

# LOG & EXTENDED README

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## Project: radseq pilot

RADseq optimization pilot experiment with several Antarctic species.

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## Log:

29/06/2021: added coverage calculation in script 05\_\_marker\_\_density.R

24/06/2021: changed and rerun empirical digestion plots, set alpha to 1 and changed plotting order to avoid overlays

23/06/2021: added digestion with a standard 1000 mb genome

10-14/06/2021: added empirical loci statistics and updated marker density calculations

05-06/2021: added new functions and code to run marker density calculations, new script: 05\_\_marker\_\_density.R

24/04/2021: added extensive documentation

29/03/2021: rerun 01\_\_digests.R again

24/03/2021: updated and rerun 01\_\_digests.R for some additional size windows

22/03/2021: updated amphipod species ID

01/03/2021: updated bird plots in 02\_\_empirical\_\_digests.R

23/02/2021: updated double digest calculations in recto\_REs\_and\_functions.R and 02\_\_empirical\_\_digests.R

22/02/2021: updated and rerun 02\_\_empirical\_\_digests.R, added input files for coverage calculations

21/02/2021: fixed some git issue with large .RDataTmp file

18/02/2021: updated and rerun 02\_\_empirical\_\_digests.R

17/02/2021: updated and consolidated code and files locally and on github, downgraded bioanalyzer to v0.5.1 again, the other version throws an error

22/01/2021: fixed xml encoding issues

20/01/2021: added new xml file for run 7, updated bioanalyzer to v0.6.2 and updated 02\_\_empirical\_\_digests.R

20/01/2021: added the project to a github repository

16/12/2020: added a modified read.bioanalyzer function to read in xml file with a missing lower marker in one sample

14/12/2020: small, mostly cosmetic updates in most scripts; updated project and input/output description; finalized 02\_empirical\_digests.R

11/12/2020: added customized plot saving function in recto\_REs\_and\_functions.R

10/12/2020: updated 02\_empirical\_digests.R, added a customized ggplot plotting function in recto\_REs\_and\_functions.R added in silico functions for comparison in recto\_REs\_and\_functions.R

12/11/2020: updated 02\_empirical\_digests.R & added a customized plotting function in recto\_REs\_and\_functions.R

11/11/2020: updated to run with R v4.0.3

07-10/2020: added script 02\_empirical\_digests.R to read results from bioanalyzer and analyze them in R, instead of cumbersome “manual” analysis in spreadsheets

20/07/2019: updated plot ratio scripts

10/07/2019: updated digest scripts

09/07/2019: updated the plots for test library 2

02/07/2019: updated digest scripts

28/06/2019: finalized coverage plot

27/06/2019: re-orderd scripts, included scripts for in silico digestion (previously stored in different R project)

23-27/06/2019: updated parameter plots (now based on correct de novo runs, with PE concatenating); included & updated coverage plot

16-22/06/2019: updated parameter plots; started script to plot targeted and realized coverage

01-14/06/2019: included scripts to plot n\_loci and n\_snps\_per\_locus from de novo parameter optimization series

04/12/2018: rerun due to updated input data (Trematomus mainly)

29/11/2018: created script 01\_plotting.R and ratio plots

28/11/2018: created R project & input data

## Input:

Various reference genomes from target species, or related species in fasta format under:

- ../refgenomes

Output files from the Agilent Bioanalyzer Software in XML format under:

- data/bioanalyzer\_results

CSV file with all metadata related to the Bioanalyzer runs, created manually:

- data/bioanalyzer\_results/run\_overview.csv

CSV/TSV files in data/test\_libraries with data from 5 test libraries:

- coverage\_stats.csv: number of loci and coverage for each library under different M values as reported in gstacks.log.distribs files

- density\_stats.csv: additional statistics for each library as reported in populations.log files
- various n\_snps\_per\_locus.tsv: files for each library/species and different r/M values with information about the loci and SNPs

R script that contains restriction enzyme information and various functions for analysis and plotting as used in the analyses scripts:

- scripts/recto\_REs\_and\_functions.R

Additional R script to export plots consistently:

- scripts/printfig.R

## Analyses:

To be run sequentially, all listed under /scripts.

- 01\_digests.R: script to perform in silico digestions for different target organisms
- 02\_empirical\_digests.R: script to read bioanalyzer results and plot output in comparison with in silico results
- 03\_plot\_n\_loci.R: script based on Rochette & Catchen 2017 to plot the number of (polymorphic) loci for different M parameters
- 04\_plot\_n\_snps\_per\_locus.R: script based on Rochette & Catchen 2017 to plot the number of SNPs per locus for different M parameters
- 05\_marker\_density.R: script to calculate how many snps are sequenced across the genome
- 06\_plot\_coverage.R: script to plot target and realized coverage

## Output:

In /data/in\_silico\_results:

- various tables.csv containing in silico digest results per target taxa

In /figures:

- genome digestion curves, empirical and in silico, created with 02\_empirical\_digests.R
- various n\_loci\_Mn plots per library, created with 03\_plot\_n\_loci.R
- various n\_snps per library/species, created with 04\_n\_snps\_per\_locus.R
- coverage plots, created by 06\_plot\_coverage.R

## Session info:

Package citations:

```
## Warning in FUN(X[[i]], ...): no date field in DESCRIPTION file of package
## 'bioanalyzeR'

## [[1]]
##
## To cite package 'here' in publications use:
##
## Kirill Müller (2020). here: A Simpler Way to Find Your Files. R
## package version 1.0.1. https://CRAN.R-project.org/package=here
##
## A BibTeX entry for LaTeX users is
##
## @Manual{,
##   title = {here: A Simpler Way to Find Your Files},
##   author = {Kirill Müller},
##   year = {2020},
##   note = {R package version 1.0.1},
##   url = {https://CRAN.R-project.org/package=here},
## }
##
##
## [[2]]
##
## To cite package 'SimRAD' in publications use:
##
## Olivier Lepais and Jason Weir (2016). SimRAD: Simulations to Predict
## the Number of RAD and GBS Loci. R package version 0.96.
## https://CRAN.R-project.org/package=SimRAD
##
## A BibTeX entry for LaTeX users is
##
## @Manual{,
##   title = {SimRAD: Simulations to Predict the Number of RAD and GBS Loci},
##   author = {Olivier Lepais and Jason Weir},
##   year = {2016},
##   note = {R package version 0.96},
##   url = {https://CRAN.R-project.org/package=SimRAD},
## }
##
##
## [[3]]
##
## To cite seqinr in publications use:
##
## Charif, D. and Lobry, J.R. (2007)
##
## A BibTeX entry for LaTeX users is
##
## @InCollection{,
##   author = {D. Charif and J.R. Lobry},
```

```

## title = {Seqin{R} 1.0-2: a contributed package to the {R} project for statistical computing devot
## booktitle = {Structural approaches to sequence evolution: Molecules, networks, populations},
## year = {2007},
## editor = {U. Bastolla and M. Porto and H.E. Roman and M. Vendruscolo},
## series = {Biological and Medical Physics, Biomedical Engineering},
## pages = {207-232},
## address = {New York},
## publisher = {Springer Verlag},
## note = {{ISBN :} 978-3-540-35305-8},
## }
##
##
## [[4]]
##
## To cite package 'bioanalyzer' in publications use:
##
## Joseph Foley (2020). bioanalyzer: Analysis of Agilent electrophoresis
## data. R package version 0.5.1.
##
## A BibTeX entry for LaTeX users is
##
## @Manual{,
## title = {bioanalyzer: Analysis of Agilent electrophoresis data},
## author = {Joseph Foley},
## year = {2020},
## note = {R package version 0.5.1},
## }
##
##
## [[5]]
##
## Wickham et al., (2019). Welcome to the tidyverse. Journal of Open
## Source Software, 4(43), 1686, https://doi.org/10.21105/joss.01686
##
## A BibTeX entry for LaTeX users is
##
## @Article{,
## title = {Welcome to the {tidyverse}},
## author = {Hadley Wickham and Mara Averick and Jennifer Bryan and Winston Chang and Lucy D'Agostini
## year = {2019},
## journal = {Journal of Open Source Software},
## volume = {4},
## number = {43},
## pages = {1686},
## doi = {10.21105/joss.01686},
## }
##
##
## [[6]]
##
## To cite package 'ggsci' in publications use:
##
## Nan Xiao (2018). ggsci: Scientific Journal and Sci-Fi Themed Color
## Palettes for 'ggplot2'. R package version 2.9.

```

```
## https://CRAN.R-project.org/package=ggsci
##
## A BibTeX entry for LaTeX users is
##
## @Manual{,
##   title = {ggsci: Scientific Journal and Sci-Fi Themed Color Palettes for
## 'ggplot2'},
##   author = {Nan Xiao},
##   year = {2018},
##   note = {R package version 2.9},
##   url = {https://CRAN.R-project.org/package=ggsci},
## }
##
##
## [[7]]
##
## To cite package 'gridExtra' in publications use:
##
## Baptiste Auguie (2017). gridExtra: Miscellaneous Functions for "Grid"
## Graphics. R package version 2.3.
## https://CRAN.R-project.org/package=gridExtra
##
## A BibTeX entry for LaTeX users is
##
## @Manual{,
##   title = {gridExtra: Miscellaneous Functions for "Grid" Graphics},
##   author = {Baptiste Auguie},
##   year = {2017},
##   note = {R package version 2.3},
##   url = {https://CRAN.R-project.org/package=gridExtra},
## }
```

R Session:

```
## R version 4.1.0 (2021-05-18)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 18.04.5 LTS
##
## Matrix products: default
## BLAS: /usr/lib/x86_64-linux-gnu/blas/libblas.so.3.7.1
## LAPACK: /usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.7.1
##
## 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=en_US.UTF-8
## [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] stats4 parallel stats graphics grDevices utils datasets
## [8] methods base
##
```

```

## other attached packages:
## [1] gridExtra_2.3          ggsci_2.9
## [3] forcats_0.5.1          stringr_1.4.0
## [5] dplyr_1.0.6            purrr_0.3.4
## [7] readr_1.4.0            tidyr_1.1.3
## [9] tibble_3.1.2           ggplot2_3.3.3
## [11] tidyverse_1.3.1        bioanalyzeR_0.5.1
## [13] seqinr_4.2-5           SimRAD_0.96
## [15] zlibbioc_1.38.0        ShortRead_1.50.0
## [17] GenomicAlignments_1.28.0 SummarizedExperiment_1.22.0
## [19] Biobase_2.52.0         MatrixGenerics_1.4.0
## [21] matrixStats_0.59.0     Rsamtools_2.8.0
## [23] GenomicRanges_1.44.0   BiocParallel_1.26.0
## [25] Biostrings_2.60.0      GenomeInfoDb_1.28.0
## [27] XVector_0.32.0         IRanges_2.26.0
## [29] S4Vectors_0.30.0       BiocGenerics_0.38.0
## [31] here_1.0.1
##
## loaded via a namespace (and not attached):
## [1] bitops_1.0-7           fs_1.5.0               lubridate_1.7.10
## [4] RColorBrewer_1.1-2     progress_1.2.2          httr_1.4.2
## [7] rprojroot_2.0.2        tools_4.1.0            backports_1.2.1
## [10] utf8_1.2.1             R6_2.5.0               DBI_1.1.1
## [13] colorspace_2.0-1       ade4_1.7-16            withr_2.4.2
## [16] tidyselect_1.1.1       prettyunits_1.1.1      compiler_4.1.0
## [19] cli_2.5.0              rvest_1.0.0            xml2_1.3.2
## [22] DelayedArray_0.18.0    scales_1.1.1           digest_0.6.27
## [25] rmarkdown_2.8          base64enc_0.1-3         jpeg_0.1-8.1
## [28] pkgconfig_2.0.3        htmltools_0.5.1.1      dbplyr_2.1.1
## [31] rlang_0.4.11           readxl_1.3.1           rstudioapi_0.13
## [34] generics_0.1.0         hwriter_1.3.2          jsonlite_1.7.2
## [37] RCurl_1.98-1.3         magrittr_2.0.1         GenomeInfoDbData_1.2.6
## [40] Matrix_1.3-4           Rcpp_1.0.6             munsell_0.5.0
## [43] fansi_0.5.0            lifecycle_1.0.0        stringi_1.6.2
## [46] yaml_2.2.1             MASS_7.3-54            grid_4.1.0
## [49] crayon_1.4.1           lattice_0.20-44        haven_2.4.1
## [52] hms_1.1.0              knitr_1.33             pillar_1.6.1
## [55] reprex_2.0.0           XML_3.99-0.6           glue_1.4.2
## [58] evaluate_0.14          latticeExtra_0.6-29    modelr_0.1.8
## [61] png_0.1-7              vctrs_0.3.8            cellranger_1.1.0
## [64] gtable_0.3.0           assertthat_0.2.1       xfun_0.23
## [67] broom_0.7.6            ellipsis_0.3.2

```