Generating an using Ensembl based annotation packages

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Package

ensembldb 2.12.1

1 Introduction

The ensembldb package provides functions to create and use transcript centric annotation databases/packages. The annotation for the databases are directly fetched from Ensembl [1] using their Perl API. The functionality and data is similar to that of the TxDb packages from the GenomicFeatures package, but, in addition to retrieve all gene/transcript models and annotations from the database, the ensembldb package provides also a filter framework allowing to retrieve annotations for specific entries like genes encoded on a chromosome region or transcript models of lincRNA genes. From version 1.7 on, EnsDb databases created by the ensembldb package contain also protein annotation data (see

Section 11 for the database layout and an overview of available attributes/columns). For more information on the use of the protein annotations refer to the *proteins* vignette.

Another main goal of this package is to generate *versioned* annotation packages, i.e. annotation packages that are build for a specific Ensembl release, and are also named according to that (e.g. `EnsDb.Hsapiens.v86` for human gene definitions of the Ensembl code database version 86). This ensures reproducibility, as it allows to load annotations from a specific Ensembl release also if newer versions of annotation packages/releases are available. It also allows to load multiple annotation packages at the same time in order to e.g. compare gene models between Ensembl releases.

In the example below we load an Ensembl based annotation package for Homo sapiens, Ensembl version 86. The `EnsDb` object providing access to the underlying SQLite database is bound to the variable name `EnsDb.Hsapiens.v86` .

```
library(EnsDb.Hsapiens.v86)

## Making a "short cut"
edb <- EnsDb.Hsapiens.v86
## print some informations for this package
edb
```

```
## EnsDb for Ensembl:
## |Backend: SQLite
## |Db type: EnsDb
## |Type of Gene ID: Ensembl Gene ID
## |Supporting package: ensembldb
## |Db created by: ensembldb package from Bioconductor
## |script_version: 0.3.0
## |Creation time: Thu May 18 16:32:27 2017
## |ensembl_version: 86
## |ensembl_host: localhost
## |Organism: homo_sapiens
## |taxonomy_id: 9606
## |genome_build: GRCh38
## |DBSCHEMAVERSION: 2.0
## | No. of genes: 63970.
## | No. of transcripts: 216741.
## |Protein data available.
```

```
## For what organism was the database generated?
organism(edb)
```

```
## [1] "Homo sapiens"
```

2 Using `ensembldb` annotation packages to retrieve specific annotations

One of the strengths of the `ensembldb` package and the related `EnsDb` databases is its implementation of a filter framework that enables to efficiently extract data sub-sets from the databases. The `ensembldb` package supports most of the filters defined in the `AnnotationFilter` Bioconductor package and defines some additional filters specific to the data stored in `EnsDb` databases. Filters can be passed directly to all methods extracting data from an `EnsDb` (such as `genes`, `transcripts` or `exons`). Alternatively it is possible with the `addFilter` or `filter` functions to add a filter directly to an `EnsDb` which will then be used in all queries on that object.

The `supportedFilters` method can be used to get an overview over all supported filter classes, each of them (except the `GRangesFilter`) working on a single column/field in the database.

```
supportedFilters(edb)
```

```
##                       filter                field
## 1               EntrezFilter               entrez
## 2             ExonEndFilter             exon_end
## 3              ExonIdFilter              exon_id
## 4            ExonRankFilter            exon_rank
## 5           ExonStartFilter           exon_start
## 6             GRangesFilter                 <NA>
## 7         GeneBiotypeFilter         gene_biotype
## 8             GeneEndFilter             gene_end
## 9              GeneIdFilter              gene_id
## 10           GeneNameFilter            gene_name
## 11          GeneStartFilter           gene_start
## 12           GenenameFilter             genename
## 13          ProtDomIdFilter          prot_dom_id
## 14     ProteinDomainIdFilter    protein_domain_id
## 15 ProteinDomainSourceFilter protein_domain_source
## 16          ProteinIdFilter            protein_id
## 17            SeqNameFilter              seq_name
## 18          SeqStrandFilter            seq_strand
## 19             SymbolFilter                symbol
## 20          TxBiotypeFilter            tx_biotype
## 21              TxEndFilter                tx_end
## 22               TxIdFilter                 tx_id
## 23             TxNameFilter               tx_name
## 24            TxStartFilter              tx_start
## 25          UniprotDbFilter            uniprot_db
## 26            UniprotFilter               uniprot
## 27  UniprotMappingTypeFilter  uniprot_mapping_type
```

These filters can be divided into 3 main filter types:

- `IntegerFilter`: filter classes extending this basic object can take a single numeric value as input and support the conditions `==`, `!=`, `>`, `<`, `>=` and `<=`. All filters that work on chromosomal coordinates, such as the `GeneEndFilter` extend `IntegerFilter`.
- `CharacterFilter`: filter classes extending this object can take a single or multiple character values as input and allow

conditions: `==`, `!=`, `"startsWith"`, `"endsWith"` and `"contains"`. All filters working on IDs extend this class.

- `GRangesFilter`: takes a `GRanges` object as input and supports all conditions that `findOverlaps` from the `IRanges` package supports (`"any"`, `"start"`, `"end"`, `"within"`, `"equal"`). Note that these have to be passed using the parameter `type` to the constructor function.

The supported filters are:

- `EntrezFilter`: allows to filter results based on NCBI Entrezgene identifiers of the genes.
- `ExonEndFilter`: filter using the chromosomal end coordinate of exons.
- `ExonIdFilter`: filter based on the (Ensembl) exon identifiers.
- `ExonRankFilter`: filter based on the rank (index) of an exon within the transcript model. Exons are always numbered from 5' to 3' end of the transcript, thus, also on the reverse strand, the exon 1 is the most 5' exon of the transcript.
- `ExonStartFilter`: filter using the chromosomal start coordinate of exons.
- `GeneBiotypeFilter`: filter using the gene biotypes defined in the Ensembl database; use the `listGenebiotypes` method to list all available biotypes.
- `GeneEndFilter`: filter using the chromosomal end coordinate of gene.
- `GeneIdFilter`: filter based on the Ensembl gene IDs.
- `GeneNameFilter`: filter based on the names (symbols) of the genes.
- `GeneStartFilter`: filter using the chromosomal start coordinate of gene.
- `GRangesFilter`: allows to retrieve all features (genes, transcripts or exons) that are either within (setting parameter `type` to "within") or partially overlapping (setting `type` to "any") the defined genomic region/range. Note that, depending on the called method (`genes`, `transcripts` or `exons`) the start and end coordinates of either the genes, transcripts or exons are used for the filter. For methods `exonsBy`, `cdsBy` and `txBy` the coordinates of `by` are used.
- `SeqNameFilter`: filter by the name of the chromosomes the genes are encoded on.
- `SeqStrandFilter`: filter for the chromosome strand on which the genes are encoded.
- `SymbolFilter`: filter on gene symbols; note that no database columns *symbol* is available in an `EnsDb` database and hence the gene name is used for filtering.
- `TxBiotypeFilter`: filter on the transcript biotype defined in Ensembl; use the `listTxbiotypes` method to list all available biotypes.
- `TxEndFilter`: filter using the chromosomal end coordinate of transcripts.
- `TxIdFilter`: filter on the Ensembl transcript identifiers.

- `TxNameFilter` : filter on the Ensembl transcript names (currently identical to the transcript IDs).
- `TxStartFilter` : filter using the chromosomal start coordinate of transcripts.

In addition to the above listed *DNA-RNA-based* filters, *protein-specific* filters are also available:

- `ProtDomIdFilter` , `ProteinDomainIdFilter` : filter by the protein domain ID.
- `ProteinDomainSourceFilter` : filter by the source of the protein domain (database or method, e.g. *pfam*).
- `ProteinIdFilter` : filter by Ensembl protein ID filters.
- `UniprotDbFilter` : filter by the name of the Uniprot database.
- `UniprotFilter` : filter by the Uniprot ID.
- `UniprotMappingTypeFilter` : filter by the mapping type of Ensembl protein IDs to Uniprot IDs.

These can however only be used on `EnsDb` databases that provide protein annotations, i.e. for which a call to `hasProteinData` returns `TRUE` .

`EnsDb` databases for more recent Ensembl versions (starting from Ensembl 87) provide also evidence levels for individual transcripts in the `tx_support_level` database column. Such databases support also a `TxSupportLevelFilter` filter to use this columns for filtering.

A simple use case for the filter framework would be to get all transcripts for the gene *BCL2L11*. To this end we specify a `GeneNameFilter` with the value *BCL2L11*. As a result we get a `GRanges` object with `start` , `end` , `strand` and `seqname` being the start coordinate, end coordinate, chromosome name and strand for the respective transcripts. All additional annotations are available as metadata columns. Alternatively, by setting `return.type` to “DataFrame”, or “data.frame” the method would return a `DataFrame` or `data.frame` object instead of the default `GRanges` .

```
Tx <- transcripts(edb, filter = GeneNameFilter("BCL2L1
1"))

Tx
```

```
## GRanges object with 28 ranges and 7 metadata column
s:
##                    seqnames              ranges stra
nd |           tx_id
##                       <Rle>           <IRanges>  <Rl
e> |     <character>
##   ENST00000432179        2 111119378-111124112
+ | ENST00000432179
##   ENST00000308659        2 111120914-111165048
+ | ENST00000308659
##   ENST00000337565        2 111120914-111128844
+ | ENST00000337565
##   ENST00000622509        2 111120914-111168445
+ | ENST00000622509
##   ENST00000619294        2 111120914-111168445
+ | ENST00000619294
##               ...      ...                 ...
... .             ...
##   ENST00000452231        2 111123746-111164231
+ | ENST00000452231
##   ENST00000361493        2 111123746-111164231
+ | ENST00000361493
##   ENST00000431217        2 111123746-111164352
+ | ENST00000431217
##   ENST00000439718        2 111123746-111164643
+ | ENST00000439718
##   ENST00000438054        2 111123752-111146284
+ | ENST00000438054
##                                tx_biotype tx_cds_se
q_start tx_cds_seq_end
##                               <character>        <i
nteger>      <integer>
##   ENST00000432179          protein_coding        11
1123746      111124112
##   ENST00000308659          protein_coding        11
1123746      111164231
##   ENST00000337565          protein_coding        11
1123746      111128751
##   ENST00000622509          protein_coding        11
1123746      111161439
##   ENST00000619294          protein_coding        11
1123746      111144501
##               ...                     ...
...             ...
##   ENST00000452231 nonsense_mediated_decay        11
1123746      111161439
##   ENST00000361493 nonsense_mediated_decay        11
1123746      111130235
##   ENST00000431217 nonsense_mediated_decay        11
1123746      111144501
##   ENST00000439718 nonsense_mediated_decay        11
1123746      111151851
##   ENST00000438054          protein_coding        11
1123752      111144491
##                           gene_id         tx_name
gene_name
##                       <character>     <character> <
character>
##   ENST00000432179 ENSG00000153094 ENST00000432179
BCL2L11
```

```
##   ENST00000308659 ENSG00000153094 ENST00000308659
BCL2L11
##   ENST00000337565 ENSG00000153094 ENST00000337565
BCL2L11
##   ENST00000622509 ENSG00000153094 ENST00000622509
BCL2L11
##   ENST00000619294 ENSG00000153094 ENST00000619294
BCL2L11
##               ...             ...             ...
...
##   ENST00000452231 ENSG00000153094 ENST00000452231
BCL2L11
##   ENST00000361493 ENSG00000153094 ENST00000361493
BCL2L11
##   ENST00000431217 ENSG00000153094 ENST00000431217
BCL2L11
##   ENST00000439718 ENSG00000153094 ENST00000439718
BCL2L11
##   ENST00000438054 ENSG00000153094 ENST00000438054
BCL2L11
##   -------
##   seqinfo: 1 sequence from GRCh38 genome
```

```
## as this is a GRanges object we can access e.g. the
 start coordinates with
head(start(Tx))
```

```
## [1] 111119378 111120914 111120914 111120914 1111209
14 111120914
```

```
## or extract the biotype with
head(Tx$tx_biotype)
```

```
## [1] "protein_coding" "protein_coding" "protein_codi
ng" "protein_coding"
## [5] "protein_coding" "protein_coding"
```

The parameter `columns` of the extractor methods (such as `exons`, `genes` or `transcripts`) allows to specify which database attributes (columns) should be retrieved. The `exons` method returns by default all exon-related columns, the `transcripts` all columns from the transcript database table and the `genes` all from the gene table. Note however that in the example above we got also a column `gene_name` although this column is not present in the transcript database table. By default the methods return also all columns that are used by any of the filters submitted with the `filter` argument (thus, because a `GeneNameFilter` was used, the column `gene_name` is also returned). Setting `returnFilterColumns(edb) <- FALSE` disables this option and only the columns specified by the `columns` parameter are retrieved.

Instead of passing a filter *object* to the method it is also possible to provide a filter *expression* written as a `formula`. The `formula` has to be written in the form `~ <field> <condition> <value>` with `<field>` being the field (database column) in the database,

`<condition>` the condition for the filter object and `<value>` its value. Use the `supportedFilter` method to get the field names corresponding to each filter class.

```
## Use a filter expression to perform the filtering.
transcripts(edb, filter = ~ gene_name == "ZBTB16")
```

```
## GRanges object with 9 ranges and 7 metadata column
s:
##                    seqnames              ranges stra
nd |           tx_id
##                       <Rle>           <IRanges>  <Rl
e> |     <character>
##   ENST00000335953       11 114059593-114250676
+ | ENST00000335953
##   ENST00000541602       11 114059725-114189764
+ | ENST00000541602
##   ENST00000544220       11 114059737-114063646
+ | ENST00000544220
##   ENST00000535700       11 114060257-114063744
+ | ENST00000535700
##   ENST00000392996       11 114060507-114250652
+ | ENST00000392996
##   ENST00000539918       11 114064412-114247344
+ | ENST00000539918
##   ENST00000545851       11 114180766-114247296
+ | ENST00000545851
##   ENST00000535379       11 114237207-114250557
+ | ENST00000535379
##   ENST00000535509       11 114246790-114250476
+ | ENST00000535509
##                                tx_biotype tx_cds_se
q_start tx_cds_seq_end
##                               <character>        <i
nteger>      <integer>
##   ENST00000335953          protein_coding        11
4063301      114250555
##   ENST00000541602         retained_intron
<NA>           <NA>
##   ENST00000544220          protein_coding        11
4063301      114063646
##   ENST00000535700          protein_coding        11
4063301      114063744
##   ENST00000392996          protein_coding        11
4063301      114250555
##   ENST00000539918 nonsense_mediated_decay        11
4064412      114121827
##   ENST00000545851    processed_transcript
<NA>           <NA>
##   ENST00000535379    processed_transcript
<NA>           <NA>
##   ENST00000535509         retained_intron
<NA>           <NA>
##                            gene_id         tx_name
gene_name
##                        <character>     <character> <
character>
##   ENST00000335953 ENSG00000109906 ENST00000335953
ZBTB16
##   ENST00000541602 ENSG00000109906 ENST00000541602
ZBTB16
##   ENST00000544220 ENSG00000109906 ENST00000544220
ZBTB16
##   ENST00000535700 ENSG00000109906 ENST00000535700
ZBTB16
##   ENST00000392996 ENSG00000109906 ENST00000392996
ZBTB16
```

```
##   ENST00000539918 ENSG00000109906 ENST00000539918
ZBTB16
##   ENST00000545851 ENSG00000109906 ENST00000545851
ZBTB16
##   ENST00000535379 ENSG00000109906 ENST00000535379
ZBTB16
##   ENST00000535509 ENSG00000109906 ENST00000535509
ZBTB16
##   -------
##   seqinfo: 1 sequence from GRCh38 genome
```

Filter expression have to be written as a formula (i.e. starting with a `~`) in the form *column name* followed by the logical condition.

Alternatively, `EnsDb` objects can be filtered directly using the `filter` function. In the example below we use the `filter` function to filter the `EnsDb` object and pass that filtered database to the `transcripts` method using the `%>%` from the `magrittr` package.

```
library(magrittr)

edb %>% filter(~ symbol == "BCL2" & tx_biotype != "pro
tein_coding") %>%
    transcripts
```

```
## GRanges object with 1 range and 6 metadata columns:
##                    seqnames            ranges strand
|           tx_id
##                       <Rle>         <IRanges>  <Rle>
|     <character>
##   ENST00000590515       18 63128212-63161869      -
| ENST00000590515
##                             tx_biotype tx_cds_seq_s
tart tx_cds_seq_end
##                            <character>        <inte
ger>      <integer>
##   ENST00000590515 processed_transcript
<NA>           <NA>
##                           gene_id         tx_name
##                       <character>     <character>
##   ENST00000590515 ENSG00000171791 ENST00000590515
##   -------
##   seqinfo: 1 sequence from GRCh38 genome
```

Adding a filter to an `EnsDb` enables this filter (globally) on all subsequent queries on that object. We could thus filter an `EnsDb` to (virtually) contain only features encoded on chromosome Y.

```
edb_y <- addFilter(edb, SeqNameFilter("Y"))

## All subsequent filters on that EnsDb will only work
on features encoded on
## chromosome Y
genes(edb_y)
```

```
## GRanges object with 523 ranges and 6 metadata colum
ns:
##                    seqnames            ranges strand
|         gene_id
##                       <Rle>         <IRanges>  <Rle>
|     <character>
##    ENSG00000251841        Y   2784749-2784853      +
| ENSG00000251841
##    ENSG00000184895        Y   2786855-2787699      -
| ENSG00000184895
##    ENSG00000237659        Y   2789827-2790328      +
| ENSG00000237659
##    ENSG00000232195        Y   2827982-2828218      +
| ENSG00000232195
##    ENSG00000129824        Y   2841486-2932000      +
| ENSG00000129824
##                ...      ...               ...    ...
.             ...
##    ENSG00000224240        Y 26549425-26549743      +
| ENSG00000224240
##    ENSG00000227629        Y 26586642-26591601      -
| ENSG00000227629
##    ENSG00000237917        Y 26594851-26634652      -
| ENSG00000237917
##    ENSG00000231514        Y 26626520-26627159      -
| ENSG00000231514
##    ENSG00000235857        Y 56855244-56855488      +
| ENSG00000235857
##                      gene_name           gene_biotyp
e seq_coord_system
##                    <character>            <character
>      <character>
##    ENSG00000251841  RNU6-1334P                  snRN
A       chromosome
##    ENSG00000184895         SRY         protein_codin
g       chromosome
##    ENSG00000237659  RNASEH2CP1   processed_pseudogen
e       chromosome
##    ENSG00000232195    TOMM22P2   processed_pseudogen
e       chromosome
##    ENSG00000129824      RPS4Y1         protein_codin
g       chromosome
##                ...         ...
...             ...
##    ENSG00000224240     CYCSP49   processed_pseudogen
e       chromosome
##    ENSG00000227629  SLC25A15P1 unprocessed_pseudogen
e       chromosome
##    ENSG00000237917     PARP4P1 unprocessed_pseudogen
e       chromosome
##    ENSG00000231514     FAM58CP   processed_pseudogen
e       chromosome
##    ENSG00000235857     CTBP2P1   processed_pseudogen
e       chromosome
##                        symbol entrezid
##                    <character>   <list>
##    ENSG00000251841  RNU6-1334P       NA
##    ENSG00000184895         SRY     6736
##    ENSG00000237659  RNASEH2CP1       NA
##    ENSG00000232195    TOMM22P2       NA
```

```
##   ENSG00000129824      RPS4Y1     6192
##               ...         ...      ...
##   ENSG00000224240     CYCSP49       NA
##   ENSG00000227629  SLC25A15P1       NA
##   ENSG00000237917     PARP4P1       NA
##   ENSG00000231514     FAM58CP       NA
##   ENSG00000235857     CTBP2P1       NA
##   -------
##   seqinfo: 1 sequence from GRCh38 genome

## Get all lincRNAs on chromosome Y
genes(edb_y, filter = ~ gene_biotype == "lincRNA")
```

```
## GRanges object with 52 ranges and 6 metadata column
s:
##                    seqnames            ranges strand
|         gene_id
##                       <Rle>         <IRanges>  <Rle>
|     <character>
##   ENSG00000278847        Y   2934406-2934771      -
| ENSG00000278847
##   ENSG00000231535        Y   3002912-3102272      +
| ENSG00000231535
##   ENSG00000229308        Y   4036497-4100320      +
| ENSG00000229308
##   ENSG00000277930        Y   4993858-4999650      -
| ENSG00000277930
##   ENSG00000237069        Y   6242446-6243610      -
| ENSG00000237069
##               ...      ...               ...    ...
.             ...
##   ENSG00000228296        Y 25063083-25099892      -
| ENSG00000228296
##   ENSG00000223641        Y 25183643-25184773      -
| ENSG00000223641
##   ENSG00000228786        Y 25378300-25394719      -
| ENSG00000228786
##   ENSG00000240450        Y 25482908-25486705      +
| ENSG00000240450
##   ENSG00000231141        Y 25728490-25733388      +
| ENSG00000231141
##                       gene_name gene_biotype seq_co
ord_system        symbol
##                     <character>  <character>      <
character>   <character>
##   ENSG00000278847 RP11-414C23.1      lincRNA
chromosome RP11-414C23.1
##   ENSG00000231535     LINC00278      lincRNA
chromosome     LINC00278
##   ENSG00000229308    AC010084.1      lincRNA
chromosome    AC010084.1
##   ENSG00000277930  RP11-122L9.1      lincRNA
chromosome  RP11-122L9.1
##   ENSG00000237069       TTTY23B      lincRNA
chromosome       TTTY23B
##               ...           ...          ...
...           ...
##   ENSG00000228296        TTTY4C      lincRNA
chromosome        TTTY4C
##   ENSG00000223641       TTTY17C      lincRNA
chromosome       TTTY17C
##   ENSG00000228786  LINC00266-4P      lincRNA
chromosome  LINC00266-4P
##   ENSG00000240450      CSPG4P1Y      lincRNA
chromosome      CSPG4P1Y
##   ENSG00000231141         TTTY3      lincRNA
chromosome         TTTY3
##                                 entrezid
##                                   <list>
##   ENSG00000278847                     NA
##   ENSG00000231535              100873962
##   ENSG00000229308                     NA
##   ENSG00000277930                     NA
```

```
##   ENSG00000237069     100101121,252955
##               ...                  ...
##   ENSG00000228296 474150,474149,114761
##   ENSG00000223641 474152,474151,252949
##   ENSG00000228786                   NA
##   ENSG00000240450               114758
##   ENSG00000231141        474148,114760
##   -------
##   seqinfo: 1 sequence from GRCh38 genome
```

To get an overview of database tables and available columns the function `listTables` can be used. The method `listColumns` on the other hand lists columns for the specified database table.

```
## list all database tables along with their columns
listTables(edb)
```

```
## $gene
## [1] "gene_id"          "gene_name"        "gene_bio
type"     "gene_seq_start"
## [5] "gene_seq_end"     "seq_name"         "seq_stra
nd"       "seq_coord_system"
## [9] "symbol"
##
## $tx
## [1] "tx_id"            "tx_biotype"       "tx_seq_s
tart"     "tx_seq_end"
## [5] "tx_cds_seq_start" "tx_cds_seq_end"   "gene_id"
"tx_name"
##
## $tx2exon
## [1] "tx_id"    "exon_id"  "exon_idx"
##
## $exon
## [1] "exon_id"        "exon_seq_start" "exon_seq_en
d"
##
## $chromosome
## [1] "seq_name"    "seq_length"  "is_circular"
##
## $protein
## [1] "tx_id"            "protein_id"       "protein_
sequence"
##
## $uniprot
## [1] "protein_id"           "uniprot_id"
"uniprot_db"
## [4] "uniprot_mapping_type"
##
## $protein_domain
## [1] "protein_id"            "protein_domain_id"
"protein_domain_source"
## [4] "interpro_accession"    "prot_dom_start"
"prot_dom_end"
##
## $entrezgene
## [1] "gene_id"  "entrezid"
##
## $metadata
## [1] "name"  "value"
```

```
## list columns from a specific table
listColumns(edb, "tx")
```

```
## [1] "tx_id"            "tx_biotype"       "tx_seq_s
tart"     "tx_seq_end"
## [5] "tx_cds_seq_start" "tx_cds_seq_end"   "gene_id"
"tx_name"
```

Thus, we could retrieve all transcripts of the biotype *nonsense_mediated_decay* (which, according to the definitions by Ensembl are transcribed, but most likely not translated in a protein, but rather degraded after transcription) along with the name of the

gene for each transcript. Note that we are changing here the `return.type` to `DataFrame`, so the method will return a `DataFrame` with the results instead of the default `GRanges`.

```
Tx <- transcripts(edb,
          columns = c(listColumns(edb , "tx"), "gene_n
ame"),
          filter = TxBiotypeFilter("nonsense_mediated_
decay"),
          return.type = "DataFrame")
nrow(Tx)
```

```
## [1] 14423
```

```
Tx
```

```
## DataFrame with 14423 rows and 9 columns
##                tx_id             tx_biotype tx_se
q_start tx_seq_end
##          <character>            <character>    <i
nteger>  <integer>
## 1     ENST00000567466 nonsense_mediated_decay
47578      49521
## 2     ENST00000397876 nonsense_mediated_decay
53887      57372
## 3     ENST00000428730 nonsense_mediated_decay
58062      65039
## 4     ENST00000417043 nonsense_mediated_decay
62973      65037
## 5     ENST00000622194 nonsense_mediated_decay
85386     138349
## ...               ...                    ...
...        ...
## 14419 ENST00000496411 nonsense_mediated_decay    24
8855728  248859018
## 14420 ENST00000483223 nonsense_mediated_decay    24
8856515  248858529
## 14421 ENST00000533647 nonsense_mediated_decay    24
8857273  248858324
## 14422 ENST00000528141 nonsense_mediated_decay    24
8857391  248859085
## 14423 ENST00000530986 nonsense_mediated_decay    24
8857469  248859085
##       tx_cds_seq_start tx_cds_seq_end         gene_
id         tx_name
##              <integer>      <integer>     <characte
r>     <character>
## 1                48546          48893 ENSG000002614
56 ENST00000567466
## 2                54017          56360 ENSG000001619
81 ENST00000397876
## 3                62884          65015 ENSG000000073
84 ENST00000428730
## 4                63904          65015 ENSG000000073
84 ENST00000417043
## 5               117330         138267 ENSG000001031
48 ENST00000622194
## ...                ...            ...
...             ...
## 14419        248857954      248858309 ENSG000001711
63 ENST00000496411
## 14420        248857954      248858309 ENSG000001711
63 ENST00000483223
## 14421        248857954      248858309 ENSG000001711
63 ENST00000533647
## 14422        248858004      248858309 ENSG000001711
63 ENST00000528141
## 14423        248858004      248858309 ENSG000001711
63 ENST00000530986
##         gene_name
##       <character>
## 1           TUBB8
## 2         SNRNP25
## 3          RHBDF1
## 4          RHBDF1
## 5           NPRL3
```

```
## ...           ...
## 14419      ZNF692
## 14420      ZNF692
## 14421      ZNF692
## 14422      ZNF692
## 14423      ZNF692
```

For protein coding transcripts, we can also specifically extract their coding region. In the example below we extract the CDS for all transcripts encoded on chromosome Y.

```
yCds <- cdsBy(edb, filter = SeqNameFilter("Y"))
yCds
```

```
## GRangesList object of length 151:
## $ENST00000155093
## GRanges object with 7 ranges and 3 metadata column
s:
##       seqnames          ranges strand |    seq_name
exon_id exon_rank
##          <Rle>       <IRanges>  <Rle> | <character>
<character> <integer>
##   [1]        Y 2953937-2953997      + |           Y
ENSE00002223884         2
##   [2]        Y 2961074-2961646      + |           Y
ENSE00003645989         3
##   [3]        Y 2975095-2975244      + |           Y
ENSE00003764421         4
##   [4]        Y 2975511-2975654      + |           Y
ENSE00003768468         5
##   [5]        Y 2976670-2976822      + |           Y
ENSE00003766362         6
##   [6]        Y 2977940-2978080      + |           Y
ENSE00003766086         7
##   [7]        Y 2978810-2979993      + |           Y
ENSE00001368923         8
##   -------
##   seqinfo: 1 sequence from GRCh38 genome
##
## $ENST00000215473
## GRanges object with 2 ranges and 3 metadata column
s:
##       seqnames          ranges strand |    seq_name
exon_id exon_rank
##          <Rle>       <IRanges>  <Rle> | <character>
<character> <integer>
##   [1]        Y 5056824-5057459      + |           Y
ENSE00001436852         1
##   [2]        Y 5098215-5100740      + |           Y
ENSE00003741448         2
##   -------
##   seqinfo: 1 sequence from GRCh38 genome
##
## $ENST00000215479
## GRanges object with 5 ranges and 3 metadata column
s:
##       seqnames          ranges strand |    seq_name
exon_id exon_rank
##          <Rle>       <IRanges>  <Rle> | <character>
<character> <integer>
##   [1]        Y 6872555-6872608      - |           Y
ENSE00001671586         2
##   [2]        Y 6870006-6870053      - |           Y
ENSE00001645681         3
##   [3]        Y 6868732-6868776      - |           Y
ENSE00000652250         4
##   [4]        Y 6868037-6868462      - |           Y
ENSE00001667251         5
##   [5]        Y 6866073-6866078      - |           Y
ENSE00001494454         6
##   -------
##   seqinfo: 1 sequence from GRCh38 genome
##
```

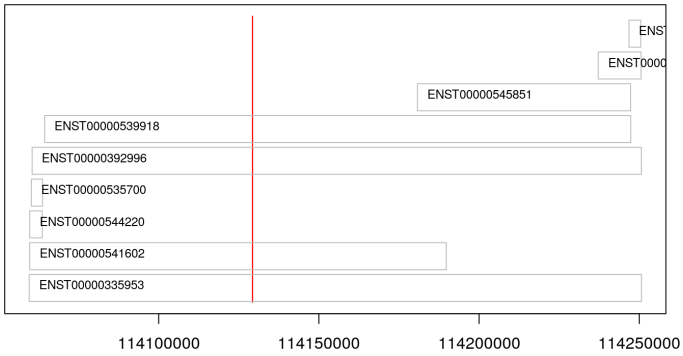
```
## ...
## <148 more elements>
```

Using a `GRangesFilter` we can retrieve all features from the database that are either within or overlapping the specified genomic region. In the example below we query all genes that are partially overlapping with a small region on chromosome 11. The filter restricts to all genes for which either an exon or an intron is partially overlapping with the region.

```
## Define the filter
grf <- GRangesFilter(GRanges("11", ranges = IRanges(11
4129278, 114129328),
                strand = "+"), type = "any")

## Query genes:
gn <- genes(edb, filter = grf)
gn
```

```
## GRanges object with 1 range and 6 metadata columns:
##                    seqnames              ranges stra
nd |         gene_id
##                       <Rle>           <IRanges>  <Rl
e> |     <character>
##   ENSG00000109906       11 114059593-114250676
+ | ENSG00000109906
##                     gene_name   gene_biotype seq_co
ord_system      symbol
##                   <character>    <character>      <
character> <character>
##   ENSG00000109906      ZBTB16 protein_coding
chromosome      ZBTB16
##                   entrezid
##                     <list>
##   ENSG00000109906     7704
##   -------
##   seqinfo: 1 sequence from GRCh38 genome
```

```
## Next we retrieve all transcripts for that gene so t
hat we can plot them.
txs <- transcripts(edb, filter = GeneNameFilter(gn$gen
e_name))
```



3

As we can see, 4 transcripts of the gene ZBTB16 are also overlapping the region. Below we fetch these 4 transcripts. Note, that a call to `exons` will not return any features from the database,

as no exon is overlapping with the region.

```
transcripts(edb, filter = grf)
```

```
## GRanges object with 4 ranges and 6 metadata column
s:
##                    seqnames              ranges stra
nd |           tx_id
##                       <Rle>           <IRanges>  <Rl
e> |     <character>
##   ENST00000335953       11 114059593-114250676
+ | ENST00000335953
##   ENST00000541602       11 114059725-114189764
+ | ENST00000541602
##   ENST00000392996       11 114060507-114250652
+ | ENST00000392996
##   ENST00000539918       11 114064412-114247344
+ | ENST00000539918
##                                tx_biotype tx_cds_se
q_start tx_cds_seq_end
##                               <character>        <i
nteger>      <integer>
##   ENST00000335953          protein_coding        11
4063301      114250555
##   ENST00000541602         retained_intron
<NA>           <NA>
##   ENST00000392996          protein_coding        11
4063301      114250555
##   ENST00000539918 nonsense_mediated_decay        11
4064412      114121827
##                           gene_id         tx_name
##                       <character>     <character>
##   ENST00000335953 ENSG00000109906 ENST00000335953
##   ENST00000541602 ENSG00000109906 ENST00000541602
##   ENST00000392996 ENSG00000109906 ENST00000392996
##   ENST00000539918 ENSG00000109906 ENST00000539918
##   -------
##   seqinfo: 1 sequence from GRCh38 genome
```

The `GRangesFilter` supports also `GRanges` defining multiple regions and a query will return all features overlapping any of these regions. Besides using the `GRangesFilter` it is also possible to search for transcripts or exons overlapping genomic regions using the `exonsByOverlaps` or `transcriptsByOverlaps` known from the `GenomicFeatures` package. Note that the implementation of these methods for `EnsDb` objects supports also to use filters to further fine-tune the query.

The functions `listGenebiotypes` and `listTxbiotypes` can be used to get an overview of allowed/available gene and transcript biotype

```
## Get all gene biotypes from the database. The GeneBi
otypeFilter
## allows to filter on these values.
listGenebiotypes(edb)
```

```
##  [1] "protein_coding"                      "unitary_
pseudogene"
##  [3] "unprocessed_pseudogene"              "processe
d_pseudogene"
##  [5] "processed_transcript"                "transcri
bed_unprocessed_pseudogene"
##  [7] "antisense"                           "transcri
bed_unitary_pseudogene"
##  [9] "polymorphic_pseudogene"              "lincRNA"
## [11] "sense_intronic"                      "transcri
bed_processed_pseudogene"
## [13] "sense_overlapping"                   "IG_V_pse
udogene"
## [15] "pseudogene"                          "TR_V_gen
e"
## [17] "3prime_overlapping_ncRNA"            "IG_V_gen
e"
## [19] "bidirectional_promoter_lncRNA"       "snRNA"
## [21] "miRNA"                               "misc_RN
A"
## [23] "snoRNA"                              "rRNA"
## [25] "Mt_tRNA"                             "Mt_rRNA"
## [27] "IG_C_gene"                           "IG_J_gen
e"
## [29] "TR_J_gene"                           "TR_C_gen
e"
## [31] "TR_V_pseudogene"                     "TR_J_pse
udogene"
## [33] "IG_D_gene"                           "ribozym
e"
## [35] "IG_C_pseudogene"                     "TR_D_gen
e"
## [37] "TEC"                                 "IG_J_pse
udogene"
## [39] "scRNA"                               "scaRNA"
## [41] "vaultRNA"                            "sRNA"
## [43] "macro_lncRNA"                        "non_codi
ng"
## [45] "IG_pseudogene"                       "LRG_gen
e"
```

```
## Get all transcript biotypes from the database.
listTxbiotypes(edb)
```

```
##  [1] "protein_coding"                      "processe
d_transcript"
##  [3] "nonsense_mediated_decay"             "retained
_intron"
##  [5] "unitary_pseudogene"                  "TEC"
##  [7] "miRNA"                               "misc_RN
A"
##  [9] "non_stop_decay"                      "unproces
sed_pseudogene"
## [11] "processed_pseudogene"                "transcri
bed_unprocessed_pseudogene"
## [13] "lincRNA"                             "antisens
e"
## [15] "transcribed_unitary_pseudogene"      "polymorp
hic_pseudogene"
## [17] "sense_intronic"                      "transcri
bed_processed_pseudogene"
## [19] "sense_overlapping"                   "IG_V_pse
udogene"
## [21] "pseudogene"                          "TR_V_gen
e"
## [23] "3prime_overlapping_ncRNA"            "IG_V_gen
e"
## [25] "bidirectional_promoter_lncRNA"       "snRNA"
## [27] "snoRNA"                              "rRNA"
## [29] "Mt_tRNA"                             "Mt_rRNA"
## [31] "IG_C_gene"                           "IG_J_gen
e"
## [33] "TR_J_gene"                           "TR_C_gen
e"
## [35] "TR_V_pseudogene"                     "TR_J_pse
udogene"
## [37] "IG_D_gene"                           "ribozym
e"
## [39] "IG_C_pseudogene"                     "TR_D_gen
e"
## [41] "IG_J_pseudogene"                     "scRNA"
## [43] "scaRNA"                              "vaultRN
A"
## [45] "sRNA"                                "macro_ln
cRNA"
## [47] "non_coding"                          "IG_pseud
ogene"
## [49] "LRG_gene"
```

Data can be fetched in an analogous way using the `exons` and `genes` methods. In the example below we retrieve `gene_name`, `entrezid` and the `gene_biotype` of all genes in the database which names start with “BCL2”.

```
## We're going to fetch all genes which names start wi
th BCL.
BCLs <- genes(edb,
          columns = c("gene_name", "entrezid", "gene_b
iotype"),
          filter = GeneNameFilter("BCL", condition =
"startsWith"),
          return.type = "DataFrame")
nrow(BCLs)
```

```
## [1] 30
```

```
BCLs
```

```
## DataFrame with 30 rows and 4 columns
##       gene_name entrezid         gene_biotype
gene_id
##     <character>   <list>          <character>     <
character>
## 1         BCL10     8915       protein_coding ENSG0
0000142867
## 2        BCL11A    53335       protein_coding ENSG0
0000119866
## 3        BCL11B    64919       protein_coding ENSG0
0000127152
## 4          BCL2      596       protein_coding ENSG0
0000171791
## 5        BCL2A1      597       protein_coding ENSG0
0000140379
## ...         ...      ...                  ...
...
## 26        BCL9L   283149       protein_coding ENSG0
0000186174
## 27       BCL9P1       NA processed_pseudogene ENSG0
0000249238
## 28       BCLAF1     9774       protein_coding ENSG0
0000029363
## 29     BCLAF1P1       NA processed_pseudogene ENSG0
0000248966
## 30     BCLAF1P2       NA processed_pseudogene ENSG0
0000279800
```

Sometimes it might be useful to know the length of genes or transcripts (i.e. the total sum of nucleotides covered by their exons). Below we calculate the mean length of transcripts from protein coding genes on chromosomes X and Y as well as the average length of snoRNA, snRNA and rRNA transcripts encoded on these chromosomes. For the first query we combine two `AnnotationFilter` objects using an `AnnotationFilterList` object, in the second we define the query using a filter expression.

```
## determine the average length of snRNA, snoRNA and r
RNA genes encoded on
## chromosomes X and Y.
mean(lengthOf(edb, of = "tx",
          filter = AnnotationFilterList(
          GeneBiotypeFilter(c("snRNA", "snoRNA", "rRN
A")),
          SeqNameFilter(c("X", "Y")))))
```

```
## [1] 118.2458
```

```
## determine the average length of protein coding gene
s encoded on the same
## chromosomes.
mean(lengthOf(edb, of = "tx",
          filter = ~ gene_biotype == "protein_coding"
 &
          seq_name %in% c("X", "Y")))
```

```
## [1] 1943.554
```

Not unexpectedly, transcripts of protein coding genes are longer than those of snRNA, snoRNA or rRNA genes.

At last we extract the first two exons of each transcript model from the database.

```
## Extract all exons 1 and (if present) 2 for all gene
s encoded on the
## Y chromosome
exons(edb, columns = c("tx_id", "exon_idx"),
      filter = list(SeqNameFilter("Y"),
                    ExonRankFilter(3, condition = "<"
)))
```

```
## GRanges object with 1294 ranges and 3 metadata colu
mns:
##                    seqnames            ranges strand
|           tx_id  exon_idx
##                       <Rle>         <IRanges>  <Rle>
|     <character> <integer>
##    ENSE00002088309        Y   2784749-2784853      +
| ENST00000516032         1
##    ENSE00001494622        Y   2786855-2787699      -
| ENST00000383070         1
##    ENSE00001772499        Y   2789827-2790328      +
| ENST00000454281         1
##    ENSE00001614266        Y   2827982-2828218      +
| ENST00000430735         1
##    ENSE00002490412        Y   2841486-2841627      +
| ENST00000250784         1
##                ...      ...               ...    ...
.             ...       ...
##    ENSE00001632993        Y 26591548-26591601      -
| ENST00000456738         1
##    ENSE00001616687        Y 26626520-26627159      -
| ENST00000435741         1
##    ENSE00001638296        Y 26633345-26633431      -
| ENST00000435945         2
##    ENSE00001797328        Y 26634523-26634652      -
| ENST00000435945         1
##    ENSE00001794473        Y 56855244-56855488      +
| ENST00000431853         1
##                           exon_id
##                         <character>
##    ENSE00002088309 ENSE00002088309
##    ENSE00001494622 ENSE00001494622
##    ENSE00001772499 ENSE00001772499
##    ENSE00001614266 ENSE00001614266
##    ENSE00002490412 ENSE00002490412
##                ...             ...
##    ENSE00001632993 ENSE00001632993
##    ENSE00001616687 ENSE00001616687
##    ENSE00001638296 ENSE00001638296
##    ENSE00001797328 ENSE00001797328
##    ENSE00001794473 ENSE00001794473
##    -------
##    seqinfo: 1 sequence from GRCh38 genome
```

3 Extracting gene/transcript/exon models for RNASeq feature counting

For the feature counting step of an RNAseq experiment, the gene or transcript models (defined by the chromosomal start and end positions of their exons) have to be known. To extract these from an Ensembl based annotation package, the `exonsBy`, `genesBy` and `transcriptsBy` methods can be used in an analogous way as in `TxDb` packages generated by the `GenomicFeatures` package. However, the `transcriptsBy` method does not, in contrast to the method in the `GenomicFeatures` package, allow to return transcripts by “cds”. While the annotation packages built by the

`ensembldb` contain the chromosomal start and end coordinates of the coding region (for protein coding genes) they do not assign an ID to each CDS.

A simple use case is to retrieve all genes encoded on chromosomes X and Y from the database.

```
TxByGns <- transcriptsBy(edb, by = "gene", filter = Se
qNameFilter(c("X", "Y")))
TxByGns
```

```
## GRangesList object of length 2922:
## $ENSG00000000003
## GRanges object with 5 ranges and 6 metadata column
s:
##       seqnames              ranges strand |
tx_id
##          <Rle>           <IRanges>  <Rle> |     <ch
aracter>
##   [1]        X 100633442-100639991      - | ENST000
00494424
##   [2]        X 100627109-100637104      - | ENST000
00612152
##   [3]        X 100632063-100637104      - | ENST000
00614008
##   [4]        X 100628670-100636806      - | ENST000
00373020
##   [5]        X 100632541-100636689      - | ENST000
00496771
##                 tx_biotype tx_cds_seq_start tx_cds_
seq_end         gene_id
##                <character>        <integer>      <i
nteger>     <character>
##   [1] processed_transcript             <NA>
<NA> ENSG00000000003
##   [2]       protein_coding        100630798      10
0635569 ENSG00000000003
##   [3]       protein_coding        100632063      10
0635569 ENSG00000000003
##   [4]       protein_coding        100630798      10
0636694 ENSG00000000003
##   [5] processed_transcript             <NA>
<NA> ENSG00000000003
##               tx_name
##           <character>
##   [1] ENST00000494424
##   [2] ENST00000612152
##   [3] ENST00000614008
##   [4] ENST00000373020
##   [5] ENST00000496771
##   -------
##   seqinfo: 2 sequences from GRCh38 genome
##
## $ENSG00000000005
## GRanges object with 2 ranges and 6 metadata column
s:
##       seqnames              ranges strand |
tx_id
##          <Rle>           <IRanges>  <Rle> |     <ch
aracter>
##   [1]        X 100584802-100599885      + | ENST000
00373031
##   [2]        X 100593624-100597531      + | ENST000
00485971
##                 tx_biotype tx_cds_seq_start tx_cds_
seq_end         gene_id
##                <character>        <integer>      <i
nteger>     <character>
##   [1]       protein_coding        100585019      10
0599717 ENSG00000000005
##   [2] processed_transcript             <NA>
```

```
<NA> ENSG00000000005
##               tx_name
##           <character>
##   [1] ENST00000373031
##   [2] ENST00000485971
##   -------
##   seqinfo: 2 sequences from GRCh38 genome
##
## $ENSG00000001497
## GRanges object with 5 ranges and 6 metadata column
s:
##       seqnames            ranges strand |
tx_id
##          <Rle>         <IRanges>  <Rle> |     <char
acter>
##   [1]        X 65512583-65534775      - | ENST00000
484069
##   [2]        X 65512582-65534756      - | ENST00000
374811
##   [3]        X 65512583-65534756      - | ENST00000
374804
##   [4]        X 65512582-65534754      - | ENST00000
374807
##   [5]        X 65520429-65523617      - | ENST00000
469091
##                    tx_biotype tx_cds_seq_start tx_c
ds_seq_end         gene_id
##                   <character>        <integer>
<integer>     <character>
##   [1] nonsense_mediated_decay         65525021
65534715 ENSG00000001497
##   [2]          protein_coding         65512775
65534715 ENSG00000001497
##   [3]          protein_coding         65512775
65534715 ENSG00000001497
##   [4]          protein_coding         65512775
65534715 ENSG00000001497
##   [5]          protein_coding         65520655
65523617 ENSG00000001497
##               tx_name
##           <character>
##   [1] ENST00000484069
##   [2] ENST00000374811
##   [3] ENST00000374804
##   [4] ENST00000374807
##   [5] ENST00000469091
##   -------
##   seqinfo: 2 sequences from GRCh38 genome
##
## ...
## <2919 more elements>
```

Since Ensembl contains also definitions of genes that are on chromosome variants (supercontigs), it is advisable to specify the chromosome names for which the gene models should be returned.

In a real use case, we might thus want to retrieve all genes encoded on the *standard* chromosomes. In addition it is advisable to use a `GeneIdFilter` to restrict to Ensembl genes only, as also *LRG*

(Locus Reference Genomic) genes[2] are defined in the database, which are partially redundant with Ensembl genes.

```
## will just get exons for all genes on chromosomes 1
 to 22, X and Y.
## Note: want to get rid of the "LRG" genes!!!
EnsGenes <- exonsBy(edb, by = "gene", filter = Annotat
ionFilterList(
                      SeqNameFilter(c(1:22, "X", "Y"
)),
                      GeneIdFilter("ENSG", "startsWit
h")))
```

The code above returns a `GRangesList` that can be used directly as an input for the `summarizeOverlaps` function from the `GenomicAlignments` package [3].

Alternatively, the above `GRangesList` can be transformed to a `data.frame` in *SAF* format that can be used as an input to the `featureCounts` function of the `Rsubread` package [4].

```
## Transforming the GRangesList into a data.frame in S
AF format
EnsGenes.SAF <- toSAF(EnsGenes)
```

Note that the ID by which the `GRangesList` is split is used in the SAF formatted `data.frame` as the `GeneID`. In the example below this would be the Ensembl gene IDs, while the start, end coordinates (along with the strand and chromosomes) are those of the the exons.

In addition, the `disjointExons` function (similar to the one defined in `GenomicFeatures`) can be used to generate a `GRanges` of non-overlapping exon parts which can be used in the `DEXSeq` package.

```
## Create a GRanges of non-overlapping exon parts.
DJE <- disjointExons(edb, filter = AnnotationFilterLis
t(
                  SeqNameFilter(c(1:22, "X", "Y")),
                  GeneIdFilter("ENSG%", "startsWith"
)))
```

4 Retrieving sequences for gene/transcript/exon models

The methods to retrieve exons, transcripts and genes (i.e. `exons`, `transcripts` and `genes`) return by default `GRanges` objects that can be used to retrieve sequences using the `getSeq` method e.g. from BSgenome packages. The basic workflow is thus identical to the one for `TxDb` packages, however, it is not straight forward to identify the BSgenome package with the matching genomic sequence. Most BSgenome packages are named according to the genome build identifier used in UCSC which does not (always) match the genome build name used by Ensembl. Using the

Ensembl version provided by the `EnsDb`, the correct genomic sequence can however be retrieved easily from the `AnnotationHub` using the `getGenomeTeoBitFile`. If no 2bit file matching the Ensembl version is available, the function tries to identify a file with the correct genome build from the *closest* Ensembl release and returns that instead.

In the code block below we retrieve first the `TwoBitFile` with the genomic DNA sequence, extract the genomic start and end coordinates for all genes defined in the package, subset to genes encoded on sequences available in the `TwoBitFile` and extract all of their sequences. Note: these sequences represent the sequence between the chromosomal start and end coordinates of the gene.

```
library(EnsDb.Hsapiens.v86)
edb <- EnsDb.Hsapiens.v86

## Get the TwoBit with the genomic sequence matching t
he Ensembl version
## using the AnnotationHub package.
dna <- ensembldb:::getGenomeTwoBitFile(edb)

## Get start/end coordinates of all genes.
genes <- genes(edb)
## Subset to all genes that are encoded on chromosomes
for which
## we do have DNA sequence available.
genes <- genes[seqnames(genes) %in% seqnames(seqinfo(d
na))]

## Get the gene sequences, i.e. the sequence including
the sequence of
## all of the gene's exons and introns.
geneSeqs <- getSeq(dna, genes)
```

To retrieve the (exonic) sequence of transcripts (i.e. without introns) we can use directly the `extractTranscriptSeqs` method defined in the `GenomicFeatures` on the `EnsDb` object, eventually using a filter to restrict the query.

```
## get all exons of all transcripts encoded on chromos
ome Y
yTx <- exonsBy(edb, filter = SeqNameFilter("Y"))

## Retrieve the sequences for these transcripts from t
he TwoBitile.
library(GenomicFeatures)
yTxSeqs <- extractTranscriptSeqs(dna, yTx)
yTxSeqs

## Extract the sequences of all transcripts encoded on
chromosome Y.
yTx <- extractTranscriptSeqs(dna, edb, filter = SeqNam
eFilter("Y"))

## Along these lines, we could use the method also to
 retrieve the coding sequence
## of all transcripts on the Y chromosome.
cdsY <- cdsBy(edb, filter = SeqNameFilter("Y"))
extractTranscriptSeqs(dna, cdsY)
```

Next we retrieve transcript sequences from genes encoded on chromosome Y using the `BSGenome` package for the human genome. Ensembl version 86 based on the `GRCh38` genome build and we thus load the corresponding `BSGenome` package.

```
library(BSgenome.Hsapiens.NCBI.GRCh38)
bsg <- BSgenome.Hsapiens.NCBI.GRCh38

## Get the genome version
unique(genome(bsg))


## [1] "GRCh38"


unique(genome(edb))


## [1] "GRCh38"


## Extract the full transcript sequences.
yTxSeqs <- extractTranscriptSeqs(
  bsg, exonsBy(edb, "tx", filter = SeqNameFilter("Y"
)))

yTxSeqs
```

```
## DNAStringSet object of length 740:
##       width seq
names
##   [1]  5239 GCCTAGTGCGCGCGCAGTAACC...AATAAATGTTTACT
TGTATATG ENST00000155093
##   [2]  4595 CTGGTGGTCCAGTACCTCCAAA...TGAGCCCTTCAGAA
GACATTCT ENST00000215473
##   [3]   802 AGAGGACCAAGCCTCCCTGTGT...CAATAAAATGTTTT
AAAAATCA ENST00000215479
##   [4]   910 TGTCTGTCAGAGCTGTCAGCCT...TAAACACTGGTATA
TTTCTGTT ENST00000250776
##   [5]  1305 TTCCAGGATATGAACTCTACAG...TAAATCCTGTGGCT
GTAGGAAA ENST00000250784
##   ...   ... ...
## [736]   792 ATGGCCCGGGGCCCCAAGAAGC...TGCCAAACAGAGCA
GTGGCTAA ENST00000629237
## [737]   344 GGTTGCCACTTCAAGGGACTAC...CTGGCTCTTCTGGC
AGTTTTTT ENST00000631331
## [738]   933 CTCTCCCAGCTTCTACCCACAG...GCATACTATAAAAA
TGCTTTAA ENST00000634531
## [739]  1832 ATGTCTGCTGCAAATCCTGAGA...AGTATTTAAATCTG
TTGGATCC ENST00000634662
## [740]   890 CTCTCCCAGCTTCTACCCACAG...GCATACTATAAAAA
TGCTTTAA ENST00000635343


## Extract just the CDS
Test <- cdsBy(edb, "tx", filter = SeqNameFilter("Y"))
yTxCds <- extractTranscriptSeqs(
  bsg, cdsBy(edb, "tx", filter = SeqNameFilter("Y")))
yTxCds


## DNAStringSet object of length 151:
##       width seq
names
##   [1]  2406 ATGGATGAAGATGAATTTGAAT...TAAAGAAGTTGGTC
TGCCCTAA ENST00000155093
##   [2]  3162 ATGTTTAGGGTTGGCTTCTTAA...AGTTTCTAACACAA
CTTTCTAA ENST00000215473
##   [3]   579 ATGGGGACCTGGATTTTGTTTG...CAAGCAGGAGGAAG
TGGATTAA ENST00000215479
##   [4]   792 ATGGCCCGGGGCCCCAAGAAGC...CACCAAACAGAGCA
GTGGCTAA ENST00000250784
##   [5]   378 ATGAGTCCAAAGCCGAGAGCCT...ATCTACTCCCCTAT
CTCCCTGA ENST00000250823
##   ...   ... ...
## [147]   387 ATGCAAAGCCAGAGAGGTCTCC...CACACTCTGTGTCC
CAAAATGA ENST00000624507
## [148]    78 ATGAGAGCCAAGTGGAGGAAGA...GATGAGGCAGAAGT
CCAAGTAA ENST00000624575
## [149]  1833 ATGGATGAAGATGAATTTGAAT...TAAAGAAGTTGGTC
TGCCCTAA ENST00000625061
## [150]   792 ATGGCCCGGGGCCCCAAGAAGC...TGCCAAACAGAGCA
GTGGCTAA ENST00000629237
## [151]  1740 ATGTCTGCTGCAAATCCTGAGA...TTTAATCCAGAGAA
GAGACTGA ENST00000634662
```

5 Integrating annotations from Ensembl based `EnsDb` packages with UCSC based annotations

Sometimes it might be useful to combine (Ensembl based) annotations from `EnsDb` packages/objects with annotations from other Bioconductor packages, that might base on UCSC annotations. To support such an integration of annotations, the `ensembldb` packages implements the `seqlevelsStyle` and `seqlevelsStyle<-` from the `GenomeInfoDb` package that allow to change the style of chromosome naming. Thus, sequence/chromosome names other than those used by Ensembl can be used in, and are returned by, the queries to `EnsDb` objects as long as a mapping for them is provided by the `GenomeInfoDb` package (which provides a mapping mostly between UCSC, NCBI and Ensembl chromosome names for the *main* chromosomes).

In the example below we change the seqnames style to UCSC.

```
## Change the seqlevels style form Ensembl (default) t
o UCSC:
seqlevelsStyle(edb) <- "UCSC"

## Now we can use UCSC style seqnames in SeqNameFilter
s or GRangesFilter:
genesY <- genes(edb, filter = ~ seq_name == "chrY")
## The seqlevels of the returned GRanges are also in U
CSC style
seqlevels(genesY)
```

```
## [1] "chrY"
```

Note that in most instances no mapping is available for sequences not corresponding to the main chromosomes (i.e. contigs, patched chromosomes etc). What is returned in cases in which no mapping is available can be specified with the global `ensembldb.seqnameNotFound` option. By default (with `ensembldb.seqnameNotFound` set to “ORIGINAL”), the original seqnames (i.e. the ones from Ensembl) are returned. With `ensembldb.seqnameNotFound` “MISSING” each time a seqname can not be found an error is thrown. For all other cases (e.g. `ensembldb.seqnameNotFound = NA`) the value of the option is returned.

```
seqlevelsStyle(edb) <- "UCSC"

## Getting the default option:
getOption("ensembldb.seqnameNotFound")
```

```
## [1] "ORIGINAL"
```

```
## Listing all seqlevels in the database.
seqlevels(edb)[1:30]
```

```
## Warning in .formatSeqnameByStyleFromQuery(x, sn, if
NotFound): More than 5
## seqnames with seqlevels style of the database (Ense
mbl) could not be mapped to
## the seqlevels style: UCSC!) Returning the orginal s
eqnames for these.
```

```
##  [1] "chr1"                          "chr10"
##  [3] "chr11"                         "chr12"
##  [5] "chr13"                         "chr14"
##  [7] "chr15"                         "chr16"
##  [9] "chr17"                         "chr18"
## [11] "chr19"                         "chr2"
## [13] "chr20"                         "chr21"
## [15] "chr22"                         "chr3"
## [17] "chr4"                          "chr5"
## [19] "chr6"                          "chr7"
## [21] "chr8"                          "chr9"
## [23] "CHR_HG107_PATCH"               "CHR_HG126_PATCH"
## [25] "CHR_HG1311_PATCH"              "CHR_HG1342_HG228
2_PATCH"
## [27] "CHR_HG1362_PATCH"              "CHR_HG142_HG150_
NOVEL_TEST"
## [29] "CHR_HG151_NOVEL_TEST"          "CHR_HG1651_PATC
H"
```

```
## Setting the option to NA, thus, for each seqname fo
r which no mapping is available,
## NA is returned.
options(ensembldb.seqnameNotFound=NA)
seqlevels(edb)[1:30]
```

```
## Warning in .formatSeqnameByStyleFromQuery(x, sn, if
NotFound): More than 5
## seqnames with seqlevels style of the database (Ense
mbl) could not be mapped to
## the seqlevels style: UCSC!) Returning NA for these.
```

```
##  [1] "chr1"  "chr10" "chr11" "chr12" "chr13" "chr1
4" "chr15" "chr16" "chr17"
## [10] "chr18" "chr19" "chr2"  "chr20" "chr21" "chr2
2" "chr3"  "chr4"  "chr5"
## [19] "chr6"  "chr7"  "chr8"  "chr9"  NA      NA
NA      NA      NA      NA
## [28] NA      NA      NA
```

```
## Resetting the option.
options(ensembldb.seqnameNotFound = "ORIGINAL")
```

At last changing the seqname style to the default value `"Ensembl"`.

```
seqlevelsStyle(edb) <- "Ensembl"
```

6 Interactive annotation lookup using the shiny web app

In addition to the genes , transcripts and exons methods it is possibly to search interactively for gene/transcript/exon annotations using the internal, shiny based, web application. The application can be started with the runEnsDbApp() function. The search results from this app can also be returned to the R workspace either as a data.frame or GRanges object.

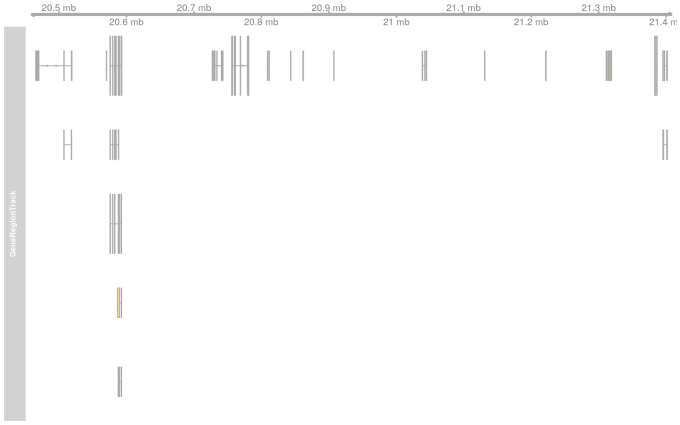
7 Plotting gene/transcript features using ensembldb and Gviz and ggbio

The Gviz package provides functions to plot genes and transcripts along with other data on a genomic scale. Gene models can be provided either as a data.frame , GRanges , TxDB database, can be fetched from biomart and can also be retrieved from ensembldb .

Below we generate a GeneRegionTrack fetching all transcripts from a certain region on chromosome Y.

Note that if we want in addition to work also with BAM files that were aligned against DNA sequences retrieved from Ensembl or FASTA files representing genomic DNA sequences from Ensembl we should change the ucscChromosomeNames option from Gviz to FALSE (i.e. by calling options(ucscChromosomeNames = FALSE)). This is not necessary if we just want to retrieve gene models from an EnsDb object, as the ensembldb package internally checks the ucscChromosomeNames option and, depending on that, maps Ensembl chromosome names to UCSC chromosome names.

```
## Loading the Gviz library
library(Gviz)
library(EnsDb.Hsapiens.v86)
edb <- EnsDb.Hsapiens.v86

## Retrieving a Gviz compatible GRanges object with al
l genes
## encoded on chromosome Y.
gr <- getGeneRegionTrackForGviz(edb, chromosome = "Y",
                start = 20400000, end = 21400000)
## Define a genome axis track
gat <- GenomeAxisTrack()

## We have to change the ucscChromosomeNames option to
FALSE to enable Gviz usage
## with non-UCSC chromosome names.
options(ucscChromosomeNames = FALSE)

plotTracks(list(gat, GeneRegionTrack(gr)))
```

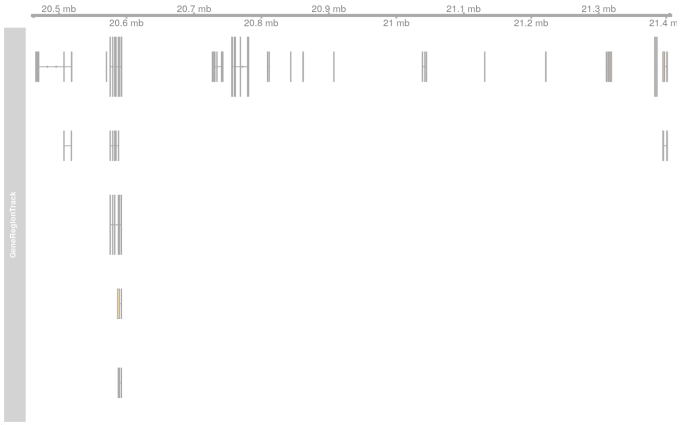



```
options(ucscChromosomeNames = TRUE)
```

Above we had to change the option `ucscChromosomeNames` to `FALSE` in order to use it with non-UCSC chromosome names. Alternatively, we could however also change the `seqnamesStyle` of the `EnsDb` object to `UCSC`. Note that we have to use now also chromosome names in the *UCSC style* in the `SeqNameFilter` (i.e. "chrY" instead of "Y").

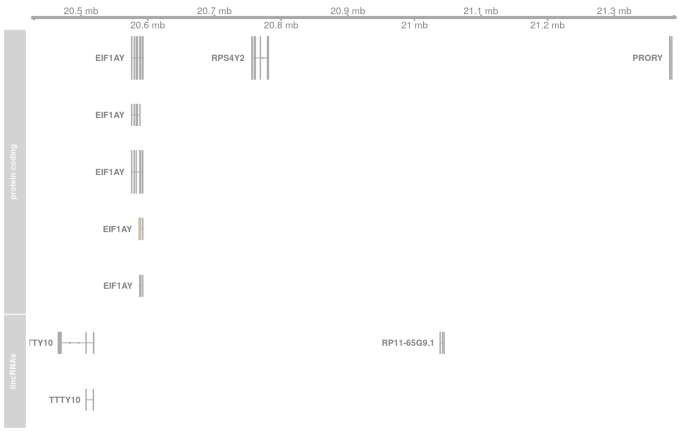
```
seqlevelsStyle(edb) <- "UCSC"
## Retrieving the GRanges objects with seqnames corres
ponding to UCSC chromosome names.
gr <- getGeneRegionTrackForGviz(edb, chromosome = "chr
Y",
                                start = 20400000, end
 = 21400000)


## Warning in .formatSeqnameByStyleForQuery(x, sn, ifN
otFound): Seqnames: Y could
## not be mapped to the seqlevels style of the databas
e (Ensembl)!Returning the
## orginal seqnames for these.


seqnames(gr)
```

```
## factor-Rle of length 89 with 1 run
##   Lengths:   89
##   Values : chrY
## Levels(1): chrY


## Define a genome axis track
gat <- GenomeAxisTrack()
plotTracks(list(gat, GeneRegionTrack(gr)))
```



We can also use the filters from the `ensembldb` package to further refine what transcripts are fetched, like in the example below, in which we create two different gene region tracks, one for protein coding genes and one for lincRNAs.

```
protCod <- getGeneRegionTrackForGviz(edb, chromosome =
"chrY",
                     start = 20400000, end = 21400000,
                     filter = GeneBiotypeFilter("prote
in_coding"))
lincs <- getGeneRegionTrackForGviz(edb, chromosome =
"chrY",
                   start = 20400000, end = 21400000,
                   filter = GeneBiotypeFilter("lincRN
A"))

plotTracks(list(gat, GeneRegionTrack(protCod, name =
"protein coding"),
        GeneRegionTrack(lincs, name = "lincRNAs")), tr
anscriptAnnotation = "symbol")
```



```
## At last we change the seqlevels style again to Ense
mbl
seqlevelsStyle <- "Ensembl"
```

Alternatively, we can also use `ggbio` for plotting. For `ggbio` we can directly pass the `EnsDb` object along with optional filters (or as in the example below a filter expression as a `formula`).

```
library(ggbio)

## Create a plot for all transcripts of the gene SKA2
autoplot(edb, ~ gene_name == "SKA2")
```

To plot the genomic region and plot genes from both strands we can use a `GRangesFilter`.

```
## Get the chromosomal region in which the gene is enc
oded
ska2 <- genes(edb, filter = ~ gene_name == "SKA2")
strand(ska2) <- "*"
autoplot(edb, GRangesFilter(ska2), names.expr = "gene_
name")
```

8 Using `EnsDb` objects in the `AnnotationDbi` framework

Most of the methods defined for objects extending the basic annotation package class `AnnotationDbi` are also defined for `EnsDb` objects (i.e. methods `columns`, `keytypes`, `keys`, `mapIds` and `select`). While these methods can be used analogously to basic annotation packages, the implementation for `EnsDb` objects also support the filtering framework of the `ensembldb` package.

In the example below we first evaluate all the available columns and keytypes in the database and extract then the gene names for all genes encoded on chromosome X.

```
library(EnsDb.Hsapiens.v86)
edb <- EnsDb.Hsapiens.v86

## List all available columns in the database.
columns(edb)
```

```
##  [1] "ENTREZID"            "EXONID"              "E
XONIDX"
##  [4] "EXONSEQEND"          "EXONSEQSTART"        "G
ENEBIOTYPE"
##  [7] "GENEID"              "GENENAME"            "G
ENESEQEND"
## [10] "GENESEQSTART"        "INTERPROACCESSION"   "I
SCIRCULAR"
## [13] "PROTDOMEND"          "PROTDOMSTART"        "P
ROTEINDOMAINID"
## [16] "PROTEINDOMAINSOURCE" "PROTEINID"           "P
ROTEINSEQUENCE"
## [19] "SEQCOORDSYSTEM"      "SEQLENGTH"           "S
EQNAME"
## [22] "SEQSTRAND"           "SYMBOL"              "T
XBIOTYPE"
## [25] "TXCDSSEQEND"         "TXCDSSEQSTART"       "T
XID"
## [28] "TXNAME"              "TXSEQEND"            "T
XSEQSTART"
## [31] "UNIPROTDB"           "UNIPROTID"           "U
NIPROTMAPPINGTYPE"
```

```
## Note that these do *not* correspond to the actual c
olumn names
## of the database that can be passed to methods like
 exons, genes,
## transcripts etc. These column names can be listed w
ith the listColumns
## method.
listColumns(edb)
```

```
##  [1] "seq_name"              "seq_length"
"is_circular"
##  [4] "gene_id"               "entrezid"
"exon_id"
##  [7] "exon_seq_start"        "exon_seq_end"
"gene_name"
## [10] "gene_biotype"          "gene_seq_start"
"gene_seq_end"
## [13] "seq_strand"            "seq_coord_system"
"symbol"
## [16] "name"                  "value"
"tx_id"
## [19] "protein_id"            "protein_sequence"
"protein_domain_id"
## [22] "protein_domain_source" "interpro_accession"
"prot_dom_start"
## [25] "prot_dom_end"          "tx_biotype"
"tx_seq_start"
## [28] "tx_seq_end"            "tx_cds_seq_start"
"tx_cds_seq_end"
## [31] "tx_name"               "exon_idx"
"uniprot_id"
## [34] "uniprot_db"            "uniprot_mapping_type"
```

```
## List all of the supported key types.
keytypes(edb)
```

```
##  [1] "ENTREZID"            "EXONID"              "G
ENEBIOTYPE"
##  [4] "GENEID"              "GENENAME"            "P
ROTDOMID"
##  [7] "PROTEINDOMAINID"     "PROTEINDOMAINSOURCE" "P
ROTEINID"
## [10] "SEQNAME"             "SEQSTRAND"           "S
YMBOL"
## [13] "TXBIOTYPE"           "TXID"                "T
XNAME"
## [16] "UNIPROTID"
```

```
## Get all gene ids from the database.
gids <- keys(edb, keytype = "GENEID")
length(gids)
```

```
## [1] 63970
```

```
## Get all gene names for genes encoded on chromosome
 Y.
gnames <- keys(edb, keytype = "GENENAME", filter = Seq
NameFilter("Y"))
head(gnames)
```

```
## [1] "KDM5D"   "DDX3Y"   "ZFY"     "TBL1Y"   "PCDH11
Y" "AMELY"
```

In the next example we retrieve specific information from the database using the `select` method. First we fetch all transcripts for the genes *BCL2* and *BCL2L11*. In the first call we provide the gene names, while in the second call we employ the filtering system to perform a more fine-grained query to fetch only the protein coding transcripts for these genes.

```
## Use the /standard/ way to fetch data.
select(edb, keys = c("BCL2", "BCL2L11"), keytype = "GE
NENAME",
       columns = c("GENEID", "GENENAME", "TXID", "TXBI
OTYPE"))
```

```
##             GENEID GENENAME            TXID
TXBIOTYPE
## 1  ENSG00000171791     BCL2 ENST00000398117
protein_coding
## 2  ENSG00000171791     BCL2 ENST00000333681
protein_coding
## 3  ENSG00000171791     BCL2 ENST00000590515    proc
essed_transcript
## 4  ENSG00000171791     BCL2 ENST00000589955
protein_coding
## 5  ENSG00000153094  BCL2L11 ENST00000432179
protein_coding
## 6  ENSG00000153094  BCL2L11 ENST00000308659
protein_coding
## 7  ENSG00000153094  BCL2L11 ENST00000393256
protein_coding
## 8  ENSG00000153094  BCL2L11 ENST00000393252
protein_coding
## 9  ENSG00000153094  BCL2L11 ENST00000433098 nonsens
e_mediated_decay
## 10 ENSG00000153094  BCL2L11 ENST00000405953
protein_coding
## 11 ENSG00000153094  BCL2L11 ENST00000415458 nonsens
e_mediated_decay
## 12 ENSG00000153094  BCL2L11 ENST00000436733 nonsens
e_mediated_decay
## 13 ENSG00000153094  BCL2L11 ENST00000437029 nonsens
e_mediated_decay
## 14 ENSG00000153094  BCL2L11 ENST00000452231 nonsens
e_mediated_decay
## 15 ENSG00000153094  BCL2L11 ENST00000361493 nonsens
e_mediated_decay
## 16 ENSG00000153094  BCL2L11 ENST00000431217 nonsens
e_mediated_decay
## 17 ENSG00000153094  BCL2L11 ENST00000439718 nonsens
e_mediated_decay
## 18 ENSG00000153094  BCL2L11 ENST00000438054
protein_coding
## 19 ENSG00000153094  BCL2L11 ENST00000337565
protein_coding
## 20 ENSG00000153094  BCL2L11 ENST00000622509
protein_coding
## 21 ENSG00000153094  BCL2L11 ENST00000619294
protein_coding
## 22 ENSG00000153094  BCL2L11 ENST00000610735
protein_coding
## 23 ENSG00000153094  BCL2L11 ENST00000622612
protein_coding
## 24 ENSG00000153094  BCL2L11 ENST00000357757
protein_coding
## 25 ENSG00000153094  BCL2L11 ENST00000615946
protein_coding
## 26 ENSG00000153094  BCL2L11 ENST00000621302
protein_coding
## 27 ENSG00000153094  BCL2L11 ENST00000620862
protein_coding
## 28         LRG_620  BCL2L11       LRG_620t1
LRG_gene
## 29         LRG_620  BCL2L11       LRG_620t2
LRG_gene
```

```
## 30         LRG_620  BCL2L11       LRG_620t3
LRG_gene
## 31         LRG_620  BCL2L11       LRG_620t4
LRG_gene
## 32         LRG_620  BCL2L11       LRG_620t5
LRG_gene
```

```
## Use the filtering system of ensembldb
select(edb, keys = ~ gene_name %in% c("BCL2", "BCL2L1
1") &
        tx_biotype == "protein_coding",
       columns = c("GENEID", "GENENAME", "TXID", "TXBI
OTYPE"))
```

```
##             GENEID GENENAME            TXID      TX
BIOTYPE
## 1  ENSG00000171791     BCL2 ENST00000398117 protein
_coding
## 2  ENSG00000171791     BCL2 ENST00000333681 protein
_coding
## 3  ENSG00000171791     BCL2 ENST00000589955 protein
_coding
## 4  ENSG00000153094  BCL2L11 ENST00000432179 protein
_coding
## 5  ENSG00000153094  BCL2L11 ENST00000308659 protein
_coding
## 6  ENSG00000153094  BCL2L11 ENST00000393256 protein
_coding
## 7  ENSG00000153094  BCL2L11 ENST00000393252 protein
_coding
## 8  ENSG00000153094  BCL2L11 ENST00000405953 protein
_coding
## 9  ENSG00000153094  BCL2L11 ENST00000438054 protein
_coding
## 10 ENSG00000153094  BCL2L11 ENST00000337565 protein
_coding
## 11 ENSG00000153094  BCL2L11 ENST00000622509 protein
_coding
## 12 ENSG00000153094  BCL2L11 ENST00000619294 protein
_coding
## 13 ENSG00000153094  BCL2L11 ENST00000610735 protein
_coding
## 14 ENSG00000153094  BCL2L11 ENST00000622612 protein
_coding
## 15 ENSG00000153094  BCL2L11 ENST00000357757 protein
_coding
## 16 ENSG00000153094  BCL2L11 ENST00000615946 protein
_coding
## 17 ENSG00000153094  BCL2L11 ENST00000621302 protein
_coding
## 18 ENSG00000153094  BCL2L11 ENST00000620862 protein
_coding
```

Finally, we use the `mapIds` method to establish a mapping between ids and values. In the example below we fetch transcript ids for the two genes from the example above.

```
## Use the default method, which just returns the firs
t value for multi mappings.
mapIds(edb, keys = c("BCL2", "BCL2L11"), column = "TXI
D", keytype = "GENENAME")
```

```
##              BCL2           BCL2L11
## "ENST00000398117" "ENST00000432179"
```

```
## Alternatively, specify multiVals="list" to return a
ll mappings.
mapIds(edb, keys = c("BCL2", "BCL2L11"), column = "TXI
D", keytype = "GENENAME",
       multiVals = "list")
```

```
## $BCL2
## [1] "ENST00000398117" "ENST00000333681" "ENST000005
90515" "ENST00000589955"
##
## $BCL2L11
##  [1] "ENST00000432179" "ENST00000308659" "ENST00000
393256" "ENST00000393252"
##  [5] "ENST00000433098" "ENST00000405953" "ENST00000
415458" "ENST00000436733"
##  [9] "ENST00000437029" "ENST00000452231" "ENST00000
361493" "ENST00000431217"
## [13] "ENST00000439718" "ENST00000438054" "ENST00000
337565" "ENST00000622509"
## [17] "ENST00000619294" "ENST00000610735" "ENST00000
622612" "ENST00000357757"
## [21] "ENST00000615946" "ENST00000621302" "ENST00000
620862" "LRG_620t1"
## [25] "LRG_620t2"       "LRG_620t3"       "LRG_620t
4"       "LRG_620t5"
```

```
## And, just like before, we can use filters to map on
ly to protein coding transcripts.
mapIds(edb, keys = list(GeneNameFilter(c("BCL2", "BCL2
L11")),
                        TxBiotypeFilter("protein_codin
g")), column = "TXID",
       multiVals = "list")
```

```
## Warning in .mapIds(x = x, keys = keys, column = col
umn, keytype = keytype, : Got
## 2 filter objects. Will use the keys of the first fo
r the mapping!
```

```
## $BCL2
## [1] "ENST00000398117" "ENST00000333681" "ENST000005
89955"
##
## $BCL2L11
##  [1] "ENST00000432179" "ENST00000308659" "ENST00000
393256" "ENST00000393252"
##  [5] "ENST00000405953" "ENST00000438054" "ENST00000
337565" "ENST00000622509"
##  [9] "ENST00000619294" "ENST00000610735" "ENST00000
622612" "ENST00000357757"
## [13] "ENST00000615946" "ENST00000621302" "ENST00000
620862"
```

Note that, if the filters are used, the ordering of the result does no longer match the ordering of the genes.

9 Important notes

These notes might explain eventually unexpected results (and, more importantly, help avoiding them):

- The ordering of the results returned by the `genes`, `exons`, `transcripts` methods can be specified with the `order.by` parameter. The ordering of the results does however **not** correspond to the ordering of values in submitted filter objects. The exception is the `select` method. If a character vector of values or a single filter is passed with argument `keys` the ordering of results of this method matches the ordering of the key values or the values of the filter.
- Results of `exonsBy`, `transcriptsBy` are always ordered by the `by` argument.
- The CDS provided by `EnsDb` objects **always** includes both, the start and the stop codon.
- Transcripts with multiple CDS are at present not supported by `EnsDb`.
- At present, `EnsDb` support only genes/transcripts for which all of their exons are encoded on the same chromosome and the same strand.
- Since a single Ensembl gene ID might be mapped to multiple NCBI Entrezgene IDs methods such as `genes`, `transcripts` etc return a `list` in the `"entrezid"` column of the resulting result object.

10 Getting or building `EnsDb` databases/packages

Some of the code in this section is not supposed to be automatically executed when the vignette is built, as this would require a working installation of the Ensembl Perl API, which is not expected to be

available on each system. Also, building `EnsDb` from alternative sources, like GFF or GTF files takes some time and thus also these examples are not directly executed when the vignette is build.

10.1 Getting `EnsDb` databases

Some `EnsDb` databases are available as `R` packages from Bioconductor and can be simply installed with the `install` function from the `BiocManager` package. The name of such annotation packages starts with *EnsDb* followed by the abbreviation of the organism and the Ensembl version on which the annotation bases. `EnsDb.Hsapiens.v86` provides thus an `EnsDb` database for homo sapiens with annotations from Ensembl version 86.

Since Bioconductor version 3.5 `EnsDb` databases can also be retrieved directly from `AnnotationHub` .

```
library(AnnotationHub)
## Load the annotation resource.
ah <- AnnotationHub()

## Query for all available EnsDb databases
query(ah, "EnsDb")
```

```
## AnnotationHub with 1530 records
## # snapshotDate(): 2020-04-27
## # $dataprovider: Ensembl
## # $species: Homo sapiens, Xiphophorus maculatus, Xe
nopus tropicalis, Vicugna...
## # $rdataclass: EnsDb
## # additional mcols(): taxonomyid, genome, descripti
on,
## #   coordinate_1_based, maintainer, rdatadateadded,
preparerclass, tags,
## #   rdatapath, sourceurl, sourcetype
## # retrieve records with, e.g., 'object[["AH5318
5"]]'
##
##             title
##   AH53185 | Ensembl 87 EnsDb for Anolis Carolinensi
s
##   AH53186 | Ensembl 87 EnsDb for Ailuropoda Melanol
euca
##   AH53187 | Ensembl 87 EnsDb for Astyanax Mexicanus
##   AH53188 | Ensembl 87 EnsDb for Anas Platyrhynchos
##   AH53189 | Ensembl 87 EnsDb for Bos Taurus
##   ...       ...
##   AH78907 | Ensembl 99 EnsDb for Vulpes vulpes
##   AH78908 | Ensembl 99 EnsDb for Xiphophorus couchi
anus
##   AH78909 | Ensembl 99 EnsDb for Xiphophorus macula
tus
##   AH78910 | Ensembl 99 EnsDb for Xenopus tropicalis
##   AH78911 | Ensembl 99 EnsDb for Zonotrichia albico
llis
```

We can simply fetch one of the databases.

```
ahDb <- query(ah, pattern = c("Xiphophorus Maculatus",
"EnsDb", 87))
## What have we got
ahDb
```

```
## AnnotationHub with 1 record
## # snapshotDate(): 2020-04-27
## # names(): AH53251
## # $dataprovider: Ensembl
## # $species: Xiphophorus maculatus
## # $rdataclass: EnsDb
## # $rdatadateadded: 2017-02-07
## # $title: Ensembl 87 EnsDb for Xiphophorus Maculatu
s
## # $description: Gene and protein annotations for Xi
phophorus Maculatus based...
## # $taxonomyid: 8083
## # $genome: Xipmac4.4.2
## # $sourcetype: ensembl
## # $sourceurl: http://www.ensembl.org
## # $sourcesize: NA
## # $tags: c("EnsDb", "Ensembl", "Gene", "Transcrip
t", "Protein",
## #   "Annotation", "87", "AHEnsDbs")
## # retrieve record with 'object[["AH53251"]]'
```

Fetch the `EnsDb` and use it.

```
ahEdb <- ahDb[[1]]
```

```
## retriebe all genes
gns <- genes(ahEdb)
```

We could even make an annotation package from this `EnsDb` object using the `makeEnsembldbPackage` and passing `dbfile(dbconn(ahEdb))` as `ensdb` argument.

10.2 Building annotation packages

10.2.1 Directly from Ensembl databases

The `fetchTablesFromEnsembl` function uses the Ensembl Perl API to retrieve the required annotations from an Ensembl database (e.g. from the main site *ensembldb.ensembl.org*). Thus, to use this functionality to build databases, the Ensembl Perl API needs to be installed (see [5] for details).

Below we create an `EnsDb` database by fetching the required data directly from the Ensembl core databases. The `makeEnsembldbPackage` function is then used to create an annotation package from this `EnsDb` containing all human genes for Ensembl version 75.

```
library(ensembldb)

## get all human gene/transcript/exon annotations from
Ensembl (75)
## the resulting tables will be stored by default to t
he current working
## directory
fetchTablesFromEnsembl(75, species = "human")

## These tables can then be processed to generate a SQ
Lite database
## containing the annotations (again, the function ass
umes the required
## txt files to be present in the current working dire
ctory)
DBFile <- makeEnsemblSQLiteFromTables()

## and finally we can generate the package
makeEnsembldbPackage(ensdb = DBFile, version = "0.99.1
2",
                     maintainer = "Johannes Rainer <jo
hannes.rainer@eurac.edu>",
                     author = "J Rainer")
```

The generated package can then be build using `R CMD build EnsDb.Hsapiens.v75` and installed with `R CMD INSTALL EnsDb.Hsapiens.v75*`. Note that we could directly generate an `EnsDb` instance by loading the database file, i.e. by calling `edb <- EnsDb(DBFile)` and work with that annotation object.

To fetch and build annotation packages for plant genomes (e.g. arabidopsis thaliana), the *Ensembl genomes* should be specified as a host, i.e. setting `host` to "mysql-eg-publicsql.ebi.ac.uk", `port` to `4157` and `species` to e.g. "arabidopsis thaliana".

10.2.2 From a GTF or GFF file

Alternatively, the `ensDbFromAH`, `ensDbFromGff`, `ensDbFromGRanges` and `ensDbFromGtf` functions allow to build EnsDb SQLite files from a `GRanges` object or GFF/GTF files from Ensembl (either provided as files or *via* `AnnotationHub`). These functions do not depend on the Ensembl Perl API, but require a working internet connection to fetch the chromosome lengths from Ensembl as these are not provided within GTF or GFF files. Also note that protein annotations are usually not available in GTF or GFF files, thus, such annotations will not be included in the generated `EnsDb` database - protein annotations are only available in `EnsDb` databases created with the Ensembl Perl API (such as the ones provided through `AnnotationHub` or as Bioconductor packages).

In the next example we create an `EnsDb` database using the `AnnotationHub` package and load also the corresponding genomic DNA sequence matching the Ensembl version. We thus first query the `AnnotationHub` package for all resources available

for `Mus musculus` and the Ensembl release 77. Next we create the `EnsDb` object from the appropriate `AnnotationHub` resource. We then use the `getGenomeTwoBitFile` method on the `EnsDb` to directly look up and retrieve the correct or best matching `TwoBitFile` with the genomic DNA sequence. At last we retrieve the sequences of all exons using the `getSeq` method.

```
## Load the AnnotationHub data.
library(AnnotationHub)
ah <- AnnotationHub()

## Query all available files for Ensembl release 77 fo
r
## Mus musculus.
query(ah, c("Mus musculus", "release-77"))

## Get the resource for the gtf file with the gene/tra
nscript definitions.
Gtf <- ah["AH28822"]
## Create a EnsDb database file from this.
DbFile <- ensDbFromAH(Gtf)
## We can either generate a database package, or direc
tly load the data
edb <- EnsDb(DbFile)


## Identify and get the TwoBit object with the genomic
DNA sequence matching
## the EnsDb annotation.
Dna <- getGenomeTwoBitFile(edb)
## We next retrieve the sequence of all exons on chrom
osome Y.
exons <- exons(edb, filter = SeqNameFilter("Y"))
exonSeq <- getSeq(Dna, exons)
```

In the example below we load a `GRanges` containing gene definitions for genes encoded on chromosome Y and generate a `EnsDb` SQLite database from that information.

```
## Generate a sqlite database from a GRanges object sp
ecifying
## genes encoded on chromosome Y
load(system.file("YGRanges.RData", package = "ensembld
b"))
Y
```

```
## GRanges object with 7155 ranges and 16 metadata col
umns:
##          seqnames            ranges strand |
source       type
##             <Rle>         <IRanges>  <Rle> |
<factor>   <factor>
##      [1]        Y   2652790-2652894      + |
snRNA          gene
##      [2]        Y   2652790-2652894      + |
snRNA          transcript
##      [3]        Y   2652790-2652894      + |
snRNA          exon
##      [4]        Y   2654896-2655740      - |
protein_coding gene
##      [5]        Y   2654896-2655740      - |
protein_coding transcript
##      ...      ...               ...    ... .
...        ...
##   [7151]        Y 28772667-28773306      - | proces
sed_pseudogene transcript
##   [7152]        Y 28772667-28773306      - | proces
sed_pseudogene exon
##   [7153]        Y 59001391-59001635      + | pseudo
gene           gene
##   [7154]        Y 59001391-59001635      + | proces
sed_pseudogene transcript
##   [7155]        Y 59001391-59001635      + | proces
sed_pseudogene exon
##              score     phase         gene_id   gene
_name    gene_source
##          <numeric> <integer>     <character> <chara
cter>    <character>
##      [1]        NA      <NA> ENSG00000251841  RNU6-
1334P        ensembl
##      [2]        NA      <NA> ENSG00000251841  RNU6-
1334P        ensembl
##      [3]        NA      <NA> ENSG00000251841  RNU6-
1334P        ensembl
##      [4]        NA      <NA> ENSG00000184895
SRY ensembl_havana
##      [5]        NA      <NA> ENSG00000184895
SRY ensembl_havana
##      ...       ...       ...             ...
...            ...
##   [7151]        NA      <NA> ENSG00000231514     FA
M58CP         havana
##   [7152]        NA      <NA> ENSG00000231514     FA
M58CP         havana
##   [7153]        NA      <NA> ENSG00000235857     CT
BP2P1         havana
##   [7154]        NA      <NA> ENSG00000235857     CT
BP2P1         havana
##   [7155]        NA      <NA> ENSG00000235857     CT
BP2P1         havana
##            gene_biotype   transcript_id transcript_
name transcript_source
##             <character>     <character>     <charac
ter>       <character>
##      [1]          snRNA            <NA>
<NA>             <NA>
```

```
##      [2]          snRNA ENST00000516032  RNU6-1334P
-201          ensembl
##      [3]          snRNA ENST00000516032  RNU6-1334P
-201          ensembl
##      [4] protein_coding            <NA>
<NA>             <NA>
##      [5] protein_coding ENST00000383070         SRY
-001    ensembl_havana
##      ...            ...             ...
...             ...
##   [7151]     pseudogene ENST00000435741     FAM58CP
-001           havana
##   [7152]     pseudogene ENST00000435741     FAM58CP
-001           havana
##   [7153]     pseudogene            <NA>
<NA>             <NA>
##   [7154]     pseudogene ENST00000431853     CTBP2P1
-001           havana
##   [7155]     pseudogene ENST00000431853     CTBP2P1
-001           havana
##          exon_number         exon_id         tag
ccds_id  protein_id
##            <numeric>     <character> <character> <c
haracter> <character>
##      [1]          NA            <NA>        <NA>
<NA>        <NA>
##      [2]          NA            <NA>        <NA>
<NA>        <NA>
##      [3]           1 ENSE00002088309        <NA>
<NA>        <NA>
##      [4]          NA            <NA>        <NA>
<NA>        <NA>
##      [5]          NA            <NA>        CCDS
CCDS14772        <NA>
##      ...         ...             ...         ...
...         ...
##   [7151]          NA            <NA>        <NA>
<NA>        <NA>
##   [7152]           1 ENSE00001616687        <NA>
<NA>        <NA>
##   [7153]          NA            <NA>        <NA>
<NA>        <NA>
##   [7154]          NA            <NA>        <NA>
<NA>        <NA>
##   [7155]           1 ENSE00001794473        <NA>
<NA>        <NA>
##   -------
##   seqinfo: 1 sequence from GRCh37 genome


## Create the EnsDb database file
DB <- ensDbFromGRanges(Y, path = tempdir(), version =
75,
              organism = "Homo_sapiens")


## Warning in ensDbFromGRanges(Y, path = tempdir(), ve
rsion = 75, organism =
## "Homo_sapiens"): I'm missing column(s): 'entrezid'.
The corresponding database
## column(s) will be empty!
```

```
## Load the database
edb <- EnsDb(DB)
edb


## EnsDb for Ensembl:
## |Backend: SQLite
## |Db type: EnsDb
## |Type of Gene ID: Ensembl Gene ID
## |Supporting package: ensembldb
## |Db created by: ensembldb package from Bioconductor
## |script_version: 0.0.1
## |Creation time: Wed May  6 21:26:00 2020
## |ensembl_version: 75
## |ensembl_host: unknown
## |Organism: Homo_sapiens
## |genome_build: GRCh37
## |DBSCHEMAVERSION: 1.0
## |source_file: GRanges object
## | No. of genes: 495.
## | No. of transcripts: 731.
```

Alternatively we can build the annotation database using the `ensDbFromGtf` `ensDbFromGff` functions, that extract most of the required data from a GTF respectively GFF (version 3) file which can be downloaded from Ensembl (e.g. from ftp://ftp.ensembl.org/pub/release-75/gtf/homo_sapiens (ftp://ftp.ensembl.org/pub/release-75/gtf/homo_sapiens) for human gene definitions from Ensembl version 75; for plant genomes etc, files can be retrieved from ftp://ftp.ensemblgenomes.org (ftp://ftp.ensemblgenomes.org)). All information except the chromosome lengths, the NCBI Entrezgene IDs and protein annotations can be extracted from these GTF files. The function also tries to retrieve chromosome length information automatically from Ensembl.

Below we create the annotation from a gtf file that we fetch directly from Ensembl.

```
library(ensembldb)

## the GTF file can be downloaded from
## ftp://ftp.ensembl.org/pub/release-75/gtf/homo_sapie
ns/
gtffile <- "Homo_sapiens.GRCh37.75.gtf.gz"
## generate the SQLite database file
DB <- ensDbFromGtf(gtf = gtffile)

## load the DB file directly
EDB <- EnsDb(DB)

## alternatively, build the annotation package
## and finally we can generate the package
makeEnsembldbPackage(ensdb = DB, version = "0.99.12",
                     maintainer = "Johannes Rainer <jo
hannes.rainer@eurac.edu>",
                     author = "J Rainer")
```

11 Database layout

The database consists of the following tables and attributes (the layout is also shown in Figure 165). Note that the protein-specific annotations might not be available in all `EnsDB` databases (e.g. such ones created with `ensembldb` version < 1.7 or created from GTF or GFF files).

- **gene**: all gene specific annotations.
  - `gene_id` : the Ensembl ID of the gene.
  - `gene_name` : the name (symbol) of the gene.
  - `gene_biotype` : the biotype of the gene.
  - `gene_seq_start` : the start coordinate of the gene on the sequence (usually a chromosome).
  - `gene_seq_end` : the end coordinate of the gene on the sequence.
  - `seq_name` : the name of the sequence (usually the chromosome name).
  - `seq_strand` : the strand on which the gene is encoded.
  - `seq_coord_system` : the coordinate system of the sequence.
  - `description` : the description of the gene.
- **entrezgene**: mapping of Ensembl genes to NCBI Entrezgene identifiers. Note that this mapping can be a one-to-many mapping.
  - `gene_id` : the Ensembl gene ID.
  - `entrezid` : the NCBI Entrezgene ID.
- **tx**: all transcript related annotations. Note that while no `tx_name` column is available in this database column, all methods to retrieve data from the database support also this column. The returned values are however the ID of the transcripts.
  - `tx_id` : the Ensembl transcript ID.
  - `tx_biotype` : the biotype of the transcript.
  - `tx_seq_start` : the start coordinate of the transcript.
  - `tx_seq_end` : the end coordinate of the transcript.
  - `tx_cds_seq_start` : the start coordinate of the coding region of the transcript (NULL for non-coding transcripts).
  - `tx_cds_seq_end` : the end coordinate of the coding region of the transcript.
  - `gc_count` : from Ensembl release 98 on, the **tx** table contains also a column `gc_count` providing the transcript's G-C content expressed as a percentage.
  - `gene_id` : the gene to which the transcript belongs.

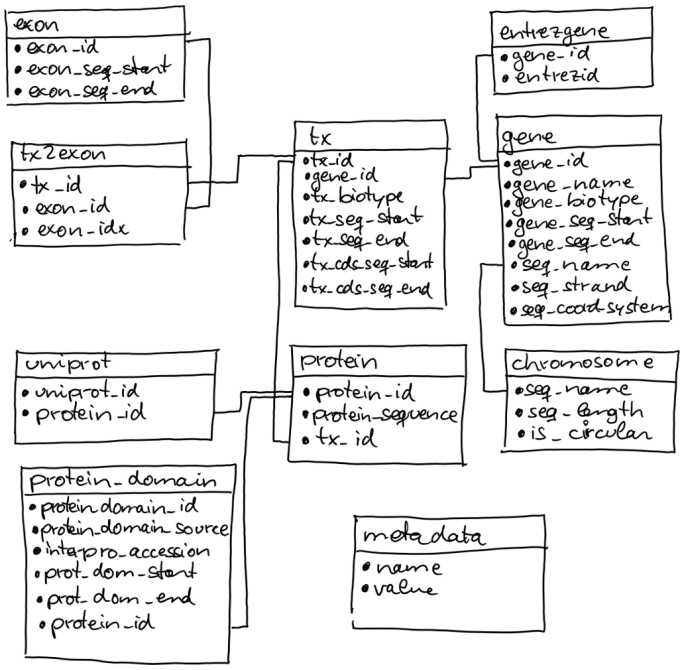
  `EnsDb` databases for more recent Ensembl releases have also a column `tx_support_level` providing the evidence level for a transcript (1 high evidence, 5 low evidence, NA no evidence calculated).
- **exon**: all exon related annotation.
  - `exon_id` : the Ensembl exon ID.

    - `exon_seq_start` : the start coordinate of the exon.
    - `exon_seq_end` : the end coordinate of the exon.
- **tx2exon**: provides the n:m mapping between transcripts and exons.
    - `tx_id` : the Ensembl transcript ID.
    - `exon_id` : the Ensembl exon ID.
    - `exon_idx` : the index of the exon in the corresponding transcript, always from 5' to 3' of the transcript.
- **chromosome**: provides some information about the chromosomes.
    - `seq_name` : the name of the sequence/chromosome.
    - `seq_length` : the length of the sequence.
    - `is_circular` : whether the sequence in circular.
- **protein**: provides protein annotation for a (coding) transcript.
    - `protein_id` : the Ensembl protein ID.
    - `tx_id` : the transcript ID which CDS encodes the protein.
    - `protein_sequence` : the peptide sequence of the protein (translated from the transcript's coding sequence after applying eventual RNA editing).
- **uniprot**: provides the mapping from Ensembl protein ID(s) to Uniprot ID(s). Not all Ensembl proteins are annotated to Uniprot IDs, also, each Ensembl protein might be mapped to multiple Uniprot IDs.
    - `protein_id` : the Ensembl protein ID.
    - `uniprot_id` : the Uniprot ID.
    - `uniprot_db` : the Uniprot database in which the ID is defined.
    - `uniprot_mapping_type` : the type of the mapping method that was used to assign the Uniprot ID to an Ensembl protein ID.
- **protein_domain**: provides protein domain annotations and mapping to proteins.
    - `protein_id` : the Ensembl protein ID on which the protein domain is present.
    - `protein_domain_id` : the ID of the protein domain (from the protein domain source).
    - `protein_domain_source` : the source/analysis method in/by which the protein domain was defined (such as pfam etc).
    - `interpro_accession` : the Interpro accession ID of the protein domain.
    - `prot_dom_start` : the start position of the protein domain within the protein's sequence.
    - `prot_dom_end` : the end position of the protein domain within the protein's sequence.
- **metadata**: some additional, internal, informations (Genome build, Ensembl version etc).
    - `name`
    - `value`
- *virtual* columns:
    - `symbol` : the database does not have such a database column, but it is still possible to use it in the `columns`

parameter. This column is *symlinked* to the `gene_name` column.

- `tx_name` : similar to the `symbol` column, this column is *symlinked* to the `tx_id` column.

The database layout: as already described above, protein related annotations (green) might not be available in each `EnsDb` database.



img

12 Session information

```
sessionInfo()
```

```
## R version 4.0.0 (2020-04-24)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 18.04.4 LTS
##
## Matrix products: default
## BLAS:   /home/biocbuild/bbs-3.11-bioc/R/lib/libRbla
s.so
## LAPACK: /home/biocbuild/bbs-3.11-bioc/R/lib/libRlap
ack.so
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=en_US.UTF-8        LC_COLLATE=C
##  [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.U
TF-8
##  [7] LC_PAPER=en_US.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
##  [1] grid      stats4    parallel  stats     graphi
cs  grDevices utils
##  [8] datasets  methods   base
##
## other attached packages:
##  [1] AnnotationHub_2.20.0
##  [2] BiocFileCache_1.12.0
##  [3] dbplyr_1.4.3
##  [4] magrittr_1.5
##  [5] BSgenome.Hsapiens.NCBI.GRCh38_1.3.1000
##  [6] BSgenome_1.56.0
##  [7] rtracklayer_1.48.0
##  [8] Biostrings_2.56.0
##  [9] XVector_0.28.0
## [10] Gviz_1.32.0
## [11] EnsDb.Hsapiens.v86_2.99.0
## [12] ensembldb_2.12.1
## [13] AnnotationFilter_1.12.0
## [14] GenomicFeatures_1.40.0
## [15] AnnotationDbi_1.50.0
## [16] Biobase_2.48.0
## [17] GenomicRanges_1.40.0
## [18] GenomeInfoDb_1.24.0
## [19] IRanges_2.22.1
## [20] S4Vectors_0.26.0
## [21] BiocGenerics_0.34.0
## [22] BiocStyle_2.16.0
##
## loaded via a namespace (and not attached):
##  [1] colorspace_1.4-1              ellipsis_0.3.0
##  [3] biovizBase_1.36.0             htmlTable_1.13.3
##  [5] base64enc_0.1-3               dichromat_2.0-0
##  [7] rstudioapi_0.11               bit64_0.9-7
##  [9] interactiveDisplayBase_1.26.0 splines_4.0.0
## [11] knitr_1.28                    Formula_1.2-3
## [13] Rsamtools_2.4.0               cluster_2.1.0
## [15] png_0.1-7                     shiny_1.4.0.2
## [17] BiocManager_1.30.10           compiler_4.0.0
## [19] httr_1.4.1                    backports_1.1.6
## [21] fastmap_1.0.1                 assertthat_0.2.1
```

```
## [23] Matrix_1.2-18                lazyeval_0.2.2
## [25] later_1.0.0                  acepack_1.4.1
## [27] htmltools_0.4.0              prettyunits_1.1.
1
## [29] tools_4.0.0                  gtable_0.3.0
## [31] glue_1.4.0                   GenomeInfoDbData
_1.2.3
## [33] dplyr_0.8.5                  rappdirs_0.3.1
## [35] Rcpp_1.0.4.6                 vctrs_0.2.4
## [37] xfun_0.13                    stringr_1.4.0
## [39] mime_0.9                     lifecycle_0.2.0
## [41] XML_3.99-0.3                 zlibbioc_1.34.0
## [43] scales_1.1.0                 VariantAnnotatio
n_1.34.0
## [45] promises_1.1.0               hms_0.5.3
## [47] ProtGenerics_1.20.0          SummarizedExperi
ment_1.18.1
## [49] RColorBrewer_1.1-2           yaml_2.2.1
## [51] curl_4.3                     memoise_1.1.0
## [53] gridExtra_2.3                ggplot2_3.3.0
## [55] biomaRt_2.44.0               rpart_4.1-15
## [57] latticeExtra_0.6-29          stringi_1.4.6
## [59] RSQLite_2.2.0                BiocVersion_3.1
1.1
## [61] highr_0.8                    checkmate_2.0.0
## [63] BiocParallel_1.22.0          rlang_0.4.6
## [65] pkgconfig_2.0.3              matrixStats_0.5
6.0
## [67] bitops_1.0-6                 evaluate_0.14
## [69] lattice_0.20-41              purrr_0.3.4
## [71] GenomicAlignments_1.24.0     htmlwidgets_1.5.
1
## [73] bit_1.1-15.2                 tidyselect_1.0.0
## [75] bookdown_0.18                R6_2.4.1
## [77] magick_2.3                   Hmisc_4.4-0
## [79] DelayedArray_0.14.0          DBI_1.1.0
## [81] pillar_1.4.4                 foreign_0.8-79
## [83] survival_3.1-12              RCurl_1.98-1.2
## [85] nnet_7.3-14                  tibble_3.0.1
## [87] crayon_1.3.4                 rmarkdown_2.1
## [89] jpeg_0.1-8.1                 progress_1.2.2
## [91] data.table_1.12.8            blob_1.2.1
## [93] digest_0.6.25                xtable_1.8-4
## [95] httpuv_1.5.2                 openssl_1.4.1
## [97] munsell_0.5.0                askpass_1.1
```

13 Footnotes

[1] http://www.ensembl.org (http://www.ensembl.org)

[2] http://www.lrg-sequence.org (http://www.lrg-sequence.org)

[3] http://www.ncbi.nlm.nih.gov/pubmed/23950696 (http://www.ncbi.nlm.nih.gov/pubmed/23950696)

[4] http://www.ncbi.nlm.nih.gov/pubmed/24227677 (http://www.ncbi.nlm.nih.gov/pubmed/24227677)

[5] http://www.ensembl.org/info/docs/api/api_installation.html (http://www.ensembl.org/info/docs/api/api_installation.html)