1 Overview

The intent of this vignette is to show how to make reasonably nice looking HTML tables for presenting the results of a microarray analysis. These tables are a very nice format because you can insert clickable links to various public annotation databases, which facilitates the downstream analysis. In addition, the format is quite compact, can be posted on the web, and can be viewed using any number of free web browsers. One caveat; an HTML table is probably not the best format for presenting the results for all of the genes on a chip. For even a small (5000 gene) chip, the file could be 10 Mb or more, which would take an inordinate amount of time to open and view. Also note that the Bioconductor project supplies annotation packages for many of the more popular Affymetrix chips, as well as for many commercial spotted cDNA chips. For chips that have annotation packages, the annaffy package is the preferred method for making HTML tables.

To make an annotated HTML table, the only requirement is that we have some sort of annotation data for the microarray that we are using. Most manufacturers supply data in various formats that can be read into $R$. For instance, Affymetrix supplies CSV files that can be read into $R$ using the read.csv() function [http://www.affymetrix.com/support/technical/byproduct.affx?cat=arrays](http://www.affymetrix.com/support/technical/byproduct.affx?cat=arrays).

Another alternative is to annotate using functionality in the biomaRt package. This allows one to get the most current annotations interactively. In addition, the output can be used directly with functions in annotate to make HTML tables. We will use these functions in this vignette.
2 Data Analysis

I will assume that the reader is familiar with the analysis of microarray data, and has a set of genes that she would like to use. In addition, I will assume that the reader is familiar enough with R that she can subset the data based on a list of genes, and reorder based on a particular statistic. For any questions about subsetting or ordering data, please see “An Introduction to R”. For questions regarding microarray analysis, please consult the vignettes for, say limma, multtest, or marray.

3 Getting Started

We first load the annotate package, as well as some data. These data will be from the Affymetrix HG-U95Av2 chip (for which we would normally use annaffy). To keep the HTML table small, we will take a subset of fifteen genes as an example.

> library("annotate")
> data(sample.ExpressionSet)
> igenes <- featureNames(sample.ExpressionSet)[246:260]

We also have to load the biomaRt package and connect to a Biomart database, using the useMart function. Note that there are two interfaces that biomaRt can use to connect to a Biomart database, using either the RCurl package to connect via http protocols, or the RMySQL package to connect via database protocols. The default is to use RCurl because it can be difficult to get RMySQL set up on Windows computers. However, I find the database connectivity to be much faster, so for those who want to annotate a large number of genes, I would recommend using the RMySQL interface. See the help file for useMart for more information.

> library("biomaRt")
> mart <- useMart("ensembl", "hsapiens_gene_ensembl")

4 Annotation Data

The htmlpage function is designed to take two sets of input; data that will be converted to clickable links to various online databases, and data that will simply be put into the HTML table as is. For the clickable links we need an list of character vectors for each database. For the data, we need a list
of either vectors, `data.frames` or a mixture of the two. We will explore this topic more later. First, we will see how to get data using `biomaRt` functionality.

We first need to see exactly what sort of data we can get from Ensembl’s Biomart. Note that some of the information can be a bit cryptic, so we can parse out a more reasonable description. We also need to see what things we can use as identifiers in our query of the Biomart server.

First, a bit of terminology. An 'attribute' is a data type that can be returned from a query of a Biomart server. A 'filter' is the identifier that we use to query the server. For instance, we can get GO terms and Entrez Gene IDs (the attributes) by querying on the Affy Probe ID (the filter).

There are too many attributes to list here, so we can parse out things that may be interesting to us. Let’s say we want to get Entrez Gene and SwissProt IDs for our set of genes.

```r
e > attributs <- listAttributes(mart)
> attributs[grep("swiss", attributs[,1]),]

<table>
<thead>
<tr>
<th>name</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>103 uniprot_swissprot</td>
<td>UniProt/SwissProt ID</td>
</tr>
<tr>
<td>104 uniprot_swissprot_accession</td>
<td>UniProt/SwissProt Accession</td>
</tr>
</tbody>
</table>

e > attributs[grep("entrez", attributs[,1]),]

<table>
<thead>
<tr>
<th>name</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 entrezgene</td>
<td>EntrezGene ID</td>
</tr>
</tbody>
</table>

So the attributes we want are `uniprot_swissprot_accession` and `entrezgene`.

The same basic idea can be used to figure out which filter to use. Since we are using the HG-U95av2 chip, we need to figure out what that filter is called.

```r
e > fltr <- listFilters(mart)
> fltr[grep("affy", fltr[,1]),]

<table>
<thead>
<tr>
<th>name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 affy_HC_g110</td>
</tr>
<tr>
<td>2 affy_hg_focus</td>
</tr>
<tr>
<td>3 affy_hg_u133a</td>
</tr>
<tr>
<td>4 affy_hg_u133a_2</td>
</tr>
</tbody>
</table>
```
5 affy_hg_u133b
6 affy_hg_u133_plus_2
7 affy_hg_u95a
8 affy_hg_u95av2
9 affy_hg_u95b
10 affy_hg_u95c
11 affy_hg_u95d
12 affy_hg_u95e
13 affy_hugenefl
14 affy_u133_x3p
96 with_affy_hc_g110
97 with_affy_hg_focus
98 with_affy_hg_u133a
99 with_affy_hg_u133a_2
100 with_affy_hg_u133b
101 with_affy_hg_u133_plus_2
102 with_affy_hg_u95a
103 with_affy_hg_u95av2
104 with_affy_hg_u95b
105 with_affy_hg_u95c
106 with_affy_hg_u95d
107 with_affy_hg_u95e
108 with_affy_huex_1_0_st_v2
109 with_affy_hugene_1_0_st_v1
110 with_affy_hugenefl
111 with_affy_u133_x3p

description
1 Affy hc g110 ID(s)
2 Affy hg focus ID(s)
3 Affy hg u133a ID(s)
4 Affy hg u133a 2 ID(s)
5 Affy hg u133b ID(s)
6 Affy hg u133 plus 2 ID(s)
7 Affy hg u95a ID(s)
8 Affy hg u95av2 ID(s)
9 Affy hg u95b ID(s)
10 Affy hg u95c ID(s)
11 Affy hg u95d ID(s)
12 Affy hg u95e ID(s)
13 Affy hugenefl ID(s)
This one is pretty obvious - we want the affy_hg_u95av2 filter.

Now to get back to the task at hand; we have 15 Affy Probeset IDs that we want to use to create our HTML table. Let's say we want to create an HTML table in which we map the Affy IDs to Entrez Gene, SwissProt, UniGene, and RefSeq IDs (all clickable links) and in addition we want to include the gene description and symbol, the Gene Ontology terms, and chromosome location, as well as the t-statistic, p-value, fold change and expression values. That would be a nice compact format for presenting the data to someone.

We need to collect all this information in two lists; one that will be used to make the hyperlinks, and one that will just be static information. First, we do the hyperlinks. By using the ideas presented above, I figured out which attributes correspond to Entrez Gene, SwissProt, UniGene, and RefSeq IDs, so I will use them here. Instead of writing them all out, I will simply select the correct terms using the listAttributes function.

```r
> genelist <- getBM(attributes = c("affy_hg_u95av2","entrezgene","uniprot_swissprot_accession","refseq_dna"),
+                  filter = "affy_hg_u95av2", values = igenes, mart = mart,
+                  output = "list", na.value = "&nbsp;")
> genelist[[1]] <- igenes
> ## let's look at genelist values
> lapply(genelist, function(x) x[5:10])
```
<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Description</th>
<th>EntrezGene ID</th>
<th>SwissProt Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>31489_at</td>
<td></td>
<td>6331 731231</td>
<td>Q14524</td>
</tr>
<tr>
<td>31490_at</td>
<td></td>
<td>841</td>
<td>Q14790</td>
</tr>
<tr>
<td>31491_s_at</td>
<td></td>
<td>1443 1442 1444</td>
<td>Q9UBQ5</td>
</tr>
<tr>
<td>31492_at</td>
<td></td>
<td>27335</td>
<td>Q01243</td>
</tr>
<tr>
<td>31493_s_at</td>
<td></td>
<td></td>
<td>P01243</td>
</tr>
</tbody>
</table>
Here we can see that genelist is a list of lists, with each sub-list being made up of a character vector. I substituted the 'igenes' vector back into the first position of the list because the Biomart server we are using doesn’t have data for all Affy IDs (including the Affy ID itself). Since we want links for all of the Affy IDs, I simply substituted the original vector into the genelist.

Two important things to note about the call to getBM. First, we have to use the argument output = “list”. Second, we have to use na.value = “&nbsp;”, which will create an empty table entry for any missing data. This is much nicer than leaving the NAs, which will tend to clutter up the table without adding any information.

Making the second list is a bit more complicated. We need to get some annotation from the Biomart database, and append that to some data we have from our experiment. The first step is to get the annotation data. As noted above, we want the gene name and symbol, as well as the GO terms
and chromosome location for these probesets. We can figure out which attribute terms to use, following the ideas presented above.

```r
> attrbuts[grep("description", attrbuts[,1]),]

<table>
<thead>
<tr>
<th>name</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>32 description</td>
<td>Description</td>
</tr>
<tr>
<td>61 interpro_description</td>
<td>Interpro Description</td>
</tr>
<tr>
<td>62 interpro_short_description</td>
<td>Interpro Short Description</td>
</tr>
<tr>
<td>65 mim_gene_description</td>
<td>MIM Gene Description</td>
</tr>
<tr>
<td>67 mim_morbid_description</td>
<td>MIM Morbid Description</td>
</tr>
<tr>
<td>302 homologs_description</td>
<td>&lt;NA&gt;</td>
</tr>
<tr>
<td>543 sequence_description</td>
<td>&lt;NA&gt;</td>
</tr>
<tr>
<td>582 snp_description</td>
<td>&lt;NA&gt;</td>
</tr>
<tr>
<td>610 structure_description</td>
<td>&lt;NA&gt;</td>
</tr>
</tbody>
</table>

> attrbuts[grep("symbol", attrbuts[,1]),]

<table>
<thead>
<tr>
<th>name</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>55 hgnc_symbol</td>
<td>HGNC symbol</td>
</tr>
</tbody>
</table>

> attrbuts[grep("go", attrbuts[,1]),]

<table>
<thead>
<tr>
<th>name</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>44 go_biological_process_id</td>
<td>GO ID</td>
</tr>
<tr>
<td>45 go_biological_process_linkage_type</td>
<td>Linkage type</td>
</tr>
<tr>
<td>46 go_cellular_component_id</td>
<td>GO ID</td>
</tr>
<tr>
<td>47 go_cellular_component_linkage_type</td>
<td>Linkage type</td>
</tr>
<tr>
<td>48 go_molecular_function_id</td>
<td>GO ID</td>
</tr>
<tr>
<td>49 go_molecular_function_linkage_type</td>
<td>Linkage type</td>
</tr>
</tbody>
</table>

> attrbuts[grep("chrom", attrbuts[,1]),]

<table>
<thead>
<tr>
<th>name</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>26 chromosome_name</td>
<td>Chromosome Name</td>
</tr>
<tr>
<td>573 chromosome_location</td>
<td>Chromosome Location (bp)</td>
</tr>
</tbody>
</table>

We want "description", "hgnc_symbol", "go_biological_process_id" and "band"

```r
eannotlist <- getBM(attributes = c("description", "hgnc_symbol", "go_biological_process_id", "band"),
                    filter = "affy_hg_u95av2", values = igenes, mart = mart,
                    output = "list", na.value = "&nbsp;")
```
> lapply(annotlist, function(x) x[5:10])

$description
$description$'31489_at'
[1] "&nbsp;"

$description$'31490_at'
[1] "Sodium channel protein type 5 subunit alpha (Sodium channel"
[2] "protein type V subunit alpha) (Voltage-gated sodium channel"
[3] "subunit alpha Nav1.5) (Sodium channel protein cardiac muscle"

$description$'31491_s_at'
[1] "Caspase-8 precursor (EC 3.4.22.61) (CASP-8) (ICE-like"
[2] "apoptotic protease 5) (MORT1-associated CED-3 homolog) (MACH)"
[3] "(FADD-homologous ICE/CED-3-like protease) (FADD-like ICE)"
[4] "(FLICE) (Apoptotic cysteine protease) (Apoptotic protease"
[5] "Mch-5) (CAP4) [Contains [Source:Uniprot/SWISSPROT;Acc:Q14790]"

$description$'31492_at'
[1] "Eukaryotic translation initiation factor 3 subunit K"
[2] "(Eukaryotic translation initiation factor 3 subunit 12) (eIF-3"

$description$'31493_s_at'
[1] "chorionic somatomammatropin hormone 2 isoform 3"
[2] "[Source:RefSeq_peptide;Acc:NP_072171]"
[3] "Chorionic somatomammatropin hormone precursor"
[4] "(Choriomammotropin) (Lactogen)."
[6] "Chorionic somatomammatropin hormone-like 1 precursor"
[7] "(Chorionic somatomammatropin-like) (Lactogen-like)."
[8] "[Source:Uniprot/SWISSPROT;Acc:Q14406]"

$description$'31494_at'
[1] "&nbsp;"

$hgnc_symbol

9
Now we have the annotation data, it is time to add in the experimental data. As an example, we will only use the first ten samples. We also use the `round` function to truncate the data to a reasonable number of decimal points.

```r
> dat <- round(exprs(sample.ExpressionSet)[igenes, 1:10], 3)
> FC <- round(rowMeans(dat[igenes, 1:5]) - rowMeans(dat[igenes, 6:10]), 2)
> pval <- round(esApply(sample.ExpressionSet[igenes, 1:10], 1,
+ function(x) t.test(x[1:5], x[6:10])$p.value), 3)
> tstat <- round(esApply(sample.ExpressionSet[igenes, 1:10], 1,
+ function(x) t.test(x[1:5], x[6:10])$statistic), 2)
```

We now need to put all this into one list.

```r
> othernames <- vector("list", length = 8)
> othernames[1:4] <- annotlist
> othernames[5:8] <- list( tstat, pval, FC, dat)
```

## 5 Build the Table

Once we have all our data in lists, it is simple to build the HTML table.
Let's start by defining the columns we need for our HTML table.

```r
> table.head <- c("Affy ID", "Entrez Gene", "SwissProt", "RefSeq",
+                  "Name", "Symbol", "GO Term", "Band",
+                  "t-statistic", "p-value", "Fold change",
+                  sampleNames(sample.ExpressionSet)[1:10])
```

Next, we'll create a list containing our repository names.

```r
> repository <- list("affy", "en", "sp", "gb")
```

Now, we can generate the HTML table using `htmlpage`.

```r
> htmlpage(genelist, "Annotated genes.html", "Annotated genes", othernames, table.head,
+          repository = repository)
```

The resulting HTML table should be in `R_HOME/library/annotate/doc`. If not, you can reproduce it using the `vExplorer` function in the `tkWidgets` package, which will allow you to step through the code in this vignette and examine all the objects that are made. Alternatively, one could use `getwd` to change the working directory to `R_HOME/library/annotate/doc`, then use `Stangle` on the vignette and then source the resulting R code (e.g., `Stangle("prettyOutput.Rnw")` followed by `source("prettyOutput.R")`).

## 6 Session Information

The version number of R and packages loaded for generating the vignette were:

```
R version 2.7.1 (2008-06-23)
x86_64-unknown-linux-gnu

locale:
LC_CTYPE=en_US;LC_NUMERIC=C;LC_TIME=en_US;LC_COLLATE=en_US;LC_MONETARY=C;LC_MESSAGES=en_US;LC_PAPER=en_US;LC_NAME=C;LC_ADDRESS=C;LC_TELEPHONE=C;LC_MEASUREMENT=en_US;LC_IDENTIFICATION=C

attached base packages:
[1] tools stats graphics grDevices utils datasets
[7] methods base

other attached packages:
[1] biomaRt_1.14.1 R Curl_0.9-4 annotate_1.18.0
[4] xtable_1.5-2 AnnotationDbi_1.2.2 RSQLite_0.6-9
[7] DBI_0.2-4 Biobase_2.0.1

loaded via a namespace (and not attached):
[1] XML_1.96-0
```