# Working with substrate information in opm

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### Abstract

This is the substrate-information tutorial of **opm** in the version of October 9, 2013.

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## 1. Introduction

A detailed description of the OmniLog® Phenotype MicroArray (PM) system, its measuring procedure and data characteristics are found in the vignette "**opm**: An R Package for Analysing OmniLog® Phenotype MicroArray Data" (called "main tutorial" in the following). The description of the methods below presupposes that the user is familiar with the usage of **opm** and has studied the main tutorial as well as the entries of the **opm** manual relevant to her or his research. Especially the concept and structure of the different object-classes should be made clear before starting with this tutorial.

In addition to visual inspection or statistical comparative analyses of Phenotype Microarray data, as described in the main manual, users might be interested in specific information on the substrates used in PM assays. The **opm** package contains a large variety of additional data on PM substrates. Beside methods for assessing these information directly, this tutorial introduces strategies for visualization of the measured PM results by mapping on pathway-maps and introduces analysis methods for modelling and identification of informative and less informative substrates.

## 2. Preparation

Before starting, install the package **pathview** from Bioconductor (http://bioconductor.org/packages/2.12/bioc/html/pathview.html) and load it together with the **opm** package, into an R session. Please note, that it is important to load **pathview** before **opm**, since otherwise some functions are not visible and the package does not work properly. In this vignette this is catched by the `if`-construct.

```
R> suppressPackageStartupMessages(library("pathview"))
R> if ("package:opm" %in% search())
     detach("package:opm")
R> library("opm")
R> data(vaas_et_al, package = "opmdata")
```

## 3. Available plate information

Currently substrate layouts of various plates are available within **opm**. An overview about the plate types available in the respective version of **opm** is obtained by entering

```
R> plate_type(full = TRUE)
```

The resulting vector of names does not only include OmniLog® plates; see the manual and the main tutorial for further details. Using other values for `full`, or additional arguments, distinct spelling variants of the plate names can be obtained.

## 4. Accessing substrate information

The **opm** package contains a number of functions suitable for accessing precomputed information on the substrates within certain wells and entire plates. In the manual and help pages these functions are explained within the family "naming-functions" with according cross-references. One usually would start a search by determining the exact spelling of an internally used name with `find_substrate()`:

```
R> substrates <- find_substrate(c("Glutamine", "Glutamic acid"))
R> substrates
```

The results is a list (of the S3 class "substrate_match") containing character vectors with the results for each query name as values. Surprisingly, nothing was found for "Glutamic acid" but several values for "Glutamine". The default `search` argument is "exact", which is exact (case-sensitive) matching of *substrings* of the names. One might want to use "glob" searching mode:

```
R> substrates <- find_substrate(c("L-Glutamine", "L-Glutamic acid"), "glob")
R> substrates
```

But with so-called wildcards, i.e. "*" for zero to many and "?" for a single arbitrary character the search is more flexible:

```
R> substrates <- find_substrate(c("*L-Glutamine", "*L-Glutamic acid"), "glob")
R> substrates
```

This fetches all terms that end in either query character string, and does so case-insensitively. Advanced users can apply the much more powerful "regex" and "approx" search modes; see the manual for details, entry `?find_substrate`.

Note that **opm** appends a concentration (or just repetition) indicator as a number after a hash sign ("#") to the substrate names wherever necessary. Thus a wildcard at the end of a name might often by the most useful search pattern.

Once the internally used names (which are not guaranteed to be stable between distinct **opm** releases) have been found, information on the substrates can be queried such as their occurrences and positions on plates:

```
R> positions <- find_positions(substrates)
R> positions
```

This yields a nested list containing two-column matrices with plate names in the first and well coordinates in the second column. References to external data resources for each substrate name can be obtained using `substrate_info()`:

```
R> subst.info <- substrate_info(substrates)
R> subst.info
```

By default this yields CAS numbers (http://www.cas.org/content/chemical-substances/faqs), but MeSH names (useful for conducting PubMed queries; see http://www.ncbi.nlm.nih.gov/mesh/) (?), ChEBI IDs (?), KEGG compound IDs, KEGG drug IDs (?) and MetaCyc IDs (?) IDs have also been collected for the majority of the substrates. Using the "browse" argument, full URLs can be created and optionally also directly opened in the default web browser. Using the "download" argument, if KEGG drug or compound IDs have been selected, these can be downloaded from the KEGG server if the **KEGGREST** is available and converted into customized objects. It is possible to nicely display all available information at once:

```
R> subst.info <- substrate_info(substrates, "all")
R> subst.info
```

Another use of `substrate_info()` is to convert substrate names to lower case but protecting name components such as abbreviations or chemical symbols. See the manual for further details, help page `?substrate_info`.

# 5. Visualisation by integration of PM data in pathway maps

In conjunction with other packages, it is possible to visualize PM results directly in pre-existing pathway maps as, for example, those from the KEGG database. These maps are essentially manually drawn pathway maps representing the currently available knowledge about genes, substrates and their connection in pathways. Depending on availability of genome and gene-annotation information within KEGG, organism-specific, individual maps are allocateable (?).

The mapping itself is simply the introduction of PM-measurement data as colour-coding of nodes (here, representing the substrates) into those maps, as it can be done similarly with several other types of *OMICS*-data. For details, please see the description on the KEGG-homepage: (http://www.genome.jp/kegg/).

Here we will use the function `pathview` from the package of same name (?). This function downloads the user-defined pathway map and subsequently maps and renders the given PM-data into it. Please note the mandatory ordering when loading the packages;`pathview` must be loaded *before* loading package **opm** into an R session.

## 5.1. suitable input-data

The workflow starts with either an OPMX object containing the agregated values, or the result from an opm_mcp analysis. The first step in both cases is to bring the PM-results in a suitable format, which is a named vector created by the function annotated.

```
R> # here provide the annotated vector from vaas_1
R> xx <- annotated(vaas_1)
R> head(xx)

    <NA>   C00721   C00208   C01083   C00185   C08240
123.4558 248.1809 284.0994 269.7548 180.7536 287.7959
```

The resulting vector basically contains the numeric values (selected parameter estimates or opm_mcp results, as explained below) as well as an annotation of the according substrates. With the what-argument, passed as eponymous argument to substrate_info, the user can indicate which kind of information should be used for the annotation.

Although annotated works directly on OPMX-objects containing aggregated data for single plates or bundles of plates, please note, that the output allows for only one value per substrate. Thus, when applying annotated to a set of plates, make sure, that only one experimental group is comprised, since the resulting values are averaged per well over all plates in the input object. Using the output-argument, one is able to choose which parameter should be adressed, for example AUC instead of maximum height:

```
R> y <- annotated(vaas_1, output = param_names()[4])
R> head(y)

     <NA>    C00721    C00208    C01083    C00185    C08240
 8918.137 18391.590 21960.080 18531.180 11831.150 19254.160
```

Further options allow for indication of categorical (different) and for the opm_glht method, binary outcomes (smaller, larger or equal).

But visualization of the results of an opm_mcp analysis is also possible, which offers more (statistically interesting) opportunities for making sense of the PM data in the context of pathways.

However, this method only makes sense, if each coefficient estimated by opm_mcp can be linked to a single substrate. This is usually only possible for the "Dunnett" and "Pairs" type of contrast if applied to the wells.

First an opm_glht object is generated from the vaas_4 exemplar object by performing an Dunnett-type multiple comparison among the first fiften wells agains well A05 as control group.

```
R> x <- opm_mcp(vaas_4[, , 1:15], output = "mcp", model = ~ Well,
     linfct = c(`Dunnett.A05` = 1), full = FALSE)
```

The resulting 95% confidence intervals for the difference of means are plotted in Figure 1.

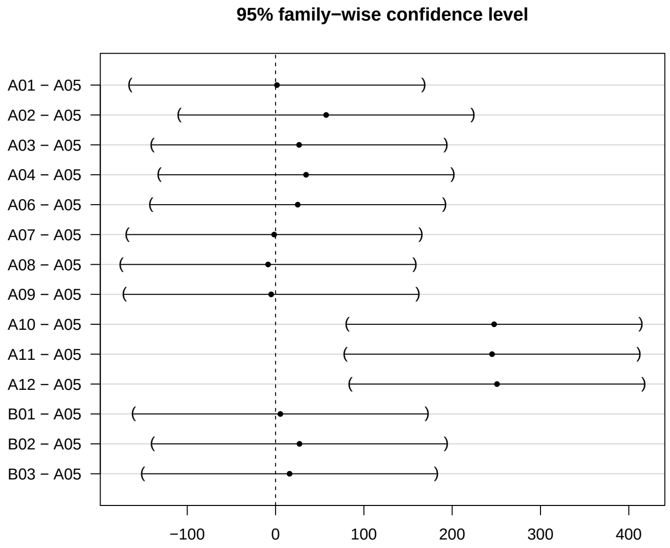


Figure 1: Point estimates and 95% confidence intervals in a manually defined comparison of group means for a specifically selected set of wells (A01 to B03) from the `vaas_4` exemplar object. In this procedure each well is compared against A05. The picture was obtained by running `opm_mcp()` and then the plotting function for the resulting `opm_glht`-object. See the main tutorial for details.

Using the above generated `opm_glht` example, the options allowing for categorical output of `annotated` will be illustrated. Obviously, only three comparisons exhibit a statistically significant difference, namely the comparisons A10 - A05, A11 - A05 and A12 - A05, all showing that A05 is smaller than A10, A11 and A12, respectively.

Using the `output`-argument users are able to get various categorical results beside simple numerical output of the respective point estimator. The options `upwards` and `downwards` result classifications in three categories (FALSE, NA, or TRUE), giving an indication, if the CI is located in the positive or negative range with resulting NA, if zero is included and thus no decission possible. The options `different`, `smaller`, `larger` and `equal` work similarly, but use only the two categories TRUE and FALSE.

```
R> anno.nu <- annotated(x, output = "numeric")
R> anno.up <- annotated(x, output = "upwards")
R> anno.do <- annotated(x, output = "downwards")
R> anno.di <- annotated(x, output = "different")
R> anno.eq <- annotated(x, output = "equal")
R> anno.sm <- annotated(x, output = "smaller")
R> anno.la <- annotated(x, output = "larger")
```

A comprehensive overview of the results is provided by bundling the results of all possible options into one dataframe:

```
R> annotab <- data.frame(Comparison = rownames(confint(x)$confint),
     numeric = anno.nu, upwards = anno.up, downward = anno.do,
     different = anno.di, equal = anno.eq, smaller = anno.sm,
     larger = anno.la)
R> annotab

   Comparison    numeric upwards downward different equal smaller larger
1   A01 - A05   1.577317      NA       NA     FALSE  TRUE   FALSE  FALSE
2   A02 - A05  57.274661      NA       NA     FALSE  TRUE   FALSE  FALSE
3   A03 - A05  26.661023      NA       NA     FALSE  TRUE   FALSE  FALSE
4   A04 - A05  34.537328      NA       NA     FALSE  TRUE   FALSE  FALSE
5   A06 - A05  25.078779      NA       NA     FALSE  TRUE   FALSE  FALSE
6   A07 - A05  -1.606236      NA       NA     FALSE  TRUE   FALSE  FALSE
7   A08 - A05  -8.458879      NA       NA     FALSE  TRUE   FALSE  FALSE
8   A09 - A05  -4.975284      NA       NA     FALSE  TRUE   FALSE  FALSE
9   A10 - A05 247.470724    TRUE    FALSE      TRUE FALSE   FALSE   TRUE
10  A11 - A05 245.163382    TRUE    FALSE      TRUE FALSE   FALSE   TRUE
11  A12 - A05 250.763650    TRUE    FALSE      TRUE FALSE   FALSE   TRUE
12  B01 - A05   5.417463      NA       NA     FALSE  TRUE   FALSE  FALSE
13  B02 - A05  27.079437      NA       NA     FALSE  TRUE   FALSE  FALSE
14  B03 - A05  15.870312      NA       NA     FALSE  TRUE   FALSE  FALSE
```

Using the argument `how = "value"` a numeric matrix containing the chosen computed values together with their abundance in pathway maps can be investigated.

The results from an `opm_mcp` procedure are treated with `annotated` as shown before with an `OPMX` object:

## 5.2. Visualisation in pathway-maps using pathview

Basically, `pathview` maps and renders pathway graphs (from KEGG) and integrates additional data provided from other sources, mainly experimental data from various OMICS-approaches. Here the functions serves to integrate and visualize PM data provided by `annotated`. The user has to specify the pathway, provide the PM-data and all necessary steps (download of pathway graph data, parsing of data file, maping user's data to the pathway, and rendering of pathway graph with the mapped data) are executed automatically by `pathview`.

Generally, a pathway map is described as "[...]a molecular interaction/reaction network diagram represented in terms of the KEGG Orthology (KO) groups [...]" (see http://www.genome.jp/kegg/kegg3a.html for further details). KEGG contains a collection of different types of pathway maps, precisely identifiable by the combination of 2-4 letter code and 5 digit number.

The letter prefixes have the following meanings:

map - Reference pathway

ko - Reference pathway (KO)

ec - Reference pathway (EC)

rn - Reference pathway (Reaction)

org - Organism-specific pathway map

Only the first reference pathway map is manually drawn; all other maps are computationally generated. The *ko*-maps contain the manually defined ortholog groups (*ko* entries) for all proteins and functional RNAs that correspond to KEGG pathway nodes, BRITE hierarchy nodes, and KEGG module nodes, whereas the (*ko* entries) are converted to gene identifiers if organism-specific pathways maps are generated. A list of the existing maps and their corresponding numbers are available under `http://www.genome.jp/kegg-bin/get_htext?query=01100&htext=br08901.keg&option=-a`.

`pathview` allows only for usage of KEGG orthology (the *ko*-maps) or species specific letter code (see `http://www.genome.jp/kegg/catalog/org_list.html` for an up-to-date list of organisms with complete genome information in KEGG).

Before you start with this section, please make sure, that you have installed the **pathview** package including all its dependencies from Bioconductor (`http://bioconductor.org/packages/2.12/bioc/html/pathview.html`).

Generally, the user has to provide a vector with the PM values which are named by the corresponding substrate IDs. This vector then serves as input for the visualization procedure based on `pathview`. The function is able to integrate other sources of *OMICS*-data, like for example, transcriptomics or proteomics, see the corresponding chapter in the `pathview`-vignette for details: `http://bioconductor.org/packages/2.12/bioc/vignettes/pathview/inst/doc/pathview.pdf`.

For demonstration purposes, we use a subset of `vaas_et_al` comprehending the *Escherichia coli* strains from the first biological repetition.

```
R> # subset the data
R> e.coli.sub <- subset(vaas_et_al, list(Species = "Escherichia coli",
     Experiment = "First replicate"))
R> e.coli.k12 <-  subset(vaas_et_al, list(Strain = "DSM18039"))
R> e.coli.typ <-  subset(vaas_et_al, list(Strain = "DSM30083T"))
```

Afterwards we provide the annotated vectors for the two groups separately

```
R> # annotated from single strains
R> anno.k12 <- annotated(e.coli.k12)
R> anno.typ <- annotated(e.coli.typ)
```

To provide more convenient functionality for mapping PM-data on KEGG-pathway maps, we wrote a wrapper for the `pathview`-function. This wrapper provides mainly fine tuned settings for the convenient application of `pathview` on PM-data. All graphics below are produced using this wrapper, however, the user is free to use the original `pathview`-function with the respectively changed arguments.

```
R> opm_path <- function(cpd.data,
     high = list(gene = "green4", cpd = "magenta4"),
     low = list(gene = "white", cpd = "white"),
     mid = list(gene = "lightsteelblue1", cpd = "lightsteelblue1"),
     pathway.id = "00052", species = "ko",  out.suffix = "non-native",
     key.pos = "bottomright", afactor = 1000,
     limit = list(gene = 2, cpd = 400), bins = list(gene = 0.5, cpd = 20),
     both.dirs = list(gene = FALSE, cpd = FALSE),
     sign.pos = "bottomleft", cpd.lab.offset = 0, same.layer = F,
     rank.dir = "TB", gene.data = NULL, ...) {

     pathview(gene.data = gene.data, cpd.data = cpd.data, na.col ="white",
       low = low, mid = mid, high = high, pathway.id = pathway.id,
       species = species, out.suffix = out.suffix, key.pos = key.pos,
       afactor = afactor, limit = limit, bins = bins,
       both.dirs = both.dirs, sign.pos = sign.pos,
       cpd.lab.offset = cpd.lab.offset, same.layer = same.layer,
       rank.dir = rank.dir, ...)
   }
```

First we show the results of the two strain on separate maps in Figure **??**.

```
R> e.coli.map.k12 <- opm_path(cpd.data = anno.k12, species = "ko",
     out.suffix = "k12.ko", pathway.id = "00052")
R> e.coli.map.typ <- opm_path(cpd.data = anno.typ, species = "ko",
     out.suffix = "typ.ko", pathway.id = "00052")
```

Now we compute an multiple comparison between the 13 substrates included in the galactose-pathway-map. The test compares the results of the type-strain vs. the K12, corresponding 95% CI for differences of means are shown in Figure **??**.

```
R> colicomp <- opm_mcp(
     e.coli.sub[, , c(7, 9, 13, 14, 15, 20, 25, 26, 27, 28, 37, 40, 41)],
     output = "mcp", model = ~ J(Well + Strain), linfct = c(`Pairs` = 1))
```

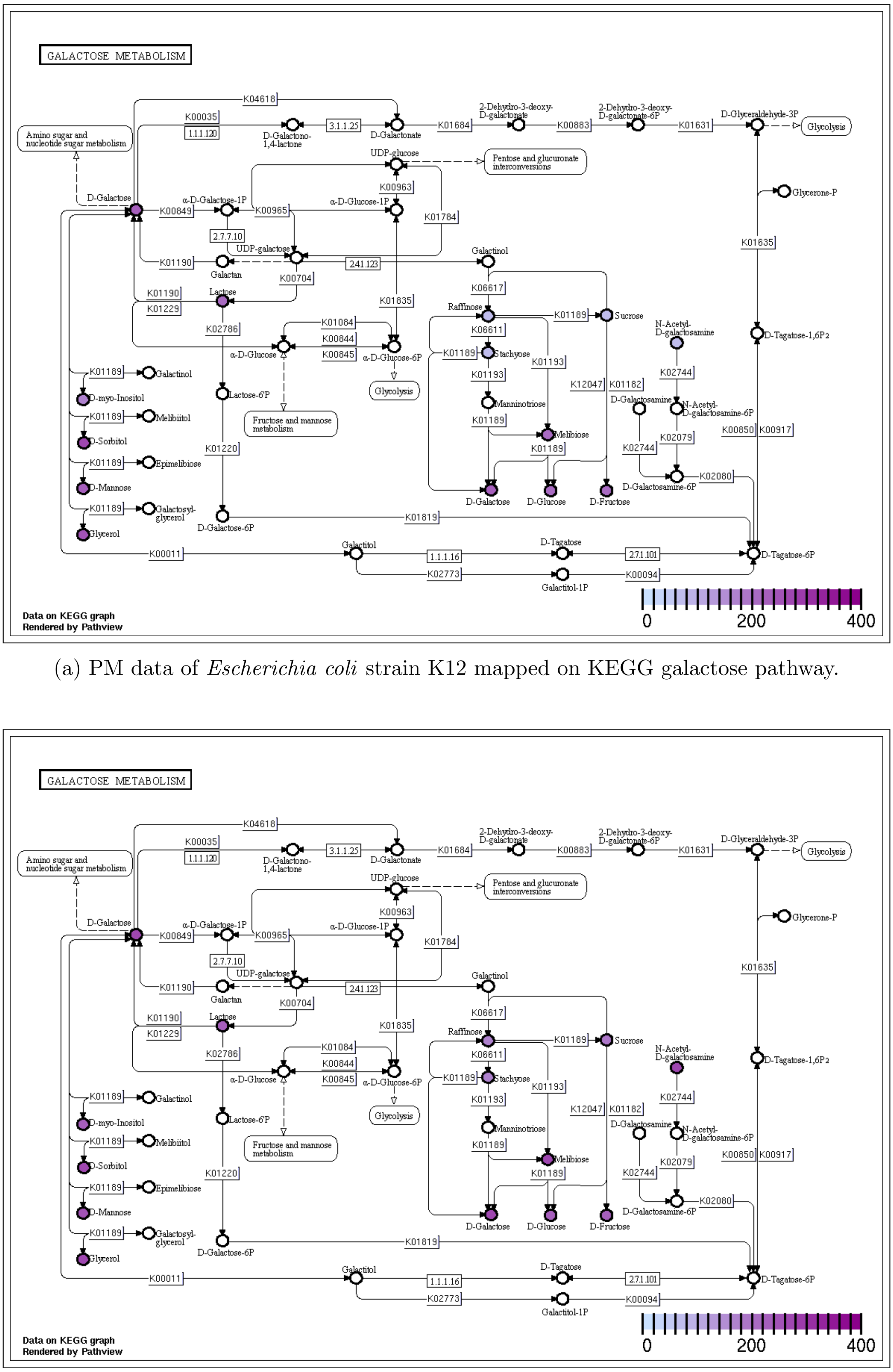
```
R> x <- ci_plot(e.coli.sub[, , c(7, 9, 13, 14, 15, 20, 25, 26, 27, 28, 37, 40, 41)],
     as.labels = list("Species", "Strain"), subset = "A", x = "bottomright",
     legend.field = NULL)
```

The annotation vector for the differences of means is provided by application of `annotated` on the `opm_glht` object, which directly facilitates the mapping of the differences on the galactose pathway, as it is shown in Figure **??**

```
R> anno.colicomp <- annotated(colicomp)
R> e.coli.comp.map <- opm_path(cpd.data = anno.colicomp, species = "ko",
     out.suffix = "e.coli.comp.ko", pathway.id = "00052")
```

## 6. Model-building approach using random forest

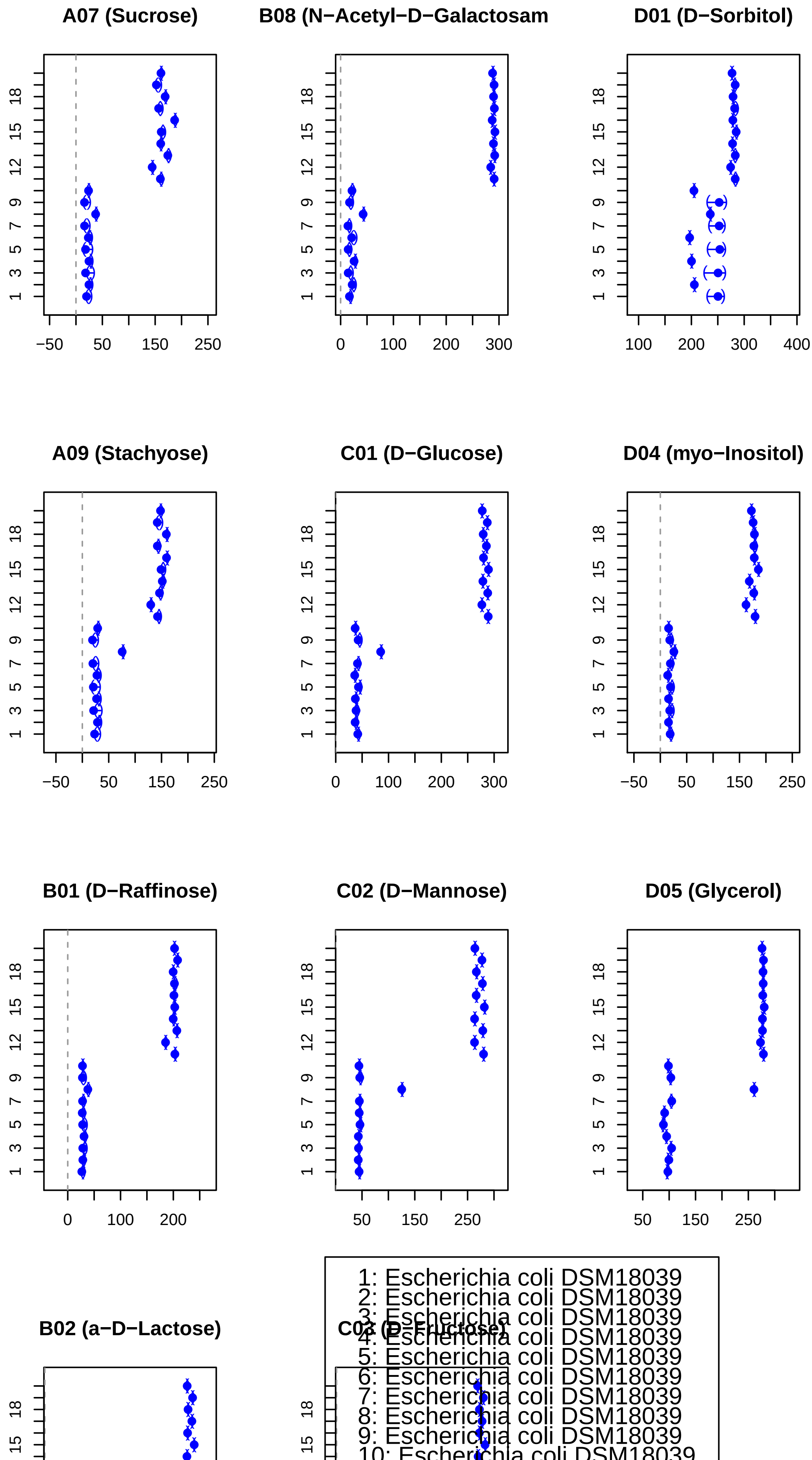
Using the argument `how= "value"` a numeric matrix containing the chosen computed values together with their abundance in pathway maps can be investigated. In analogy to the

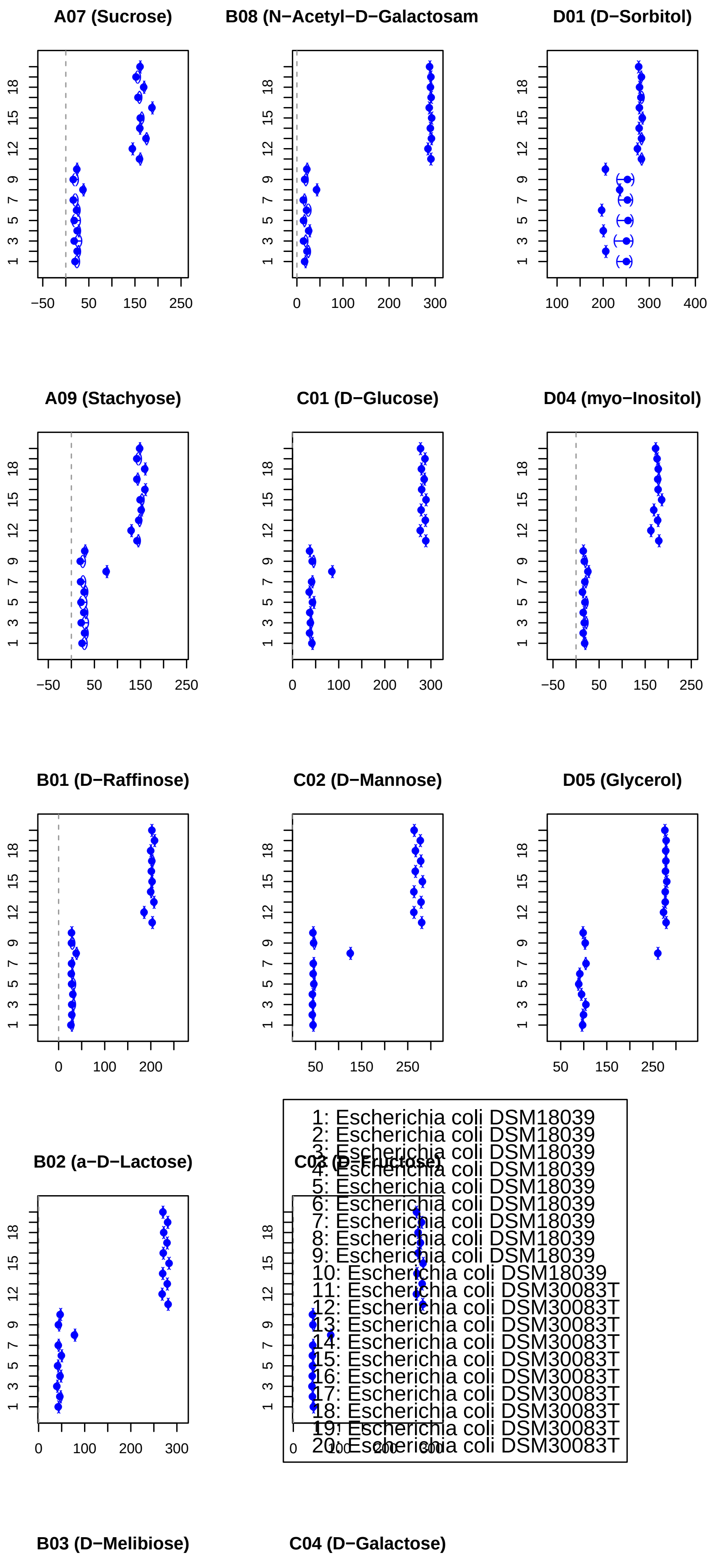


(a) PM data of *Escherichia coli* strain K12 mapped on KEGG galactose pathway.

(b) PM data of *Escherichia coli* type strain DSM30083T mapped on KEGG galactose pathway.

Figure 2: TODO: write caption, draw attention to the substrates Raffinose, Stachyose and Sucrose (in the middle of the map), which exhibit


A07 (Sucrose)
B08 (N−Acetyl−D−Galactosam
D01 (D−Sorbitol)
A09 (Stachyose)
C01 (D−Glucose)
D04 (myo−Inositol)
B01 (D−Raffinose)
C02 (D−Mannose)
D05 (Glycerol)
B02 (a−D−Lactose)
C03 (D−Fructose)
1 3 5 7 9 12 15 18
−50 50 150 250
0 100 200 300
100 200 300 400
0 100 200
50 150 250
1: Escherichia coli DSM18039
2: Escherichia coli DSM18039
3: Escherichia coli DSM18039
4: Escherichia coli DSM18039
5: Escherichia coli DSM18039
6: Escherichia coli DSM18039
7: Escherichia coli DSM18039
8: Escherichia coli DSM18039
9: Escherichia coli DSM18039
10: Escherichia coli DSM18039


A07 (Sucrose)
B08 (N−Acetyl−D−Galactosam
D01 (D−Sorbitol)
A09 (Stachyose)
C01 (D−Glucose)
D04 (myo−Inositol)
B01 (D−Raffinose)
C02 (D−Mannose)
D05 (Glycerol)
B02 (a−D−Lactose)
C03 (D−Fructose)
B03 (D−Melibiose)
C04 (D−Galactose)
1 3 5 7 9 12 15 18
−50 50 150 250
0 100 200 300
100 200 300 400
0 100 200
50 150 250
1: Escherichia coli DSM18039
2: Escherichia coli DSM18039
3: Escherichia coli DSM18039
4: Escherichia coli DSM18039
5: Escherichia coli DSM18039
6: Escherichia coli DSM18039
7: Escherichia coli DSM18039
8: Escherichia coli DSM18039
9: Escherichia coli DSM18039
10: Escherichia coli DSM18039
11: Escherichia coli DSM30083T
12: Escherichia coli DSM30083T
13: Escherichia coli DSM30083T
14: Escherichia coli DSM30083T
15: Escherichia coli DSM30083T
16: Escherichia coli DSM30083T
17: Escherichia coli DSM30083T
18: Escherichia coli DSM30083T
19: Escherichia coli DSM30083T
20: Escherichia coli DSM30083T

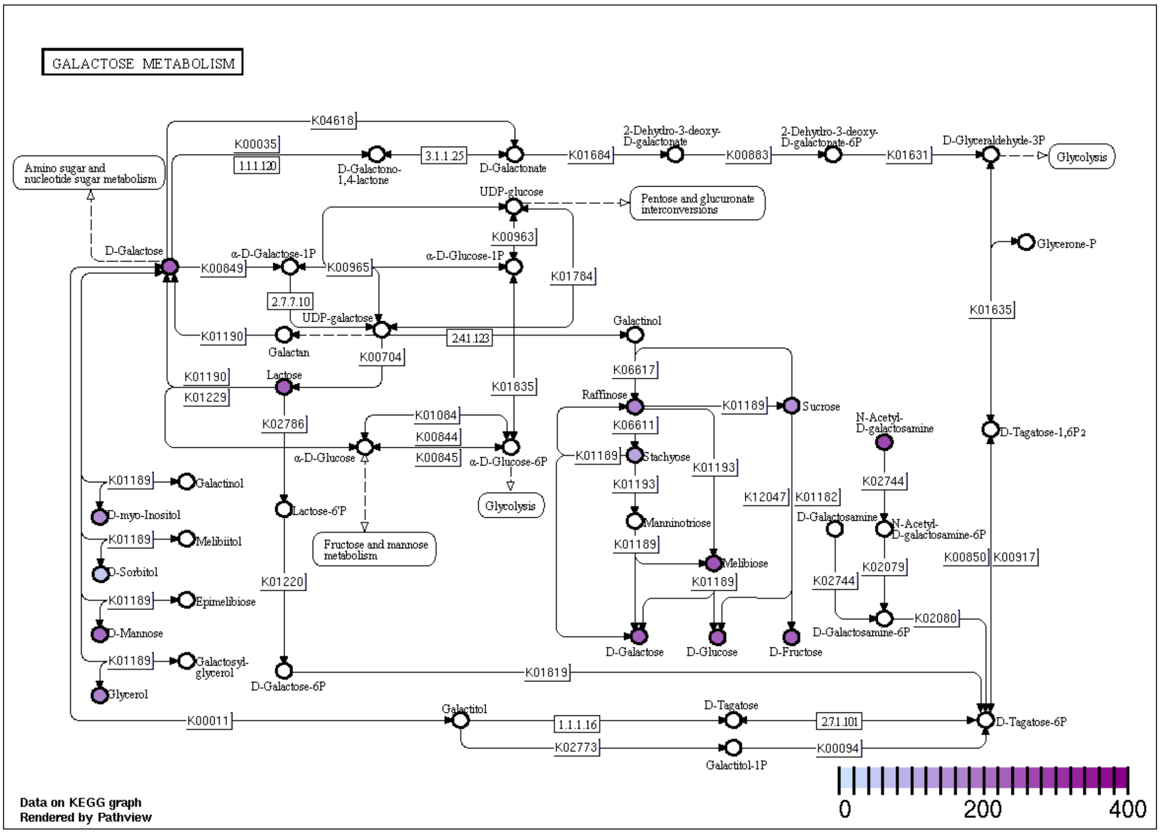


Figure 5: Mapping of differences between *E. coli* K12 and type strain DSM30083T galactose pathway.

download argument of `substrate_info` the information are convertet into a numeric matrix. Please note, that this option is not available for all values of `what` and requires additional libraries. See `substrate_info` for details.

The obtained information about the pathway certain substrates are involved in can be used to determine manually which pathways are actually of interest or can serve as input for model-building procedures as, for example, random forest approaches, which is demonstrated below.

## 7. Acknowledgements

The integration of missing OmniLog® substrates into ChEBI by the ChEBI staff is gratefully acknowledged.

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