

# 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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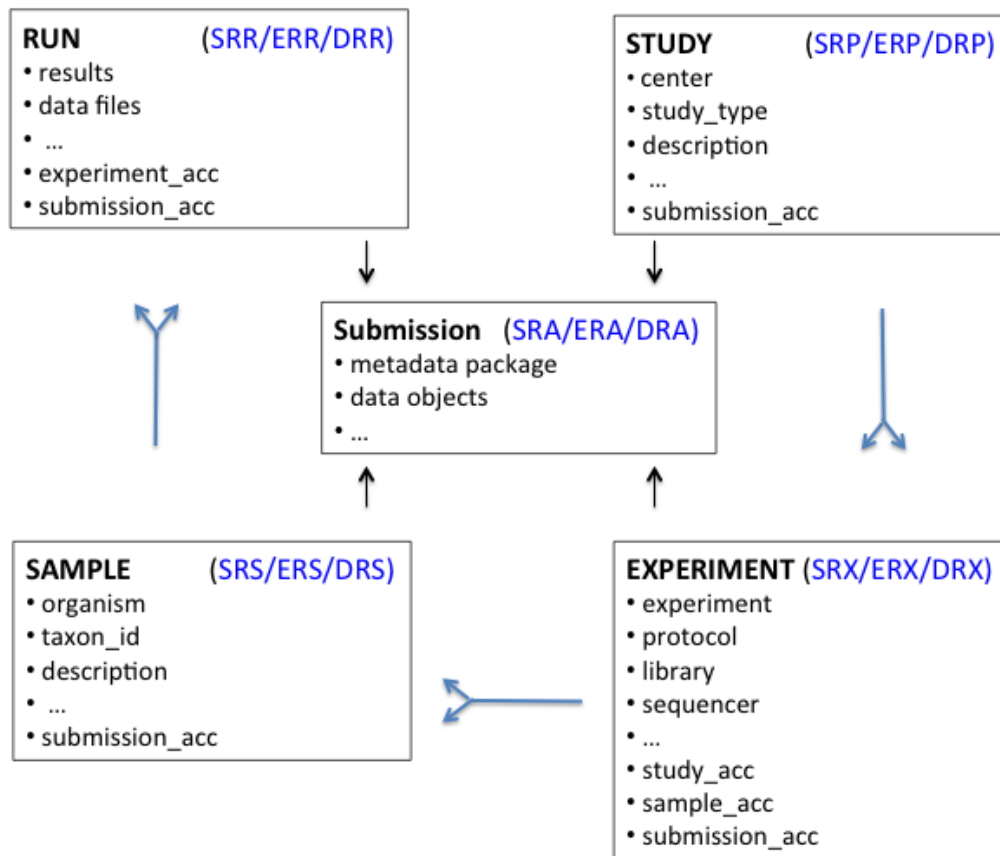


Figure 1: A graphical representation (sometimes called an *Entity-Relationship Diagram*) of the relationships between the main tables in the SRADB package.

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

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 few moments due to file size, depending 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
30.93   10.73   383.16
```

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 5951075328 FALSE 666
      mtime
SRAmetadb.sqlite 2014-04-12 02:28:04
      ctime
SRAmetadb.sqlite 2014-04-12 02:20:51
      atime exe
SRAmetadb.sqlite 2014-04-12 02:20:51 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(RSQLite(), 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.

```

> 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	

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

```
> colDesc <- colDescriptions(sra_con=sra_con)[1:5,]
> colDesc[, 1:4]
```

	col_desc_ID	table_name	field_name
1	1	submission	ID
2	2	submission	accession
3	3	submission	alias

```

4          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:

```

> rs <- dbGetQuery(sra_con,"select * from study limit 3")
> rs[, 1:3]

```

```

      study_ID      study_alias
1          1      Natto BEST195
2          2 Resequene 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      ERP000233
2      ERP000350
3      ERP000527

```

```

1 Identification of the expression profile of Staphylococcus aureus grown in the presence of
2
3

```

Transcriptome Analysis of the

Of course, we can combine programming and data access. A simple `summary` 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,apply(sra_tables[c(2,4,5,11,12)],
+   getTableCounts, sra_con, simplify=FALSE))

      [,1]
experiment 562876
run         732910
sample      575083
study       37892
submission  224190

```

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	16472
2	<NA>	9046
3	Other	3613
4	Transcriptome Analysis	3081
5	Metagenomics	2152
6	Epigenetics	1607
7	RNASeq	1254
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:

```

> rs <- dbGetQuery(sra_con, paste( "SELECT instrument_model AS
+   'Instrument Model', count( * ) AS Experiments FROM `experiment`
+   GROUP BY instrument_model order by Experiments DESC", sep=""))
> rs

```

	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>
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
33	AB 3730 Genetic Analyzer
34	AB 3500xL Genetic Analyzer
35	AB SOLiD PI System

#### 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
25	RIP-Seq	4
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 )
> 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",
+               out_types = c('run', 'study'), sra_con )
> dim(rs)

```

```

[1] 6555    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
              6555              4851
      study_type              platform
              8                  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":

```

> rs <- getSRA( search_terms = 'MCF7 OR "MCF-7"',
+               out_types = c('sample'), sra_con )
> dim(rs)

[1] 931    10

```

Find all submissions by GEO:

```

> rs <- getSRA( search_terms = 'submission_center: GEO',
+               out_types = c('submission'), sra_con )
> dim(rs)

[1] 4520    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] 238    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:

```
> 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      run
1 SRX000122 SRP000098 SRS000290 SRR000648
2 SRX000122 SRP000098 SRS000290 SRR000649
3 SRX000122 SRP000098 SRS000290 SRR000650
  size(KB) date
1      <NA> <NA>
2      <NA> <NA>
3      <NA> <NA>
```

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

### 3.6 Download SRA data files using fasp protocol

Currently both NCBI and EBI supports fasp protocol for downloading 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 (fasp protocol) to download SRA data files from either the NCBI or EBI, 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 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 -l 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 -l 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 message 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'

```
> library(SRADb)
> library(Rgraphviz)
> g <- sraGraph('primary thyroid cell line', sra_con)
> attrs <- getDefaultAttrs(list(node=list(
+   fillcolor='lightblue', shape='ellipse'))))
> plot(g, attrs=attrs)
> ## similiar search as the above, returned much larger data.frame and graph is too cl
> g <- sraGraph('Ewing Sarcoma', sra_con)
> plot(g)
>
```

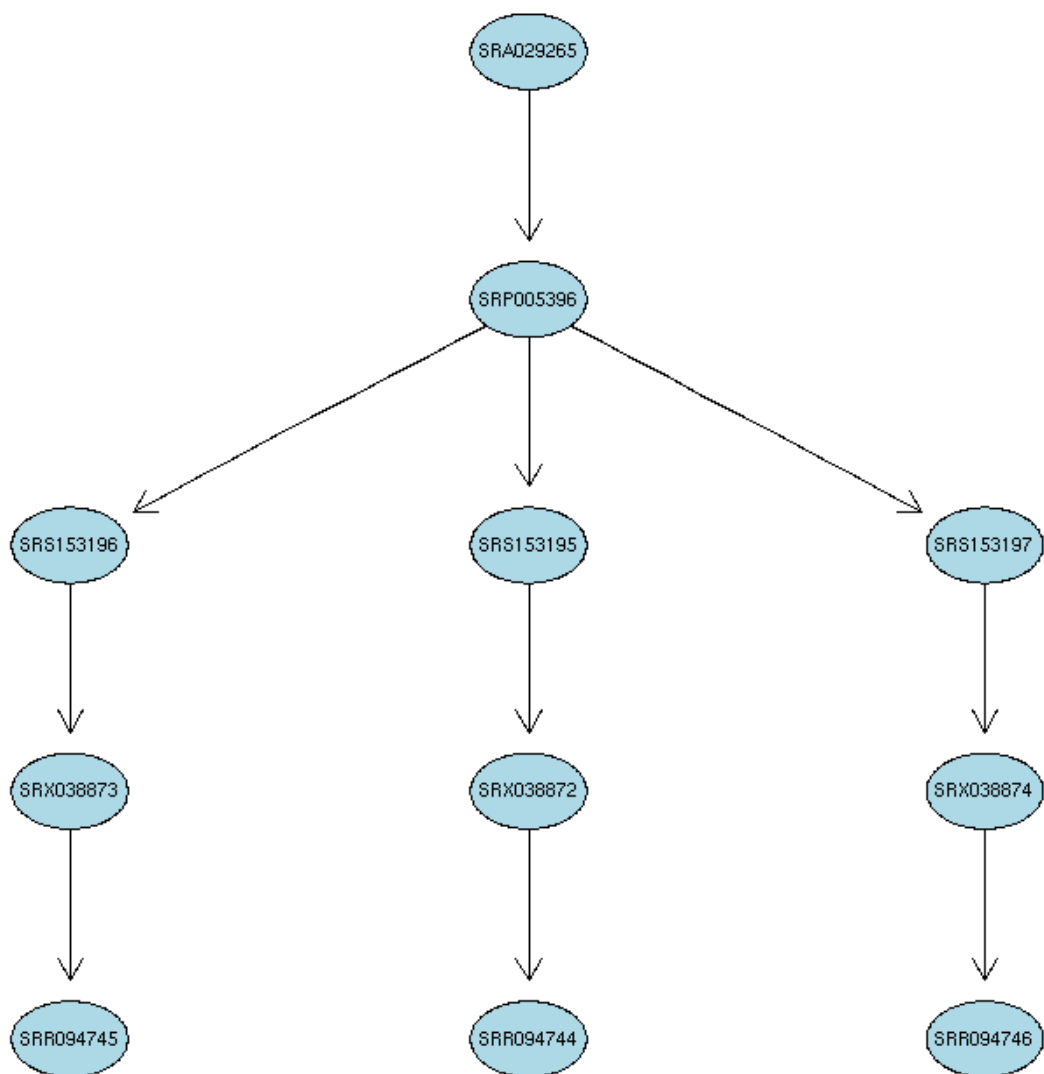


Figure 2: A graphical representation (sometimes called an *Entity-Relationship Diagram*) of the relationships between the SRA entities.



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)
```

```
[1] TRUE
```

## 6 Example use case

This section 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 snapshots programmatically from R

```
> library(SRADB)
> setwd('1000g')
> if( ! file.exists('SRAMetadb.sqlite') ) {
+     sqlfile <- getSRADBFile()
+ } else {
+     sqlfile <- 'SRAMetadb.sqlite'
+ }
> sra_con <- dbConnect(SQLite(),sqlfile)
> ## 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)
> 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 -l 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 completed.

## 7 sessionInfo

```
> toLatex(sessionInfo())
```

- R version 3.1.0 RC (2014-04-02 r65358), i386-w64-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 0.2-7, 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