opm software: an R package for analyzing Phenotype MicroArray data

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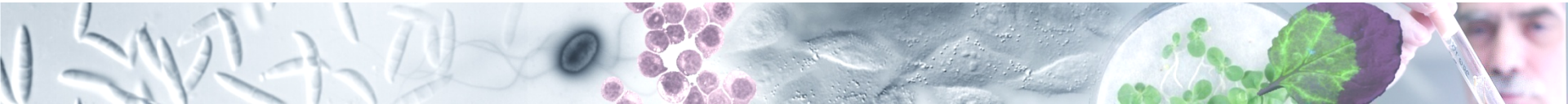
Benjamin Hofner, FAU, Germany



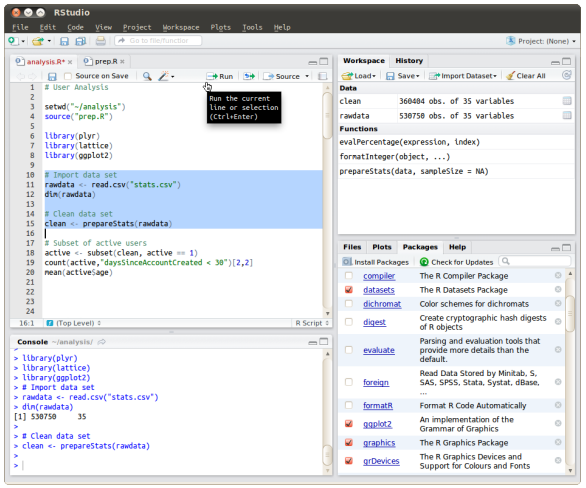
Motivation

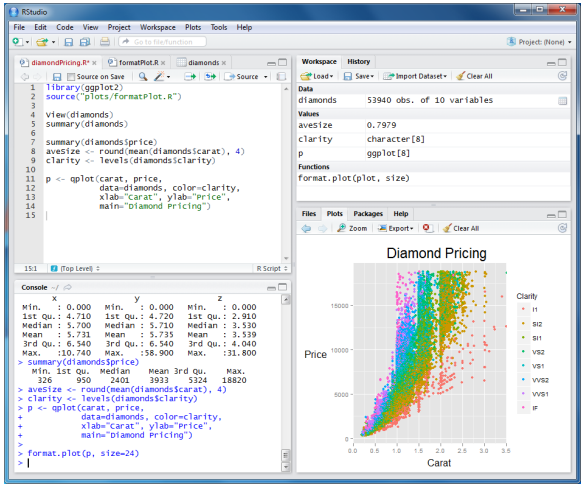
- robust statistical analysis of PM data
- flexible metadata management
- flexible production of high-quality graphics
- no restrictions regarding user-defined analyses
- reproducible research
- easy interaction with other software
- easily extendable by the user
- interactive or fully automated usage possible

www.r-project.org

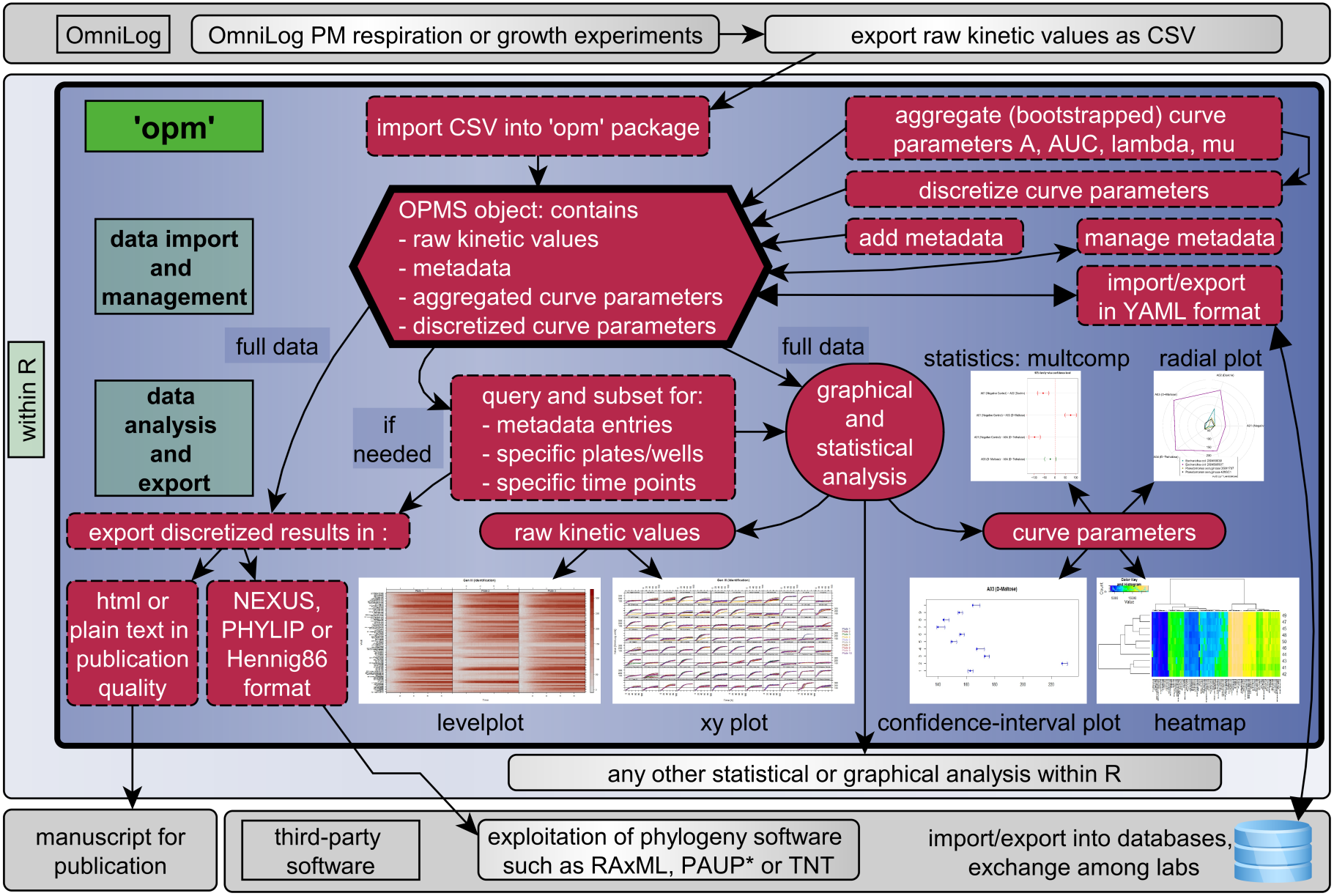
Why R?

- ***de facto* standard for free-software statistical computing**
- **all operating systems**
- **flexible and clean coding**
- **non-interactive and interactive use**
- **powerful GUIs/IDEs (e.g. RStudio™)**





www.rstudio.com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discretize curve parameters
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statistics: multcomp
radial plot
data analysis and export
if needed
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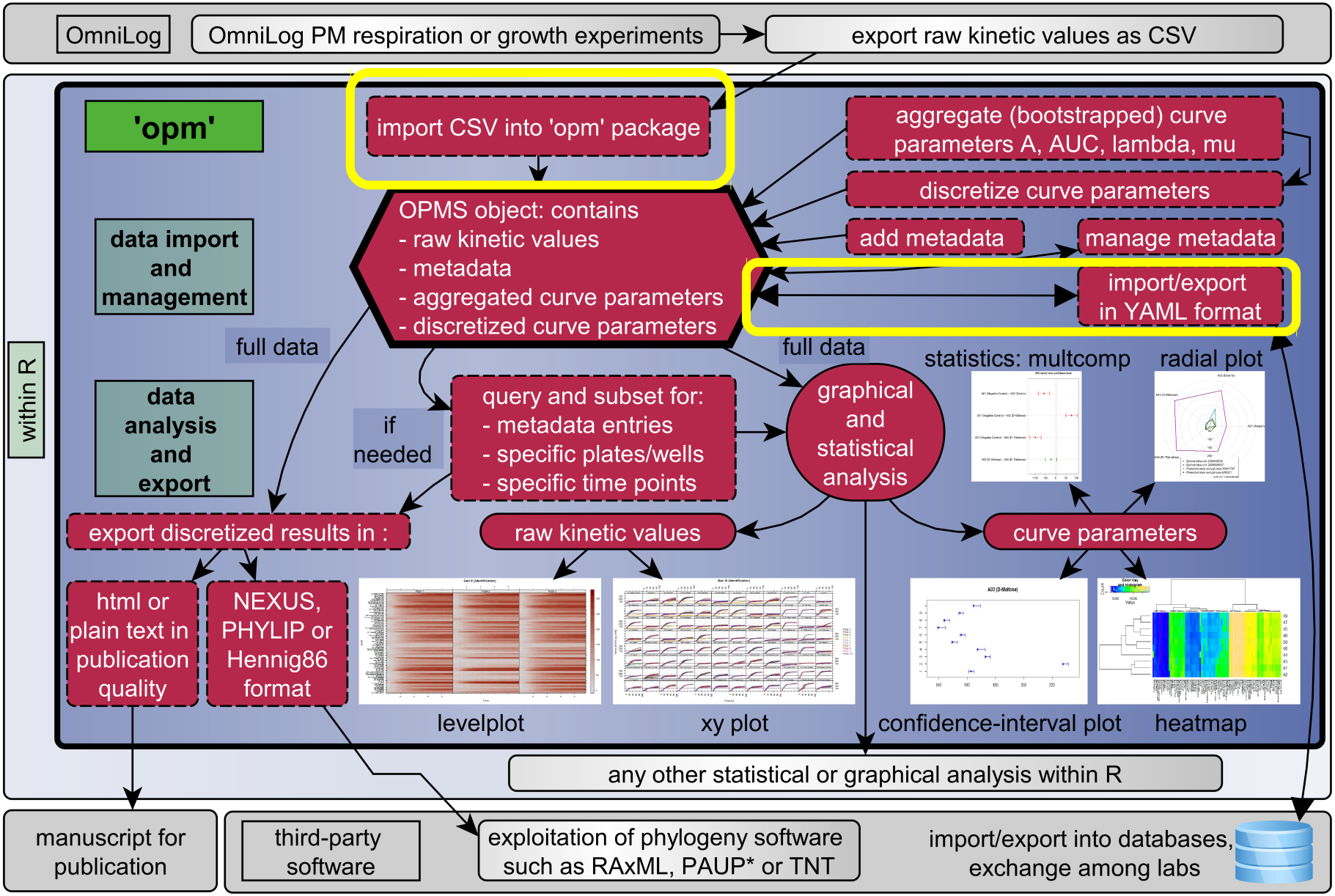
any other statistical or graphical analysis within R
manuscript for publication
third-party software
exploitation of phylogeny software such as RAxML, PAUP* or TNT
import/export into databases, exchange among labs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Input formats

- **CSV from *OmniLog® PM File Management/Kinetic Analysis* software**
- **CSV from *MicroStation®* software**
- **YAML (produced by opm itself)**

Facilities

- **Single command reads entire directory structures**
- **All plate types can be input**
- **Automated split into plate types**
- **Batch conversion of large numbers of files**

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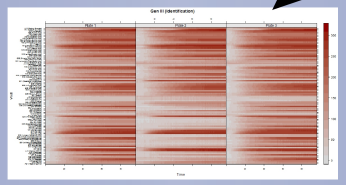

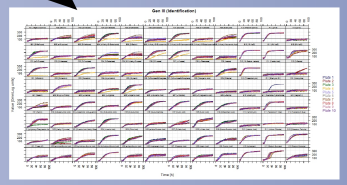

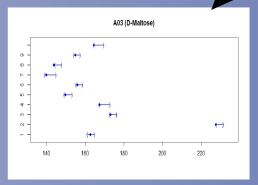

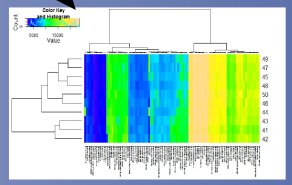

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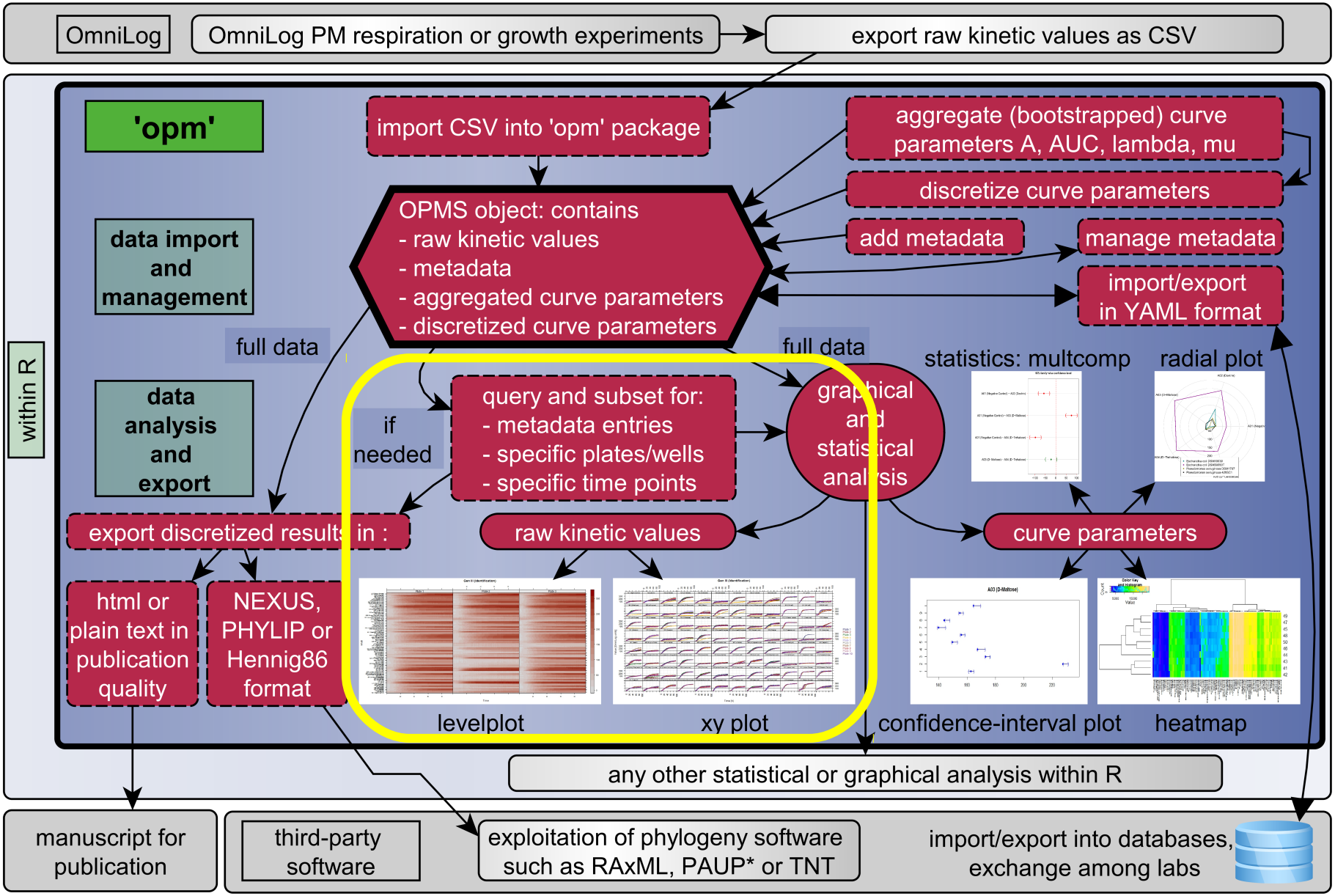
Metadata addition & manipulation

Goal: self-describing objects that carry all relevant information (data and metadata)

- if well-structured information from CSV files is present, it can be converted to metadata in 1 line of code
- unique plate identifiers assist in adding other meta-information from tabular input
- no limits regarding structure and amount of metadata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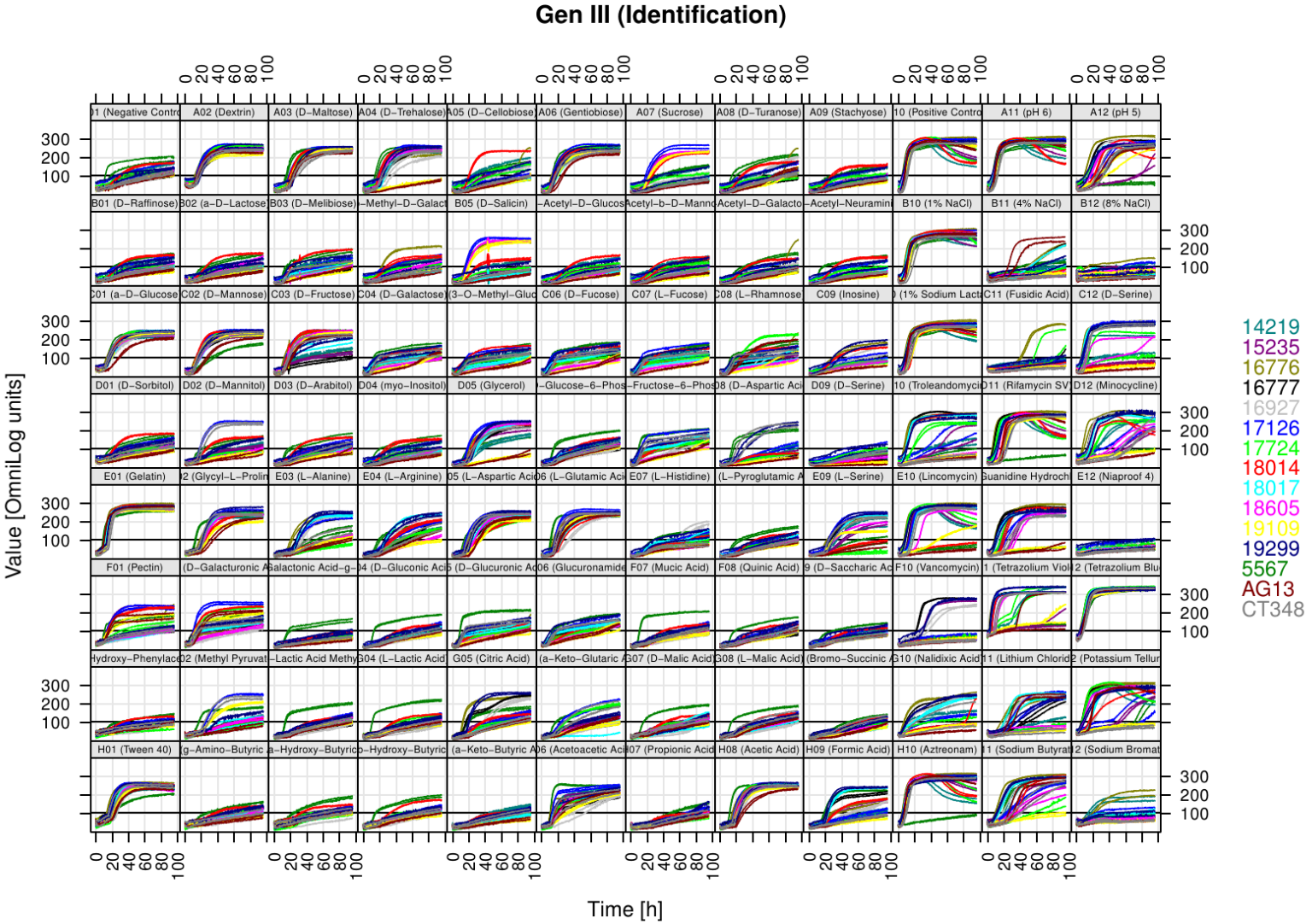
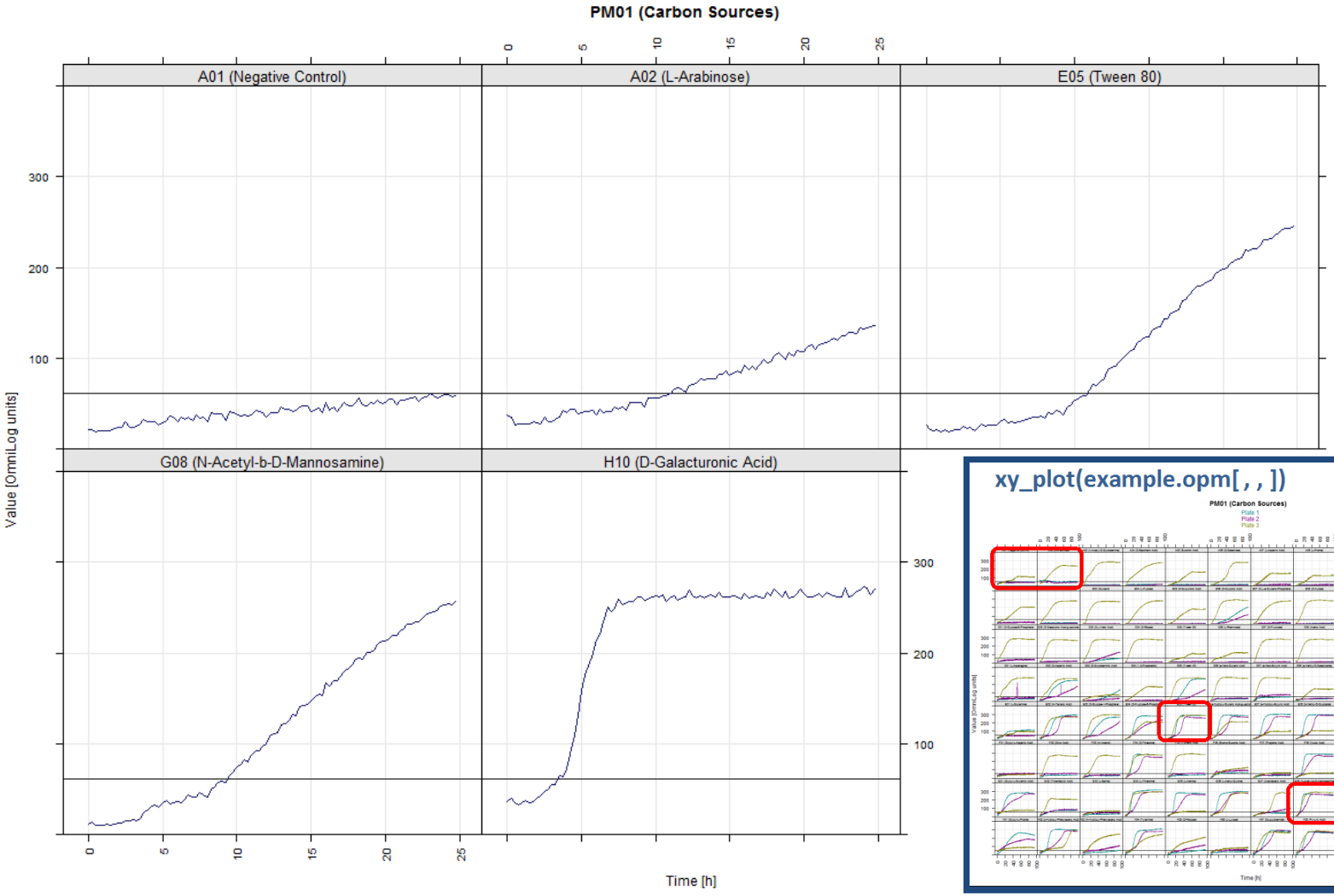
Plotting raw data: x/y plots



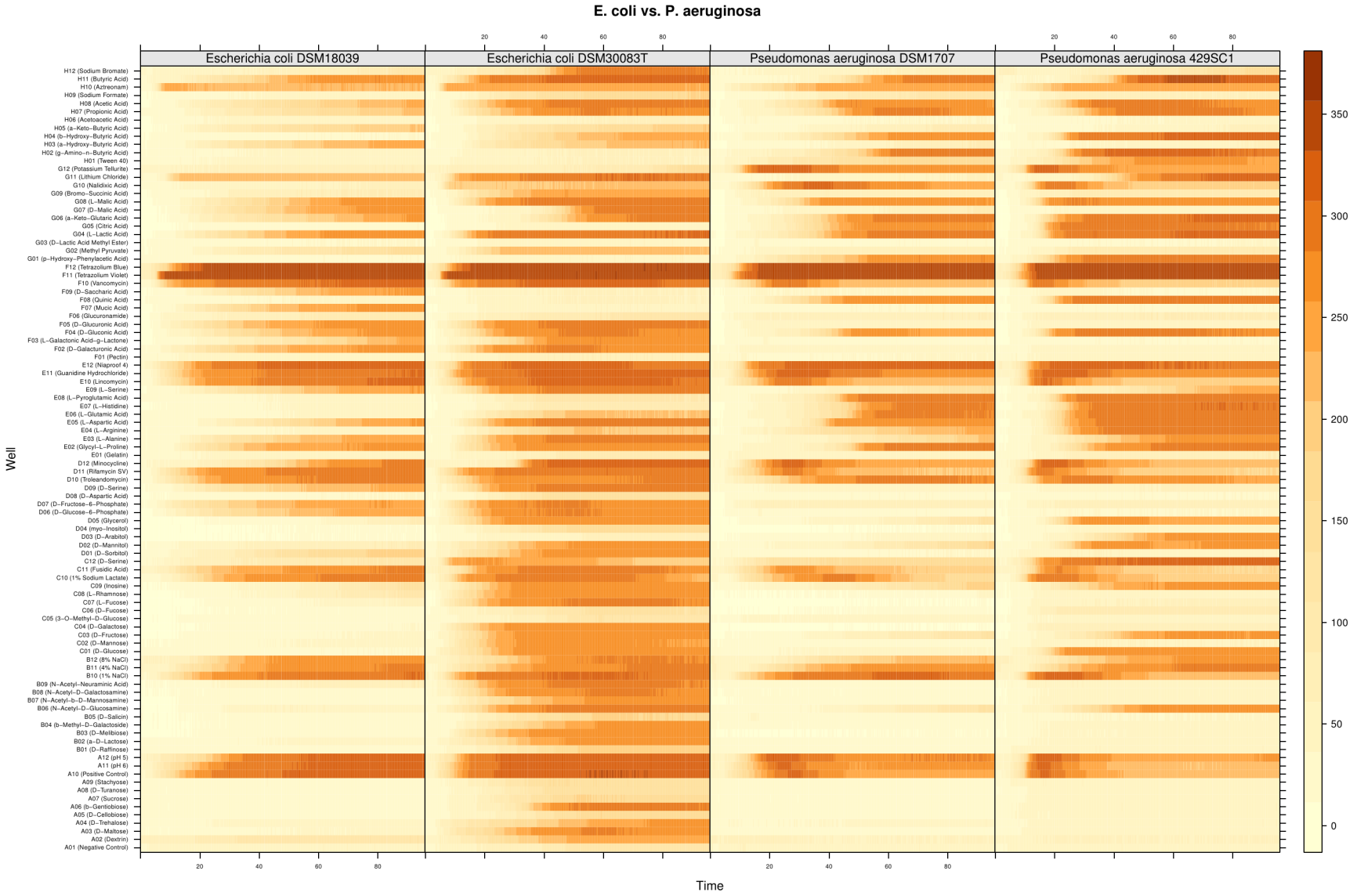
Plotting raw data: x/y plots (after subsetting)

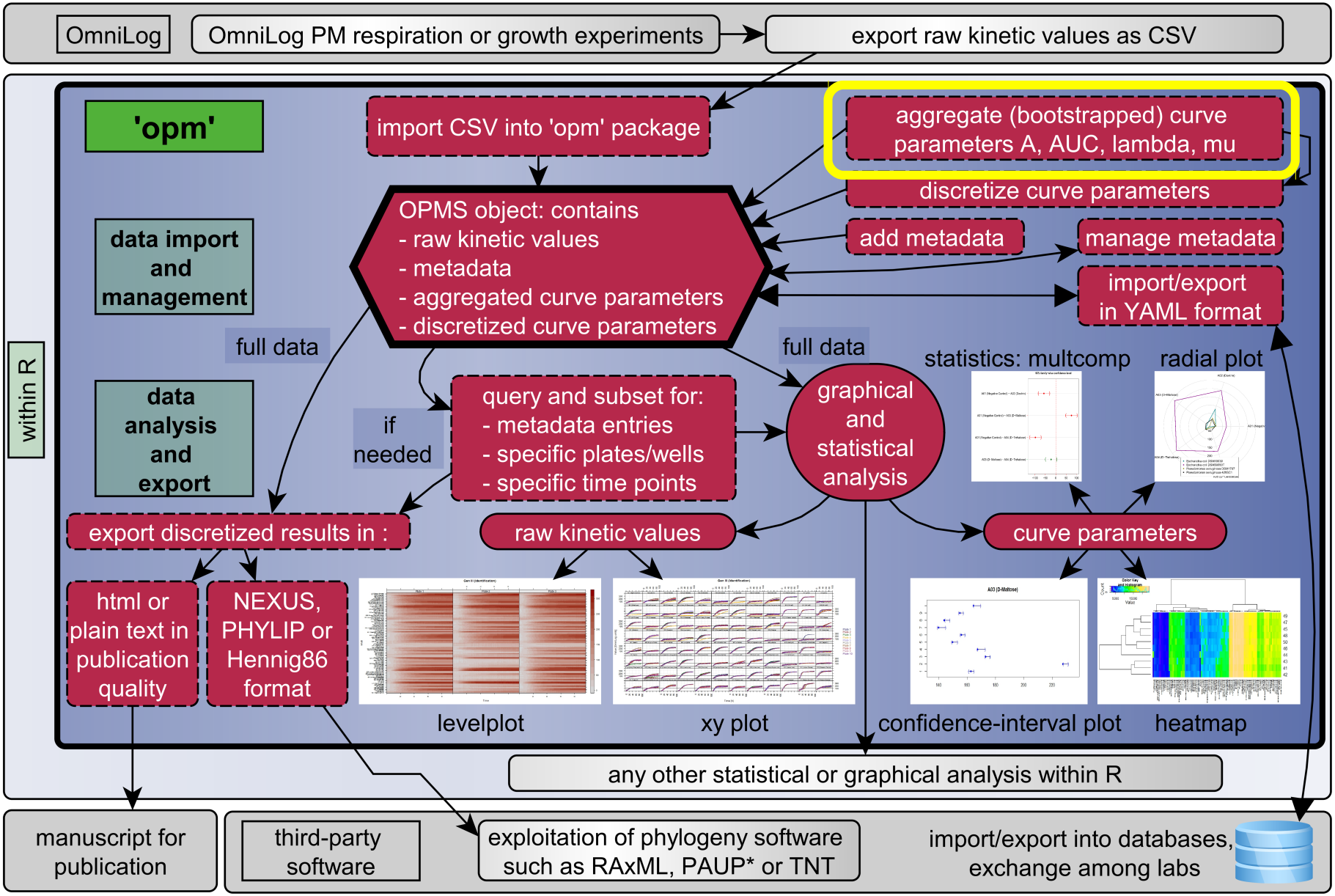


Stored substrate information

- mapping to substrate names for almost all plates (PM21-25 to be added)
- KEGG Ids (=> Bioconductor/KEGGGraph)
- Metacyc IDs
- MeSH IDs
- CAS IDs
- error-tolerant search for names, plates, positions
- user-defined modifications (abbreviations etc.) for plots

Plotting raw data: level plots

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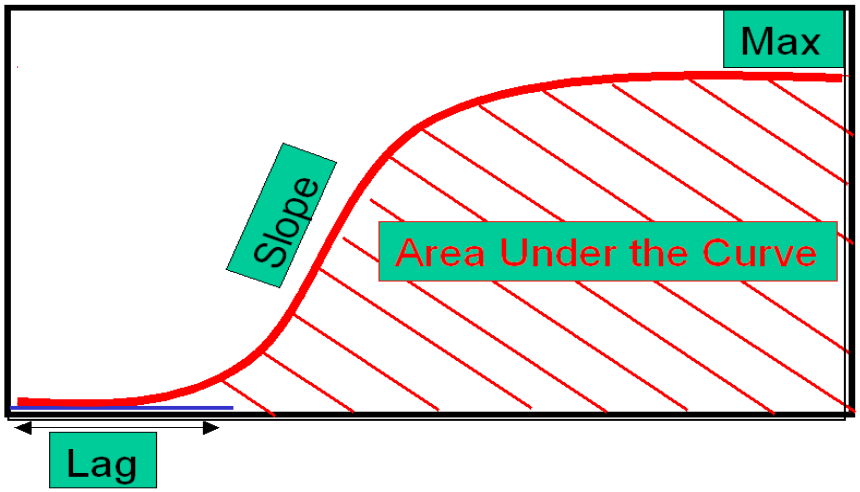
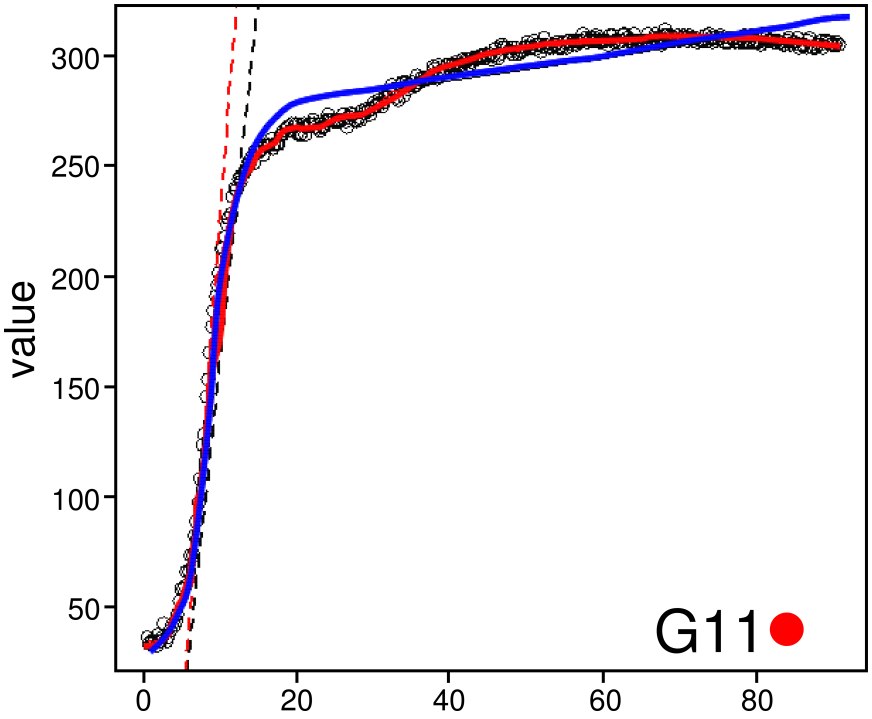
Aggregating: estimating curve parameters





- **parametric models** (Gompertz, logistic etc.) work only well for rather regular curve shapes
- **splines** outperform them in other cases

Vaas et al. PLoS ONE 7: e34846, 2012

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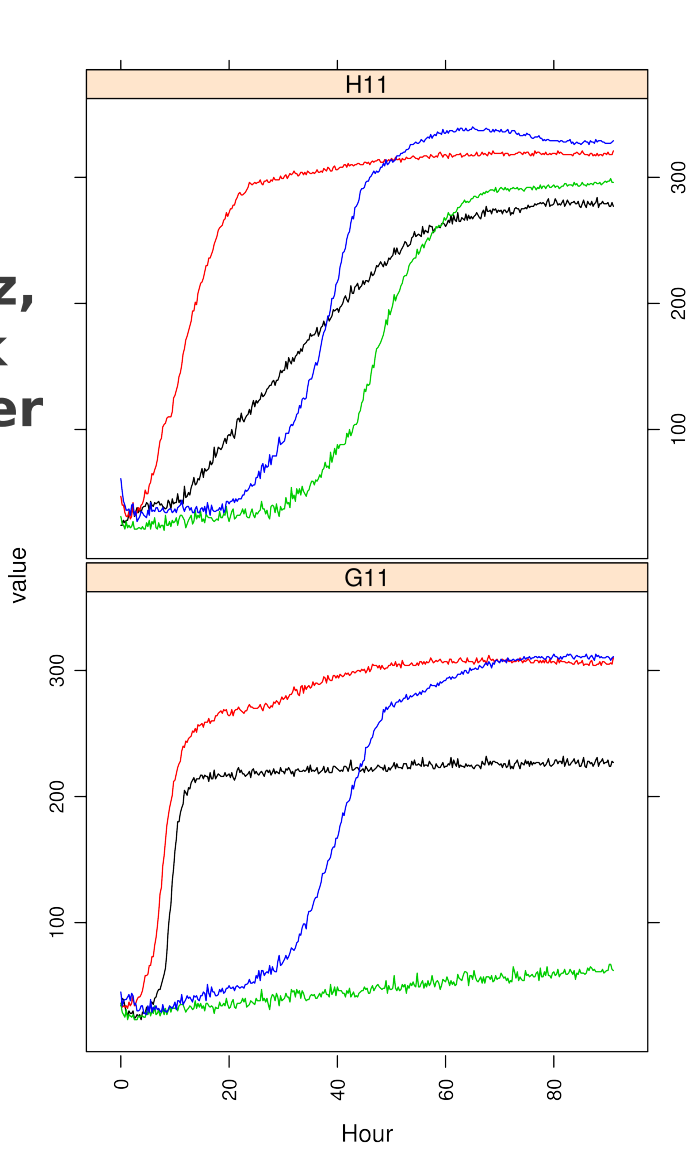
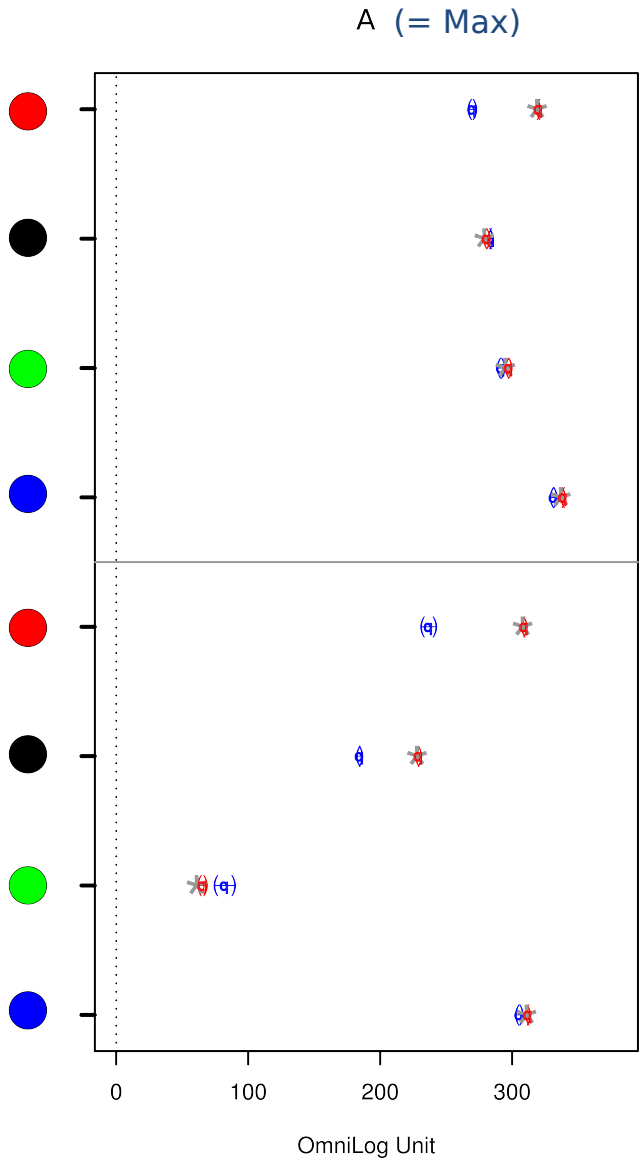
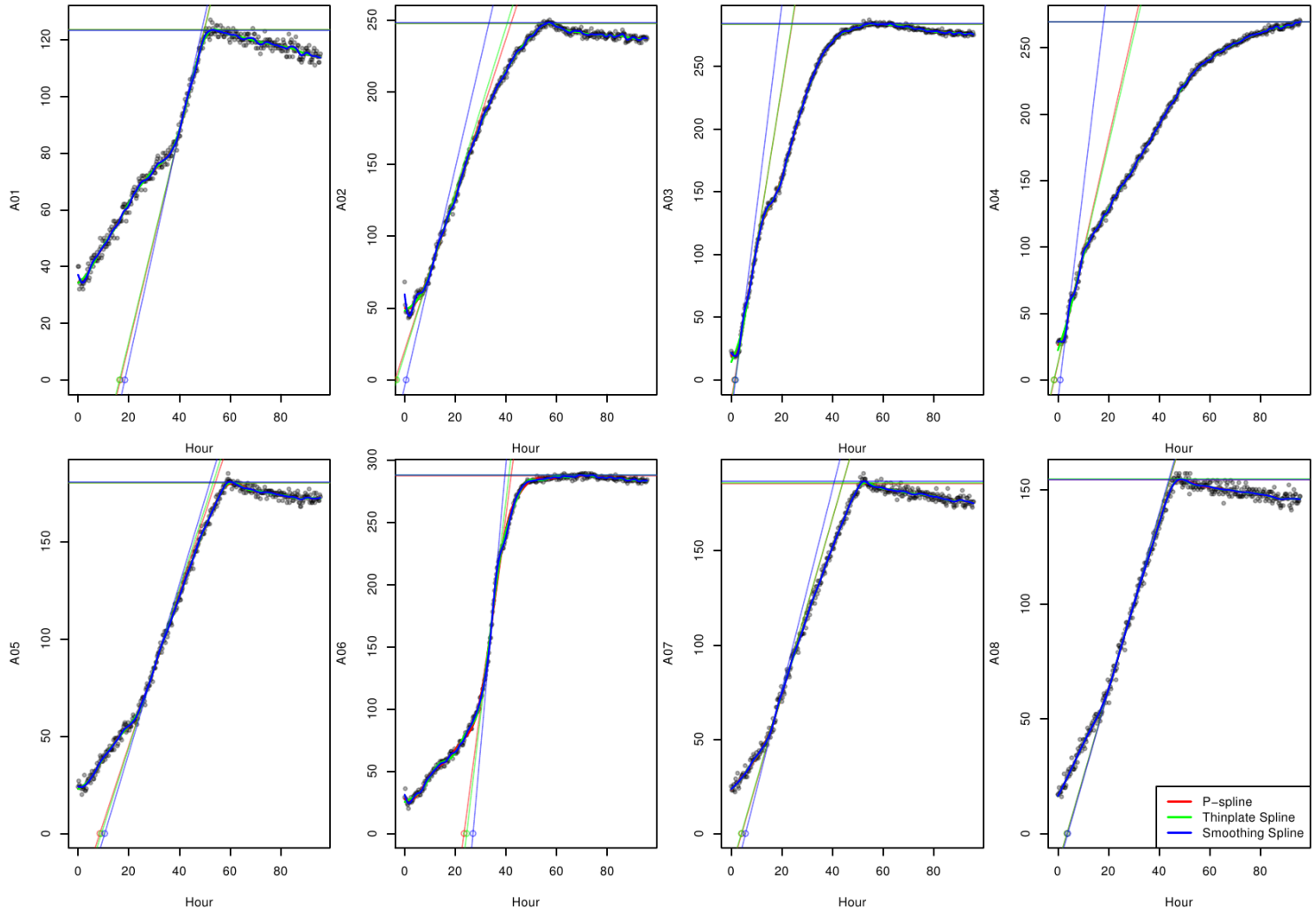
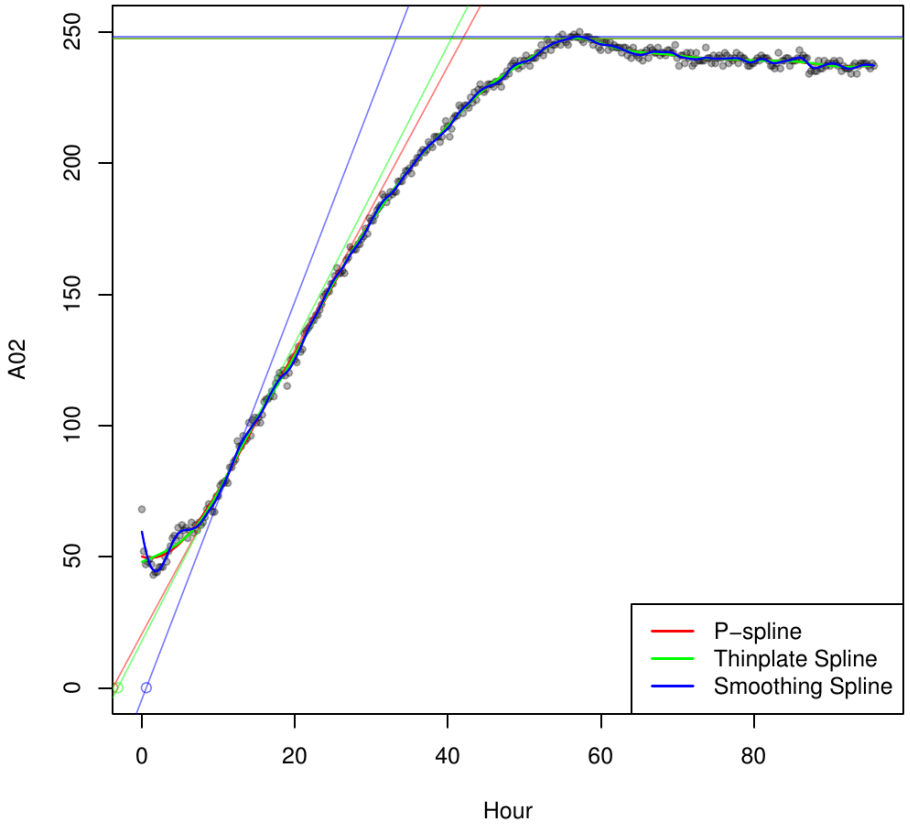


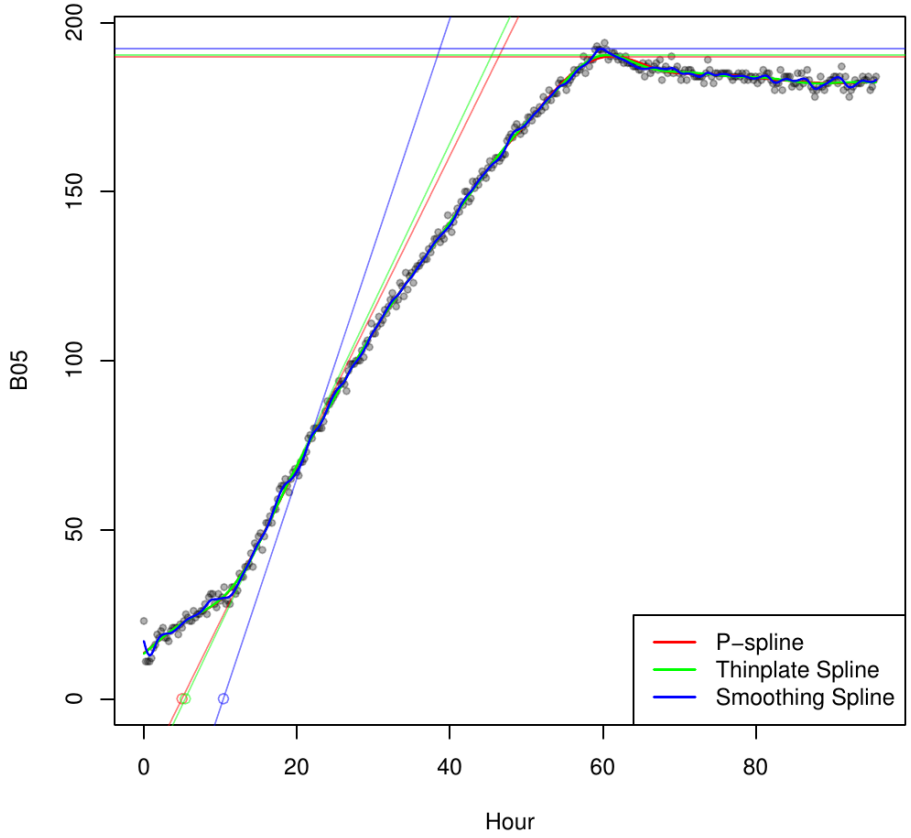


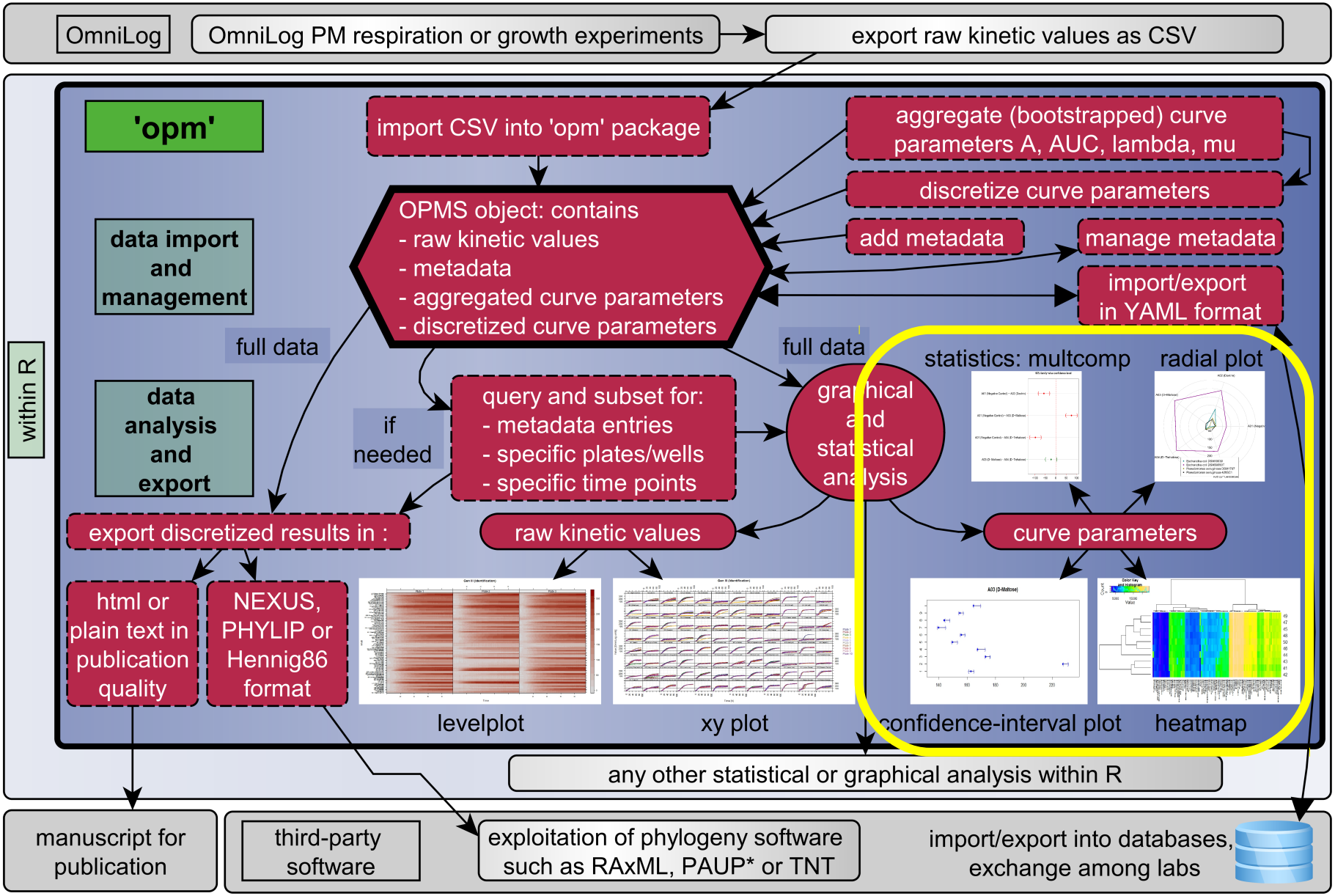
Aggregating: estimating curve parameters …from splines



Aggregating: estimating curve parameters from splines
…but splines must be tuned



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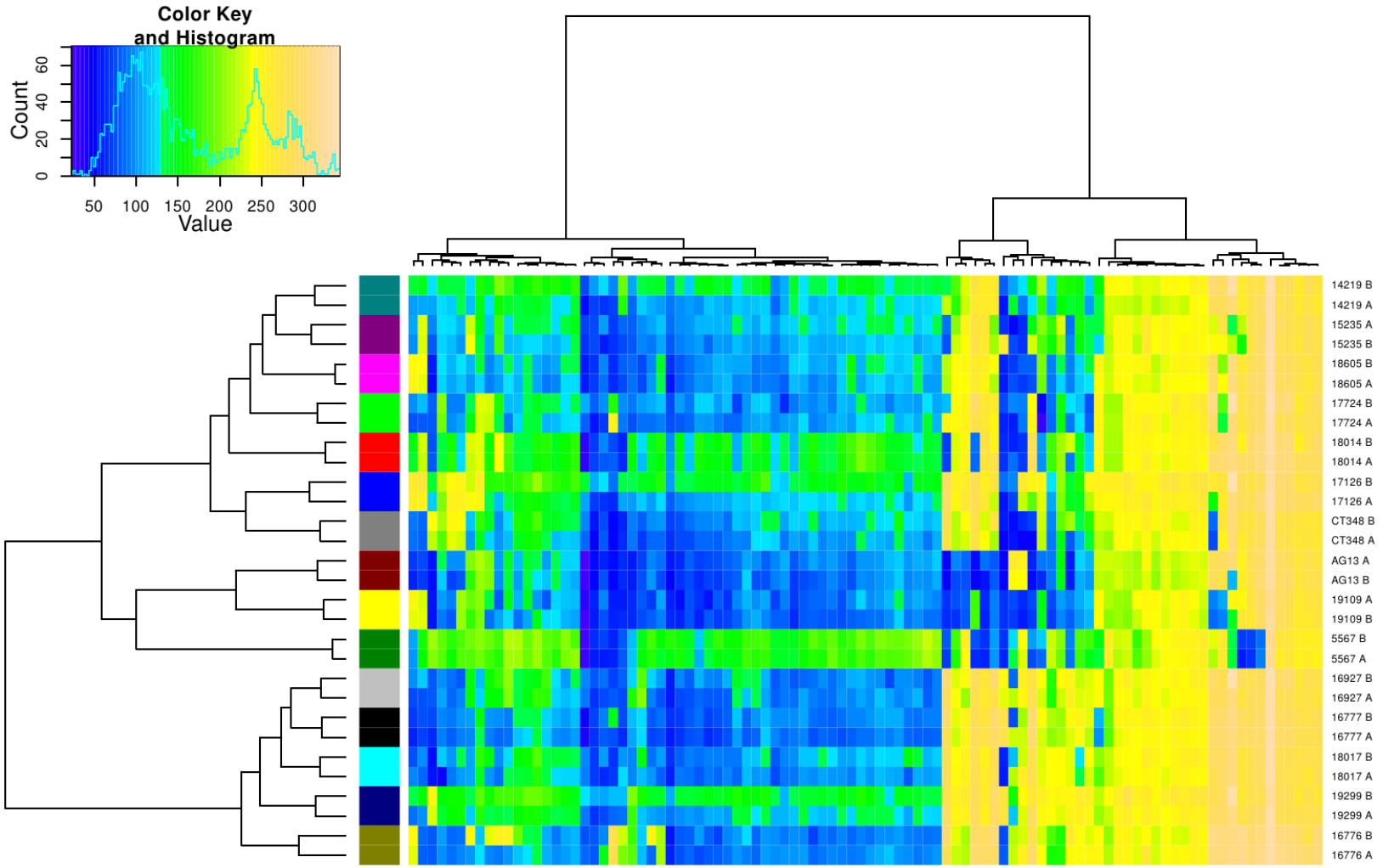
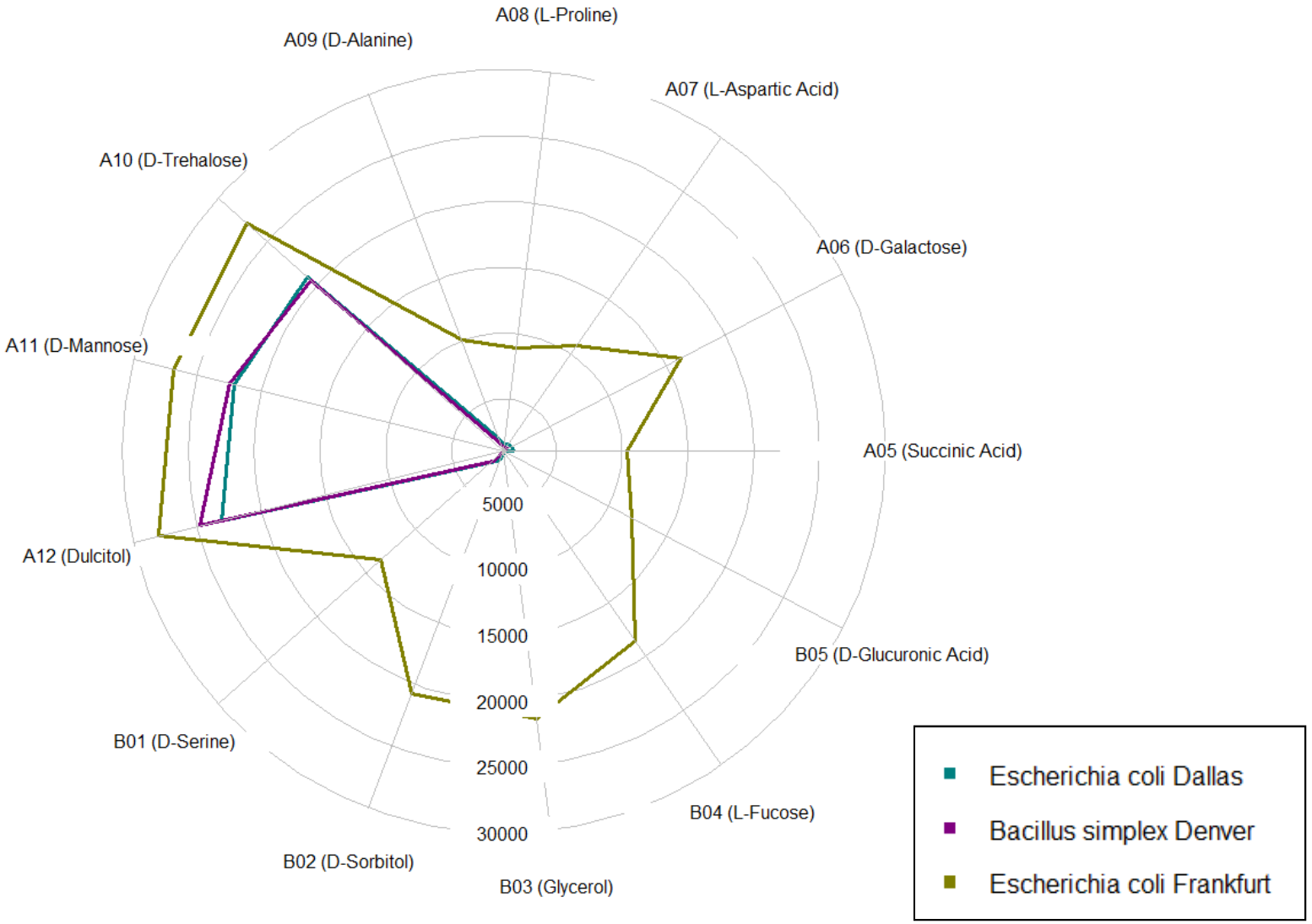
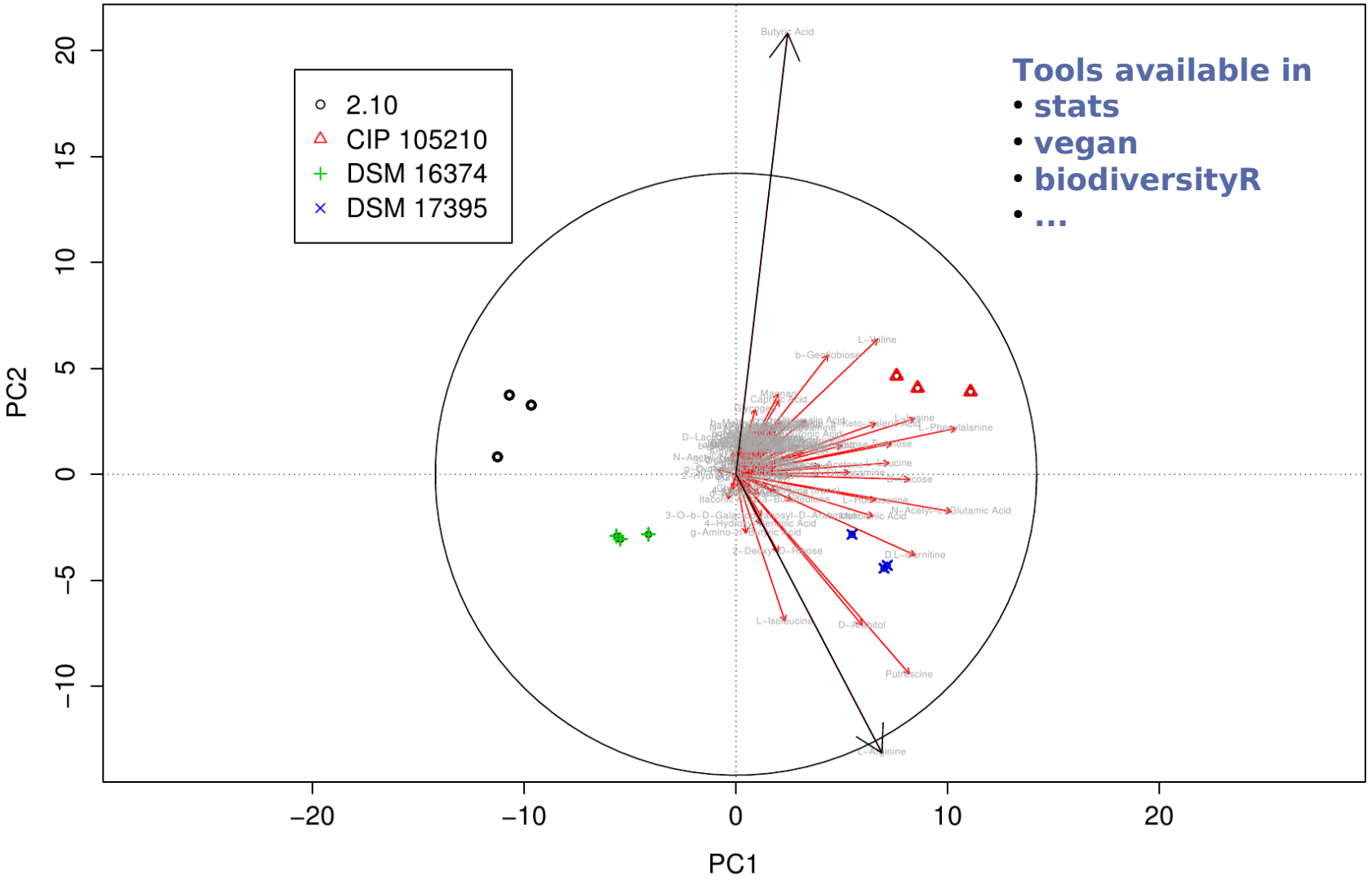
Plotting curve parameters: heat map



Plotting curve parameters: radial plot



Plotting curve parameters: principal-component analysis & biplot



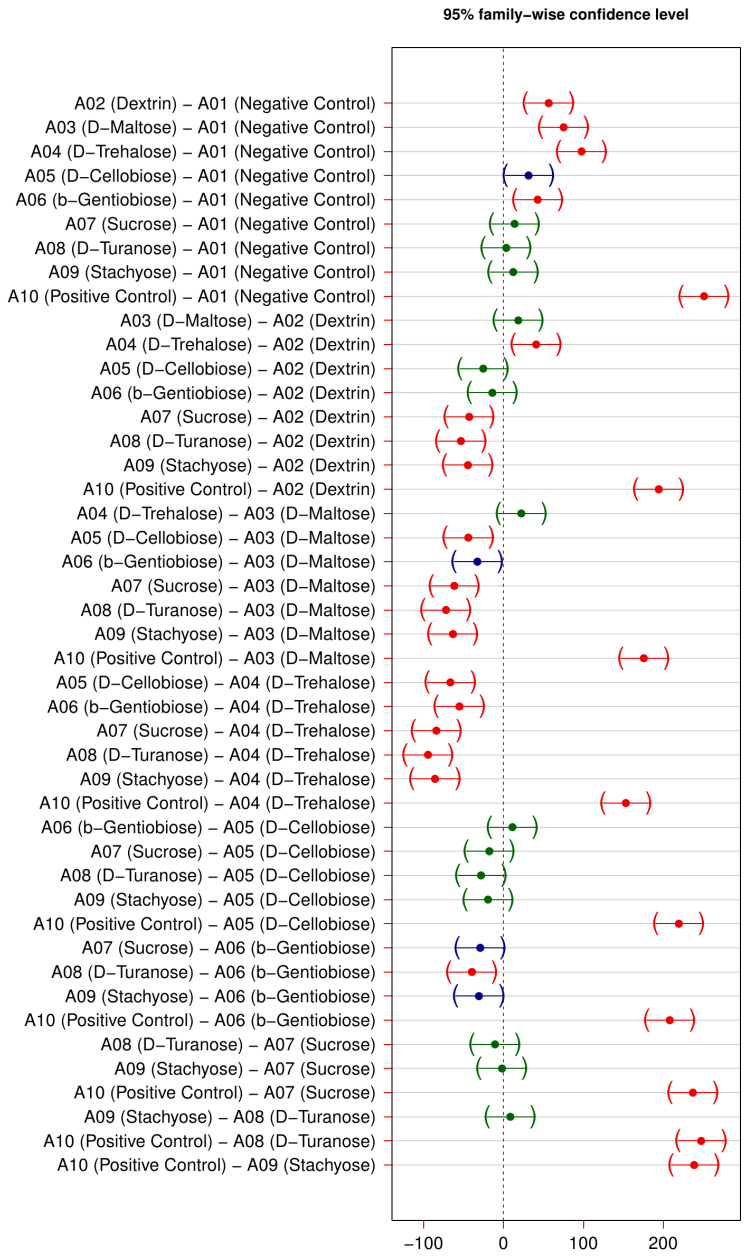
Tools available in

- **stats**
- **vegan**
- **biodiversityR**
- **…**

Multiple comparison of means

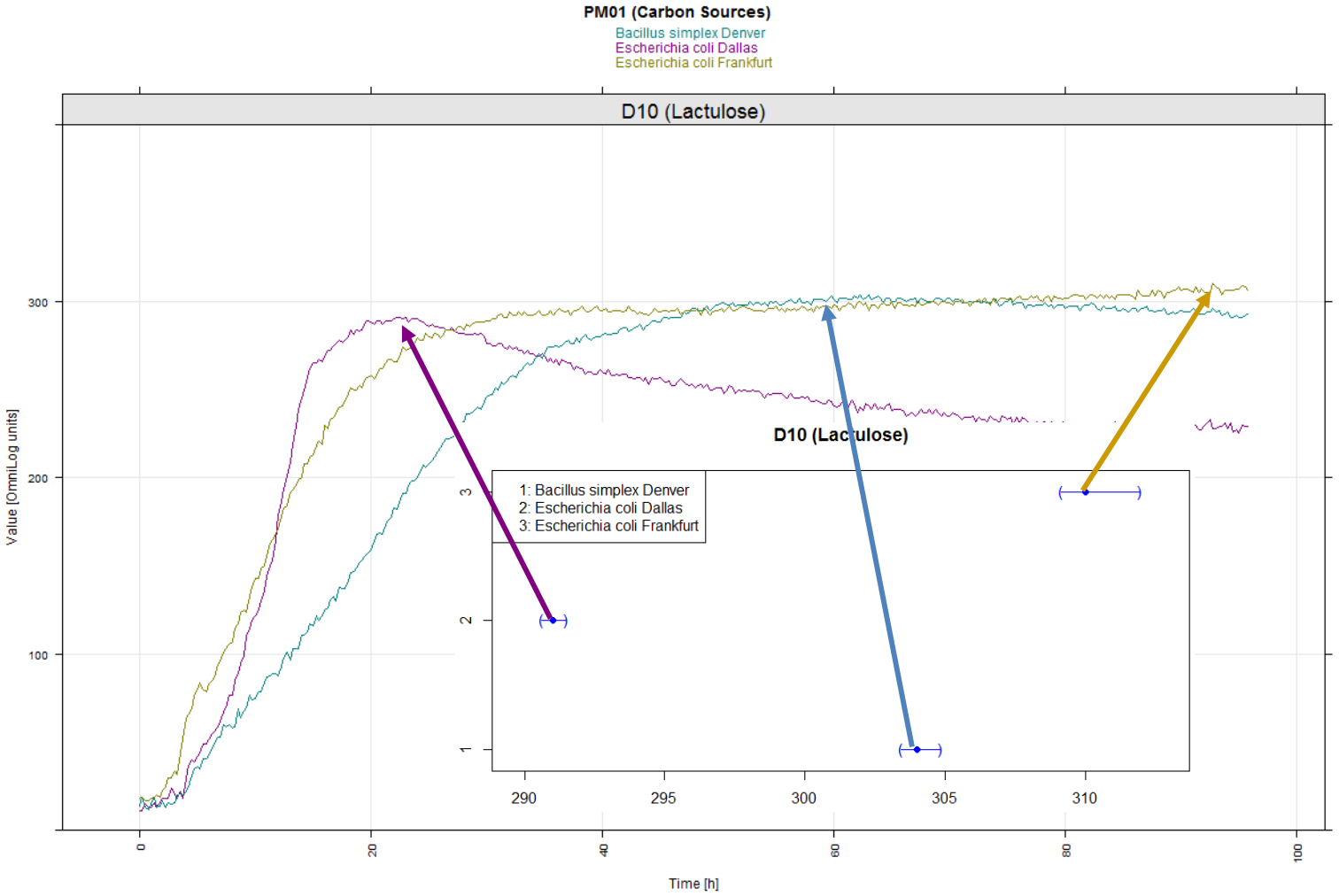
- **user-defined comparisons**
- **inherent multiplicity adjustment**
- **significance of difference**
- ***and* effect size visible**
- **on original scale**

Hothorn,T. et al. (2008) Simultaneous inference in general parametric models. Biometr. J. 50. 346–363.



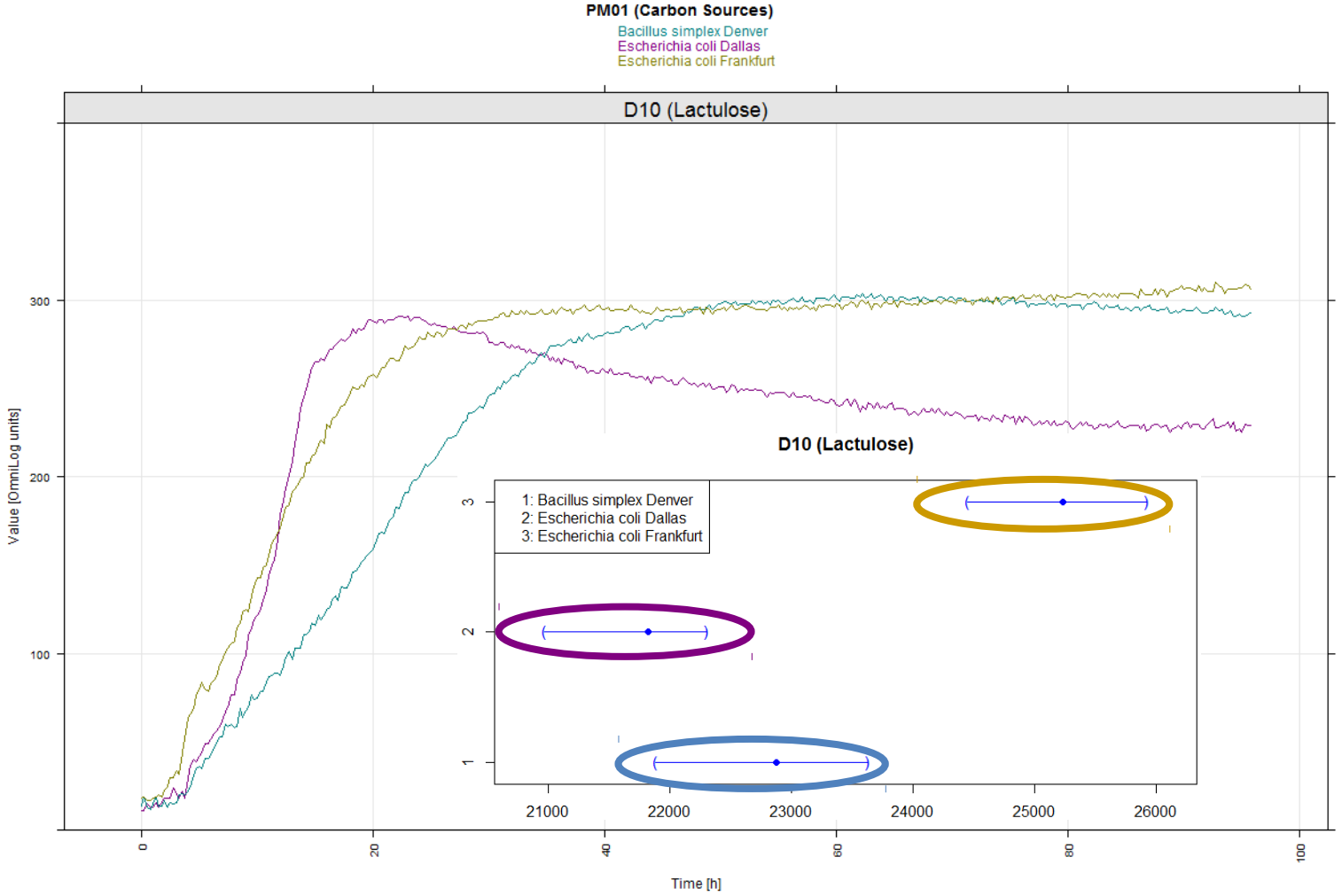
Subsetting and exploration of details

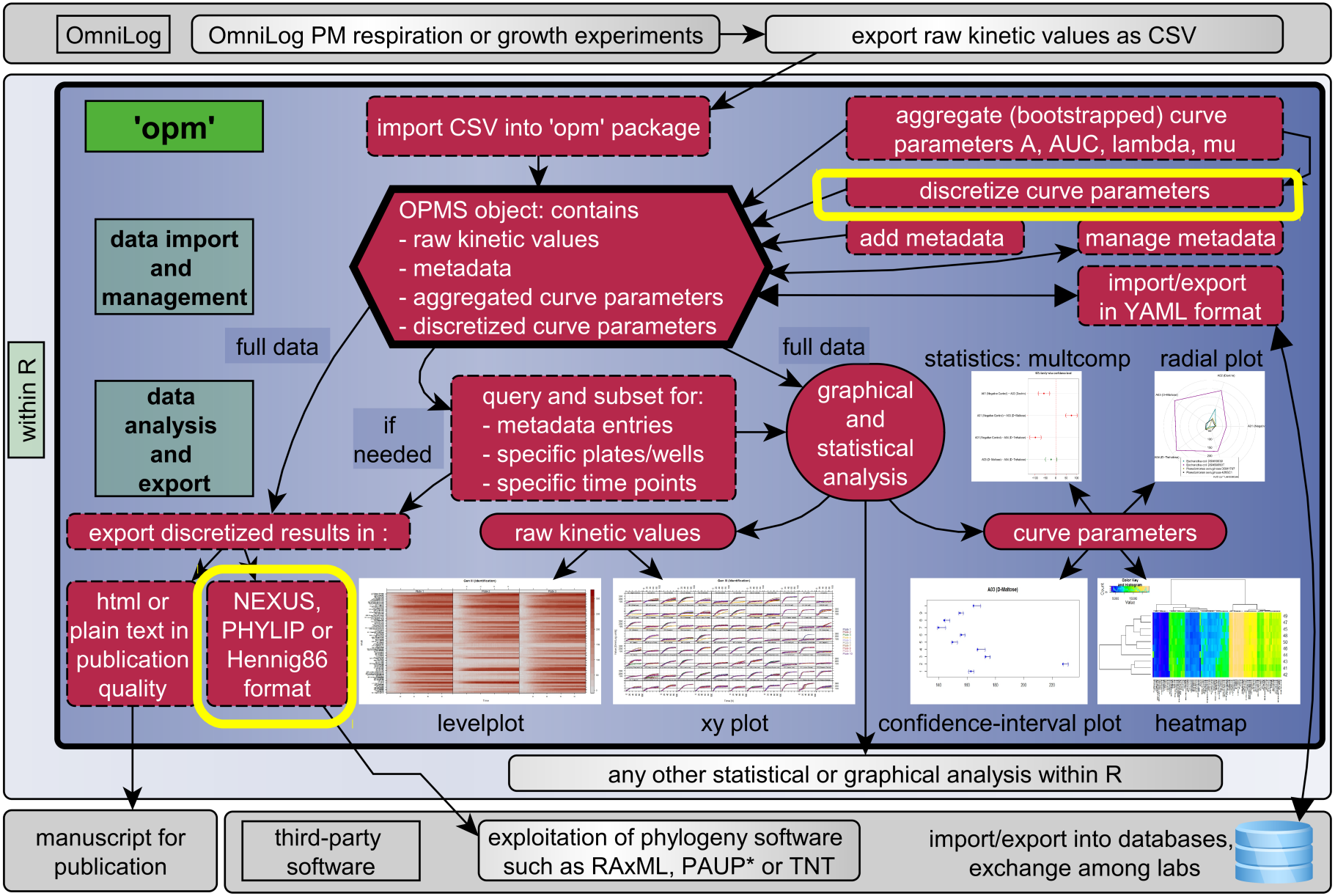
Example: A (maximum)



Subsetting and exploration of details

Example: lambda (lag phase)

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Discretization

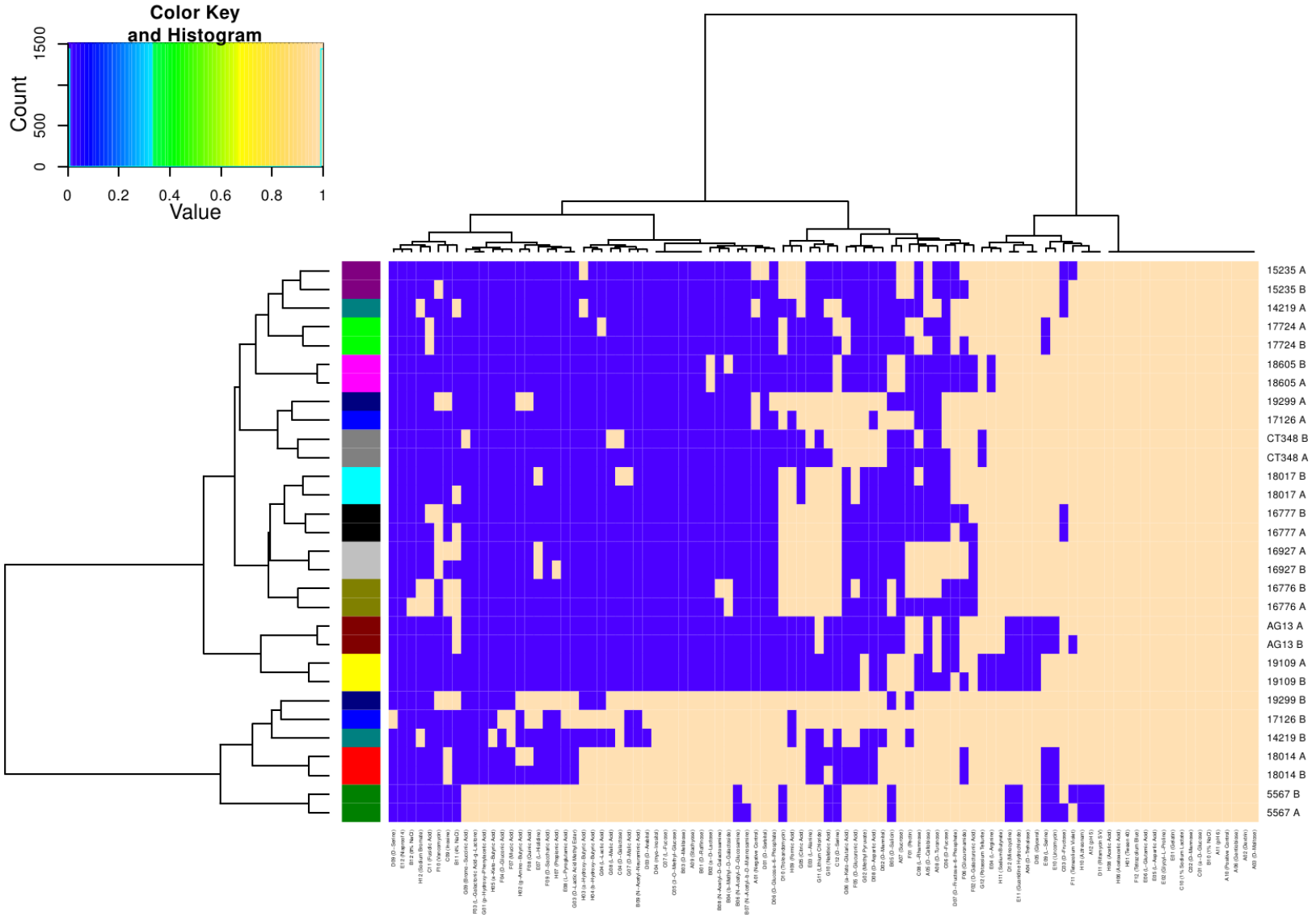
- **multi-state characters**

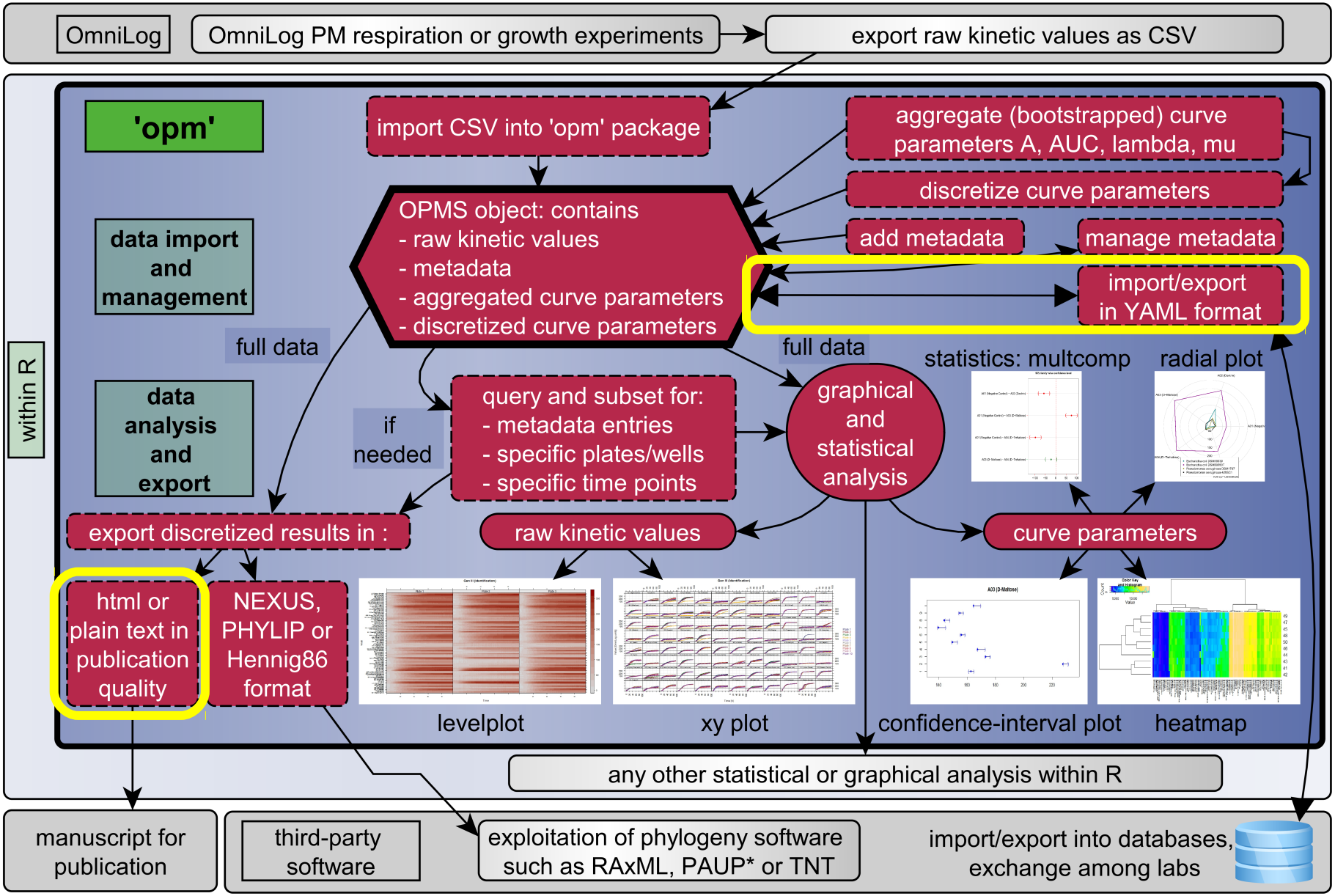
```
#NEXUS

begin data;
   dimensions ntax = 4 nchar = 96;
   format datatype = standard missing = ?;
   format symbols = "0123456789ABCDEFGHIJKLMNOPQRSTUV";
   charlabels 'A01 (Negative Control)' 'A02 (Dextrin)' 'A03 (D-Maltose)' 'A04 (D-Trehalose)' 'A05 (D-Cellobiose)' 'A0
   matrix
'Escherichia coli DSM18039'         4B2311111TSS131104106RPN23220059DQP9H6007MM2LQQQ3NL3M332MRRS3NLNN8N2NRVV2E2O1NNO7
'Escherichia coli DSM30083T'        ALPNFPGDDTSTHNOOGQOPQRPPONNOBBPOOQPNON5FOPPDQPRT8OQIQI89QSSS6PPPQA444SVV4J4R3PQQM
'Pseudomonas aeruginosa DSM1707'    450421111RRS11012C001ROK30B03003IQOF2G40C2523PPS3PKLOPPQERST232N362O3RVVO62QNP7O6
'Pseudomonas aeruginosa 429SC1'     453333334RRT43232O334SQNO4N53754NRPS4NN3O2525QPS6QOQQRRRLRST332P372Q2QVVQ82SPR2P8
   ;
end;
```

- **binary characters (positive, negative, weak/ambiguous)**
- **two partitioning algorithms**
- **optionally applied group-wise**
- **two concepts of “ambiguity”**

Plotting discretized curve parameters: heat map

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Output: text and tables

13-10

Positive for γ-amino-n-butyric acid, δ-amino-valeric acid, butyric acid, capric acid, caproic acid, 4-hydroxy-benzoic acid, β-hydroxy-butyric acid, malonic acid, quinic acid, L-alaninamide, L-arginine, L-histidine, L-homoserine, 4-hydroxy-L-proline (trans), L-isoleucine, L-leucine, L-lysine, L-ornithine, L-pyroglutamic acid, L-valine, D,L-carnitine, putrescine and dihydroxy-acetone.

Negative for negative control, chondroitin sulfate C, α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, dextrin, gelatin, glycogen, inulin, laminarin, mannan, pectin, N-acetyl-D-galactosamine, N-acetyl-neuraminic acid, β-D-allose, amygdalin, D-arabinose, D-arabitol, L-arabitol, arbutin, 2-deoxy-D-ribose, m-erythritol, D-fucose, 3-O-β-D-galactopyranosyl-D-arabinose, β-gentiobiose, L-glucose, D-lactitol, D-melezitose, maltitol, α-methyl-D-glucoside, β-methyl-D-galactoside, 3-O-methyl-D-glucose, β-methyl-D-glucuronic acid, α-methyl-D-mannoside, β-methyl-D-xylopyranoside, palatinose, D-raffinose, D-salicin, sedoheptulosan, L-sorbose, stachyose, D-tagatose, turanose, xylitol, N-acetyl-D-glucosaminitol, citraconic acid, D-citramalic acid, D-glucosamine, 2-hydroxy-benzoic acid, γ-hydroxy-butyric acid, α-keto-valeric acid, itaconic acid, 5-keto-D-gluconic acid, D-lactic acid methyl ester, melibionic acid, oxalic acid, oxalomalic acid, D-ribono-1,4-lactone, sebacic acid, succinamic acid, D-tartaric acid, L-tartaric acid, acetamide, N-acetyl-L-glutamic acid, glycine, L-methionine, L-phenylalanine, butylamine (sec), D,L-octopamine, 2,3-butanediol, 2,3-butanedione and 3-hydroxy-2-butanone.

Ambiguous for sorbic acid.

13-9

Positive for γ-amino-n-butyric acid, δ-amino-valeric acid, caproic acid, 4-hydroxy-benzoic acid, β-hydroxy-butyric acid, malonic acid, quinic acid, L-arginine, 4-hydroxy-L-proline (trans), L-isoleucine, L-pyroglutamic acid, L-valine, D,L-carnitine and putrescine.

Negative for negative control, chondroitin sulfate C, α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, dextrin, gelatin, glycogen, inulin, laminarin, mannan, pectin, N-acetyl-D-galactosamine, N-acetyl-neuraminic acid, β-D-allose, amygdalin, D-arabinose, D-arabitol, L-arabitol, arbutin, 2-deoxy-D-ribose, m-erythritol, D-fucose, 3-O-β-D-galactopyranosyl-D-arabinose, β-gentiobiose, L-glucose, D-lactitol, D-melezitose, maltitol, α-methyl-D-glucoside, β-methyl-D-galactoside, 3-O-methyl-D-glucose, β-methyl-D-glucuronic acid, α-methyl-D-mannoside, β-methyl-D-xylopyranoside, palatinose, D-raffinose, D-salicin, sedoheptulosan, L-sorbose, stachyose, D-tagatose, turanose, xylitol, N-acetyl-D-glucosaminitol, citraconic acid, D-citramalic acid, D-glucosamine, 2-hydroxy-benzoic acid, γ-hydroxy-butyric acid, α-keto-valeric acid, itaconic acid, 5-keto-D-gluconic acid, D-lactic acid

Characters exported by opm version 0.8.17

Organisms: 1, *13-10*; 2, *13-9*.

Symbols: -, negative reaction; w, weak reaction; +, positive reaction.

	1	2
A01 (Negative Control)	-	-
A02 (L-Arabinose)	-/+	-
A03 (N-Acetyl-D-Glucosamine)	-	-
A04 (D-Saccharic Acid)	+	+
A05 (Succinic Acid)	+	+
A06 (D-Galactose)	-	-
A07 (L-Aspartic Acid)	+	+
A08 (L-Proline)	+	+
A09 (D-Alanine)	+	+
A10 (D-Trehalose)	-	-
A11 (D-Mannose)	-	-
A12 (Dulcitol)	-	-
B01 (D-Serine)	-	-
B02 (D-Sorbitol)	-	-
B03 (Glycerol)	+	+
B04 (L-Fucose)	-	-
B05 (D-Glucuronic Acid)	+	+
B06 (D-Gluconic Acid)	+	+
B07 (D,L-α-Glycerol-Phosphate)	-	-
B08 (D-Xylose)	+	+
B09 (L-Lactic Acid)	+	+

Output: YAML (cross-language serialization)

```
---
- metadata:
    Strain: 13-10
    Replicate: II
  csv_data:
[...]
  measurements:
    Hour:
    - 0.00
    - 0.25
[...]
    A01:
    - 30.0
    - 24.0
[...]
  aggregated:
    A01:
      mu: 4.910216
      lambda: -2.095738
      A: 52.18975
      AUC: 4422.683
[...]
```

- stored metadata
- raw measurements
- estimated curve parameters

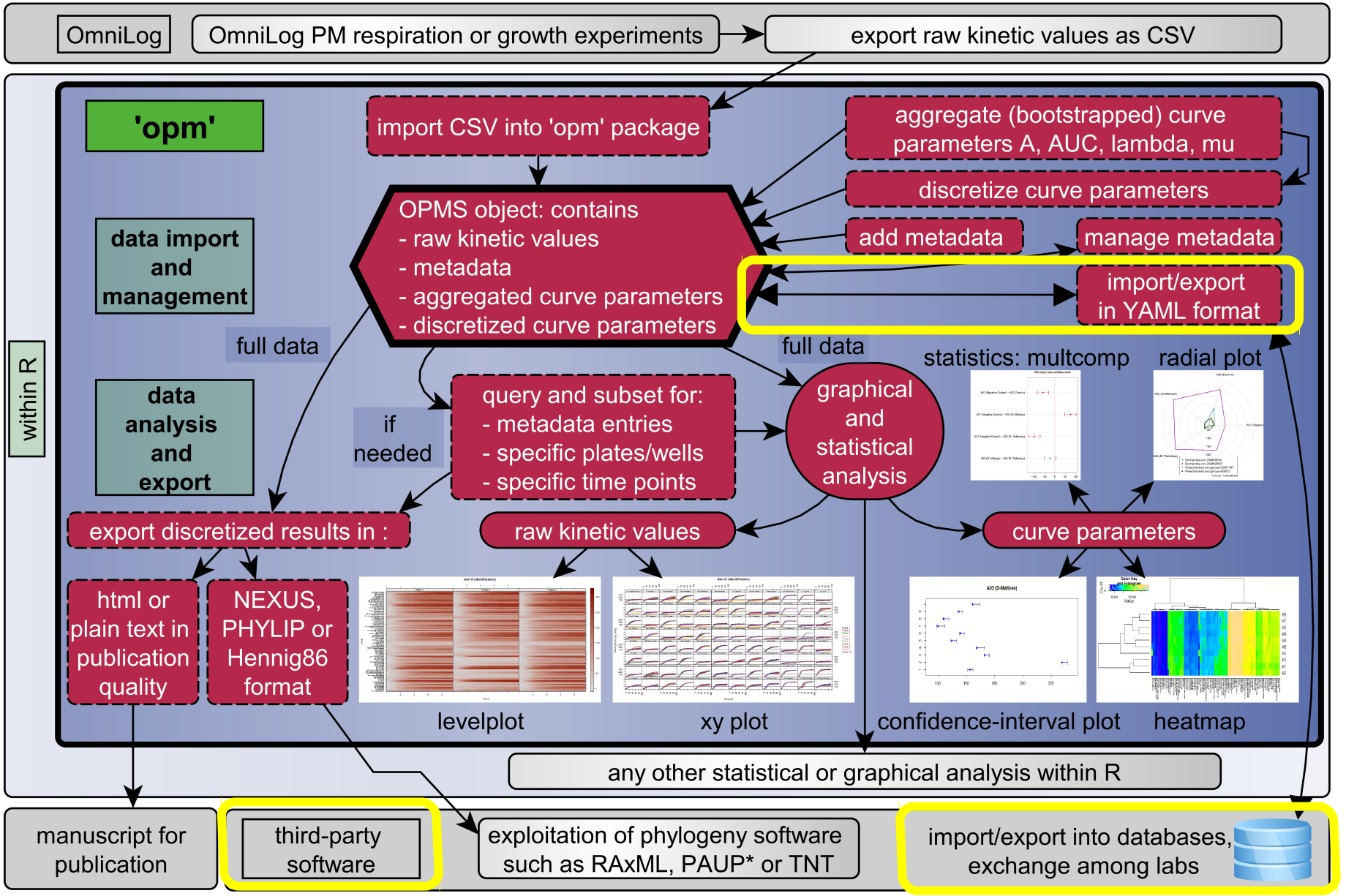
```
[...]
  aggr_settings:
    method: grofit
    options:
      neg.nan.act: FALSE
      clean.bootstrap: TRUE
[...]
    software: opm
    version: 0.8.17
  discretized:
    A01: FALSE
    A02: FALSE
[...]
  disc_settings:
    method: kmeans
    options:
      cutoffs: 157.1985
      datasets: 4
    software: opm
    version: 0.8.17
```

- parameter estimation settings
- discretized values
- discretization settings

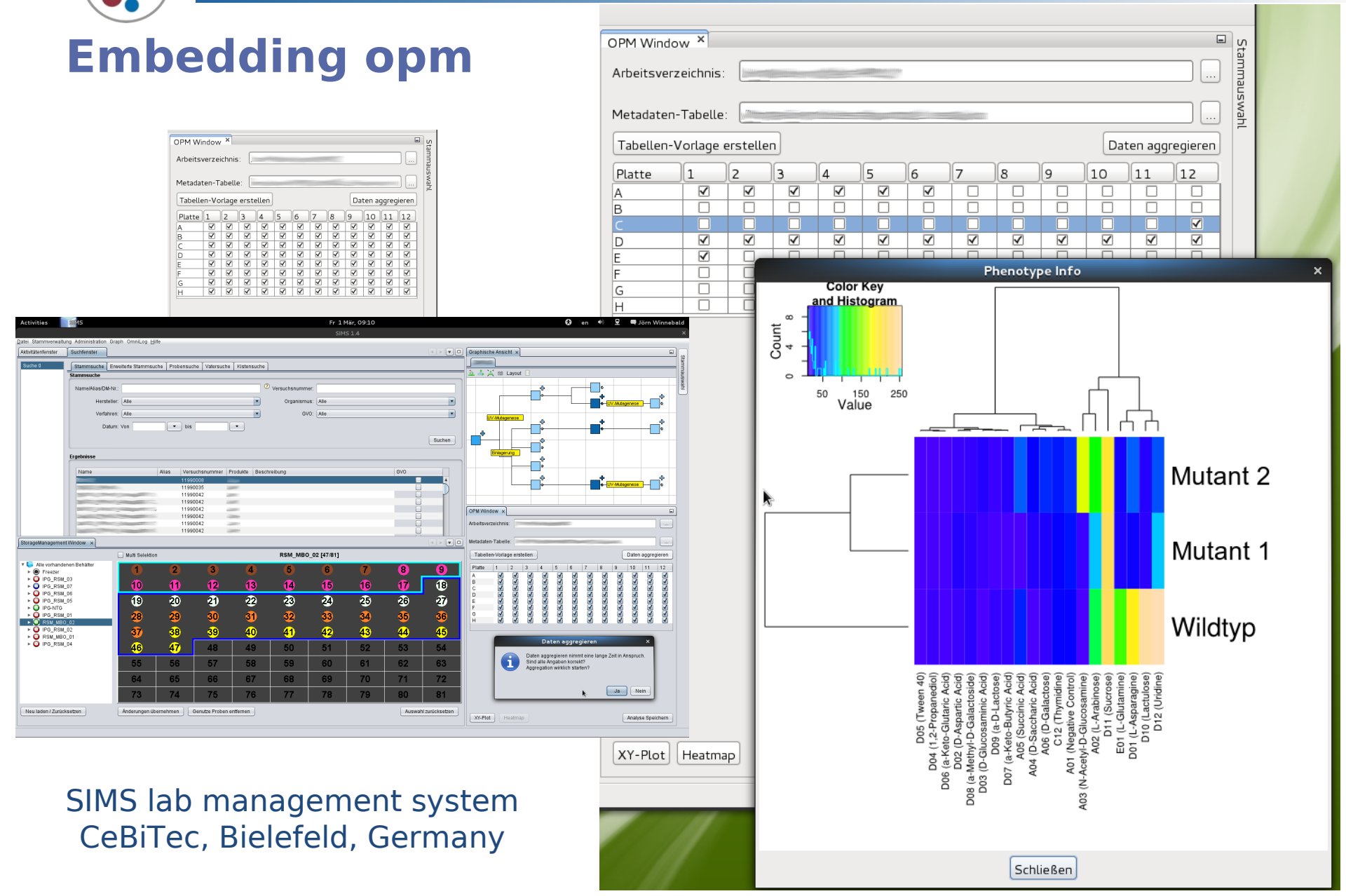
Code example

```
library(opm)

x <- read_opm(getwd(), convert = "grp", include = list("csv"))
x <- lapply(x, function(item) {
  md <- to_metadata(csv_data(item))
  md <- do.call(rbind, strsplit(md$`Strain Number`, " ", fixed = TRUE))
  colnames(md) <- c("Strain", "Replicate")
  metadata(item) <- to_metadata(md)
  item
})

x <- lapply(lapply(x, do_aggr, boot = 0L, cores = 8L), do_disc, cutoff = FALSE)

file.copy(grep("[.]css$", opm_files("auxiliary"), value = TRUE),
  "opm_styles.css", overwrite = TRUE)
opm_opt(css.file = "opm_styles.css")

for (plate in names(x)) {
  write(to_yaml(x[[plate]]), file = sprintf("Data_%s.yml", plate))
  text <- listing(x[[plate]], as.groups = "Strain", html = TRUE)
  write(phylo_data(text), sprintf("Description_%s.html", plate))
  text <- phylo_data(x[[plate]], format = "html", as.labels = "Strain")
  write(text, sprintf("Table_%s.html", plate))
  pkgutils::mypdf(sprintf("Plot_%s.pdf", plate))
  print(xy_plot(x[[plate]], include = list("Strain", "Replicate")))
  dev.off()
}
```

- **read files**
- **set up metadata**
- **aggregate & discretize**
- **for all plate types in input: create formatted text, table, and plots**


OmniLog
OmniLog PM respiration or growth experiments
export raw kinetic values as CSV
'opm'
import CSV into 'opm' package
aggregate (bootstrapped) curve parameters A, AUC, lambda, mu
discretize curve parameters
OPMS object: contains
- raw kinetic values
- metadata
- aggregated curve parameters
- discretized curve parameters
add metadata
manage metadata
import/export in YAML format
data import and management
within R
full data
full data
statistics: multcomp
radial plot
data analysis and export
if needed
query and subset for:
- metadata entries
- specific plates/wells
- specific time points
graphical and statistical analysis
export discretized results in :
raw kinetic values
curve parameters
html or plain text in publication quality
NEXUS, PHYLIP or Hennig86 format
levelplot
xy plot
confidence-interval plot
heatmap
any other statistical or graphical analysis within R
manuscript for publication
third-party software
exploitation of phylogeny software such as RAxML, PAUP* or TNT
import/export into databases, exchange among labs

Embedding opm



SIMS lab management system
CeBiTec, Bielefeld, Germany

Summary: opm

- robust statistical analysis of PM data
- flexible metadata management
- flexible production of high-quality graphics
- no restrictions regarding user-defined analyses
- reproducible research
- easy interaction with other software
- easily extendable by the user
- interactive or fully automated usage possible

opm availability

- **http://opm.dsmz.de/**
- **manual, tutorial, mailing list etc.**
- **open project, hosted at R-Forge**
- **programmers and advanced users are invited to join**