

yriMulti – HapMap YRI population, multias- say interfaces

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1 Introduction

The EBV-transformed B-cells from Yoruban donors are assayed for genotype and various genomic features in a number of prominent studies. This package helps work with relevant datasets and data structures as use cases for MultiAssayExperiment package development. A particular concern is accommodation of distributed genotype data, in this case, based on the 1000 genomes VCF files in an S3 bucket.

2 Basic data resources

2.1 Expression data

We will use the RNA-seq expression data in the *geuvPack* package.

```
library(geuvPack)
data(geuFPKM)
```

```
geuFPKM
## class: RangedSummarizedExperiment
## dim: 23722 462
## metadata(3): MIAME constrHist colDataSource
## assays(1): exprs
## rownames(23722): ENSG00000152931.6 ENSG00000183696.9 ...
##   ENSG00000257337.1 ENSG00000177494.5
## rowData names(18): source type ... tag ccidsid
## colnames(462): HG000096 HG000097 ... NA20826 NA20828
## colData names(35): Source.Name Comment.ENA_SAMPLE. ...
##   Factor.Value.laboratory. popcode
```

2.2 Methylation data

We have added 450k data from Banovich, Lan, McVicker, van de Geijn, Degner, Blischak, Roux, Pritchard, and Gilad (2014) paper to the yriMulti package.

```
library(yriMulti)
data(banovichSE)
```

```
banovichSE
## 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
```

2.3 DnaseI hypersensitivity data

```
library(dsQTL)
if (!exists("DHStop5_hg19")) data(DHStop5_hg19)
```

```
DHStop5_hg19
## class: RangedSummarizedExperiment
## dim: 1465442 70
## metadata(2): MIAME annotation
## assays(1): scores
## rownames(1465442): dhs_chr1_10402 dhs_chr1_10502 ... dhs_chr22_51228236
##   dhs_chr22_51234736
## rowData names(0):
## colnames(70): NA18486 NA18498 ... NA19239 NA19257
## colData names(9): naid one ... male isFounder
```

2.4 Genotype data

We take advantage of a function (`gtpath`) that generates paths to S3-resident VCF from the 1000 genomes project.

```
litvcf = readVcf(gtpath(20),
  param=ScanVcfParam(which=GRanges("20",
    IRanges(3.7e7,3.701e7))), genome="hg19")
```

```
litvcf
## class: CollapsedVCF
## dim: 347 2504
## rowRanges(vcf):
##   GRanges with 5 metadata columns: paramRangeID, REF, ALT, QUAL, FILTER
## info(vcf):
##   DataFrame with 27 columns: CIEND, CIPOS, CS, END, IMPRECISE, MC, MEINFO, M...
## info(header(vcf)):
##           Number Type   Description
## CIEND      2      Integer Confidence interval around END for imprecise...
## CIPOS      2      Integer Confidence interval around POS for imprecise...
## CS         1      String   Source call set.
## END        1      Integer End coordinate of this variant
## IMPRECISE  0      Flag    Imprecise structural variation
## MC         .      String   Merged calls.
## MEINFO     4      String   Mobile element info of the form NAME,START,E...
## MEND       1      Integer Mitochondrial end coordinate of inserted seq...
## MLEN       1      Integer Estimated length of mitochondrial insert
## MSTART     1      Integer Mitochondrial start coordinate of inserted s...
## SVLEN      .      Integer SV length. It is only calculated for structu...
## SVTYPE     1      String   Type of structural variant
## TSD        1      String   Precise Target Site Duplication for bases, i...
## AC         A      Integer Total number of alternate alleles in called ...
## AF         A      Float   Estimated allele frequency in the range (0,1)
```

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```
## NS 1 Integer Number of samples with data
## AN 1 Integer Total number of alleles in called genotypes
## EAS_AF A Float Allele frequency in the EAS populations calc...
## EUR_AF A Float Allele frequency in the EUR populations calc...
## AFR_AF A Float Allele frequency in the AFR populations calc...
## AMR_AF A Float Allele frequency in the AMR populations calc...
## SAS_AF A Float Allele frequency in the SAS populations calc...
## DP 1 Integer Total read depth; only low coverage data wer...
## AA 1 String Ancestral Allele. Format: AA|REF|ALT|IndelTy...
## VT . String indicates what type of variant the line repr...
## EX_TARGET 0 Flag indicates whether a variant is within the ex...
## MULTI_ALLELIC 0 Flag indicates whether a site is multi-allelic
## geno(vcf):
## List of length 1: GT
## geno(header(vcf)):
## Number Type Description
## GT 1 String Genotype
length(colnames(litvcf))
## [1] 2504
length(intersect(colnames(litvcf), colnames(banovichSE)))
## [1] 52
length(intersect(colnames(litvcf), colnames(geuFPKM)))
## [1] 445
length(intersect(colnames(litvcf), colnames(DHStop5_hg19)))
## [1] 59
```

3 Some computations focused on methylation-expression association

The `yriMulti` package is currently a scratch-pad for some integrative infrastructure thoughts.

3.1 Gene-centric selection

With `mexGR`, a `GRanges` instance is formed with methylation scores for CpG near a gene. The assay data are placed in the `mcols`, with one range devoted to the expression measures.

```
m1 = mexGR(banovichSE, geuFPKM, symbol="ORMDL3")
m1
## mexGR instance with 44 metadata columns:
## NA18498, NA18499, ..., NA18909, type
## and 7 ranges
mcols(m1)[1:4,1:4]
## DataFrame with 4 rows and 4 columns
## NA18498 NA18499 NA18502 NA18517
## <numeric> <numeric> <numeric> <numeric>
## cg01482279 -0.592058 -0.177503 -0.00553931 -1.566010
## cg02305874 -0.463355 0.287661 0.55386303 -1.096807
```

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```
## cg04145193 -0.224534 -1.179222 1.09071357 0.453933
## cg12655416 -0.158362 -0.899116 1.02087023 0.539971
table(mcols(m1)$type)
##
## expr meth
## 1 6
```

3.2 DNA-methylation association with expression

`bindelms` computes the regressions of the selected gene's expression values on the methylation scores. We have options to transform the expression value (parameter `ytx` is a function) and can indicate the radius around the gene coding region to search for CpG (parameter `gradius`).

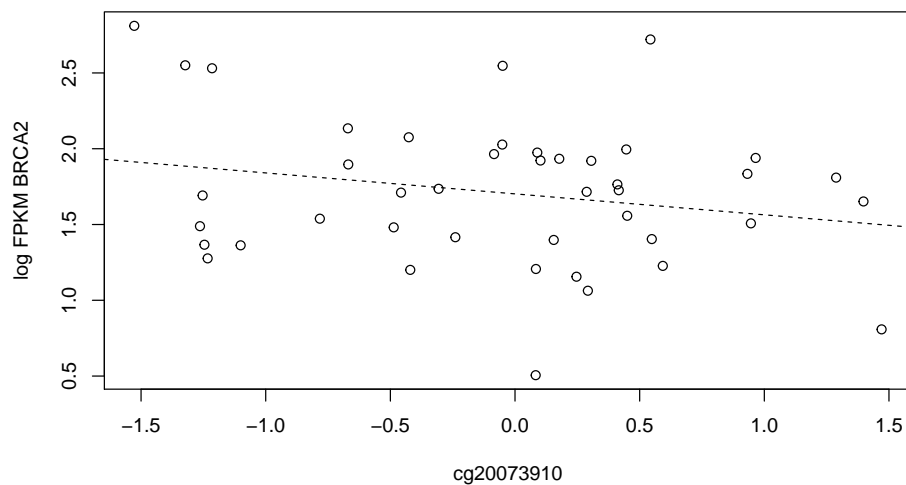
We'll examine a region around gene BRCA2 for a CpG whose methylation score is negatively associated with BRCA2 expression.

```
b1 = bindelms(geuFPKM, banovichSE, symbol="BRCA2", ytx=log,
             radius=20000)
b1
## class: RangedSummarizedExperiment
## dim: 29 43
## metadata(6): theCall symbol ... pwd txexpr
## assays(1): betas
## rownames(29): cg00031759 cg00214044 ... cg26458617 cg26941801
## rowData names(15): addressA addressB ... t p
## colnames(43): NA18498 NA18499 ... NA18489 NA18909
## colData names(35): title geo_accession ... data_row_count naid
mcols(b1)[1:3,]
## DataFrame with 3 rows and 15 columns
##           addressA   addressB channel platform percentGC
##           <character> <character> <Rle>   <Rle> <numeric>
## cg00031759 11602350                Both   HM450    0.50
## cg00214044 33707391                Both   HM450    0.62
## cg00785980 12675375                Both   HM450    0.54
##           sourceSeq probeType probeStart probeEnd
##           <DNAStrngSet> <Rle> <character> <character>
## cg00031759 CGGGTATTTTC...GCATCCCAAC      cg   32889486 32889535
## cg00214044 CGGGCACCAG...ACCCATATTT      cg   32885906 32885955
## cg00785980 GCCCACCTGA...TTCATTCCCG      cg   32984237 32984286
##           probeTarget
##           <numeric>
## cg00031759 32889534
## cg00214044 32885906
## cg00785980 32984237
##
## cg00031759 1.71061243,0.00740907, 0.219326, 1.012209,-0.547747,...,-11.2155822, 0.0360572, -0.7717264,..
## cg00214044 1.7061959,0.0396189, 0.233507, 1.023472,-0.564099,...,-11.215582, 0.188516, -0.542267,..
## cg00785980 1.7112058,-0.0241224, 0.216715, 0.982081,-0.555507,...,-11.215582, -0.138971, -0.583150,..
##           slope          se          t          p
```

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```
##           <numeric> <numeric> <numeric> <numeric>
## cg00031759 0.00740907 0.1006508 0.0736116 0.941677
## cg00214044 0.03961891 0.1027646 0.3855307 0.701837
## cg00785980 -0.02412238 0.0849465 -0.2839713 0.777862
summary(mcols(b1)$t)
##   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
## -1.4738 -0.3809  0.3426  0.2091  0.5894  1.6641
mintind = which.min(mcols(b1)$t)
mincpG = names(b1)[mintind]
mincpG
## [1] "cg20073910"
```

```
plotEvM(b1)
```



4 Using the MultiAssayExperiment infrastructure

4.1 Construction of the MultiAssayExperiment

We will use a unified object design to reproduce the BRCA2 display just obtained.

We need a list of relevant objects and a phenodata component.

```
library(MultiAssayExperiment)
myobjs = list(geuvRNAseq=geuFPKM, yri450k=banovichSE, yriDHS=DHStop5_hg19)
cold = colData(geuFPKM)
suppressWarnings({
  library(MultiAssayExperiment)
  mm = MultiAssayExperiment(myobjs, as.data.frame(cold))
})
mm
## A MultiAssayExperiment object of 3 listed
## experiments with user-defined names and respective classes.
## Containing an ExperimentList class object of length 3:
```

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```
## [1] geuvRNAseq: RangedSummarizedExperiment with 23722 rows and 462 columns
## [2] yri450k: RangedSummarizedExperiment with 329469 rows and 43 columns
## [3] yriDHS: RangedSummarizedExperiment with 1465442 rows and 50 columns
## Features:
## experiments() - obtain the ExperimentList instance
## colData() - the primary/phenotype DFrame
## sampleMap() - the sample availability DFrame
## `$$`, `[`, `[[]` - extract colData columns, subset, or experiment
## *Format() - convert into a long or wide DFrame
## assays() - convert ExperimentList to a SimpleList of matrices
```

4.2 Restriction by range

We compute the BRCA2 'gene range'.

```
library(erma)
brr = range(genemodel("BRCA2"))
## 'select()' returned 1:many mapping between keys and columns
brr
## GRanges object with 1 range and 0 metadata columns:
##      seqnames      ranges strand
##      <Rle>         <IRanges> <Rle>
## [1] chr13 32889617-32973809      +
## -----
## seqinfo: 1 sequence from hg19 genome
```

Subset the multiassay structure to features in the vicinity of this range.

```
.subsetByRanges = function(ma, r) {
  subsetByRow(ma, r)
}
newmm = .subsetByRanges(mm, brr+20000)
newmm
## A MultiAssayExperiment object of 3 listed
## experiments with user-defined names and respective classes.
## Containing an ExperimentList class object of length 3:
## [1] geuvRNAseq: RangedSummarizedExperiment with 3 rows and 462 columns
## [2] yri450k: RangedSummarizedExperiment with 29 rows and 43 columns
## [3] yriDHS: RangedSummarizedExperiment with 32 rows and 50 columns
## Features:
## experiments() - obtain the ExperimentList instance
## colData() - the primary/phenotype DFrame
## sampleMap() - the sample availability DFrame
## `$$`, `[`, `[[]` - extract colData columns, subset, or experiment
## *Format() - convert into a long or wide DFrame
## assays() - convert ExperimentList to a SimpleList of matrices
```

We now have all the relevant features and samples. In fact we have more genes than we really wanted. But we will proceed with this selection.

4.3 All pairwise regressions

We will introduce a formula idiom to specify a collection of models of interest.

```

library(doParallel)
## Loading required package: foreach
## Loading required package: iterators
registerDoSEQ()
allLM_pw = function(fmla, mae, xtx=force, ytx=force) {
#
# formula specifies dependent and independent assays
# form all regressions of ytx(dep) on xtx(indep) for all
# pairs of dependent and independent variables defined by
# assays for samples held in common
#
lf = as.list(fmla)
nms = lapply(lf, as.character)
yel = experiments(mae)[[nms[[2]]]]
xel = experiments(mae)[[nms[[3]]]]
sy = colnames(yel)
sx = colnames(xel)
sb = intersect(sy,sx)
yel = yel[,sb]
xel = xel[,sb]
vdf = as.matrix(expand.grid( rownames(yel),
                             rownames(xel), stringsAsFactors=FALSE ))
allf = apply(vdf, 1, function(x) as.formula(paste(x, collapse="~")))
alllm = foreach (i = 1:length(allf)) %dopar% {
  df = data.frame(ytx(assay(yel)[vdf[i,1],]), xtx(assay(xel)[vdf[i,2],]))
  names(df) = vdf[i,]
  lm(allf[[i]], data=df)
}
names(alllm) = apply(vdf,1,function(x) paste(x, collapse="~"))
allts = lapply(alllm, function(x) summary(x)$coef[2, "t value"])
list(mods=alllm, tslopes=allts)
}
pwplot = function(fmla1, fmla2, mae, ytx=force, xtx=force, ...) {
#
# use fmla1 with assays as components to identify
# two assays to regard as sources of y and x
# fmla2 indicates which features to plot
#
lf = as.list(fmla1)
nms = lapply(lf, as.character)
yel = experiments(mae)[[nms[[2]]]]
xel = experiments(mae)[[nms[[3]]]]
sy = colnames(yel)
sx = colnames(xel)
sb = intersect(sy,sx)
yel = yel[,sb]
xel = xel[,sb]

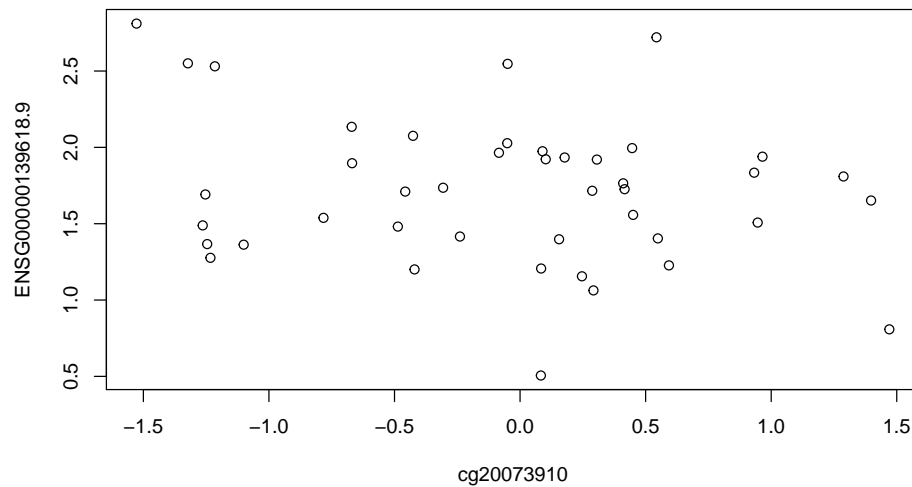
```


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```
## Warning in ytx(assay(yel)[vdf[i, 1], ]): NaNs produced
## Warning in ytx(assay(yel)[vdf[i, 1], ]): NaNs produced
## Warning in ytx(assay(yel)[vdf[i, 1], ]): NaNs produced
## Warning in ytx(assay(yel)[vdf[i, 1], ]): NaNs produced
## Warning in ytx(assay(yel)[vdf[i, 1], ]): NaNs produced
## Warning in ytx(assay(yel)[vdf[i, 1], ]): NaNs produced
## Warning in ytx(assay(yel)[vdf[i, 1], ]): NaNs produced
names(pp)
## [1] "mods"      "tslopes"
summary(pp[[1]][[1]])
##
## Call:
## lm(formula = allf[[i]], data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.20112 -0.30856  0.00553  0.24330  1.10330
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  1.710612   0.074777  22.876  <2e-16 ***
## cg00031759   0.007409   0.100651   0.074   0.942
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.4898 on 41 degrees of freedom
## Multiple R-squared:  0.0001321, Adjusted R-squared: -0.02425
## F-statistic: 0.005419 on 1 and 41 DF,  p-value: 0.9417
which.min(unlist(pp[[2]])) # not BRCA2 but FRY
## ENSG00000073910.13~cg05918473
##
##              23
```

The formula idiom can be used to isolate assays and features.

```
pwplot(geuvRNAseq~yri450k, ENSG00000139618.9~cg20073910, newmm, ytx=log)
```



4.4 Integrating dense genotypes in a VcfStack instance

We set up a reference to a collection of VCF. We'll use 1000 genomes VCF for chr21, and chr22. At present (16 Oct 2016) chrY must be kept out of the stack as it does not have the full set of samples for other chromosomes.

```
library(gQTLstats)
library(VariantAnnotation)
library(GenomicFiles)
pa = paths1kg(paste0("chr", c(21:22))) #, "Y"))
sn = vcfSamples(scanVcfHeader(TabixFile(pa[1])))
library(Homo.sapiens) # necessary?
stopifnot(requireNamespace("GenomeInfoDb")) # indexVcf with S3 bucket? ->
ob = RangedVcfStack(VcfStack2(pa, seqinfo(Homo.sapiens)))
cd = DataFrame(id1kg=sn)
rownames(cd) = sn
colData(ob) = cd
```

Now we set up a region of interest and bind it to the stack. This yields an instance of Ranged VcfStack, for which we have `samples`, `features`, and `assay` methods defined in `gQTLstats`.

```
myr = GRanges("chr22", IRanges(20e6, 20.01e6))
rowRanges(ob) = myr
colData(ob) = DataFrame(colData(ob), zz=runif(nrow(colData(ob))))
hasInternetConnectivity = function()
  !is.null(nsl("www.r-project.org"))
#if (hasInternetConnectivity()) lka = assay(ob)
myobs = list(geuvRNAseq=geuFPKM, yri450k=banovichSE, yriDHS=DHStop5_hg19,
  yriGeno=ob)
suppressWarnings({
mm = MultiAssayExperiment(myobs)
})
mm
## A MultiAssayExperiment object of 4 listed
## experiments with user-defined names and respective classes.
```

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```
## Containing an ExperimentList class object of length 4:  
## [1] geuvRNAseq: RangedSummarizedExperiment with 23722 rows and 462 columns  
## [2] yri450k: RangedSummarizedExperiment with 329469 rows and 64 columns  
## [3] yriDHS: RangedSummarizedExperiment with 1465442 rows and 70 columns  
## [4] yriGeno: RangedVcfStack with 2 rows and 2504 columns  
## Features:  
## experiments() - obtain the ExperimentList instance  
## colData() - the primary/phenotype DFrame  
## sampleMap() - the sample availability DFrame  
## `$`, `[`, `[[]` - extract colData columns, subset, or experiment  
## *Format() - convert into a long or wide DFrame  
## assays() - convert ExperimentList to a SimpleList of matrices
```

References

[1] N. E. Banovich, X. Lan, G. McVicker, et al. "Methylation QTLs Are Associated with Coordinated Changes in Transcription Factor Binding, Histone Modifications, and Gene Expression Levels". In: *PLoS Genetics* 10.9 (2014), p. e1004663. ISSN: 1553-7404. DOI: 10.1371/journal.pgen.1004663. <URL: <http://dx.plos.org/10.1371/journal.pgen.1004663>>.