



8. Bioconductor Intro and Annotation

Ken Rice
Thomas Lumley

Universities of Washington and Auckland

Lausanne, September 2014

What is Bioconductor?

The screenshot shows the Bioconductor website homepage. At the top left is the Bioconductor logo with the tagline "OPEN SOURCE SOFTWARE FOR BIOINFORMATICS". To the right is a search bar and a navigation menu with links for Home, Install, Help, Developers, and About. The main content area is divided into three columns. The left column is titled "About Bioconductor" and describes the software's purpose and development. The middle column is titled "Use Bioconductor for..." and lists three categories: Microarrays, High Throughput Assays, and Sequence Data, each with a brief description of supported data types and analysis tools. The right column is titled "Annotation" and describes the types of annotations supported. Below the main content are three sections: "Mailing Lists" with a "Subscribe" button, "Events" with a calendar icon, and "News" with a speech bubble icon. Each section contains a list of recent updates or announcements with links and timestamps.

Bioconductor
OPEN SOURCE SOFTWARE FOR BIOINFORMATICS

Search:

[Home](#) [Install](#) [Help](#) [Developers](#) [About](#)

About Bioconductor

Bioconductor provides tools for the analysis and comprehension of high-throughput genomic data. Bioconductor uses the R statistical programming language, and is open source and open development. It has two releases each year, more than [460 packages](#), and an active user community.

Use Bioconductor for...

- ➔ **Microarrays**
Import Affymetrix, Illumina, Nimblegen, Agilent, and other platforms. Perform quality assessment, normalization, differential expression, clustering, classification, gene set enrichment, genetical genomics and other workflows for expression, exon, copy number, SNP, methylation and other assays. Access GEO, ArrayExpress, Biomart, UCSC, and other community resources.
- ➔ **High Throughput Assays**
Import, transform, edit, analyze and visualize flow cytometric, mass spec, HTqPCR, cell-based, and other assays.
- ➔ **Sequence Data**
Import fasta, fastq, ELAND, MAQ, BWA, Bowtie, BAM, gff, bed, wig, and other sequence formats. Trim, transform, align, and manipulate sequences. Perform quality assessment, ChIP-seq, differential expression, RNA-seq, and other workflows. Access the Sequence Read Archive.
- ➔ **Annotation**
Use microarray probe, gene, pathway, gene ontology, homology and other annotations. Access GO, KEGG, NCBI, Biomart, UCSC, vendor, and other sources.

Mailing Lists

[Subscribe »](#)

- [Re: views on Rle using GRanges object](#)
about an hour ago
- [How to output Normalised count data f...](#)
about 2 hours ago
- [Re: EBS volumes with the Bioconductor...](#)
about 7 hours ago

Events

- [useR! 2011](#)
16 - 18 August 2011 — University of Warwick, Coventry, UK
- [Statistical Analyses for Next Generation Sequencing](#)
26 - 27 September 2011 — Birmingham, AL, USA

[See all events »](#)

News

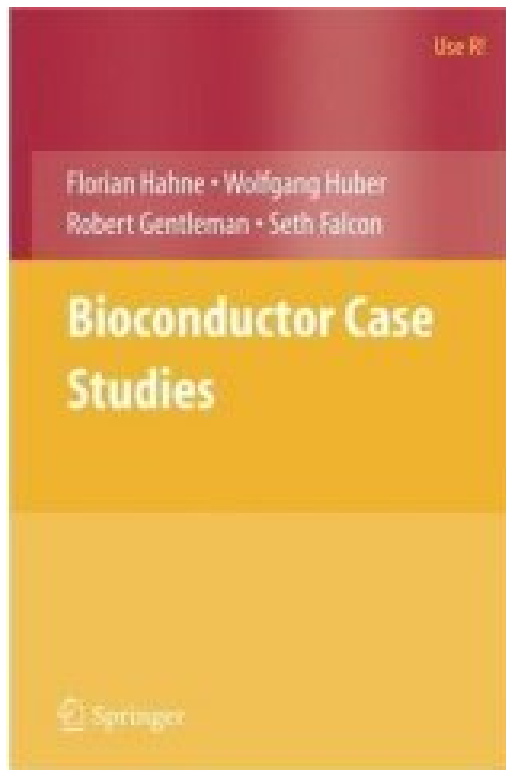
- [BioC 2011 conference material](#)
BioC 2011 conference material is now available.
- [Bioconductor 2.8 released](#)
Following the usual 6-month cycle, the Bioconductor community released Bioconductor 2.8 on April 14th, 2011. This release comprises 466 software packages and more than 500 up-to-date annotation packages. It has been expressly designed to work with R 2.13.

What is Bioconductor?

- `www.bioconductor.org`
- Software project for analysis of genomic data – and related tools, resources/datasets
- **Open source** and **Open development**
- **Free**

You **could** use commercial software; but experts typically write R code first. Also, the help manuals are not a sales pitch and encourage appropriate use.

Bioconductor basics



- Begun in 2001, based at Harvard and now FHCRC (Seattle)
- A large collection of R packages (they also convert good software to R)
- Far too much for our little course!

We'll give examples of what Bioconductor can do, and how to learn more. Hahne et al (above) is a helpful reference text

Bioconductor basics

Getting started...

[Home](#) » [Install](#)

• [Install Packages](#) • [Find Packages](#) • [Update Packages](#) • [Install R](#)

Install Bioconductor Packages

Use the `biocLite.R` script to install Bioconductor packages. To install a particular package, e.g., `limma`, type the following in an R command window:

```
source("http://bioconductor.org/biocLite.R")
biocLite("limma")
```

After downloading and installing this package, the script prints "Installation complete" and "TRUE". Install several packages, e.g., "GenomicFeatures" and "AnnotationDbi", with

```
biocLite(c("GenomicFeatures", "AnnotationDbi"))
```

To install a selection of core Bioconductor packages, use

```
biocLite()
```

Packages and their dependencies installed by this usage are: `affy`, `affydata`, `affyPLM`, `affyQCReport`, `annaffy`, `annotate`, `Biobase`, `biomaRt`, `Biostings`, `DynDoc`, `gcrma`, `genefilter`, `genefilter`, `GenomicRanges`, `hgu95av2.db`, `limma`, `marray`, `multtest`, `vsn`, and `xtable`. After downloading and installing these packages, the script prints "Installation complete" and "TRUE".

The `biocLite.R` script has arguments that change its default behavior:

```
pkgs      Character vector of Bioconductor packages to install.
destdir   File system directory for downloaded packages.
lib       R library where packages are installed.
```

[[Back to top](#)]

Bioconductor Release »

Packages in the stable, semi-annual release:

- [BiocViews](#) package discovery
- [Software](#)
- [Metadata](#) (Annotation, CDF and Probe)
- [Experiment Data](#)

Bioconductor is also available as an [Amazon Machine Image](#) (AMI).

Workflows »

Common Bioconductor workflows include:

- [Oligonucleotide Arrays](#)
- [High-throughput Sequencing](#)
- [Annotation](#)
- [Flow Cytometry](#) and other assays

Previous Versions »

For use with Bioconductor (R):

- [2.7 \(2.12\)](#) • [2.6 \(2.11\)](#) • [2.5 \(2.10\)](#)
- [2.4 \(2.9\)](#) • [2.3 \(2.8\)](#) • [2.2 \(2.7\)](#) • [2.1 \(2.6\)](#) • [2.0 \(2.5\)](#) • [1.9 \(2.4\)](#) • [1.8 \(2.3\)](#)
- [1.7 \(2.2\)](#) • [1.6 \(2.1\)](#)

Bioconductor basics

```
> source("http://bioconductor.org/biocLite.R")  
> biocLite()
```

installs the following general-purpose libraries;

Biobase, IRanges, AnnotationDbi

... then you use e.g. `library("Biobase")` as before. (NB older versions used to download much more than this)

`vignette(package="Biobase")` tells you to look at `vignette("esApply")` for a worked example – a very helpful introduction. (Or use e.g. `openVignette()`, which is in the Biobase package itself)

Bioconductor basics

To get other packages, use the `source()` command as before, then use e.g.

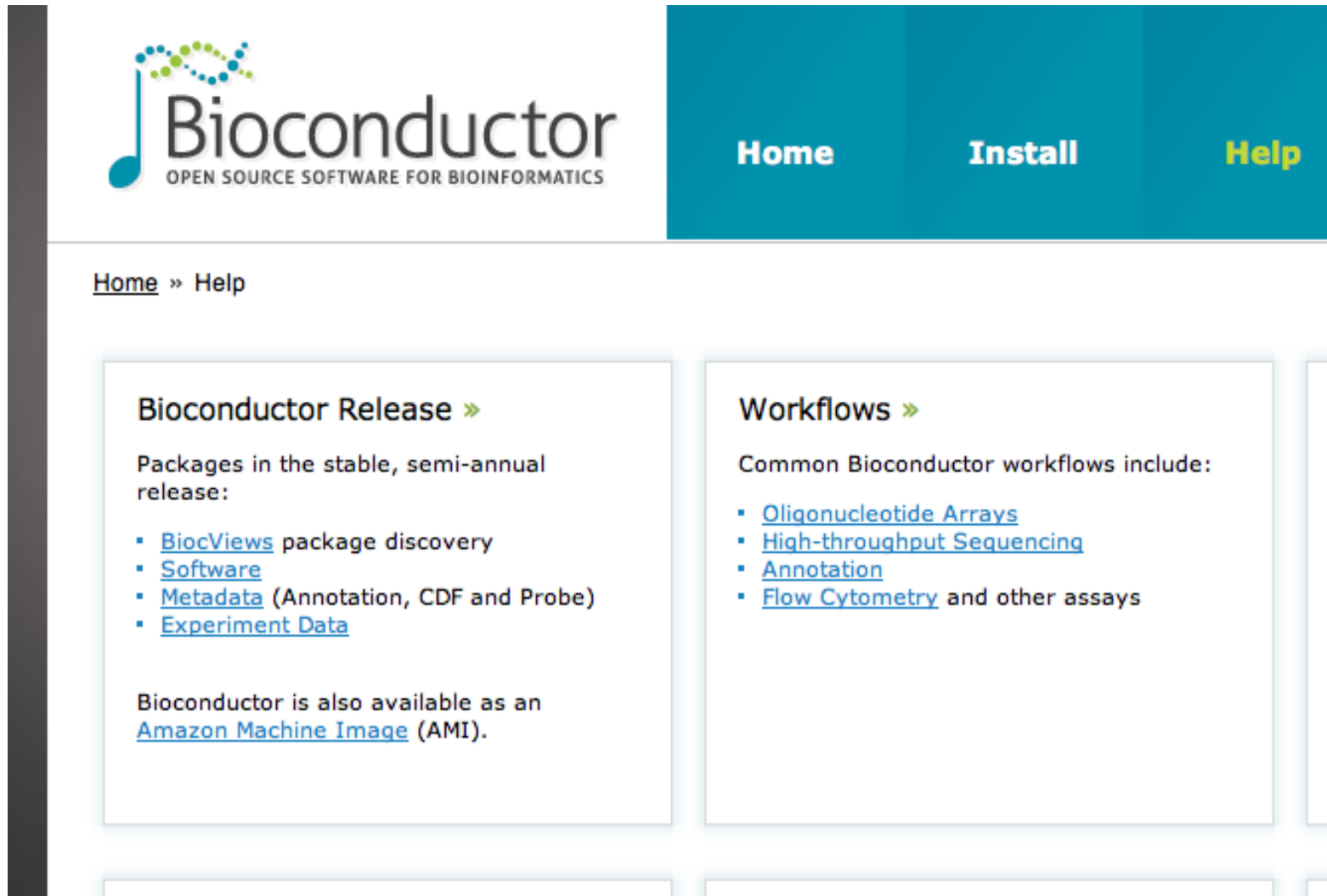
```
biocLite("SNPchip")  
biocLite(c("limma", "siggenes"))
```

You do not need to type `biocLite()` again (even in a new R session). This would install the general-purpose packages again – which is harmless, but a waste of time.

Note; if, due to access privileges, you need to write to non-default directories, follow the onscreen commands and then start again. On Windows, ‘Run as Administrator’ may cut out this step.

What to install?

Back to the front page – click ‘Help’



The screenshot shows the Bioconductor website interface. At the top left is the Bioconductor logo, which consists of a stylized DNA double helix in blue and green above the text "Bioconductor" and "OPEN SOURCE SOFTWARE FOR BIOINFORMATICS". To the right of the logo is a teal navigation bar with three buttons: "Home", "Install", and "Help" (highlighted in yellow). Below the navigation bar, the breadcrumb "Home » Help" is visible. The main content area is divided into two columns. The left column features a section titled "Bioconductor Release »" with the text "Packages in the stable, semi-annual release:" followed by a bulleted list of links: "BiocViews package discovery", "Software", "Metadata (Annotation, CDF and Probe)", and "Experiment Data". Below this list, it states "Bioconductor is also available as an Amazon Machine Image (AMI)". The right column features a section titled "Workflows »" with the text "Common Bioconductor workflows include:" followed by a bulleted list of links: "Oligonucleotide Arrays", "High-throughput Sequencing", "Annotation", and "Flow Cytometry and other assays".

Bioconductor
OPEN SOURCE SOFTWARE FOR BIOINFORMATICS

Home **Install** **Help**

[Home](#) » [Help](#)

Bioconductor Release »

Packages in the stable, semi-annual release:

- [BiocViews](#) package discovery
- [Software](#)
- [Metadata](#) (Annotation, CDF and Probe)
- [Experiment Data](#)

Bioconductor is also available as an [Amazon Machine Image](#) (AMI).

Workflows »

Common Bioconductor workflows include:

- [Oligonucleotide Arrays](#)
- [High-throughput Sequencing](#)
- [Annotation](#)
- [Flow Cytometry](#) and other assays

What to install?

- **Software** – probably what you want
- **Metadata** – e.g. annotation data, probe sequence data for microarrays of different types
- **Experiment data** – e.g. datasets from hapmap.org, some expression datasets

Simple QC graphics

The "splots" package plots values from 96 or 384-well plates, for QC purposes

First, install it

```
biocLite("splots")
```

Then load into R

```
library("splots")
```

There is a single function: `plotScreen()` for displaying the results

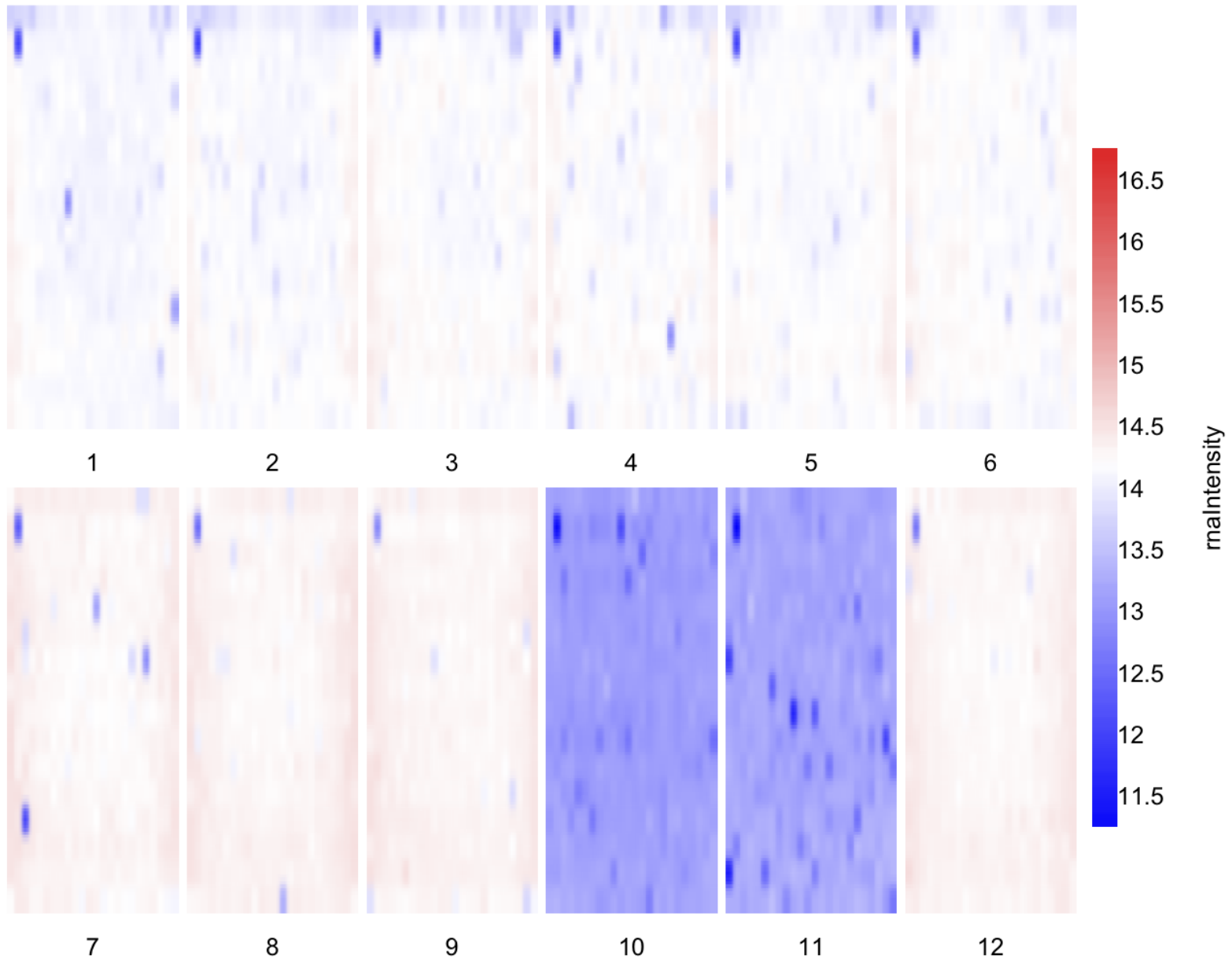
Example

The file "drosophila.rda" contains 12 of 114 plates from a RNAi gene-knockout study in fruit flies. Each spot represents a gene, and the intensity is low if knockout of that gene is lethal (data from the "RNAiR" package)

```
load("drosophila.rda")  
plotScreen(rnai)
```

The positive controls in the same position each plate are clear, and there are obvious plate effects that you might need to correct by normalization.

Example



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 (and called Nox1)

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
      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)
...
30     pvampyrus_gene_ensembl   Pteropus vampyrus genes (pteVam1)
...
58     btaurus_gene_ensembl     Bos taurus genes (UMD3.1)
59     meugenii_gene_ensembl    Macropus eugenii genes (Meug_1.0)
60     sharrisii_gene_ensembl   Sarcophilus harrisii genes (DEVIL7.0)
61     cfamiliaris_gene_ensembl  Canis familiaris genes (CanFam3.1)
> 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
                                     description
1 colony stimulating factor 3 (granulocyte) [Source:HGNC Symbol;Acc:2438]
  chromosome_name  band strand start_position end_position ensembl_gene_id
1                17 q21.1      1       38171614      38174066 ENSG00000108342
```

```
> getGene(id=c("AGT","AGTR1"), type="hgnc_symbol", mart=hsap)
```

```
hgnc_symbol hgnc_symbol
1          AGT          AGT
2          AGTR1        AGTR1
1 angiotensinogen (serpin peptidase inhibitor, clade A, member 8) [Source:HGNC Sym
2                                angiotensin II receptor, type 1 [Source:HGNC Sym
  chromosome_name  band strand start_position end_position ensembl_gene_id
1                1 q42.2      -1       230838269      230850043 ENSG00000135744
2                3  q24       1       148415571      148460795 ENSG00000144891
```

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", "external_gene_id"),
+ martL = mouse)
      V1 V2          V3 V4          V5  V6
1 NOX1  X 100098313  X 134086421 Nox1
```

The mouse gene name is the same as the human one apart from capitalisation.

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
PRSLDFTELDVAAEKIDRFMQAVTGWKTGCSLMGASVDSTLAFNTYVHFQGMKGFSLLAEPQEFWVDNSTSVSVPMLS
GMGTFQHWSDIQDNFSVTQVPFTESACLLLIQPHYASDLKVEGLTFQQNSLNMKKLSPRTIHLTMPQLVLQGSYDLQ
DLLAQAEPAIILHTELNQKLSNDRIRVGEVLNSIFFELEADEREPTTESTQQLNKPEVLEVTLNRPFLFAVYDQSATAL
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 (and short indels) 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	rs7054049	T/A	99985184	1
2	rs60975472	G/T	99985304	1
3	rs58902780	A/G	99985571	1
4	rs182188185	G/A	99985618	1
5	rs186748080	A/G	99985798	1

More metadata

The `citation()` function prints out information about how to cite a package

```
> citation("biomaRt")
```

To cite the `biomaRt` package in publications use:

```
Mapping identifiers for the integration of genomic datasets with the  
R/Bioconductor package biomaRt. Steffen Durinck, Paul T. Spellman,  
Ewan Birney and Wolfgang Huber, Nature Protocols 4, 1184-1191 (2009).
```

```
BioMart and Bioconductor: a powerful link between biological  
databases and microarray data analysis. Steffen Durinck, Yves Moreau,  
Arek Kasprzyk, Sean Davis, Bart De Moor, Alvis Brazma and Wolfgang  
Huber, Bioinformatics 21, 3439-3440 (2005).
```

Citations are one way academic software authors can prove to funders and promotion committees that software is worthwhile.

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

