restfulSE – experiments with HDF5 server content wrapped in SummarizedExperiment

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This R package includes proof-of-concept code illustrating several approaches to SummarizedExperiment design with assays stored out-of-memory.

1.1 HDF5 server backed SummarizedExperiment

HDF Server "extends the HDF5 data model to efficiently store large data objects (e.g. up to multi-TB data arrays) and access them over the web using a RESTful API." In this `restfulSE` package, several data structures are introduced

- to model the server data architecture and
- to perform targeted extraction of numerical data from HDF5 arrays stored on the server.

We maintain, thanks to a grant from the National Cancer Institute, the server http://h5s.channingremotedata.org:5000/. Visit this URL to get a flavor of the server structure: datasets, groups, and datatypes are high-level elements to be manipulated to work with data values from the server.

1.1.1 Illustration with 10x genomics 1.3 million neurons

We used Martin Morgan's TENxGenomics package to transform the sparse-formatted HDF5 supplied by 10x into a dense HDF5 matrix to support natural slicing. Thanks to native compression in HDF5, the data volume expansion is modest.

A helper function in the restfulSE package creates a `RESTfulSummarizedExperiment` instance that points to the full numerical dataset.

```
library(restfulSE)
my10x = se1.3M()
## analyzing groups for their links...
## done
## snapshotDate(): 2018-04-27
## see ?restfulSEData and browseVignettes('restfulSEData') for documentation
## downloading 0 resources
## loading from cache
##     '/home/biocbuild//.ExperimentHub/1656'
my10x
## class: SummarizedExperiment
## dim: 27998 1306127
## metadata(0):
## assays(1): counts
## rownames(27998): ENSMUSG00000051951 ENSMUSG00000089699 ...
##   ENSMUSG00000096730 ENSMUSG00000095742
## rowData names(12): ensid seqnames ... symbol entrezid
## colnames(1306127): AAACCTGAGATAGGAG-1 AAACCTGAGCGGCTTC-1 ...
##   TTTGTCAGTTAAAGTG-133 TTTGTCATCTGAAAGA-133
## colData names(4): Barcode Sequence Library Mouse
```

As an exercise, we acquire the ENSEMBL identifiers for mouse genes annotated to hippocampus development, which has GO ID GO:0021766, and check counts for 10 genes on 6 samples:

```
library(org.Mm.eg.db)
##
hippdev = select(org.Mm.eg.db,
    keys="GO:0021766", keytype="GO", column="ENSEMBL")$ENSEMBL
## 'select()' returned 1:many mapping between keys and columns
hippdev = intersect(hippdev, rownames(my10x))
unname(assay(my10x[ hippdev[1:10], 10001:10006]))
## <10 x 6> DelayedMatrix object of type "double":
##       [,1] [,2] [,3] [,4] [,5] [,6]
##  [1,]    0    0    0    0    0    0
##  [2,]    0    0    0    0    0    0
##  [3,]    0    0    0    1    0    0
##  [4,]    0    1    2    6    5    0
##  [5,]    0    0    0    0    0    0
##  [6,]    1    2    4    8    7    3
##  [7,]    0    0    0    0    0    0
##  [8,]    0    0    0    0    0    2
##  [9,]    0    0    0    0    0    0
## [10,]    3    0    3    0    1    9
```

The result:

```
      [,1] [,2] [,3] [,4] [,5] [,6]
 [1,]    0    0    0    0    0    0
 [2,]    0    0    0    0    0    0
 [3,]    0    0    0    1    0    0
 [4,]    0    1    2    6    5    0
 [5,]    0    0    0    0    0    0
 [6,]    1    2    4    8    7    3
 [7,]    0    0    0    0    0    0
 [8,]    0    0    0    0    0    2
 [9,]    0    0    0    0    0    0
[10,]    3    0    3    0    1    9
```

1.1.2 Illustration with GTEx tissue expression

We exported the content of the recount2 GTEx gene-level quantifications to our HDF5 server. A convenience function is available:

```
tiss = gtexTiss()
## analyzing groups for their links...
## done
## snapshotDate(): 2018-04-27
## see ?restfulSEData and browseVignettes('restfulSEData') for documentation
## downloading 0 resources
## loading from cache
##     '/home/biocbuild//.ExperimentHub/556'
tiss
## class: RangedSummarizedExperiment
## dim: 58037 9662
## metadata(0):
## assays(1): recount
## rownames(58037): ENSG00000000003.14 ENSG00000000005.5 ...
##   ENSG00000283698.1 ENSG00000283699.1
## rowData names(3): gene_id bp_length symbol
## colnames(9662): SRR660824 SRR2166176 ... SRR612239 SRR615898
## colData names(82): project sample ... title characteristics
```

We'll use this remote data as a tool for investigating transcriptional patterns in brain anatomy. We can identify the samples from brain using the 'smtsd' colData element:

```
binds = grep("Brain", tiss$smtsd)
table(tiss$smtsd[binds][1:100]) # check diversity in 100 samples
##
##                           Brain - Amygdala
##                                          4
##  Brain - Anterior cingulate cortex (BA24)
##                                          5
##           Brain - Caudate (basal ganglia)
##                                         10
##             Brain - Cerebellar Hemisphere
##                                          9
##                        Brain - Cerebellum
##                                         13
##                            Brain - Cortex
##                                         13
##              Brain - Frontal Cortex (BA9)
##                                         10
##                       Brain - Hippocampus
##                                          8
##                      Brain - Hypothalamus
##                                          5
## Brain - Nucleus accumbens (basal ganglia)
##                                          7
##           Brain - Putamen (basal ganglia)
##                                          4
##        Brain - Spinal cord (cervical c-1)
##                                          3
##                  Brain - Substantia nigra
##                                          9
```

We'll identify genes annotated to neurotrophic functions using another convenience function in this package:

```
ntgenes = goPatt(termPattern="neurotroph")
## 'select()' returned 1:1 mapping between keys and columns
## 'select()' returned 1:many mapping between keys and columns
head(ntgenes)
##           GO EVIDENCE ONTOLOGY         ENSEMBL SYMBOL
## 1 GO:0004897      IDA       MF ENSG00000122756  CNTFR
## 2 GO:0004897      IMP       MF ENSG00000160712   IL6R
## 3 GO:0004897      IDA       MF ENSG00000134352  IL6ST
## 4 GO:0004897      IDA       MF ENSG00000113594   LIFR
## 5 GO:0043121      ISS       MF ENSG00000064300   NGFR
## 6 GO:0043121      IBA       MF ENSG00000198400  NTRK1
```

2 Some details

2.1 Motivation

Extensive human and computational effort is expended on downloading and managing large genomic data at site of analysis. Interoperable formats that are accessible via generic operations like those in RESTful APIs may help to improve cost-effectiveness of genome-scale analyses.

In this report we examine the use of HDF5 server as a back end for assay data, mediated through the RangedSummarizedExperiment API for interactive use.

A modest server configured to deliver HDF5 content via a RESTful API has been prepared and is used in this vignette.

2.2 Executive summary

We want to provide rapid access to array-like data. We'll work with the Banovich 450k data as there is a simple check against an in-memory representation.

```
library(restfulSE)
bigec2 = H5S_source("http://h5s.channingremotedata.org:5000")
## analyzing groups for their links...
## done
bigec2
## H5serv server url :  http://h5s.channingremotedata.org:5000
##  There are 2 groups.
##  Use groups(), links(), ..., to probe and access metadata.
##  Use dsmeta() to get information on datasets within groups.
##  Use [[ [dsname] ]]  to get a reference suitable for [i, j] subsetting.
dsmeta(bigec2)[1:2,] # two groups
## DataFrame with 2 rows and 3 columns
##    groupnum                            dsnames
##   <integer>                    <CharacterList>
## 1         1 tenx_400k_sorted,mike,darmgcls,...
## 2         2                        tall.public
##                               grp.uuid
##                            <character>
## 1 9daeb1ae-c279-11e8-b09f-1678ea0f979a
## 2 9daee0d4-c279-11e8-b09f-1678ea0f979a
dsmeta(bigec2)[1,2][[1]] # all dataset candidates in group 1
##  [1] "tenx_400k_sorted" "mike"             "darmgcls"
##  [4] "test3darray"      "tabulamuris"      "neurons400k"
##  [7] "tenx_full"        "tissues"          "assays"
## [10] "patelGBMSC"       "neurons100k"      "tenx_100k_sorted"
```

We use double-bracket subscripting to grab a reference to a dataset from an H5S source.

```
banref = bigec2[["assays"]] # arbitrary name assigned long ago
banref
## H5S_dataset instance:
##   dsname intl.dim1 intl.dim2              created      type.base
## 1 assays        64    329469 2017-04-05T18:02:37Z H5T_IEEE_F64LE
```

We build a SummarizedExperiment by combining an assay-free RangedSummarizedExperiment with this reference.

```
ehub = ExperimentHub::ExperimentHub()
## snapshotDate(): 2018-04-27
tag = names(AnnotationHub::query(ehub, "banoSEMeta"))
banoSE = ehub[[tag[1]]]
## see ?restfulSEData and browseVignettes('restfulSEData') for documentation
## downloading 0 resources
## loading from cache
##     '/home/biocbuild//.ExperimentHub/551'
ds = H5S_Array("http://h5s.channingremotedata.org:5000", "assays")
## analyzing groups for their links...
## done
assays(banoSE) = SimpleList(betas=ds)
banoSE
## class: RangedSummarizedExperiment
## dim: 329469 64
## metadata(0):
## assays(1): betas
## rownames(329469): cg00000029 cg00000165 ... ch.9.98989607R
##   ch.9.991104F
## rowData names(10): addressA addressB ... probeEnd probeTarget
## colnames(64): NA18498 NA18499 ... NA18489 NA18909
## colData names(35): title geo_accession ... data_row_count naid
```

We can update the SummarizedExperiment metadata through subsetting operations, and then extract the relevant assay data. The data are retrieved from the remote server with the `assay` method.

```
rbanoSub = banoSE[5:8, c(3:9, 40:50)]
## Loading required package: Biostrings
## Loading required package: XVector
##
## Attaching package: 'Biostrings'
## The following object is masked from 'package:DelayedArray':
##
##     type
## The following object is masked from 'package:base':
##
##     strsplit
assay(rbanoSub)
## <4 x 18> DelayedMatrix object of type "double":
##                 NA18501      NA18502      NA18516 ...      NA19138
## cg00000363  0.325433263  1.377820005  0.596699897   .  0.966695669
## cg00000622  0.003436888 -0.668499289 -1.210634762   .  0.076062477
## cg00000714 -1.184443665 -1.654047967 -0.174729357   .  0.325742947
## cg00000734  0.153831565 -1.299289359  1.903976827   .  1.185320424
##                 NA19140
## cg00000363  1.203765271
## cg00000622  0.958031578
## cg00000714 -0.008202908
## cg00000734  0.319937329
```

2.3 10xGenomics examples

2.3.1 t-SNE for a set of genes annotated to hippocampus

We have used Martin Morgan's TENxGenomics package to create a dense HDF5 representation of the assay data, and placed it on the `bigec2` server. The metadata are available as `se100k` in this package; we have used EnsDb.Mmusculus.v79 to supply gene ranges where available; genes reported but without addresses are addressed at chr1:2 with width 0. The rows are sorted by genomic address within chromosomes.

```
tenx100k = se100k()
## analyzing groups for their links...
## done
## snapshotDate(): 2018-04-27
## see ?restfulSEData and browseVignettes('restfulSEData') for documentation
## downloading 0 resources
## loading from cache
##     '/home/biocbuild//.ExperimentHub/552'
tenx100k
## class: RangedSummarizedExperiment
## dim: 27998 100000
## metadata(1): source
## assays(1): counts
## rownames(27998): ENSMUSG00000109048 ENSMUSG00000109510 ...
##   ENSMUSG00000096768 ENSMUSG00000096850
## rowData names(6): gene_id gene_name ... seq_coord_system symbol
## colnames(100000): AAACCTGAGATAGGAG-1 AAACCTGAGCGGCTTC-1 ...
##   GACGTTAGTCATACTG-11 GACGTTAGTCCGTGAC-11
## colData names(4): Barcode Sequence Library Mouse
```

We will subset genes annotated to hippocampus development. Here are some related categories:

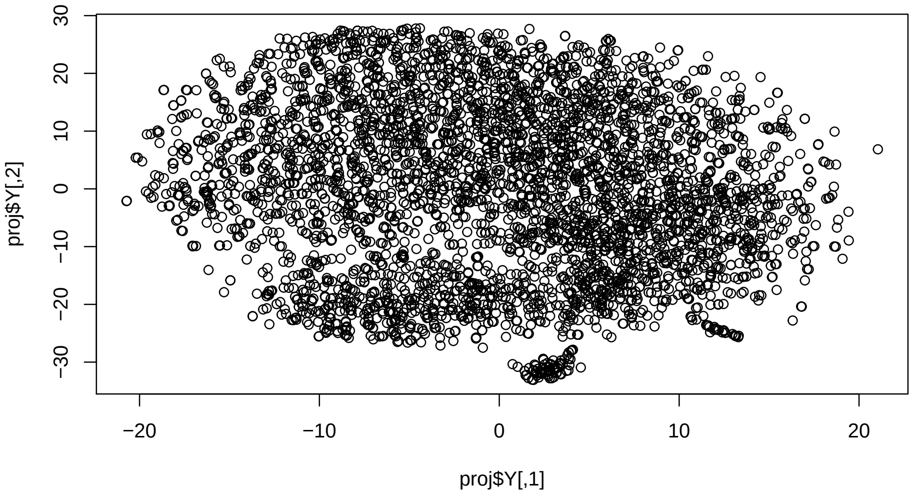
```
12092 GO:0021766                      hippocampus development
12096 GO:0021770            parahippocampal gyrus development
34609 GO:0097410      hippocampal interneuron differentiation
34631 GO:0097432 hippocampal pyramidal neuron differentiation
34656 GO:0097457                      hippocampal mossy fiber
35169 GO:0098686       hippocampal mossy fiber to CA3 synapse
42398 GO:1990026            hippocampal mossy fiber expansion
```

```
library(org.Mm.eg.db)
atab = select(org.Mm.eg.db, keys="GO:0021766", keytype="GO", columns="ENSEMBL")
## 'select()' returned 1:many mapping between keys and columns
hg = atab[,"ENSEMBL"]
length(hgok <- intersect(hg, rownames(tenx100k)))
## [1] 50
```

This is a very scattered collection of rows in the matrix. We acquire expression measures for genes annotated to hippocampus on 4000 samples. t-SNE is then used to project the log-transformed measures to the plane.

```
hipn = assay(tenx100k[hgok,1:4000])  # slow
d = dist(t(log(1+hipn)), method="manhattan")
proj = Rtsne(d)
```

```
plot(proj$Y)
```



2.3.2 A set of genes related to the visual cortex

Tasic et al. (Nature neuro 2016, DOI 10.1038/nn.4216) describe single cell analysis of the adult murine brain, identify clusters of cells with distinct transcriptional profiles and anatomic location, and enumerate lists of genes that discriminate these clusters. The tasicST6 DataFrame provides details.

```
#data("tasicST6", package = "restfulSEData")
ehub = ExperimentHub::ExperimentHub()
## snapshotDate(): 2018-04-27
tag = names(AnnotationHub::query(ehub, "tasicST6"))
tasicST6 = ehub[[tag[1]]]
## see ?restfulSEData and browseVignettes('restfulSEData') for documentation
## downloading 0 resources
## loading from cache
##     '/home/biocbuild//.ExperimentHub/557'
tasicST6
```

```
## DataFrame with 49 rows and 4 columns
##            clid     txtype1        txtype2
##     <character> <character>    <character>
## 1           f01         Vip         Mybpc1
## 2           f02         Vip          Parm1
## 3           f03         Vip           Sncg
## 4           f04         Vip           Chat
## 5           f05         Vip           Gpc3
## ...         ...         ...            ...
## 45          f45       Oligo 9630013A20Rik
## 46          f46       Oligo         Opalin
## 47          f47       Micro           Ctss
## 48          f48        Endo            Xdh
## 49          f49         SMC           Myl9
##
##
## 1
## 2
## 3
## 4
## 5
## ...
## 45                                                              c("Brca1", "Rnf122", "Mbp", "Zcchc12", "E
## 46
## 47                                         c("Cx3cr1", "C1qb", "Cd53", "Csf1r", "Itgam", "Abi3", "C1qa", "
## 48                                        c("Tbc1d4", "AI467606", "Exosc7", "Eltd1", "Fas", "Hmgcs2", "No
## 49  c("Bgn", "Nupr1", "Casq2", "Mylk", "Gprc5c", "Slc38a11", "Slc6a20a", "Pcolce", "Vtn", "Cnn2", "Nid1",
```

Key high-level discrimination concerns cells regarded as GABAergic vs. glutamatergic (inhibitory vs excitatory neurotransmission).

2.4 Background

Banovich et al. published a subset of DNA methylation measures assembled on 64 samples of immortalized B-cells from the YRI HapMap cohort.

```
library(restfulSE)
#data("banoSEMeta", package = "restfulSEData")
ehub = ExperimentHub::ExperimentHub()
## snapshotDate(): 2018-04-27
tag = names(AnnotationHub::query(ehub, "banoSEMeta"))
banoSEMeta = ehub[[tag[1]]]
## see ?restfulSEData and browseVignettes('restfulSEData') for documentation
## downloading 0 resources
## loading from cache
##     '/home/biocbuild//.ExperimentHub/551'
banoSEMeta
## class: RangedSummarizedExperiment
## dim: 329469 64
## metadata(0):
```

```
## assays(0):
## rownames(329469): cg00000029 cg00000165 ... ch.9.98989607R
##   ch.9.991104F
## rowData names(10): addressA addressB ... probeEnd probeTarget
## colnames(64): NA18498 NA18499 ... NA18489 NA18909
## colData names(35): title geo_accession ... data_row_count naid
```

The numerical data have been exported using H. Pages' saveHDF5SummarizedExperiment applied to the banovichSE SummarizedExperiment in the yriMulti package. The HDF5 component is simply copied into the server data space on the remote server.

2.5 Hierarchy of server resources

2.5.1 Server

Given the URL of a server running HDF5 server, we create an instance of `H5S_source`:

```
mys = H5S_source(serverURL="http://h5s.channingremotedata.org:5000")
## analyzing groups for their links...
## done
mys
## H5serv server url :  http://h5s.channingremotedata.org:5000
##  There are 2 groups.
##  Use groups(), links(), ..., to probe and access metadata.
##  Use dsmeta() to get information on datasets within groups.
##  Use [[ [dsname] ]]  to get a reference suitable for [i, j] subsetting.
```

2.5.2 Groups

The server identifies a collection of 'groups'. For the server we are working with, only one group, at the root, is of interest.

```
groups(mys)
## DataFrame with 2 rows and 2 columns
##                                  groups    nlinks
##                             <character> <integer>
## 1 9daeb1ae-c279-11e8-b09f-1678ea0f979a        13
## 2 9daee0d4-c279-11e8-b09f-1678ea0f979a         1
```

2.5.3 Links for a group

There is a class to hold the link set for any group:

```
lin1 = rhdf5client::links(mys,1)
lin1
## HDF5 server link set for group 9daeb1ae-c279-11e8-b09f-1678ea0f979a
##  There are 13 links.
##  Use targets([linkset]) to extract target URLs.
```