restfulSE – experiments with HDF5 server content wrapped in SummarizedExperiment

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```
## Warning: replacing previous import 'utils::findMatches' by
## 'S4Vectors::findMatches' when loading 'AnnotationDbi'
```

1 restfulSE

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

1.1 HSDS-backed SummarizedExperiment

Several data structures are introduced

- to model the HDF Scalable Data Service (HSDS) server data architecture and
- to perform targeted extraction of numerical data from HDF5 arrays stored on the server.

We work with HDF Object store (https://www.hdfgroup.org/solutions/hdf-cloud/).

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()
## see ?restfulSEData and browseVignettes('restfulSEData') for documentation
## loading from cache
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]))
```

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()
## see ?restfulSEData and browseVignettes('restfulSEData') for documentation
## loading from cache
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      IBA       MF ENSG00000122756  CNTFR
## 2 GO:0004897      IDA       MF ENSG00000122756  CNTFR
## 3 GO:0004897      IMP       MF ENSG00000160712   IL6R
## 4 GO:0004897      IDA       MF ENSG00000134352  IL6ST
## 5 GO:0004897      IDA       MF ENSG00000113594   LIFR
## 6 GO:0005030      IEA       MF ENSG00000151892  GFRA1
```

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.

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

```
ehub = ExperimentHub::ExperimentHub()
tag = names(AnnotationHub::query(ehub, "banoSEMeta"))
banoSE = ehub[[tag[1]]]
## see ?restfulSEData and browseVignettes('restfulSEData') for documentation
## loading from cache
ds = HSDSArray(endpoint=URL_hsds(),svrtype="hsds",
               domain="/shared/bioconductor/bano_meQTLex.h5",dsetname="/assay001")
assays(banoSE, withDimnames=FALSE) = 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)]
assay(rbanoSub)
## <4 x 18> matrix of class DelayedMatrix and type "double":
##                NA18501      NA18502      NA18516 ...      NA19138      NA19140
## cg00000363  0.325433263  1.377820005  0.596699897   .  0.966695669  1.203765271
## cg00000622  0.003436888 -0.668499289 -1.210634762   .  0.076062477  0.958031578
## cg00000714 -1.184443665 -1.654047967 -0.174729357   .  0.325742947 -0.008202908
## cg00000734  0.153831565 -1.299289359  1.903976827   .  1.185320424  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 in HSDS.

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
```

Oct 2022: Following code needs revision.

```
library(org.Mm.eg.db)
atab = select(org.Mm.eg.db, keys="GO:0021766", keytype="GO", columns="ENSEMBL")
hg = atab[,"ENSEMBL"]
ehub = ExperimentHub::ExperimentHub()
lkst100k = AnnotationHub::query(ehub, "st100k")
tenx100k = ehub[[names(lkst100k)]]
length(hgok <- intersect(hg, rownames(tenx100k)))
```

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()
tag = names(AnnotationHub::query(ehub, "tasicST6"))
tasicST6 = ehub[[tag[1]]]
## see ?restfulSEData and browseVignettes('restfulSEData') for documentation
## loading from cache
tasicST6
## DataFrame with 49 rows and 4 columns
##              clid     txtype1       txtype2                      genes
##         <character> <character>   <character>                    <List>
## 1            f01         Vip       Mybpc1    Crispld2,Cxcl14,Tpm2,...
## 2            f02         Vip        Parm1          Cxcl14,Car4,Tac2
## 3            f03         Vip         Sncg    Reln,Npy2r,Tnfaip8l3,...
## 4            f04         Vip         Chat     Aebp1,Slc18a3,Pvrl4,...
## 5            f05         Vip         Gpc3       Bcar3,Mab21l1,Pbx3,...
## ...         ...         ...          ...                        ...
## 45           f45       Oligo 9630013A20Rik        Brca1,Rnf122,Mbp,...
## 46           f46       Oligo        Opalin         Mbp,Mog,Aspa,...
## 47           f47       Micro         Ctss      Cx3cr1,C1qb,Cd53,...
## 48           f48        Endo          Xdh Tbc1d4,AI467606,Exosc7,...
## 49           f49         SMC         Myl9       Bgn,Nupr1,Casq2,...
```

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)
ehub = ExperimentHub::ExperimentHub()
tag = names(AnnotationHub::query(ehub, "banoSEMeta"))
banoSEMeta = ehub[[tag[1]]]
## see ?restfulSEData and browseVignettes('restfulSEData') for documentation
## loading from cache
banoSEMeta
## 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
```

The numerical data have been exported using H. Pages' saveHDF5SummarizedExperiment applied to the banovichSE SummarizedExperiment in the yriMulti package. The HDF5 component is imported to HSDS with the hsload utility disributed with the python package h5pyd.