Making 3'UTR files for additional species

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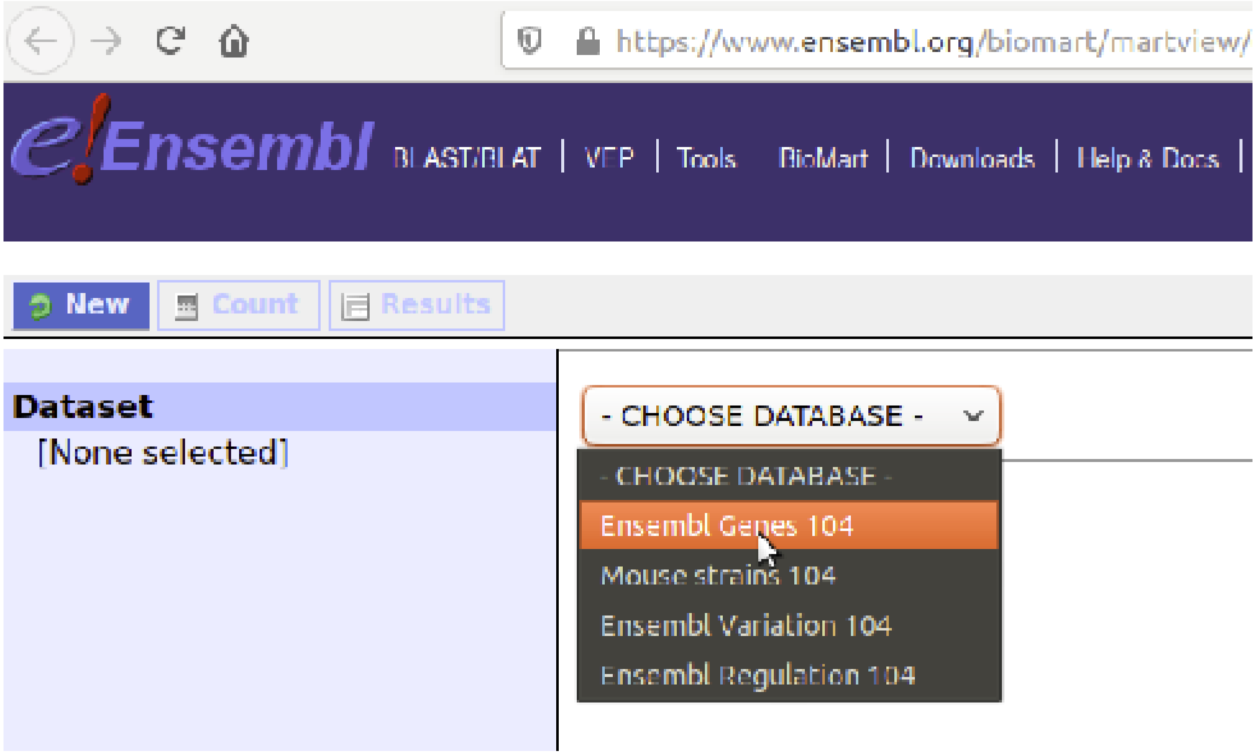
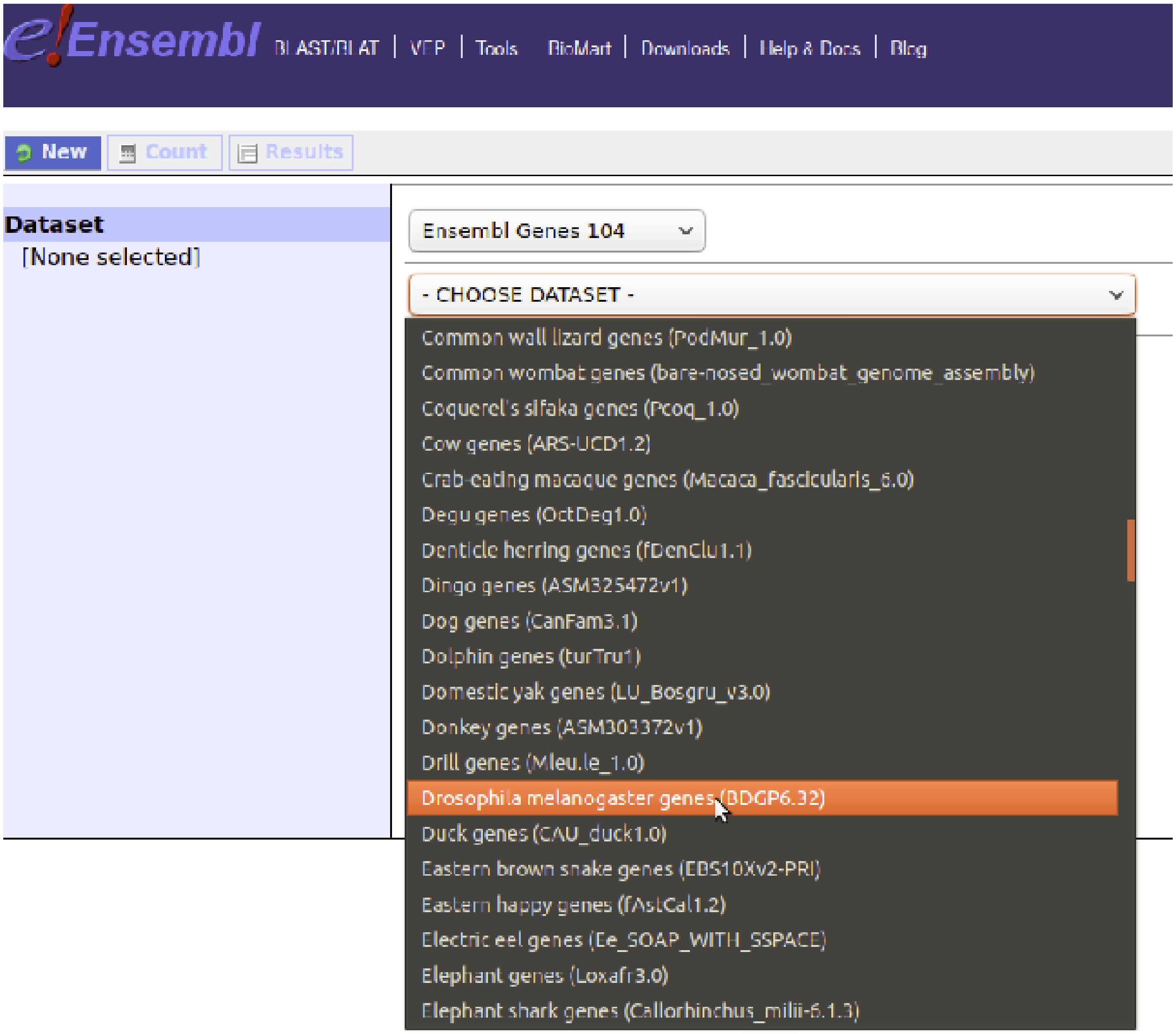
Obtain sequences from Ensembl

In a browser, navigate to https://www.ensembl.org/biomart/martview/.

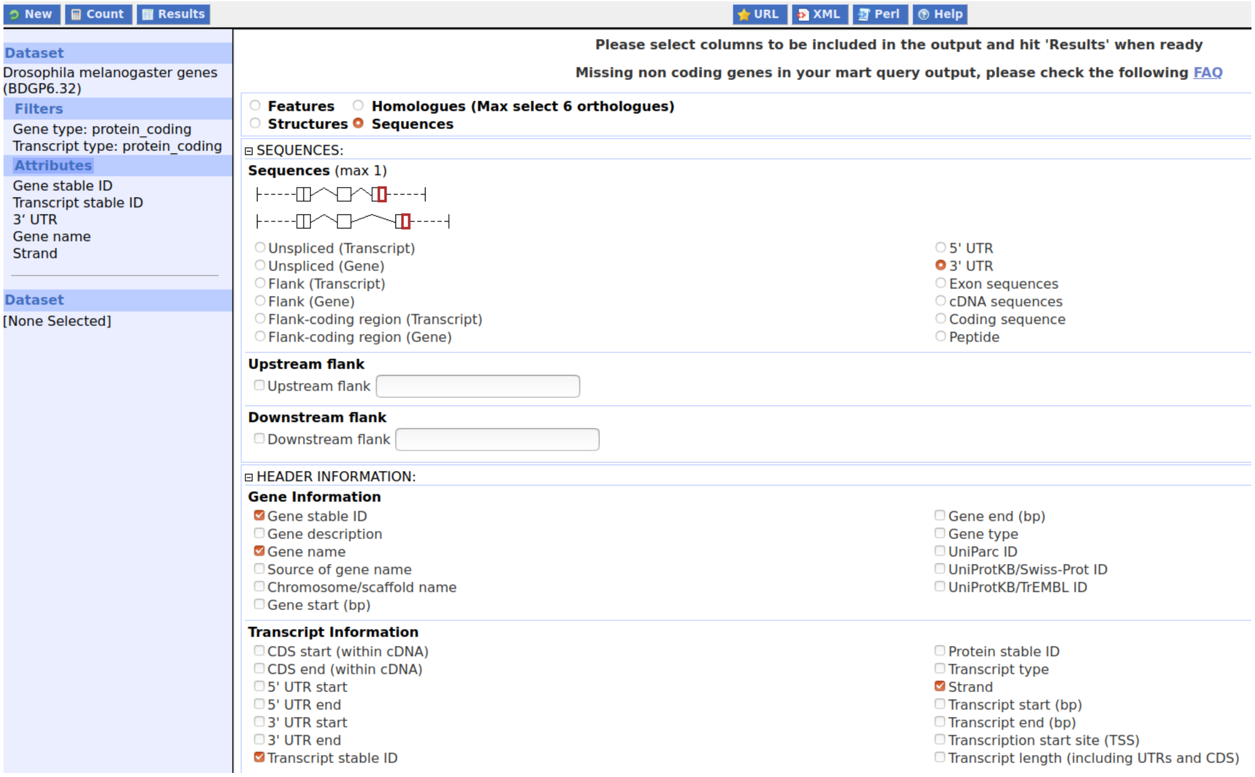
Choose 'Ensembl Genes' under 'CHOOSE DATABASE'. At the time of writing, the version was 104.



Now choose the species of interest:


e!Ensembl
BLAST/BLAT | VEP | Tools | BioMart | Downloads | Help & Docs | Blog
New
Count
Results
Dataset
[None selected]
Ensembl Genes 104
- CHOOSE DATASET -
Common wall lizard genes (PodMur_1.0)
Common wombat genes (bare-nosed_wombat_genome_assembly)
Coquerel's sifaka genes (Pcoq_1.0)
Cow genes (ARS-UCD1.2)
Crab-eating macaque genes (Macaca_fascicularis_6.0)
Degu genes (OctDeg1.0)
Denticle herring genes (fDenClu1.1)
Dingo genes (ASM325472v1)
Dog genes (CanFam3.1)
Dolphin genes (turTru1)
Domestic yak genes (LU_Bosgru_v3.0)
Donkey genes (ASM303372v1)
Drill genes (Mleu.le_1.0)
Drosophila melanogaster genes (BDGP6.32)
Duck genes (CAU_duck1.0)
Eastern brown snake genes (EBS10Xv2-PRI)
Eastern happy genes (fAstCal1.2)
Electric eel genes (Ee_SOAP_WITH_SSPACE)
Elephant genes (Loxafr3.0)
Elephant shark genes (Callorhinchus_milii-6.1.3)

Add a filter so that only protein coding genes and transcriptsare returned, and choose the attributes ‘3’UTR sequence’, ‘Gene Stable ID’, ‘Transcript Stable ID’, ‘Strand’ and ‘Gene Name’.



Dataset
Drosophila melanogaster genes (BDGP6.32)
Filters
Gene type: protein_coding
Transcript type: protein_coding
Attributes
Gene stable ID
Transcript stable ID
3' UTR
Gene name
Strand

Dataset
[None Selected]

Please select columns to be included in the output and hit 'Results' when ready

Missing non coding genes in your mart query output, please check the following FAQ

○ Features ○ Homologues (Max select 6 orthologues)
○ Structures ● Sequences

⊟ SEQUENCES:

Sequences (max 1)

○ Unspliced (Transcript)
○ Unspliced (Gene)
○ Flank (Transcript)
○ Flank (Gene)
○ Flank-coding region (Transcript)
○ Flank-coding region (Gene)
○ 5' UTR
● 3' UTR
○ Exon sequences
○ cDNA sequences
○ Coding sequence
○ Peptide

Upstream flank
□ Upstream flank

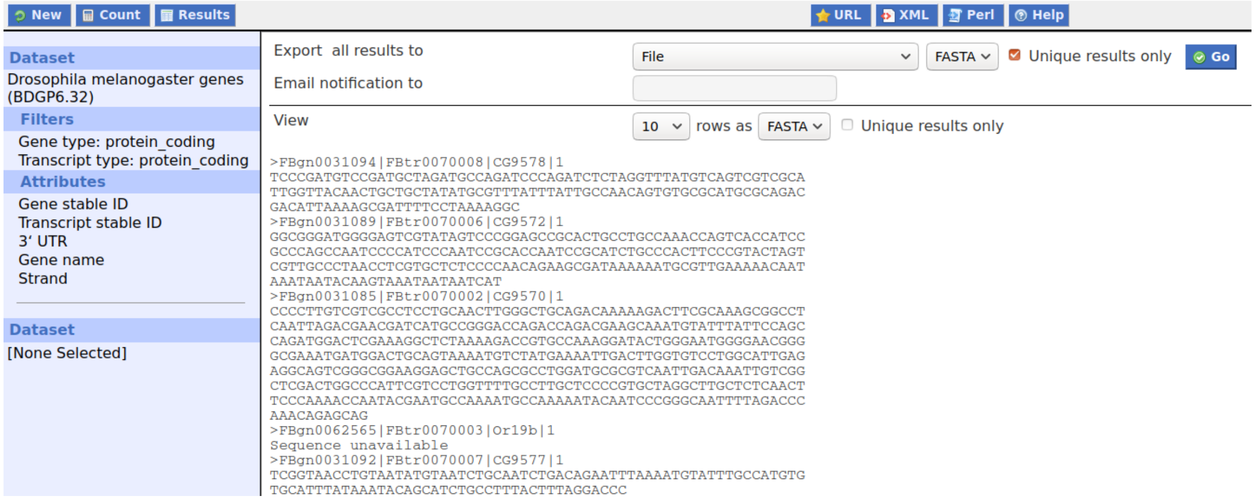
Downstream flank
□ Downstream flank

⊟ HEADER INFORMATION:

Gene Information
☑ Gene stable ID
□ Gene description
☑ Gene name
□ Source of gene name
□ Chromosome/scaffold name
□ Gene start (bp)
□ Gene end (bp)
□ Gene type
□ UniParc ID
□ UniProtKB/Swiss-Prot ID
□ UniProtKB/TrEMBL ID

Transcript Information
□ CDS start (within cDNA)
□ CDS end (within cDNA)
□ 5' UTR start
□ 5' UTR end
□ 3' UTR start
□ 3' UTR end
☑ Transcript stable ID
□ Protein stable ID
□ Transcript type
☑ Strand
□ Transcript start (bp)
□ Transcript end (bp)
□ Transcription start site (TSS)
□ Transcript length (including UTRs and CDS)

Export the results as a fasta file by pressing the 'Results' button. You can now download the fasta file by pressing 'Go'. You may choose to export a zipped file and get notified by mail when it is ready.



Dataset
Drosophila melanogaster genes (BDGP6.32)
Filters
Gene type: protein_coding
Transcript type: protein_coding
Attributes
Gene stable ID
Transcript stable ID
3' UTR
Gene name
Strand

Dataset
[None Selected]

Export all results to File FASTA ☑ Unique results only
Email notification to
View 10 rows as FASTA □ Unique results only

```
>FBgn0031094|FBtr0070008|CG9578|1
TCCCGATGTCCGATGCTAGATGCCAGATCCCAGATCTCTAGGTTTATGTCAGTCGTCGCA
TTGGTTACAACTGCTGCTATATGCGTTTATTTATTGCCAACAGTGTGCGCATGCGCAGAC
GACATTAAAAGCGATTTTCCTAAAAGGC
>FBgn0031089|FBtr0070006|CG9572|1
GGCGGGATGGGGAGTCGTATAGTCCCGGAGCCGCACTGCCTGCCAAACCAGTCACCATCC
GCCCAGCCAATCCCCATCCCAATCCGCACCAATCCGCATCTGCCCACTTCCCGTACTAGT
CGTTGCCCTAACCTCGTGCTCTCCCCAACAGAAGCGATAAAAAATGCGTTGAAAAACAAT
AAATAATACAAGTAAATAATAATCAT
>FBgn0031085|FBtr0070002|CG9570|1
CCCCTTGTCGTCGCCTCCTGCAACTTGGGCTGCAGACAAAAAGACTTCGCAAAGCGGCCT
CAATTAGACGAACGATCATGCCGGGACCAGACCAGACGAAGCAAATGTATTTATTCCAGC
CAGATGGACTCGAAAGGCTCTAAAAGACCGTGCCAAAGGATACTGGGAATGGGGAACGGG
GCGAAATGATGGACTGCAGTAAAATGTCTATGAAAATTGACTTGGTGTCCTGGCATTGAG
AGGCAGTCGGGCGGAAGGAGCTGCCAGCGCCTGGATGCGCGTCAATTGACAAATTGTCGG
CTCGACTGGCCCATTCGTCCTGGTTTTGCCTTGCTCCCCGTGCTAGGCTTGCTCTCAACT
TCCCAAAACCAATACGAATGCCAAAATGCCAAAAATACAATCCCGGGCAATTTTAGACCC
AAACAGAGCAG
>FBgn0062565|FBtr0070003|Or19b|1
Sequence unavailable
>FBgn0031092|FBtr0070007|CG9577|1
TCGGTAACCTGTAATATGTAATCTGCAATCTGACAGAATTTAAAATGTATTTGCCATGTG
TGCATTTATAAATACAGCATCTGCCTTTACTTTAGGACCC
```

Save the file to an appropriate location. Now use the command line to create a one line fasta file:

```
cat /path/to/mart_export.txt | awk '/^>/&&NR>1{print "";}{ printf "%s",/^>/ ? $0" ":$0 }'
| awk '{print($1" "$2)}'> /desired/path/to/oneLineFasta.fa
# the cryptic step is to avoid three fields when sequence unavailable.
```

Now do the following in R to produce the seq dataframe and seqlist needed in miReact:

```
####################################
fastafile <- "/path/to/oneLineFasta.fa"
seqs <- read.table(as.is=TRUE, sep="|", comment.char = "", quote = "", header=F, fastafile)
dim(seqs)
```

```r
# checkup that there are four columns,
# of which the last contains the sequence as well as the strand direction.
colnames(seqs) <- c("gid","tid","gsym","sequence")
head(seqs$gid) # check
seqs$gid<-sub(">","",seqs$gid) # remove the fasta '>' separator.
seqs$strand <- sub(" .*","",seqs$sequence) # Fetch the strand from the 'sequence' col.
table(seqs$strand) # should be '1' and '-1' only
seqs$sequence <- sub("^-","",seqs$sequence) # remove strand info from sequence field.
seqs$sequence <- sub("1 ","",seqs$sequence)
seqs$sequence<-toupper(seqs$sequence) # sequence to upper case.
seqs$nchar <- nchar(seqs$sequence) # sequence lengths
hist(seqs$nchar) # look at seq length distribution
min(seqs$nchar)
sum(seqs$nchar==1) # 44
head(seqs[grep("S",seqs$sequence),]) # See if some have 'SEQUENCE' dummy annotation.
sum(!grepl("S",seqs$sequence))
seqs$sequence <- sub("^SEQUENCE","",seqs$sequence) # make these have length 0
seqs$nchar <- nchar(seqs$sequence) # re-define sequence lengths accordingly.
# Sequences with Ns will interfere with downstream processes
sum(grepl("N",seqs$sequence)) #
# remove if neccesary
seqs <- seqs[!grepl("N",seqs$sequence),]
hist(seqs$nchar) # checks...
min(seqs$nchar)
sum(seqs$nchar==0)
# get rid og seqs < 20 and > 10000
seqs <- seqs[seqs$nchar>20&seqs$nchar<10000,]
dim(seqs) # check reduction in size.
# order by gene ID and length
seqs <- seqs[order(seqs$gid,-seqs$nchar),]
# and save
saveRDS(seqs, file="/path/to/miReact/installation/folder/seqs/dm.utr3.seqs.rds")
# save to the miReact installation folder in the sub folder 'seqs'
# Note that the species name. here 'dm', for drosophila melanogaster,
# is needed as input in the 'species' parameter in miReact in the downstream process...

# Produce the seqlist, list of 3'UTR sequences with nucleotide probabilities
# This requires the Regmex package installed:
# install it with devtools::install_github("muhligs/Regmex")
require(Regmex)
require(parallel) # to speed up, run parallel by specifying cores > 1 below
seqlist <- Regmex::seq.list.con(seqlist = seqs$sequence, cores=1)
# it is imperative that the seqlist exactly corresponds to the seqs object above,
# i.e. the sequences must match.

# Now save to the seqs installation folder.
saveRDS(seqlist, file="/path/to/miReact/installation/folder/seqs/dm.utr3.seqlist.rds")
#################################
```

And thats basically it…