PH525.5x Section 4: Genomic annotation with Bioconductor

Lauren Kemperman

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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
##   4.889   3.603  11.740
```

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")
```

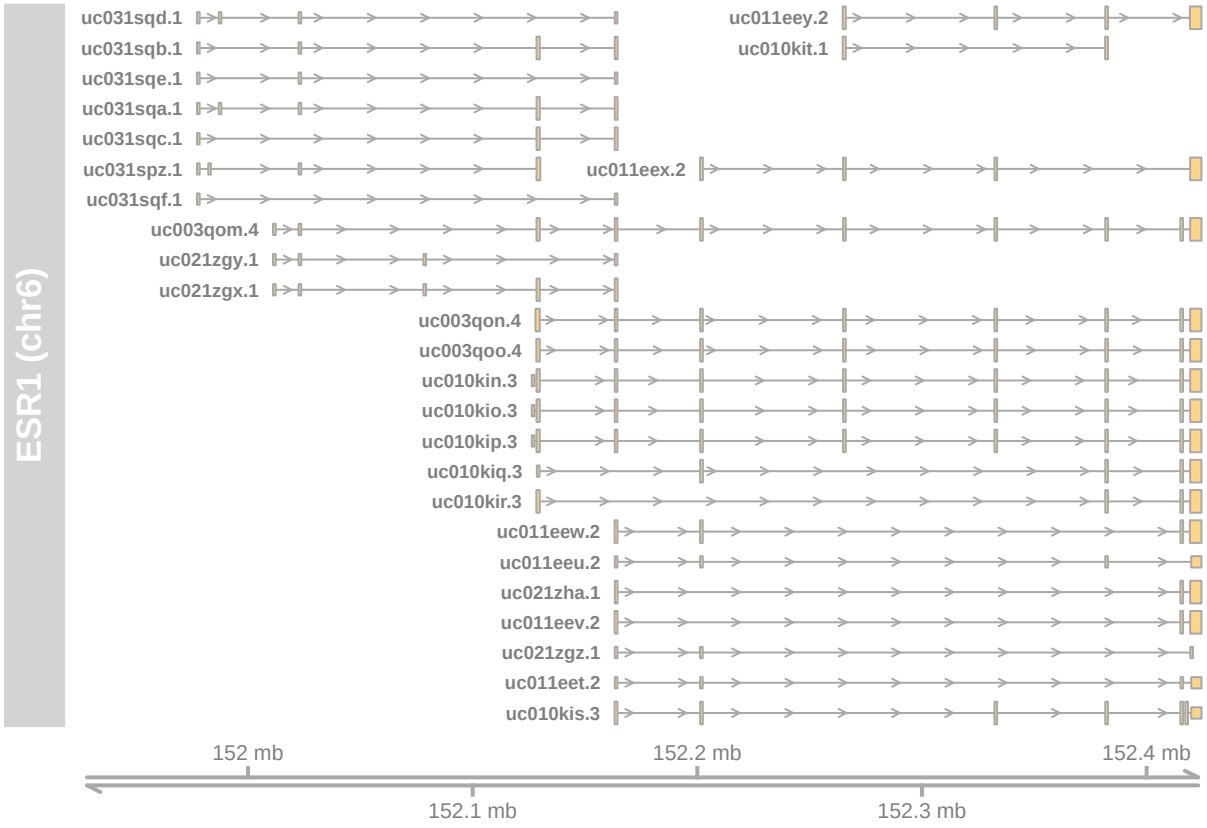
```
## Skipping install of 'ph525x' from a github remote, the SHA1 (e83c0d57) has not changed since last ins
##   Use `force = TRUE` to force installation
```

```
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"]]
```

```
## 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
```

Assessment: AnnotationHub

```
library(AnnotationHub)
ah = AnnotationHub()
```

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

```
mah = mcols(ah)
names(mah)
```

```
##  [1] "title"              "dataprovider"       "species"
##  [4] "taxonomyid"         "genome"             "description"
##  [7] "coordinate_1_based" "maintainer"         "rdatadateadded"
## [10] "preparerclass"      "tags"               "rdataclass"
## [13] "rdatapath"          "sourceurl"          "sourcetype"
```

```
sort(table(mah$species), decreasing=TRUE)[1:10]
```

```
##
##            Homo sapiens            Mus musculus Drosophila melanogaster
##                   26461                    1617                     422
##              Bos taurus         Pan troglodytes       Rattus norvegicus
##                     318                     306                     305
##             Danio rerio           Gallus gallus   Monodelphis domestica
##                     297                     265                     242
```

```
##             Felis catus
##                     235
```

```
n_ctcf_binding_hepg2 <- length(names(query(query(ah, "HepG2"), "CTCF")))
paste("Number of entries addressing CTCF binding in HepG2: ", n_ctcf_binding_hepg2)
```

```
## [1] "Number of entries addressing CTCF binding in HepG2:  13"
```

liftOver: Translating between reference builds

- Genomic annotations typically defined for fixed genome build
- Human is often hg19
- When analysis is performed on different genome build, annotations must be translated to the coordinates of the new build before use
- Process of translating called **lifting**
- Implemented in **liftOver()** function of **rtracklayer** Bioconductor package
- Tutorial will move features from genome build hg38 -> hg19

```
# liftOver from rtracklayer
library(rtracklayer)
?liftOver

# chromosome 1 gene locations in hg38
library(TxDb.Hsapiens.UCSC.hg38.knownGene)
tx38 <- TxDb.Hsapiens.UCSC.hg38.knownGene
seqlevels(tx38, pruning.mode="coarse") = "chr1"
g1_38 <- genes(tx38)
```

```
##   12 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.
```

```
# Download hg38 to hg19 chain file
library(AnnotationHub)
ah <- AnnotationHub()
```

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

```
ah.chain <- subset(ah, rdataclass == "ChainFile" & species == "Homo sapiens")
query(ah.chain, c("hg19", "hg38"))
```

```
## AnnotationHub with 4 records
## # snapshotDate(): 2020-10-27
## # $dataprovider: UCSC, NCBI
## # $species: Homo sapiens
## # $rdataclass: ChainFile
## # additional mcols(): taxonomyid, genome, description,
## #   coordinate_1_based, maintainer, rdatadateadded, preparerclass, tags,
## #   rdatapath, sourceurl, sourcetype
## # retrieve records with, e.g., 'object[["AH14108"]]'
##
##             title
##   AH14108 | hg38ToHg19.over.chain.gz
##   AH14150 | hg19ToHg38.over.chain.gz
```

```
##   AH78915 | Chain file for Homo sapiens rRNA hg19 to hg38
##   AH78916 | Chain file for Homo sapiens rRNA hg38 to hg19
```

```
ch <- ah [["AH14108"]]
```

```
## loading from cache
```

```
# perform the liftOver
g1_19L <- liftOver(g1_38, ch)
g1_19L
```

```
## GRangesList object of length 2696:
## $`10000`
## GRanges object with 1 range and 1 metadata column:
##       seqnames              ranges strand |     gene_id
##          <Rle>           <IRanges>  <Rle> | <character>
##   [1]     chr1 243651535-244014381      - |       10000
##   -------
##   seqinfo: 19 sequences from an unspecified genome; no seqlengths
##
## $`100034743`
## GRanges object with 3 ranges and 1 metadata column:
##       seqnames              ranges strand |     gene_id
##          <Rle>           <IRanges>  <Rle> | <character>
##   [1]     chr1 147466094-147484530      - |   100034743
##   [2]     chr1 147484532-147484551      - |   100034743
##   [3]     chr1 147484553-147487188      - |   100034743
##   -------
##   seqinfo: 19 sequences from an unspecified genome; no seqlengths
##
## $`100126331`
## GRanges object with 1 range and 1 metadata column:
##       seqnames              ranges strand |     gene_id
##          <Rle>           <IRanges>  <Rle> | <character>
##   [1]     chr1 117637265-117637350      + |   100126331
##   -------
##   seqinfo: 19 sequences from an unspecified genome; no seqlengths
##
## ...
## <2693 more elements>
```

Assessment: liftOver

```
if(!file.exists("hg19ToHg38.over.chain")){
  download.file("http://hgdownload.cse.ucsc.edu/goldenPath/hg19/liftOver/hg19ToHg38.over.chain.gz", "hg
  library(R.utils)
  gunzip("hg19ToHg38.over.chain.gz")
}

library(ERBS)
data(HepG2)
library(rtracklayer)
ch = import.chain("hg19ToHg38.over.chain")
nHepG2 = liftOver(HepG2, ch)
```

```
s1 <- start(HepG2[1])
s2 <- start(nHepG2[1])[[1]]

abs_diff_bases <- abs(s2 - s1)
paste("Number of bases moved upstream in first range of HepG2 to hg38: ", abs_diff_bases)
```

```
## [1] "Number of bases moved upstream in first range of HepG2 to hg38:  199761"
```

Data import and export with rtracklayer

- **rtracklayer** package parses data into common formats so they can easily be used as annotations in future analysis

```
library(devtools)
install_github("genomicsclass/ERBS") # install ERBS package
```

```
## Skipping install of 'ERBS' from a github remote, the SHA1 (9f16eb6a) has not changed since last insta
##   Use `force = TRUE` to force installation
```

```
f1 = dir(system.file("extdata",package="ERBS"), full=TRUE)[1] # access dat a
readLines(f1, 4) # preview a few lines
```

```
## [1] "chrX\t1509354\t1512462\t5\t0\t.\t157.92\t310\t32.000000\t1991"
## [2] "chrX\t26801421\t26802448\t6\t0\t.\t147.38\t310\t32.000000\t387"
## [3] "chr19\t11694101\t11695359\t1\t0\t.\t99.71\t311.66\t32.000000\t861"
## [4] "chr19\t4076892\t4079276\t4\t0\t.\t84.74\t310\t32.000000\t1508"
```

```
library(rtracklayer)
imp = import(f1, format="bedGraph") # import as bedGraph format
imp
```

```
## GRanges object with 1873 ranges and 7 metadata columns:
##          seqnames            ranges strand |     score       NA.      NA.1
##             <Rle>         <IRanges>  <Rle> | <numeric> <integer> <logical>
##      [1]     chrX   1509355-1512462      * |         5         0      <NA>
##      [2]     chrX 26801422-26802448      * |         6         0      <NA>
##      [3]    chr19 11694102-11695359      * |         1         0      <NA>
##      [4]    chr19   4076893-4079276      * |         4         0      <NA>
##      [5]     chr3 53288568-53290767      * |         9         0      <NA>
##      ...      ...               ...    ... .       ...       ...       ...
##   [1869]    chr19 11201120-11203985      * |      8701         0      <NA>
##   [1870]    chr19   2234920-2237370      * |       990         0      <NA>
##   [1871]     chr1 94311336-94313543      * |      4035         0      <NA>
##   [1872]    chr19 45690614-45691210      * |     10688         0      <NA>
##   [1873]    chr19   6110100-6111252      * |      2274         0      <NA>
##               NA.2      NA.3      NA.4      NA.5
##          <numeric> <numeric> <numeric> <integer>
##      [1]    157.92   310.000        32      1991
##      [2]    147.38   310.000        32       387
##      [3]     99.71   311.660        32       861
##      [4]     84.74   310.000        32      1508
##      [5]     78.20   299.505        32      1772
##      ...       ...       ...       ...       ...
##   [1869]      8.65     7.281   0.26576      2496
##   [1870]      8.65    26.258   1.99568      1478
```

```
##   [1871]      8.65    12.511   1.47237      1848
##   [1872]      8.65     6.205   0.00000       298
##   [1873]      8.65    17.356   2.01323       496
##   -------
##   seqinfo: 23 sequences from an unspecified genome; no seqlengths
```

```
genome(imp) # genome identifier tag not set, but can be set manually
```

```
##  chrX chr19  chr3 chr17  chr8 chr11 chr16  chr1  chr2  chr6  chr9  chr7  chr5
##    NA    NA    NA    NA    NA    NA    NA    NA    NA    NA    NA    NA    NA
## chr12 chr20 chr21 chr22 chr18 chr10 chr14 chr15  chr4 chr13
##    NA    NA    NA    NA    NA    NA    NA    NA    NA    NA
```

```
genome(imp) = "hg19"
genome(imp)
```

```
##   chrX  chr19   chr3  chr17   chr8  chr11  chr16   chr1   chr2   chr6   chr9
## "hg19" "hg19" "hg19" "hg19" "hg19" "hg19" "hg19" "hg19" "hg19" "hg19" "hg19"
##   chr7   chr5  chr12  chr20  chr21  chr22  chr18  chr10  chr14  chr15   chr4
## "hg19" "hg19" "hg19" "hg19" "hg19" "hg19" "hg19" "hg19" "hg19" "hg19" "hg19"
##  chr13
## "hg19"
```

```
export(imp, "demoex.bed") # export as BED format
cat(readLines("demoex.bed", n=5), sep="\n") # check output file
```

```
## chrX 1509354 1512462 .   5   .
## chrX 26801421    26802448    .   6   .
## chr19    11694101    11695359    .   1   .
## chr19    4076892 4079276 .   4   .
## chr3 53288567    53290767    .   9   .
```