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differentialabundance workflow version/1.3.0-g3a03f5f

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Abstract

This report summarises differential gene analysis as performed by the nf-core/differentialabundance pipeline.

Data

Samples

A total number of samples metadata is below:

Sample metadata

Sample	Treatment	Sample number	Sample title	Strandedness
SRR8482582	mCherry	sample1	mCherry_1	unstranded
SRR8482583	mCherry	sample2	mCherry_2	unstranded
SRR8482584	mCherry	sample3	mCherry_3	unstranded
SRR8482585	H9DE	sample1	H9DE_1	unstranded
SRR8482586	H9DE	sample2	H9DE_2	unstranded
SRR8482587	H9DE	sample3	H9DE_3	unstranded

Contrasts

Comparisons were made between sample groups defined using metadata columns, as described in the following table of contrasts:

Table of contrasts

id	variable	Reference	Target	Blocking	model
treatment_mCherry_H9DE	treatment	mCherry	H9DE	sample_number	~ 0 + treatment
treatment_mCherry_H9DE_sample_number	treatment	mCherry	H9DE	sample_number	~ 0 + treatment + sample_number

Results

Counts

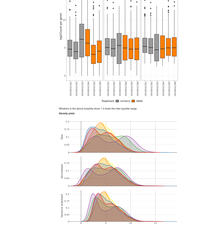
Following a matrix of 1000 genes for 6 samples, reduced to 985 genes after filtering for low abundance.

Exploratory analysis

Uncentered view distributions

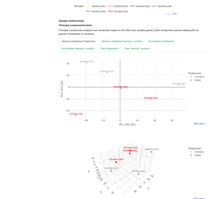
The following plots show the uncentered value distributions of input matrices. A log2 transformation is applied where not already performed.

Raw plots



Windows in the above boxplots show 1.5 times the inter-quartile range.

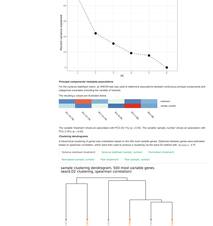
Density plots



Sample relationships

Principal components plots

Principal components analysis was conducted based on the most variable 500 count genes. Datasets between genes were normalised and log2 transformed.



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Arrow plot

The following scores plot visualises pairwise groupings of inter-variation in the data can be explained by each of the principal components above.

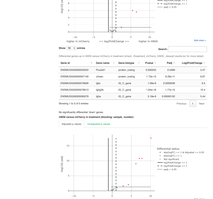


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Principal component variables associations

In the scores scatterplot matrix, an ANOVA test was used to determine associations between continuous principal components and categorical covariates (including the variable of interest).

The following plots show the Variance explained:

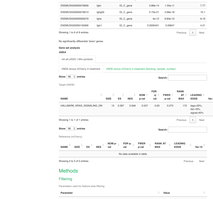


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The variable 'treatment' stands out associated with PC1 (R2 of 76.3%, p: 0.008). The variable 'sample_number' shows an association with PC2 (15.3%, p: 0.0496).

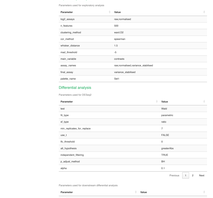
Sample clustering dendrogram

A hierarchical clustering of genes was conducted based on the 500 most variable genes. Distances between genes were normalised based on spearman correlation, which were then used to produce a clustering via the ward.D2 method (R: hclust). A R



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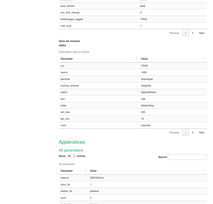
Sample clustering: 500 most variable genes (ward.D2 clustering, spearman correlation)



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Outlier detection

Outlier detection based on [human absolute deviation](#) was undertaken, the outlier scoring is plotted below.



Edit trace >

No outlying samples were detected in groups defined by treatment.

Differential analysis

The design [] settings were used to test differential analysis. p-values were adjusted with the BH method to reduce the number of false positives. Genes were considered differential if, for the respective contrast, the adjusted p-value was equal to or lower than 0.1 and the absolute log2 fold change was greater than or equal to 0.

Differential gene sets tested

Adjusted Unadjusted

Differential gene thresholds (defined by reference)

up	down
1	-1
0	0

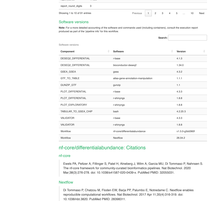
H9DE versus mCherry in treatment

H9DE versus mCherry in treatment blocking: sample_number

Differential gene sets tested

Adjusted p-values Unadjusted p-values

H9DE versus mCherry in treatment blocking: sample_number



Edit trace >

Show 10 entries Search:

Differential genes at H9DE versus mCherry in treatment blocking: sample_number, adjusted p-values, Wald test, shrunken log2 fold change

Gene id	Gene name	Gene biotype	Pvalue	Padj	Log2FoldChange
ENSMUSG00000000562	Pclaf	protein_coding	0.000144	0.0296	1.66
ENSMUSG00000001749	Ccbe1	protein_coding	0.756-14	1.55-11	1.67
ENSMUSG00000019810	Kif22	KI_C_gene	0.756-14	1.55-11	1.67
ENSMUSG00000019810	Kif22	KI_C_gene	0.756-14	1.55-11	1.67
ENSMUSG00000020878	Ighg3b	KI_C_gene	0.756-14	1.55-11	1.67
ENSMUSG00000020878	Ighg3b	KI_C_gene	0.756-14	1.55-11	1.67
ENSMUSG00000020878	Ighg3b	KI_C_gene	0.756-14	1.55-11	1.67
ENSMUSG00000020878	Ighg3b	KI_C_gene	0.756-14	1.55-11	1.67
ENSMUSG00000020878	Ighg3b	KI_C_gene	0.756-14	1.55-11	1.67
ENSMUSG00000020878	Ighg3b	KI_C_gene	0.756-14	1.55-11	1.67

Showing 1 to 10 of 10 entries Previous 1 Next

No significantly differential 'down' genes.

Gene set analysis
GSEA

1 of 1 (2023 / 2K) permutations

H9DE versus mCherry in treatment

H9DE versus mCherry in treatment blocking: sample_number

Show 10 entries Search:

Target GSEA:

Name	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE	Tar id
HALLMARK_MYC_TARGETS_V1	200	0.567	1.046	0.421	0.421	0.973	4,970	tags=42%, list=40%, signal=70%	

Showing 1 to 1 of 1 entries Previous 1 Next

Show 10 entries Search:

Reference GO terms:

Name	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE	Tar id

No data available in table

Showing 0 to 0 of 0 entries Previous Next

Methods

Filtering

Parameters used for feature-wise filtering

Parameter	Value
min_samples	3
min_abundance	10
min_proportion_cpm_int	0

Filtering was carried out by collecting genes with an abundance of at least 10 in at least a proportion of 0.5 of samples.

Exploratory analysis

Parameters used for exploratory analysis

Parameter	Value
legX_anchor	topcrossfollowed
n_features	500
clustering_method	ward.D2
cor_method	spearman
whiskers_distance	1.5
max_threshold	0
heatmap_samples	contrasts
variable_clustering	topcrossfollowed_centroidfollowed
final_sweep	sensitive_stabilised
pattern_matrix	topcrossfollowed

Differential analysis

Parameters used for DESeq2

Parameter	Value
deseq2	TRUE
fit_type	parametric
cooks_cutoff	FALSE
independent_filtering	FALSE
min_replicates_for_replace	7
vst	FALSE
fit_threshold	0
vst_nsub	1000
shrink_lfc	TRUE
lfc_shrinkage	apeglm
shrink_type	normal
p_adjust_method	BH
alpha	0.1

Parameters used for recommended differential analysis

Parameter	Value
tximport	TRUE
tximport_ignore_after_bar	TRUE
tximport_counts_from_abundance	scaledTPM
gene_to_symbol	FALSE
gene_id_column	gene_id
gene_name_column	gene_name
filter_mincount	FALSE
filter_minprop	0.01
filter_mingroup	2
filter_large	Inf
normalisation_method	TMM
limma_voom	FALSE
limma_voom_plots	FALSE
limma_duplicate_correlation	FALSE
limma_ndups	2
limma_spacing	1
limma_trend	FALSE
limma_robust	FALSE
limma_winsor_tail_p	0.05,0.1
deseq2_vst	TRUE
deseq2_fit_type	parametric
deseq2_sf_type	ratio
deseq2_min_replicates_for_replace	7
deseq2_use_t	FALSE
deseq2_lfc_shrink	TRUE
deseq2_lfc_shrink_type	apeglm
deseq2_res_type	ashr
deseq2_res_threshold	0.1
deseq2_independent_filtering	TRUE
deseq2_cooks_cutoff	FALSE
deseq2_min_mu_threshold	0.5
deseq2_alt_hypothesis	greaterAbs
deseq2_shrink_apeglm_coef	2
deseq2_shrink_ashr_mixcompdist	normal
deseq2_vs_method	rlog
deseq2_blind_dispersions_for_vst	FALSE
deseq2_fit_local_for_vst	FALSE
deseq2_nsub_for_vst	1000
deseq2_use_quiet_mode	TRUE
deseq2_use_parallel	FALSE
deseq2_num_cores	1
deseq2_use_beta_prior	FALSE
deseq2_use_weights	TRUE
deseq2_weight_threshold	1
deseq2_use_reduced_model	FALSE
deseq2_use_custom_formula	FALSE
deseq2_custom_formula	~ 0 + treatment
deseq2_use_custom_reduced_formula	FALSE
deseq2_custom_reduced_formula	~ 1
deseq2_use_custom_contrasts	FALSE
deseq2_custom_contrasts	treatment_mCherry_H9DE
deseq2_use_custom_contrasts_variable	FALSE
deseq2_custom_contrasts_variable	
deseq2_use_custom_contrasts_reference	FALSE
deseq2_custom_contrasts_reference	
deseq2_use_custom_contrasts_target	FALSE
deseq2_custom_contrasts_target	
deseq2_use_custom_contrasts_blocking	FALSE
deseq2_custom_contrasts_blocking	
deseq2_use_custom_contrasts_model	FALSE
deseq2_custom_contrasts_model	
deseq2_use_custom_contrasts_name	FALSE
deseq2_custom_contrasts_name	

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Appendices

All parameters

Show 10 entries Search:

All parameters

Parameter	Value
input	SRP204618
input_id	s
settings_id	default
save	TRUE
study_type	rnaseq
study_name	SRP204618
study_abundance_type	counts
report_contributors	Jose Sanz,Institute of Microbiology University of Innsbruck,Austria,0000-0001-7523-3822,Jack Monahan,Pirbright Institute,0000-0002-5205-2555
report_scope	TRUE
report_count_scale	S
report_de	TRUE

Showing 1 to 10 of 21 entries Previous 1 2 3 4 5 Next

Software versions

Note For a more detailed processing of the software and commands used (including containers), consult the execution report provided in the output directory with the file extension `.html`.

Search:

Software versions

Category	Software	Version
GENERAL_DIFFERENTIAL	r-base	4.4.3
GENERAL_DIFFERENTIAL	r-optparse	1.7.3
DESEQ_NORM	gawk	5.3.0
DTE_TPD_TMM	bioconductor-rnaseqmethods	1.14.0
SUMARY_DTP	gawk	5.3.1
PLOT_DIFFERENTIAL	r-base	4.3.3
PLOT_DIFFERENTIAL	r-ggrepel	1.0.6
PLOT_EXPLORATORY	r-ggrepel	0.9.6
TIBCLUST_TO_CSV_CHP	bash	4.2.25-1
VALIDATOR	r-base	4.4.0
VALIDATOR	r-stringr	1.5.1
Workflow	nf-core/differentialabundance	1.3.0
Workflow	Nextflow	25.04.2

nf-core/differentialabundance: Citations

nf-core

Ewels PA, Peltzer A, Fillinger S, Patel H, Alneberg J, Wilm A, Garcia MU, Di Tommaso P, Nahnsen S. nf-core: the community-curated workflows for reproducible omics data analysis. Nat Biotechnol. 2020 Feb;38(2):276-278. doi: 10.1038/s41587-020-0439-x. PubMed PMID: 32055031.

Nextflow

Di Tommaso P, Chatzou M, Floden EW, Barja PP, Palumbo E, Notredame C. Nextflow enables reproducible computational workflows. Nat Biotechnol. 2017 Apr 11;35(4):316-319. doi: 10.1038/nbt.3820. PubMed PMID: 28398311.

Pipeline tools

- Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A. 2005;102(43):15545-15550.

R packages

- ashr
  - Stephens, M., (2016) Biostatistics, doi:10.1093/biostatistics/kxw041
- base
  - R Core Team (2024). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
- BiocGenerics
  - Bioconductor Core Team and Bioconductor Package Maintainer (2024). BiocGenerics: S4 generic functions used in Bioconductor. R package version 0.50.0.
- biocparallel
  - Morgan M, Shepherd L (2024). BiocParallel: Bioconductor facilities for parallel evaluation. R package version 1.38.0.
- biocstyle
  - Oleś A, Morgan M, Huber W (2024). BiocStyle: Standard styles for vignettes and other Bioconductor documents. R package version 2.32.1.
- circlize
  - Gu Z, Gu L, Eils R, Schlesner M, Brors B (2014). circlize implements and enhances circular visualization in R. Bioinformatics, 30, 2811-2812.
- cli
  - Wickham H (2024). cli: Helpers for Developing Command Line Interfaces. R package version 3.6.3.
- cluster
  - Maechler M, Rousseeuw P, Struyf A, Hubert M, Hornik K (2024). cluster: Cluster Analysis Basics and Extensions. R package version 2.1.6.
- colorspace
  - Ihaka R, Murrell P, Hornik K, Fisher JC, Stauffer R, Wilke CO, McWhite CD, Zeileis A (2024). colorspace: Color Space Manipulation. R package version 2.1-1.
- crayon
  - Csardi G (2024). crayon: Colored Terminal Output. R package version 1.5.3.
- data.table
  - Dowle M, Srinivasan A (2024). data.table: Extension of `data.frame`. R package version 1.16.4.
- dbplyr
  - Wickham H, Girlich M (2024). dbplyr: A 'dplyr' Back End for Databases. R package version 2.5.0.
- deseq2
  - Love MI, Huber W, Anders S (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology, 15(12):550. PubMed PMID: 25516281; PubMed Central PMCID: PMC4302049.
- digest
  - Dowle M, Bolker BM, Lianoglou S, Antonyan E, Simpson G (2024). digest: Create Compact Hash Digests of R Objects. R package version 0.6.37.
- dplyr
  - Wickham H, François R, Henry L, Müller K, Vaughan D (2024). dplyr: A Grammar of Data Manipulation. R package version 1.1.4.
- dtplyr
  - Wickham H, Vaughan D (2024). dtplyr: Data Table Back End for 'dplyr'. R package version 1.3.1.
- dynamictreecut
  - Langfelder P, Zhang B, Horvath S (2016). dynamicTreeCut: Methods for Detection of Clusters in Hierarchical Clustering Dendrograms. R package version 1.63-1.
- edger
  - Robinson MD, McCarthy DJ, Smyth GK (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139-140.
- evaluate
  - Hester J, Csardi G (2024). evaluate: Parsing and Evaluation Tools that Provide More Details than the Default. R package version 1.0.3.
- fansi
  - Brodie Gaslam, Elliott Sales de Andrade (2024). fansi: ANSI Control Sequence Aware String Functions. R package version 1.0.6.
- farver
  - Pedersen T (2024). farver: High Performance Colour Space Manipulation. R package version 2.1.2.
- fastmap
  - Wickham H, Chang W, Flight R, Müller K, Hester J (2024). fastmap: Fast Implementation of Key-Value Stores. R package version 1.2.0.
- fdrtool
  - Strimmer K (2008). fdrtool: a versatile R package for estimating local and tail area-based false discovery rates. Bioinformatics 24, 1461-1462.
- fgsea
  - Sergushichev A (2016). An algorithm for fast preranked gene set enrichment analysis using cumulative statistic calculation. bioRxiv 060012.
- forcats
  - Wickham H (2024). forcats: Tools for Working with Categorical Variables (Factors). R package version 1.0.0.
- fs
  - Hester J, Bryan J, Csardi G, Wickham H, Chang W, Müller K, Ooms J (2024). fs: Cross-Platform File System Operations Based on 'libuv'. R package version 1.6.5.
- generics
  - Wickham H, Vaughan D, Kuhn M (2024). generics: Common S3 Generics not Provided by Base R Methods Related to Model Fitting. R package version 0.1.3.
- ggbeeswarm
  - Clarke E, Sherrill-Mix S (2023). ggbeeswarm: Categorical Scatter (Violin Point) Plots. R package version 0.7.2.
- ggplot2
  - Wickham H (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.
- ggrepel
  - Kolberg L, Raudvere U, Kuzmin I, Vilo J, Peterson H (2020). "gprofiler2- an R package for gene list functional enrichment analysis and namespace conversion toolset g:Profiler". F1000Research, 9 (ELIXIR)(709). R package version 0.2.2.
- glue
  - Hester J, Wickham H (2024). glue: Interpreted String Literals. R package version 1.8.0.
- gplots
  - Warnes GR, Bolker B, Bonebakker L, Gentleman R, Huber W, Liaw A, Lumley T, Maechler M, Magnusson A, Moeller S, Schwartz M, Venables B (2024). gplots: Various R Programming Tools for Plotting Data. R package version 3.2.0.
- gridextra
  - Auguie B (2017). gridExtra: Miscellaneous Functions for "Grid" Graphics. R package version 2.3.
- gtable
  - Wickham H, Pedersen T (2024). gtable: Arrange 'Grobs' in Tables. R package version 0.3.6.
- hms
  - Müller K (2024). hms: Pretty Time of Day. R package version 1.1.3.
- htmltools
  - Cheng J, Sievert C, Chang W, Xie Y, Allaire J (2024). htmltools: Tools for HTML. R package version 0.5.8.1.
- htmlwidgets
  - Vaidyanathan R, Xie Y, Allaire J, Cheng J, Sievert C, Russell K (2024). htmlwidgets: HTML Widgets for R. R package version 1.6.4.
- httpuv
  - Cheng J, Luraschi J, Allaire J, Corenthin R, Ushey K, Atkins A, Kuo F, Borges B, Chang W (2024). httpuv: HTTP and WebSocket Server Library. R package version 1.6.15.
- httr
  - Wickham H (2024). httr: Tools for Working with URLs and HTTP. R package version 1.4.7.
- ihaka
  - Ihaka R, Murrell P, Hornik K, Fisher JC, Stauffer R, Wilke CO, McWhite CD, Zeileis A (2024). colorspace: Color Space Manipulation. R package version 2.1-1.
- isoband
  - Wickham H, Pedersen T, Luraschi J (2024). isoband: Generate Isolines and Isobands from Regularly Spaced Elevation Grids. R package version 0.2.7.
- jquerylib
  - Cheng J, Sievert C (2024). jquerylib: Add 'jQuery' Dependencies to 'htmltools' Output. R package version 0.5.1.
- jsonlite
  - Ooms J (2024). jsonlite: A Simple and Robust JSON Parser and Generator for R. R package version 1.8.9.
- knitr
  - Xie Y (2024). knitr: A General-Purpose Package for Dynamic Report Generation in R. R package version 1.49.
- labeling
  - Nuno Sempere (2024). labeling: Axis Labeling. R package version 0.4.3.
- lattice
  - Sarkar D (2024). lattice: Trellis Graphics for R. R package version 0.22-6.
- lifecycle
  - Wickham H (2024). lifecycle: Manage the Life Cycle of your Package Functions. R package version 1.0.4.
- limma
  - Ritchie ME, Phipson B, Wu D, et al. Limma powers differential expression analyses for rna-sequencing and microarray studies. Nucleic Acids Res. 2015;43(7):e47.
- locfit
  - Loader C (2024). locfit: Local Regression, Likelihood and Density Estimation. R package version 1.5-9.8.
- magrittr
  - Bache S, Wickham H (2024). magrittr: A Forward-Pipe Operator for R. R package version 2.0.3.
- markdown
  - Xie Y, Allaire J, Grolemund G (2024). markdown: Rendering R Markdown. R package version 1.13.
- mass
  - Venables WN, Ripley BD (2024). MASS: Support Functions and Datasets for Venables and Ripley's MASS. R package version 7.3-61.
- matrix
  - Bates D, Maechler M (2024). Matrix: Sparse and Dense Matrix Classes and Methods. R package version 1.7-1.
- matrixstats
  - Bengtsson H (2024). matrixStats: Functions that Apply to Rows and Columns of Matrices (and to Vectors). R package version 1.4.1.
- memoise
  - Wickham H, Hester J, Ooms J (2024). memoise: Memoisation of Functions. R package version 2.0.1.
- mgcv
  - Wood SN (2024). mgcv: Mixed GAM Computation Vehicle with Automatic Smoothness Estimation. R package version 1.9-1.
- mime
  - Ooms J (2024). mime: Map Filenames to MIME Types. R package version 0.12.
- multcomp
  - Hothorn T, Bretz F, Westfall P (2008). Simultaneous Inference in General Parametric Models. Biometrical Journal 50(3), 346-363.
- munsell
  - Stauffer R, Mair P, Murrell P, Ihaka R (2024). munsell: Utilities for Using Munsell Colours. R package version 0.5.1.
- mvtnorm
  - Genz A, Bretz F, Miwa T, Mi X, Leisch F, Scheipl F, Hothorn T (2024). mvtnorm: Multivariate Normal and t Distributions. R package version 1.3-2.
- nlme
  - Pinheiro J, Bates D, R Core Team (2024). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-166.
- openssl
  - Ooms J (2024). openssl: Toolkit for Encryption, Signatures and Certificates Based on OpenSSL. R package version 2.2.2.
- optparse
  - Davis S, Meyer PS, Morgan M (2024). optparse: Command Line Option Parser. R package version 1.7.5.
- pheatmap
  - Kolde R (2024). pheatmap: Pretty Heatmaps. R package version 1.0.12.
- pillar
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- pkgconfig
  - Hester J (2024). pkgconfig: Private Configuration for 'R' Packages. R package version 2.0.3.
- plogr
  - Csardi G (2024). plogr: The Simple Logging Framework I Always Wanted. R package version 0.2.0.
- plyr
  - Wickham H (2024). plyr: Tools for Splitting, Applying and Combining Data. R package version 1.8.9.
- png
  - Urbanek S (2024). png: Read and write PNG images. R package version 0.1-8.
- prettyunits
  - Csardi G (2024). prettyunits: Pretty, Human Readable Formatting of Quantities. R package version 1.2.0.
- progress
  - Csardi G, Hester J (2024). progress: Terminal Progress Bars. R package version 1.2.3.
- promises
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