Exploration of a genomic annotations table (GTF)

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Goal of the practical session

During this practical session, we will cover the following items:

1. Manipulate a table containing genomics data (*E. Coli* genome annotations).
2. Select a subset of the data/rows according to a given criterion.
3. Generate different graphical representations of these data.
4. Compute statistics describing the different types of annotations.

The GTF file format

The **GTF** (**General Transfer Format**) file format is extensively used to provide easily readable genomics annotations while being very handy with a computer.

Text files,

- one row per genomic "object" (gene, transcript, exon, intron, CDS, . . .)
- one column per attribute (name, source, object type, genomic coordinates, description).

The GTF format is described on the following websites:

- http://www.ensembl.org/info/website/upload/gff.html
- https://genome.ucsc.edu/FAQ/FAQformat.html#format4

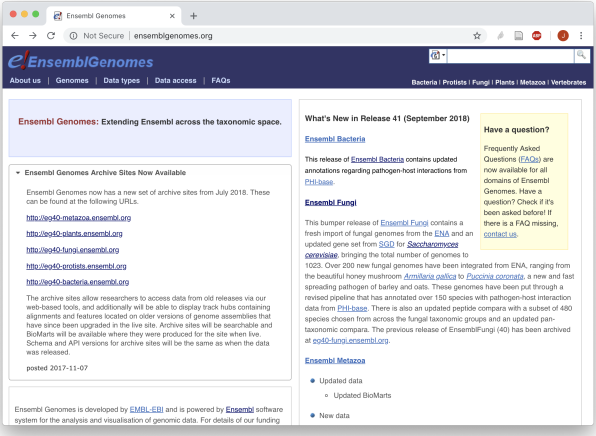
Find the GTF file of your favorite organism (on Ensembl)

1. Visit http://ensemblgenomes.org/.
2. Click on the link Bacteria.
3. Click on Download
4. In the **Filter** box, write *Escherichia coli*. The list of the proposed organisms is changing while you are writing.

For this session we will use the *E. Coli* GTF annotation file available here.

Page d'accueil d'EnsemblGenomes

http://ensemblgenomes.org/



Ensembl Genomes
Not Secure | ensemblgenomes.org
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About us | Genomes | Data types | Data access | FAQs
Bacteria | Protists | Fungi | Plants | Metazoa | Vertebrates

Ensembl Genomes: Extending Ensembl across the taxonomic space.

Ensembl Genomes Archive Sites Now Available

Ensembl Genomes now has a new set of archive sites from July 2018. These can be found at the following URLs.

http://eg40-metazoa.ensembl.org

http://eg40-plants.ensembl.org

http://eg40-fungi.ensembl.org

http://eg40-protists.ensembl.org

http://eg40-bacteria.ensembl.org

The archive sites allow researchers to access data from old releases via our web-based tools, and additionally will be able to display track hubs containing alignments and features located on older versions of genome assemblies that have since been upgraded in the live site. Archive sites will be searchable and BioMarts will be available where they were produced for the site when live. Schema and API versions for archive sites will be the same as when the data was released.

posted 2017-11-07

Ensembl Genomes is developed by EMBL-EBI and is powered by Ensembl software system for the analysis and visualisation of genomic data. For details of our funding

What's New in Release 41 (September 2018)

Ensembl Bacteria

This release of Ensembl Bacteria contains updated annotations regarding pathogen-host interactions from PHI-base.

Ensembl Fungi

This bumper release of Ensembl Fungi contains a fresh import of fungal genomes from the ENA and an updated gene set from SGD for *Saccharomyces cerevisiae*, bringing the total number of genomes to 1023. Over 200 new fungal genomes have been integrated from ENA, ranging from the beautiful honey mushroom *Armillaria gallica* to *Puccinia coronata*, a new and fast spreading pathogen of barley and oats. These genomes have been put through a revised pipeline that has annotated over 150 species with pathogen-host interaction data from PHI-base. There is also an updated peptide compara with a subset of 480 species chosen from across the fungal taxonomic groups and an updated pan-taxonomic compara. The previous release of EnsemblFungi (40) has been archived at eg40-fungi.ensembl.org.

Ensembl Metazoa

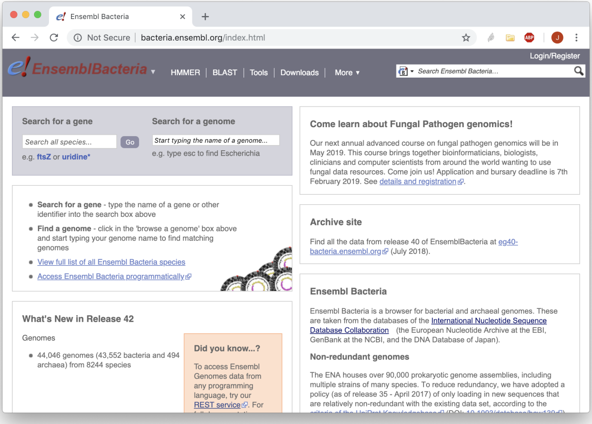
- Updated data
  - Updated BioMarts
- New data

Have a question?

Frequently Asked Questions (FAQs) are now available for all domains of Ensembl Genomes. Have a question? Check if it's been asked before! If there is a FAQ missing, contact us.

EnsemblGenomes Bacteria

http://bacteria.ensembl.org/



Search for a gene

Search all species... Go

e.g. ftsZ or uridine*

Search for a genome

Start typing the name of a genome...

e.g. type esc to find Escherichia

- **Search for a gene** - type the name of a gene or other identifier into the search box above
- **Find a genome** - click in the 'browse a genome' box above and start typing your genome name to find matching genomes
- View full list of all Ensembl Bacteria species
- Access Ensembl Bacteria programmatically

What's New in Release 42

Genomes

- 44,046 genomes (43,552 bacteria and 494 archaea) from 8244 species

Did you know...?

To access Ensembl Genomes data from any programming language, try our REST service. For

Come learn about Fungal Pathogen genomics!

Our next annual advanced course on fungal pathogen genomics will be in May 2019. This course brings together bioinformaticians, biologists, clinicians and computer scientists from around the world wanting to use fungal data resources. Come join us! Application and bursary deadline is 7th February 2019. See details and registration.

Archive site

Find all the data from release 40 of EnsemblBacteria at eg40-bacteria.ensembl.org (July 2018).

Ensembl Bacteria

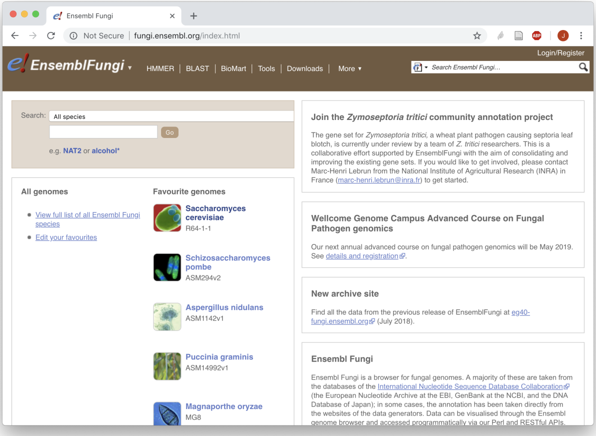
Ensembl Bacteria is a browser for bacterial and archaeal genomes. These are taken from the databases of the International Nucleotide Sequence Database Collaboration (the European Nucleotide Archive at the EBI, GenBank at the NCBI, and the DNA Database of Japan).

Non-redundant genomes

The ENA houses over 90,000 prokaryotic genome assemblies, including multiple strains of many species. To reduce redundancy, we have adopted a policy (as of release 35 - April 2017) of only loading in new sequences that are relatively non-redundant with the existing data set, according to the criteria of the UniProt Knowledgebase (DOI: 10.1093/database/bau130)

EnsemblGenomes Fungi

http://fungi.ensembl.org/


Ensembl Fungi
Not Secure
fungi.ensembl.org/index.html
Login/Register
EnsemblFungi
HMMER | BLAST | BioMart | Tools | Downloads | More
Search Ensembl Fungi…

Search: All species

Go

e.g. NAT2 or alcohol*

All genomes

- View full list of all Ensembl Fungi species
- Edit your favourites

Favourite genomes

Saccharomyces cerevisiae
R64-1-1

Schizosaccharomyces pombe
ASM294v2

Aspergillus nidulans
ASM1142v1

Puccinia graminis
ASM14992v1

Magnaporthe oryzae
MG8

Join the *Zymoseptoria tritici* community annotation project

The gene set for *Zymoseptoria tritici*, a wheat plant pathogen causing septoria leaf blotch, is currently under review by a team of *Z. tritici* researchers. This is a collaborative effort supported by EnsemblFungi with the aim of consolidating and improving the existing gene sets. If you would like to get involved, please contact Marc-Henri Lebrun from the National Institute of Agricultural Research (INRA) in France (marc-henri.lebrun@inra.fr) to get started.

Wellcome Genome Campus Advanced Course on Fungal Pathogen genomics

Our next annual advanced course on fungal pathogen genomics will be May 2019. See details and registration.

New archive site

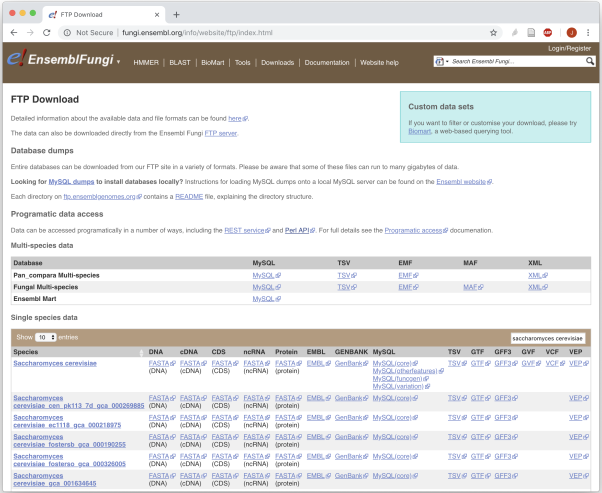
Find all the data from the previous release of EnsemblFungi at eg40-fungi.ensembl.org (July 2018).

Ensembl Fungi

Ensembl Fungi is a browser for fungal genomes. A majority of these are taken from the databases of the International Nucleotide Sequence Database Collaboration (the European Nucleotide Archive at the EBI, GenBank at the NCBI, and the DNA Database of Japan); in some cases, the annotation has been taken directly from the websites of the data generators. Data can be visualised through the Ensembl genome browser and accessed programmatically via our Perl and RESTful APIs.

EnsemblGenomes Fungi Download page

http://fungi.ensembl.org/info/website/ftp/


FTP Download
Not Secure
fungi.ensembl.org/info/website/ftp/index.html
Login/Register
EnsemblFungi
HMMER | BLAST | BioMart | Tools | Downloads | Documentation | Website help
Search Ensembl Fungi…

FTP Download

Detailed information about the available data and file formats can be found here.

The data can also be downloaded directly from the Ensembl Fungi FTP server.

Custom data sets

If you want to filter or customise your download, please try Biomart, a web-based querying tool.

Database dumps

Entire databases can be downloaded from our FTP site in a variety of formats. Please be aware that some of these files can run to many gigabytes of data.

Looking for MySQL dumps to install databases locally? Instructions for loading MySQL dumps onto a local MySQL server can be found on the Ensembl website.

Each directory on ftp.ensemblgenomes.org contains a README file, explaining the directory structure.

Programatic data access

Data can be accessed programatically in a number of ways, including the REST service and Perl API. For full details see the Programatic access documenation.

Multi-species data

Database	MySQL	TSV	EMF	MAF	XML
Pan_compara Multi-species	MySQL	TSV	EMF		XML
Fungal Multi-species	MySQL	TSV	EMF	MAF	XML
Ensembl Mart	MySQL				

Single species data

Show 10 entries

saccharomyces cerevisiae

Species	DNA	cDNA	CDS	ncRNA	Protein	EMBL	GENBANK	MySQL	TSV	GTF	GFF3	GVF	VCF	VEP
Saccharomyces cerevisiae	FASTA (DNA)	FASTA (cDNA)	FASTA (CDS)	FASTA (ncRNA)	FASTA (protein)	EMBL	GenBank	MySQL(core) MySQL(otherfeatures) MySQL(funcgen) MySQL(variation)	TSV	GTF	GFF3	GVF	VCF	VEP
Saccharomyces cerevisiae_cen_pk113_7d_gca_000269885	FASTA (DNA)	FASTA (cDNA)	FASTA (CDS)	FASTA (ncRNA)	FASTA (protein)	EMBL	GenBank	MySQL(core)	TSV	GTF	GFF3			VEP
Saccharomyces cerevisiae_ec1118_gca_000218975	FASTA (DNA)	FASTA (cDNA)	FASTA (CDS)	FASTA (ncRNA)	FASTA (protein)	EMBL	GenBank	MySQL(core)	TSV	GTF	GFF3			VEP
Saccharomyces cerevisiae_fostersb_gca_000190255	FASTA (DNA)	FASTA (cDNA)	FASTA (CDS)	FASTA (ncRNA)	FASTA (protein)	EMBL	GenBank	MySQL(core)	TSV	GTF	GFF3			VEP
Saccharomyces cerevisiae_fosterso_gca_000326005	FASTA (DNA)	FASTA (cDNA)	FASTA (CDS)	FASTA (ncRNA)	FASTA (protein)	EMBL	GenBank	MySQL(core)	TSV	GTF	GFF3			VEP
Saccharomyces cerevisiae_gca_001634645	FASTA (DNA)	FASTA (cDNA)	FASTA (CDS)	FASTA (ncRNA)	FASTA (protein)	EMBL	GenBank	MySQL(core)	TSV	GTF	GFF3			VEP

Define and create your working directory

Exercise: create a working directory named `workDir` in your home folder and go inside.

```
## Define the working directory
workDir <- "~/intro_R/explorer_un_GTF"

## Create the working directory
dir.create(workDir, recursive = TRUE, showWarnings = FALSE

## Go to the working directory
setwd(workDir)
getwd()        ## Check your current location
list.files()  ## List files (should be empty if just create
```

Downloading the GTF file

Exercise: download the GTF file in the working directory (optionally, adapt the command to load a GTF of your interest). Before downloading the file we check if it is already present in the working directory. If yes, we skip the download.

Tip: use the commands `file.exists`, `download.file`.

Downloading the GTF file: solution

```
## Define the URL of the file to download
gtf.url <- "ftp://ftp.ensemblgenomes.org/pub/bacteria/relea
## create a directory to store the file
dir.create("data", showWarnings = FALSE)
## create a local filename
destfile <- paste0("data/", basename(gtf.url))
print(destfile)
```

```
[1] "data/Escherichia_coli_str_k_12_substr_mg1655.ASM584v2.
```

```
## Download the file, but only if not yet there
if (file.exists(destfile)) {
  message("GTF annotation file already there: ", destfile)
} else {
  message("Downloading GTF annotation file")
  download.file(url = gtf.url, destfile = destfile)
```

Loading a data table in R

Commands: `read.table`, `read.delim`, `read.csv`.

R includes several types of tabular structures (matrix, data.frame, table). The most widely used is `data.frame()`, which consists in a table of values with a type (strings, integer, ..) attached to each column, and names associated to rows and columns.

The function `read.table()` enables to read a text file containing tabular data, and to store its content in a variable.

Several finctions derived from `read.table()` facilitate the loading of different formats.

- `read.delim()` for files where a particular charcater is used as column separator (by default the tab character “”̑).
- `read.csv()` for “comma-searated values”.

Loading the GTF file

Load the GTF file in a variable named featureTable.

**Tip:∗∗ command read.delim.

```
## Load GTF file in a data.frame
featureTable <- read.delim(destfile, comment.char = "#", se
                           header=FALSE, row.names = NULL)

## The GTF format has no header, but we can define it based
names(featureTable) <- c("seqname", "source", "feature", "s
                         "score", "strand", "frame", "attri
```

Exploring the content of a data table

Immediately after having loaded a data table, check its dimensions.

```
dim(featureTable) ## Dimensions of the tbale
```

```
[1] 25979     9
```

```
nrow(featureTable) ## Number of rows
```

```
[1] 25979
```

```
ncol(featureTable) ## Number of columns
```

```
[1] 9
```

Checking heads and tails

Displaying the full annotation table would not be very convenient, since it contains tens of thousands of rows.

We can display the first rows of the file with the function `head()`, and the last rows with `tail()`.

```r
## Display the 5 first rows of the feature table
head(featureTable, n = 5)

## Display the 5 last rows of the feature table
tail(featureTable, n = 5)
```

Viewing a table

If you are using the **RStudio** environment, you can display the table in a dynamic viewer pane with the function `View()`.

```
## In RStudio, display the table in a separate tab
View(featureTable)
```

The `View()` function is interactive, so it should not be used in a script because it would perturbate its execution.

Selecting columns

The last column of GTF files is particularly heavy, it contains a lof of semi-structured information.

We can select the 8 first columns and display the 5 first rows of this sub-table.

```
## Column selection + head
head(featureTable[,1:8], n=5)
```

```
     seqname source     feature start end score strand fran
1 Chromosome    ena        gene   190 255     .      +
2 Chromosome    ena  transcript   190 255     .      +
3 Chromosome    ena        exon   190 255     .      +
4 Chromosome    ena         CDS   190 252     .      +
5 Chromosome    ena start_codon   190 192     .      +
```

```
## Equivalent: selecting subsets of rows and columns
```

Feature types

Exercise: the column *feature* of the GTF indicates the feature table.

- List the feature types found in the GTF
- Count the number of features per type, and sort them by decreasing values.

Tip: commands `unique`, `table` and `sort`.

```
## List the types of features
unique(featureTable$feature)
```

```
[1] gene        transcript  exon        CDS         start_c
Levels: CDS exon gene start_codon stop_codon transcript
```

```
## Count the number of features per type
sort(table(featureTable$feature), decreasing = TRUE)
```

Décompte par valeur

The `table()` function allows to count the frequency of each value in a qualitative variable:

```r
## Count the number of features per chromosome
table(featureTable$seqname)
```

```
Chromosome
     25979
```

```r
## Count the number of features per strand
table(featureTable$strand)
```

```
    -     +
13246 12733
```

Contingency table

We can compute the number of combinations between two qualitatives variables:

```
##  Table with two vectors
table(featureTable$strand, featureTable$feature)
```

```
     CDS exon gene start_codon stop_codon transcript
  - 2129 2307 2277        2128       2128       2277
  + 2012 2257 2220        2012       2012       2220
```

```
## Same result with a 2-column data frame
table(featureTable[, c("strand", "feature")])
```

```
      feature
strand  CDS exon gene start_codon stop_codon transcript
       2129 2307 2277        2128       2128       2277
```

Computing feature lengths

- Add a column with feature lengths.

Note about feature length computation (explain why) :

$$L = \text{end} - \text{start} + 1$$

```
## Add a column to the table with genes lengths
featureTable$length <- featureTable$end - featureTable$star
```

Filtering rows based on a column content

The function `subset()` enables to select a subset of rows based on a filter applied to the content of one or several columns.

We can use it to select the subset of features corresponding to genes.

Selecting genes from the GTF table

- Select of genes from the GTF table and store them in a separate variable named genes.
- Compute summary statistics about gene lengthhs

Tip: commands `subset`, `summary`.

```
## Select subset of features having "CDS" as "feature" attr
genes <- subset(featureTable, feature == "gene")

## Print a message with the number of genes
message("Number of genes: ", nrow(genes))

## Compute basic statistics on genes lengths
summary(genes$length)
```

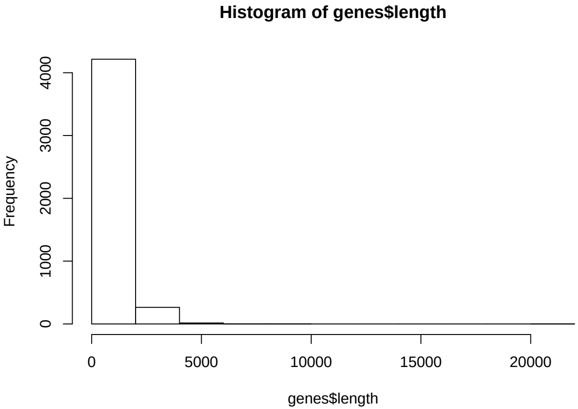
```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
   14.0   462.0   813.0   929.4  1221.0 21837.0
```

Exercices

1. Draw an histogram with gene length distribution. Choose a relevant number of breaks to display an informative histogram.
2. Draw a boxplot of gene lengths per strand. Are gene longer on the minus or plus strand?

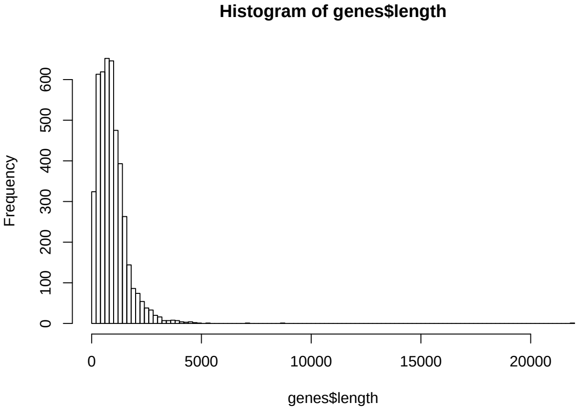
Gene length histogram

```r
hist(genes$length)
```



Setting a relevant number of breaks

```
## Take more or less 100 bins
hist(genes$length, breaks = 100)
```



Gene length distribution – improving the output

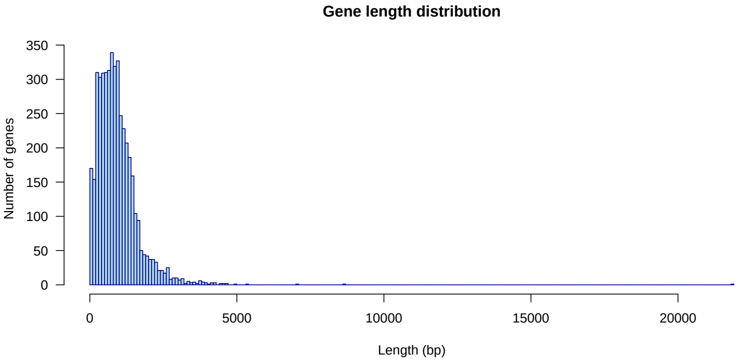
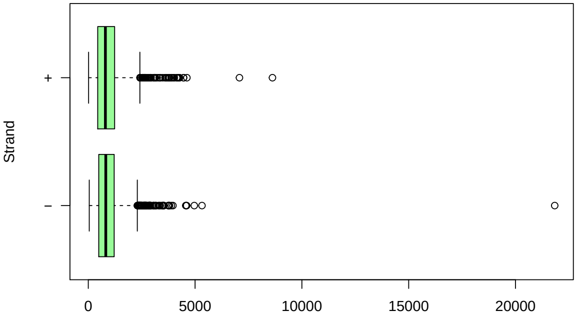


Figure 1: Distribution of gene lengths for E. Coli.

Gene length box plot

Other types of plots allow to explore the distribution of some data. In particular, boxplots display the median, the first and third quartiles and outlier values.

```
boxplot(length ~ strand, data = genes, col="palegreen", hor
         las=1, xlab="Gene length", ylab="Strand")
```


Strand