

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 <- file.path(system.file('extdata', package='SRAdb'), 'SRAmetadb_demo.sqlite')
```

Note: the above "SRAmetadb_demo.sqlite" is a down-sized demo SRAmetadb sqlite database. The actual SRAmetadb sqlite database can be downloaded using function: `getSRAdbFile`. Warning: the actual SRAmetadb sqlite database is pretty large (> 35GB as of May, 2018) after uncompression. So, downloading and uncompressing of the actual SRAmetadb sqlite could take quite a few minutes depending on your network bandwidth. Direct links for downloading the SRAmetadb sqlite database: https://s3.amazonaws.com/starbuck1/sradb/SRAmetadb_demo.sqlite <https://gbnci-abcc.ncifcrf.gov/backup/SRAmetadb.sqlite.gz> . 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.

```
> file.info(sqlfile)
```

	size	
/tmp/RtmpRPtNBz/Rinst629f32464a21/SRAdb/extdata/SRAmetadb_demo.sqlite	4534272	
	isdir	
/tmp/RtmpRPtNBz/Rinst629f32464a21/SRAdb/extdata/SRAmetadb_demo.sqlite	FALSE	
	mode	
/tmp/RtmpRPtNBz/Rinst629f32464a21/SRAdb/extdata/SRAmetadb_demo.sqlite	755	
		mtim
/tmp/RtmpRPtNBz/Rinst629f32464a21/SRAdb/extdata/SRAmetadb_demo.sqlite	2018-05-11 19:07:0	
		ctim
/tmp/RtmpRPtNBz/Rinst629f32464a21/SRAdb/extdata/SRAmetadb_demo.sqlite	2018-05-11 19:07:0	
		atim
/tmp/RtmpRPtNBz/Rinst629f32464a21/SRAdb/extdata/SRAmetadb_demo.sqlite	2018-05-11 19:07:0	
	uid	
/tmp/RtmpRPtNBz/Rinst629f32464a21/SRAdb/extdata/SRAmetadb_demo.sqlite	1005	
	gid	
/tmp/RtmpRPtNBz/Rinst629f32464a21/SRAdb/extdata/SRAmetadb_demo.sqlite	1005	
	uname	
/tmp/RtmpRPtNBz/Rinst629f32464a21/SRAdb/extdata/SRAmetadb_demo.sqlite	biocbuild	
	grname	
/tmp/RtmpRPtNBz/Rinst629f32464a21/SRAdb/extdata/SRAmetadb_demo.sqlite	biocbuild	

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.

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

```
[1] "col_desc"      "experiment"
[3] "fastq"         "metaInfo"
[5] "run"           "sample"
[7] "sra"           "sra_ft"
[9] "sra_ft_content" "sra_ft_segdir"
[11] "sra_ft_segments" "study"
[13] "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. 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	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 field name, type, description 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	study_accession
1	865	DRP002494	DRP002494
2	2297	DRP002820	DRP002820
3	2403	DRP002612	DRP002612

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	study_title
1	DRP002494	
2	DRP002820	
3	DRP002612	
1		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)
+   return(dbGetQuery(conn,sql)[1,1])
+ }
> do.call(rbind,sapply(sra_tables[c(2,4,5,11,12)],
+   getTableCounts, sra_con, simplify=FALSE))

              [,1]
experiment      712
metaInfo         2
run             756
sra_ft_segments 186
study           31
```

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      Other      15
2 Transcriptome Analysis 11
3      <NA>       2
4 Whole Genome Sequencing 2
5      Metagenomics   1
```

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	Experiments
1	<NA>	430
2	Illumina HiSeq 2000	197
3	Illumina Genome Analyzer II	29
4	NextSeq 500	11
5	Illumina HiSeq 1500	10
6	Illumina HiSeq 2500	10
7	Illumina Genome Analyzer	8
8	454 GS FLX Titanium	6
9	454 GS FLX	4
10	454 GS FLX+	3
11	Illumina MiSeq	3
12	unspecified	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	<NA>	430
2	RNA-Seq	215
3	OTHER	32
4	WGS	17
5	WXS	11
6	RIP-Seq	4
7	FL-cDNA	2
8	EST	1

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_demo.sqlite`:


```
> conversion <- sraConvert( c('SRP001007','SRP000931'), sra_con = sra_con )
> conversion[1:3,]
```

```
      study submission      sample experiment
1 SRP000931  SRA009053 SRS003458  SRX006127
2 SRP000931  SRA009053 SRS003455  SRX006124
3 SRP000931  SRA009053 SRS003462  SRX006133
      run
1 SRR018261
2 SRR018258
3 SRR018267
```

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

```
$experiment
```

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

```
$run
```

```
[1] "SRR018261" "SRR018258" "SRR018267"
[4] "SRR018265" "SRR018260" "SRR018262"
[7] "SRR018268" "SRR018263" "SRR018259"
[10] "SRR018266" "SRR018256" "SRR018269"
[13] "SRR018257" "SRR018264" "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] 487  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
      487       104
study_type platform
      5         2
instrument_model
      4

>
```

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] 487  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] 12 10
```

Find all submissions by GEO:

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

```
[1] 8 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] 4 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/ByRun/sra/SRR/SRR000/SRR000648/
2 ftp://ftp-trace.ncbi.nlm.nih.gov/sra/sra-instant/reads/ByRun/sra/SRR/SRR000/SRR000649/
3 ftp://ftp-trace.ncbi.nlm.nih.gov/sra/sra-instant/reads/ByRun/sra/SRR/SRR000/SRR000650/
  experiment      study      sample      run
1 SRX000122 SRP000098 SRS000290 SRR000648
2 SRX000122 SRP000098 SRS000290 SRR000649
3 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' )
```

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.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

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"), 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 -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 remote 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'

```
> 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)
>
```

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 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

- R version 3.5.0 (2018-04-23), 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 16.04.4 LTS
- Matrix products: default
- BLAS: /home/biocbuild/bbs-3.7-bioc/R/lib/libRblas.so
- LAPACK: /home/biocbuild/bbs-3.7-bioc/R/lib/libRlapack.so
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, utils
- Other packages: BiocGenerics 0.26.0, RCurl 1.95-4.10, RSQLite 2.1.1, SRAdb 1.42.1, bitops 1.0-6, graph 1.58.0

- Loaded via a namespace (and not attached): Biobase 2.40.0, DBI 1.0.0, GEOquery 2.48.0, R6 2.2.2, Rcpp 0.12.16, assertthat 0.2.0, bindr 0.1.1, bindrcpp 0.2.2, bit 1.1-12, bit64 0.9-7, blob 1.1.1, compiler 3.5.0, digest 0.6.15, dplyr 0.7.4, glue 1.2.0, hms 0.4.2, limma 3.36.1, magrittr 1.5, memoise 1.1.0, pillar 1.2.2, pkgconfig 2.0.1, purrr 0.2.4, readr 1.1.1, rlang 0.2.0, stats4 3.5.0, tibble 1.4.2, tidyr 0.8.0, tools 3.5.0, xml2 1.2.0