Using ReportingTools in an Analysis of Microarray Data

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1 Introduction

The ReportingTools package is particularly useful in displaying results from a microarray experiment. In this vignette we show how to display results from differential gene expression, Gene Ontology (GO), protein families (PFAM) and gene set enrichment analyses. In the final section, we create an index page where the user can easily access any of these results.

2 Differential expression analysis using limma

For this vignette we will examine the ALL dataset. First we load our ReportingTools package and the data. This dataset is from a clinical trial in acute lymphoblastic leukemia (ALL) and is available from Bioconductor.

```
> library(ReportingTools)
> library(ALL)
> library(hgu95av2.db)
> library(genefilter)
> data(ALL)
```

We will compare the gene expression between the BCR/ABL and NEG samples. We use featureFilter to remove most of the unexpressed genes.

```
> ALL <- ALL[, ALL$mol.biol %in% c('NEG', 'BCR/ABL') &
+ !is.na(ALL$sex)]
> ALL$mol.biol <- factor(ALL$mol.biol,
+ levels = c('NEG', 'BCR/ABL'))
> ALL <- featureFilter(ALL)</pre>
```

Next we use limma to find statistical evidence of differentially expressed genes.

```
> library(limma)
> model <- model.matrix(~mol.biol+sex, ALL)
> fit <- eBayes(lmFit(ALL, model))</pre>
```

With the limma output we can make our differential analysis report. To publish MArrayLM objects, we supply the eSet and factor used in our analysis.

The resulting output is displayed on an .html page and includes several statistics of interest as well as an image of the data.

If we want to change our images, we can do so with .modifyDF (see the basic vignette for more examples of how to use this feature). In this example, we make lattice plots of the expression of each gene in our table stratified by mol.biol and sex . Note that .modifyDF uses the basic data frame (output from topTable) as its default object and then modifies it with the corresponding function. To modify the decorated ReportingToolsdata.frame, let .modifyDF=list(modifyReportDF, makeNewImages) .

Analysis of BCR/ABL translocation differential expression

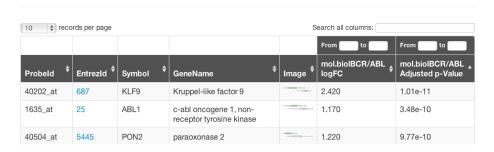


Figure 1: Resulting page created by publish for fit.

```
> library(hwriter)
> makeNewImages <- function(df,...) {</pre>
          imagename <- c()
+
          for (i in 1:nrow(df)) {
                   probeId <- df$ProbeId[i]</pre>
                   y_at <- pretty(exprs(ALL)[probeId,])</pre>
                   y_labels <- formatC(y_at, digits = 1, format = 'f')</pre>
                   imagename[i] <- paste0("plot", probeId, ".png")</pre>
                   png(filename = paste0("./reports/figuresde_analysis/",
                          imagename[i]))
                   print (stripplot (exprs (ALL) [probeId,]~ALL$mol.biol|ALL$sex))
                   dev.off()
          df$Image <- hwriteImage(paste0("figuresde_analysis/", imagename),</pre>
                                    link=paste0("figuresde_analysis/", imagename),
                                    table=FALSE, width=100)
+
          return (df)
 deReport2 <- HTMLReport(shortName='de analysis2',</pre>
                            title = 'Analysis of BCR/ABL translocation differential expression
                            reportDirectory = "./reports")
 publish (fit, deReport2, eSet = ALL, factor = ALL$mol.biol, coef=2,
          n=100,
         ##.modifyDF=list(modifyReportDF, makeNewImages) ) ##to add new images to default R
          .modifyDF=makeNewImages)
> finish(deReport2)
```

3 GO analysis using GOstats

In this section, we show how to use ReportingTools to publish a GO analysis to an html file. First we select the top 100 differential genes and then run the hyperGTest from the GOstats package.

```
> library(GOstats)
> tt <- topTable(fit, coef = 2, n = 100)
> selectedIDs <- unlist(mget(rownames(tt), hgu95av2ENTREZID))</pre>
```

Analysis of BCR/ABL translocation differential expression with new plots

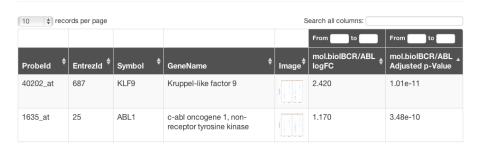


Figure 2: Resulting page created by makeNewImages

With these results, we can then make the GO report. We must supply publish with the genes of interest and the species annotation for this dataset. The default p-value cutoff is 0.01 and the minimum category size is 10 genes.

The resulting output is displayed on an .html page and includes several statistics of interest as well as an image of the overlap for each category.

4 PFAM analysis

In this section, we show how to use ReportingTools to write a table of PFAM analysis results to an html file. First we run the hyperGTest from the Category package.

GO analysis of BCR/ABL translocation

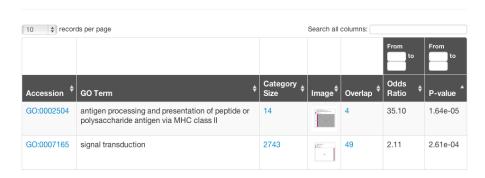


Figure 3: Resulting page created by publish for goResults.

Then we make the PFAM report. Again we supply publish with the genes of interest and the species annotation for this dataset. We set the minimum category size to 3 genes.

The resulting output is displayed on an .html page and includes several statistics of interest as well as an image of the overlap for each category.

5 GSEA analysis

In this section we show how to use publish to display GeneSetCollection objects and their corresponding gene set enrichment statistics. For this example, we will randomly select our gene sets and create our collection.

```
> library (GSEAlm)
> library(GSEABase)
> mapped genes <- mappedkeys(org.Hs.egSYMBOL)</pre>
> eidsAndSymbols <- as.list(org.Hs.egSYMBOL[mapped_genes])</pre>
> geneEids <- names(eidsAndSymbols)</pre>
> set.seed(123)
> set1 <- GeneSet(geneIds=sample(geneEids, 100, replace=FALSE), setName="set1",
                  shortDescription = "This is set1")
>
 set2 <- GeneSet (geneIds=sample (geneEids, 10, replace=FALSE), setName="set2",
                  shortDescription = "This is set2")
 set3 <- GeneSet (geneIds=sample (geneEids, 37, replace=FALSE), setName="set3",
                   shortDescription = "This is set3")
> set4 <- GeneSet(geneIds=sample(geneEids, 300, replace=FALSE), setName="set4",
                  shortDescription = "This is set4")
> geneSets <- GeneSetCollection(c(set1, set2, set3, set4))
```

We can now make a very simple GeneSetCollection html table with ReportingTools .

The resulting output is displayed on an .html page and includes the gene sets and links to pages listing the genes within the corresponding set.

Often, investigators would like more information about the enrichment of certain gene sets. Thus, we will proceed with gene set enrichment analysis (GSEA). To begin, we determine the overlap between our sets and our genes of interest by creating an incidence matrix.

```
> mat <- matrix(data=0, ncol=length(universeIDs),nrow=length(geneSets))</pre>
> for(i in 1:length(geneSets)){
    geneIdEntrez <- unlist(geneIds(geneSets[[i]]))</pre>
    mat[i,match(geneIdEntrez, universeIDs)] <- 1</pre>
+ }
> colnames(mat) <- universeIDs</pre>
> rownames(mat) <- sapply(geneSets, function(x) x@setName)
  Now we can run the GSEA and obtain set-specific statistics and p-values.
> lm <- lmPerGene(ALL, ~mol.biol+sex, na.rm=TRUE)</pre>
> GSNorm <- GSNormalize(lm$tstat[2,], mat)
> #one-sided p-values
> pVals <- gsealmPerm(ALL,~mol.biol+sex, mat, nperm=100)
> bestPval <- apply(pVals, 1, min)</pre>
  We can add these statistics to our report page.
> gseaReport <- HTMLReport(shortName = "gsea_analysis",</pre>
                             title = "GSEA analysis",
                             reportDirectory = "./reports")
> publish(geneSets, gseaReport, annotation.db = "org.Hs.eg",
           setStats = GSNorm, setPValues = 2*bestPval)
```

> finish(gseaReport)

The resulting output is displayed on an .html page and includes our set statistics and p-values. Links to set-specific pages are also created.

We can also add the same statistics via .modifyDF . As demonstrated in the basic vignette, .modifyDF allows us to manipulate the output published to our html pages.

```
> runGSEA <- function(df,...){
+ mat <- matrix(data = 0, ncol = length(universeIDs), nrow = length(geneSets))
+ for(i in 1:length(geneSets)){
+ geneIdEntrez <- unlist(geneIds(geneSets[[i]]))
+ mat[i,match(geneIdEntrez, universeIDs)] <- 1
+ }
+ colnames(mat) <- universeIDs
+ rownames(mat) <- sapply(geneSets, function(x) x@setName)
+ lm <- lmPerGene(ALL, ~mol.biol+sex, na.rm=TRUE)
+ GSNorm <- GSNormalize(lm$tstat[2,], mat)
+ pVals <- gsealmPerm(ALL, ~mol.biol+sex, mat, nperm = 100)</pre>
```

GSEA analysis



Figure 4: Resulting updated page created by publish for geneSets after we include the set statistics and p-values.

Genes in set1 -- This is set1

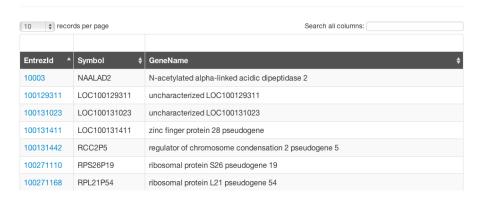


Figure 5: The set-specific page.

Analysis of ALL Gene Expression

Analysis of BCR/ABL translocation differential expression GO analysis of BCR/ABL translocation PFAM analysis of BCR/ABL translocation GSEA report has a new title

Figure 6: The page created from calling publish on all of our previous pages.

```
+ bestPval <- apply(pVals,1, min)
+ df <- cbind(df, GSNorm, bestPval)
+ return(df)
+ }
> gseaReport2 <- HTMLReport(shortName = "gsea_analysis2",
+ title = "GSEA analysis",
+ reportDirectory = "./reports")
> publish(geneSets, gseaReport2, annotation.db = "org.Hs.eg",
+ .modifyDF = runGSEA)
> finish(gseaReport2)
```

6 Putting it all together

We now make an index page to put all the output together.

7 References

Huntley, M.A., Larson, J.L., Chaivorapol, C., Becker, G., Lawrence, M., Hackney, J.A., and J.S. Kaminker. (2013). Reporting Tools: an automated results processing and presentation toolkit for high throughput genomic analyses. *Bioinformatics*. **29**(24): 3220-3221.