

# HowTo: get pretty HTML output for my gene list

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## 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>.

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.

```
> attrbuts <- listAttributes(mart)
> attrbuts[grep("swiss", attrbuts[,1]),]

      name                description
89      uniprot_swissprot      UniProt/Swiss-Prot ID
90 uniprot_swissprot_accession UniProt/Swiss-Prot Accession

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

      name      description
32 entrezgene EntrezGene ID
```

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.

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

      name                description
1      affy_hc_g110      Affy hc g 110 ID(s)
2      affy_hg_focus      Affy hg focus ID(s)
3      affy_hg_u133_plus_2 Affy hg u133 plus 2 ID(s)
4      affy_hg_u133a      Affy hg u133a ID(s)
```

5	affy_hg_u133a_2	Affy hg u133a 2 ID(s)
6	affy_hg_u133b	Affy hg u133b ID(s)
7	affy_hg_u95a	Affy hg u95a ID(s)
8	affy_hg_u95av2	Affy hg u95av2 ID(s)
9	affy_hg_u95b	Affy hg u95b ID(s)
10	affy_hg_u95c	Affy hg u95c ID(s)
11	affy_hg_u95d	Affy hg u95d ID(s)
12	affy_hg_u95e	Affy hg u95e ID(s)
13	affy_hugene1	Affy hugene1 ID(s)
14	affy_u133_x3p	Affy u133 x3p ID(s)
93	with_affy_hc_g110	with affy HC G110 ID(s)
94	with_affy_hg_focus	with affy hg focus ID(s)
95	with_affy_hg_u133_plus_2	with affy hg u133 plus 2 ID(s)
96	with_affy_hg_u133a	with affy hg u133a ID(s)
97	with_affy_hg_u133a_2	with affy hg u133a 2 ID(s)
98	with_affy_hg_u133b	with affy hg u133b ID(s)
99	with_affy_hg_u95a	with affy hg u95a ID(s)
100	with_affy_hg_u95av2	with affy hg u95av2 ID(s)
101	with_affy_hg_u95b	with affy hg u95b ID(s)
102	with_affy_hg_u95c	with affy hg u95c ID(s)
103	with_affy_hg_u95d	with affy hg u95d ID(s)
104	with_affy_hg_u95e	with affy hg u95e ID(s)
105	with_affy_hugene1	with affy hugene1 ID(s)
106	with_affy_u133_x3p	with affy u133 x3p 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.

```

> genelist <- getBM(attributes = c("affy_hg_u95av2", "entrezgene", "uniprot_swissprot_a
+                               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])

$affy_hg_u95av2
[1] "31489_at" "31490_at" "31491_s_at" "31492_at" "31493_s_at"
[6] "31494_at"

$entrezgene
$entrezgene$`31489_at`
[1] "&nbsp;"

$entrezgene$`31490_at`
[1] 6331 650400 731231

$entrezgene$`31491_s_at`
[1] 841

$entrezgene$`31492_at`
[1] 27335

$entrezgene$`31493_s_at`
[1] 1443 1442 1444

$entrezgene$`31494_at`
[1] "&nbsp;"

$uniprot_swissprot_accession
$uniprot_swissprot_accession$`31489_at`
[1] "&nbsp;"

$uniprot_swissprot_accession$`31490_at`
[1] "Q14524"

$uniprot_swissprot_accession$`31491_s_at`
[1] "Q14790"

```

```

$uniprot_swissprot_accession$`31492_at`
[1] "Q9UBQ5"

$uniprot_swissprot_accession$`31493_s_at`
[1] "P01243"

$uniprot_swissprot_accession$`31494_at`
[1] "&nbsp;"

$refseq_dna
$refseq_dna$`31489_at`
[1] "&nbsp;"

$refseq_dna$`31490_at`
[1] "NM_000335"      "NM_198056"      "NM_001099404"

$refseq_dna$`31491_s_at`
[1] "NM_001080125" "NM_001228"      "NM_001080124" "NM_033355"
[5] "NM_033356"      "NM_033358"

$refseq_dna$`31492_at`
[1] "NM_013234"

$refseq_dna$`31493_s_at`
[1] "NM_020991" "NM_022644" "NM_001317" "NM_022641" "NM_022640"
[6] "NM_022579" "NM_022580" "NM_001318"

$refseq_dna$`31494_at`
[1] "&nbsp;"

```

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.

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

	name	description
22	description	Description
37	family_description	Family Description
46	go_description	GO description
54	interpro_description	Interpro Description
55	interpro_short_description	Interpro Short Description
336	homologs_description	<NA>
573	sequence_description	<NA>
615	snp_description	<NA>
639	structure_description	<NA>

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

	name	description
48	hgnc_symbol	HGNC symbol

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

	name	description
45	go	GO ID
46	go_description	GO description

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

	name	description
20	chromosome_name	Chromosome Name
603	chromosome_location	Chromosome Location (bp)

We want “description”, “hgnc\_symbol”, “go\_description” and “band”

```

> annotlist <- getBM(attributes = c("description", "hgnc_symbol", "go_description", "
+                               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"
[4] "alpha-subunit) (HH1). [Source:Uniprot/SWISSPROT;Acc:Q14524]"

$description$`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 12 (eIF-3"
[2] "p25) (eIF-3 p28) (eIF3k) (Muscle-specific gene M9 protein)"
[3] "(PLAC-24). [Source:Uniprot/SWISSPROT;Acc:Q9UBQ5]"

$description$`31493_s_at`
[1] "chorionic somatomammotropin hormone 2 isoform 3"
[2] "[Source:RefSeq_peptide;Acc:NP_072171]"
[3] "Chorionic somatomammotropin hormone precursor"
[4] "(Choriomammotropin) (Lactogen).]"
[5] "[Source:Uniprot/SWISSPROT;Acc:P01243]"
[6] "chorionic somatomammotropin hormone-like 1 isoform 3"
[7] "[Source:RefSeq_peptide;Acc:NP_001309]"

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

```



```

$hgnc_symbol
$hgnc_symbol$`31489_at`
[1] "&nbsp;"

$hgnc_symbol$`31490_at`
[1] "SCN5A"

$hgnc_symbol$`31491_s_at`
[1] "CASP8"

$hgnc_symbol$`31492_at`
[1] "EIF3K"

$hgnc_symbol$`31493_s_at`
[1] "CSH2" "CSHL1"

$hgnc_symbol$`31494_at`
[1] "&nbsp;"

$go_description
$go_description$`31489_at`
[1] "&nbsp;"

$go_description$`31490_at`
[1] "voltage-gated sodium channel complex"
[2] "ion channel activity"
[3] "voltage-gated ion channel activity"
[4] "voltage-gated calcium channel activity"
[5] "voltage-gated sodium channel activity"
[6] "voltage-gated potassium channel activity"
[7] "membrane fraction"
[8] "voltage-gated calcium channel complex"
[9] "ion transport"
[10] "potassium ion transport"
[11] "sodium ion transport"
[12] "calcium ion transport"
[13] "muscle contraction"
[14] "circulation"

```

- [15] "regulation of heart contraction"
- [16] "voltage-gated potassium channel complex"
- [17] "membrane"
- [18] "integral to membrane"
- [19] "sodium ion binding"

\$go\_description\$`31491\_s\_at`

- [1] "signal transducer activity"
- [2] "protein binding"
- [3] "cytoplasm"
- [4] "mitochondrion"
- [5] "cytoskeleton"
- [6] "proteolysis"
- [7] "cysteine-type peptidase activity"
- [8] "apoptotic program"
- [9] "caspase activity"
- [10] "identical protein binding"
- [11] "regulation of apoptosis"
- [12] "positive regulation of I-kappaB kinase/NF-kappaB cascade"
- [13] "angiogenesis"
- [14] "neural tube formation"
- [15] "nucleus"
- [16] "heart development"
- [17] "macrophage differentiation"
- [18] "Noc1p-Noc2p complex"

\$go\_description\$`31492\_at`

- [1] "translation initiation factor activity"
- [2] "protein binding"
- [3] "nucleus"
- [4] "cytoplasm"
- [5] "translational initiation"

\$go\_description\$`31493\_s\_at`

- [1] "hormone activity"
- [2] "extracellular region"
- [3] "serine carboxypeptidase activity"
- [4] "growth hormone receptor binding"
- [5] "extracellular space"
- [6] "proteolysis"

```
[7] "signal transduction"
[8] "cell-cell signaling"
[9] "female pregnancy"
```

```
$go_description$`31494_at`
[1] "&nbsp;"
```

```
[[4]]
[[4]]$`31489_at`
[1] "&nbsp;"
```

```
[[4]]$`31490_at`
[1] "p22.2"
```

```
[[4]]$`31491_s_at`
[1] "q33.1"
```

```
[[4]]$`31492_at`
[1] "q13.2"
```

```
[[4]]$`31493_s_at`
[1] "q23.3"
```

```
[[4]]$`31494_at`
[1] "&nbsp;"
```

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.

```
> 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.

```
> 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.

```
> 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])
> repository <- list("affy", "en", "sp", "gb")
> 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 2.6.0 (2007-10-03)
i386-pc-mingw32
```

```
locale:
```

```
LC_COLLATE=English_United States.1252;LC_CTYPE=English_United States.1252;LC_MONETARY
```

```
attached base packages:
```

```
[1] tools      stats      graphics  grDevices  utils      datasets
[7] methods    base
```

```
other attached packages:
```

```
[1] biomaRt_1.12.2      RCurl_0.8-1          annotate_1.16.1
[4] xtable_1.5-2        AnnotationDbi_1.0.6  RSQLite_0.6-4.01
[7] DBI_0.2-4           Biobase_1.16.1
```

loaded via a namespace (and not attached):

```
[1] XML_1.93-2.1
```