Using the SRAdb Package to Query the Sequence Read Archive

Jack Zhu*and Sean Davis[†]

Genetics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health

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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.

^{*}zhujack@mail.nih.gov

[†]sdavis2@mail.nih.gov

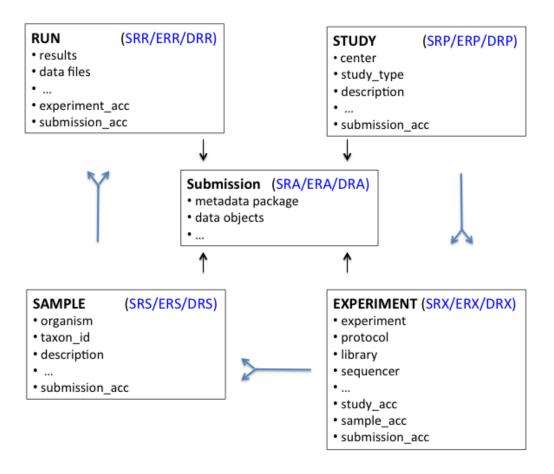


Figure 1: A graphical representation (sometimes called an *Entity-Relationship Diagram*) of the relationships between the main tables in the SRAdb package.

```
> 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
1
   Whole Genome Sequencing
                             26121
2
                     Other
                             11282
3
    Transcriptome Analysis
                              6622
              Metagenomics
4
                              4144
5
               Epigenetics
                              2064
6
       Population Genomics
                               680
7
                      < NA >
                               510
8
          Exome Sequencing
                               135
9
           Cancer Genomics
                                73
10 Pooled Clone Sequencing
                                32
        Synthetic Genomics
                                 9
11
```

8

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

Instrument Model Illumina HiSeq 2000

RNASeq

12

1

2	Illumina MiSeq
3	454 GS FLX Titanium
4	Illumina Genome Analyzer II
5	Illumina Genome Analyzer IIx
6	Illumina HiSeq 2500
7	unspecified
8	454 GS FLX
9	
10	Illumina Genome Analyzer <na></na>
11	454 GS Junior
12	AB SOLiD 4 System
13	PacBio RS II
14	Ion Torrent PGM
15	Illumina HiSeq 1000
16	454 GS FLX+
17	Helicos HeliScope
18	Complete Genomics
19	PacBio RS
20	AB SOLiD System 3.0
21	AB 5500xl Genetic Analyzer
22	AB 5500 Genetic Analyzer
23	Illumina HiScanSQ
24	454 GS 20
25	Illumina HiSeq 1500
26	454 GS
27	AB SOLiD System 2.0
28	AB SOLiD System
29	Ion Torrent Proton
30	AB 3730xL Genetic Analyzer
31	HiSeq X Ten
32	AB SOLiD 4hq System
33	AB SOLID 3 Plus System
34	NextSeq 500
35	MinION
36	AB 5500x1-W Genetic Analysis System
37	454 GS FLX
38	AB 3500xL Genetic Analyzer
39	AB 3730 Genetic Analyzer
40	Illumina NextSeq 500
41	AB 3130xL Genetic Analyzer
42	AB SOLiD PI System
43	AB 310 Genetic Analyzer

```
44
              AB 3500 Genetic Analyzer
45
                    Illumina HiSeq 4000
   Experiments
        576428
1
2
         81112
3
         79416
4
         74743
5
         42277
6
         40181
7
         31765
8
         25955
9
         16617
10
         14054
11
         11120
12
          9464
13
          5616
14
          5469
15
          5060
16
          3806
17
          3777
          3056
18
19
          2934
20
          2377
21
          1804
22
          1622
23
          1142
24
           891
25
           840
26
           503
           464
27
28
           378
29
           270
30
           199
31
           179
32
           158
33
           138
34
           126
35
            39
            22
36
37
            10
             6
38
39
             5
```

```
40
                4
                3
41
42
                2
43
                1
44
                1
45
                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 375890
2
                  AMPLICON 176811
3
                       WXS 167794
4
                   RNA-Seq 148720
5
                     OTHER 79598
6
                  ChIP-Seq
                            36579
7
                      <NA>
                            14054
8
                 POOLCLONE
                             8240
9
            Bisulfite-Seq
                             6635
10
                       WGA
                             5320
                     CLONE
11
                             4636
12
                 miRNA-Seq
                             4437
13
                       EST
                             3328
14
                VALIDATION
                             3266
15
                   FL-cDNA
                             1303
                 MeDIP-Seq
                             1255
16
17 DNase-Hypersensitivity
                             1218
                 MNase-Seq
                              861
18
19
                    Tn-Seq
                              792
20
                   MBD-Seq
                               712
21
                   MRE-Seq
                               565
22
                   RAD-Seq
                               539
23
                 ncRNA-Seq
                               482
24
                   RIP-Seq
                               354
25
                       WCS
                               328
                       CTS
26
                               104
27
                 FAIRE-seq
                               94
                  CLONEEND
28
                                51
29
                     SELEX
                                32
```

30	FINISHING	20
31	ChIA-PET	10
32	Synthetic-Long-Read	7

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:

2 SRR018259

3 SRR018258

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] "SRS003457" "SRS003456" "SRS003455"
```

- [7] "SRS003462" "SRS003454" "SRS003460"
- [10] "SRS003463" "SRS003459" "SRS003464"
- [13] "SRS004650"

\$experiment

- [1] "SRX006126" "SRX006125" "SRX006124"
- [4] "SRX006130" "SRX006129" "SRX006122"

^{[4] &}quot;SRS003453" "SRS003458" "SRS003461"

```
[7] "SRX006127" "SRX006132" "SRX006133" [10] "SRX006123" "SRX006131" "SRX006134" [13] "SRX006128" "SRX006135" "SRX007396" "SRX006135" "SRX007396" "SRX007396" "SRR018260" "SRR018259" "SRR018258" [4] "SRR018264" "SRR018263" "SRR018256" [7] "SRR018261" "SRR018266" "SRR018267" [10] "SRR018257" "SRR018265" "SRR018268" [13] "SRR018262" "SRR018269" "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] 10269
             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
           10269
                             7269
                         platform
      study_type
instrument_model
              23
```

>

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] 9021
           23
   Find all sample records containing words of either "MCF7" or "MCF-7":
> rs <- getSRA( search_terms ='MCF7 OR "MCF-7"',
          out_types = c('sample'), sra_con )
> dim(rs)
[1] 1796
           10
   Find all submissions by GEO:
> rs <- getSRA( search_terms ='submission_center: GEO',</pre>
       out_types = c('submission'), sra_con )
> dim(rs)
[1] 7371
            6
   Find study records containing a word beginning with 'Carcino':
> rs <- getSRA( search_terms ='Carcino*',</pre>
       out_types = c('study'), sra_con=sra_con )
> dim(rs)
[1] 423 12
```

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:

```
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
1 SRX000122 SRP000098 SRS000290 SRR000648
 SRX000122 SRP000098 SRS000290 SRR000649
  SRX000122 SRP000098 SRS000290 SRR000650
 size(KB)
                   date
1
       281 Jan 19
                   2012
2
    130940 Jan 19
                   2012
3
       844 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")
```

```
> ## 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 indcluded 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 funcitons 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'
> ## 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
```

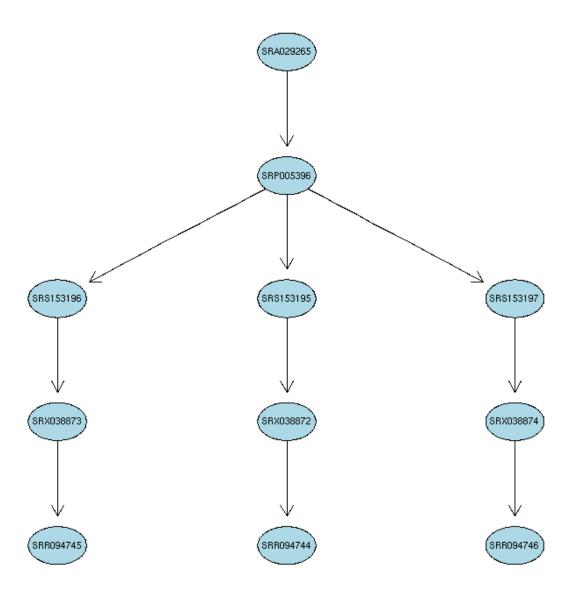


Figure 2: A graphical representation of the relationships between the SRA entities.

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

> fs <- getSRAinfo(runs, sra_con, sraType = "sra")</pre>

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:

... to be compeleted.

+ }

> runs <- tail(rs\$run)</pre>

7 sessionInfo

- R version 3.1.3 (2015-03-09), 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 0.3.1, RCurl 1.95-4.5, RSQLite 1.0.0, SRAdb 1.20.13, bitops 1.0-6, graph 1.44.1
- Loaded via a namespace (and not attached): Biobase 2.26.0, BiocGenerics 0.12.1, GEOquery 2.32.0, XML 3.98-1.1, parallel 3.1.3, stats4 3.1.3, tools 3.1.3