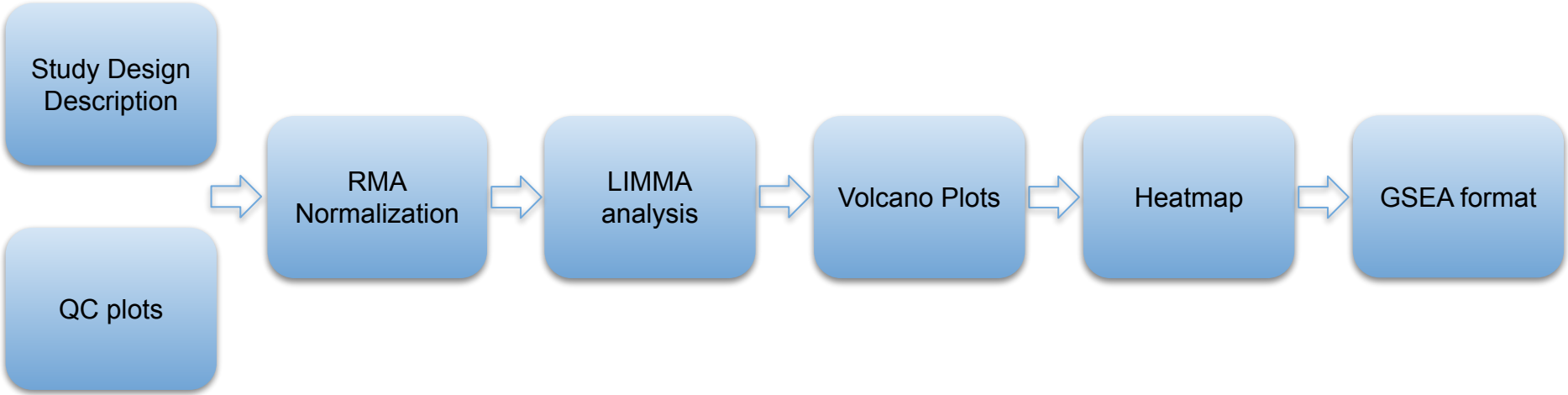
GSE46495
Transcriptome signature of white adipose tissue, liver, and skeletal muscle in 24 hours fasted mice (C57Bl/6J)

➢ CEL files



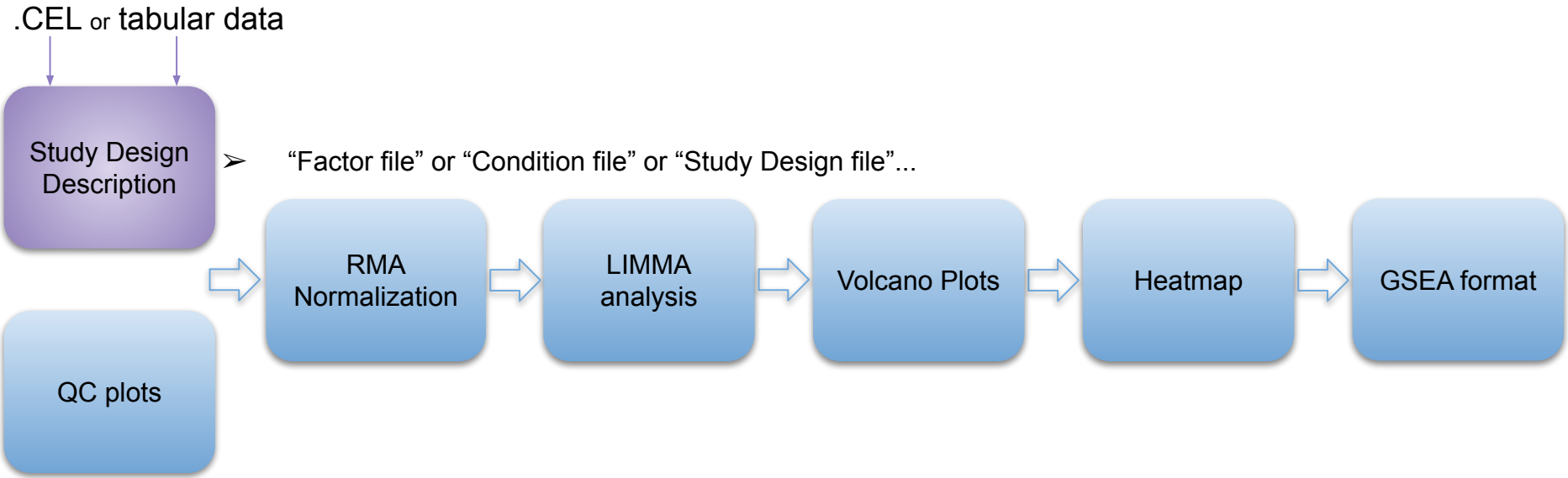
GIANT - Tools Suite

Microarray analysis pipeline: “ from .CEL to GSEA “



GIANT - Tools Suite

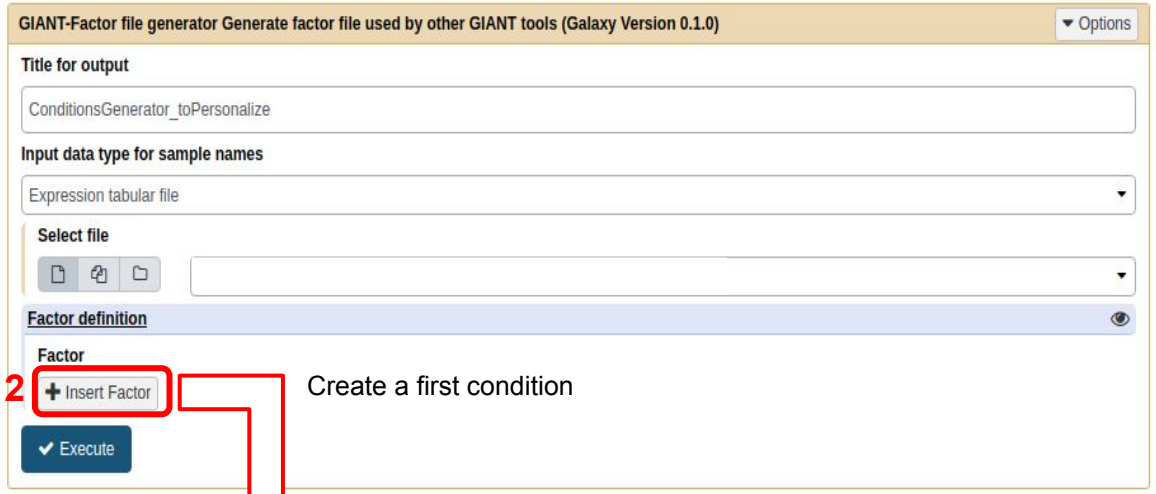
Microarray analysis pipeline: “ from .CEL to GSEA “

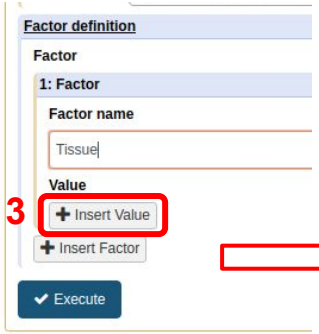


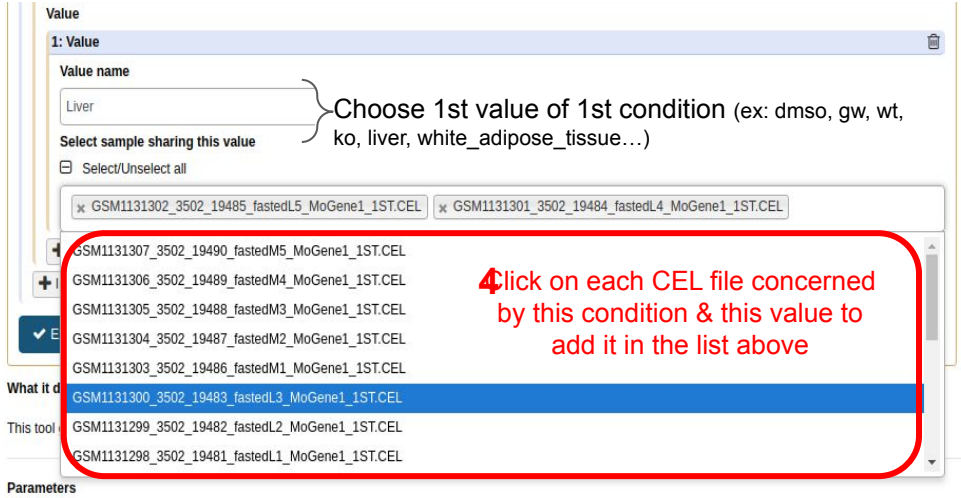
Condition File Generator

GIANT

- GIANT-QC Plots Descriptive plots of .CEL collections or normalized expression data
- GIANT-Heatmap and Hierarchical clustering Run hierarchical clustering and plot heatmap from expression data and/or differential expression analysis
- GIANT-Normalization with APT Summarize Apply Affymetrix Power Tool summarize function to .CEL collection
- GIANT-GSEA Formating Format input files for GSEA software
- GIANT-Differential Expression with LIMMA Use LIMMA to detect differentially expressed genes
- **1** GIANT-Factor file generator Generate factor file used by other GIANT tools
- GIANT-Plot volcanos Plot volcano from tabular file







5 Add Value as much as useful for your first condition and concerned files + Add Conditions as much as useful

And execute !

Condition File Generator

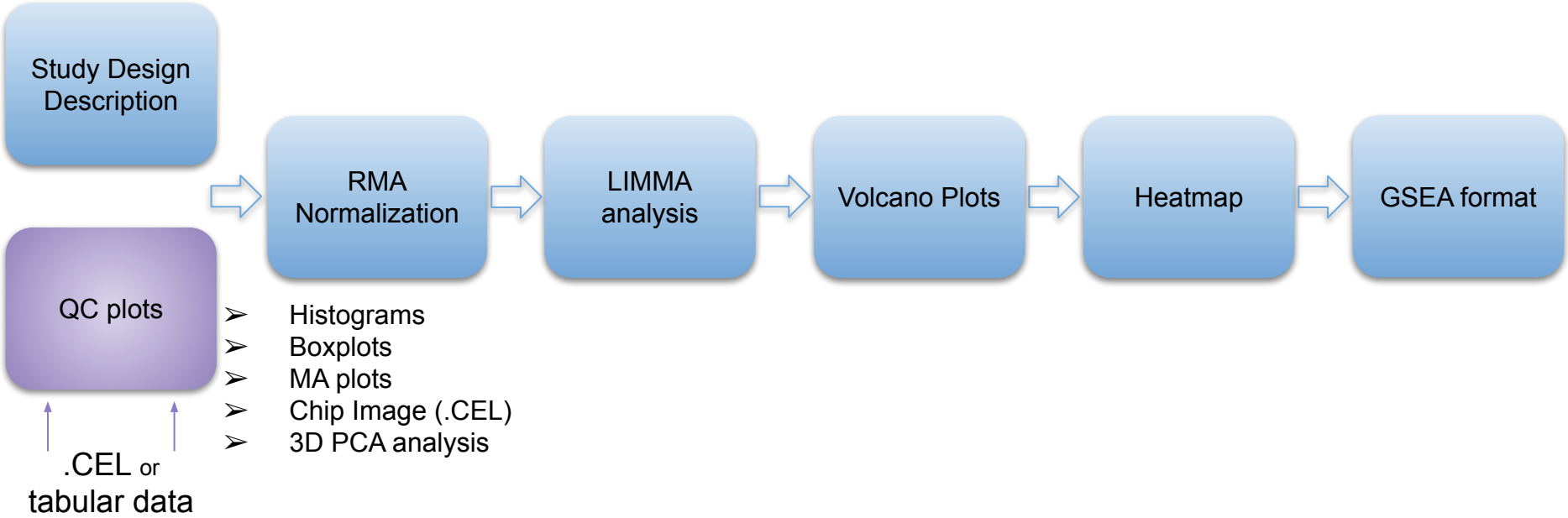
2 results in history :

- log file
- Tabular file

1	2	3
Conditions	Tissue	FastFed
GSM1131307_3502_19490_fastedM5_MoGene1_1ST.CEL	Muscle	Fasted
GSM1131306_3502_19489_fastedM4_MoGene1_1ST.CEL	Muscle	Fasted
GSM1131305_3502_19488_fastedM3_MoGene1_1ST.CEL	Muscle	Fasted
GSM1131304_3502_19487_fastedM2_MoGene1_1ST.CEL	Muscle	Fasted
GSM1131303_3502_19486_fastedM1_MoGene1_1ST.CEL	Muscle	Fasted
GSM1131302_3502_19485_fastedL5_MoGene1_1ST.CEL	Liver	Fasted
GSM1131301_3502_19484_fastedL4_MoGene1_1ST.CEL	Liver	Fasted
GSM1131300_3502_19483_fastedL3_MoGene1_1ST.CEL	Liver	Fasted
GSM1131299_3502_19482_fastedL2_MoGene1_1ST.CEL	Liver	Fasted
GSM1131298_3502_19481_fastedL1_MoGene1_1ST.CEL	Liver	Fasted
GSM1131297_3502_19480_fastedF5_MoGene1_1ST.CEL	WAT	Fasted
GSM1131296_3502_19479_fastedF4_MoGene1_1ST.CEL	WAT	Fasted
GSM1131295_3502_19478_fastedF3_MoGene1_1ST.CEL	WAT	Fasted
GSM1131294_3502_19477_fastedF2_MoGene1_1ST.CEL	WAT	Fasted
GSM1131293_3502_19476_fastedF1_MoGene1_1ST.CEL	WAT	Fasted
GSM1131292_3502_19475_fedM5_MoGene1_1ST.CEL	Muscle	Fed
GSM1131291_3502_19474_fedM4_MoGene1_1ST.CEL	Muscle	Fed
GSM1131290_3502_19473_fedM3_MoGene1_1ST.CEL	Muscle	Fed
GSM1131289_3502_19472_fedM2_MoGene1_1ST.CEL	Muscle	Fed
GSM1131288_3502_19471_fedM1_MoGene1_1ST.CEL	Muscle	Fed
GSM1131287_3502_19470_fedL5_MoGene1_1ST.CEL	Liver	Fed
GSM1131286_3502_19469_fedL4_MoGene1_1ST.CEL	Liver	Fed
GSM1131285_3502_19468_fedL3_MoGene1_1ST.CEL	Liver	Fed
GSM1131284_3502_19467_fedL2_MoGene1_1ST.CEL	Liver	Fed
GSM1131283_3502_19466_fedL1_MoGene1_1ST.CEL	Liver	Fed
GSM1131282_3502_19465_fedF5_MoGene1_1ST.CEL	WAT	Fed
GSM1131281_3502_19464_fedF4_MoGene1_1ST.CEL	WAT	Fed
GSM1131280_3502_19463_fedF3_MoGene1_1ST.CEL	WAT	Fed
GSM1131279_3502_19462_fedF2_MoGene1_1ST.CEL	WAT	Fed
GSM1131278_3502_19461_fedF1_MoGene1_1ST.CEL	WAT	Fed

GIANT - Tools Suite

Microarray analysis pipeline: “ from .CEL to GSEA “



QC plots

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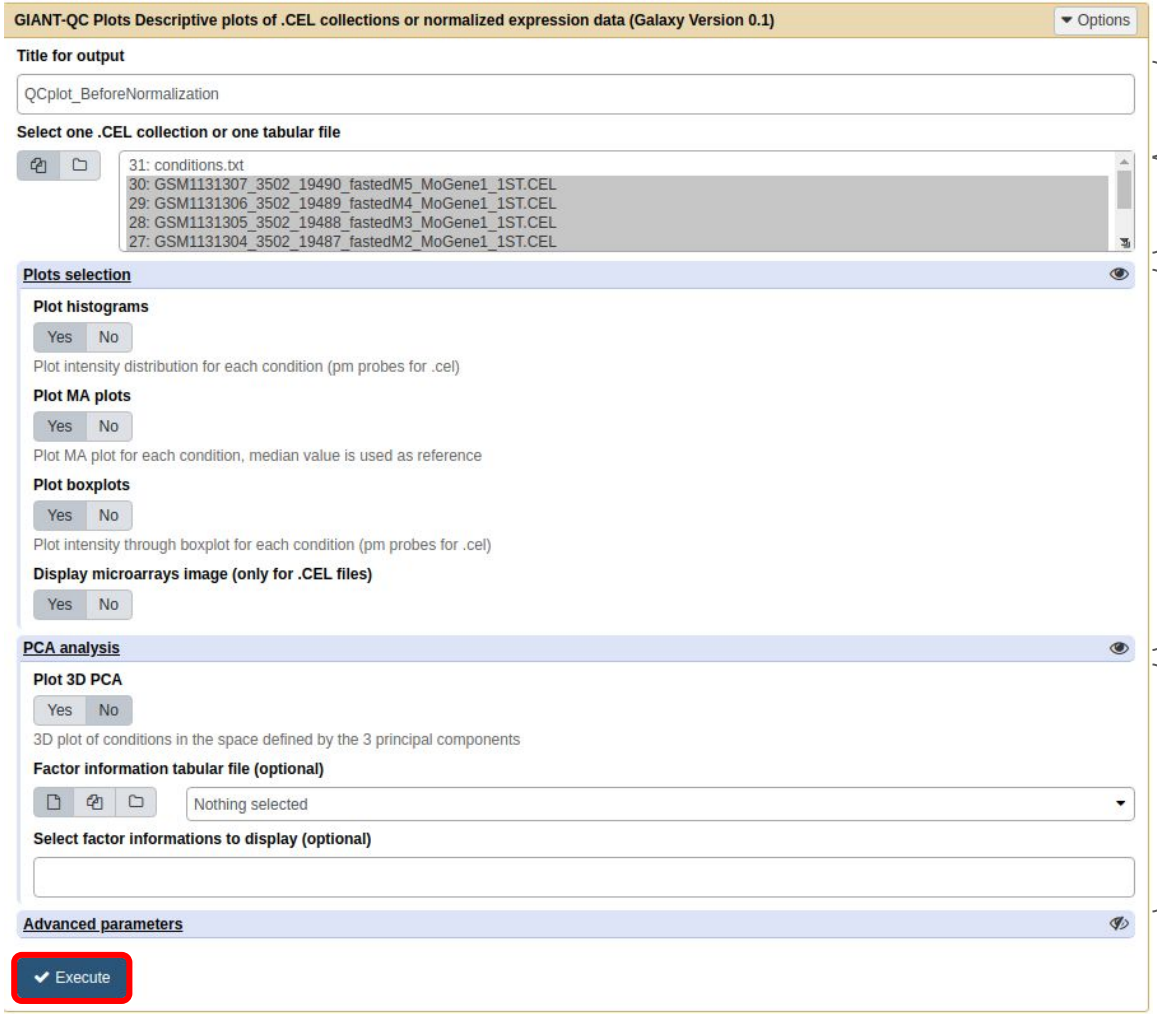


Choose a title

Select CEL files

Select desired plots

Microarrays images

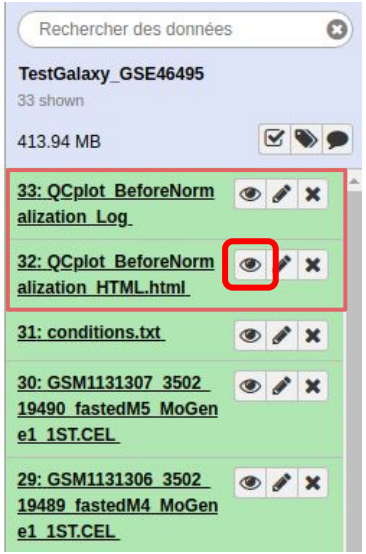
PCA analysis with conditions infos

And execute !

QC plots

2 results in history :

- log file
- HTML page



Html page

Histograms

Histograms1

Boxplots

Boxplots1

MA plots (show/hide)

MAplot GSM1131287_3502_19470_fedL5_MoGene1_1ST.CEL

MAplot GSM1131288_3502_19471_fedM1_MoGene1_1ST.CEL

MAplot GSM1131289_3502_19472_fedM2_MoGene1_1ST.CEL

MAplot GSM1131290_3502_19473_fedM3_MoGene1_1ST.CEL

MAplot GSM1131291_3502_19474_fedM4_MoGene1_1ST.CEL

MAplot GSM1131292_3502_19475_fedM5_MoGene1_1ST.CEL

MAplot GSM1131293_3502_19476_fastedF1_MoGene1_1ST.CEL

MAplot GSM1131294_3502_19477_fastedF2_MoGene1_1ST.CEL

MAplot GSM1131295_3502_19478_fastedF3_MoGene1_1ST.CEL

Microarray

Microarray GSM1131287_3502_19470_fedL5_MoGene1_1ST.CEL

Microarray GSM1131288_3502_19471_fedM1_MoGene1_1ST.CEL

Microarray GSM1131289_3502_19472_fedM2_MoGene1_1ST.CEL

Microarray GSM1131290_3502_19473_fedM3_MoGene1_1ST.CEL

Microarray GSM1131291_3502_19474_fedM4_MoGene1_1ST.CEL

Microarray GSM1131292_3502_19475_fedM5_MoGene1_1ST.CEL

Microarray GSM1131293_3502_19476_fastedF1_MoGene1_1ST.CEL

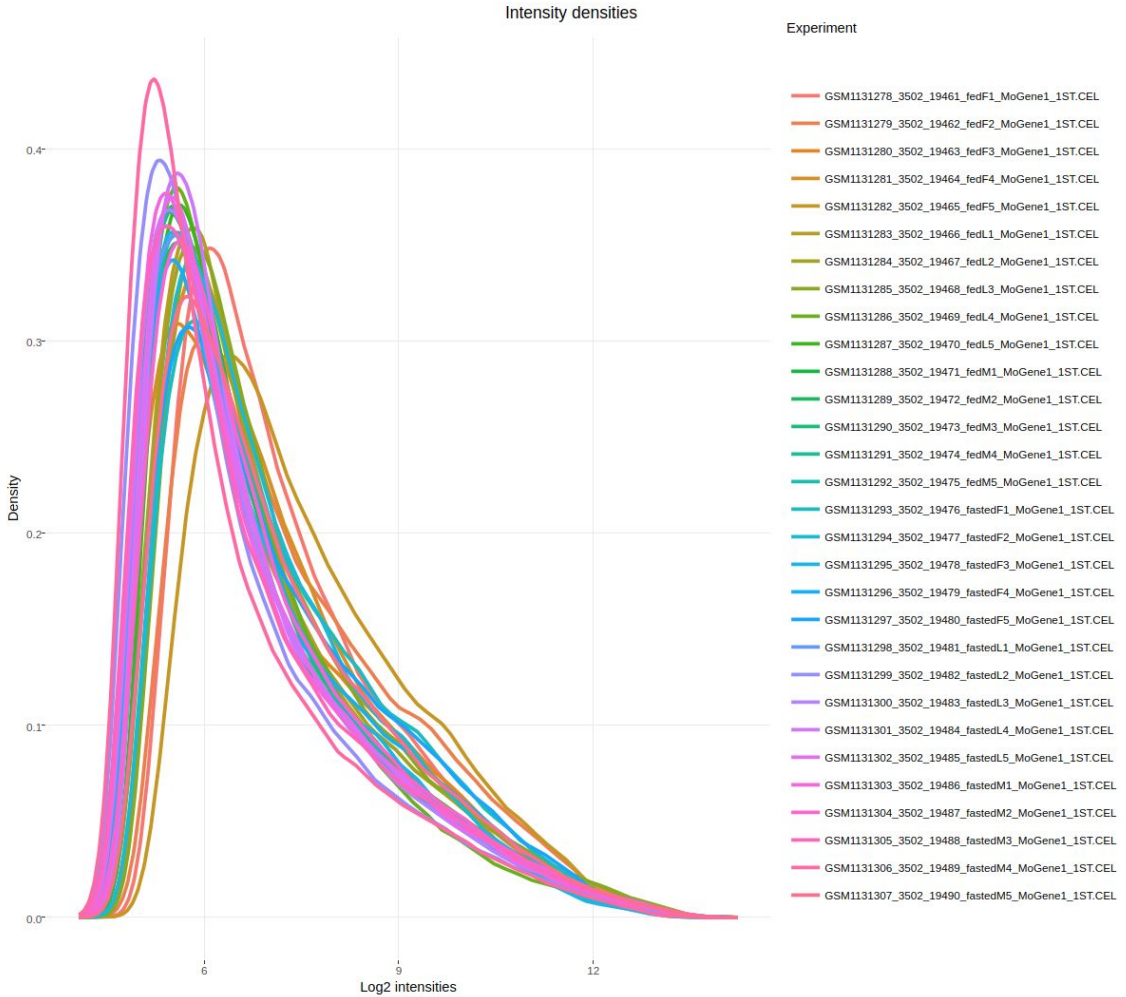
Microarray GSM1131294_3502_19477_fastedF2_MoGene1_1ST.CEL

Microarray GSM1131295_3502_19478_fastedF3_MoGene1_1ST.CEL

QC plots

Histograms

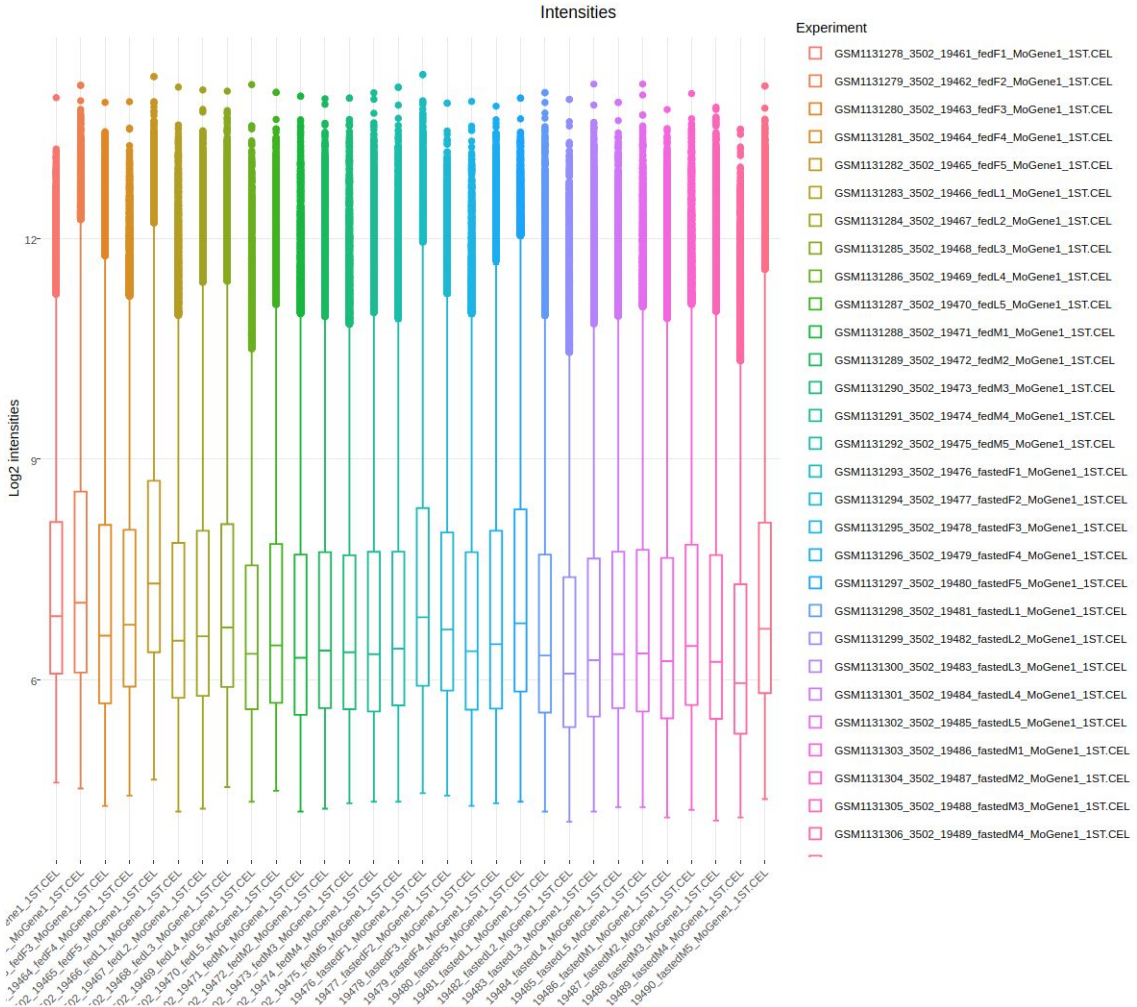
Histograms1



QC plots

Boxplots

Boxplots1



QC plots

MA plots (show/hide)

MAplot GSM1131287_3502_19470_fedL5_MoGene1_1ST.CEL

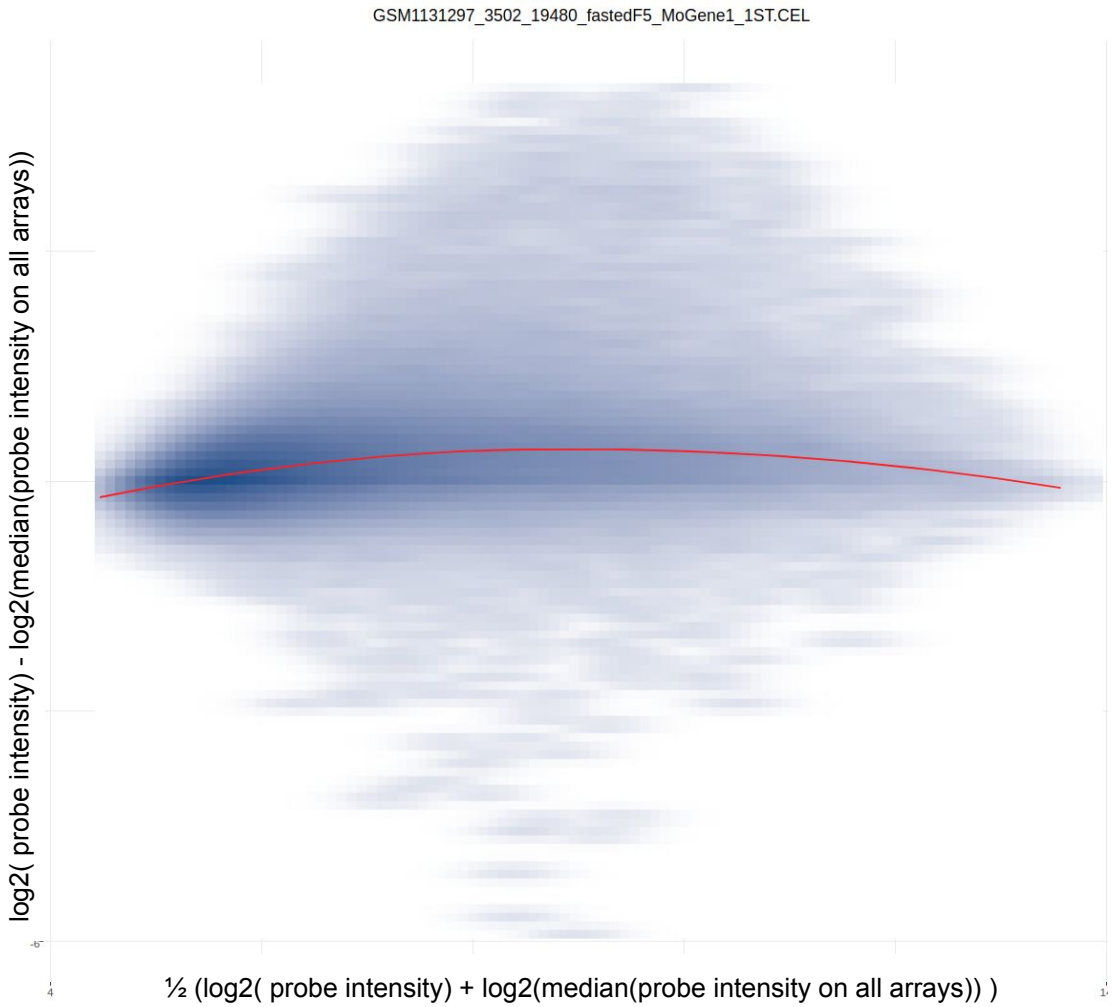
MAplot GSM1131288_3502_19471_fedM1_MoGene1_1ST.CEL

MAplot GSM1131289_3502_19472_fedM2_MoGene1_1ST.CEL

MAplot GSM1131290_3502_19473_fedM3_MoGene1_1ST.CEL

MAplot GSM1131291_3502_19474_fedM4_MoGene1_1ST.CEL

MAplot GSM1131292_3502_19475_fedM5_MoGene1_1ST.CEL



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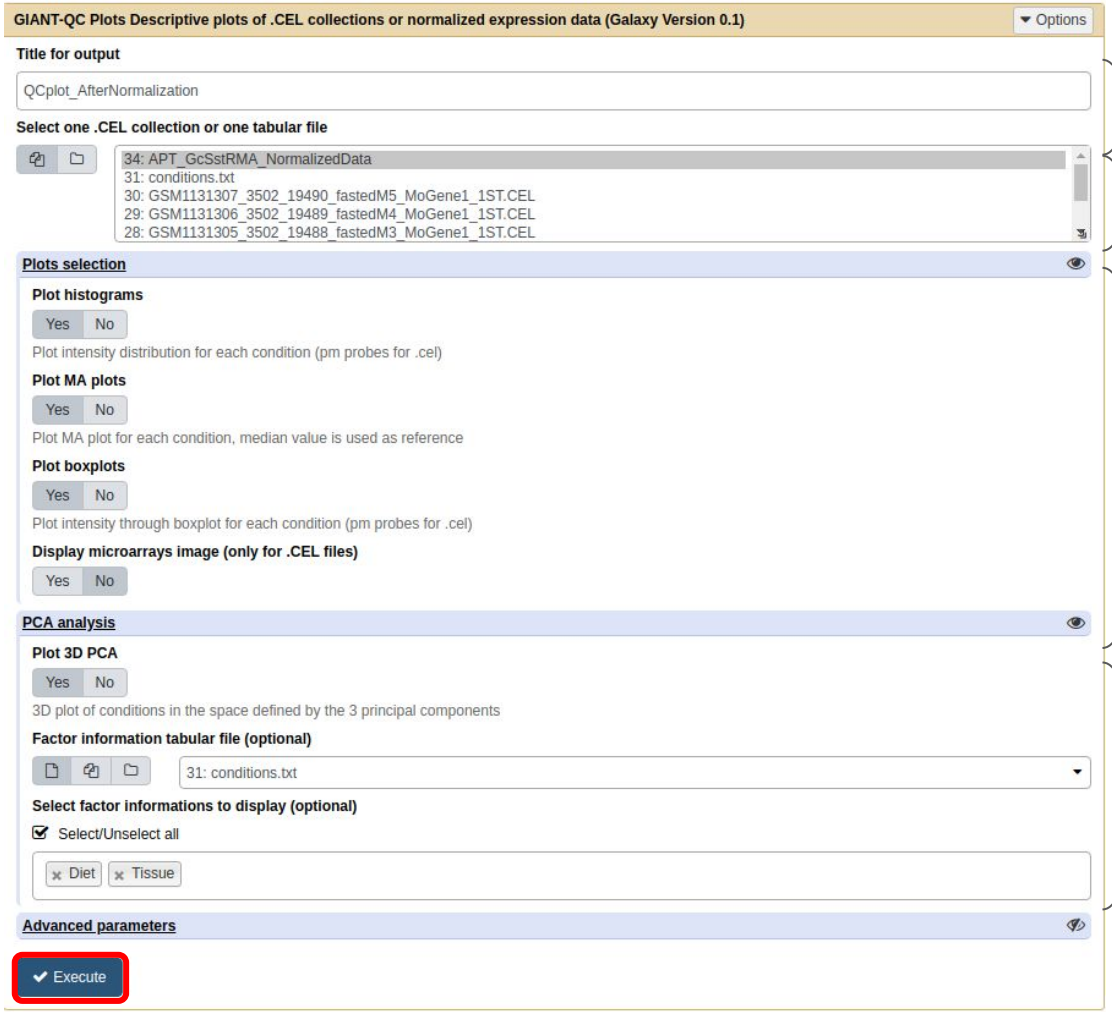


Choose a title

Select normalized data

Select desired plots

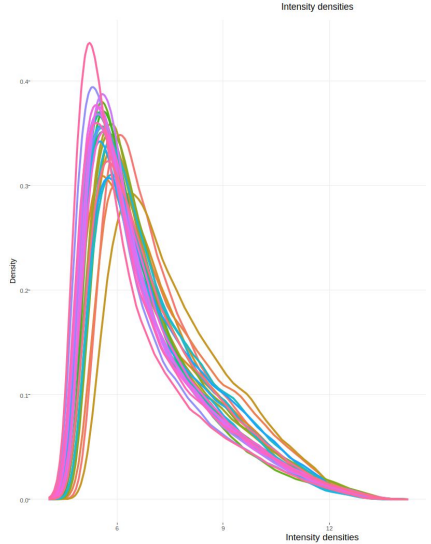
⚠ *Microarrays images*

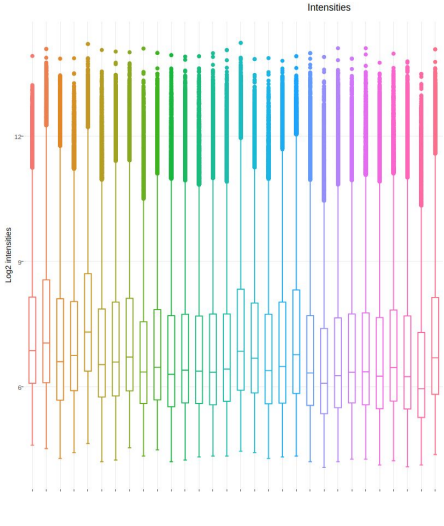
PCA analysis with conditions infos

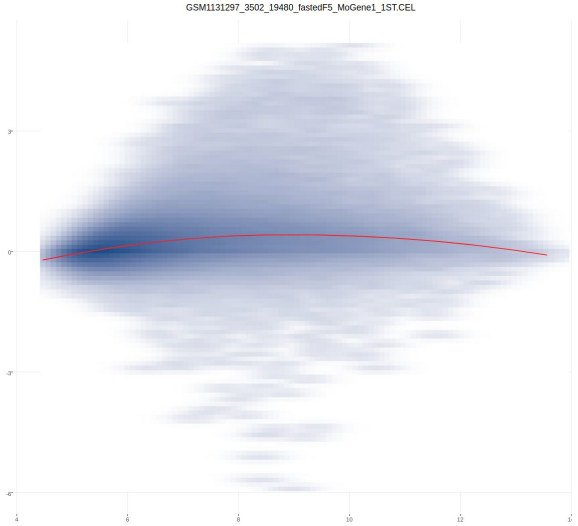
And execute !

QC plots

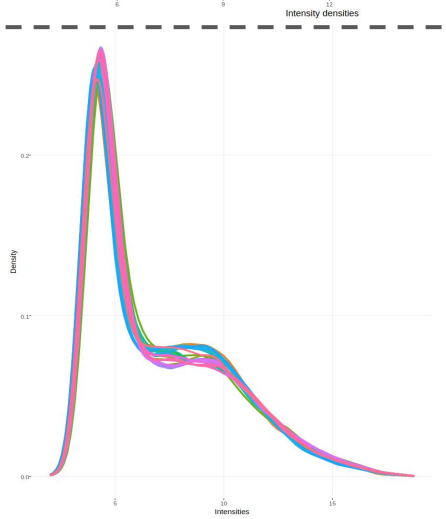
RAW data

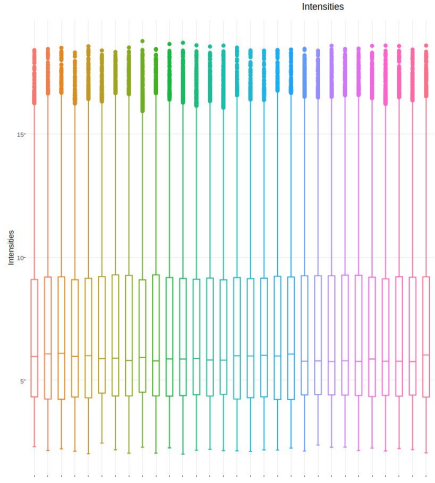


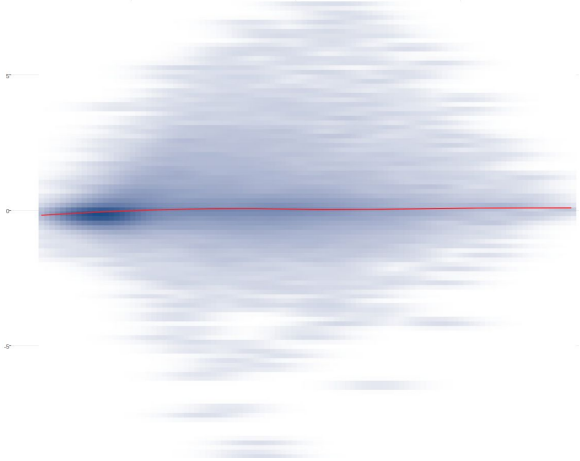




Normalized data





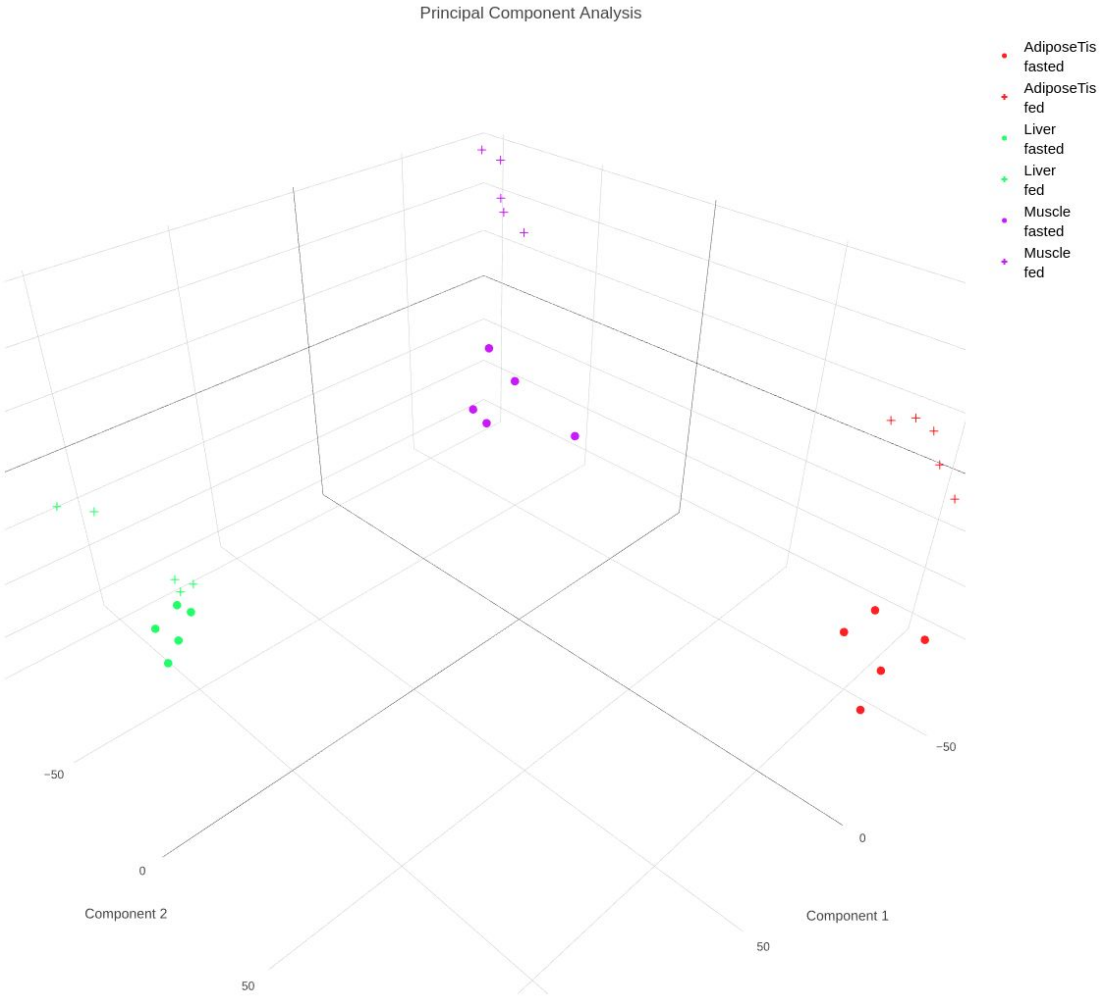


QC plots

PCA

PCA Tissue * Diet

Scree plot



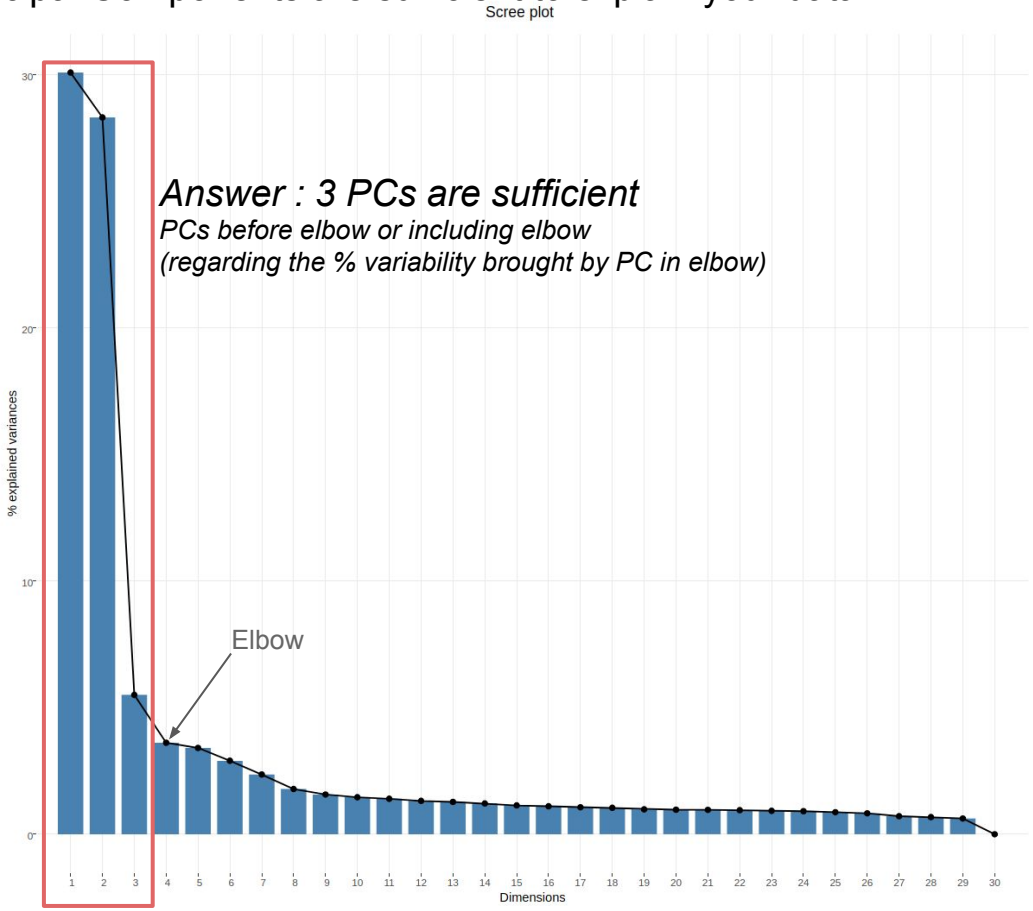
QC plots

How many Principal Components are sufficient to explain your data ?

PCA

PCA Tissue * Diet

Scree plot

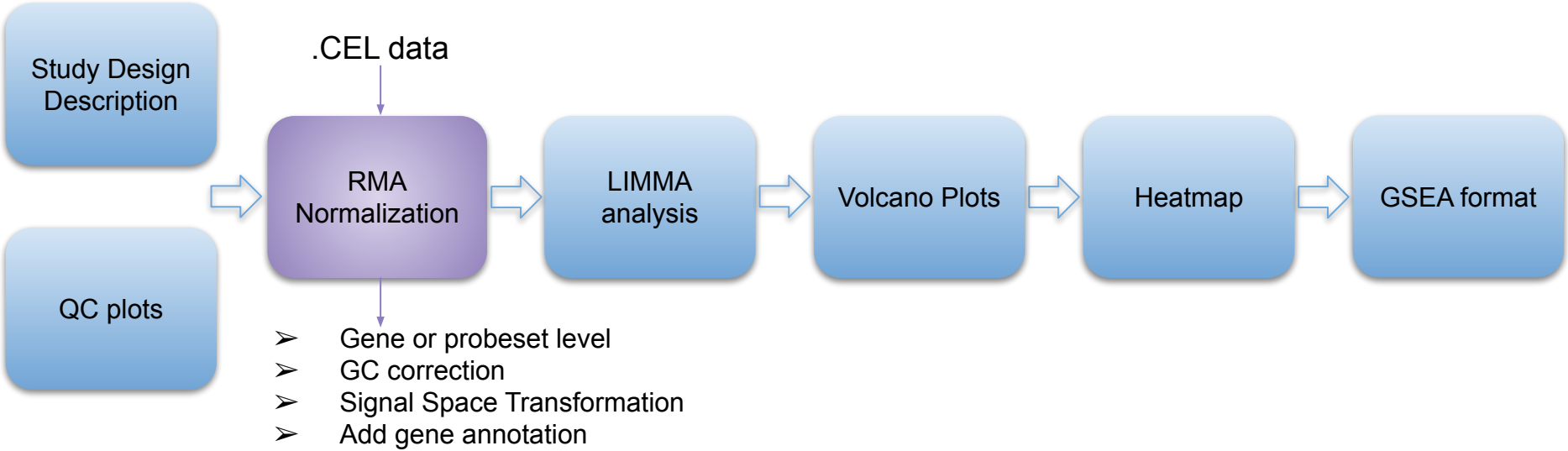


Answer : 3 PCs are sufficient

PCs before elbow or including elbow
(regarding the % variability brought by PC in elbow)

GIANT - Tools Suite

Microarray analysis pipeline: “ from .CEL to GSEA “



RMA Normalization

What is a Normalization in GIANT ?

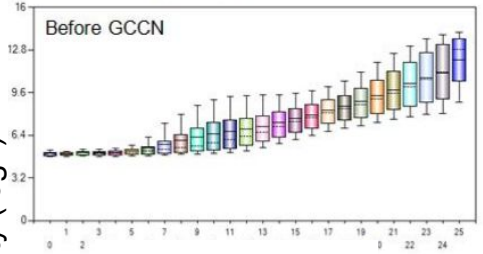
GCCN
GC correction

SST
scaling

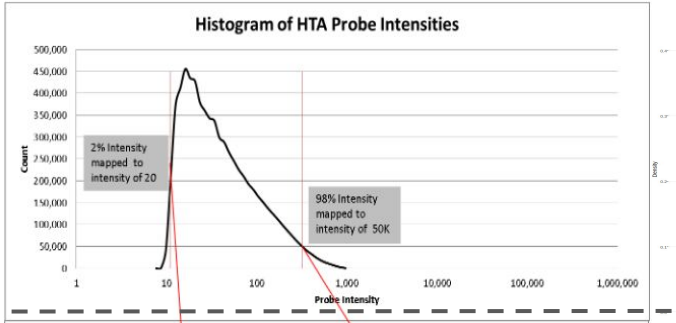
RMA Normalization

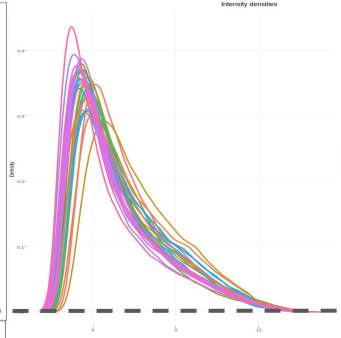
- RMA background
- Quantile Normalization
- Median Polish

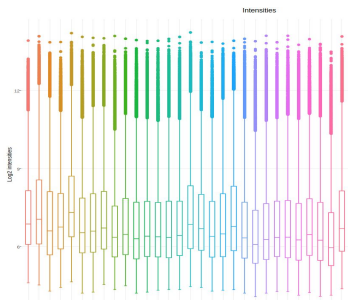
Probe intensity (log2)

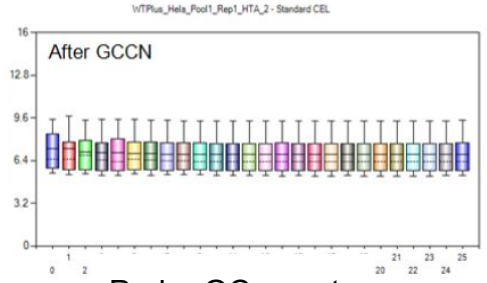


Probe GC-count

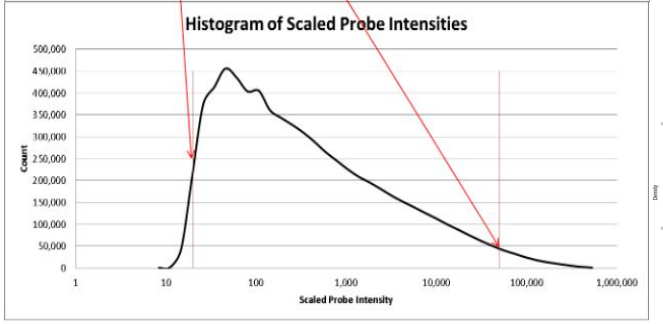


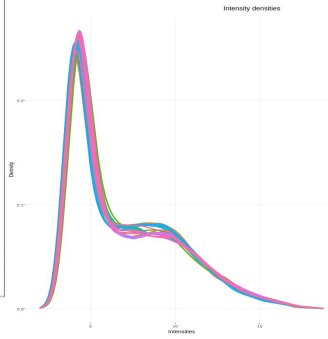


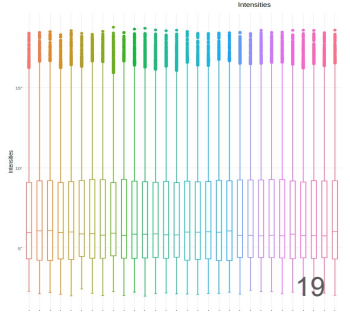




Probe GC-count







https://tools.thermofisher.com/content/sfs/brochures/sst_gccn_whitepaper.pdf - affymetrix documentation

RMA Normalization

Normalization in GIANT - recommendations

1. HTA/MTA & Hu/MoGene : use GCcorrection+Scaling+RMA
   a. Advantages : scaled data are comparable to QPCR & RNA-seq signal level
   b. Warning for Hu/MoGene, to be similar with an old analysis, you should use RMA only
2. MOE 430 : use Scaling+RMA
   a. Advantages : scaled data are comparable to QPCR & RNA-seq signal level
   b. Warning : no available information about GC content, so GC correction option has no effect
3. No Affymetrix data : use a dataset already normalized to perform further analysis steps (differential analysis...)

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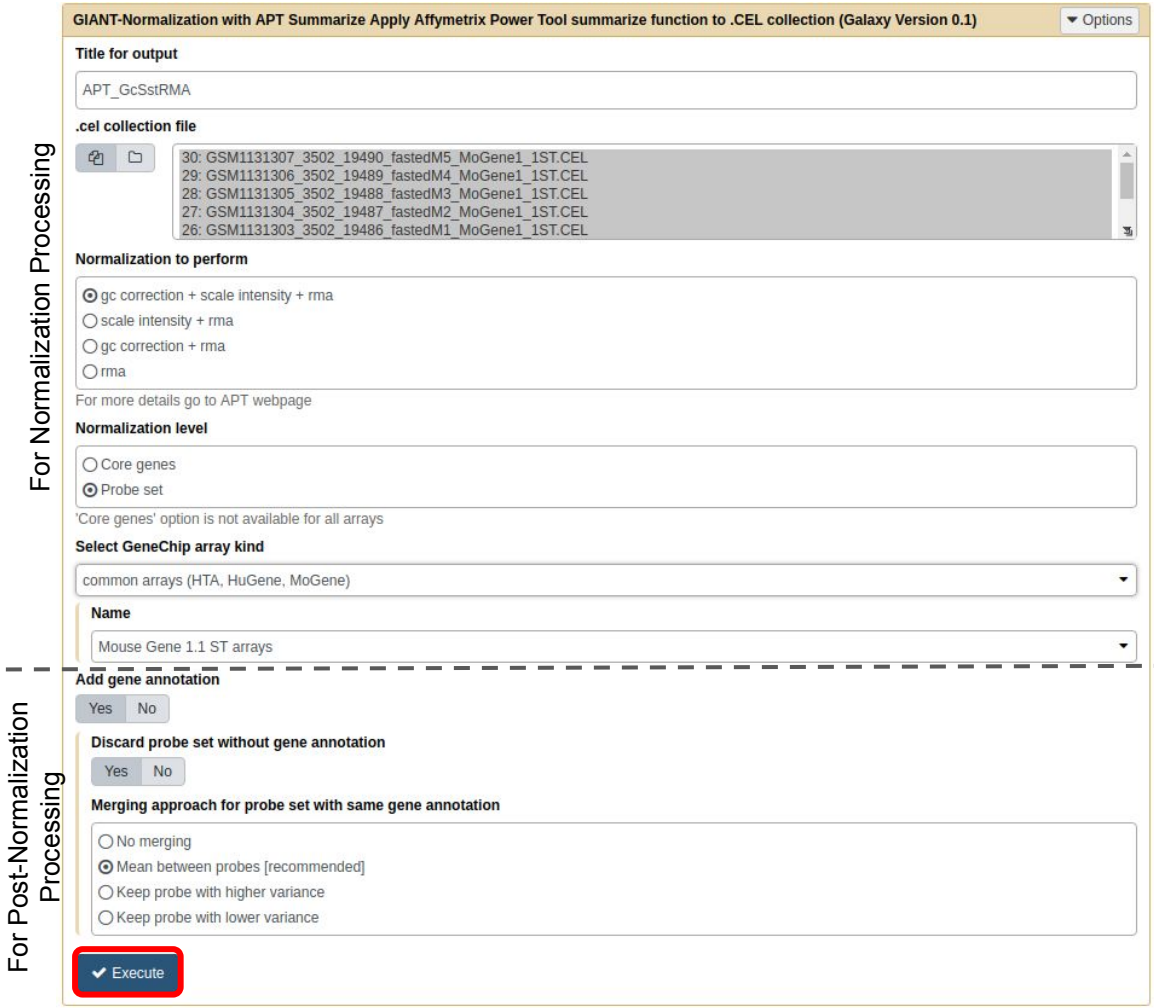


Choose a title

Select CEL files

Select normalization options

GC correction

Select GeneChip kind

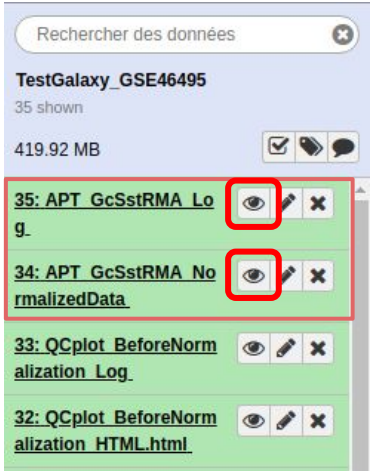
Add gene annotation

And execute !

RMA Normalization

2 results in history :

- log file
- normalized data



35: APT_GcSstRMA_Log

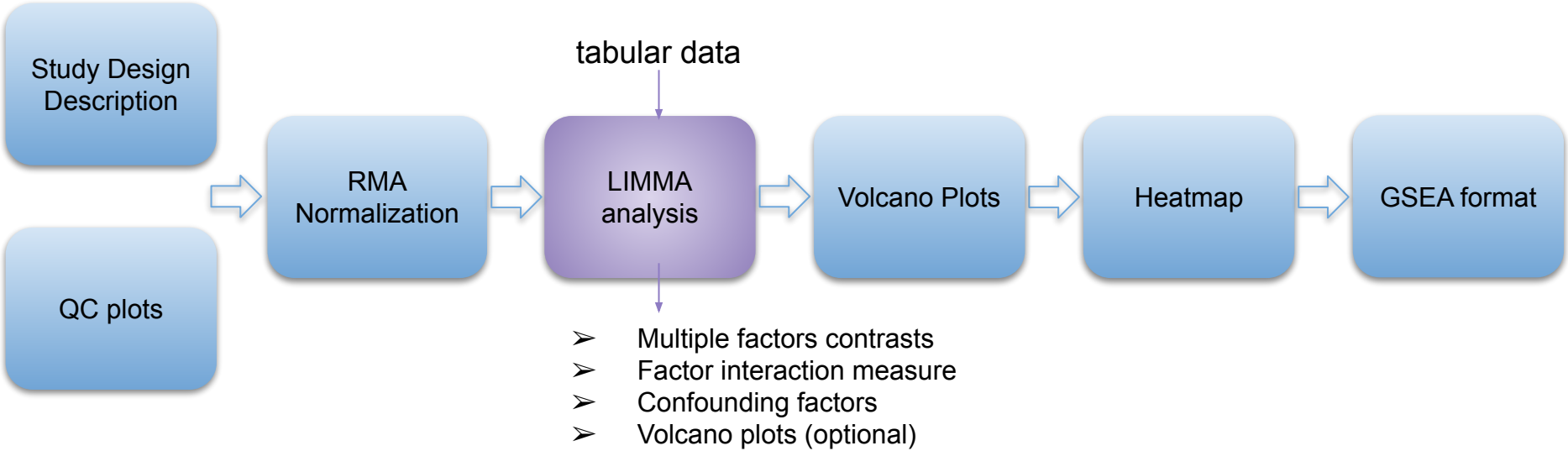
```
Running ProbesetSummarizeEngine...
Opening clf file: MoGene1.1ST.clf
Opening pgf file: MoGene1.1ST.pgf
Reading 241576 probesets.....................................Done. (0.06 min)
Setting analysis info.
Reading and pre-processing 30 cel files.....................Done. (0.08 min)
Processing 1 chipstream.
Applying GCCorrection to 30 cel datasets.....................Done. (0.13 min)
Applying ScaleIntensities to 30 cel datasets.................Done. (0.16 min)
Applying RMA background transformation 30 cel datasets.......Done. (0.23 min)
Computing sketch normalization for 30 cel datasets...........Done. (0.03 min)
Applying sketch normalization to 30 cel datasets.............Done. (0.13 min)
Finalizing 1 chipstream.
Processing Probesets.....................Done. (0.21 min)
Flushing output reporters. Finalizing output.
Done.
```

34: APT_GcSstRMA_NormalizedData

1	2	3	4
Conditions	GSM1131278_3502_19461_fedF1_MoGene1_1ST.CEL	GSM1131279_3502_19462_fedF2_MoGene1_1ST.CEL	GSM1131280_3502_19463_fedF3_MoGene1_1ST.CEL
Cep72	5.79442	6.13562	5.84982
Unc93b1	10.344	10.3944	10.2048
n-R5s217	4.32256	4.14826	4.35658
Gm28388	4.16515	4.16639	4.18478
C920025E04Rik	4.71314	5.56514	5.28329
Fdxr	5.41385	5.48893	5.36192
Cep76	6.91781	6.92847	6.72883
Uba1	13.7473	13.8369	13.596
Cep78	8.03659	8.1785	8.2553

GIANT - Tools Suite

Microarray analysis pipeline: “ from .CEL to GSEA “



Limma Analysis

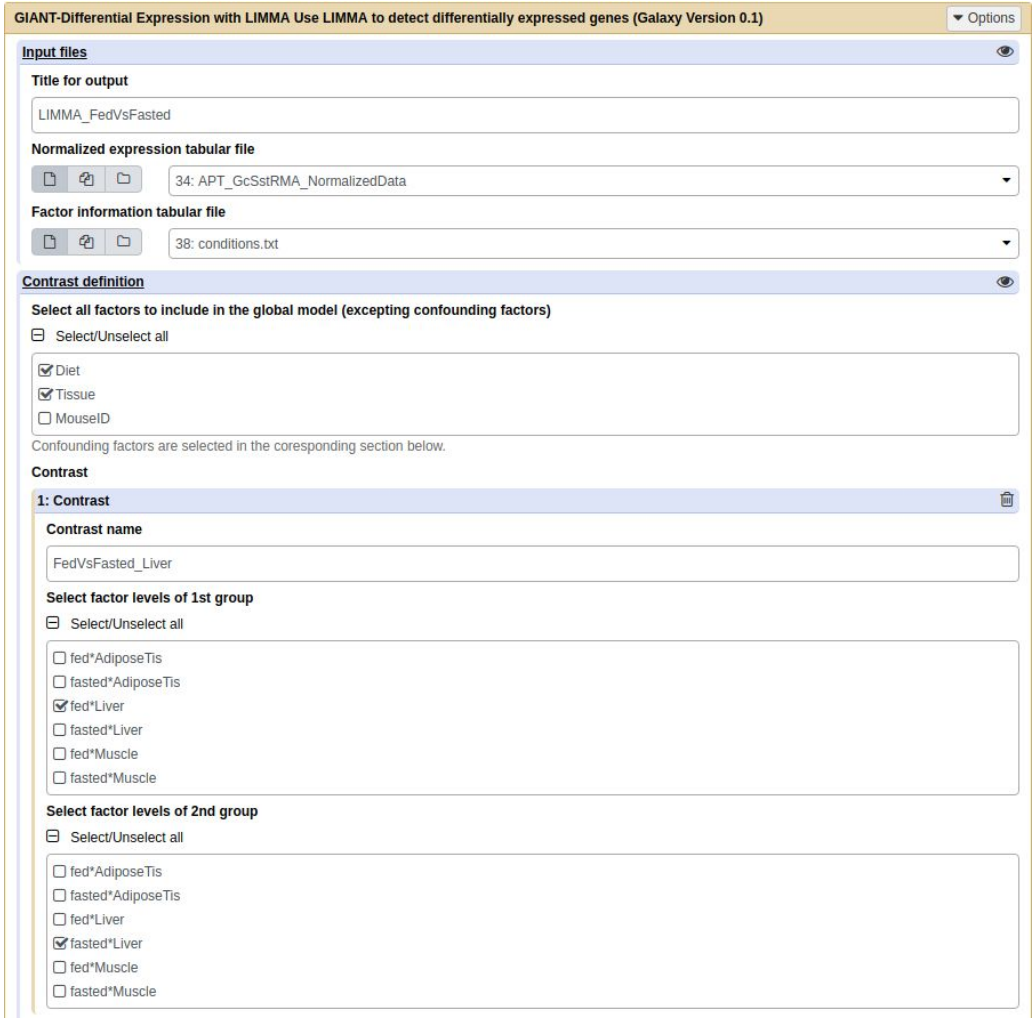
Differential Analysis - Confounding factors

1. What is a confounding factor ?
   a. Paired-analysis (same individual for 2 or more samples)
   b. Batch effect : scan dates for example
   c. Any “blocking” effect of samples not confounded with principal factors
2. How deal with them ?
   a. In differential analysis : add a column in conditionFile.txt and use the form “confounding factor” option. Possibility to use multiple confounding factors.
   b. Why we doesn’t use existing removeBatch functions or Combat package ?
      i. It’s better to make a global modelisation for the differential analysis. So to take into account all potential effects (principal & confounding) in the model.
      ii. This kind of data pre-treatment is only dedicated for unbiased data exploratory (as clustering/network inference/co-module expression).

LIMMA analysis

GIANT

- GIANT-QC Plots Descriptive plots of .CEL collections or normalized expression data
- GIANT-Heatmap and Hierarchical clustering Run hierarchical clustering and plot heatmap from expression data and/or differential expression analysis
- GIANT-Normalization with APT Summarize Apply Affymetrix Power Tool summarize function to .CEL collection
- GIANT-GSEA Formating Format input files for GSEA software
- GIANT-Differential Expression with LIMMA Use LIMMA to detect differentially expressed genes



Choose a title

Select normalized data and factor information

Select factors needed for contrasts

⚠ *No confounding*

Define a first contrast :
- name
- first group members
- second group members

LIMMA analysis

GIANT

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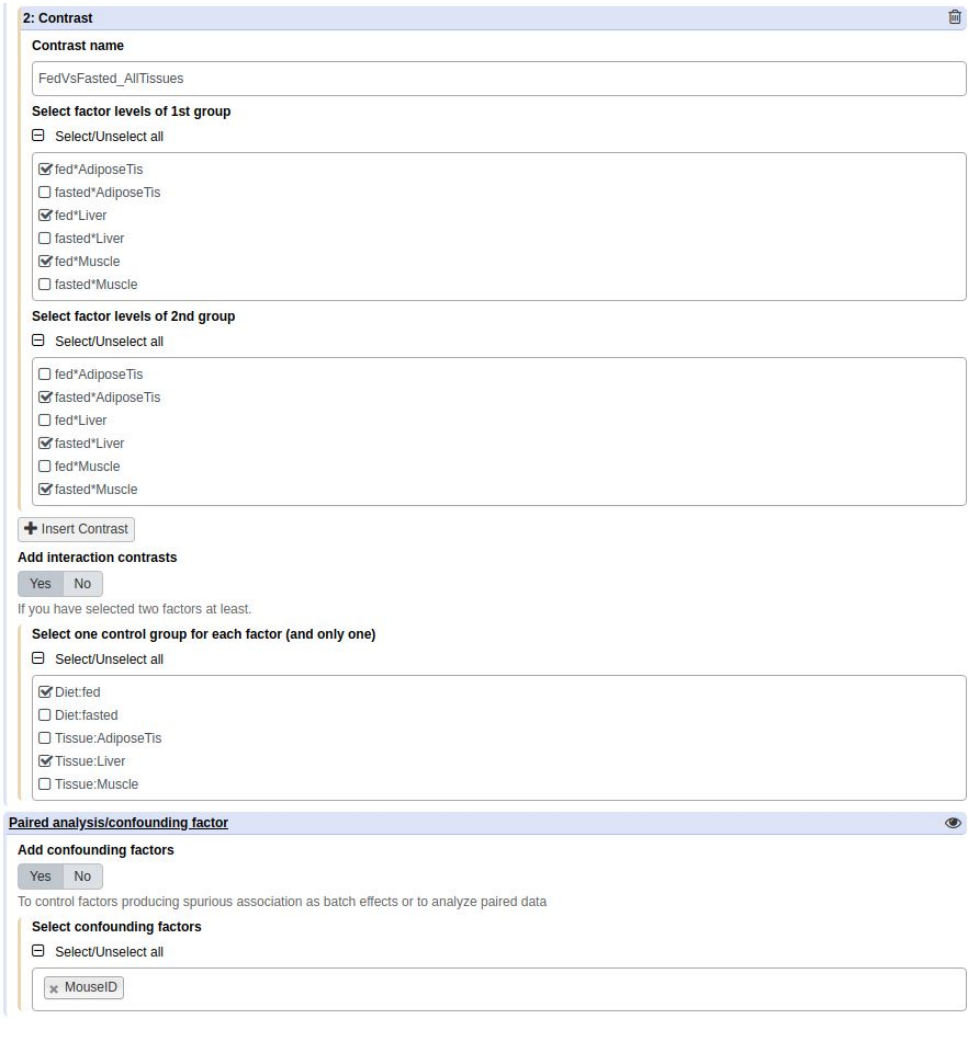


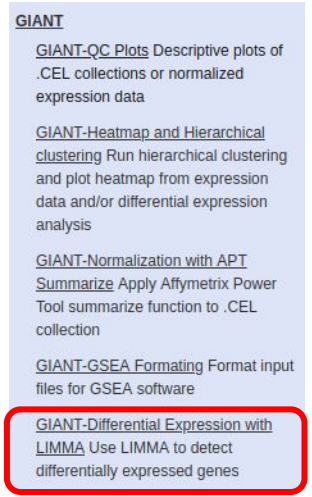
Define a second contrast :
- name
- first group members
- second group members

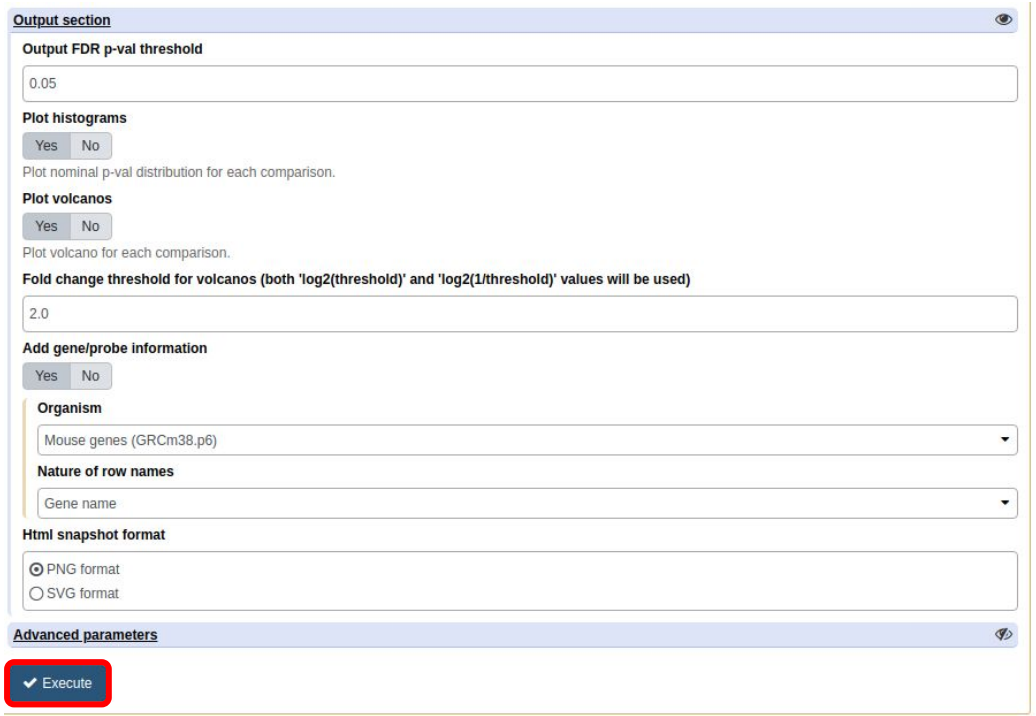
As many as you need !

To compute interaction contrast you need control group **for each factor**

Precise potential confounding factors as paired analysis...

LIMMA analysis





Define graphic and output filtering options

⚠ *FDR p-val / FC thres.*

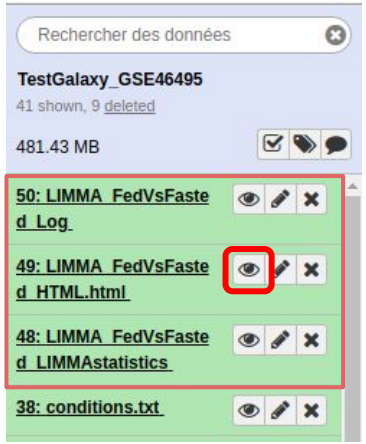
Select studied organism to add gene infos in output files

And execute !

LIMMA analysis

4 results in history :

- log file
- HTML page
- *Tabular LIMMAdetailed* **For future developpments, don't use it**
- Tabular LIMMAstatistics



Html page

LIMMA statistics (p.val, FC)

LIMMA results

P-val histograms

Histogram Diet_fasted:Tissue_AdiposeTis

Histogram Diet_fasted:Tissue_Muscle

Histogram FedVsFasted_AllTissues

Histogram FedVsFasted_Liver

Source of variation

F-ratio barplot

Volcanos

Volcano Diet_fasted:Tissue_AdiposeTis

Volcano Diet_fasted:Tissue_Muscle

Volcano FedVsFasted_AllTissues

Volcano FedVsFasted_Liver

LIMMA analysis

Can I continue my analysis ?

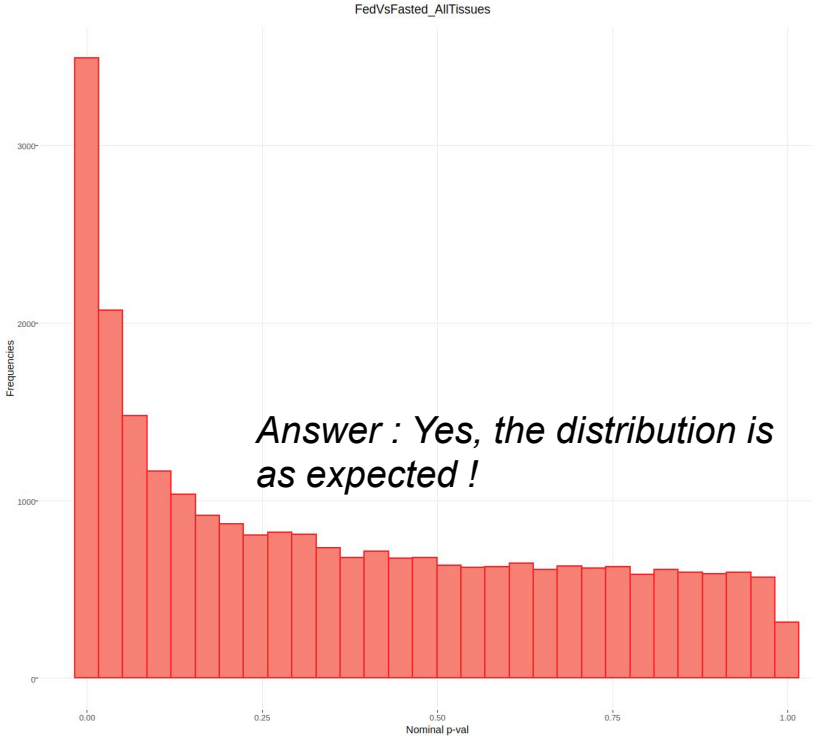
P-val histograms

Histogram Diet_fasted:Tissue_AdiposeTis

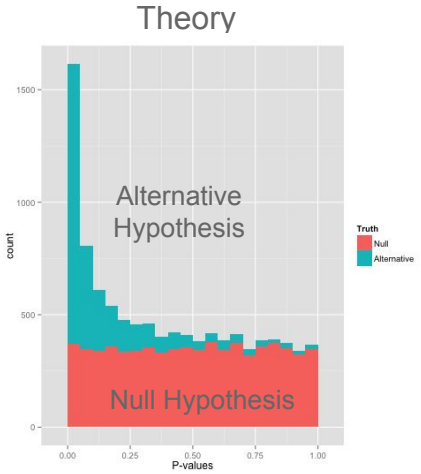
Histogram Diet_fasted:Tissue_Muscle

Histogram FedVsFasted_AllTissues

Histogram FedVsFasted_Liver



Answer : Yes, the distribution is as expected !



LIMMA analysis

Can I continue my analysis ?

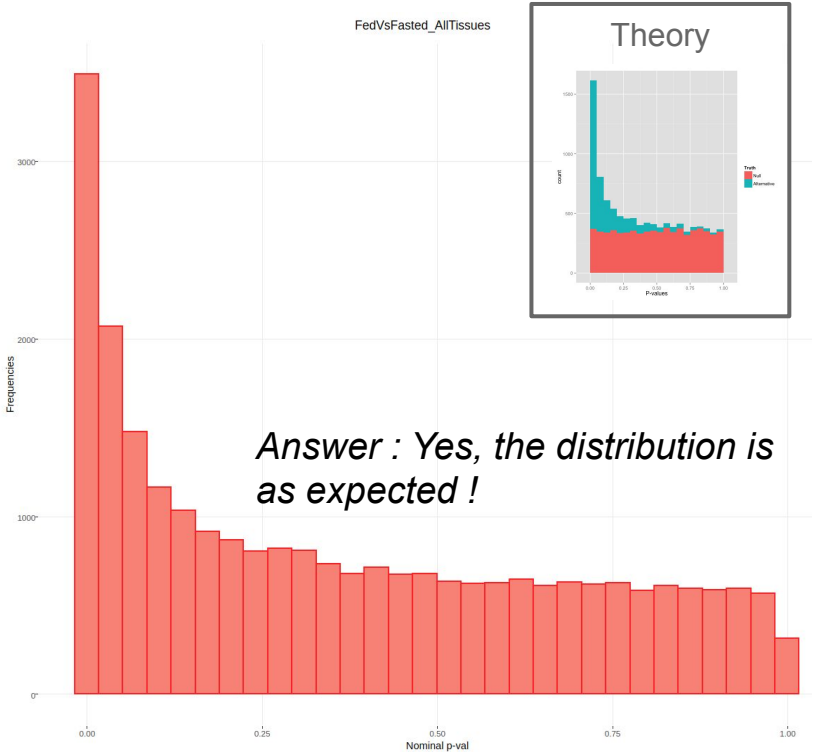
P-val histograms

Histogram Diet_fasted:Tissue_AdiposeTis

Histogram Diet_fasted:Tissue_Muscle

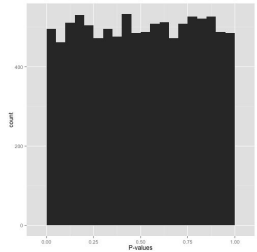
Histogram FedVsFasted_AllTissues

Histogram FedVsFasted_Liver



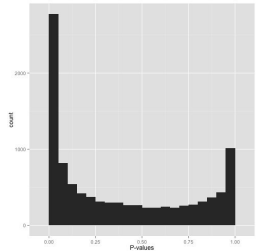
Answer : Yes, the distribution is as expected !

BAD examples & solutions… or not



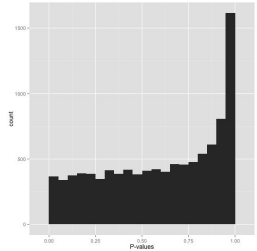
***Small % hypothesis Not Null**, apply FDR to find them

* DON'T :"Accept everything with p-values < 0.05"



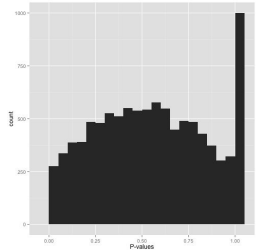
*DON'T apply FDR, **Correct & Rerun**

* Can be due to very low signal in most of conditions for some genes (filter ?)



*Something is **wrong with your test** (assumption on signal distribution ?)

* Find a statistician



***"What the…?!?"**

* Find a statistician

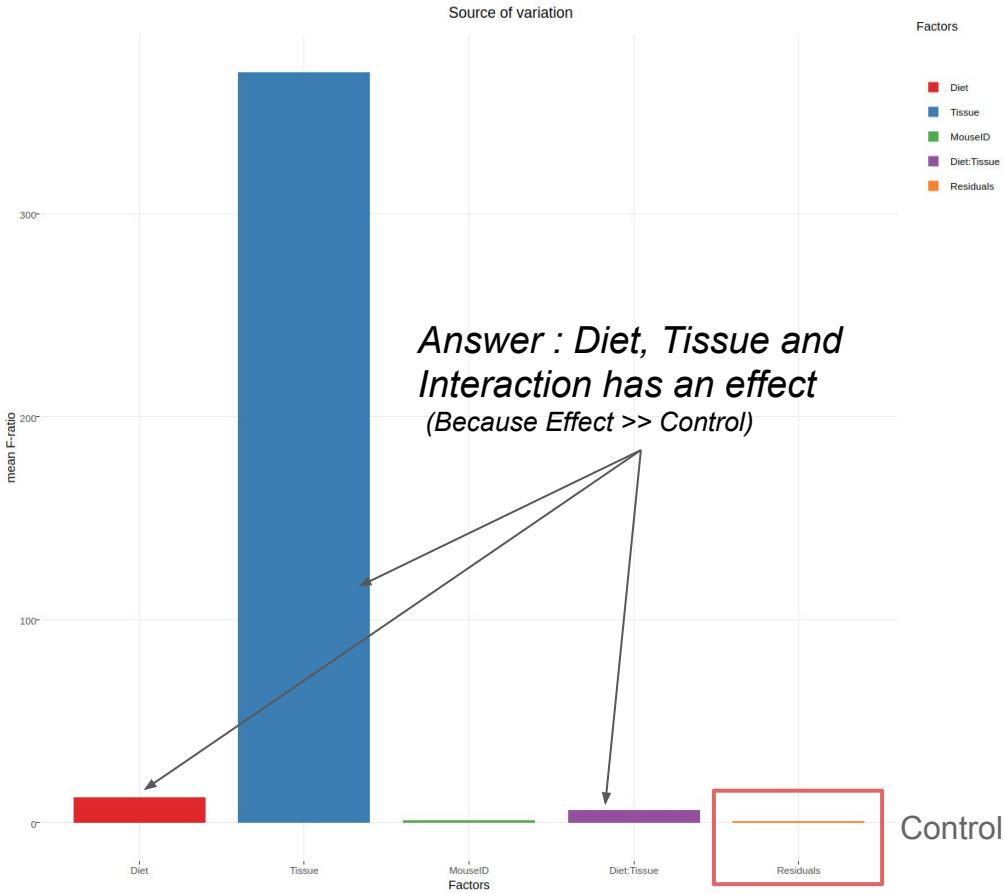
We expect a uniform distribution of p-value except for low p-values. If it's not the case, there is a problem with your dataset. http://varianceexplained.org/statistics/interpreting-pvalue-histogram/

LIMMA analysis

Which factor has an effect in my study ?

Source of variation

F-ratio barplot



Answer : Diet, Tissue and Interaction has an effect
(Because Effect >> Control)

Control set to 1

Inspect the graph and see if there is an "effect". Generally, the effect here is retrieve in the number of differential expressed genes

LIMMA analysis

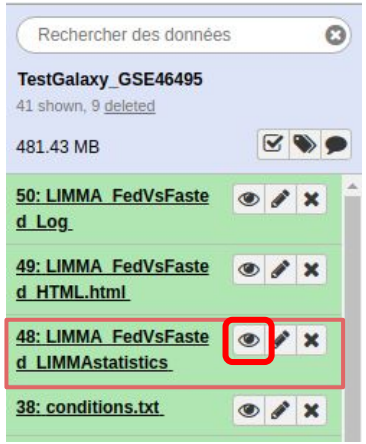
LIMMA statistics (p.val, FC)

LIMMA results

Show 10 entries

Gene	Info	Fed vs Fasted inLiver					Fed vs Fasted Global				
		p-val	FDR.p-val	FC	log2(FC)	t-stat	p-val	FDR.p-val	FC	log2(FC)	t-stat
0610007P14Rik	NA	0.1767	0.5372	1.258	0.3315	1.4	0.4394	0.72	1.116	0.1578	0.7889
0610009B22Rik	RIKEN cDNA 0610009B22 gene	0.198	0.5611	0.77	-0.3771	-1.332	0.1894	0.481	1.253	0.3252	1.359
0610009L18Rik	RIKEN cDNA 0610009L18 gene	0.5914	0.835	1.063	0.08881	0.5456	0.08914	0.3214	1.186	0.2458	1.787
0610009O20Rik	NA	0.2534	0.6133	1.122	0.1656	1.176	0.08749	0.318	1.16	0.2138	1.797
0610010B08Rik	NA	0.5333	0.8042	0.889	-0.1698	-0.634	0.172	0.456	1.249	0.3207	1.417
0610010F05Rik	RIKEN cDNA 0610010F05 gene	0.9759	0.9926	1.003	0.004895	0.03063	0.4799	0.7483	1.07	0.09725	0.72
0610010K14Rik	RIKEN cDNA 0610010K14 gene	0.2977	0.6523	0.8151	-0.295	-1.069	0.6805	0.8689	1.07	0.09743	0.4179
0610011F06Rik	NA	0.1188	0.4544	1.242	0.3128	1.63	0.08007	0.302	1.23	0.2991	1.844
0610012G03Rik	RIKEN cDNA 0610012G03 gene	0.6663	0.8732	1.043	0.06077	0.4376	0.003222	0.04594	1.313	0.3927	3.346
0610025J13Rik	RIKEN cDNA 0610025J13 gene	0.1016	0.4249	1.248	0.3198	1.716	0.1001	0.3418	1.207	0.2716	1.724
Gene	Info	p-val	FDR.p-val	FC	log2(FC)	t-stat	p-val	FDR.p-val	FC	log2(FC)	t-stat

LIMMA analysis

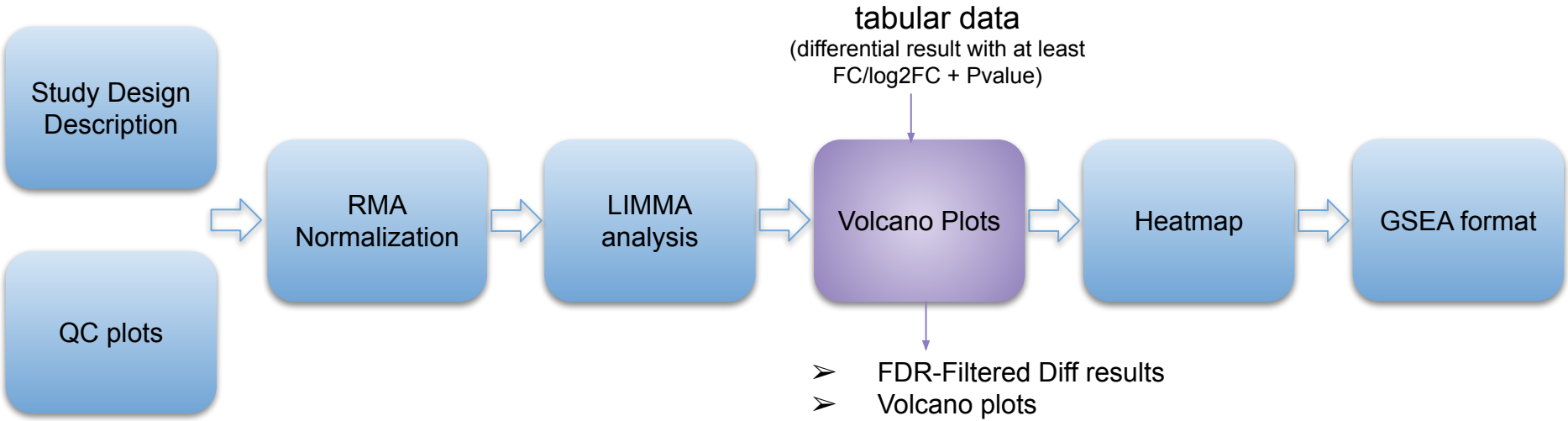


1	2	3	4	5	6	7
LIMMA	Comparison	FastVSFed_inLiver	FastVSFed_inLiver	FastVSFed_inLiver	FastVSFed_inLive	FastVSFed_inLiver
Gene	Info	p-val	FDR.p-val	FC	log2(FC)	t-stat
Gm15998	na	3.534e-24	9.091e-20	0.0371	-4.752	-33.71
Serpina7	na	3.088e-21	3.972e-17	0.04525	-4.466	-26.26
Pcsk9	na	1.444e-20	1.238e-16	0.09787	-3.353	-24.79
Hsd3b5	na	1.105e-19	7.108e-16	0.01318	-6.246	-22.97
Cdkn1a	na	1.671e-18	8.596e-15	26.95	4.752	20.73
Lss	na	1.607e-17	6.89e-14	0.1578	-2.663	-19.01
Hsd3b4	na	6.914e-17	2.307e-13	0.09852	-3.344	-17.97
Rgn	na	7.176e-17	2.307e-13	0.3125	-1.678	-17.94
Slc16a5	na	8.689e-17	2.483e-13	8.548	3.096	17.81
Mfsd2a	na	1.024e-16	2.634e-13	18.19	4.185	17.7
Zbtb16	na	1.163e-16	2.719e-13	8.234	3.042	17.61
Insig1	na	1.296e-16	2.779e-13	0.1683	-2.571	-17.53
Apex2	na	2.46e-16	4.868e-13	4.662	2.221	17.1
Usp2	na	2.				16.99
Acot1	na	3.				16.78
Rdh11	na	4.				-16.65
Sdsl	na	5.				16.61
Cyp17a1	na	6.				16.45
Cyp4a31	na	1.				15.91
Plin5	na	2.				15.62
Asl	na	3.				15.49
Id3	na	4.				-15.26
Fkbp5	na	5.				15.15
Slc27a1	na	5.				15.17
Slc22a5	na	5.08e-15	5.477e-12	3.588	1.843	15.17
Gm15441	na	7e-15	6.925e-12	17.55	4.133	14.98
Gm38481	na	8e-15	7.622e-12	0.1291	-2.953	-14.9
Aqp8	na	8.654e-15	7.95e-12	0.1235	-3.017	-14.85
Hmgcr	na	1.042e-14	9.242e-12	0.2544	-1.975	-14.74
Keg1	na	1.199e-14	1.028e-11	0.1545	-2.694	-14.66
1810055G02Rik	na	1.239e-14	1.028e-11	4.194	2.068	14.64
Adgrf1	na	1.651e-14	1.282e-11	0.149	-2.747	-14.47
Mvk	na	1.695e-14	1.282e-11	0.2556	-1.968	-14.46
Cyp2c70	na	1.672e-14	1.282e-11	0.137	-2.867	-14.46
Nsdhl	na	1.815e-14	1.334e-11	0.1722	-2.538	-14.42
Gstm3	na	2.106e-14	1.435e-11	0.1146	-3.126	-14.33
Eif4ebp3	na	2.105e-14	1.435e-11	4.46	2.157	14.33
Nudt7	na	2.142e-14	1.435e-11	0.1682	-2.572	-14.32
Fdps	na	2.176e-14	1.435e-11	0.1778	-2.492	-14.31
Myd	na	2.737e-14	1.717e-11	0.1435	-2.801	-14.18

Column
Useful only for GSEA pre-ranking.
Don't use for results interpretation

GIANT - Tools Suite

Microarray analysis pipeline: “ from .CEL to GSEA “



Volcano Plot

GIANT

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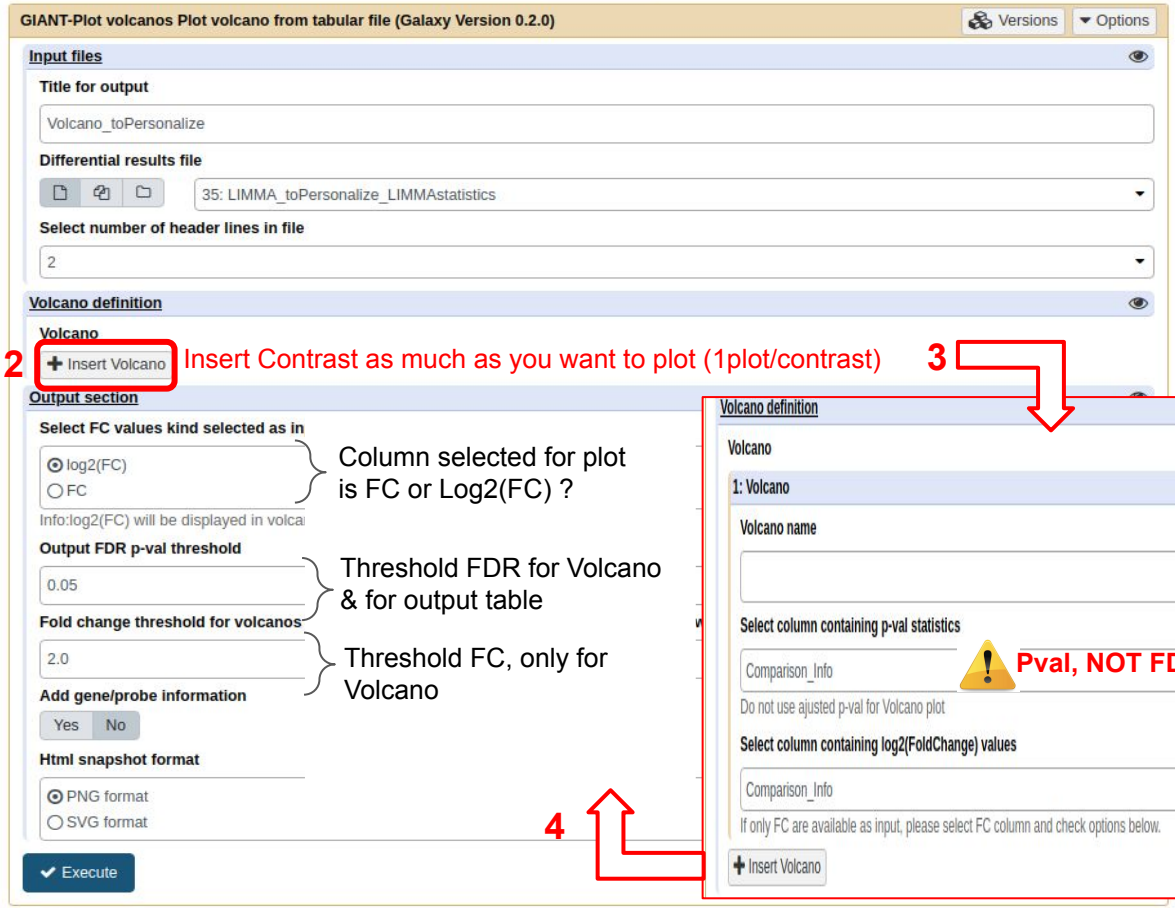
- GIANT-GSEA Formating Format input files for GSEA software
- GIANT-Differential Expression with LIMMA Use LIMMA to detect differentially expressed genes
- GIANT-Factor file generator Generate factor file used by other GIANT tools
- GIANT-Plot volcanos Plot volcano from tabular file

1



Choose a title

Select LIMMA results file
OR
Differential table (ex: RNA-seq)

= 2 if it's a LIMMA result

Column selected for plot is FC or Log2(FC) ?

Threshold FDR for Volcano & for output table

Threshold FC, only for Volcano

Choose a Plot Title

Select column of differential file containing p-val for contrast of interest

Select column of differential file containing FC or log2(FC) for contrast of interest

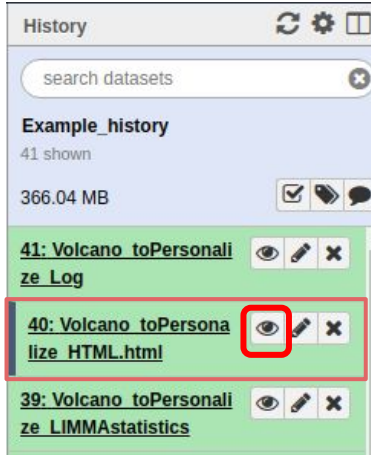
5 Add Volcano as much as contrasts you want to plot **And execute !**

COMPATIBILITY with ANY Differential Results (from GIANT or not, From microarrays or RNA-seq ...) in tabular format

Volcano Plot

3 results in history :

- log file
- Tabular file (with selected genes regarding FDR threshold)
- Html page



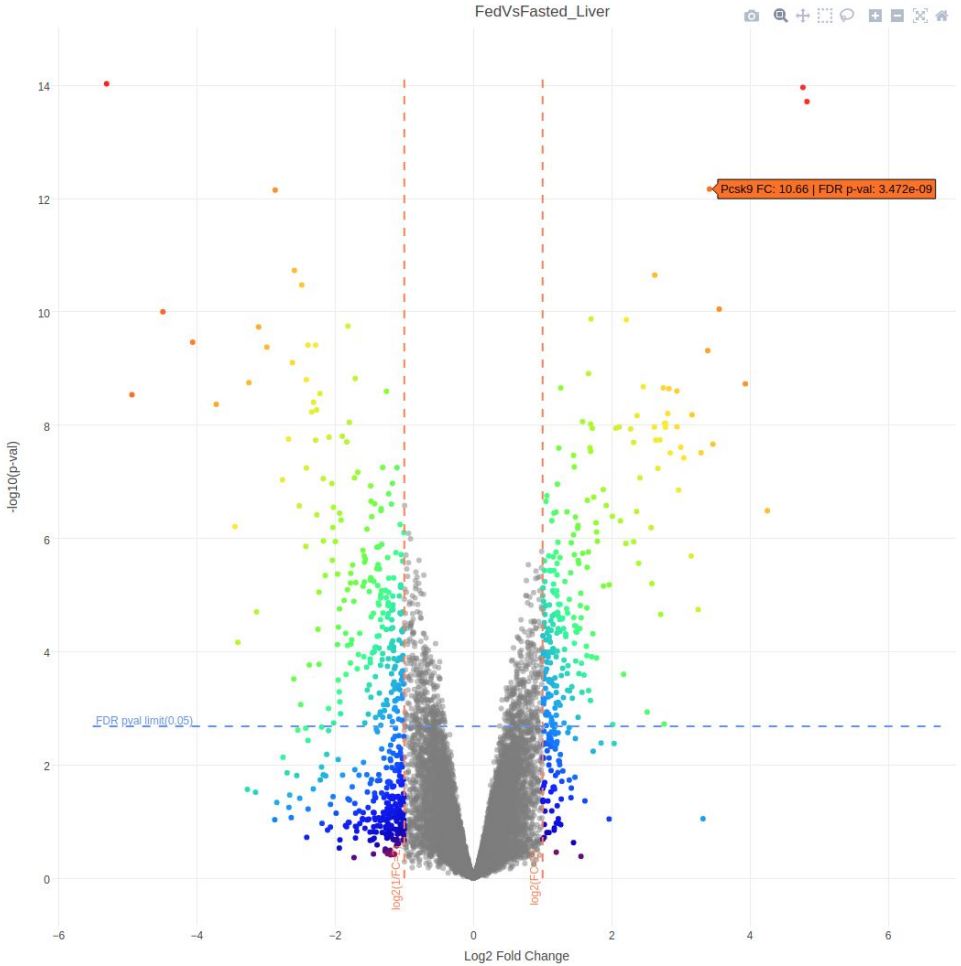
Volcanos

Volcano Diet_fasted:Tissue_AdiposeTis

Volcano Diet_fasted:Tissue_Muscle

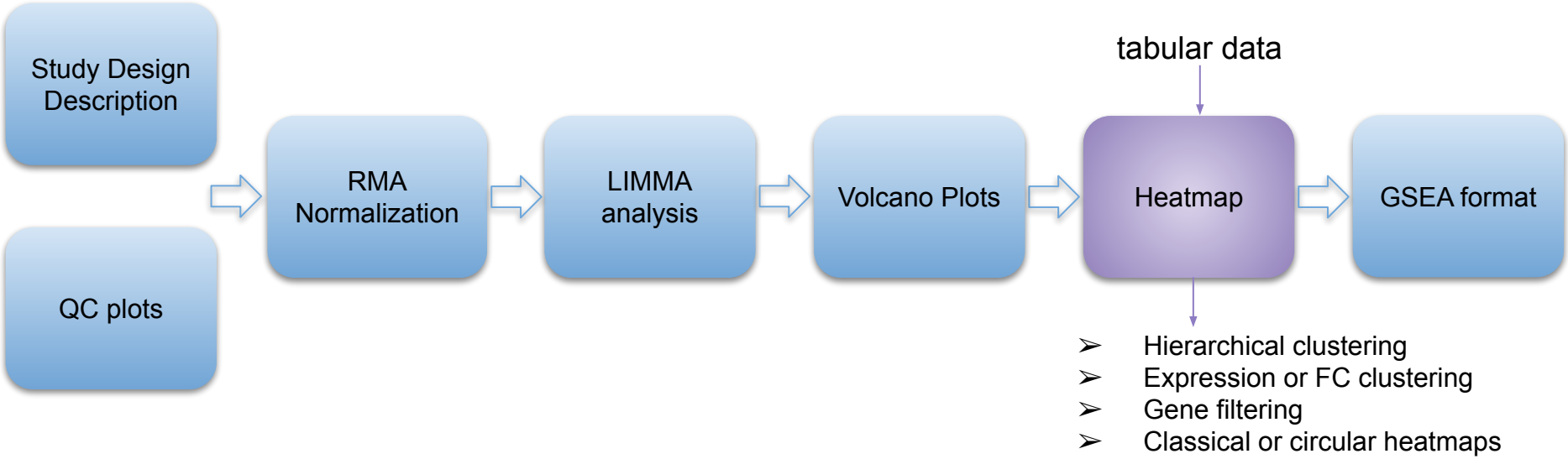
Volcano FedVsFasted_AllTissues

Volcano FedVsFasted_Liver



GIANT - Tools Suite

Microarray analysis pipeline: “ from .CEL to GSEA “



Heatmap

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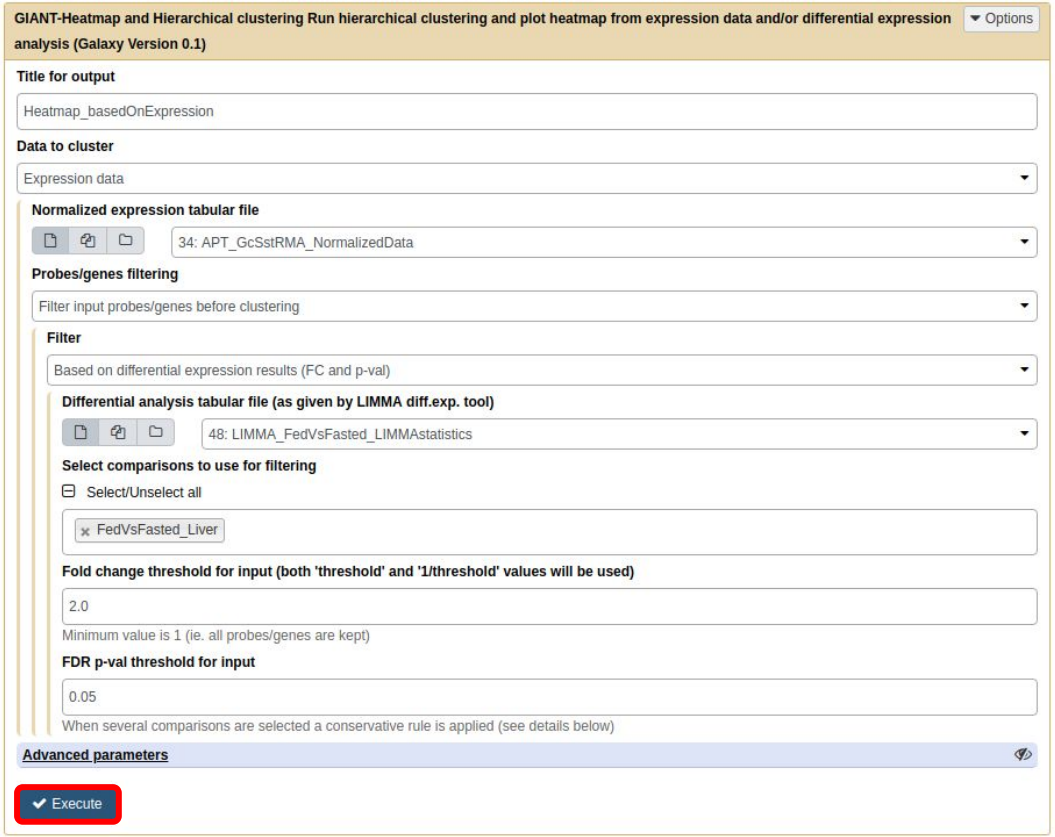


Choose a title

Choose data to cluster, here expression data

Select optional filtering policy

Select filtering options corresponding to chosen policy (here contrast to use and thresholds on FC and FDR p-val)

And execute !

Heatmap

3 results in history :

- log file
- HTML page
- tabular results

Html page

Clustering tabular

Clustering results

Heatmap plot

Heatmap

Scree plot

Scree plot

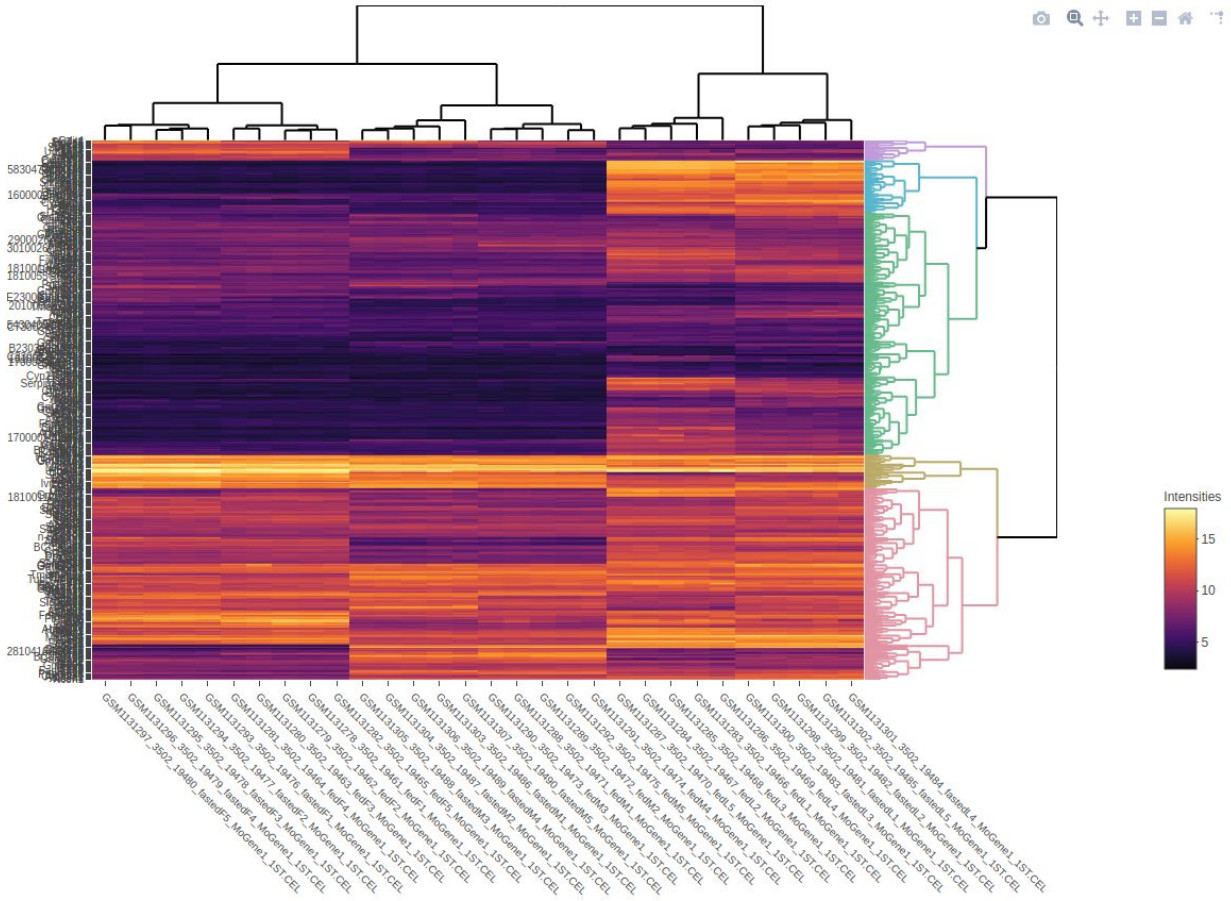
Circular plot

Circular plot

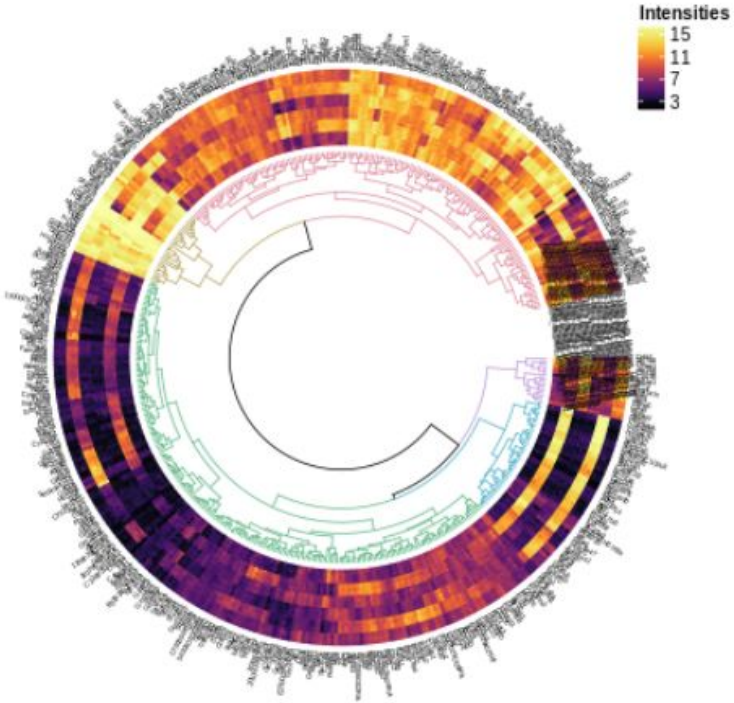
Heatmap

Heatmap plot

Heatmap



Heatmap

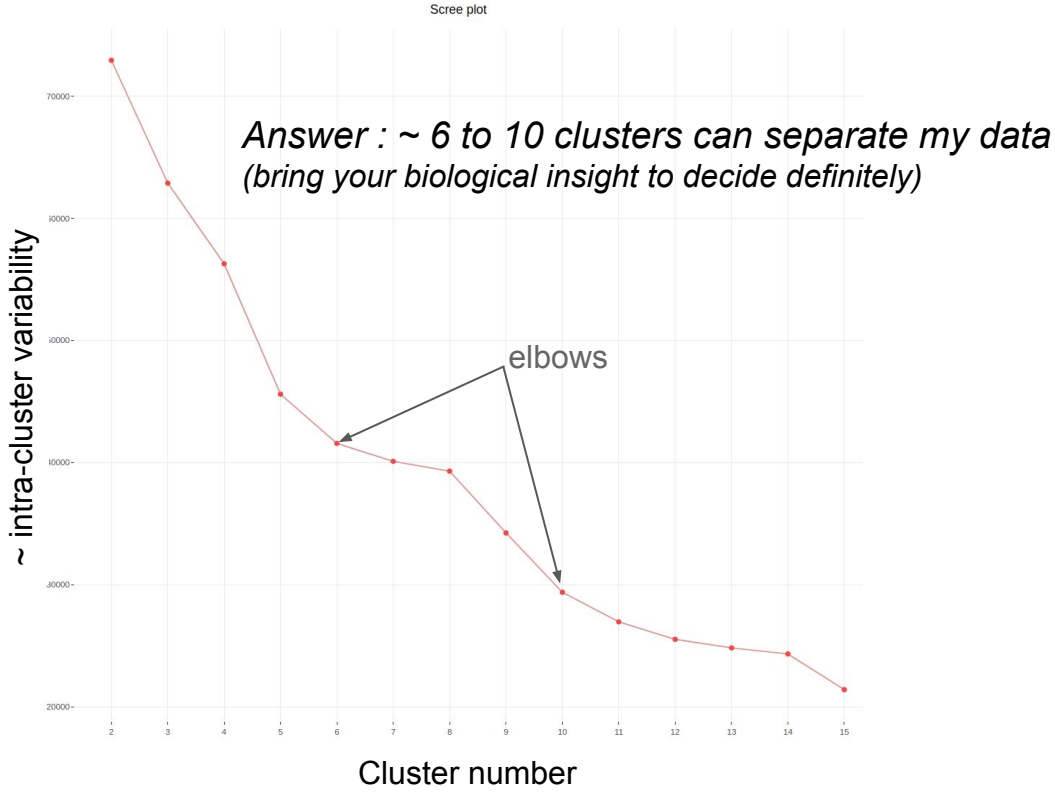


Circular plot

Circular plot

Heatmap

How many clusters are informative/useful ?



Answer : ~ 6 to 10 clusters can separate my data
(bring your biological insight to decide definitely)

Scree plot

Scree plot

Heatmap

Clustering tabular

Clustering results

Show 10 entries

Search:

Gene	Info	Cluster	FedVsFasted_Liver			
			p-val	FDR.p-val	FC	log2(FC)
Cdkn1a	cyclin-dependent kinase inhibi	4	9.414e-15	1.404e-10	0.02529	-5.306
Serpina7	serine (or cysteine) peptidase	1	1.092e-14	1.404e-10	27.18	4.765
Gm15998	predicted gene 15998	1	1.943e-14	1.666e-10	28.31	4.823
Hsd3b5	hydroxy-delta-5-steroid dehydr	1	8.1e-13	3.472e-09	94.14	6.557
Pcsk9	proprotein convertase subtilis	1	6.793e-13	3.472e-09	10.66	3.414
Zbtb16	zinc finger and BTB domain con	2	7.096e-13	3.472e-09	0.137	-2.868
Usp2	ubiquitin specific peptidase 2	4	1.855e-11	6.816e-08	0.1662	-2.589
Lss	lanosterol synthase	1	2.245e-11	7.219e-08	6.157	2.622
Per1	period circadian clock 1	4	3.353e-11	9.583e-08	0.1789	-2.483
Srebf1	sterol regulatory element bind	4	8.962e-11	2.305e-07	11.75	3.554
Gene	Info	Cluster	p-val	FDR.p-val	FC	log2(FC)

Showing 1 to 10 of 465 entries

Previous 1 2 3 4 5 … 47 Next

Heatmap

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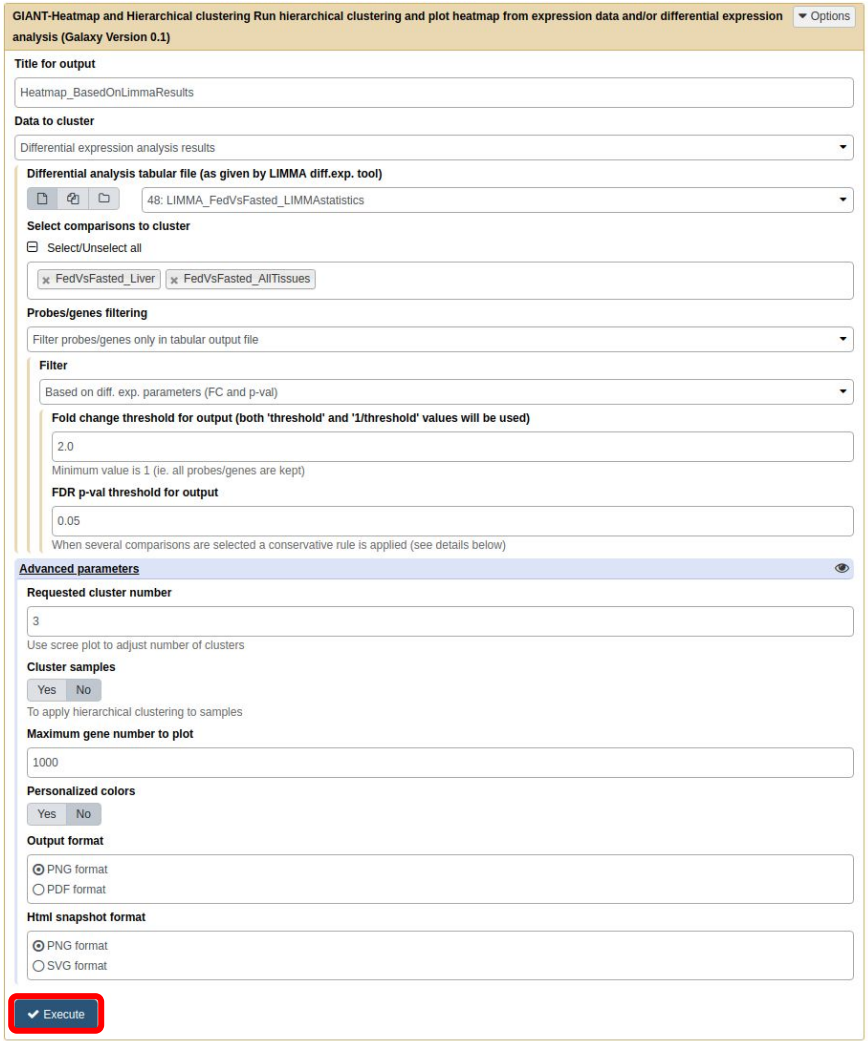


Choose a title

Choose data to cluster, here contrast FC from differential analysis results

Select optional filtering policy and corresponding options

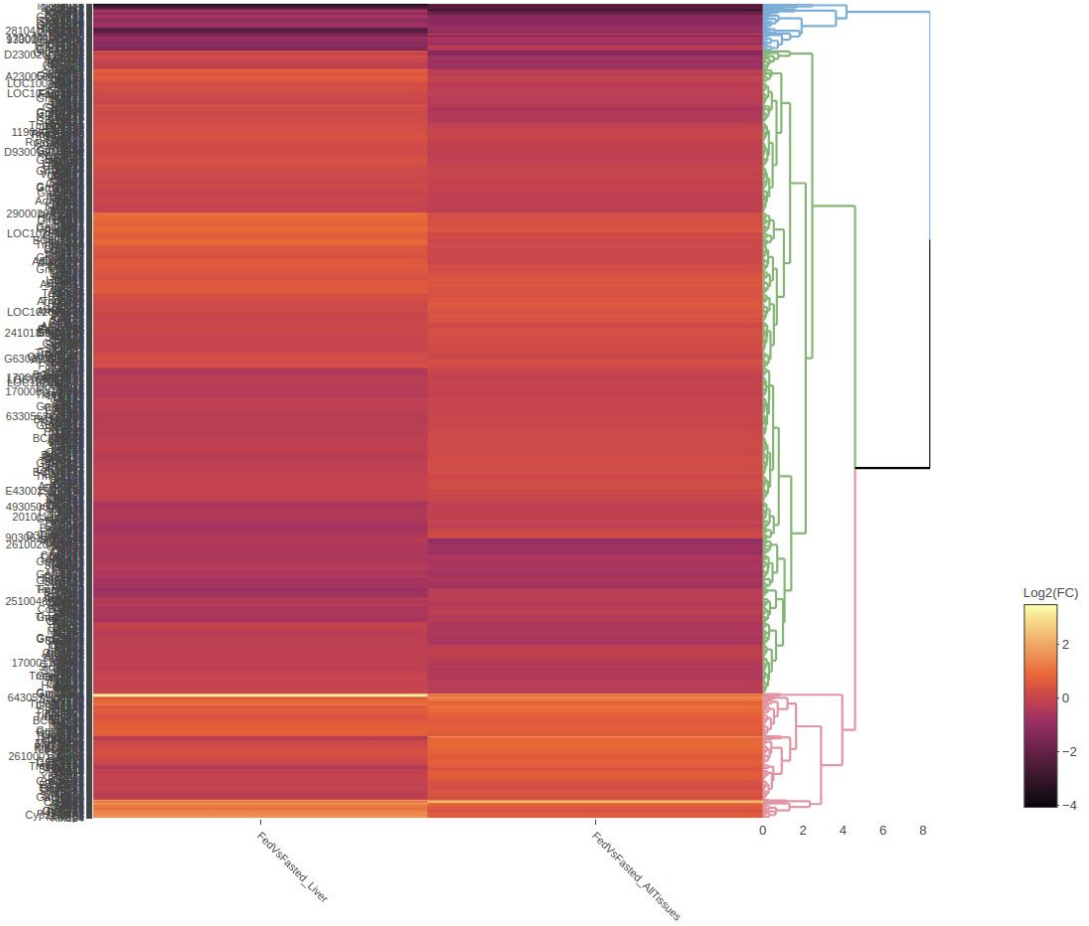
Define clustering options :
- cluster number
- cluster samples/contrasts
- color gradient

And execute !

Heatmap

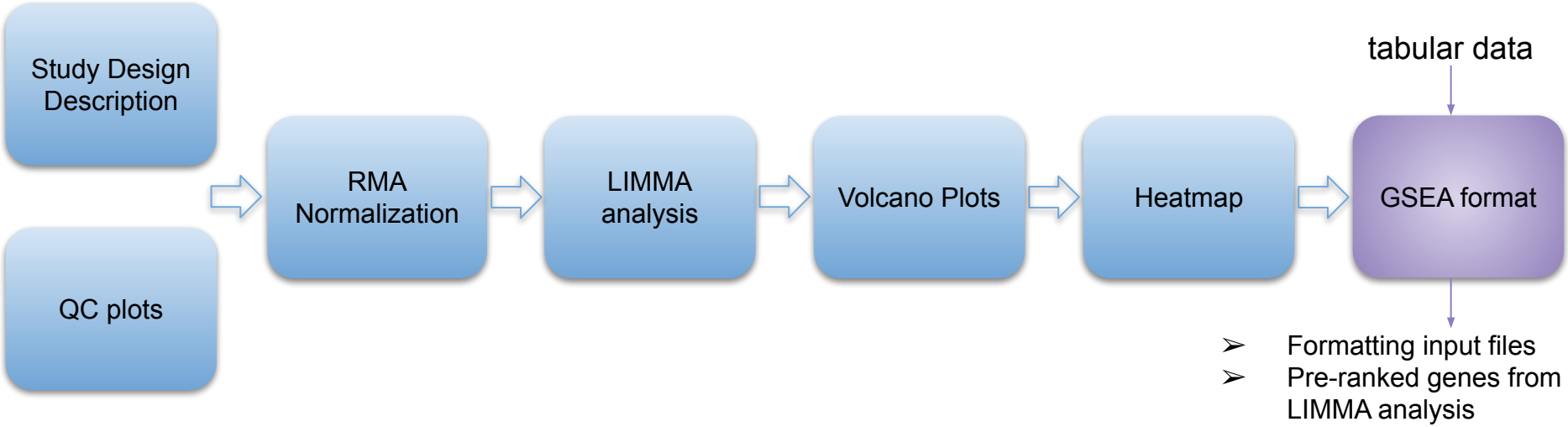
Heatmap plot

Heatmap

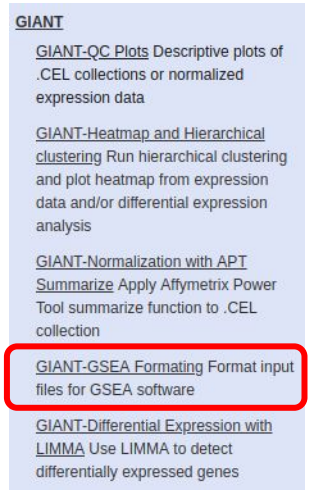


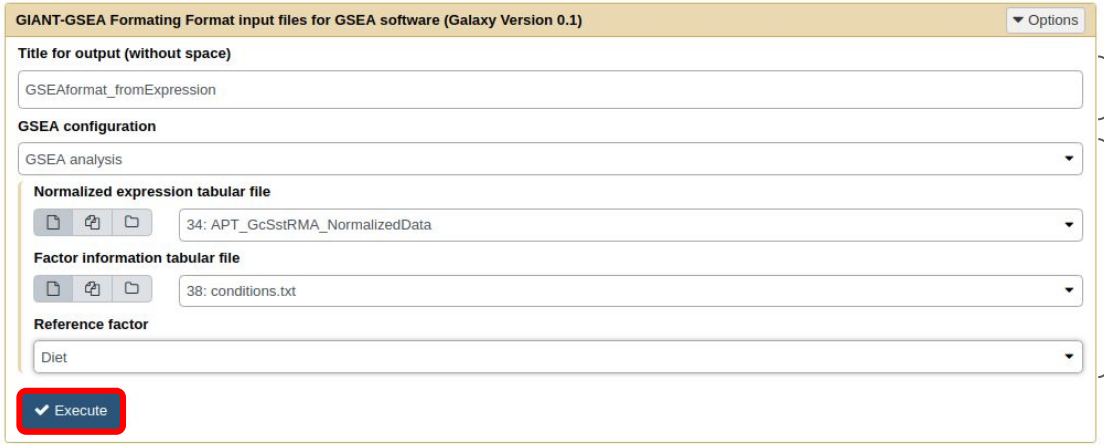
GIANT - Tools Suite

Microarray analysis pipeline: “ from .CEL to GSEA “



GSEA format





Choose a title

For GSEA analysis, select expression and condition files with phenotype factor

And execute !

GSEA format

3 results in history :

- log file
- phenotype file
- expression file

61: GSEAformat_fromExpression_Phenotypes.cls

```
30 2 1
# fed fasted
fed fed fed fed fed fed fed fed fed fed fed fed fed fed fed fed fasted fasted
```

60: GSEAformat_fromExpression_Phenotypes.gct

```
#1.2
25723   30
NAME    DESCRIPTION     GSM1131278_3502_19461_fedF1_MoGene1_1ST.CEL     GSM1131279_3502_19462_fedF2_MoGene1_1ST.CEL     GSM1131280_3502_19463_fe
Cep72   na      5.79442 6.13562 5.84982 5.98774 5.92123 5.33125 5.58054 5.55378 5.46471 5.23132 4.93863 4.99035 5.00348 4.70104 5.28328 5.8962
Unc93b1 na      10.344  10.3944 10.2048 10.4642 10.4454 9.47205 9.77977 9.77173 9.96121 9.7751  7.11397 7.33832 6.99454 7.05715 7.10572 10.4969
n-R5s217        na      4.32256 4.14826 4.35658 4.24367 4.56956 4.53336 4.57694 4.39047 4.36536 4.54664 4.44205 4.48468 4.40446 4.71065 4.57785
Gm28388 na      4.16515 4.16639 4.18478 3.98049 4.40679 4.33571 3.99504 4.39457 4.32379 4.24779 4.31598 4.78819 4.47686 4.65536 4.34895 3.96071
C920025E04Rik   na      4.71314 5.56514 5.28329 5.36201 5.81648 7.3407  6.80435 7.23555 7.97076 7.0976  4.14262 4.55554 4.70368 4.45674 4.42379
Fdxr    na      5.41385 5.48893 5.36192 5.31349 5.25819 7.04597 6.97918 6.95787 7.15202 7.05526 5.31033 5.05699 5.46236 5.04118 5.21375 5.22125
Cep76   na      6.91781 6.92847 6.72883 6.63956 6.92951 7.17886 7.13444 7.31411 6.83268 7.31083 8.05704 8.0441  8.10768 8.30292 7.8898  6.98429
Uba1    na      13.7473 13.8369 13.596  13.6206 13.723  13.6431 13.7014 13.7335 13.5518 13.5609 13.4516 13.3693 13.6276 13.3863 13.5045 13.3821
Cep78   na      8.03659 8.1785  8.2553  8.24717 7.8063  6.88205 6.66618 6.84899 6.24082 6.97469 7.97384 7.99195 7.81602 8.0196  8.16906 7.89462
Uba2    na      10.9947 10.9617 11.0736 10.6946 11.0001 9.95672 10.1506 10.2292 9.92522 10.1729 11.2999 11.2354 11.2891 11.2002 11.3825 10.3875
Spo11   na      3.7536  3.76609 3.76075 4.15384 3.8664  3.99895 4.07094 3.89099 4.39034 4.04493 3.86349 4.10587 4.16552 4.06177 4.21527 3.67195
Uba3    na      11.775  11.9832 11.9057 11.6576 11.8011 12.0993 11.9783 12.1653 11.337  12.1241 12.7865 12.5603 12.6863 12.545  12.81   11.1022
Dmpk    na      12.4793 12.4088 12.3493 12.5491 12.4996 9.40599 8.93488 9.3373  9.48732 9.33554 14.362  14.3146 14.4238 14.2209 14.2243 9.76844
1700084C06Rik   na      4.02575 4.19404 4.34464 4.08788 4.22004 3.94578 4.30068 4.2995  4.42673 4.75624 4.31217 4.17765 4.05961 4.46004 4.56209
Uba5    na      9.94948 9.95413 9.57235 9.80935 9.75292 9.94691 9.88339 10.2151 9.60875 10.1696 9.318   8.93312 9.21813 8.84039 9.1798  9.01907
Fubp1   na      11.7124 11.8233 11.9381 11.8558 11.7228 11.2408 11.2254 11.3883 10.1782 11.2232 10.9961 10.5842 10.8039 10.7685 10.7047 11.9859
```

GSEA format

GIANT

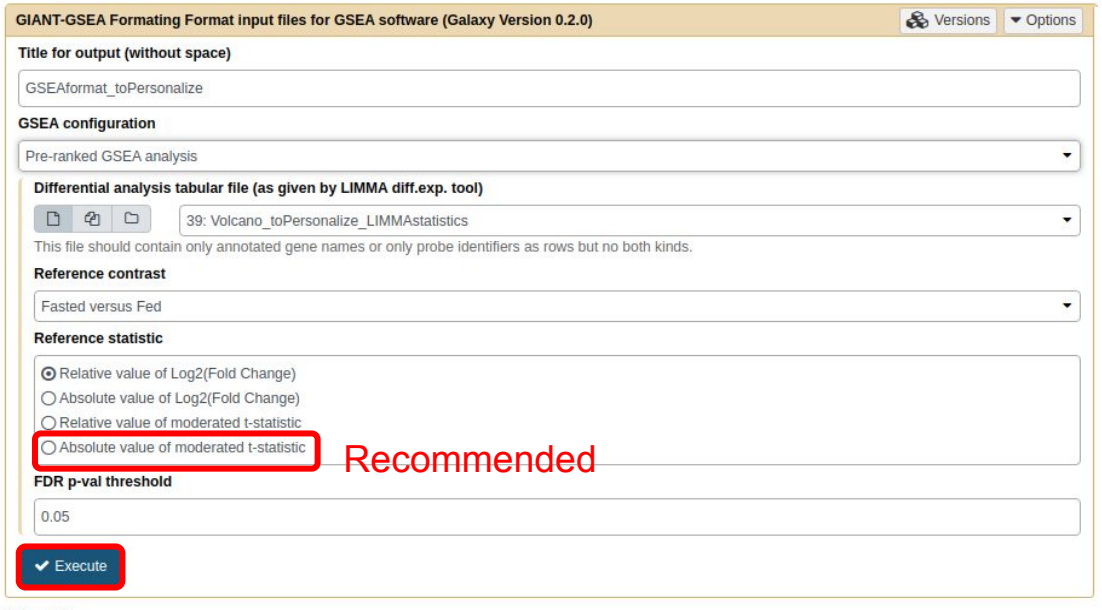
GIANT-QC Plots Descriptive plots of .CEL collections or normalized expression data

GIANT-Heatmap and Hierarchical clustering Run hierarchical clustering and plot heatmap from expression data and/or differential expression analysis

GIANT-Normalization with APT Summarize Apply Affymetrix Power Tool summarize function to .CEL collection

GIANT-GSEA Formating Format input files for GSEA software

GIANT-Differential Expression with LIMMA Use LIMMA to detect differentially expressed genes



Choose a title

For pre-ranked analysis, select diff. analysis results with contrast and sorting parameter to use

And execute !

This new option and recommendation is based on litterature & discussions with Biostatisticians,but can be adapted. Be AWARE of what you want to do with GSEA before selecting such option and adapt according your question and your expertise.