

Arrays Analysis With GIANT

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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)**

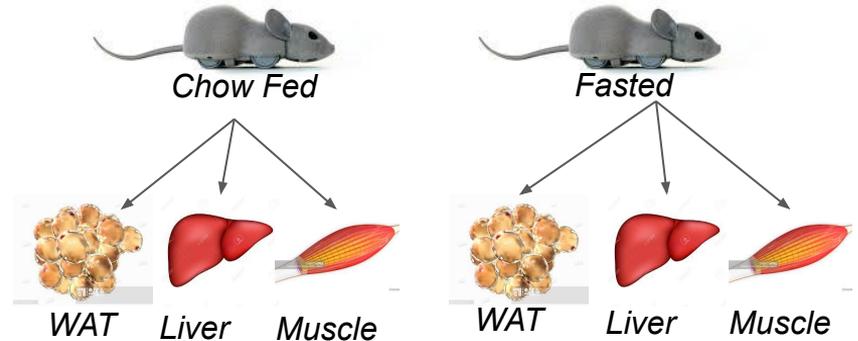
Example Dataset

Expression

➤ CEL files

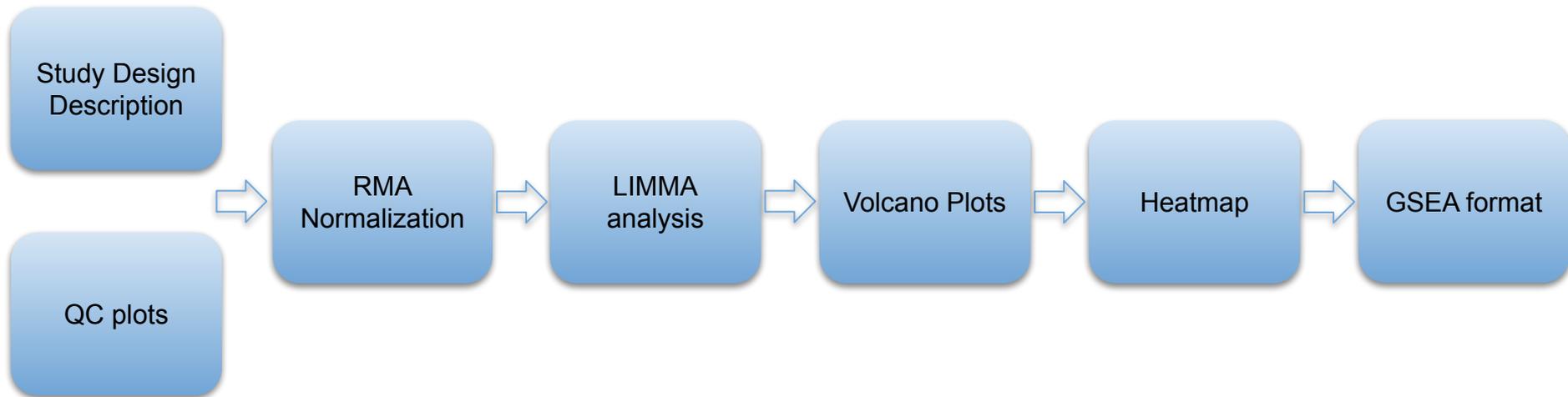
GSE46495

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



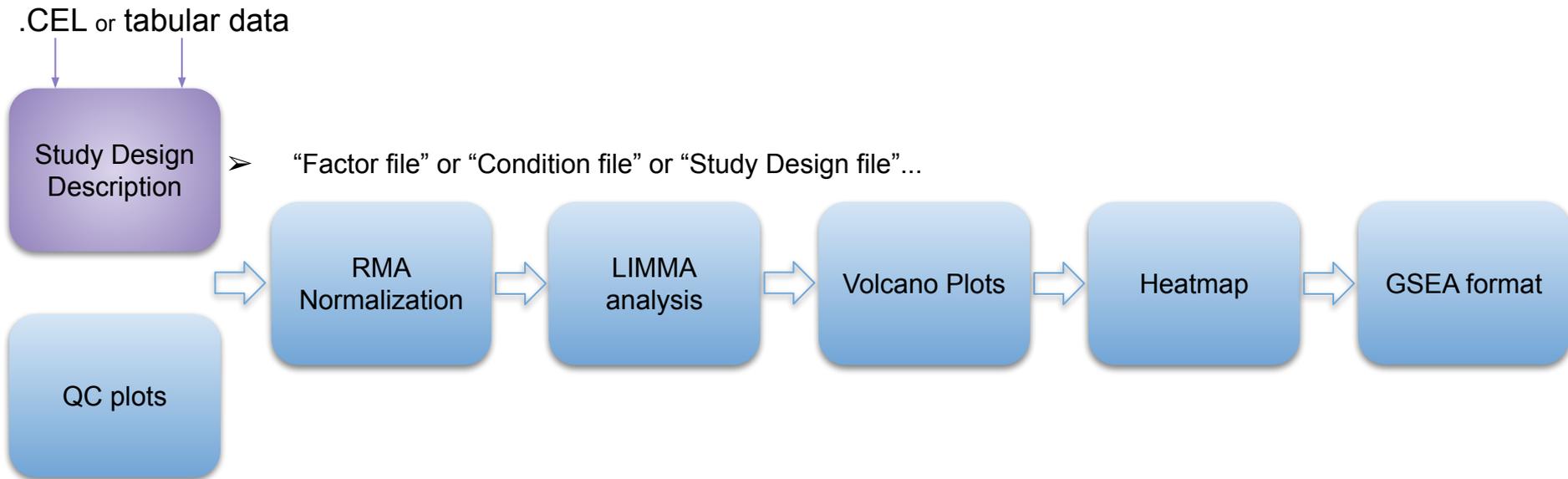
GIANT - Tools Suite

Microarray analysis pipeline: “ from .CEL to GSEA “



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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 Formatting](#) 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

GIANT-Factor file generator Generate factor file used by other GIANT tools (Galaxy Version 0.1.0) Options

Title for output
ConditionsGenerator_toPersonalize

Input data type for sample names
Expression tabular file

Select file

Factor definition

Factor

2 + Insert Factor

Execute

Create a first condition

Choose a title

Expression tabular file
.CEL files

Choose to select Normalized data or .CEL
Select file(s) of study

Factor definition

Factor

1: Factor

Factor name
Tissue

Value

3 + Insert Value

+ Insert Factor

Execute

Choose 1st condition name (ex: Tissue ,Strain, Treatment...)

Value

1: Value

Value name
Liver

Select sample sharing this value
Select/Unselect all

GSM1131302_3502_19485_fastedL5_MoGene1_1ST.CEL GSM1131301_3502_19484_fastedL4_MoGene1_1ST.CEL

GSM1131307_3502_19490_fastedM5_MoGene1_1ST.CEL

GSM1131306_3502_19489_fastedM4_MoGene1_1ST.CEL

GSM1131305_3502_19488_fastedM3_MoGene1_1ST.CEL

GSM1131304_3502_19487_fastedM2_MoGene1_1ST.CEL

GSM1131303_3502_19486_fastedM1_MoGene1_1ST.CEL

4 Click on each CEL file concerned by this condition & this value to add it in the list above

What it d
GSM1131300_3502_19483_fastedL3_MoGene1_1ST.CEL

This tool
GSM1131299_3502_19482_fastedL2_MoGene1_1ST.CEL
GSM1131298_3502_19481_fastedL1_MoGene1_1ST.CEL

Parameters

Choose 1st value of 1st condition (ex: dmsso, gw, wt, ko, liver, white_adipose_tissue...)

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

History

search datasets

Example_history

32 shown

337.7 MB

32: ConditionsGenerator toPersonalize Log

31: ConditionsGenerator or toPersonalize conditionsFile **View data**

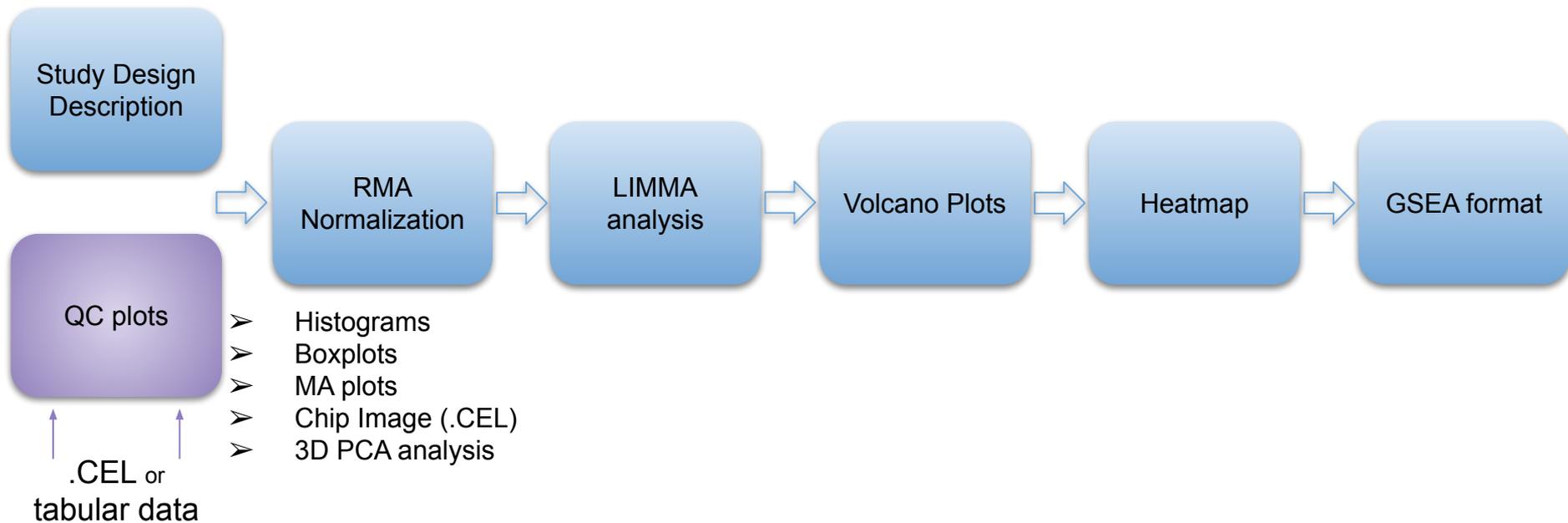
30: GSM1131278 3502 19461 fedF1 MoGene1_1ST.CEL

29: GSM1131279 3502 19462 fedF2 MoGene1_1ST.CEL

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

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

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[GIANT-Differential Expression with LIMMA](#) Use LIMMA to detect differentially expressed genes

GIANT-QC Plots Descriptive plots of .CEL collections or normalized expression data (Galaxy Version 0.1)

Options

Title for output

QCplot_BeforeNormalization

Select one .CEL collection or one tabular file

31: conditions.txt
30: GSM1131307_3502_19490_fastedM5_MoGene1_1ST.CEL
29: GSM1131306_3502_19489_fastedM4_MoGene1_1ST.CEL
28: GSM1131305_3502_19488_fastedM3_MoGene1_1ST.CEL
27: GSM1131304_3502_19487_fastedM2_MoGene1_1ST.CEL

Plots selection

Plot histograms

Yes No

Plot intensity distribution for each condition (pm probes for .cel)

Plot MA plots

Yes No

Plot MA plot for each condition, median value is used as reference

Plot boxplots

Yes No

Plot intensity through boxplot for each condition (pm probes for .cel)

Display microarrays image (only for .CEL files)

Yes No

PCA analysis

Plot 3D PCA

Yes No

3D plot of conditions in the space defined by the 3 principal components

Factor information tabular file (optional)

Nothing selected

Select factor informations to display (optional)

Advanced parameters

Execute

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

Rechercher des données

TestGalaxy_GSE46495
33 shown
413.94 MB

- 33: QCplot BeforeNormalization Log
- 32: QCplot BeforeNormalization HTML.html
- 31: conditions.txt
- 30: GSM1131307 3502 19490 fastedM5 MoGene1 1ST.CEL
- 29: GSM1131306 3502 19489 fastedM4 MoGene1 1ST.CEL

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](#)

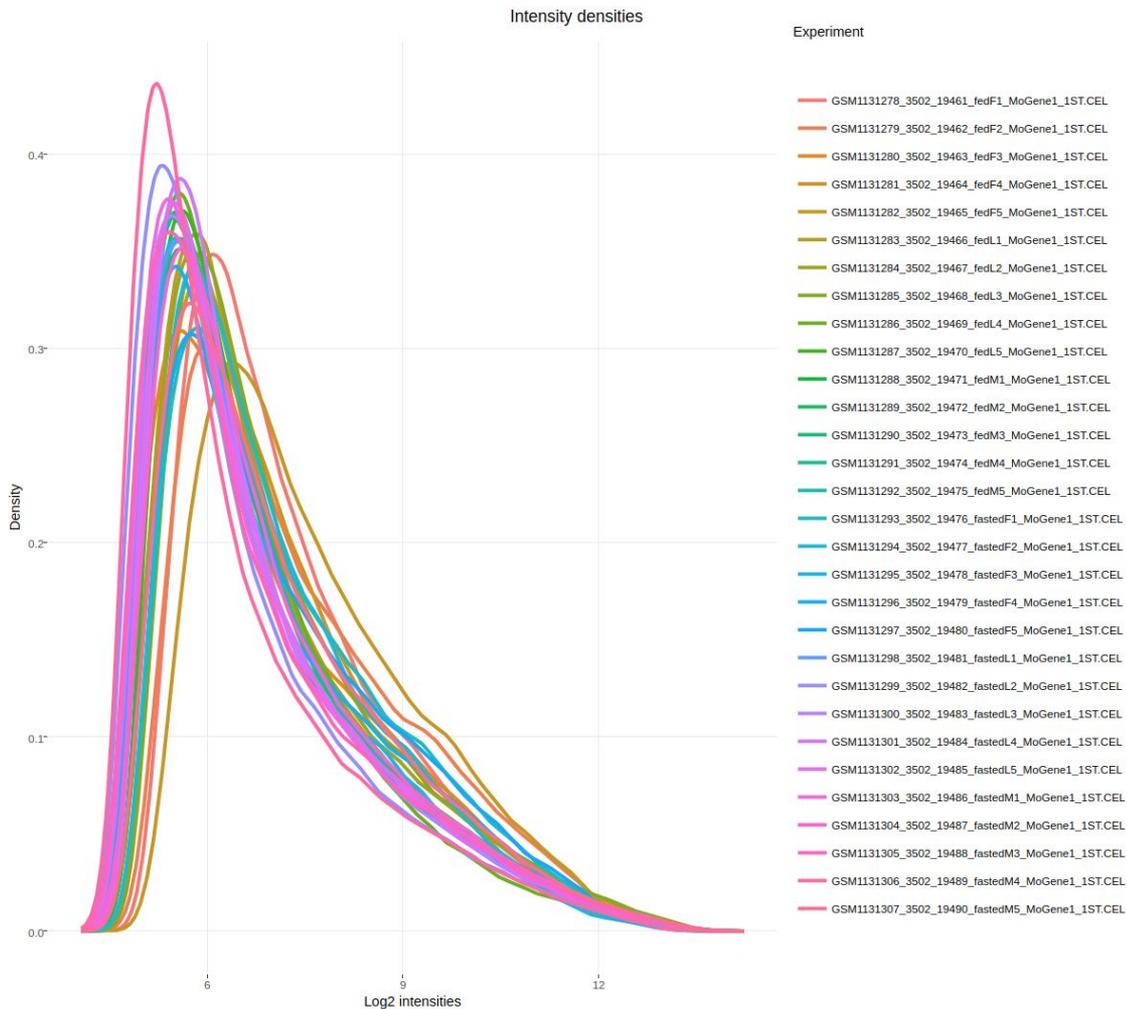
[Microarray GSM1131294 3502 19477 fastedF2 MoGene1 1ST.CEL](#)

[Microarray GSM1131295 3502 19478 fastedF3 MoGene1 1ST.CEL](#)

QC plots

Histograms

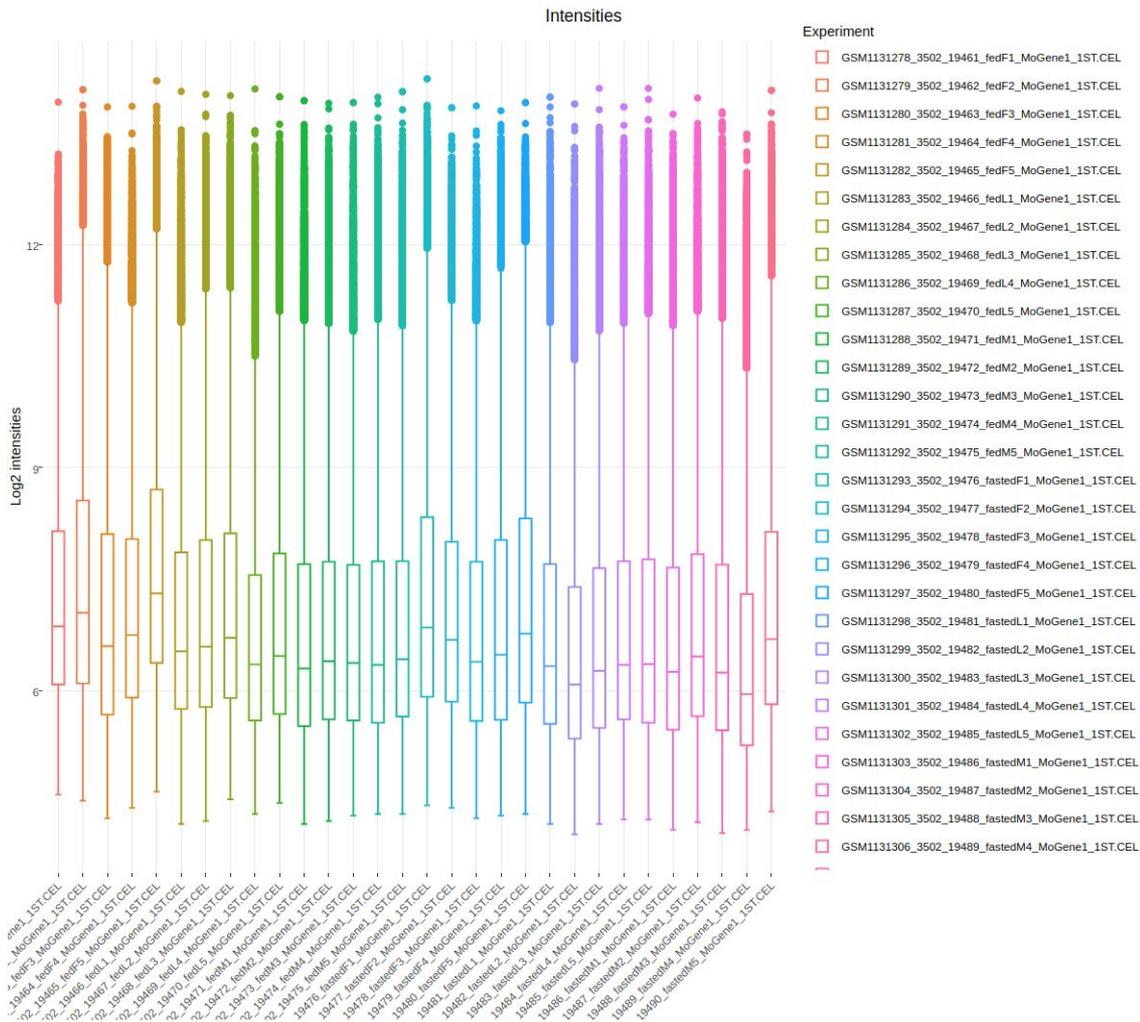
[Histograms1](#)



QC plots

Boxplots

[Boxplots 1](#)



QC plots

MA plots (show/hide)

[MAplot GSM1131287_3502_19470_fedL5_MoGene1_1ST.CEL](#)

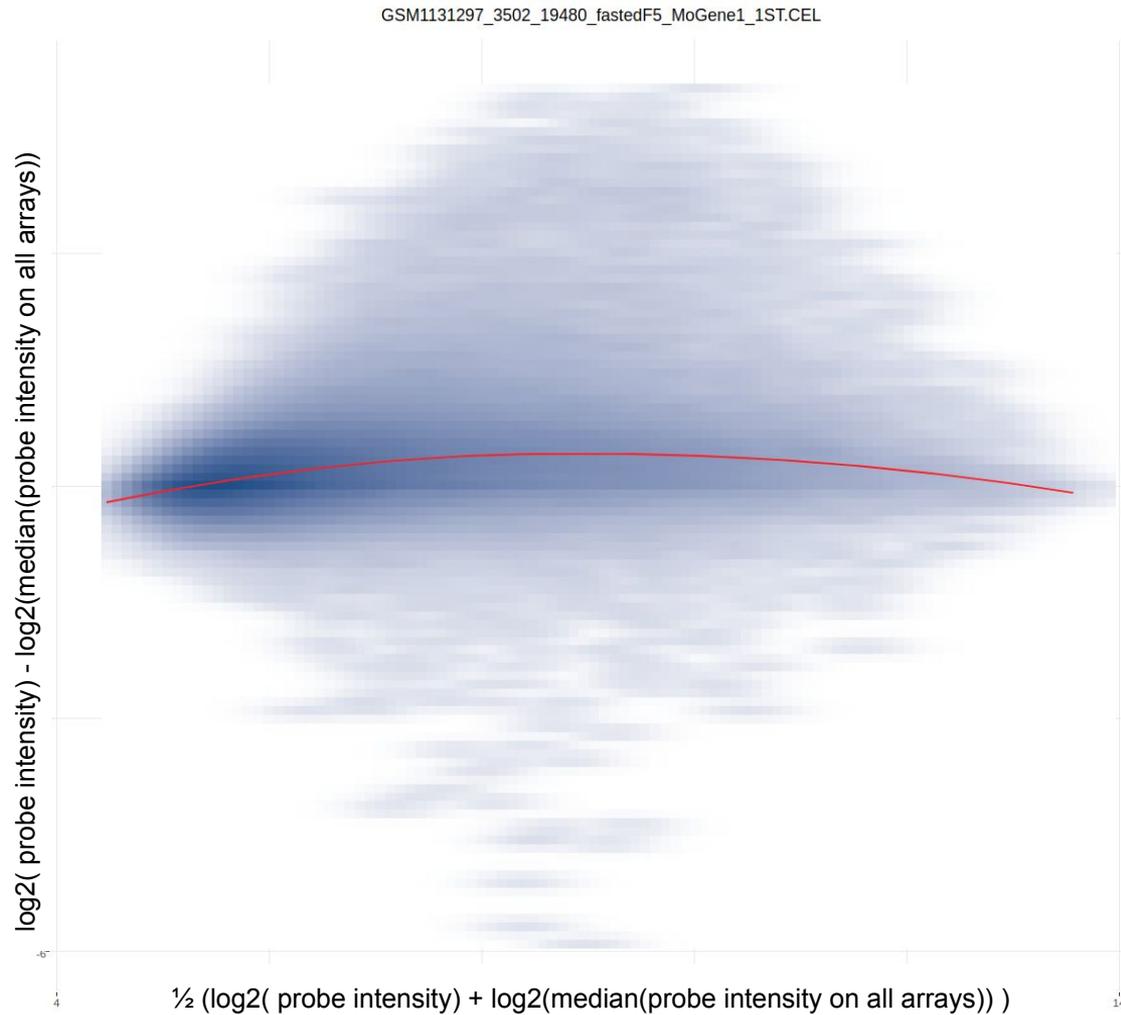
[MAplot GSM1131288_3502_19471_fedM1_MoGene1_1ST.CEL](#)

[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](#)



QC plots

GIANT

[GIANT-QC Plots](#) Descriptive plots of .CEL collections or normalized expression data

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GIANT-QC Plots Descriptive plots of .CEL collections or normalized expression data (Galaxy Version 0.1)

Options

Title for output

QCplot_AfterNormalization

Select one .CEL collection or one tabular file

34: APT_GcSstRMA_NormalizedData
31: conditions.txt
30: GSM1131307_3502_19490_fastedM5_MoGene1_1ST.CEL
29: GSM1131306_3502_19489_fastedM4_MoGene1_1ST.CEL
28: GSM1131305_3502_19488_fastedM3_MoGene1_1ST.CEL

Plots selection

Plot histograms

Yes No

Plot intensity distribution for each condition (pm probes for .cel)

Plot MA plots

Yes No

Plot MA plot for each condition, median value is used as reference

Plot boxplots

Yes No

Plot intensity through boxplot for each condition (pm probes for .cel)

Display microarrays image (only for .CEL files)

Yes No

PCA analysis

Plot 3D PCA

Yes No

3D plot of conditions in the space defined by the 3 principal components

Factor information tabular file (optional)

31: conditions.txt

Select factor informations to display (optional)

Select/Unselect all

Diet Tissue

Advanced parameters

Execute

Choose a title

Select normalized data

Select desired plots

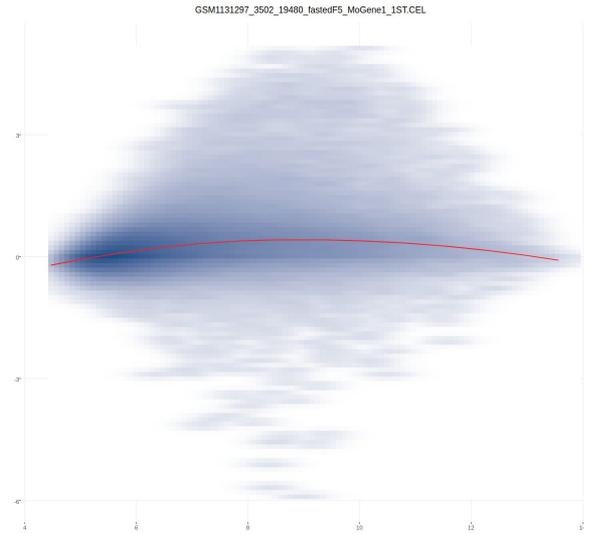
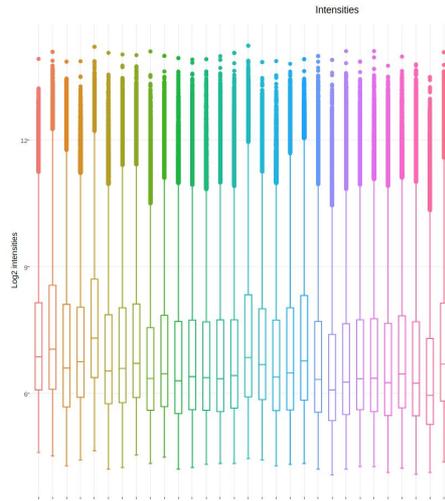
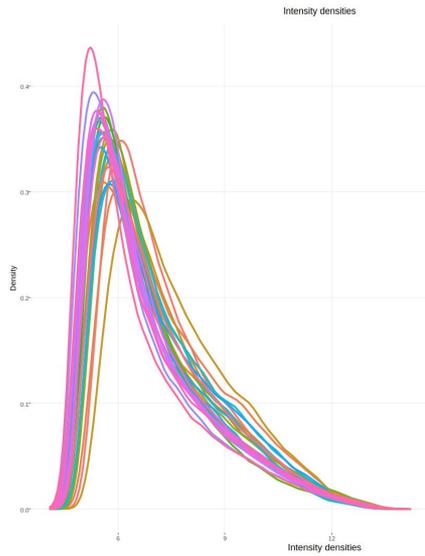
 *Microarrays images*

PCA analysis with conditions infos

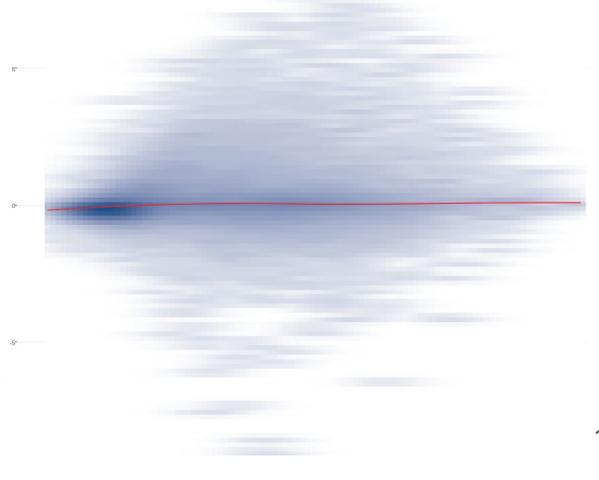
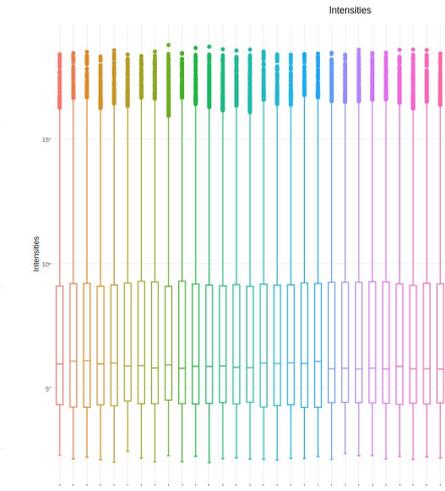
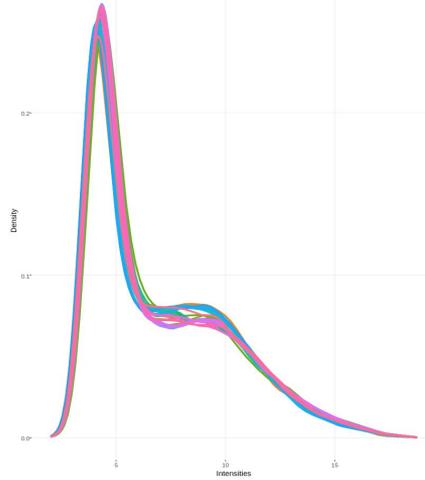
And execute !

QC plots

RAW data

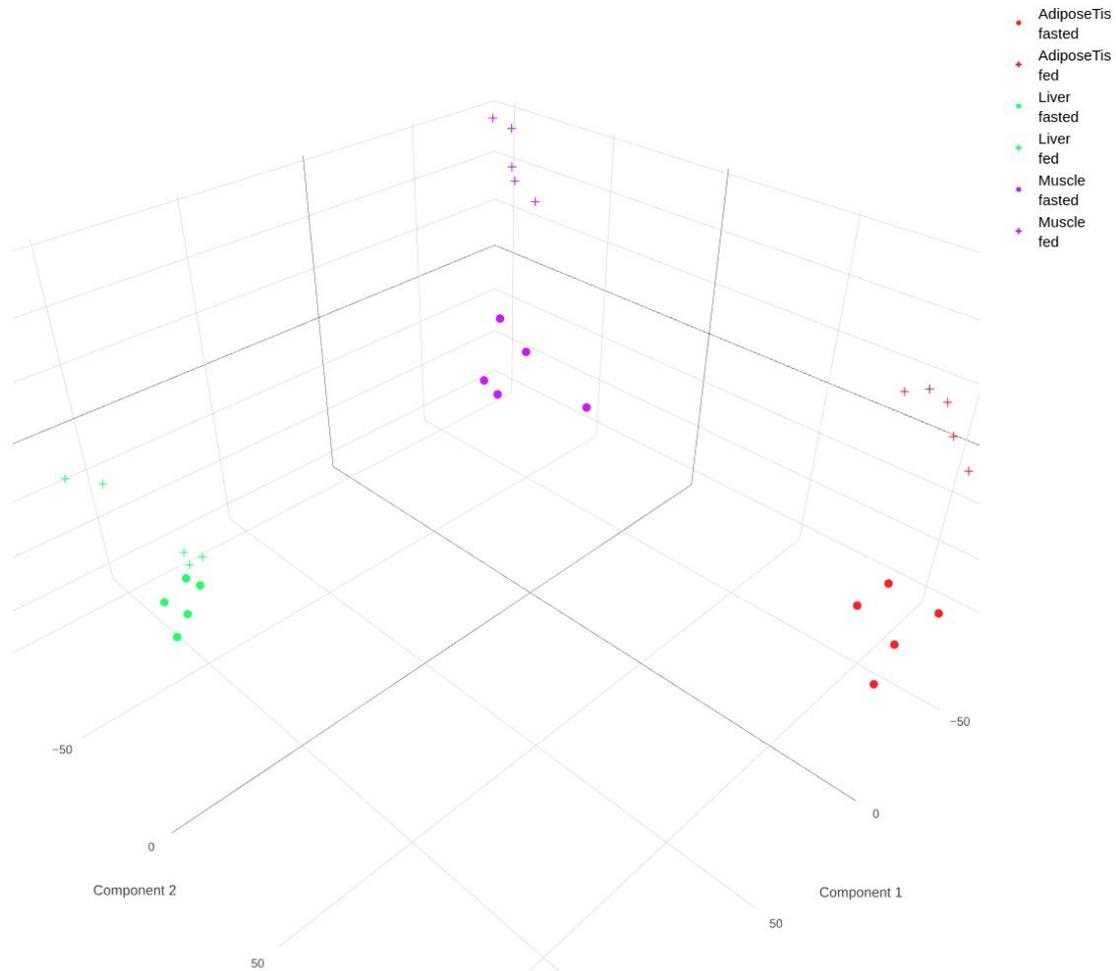


Normalized data



QC plots

Principal Component Analysis



PCA

PCA Tissue * Diet

[Scree plot](#)

QC plots

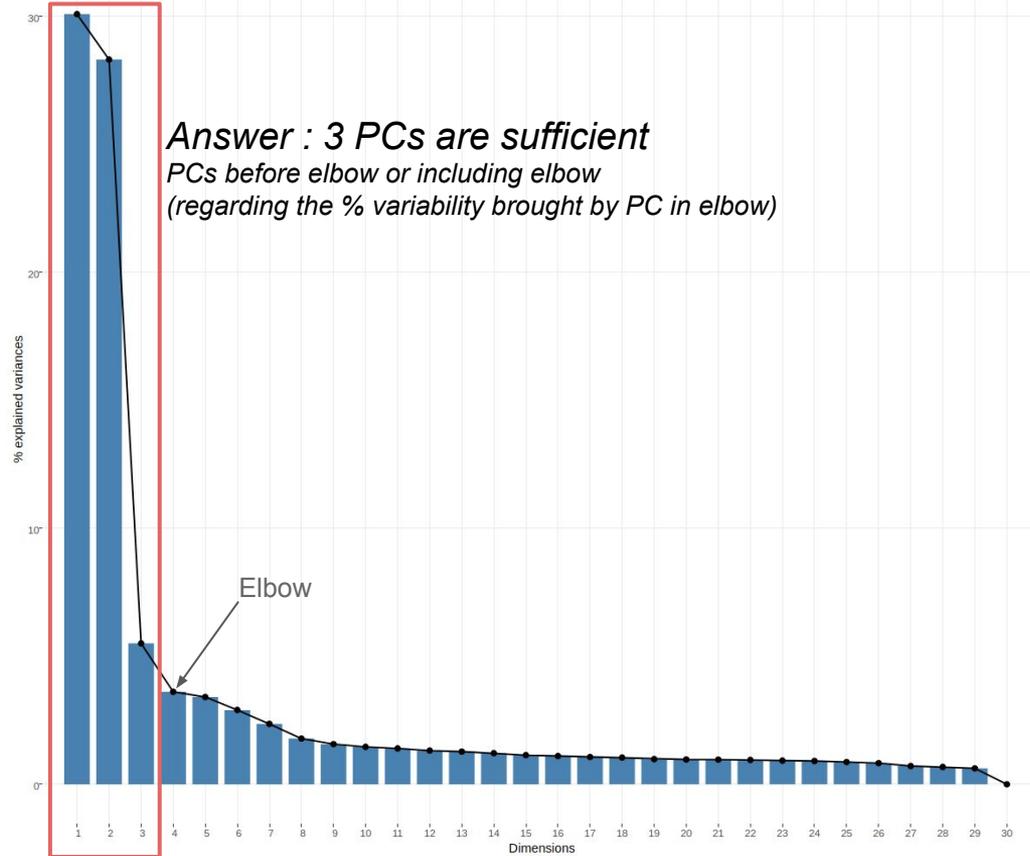
How many Principal Components are sufficient to explain your data ?

Scree plot

PCA

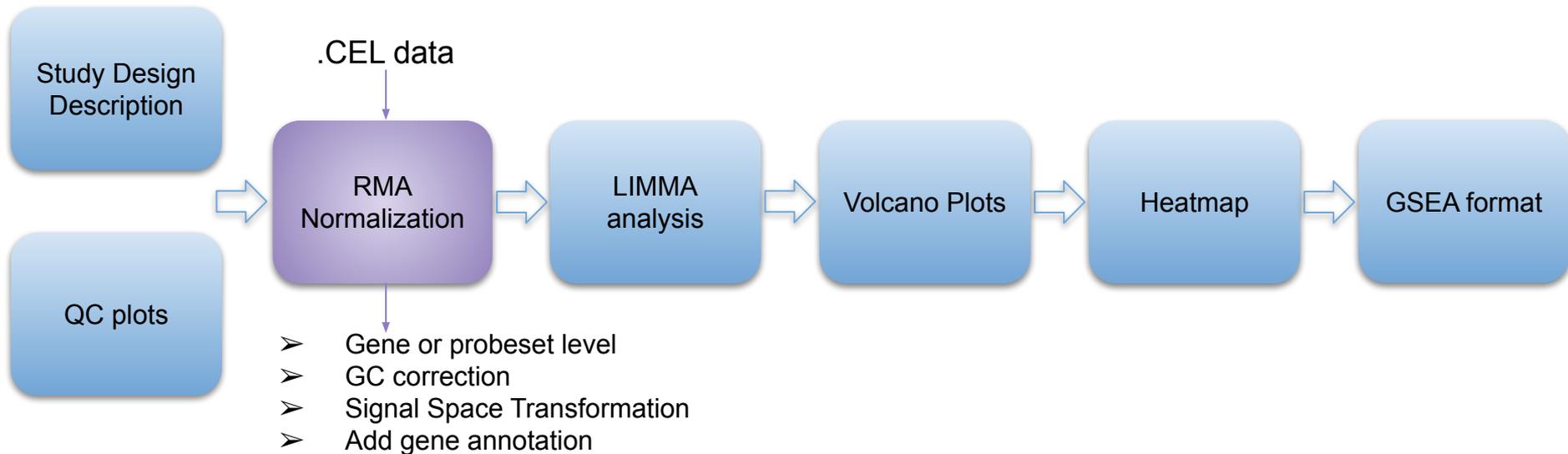
[PCA Tissue * Diet](#)

[Scree plot](#)



GIANT - Tools Suite

Microarray analysis pipeline: “ from .CEL to GSEA “



RMA Normalization

What is a Normalization in GIANT ?

GCCN
GC correction

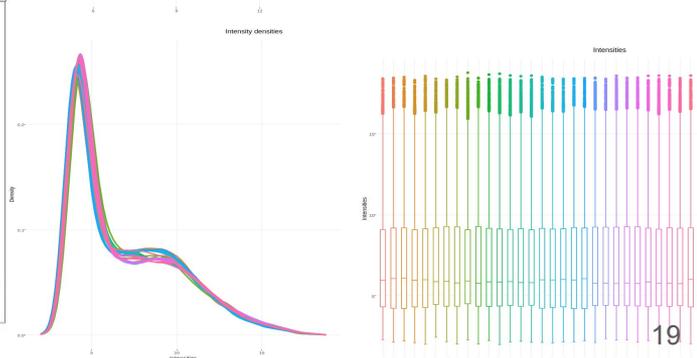
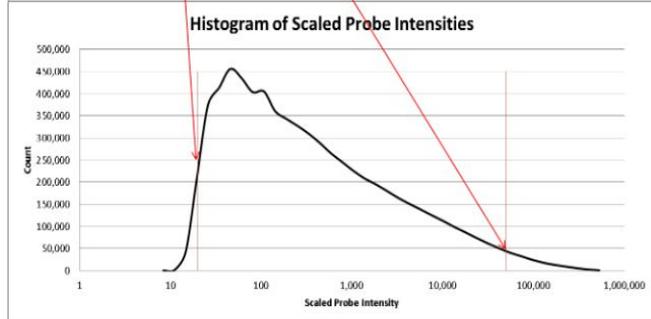
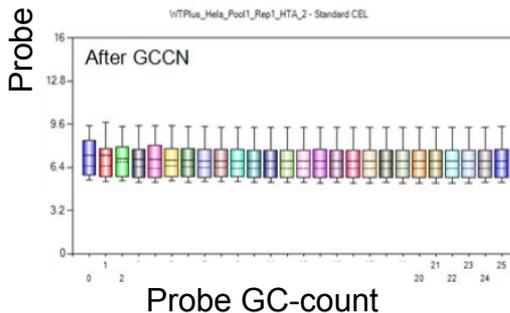
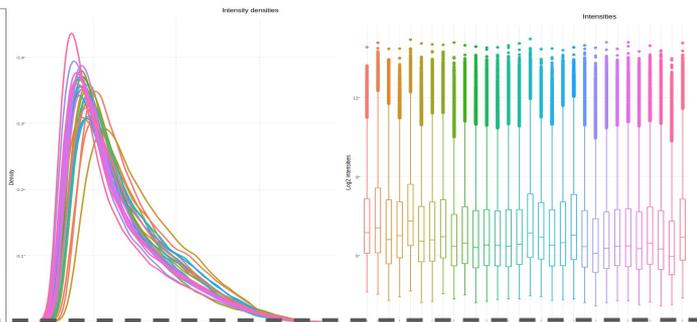
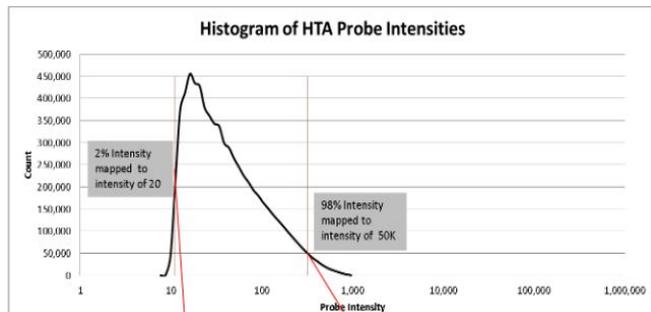
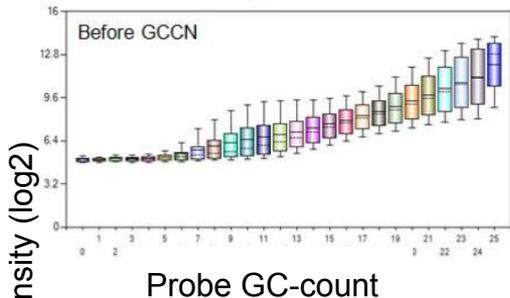


SST
scaling



RMA
Normalization

➤ RMA background
➤ Quantile Normalization
➤ Median Polish



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...)

RMA Normalization

GIANT

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For Normalization Processing

For Post-Normalization Processing

GIANT-Normalization with APT Summarize Apply Affymetrix Power Tool summarize function to .CEL collection (Galaxy Version 0.1) Options

Title for output
APT_GcSstRMA

.cel collection file
30: GSM1131307_3502_19490_fastedM5_MoGene1_1ST.CEL
29: GSM1131306_3502_19489_fastedM4_MoGene1_1ST.CEL
28: GSM1131305_3502_19488_fastedM3_MoGene1_1ST.CEL
27: GSM1131304_3502_19487_fastedM2_MoGene1_1ST.CEL
26: GSM1131303_3502_19486_fastedM1_MoGene1_1ST.CEL

Normalization to perform
 gc correction + scale intensity + rma
 scale intensity + rma
 gc correction + rma
 rma

For more details go to APT webpage

Normalization level
 Core genes
 Probe set

'Core genes' option is not available for all arrays

Select GeneChip array kind
common arrays (HTA, HuGene, MoGene)

Name
Mouse Gene 1.1 ST arrays

Add gene annotation
Yes No

Discard probe set without gene annotation
Yes No

Merging approach for probe set with same gene annotation
 No merging
 Mean between probes [recommended]
 Keep probe with higher variance
 Keep probe with lower variance

Execute

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

Rechercher des données

TestGalaxy_GSE46495
35 shown
419.92 MB

35: APT_GcSstRMA Log   

34: APT_GcSstRMA NormalizedData   

33: QCplot BeforeNormalization Log   

32: QCplot BeforeNormalization HTML.html   

35: APT_GcSstRMA_Log

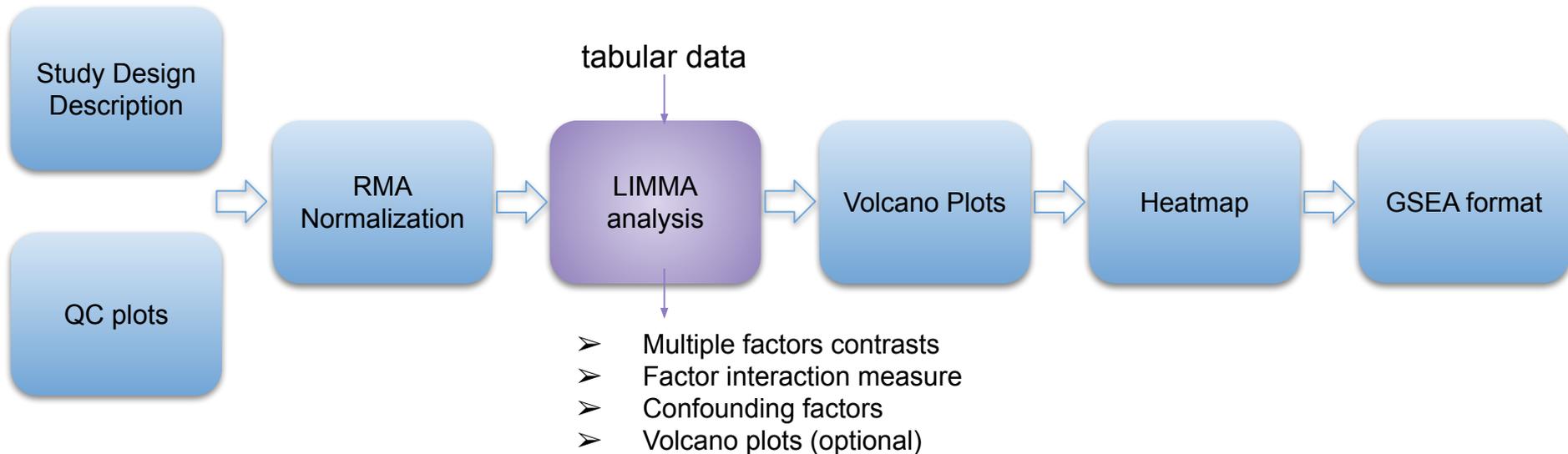
```
Running ProbesetSummarizeEngine...
Opening clf file: MoGene1.1ST.clf
Opening pgf file: MoGene1.1ST.pgf
Reading 241576 probesets.....Done. (0.06 min)
Setting analysis info.
Reading and pre-processing 30 cel files.....Done. (0.08 min)
Processing 1 chipstream
Applying GCcorrection to 30 cel datasets.....Done. (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 “



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

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GIANT-Differential Expression with LIMMA Use LIMMA to detect differentially expressed genes (Galaxy Version 0.1) Options

Input files

Title for output
LIMMA_FedVsFasted

Normalized expression tabular file
34: APT_GcSstRMA_NormalizedData

Factor information tabular file
38: conditions.txt

Contrast definition

Select all factors to include in the global model (excepting confounding factors)

Select/Unselect all

Diet
 Tissue
 MouseID

Confounding factors are selected in the corresponding section below.

Contrast

1: Contrast

Contrast name
FedVsFasted_Liver

Select factor levels of 1st group

Select/Unselect all

fed*AdiposeTis
 fasted*AdiposeTis
 fed*Liver
 fasted*Liver
 fed*Muscle
 fasted*Muscle

Select factor levels of 2nd group

Select/Unselect all

fed*AdiposeTis
 fasted*AdiposeTis
 fed*Liver
 fasted*Liver
 fed*Muscle
 fasted*Muscle

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

[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 Formatting](#) Format input files for GSEA software

[GIANT-Differential Expression with LIMMA](#) Use LIMMA to detect differentially expressed genes

2: Contrast

Contrast name
FedVsFasted_AllTissues

Select factor levels of 1st group
 Select/Unselect all

fed*AdiposeTis
 fasted*AdiposeTis
 fed*Liver
 fasted*Liver
 fed*Muscle
 fasted*Muscle

Select factor levels of 2nd group
 Select/Unselect all

fed*AdiposeTis
 fasted*AdiposeTis
 fed*Liver
 fasted*Liver
 fed*Muscle
 fasted*Muscle

+ Insert Contrast

Add interaction contrasts
 Yes No

If you have selected two factors at least.

Select one control group for each factor (and only one)
 Select/Unselect all

Diet:fed
 Diet:fasted
 Tissue:AdiposeTis
 Tissue:Liver
 Tissue:Muscle

Paired analysis/confounding factor

Add confounding factors
 Yes No

To control factors producing spurious association as batch effects or to analyze paired data

Select confounding factors
 Select/Unselect all

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

GIANT

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Output section

Output FDR p-val threshold

Plot histograms
 Yes No
Plot nominal p-val distribution for each comparison.
Plot volcanos
 Yes No
Plot volcano for each comparison.
Fold change threshold for volcanos (both 'log2(threshold)' and 'log2(1/threshold)' values will be used)
Add gene/probe information
 Yes No
Organism
Mouse genes (GRCm38.p6)
Nature of row names
Gene name
Html snapshot format
 PNG format
 SVG format
Advanced parameters

Define graphic and output filtering options

 *FDR p-val / FC thres.*

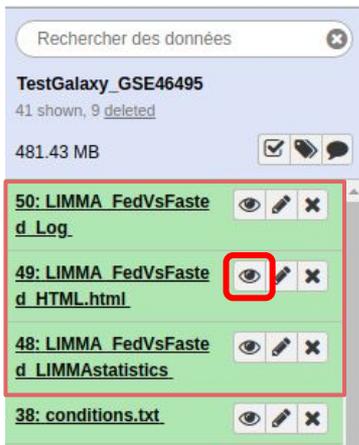
Select studied organism to add gene infos in output files

And execute !

LIMMA analysis

4 results in history :

- log file
- HTML page
- **Tabular LIMMA detailed** For future developments, don't use it
- Tabular LIMMA statistics



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 ?

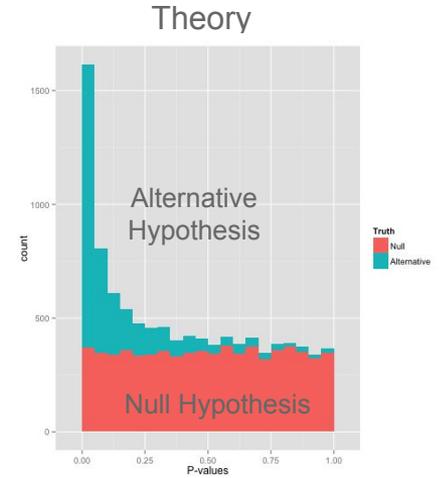
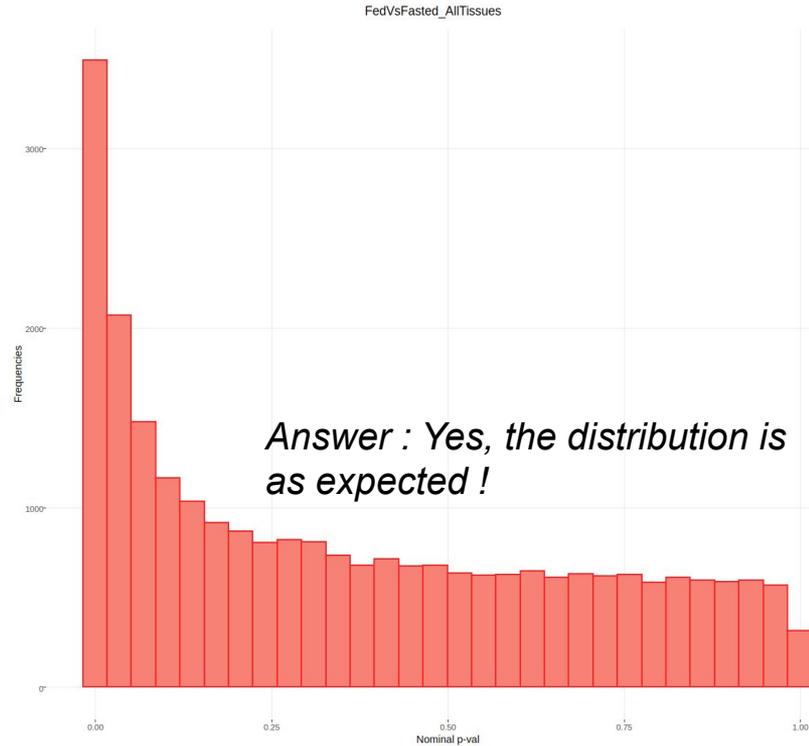
P-val histograms

[Histogram Diet_fasted:Tissue_AdiposeTis](#)

[Histogram Diet_fasted:Tissue_Muscle](#)

[Histogram FedVsFasted_AllTissues](#)

[Histogram FedVsFasted_Liver](#)



LIMMA analysis

Can I continue my analysis ?

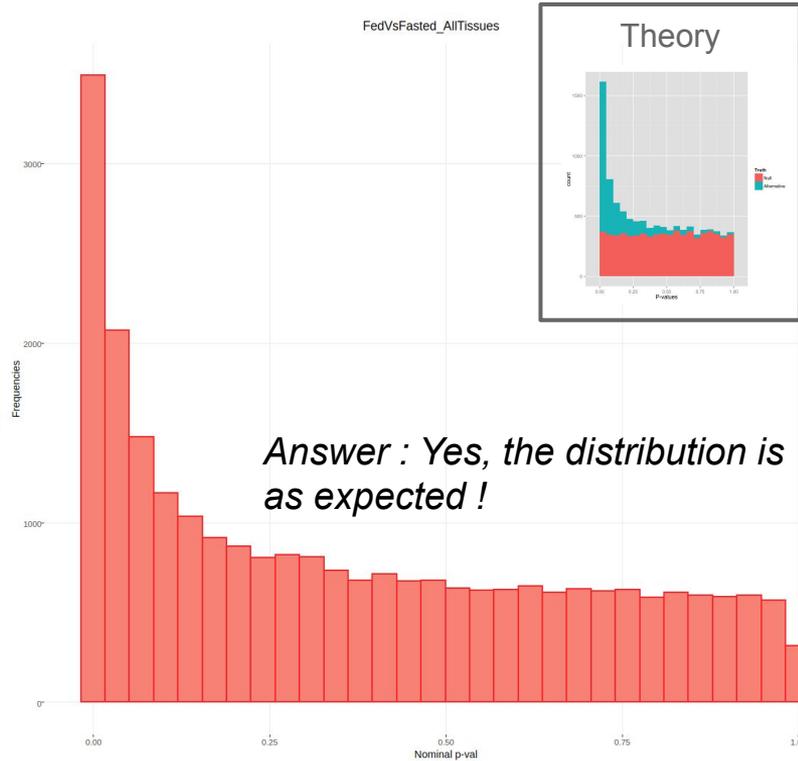
P-val histograms

[Histogram Diet fasted:Tissue AdiposeTis](#)

[Histogram Diet fasted:Tissue Muscle](#)

[Histogram FedVsFasted AllTissues](#)

[Histogram FedVsFasted Liver](#)



BAD examples & solutions... or not

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

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

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

*** Find a statistician**

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

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

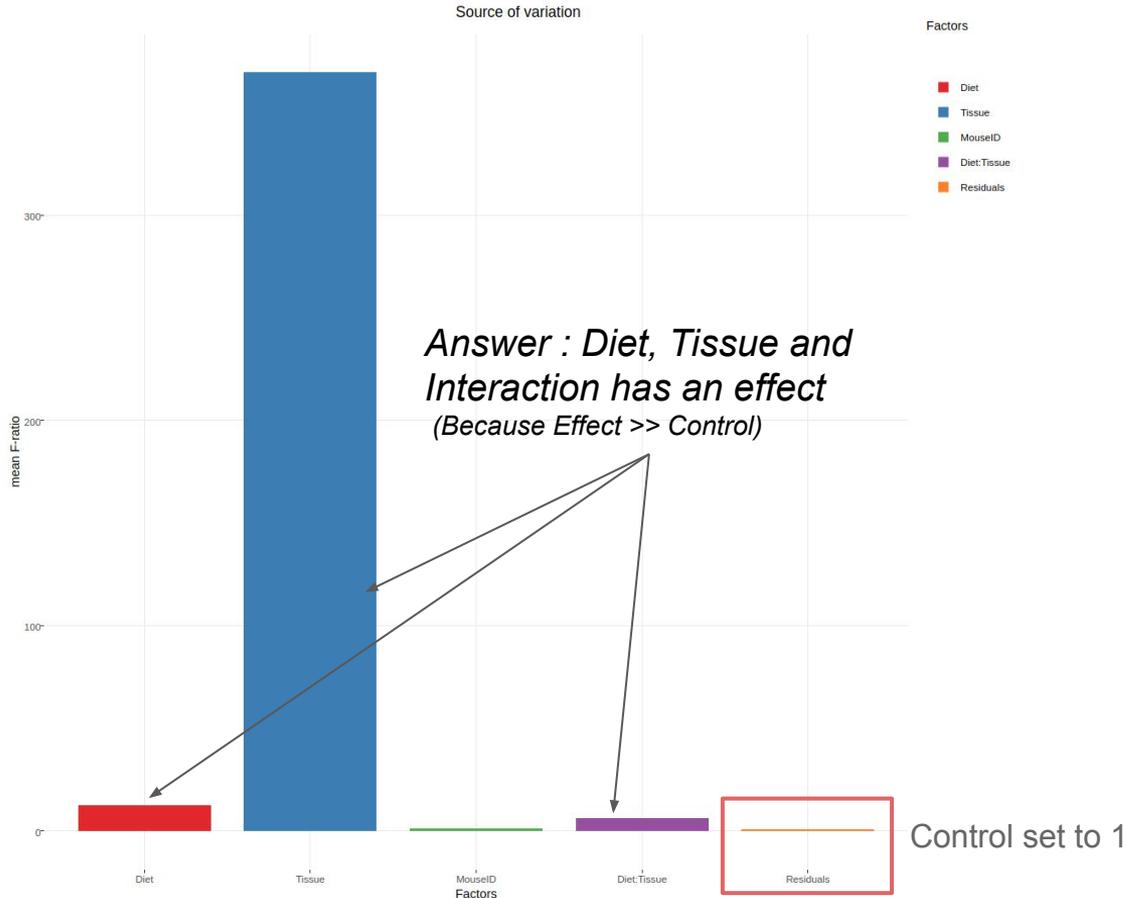
****"What the...!?"**

*** 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

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

Rechercher des données

TestGalaxy_GSE46495
41 shown, 9 deleted

481.43 MB

50: LIMMA FedVsFasted Log

49: LIMMA FedVsFasted HTML.html

48: LIMMA FedVsFasted LIMMAstatistics

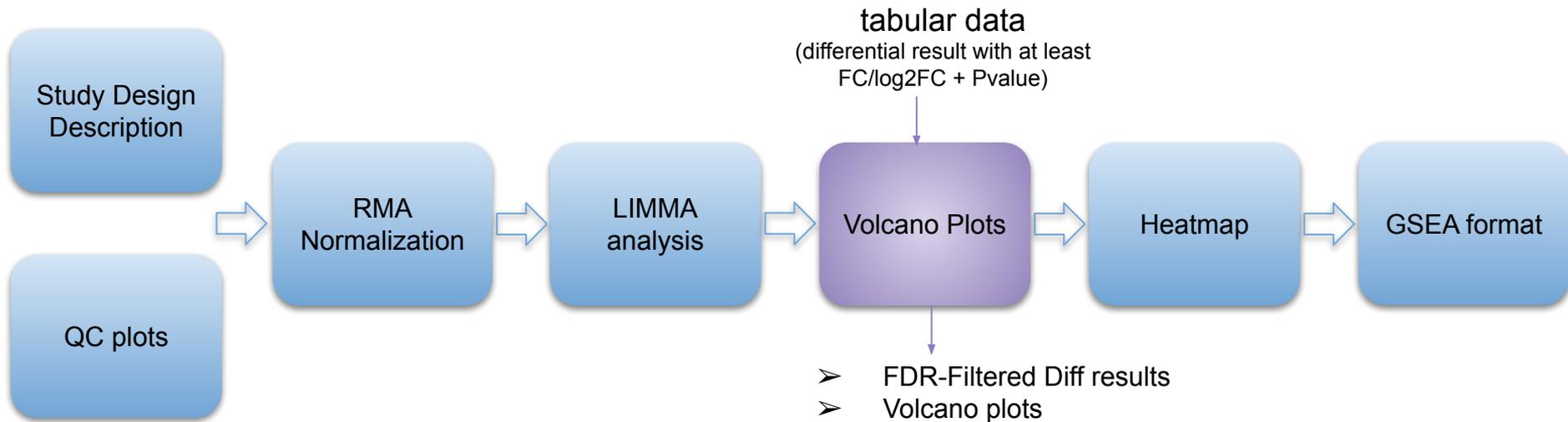
38: conditions.txt

1	2	3	4	5	6	7
LIMMA	Comparison	FastVSVFed_inLiver	FastVSVFed_inLiver	FastVSVFed_inLiver	FastVSVFed_inLiver	FastVSVFed_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	2.46e-16	4.868e-13	4.662	2.221	16.99
Acot1	na	3.11e-16	6.22e-13	1.578	0.789	16.78
Rdh11	na	4.11e-16	8.22e-13	1.578	0.789	-16.65
Sdsl	na	5.11e-16	1.022e-12	1.578	0.789	16.61
Cyp17a1	na	6.11e-16	1.222e-12	1.578	0.789	16.45
Cyp4a31	na	1.11e-15	2.22e-12	1.578	0.789	15.91
Plin5	na	2.11e-15	4.22e-12	1.578	0.789	15.62
Asl	na	3.11e-15	6.22e-12	1.578	0.789	15.49
Id3	na	4.11e-15	8.22e-12	1.578	0.789	-15.26
Fkbp5	na	5.11e-15	1.022e-11	1.578	0.789	15.15
Slc27a1	na	5.11e-15	1.022e-11	1.578	0.789	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
Mvl	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

[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

[GIANT-Factor file generator](#) Generate factor file used by other GIANT tools

[GIANT-Plot volcanos](#) Plot volcano from tabular file

GIANT-Plot volcanos Plot volcano from tabular file (Galaxy Version 0.2.0) Versions Options

Input files

Title for output
Volcano_toPersonalize

Differential results file
35: LIMMA_toPersonalize_LIMMAstatistics

Select number of header lines in file
2

Volcano definition

Volcano

2 **+ Insert Volcano** Insert Contrast as much as you want to plot (1plot/contrast) 3

Output section

Select FC values kind selected as in
 log₂(FC) } Column selected for plot is FC or Log₂(FC) ?
 FC

Info:log₂(FC) will be displayed in volca

Output FDR p-val threshold
0.05 } Threshold FDR for Volcano & for output table

Fold change threshold for volcanos
2.0 } Threshold FC, only for Volcano

Add gene/probe information
 Yes No

Html snapshot format
 PNG format
 SVG format

Execute

Volcano definition

Volcano

1: Volcano

Volcano name
} Choose a Plot Title

Select column containing p-val statistics
Comparison_Info **! Pval, NOT FDR** } Select column of differential file containing p-val for contrast of interest

Do not use adjusted p-val for Volcano plot

Select column containing log₂(FoldChange) values
Comparison_Info } Select column of differential file containing FC or log₂(FC) for contrast of interest

If only FC are available as input, please select FC column and check options below.

+ Insert Volcano

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

History

search datasets

Example_history
41 shown
366.04 MB

41: Volcano toPersonalize Log

40: Volcano toPersonalize HTML.html

39: Volcano toPersonalize LIMMAstatistics

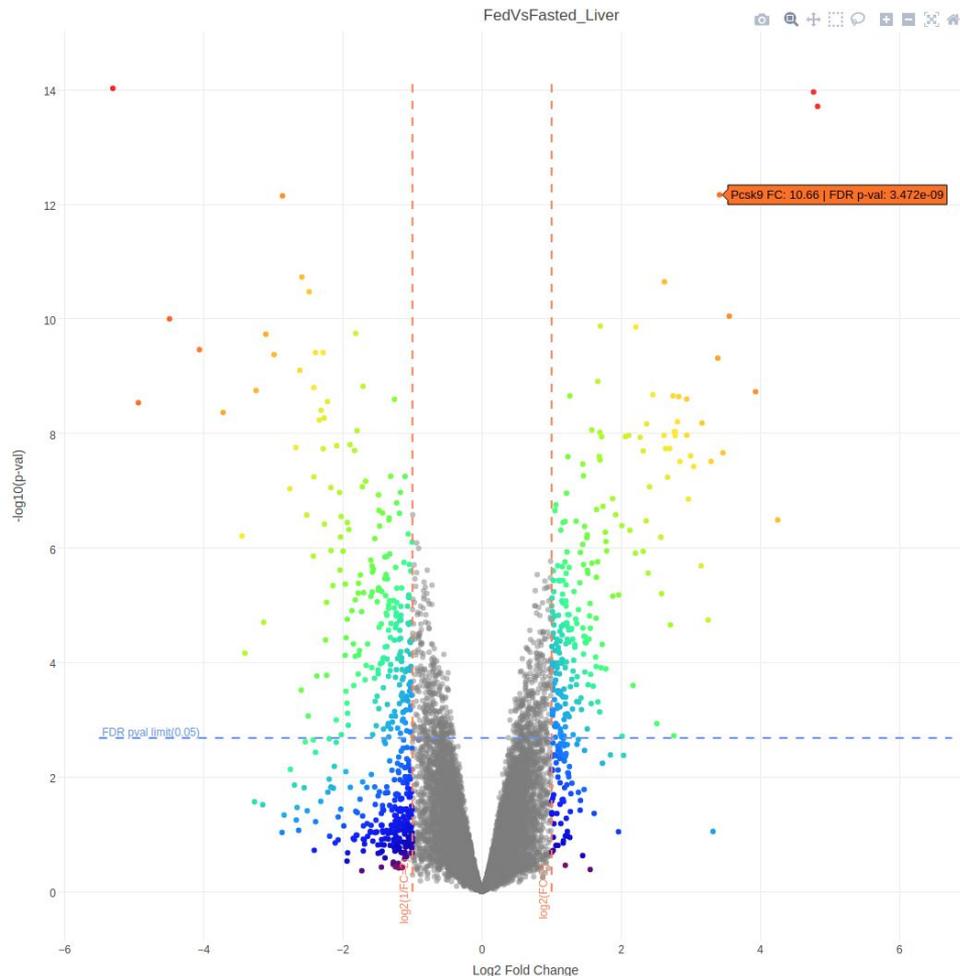
Volcanos

[Volcano Diet_fasted:Tissue_AdiposeTis](#)

[Volcano Diet_fasted:Tissue_Muscle](#)

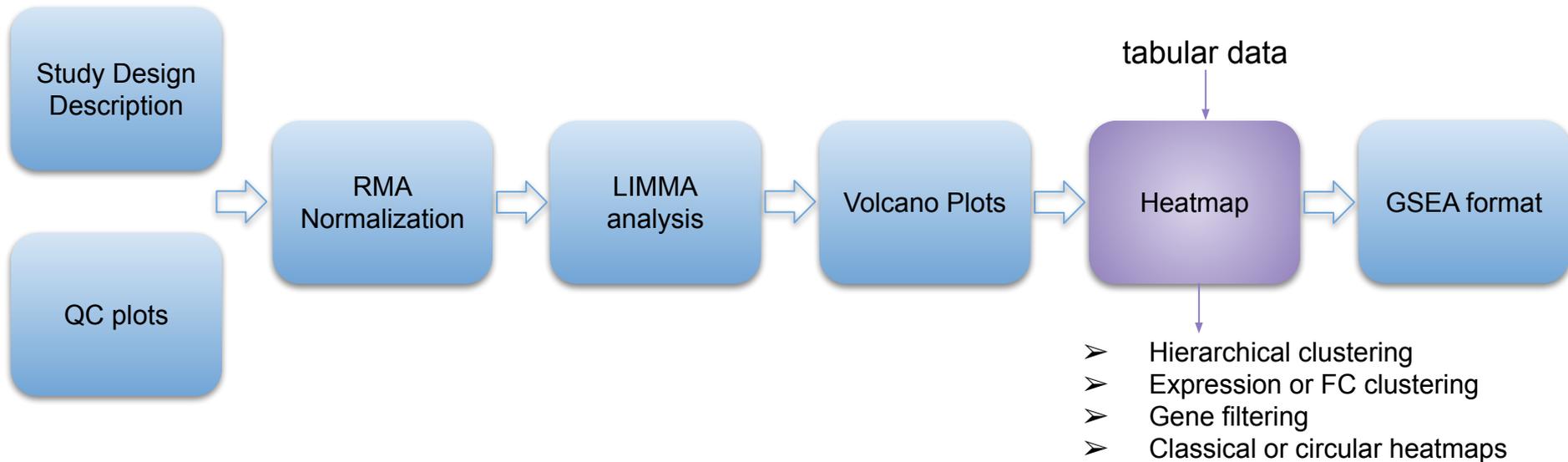
[Volcano FedVsFasted_AllTissues](#)

[Volcano FedVsFasted_Liver](#)



GIANT - Tools Suite

Microarray analysis pipeline: “ from .CEL to GSEA “



Heatmap

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 Formatting](#) Format input files for GSEA software

[GIANT-Differential Expression with LIMMA](#) Use LIMMA to detect differentially expressed genes

GIANT-Heatmap and Hierarchical clustering Run hierarchical clustering and plot heatmap from expression data and/or differential expression analysis (Galaxy Version 0.1) Options

Title for output
Heatmap_basedOnExpression

Data to cluster
Expression data

Normalized expression tabular file
34: APT_GcSstrMA_NormalizedData

Probes/genes filtering
Filter input probes/genes before clustering

Filter
Based on differential expression results (FC and p-val)

Differential analysis tabular file (as given by LIMMA diff.exp. tool)
48: LIMMA_FedVsFasted_LIMMAstatistics

Select comparisons to use for filtering
 Select/Unselect all
* FedVsFasted_Liver

Fold change threshold for input (both 'threshold' and '1/threshold' values will be used)
2.0
Minimum value is 1 (ie. all probes/genes are kept)

FDR p-val threshold for input
0.05
When several comparisons are selected a conservative rule is applied (see details below)

Advanced parameters

Execute

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

Rechercher des données

TestGalaxy_GSE46495
44 shown, 12 deleted
493.9 MB

56: Heatmap BasedOnExpression Log

55: Heatmap BasedOnExpression HTML.html

54: Heatmap BasedOnExpression ClusteringResults

50: LIMMA FedVsFasted Log

Html page

Clustering tabular

[Clustering results](#)

Heatmap plot

[Heatmap](#)

Scree plot

[Scree plot](#)

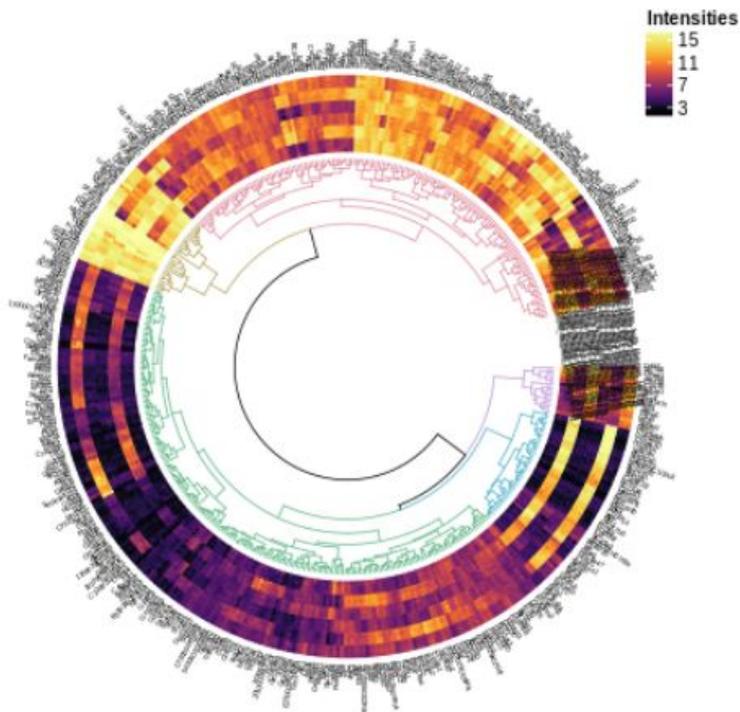
Circular plot

[Circular plot](#)

Heatmap

Circular plot

[Circular plot](#)

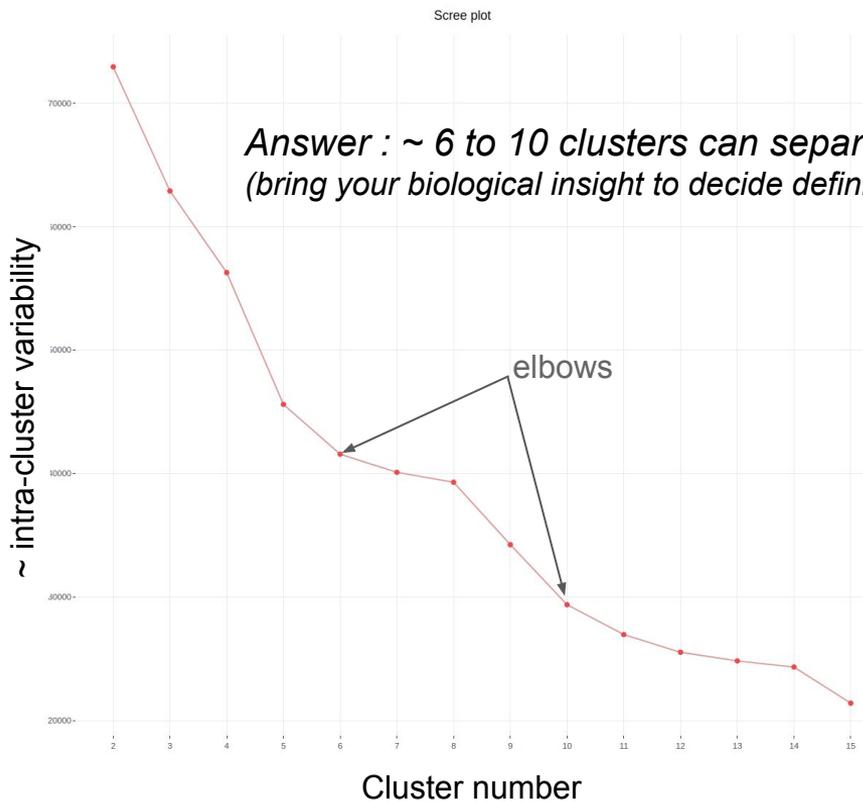


How many clusters are informative/useful ?

Heatmap

Scree plot

[Scree plot](#)



Heatmap

Clustering tabular

Clustering results

Show 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 2 3 4 5 ... 47 Next

Heatmap

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

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[GIANT-GSEA Formatting](#) Format input files for GSEA software

[GIANT-Differential Expression with LIMMA](#) Use LIMMA to detect differentially expressed genes

GIANT-Heatmap and Hierarchical clustering Run hierarchical clustering and plot heatmap from expression data and/or differential expression analysis (Galaxy Version 0.1) Options

Title for output
Heatmap_BasedOnLimmaResults

Data to cluster
Differential expression analysis results

Differential analysis tabular file (as given by LIMMA diff.exp. tool)
48: LIMMA_FedVsFasted_LIMMAstatistics

Select comparisons to cluster
 Select/Unselect all
x FedVsFasted_Liver x FedVsFasted_AllTissues

Probes/genes filtering
Filter probes/genes only in tabular output file

Filter
Based on diff. exp. parameters (FC and p-val)

Fold change threshold for output (both 'threshold' and 'lthreshold' values will be used)
2.0
Minimum value is 1 (ie. all probes/genes are kept)

FDR p-val threshold for output
0.05
When several comparisons are selected a conservative rule is applied (see details below)

Advanced parameters

Requested cluster number
3
Use scree plot to adjust number of clusters

Cluster samples
Yes No
To apply hierarchical clustering to samples

Maximum gene number to plot
1000

Personalized colors
Yes No

Output format
 PNG format
 PDF format

Html snapshot format
 PNG format
 SVG format

Execute

Choose a title

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

Select optional filtering policy and corresponding options

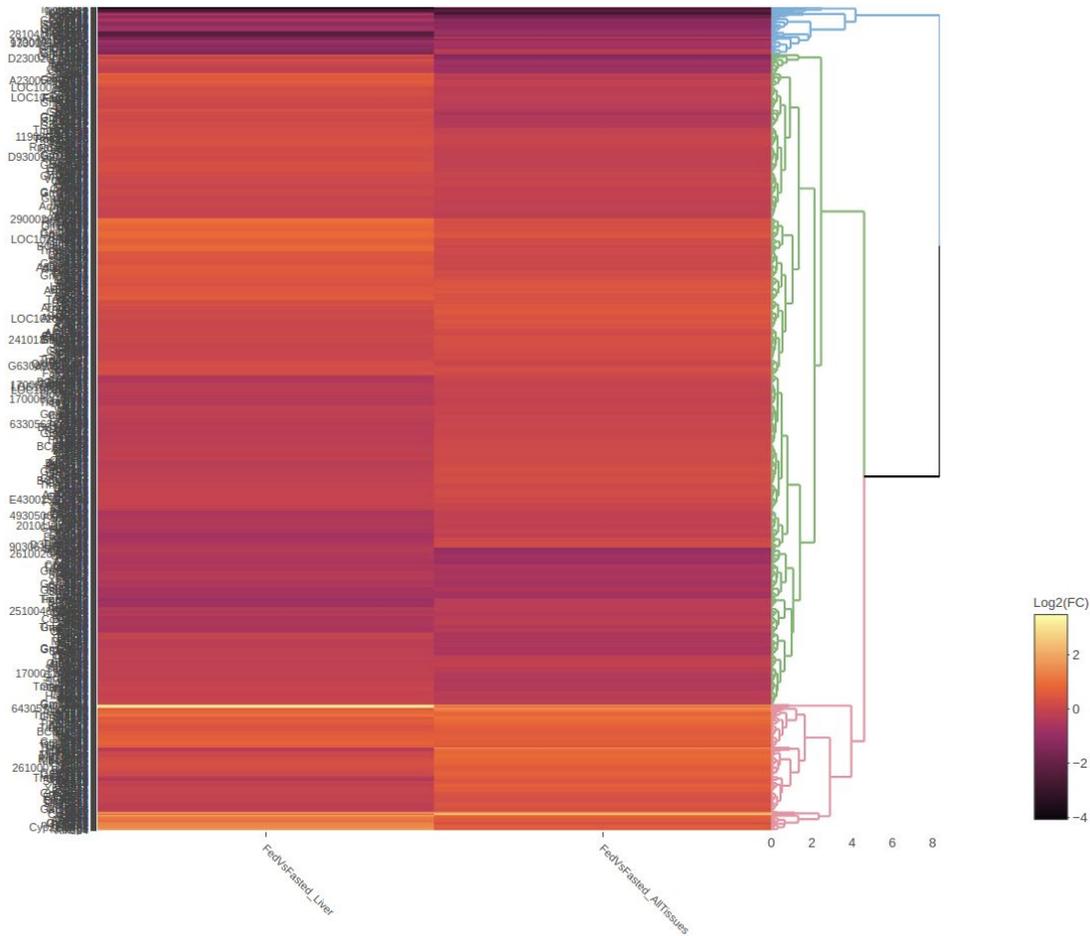
Define clustering options :
- cluster number
- cluster samples/contrasts
- color gradient

And execute !

Heatmap

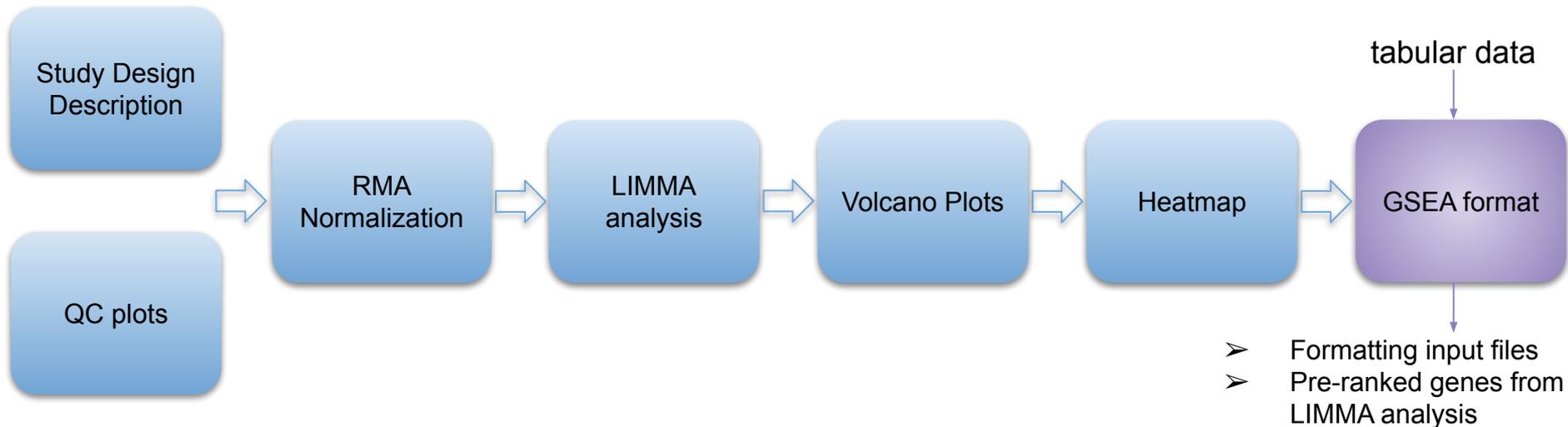
Heatmap plot

Heatmap



GIANT - Tools Suite

Microarray analysis pipeline: “ from .CEL to GSEA “



GSEA format

GIANT

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[GIANT-GSEA Formatting](#) Format input files for GSEA software

[GIANT-Differential Expression with LIMMA](#) Use LIMMA to detect differentially expressed genes

GIANT-GSEA Formatting Format input files for GSEA software (Galaxy Version 0.1) Options

Title for output (without space)
GSEFormat_fromExpression

GSEA configuration
GSEA analysis

Normalized expression tabular file
34: APT_GcSstRMA_NormalizedData

Factor information tabular file
38: conditions.txt

Reference factor
Diet

Execute

Choose a title

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

And execute !

GSEA format

61: GSEAformat_fromExpression_Phenotypes.cls

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

3 results in history :

- log file
- phenotype file
- expression file

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_fedF3_MoGene1_1ST.CEL
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-R55217 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 3.99805 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 4.05061 4.46004 4.56209
Uba5 na 9.94948 9.95413 9.57235 9.80935 9.75292 9.94601 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
```

Rechercher des données

TestGalaxy_GSE46495
50 shown, 12 deleted

507.97 MB

- 62: GSEAformat_fromExpression_Log
- 61: GSEAformat_fromExpression_Phenotypes
- 60: GSEAformat_fromExpression_Expressions
- 59: Heatmap BasedOnLlmmaResults_Log

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 Formatting](#) Format input files for GSEA software

[GIANT-Differential Expression with LIMMA](#) Use LIMMA to detect differentially expressed genes

GIANT-GSEA Formatting Format input files for GSEA software (Galaxy Version 0.2.0) Versions Options

Title for output (without space)
GSEAformat_toPersonalize

GSEA configuration
Pre-ranked GSEA analysis

Differential analysis tabular file (as given by LIMMA diff.exp. tool)
39: Volcano_toPersonalize_LIMMAstatistics
This file should contain only annotated gene names or only probe identifiers as rows but no both kinds.

Reference contrast
Fasted versus Fed

Reference statistic
 Relative value of Log2(Fold Change)
 Absolute value of Log2(Fold Change)
 Relative value of moderated t-statistic
 Absolute value of moderated t-statistic

FDR p-val threshold
0.05

Execute

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 literature & 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.