Supplementary data for: *ensemblpdb: an R package to create and use Ensembl-based annotation resources*

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This document describes three use cases for *ensembldb*: the first two examples illustrate the filter framework and the coordinate system mapping functionality of *ensembldb*, the latter shows how *ensembldb* annotation resources can be used in an RNA-seq data analysis workflow.

In the last section we compare the performance of queries with and without the filter framework from *ensembldb*.

1 Query for helix-loop-helix transcription factors on chromosome 21

Down syndrome is a genetic disorder characterized by the presence of all or parts of a third copy of chromosome 21. It is associated, among other, with characteristic facial features and mild to moderate intellectual disability. The phenotypes are currently believed to be the result from a gene dosage-dependent increased expression of the genes encoded on chromosome 21 (Lana-Elola et al. 2011). Compared to other gene classes, transcription factors are more likely to have an immediate impact, even due to a moderate over-expression (which might be the result from gene duplication). One of the largest dimerizing transcription factor families is characterized by a basic helix-loop-helix domain (Massari and Murre 2000), a protein structural motif facilitating DNA binding.

The example below aims at identifying transcription factors with a basic helix-loop-helix domain (Pfam ID PF00010) that are encoded on chromosome 21. To this end we first load an R-library providing human annotations from Ensembl release 86 and pass the loaded *EnsDb* object along with a filter expression to the *genes* method that retrieves the corresponding genes. Filter expressions have to be written in the form `~ <field> <condition> <value>` with `<field>` representing the database column to be used for the filter. Several such filter expressions can be concatenated with standard R logical expressions (such as & or |). To get a list of all available filters and their corresponding fields, the *supportedFilters(edb)* function could be used.

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

## Retrieve the genes
gns <- genes(edb, filter = ~ protein_domain_id == "PF00010" & seq_name == "21")
```

The function returned a GRanges object with the genomic position of the genes and additional gene-related annotations stored in *metadata* columns.

```r
# GRanges object with 3 ranges and 7 metadata columns:
# seqnames ranges strand | gene_id
# ENSG00000205927 21 33025845-33029196 + | ENSG00000205927
# ENSG00000184221 21 33070144-33072420 + | ENSG00000184221
# ENSG00000159263 21 36699133-36749917 + | ENSG00000159263
# gene_name gene_biotype seq_coord_system symbol
# OLIG2 protein_coding chromosome OLIG2
# OLIG1 protein_coding chromosome OLIG1
```
Three transcription factors with a helix-loop-helix domain are encoded on chromosome 21: SIM2, which is a master regulator of neurogenesis and is thought to contribute to some specific phenotypes of Down syndrome (Gardiner and Costa 2006) and the two genes OLIG1 and OLIG2 for which genetic triplication was shown to cause developmental brain defects (Chakrabarti et al. 2010). To visualize the exonic regions encoding the helix-loop-helix domain of these genes we next retrieve their transcript models and the positions of all Pfam protein domains within the amino acid sequences encoded by these transcripts. We process SIM2 separately from OLIG1 and OLIG2 because the latter are encoded in a narrow region on chromosome 21 and can thus be visualized easily within the same plot. We extract the transcript models for OLIG1 and OLIG2 that encode the protein domain using the getGeneRegionTrackForGviz function which returns the data in a format that can be directly passed to functions from the Gviz Bioconductor package (Hahne and Ivanek 2016) for plotting. Since Gviz expects UCSC-style chromosome names instead of the Ensembl chromosome names (e.g. chr21 instead of 21), we change the format in which chromosome names are returned by ensembldb with the seqlevelsStyle method. All subsequent queries to the EnsDb database will return chromosome names in UCSC format.

## Change chromosome naming style to UCSC
```
seqlevelsStyle(edb) <- "UCSC"
```

Next we fetch the coordinates of all Pfam protein domains encoded by these transcripts with the proteins method, asking for columns "prot_dom_start", "prot_dom_end" and "protein_domain_id" to be returned by the function. Note that we restrict the results in addition to protein domains defined in Pfam by using an additional filter.

```
pdoms <- proteins(edb, filter = ~ tx_id %in% txs$transcript & protein_domain_source == "pfam", columns = c("protein_domain_id", "prot_dom_start", "prot_dom_end"))
```

- Table: `pdoms` with 3 rows and 6 columns
  - `protein_domain_id`<character>
  - `prot_dom_start`<integer>
  - `prot_dom_end`<integer>
  - `protein_id`<character>
  - 1 PF00010 107 164 ENSP00000371785
  - 2 PF00010 110 162 ENSP00000371794
  - 3 PF00010 110 162 ENSP00000331040

---
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| tx_id  | protein_domain_source |
|--------|------------------------|
| ENST00000382348 | pfam |
| ENST00000382357 | pfam |
| ENST00000333337 | pfam |

We next map these protein-relative positions to the genome. We define first an IRanges object with the coordinates and submit this to the proteinToGenome function for mapping. Besides coordinates, the function requires also the respective protein identifiers which we supply as names.

```r
pdoms_rng <- IRanges(start = pdoms$prot_dom_start, end = pdoms$prot_dom_end, names = pdoms$protein_id)
pdoms_gnm <- proteinToGenome(pdoms_rng, edb)
```

The result is a list of GRanges objects with the genomic coordinates at which the protein domains are encoded, one for each of the input protein domains. Additional information such as the protein ID, the encoding transcript and the exons of the respective transcript in which the domain is encoded are provided as metadata columns.

```r
pdoms_gnm
## $ENSP00000371785
## GRanges object with 1 range and 7 metadata columns:
## seqnames ranges strand | protein_id tx_id
## <Rle> <IRanges> <Rle> | <character> <character>
## [1] chr21 33070565-33070738 + | ENSP00000371785 ENST00000382348
## exon_id exon_rank cds_ok protein_start protein_end
## <character> <integer> <logical> <integer> <integer>
## [1] ENSE00001491811 1 TRUE 107 164
## -------
## seqinfo: 1 sequence from GRCh38 genome
##
## $ENSP00000371794
## GRanges object with 1 range and 7 metadata columns:
## seqnames ranges strand | protein_id tx_id
## <Rle> <IRanges> <Rle> | <character> <character>
## [1] chr21 33027190-33027348 + | ENSP00000371794 ENST00000382357
## exon_id exon_rank cds_ok protein_start protein_end
## <character> <integer> <logical> <integer> <integer>
## [1] ENSE00001491833 2 TRUE 110 162
## -------
## seqinfo: 1 sequence from GRCh38 genome
##
## $ENSP00000331040
## GRanges object with 1 range and 7 metadata columns:
## seqnames ranges strand | protein_id tx_id
## <Rle> <IRanges> <Rle> | <character> <character>
## [1] chr21 33027190-33027348 + | ENSP00000331040 ENST00000333337
## exon_id exon_rank cds_ok protein_start protein_end
## <character> <integer> <logical> <integer> <integer>
```
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Column `cds_ok` in the result object indicates whether the length of the CDS of the encoding transcript matches the length of the protein sequence. For transcripts with unknown 3' and/or 5' CDS ends these will differ. The mapping result has to be re-organized before being plotted: `Gviz` expects a single `GRanges` object, with specific metadata columns for the grouping of the individual genomic regions. This is performed in the code block below.

```r
## Convert the list to a GRanges with grouping information
pdoms_gnm_grng <- unlist(GRangesList(pdoms_gnm))
pdoms_gnm_grng$id <- rep(pdoms$protein_domain_id, lengths(pdoms_gnm))
pdoms_gnm_grng$grp <- rep(1:nrow(pdoms), lengths(pdoms_gnm))
pdoms_gnm_grng
```

We next define the individual tracks we want to visualize and plot them with the `plotTracks` function from the `Gviz` package.
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```r
just.group = "right", shape = "box",
name = "Protein domains", size = 0.5)

## Generate the plot
plotTracks(list(ideo_track, gaxis_track, gene_track, pdom_track))
```

![Figure 1: Transcripts of genes OLIG1 and OLIG2 encoding a helix-loop-helix protein domain](image1)

**Figure 1:** Transcripts of genes OLIG1 and OLIG2 encoding a helix-loop-helix protein domain
Shown are all transcripts of the genes OLIG1 and OLIG2 that encode a protein with a helix-loop-helix protein domain (PF00010). Genomic positions encoding protein domains defined in Pfam are shown in light blue.

All transcripts are relatively short with the full coding region being in a single exon. Also, both transcripts encode a protein with a single protein domain, the helix-loop-helix domain PF00010.

Next we repeat the analysis for SIM2 by first fetching all of its transcript variants encoding the PF00010 Pfam protein domain from the database. Subsequently we retrieve all Pfam protein domains encoded in these transcripts.

```r
## Fetch all SIM2 transcripts encoding PF00010
txs <- getGeneRegionTrackForGviz(edb, filter = ~ genename == "SIM2" &
protein_domain_id == "PF00010")

## Fetch all Pfam protein domains within these transcripts
pdoms <- proteins(edb, filter = ~ tx_id %in% txs$transcript &
protein_domain_source == "pfam",
columns = c("protein_domain_id", "prot_dom_start",
"prot_dom_end"))
```

At last we have to map the protein domain coordinates to the genome and prepare the data for the plot. Since the code is essentially identical to the one for OLIG1 and OLIG2 it is not displayed.

![Figure 2: Transcripts of the gene SIM2 encoding a helix-loop-helix domain](image2)

**Figure 2:** Transcripts of the gene SIM2 encoding a helix-loop-helix domain
Shown are all transcripts of SIM2 encoding a protein with a helix-loop-helix protein domain (PF00010). Genomic positions encoding protein domains defined in Pfam are shown in light blue.

The SIM2 transcript encodes a protein with in total 4 protein domains. The helix-loop-helix domain PF00010 is encoded in its first exon.
Mapping of genomic coordinates to protein-relative positions

One of the known mutations for human red hair color is located at position 16:89920138 (dbSNP ID rs1805009) on the human genome (version GRCh38). Below we map this genomic coordinate to the respective coordinate within the protein sequence encoded at that location using the `genomeToProtein` function. Note that we use "chr16" as the name of the chromosome, since we changed the chromosome naming style to UCSC in the previous example.

```r
# Genomic position
gnm_pos <- GRanges("chr16", IRanges(89920138, width = 1))

# Convert to protein positions
prt_pos <- genomeToProtein(gnm_pos, edb)

# Print results
prt_pos
```

The genomic position could thus be mapped to the amino acid 294 in each of the 3 proteins listed above. Using the `select` function we retrieve the HGNC symbol of the gene for these 3 proteins.

```r
# Select HGNC symbols
select(edb, keys = ~ protein_id == names(prt_pos[[1]]), columns = "SYMBOL")
```

Two proteins are from the `MC1R` gene and one from `RP11-566K11.2` (ENSG00000198211) a gene which exons overlap exons from `MC1R` as well as exons of the more downstream located gene `TUBB3`. To visualize this we first fetch transcripts overlapping the genomic position of interest and subsequently all additional transcripts within the region defined by the most downstream and upstream exons of the transcripts.

```r
# Get transcripts overlapping the genomic position.
txs <- getGeneRegionTrackForGviz(edb, filter = GRangesFilter(gnm_pos))
```
## Get all transcripts within the region from the start of the most 5' and end of the most 3' exon.

```r
all_txs <- getGeneRegionTrackForGviz(edb, filter = GRangesFilter(range(txs), type = "within"))
```

## Plot the data

```r
# - Ideogram
ideo_track <- IdeogramTrack(genome = "hg38", chromosome = "chr16")
# - Genome axis
gaxis_track <- GenomeAxisTrack()
# - Transcripts
gene_track <- GeneRegionTrack(all_txs, showId = TRUE, just.group = "right", name = "", geneSymbol = TRUE, size = 0.5)
# - highlight the region.
hl_track <- HighlightTrack(list(gaxis_track, gene_track), range = gnm_pos)

## Generate the plot
plotTracks(list(ideo_track, hl_track))
```

**Figure 3: Transcripts overlapping, or close to, the genomic position of interest**

Shown are all transcripts. The genomic position of the variant is highlighted in red.

The plot above visualizes the expanded genomic region of the variant (indicated with a vertical red line) including all transcripts (from both strands) encoded in the region. In total 4 genes are present in the region: MC1R, the MC1R-TUBB3 read-through gene RP11-566K11.2, the non-coding gene RP11-566K11.4 located on the reverse strand and TUBB3. Exons of transcripts from the former 3 genes overlap the genomic position of the variant. Using the proteins method we next extract the sequences of the proteins encoded by the 3 transcripts (two of MC1R and one of RP11-566K11.2) and determine the amino acid at position 294 in these. To retrieve the results in a format most suitable for the representation of amino acid sequences we specify `return.type = "AAStringSet"` in the proteins call.

```r
## Get the amino acid sequences for the 3 transcripts
prt_seq <- proteins(edb, return.type = "AAStringSet",
                    filter = ~ protein_id == names(prt_pos[[1]]))

## Extract the amino acid at position 294
subseq(prt_seq, start = 294, end = 294)
```
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## A AAStringSet instance of length 3
## width seq names
## [1] 1 D ENSP00000451560
## [2] 1 D ENSP00000451605
## [3] 1 D ENSP00000451760

The amino acid at position 294 is for all an aspartic acid (“D”) which is in agreement with the reference amino acid of mutation Asp294His (Valverde et al. 1995) described by the dbSNP ID of this example.

### 3 Using ensemblldb in a standard gene-level RNA-seq workflow

This workflow is based on the RNA-seq workflow (Love et al. 2015) and shows how the ensemblldb package can be used for all annotation-related tasks in that analysis. In brief, the workflow describes the analysis of an RNA-seq data set after alignment of the reads to the genome with the aim to identify genes that are differentially expressed in airway smooth muscle cells after treatment with the synthetic glucocorticoid dexamethasone, a drug with anti-inflammatory effects. Glucocorticoids are used, for example, by people with asthma to reduce inflammation of the airways. Note that here we focus only on the parts of the analysis in which ensemblldb functionality and data are used, i.e. the gene quantification step and the annotation of the analysis results. Other topics from the workflow such as the visualization and exploratory data analysis are not covered here. Also, please refer to the original workflow for background information and detailed descriptions of the methodology used.

Below we load the airway data package that provides all required files for the present analysis (including BAM files with a subset of the alignment results of the raw reads to the genome GRCh37) and load a text file with sample descriptions from this package.

```r
library(airway)
sampleTable <- read.csv(system.file("extdata/sample_table.csv", package = "airway"),
                        row.names = 1)
```

#### 3.1 Gene quantification

After alignment of the reads to the genome, gene abundance estimates can be generated by counting the number of reads falling within the exon boundaries of a gene. The BAM files with the alignment results of the present data set are provided by the airway package. Below we load these into a BamFileList object defined in the Rsamtools package which enables reading alignment information from BAM files.

```r
library(Rsamtools)
bamfiles <- BamFileList(paste0(system.file("extdata", package = "airway"),
                            "/", sampleTable$Run, "._subset.bam"),
                        yieldSize = 2000000)
```
For gene quantification we use the `summarizeOverlaps` function from the `GenomicAlignments` package (Lawrence et al. 2013) that requires, apart from the aligned reads, also the genomic positions of all exons of all genes. In contrast to the original workflow, we retrieve these directly from the appropriate `ensembldb` EnsDb database that we load in the code block below. Since the reads in this RNA-seq data set were aligned against GRCh37, we load an `ensembldb` resource for an Ensembl release matching that genome version (i.e. release 75). The genomic positions of all exons grouped by gene are extracted from the database with the `exonsBy` function.

```r
library(ensembldb)
library(EnsDb.Hsapiens.v75)
ebg <- exonsBy(EnsDb.Hsapiens.v75, "gene")
```

With this we can now proceed to the read counting with the `summarizeOverlaps` function using the same settings as in the original workflow.

```r
library(GenomicAlignments)
se <- summarizeOverlaps(features = ebg, reads = bamfiles,
                         mode = "Union", singleEnd = FALSE,
                         ignore.strand = TRUE, fragments = TRUE)
```

At last we add the sample metadata to the result object (a `SummarizedExperiment`) and ensure that `untrt` is the reference level for the variable specifying the treatment of the samples (i.e. `dex`).

```r
# Add sample metadata
colData(se) <- DataFrame(sampleTable)
se$dex <- relevel(se$dex, "untrt")
```

### 3.2 Differential expression analysis

We use `DESeq2` (Love, Huber, and Anders 2014) for the differential expression analysis to identify genes that are deregulated upon treatment with the synthetic glucocorticoid dexamethasone (`dex`). The `SummarizedExperiment` with the gene quantifications generated in the previous section contains only reads aligned against chromosome 1. Thus, as in the original workflow, we load a prepared `SummarizedExperiment` containing read counts for genes from all chromosomes (which is also provided in the `airway` package).

```r
data("airway")
se <- airway
se$dex <- relevel(se$dex, "untrt")
```

We next build a `DESeqDataSet` from this object also specifying the experimental layout with the `design` argument.

```r
library(DESeq2)
dds <- DESeqDataSet(se, design = ~ cell + dex)
```

Before proceeding to the differential expression analysis we remove rows (genes) with only zeros or very low read counts.
As we have already specified an experimental design when creating the DESeqDataSet, we can run the differential expression analysis on the raw counts with a single call to the function DESeq:

```r
dds <- DESeq(dds)
```

We can now extract the estimated log2 fold changes and p values for the last variable in the design formula.

```r
res <- results(dds)
head(res[order(res$pvalue), ])
## log2 fold change (MLE): dex trt vs untrt
## Wald test p-value: dex trt vs untrt
## DataFrame with 6 rows and 6 columns
## baseMean log2FoldChange lfcSE
## <numeric> <numeric> <numeric>
## ENSG00000152583 997.439773207048 4.57491904614571 0.184056290144421
## ENSG00000165995 495.092906698546 3.29106191054141 0.13317371150066
## ... ... ... ...
## ENSG00000189221 2341.76725275591 3.3535801702967 0.141782454259944
## ENSG00000211445 12285.6151498691 3.73040303801649 0.165830587883941
## # # # # # # # # # # # #
## stat pvalue padj
## <numeric> <numeric> <numeric>
## ENSG00000152583 24.8560863774662 2.22231970363871 3.99884207472749e-132
## ENSG00000165995 24.7125492971269 7.83975707156014e-135 7.05342943728266e-108
## ... ... ... ...
## ENSG00000189221 23.6529984461141 1.09937071872851 3.95641534256016e-120
## ENSG00000211445 22.4952651113274 6.1813770361409e-112 1.38497949731387e-108
```

### 3.3 Annotating results

As a last step we want to annotate our result table. In contrast to the original workflow, we extract annotations for the Ensembl gene identifiers from the EnsDb database we have already used in the gene quantification step above. This simplifies the analysis and additionally ensures that both annotations as well as positional information used for the read counting are from the same data release.

Below we use ensembldb’s `genes` function to extract all gene annotations available in the EnsDb database and return the results as a DataFrame.

```r
anns <- genes(EnsDb.Hsapiens.v75, return.type = "DataFrame")
anns
## # A tibble: 64102 x 10
## # Groups:   sequence, gene [64102]
## #
## # baseMean log2FoldChange lfcSE
## # <numeric> <numeric> <numeric>
## # ENSG00000152583 997.439773207048 4.57491904614571 0.184056290144421
## # ENSG00000165995 495.092906698546 3.29106191054141 0.13317371150066
## # ... ... ... ...
## # ENSG00000189221 2341.76725275591 3.3535801702967 0.141782454259944
## # ENSG00000211445 12285.6151498691 3.73040303801649 0.165830587883941
## # # # # # # # # # # # #
## # stat pvalue padj
## # <numeric> <numeric> <numeric>
## # ENSG00000152583 24.8560863774662 2.22231970363871 3.99884207472749e-132
## # ENSG00000165995 24.7125492971269 7.83975707156014e-135 7.05342943728266e-108
## # ... ... ... ...
## # ENSG00000189221 23.6529984461141 1.09937071872851 3.95641534256016e-120
## # ENSG00000211445 22.4952651113274 6.1813770361409e-112 1.38497949731387e-108
```
Annotations include the positional information of the genes, their name/symbol, their biotype and all NCBI Entrez gene identifiers associated with them. The mapping from Ensembl gene IDs to Entrez gene IDs can be one to many. We thus collapse multiple Entrez gene IDs for one Ensembl gene into a comma separated string.

```r
anns$entrezid <- vapply(anns$entrezid,
  function(z) {
    if (all(is.na(z)))
      NA_character_
    else paste(z, collapse = ",")
  }, character(1))
```

```r
anns
## DataFrame with 64102 rows and 10 columns
## gene_id gene_name gene_biotype gene_seq_start gene_seq_end
## <character> <character> <character> <integer> <integer>
## 1 ENSG00000223972 DDX11L1 pseudogene 11869 14412
## 2 ENSG00000227232 WASH7P pseudogene 14363 29806
## ... ... ... ... ... ...
## 64101 ENSG00000231514 FAM58CP pseudogene 28772667 28773306
## 64102 ENSG00000235857 CTBP2P1 pseudogene 59001391 59001635
## seq_name seq_strand seq_coord_system symbol
## <character> <integer> <character> <character>
## 1 1 1 chromosome DDX11L1
## 2 1 -1 chromosome WASH7P
## ... ... ... ... ...
## 64101 Y -1 chromosome FAM58CP
## 64102 Y 1 chromosome CTBP2P1
## entrezid
## <list>
## 1 c(100287596, 100287102)
## 2 c(100287171, 653635)
## ... ...
## 64101 NA
## 64102 NA
```
At last we combine the results and annotation tables based on the Ensembl gene identifier.

```r
rownames(anns) <- anns$gene_id
res <- cbind(anns[rownames(res), ], res)
head(res[order(res$pvalue), c("gene_name", "log2FoldChange", "padj")])
```

### DataFrame with 6 rows and 3 columns

| gene_name | log2FoldChange | padj           |
|-----------|---------------|---------------|
| ENSG00000152583 | 4.57491904614571 | 3.99884207472749e-132 |
| ENSG00000165995 | 3.29106191054141 | 7.05342943728266e-131 |
| ENSG00000189221 | 3.3535801702967  | 3.95641534256016e-120 |
| ENSG00000211445 | 3.73040303801649 | 1.38497949731387e-108 |

## Benchmarking the filter framework

Filters in `ensemblldb` are translated into the underlying SQL commands which leads to a significant performance gain on data retrieval if only subsets of the data are fetched. In this section we compare the performances of data retrieval in the classical way, i.e. by first extracting the full data from the database with subsequent sub-setting in R, and data retrieval using the `ensemblldb` filter framework.

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

The first use case is to retrieve all gene-related annotations for the `SIM2` gene from Section 1. The classical approach is to get all gene annotations and subset the results to those for which the gene name matches `SIM2`.

```r
gns <- genes(edb)
gns[gns$gene_name == "SIM2", ]
```

### GRanges object with 1 range and 6 metadata columns:

| seqnames | ranges | strand | gene_id | gene_name | gene_biotype | seq_coordsystem | symbol |
|----------|--------|--------|---------|-----------|--------------|----------------|--------|
| ENSG00000159263 | 21 36699133-36749917 + | ENSG00000159263 | SIM2 | protein_coding | chromosome | SIM2 |

```

## seqinfo: 357 sequences from GRCh38 genome
Using the filter framework this is simplified to:

```r
genes(edb, filter = ~ gene_name == "SIM2")
```

Next we compare the performance of both approaches using the `microbenchmark` function evaluating each expression 8 times. The output from the benchmark with a summary of the evaluation times is displayed below. The calculations were performed on a MacBook Pro (15-inch 2016) with a quad-core 2.9 GHz Intel Core i7 CPU and 16 GB memory.

```r
microbenchmark(
  {
    gns <- genes(edb)
    gns[gns$gene_name == "SIM2"]
  },
  genes(edb, filter = ~ gene_name == "SIM2"), times = 8)
```

We see a clear performance advantage of the filter framework. With the classical approach it took on average 1 second to extract the relevant annotation, while filtering the data with the filter framework reduced this to less than 20 milliseconds which represents an about 60-fold performance increase.

In the next example we want to get the exon positions, grouped by gene, for genes that are encoded on chromosome X. First we use again the classical approach fetching all information and sub-setting later.

```r
exns <- exonsBy(edb, "gene")
exns[seqnames(exns) == "X"]
```
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|   | X 100639945-100639991 |   | ENSE00001828996 |
|---|----------------------|---|------------------|
| 2 | X 100636793-100637104 |   | ENSE00001863395  |
|   | ...                  |   | ...              |
| 19| X 100628670-100629986 |   | ENSE00001459322  |
| 20| X 100627109-100629986 |   | ENSE00003730948  |
|   | ...                  |   | <63969 more elements> |
|   | seqinfo: 357 sequences from GRCh38 genome |

With the filter framework the task is simplified to:

```r
exonsBy(edb, "gene", filter = ~ seq_name == "X")
```

Next we compare the performance of both approaches.

```r
microbenchmark(
  { exns <- exonsBy(edb, "gene") exns[seqnames(exns) == "X"] },
  exonsBy(edb, "gene", filter = ~ seq_name == "X"), times = 8)
```

The performance gain when using the filter framework was again remarkable (on average about 13 seconds with the classical approach compared to approximately 0.25 seconds with the filter framework).
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Summarizing, filters in *ensemblldb* allow to retrieve only subsets of the full annotations available in *EnsDb* databases and their use leads to a significant performance gain in such cases. In addition, the filtering framework simplifies the sub-setting workflow considerably and provides a clearer syntax.

### 5 Session information

```r
sessionInfo()
## R version 3.5.1 (2018-07-02)
## Platform: x86_64-apple-darwin18.2.0/x86_64 (64-bit)
## Running under: macOS 10.14.3
##
## Matrix products: default
## BLAS: /System/Library/Frameworks/Accelerate.framework/Versions/A/Frameworks/vecLib.framework/Versions/A/libBLAS.dylib
## LAPACK: /System/Library/Frameworks/Accelerate.framework/Versions/A/Frameworks/vecLib.framework/Versions/A/libLAPACK.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] grid stats4 parallel stats graphics grDevices utils
## [8] datasets methods base
##
## other attached packages:
## [1] microbenchmark_1.4-6 DESeq2_1.22.1
## [3] GenomicAlignments_1.18.0 EnsDb.Hsapiens.v75_2.99.0
## [5] Rsamtools_1.34.0 Biostrings_2.50.1
## [7] XVector_0.22.0 airway_1.2.0
## [9] SummarizedExperiment_1.12.0 DelayedArray_0.8.0
## [11] BiocParallel_1.16.2 matrixStats_0.54.0
## [13] Gviz_1.26.3 EnsDb.Hsapiens.v86_2.99.0
## [15] ensembldb_2.6.3 AnnotationFilter_1.6.0
## [17] GenomicFeatures_1.34.1 AnnotationDbi_1.44.0
## [19] Biobase_2.42.0 GenomicRanges_1.34.0
## [21] GenomeInfoDb_1.18.1 IRanges_2.16.0
## [23] S4Vectors_0.20.1 BiocGenerics_0.28.0
## [25] BioStyle_2.10.0 rmarkdown_1.11
##
## loaded via a namespace (and not attached):
## [1] TH.data_1.0-9 colorspace_1.3-2
## [3] biovizBase_1.30.0 htmlTable_1.12
## [5] base64enc_0.1-3 dichromat_2.0-0
## [7] rstudioapi_0.8 bit64_0.9-7
## [9] mvtnorm_1.0-8 codetools_0.2-15
## [11] splines_3.5.1 geneplotter_1.60.0
## [13] knitr_1.21 Formula_1.2-3
## [15] annotate_1.60.0 cluster_2.0.7-1
## [17] BiocManager_1.30.4 compiler_3.5.1
## [19] httr_1.4.0 backports_1.1.2
```
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```r
## [21] assertthat_0.2.0 Matrix_1.2-15
## [23] lazyeval_0.2.1 acepack_1.4.1
## [25] htmltools_0.3.6 prettyunits_1.0.2
## [27] tools_3.5.1 bindrcpp_0.2.2
## [29] gtable_0.2.0 glue_1.3.0
## [31] GenomeInfoDbData_1.2.0 dplyr_0.7.8
## [33] tinytex_0.9 Rcpp_1.0.0
## [35] rtracklayer_1.42.1 xfun_0.4
## [37] stringr_1.3.1 XML_3.98-1.16
## [39] zlibbioc_1.28.0 MASS_7.3-51.1
## [41] zoo_1.8-4 scales_1.0.0
## [43] BSgenome_1.50.0 VariantAnnotation_1.28.3
## [45] hms_0.4.2 ProtGenerics_1.14.0
## [47] sandwich_2.5-0 RColorBrewer_1.1-2
## [49] yaml_2.2.0
## [51] memoise_1.1.0 gridExtra_2.3
## [53] ggplot2_3.1.0 biomaRt_2.38.0
## [55] rpart_4.1-13 latticeExtra_0.6-28
## [57] stringr_1.2.4 RSQLite_2.1.1
## [59] genefilter_1.64.0 checkmate_1.8.5
## [61] rlang_0.3.0.1 pkgconfig_2.0.2
## [63] bitops_1.0-6 evaluate_0.12
## [65] lattice_0.20-38 purrr_0.2.5
## [67] bindr_0.1.1 htmlwidgets_1.3
## [69] bit_1.1-14 tidyselect_0.2.5
## [71] plyr_1.8.4 magrittr_1.5
## [73] bookdown_0.8 R6_2.3.0
## [75] Hmisc_4.1-1 multcomp_1.4-8
## [77] DBI_1.0.0 pillar_1.3.0
## [79] foreign_0.8-71 survival_2.43-3
## [81] Rcurl_1.95-4.11 nnet_7.3-12
## [83] tibble_1.4.2 crayon_1.3.4
## [85] progress_1.2.0 locfit_1.5-9.1
## [87] data.table_1.11.8 blob_1.1.1
## [89] digest_0.6.18 xtable_1.8-3
## [91] munsell_0.5.0
```

**References**

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