# 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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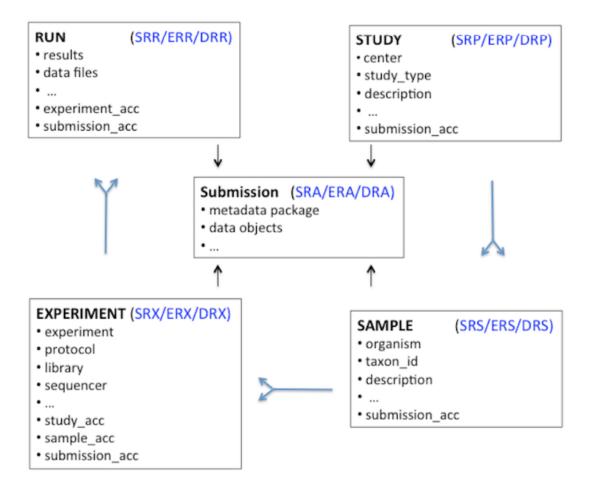


Figure 1: A graphical representation (sometimes called an *Entity-Relationship Diagram*) of the relationships between the main tables in the SRAdb package.

```
> 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
16
    15
           study_entrez_link TEXT
                                            0
17
    16
                    ddbj_link TEXT
                                            0
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
2
                0
            NA
3
            NA
                0
4
            NA
                0
5
                0
            NA
6
            NA
                0
7
                0
            NA
8
            NA
                0
9
            NA
                0
10
            NA
                0
11
            NA
                0
12
            NA
                0
13
            NA
                0
14
                0
            NA
15
            NA
                0
16
            NA
                0
17
                0
            NA
18
            NA
                0
19
                0
            NA
20
            NA
                0
21
            NA
                0
```

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

```
4 submission submission_comment
5 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.

```
> rs <- dbGetQuery(sra_con, paste( "select study_accession,
          study_title from study where",
         "study_description like 'Transcriptome%'", sep=" "))
> rs[1:3,]
  study_accession
1
        DRP002494
2
        DRP002820
3
        DRP002612
                                            study_title
            Allium fistulosum transcriptome sequencing
2 Transcriptome sequence of planarian Dugesia japonica
3
              Bursaphelenchus xylophilus transcriptome
```

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
                              42832
2
                       Other
                              30964
3
     Transcriptome Analysis
                              13857
4
               Metagenomics
                              11196
5
                        <NA>
                               1037
6
        Population Genomics
                                766
7
                Epigenetics
                                637
           Exome Sequencing
8
                                213
9
            Cancer Genomics
                                110
                                 33
10
   Pooled Clone Sequencing
         Synthetic Genomics
                                  9
11
                                  2
12 Transcriptome Sequencing
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	Illumina Genome Analyzer IIx		
7	NextSeq 500		
8	454 GS FLX		
9	HiSeq X Ten		
10	unspecified		
11	Ion Torrent PGM		
12	454 GS Junior		
13	Illumina Genome Analyzer		
14	454 GS FLX+		
15	<na></na>		
16	Illumina HiSeq 1000		
17	PacBio RS II		
18	PacBio RS		
19	AB SOLiD 4 System		
20	Illumina HiSeq 4000		
21	454 GS		
22	Illumina HiSeq 1500		
23	Ion Torrent Proton		
24	AB 5500xl Genetic Analyzer		
25	Complete Genomics		
26	Helicos HeliScope		
27	Illumina HiScanSQ		
28	NextSeq 550		
29	AB SOLiD System 3.0		
30	Illumina HiSeq 3000		
31	AB 5500 Genetic Analyzer		
32	454 GS 20		
33	AB 3730xL Genetic Analyzer		
34	MinION		
35	AB SOLiD System 2.0		
36	AB SOLiD System		
37	AB SOLiD 3 Plus System		
38	AB 3730 Genetic Analyzer		
39	AB SOLiD 4hq System		
40	AB 5500xl-W Genetic Analysis System		
41	AB 3130 Genetic Analyzer		

```
42
               AB 3500 Genetic Analyzer
43
                              BGISEQ-500
44
            AB 3130xL Genetic Analyzer
45
            AB 3500xL Genetic Analyzer
46
                       Illumina MiniSeq
47
                             454 GS FLX
48
                            HiSeq X Five
49
                     AB SOLiD PI System
         Illumina Genome Analyzer IIx
50
51
                AB 310 Genetic Analyzer
52
                        Illumina MiSeq
   Experiments
1
       1255685
2
        464419
3
        444732
4
        141191
5
        105397
6
         60137
7
         48628
8
         47127
9
         42880
10
         31948
11
         26305
12
         22179
13
         18751
14
         15385
15
         15095
16
         12471
17
         12013
18
         11221
19
         10834
20
         10313
21
          7280
22
          6693
23
          5991
24
          4644
25
          4120
          3902
26
27
          3135
28
          2552
29
          2537
30
          2535
```

```
31
            2046
32
             984
33
             891
34
             669
35
             480
             468
36
37
             316
             245
38
39
             153
40
             144
41
             108
42
              71
43
              68
44
              33
45
              14
46
              13
47
              10
48
              10
49
               3
50
               2
51
               1
52
               1
```

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

> rs <- dbGetQuery(sra\_con, paste( "SELECT library\_strategy AS

```
'Library Strategy', count( * ) AS Runs FROM `experiment`
          GROUP BY library_strategy order by Runs DESC", sep=""))
+
> rs
         Library Strategy
                            Runs
                      WGS 941137
1
2
                 AMPLICON 545554
3
                  RNA-Seq 521021
4
                    OTHER 266283
5
                      WXS 222083
6
                    CLONE
                           89306
7
                 ChIP-Seq
                           72459
                POOLCLONE 50788
8
            Bisulfite-Seq
9
                           23702
10
                miRNA-Seq
                           17815
11
                    SELEX
                           17436
```

WGA

<NA>

```
14
                   RAD-Seq
                               7864
15
         Targeted-Capture
                               7152
16
                  ATAC-seq
                               4944
17
                 ncRNA-Seq
                               4125
18
                        EST
                               3583
19
                   RIP-Seq
                               2671
20 DNase-Hypersensitivity
                               2647
                 MNase-Seq
21
                               2157
22
                 MeDIP-Seq
                               2148
23
                   FL-cDNA
                               1957
24
                   MRE-Seq
                               1889
25
                    Tn-Seq
                               1622
26
                   MBD-Seq
                               1620
27
                        WCS
                               1419
28
                  CLONEEND
                                505
29
                                352
                 FAIRE-seq
30
                        CTS
                                273
31
                      other
                                214
32
      Synthetic-Long-Read
                                175
33
                       Hi-C
                                 83
34
                                 37
                 FINISHING
35
                  ChIA-PET
                                 30
36
                                 22
                VALIDATION
```

### 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 ) > conversion[1:3,]
```

```
study submission sample experiment
1 SRP000931 SRA009053 SRS003464 SRX006135
2 SRP000931 SRA009053 SRS003455 SRX006124
3 SRP000931 SRA009053 SRS003463 SRX006134
run
1 SRR018269
2 SRR018258
```

3 SRR018268

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

#### 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] 30878
             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))} )
                            sample
             run
                             21953
           30878
      study_type
                          platform
                                  7
instrument_model
>
   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] 22089
             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] 4141
           10
  Find all submissions by GEO:
> rs <- getSRA( search_terms = 'submission_center: GEO',
       out_types = c('submission'), sra_con )
> dim(rs)
```

```
[1] 20285 6
```

2

130940 Jan 19

844 Jan 19

2012

2012

Find study records containing a word beginning with 'Carcino':

#### 3.5 Download SRA data files

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
                          sample
                 study
1 SRX000122 SRP000098 SRS000290 SRR000648
2 SRX000122 SRP000098 SRS000290 SRR000649
3 SRX000122 SRP000098 SRS000290 SRR000650
 size(KB)
                   date
1
       281 Jan 19
                   2012
```

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

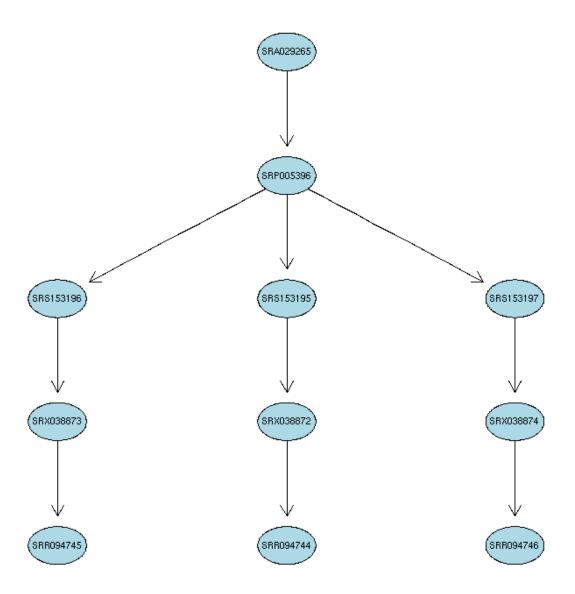


Figure 2: A graphical representation of the relationships between the SRA entities.

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'

Please see the Figure 2 for an example diagram.

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

> dbDisconnect(sra\_con)

## 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

### 7 sessionInfo

... to be compeleted.

- R version 3.4.0 (2017-04-21), 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
- Running under: Ubuntu 16.04.2 LTS

> ## get counts for each data types

- Matrix products: default
- BLAS: /home/biocbuild/bbs-3.6-bioc/R/lib/libRblas.so
- LAPACK: /home/biocbuild/bbs-3.6-bioc/R/lib/libRlapack.so
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, utils
- Other packages: BiocGenerics 0.23.0, RCurl 1.95-4.8, RSQLite 1.1-2, SRAdb 1.35.0, bitops 1.0-6, graph 1.55.0
- Loaded via a namespace (and not attached): Biobase 2.37.0, DBI 0.6-1, GEOquery 2.43.0, R6 2.2.0, Rcpp 0.12.10, XML 3.98-1.6, compiler 3.4.0, digest 0.6.12, httr 1.2.1, memoise 1.1.0, stats4 3.4.0, tools 3.4.0