EDA organoid partition

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Loading packages

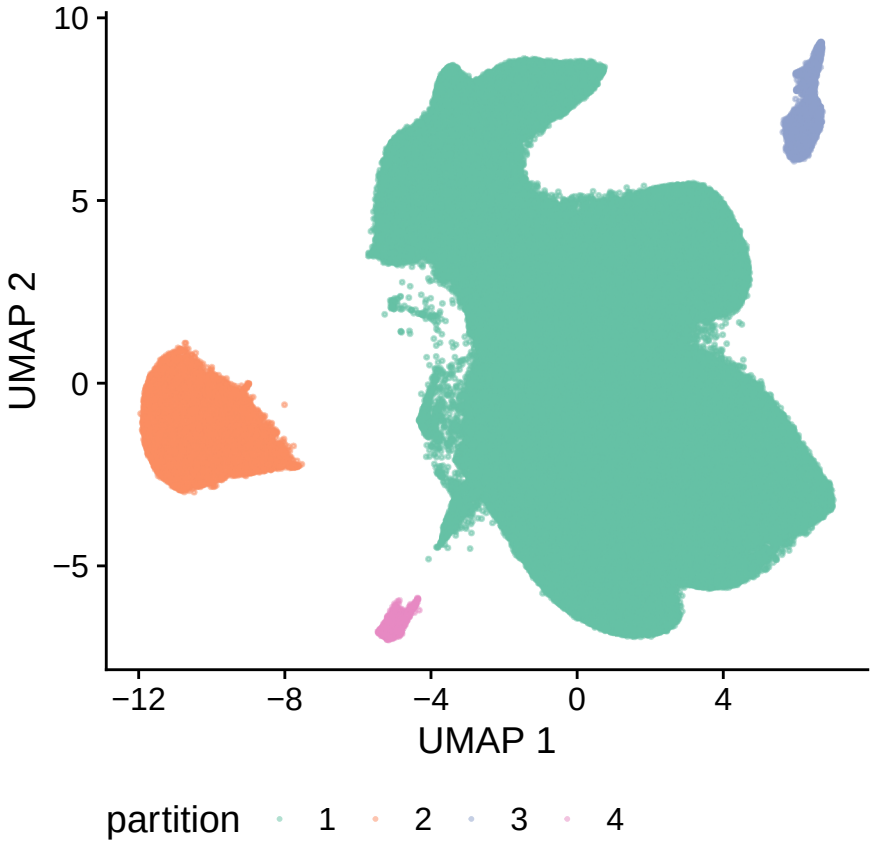
```
## [1] "parameter input:"
```

```
## [1] "data/processed/PhenotypeSpectrum/filtered_lenient/umap_absolute_all_drugs_sampled.Rds"
```

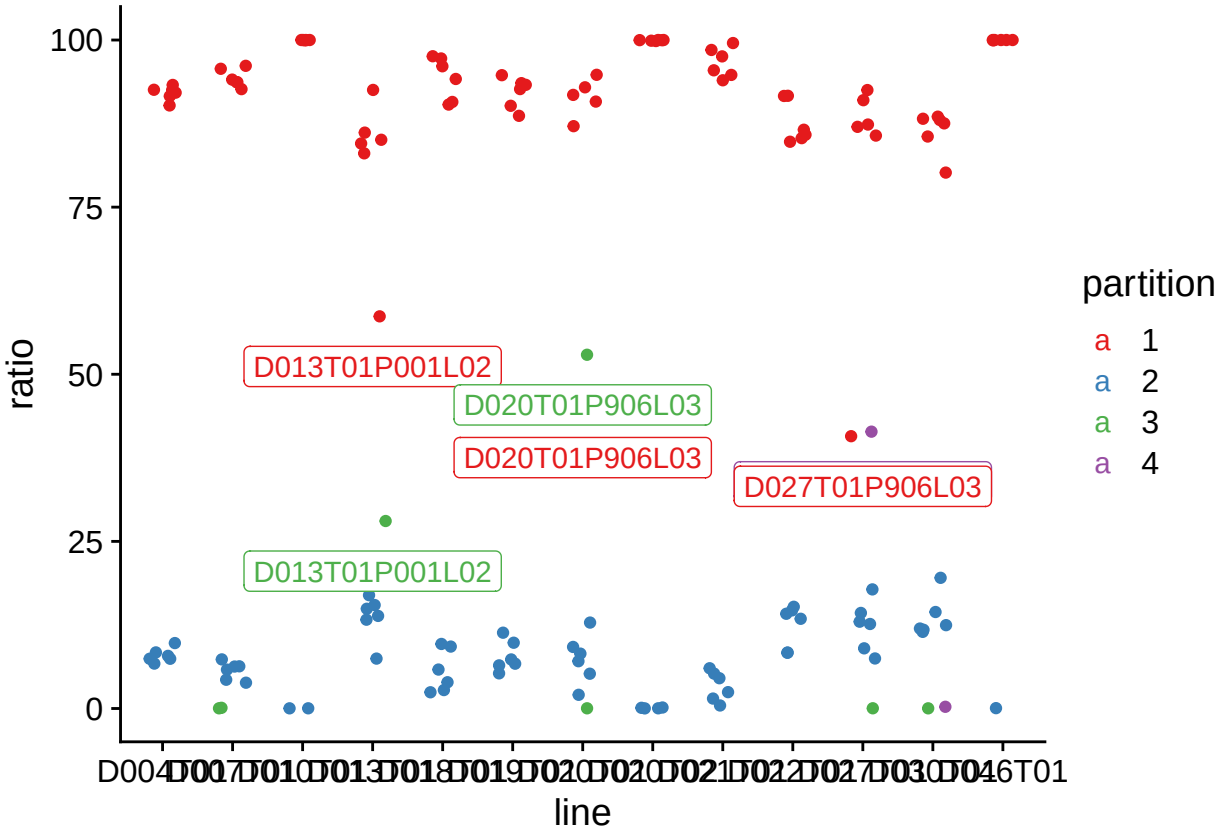
loading input data and annotation. Note that on the central cluster, with access to the complete data table, the definition of the input can easily be changed. For remote work, the subsampled dataset "umap_drugs_sampled.Rds" is the default choice.

Partition inspection

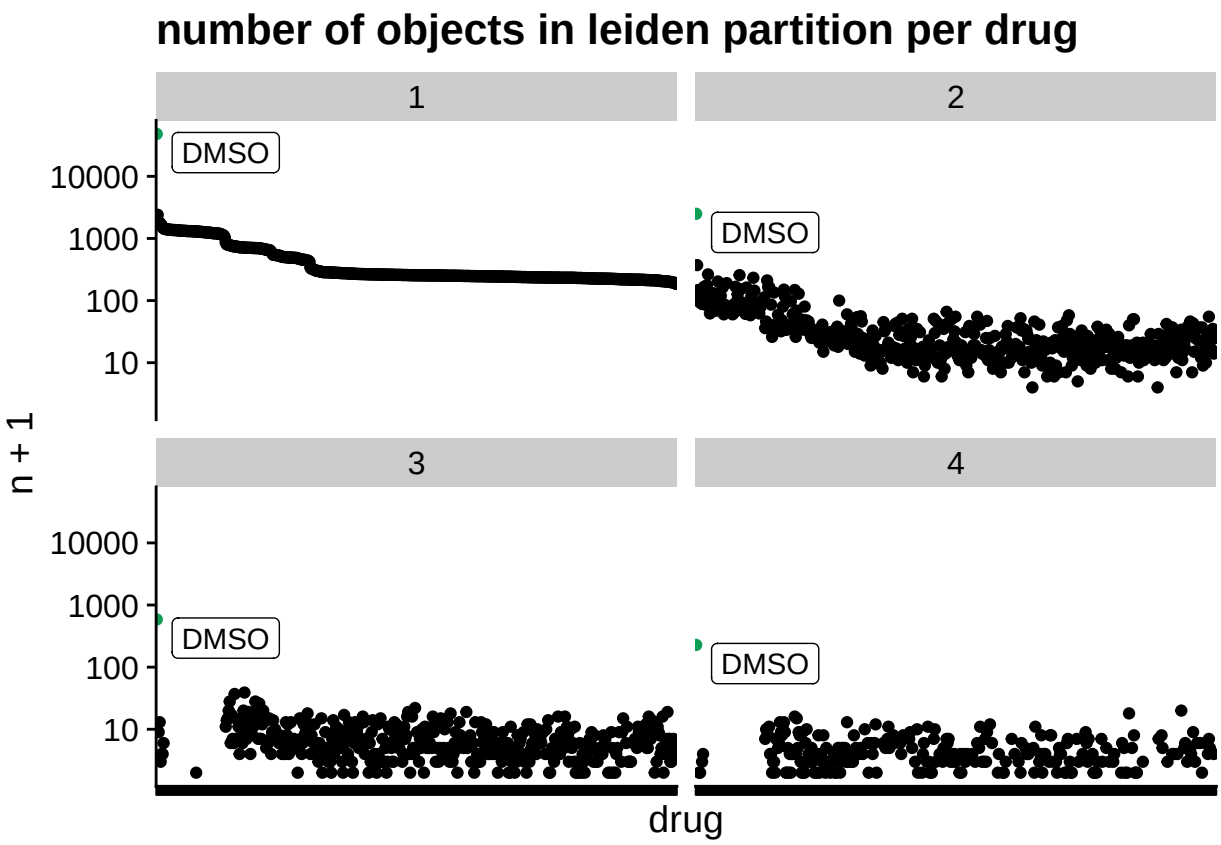
We are able to observe 4 partitions in our data. After manual inspection, it becomes cleat that the two smallest partitions are mostly consisting of

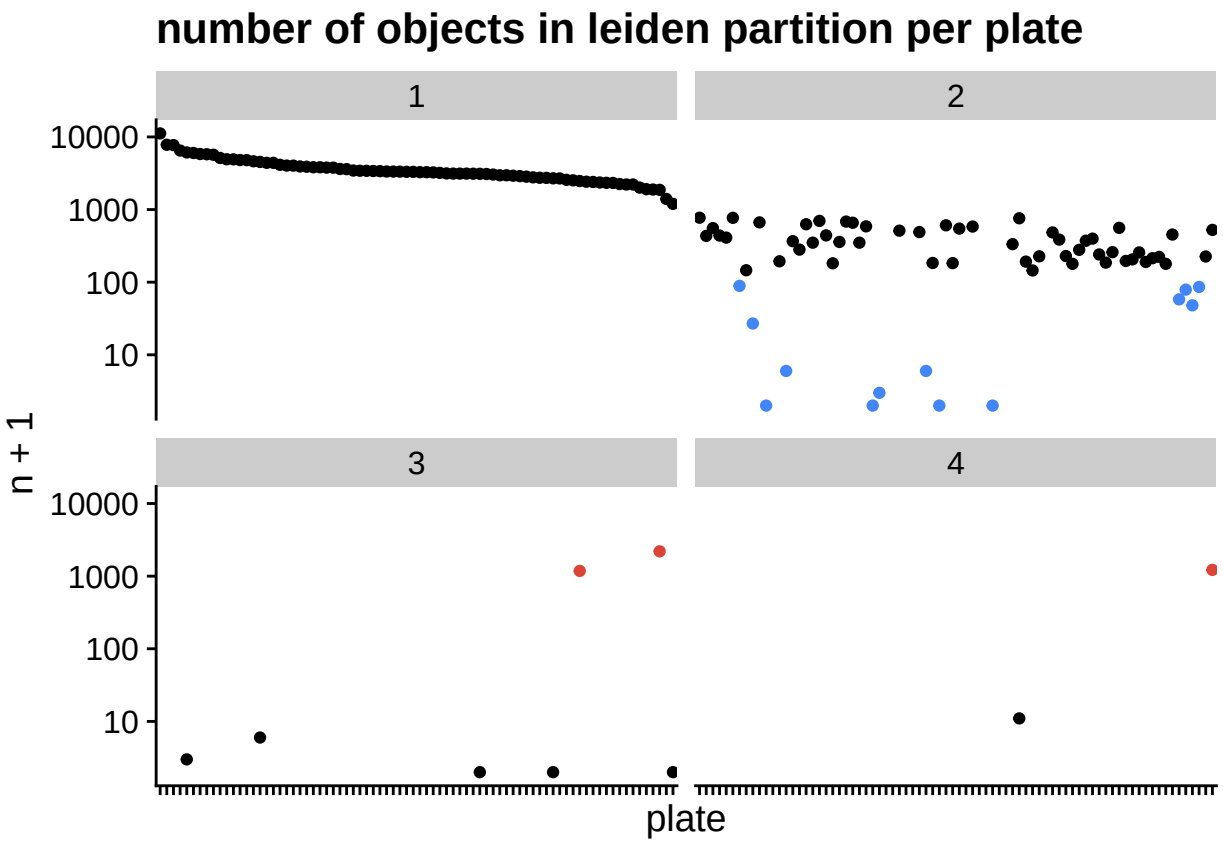


partition	n	ratio	min_ratio	max_ratio
1	283583	91.621	40.734	100.000
2	21320	6.888	0.020	19.551
3	3385	1.094	0.026	52.917
4	1228	0.397	0.258	41.414


100
75
50
25
0
ratio
D013T01P001L02
D020T01P906L03
D020T01P906L03
D027T01P906L03
D013T01P001L02
line
partition
a 1
a 2
a 3
a 4

drug overrepresentation





chi-square

I wonder wether certain batches or organoid lines are overrepresented in each section.

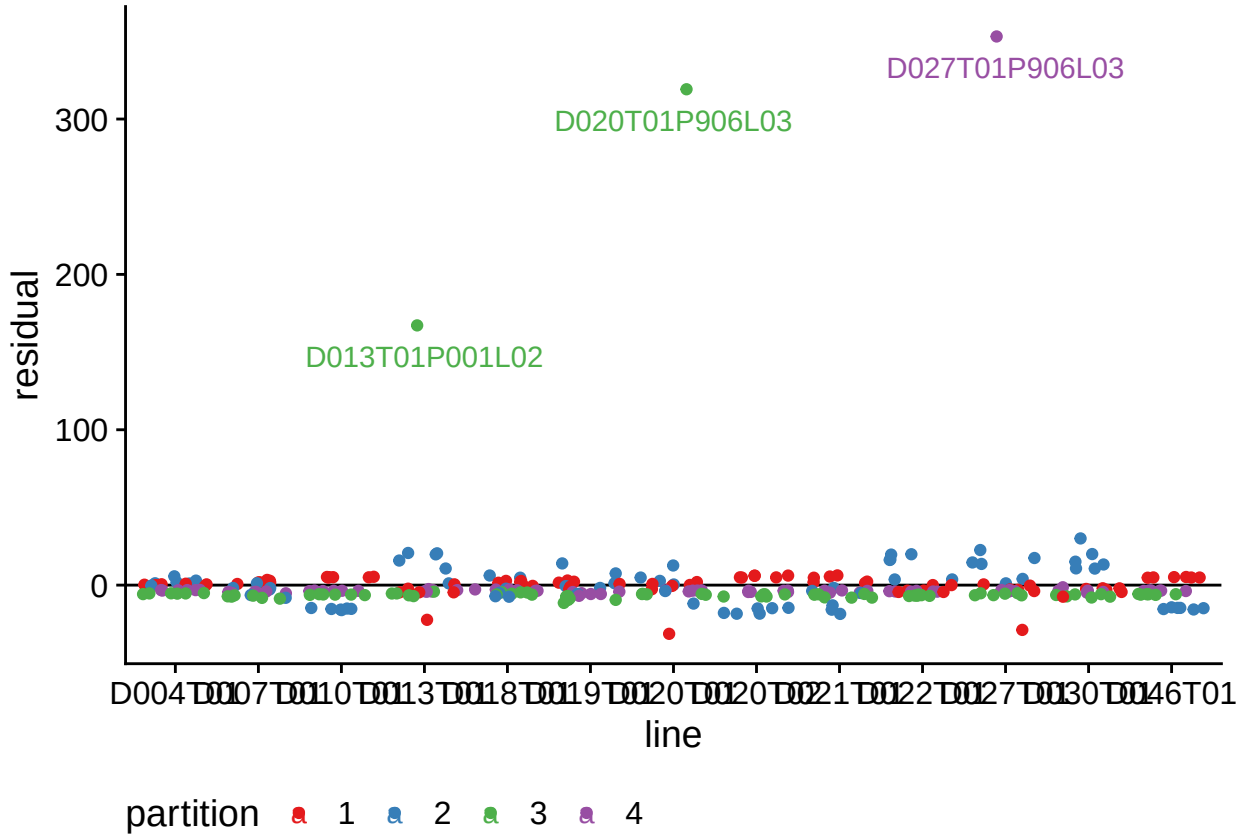
drug	partition	residual
DMSO	2	−17.612616
Staurosporine_500nM	3	−5.507963
AT9283	1	−4.715373
Bortezomib	3	−4.249241
AZD2858	1	−4.246050
Bortezomib	2	13.997972
Irinotecan / SN−38	2	14.602106
BGT226 (NVP−BGT226)	4	14.805251
AT9283	2	16.524639
IKK−16 (IKK Inhibitor VII)	4	17.110454

line overrepresentation

plate	partition	residual
D027T01P906L03	4	353.15192
D020T01P906L03	3	319.15945
D013T01P001L02	3	167.18040
D030T01P906L03	2	30.04231
D027T01P906L03	2	22.58248
D020T02P013L02	2	−18.39796
D021T01P003L08	2	−18.52301
D013T01P001L02	1	−22.33260
D027T01P906L03	1	−28.83074
D020T01P906L03	1	−31.34671

I plot chisq residuals for each plate

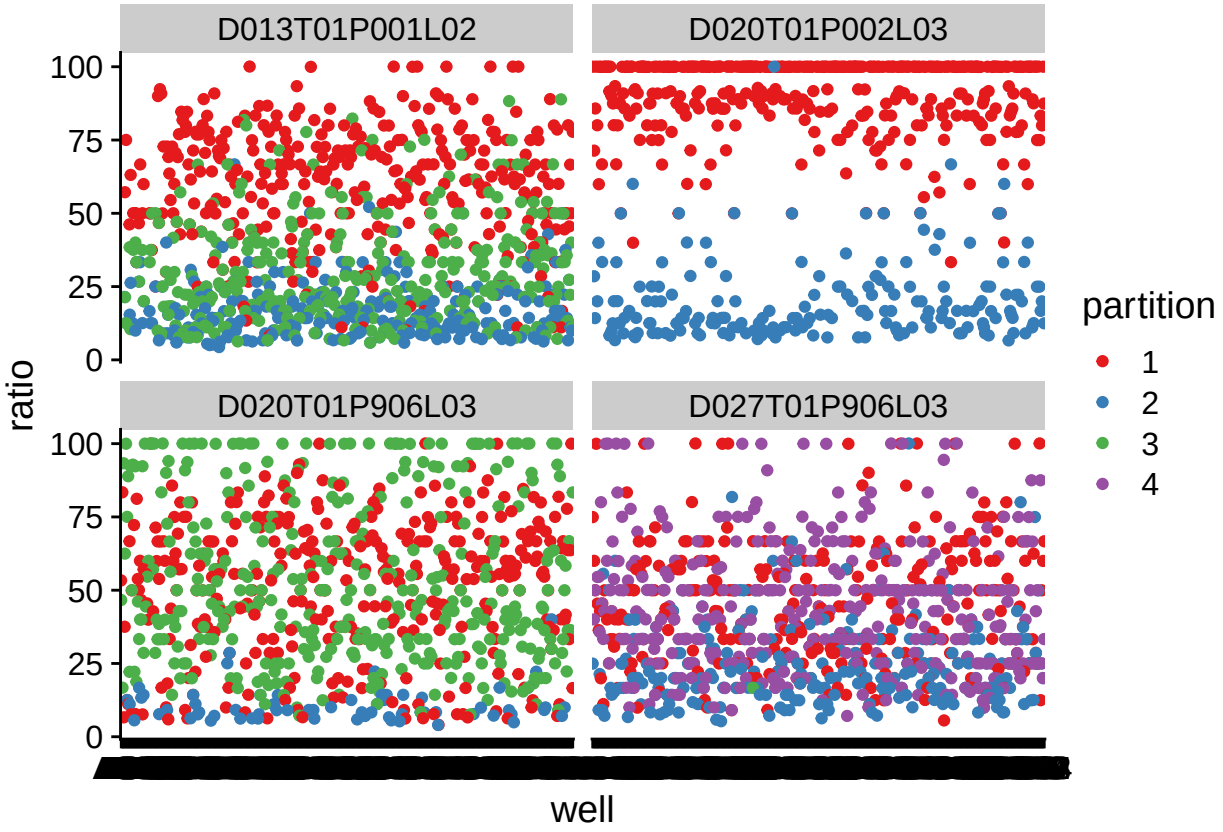
I recognize no difference between reimaged plates (leading digit is “9”, plates were reimaged due to errors during the first pass) and plates that were not reimaged.



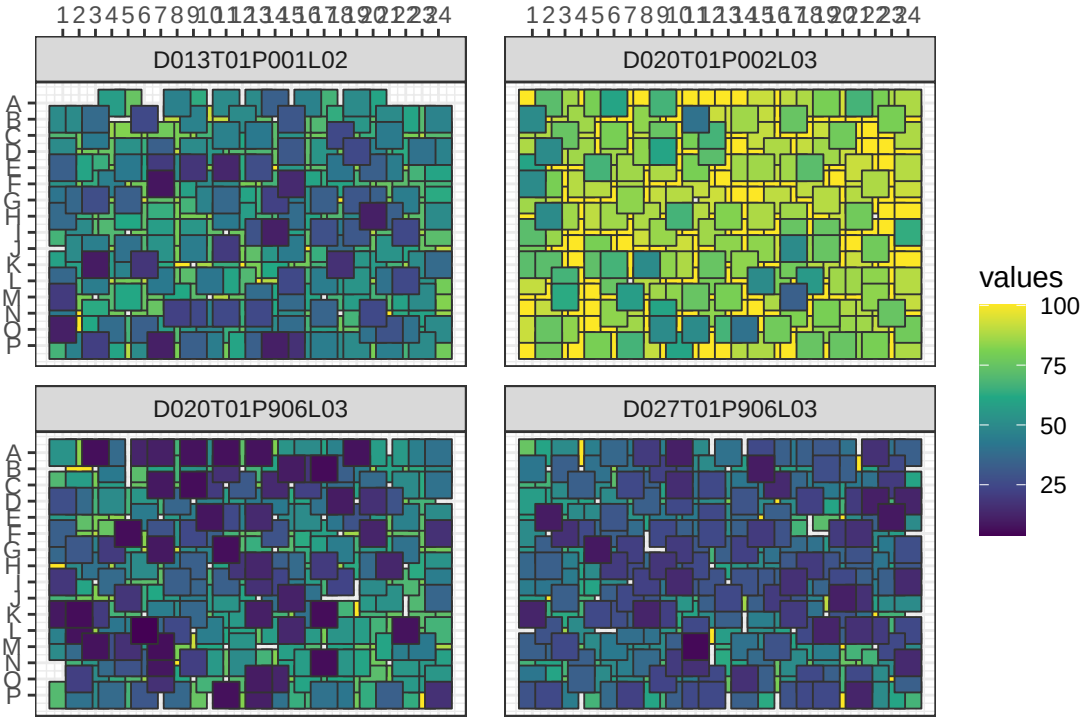
multinomial regression

We run a multinomial regression using the *nnet* package. I loaded the models in the beginning of the vignette.

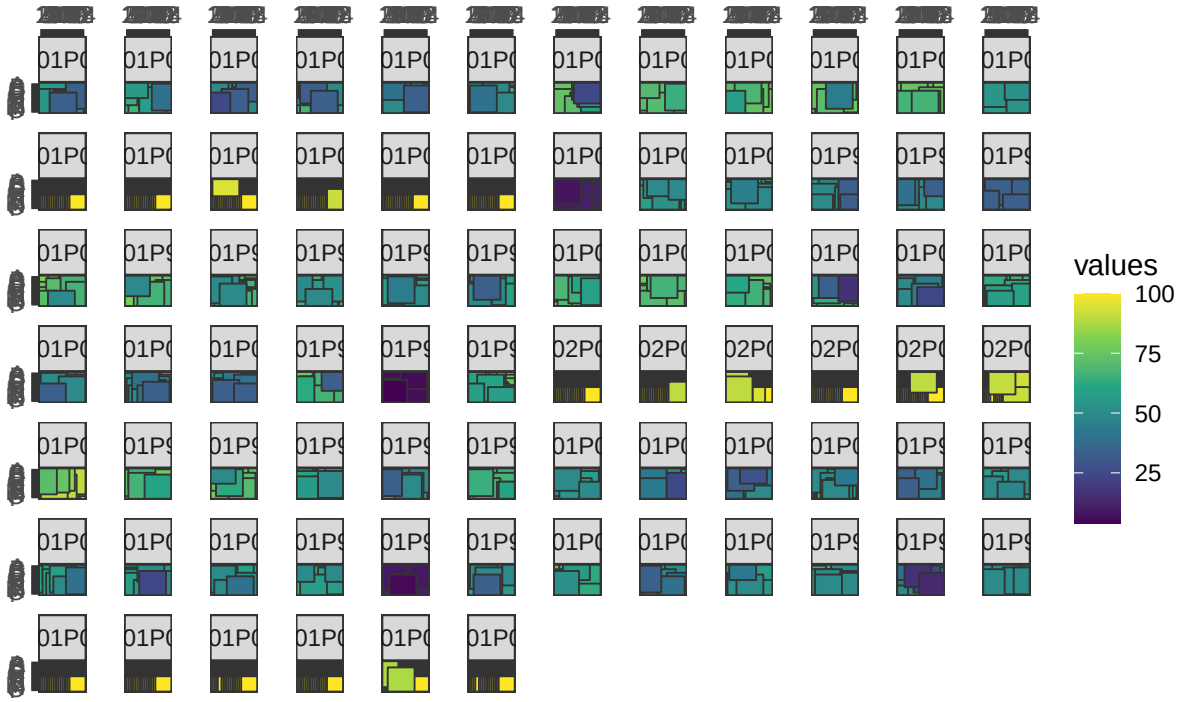
plate inspection



proportion of objects belonging to partition 1



D020T01P002L03 is a plate with one of the lowest chi−square residues



There appear to be three groups: * almost all organoids are within partition one, holds true for D010, D020 and D046 * 75% of objects are within partition one * almost no organoids are within partition one, this is true for the three plates previously identified and discussed below.

observation

- after processing, organoids organize in 4 distinct phenotype partitions
- the distribution of organoids across these partitions is non-random
  - the screening plate influences the distribution of organoids across partitions, 3 plates show strong deviation from the expected distribution, both in a chi-square test **and** in a multinomial regression. The feature **plate** is more predictive than the organoid **line** or **screen_id**
    - D027T01P906L03
    - D020T01P906L03
    - D013T01P001L02
  - drug treatment influences the distribution of organoids across the partitions
    - DMSO control treatment are depleted in sector 2, sector 2 has previously been shown to contain dead and small organoids
    - SN38 and bortezomib are enriched in sector 2

conclusion

- given the patterns above, I believe it is most likely we are seeing systematic errors (not dependent on the biological axes of line and drug) in plates:

  - D027T01P906L03
  - D020T01P906L03
  - D013T01P001L02
- in addition we are observing deviations that are drug dependent, that I consider signal

next steps

- inspect the three plates manually
- remove data in case there is more evidence for otherwise non-recoverable systematic noise

figure

```
## R version 4.0.0 (2020-04-24)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 20.04.2 LTS
##
## Matrix products: default
## BLAS/LAPACK: /usr/lib/x86_64-linux-gnu/openblas-pthread/libopenblasp-r0.3.8.so
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=en_US.UTF-8        LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=C
##  [7] LC_PAPER=en_US.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
##  [1] nnet_7.3-13      platetools_0.1.3 gridExtra_2.3    ggridges_0.5.2
##  [5] scico_1.1.0      princurve_2.1.4  cowplot_1.0.0    ggrastr_0.2.3
##  [9] here_0.1         forcats_0.5.0    stringr_1.4.0    dplyr_1.0.0
## [13] purrr_0.3.4      readr_1.3.1      tidyr_1.1.0      tibble_3.0.1
## [17] ggplot2_3.3.1    tidyverse_1.3.0
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_1.0.4.6       lubridate_1.7.8    lattice_0.20-41    assertthat_0.2.1
##  [5] rprojroot_1.3-2    digest_0.6.25      R6_2.4.1           cellranger_1.1.0
##  [9] plyr_1.8.6         backports_1.1.7    reprex_0.3.0       evaluate_0.14
## [13] httr_1.4.1         pillar_1.4.4       rlang_0.4.6        readxl_1.3.1
## [17] rstudioapi_0.11    blob_1.2.1         rmarkdown_2.2      labeling_0.3
## [21] munsell_0.5.0      broom_0.5.6        compiler_4.0.0     vipor_0.4.5
## [25] modelr_0.1.8       xfun_0.14          pkgconfig_2.0.3    ggbeeswarm_0.6.0
## [29] htmltools_0.4.0    tidyselect_1.1.0   viridisLite_0.3.0  fansi_0.4.1
## [33] crayon_1.3.4       dbplyr_1.4.4       withr_2.2.0        grid_4.0.0
## [37] nlme_3.1-147       jsonlite_1.6.1     gtable_0.3.0       lifecycle_0.2.0
## [41] DBI_1.1.0          magrittr_1.5       scales_1.1.1       cli_2.0.2
## [45] stringi_1.4.6      farver_2.0.3       fs_1.4.1           xml2_1.3.2
```

```
## [49] ellipsis_0.3.1     generics_0.0.2     vctrs_0.3.1        RColorBrewer_1.1-2
## [53] Cairo_1.5-12       tools_4.0.0        glue_1.4.1         beeswarm_0.2.3
## [57] hms_0.5.3          yaml_2.2.1         colorspace_1.4-1   rvest_0.3.5
## [61] knitr_1.28         haven_2.3.1
```