Practical: Annotations

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1 Gene annotation

1.1 Data packages

Organism-level ('org') packages contain mappings between a central identifier (e.g., Entrez gene ids) and other identifiers (e.g. GenBank or Uniprot accession number, RefSeq id, etc.). The name of an org package is always of the form `org.<Sp>.<id>.db` (e.g. *org.Hs.eg.db*) where `<Sp>` is a 2-letter abbreviation of the organism (e.g. `Hs` for *Homo spaiens*) and `<id>` is an abbreviation (in lower-case) describing the type of central identifier (e.g. `eg` for ENTREZ gene identifiers). The "How to use the '.db' annotation packages" vignette in the *AnnotationDbi* package (org packages are only one type of ".db" annotation packages) is a key reference. The '.db' and most other *Bioconductor* annotation packages are updated every 6 months.

Annotation packages usually contain an object named after the package itself. These objects are collectively called *AnnotationDb* objects, with more specific classes named *OrgDb*, *ChipDb* or *TranscriptDb* objects. Methods that can be applied to these objects include `cols`, `keys`, `keytypes` and `select`. Common operations for retrieving annotations are summarized in Table˜1.

Exercise 1 *This exercise illustrates basic use of the 'select' interface to annotation packages.*

a. *What is the name of the org package for* Homo sapiens*? Load it. Display the OrgDb object for the org.Hs.eg.db package. Use the `keytypes` and `columns` methods to discover which sorts of annotations can be queried and extracted.*
b. *Here are some ENTREZID values.*

```
egids <- c("3183", "91828", "81537", "4776", "283624", "4053", "85446",
           "10484", "55701", "1112")
```

These are the most strongly differentially expressed genes from a subset of an RNA-seq differential expression analysis that you will encounter later in the course. The biological background is provided in [1]; see the ArrayExpress entry for E-MTAB-1147. The data are from chromosome 14 only
Use the ENTREZIDs in the `select` method in such a way that you extract the SYMBOL (gene symbol) and GENENAME information for each. To what extent do the differentially expressed genes make biological sense?

Table 1: Common operations for retrieving and manipulating annotations.

Category	Function	Description
Discover	`columns`	List the kinds of columns that can be returned
	`keytypes`	List columns that can be used as keys
	`keys`	List values that can be expected for a given keytype
	`select`	Retrieve annotations matching `keys`, `keytype` and `columns`
Manipulate	`setdiff`, `union`, `intersect`	Operations on sets
	`duplicated`, `unique`	Mark or remove duplicates
	`%in%`, `match`	Find matches
	`any`, `all`	Are any `TRUE`? Are all?
	`merge`	Combine two different `data.frames` based on shared keys
*GRanges**	`transcripts`, `exons`, `cds`	Features (transcripts, exons, coding sequence) as *GRanges*.
	`transcriptsBy` , `exonsBy` `cdsBy`	Features group by gene, transcript, etc., as *GRangesList*.

Solution: The 'org' package for humans (*Homo sapiens*) is *org.Hs.eg.db*. Load the *org.Hs.eg.db* package.

```
library(org.Hs.eg.db)
```

Discover the key types and columns in the annotation package.

```
keytypes(org.Hs.eg.db)
```

```
##  [1] "ENTREZID"     "PFAM"         "IPI"          "PROSITE"      "ACCNUM"
##  [6] "ALIAS"        "CHR"          "CHRLOC"       "CHRLOCEND"    "ENZYME"
## [11] "MAP"          "PATH"         "PMID"         "REFSEQ"       "SYMBOL"
## [16] "UNIGENE"      "ENSEMBL"      "ENSEMBLPROT"  "ENSEMBLTRANS" "GENENAME"
## [21] "UNIPROT"      "GO"           "EVIDENCE"     "ONTOLOGY"     "GOALL"
## [26] "EVIDENCEALL"  "ONTOLOGYALL"  "OMIM"         "UCSCKG"
```

```
columns(org.Hs.eg.db)
```

```
##  [1] "ENTREZID"     "PFAM"         "IPI"          "PROSITE"      "ACCNUM"
##  [6] "ALIAS"        "CHR"          "CHRLOC"       "CHRLOCEND"    "ENZYME"
## [11] "MAP"          "PATH"         "PMID"         "REFSEQ"       "SYMBOL"
## [16] "UNIGENE"      "ENSEMBL"      "ENSEMBLPROT"  "ENSEMBLTRANS" "GENENAME"
## [21] "UNIPROT"      "GO"           "EVIDENCE"     "ONTOLOGY"     "GOALL"
## [26] "EVIDENCEALL"  "ONTOLOGYALL"  "OMIM"         "UCSCKG"
```

```
cols <- c("SYMBOL", "GENENAME")
select(org.Hs.eg.db, keys=egids, columns=cols, keytype="ENTREZID")
```

```
##    ENTREZID    SYMBOL
## 1      3183    HNRNPC
## 2     91828   EXOC3L4
## 3     81537     SGPP1
## 4      4776    NFATC4
## 5    283624 LINC00641
## 6      4053     LTBP2
## 7     85446     ZFHX2
## 8     10484    SEC23A
## 9     55701  ARHGEF40
## 10     1112     FOXN3
##                                                        GENENAME
## 1                        heterogeneous nuclear ribonucleoprotein C (C1/C2)
## 2                                     exocyst complex component 3-like 4
```

```
## 3                                      sphingosine-1-phosphate phosphatase 1
## 4  nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4
## 5                                  long intergenic non-protein coding RNA 641
## 6                   latent transforming growth factor beta binding protein 2
## 7                                                    zinc finger homeobox 2
## 8                                         Sec23 homolog A (S. cerevisiae)
## 9                            Rho guanine nucleotide exchange factor (GEF) 40
## 10                                                        forkhead box N3
```

Exercise 2 *This exercise annotates a larger selection of the differential expression results, merging the statistics of differential expression with annotation.*

a. *Read the comma-separate value file* `E-MTAB-1147-toptable.csv` *in to R using* `read.csv`*; include the argument* `row.names=1` *to name the rows of the data (these are ENTREZIDs). Perform basic R operations to discover the object dimensions, and to view the head (first 6 rows) of the data frame. The columns of this data frame are statistics of differential representation, to be discussed later in the course.*
b. *Use* `select` *to map* all *ENTREZIDs (*`rownames`*) to their CHR (chromosome), SYMBOL and GENENAME. Verify that in fact all genes are located on chromosome 14.*
c. *Use* `merge` *to add the SYMBOL and GENENAME annotations to the differential expression statistics.*
d. *Print out the 10 rows of the merged data frame with highest absolute log fold change. To do this, you'll need to (a) take the absolute value of the log fold change column; (b) determine the order, decreasing from largest to smallest, of the absolute values; (c) select the rows of the merged data frame in decreasing order of fold change; and (d) select, using* `head`*, just the first 10 rows of the ordered data frame.*

Solution:

a. Find the file `E-MTAB-1147-toptable.csv` of differentially expressed genes.

```
fl <- file.choose()
```

Verify that the file exists!

```
file.exists(fl)
## [1] TRUE
```

Input the file, specifying that the the first column should be used for row names; look at basic properties of the data.

```
csv <- read.csv(fl, row.names=1)
class(csv)                              # data.frame
## [1] "data.frame"
dim(csv)                                # 528 genes x 6 columns
## [1] 528   6
head(csv)                               # log (base 2) fold change, # adjusted P-values
##        baseMean log2FoldChange  lfcSE    stat     pvalue       padj
## 3183    1432.40         -4.523 0.1426 -31.710 1.114e-220 4.992e-218
## 91828     58.28         -3.478 0.3757  -9.259  2.071e-20  8.434e-19
## 81537   1106.57         -2.758 0.1301 -21.195  1.061e-99  2.377e-97
## 4776      97.46         -2.246 0.2557  -8.785  1.568e-18  4.943e-17
## 283624    29.54         -2.240 0.4058  -5.520  3.394e-08  4.344e-07
## 4053     135.52         -2.114 0.2408  -8.779  1.655e-18  4.943e-17
```

b. Select CHR, SYMBOL, and GENENAME annotations for each ENTREZID, and verify that all genes are from CHR 14.

```
cols <- c("CHR", "SYMBOL", "GENENAME")
anno <- select(org.Hs.eg.db, rownames(csv), cols)
class(anno)
```

```
## [1] "data.frame"
dim(anno)
## [1] 528   4
head(anno)
##   ENTREZID CHR    SYMBOL
## 1     3183  14    HNRNPC
## 2    91828  14   EXOC3L4
## 3    81537  14     SGPP1
## 4     4776  14    NFATC4
## 5   283624  14 LINC00641
## 6     4053  14     LTBP2
##                                                                GENENAME
## 1                        heterogeneous nuclear ribonucleoprotein C (C1/C2)
## 2                                     exocyst complex component 3-like 4
## 3                                  sphingosine-1-phosphate phosphatase 1
## 4 nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4
## 5                                long intergenic non-protein coding RNA 641
## 6                   latent transforming growth factor beta binding protein 2
all(anno$CHR %in% "14")                 # all on CHR 14?
## [1] TRUE
```

c. Merge the differential expression and annotation data frames, specifying that rows are to be matched based on row names in the first data frame (`by.x=0`) and by the ENTREZID in the second data frame (`by.y="ENTREZID"`). Verify that the result is as expected using standard *R* commands.

```
annotated <- merge(csv, anno, by.x=0, by.y="ENTREZID")
class(annotated)
## [1] "data.frame"
dim(annotated)
## [1] 528  10
head(annotated)
##   Row.names baseMean log2FoldChange  lfcSE   stat    pvalue      padj CHR       SYMBOL
## 1     10001  141.222         0.4709 0.1891  2.490 1.276e-02 2.962e-02  14         MED6
## 2 100128927  236.118        -0.4525 0.1706 -2.652 8.007e-03 2.062e-02  14       ZBTB42
## 3 100129075   31.483         0.3801 0.3206  1.186 2.357e-01 3.418e-01  14     KTN1-AS1
## 4 100129794   21.091        -0.5833 0.4009 -1.455 1.457e-01 2.282e-01  14 SLC25A21-AS1
## 5 100288846    6.135        -1.3658 0.6248 -2.186 2.880e-02 5.865e-02  14 LOC100288846
## 6 100289511   87.824        -1.5688 0.2793 -5.617 1.948e-08 2.645e-07  14 LOC100289511
##                                 GENENAME
## 1              mediator complex subunit 6
## 2 zinc finger and BTB domain containing 42
## 3                    KTN1 antisense RNA 1
## 4                SLC25A21 antisense RNA 1
## 5            uncharacterized LOC100288846
## 6            uncharacterized LOC100289511
```

d. Re-organize the annotated data frame by (a) taking the absolute value of the log fold change column and (b) determine the order, decreasing from largest to smallest, of the absolute values of the fold change. Here is a 'one-liner'; explain it to your neighbor. What is `o`?

```
o <- order(abs(annotated$log2FoldChange), decreasing=TRUE)
```

Finally, order the annotated data frame and view the 10 genes with largest differential expression.

```
annotated[head(o),]
##     Row.names baseMean log2FoldChange  lfcSE    stat     pvalue       padj CHR    SYMBOL
## 216      3183  1432.40         -4.523 0.1426 -31.710 1.114e-220 4.992e-218  14    HNRNPC
```

Table 2: Selected packages querying web-based annotation services.

Package	Description
AnnotationHub	Ensembl, Encode, dbSNP, UCSC data objects
biomaRt	http://biomart.org, Ensembl and other annotations
PSICQUIC	https://code.google.com/p/psicquic.org, protein interactions
uniprot.ws	http://uniprot.org, protein annotations
KEGGREST	http://www.genome.jp/kegg, KEGG pathways
SRAdb	http://www.ncbi.nlm.nih.gov/sra, sequencing experiments.
rtracklayer	http://genome.ucsc.edu, genome tracks.
GEOquery	http://www.ncbi.nlm.nih.gov/geo/, array and other data
ArrayExpress	http://www.ebi.ac.uk/arrayexpress/, array and other data

```
## 502     91828    58.28         -3.478 0.3757  -9.259  2.071e-20  8.434e-19  14   EXOC3L4
## 453     81537  1106.57         -2.758 0.1301 -21.195  1.061e-99  2.377e-97  14     SGPP1
## 261      4776    97.46         -2.246 0.2557  -8.785  1.568e-18  4.943e-17  14    NFATC4
## 195    283624    29.54         -2.240 0.4058  -5.520  3.394e-08  4.344e-07  14 LINC00641
## 242      4053   135.52         -2.114 0.2408  -8.779  1.655e-18  4.943e-17  14     LTBP2
##                                                                              GENENAME
## 216                       heterogeneous nuclear ribonucleoprotein C (C1/C2)
## 502                                    exocyst complex component 3-like 4
## 453                                 sphingosine-1-phosphate phosphatase 1
## 261 nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4
## 195                                long intergenic non-protein coding RNA 641
## 242                 latent transforming growth factor beta binding protein 2
```

Verify that the *most* differentially expressed genes are at the top of your table!

1.2 Internet resources

A short summary of select *Bioconductor* packages enabling web-based queries is in Table˜2.

Using biomaRt The *biomaRt* package offers access to the online biomart resource. this consists of several data base resources, referred to as 'marts'. Each mart allows access to multiple data sets; the *biomaRt* package provides methods for mart and data set discovery, and a standard method `getBM` to retrieve data.

Exercise 3 *warning: This exercise requires INTERNET ACCESS*

a. *Load the biomaRt package and list the available marts. Choose the* ensembl *mart and list the datasets for that mart. Set up a mart to use the* ensembl *mart and the* hsapiens_gene_ensembl *dataset.*
b. *A biomaRt dataset can be accessed via `getBM`. In addition to the mart to be accessed, this function takes filters and attributes as arguments. Use `filterOptions` and `listAttributes` to discover values for these arguments. Call `getBM` using filters and attributes of your choosing.*

Solution:

```
## NEEDS INTERNET ACCESS !!
library(biomaRt)
head(listMarts(), 3)                       ## list the marts
head(listDatasets(useMart("ensembl")), 3) ## mart datasets
ensembl <-                                 ## fully specified mart
```

```
    useMart("ensembl", dataset = "hsapiens_gene_ensembl")

head(listFilters(ensembl), 3)             ## filters
myFilter <- "chromosome_name"
head(filterOptions(myFilter, ensembl), 3) ## return values
myValues <- c("21", "22")
head(listAttributes(ensembl), 3)          ## attributes
myAttributes <- c("ensembl_gene_id","chromosome_name")

## assemble and query the mart
res <- getBM(attributes =  myAttributes, filters =  myFilter,
             values =  myValues, mart = ensembl)
```

Use `head(res)` to see the results.

Exercise 4 *As an optional exercise, annotate the genes that are differentially expressed in the DESeq2 laboratory, e.g., find the `GENENAME` associated with the five most differentially expressed genes. Do these make biological sense? Can you `merge` the annotation results with the 'top table' results to provide a statistically and biologically informative summary?*

Using PSICQUIC PSICQUIC is a really useful effort to provide programmatic access to molecular interaction data bases. The *PSICQUIC* package provides an *R* / *Bioconductor* interace to PSICQUIC.

1. Follow instructions on the PSICQUIC package landing page[1] to install the package.
2. Work through section 2 'Quick Start' of the PSICQUIC vignette[2], discovering documented interactions between Myc and TP53.
3. If interested, explore more of the PSICQUIC vignette. Save yourself typing by using the R script from the package landing page.

2 Genome annotation

There are a diversity of packages and classes available for representing large genomes. Several include:

TxDb.* For transcript and other genome / coordinate annotation.
BSgenome For whole-genome representation. See `available.packages` for pre-packaged genomes, and the vignette 'How to forge a BSgenome data package' in the
Homo.sapiens For integrating *TxDb** and *org.** packages.
SNPlocs.* For model organism SNP locations derived from dbSNP.
`FaFile` (*Rsamtools*) for accessing indexed FASTA files.
SIFT.**, PolyPhen, ensemblVEP Variant effect scores.

2.1 Transcript annotation packages

Genome-centric packages are very useful for annotations involving genomic coordinates. It is straight-forward, for instance, to discover the coordinates of coding sequences in regions of interest, and from these retrieve corresponding DNA or protein coding sequences. Other examples of the types of operations that are easy to perform with genome-centric annotations include defining regions of interest for counting aligned reads in RNA-seq

[1] http://bioconductor.org/packages/release/bioc/html/PSICQUIC.html
[2] http://bioconductor.org/packages/release/bioc/vignettes/PSICQUIC/inst/doc/PSICQUIC.pdf

experiments and retrieving DNA sequences underlying regions of interest in ChIP-seq analysis, e.g., for motif characterization.

Exercise 5 *This exercise uses annotation packages to go from gene identifiers to coding sequences.*

a. *Map from an informal gene SYMBOL, e.g., BRCA1, to ENTREZID gene identifiers using the org.Hs.eg.db package and the `select` function, use the TxDb.Hsapiens.UCSC.hg19.knownGene package and a second map to go from ENTREZID to TXNAME.*
b. *Extract the coding sequence grouped by transcript using the TxDb.Hsapiens.UCSC.hg19.knownGene package and `cdsBy` function; select just those transcripts we are interested in.*
c. *Retrieve the nucleotide sequence from the BSgenome.Hsapiens.UCSC.hg19 package using the function `extractTranscriptsFromGenome`.*
d. *Verify that the coding sequences are all multiples of 3, and translate from nucleotide to amino acid sequence.*

Solution: Map from gene SYMBOL to ENTREZID, and from ENTREZID to TXNAME

```
library(org.Hs.eg.db)
egid <- select(org.Hs.eg.db, "BRCA1", "ENTREZID", "SYMBOL")$ENTREZID
library(TxDb.Hsapiens.UCSC.hg19.knownGene)
txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene
egToTx <- select(txdb, egid, "TXNAME", "GENEID")
```

```
## Warning:  'select' resulted in 1:many mapping between keys and return rows
```

Extract the releveant coding sequence, grouped by transcript

```
cdsByTx <- cdsBy(txdb, "tx", use.names=TRUE)[egToTx$TXNAME]
```

Retrieve the sequence

```
library(BSgenome.Hsapiens.UCSC.hg19)
txx <- extractTranscriptsFromGenome(Hsapiens, cdsByTx)
```

Translate to amino acid sequence

```
all(width(txx) %% 3 == 0)  # sanity check
```

```
## [1] TRUE
```

```
translate(txx)             # amino acid sequence
```

```
##   A AAStringSet instance of length 20
##      width seq
##  [1]   760 MDLSALRVEEVQNVINAMQKILECPICLELIKEPVSTK...MCEAPVVTREWVLDSVALYQCQELDTYLIPQIPHSHY*
##  [2]  1793 MSLQESTRFSQLVEELLKIICAFQLDTGLEYANSYNFA...MCEAPVVTREWVLDSVALYQCQELDTYLIPQIPHSHY*
##  [3]   174 MDAEFVCERTLKYFLGIAGGKWVVSYFWVTQSIKERKM...MCEAPVVTREWVLDSVALYQCQELDTYLIPQIPHSHY*
##  [4]   700 MDLSALRVEEVQNVINAMQKILECPICLELIKEPVSTK...CCYGPFTNMPTGCPPNCGCAARCLDRGQWLPCNWADV*
##  [5]  1817 MLKLLNQKKGPSQCPLCKNDITKRSLQESTRFSQLVEE...MCEAPVVTREWVLDSVALYQCQELDTYLIPQIPHSHY*
##  ...   ... ...
## [16]  1365 MDLSALRVEEVQNVINAMQKILECPICLELIKEPVSTK...SESQGVGLSDKELVSDDEERGTGLEENNQEEQSMDSNL
## [17]  1365 MDLSALRVEEVQNVINAMQKILECPICLELIKEPVSTK...SESQGVGLSDKELVSDDEERGTGLEENNQEEQSMDSNL
## [18]  1318 MLKLLNQKKGPSQCPLCKNDITKRSLQESTRFSQLVEE...SESQGVGLSDKELVSDDEERGTGLEENNQEEQSMDSNL
## [19]  1339 MDLSALRVEEVQNVINAMQKILECPICLELIKEPVSTK...SESQGVGLSDKELVSDDEERGTGLEENNQEEQSMDSNL
## [20]  1069 MNVEKAEFCNKSKQPGLARSQHNRWAGSKETCNDRRTP...SESQGVGLSDKELVSDDEERGTGLEENNQEEQSMDSNL
```

2.2 *rtracklayer*

The *rtracklayer* package allows us to query the UCSC genome browser, as well as providing `import` and `export` functions for common annotation file formats like GFF, GTF, and BED.

Exercise 6 *warning: This exercise requires INTERNET ACCESS*

Here we use rtracklayer to retrieve estrogen receptor binding sites identified across cell lines in the ENCODE project. We focus on binding sites in the vicinity of a particularly interesting region of interest.

a. *Define our region of interest by creating a GRanges instance with appropriate genomic coordinates. Our region corresponds to 10Mb up- and down-stream of a particular gene.*
b. *Create a session for the UCSC genome browser*
c. *Query the UCSC genome browser for ENCODE estrogen receptor ERalpha$_a$ transcription marks; identifying the appropriate track, table, and transcription factor requires biological knowledge and detective work.*
d. *Visualize the location of the binding sites and their scores; annotate the mid-point of the region of interest.*

Solution: Define the region of interest

```
roi <- GRanges("chr10", IRanges(92106877, 112106876, names="ENSG00000099194"))
```

Create a session

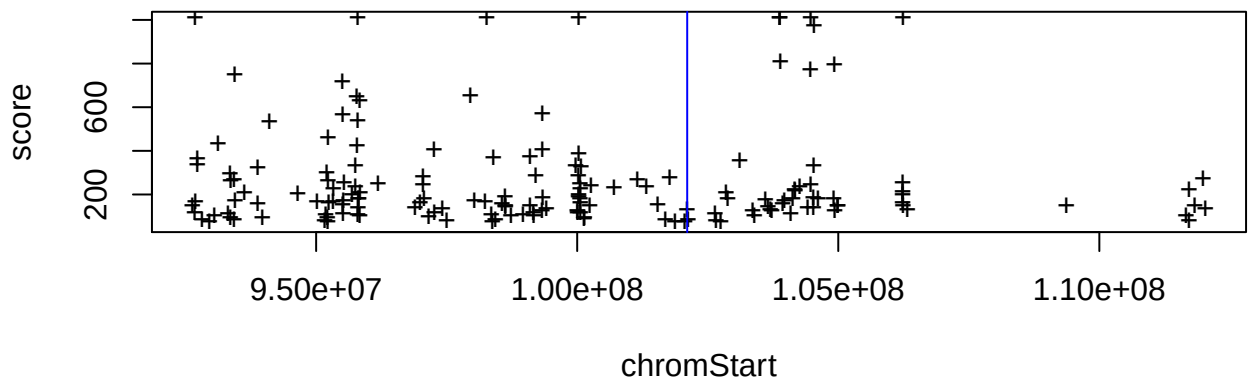
```
library(rtracklayer)
session <- browserSession()
```

Query the UCSC for a particular track, table, and transcription factor, in our region of interest

```
trackName <- "wgEncodeRegTfbsClusteredV2"
tableName <- "wgEncodeRegTfbsClusteredV2"
trFactor <- "ERalpha_a"
ucscTable <- getTable(ucscTableQuery(session, track=trackName,
    range=roi, table=tableName, name=trFactor))
```

Visualize the result

```
plot(score ~ chromStart, ucscTable, pch="+")
abline(v=start(roi) + (end(roi) - start(roi) + 1) / 2, col="blue")
```



References

[1] K.˜Zarnack, J.˜Konig, M.˜Tajnik, I.˜Martincorena, S.˜Eustermann, I.˜Stevant, A.˜Reyes, S.˜Anders, N.˜M. Luscombe, and J.˜Ule. Direct competition between hnRNP C and U2AF65 protects the transcriptome from the exonization of Alu elements. *Cell*, 152(3):453–466, Jan 2013.