

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

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

```
> file.info('SRAmetadb.sqlite')

              size isdir mode
SRAmetadb.sqlite 3853249536 FALSE  666
              mtime
SRAmetadb.sqlite 2012-03-31 01:52:58
              ctime
SRAmetadb.sqlite 2012-03-31 01:52:34
              atime exe
SRAmetadb.sqlite 2012-03-31 01:52:34  no
```

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.

```
> sra_tables <- dbListTables(sra_con)
> sra_tables

[1] "col_desc"      "experiment"
[3] "metaInfo"     "run"
[5] "sample"       "sra"
[7] "sra_ft"       "sra_ft_content"
[9] "sra_ft_segdir" "sra_ft_segments"
[11] "study"        "submission"
```

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"
[17] "ddbj_link"          "ena_link"
[19] "study_attribute"    "submission_accession"
[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	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	center_project_name	TEXT	0
10	9	study_description	TEXT	0
11	10	related_studies	TEXT	0
12	11	primary_study	TEXT	0
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>	0		
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		
17	<NA>	0		
18	<NA>	0		
19	<NA>	0		
20	<NA>	0		
21	<NA>	0		

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
1	1	Natto BEST195
2	2	Resequencing B. subtilis 168
3	3	DLD1_normoxia_nucleosome

	study_accession
1	DRP000001
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.

```
> rs <- dbGetQuery(sra_con, paste( "select study_accession,
+      study_title from study where",
+      "study_description like 'Transcriptome%'",sep=" "))
> rs[1:3,]

  study_accession
1      ERP000350
2      ERP000527
3      SRP000568

                                study_title
1                                RNA sequencing in E. coli K12
2      Transcriptome Analysis of the potato (genotype RH89-039-16)
3 Highly integrated epigenome maps in Arabidopsis - transcriptome sequencing
```

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)
+   return(dbGetQuery(conn,sql)[1,1])
+ }
> do.call(rbind,sapply(sra_tables[1:3],
+   getTableCounts, sra_con, simplify=FALSE))

      [,1]
col_desc      129
experiment 128055
metaInfo       2
```

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'. Function `sraConvert` does the conversion with a very fast mapping between entity types.

Covert 'SRP001007' 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 SRS003460  SRX006131
3 SRP000931  SRA009053 SRS003458  SRX006127
```

```

run
1 SRR018269
2 SRR018265
3 SRR018261

```

Check what SRA types and how many entities in each type in the conversion.

```

> apply(conversion, 2, unique)

$study
[1] "SRP000931" "SRP001007"

$submission
[1] "SRA009053" "SRA009276"

$sample
[1] "SRS003464" "SRS003460" "SRS003458"
[4] "SRS003453" "SRS003461" "SRS003456"
[7] "SRS003457" "SRS003454" "SRS003459"
[10] "SRS003462" "SRS003463" "SRS003455"
[13] "SRS004650"

$experiment
[1] "SRX006135" "SRX006131" "SRX006127"
[4] "SRX006129" "SRX006130" "SRX006132"
[7] "SRX006125" "SRX006126" "SRX006122"
[10] "SRX006123" "SRX006128" "SRX006133"
[13] "SRX006134" "SRX006124" "SRX007396"

$run
[1] "SRR018269" "SRR018265" "SRR018261"
[4] "SRR018263" "SRR018264" "SRR018266"
[7] "SRR018259" "SRR018260" "SRR018256"
[10] "SRR018257" "SRR018262" "SRR018267"
[13] "SRR018268" "SRR018258" "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=sra_con)  
> dim(rs)  
  
[1] 1473   23
```

If you only wants records containing exact phrase of 'breast cancer', in which 'breast' and 'cancer' have other characters between other than a space:

```
> rs <- getSRA (search_terms = '"breast cancer"',  
+             out_types=c('run', 'study'), sra_con=sra_con)  
> dim(rs)  
  
[1] 1336   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=sra_con)  
> dim(rs)  
  
[1] 321   10
```

Find all submissions by GEO:

```
> rs <- getSRA (search_terms = 'submission_center: GEO',  
+             out_types=c('submission'), sra_con=sra_con)  
> dim(rs)  
  
[1] 1373    6
```

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] 80 12
```

3.5 Get sra or sra-lite data file information

List fastq file ftp or fasp addresses associated with "SRX000122":

```
> listSRAfile (in_acc = c("SRX000122"),
+             sra_con = sra_con, fileType = 'sra')
> listSRAfile (in_acc = c("SRX000122"),
+             sra_con = sra_con, fileType = 'sra', srcType='fasp')
```

The above function does not check file availability, size and date of the sra or sra-lite 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 (in_acc=c("SRX000122"),
+                  sra_con=sra_con)
> rs[1:3,]
```

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

```
> getSRAfile( in_acc = c("SRR000648", "SRR000657"),
+             sra_con = sra_con, destDir = getwd(),
+             fileType = 'litesra' )
```

Then downloaded .sra or .lite.sra can be easily converted into fastq files using fastq-dump in SRA Toolkit (<http://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?cmd=show&f=software&m=software&s>);

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

Or users can directly download fastq files from EBI using ftp protocol:

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

3.6 Download SRA data files using fasp protocol

Currently both NCBI and EBI supports fasp downloading of SRA data files, which has several advantages over ftp protocol, including high-speed transferring large files over long distance. Please check EBI or NCBI web site or Aspera (<http://www.asperasoft.com/>) for details. SRADB has included two wrapper functions for using ascp command line program to download files by fasp protocol, which is included 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 commands.

Download fastq files from EBI ftp site using fasp protocol:


```

> ## List fasp addresses of SRA fastq files associated with "SRX000122"
> listSRAfile (in_acc = c("SRX000122"), sra_con = sra_con,
+   fileType = 'fastq', srcType='fasp')
> ## download fastq files using fasp protocol:
> ascpCMD <- 'ascp -QT -l 300m -i
+   /usr/local/aspera/connect/etc/asperaweb_id_dsa.putty'
> getSRAfile( in_acc = c("SRX000122"), sra_con,
+   fileType = 'fastq', srcType = 'fasp', ascpCMD = ascpCMD )

```

Download .lite.sra files from NCBI ftp site using fasp protocol:

```

> ## List fasp addresses of SRA litesra files associated with "SRX000122"
> listSRAfile (in_acc = c("SRX000122"),
+   sra_con = sra_con, fileType = 'litesra', srcType='fasp')
> ## download litesra files using fasp protocol:
> ascpCMD <- 'ascp -QT -l 300m -i
+   /usr/local/aspera/connect/etc/asperaweb_id_dsa.putty'
> ## common ascpCMD for a system with Mac OS X:
> #ascpCMD <- "'/Applications/Aspera Connect.app
+   #/Contents/Resources/ascp' -QT -l 300m -i
+   #'/Applications/Aspera Connect.app/Contents/
+   #Resources/asperaweb_id_dsa.putty'"
> sraFiles <- ascpSRA( in_acc = c("SRX000122"),
+   sra_con, ascpCMD, fileType = 'litesra', destDir=getwd() )

```

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 graph.

Create a graphNEL object from SRA accessions, which are full text search results of terms 'primary thyroid cell line'

```

> acc <- getSRA (search_terms = 'primary thyroid cell line',
+   out_types=c('sra'), sra_con=sra_con, acc_only=TRUE)
> g <- entityGraph(acc)
> attrs <- getDefaultAttrs(list(node=list(
+   fillcolor='lightblue', shape='ellipse'))))
> plot(g, attrs= attrs)

```

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

```

> g <- sraGraph('primary thyroid cell line', sra_con)
> library(Rgraphviz)
> attrs <- getDefaultAttrs(list(node=list(
+   fillcolor='lightblue', shape='ellipse'))))
> plot(g, attrs=attrs)

```

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

```

> dbDisconnect(sra_con)

```

```

[1] TRUE

```

6 sessionInfo

```

> toLatex(sessionInfo())

```

- R version 2.15.0 (2012-03-30), i386-pc-mingw32
- Locale: LC_COLLATE=C, LC_CTYPE=English_United States.1252, LC_MONETARY=English_United States.1252, LC_NUMERIC=C, LC_TIME=English_United States.1252
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: DBI 0.2-5, RCurl 1.91-1.1, RSQLite 0.11.1, SRADB 1.10.0, bitops 1.0-4.1, graph 1.34.0
- Loaded via a namespace (and not attached): Biobase 2.16.0, BiocGenerics 0.2.0, GEOquery 2.22.0, XML 3.9-4.1, tools 2.15.0