



9. Bioconductor: annotation databases

Thomas Lumley
Ken Rice

Universities of Washington and Auckland

Seattle, July 2012

Outline

One goal of Bioconductor is to provide efficient access inside R to the genome databases that are vital to interpreting associations.

We will look at a few of these

- `annotate`
- `biomaRt`
- `genomeGraphs`

The reason to have an R interface to these databases is to be able to analyze annotation data for many SNPs or RNA transcripts.

Online or stored data

Annotation data can be downloaded in a single file or retrieved for each query from an online database.

Local storage is faster, but may require too much space (eg Ensembl) or become obsolete too quickly.

Local storage is ideal for fixed annotation data such as gene names for a microarray or SNP chip.

Types of database

Translations of names: Affy probe 32972_at is the gene **NADPH oxidase 1** with symbol **NOX1** and Ensembl gene id **ENSG00000007952**

Location: NOX1 is on Xq22.1, from 99984969 to 100015990, coded on the negative strand. There are 120 known polymorphisms (SNPs or indels) in this range.

Homology: The mouse version of NOX1 is also on the X chromosome, starting at 130621066.

Structure and function: **NOX1** is a membrane protein (location), involved in voltage-gated ion channel activity (molecular function), and involved in signal transduction (biological process).

Annotate

Bioconductor distributes annotation packages for a wide range of gene expression microarrays. The `annotate` package is one way to use this annotation information.

```
> library("annotate")  
> library("hgu95av2.db")  
> library("GO.db")
```

loads the `annotate` package and the databases for the Gene Ontology and one of the Affymetrix human microarray chips.

Lookups

The databases are queried with `get()` or `mget()` for multiple queries

```
> mget(c("738_at", "40840_at", "32972_at"), envir=hgu95av2GENENAME)
```

```
$'738_at'
```

```
[1] "5'-nucleotidase, cytosolic II"
```

```
$'40840_at'
```

```
[1] "peptidylprolyl isomerase F (cyclophilin F)"
```

```
$'32972_at'
```

```
[1] "NADPH oxidase 1"
```

```
> go<-get("738_at", envir=hgu95av2GO)
```

```
> names(go)
```

```
[1] "GO:0009117" "GO:0005829" "GO:0005737" "GO:0000166" "GO:0000287"
```

```
[6] "GO:0008253" "GO:0008253" "GO:0016787"
```

Lookups

```
> get("GO:0009117", envir=GOTERM)
```

```
GOID: GO:0009117
```

```
Term: nucleotide metabolic process
```

```
Ontology: BP
```

```
Definition: The chemical reactions and pathways involving a  
nucleotide, a nucleoside that is esterified with (ortho)phosphate  
or an oligophosphate at any hydroxyl group on the glucose  
moiety; may be mono-, di- or triphosphate; this definition  
includes cyclic nucleotides (nucleoside cyclic phosphates).
```

```
Synonym: nucleotide metabolism
```

What lookups are there?

```
> library(help="hgu95av2.db")
```

```
hgu95av2ALIAS2PROBE  Map between Common Gene Symbol Identifiers and  
                    Manufacturer Identifiers
```

```
> get("NOX1", envir=hgu95av2ALIAS2PROBE)
```

```
[1] "32972_at"    "32973_s_at"
```

You can also reverse a lookup table with `revmap()`

```
> get("NOX1", envir=revmap(hgu95av2SYMBOL))
```

```
[1] "32972_at"    "32973_s_at"
```

```
> get("X", revmap(hgu95av2CHR))
```

```
[1] "1016_s_at"  "107_at"      "1100_at"     "112_g_at"    "1155_at"
```

```
.... and lots more
```


BioMart

BioMart (www.biomart.org) is a query-oriented data management system developed jointly by the European Bioinformatics Institute (EBI) and Cold Spring Harbor Laboratory (CSHL).

`biomaRt` is an R interface to BioMart systems, in particular to Ensembl (www.ensembl.org). Ensembl is a joint project between EMBL - European Bioinformatics Institute (EBI) and the Wellcome Trust Sanger Institute (WTSI) to develop a software system which produces and maintains automatic annotation on selected eukaryotic genomes.

BioMart

We begin by choosing which BioMart to use

```
> library(biomaRt)
Loading required package: RCurl
> listMarts()

          biomart
1          ensembl          ENSEMBL GENES 63 (SANGER U
2             snp          ENSEMBL VARIATION 63 (SANGER U
3 functional_genomics      ENSEMBL REGULATION 63 (SANGER U
4             vega          VEGA 43 (SANGER U
5 bacteria_mart_10        ENSEMBL BACTERIA 10 (EBI U
6      fungi_mart_10        ENSEMBL FUNGI 10 (EBI U
7 fungi_variations_10      ENSEMBL FUNGI VARIATION 10 (EBI U
8      metazoa_mart_10        ENSEMBL METAZOA 10 (EBI U
9 metazoa_variations_10    ENSEMBL METAZOA VARIATION 10 (EBI U
...
60 ENSEMBL_MART_PLANT        GRAMENE 30 ENSEMBL GENES (CSHL/CORNELL U
61 ENSEMBL_MART_PLANT_SNP    GRAMENE 30 VARIATION (CSHL/CORNELL U
62 GRAMENE_MARKER_30        GRAMENE 30 MARKERS (CSHL/CORNELL U
63 GRAMENE_MAP_30          GRAMENE 30 MAPPINGS (CSHL/CORNELL U
64 QTL_MART                GRAMENE 32 QTL DB (CSHL/CORNELL U
65 salmosalar2_mart        UNIGENE SALMO SALAR DATABASE (CMM CHIL
66 trucha_mart            UNIGENE ONCORHYNCHUS MYKISS DATABASE (CMM CHIL
> ens <- useMart("ensembl")
```

BioMart

We then choose a database to use

```
> listDatasets(ens)
```

	dataset	description
1	oanatinus_gene_ensembl	Ornithorhynchus anatinus genes (OANA5)
2	tguttata_gene_ensembl	Taeniopygia guttata genes (taeGut3.2.4)
3	cporcellus_gene_ensembl	Cavia porcellus genes (cavPor3)
4	gaculeatus_gene_ensembl	Gasterosteus aculeatus genes (BROADS1)
5	lafricana_gene_ensembl	Loxodonta africana genes (loxAfr3)
6	mlucifugus_gene_ensembl	Myotis lucifugus genes (myoLuc2)
7	hsapiens_gene_ensembl	Homo sapiens genes (GRCh37.p3)
...		
26	pvampyrus_gene_ensembl	Pteropus vampyrus genes (pteVam1)
27	mdomestica_gene_ensembl	Monodelphis domestica genes (monDom5)
28	vpacos_gene_ensembl	Vicugna pacos genes (vicPac1)
...		
52	meugenii_gene_ensembl	Macropus eugenii genes (Meug_1.0)
53	cfamiliaris_gene_ensembl	Canis familiaris genes (CanFam_2.0)

```
> hsap <- useDataset("hsapiens_gene_ensembl",mart=ens)
```

BioMart

The `getGene` function queries the database for gene information. It accepts many forms of gene identifier, eg Entrez, HUGO, Affy transcript

```
> getGene(id=1440, type="entrezgene", mart=hsap)
  entrezgene hgnc_symbol
1      1440      CSF3

1 Granulocyte colony-stimulating factor Precursor (G-CSF)(Pluripoietin)
(Filgrastim)(Lenograstim) [Source:UniProtKB/Swiss-Prot;Acc:P09919]
  chromosome_name band strand start_position end_position ensembl_gene_id
1              17 q21.1      1      35425140      35427592 ENSG00000108342

> getGene(id=c("AGT","AGTR1"), type="hgnc_symbol", mart=ens)
  hgnc_symbol hgnc_symbol
1      AGTR1      AGTR1
2      AGT      AGT
1
2 Angiotensinogen Precursor (Serpin A8) [Contains Angiotensin-1(Angiotensin I)
(Ang I);Angiotensin-2(Angiotensin II)(Ang II);Angiotensin-3(Angiotensin III)
(Ang III)(Des-Asp[1]-angiotensin II)] [Source:UniProtKB/Swiss-Prot;Acc:P01019]
  chromosome_name band strand start_position end_position ensembl_gene_id
1              3  q24      1      149898348      149943478 ENSG00000144891
2              1 q42.2     -1      228904897      228916564 ENSG00000135744
```

BioMart

getBM is more general than getGene. It specifies a list of **filters** for selecting genes or SNPs and **attributes** to return from the database.

```
> affyids <- c("202763_at", "209310_s_at", "207500_at")
> getBM(attributes = c("affy_hg_u133_plus_2", "hgnc_symbol", "chromosome_name",
  "start_position", "end_position", "band"), filters = "affy_hg_u133_plus_2",
  values = affyids, mart = hsap)
```

	affy_hg_u133	hgnc	chromosome_name	start_position	end_position	band
1	202763_at	CASP3	4	185785844	185807623	q35.1
2	207500_at	CASP5	11	104370180	104384957	q22.3
3	209310_s_at	CASP4	11	104318804	104344535	q22.3

listAttributes(hsap) and listFilters(hsap) list the available attributes and filters (hundreds)

BioMart

```
> getBM(mart=hsap, attributes=c("band","hgnc_symbol"),
        filters=c("band_start","band_end","chromosome_name"),
        values=list("p21.33","p21.33",6))
```

```
   band hgnc_symbol
1  p21.33
2  p21.33  SNORD117
3  p21.33  SNORA38
4  p21.33  SNORD48
5  p21.33  SNORD52
6  p21.33  MIR877
7  p21.33  MIR1236
8  p21.33  GTF2H4
9  p21.33  VARS2
10 p21.33  SFTA2
11 p21.33  DPCR1
12 p21.33  MUC21
...
121 p21.33  HSPA1A
122 p21.33  TNXB
123 p21.33  STK19
124 p21.33  C4A
125 p21.33  C4B
```

Homology

`getLDS()` combines two data marts, for example to homologous genes in other species. We can look up the mouse equivalents of a particular Affy transcript, or of the NOX1 gene.

```
> human = useMart("ensembl", dataset = "hsapiens_gene_ensembl")
> mouse = useMart("ensembl", dataset = "mmusculus_gene_ensembl")
> getLDS(attributes = c("hgnc_symbol", "chromosome_name", "start_position"),
  filters = "hgnc_symbol", values = "NOX1", mart = human,
  attributesL = c("chromosome_name", "start_position"),
  martL = mouse)
  V1 V2      V3 V4      V5
1 NOX1 X 99984969 X 130621066
```

Homology

The `getSequence` function looks up DNA or protein sequences by chromosome position or gene identifiers

```
> agt<-getSequence(id="AGT",type="hgnc_symbol", seqType="peptide",mart=hsap)
> agt
```

```
1 MRKRAPQSEMAPAGVSLRATILCLLAWAGLAAGDRVYIHPFHLVIHNESTCEQLAKANAGKPKDPTFIPAPIQAKTS
PVDEKALQDQLVLVAAKLDTEDKLRAAMVGMLANFLGFRIYGMHSELWGVVHGATVLSPTAVFGTLASLYLGALDHTAD
RLQAILGVPWKDKNCTSRLDAHKVLSALQAVQGLLVAQGRADSQAQLLLSTVVGVFTAPGLHLKQPFVQGLALYTPVVL
PRSLDFTELDVAAEKIDRFMQAVTGWKTGCSLMGASVDSTLAFNTYVHFQGMKGFSLLAEPQEFWVDNSTS SVSVPMLS
GMGTFQHWSDIQDNFSVTQVPFTESACLLLIQPHYASDLKVEGLTFQQNSLNMKKLSPRTIHLTMPQLVLQGSYDLQ
DLLAQAE LPA I LHTELNLQKLSNDRIRVGEVLNSIFFE LEADEREPTTESTQQLNKPEVLEVT LNRPFLFAVYDQSATAL
HFLGRVANPLSTA*
```


Example: finding chromosomes

We had a set 1524 SNPs, of which 409 did not have their chromosome listed.

I needed to know which SNPs were on the X chromosome, to estimate sex from DNA intensity and heterozygous X-chromosome loci, for QC.

```
> head(unknown)
[1] "UGT1A3-001449-0_B_R_1538822" "LIPC-002761-0_B_R_1538453"
[3] "CETP-001265-0_B_R_1538254"  "F8-165293-0_T_F_1538626"
[5] "CPB2-051208-0_B_F_1539402"  "VDRDIL-1355-0_T_F_1539404"
```

A hand-search would be easy but tedious, so we want an automated approach

Example: finding chromosomes

First extract the gene names

```
genes <- sapply(unknown, function(snp) strsplit(snp, "-")[[1]][1])
ugenes <- unique(genes)
```

Now call out to Ensembl

```
getBM(attributes="chromosome_name", filters="hgnc_symbol", values=ugenes,
      mart=hsap)
```

works for all except VRDIL, which isn't recognized.

Finding SNPs

Human SNPs are in a separate database from gene information. We can look up known SNPs and other polymorphisms for the NOX1 gene

```
> snpmart = useMart("snp", dataset = "hsapiens_snp")
Checking attributes ... ok
Checking filters ... ok
> getBM(c("refsnp_id", "allele", "chrom_start", "chrom_strand"),
        filters = c("chr_name", "chrom_start", "chrom_end"),
        values = list("X", 99984969, 100015990), mart = snpmart)
```

	refsnp_id	allele	chrom_start	chrom_strand
1	rs35477500	AAG/-	99985012	1
2	rs41310727	G/T	99985014	1
3	rs61639376	GAA/-	99985014	1
4	rs34181451	G/A	99985489	1
5	rs34009592	G/A	99985531	1

GenomeGraphs

This package makes pretty pictures from the annotation data.

For example, a pictures showing the standard and alternative splices for the NOX1 gene and the location of the gene on the X chromosome

```
> library(GenomeGraphs)
> gene <- makeGene(id = "NOX1", type = "hgnc_symbol",
  biomart = hsap)
> transcript<-makeTranscript(id="NOX1",type="hgnc_symbol",
  biomart=hsap)
> ideogram <- makeIdeogram(chromosome ="X")
> gdPlot(list(ideogram, gene, transcript))
```

GenomeGraphs

