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### **► To cite this version:**

Nicolas Servant, Eleonore Gravier, Pierre Gestraud, Cecile Laurent, Caroline Paccard, et al.. EMA - A R package for Easy Microarray data analysis.. BMC Research Notes, BioMed Central, 2010, 3 (1), pp.277. 10.1186/1756-0500-3-277 . inserm-00984710

**HAL Id: inserm-00984710**

**<https://www.hal.inserm.fr/inserm-00984710>**

Submitted on 28 Apr 2014

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TECHNICAL NOTE

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# EMA - A R package for Easy Microarray data analysis

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## Abstract

**Background:** The increasing number of methodologies and tools currently available to analyse gene expression microarray data can be confusing for non specialist users.

**Findings:** Based on the experience of biostatisticians of Institut Curie, we propose both a clear analysis strategy and a selection of tools to investigate microarray gene expression data. The most usual and relevant existing R functions were discussed, validated and gathered in an easy-to-use R package (EMA) devoted to gene expression microarray analysis. These functions were improved for ease of use, enhanced visualisation and better interpretation of results.

**Conclusions:** Strategy and tools proposed in the EMA R package could provide a useful starting point for many microarrays users. EMA is part of Comprehensive R Archive Network and is freely available at <http://bioinfo.curie.fr/projects/ema/>.

## Findings

Numerous analysis methods and tools have been developed to study microarray, many of them being implemented as free R [1] and/or Bioconductor [2] packages. This abundance of methods makes choosing the best approach difficult for newcomers and non-specialist users.

Based on the experience of the biostatisticians of Institut Curie, we propose a clear analysis strategy combining a large variety of standard methodologies. The most usual and relevant R functions needed to perform these analyses were selected and gathered in the R package EMA (Easy Microarray data Analysis). EMA covers an entire analysis process including quality control, normalisation, exploratory analysis, unsupervised and supervised classification, functional analysis and censored data exploration. The package can be used for both one or two-colours gene expression micrarrays and for exon expression experiments.

## Analysis strategy

Firstly, the quality of the data must be assessed in order to detect problematic raw probe-level data, such as spatial artifacts on the chip or poor quality hybridisation. Indeed, gene expression experiments suffer from many sources of technical and experimental variation. Removing noise and systematic biases is performed in order to both improve the biological signal and make all the arrays comparable. This is the so-called normalisation step. Secondly, we propose to discard the probesets with very low signal across the samples (*i.e.* genes unexpressed or below detection threshold). This filtering step leads to both a noise reduction in the data and an increase in the statistical power of the subsequent analysis.

Then, exploratory approaches are classically used to find clusters of genes (or samples) with similar profiles. Note that here, biological interpretation depends on the choice of the similarity metrics. These approaches potentially highlight outliers and/or non relevant effects (batch effect for example), which can be subsequently estimated and/or removed from the data thanks to appropriate methods.

Finally, supervised approaches aim at the identification of differentially expressed genes (DEG), or deregulated

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pathways by taking into account the multiple testing issues. The biological interpretation of the differential analysis results can be performed thanks to functional and gene set enrichment analyses. Sample class prediction (eg good vs poor clinical outcome) based on supervised classification methods can also be performed to highlight genes signatures.

### Selected tools

For the data quality assessment, we recommend to use the `arrayQualityMetrics` package [3], which performs a powerful, easy-to-use and comprehensive data quality estimation as well as an automatic html report. The EMA package proposes the most famous techniques for Affymetrix GeneChip normalisation: MAS5.0 [4], RMA [5] and GCRMA [6]. We recommend to use GCRMA because it outperforms the other approaches (by ignoring the mismatch intensities and taking into account the probe sequence information) and allows an efficient filtering of irrelevant probesets thanks to its bimodal distribution of probesets expression values (Figure 1a). Other packages such as `limma` [7], `vsn` [8] or `lumi` [9] can be used to normalise non Affymetrix data. After this first step, the main EMA functions can be used for any type of expression data, using a simple data expression matrix as input.

The EMA package provides functions to perform exploratory analyses such as Principal Component Analysis (PCA, Figure 1b), hierarchical clustering (Figure 1c) or Multiple Factor Analysis. They are based on R packages such as `FactoMineR` [10], `cluster` [11], or `multclust` [12]. The use of linear model is proposed to estimate and to remove the non relevant effects potentially detected.

Various methods are proposed to perform differential analysis and their choice depends on the sample size. The `multtest` package provides standard approaches like Student or Mann-Whitney test associated with multiple testing correction methods. The Significance Analysis of Microarrays (SAM) approach [13] (`siggenes` package) is also very interesting because it both estimates the null distribution and takes into account the correlation between probesets (Figure 1d). The rank product method [14] (`RankProd` package) dedicated to small sample size dataset is also offered, as well as some linear model (ANOVA) functions. Alternatively, the user can apply the `limma` package which is a very powerful tool to assess differential expression by linear models.

The functional enrichment of the DEG list is assessed based on the GeneOntology [15], and KEGG [16] pathways annotation terms. The hyper-geometric test of the `GOstats` package is used to test the over-representation of the functional terms in the gene list.

For sample class prediction, we suggest to use the `CMA` package [17] including the most popular machine learning and gene selection algorithms. In the context of censored data, the EMA package supports Kaplan Meier and log-rank analyses using the survival package.

### Example

The proposed analysis strategy was applied to the breast cancer gene expression dataset [18] comparing 12 Basal-like carcinomas (BLCs) and 11 HER2 positive carcinomas (HER2+). Some graphical outputs for data preprocessing, exploratory analysis and differential analysis steps are displayed in Figure 1. The RNA profiles were analysed using U133 plus 2.0 Affymetrix GeneChip. Three genes (*P-cadherin*, *v-kit*, *FOXC1*) were reported by the authors to be associated to a genes cluster over-expressed in the basal-like carcinomas and three genes (*PTEN*, *Her2* and *GRB7*) to a genes cluster over-expressed in the Her2+ carcinomas. All these genes but one (*v-kit*) were found to be differentially expressed using the EMA package. This discrepancy is easily explained because in spite of *v-kit* belongs to a basal-like expression cluster, no change in *v-kit* expression can be observed between the two groups in this clustering analysis. This is because the hierarchical clustering was performed on genes (such as *v-kit*) not necessary differentially expressed between the two populations.

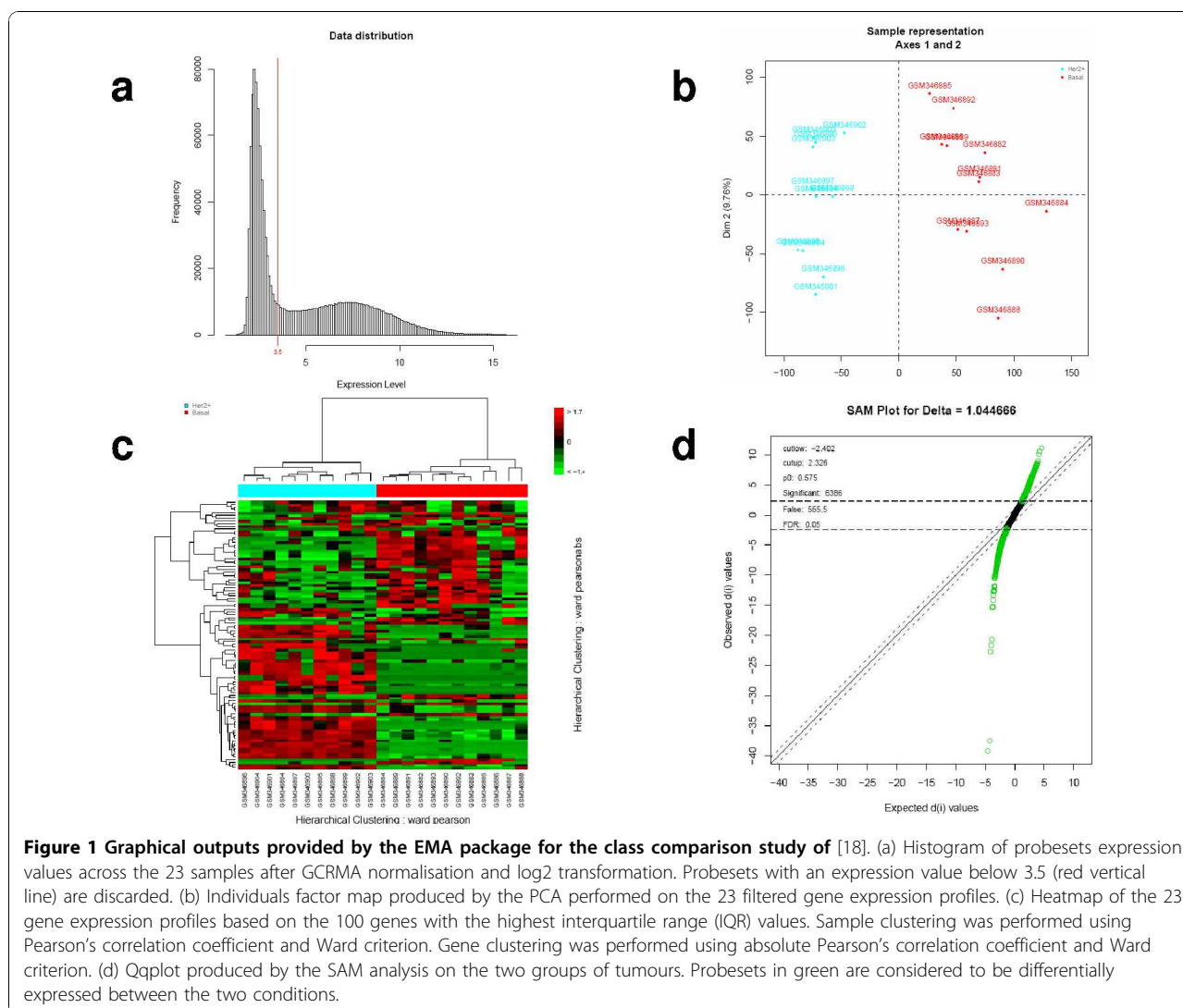
The R scripts used to analyse this gene expression dataset can be found in [Additional file 1]. Transcriptional data used in this application are publicly available at Gene Expression Omnibus (Accession number: [GSE13787]) and are part of the package.

### Conclusions

EMA is a freely available R package which implements a complete strategy for expression microarray analysis. The package includes a vignette [Additional file 2] which describes the detailed biological/clinical analysis strategy used at Institut Curie. Most of the functions were improved for ease of use (fewer command lines, default parameters tested and chosen to be optimal). Relevant, enhanced and easy-to-interpret text and graphic outputs are offered. The package is available on The Comprehensive R Archive Network repository [19].

### Availability and requirements

- Project Name: EMA
- Project home page:  
<http://bioinfo.curie.fr/projects/ema/>  
<http://cran.r-project.org/>
- Operating systems: Linux, Windows
- Programming language: R
- Other requirements: R version  $\geq 2.10$ . R packages: `cluster`, `Hmisc`, `heatmap.plus`, `FactoMineR`, `GOstats`,



survival, multtest, affy, gcrma, rgl, GSA, RankProd, siggenes, MASS, hgu133plus2.db, xtable, biomaRt.

- License: GNU GPL
- Any restrictions to use by non-academics: none

### Additional material

**Additional file 1: R scripts applied to the breast cancer gene expression dataset [18].** R script used to analyse the breast cancer gene expression data set [18].

**Additional file 2: EMA vignette.** The vignette discuss the detailed biological/clinical analysis strategy used at Institut Curie and presents an application to a gene expression dataset.

### Acknowledgements

We thank all the persons from the ABCIS methodology group for their collaboration and fruitful discussions. This work was supported by a grant from the Institut Curie "Programmes incitatifs et coopératifs Bioinformatique

et Biostatistique". EB and PH are members of the team Systems Biology of Cancer "équipes labellisées par la Ligue Nationale Contre le Cancer".

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### Authors' contributions

NS and EG discussed the choice of the strategy and tools, participated to the development of the EMA package and wrote the paper. PG, CL, CP, AB, IB, JM discussed the choice of the strategy and tools and participated to the development of the EMA package. BA, EB and PH discussed the choice of the strategy and tools and supervised the work group. All authors read and approved the final manuscript.

### Competing interests

The authors declare that they have no competing interests.

Received: 15 July 2010 Accepted: 3 November 2010

Published: 3 November 2010

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doi:10.1186/1756-0500-3-277

**Cite this article as:** Servant et al.: **EMA - A R package for Easy Microarray data analysis**. *BMC Research Notes* 2010 **3**:277.

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