PH525.5x Section 4: Genomic annotation with Bioconductor

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4/13/2021

Representing Reference Sequence

- Annotation concept hierarchy
- Base - reference genomic sequence for an organism
- Above this, organize the chromosomal sequence into regions of interest - i.e. genes, transcripts
- SNPs and CpG sites are also regions of interest
- SNPS are single nucleotide
- Other varients – indels, structural variants, fusions can constitute regions of interest but are more complicated to express + represent
- Within ROI, identify platform oriented annotation provided by assay manufacturer
- Once manufacturing happens, genomic annotation proceeds and annotations must be updated to account for ambiguities or updates for assay probe elements
- Above genomic sequence ROIs, annotations concerning groups with shared structural or functional properties
- Pathways with nodes being genes and paths being relationships between gene products, i.e. protein protein interaction, promotion, enhancement, repression (3rd level of hierarchy)
- Begin with reference genomes
- Biostrings package - **available.genomes** - packages that represent reference genomic sequences for many different organisms
- Homo sapiens reference - some have repeat masking and there are versions which include the masked regions
  - different numbers of sequences in the two builds due to contigs that haven't been placed on chromosomes yet
- Operations defined for BSGenome objects - substring, extract chromosomal information
- Bases in full sequence aren't completely resolved
- Application of iteration - count the number of bases in a number of chromosomes
- If you have enough RAM, it is possible to operate on chromosomes in parallel and performing operations using multicore programming

```
library(BSgenome)
```

```
## Loading required package: BiocGenerics
```

```
## Loading required package: parallel
```

```
##
## Attaching package: 'BiocGenerics'

## The following objects are masked from 'package:parallel':
##
##     clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##     clusterExport, clusterMap, parApply, parCapply, parLapply,
##     parLapplyLB, parRapply, parSapply, parSapplyLB

## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs

## The following objects are masked from 'package:base':
##
##     anyDuplicated, append, as.data.frame, basename, cbind, colnames,
##     dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
##     grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
##     order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
##     rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
##     union, unique, unsplit, which.max, which.min

## Loading required package: S4Vectors

## Loading required package: stats4

##
## Attaching package: 'S4Vectors'

## The following object is masked from 'package:base':
##
##     expand.grid

## Loading required package: IRanges

## Loading required package: GenomeInfoDb

## Warning: package 'GenomeInfoDb' was built under R version 4.0.5

## Loading required package: GenomicRanges

## Loading required package: Biostrings

## Loading required package: XVector

##
## Attaching package: 'Biostrings'

## The following object is masked from 'package:base':
##
##     strsplit

## Loading required package: rtracklayer
```

```
library(Biostrings)
ag = available.genomes()
grep("Scerev", ag, value=TRUE)
```

```
## [1] "BSgenome.Scerevisiae.UCSC.sacCer1" "BSgenome.Scerevisiae.UCSC.sacCer2"
## [3] "BSgenome.Scerevisiae.UCSC.sacCer3"
```

```
grep("Hsap", ag, value=TRUE)
```

```
##  [1] "BSgenome.Hsapiens.1000genomes.hs37d5"
##  [2] "BSgenome.Hsapiens.NCBI.GRCh38"
##  [3] "BSgenome.Hsapiens.UCSC.hg17"
##  [4] "BSgenome.Hsapiens.UCSC.hg17.masked"
##  [5] "BSgenome.Hsapiens.UCSC.hg18"
##  [6] "BSgenome.Hsapiens.UCSC.hg18.masked"
##  [7] "BSgenome.Hsapiens.UCSC.hg19"
##  [8] "BSgenome.Hsapiens.UCSC.hg19.masked"
##  [9] "BSgenome.Hsapiens.UCSC.hg38"
## [10] "BSgenome.Hsapiens.UCSC.hg38.masked"
```

```
# inspect the human genome
library(BSgenome.Hsapiens.UCSC.hg19)
Hsapiens
```

```
## Human genome:
## # organism: Homo sapiens (Human)
## # genome: hg19
## # provider: UCSC
## # release date: June 2013
## # 298 sequences:
## #   chr1                 chr2                 chr3
## #   chr4                 chr5                 chr6
## #   chr7                 chr8                 chr9
## #   chr10                chr11                chr12
## #   chr13                chr14                chr15
## #   ...                  ...                  ...
## #   chr19_gl949749_alt   chr19_gl949750_alt   chr19_gl949751_alt
## #   chr19_gl949752_alt   chr19_gl949753_alt   chr20_gl383577_alt
## #   chr21_gl383578_alt   chr21_gl383579_alt   chr21_gl383580_alt
## #   chr21_gl383581_alt   chr22_gl383582_alt   chr22_gl383583_alt
## #   chr22_kb663609_alt
## # (use 'seqnames()' to see all the sequence names, use the '$' or '[[' operator
## # to access a given sequence)
```

```
length(Hsapiens)
```

```
## [1] 298
```

```
class(Hsapiens)
```

```
## [1] "BSgenome"
## attr(,"package")
## [1] "BSgenome"
```

```
methods(class="BSgenome")
```

```
##  [1] [[              $               as.list         bsgenomeName
##  [5] coerce          commonName      countPWM        export
##  [9] extractAt       getSeq          injectSNPs      length
## [13] masknames       matchPWM        metadata        metadata<-
## [17] mseqnames       names           organism        provider
## [21] providerVersion releaseDate     releaseName     seqinfo
## [25] seqinfo<-       seqnames        seqnames<-      show
## [29] snpcount        SNPlocs_pkgname snplocs         sourceUrl
## [33] vcountPattern   vcountPDict     Views           vmatchPattern
## [37] vmatchPDict
```

```
## see '?methods' for accessing help and source code
```

```
# inspect human genome
Hsapiens$chrX
```

```
## 155270560-letter DNAString object
## seq: NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN...NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
```

```
substr(Hsapiens$chrX, 5e6, 5.1e6)
```

```
## 100001-letter DNAString object
## seq: GCCTCAATGTCAGAATTATGCTGTTGCCCAAAATTG...TACTAAAAATACAAAAATTAGCTGGGCATGGTGGTG
```

```
nchar(Hsapiens$chrY)
```

```
## [1] 59373566
```

```
nchar(Hsapiens[[24]])
```

```
## [1] 59373566
```

```
library(parallel)
options(mc.cores=detectCores())

system.time(sum(unlist(mclapply(18:24, function(x) nchar(Hsapiens[[x]])))))
```

```
##    user  system elapsed
##   4.888   3.591   9.042
```

Assessment: Reference Genomes

```
library(BSgenome)
library(Biostrings)
ag = available.genomes()
library(BSgenome)
grep("mask", grep("Drerio", available.genomes(), value=TRUE), invert=TRUE, value=TRUE) # exclude masked
```

```
## [1] "BSgenome.Drerio.UCSC.danRer10" "BSgenome.Drerio.UCSC.danRer11"
## [3] "BSgenome.Drerio.UCSC.danRer5"  "BSgenome.Drerio.UCSC.danRer6"
## [5] "BSgenome.Drerio.UCSC.danRer7"
```

```
library(BSgenome.Hsapiens.UCSC.hg19.masked)
c17m = BSgenome.Hsapiens.UCSC.hg19.masked$chr17

c22m = BSgenome.Hsapiens.UCSC.hg19.masked$chr22
round(100*sum(width(masks(c22m)$AGAPS))/length(c22m),0)
```

```
## [1] 32
```

Gene, Transcript and Exon Databases

- Can find information about reference genome regions such as genes, transcripts and exons on annotation packages
- UCSC Genome Browser - major source of reference genome structure annotation
- **TxDb.Hsapiens.UCSC.hg19** - collection of well documented protein coding genes, transcripts and exons on the hg19 build of the human genome. Additional TxDb packages exist for other organisms and genome builds

- Introduction to TxDb package architecture

```
# Import TxDb transcript database
library(TxDb.Hsapiens.UCSC.hg19.knownGene)
```

```
## Loading required package: GenomicFeatures
```

```
## Warning: package 'GenomicFeatures' was built under R version 4.0.4
```

```
## Loading required package: AnnotationDbi
```

```
## Loading required package: Biobase
```

```
## Welcome to Bioconductor
##
##     Vignettes contain introductory material; view with
##     'browseVignettes()'. To cite Bioconductor, see
##     'citation("Biobase")', and for packages 'citation("pkgname")'.
```

```
txdb = TxDb.Hsapiens.UCSC.hg19.knownGene
class(txdb)
```

```
## [1] "TxDb"
## attr(,"package")
## [1] "GenomicFeatures"
```

```
methods(class="TxDb")
```

```
##  [1] $                     $<-                   annotatedDataFrameFrom
##  [4] as.list               asBED                 asGFF
##  [7] assayData             assayData<-           cds
## [10] cdsBy                 cdsByOverlaps         coerce
## [13] columns               combine               contents
## [16] dbconn                dbfile                dbInfo
## [19] dbmeta                dbschema              disjointExons
## [22] distance              exons                 exonsBy
## [25] exonsByOverlaps       ExpressionSet         extractUpstreamSeqs
## [28] featureNames          featureNames<-        fiveUTRsByTranscript
## [31] genes                 initialize            intronsByTranscript
## [34] isActiveSeq           isActiveSeq<-         isNA
## [37] keys                  keytypes              mapIds
## [40] mapIdsToRanges        mappedkeys            mapRangesToIds
## [43] mapToTranscripts      metadata              microRNAs
## [46] nhit                  organism              promoters
## [49] revmap                sample                sampleNames
## [52] sampleNames<-         saveDb                select
## [55] seqinfo               seqinfo<-             seqlevels<-
## [58] seqlevels0            show                  species
## [61] storageMode           storageMode<-         taxonomyId
## [64] threeUTRsByTranscript transcripts           transcriptsBy
## [67] transcriptsByOverlaps tRNAs                 updateObject
## see '?methods' for accessing help and source code
```

```
# extract and inspect genes from TxDb
genes(txdb)
```

```
##   403 genes were dropped because they have exons located on both strands
##   of the same reference sequence or on more than one reference sequence,
##   so cannot be represented by a single genomic range.
```

```
##   Use 'single.strand.genes.only=FALSE' to get all the genes in a
##   GRangesList object, or use suppressMessages() to suppress this message.
```

```
## GRanges object with 23056 ranges and 1 metadata column:
##         seqnames              ranges strand |     gene_id
##            <Rle>           <IRanges>  <Rle> | <character>
##       1    chr19   58858172-58874214      - |           1
##      10     chr8   18248755-18258723      + |          10
##     100    chr20   43248163-43280376      - |         100
##    1000    chr18   25530930-25757445      - |        1000
##   10000     chr1 243651535-244006886      - |       10000
##     ...      ...                 ...    ... .         ...
##    9991     chr9 114979995-115095944      - |        9991
##    9992    chr21   35736323-35743440      + |        9992
##    9993    chr22   19023795-19109967      - |        9993
##    9994     chr6   90539619-90584155      + |        9994
##    9997    chr22   50961997-50964905      - |        9997
##   -------
##   seqinfo: 93 sequences (1 circular) from hg19 genome
```

```
table(strand(genes(txdb)))
```

```
##   403 genes were dropped because they have exons located on both strands
##   of the same reference sequence or on more than one reference sequence,
##   so cannot be represented by a single genomic range.
##   Use 'single.strand.genes.only=FALSE' to get all the genes in a
##   GRangesList object, or use suppressMessages() to suppress this message.
```

```
##
##     +     -     *
## 11737 11319     0
```

```
summary(width(genes(txdb)))
```

```
##   403 genes were dropped because they have exons located on both strands
##   of the same reference sequence or on more than one reference sequence,
##   so cannot be represented by a single genomic range.
##   Use 'single.strand.genes.only=FALSE' to get all the genes in a
##   GRangesList object, or use suppressMessages() to suppress this message.
```

```
##     Min.  1st Qu.   Median     Mean  3rd Qu.     Max.
##       20     5666    20116    60660    58175 24187703
```

```
# inspect larges gene in genome
id = which.max(width(genes(txdb)))
```

```
##   403 genes were dropped because they have exons located on both strands
##   of the same reference sequence or on more than one reference sequence,
##   so cannot be represented by a single genomic range.
##   Use 'single.strand.genes.only=FALSE' to get all the genes in a
##   GRangesList object, or use suppressMessages() to suppress this message.
```

```
genes(txdb)[id]
```

```
##   403 genes were dropped because they have exons located on both strands
##   of the same reference sequence or on more than one reference sequence,
##   so cannot be represented by a single genomic range.
##   Use 'single.strand.genes.only=FALSE' to get all the genes in a
```

```
##   GRangesList object, or use suppressMessages() to suppress this message.
```

```
## GRanges object with 1 range and 1 metadata column:
##          seqnames            ranges strand |     gene_id
##             <Rle>         <IRanges>  <Rle> | <character>
##   286297     chr9 42844370-67032072      - |      286297
##   -------
##   seqinfo: 93 sequences (1 circular) from hg19 genome
```

```
library(org.Hs.eg.db)
```

```
##
```

```
select(org.Hs.eg.db, keys="286297", keytype="ENTREZID", columns=c("SYMBOL", "GENENAME"))
```

```
## 'select()' returned 1:1 mapping between keys and columns
```

```
##   ENTREZID    SYMBOL
## 1   286297 LOC286297
##                                                                  GENENAME
## 1 methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1 like pseudogene
```

```
# compare total size of exons to total size of genes
ex = exons(txdb)
rex = reduce(ex)
ex_width = sum(width(rex)) # bases in exons
gene_width = sum(width(genes(txdb))) # bases in genes
```

```
##   403 genes were dropped because they have exons located on both strands
##   of the same reference sequence or on more than one reference sequence,
##   so cannot be represented by a single genomic range.
##   Use 'single.strand.genes.only=FALSE' to get all the genes in a
##   GRangesList object, or use suppressMessages() to suppress this message.
```

```
ex_width/gene_width
```

```
## [1] 0.06380062
```

ensembldb, EnsDb: annotation from EMBL

- European initiative for annotating genome called ensembl
- Ensemble-based representations managed in package called EmsembleDb
- Different packages representing different builds of ensembl annotation for different organisms
- More direct relationship to database and database tables - gene, transcipt, transcript to exon mapping tables.
- More details provided to user through Ensembl transcripts method - get info on transcripts but also associated proteins, genes and biotype

```
# inspect data available from Ensembl
library(ensembldb)
```

```
## Loading required package: AnnotationFilter
```

```
##
## Attaching package: 'ensembldb'
```

```
## The following object is masked from 'package:stats':
##
##     filter
```

```
library(EnsDb.Hsapiens.v75)
names(listTables(EnsDb.Hsapiens.v75))
```

```
##  [1] "gene"           "tx"             "tx2exon"        "exon"
##  [5] "chromosome"     "protein"        "uniprot"        "protein_domain"
##  [9] "entrezgene"     "metadata"
```

```
# extract Ensembl transcripts
edb = EnsDb.Hsapiens.v75 # abbreviate
txs <- transcripts(edb, filter = GeneNameFilter("ZBTB16"),
                   columns = c("protein_id", "uniprot_id", "tx_biotype"))
txs
```

```
## GRanges object with 20 ranges and 5 metadata columns:
##                    seqnames              ranges strand |      protein_id
##                       <Rle>           <IRanges>  <Rle> |     <character>
##   ENST00000335953       11 113930315-114121398      + | ENSP00000338157
##   ENST00000335953       11 113930315-114121398      + | ENSP00000338157
##   ENST00000335953       11 113930315-114121398      + | ENSP00000338157
##   ENST00000335953       11 113930315-114121398      + | ENSP00000338157
##   ENST00000335953       11 113930315-114121398      + | ENSP00000338157
##               ...      ...                 ...    ... .             ...
##   ENST00000392996       11 113931229-114121374      + | ENSP00000376721
##   ENST00000539918       11 113935134-114118066      + | ENSP00000445047
##   ENST00000545851       11 114051488-114118018      + |            <NA>
##   ENST00000535379       11 114107929-114121279      + |            <NA>
##   ENST00000535509       11 114117512-114121198      + |            <NA>
##                     uniprot_id            tx_biotype           tx_id
##                    <character>           <character>     <character>
##   ENST00000335953  ZBT16_HUMAN         protein_coding ENST00000335953
##   ENST00000335953 Q71UL7_HUMAN         protein_coding ENST00000335953
##   ENST00000335953 Q71UL6_HUMAN         protein_coding ENST00000335953
##   ENST00000335953 Q71UL5_HUMAN         protein_coding ENST00000335953
##   ENST00000335953 F5H6C3_HUMAN         protein_coding ENST00000335953
##               ...          ...                    ...             ...
##   ENST00000392996 F5H5Y7_HUMAN         protein_coding ENST00000392996
##   ENST00000539918         <NA> nonsense_mediated_de.. ENST00000539918
##   ENST00000545851         <NA>   processed_transcript ENST00000545851
##   ENST00000535379         <NA>   processed_transcript ENST00000535379
##   ENST00000535509         <NA>        retained_intron ENST00000535509
##                     gene_name
##                    <character>
##   ENST00000335953      ZBTB16
##   ENST00000335953      ZBTB16
##   ENST00000335953      ZBTB16
##   ENST00000335953      ZBTB16
##   ENST00000335953      ZBTB16
##               ...         ...
##   ENST00000392996      ZBTB16
##   ENST00000539918      ZBTB16
##   ENST00000545851      ZBTB16
##   ENST00000535379      ZBTB16
##   ENST00000535509      ZBTB16
##   -------
##   seqinfo: 1 sequence from GRCh37 genome
```

```
# compare Ensembl and UCSC transcripts
alltx = transcripts(edb) # Ensembl is larger
utx = transcripts(txdb) # UCSC is smaller

# table of biological types of transcripts
table(alltx$tx_biotype)
```

```
##
##           3prime_overlapping_ncrna                           antisense
##                                 29                               10058
##                           IG_C_gene                     IG_C_pseudogene
##                                 31                                  13
##                           IG_D_gene                           IG_J_gene
##                                 64                                  24
##                     IG_J_pseudogene                           IG_V_gene
##                                  6                                 185
##                     IG_V_pseudogene                             lincRNA
##                                264                               12101
##                            LRG_gene                             miRNA
##                                477                                3424
##                            misc_RNA                            Mt_rRNA
##                                2190                                  2
##                             Mt_tRNA                       non_stop_decay
##                                  22                                 63
##             nonsense_mediated_decay                polymorphic_pseudogene
##                               13812                                70
##                processed_pseudogene                  processed_transcript
##                               11321                               31417
##                      protein_coding                           pseudogene
##                               90273                                664
##                     retained_intron                                rRNA
##                               28579                                570
##                      sense_intronic                    sense_overlapping
##                                827                                342
##                             snoRNA                               snRNA
##                                1621                               2074
##                           TR_C_gene                           TR_D_gene
##                                  6                                  3
##                           TR_J_gene                     TR_J_pseudogene
##                                 82                                  4
##                           TR_V_gene                     TR_V_pseudogene
##                                150                                 40
##   transcribed_processed_pseudogene transcribed_unprocessed_pseudogene
##                                476                                986
##    translated_processed_pseudogene                  unitary_pseudogene
##                                  1                                189
##              unprocessed_pseudogene
##                               3187
```

Assessment: Gene and transcript model

```
library(devtools)
```

```
## Loading required package: usethis
```

```
install_github("genomicsclass/ph525x")
```

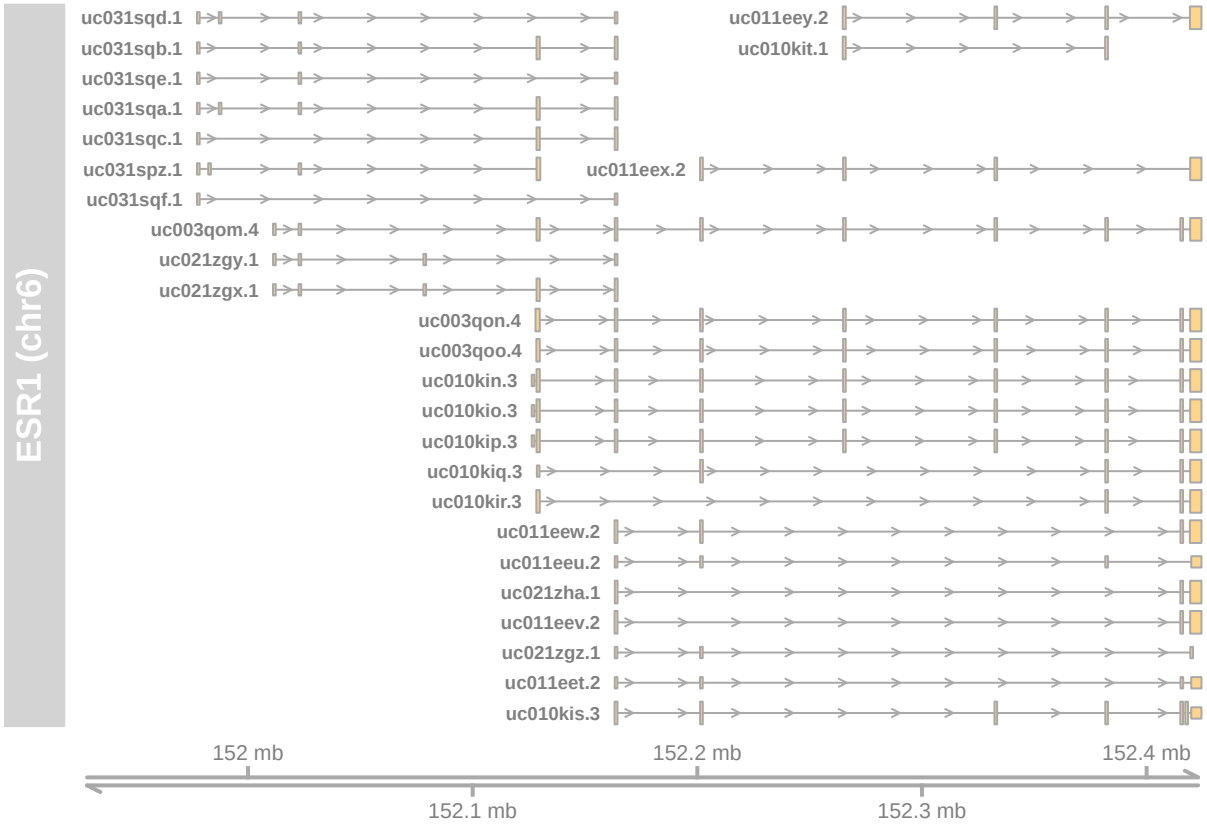
```
## Skipping install of 'ph525x' from a github remote, the SHA1 (e83c0d57) has not changed since last ins
##   Use `force = TRUE` to force installation
```

```
library(ph525x)
```

```
## Loading required package: png
## Loading required package: grid
## Loading required package: Homo.sapiens
## Loading required package: OrganismDbi
## Loading required package: GO.db
##
```

```
stopifnot(packageVersion("ph525x") >= "0.0.16") # do over if fail
modPlot("ESR1", useGeneSym=FALSE, collapse=FALSE)
```

```
## Loading required package: Gviz
## Warning: package 'Gviz' was built under R version 4.0.4
##
## Attaching package: 'Gviz'
## The following object is masked from 'package:AnnotationFilter':
##
##     feature
## 'select()' returned 1:many mapping between keys and columns
```




```
library(TxDb.Hsapiens.UCSC.hg19.knownGene)
txdb = TxDb.Hsapiens.UCSC.hg19.knownGene
e_id <- select(edb, keys="ESR1", keytype="GENENAME", columns=c("ENTREZID"))[1, "ENTREZID"]
n_transcripts <- length(transcripts(txdb, filter=list(gene_id=e_id)))
paste("Number of transcripts comprimising model of ESR1: ", n_transcripts)
```

```
## [1] "Number of transcripts comprimising model of ESR1:  27"
```

AnnotationHub: finding and caching important information

- Central hub for genomic annotation files maintained by Bioconductor community
- Includes annotation files from UCSC, ENSEMBL, and the Broad Institute
- **AnnotationHub** allows you to search and download resources from inside R session

```
library(AnnotationHub)
```

```
## Loading required package: BiocFileCache
```

```
## Loading required package: dbplyr
```

```
##
## Attaching package: 'AnnotationHub'
```

```
## The following object is masked from 'package:Biobase':
##
##     cache
```

```
ah <- AnnotationHub()
```

```
## snapshotDate(): 2020-10-27
```

```
ah
```

```
## AnnotationHub with 57231 records
## # snapshotDate(): 2020-10-27
## # $dataprovider: Ensembl, BroadInstitute, UCSC, ftp://ftp.ncbi.nlm.nih.gov/g...
## # $species: Homo sapiens, Mus musculus, Drosophila melanogaster, Bos taurus,...
## # $rdataclass: GRanges, TwoBitFile, BigWigFile, EnsDb, Rle, OrgDb, ChainFile...
## # additional mcols(): taxonomyid, genome, description,
## #   coordinate_1_based, maintainer, rdatadateadded, preparerclass, tags,
## #   rdatapath, sourceurl, sourcetype
## # retrieve records with, e.g., 'object[["AH5012"]]'
##
##             title
##   AH5012  | Chromosome Band
##   AH5013  | STS Markers
##   AH5014  | FISH Clones
##   AH5015  | Recomb Rate
##   AH5016  | ENCODE Pilot
##   ...       ...
##   AH91566 | Zonotrichia_albicollis.Zonotrichia_albicollis-1.0.1.ncrna.2bit
##   AH91567 | Zosterops_lateralis_melanops.ASM128173v1.cdna.all.2bit
##   AH91568 | Zosterops_lateralis_melanops.ASM128173v1.dna_rm.toplevel.2bit
##   AH91569 | Zosterops_lateralis_melanops.ASM128173v1.dna_sm.toplevel.2bit
##   AH91570 | Zosterops_lateralis_melanops.ASM128173v1.ncrna.2bit
```

```
length(unique(ah$species))
```

```
## [1] 2643
```

```
ah_human <- subset(ah, species == "Homo sapiens")
ah_human
```

```
## AnnotationHub with 26461 records
## # snapshotDate(): 2020-10-27
## # $dataprovider: BroadInstitute, UCSC, Ensembl, GENCODE, UWashington, Stanfo...
## # $species: Homo sapiens
## # $rdataclass: GRanges, BigWigFile, Rle, ChainFile, TwoBitFile, list, data.f...
## # additional mcols(): taxonomyid, genome, description,
## #   coordinate_1_based, maintainer, rdatadateadded, preparerclass, tags,
## #   rdatapath, sourceurl, sourcetype
## # retrieve records with, e.g., 'object[["AH5012"]]'
##
##             title
##   AH5012  | Chromosome Band
##   AH5013  | STS Markers
##   AH5014  | FISH Clones
##   AH5015  | Recomb Rate
##   AH5016  | ENCODE Pilot
##   ...       ...
##   AH83216 | Ensembl 101 EnsDb for Homo sapiens
##   AH83362 | Sequences of snoRNA targets of Homo sapiens hg38
##   AH84122 | org.Hs.eg.db.sqlite
##   AH89180 | Ensembl 102 EnsDb for Homo sapiens
##   AH89426 | Ensembl 103 EnsDb for Homo sapiens
```

```
query(ah, "HepG2")
```

```
## AnnotationHub with 440 records
## # snapshotDate(): 2020-10-27
## # $dataprovider: UCSC, BroadInstitute, Pazar
## # $species: Homo sapiens, NA
## # $rdataclass: GRanges, BigWigFile
## # additional mcols(): taxonomyid, genome, description,
## #   coordinate_1_based, maintainer, rdatadateadded, preparerclass, tags,
## #   rdatapath, sourceurl, sourcetype
## # retrieve records with, e.g., 'object[["AH22246"]]'
##
##             title
##   AH22246 | pazar_CEBPA_HEPG2_Schmidt_20120522.csv
##   AH22249 | pazar_CTCF_HEPG2_Schmidt_20120522.csv
##   AH22273 | pazar_HNF4A_HEPG2_Schmidt_20120522.csv
##   AH22309 | pazar_STAG1_HEPG2_Schmidt_20120522.csv
##   AH22348 | wgEncodeAffyRnaChipFiltTransfragsHepg2CytosolLongnonpolya.broadP...
##   ...       ...
##   AH41564 | E118-H4K5ac.imputed.pval.signal.bigwig
##   AH41691 | E118-H4K8ac.imputed.pval.signal.bigwig
##   AH41818 | E118-H4K91ac.imputed.pval.signal.bigwig
##   AH46971 | E118_15_coreMarks_mnemonics.bed.gz
##   AH49484 | E118_RRBS_FractionalMethylation.bigwig
```

```
query(ah, c("HepG2", "H3K4me3"))
```

```
## AnnotationHub with 11 records
## # snapshotDate(): 2020-10-27
## # $dataprovider: BroadInstitute, UCSC
## # $species: Homo sapiens
## # $rdataclass: GRanges, BigWigFile
## # additional mcols(): taxonomyid, genome, description,
## #   coordinate_1_based, maintainer, rdatadateadded, preparerclass, tags,
## #   rdatapath, sourceurl, sourcetype
## # retrieve records with, e.g., 'object[["AH23311"]]'
##
##             title
##   AH23311 | wgEncodeBroadHistoneHepg2H3k4me3StdPk.broadPeak.gz
##   AH27201 | wgEncodeUwHistoneHepg2H3k4me3StdHotspotsRep1.broadPeak.gz
##   AH27202 | wgEncodeUwHistoneHepg2H3k4me3StdHotspotsRep2.broadPeak.gz
##   AH27203 | wgEncodeUwHistoneHepg2H3k4me3StdPkRep1.narrowPeak.gz
##   AH27204 | wgEncodeUwHistoneHepg2H3k4me3StdPkRep2.narrowPeak.gz
##   ...       ...
##   AH30771 | E118-H3K4me3.narrowPeak.gz
##   AH31712 | E118-H3K4me3.gappedPeak.gz
##   AH32893 | E118-H3K4me3.fc.signal.bigwig
##   AH33925 | E118-H3K4me3.pval.signal.bigwig
##   AH40296 | E118-H3K4me3.imputed.pval.signal.bigwig
```

```
hepg2 <- query(ah, "HepG2")
hepg2_h3k4me3 <- query(hepg2, c("H3k4me3"))
hepg2_h3k4me3
```

```
## AnnotationHub with 11 records
```

```
## # snapshotDate(): 2020-10-27
## # $dataprovider: BroadInstitute, UCSC
## # $species: Homo sapiens
## # $rdataclass: GRanges, BigWigFile
## # additional mcols(): taxonomyid, genome, description,
## #   coordinate_1_based, maintainer, rdatadateadded, preparerclass, tags,
## #   rdatapath, sourceurl, sourcetype
## # retrieve records with, e.g., 'object[["AH23311"]]'
##
##             title
##   AH23311 | wgEncodeBroadHistoneHepg2H3k4me3StdPk.broadPeak.gz
##   AH27201 | wgEncodeUwHistoneHepg2H3k4me3StdHotspotsRep1.broadPeak.gz
##   AH27202 | wgEncodeUwHistoneHepg2H3k4me3StdHotspotsRep2.broadPeak.gz
##   AH27203 | wgEncodeUwHistoneHepg2H3k4me3StdPkRep1.narrowPeak.gz
##   AH27204 | wgEncodeUwHistoneHepg2H3k4me3StdPkRep2.narrowPeak.gz
##   ...       ...
##   AH30771 | E118-H3K4me3.narrowPeak.gz
##   AH31712 | E118-H3K4me3.gappedPeak.gz
##   AH32893 | E118-H3K4me3.fc.signal.bigwig
##   AH33925 | E118-H3K4me3.pval.signal.bigwig
##   AH40296 | E118-H3K4me3.imputed.pval.signal.bigwig
```

```
hepg2_h3k4me3$tags
```

```
##  [1] "wgEncode, ChipSeq, broadPeak, HepG2 cell, Bernstein grant"
##  [2] "wgEncode, ChipSeq, broadPeak, HepG2 cell, Stam grant"
##  [3] "wgEncode, ChipSeq, broadPeak, HepG2 cell, Stam grant"
##  [4] "wgEncode, ChipSeq, narrowPeak, HepG2 cell, Stam grant"
##  [5] "wgEncode, ChipSeq, narrowPeak, HepG2 cell, Stam grant"
##  [6] "EpigenomeRoadMap, peaks, consolidated, broadPeak, E118, ENCODE2012, LIV.HEPG2.CNCR, HepG2 Hepa
##  [7] "EpigenomeRoadMap, peaks, consolidated, narrowPeak, E118, ENCODE2012, LIV.HEPG2.CNCR, HepG2 Hepa
##  [8] "EpigenomeRoadMap, peaks, consolidated, gappedPeak, E118, ENCODE2012, LIV.HEPG2.CNCR, HepG2 Hepa
##  [9] "EpigenomeRoadMap, signal, consolidated, macs2signal, E118, ENCODE2012, LIV.HEPG2.CNCR, HepG2 He
## [10] "EpigenomeRoadMap, signal, consolidated, macs2signal, E118, ENCODE2012, LIV.HEPG2.CNCR, HepG2 He
## [11] "EpigenomeRoadMap, signal, consolidatedImputed, H3K4me3, E118, ENCODE2012, LIV.HEPG2.CNCR, HepG2
```

```
# display(query(ah, "HepG2"))

e118_broadpeak <- query(hepg2_h3k4me3, c("E118", "broadPeak"))
id <- e118_broadpeak$ah_id
id
```

```
## [1] "AH29728"
```

```
hepg2_h3k4me3_broad <- ah[["AH29728"]]
```

```
## downloading 1 resources
```

```
## retrieving 1 resource
```

```
## loading from cache
```

```
hepg2_h3k4me3_broad
```

```
## GRanges object with 60638 ranges and 5 metadata columns:
##           seqnames            ranges strand |        name     score signalValue
##              <Rle>         <IRanges>  <Rle> | <character> <numeric>   <numeric>
##       [1]    chr14 24614467-24618166      * |      Rank_1       850     20.3233
```

```
##       [2]    chr20   3183140-3185609      * |      Rank_2       830     25.7534
##       [3]    chr14 24700096-24704098      * |      Rank_3       811     17.2931
##       [4]    chr14 24766070-24770499      * |      Rank_4       763     18.9677
##       [5]    chr20 44420138-44421910      * |      Rank_5       755     24.0763
##       ...      ...               ...    ... .         ...       ...         ...
##   [60634]     chr2 11928736-11929617      * |  Rank_60634         0     1.73093
##   [60635]    chr10 97229724-97230412      * |  Rank_60635         0     1.73015
##   [60636]     chr2 39896310-39896946      * |  Rank_60636         0     1.73014
##   [60637]     chr6   3978391-3978677      * |  Rank_60637         0     1.73015
##   [60638]     chr6 49433554-49434110      * |  Rank_60638         0     1.73014
##              pValue    qValue
##           <numeric> <numeric>
##       [1]   88.3475   85.0287
##       [2]   86.2138   83.0301
##       [3]   84.3213   81.1706
##       [4]   79.3876   76.3449
##       [5]   78.6304   75.5947
##       ...       ...       ...
##   [60634]   1.00441         0
##   [60635]   1.00357         0
##   [60636]   1.00357         0
##   [60637]   1.00357         0
##   [60638]   1.00357         0
##   -------
##   seqinfo: 298 sequences (2 circular) from hg19 genome
```

```
alt_format <- ah[[id]]
```

```
## loading from cache
```

```
identical(hepg2_h3k4me3_broad, alt_format)
```

```
## [1] TRUE
```