# Using the SRAdb Package to Query the Sequence Read Archive

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### 1 Introduction

High throughput sequencing technologies have very rapidly become standard tools in biology. The data that these machines generate are large, extremely rich. As such, the Sequence Read Archives (SRA) have been set up at NCBI in the United States, EMBL in Europe, and DDBJ in Japan to capture these data in public repositories in much the same spirit as MIAME-compliant microarray databases like NCBI GEO and EBI ArrayExpress.

Accessing data in SRA requires finding it first. This R package provides a convenient and powerful framework to do just that. In addition, SRAdb features functionality to determine availability of sequence files and to download files of interest.

SRA currently store aligned reads or other processed data that relies on alignment to a reference genome. Please refer to the SRA handbook (http://www.ncbi.nlm.nih.gov/books/NBK47537/) for details. NCBI GEO also often contain aligned reads for sequencing experiments and the SRAdb package can help to provide links to these data as well. In combination with the GEOmetadb and GEOquery packages, these data are also, then, accessible.

## 2 Getting Started

Since SRA is a continuously growing repository, the SRAdb SQLite file is updated regularly. The first step, then, is to get the SRAdb SQLite file from the online location. The download and uncompress steps are done automatically with a single command, getSRAdbFile.

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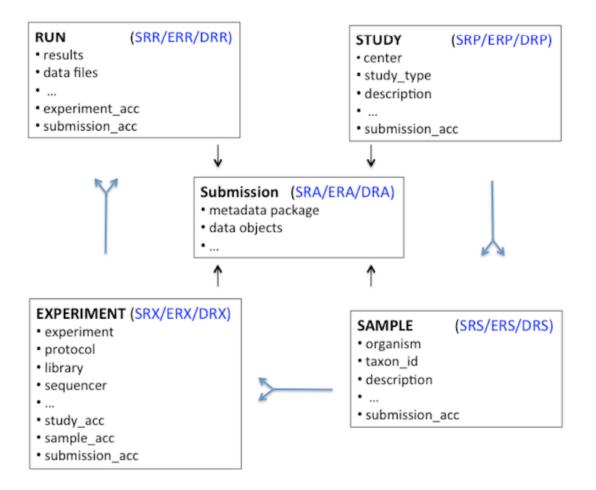


Figure 1: A graphical representation (sometimes called an *Entity-Relationship Diagram*) of the relationships between the main tables in the SRAdb package.

```
> library(SRAdb)
> sqlfile <- 'SRAmetadb.sqlite'
> if(!file.exists('SRAmetadb.sqlite')) sqlfile <<- getSRAdbFile()</pre>
```

The default storage location is in the current working directory and the default filename is "SRAmetadb.sqlite"; it is best to leave the name unchanged unless there is a pressing reason to change it. Note: the above downloading and uncompressing steps could take quite a fews moments due to file size, depdending on your network bandwidth. If interested, it can be timed using the following commands:

```
> timeStart <- proc.time()
> sqlfile <- getSRAdbFile()
> proc.time() - timeStart
```

Since this SQLite file is of key importance in SRAdb, it is perhaps of some interest to know some details about the file itself.

Then, create a connection for later queries. The standard DBI functionality as implemented in RSQLite function dbConnect makes the connection to the database. The dbDisconnect function disconnects the connection.

```
> sra_con <- dbConnect(SQLite(),sqlfile)
For further details, at this time see help('SRAdb-package').</pre>
```

## 3 Using the SRAdb package

### 3.1 Interacting with the database

The functionality covered in this section is covered in much more detail in the DBI and RSQLite package documentation. We cover enough here only to be useful. The dbListTables function lists all the tables in the SQLite database handled by the connection object sra\_con created in the previous section. A simplified illustration of the relationship between the SRA main data types is shown in the Figure 1.

There is also the dbListFields function that can list database fields associated with a table.

#### > dbListFields(sra\_con, "study")

```
[1] "study_ID"
                             "study_alias"
 [3] "study_accession"
                             "study_title"
 [5] "study_type"
                             "study_abstract"
 [7] "broker_name"
                             "center_name"
 [9] "center_project_name"
                             "study_description"
[11] "related_studies"
                             "primary_study"
[13] "sra_link"
                             "study_url_link"
[15] "xref_link"
                             "study_entrez_link"
                             "ena_link"
[17] "ddbj_link"
                             "submission_accession"
[19] "study_attribute"
[21] "sradb_updated"
```

Sometimes it is useful to get the actual SQL schema associated with a table. Here, we get the table schema for the study table:

#### > dbGetQuery(sra\_con, 'PRAGMA TABLE\_INFO(study)')

	cid	name	type	notnull
1	0	study_ID	REAL	0
2	1	study_alias	TEXT	0
3	2	study_accession	TEXT	0
4	3	study_title	TEXT	0
5	4	study_type	TEXT	0
6	5	study_abstract	TEXT	0
7	6	broker_name	TEXT	0
8	7	center_name	TEXT	0
9	8	<pre>center_project_name</pre>	TEXT	0
10	9	study_description	TEXT	0

```
11
    10
             related_studies TEXT
                                            0
12
                                            0
    11
               primary_study TEXT
13
    12
                     sra_link TEXT
                                            0
14
    13
              study_url_link TEXT
                                            0
15
    14
                    xref_link TEXT
                                            0
           study_entrez_link TEXT
16
    15
                                            0
17
    16
                                            0
                    ddbj_link TEXT
18
    17
                     ena_link TEXT
                                            0
19
    18
             study_attribute TEXT
                                            0
20
    19 submission_accession TEXT
                                            0
21
    20
                sradb_updated TEXT
                                            0
   dflt_value pk
1
          <NA>
                 0
2
          <NA>
                 0
3
          <NA>
                 0
4
          <NA>
                 0
          < NA >
                 0
5
6
          <NA>
                 0
7
          <NA>
                 0
8
          <NA>
                 0
9
          <NA>
                 0
10
          <NA>
                 0
11
          <NA>
                 0
12
          <NA>
                 0
13
          <NA>
                 0
14
          <NA>
                 0
15
          <NA>
                 0
16
          <NA>
                 0
17
          <NA>
                 0
18
          <NA>
                 0
19
          <NA>
                 0
                 0
20
          <NA>
21
          <NA>
                 0
```

The table "col\_desc" contains information of filed name, type, descritption and default values:

```
4 submission submission_comment
5 submission files
type
1 int
2 varchar
3 varchar
4 text
5 text
```

#### 3.2 Writing SQL queries and getting results

Select 3 records from the *study* table and show the first 5 columns:

Get the SRA study accessions and titles from SRA study that study\_type contains "Transcriptome". The "%" sign is used in combination with the "like" operator to do a "wildcard" search for the term "Transcriptome" with any number of characters after it.

Of course, we can combine programming and data access. A simple sapply example shows how to query each of the tables for number of records.

```
> getTableCounts <- function(tableName,conn) {
+ sql <- sprintf("select count(*) from %s",tableName)</pre>
```

Get some high-level statistics could be to helpful to get overall idea about what data are available in the SRA database. List all study types and number of studies contained for each of the type:

```
> rs <- dbGetQuery(sra_con, paste( "SELECT study_type AS StudyType,
          count( * ) AS Number FROM `study` GROUP BY study_type order
          by Number DESC ", sep=""))
> rs
                  StudyType Number
    Whole Genome Sequencing
1
                              29003
2
                      Other
                              20925
3
     Transcriptome Analysis
                               9575
4
               Metagenomics
                               6092
5
        Population Genomics
                                753
6
                Epigenetics
                                651
7
                       <NA>
                                374
           Exome Sequencing
8
                                181
9
            Cancer Genomics
                                 96
10
   Pooled Clone Sequencing
                                 31
         Synthetic Genomics
                                  9
11
12 Transcriptome Sequencing
                                  1
13 Whole Genome Sequencing
                                  1
```

List all Instrument Models and number of experiments for each of the Instrument Models:

		Instrument Model
1		Illumina HiSeq 2000
2		Illumina MiSeq
3		Illumina HiSeq 2500
4		454 GS FLX Titanium
5		Illumina Genome Analyzer II
6		<pre></pre>
7		Illumina Genome Analyzer IIx
8		unspecified
9		454 GS FLX
10		Illumina Genome Analyzer
11		454 GS Junior
12		Ion Torrent PGM
13		AB SOLiD 4 System
14		Illumina HiSeq 1000
15		454 GS FLX+
16		NextSeq 500
17		PacBio RS II
		PacBio RS
18 19		
		454 GS
20		Helicos HeliScope
21		Complete Genomics
22		Illumina HiSeq 1500
23		AB 5500xl Genetic Analyzer
24		AB SOLID System 3.0
25		HiSeq X Ten
26		AB 5500 Genetic Analyzer
27		Illumina HiScanSQ
28		Ion Torrent Proton
29		454 GS 20
30		AB 3730xL Genetic Analyzer
31		AB SOLiD System 2.0
32		AB SOLiD System
33		MinION
34		Illumina HiSeq 4000
35		AB SOLiD 3 Plus System
36	4.5	AB SOLiD 4hq System
37	AB	5500xl-W Genetic Analysis System
38		Illumina HiSeq 3000
39		AB 3130 Genetic Analyzer
40		AB 3130xL Genetic Analyzer
41		Illumina NextSeq 500

```
42
               AB 3730 Genetic Analyzer
                            NextSeq 550
43
44
                            454 GS FLX
45
            AB 3500xL Genetic Analyzer
46
                     AB SOLiD PI System
47
                           HiSeq X Five
48
         Illumina Genome Analyzer IIx
49
                AB 310 Genetic Analyzer
50
               AB 3500 Genetic Analyzer
   Experiments
1
        878142
2
        197916
3
        141300
4
        109563
5
        100263
6
         78674
7
         50052
8
         36309
9
         30628
10
         17744
11
         14075
12
         13558
13
         10027
14
          9192
15
          8103
16
          8016
17
          6513
18
          5338
19
          4392
20
          3831
21
          3165
22
          2656
23
          2632
24
          2511
25
          1890
26
          1811
27
          1713
28
          1472
           980
29
30
           602
           466
31
32
           426
```

```
33
            350
34
            269
35
            194
36
            158
37
            109
38
             81
             70
39
40
             27
41
             20
42
             19
43
             14
44
             10
              9
45
              2
46
47
              2
48
              2
49
              1
50
              1
```

List all types of library strategies and number of runs for each of them:

	Library Strategy	Runs
1	WGS	579021
2	AMPLICON	297316
3	RNA-Seq	255068
4	WXS	214611
5	OTHER	139515
6	<na></na>	78674
7	POOLCLONE	49541
8	ChIP-Seq	48682
9	SELEX	21657
10	Bisulfite-Seq	12260
11	miRNA-Seq	9660
12	CLONE	8923
13	WGA	7938
14	EST	3461
15	VALIDATION	3284
16	RAD-Seq	1816
17	ncRNA-Seq	1672

```
18
                 MeDIP-Seq
                              1492
19 DNase-Hypersensitivity
                              1486
20
                   FL-cDNA
                              1478
21
                    Tn-Seq
                              1358
22
                 MNase-Seq
                              1330
23
                   RIP-Seq
                              1131
24
                   MBD-Seq
                              1078
25
                   MRE-Seq
                              1051
26
                        WCS
                               513
27
         Targeted-Capture
                               453
28
                  CLONEEND
                               326
29
                        CTS
                                186
30
                 FAIRE-seq
                                174
31
                       Hi-C
                                 78
32
                  ChIA-PET
                                 29
33
                 FINISHING
                                 24
34
      Synthetic-Long-Read
                                 12
```

### 3.3 Conversion of SRA entity types

Large-scale consumers of SRA data might want to convert SRA entity type from one to others, e.g. finding all experiment accessions (SRX, ERX or DRX) and run accessions (SRR, ERR or DRR) associated with "SRP001007" and "SRP000931". Function sraConvert does the conversion with a very fast mapping between entity types.

Covert "SRP001007" and "SRP000931" to other possible types in the SRAmetadb.sqlite:

```
> conversion <- sraConvert( c('SRP001007','SRP000931'), sra_con = sra_con )</pre>
> conversion[1:3,]
      study submission
                           sample experiment
1 SRP000931
             SRA009053 SRS003460
                                   SRX006131
2 SRP000931
             SRA009053 SRS003456
                                   SRX006125
3 SRP000931
             SRA009053 SRS003453
                                   SRX006122
        run
1 SRR018265
2 SRR018259
3 SRR018256
```

Check what SRA types and how many entities for each type:

> apply(conversion, 2, unique)

```
$study
[1] "SRP000931" "SRP001007"
```

```
$submission
[1] "SRA009053" "SRA009276"
$sample
 [1] "SRS003460" "SRS003456" "SRS003453"
 [4] "SRS003458" "SRS003455" "SRS003454"
 [7] "SRS003462" "SRS003461" "SRS003459"
[10] "SRS003457" "SRS003464" "SRS003463"
[13] "SRS004650"
$experiment
 [1] "SRX006131" "SRX006125" "SRX006122"
 [4] "SRX006127" "SRX006124" "SRX006123"
 [7] "SRX006133" "SRX006132" "SRX006129"
[10] "SRX006130" "SRX006128" "SRX006126"
[13] "SRX006135" "SRX006134" "SRX007396"
$run
 [1] "SRR018265" "SRR018259" "SRR018256"
 [4] "SRR018261" "SRR018258" "SRR018257"
 [7] "SRR018267" "SRR018266" "SRR018263"
[10] "SRR018264" "SRR018262" "SRR018260"
[13] "SRR018269" "SRR018268" "SRR020739"
[16] "SRR020740"
```

#### 3.4 Full text search

Searching by regular table and field specific SQL commands can be very powerful and if you are familiar with SQL language and the table structure. If not, SQLite has a very handy module called Full text search (fts3), which allow users to do Google like search with terms and operators. The function getSRA does Full text search against all fields in a fts3 table with terms constructed with the Standard Query Syntax and Enhanced Query Syntax. Please see http://www.sqlite.org/fts3.html for detail.

Find all run and study combined records in which any given fields has "breast" and "cancer" words, including "breast" and "cancer" are not next to each other:

```
> rs <- getSRA( search_terms = "breast cancer",
+          out_types = c('run', 'study'), sra_con )
> dim(rs)
[1] 17688 23
```

```
> rs <- getSRA( search_terms = "breast cancer",
          out_types = c("submission", "study", "sample",
           "experiment", "run"), sra_con )
> # get counts for some information interested
> apply( rs[, c('run', 'sample', 'study_type', 'platform',
           'instrument_model')], 2, function(x)
          {length(unique(x))} )
             run
                            sample
                             12149
           17688
                          platform
      study_type
                                  6
instrument_model
              27
   If you only want SRA records containing exact phrase of "breast cancer", in which "breast"
and "cancer" do not have other characters between other than a space:
> rs <- getSRA (search_terms = "breast cancer",
          out_types=c('run','study'), sra_con)
> dim(rs)
[1] 14731
             23
  Find all sample records containing words of either "MCF7" or "MCF-7":
> rs <- getSRA( search_terms ='MCF7 OR "MCF-7"',</pre>
          out_types = c('sample'), sra_con )
> dim(rs)
[1] 2494
           10
  Find all submissions by GEO:
> rs <- getSRA( search_terms = 'submission_center: GEO',
       out_types = c('submission'), sra_con )
> dim(rs)
[1] 11157
  Find study records containing a word beginning with 'Carcino':
> rs <- getSRA( search_terms ='Carcino*',
       out_types = c('study'), sra_con=sra_con )
> dim(rs)
[1] 675 12
```

#### 3.5 Download SRA data files

844 Jan 19 2012

List ftp addresses of the fastq files associated with "SRX000122":

```
> rs = listSRAfile( c("SRX000122"), sra_con, fileType = 'sra' )
```

The above function does not check file availability, size and date of the sra data files on the server, but the function getSRAinfo does this, which is good to know if you are preparing to download them:

```
> rs = getSRAinfo ( c("SRX000122"), sra_con, sraType = "sra" )
> rs[1:3,]
1 ftp://ftp-trace.ncbi.nlm.nih.gov/sra/sra-instant/reads/ByExp/sra/SRX/SRX000/SRX000122/
2 ftp://ftp-trace.ncbi.nlm.nih.gov/sra/sra-instant/reads/ByExp/sra/SRX/SRX000/SRX000122/
3 ftp://ftp-trace.ncbi.nlm.nih.gov/sra/sra-instant/reads/ByExp/sra/SRX/SRX000/SRX000122/
 experiment
                 study
                          sample
  SRX000122 SRP000098 SRS000290 SRR000648
  SRX000122 SRP000098 SRS000290 SRR000649
  SRX000122 SRP000098 SRS000290 SRR000650
 size(KB)
                   date
1
       281 Jan 19 2012
2
                  2012
    130940 Jan 19
```

Next you might want to download sra data files from the ftp site. The getSRAfile function will download all available sra data files associated with "SRR000648" and "SRR000657" from the NCBI SRA ftp site to the current directory:

```
> getSRAfile( c("SRR000648", "SRR000657"), sra_con, fileType = 'sra' )
    run    study    sample experiment
1 SRR000648 SRP000098 SRS000290    SRX000122
2 SRR000657 SRP000098 SRS000290    SRX000122

1 ftp://ftp-trace.ncbi.nlm.nih.gov/sra/sra-instant/reads/ByExp/sra/SRX/SRX000/SRX000122/
2 ftp://ftp-trace.ncbi.nlm.nih.gov/sra/sra-instant/reads/ByExp/sra/SRX/SRX000/SRX000122/
    Then downloaded sra data files can be easily converted into fastq files using fastq-dump in SRA Toolkit ( http://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?view=software ):
```

```
> ## system ("fastq-dump SRR000648.lite.sra")
```

Or directly download fastq files from EBI using ftp protocol:

```
> getFASTQinfo( c("SRR000648","SRR000657"), sra_con, srcType = 'ftp' )
> getSRAfile( c("SRR000648","SRR000657"), sra_con, fileType = 'fastq' )
```

#### 3.6 Download SRA data files using fasp protocol

Curretly both NCBI and EBI supports fasp protocol for downloading SRA data files, which has several advantages over ftp protocol, including high-speed transfering large files over long distance. Please check EBI or NCBI web site or Aspera (http://www.asperasoft.com/) for details. SRAdb has indeluded two wraper functions for using ascp command line program (fasp protocol) to download SRA data files frm either the NCBI or EBI, which is included in in Aspera Connect software. But, due to complexity of installation of the software and options within it, the functions developed here ask users to supply main ascp comands.

Download fastq files from EBI ftp siteusing fasp protocol:

```
> ## List fasp addresses for associated fastq files:
> listSRAfile ( c("SRX000122"), sra_con, fileType = 'fastq', srcType='fasp')
> ## get fasp addresses for associated fastq files:
> getFASTQinfo( c("SRX000122"), sra_con, srcType = 'fasp' )
> ## download fastq files using fasp protocol:
> # the following ascpCMD needs to be constructed according custom
> # system configuration
> # common ascp installation in a Linux system:
> ascpCMD <- 'ascp -QT -1 300m -i
+ /usr/local/aspera/connect/etc/asperaweb_id_dsa.putty'
> ## common ascpCMD for a Mac OS X system:
> # ascpCMD <- "'/Applications/Aspera Connect.app/Contents/
> # Resources/ascp' -QT -1 300m -i '/Applications/
> # Aspera Connect.app/Contents/Resources/asperaweb_id_dsa.putty'"
>
> getSRAfile( c("SRX000122"), sra_con, fileType = 'fastq',
          srcType = 'fasp', ascpCMD = ascpCMD )
  Download sra files from NCBI using fasp protocol:
> ## List fasp addresses of sra files associated with "SRX000122"
> listSRAfile( c("SRX000122"), sra_con, fileType = 'sra', srcType='fasp')
> ## download sra files using fasp protocol
> getSRAfile( c("SRX000122"), sra_con, fileType = 'sra',
  srcType = 'fasp', ascpCMD = ascpCMD )
```

The downloading messege will show significant faster downloading speed than the ftp protocol:

'SRR000658.sra 100Completed: 159492K bytes transferred in 5 seconds (249247K bits/sec), in 1 file. ... '

## 4 Interactive views of sequence data

Working with sequence data is often best done interactively in a genome browser, a task not easily done from R itself. We have found the Integrative Genomics Viewer (IGV) a high-performance visualization tool for interactive exploration of large, integrated datasets, increasing usefully for visualizing sequence alignments. In SRAdb, functions startIGV, load2IGV and load2newIGV provide convenient functionality for R to interact with IGV. Note that for some OS, these functions might not work or work well.

Launch IGV with 2 GB maximum usable memory support:

```
> startIGV("mm")
```

IGV offers a remort control port that allows R to communicate with IGV. The current command set is fairly limited, but it does allow for some IGV operations to be performed in the R console. To utilize this functionality, be sure that IGV is set to allow communication via the "enable port" option in IGV preferences. To load BAM files to IGV and then manipulate the window:

```
> exampleBams = file.path(system.file('extdata',package='SRAdb'),
+    dir(system.file('extdata',package='SRAdb'),pattern='bam$'))
> sock <- IGVsocket()
> IGVgenome(sock, 'hg18')
> IGVload(sock, exampleBams)
> IGVgoto(sock, 'chr1:1-1000')
> IGVsnapshot(sock)
```

## 5 Graphic view of SRA entities

Due to the nature of SRA data and its design, sometimes it is hard to get a whole picture of the relationship between a set of SRA entities. Functions of entityGraph and sraGraph in this package generate graphNEL objects with edgemode='directed' from input data.frame or directly from search terms, and then the plot function can easily draw a diagram.

Create a graphNEL object directly from full text search results of terms 'primary thyroid cell line'

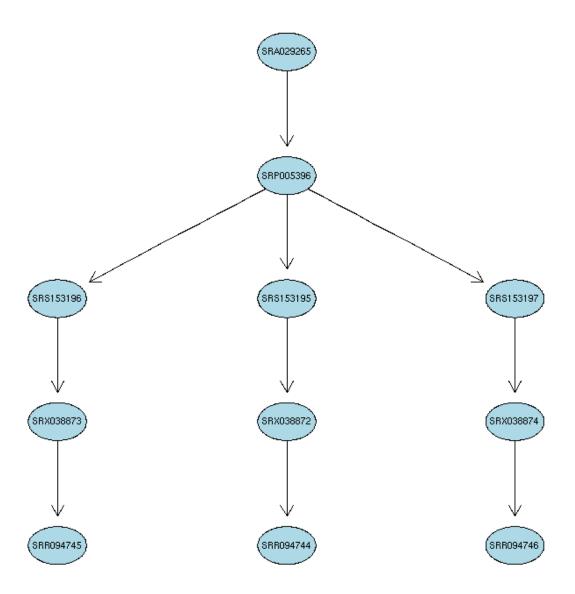


Figure 2: A graphical representation of the relationships between the SRA entities.

Please see the Figure 2 for an example diagram.

It's considered good practise to explicitely disconnect from the database once we are done with it:

```
> dbDisconnect(sra_con)
[1] TRUE
```

### 6 Example use case

This sesection will use the functionalities in the SRAdb package to explore data from the 1000 genomes project. Mainly,

1. Get some statistics of meta data and data files from the 1000 genomes project using the SRAdb 2. Download data files 3. Load bam files into the IGV from R 4. Create some snapshoots programmtically from R

After you decided what data from the 1000 Genomes, you would like to download data files from the SRA. But, it might be helpful to know file size before downloading them:

```
> runs <- tail(rs$run)
> fs <- getSRAinfo( runs, sra_con, sraType = "sra" )

Now you can download the files through ftp protocol:
> getSRAfile( runs, sra_con, fileType ='sra', srcType = "ftp" )
Or, you can download them through fasp protocol:
```

```
> ascpCMD <- "'/Applications/Aspera Connect.app/Contents/Resources/ascp' -QT -1 300m -
> sra_files = getSRAfile( runs, sra_con, fileType = 'sra', srcType = "fasp", ascpCMD =
    Next you might want to convert the downloaded sra files into fastq files:
> for( fq in basename(sra_files$fasp) ) {
        system ("fastq-dump SRR000648.lite.sra")
+ }
... to be compeleted.
```

## 7 sessionInfo

- R version 3.2.4 Revised (2016-03-16 r70336), x86\_64-pc-linux-gnu
- Locale: LC\_CTYPE=en\_US.UTF-8, LC\_NUMERIC=C, LC\_TIME=en\_US.UTF-8, LC\_COLLATE=C, LC\_MONETARY=en\_US.UTF-8, LC\_MESSAGES=en\_US.UTF-8, LC\_PAPER=en\_US.UTF-8, LC\_NAME=C, LC\_ADDRESS=C, LC\_TELEPHONE=C, LC\_MEASUREMENT=en\_US.UTF-8, LC\_IDENTIFICATION=C
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: DBI 0.3.1, RCurl 1.95-4.8, RSQLite 1.0.0, SRAdb 1.28.1, bitops 1.0-6, graph 1.48.0
- Loaded via a namespace (and not attached): Biobase 2.30.0, BiocGenerics 0.16.1, GEOquery 2.36.0, XML 3.98-1.4, parallel 3.2.4, stats4 3.2.4, tools 3.2.4