# 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 does not currently store aligned reads or any other processed data that might rely on alignment to a reference genome. However, NCBI GEO does 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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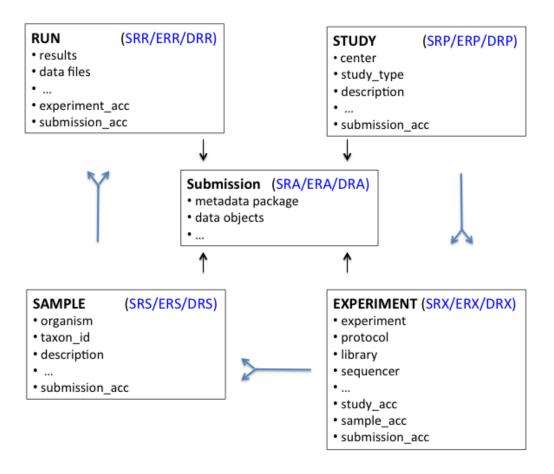


Figure 1: A graphical representation (sometimes called an *Entity-Relationship Diagram*) of the relationships between the main tables in the SRAdb package.

```
> library(SRAdb)
> sqlfile <- 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

   user system elapsed
31.150  8.012 388.761
```

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').

## 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"
                             "study_url_link"
[13] "sra_link"
[15] "xref_link"
                             "study_entrez_link"
[17] "ddbj_link"
                             "ena_link"
                             "submission_accession"
[19] "study_attribute"
[21] "sradb_updated"
```

Sometimes it is useful to get the actual SQL schema associated with a table. As an example of doing this and using an *RSQLite* shortcut function, sqliteQuickSQL, we can get the table schema for the *study* table:

```
> sqliteQuickSQL(sra_con, 'PRAGMA TABLE_INFO(study)')
```

```
cid
                         name type notnull
1
     0
                    study_ID REAL
2
     1
                 study_alias TEXT
                                           0
     2
                                           0
3
             study_accession TEXT
4
     3
                 study_title TEXT
                                           0
5
     4
                                           0
                  study_type TEXT
6
     5
                                           0
              study_abstract TEXT
7
                                           0
     6
                 broker_name TEXT
     7
                                           0
8
                 center_name TEXT
9
     8
        center_project_name TEXT
                                           0
10
     9
           study_description TEXT
                                           0
11
    10
                                           0
             related_studies TEXT
12
    11
                                           0
               primary_study TEXT
    12
13
                     sra_link TEXT
                                           0
14
    13
                                           0
              study_url_link TEXT
15
    14
                   xref_link TEXT
                                           0
16
    15
           study_entrez_link TEXT
                                           0
17
    16
                                           0
                   ddbj_link TEXT
18
    17
                    ena_link TEXT
                                           0
19
    18
                                           0
             study_attribute TEXT
                                           0
20
    19 submission_accession TEXT
21
    20
                                           0
               sradb_updated TEXT
   dflt_value pk
1
          <NA>
2
         <NA>
                0
3
         <NA>
                0
4
         <NA>
                0
5
         <NA>
                0
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
                0
17
         <NA>
18
         <NA>
                0
                0
19
         <NA>
```

```
20 <NA> 0
21 <NA> 0
```

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

```
> colDesc <- colDescriptions(sra_con=sra_con)[1:5,]</pre>
> colDesc[, 1:4]
  col_desc_ID table_name
                                   field_name
             1 submission
1
                                            TD
2
             2 submission
                                    accession
             3 submission
3
                                         alias
4
             4 submission submission_comment
             5 submission
5
     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:

```
> rs <- dbGetQuery(sra_con, "select * from study limit 3")
> rs[, 1:3]
  study_ID
                           study_alias
                        Natto BEST195
1
2
         2 Resequence B. subtilis 168
3
             DLD1_normoxia_nucleosome
  study_accession
1
        DRP00001
2
        DRP000002
3
        DRP000003
```

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.

```
study_accession

ERP000233

ERP000350

ERP000527

Identification of the expression profile of Staphylococcus aureus grown in the presence

Transcriptome Analysis of the
```

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.

Get some high-level statistics could be to helpful to get overall idea about what data are availble 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
                              16472
1
2
                       <NA>
                               9046
3
                      Other
                               3613
4
     Transcriptome Analysis
                              3081
               Metagenomics
5
                               2152
                Epigenetics
6
                               1607
7
                               1254
                     RNASeq
8
        Population Genomics
                                403
```

9	Pooled Clone Sequencing	83
10	Exome Sequencing	79
11	Cancer Genomics	54
12	Transcriptome Sequencing	39
13	Synthetic Genomics	9

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

	Instrument Model
1	Illumina HiSeq 2000
2	Illumina Genome Analyzer II
3	454 GS FLX Titanium
4	Illumina Genome Analyzer IIx
5	Illumina MiSeq
6	<na></na>
7	454 GS FLX
8	Illumina Genome Analyzer
9	unspecified
10	AB SOLiD 4 System
11	Complete Genomics
12	Illumina HiSeq 2500
13	PacBio RS II
14	AB SOLiD System 3.0
15	454 GS Junior
16	Illumina HiSeq 1000
17	Ion Torrent PGM
18	454 GS FLX+
19	PacBio RS
20	454 GS 20
21	Helicos HeliScope
22	AB 5500xl Genetic Analyzer
23	AB SOLiD System 2.0
24	AB 5500 Genetic Analyzer
25	454 GS
26	AB SOLiD System
27	Illumina HiScanSQ
28	AB SOLiD 4hq System
29	Illumina HiSeq 1500

```
30
                AB SOLiD 3 Plus System
31
                     Ion Torrent Proton
32 AB 5500xl-W Genetic Analysis System
              AB 3730 Genetic Analyzer
33
            AB 3500xL Genetic Analyzer
34
                     AB SOLiD PI System
35
   Experiments
1
        312580
2
         62362
3
         47925
4
         30265
5
         22791
6
         22583
7
         17239
8
         14107
9
          7572
10
          4278
11
          2796
12
          2756
13
          2745
14
          2008
15
          1629
16
          1505
17
          1416
18
          1250
19
          1107
20
           847
21
           659
22
           590
23
           424
24
           290
25
           275
26
           240
27
           231
28
           150
29
           109
30
            77
31
            51
32
            12
33
             5
34
             1
35
             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
1
                       WGS 212845
2
                       WXS
                             95715
3
                   RNA-Seq
                            74671
4
                  AMPLICON
                             73170
5
                     OTHER
                             43672
6
                  ChIP-Seq
                             23487
7
                      < NA >
                             22583
8
            Bisulfite-Seq
                              3450
9
                       EST
                              3136
10
                     CLONE
                              1899
11
                   FL-cDNA
                              1240
12
                 POOLCLONE
                              1120
13 DNase-Hypersensitivity
                              1099
14
                 MeDIP-Seq
                               955
15
                 miRNA-Seq
                               933
16
                    Tn-Seq
                               686
17
                       WGA
                               564
18
                   MBD-Seq
                               513
19
                 MNase-Seq
                               493
20
                       WCS
                               302
21
                   MRE-Seq
                               151
22
                       CTS
                                95
23
                 FAIRE-seq
                                51
24
                  CLONEEND
                                36
                                 4
25
                   RIP-Seq
26
                 FINISHING
                                 3
27
                 ncRNA-Seq
                                 3
```

## 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 SRS003455 SRX006124
2 SRP000931 SRA009053 SRS003462 SRX006133
3 SRP000931 SRA009053 SRS003463 SRX006134
        run
1 SRR018258
2 SRR018267
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] "SRS003455" "SRS003462" "SRS003463"
 [4] "SRS003458" "SRS003459" "SRS003461"
 [7] "SRS003453" "SRS003457" "SRS003464"
[10] "SRS003460" "SRS003456" "SRS003454"
[13] "SRS004650"
$experiment
 [1] "SRX006124" "SRX006133" "SRX006134"
 [4] "SRX006127" "SRX006128" "SRX006132"
 [7] "SRX006130" "SRX006126" "SRX006135"
[10] "SRX006122" "SRX006129" "SRX006131"
[13] "SRX006125" "SRX006123" "SRX007396"
$run
 [1] "SRR018258" "SRR018267" "SRR018268"
 [4] "SRR018261" "SRR018262" "SRR018266"
 [7] "SRR018264" "SRR018260" "SRR018269"
[10] "SRR018256" "SRR018263" "SRR018265"
[13] "SRR018259" "SRR018257" "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",</pre>
          out_types = c('run', 'study'), sra_con )
> dim(rs)
[1] 6555
           23
> rs <- getSRA( search_terms = "breast cancer",</pre>
          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
                              4851
            6555
      study_type
                          platform
                                 5
instrument_model
              15
>
```

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] 5745 23
```

Find all sample records containing words of either "MCF7" or "MCF-7":

#### 3.5 Download SRA data files

281 Jan 19 2012

844 Jan 19 2012

2012

130940 Jan 19

1 2

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:

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' )
                study
        run
                          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"), 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"), 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'</pre>
```

```
> ## 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'

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

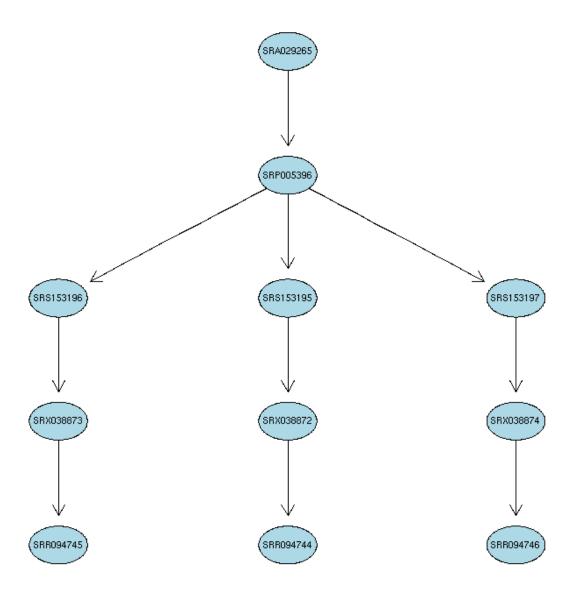


Figure 2: A graphical representation (sometimes called an  $Entity-Relationship\ Diagram)$  of the relationships between the SRA entities.

```
> ## get all related accessions
> rs <- getSRA( search_terms = '"1000 Genomes Project"',
          sra_con=sra_con, acc_only=TRUE)
> dim(rs)
> head(rs)
> ## get counts for each data types
> apply( rs, 2, function(x) {length(unique(x))} )
   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$run)</pre>
> fs <- getSRAinfo( runs, sra_con, sraType = "sra" )</pre>
   Now you can download the files through ftp protocol:
> getSRAfile( runs, sra_con, fileType = 'sra', srcType = "ftp" )
   Or, you can download them through fasp protocol:
> ascpCMD <- "'/Applications/Aspera Connect.app/Contents/Resources/ascp' -QT -1 300m -
> sra_files = getSRAfile( runs, sra_con, fileType = 'sra', srcType = "fasp", ascpCMD =
   Next you might want to convert the downloaded sra files into fastq files:
> for( fq in basename(sra_files$fasp) ) {
          system ("fastq-dump SRR000648.lite.sra")
+ }
```

## 7 sessionInfo

> toLatex(sessionInfo())

... to be compeleted.

> sra\_con <- dbConnect(SQLite(),sqlfile)

- R version 3.1.0 (2014-04-10), x86\_64-unknown-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 0.2-7, RCurl 1.95-4.1, RSQLite 0.11.4, SRAdb 1.18.0, bitops 1.0-6, graph 1.42.0
- Loaded via a namespace (and not attached): Biobase 2.24.0, BiocGenerics 0.10.0, GEOquery 2.30.0, XML 3.98-1.1, parallel 3.1.0, stats4 3.1.0, tools 3.1.0