PECA: Probe-level Expression Change Averaging

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Contents

1 Introduction

PECA determines differential gene/protein expression using directly the probe/peptide-level measurements from Affymetrix gene expression microarrays or proteomic datasets, instead of the common practice of using precalculated gene/protein-level values. An expression change between two groups of samples is first calculated for each measured probe/peptide. The gene/protein-level expression changes are then defined as medians over the probe/peptide-level changes. This is illustrated in fig 1. For more details about the probe-level expression change averaging (PECA) procedure, see Elo et al. (2005), Laajala et al. (2009) and Suomi et al.

PECA calculates the probe/peptide-level expression changes using the ordinary or modified t-statistic. The ordinary t-statistic is calculated using the function rowttests in the Bioconductor genefilter package. The modified t-statistic is calculated using the linear modeling approach in the Bioconductor limma package. Both paired and unpaired tests are supported.

The significance of an expression change is determined based on the analytical p-value of the gene-level test statistic. Unadjusted p-values are reported along with the corresponding p-values looked up from beta distribution taking into account the number of probes/peptide per gene/protein. The quality control and filtering of the data (e.g. based on low intensity or probe specificity) is left to the user.

2 Example

We start by loading PECA and spike-in example data.

```
> library(PECA)
> library(SpikeIn)
> data(SpikeIn133)
```

We subset the original spike-in for our purposes, including two groups with three replicates.

```
> data <- SpikeIn133[,c(1,15,29,2,16,30)]
```

Calculate p-values using PECA.

```
> peca_results <- PECA_AffyBatch(normalize="true",affy=data)
```

Calculate p-values using MAS5.

```
> library(multtest)
> mas5 <- mas5(data)

background correction: mas
PM/MM correction : mas
expression values: mas
```
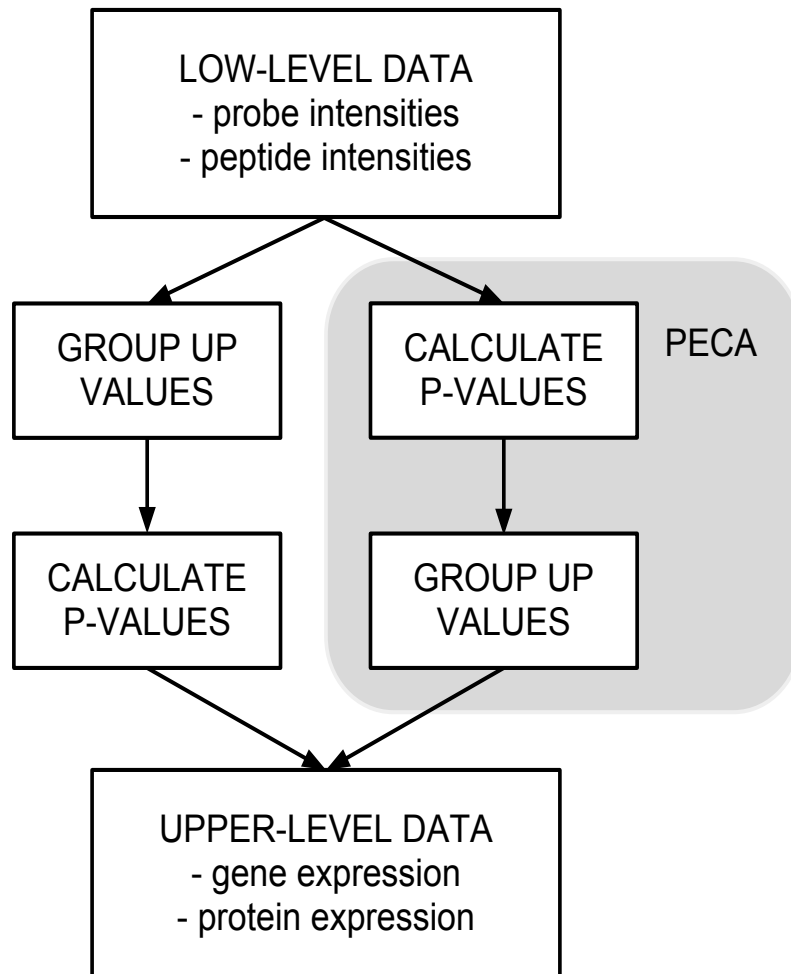
Figure 1: Probe-level expression change averaging

```
background correcting...done.
22300 ids to be processed
|                   |
|###################|

> groups=c(1,1,1,0,0,0)
> stats=mt.teststat(exprs(mas5), groups, test="t")
> mas5_results=2*(1-pnorm(abs(stats)))
```

Calculate p-values using RMA.

```
> rma <- rma(data)

Background correcting
Normalizing
Calculating Expression

> groups=c(1,1,1,0,0,0)
> stats=mt.teststat(exprs(rma), groups, test="t")
> rma_results=2*(1-pnorm(abs(stats)))
```

Combine and preview results.

```
> results <- cbind(peca=peca_results[,"p"],mas5=mas5_results,rma=rma_results,spike=0)
> rownames(results) <- rownames(peca_results)
> results[,4][rownames(results) %in% colnames(pData(data))] <- 1
> head(results)

                peca      mas5       rma spike
1007_s_at 0.4185863 0.6620376 0.1720956     0
1053_at   0.9974262 0.1478299 0.8738921     0
117_at    0.9998561 0.6182159 0.5277216     0
121_at    1.0000000 0.5922030 0.4554895     0
1255_g_at 0.9999160 0.9854084 0.4150493     0
1294_at   0.9181849 0.9342097 0.7798362     0
```

ROC Curves from these results show that PECA performs well at low false positive rates, which in practice is of relevance to the user.

```
> library(ROCR)
> results_roc <- results
> results_roc[,1:3] <- 1-results_roc[,1:3]
> pred.peca <- prediction(results_roc[,1],results_roc[,4])
> pred.mas5 <- prediction(results_roc[,2],results_roc[,4])
> pred.rma <- prediction(results_roc[,3],results_roc[,4])
> perf.peca <- performance(pred.peca,"tpr","fpr")
> perf.mas5 <- performance(pred.mas5,"tpr","fpr")
> perf.rma <- performance(pred.rma,"tpr","fpr")
```

```
> plot(perf.mas5, lwd=3, col="green")
> plot(perf.rma, lwd=3, col="blue", add=TRUE)
> plot(perf.peca, lwd=3, col="red", add=TRUE)
> legend(0.7,0.2,c('PECA', 'RMA', 'MAS'), col=c('red','blue','green'), lwd=3)
```