

# restfulSE – experiments with HDF5 server content wrapped in SummarizedExperiment

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# 1 restfulSE

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This R package includes proof-of-concept code illustrating several approaches to Summarized-Experiment 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 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()
## snapshotDate(): 2020-10-02
## see ?restfulSEData and browseVignettes('restfulSEData') for documentation
## loading from cache
my10x
## class: SummarizedExperiment
## dim: 27998 1306127
## metadata(0):
## assays(1): counts
## rownames(27998): ENSMUSG000000051951 ENSMUSG000000089699 ...
##   ENSMUSG000000096730 ENSMUSG000000095742
## rowData names(12): ensid seqnames ... symbol entrezid
## colnames(1306127): AACCTGAGATAGGAG-1 AACCTGAGCGGCTTC-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
```

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```
hippdev = intersect(hippdev, rownames(my10x))
#unnname(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()
## snapshotDate(): 2020-10-02
## 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
```

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```
##          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      IBA          MF ENSG00000198400  NTRK1
```

## 2 Some details

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### 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()
## snapshotDate(): 2020-10-02
tag = names(AnnotationHub::query(ehub, "banoSEMeta"))
banoSE = ehub[[tag[1]]]
## see ?restfulSEData and browseVignettes('restfulSEData') for documentation
## loading from cache
ds = HSDSArray(endpoint=URL_hsd(),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)]
## Loading required package: Biostrings
## Loading required package: XVector
##
## Attaching package: 'Biostrings'
## The following object is masked from 'package:base':
##
##   strsplit
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 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()
## snapshotDate(): 2020-10-02
## see ?restfulSEData and browseVignettes('restfulSEData') for documentation
## loading from cache
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 G0:0021766 hippocampus development
12096 G0:0021770 parahippocampal gyrus development
34609 G0:0097410 hippocampal interneuron differentiation
34631 G0:0097432 hippocampal pyramidal neuron differentiation
34656 G0:0097457 hippocampal mossy fiber
35169 G0:0098686 hippocampal mossy fiber to CA3 synapse
42398 G0:1990026 hippocampal mossy fiber expansion
```

```
library(org.Mm.eg.db)
atab = select(org.Mm.eg.db, keys="G0:0021766", keytype="GO", columns="ENSEMBL")
## 'select()' returned 1:many mapping between keys and columns
hg = atab[, "ENSEMBL"]
length(hgok <- intersect(hg, rownames(tenx100k)))
## [1] 61
```

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(): 2020-10-02
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      ReIn,Npy2r,Tnfaip8l3,...
## 4      f04      Vip      Chat      Aebp1,Slc18a3,Pvr14,...
## 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)
#data("banoSEMeta", package = "restfulSEData")
ehub = ExperimentHub::ExperimentHub()
## snapshotDate(): 2020-10-02
tag = names(AnnotationHub::query(ehub, "banoSEMeta"))
banoSEMeta = ehub[[tag[1]]]
## see ?restfulSEData and browseVignettes('restfulSEData') for documentation
## loading from cache
banoSEMeta
```

## restfulSE – experiments with HDF5 server content wrapped in SummarizedExperiment

```
## class: RangedSummarizedExperiment
## dim: 329469 64
## metadata(0):
## assays(1): betas
## rownames(329469): cg000000029 cg000000165 ... 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.