An Introduction to R and Bioconductor

Robert Gentleman

The R Language

- R is a fully functional programming language and analysis environment for scientific computing
- it contains an essentially complete set of routines for numerical computations, statistical analysis and has extensive graphics capabilities
- computations/algorithms are organized by packages (there are over 3000) and these can easily be downloaded and installed on your computer
- users can create and share their own packages
  - two main repositories are CRAN and Bioconductor
  - packages will contain source code, documentation etc

R Language

- R has a new release once per year with patch releases somewhat more often
  - you should keep your local versions of R and Bioconductor up to date
- you should always use biocLite in the biocInstaller package for Bioconductor packages and install.packages, or update.packages for R
  - this will ensure you have compatible versions of software
- packages contain source code, documentation
  - man pages with examples
  - vignettes: self-contained runnable documents that describe how the code in the package can be used on an analysis problem

Bioconductor

- Bioconductor is an open source and open development software project for the analysis of biomedical and genomic data.
- The project was started in the Fall of 2001 and includes developers in many countries
- R and the R package system are used to design and distribute software.
- A goal of the project is to develop integrated and interoperable software modules to provide comprehensive software solutions to relevant problems.
- we largely achieve that goal by using common data structures

Why are we Open Source

- so that you can find out what algorithm is being used, and how it is being used
- so that you can modify these algorithms to try out new ideas or to accommodate local conditions or needs
- so you can read the code, find bugs, suggest improvements etc.
- so that they can be used as components (potentially modified) in other peoples software

Overview

- biology is a computational science
- problems of data analysis, data generation, reproducibility require computational support and computational solutions
- we value code reuse
  - many of the tasks have already been solved
  - if we use those solutions we can put effort into new research
- well designed, self-describing data structures help us deal with complex data

Goals

- Provide access to powerful statistical and graphical methods for the analysis of genomic data.
- Facilitate the integration of biological metadata (GenBank, GO, Entrez Gene, PubMed) in the analysis of experimental data.
- Allow the rapid development of extensible, interoperable, and scalable software.
- Promote high-quality documentation and reproducible research.
- Provide training in computational and statistical methods.

Bioconductor packages

Release 2.10, 554 Software Packages!

- **General infrastructure**
  `Biobase, Biostrings, biocViews`
- **Annotation:**
  `annotate, annaffy, biomaRt, AnnotationDbi` → data packages.
- **Graphics/GUIs:**
  `geneplotter, hexbin, limmaGUI, exploRase`
- **Pre-processing:**
  `affy, affycomp, oligo, makecdfenv, vsn, gcrm, limma`
- **Differential gene expression:**
  `genefilter, limma, ROC, siggenes, EBArrays, factDesign`
- **GSEA/Hypergeometric Testing**
  `GSEABase, Category, GOstats, topGO`
- **Graphs and networks:**
  `graph, RBGL, Rgraphviz`
- **Flow Cytometry:**
  `flowCore, flowViz, flowUtils`
- **Protein Interactions:**
  `ppiData, ppiStats, ScISI, Rintact`
- **Sequence Data:**
  `Biostrings, ShortRead, rtracklayer, IRanges, GenomicFeatures, VariantAnnotation`
- **Other data:**

Component software

- most interesting problems will require the coordinated application of many different techniques
- thus we need integrated interoperable software
- of primary importance is well designed and shared data structures
- you should design your contributions to be a cog in a big machine

Data complexity

- Dimensionality.
- Dynamic/evolving data: e.g., gene annotation, sequence, literature.
- Multiple data sources and locations: in-house, WWW.
- Multiple data types: numeric, textual, graphical.

No longer X_{nxp}!

We distinguish between biological metadata and experimental metadata.

Experimental metadata

- when were the samples processed and how
- what arrays were used/what kits
- if size selection of some sort (eg. fractionation for proteomics experiments) was used
- date the samples were run
- lane or chip information
- treatments

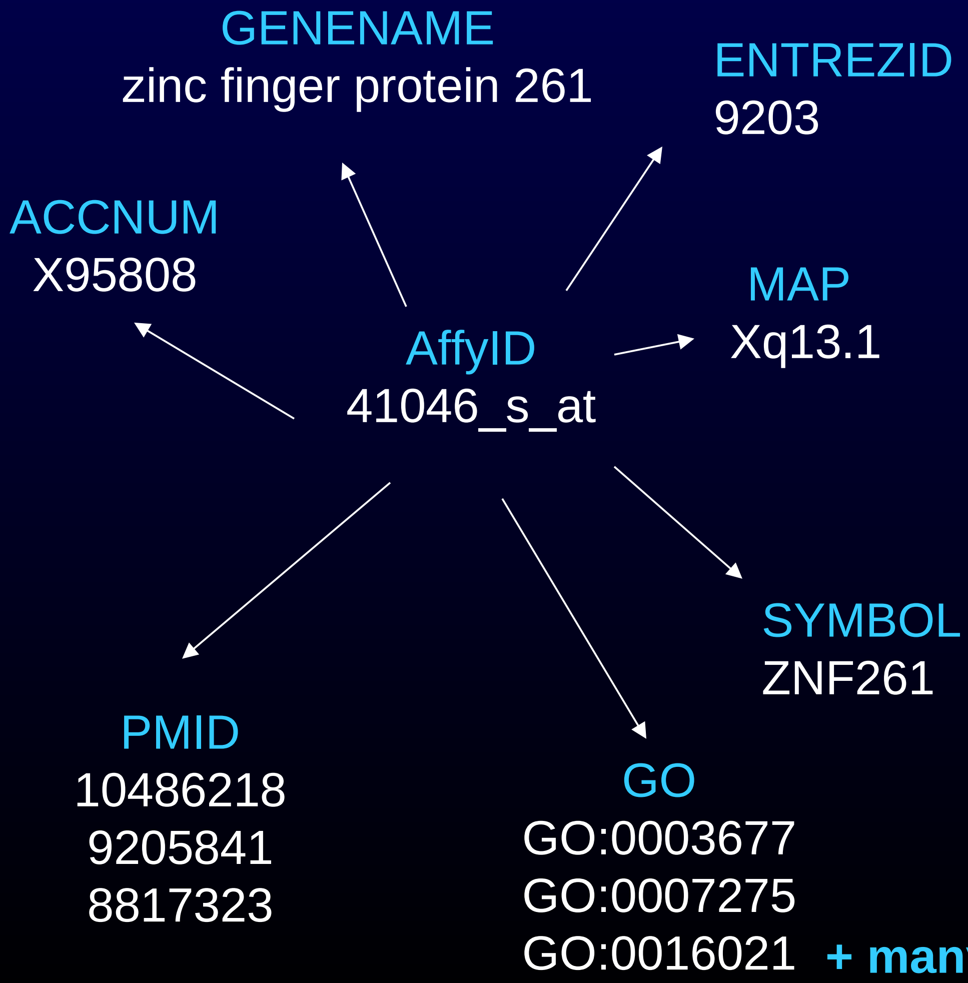
Biological metadata

- **Biological attributes that can be applied to the experimental data.**
- **E.g. for genes**
  - **chromosomal location;**
  - **gene annotation (Entrez Gene, GO);**
  - **gene models**
  - **relevant literature (PubMed)**
- **Biological metadata sets are large, evolving rapidly, and typically distributed via the WWW.**
- **Tools:** `annotate`, `biomaRt`, **and** `AnnotationDbi`, `GenomicFeatures` **packages, and annotation data packages.**

Annotation packages

annotate, annafy, biomaRt, and AnnotationDbi

Metadata package hgu95av2 mappings between different gene IDs for this chip.



+ many other mappings

- **Assemble and process genomic annotation data from public repositories.**
- **Build annotation data packages.**
- **Associate experimental data in real time to biological metadata from web databases such as GenBank, GO, KEGG, Entrez Gene, and PubMed.**
- **Process and store query results: e.g., search PubMed abstracts.**
- **Generate HTML reports of analyses.**

Sequence Annotation

- for a given gene:
  - gene models
  - sequence
  - exon/intron boundaries
  - location
  - conservation
- often in the form of tracks
- it is important to keep track of the reference genome being used

Vignettes

- Bioconductor developed a new documentation paradigm, the vignette.
- A vignette is an executable document consisting of a collection of documentation text and code chunks.
- Vignettes form dynamic, integrated, and reproducible statistical documents that can be automatically updated if either data or analyses are changed.
- Vignettes can be generated using the `Sweave` function from the R `tools` package.

Short Courses/Conferences

- we have given many short courses
  - see bioconductor.org for more details on upcoming courses

- BioC2012 - Seattle, July 24-25
- European Developers' workshop
  - Zurich, 13-14 December, 2012

Bioconductor Software

- concentrate development resources on a few important aspects
- Biobase: core classes and definitions that allow for succinct description and handling of the data
- annotate: generic functions for annotation that can be specialized
- genefilter/limma/DESeq/DEXSeq: differential expression
- ShortRead/IRanges/GenomicFeatures/Variant Annotation: string manipulations, sequence analysis

Quality Assessment

- ensuring that the data are of sufficient quality is an essential first step
- arrayQuality Metrics: comprehensive QA assessment of microarrays (one color or two color)
  - modifications are coming to make it more suitable for sequence data
- ShortRead: tools for QA of short reads, primarily Illumina

Biobase:ExpressionSet

- software should help organize and manipulate your data
- the data need to be assembled correctly once, and then they can be processed, subset etc without worrying about them
- we developed the ExpressionSet class
- SummarizedExperiment class is the next iteration in this process (in the GenomicRanges package)

Microarray data analysis

Pre-processing

CEL, CDF

affy
vsn

.gpr, .Spot

marray
limma
vsn

ExpressionSet

Differential expression

edd
genefilter
limma
multtest
ROC
+ CRAN

Graphs & networks

graph
RBGL
Rgraphviz

Cluster analysis

CRAN
class
cluster
MASS
mva

Prediction

CRAN
class
e1071
ipred
LogitBoost
MASS
nnet
randomForest
rpart

Annotation

annotate
annaffy
biomaRt
+ metadata packages

Graphics

geneplotter
hexbin
+ CRAN

Differential Expression

- limma: provides a linear models interface for DE
  - uses a moderated variance
  - a variety of p-value correction methods are provided
- DESeq and edgeR: for sequence data
  - similar approach to limma
  - make use of count data (Neg Binomial)
- DEXSeq for exon level differential expression

Machine Learning

- In R software for machine learning has been written by many different people
  - the calling sequences and return values are unique to each method
- MLInterfaces
- provides uniform calling sequences and return values for all machine learning algorithms
- MLearn is the main wrapper function
  - methods, eg knni, are passed to the wrapper
- return values are of class MLOutput
- see the MLInterfaces vignette for more details

Publications

- Bioconductor: Open software development for computational biology and bioinformatics, Genome Biology 2004, 5:R80, http://genomebiology.com/2004/5/10/R80
- Bioinformatics and Computational Biology Solutions using R and Bioconductor, Springer, 2005, R. Gentleman, V. Carey, W. Huber, R. Irizarry, S. Dudoit eds.
- Bioconductor Case Studies, Springer
- R Programming for Bioinformatics, Chapman Hall

References

- R www.r-project.org, cran.r-project.org
  - software (CRAN);
  - documentation;
  - newsletter: R News;
  - mailing list.
- Bioconductor www.bioconductor.org
  - software, data, and documentation (vignettes);
  - training materials from short courses;
  - mailing list (please read the posting guide)