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Meta-analysis of RNA-Seq data

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Meta-analysis of RNA-Seq data

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28 June 2022

Overview

- 1 Introduction
- 2 Statistical analysis of one study
- 3 Meta-analysis
- 4 metaRNASeq and SMAGEXP
- 5 Conclusion

Meta-analysis

Particular context :

- Differential expression analysis
- Several studies available but **direct comparison impossible**
- Very few individuals in each individual study, a lot of genes or transcripts.

Objectives of meta-analysis

- Increase of sensitivity, the proportion of truly declared differentially expressed (DE) genes
- Eliminate false positives (genes declared DE but not DE in reality)

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Statistical analysis of one study

Research of differentially expressed genes

- Normalisation
- Use of a test statistic appropriate for differential expression analysis
- Correction for multiple testing

	Microarrays	RNA-Seq
Information	Intensities	Counts of reads
Modelling	Normal distribution	Poisson, Negative binomial
Tests	Moderated t-tests	Likelihood ratio tests

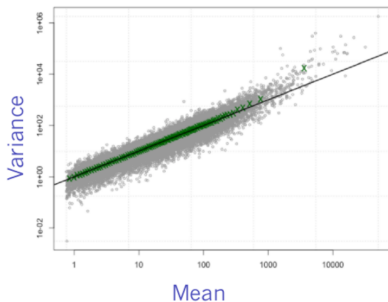
Adaptation of usual test statistics to find a compromise between a gene-by-gene analysis and a "common parameter" analysis

Mean-variance relationship in RNA-Seq data

The Poisson distribution to model counts

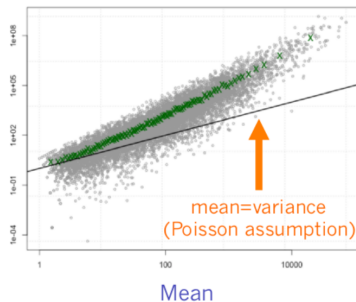
- Describes the number of occurrences of rare events during a given time interval
- Property : Mean = Variance

Technical replicates



data from Marioni et al. *Gen Res* 2008

Biological replicates

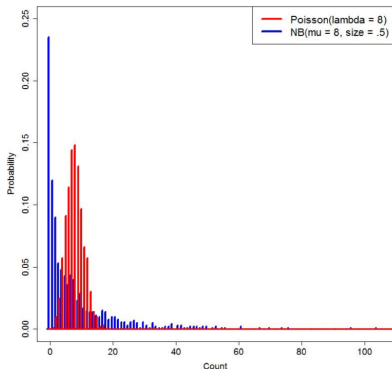


data from Parikh et al. *Genome Bio* 2010

Negative Binomial Models

A supplementary dispersion parameter ϕ to model the variance

Poisson vs Negative Binomial models



Technical variability is the main source of variability in low counts, whereas biological variability is dominant in high counts

Estimating the dispersion : the key question

Problem

Estimate a reliable dispersion from a very small number of replicates (sometimes less than 5)

Why using sophisticated approaches ?

- gene-specific tests \Rightarrow lack of sensitivity (proportion of true positives among positives) due to the lack of information
- common dispersion parameter for all tests \Rightarrow many false positives

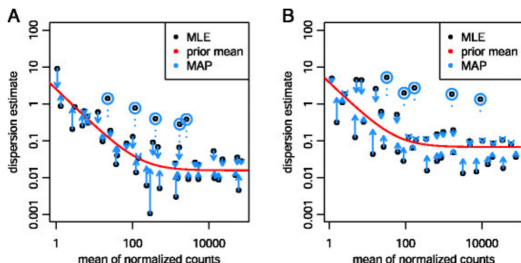
Empirical bayesian approaches = compromise between gene-specific and common dispersion parameter estimation

Exemple : edgeR, DESeq2

Dispersion estimation with DESeq2

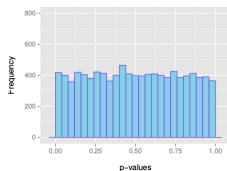
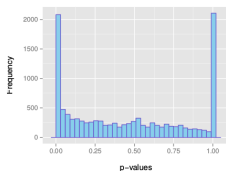
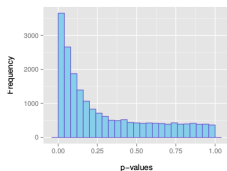
Hypothesis : genes of similar average expression strength have similar dispersion

- 1 Estimate **gene-wise dispersion** estimates using maximum likelihood (ML) (black dots)
- 2 Fit a **smooth curve** (red line)
- 3 **Shrink** the gene-wise dispersion estimates (empirical Bayes approach) toward the values predicted by the curve to obtain final dispersion values (blue arrow heads).



p-values histograms for diagnosis

Examples of expected overall distribution



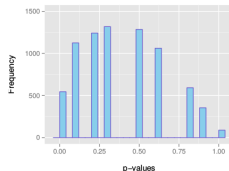
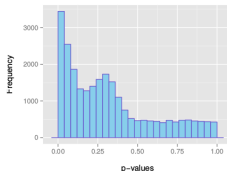
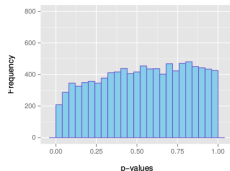
(a) : the most desirable shape

(b) : very low counts genes usually have large p-values

(c) : do not expect positive tests after correction

p-values histograms for diagnosis

Examples of not expected overall distribution



- (a) : indicates a batch effect (confounding hidden variables)
- (b) : the test statistics may be inappropriate (due to strong correlation structure for instance)
- (c) : discrete distribution of p-values : unexpected

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Back to microarray meta-analysis

(Marot et al., Bioinformatics, 2009)

⇒ p-value combination showed better performance in terms of sensitivity and AUC than effect size combination.

p-value combination was suggested with the inverse normal method (Liptak, 1958)

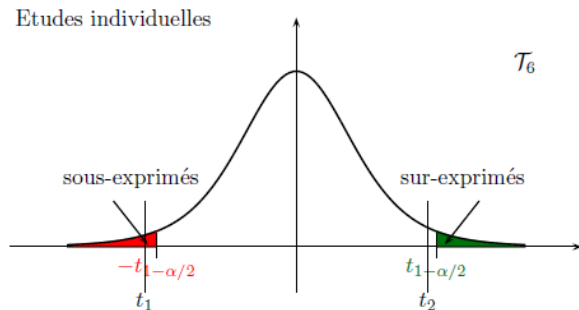
$$N_g = \sum_{s=1}^S w_s \Phi^{-1}(1 - p_{gs})$$

$$w_s = \sqrt{\frac{\sum_c R_{cs}}{\sum_l \sum_c R_{cl}}}$$

$$N_g \sim \mathcal{N}(0, 1)$$

Trick for microarrays : use unilateral p-values to avoid conflicts.

Back to microarray meta-analysis

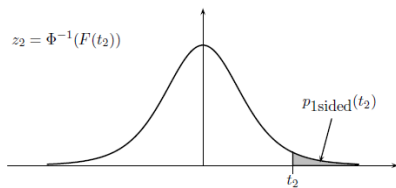
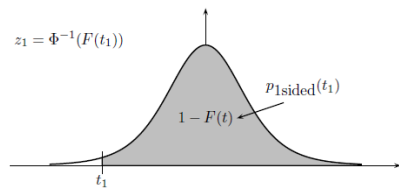


Gene for which we observe a conflict of expression
 $t_1 < 0$ observed value of test statistic for study 1
 $t_2 > 0$ observed value of test statistic for study 2

Back to microarray meta-analysis

scores from unilateral p-values

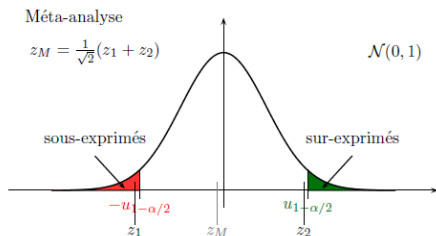
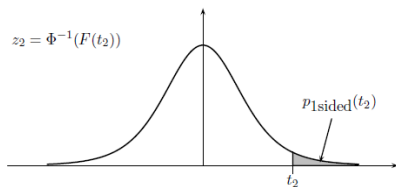
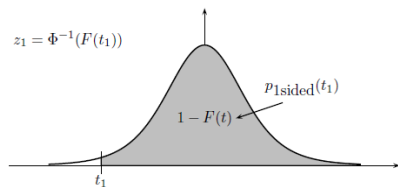
$$z = \Phi^{-1}(1 - p_{1\text{sided}}) = \Phi^{-1}(F(t))$$



Back to microarray meta-analysis

scores from unilateral p-values

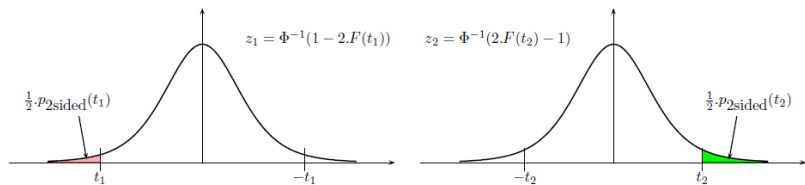
$$z = \Phi^{-1}(1 - p_{1\text{sided}}) = \Phi^{-1}(F(t))$$



Back to microarray meta-analysis

scores from bilateral p-values

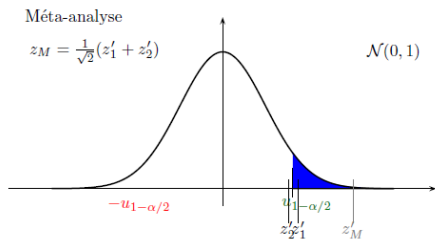
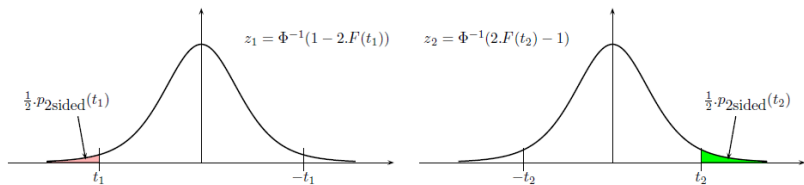
$$z = \Phi^{-1}(1 - p_{2\text{sided}}) = \Phi^{-1}(1 - 2 \cdot (1 - F(|t|)))$$



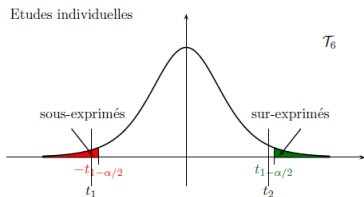
Back to microarray meta-analysis

scores from bilateral p-values

$$z = \Phi^{-1}(1 - p_{2\text{sided}}) = \Phi^{-1}(1 - 2 \cdot (1 - F(|t|)))$$



Back to microarray meta-analysis

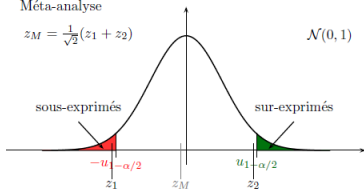


Building of individual scores from p-values

Méta-analyse

$$z_M = \frac{1}{\sqrt{2}}(z_1 + z_2)$$

$\mathcal{N}(0, 1)$

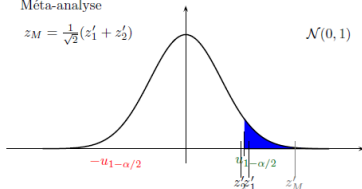


unilateral
then bilateral test

Méta-analyse

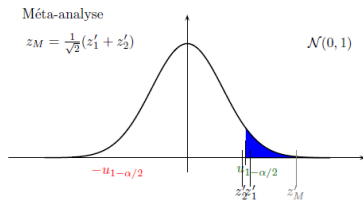
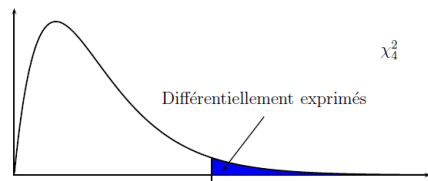
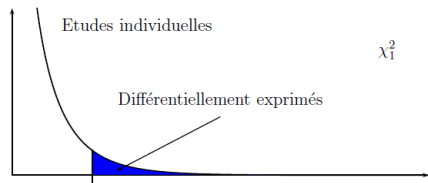
$$z_M = \frac{1}{\sqrt{2}}(z'_1 + z'_2)$$

$\mathcal{N}(0, 1)$



bilateral
then uni(bi)lateral test

RNASeq data meta-analysis



RNA-seq data meta-analysis

Inverse normal combination (Liptak, 1958)

$$N_g = \sum_{s=1}^S w_s \Phi^{-1}(1 - p_{gs})$$
$$N_g \sim \mathcal{N}(0, 1)$$

Fisher's method (1932)

$$F_g = -2 \sum_{s=1}^S \ln(p_{gs})$$
$$F_g \sim \chi_{2S}^2$$

Conflicts to be treated a posteriori

Global differential analysis (DESeq)

Gene counts : $Y_{gcrs} \sim \mathcal{NB}(\eta_{gcrs}, \phi_{gs})$

Full model $\log(\eta_{gcrs}) = \beta_g + \lambda_{gc} + \delta_{gs} + \log(\ell_{crs})$

where β_g is an intercept, λ_{gc} is a fixed condition effect, δ_{gs} a fixed **study effect**, ℓ_{crs} library size normalization factor

Reduced model $\log(\eta_{gcrs}) = \beta_g + \delta_{gs} + \log(\ell_{crs})$

Likelihood ratio test

$H_{0,g} : \forall c, \lambda_{gc} = 0$ vs $H_{1,g} : \exists c \mid \lambda_{gc} \neq 0$.

$\sim \chi^2$ with degrees of freedom equal to the number of conditions minus 1

Simulations

Data simulated according to a negative binomial distribution,

$$Y_{gcrs} \sim \mathcal{NB}(\mu_{gcs}, \phi_{gs})$$

where μ_{gcs} and ϕ_{gs} represent the mean and dispersion, respectively

Mean-variance relationship defined by

$$\text{Var}(Y_{gcrs}) = \mu_{gcs} + \frac{\mu_{gcs}^2}{\phi_{gs}}.$$

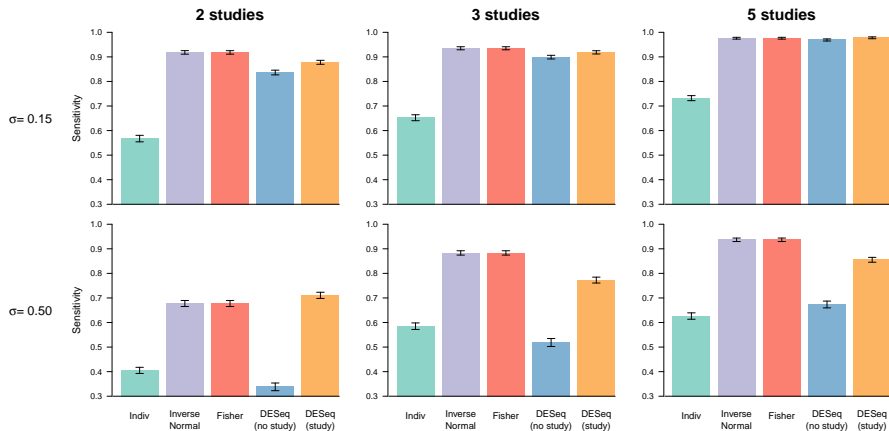
To incorporate inter-study variability :

$$\log(\mu_{gcs}) = \theta_{gc} + \varepsilon_{gcs}, \text{ and } \varepsilon_{gcs} \sim \mathcal{N}(0, \sigma^2),$$

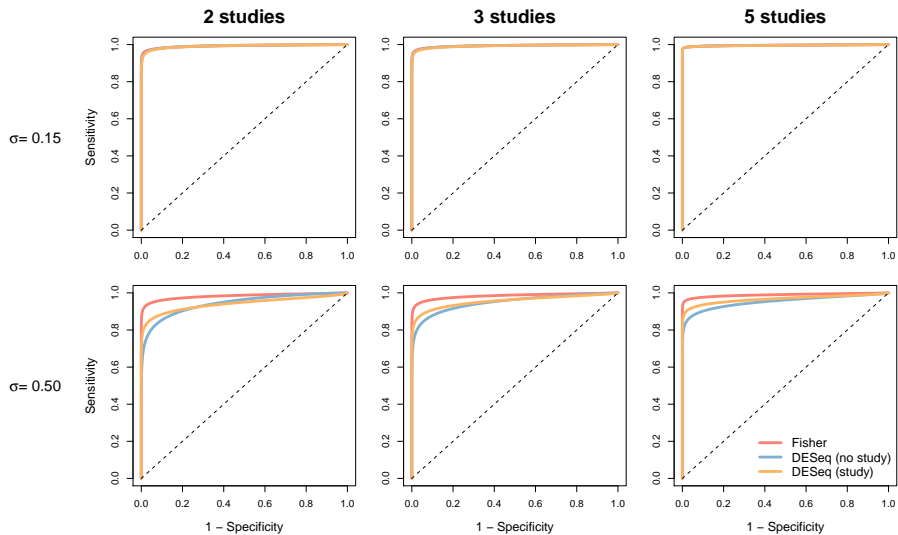
where σ^2 is the size of the inter-study variability.

Meta-analysis

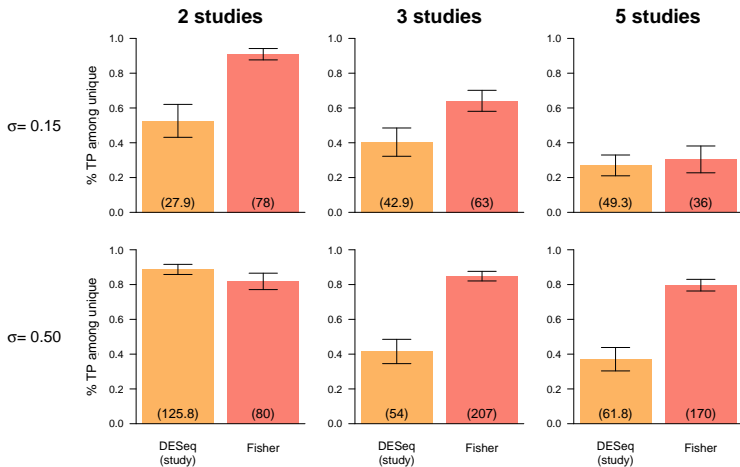
$$\text{Sensitivity} : E\left(\frac{VP}{VP+FN}\right)$$



Meta-analysis



Meta-analysis



Proportion of true positives among unique discoveries.

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R package

R package [metaRNASeq](#) available on CRAN

Publication : Rau, Marot and Jaffrézic, BMC Bioinformatics (2014)

```
library(metaRNASeq)  
vignette("metaRNASeq")
```

- data simulation with `sim.function`
- single individual analyses with `DESeq2`
- use of `HTSFilter` (if needed) to validate the p-value uniform distribution assumption
- p-value combination with `fishercomb` and `invnorm`
- treatment of conflicts from the extraction of fold changes

Key figures

- DE (differentially expressed) : number of DE genes
- IDD (integration-driven discoveries) : number of genes that are declared DE in the meta-analysis that were not identified in any of the single studies alone
- Loss : number of genes that are identified DE in single studies but not in meta-analysis
- IDR (integration-driven discovery rate) : corresponding proportion of IDD
- IRR (integration-driven revision) : corresponding proportion of loss

SMAGEXP

SMAGEXP available on Galaxy main tool shed or in a dockerised instance
 Publication : Blanck and Marot, Gigascience, (2019)

Galaxy / Galaxy SMAGEXP Analyse de données | [Workflows](#) | [Données partagées](#) | [Documentation](#) | [Aide](#) | [Authentication et Enregistrement](#) | [Help](#) | Using 10.0 KB

Tools

search tools

[Filter and Sort](#)

[Join, Subtract and Group](#)

[Convert Formats](#)

[Extract Features](#)

[Fetch Sequences](#)

[Fetch Alignments](#)

[Statistics](#)

[Graph / Display Data](#)

SMAGEXP

[Limma analysis](#) Performs gene expression analysis thanks to limma

[Microarray data meta-analysis](#) Performs meta-analysis thanks to metaMA

[QCnormalization](#) Quality control and normalization of affymatrix expression data

[GEOQuery](#) GEOQuery wrapper

[Recount](#) Get rna-seq count data with R recount Package

[Import custom data](#) Quality control and normalization of a custom matrix expression data

[RNA-seq data meta-analysis](#) Performs meta-analysis thanks to metaRNAseq

DESeq2

Workflows

- All workflows

RNA-seq data meta-analysis Performs meta-analysis thanks to metaRNAseq (Galaxy Version 1.1.0) Options

Study results

1: Study results

DESeq2 result file

68: Summary of meta-analysis and single studie analysis from RNA-seq data meta-analysis on data 44, data 46, an...

Must have the same number of row in each study

Number of replicates

10

Number of replicates of the study

2: Study results

DESeq2 result file

68: Summary of meta-analysis and single studie analysis from RNA-seq data meta-analysis on data 44, data 46, an...

Must have the same number of row in each study

Number of replicates

10

Number of replicates of the study

[Insert Study results](#)

DESeq2 Result file and number of replicate of the study

PDR

0.05

Adjusted p-value threshold to be declared differentially expressed

[Execute](#)

What it does

Given several DESeq2 results this tool runs a meta-analysis using the metaRNAseq R package.

Inputs

- At least 2 studies, and for each study
 - Results of DESeq2 study
 - Number of replicates of the study

History

Rechercher des données

Imported: Example of RNA-seq meta-analysis
69 rows
10.01 KB

- 69: Charts for RNA-seq data meta-analysis on data 44, data 46, and data 66
- 68: Summary of meta-analysis and single studie analysis from RNA-seq data meta-analysis on data 44, data 46, and data 66
- 67: DESeq2 plots on data 65, data 64, and others
- 66: Results_SRP058237
- 65: Recount (SRR2016920_Adj-Epi02)
- 64: Recount (SRR2016919_Adj-Epi02)
- 63: Recount (SRR2016918_Adj-Epi01)
- 62: Recount (SRR2016917_Adj-Neu04)
- 61: Recount (SRR2016916_Adj-Neu03)
- 60: Recount (SRR2016915_Adj-Neu02)

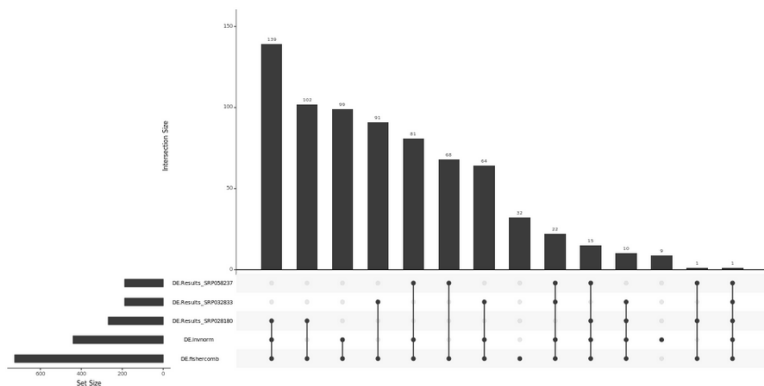
SMAGEXP

Source code, help, and installation instructions available on Github :
<https://github.com/sblanck/smagexp>

- Step by step example of a RNA-seq meta-analysis
 - Data used in this example
 - First Analysis
 - Run the recount tool
 - Run a DESeq2 analysis
 - Second Analysis
 - Run the recount tool
 - Run a DESeq2 analysis
 - Third Analysis
 - Run the recount tool
 - Run a DESeq2 analysis
 - Run the Meta-analysis with metaRNASeq

SMAGEXP

UPSETR DIAGRAM



Fisher combination summary

DE	IDD	Loss	IDR	IRR
725	131	0	18.07	0

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Conclusion - Discussion

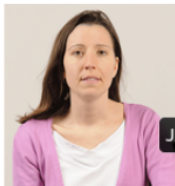
- Meta-analysis useful when strong inter-study effect and more than 3 studies
- p-value combinations enable to take advantage of empirical bayesian approaches - especially appropriate when few replicates
- with RNA-Seq data, necessity to treat conflicts a posteriori
- p-values histograms and PCA graphs enable to decide whether using or not metaRNASeq.
- metaRNASeq available on CRAN
- SMAGEXP available on Galaxy tool shed, Docker, Github.

Acknowledgements

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Claus-Dieter Mayer



Jean-Louis Foulley