

Exploring the MAQC data with Bioconductor

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1 Introduction

See the Sept 2006 issue of Nature Biotechnology for several articles about the MAQC initiative. The *MAQCsubset* package includes excerpts from the data published at GEO GSE5350.

```
> library(MAQCsubset)
> data(afxsubRMAES)
> afxsubRMAES
```

```
ExpressionSet (storageMode: lockedEnvironment)
assayData: 54675 features, 24 samples
  element names: exprs
protocolData: none
phenoData
  sampleNames: AFX_1_A1.CEL AFX_1_A2.CEL ... AFX_3_D2.CEL (24 total)
  varLabels: site samp repl pctBrain
  varMetadata: labelDescription
featureData: none
experimentData: use 'experimentData(object)'
  pubMedIds: 16964226
Annotation: hgu133plus2
```

```
> pd = pData(afxsubRMAES)
> table(pd$site, pd$samp)
```

	A	B	C	D
1	2	2	2	2
2	2	2	2	2
3	2	2	2	2

Samples labeled "A" have 100% stratagene universal human RNA, while samples labeled "B" have 100% Ambion human brain RNA. Samples labeled C have .75A+.25B, and samples labeled D have .75B+.25A.

2 The proboscis plot

For Figure 2 of Shippy et al., *Using RNA sample titrations* (Nat Biotech, 24(9):1123-1131, Sep 2006), genes differentially expressed between samples A and B using t tests at $p = 0.001$ are identified. If, for such genes, the A samples are up-regulated relative to the B samples, then a self-consistent monotone titration (SCMT) is declared if the C samples for such genes are up-regulated relative to the D samples. For genes upregulated on B samples relative to A samples, then SCMT occurs if the D samples are up-regulated relative to the C samples.

Figure 2 of Shippy et al. plots the proportion of genes exhibiting SCMT against the intensity ratios (A/B or B/A as appropriate). These plots, formed for each manufacturer/normalization combination and for each site, have the appearance of long pointy noses and are thus called proboscis plots. The following code computes the necessary quantities:

```
> #setClass("proboStruct", representation(call="call"),
> # contains="list")
> #setMethod("show", "proboStruct", function(object) {
> #   cat("proboStruct instance created by:\n")
> #   print(object@call)
> #})
> #setMethod("plot", "proboStruct", function(x, y, xlim=c(-3,3),
> #   col="black", ...) {
> #   plot(x[[1]][x$leftinds], x[[2]][x$leftinds], xlab=names(x)[1],
> #   ylab=names(x)[2], type="l", col=col, xlim=xlim, ...)
> #   lines(x[[1]][-x$leftinds], x[[2]][-x$leftinds], col=col, ...)
> #})
> proboscis = function(es, site=1, ABp=0.001, CDp=.01,
+   mmrad=100) {
+   require(genefilter)
+   mcall = match.call()
+   # assumes samples labeled A, B, C, D as in MAQC
+   mm = function(x,rad) {
+     # moving mean
+     start = ceiling(rad/2)
+     stop = floor(length(x)-(rad/2))
+     sapply(start:stop, function(i) mean(x[(i-floor(rad/2)):(i+floor(rad/2))]))
+   }
+   ess = es[,es$site==site]
+   essab = ess[, ess$samp %in% c("A", "B")]
+   essab$samp = factor(essab$samp)
+   esscd = ess[, ess$samp %in% c("C", "D")]
+   esscd$samp = factor(esscd$samp)
```

```

+ tt = rowttests( exprs(essab), essab$samp )
+ L = which( tt$p < ABp & tt$dm < 0 )
+ R = which( tt$p < ABp & tt$dm > 0 )
+ ttcd = rowttests( exprs(esscd), esscd$samp )
+ ABL = tt$dm[L]
+ CDL = ttcd$dm[L]
+ ABR = tt$dm[R]
+ CDR = ttcd$dm[R]
+ NN = list(ttab=tt,ttcd=ttcd,ABL=sort(ABL),cdokL=1*(CDL<0)[order(ABL)],
+   ABR=sort(ABR),dcokR=1*(CDR>0)[order(ABR)])
+ `A-B` = c(ONR <- mm(NN$ABL,mmrad), mm(NN$ABR,mmrad))
+ `P(SCMT|A-B)` = c(mm(NN$cdokL,mmrad), mm(NN$dcokR,mmrad))
+ new("proboStruct", call=mcall,
+   list("A-B"=`A-B`, "P(SCMT|A-B)"=`P(SCMT|A-B)` ,
+   leftinds=1:length(ONR)))
+ }
> NN1 = proboscis(afxsubRMAES)
> NN2 = proboscis(afxsubRMAES, site=2)
> NN3 = proboscis(afxsubRMAES, site=3)

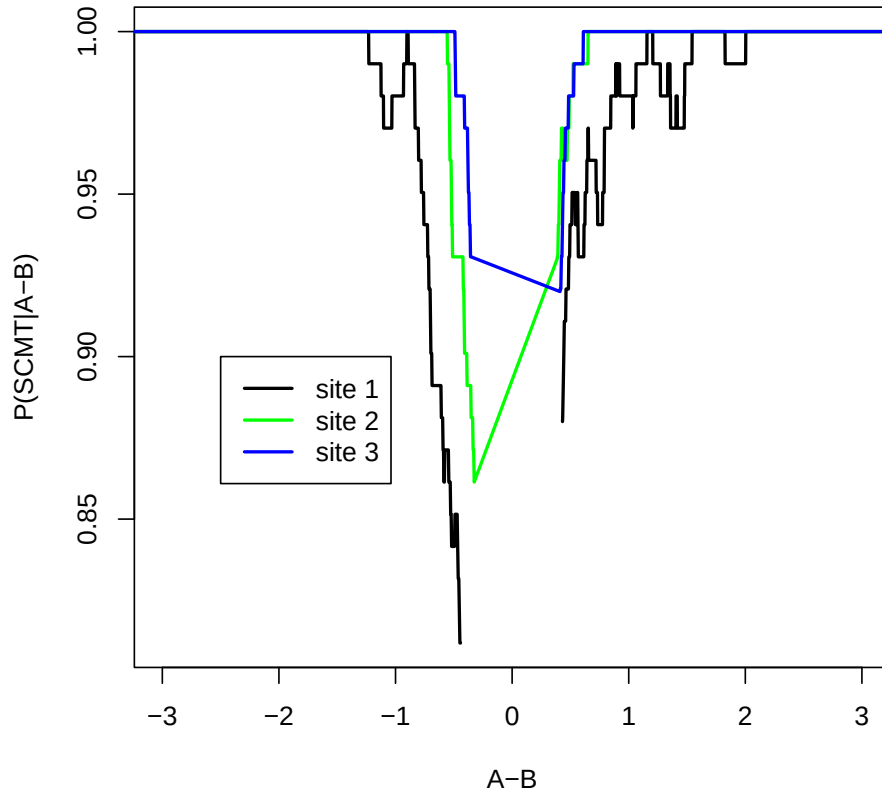
```

There are simple graphical methods:

```

> plot(NN1, lwd=2)
> lines(NN2[[1]], NN2[[2]], col="green", lwd=2)
> lines(NN3[[1]], NN3[[2]], col="blue", lwd=2)
> legend(-2.5, .9, lty=1, lwd=2, legend=c("site 1",
+ "site 2", "site 3"), col=c("black", "green", "blue"))
>

```



These do not look exactly like the plots in the Shippy paper, presumably because only two replicates per site are in use in this display.