BgeeDB: an R package for retrieval of curated expression datasets and for gene list expression localization enrichment tests

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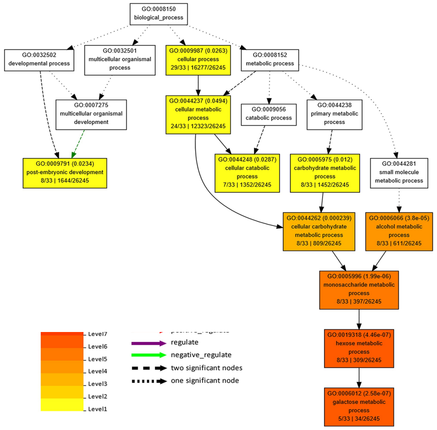
How to characterize gene lists?

- Functional categories enriched among these genes
  - Gene Ontology enrichment test
  - GSEA
  - Pathways analysis
  - …



Gene Ontology enrichment test

- For each functional category:

	Gene list	Other genes
Annotated	n_1	n_3
Not annotated	n_2	n_4

- Fisher / Hypergeometric test
- Bioconductor: topGO, GOstats, goseq,…

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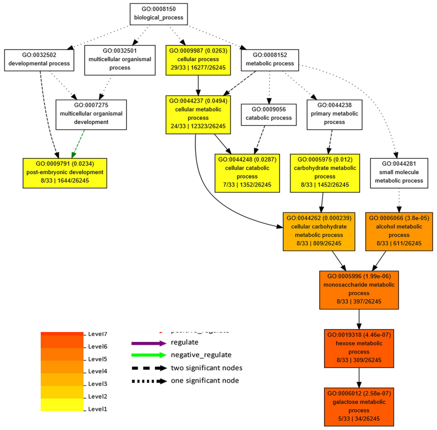
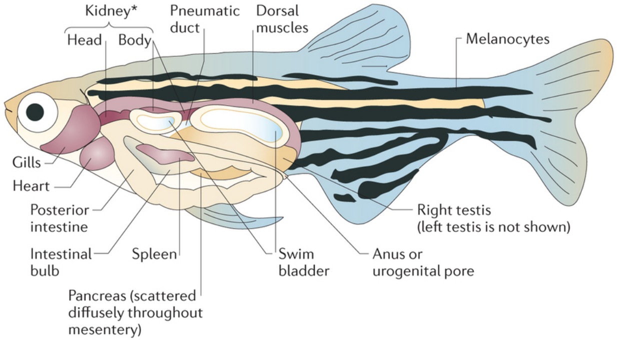
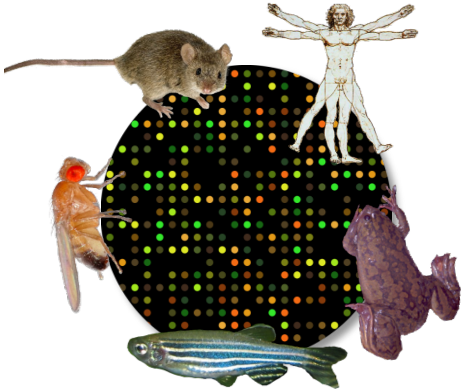


- Tissues enriched for expression of these genes?
  - Gene expression atlases
  - TopAnat





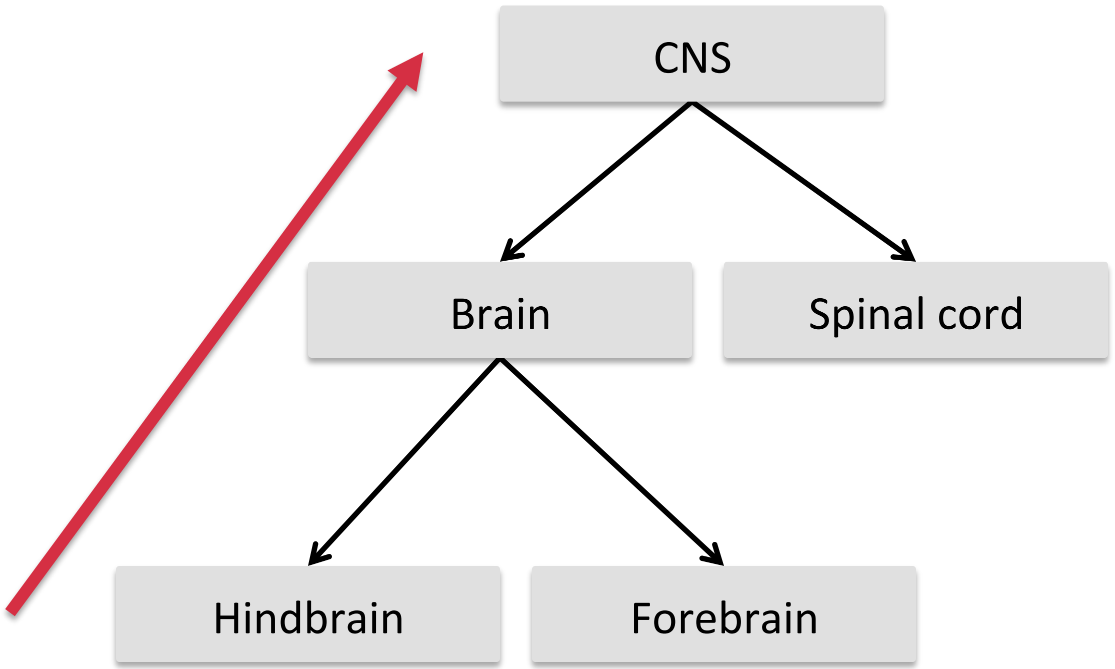
Bgee

http://bgee.org

Quick reminder:

- Only “normal” samples: no tumors, no mutants, no treatments
- RNA-seq, microarray, EST, in situ hybridization data from 17 animal species
- Manual mapping to Uberon ontology of anatomy and development

Uberon anatomical ontology



Bgee

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Quick reminder:

- Only “normal” samples: no tumors, no mutants, no treatments
- RNA-seq, microarray, EST, in situ hybridization data from 17 animal species
- Manual mapping to Uberon ontology of anatomy and development
- Data reprocessed as presence/absence calls

Gene Ontology enrichment test

- For each functional category:

	Gene list	Other genes
Annotated	n_1	n_3
Not annotated	n_2	n_4

- Fisher / Hypergeometric test

TopAnat test

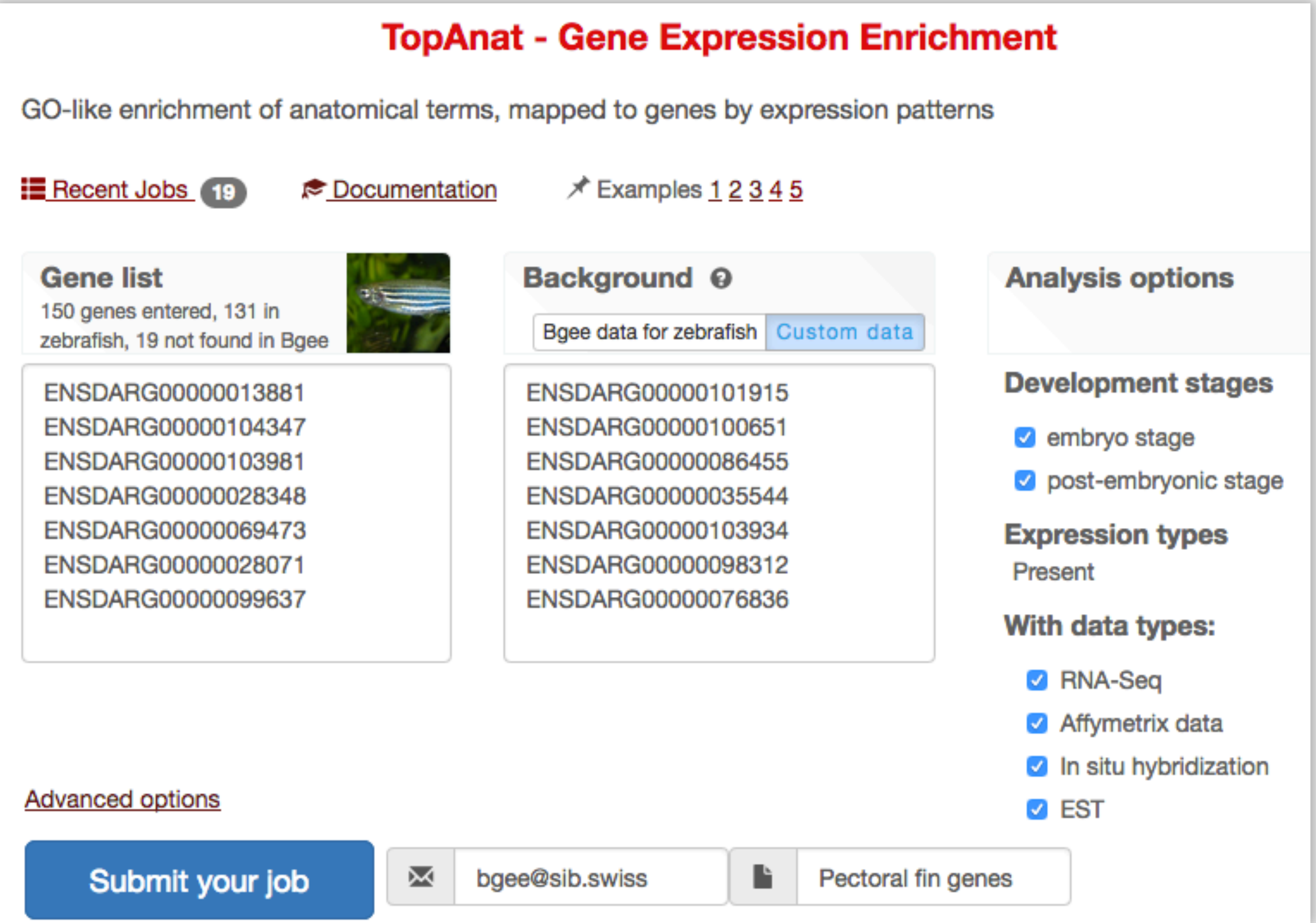
- For each anatomical structure:

	Gene list	Other genes
Expressed	n_1	n_3
Not expressed	n_2	n_4

- Fisher / Hypergeometric test

Implementation

- Based on topGO package
- Extension of *topGOdata* class
  - Accommodate Uberon Ontology
  - Use custom gene mapping



TopAnat - Gene Expression Enrichment

GO-like enrichment of anatomical terms, mapped to genes by expression patterns

Gene list

150 genes entered, 131 in zebrafish, 19 not found in Bgee

```
ENSDARG00000013881
ENSDARG00000104347
ENSDARG00000103981
ENSDARG00000028348
ENSDARG00000069473
ENSDARG00000028071
ENSDARG00000099637
```

Background

```
ENSDARG00000101915
ENSDARG00000100651
ENSDARG00000086455
ENSDARG00000035544
ENSDARG00000103934
ENSDARG00000098312
ENSDARG00000076836
```

Analysis options

Development stages

☑ embryo stage

☑ post-embryonic stage

Expression types

Present

With data types:

☑ RNA-Seq

☑ Affymetrix data

☑ In situ hybridization

☑ EST

✉ bgee@sib.swiss

Pectoral fin genes

http://bgee.org/?page=top_anat

BgeeDB

- http://www.bioconductor.org/packages/BgeeDB/

- Komljenovic*, Roux*, Robinson-Rechavi and Bastian (2016) BgeeDB, an R package for retrieval of curated expression datasets and for gene list expression localization enrichment tests. *F1000Research*, 5:2748

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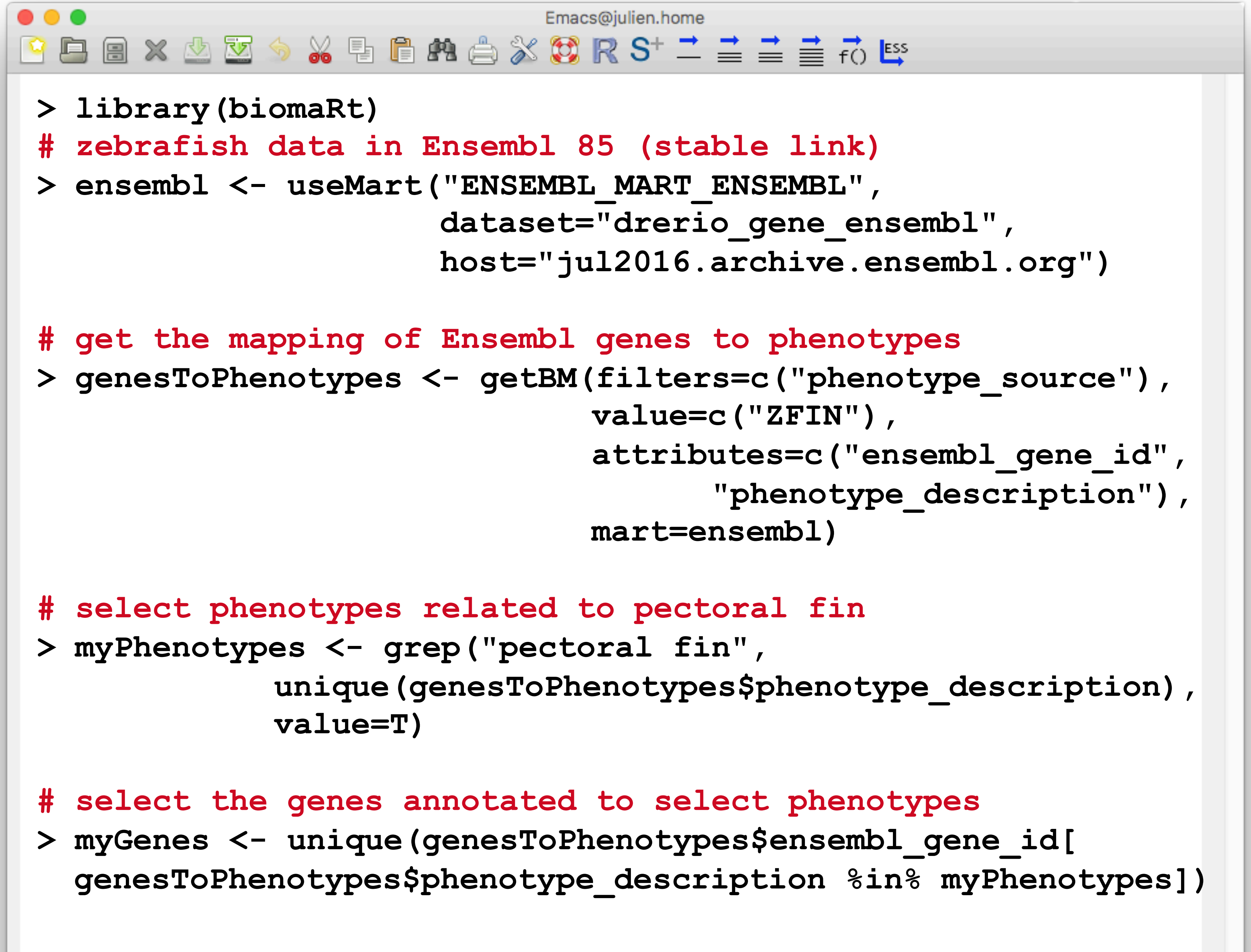
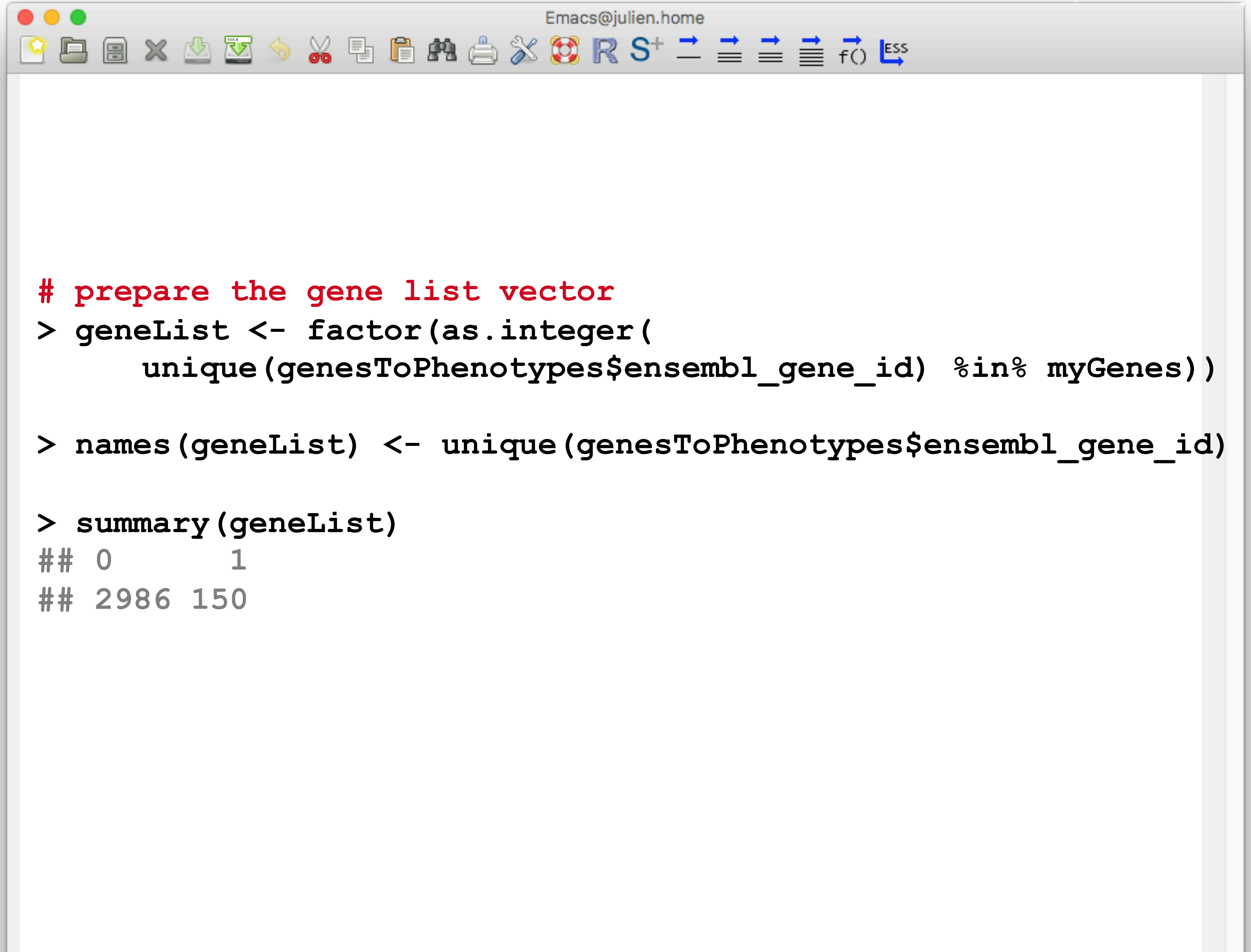
BgeeDB use case

TopAnat test:

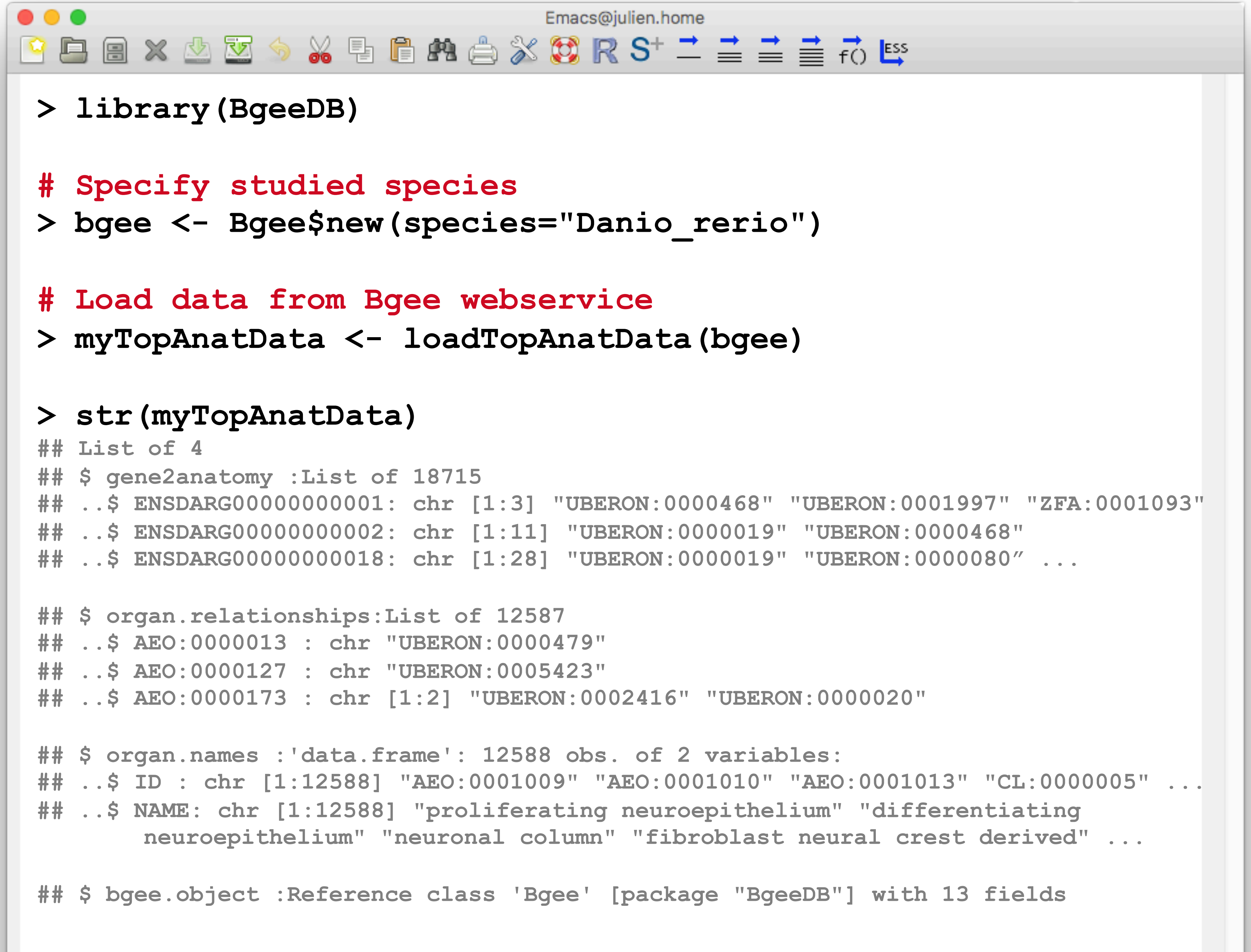
- **Foreground**: 150 Ensembl genes with *phenotype related to pectoral fin*, retrieved from ZFIN database
- **Background**: 3,136 Ensembl genes with an annotated phenotype in ZFIN



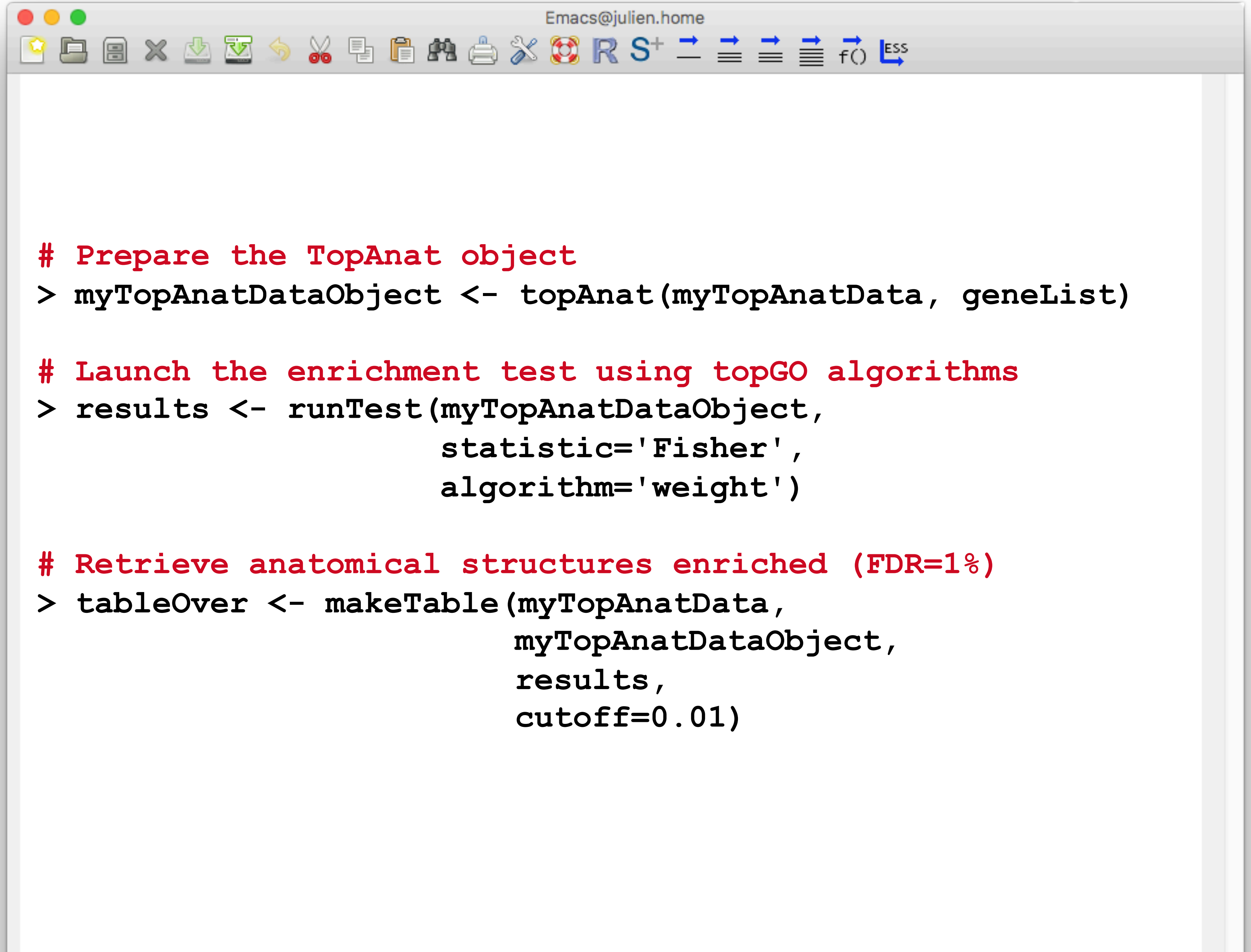
```
> library(biomaRt)
# zebrafish data in Ensembl 85 (stable link)
> ensembl <- useMart("ENSEMBL_MART_ENSEMBL",
                     dataset="drerio_gene_ensembl",
                     host="jul2016.archive.ensembl.org")

# get the mapping of Ensembl genes to phenotypes
> genesToPhenotypes <- getBM(filters=c("phenotype_source"),
                             value=c("ZFIN"),
                             attributes=c("ensembl_gene_id",
                                          "phenotype_description"),
                             mart=ensembl)

# select phenotypes related to pectoral fin
> myPhenotypes <- grep("pectoral fin",
                  unique(genesToPhenotypes$phenotype_description),
                  value=T)

# select the genes annotated to select phenotypes
> myGenes <- unique(genesToPhenotypes$ensembl_gene_id[
  genesToPhenotypes$phenotype_description %in% myPhenotypes])
```



```
# prepare the gene list vector
> geneList <- factor(as.integer(
      unique(genesToPhenotypes$ensembl_gene_id) %in% myGenes))

> names(geneList) <- unique(genesToPhenotypes$ensembl_gene_id)

> summary(geneList)
## 0       1
## 2986 150
```



```
> library(BgeeDB)

# Specify studied species
> bgee <- Bgee$new(species="Danio_rerio")

# Load data from Bgee webservice
> myTopAnatData <- loadTopAnatData(bgee)

> str(myTopAnatData)
## List of 4
## $ gene2anatomy :List of 18715
## ..$ ENSDARG00000000001: chr [1:3] "UBERON:0000468" "UBERON:0001997" "ZFA:0001093"
## ..$ ENSDARG00000000002: chr [1:11] "UBERON:0000019" "UBERON:0000468"
## ..$ ENSDARG00000000018: chr [1:28] "UBERON:0000019" "UBERON:0000080” ...

## $ organ.relationships:List of 12587
## ..$ AEO:0000013 : chr "UBERON:0000479"
## ..$ AEO:0000127 : chr "UBERON:0005423"
## ..$ AEO:0000173 : chr [1:2] "UBERON:0002416" "UBERON:0000020"

## $ organ.names :'data.frame': 12588 obs. of 2 variables:
## ..$ ID : chr [1:12588] "AEO:0001009" "AEO:0001010" "AEO:0001013" "CL:0000005" ...
## ..$ NAME: chr [1:12588] "proliferating neuroepithelium" "differentiating
        neuroepithelium" "neuronal column" "fibroblast neural crest derived" ...

## $ bgee.object :Reference class 'Bgee' [package "BgeeDB"] with 13 fields
```



```
# Prepare the TopAnat object
> myTopAnatDataObject <- topAnat(myTopAnatData, geneList)

# Launch the enrichment test using topGO algorithms
> results <- runTest(myTopAnatDataObject,
                     statistic='Fisher',
                     algorithm='weight')

# Retrieve anatomical structures enriched (FDR=1%)
> tableOver <- makeTable(myTopAnatData,
                         myTopAnatDataObject,
                         results,
                         cutoff=0.01)
```

Organ name	Enrichment fold	P-value	FDR
pectoral appendage field	12.7	4.00E-10	7.14E-08
pectoral appendage cartilage tissue	10.7	2.41E-08	3.58E-06
ceratohyal cartilage	7.6	4.76E-08	6.06E-06
median fin fold	7.1	7.17E-12	2.13E-09
fin bone	6.5	4.29E-06	0.000478091
bone of free limb or fin	6.1	7.95E-05	0.004168941
irregular bone	6.0	8.17E-06	0.000659745
dorsal hyoid arch skeleton	5.9	0.000107699	0.004817668
paired limb/fin bud	5.7	1.62E-22	1.45E-19
endochondral bone	5.4	7.11E-06	0.000633841
dermal bone	4.8	8.89E-06	0.000659745
mouth	4.4	0.000101104	0.004817668
pharyngeal epithelium	4.0	4.89E-06	0.000483901
hypoblast (generic)	3.6	1.12E-05	0.000713807
pectoral fin	3.4	1.04E-18	4.62E-16
germ ring	3.2	3.95E-05	0.002344194
skin epidermis	3.0	6.22E-05	0.003463408
ear vesicle	2.7	3.14E-10	6.98E-08
endoderm	2.7	0.000108141	0.004817668
pharyngeal arch	2.4	1.08E-05	0.000713807
cranium	2.2	0.000129452	0.005492447
immature eye	1.8	0.000190297	0.007707016

Conclusions

- TopAnat is a new way to make biological sense of gene lists
- Gene annotation entirely experimental!
- BgeeDB is a versatile way of running TopAnat analyses

Add BgeeDB to your toolbox!

Thanks!

- http://www.bioconductor.org/packages/BgeeDB/
- Komljenovic*, Roux*, Robinson-Rechavi and Bastian (2016) BgeeDB, an R package for retrieval of curated expression datasets and for gene list expression localization enrichment tests. *F1000Research*, 5:2748

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