Extending ensembldb: MySQL backend and protein annotations

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Introduction

- `ensembldb`: retrieve gene & transcript annotations.
- `ensembldb` package defines the `EnsDb` class:
  - Wrapper to access annotations from an SQLite database.
  - Same functionality than the `GenomicFeatures` package (`TxDb` object), plus:
  - Filter framework to enable specific and fast queries.
  - NEW: *MySQL* backend support.
  - NEW: provide protein annotations.

Basic usage

- Available methods to extract data: `genes`, `transcripts`, `transcriptsBy`, `exons`, `exonsBy`, `cdsBy`, `fiveUTRsByTranscripts`, `threeUTRsByTranscripts`, `proteins` (NEW).
- Example: get all lincRNA genes encoded on chromosome Y.

```r
## Load an EnsDb package matching Ensembl version 86
library(EnsDb.Hsapiens.v86)
edb <- EnsDb.Hsapiens.v86

## Retrieve all lincRNAs encoded on chromosome Y.
## Create the filter objects
sf <- SeqnameFilter("Y")
gbf <- GenebiotypeFilter("lincRNA")
```

Basic usage

- Example: (continued)

```r
## Retrieve the data.
genes(edb, filter = list(sf, gbf))
```

```
GRanges object with 52 ranges and 6 metadata columns:
  seqnames                ranges strand |         gene_id
     <Rle>             <IRanges>  <Rle> |     <character>
  ENSG00000278847        Y   [2934406, 2934771]      - | ENSG00000278847
  ENSG00000231535        Y   [3002912, 3102272]      + | ENSG00000231535
  ENSG00000229308        Y   [4036497, 4100320]      + | ENSG00000229308
      ...       ...                 ...    ... .            ...
  ENSG00000228786        Y [25378300, 25394719]      - | ENSG00000228786
  ENSG00000240450        Y [25482908, 25486705]      + | ENSG00000240450
  ENSG00000231141        Y [25728490, 25733388]      + | ENSG00000231141
      gene_name      entrezid gene_biotype seq_coord_system
    <character>   <character>  <character>      <character>
  ENSG00000278847 RP11-414C23.1                    lincRNA       chromosome
  ENSG00000231535     LINC00278     100873962      lincRNA       chromosome
  ENSG00000229308     AC010084.1                   lincRNA       chromosome
      ...             ...           ...          ...              ...
  ENSG00000228786  LINC00266-4P                    lincRNA       chromosome
  ENSG00000240450       CSPG4P1Y        114758      lincRNA       chromosome
  ENSG00000231141          TTTY3 474148;114760      lincRNA       chromosome
 symbol
    <character>
  ENSG00000278847 RP11-414C23.1
  ENSG00000231535     LINC00278
  ENSG00000229308     AC010084.1
```

Available filters

- For genes: `GeneidFilter`, `GenenameFilter`, `EntrezidFilter`, `GenebiotypeFilter`, (`SymbolFilter`).
- For transcripts: `TxidFilter`, `TxbiotypeFilter`.
- For exons: `ExonidFilter`, `ExonrankFilter`.
- NEW: for proteins: `ProteinidFilter`, `UniprotidFilter`, `UniprotdbFilter`, `UniprotmappingtypeFilter`, `ProtdomidFilter`.
- Based on chromosomal coordinates: `SeqnameFilter`, `SeqstrandFilter`, `SeqstartFilter`, `SeqendFilter`, `GRangesFilter`: condition can be *within* or *overlapping*.
- Multiple filters are combined with a logical *AND*.
- Each filter supports 1:n values, =, *!=* and also a *like* condition.
- Filters speed up queries.

Building annotation databases

- Option A): from GTF/GFF files or AnnotationHub.
- Example: create an EnsDb using AnnotationHub.

```r
library(AnnotationHub)
ah <- AnnotationHub()
## Query for available Ensembl gtf files for release 83.
query(ah, pattern=c("ensembl", "release-83", "gtf"))

## Select one; in this case: Anolis carolinensis (lizard)
edbSql83 <- ensDbFromAH(ah=ah["AH50353"])
## BUT: DB lacks NCBI Entrezgene IDs and protein annotation.

## Load the database.
db <- EnsDb(edbSql83)

## Optional, make a package.
makeEnsembldbPackage(ensdb=edbSql83, version="1.0.0", author="J Rainer",
                     maintainer="Johannes Rainer <johannes.rainer@eurac.edu>")
```

- Option B) (preferred): using the Ensembl Perl API:
  - fetchTablesFromEnsembl and makeEnsemblSQLiteFromTables.
  - Fetches also protein annotations.
  - Requirements: Perl, Bioperl, Ensembl Perl API.

MySQL backend

- Example: `listEnsDb` list all available databases, useMySQL to insert an EnsDb to a MySQL server.

```
library(RMySQL)
dbc <- dbConnect(MySQL(), host = "localhost", user = "anonuser", pass = "")

## list all available EnsDb databases.
listEnsDbs(dbc)
```

```
                   dbname      organism ensembl_version
1 ensdb_acarolinensis_v83 acarolinensis              83
2     ensdb_bvulgaris_v86     bvulgaris              86
3 ensdb_dmelanogaster_v86 dmelanogaster              86
4      ensdb_hsapiens_v75      hsapiens              75
```

```
## Connect to a database.
dbc <- dbConnect(MySQL(), host = "localhost", user = "anonuser", pass = "",
                 dbname = "ensdb_dmelanogaster_v86")
edb_2 <- EnsDb(dbc)

## To insert an EnsDb to a MySQL: useMySQL
db_my <- useMySQL(edb, host = "localhost", user = "anonuser", pass = "")
```

- Enables a central, MySQL-based annotation server.

Protein data: fetch protein data from an EnsDb

- Example: add protein columns to the columns parameter.

```
## Get all genes with a C2H2 Zinc finger domain and
## return all of their Uniprot IDs
pfam <- ProtdomidFilter("PF13912")
genes(edb, filter = pfam, return.type = "DataFrame",
    columns = c("gene_name", "uniprot_id"))
```

```
DataFrame with 583 rows and 4 columns
gene_name  uniprot_id         gene_id protein_domain_id
      <character> <character>     <character>       <character>
1      AC002310.11      B7Z5R0 ENSG00000261459           PF13912
2       AC092835.2  A0A087WUV0 ENSG00000233757           PF13912
3    CTD-2006C1.13      F5H0A9 ENSG00000267179           PF13912
...            ...         ...             ...               ...
581         ZSCAN9      O15535 ENSG00000137185           PF13912
582         ZSCAN9  A0A024RCK9 ENSG00000137185           PF13912
583         ZSCAN9      E9PQL7 ENSG00000137185           PF13912
```

Protein data: fetch protein data from an EnsDb

- Example: use proteins method to specifically fetch protein data.

```
## Return the protein annotation as a AAStringSet:
prts <- proteins(edb, filter = GenenameFilter("ZBTB16"),
                 columns = c("tx_id", "tx_biotype"),
                 return.type = "AAStringSet")
prts
```

```
  A AAStringSet instance of length 5
    width seq                                                names
[1]   673 MDLTKMGMIQLQNPSHPTGLLCK...GHKPEEIPPDWRIEKTYLYLCYV ENSP00000338157
[2]   115 MDLTKMGMIQLQNPSHPTGLLCK...QAKAEDLDDLLYAAEILEIEYLE ENSP00000437716
[3]   148 MDLTKMGMIQLQNPSHPTGLLCK...QASDDNDTEATMADGGAEEEEDR ENSP00000443013
[4]   673 MDLTKMGMIQLQNPSHPTGLLCK...GHKPEEIPPDWRIEKTYLYLCYV ENSP00000376721
[5]    55 XGGLLPQGFIQRELFSKLGELAV...GEQCSVCGVELPDNEAVEQHRVF ENSP00000445047
```

```
## Additional columns are available as mcols:
mcols(prts)
```

```
DataFrame with 5 rows and 4 columns
            tx_id              tx_biotype      protein_id   gene_name
      <character>             <character>     <character> <character>
1 ENST00000335953          protein_coding ENSP00000338157      ZBTB16
2 ENST00000544220          protein_coding ENSP00000437716      ZBTB16
3 ENST00000535700          protein_coding ENSP00000443013      ZBTB16
4 ENST00000392996          protein_coding ENSP00000376721      ZBTB16
5 ENST00000539918 nonsense_mediated_decay ENSP00000445047      ZBTB16
```

Protein data: use ensembldb with Pbase

- Pbase: (Laurent Gatto and Sebastian Gibb): provides classes and functions for the analysis of protein sequence data in proteomics experiments.
- The Proteins object: container for proteins and peptide ranges within the AA sequences.
- Example: fetch a Proteins object for all ZBTB16 proteins including their protein domains.

```
## load Pbase - we need the "ensembldb" branch.
library(Pbase)

## Fetch proteins including protein domains for ZBTB16
prts <- Proteins(edb, filter = GenenameFilter("ZBTB16"))

## Amino acid sequence:
aa(prts)
```

```
  A AAStringSet instance of length 5
    width seq                                               names
[1]   673 MDLTKMGMIQLQNPSHPTGLLCK...GHKPEEIPPDWRIEKTYLYLCYV ENSP00000338157
[2]   115 MDLTKMGMIQLQNPSHPTGLLCK...QAKAEDLDDLLYAAEILEIEYLE ENSP00000437716
[3]   148 MDLTKMGMIQLQNPSHPTGLLCK...QASDDNDTEATMADGGAEEEEDR ENSP00000443013
[4]   673 MDLTKMGMIQLQNPSHPTGLLCK...GHKPEEIPPDWRIEKTYLYLCYV ENSP00000376721
[5]    55 XGGLLPQGFIQRELFSKLGELAV...GEQCSVCGVELPDNEAVEQHRVF ENSP00000445047
```

Protein data: use ensembldb with Pbase

- Example: fetch a Proteins object for all ZBTB16 proteins including their protein domains (continued).

```
## Peptide features:
pranges(prts)
```

```
IRangesList of length 5
$ENSP00000338157
IRanges object with 36 ranges and 3 metadata columns:
          start       end     width |      protein_id protein_domain_source
      <integer> <integer> <integer> |     <character>           <character>
  PS50157       602       629        28 | ENSP00000338157                pfscan
  PS50157       490       517        28 | ENSP00000338157                pfscan
  PS50157       630       657        28 | ENSP00000338157                pfscan
      ...       ...       ...       ... .             ...                   ...
  SM00355       432       454        23 | ENSP00000338157                 smart
  SM00355       574       596        23 | ENSP00000338157                 smart
  SM00225        34       126        93 | ENSP00000338157                 smart
  interpro_accession
 <character>
  PS50157          IPR007087
  PS50157          IPR007087
  PS50157          IPR007087
      ...                ...
  SM00355          IPR015880
  SM00355          IPR015880
  SM00225          IPR000210

...
<4 more elements>
```

Protein data: use ensembldb with Pbase

- Example: use ensembldb to map peptide features to the genome.

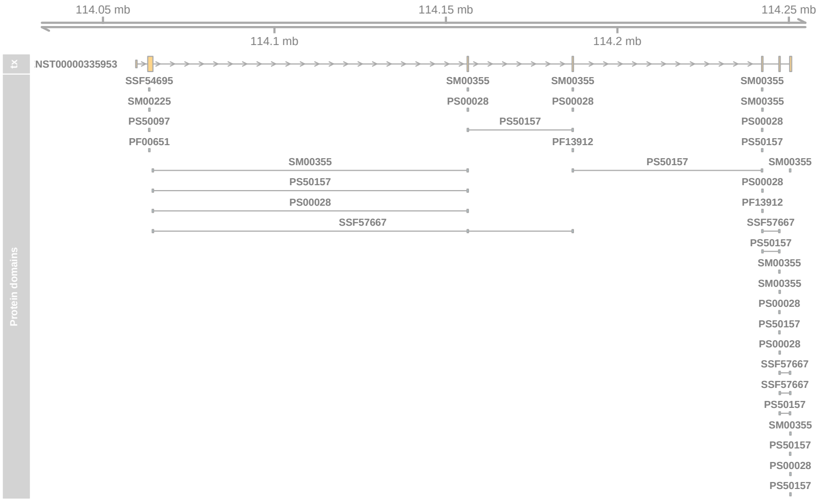
```r
## Map all protein domains to the genome
gen_map <- mapToGenome(prts, edb)

## Plot the results for the first protein (transcript)
txid <- gen_map[[1]]$tx_id
## Get the gene region track for the first transcript
tx <- getGeneRegionTrackForGviz(edb, filter = TxidFilter(txid))

## Add a protein ID column
map_1 <- gen_map[[1]]
map_1$id <- names(map_1)

## Plot using Gviz
library(Gviz)
plotTracks(list(GenomeAxisTrack(),
                GeneRegionTrack(tx, name = "tx"),
                AnnotationTrack(map_1, groupAnnotation = "id",
                                just.group = "above",
                                name = "Protein domains")),
           transcriptAnnotation = "transcript")
```

Protein data: use ensembldb with Pbase



Things not covered

- ensembldb provides full AnnotationDbi support.
- Example: use AnnotationDbi's select method to fetch annotations: keys can be a character list of IDs or a list of filter objects.

```r
## Get all data for the gene SKA2
Res <- select(edb, keys="SKA2", keytype="GENENAME")
head(Res, n=3)
```

```
  ENTREZID          EXONID EXONIDX EXONSEQEND EXONSEQSTART    GENEBIOTYPE
1   348235 ENSE00001324111       1   59155269     59155131 protein_coding
2   348235 ENSE00003636954       2   59131367     59131281 protein_coding
3   348235 ENSE00003478713       3   59119495     59119319 protein_coding
   GENEID GENENAME GENESEQEND GENESEQSTART INTERPROACCESSION ISCIRCULAR
1 ENSG00000182628     SKA2   59155269     59109951         IPR026762          0
2 ENSG00000182628     SKA2   59155269     59109951         IPR026762          0
3 ENSG00000182628     SKA2   59155269     59109951         IPR026762          0
  PROTDOMEND PROTDOMSTART PROTEINDOMAINID PROTEINDOMAINSOURCE       PROTEINID
1        115            2         PF16740                pfam ENSP00000333433
2        115            2         PF16740                pfam ENSP00000333433
3        115            2         PF16740                pfam ENSP00000333433
    PROTEINSEQUENCE
1 MEAEVDKLELMFQKAESDLDYIQYRLEYEIKTNHPDSASEKNPVTLLKELSVIKSRYQTLYARFKPVAVEQKESKSRICATVKKTMNMI
2 MEAEVDKLELMFQKAESDLDYIQYRLEYEIKTNHPDSASEKNPVTLLKELSVIKSRYQTLYARFKPVAVEQKESKSRICATVKKTMNMI
3 MEAEVDKLELMFQKAESDLDYIQYRLEYEIKTNHPDSASEKNPVTLLKELSVIKSRYQTLYARFKPVAVEQKESKSRICATVKKTMNMI
  SEQCOORDSYSTEM SEQLENGTH SEQNAME SEQSTRAND SYMBOL      TXBIOTYPE TXCDSSEQEND
1     chromosome  83257441      17        -1   SKA2 protein_coding    59155163
2     chromosome  83257441      17        -1   SKA2 protein_coding    59155163
3     chromosome  83257441      17        -1   SKA2 protein_coding    59155163
```

Things not covered

- Easy integration of UCSC and Ensembl annotations: use `seqlevelsStyle` to change chromosome naming scheme in `EnsDb`.
- Example: How to integrate Ensembl based annotation with UCSC data?

```
## Get chromosome names, they are "Ensembl-formatted"
head(seqlevels(edb))
```

```
[1] "1"  "10" "11" "12" "13" "14"
```

```
## Get genes on chromosome Y, UCSC style.
genes(edb, filter=SeqnameFilter("chrY"))
```

```
GRanges object with 0 ranges and 6 metadata columns:
   seqnames    ranges strand |     gene_id   gene_name    entrezid gene_biotype
      <Rle> <IRanges>  <Rle> | <character> <character> <character>  <character>
   seq_coord_system      symbol
        <character> <character>
  -------
  seqinfo: no sequences
```

```
## Solution: change the chromosome naming style:
seqlevelsStyle(edb) <- "UCSC"
## Get chromosome names
head(seqlevels(edb))
```

```
[1] "chr1"  "chr10" "chr11" "chr12" "chr13" "chr14"
Warning message:
```

Finally

Thank you for your attention!

`https://github.com/jotsetung/EuroBioC2016-ensembldb.git`