

An oxybenzone exposition study on gilt-head bream

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Package

FELLA 1.22.0

Contents

1	Introduction	2
1.1	Building the database.	2
1.2	Note on reproducibility	3
2	Enrichment analysis on liver tissue.	3
2.1	Defining the input and running the enrichment	3
2.2	Examining the pathways	6
2.3	Examining the metabolites	6
3	Enrichment analysis on plasma.	7
3.1	Defining the input and running the enrichment	7
3.2	Examining the pathways	10
3.3	Examining the metabolites	10
4	Conclusions	11
5	Reproducibility	11
	References	13

1 Introduction

This vignette contains a case study of the effects of environmental contamination on gilt-head bream (*Sparus aurata*) (Ziarrusta et al. 2018). Fish were exposed over 14 days to *oxybenzone* and changes were sought in their brain, liver and plasma using untargeted metabolomics. Samples were processed using Ultra-performance liquid chromatography mass-spectrometry (UHPLC-qOrbitrap MS) in positive and negative modes with both C18 and HILIC separation.

The mortality of exposed fish was not altered, as well as the brain-related metabolites. However, liver and plasma showed perturbations, proving that adverse effects beyond the well-studied hormonal activity were present.

The enrichment procedure implemented in `FELLA` (Picart-Armada et al. 2017) was used in the study for a deeper understanding of the dysregulated metabolites in both tissues.

1.1 Building the database

At the time of publication, the KEGG database (Kanehisa et al. 2016) –upon which `FELLA` is based– did not have pathway annotations for the *Sparus aurata* organism. It is common, however, to use the zebrafish (*Danio rerio*) pathways as a good approximation. KEGG provides pathway annotations for it under the organismal code `dre`, which will be used to build the `FELLA.DATA` object.

```
library(FELLA)

library(igraph)
library(magrittr)

set.seed(1)
# Filter the dre01100 overview pathway, as in the article
graph <- buildGraphFromKEGGREST(
  organism = "dre",
  filter.path = c("01100"))

tmpdir <- paste0(tempdir(), "/my_database")
# Make sure the database does not exist from a former vignette build
# Otherwise the vignette will rise an error
# because FELLA will not overwrite an existing database
unlink(tmpdir, recursive = TRUE)
buildDataFromGraph(
  keggdata.graph = graph,
  databaseDir = tmpdir,
  internalDir = FALSE,
  matrices = "none",
  normality = "diffusion",
  niter = 100)
```

We load the `FELLA.DATA` object to run both analyses:

```
fella.data <- loadKEGGdata(
  databaseDir = tmpdir,
  internalDir = FALSE,
```

An oxybenzone exposition study on gilt-head bream

```
loadMatrix = "none"  
)
```

Given the 11-month temporal gap between the study and this vignette, small changes to the amount of nodes in each category are expected (see section 2.4 *Data handling and statistical analyses* from the study). Please see the [Note on reproducibility](#) to understand why.

```
fella.data  
## General data:  
## - KEGG graph:  
##   * Nodes: 11588  
##   * Edges: 34592  
##   * Density: 0.0002576298  
##   * Categories:  
##     + pathway [170]  
##     + module [190]  
##     + enzyme [1064]  
##     + reaction [5864]  
##     + compound [4300]  
##   * Size: 5.7 Mb  
## - KEGG names are ready.  
## -----  
## Hypergeometric test:  
## - Matrix not loaded.  
## -----  
## Heat diffusion:  
## - Matrix not loaded.  
## - RowSums are ready.  
## -----  
## PageRank:  
## - Matrix not loaded.  
## - RowSums not loaded.
```

1.2 Note on reproducibility

We want to emphasise that each time this vignette is built, `FELLA` constructs its `FELLA.DAT` object using the most recent version of the KEGG database. KEGG is frequently updated and therefore small changes can take place in the knowledge graph between different releases. The discussion on our findings was written at the date specified in the vignette header and using the KEGG release in the [Reproducibility](#) section.

2 Enrichment analysis on liver tissue

2.1 Defining the input and running the enrichment

Table 1 from the main body in (Ziarrusta et al. 2018) contains 5 KEGG identifiers associated to metabolic changes in liver tissue and 12 in plasma. Our first enrichment analysis with `FELLA` will be based on the liver-derived metabolites. Also note that we use the faster `approx =`

An oxybenzone exposition study on gilt-head bream

"normality" approach, whereas the original article uses `approx = "simulation"` with `niter = 15000`. This is not only intended to keep the building time of this vignette as low as possible, but also to demonstrate that the findings using both statistical approaches are consistent.

```
cpd.liver <- c(
  "C12623",
  "C01179",
  "C05350",
  "C05598",
  "C01586"
)

analysis.liver <- enrich(
  compounds = cpd.liver,
  data = fella.data,
  method = "diffusion",
  approx = "normality")
## No background compounds specified. Default background will be used.
## Warning in defineCompounds(compounds = compounds, compoundsBackground =
## compoundsBackground, : Some compounds were introduced as affected but they do
## not belong to the background. These compounds will be excluded from the
## analysis. Use 'getExcluded' to see them.
## Running diffusion...
## Computing p-scores through the specified distribution.
## Done.
```

All the metabolites are successfully mapped:

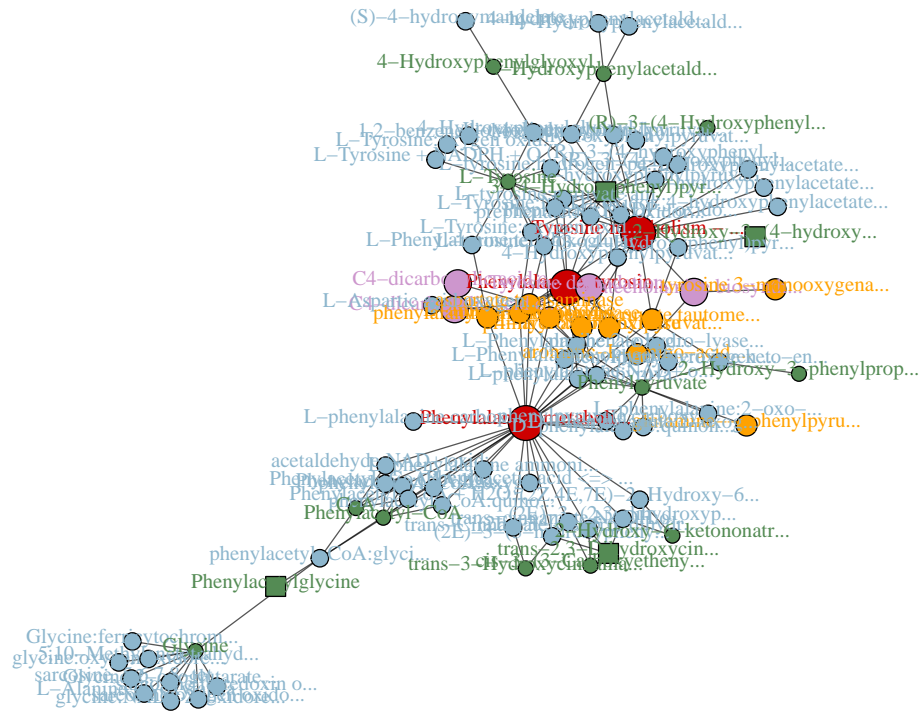
```
analysis.liver %>%
  getInput %>%
  getName(data = fella.data)
## $C01179
## [1] "3-(4-Hydroxyphenyl)pyruvate" "4-Hydroxyphenylpyruvate"
## [3] "p-Hydroxyphenylpyruvic acid"
##
## $C05350
## [1] "2-Hydroxy-3-(4-hydroxyphenyl)propenoate"
## [2] "4-Hydroxy-enol-phenylpyruvate"
##
## $C05598
## [1] "Phenylacetyl glycine"
##
## $C12623
## [1] "trans-2,3-Dihydroxycinnamate"
## [2] "(2E)-3-(2,3-Dihydroxyphenyl)prop-2-enoate"
```

Below is a plot of the reported sub-network using the default parameters. The five metabolites are present and lie within the same connected component.

```
plot(
  analysis.liver,
  method = "diffusion",
  data = fella.data,
```

An oxybenzone exposition study on gilt-head bream

```
nlimit = 250,  
plotLegend = FALSE)
```



We will examine the `igraph` object with the reported sub-network and some of its reported entities in tabular format:

```
g.liver <- generateResultsGraph(
  object = analysis.liver,
  data = fella.data,
  method = "diffusion")

tab.liver <- generateResultsTable(
  object = analysis.liver,
  data = fella.data,
  method = "diffusion")

## Writing diffusion results...
## Done.
```

The reported sub-network contains around 100 nodes and can be manually inquired:

```
g.liver
## IGRAPH b15aa18 UNW- 96 153 --
## + attr: organism (g/c), name (v/c), com (v/n), NAME (v/x), entrez
## | (v/x), label (v/c), input (v/l), weight (e/n)
## + edges from b15aa18 (vertex names):
## [1] dre00350--M00042      dre00350--M00044      dre00360--1.13.11.27
## [4] M00044    --1.13.11.27 dre00360--1.14.16.1  dre00400--1.14.16.1
## [7] M00042    --1.14.16.2 dre00360--1.4.3.2    dre00400--1.4.3.2
## [10] M00044    --1.4.3.2    dre00350--1.4.3.21  dre00360--1.4.3.21
```

An oxybenzone exposition study on gilt-head bream

```
## [13] dre00350--2.6.1.1    dre00360--2.6.1.1    dre00400--2.6.1.1
## [16] M00170  --2.6.1.1    M00171  --2.6.1.1    dre00360--2.6.1.5
## [19] dre00400--2.6.1.5    M00044  --2.6.1.5    dre00360--4.1.1.28
## + ... omitted several edges
```

2.2 Examining the pathways

Figure 2 from the original study frames the five metabolites in the input around *Phenylalanine metabolism*. We can verify that FELLA finds such pathway and two closely related suggestions: *Tyrosine metabolism* and *Phenylalanine, tyrosine and tryptophan biosynthesis*.

```
path.fig2 <- "dre00360" # Phenylalanine metabolism
path.fig2 %in% V(g.liver)$name
## [1] TRUE
```

These are the reported pathways:

```
tab.liver[tab.liver$Entry.type == "pathway", ]
##      KEGG.id Entry.type                                     KEGG.name
## 1 dre00350    pathway    Tyrosine metabolism - Danio rerio (zebrafish)
## 2 dre00360    pathway    Phenylalanine metabolism - Danio rerio (zebra...
## 3 dre00400    pathway    Phenylalanine, tyrosine and tryptophan biosyn...
##      p.score
## 1 0.000001000
## 2 0.000001000
## 3 0.005302101
```

2.3 Examining the metabolites

Figure 2 also gathers two types of metabolites: metabolites in the input (inside shaded frames) and other contextual metabolites (no frames) that link the input metabolites.

First of all, we can check that all the input metabolites appear in the suggested sub-network. While it's expected that most of the input metabolites appear as relevant, it is an important property of our method, in order to elaborate a sensible biological justification of the experimental differences.

```
cpd.liver %in% V(g.liver)$name
## [1] TRUE TRUE TRUE TRUE FALSE
```

On the other hand, one of the two contextual metabolites is also suggested by FELLA, proving its usefulness to fill the gaps between the input metabolites.

```
cpd.fig2 <- c(
  "C00079", # Phenylalanine
  "C00082" # Tyrosine
)
cpd.fig2 %in% V(g.liver)$name
## [1] FALSE TRUE
```

3 Enrichment analysis on plasma

3.1 Defining the input and running the enrichment

As shown in section [Defining the input and running the enrichment](#), 12 KEGG identifiers (one ID is repeated) are related to the experimental changes observed in plasma, which are the starting point of the enrichment:

```
cpd.plasma <- c(
  "C16323",
  "C00740",
  "C08323",
  "C00623",
  "C00093",
  "C06429",
  "C16533",
  "C00740",
  "C06426",
  "C06427",
  "C07289",
  "C01879"
) %>% unique

analysis.plasma <- enrich(
  compounds = cpd.plasma,
  data = fella.data,
  method = "diffusion",
  approx = "normality")
## No background compounds specified. Default background will be used.
## Running diffusion...
## Computing p-scores through the specified distribution.
## Done.
```

The totality of the 11 unique metabolites map to the FELLA.DATA object:

```
analysis.plasma %>%
  getInput %>%
  getName(data = fella.data)
## $C16323
## [1] "3,6-Nonadienal"
##
## $C00740
## [1] "D-Serine"
##
## $C08323
## [1] "(15Z)-Tetracosenoic acid" "Nervonic acid"
## [3] "(Z)-15-Tetracosenoic acid"
##
## $C00623
## [1] "sn-Glycerol 1-phosphate" "sn-Gro-1-P"
## [3] "L-Glycerol 1-phosphate"
##
```

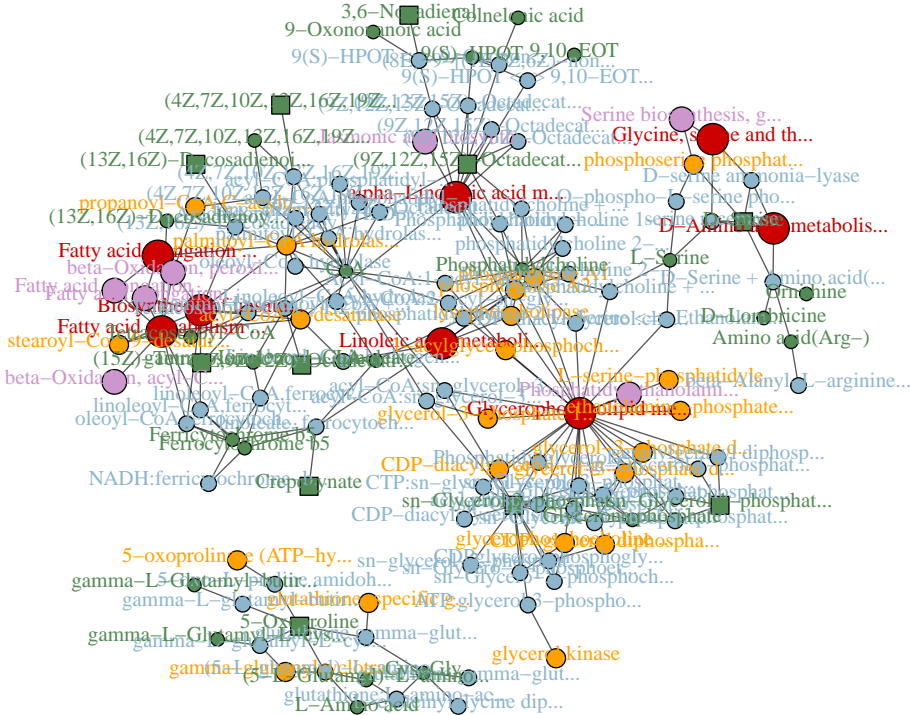
An oxybenzone exposition study on gilt-head bream

```
## $C00093
## [1] "sn-Glycerol 3-phosphate" "Glycerophosphoric acid"
## [3] "D-Glycerol 1-phosphate"
##
## $C06429
## [1] "(4Z,7Z,10Z,13Z,16Z,19Z)-Docosahexaenoic acid"
## [2] "4,7,10,13,16,19-Docosahexaenoic acid"
## [3] "Docosahexaenoic acid"
## [4] "Docosahexaenoate"
## [5] "4Z,7Z,10Z,13Z,16Z,19Z-Docosahexaenoic acid"
## [6] "(4Z,7Z,10Z,13Z,16Z,19Z)-Docosa-4,7,10,13,16,19-hexaenoic acid"
##
## $C16533
## [1] "(13Z,16Z)-Docosadienoic acid" "(13Z,16Z)-Docosa-13,16-dienoic acid"
## [3] "13Z,16Z-Docosadienoic acid"
##
## $C06426
## [1] "(6Z,9Z,12Z)-Octadecatrienoic acid" "6,9,12-Octadecatrienoic acid"
## [3] "gamma-Linolenic acid" "Gamolenic acid"
##
## $C06427
## [1] "(9Z,12Z,15Z)-Octadecatrienoic acid" "alpha-Linolenic acid"
## [3] "9,12,15-Octadecatrienoic acid" "Linolenate"
## [5] "alpha-Linolenate"
##
## $C07289
## [1] "Crepenynate" "(9Z)-Octadec-9-en-12-ynoate"
## [3] "(Z)-9-Octadecen-12-ynoic acid" "Crepenynic acid"
##
## $C01879
## [1] "5-Oxoproline" "Pidolic acid"
## [3] "Pyroglutamic acid" "5-Pyrrolidone-2-carboxylic acid"
## [5] "Pyroglutamate" "5-Oxo-L-proline"
## [7] "L-Pyroglutamic acid" "L-5-Pyrrolidone-2-carboxylic acid"
```

Again, the reported sub-network consists of a large connected component encompassing most input metabolites:

```
plot(
  analysis.plasma,
  method = "diffusion",
  data = fella.data,
  nlimit = 250,
  plotLegend = FALSE)
```


An oxybenzone exposition study on gilt-head bream



We will export the results as a network and as a table:

```
g.plasma <- generateResultsGraph(
  object = analysis.plasma,
  data = fella.data,
  method = "diffusion")

tab.plasma <- generateResultsTable(
  object = analysis.plasma,
  data = fella.data,
  method = "diffusion")

## Writing diffusion results...
## Done.
```

The reported sub-network is a bit larger than the one from liver, containing roughly 120 nodes:

```
g.plasma
## IGRAPH 0204ea0 UNW- 139 226 --
## + attr: organism (g/c), name (v/c), com (v/n), NAME (v/x), entrez
## | (v/x), label (v/c), input (v/l), weight (e/n)
## + edges from 0204ea0 (vertex names):
## [1] dre00260--M00020      dre00062--M00085      dre01212--M00085
## [4] dre01212--M00086      dre00564--M00093      dre00592--M00113
## [7] dre00062--M00415      dre01040--M00415      dre01212--M00415
## [10] dre01040--M00861      dre01212--M00861      dre00564--1.1.1.8
## [13] dre00564--1.1.5.3      dre01040--1.14.19.1   dre01212--1.14.19.1
## [16] dre00592--1.14.19.3   dre01040--1.14.19.3   dre01212--1.14.19.3
## [19] dre00564--2.3.1.15   M00861 --2.3.1.176   dre00564--2.3.1.23
## + ... omitted several edges
```

3.2 Examining the pathways

Figure 3 from the original study is a holistic view of the affected metabolites found in plasma, based on literature and on an analysis with FELLA. The 11 metabolites are depicted within their core metabolic pathways. We will check whether FELLA is able to highlight them, by first showing the reported metabolic pathways:

```
tab.plasma[tab.plasma$Entry.type == "pathway", ]
##      KEGG.id Entry.type                                     KEGG.name
## 1 dre00062    pathway Fatty acid elongation - Danio rerio (zebrafis...
## 2 dre00260    pathway Glycine, serine and threonine metabolism - Da...
## 3 dre00470    pathway D-Amino acid metabolism - Danio rerio (zebraf...
## 4 dre00564    pathway Glycerophospholipid metabolism - Danio rerio ...
## 5 dre00591    pathway Linoleic acid metabolism - Danio rerio (zebra...
## 6 dre00592    pathway alpha-Linolenic acid metabolism - Danio rerio...
## 7 dre01040    pathway Biosynthesis of unsaturated fatty acids - Dan...
## 8 dre01212    pathway Fatty acid metabolism - Danio rerio (zebrafis...
##      p.score
## 1 1.000000e-06
## 2 3.832060e-03
## 3 6.455608e-03
## 4 3.688055e-06
## 5 2.395330e-02
## 6 1.000000e-06
## 7 1.000000e-06
## 8 7.450344e-06
```

And then comparing against the ones in Figure 3:

```
path.fig3 <- c(
  "dre00591", # Linoleic acid metabolism
  "dre01040", # Biosynthesis of unsaturated fatty acids
  "dre00592", # alpha-Linolenic acid metabolism
  "dre00564", # Glycerophospholipid metabolism
  "dre00480", # Glutathione metabolism
  "dre00260" # Glycine, serine and threonine metabolism
)
path.fig3 %in% V(g.plasma)$name
## [1] TRUE TRUE TRUE TRUE FALSE TRUE
```

All of them but *Glutathione metabolism* are recovered, showing how FELLA can help gaining perspective on the input metabolites.

3.3 Examining the metabolites

As in the [analogous section for liver](#), we will quantify how many input metabolites, drawn within a shaded frame in Figure 3, are reported in the sub-network:

```
cpd.plasma %in% V(g.plasma)$name
## [1] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
```

From the 11 highlighted metabolites, only one is not reported by FELLA: *5-Oxo-L-proline*.

An oxybenzone exposition study on gilt-head bream

Conversely, two out of the three contextual metabolites from the same figure are reported:

```
cpd.fig3 <- c(
  "C01595", # Linoleic acid
  "C00157", # Phosphatidylcholine
  "C00037" # Glycine
)
cpd.fig3 %in% V(g.plasma)$name
## [1] TRUE TRUE FALSE
```

As *Figure 3* shows, the addition of *linoleic acid* and *phosphatidylcholine*, backed up by FELLA, helps connecting almost all the metabolites found in blood.

FELLA misses *glycine* and, in fact, stays consistent with the pathway (*Glutathione metabolism*) and the input metabolite (*5-Oxo-L-proline*) that it left out from *Figure 3*. The fact that FELLA does not suggest such pathway seems to happen at several molecular levels and therefore none of its metabolites are pinpointed.

Even if the glutathione pathway was not reported, FELLA can greatly ease the creation of elaborated contextual figures, such as *Figure 3*, by suggesting the intermediate metabolites and the metabolic pathways that link the input compounds.

4 Conclusions

In this vignette, we apply FELLA to an untargeted metabolic study of gilt-head bream exposed to an environmental contaminant (oxybenzone). This study is an example of how FELLA can be useful for (1) organisms not limited to *Homo sapiens*, and (2) conditions not limited to a specific disease.

On one hand, FELLA helps creating complex contextual interpretations of the data, such as the comprehensive *Figure 3* from the original article (Ziarrusta et al. 2018). This material would be challenging to build through regular over-representation analysis of the input metabolites. On the other hand, metabolites and pathways suggested by FELLA were also mentioned in the literature and supported the main findings in the study. In particular, it helped identify key processes such as *phenylalanine metabolism*, *alpha-linoleic acid metabolism* and *serine metabolism*, which ultimately pointed to alterations in *oxidative stress*.

5 Reproducibility

This is the result of running `sessionInfo()`

```
sessionInfo()
## R version 4.3.1 Patched (2023-06-17 r84564)
## Platform: x86_64-apple-darwin20 (64-bit)
## Running under: macOS Monterey 12.6.5
##
## Matrix products: default
## BLAS: /Library/Frameworks/R.framework/Versions/4.3-x86_64/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.3-x86_64/Resources/lib/libRlapack.dylib; LAPACK version 3.11.0
##
## locale:
## [1] C/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
```

An oxybenzone exposition study on gilt-head bream

```
##
## time zone: America/New_York
## tzcode source: internal
##
## attached base packages:
## [1] stats4      stats      graphics  grDevices  utils      datasets  methods
## [8] base
##
## other attached packages:
## [1] magrittr_2.0.3      igraph_1.5.1      KEGGREST_1.42.0
## [4] org.Mm.eg.db_3.18.0 AnnotationDbi_1.64.0 IRanges_2.36.0
## [7] S4Vectors_0.40.0    Biobase_2.62.0     BiocGenerics_0.48.0
## [10] FELLA_1.22.0        BiocStyle_2.30.0
##
## loaded via a namespace (and not attached):
## [1] sass_0.4.7          bitops_1.0-7       RSQLite_2.3.1
## [4] lattice_0.22-5      digest_0.6.33      evaluate_0.22
## [7] grid_4.3.1          bookdown_0.36      fastmap_1.1.1
## [10] blob_1.2.4          plyr_1.8.9         jsonlite_1.8.7
## [13] Matrix_1.6-1.1      GenomeInfoDb_1.38.0 DBI_1.1.3
## [16] tinytex_0.48        BiocManager_1.30.22 httr_1.4.7
## [19] Biostrings_2.70.0   jquerylib_0.1.4     cli_3.6.1
## [22] rlang_1.1.1         crayon_1.5.2       XVector_0.42.0
## [25] bit64_4.0.5         cachem_1.0.8       yaml_2.3.7
## [28] tools_4.3.1         memoise_2.0.1      GenomeInfoDbData_1.2.11
## [31] curl_5.1.0          vctrs_0.6.4        R6_2.5.1
## [34] png_0.1-8           magick_2.8.1       zlibbioc_1.48.0
## [37] bit_4.0.5           pkgconfig_2.0.3    bslib_0.5.1
## [40] Rcpp_1.0.11         xfun_0.40          knitr_1.44
## [43] htmltools_0.5.6.1   rmarkdown_2.25     compiler_4.3.1
## [46] RCurl_1.98-1.12
```

KEGG version:

```
cat(getInfo(fella.data))
## T01004      Danio rerio (zebrafish) KEGG Genes Database
## dre         Release 108.0+/10-24, Oct 23
##             Kanehisa Laboratories
##             27,003 entries
##
## linked db   pathway
##             brite
##             module
##             ko
##             genome
##             enzyme
##             ncbi-geneid
##             ncbi-proteinid
##             uniprot
```

Date of generation:

An oxybenzone exposition study on gilt-head bream

```
date()  
## [1] "Tue Oct 24 17:19:22 2023"
```

Image of the workspace (for submission):

```
tempfile(pattern = "vignette_dre_", fileext = ".RData") %T>%  
  message("Saving workspace to ", .) %>%  
  save.image(compress = "xz")  
## Saving workspace to /tmp/Rtmp2STcsz/vignette_dre_f56e39461fc4.RData
```

References

- Kanehisa, Minoru, Miho Furumichi, Mao Tanabe, Yoko Sato, and Kanae Morishima. 2016. "KEGG: New Perspectives on Genomes, Pathways, Diseases and Drugs." *Nucleic Acids Research* 45 (D1): D353–D361.
- Picart-Armada, Sergio, Francesc Fernández-Albert, Maria Vinaixa, Miguel A Rodríguez, Suvi Aivio, Travis H Stracker, Oscar Yanes, and Alexandre Perera-Lluna. 2017. "Null Diffusion-Based Enrichment for Metabolomics Data." *PloS One* 12 (12): e0189012.
- Ziarrusta, Haizea, Leire Mijangos, Sergio Picart-Armada, Mireia Irazola, Alexandre Perera-Lluna, Aresatz Usobiaga, Ailette Prieto, Nestor Etxebarria, Maitane Olivares, and Olatz Zuloaga. 2018. "Non-Targeted Metabolomics Reveals Alterations in Liver and Plasma of Gilt-Head Bream Exposed to Oxybenzone." *Chemosphere* 211: 624–31.