

Package ‘BatchQC’

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Type Package

Title Batch Effects Quality Control Software

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Description Sequencing and microarray samples often are collected or processed in multiple batches or at different times. This often produces technical biases that can lead to incorrect results in the downstream analysis. BatchQC is a software tool that streamlines batch preprocessing and evaluation by providing interactive diagnostics, visualizations, and statistical analyses to explore the extent to which batch variation impacts the data. BatchQC diagnostics help determine whether batch adjustment needs to be done, and how correction should be applied before proceeding with a downstream analysis. Moreover, BatchQC interactively applies multiple common batch effect approaches to the data and the user can quickly see the benefits of each method. BatchQC is developed as a Shiny App. The output is organized into multiple tabs and each tab features an important part of the batch effect analysis and visualization of the data. The BatchQC interface has the following analysis groups: Summary, Differential Expression, Median Correlations, Heatmaps, Circular Dendrogram, PCA Analysis, Shape, ComBat and SVA.

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URL <https://github.com/wejlab/BatchQC>

BugReports <https://github.com/wejlab/BatchQC/issues>

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BatchQC

Run BatchQC shiny app

Description

Run BatchQC shiny app

Usage

```
BatchQC(dev = FALSE)
```

Arguments

dev Run the application in developer mode

Value

The shiny app will open

Examples

```
if(interactive()){
  BatchQC()
}
```

batchqc_explained_variation

Returns a list of explained variation by batch and condition combinations

Description

Returns a list of explained variation by batch and condition combinations

Usage

```
batchqc_explained_variation(se, batch, condition = NULL, assay_name)
```

Arguments

se	Summarized experiment object
batch	Batch covariate
condition	Condition covariate(s) of interest if desired, default is NULL
assay_name	Assay of choice

Value

List of explained variation by batch and condition

Examples

```
library(scran)
se <- mockSCE()
batchqc_explained_variation <- BatchQC::batchqc_explained_variation(se,
  batch = "Mutation_Status",
  condition = "Treatment",
  assay_name = "counts")

batchqc_explained_variation
```

batch_correct

Batch Correct This function allows you to Add batch corrected count matrix to the SE object

Description

Batch Correct This function allows you to Add batch corrected count matrix to the SE object

Usage

```
batch_correct(se, method, assay_to_normalize, batch, group = NULL,
  covar, output_assay_name)
```

Arguments

se	SummarizedExperiment object
method	Normalization Method
assay_to_normalize	Which assay use to do normalization
batch	The batch
group	The group variable
covar	Covariate Matrix
output_assay_name	name of results assay

Value

a summarized experiment object with normalized assay appended

Examples

```
library(scran)
se <- mockSCE()
se <- BatchQC::batch_correct(se, method = "ComBat-Seq",
                             assay_to_normalize = "counts",
                             batch = "Mutation_Status",
                             covar = "Treatment",
                             output_assay_name =
                               "ComBat_Seq_Corrected")
se <- BatchQC::batch_correct(se, method = "Combat",
                             assay_to_normalize = "counts",
                             batch = "Mutation_Status",
                             covar = "Treatment",
                             output_assay_name =
                               "Combat_Corrected")

se
```

batch_design

This function allows you to make a batch design matrix

Description

This function allows you to make a batch design matrix

Usage

```
batch_design(se, batch, covariate)
```

Arguments

se	summarized experiment
batch	batch variable
covariate	biological covariate

Value

design table

Examples

```
library(scran)
se <- mockSCE()
batch_design_tibble <- batch_design(se, batch = "Mutation_Status",
                                   covariate = "Treatment")

batch_design_tibble
```

batch_indicator	<i>Batch and Condition indicator for signature data</i>
-----------------	---

Description

This dataset is from signature data captured when activating different growth pathway genes in human mammary epithelial cells (GEO accession: GSE73628). This data consists of three batches and ten different conditions corresponding to control and nine different pathways.

Usage

```
data(batch_indicator)
```

Format

A data frame with 89 rows and 2 variables:

batch batch
condition condition

bladder_data_upload	<i>Bladder data upload This function uploads the Bladder data set from the bladderbatch package. This dataset is from bladder cancer data with 22,283 different microarray gene expression data. It has 57 bladder samples with 3 metadata variables (batch, outcome and cancer). It contains 5 batches, 3 cancer types (cancer, biopsy, control), and 5 outcomes (Biopsy, mTCC, sTCC-CIS, sTCC+CIS, and Normal). Batch 1 contains only cancer, 2 has cancer and controls, 3 has only controls, 4 contains only biopsy, and 5 contains cancer and biopsy</i>
---------------------	--

Description

Bladder data upload This function uploads the Bladder data set from the bladderbatch package. This dataset is from bladder cancer data with 22,283 different microarray gene expression data. It has 57 bladder samples with 3 metadata variables (batch, outcome and cancer). It contains 5 batches, 3 cancer types (cancer, biopsy, control), and 5 outcomes (Biopsy, mTCC, sTCC-CIS, sTCC+CIS, and Normal). Batch 1 contains only cancer, 2 has cancer and controls, 3 has only controls, 4 contains only biopsy, and 5 contains cancer and biopsy

Usage

```
bladder_data_upload()
```

Value

a SE object with counts data and metadata

Examples

```
library(bladderbatch)
se_object <- bladder_data_upload()
```

check_valid_input	<i>Helper function to save variables as factors if not already factors</i>
-------------------	--

Description

Helper function to save variables as factors if not already factors

Usage

```
check_valid_input(se, batch, condition)
```

Arguments

se	se object
batch	batch
condition	condition

Value

se se object

color_palette	<i>Color palette</i>
---------------	----------------------

Description

This function creates the base color palette used in BatchQC

Usage

```
color_palette(n, first_hue = 25, last_hue = 360)
```

Arguments

n	numeric object representing number of colors to be created
first_hue	numeric object to set the first hue value
last_hue	numeric object to set the final hue value

Value

color_list list of colors generated

Examples

```
library(scran)
n <- 100
color_list <- color_palette(n)
color_list
```

combat_correction	<i>Combat Correction This function applies combat correction to your summarized experiment object</i>
-------------------	---

Description

Combat Correction This function applies combat correction to your summarized experiment object

Usage

```
combat_correction(se, assay_to_normalize, batch, covar, output_assay_name)
```

Arguments

se	SummarizedExperiment object
assay_to_normalize	Assay that should be corrected
batch	The variable that represents batch
covar	Covariate Matrix
output_assay_name	name of results assay

Value

SE object with an added combat corrected array

combat_seq_correction	<i>Combat-Seq Correction This function applies combat-seq correction to your summarized experiment object</i>
-----------------------	---

Description

Combat-Seq Correction This function applies combat-seq correction to your summarized experiment object

Usage

```
combat_seq_correction(se, assay_to_normalize, batch, group, covar,
output_assay_name)
```


Arguments

se SummarizedExperiment object
assay_to_normalize Assay that should be corrected
batch The variable that represents batch
group The group variable
covar Covariate Matrix
output_assay_name name of results assay

Value

SE object with an added combat-seq corrected array

confound_metrics *Combine std. Pearson correlation coefficient and Cramer's V*

Description

Combine std. Pearson correlation coefficient and Cramer's V

Usage

```
confound_metrics(se, batch)
```

Arguments

se summarized experiment
batch batch variable

Value

metrics of confounding

Examples

```
library(scran)  
se <- mockSCE()  
confound_table <- BatchQC::confound_metrics(se, batch = "Mutation_Status")  
confound_table
```

cor_props	<i>This function allows you to calculate correlation properties</i>
-----------	---

Description

This function allows you to calculate correlation properties

Usage

```
cor_props(bd)
```

Arguments

bd	batch design
----	--------------

Value

correlation properties

Examples

```
library(scran)
se <- mockSCE()
batch_design_tibble <- batch_design(se, batch = "Mutation_Status",
                                   covariate = "Treatment")
correlation_property <- BatchQC::cor_props(batch_design_tibble)
correlation_property
```

counts2pvalue	<i>This function calculates p-values for each gene given counts, estimated NB size, and estimated NB mean</i>
---------------	---

Description

This function calculates p-values for each gene given counts, estimated NB size, and estimated NB mean

Usage

```
counts2pvalue(counts, size, mu)
```

Arguments

counts	a vector of gene expression values (in counts)
size	an estimated size parameter of the NB distributions for the gene
mu	a vector of estimated mu parameter of the NB distributions for different samples of the gene

Value

a p-value based on estimated NB size and mean

`covariates_not_confounded`

Returns list of covariates not confounded by batch; helper function for explained variation and for populating shiny app condition options

Description

Returns list of covariates not confounded by batch; helper function for explained variation and for populating shiny app condition options

Usage

```
covariates_not_confounded(se, batch)
```

Arguments

<code>se</code>	Summarized experiment object
<code>batch</code>	Batch variable

Value

List of explained variation by batch and condition

Examples

```
library(scran)
se <- mockSCE()
covariates_not_confounded <- BatchQC::covariates_not_confounded(se,
                                                                batch = "Mutation_Status")
covariates_not_confounded
```

`cramers_v`

This function allows you to calculate Cramer's V

Description

This function allows you to calculate Cramer's V

Usage

```
cramers_v(bd)
```

Arguments

<code>bd</code>	batch design
-----------------	--------------

Value

Cramer's V

Examples

```
library(scran)
se <- mockSCE()
batch_design_tibble <- batch_design(se, batch = "Mutation_Status",
                                   covariate = "Treatment")
cramers_v_result <- BatchQC::cramers_v(batch_design_tibble)
cramers_v_result
```

dendrogram_alpha_numeric_check

Dendrogram alpha or numeric checker

Description

This function checks if there is any numeric or strings for plotting legend

Usage

```
dendrogram_alpha_numeric_check(dendro_var)
```

Arguments

dendro_var column from dendrogram object representing category

Value

geom_label label for the legend of category variable

Examples

```
library(scran)
se <- mockSCE()
dendro_alpha_numeric_check <- dendrogram_alpha_numeric_check(
  dendro_var = "Treatment")
dendro_alpha_numeric_check
```

dendrogram_color_palette

Dendrogram color palette

Description

This function creates the color palette used in the dendrogram plotter

Usage

```
dendrogram_color_palette(col, dendrogram_info)
```

Arguments

col string object representing color of the label
 dendrogram_info dendrogram_ends object

Value

annotation_color vector of colors corresponding to col variable

Examples

```
library(scran)
se <- mockSCE()
process_dendro <- BatchQC::process_dendrogram(se, "counts")
dendrogram_ends <- process_dendro$dendrogram_ends
col <- process_dendro$condition_var
dendro_colors <- dendrogram_color_palette(col = "Treatment",
                                          dendrogram_info = dendrogram_ends)
dendro_colors
```

dendrogram_plotter *Dendrogram Plot*

Description

This function creates a dendrogram plot

Usage

```
dendrogram_plotter(se, assay, batch_var, category_var)
```

Arguments

se SummarizedExperiment object
 assay assay to plot
 batch_var sample metadata column representing batch
 category_var sample metadata column representing category of interest

Value

named list of dendrogram plots
 dendrogram is a dendrogram ggplot
 circular_dendrogram is a circular dendrogram ggplot

Examples

```
library(scran)
se <- mockSCE()
dendrogram_plot <- BatchQC::dendrogram_plotter(se,
                                                "counts",
                                                "Mutation_Status",
                                                "Treatment")

dendrogram_plot$dendrogram
dendrogram_plot$circular_dendrogram
```

DE_analyze

*Differential Expression Analysis***Description**

This function runs DE analysis on a count matrix (DESeq) or a normalized log or log-CPM matrix (limma) contained in the se object

Usage

```
DE_analyze(se, method, batch, conditions, assay_to_analyze)
```

Arguments

se	SummarizedExperiment object
method	DE analysis method option (either 'DESeq2' or 'limma')
batch	metadata column in the se object representing batch
conditions	metadata columns in the se object representing additional analysis covariates
assay_to_analyze	Assay in the se object (either counts for DESeq2 or normalized data for limma) for DE analysis

Value

A named list containing the log2FoldChange, pvalue and adjusted pvalue (padj) for each analysis returned by DESeq2 or limma

Examples

```
library(scran)
se <- mockSCE()
differential_expression <- BatchQC::DE_analyze(se = se,
                                              method = "DESeq2",
                                              batch = "Treatment",
                                              conditions = c(
                                                "Mutation_Status"),
                                              assay_to_analyze = "counts")

pval_summary(differential_expression)
pval_plotter(differential_expression)
```

EV_plotter

This function allows you to plot explained variation

Description

This function allows you to plot explained variation

Usage

```
EV_plotter(batchqc_ev)
```

Arguments

batchqc_ev table of explained variation from batchqc_explained_variation

Value

boxplot of explained variation

Examples

```
library(scran)
se <- mockSCE()
se$Mutation_Status <- as.factor(se$Mutation_Status)
se$Treatment <- as.factor(se$Treatment)
expl_var_result <- batchqc_explained_variation(se, batch = "Mutation_Status",
                                             condition = "Treatment", assay_name = "counts")
EV_boxplot <- BatchQC::EV_plotter(expl_var_result[[1]])
EV_boxplot
```

EV_table

EV Table Returns table with percent variation explained for specified number of genes

Description

EV Table Returns table with percent variation explained for specified number of genes

Usage

```
EV_table(batchqc_ev)
```

Arguments

batchqc_ev explained variation results from batchqc_explained_variation

Value

List of explained variation by batch and condition

Examples

```

library(scran)
se <- mockSCE()
se$Mutation_Status <- as.factor(se$Mutation_Status)
se$Treatment <- as.factor(se$Treatment)
exp_var_result <- BatchQC::batchqc_explained_variation(se,
  batch = "Mutation_Status",
  condition = "Treatment",
  assay_name = "counts")
EV_table <- BatchQC::EV_table(exp_var_result[[1]])

EV_table

```

get.res

Helper function to get residuals

Description

Helper function to get residuals

Usage

```
get.res(y, X)
```

Arguments

y	assay
X	model matrix design

Value

residuals

goodness_of_fit_DESeq2

This function calculates goodness-of-fit pvalues for all genes by looking at how the NB model by DESeq2 fit the data

Description

This function calculates goodness-of-fit pvalues for all genes by looking at how the NB model by DESeq2 fit the data

Usage

```
goodness_of_fit_DESeq2(
  se,
  count_matrix,
  condition,
  other_variables = NULL,
  num_genes = 500,
  seeding = 13
)
```

Arguments

se	the se object where all the data is contained
count_matrix	name of the assay with gene expression matrix (in counts)
condition	name of the se colData with the condition status
other_variables	name of the se colData containing other variables of interest that should be considered in the DESeq2 model
num_genes	downsample value, default is 500 (or all genes if less)
seeding	integer to set the seed to for reproducibility; default is 13

Value

a matrix of pvalues where each row is a gene and each column is a level within the condition of interest

Examples

```
# example code
library(scran)
se <- mockSCE(ncells = 20)
se$Treatment <- as.factor(se$Treatment)
se$Mutation_Status <- as.factor(se$Mutation_Status)
nb_results <- goodness_of_fit_DESeq2(se = se, count_matrix = "counts",
  condition = "Treatment", other_variables = "Mutation_Status")
nb_results[1]
nb_results[2]
nb_results[3]
```

heatmap_num_to_char_converter

Heatmap numeric to character converter

Description

This function converts any found numerics to characters

Usage

```
heatmap_num_to_char_converter(ann_col)
```



```
correlation_heatmap <- heatmaps$correlation_heatmap
correlation_heatmap

heatmap <- heatmaps$topn_heatmap
heatmap
```

nb_histogram	<i>This function creates a histogram from the negative binomial goodness-of-fit pvalues.</i>
--------------	--

Description

This function creates a histogram from the negative binomial goodness-of-fit pvalues.

Usage

```
nb_histogram(p_val_table)
```

Arguments

p_val_table table of p-values from the nb test

Value

a histogram of the number of genes within a p-value range

nb_proportion	<i>This function determines the proportion of p-values below a specific value and compares to the previously determined threshold of 0.42 for extreme low values.</i>
---------------	---

Description

This function determines the proportion of p-values below a specific value and compares to the previously determined threshold of 0.42 for extreme low values.

Usage

```
nb_proportion(p_val_table, low_pval = 0.01, threshold = 0.42, num_samples)
```

Arguments

p_val_table table of p-values from the nb test
low_pval value of the p-value cut off to use in proportion
threshold the value to compare the proportion of p-values to for data sets less than 20, default is 0.42
num_samples the number of samples in the analysis

Value

a statement about whether DESeq2 is appropriate to use for analysis

normalize_SE	<i>This function allows you to add normalized count matrix to the SE object</i>
--------------	---

Description

This function allows you to add normalized count matrix to the SE object

Usage

```
normalize_SE(se, method, log_bool, assay_to_normalize, output_assay_name)
```

Arguments

se	SummarizedExperiment Object
method	Normalization Method, either 'CPM' or 'DESeq' or 'none' for log only
log_bool	True or False; True to log normalize the data set after normalization method
assay_to_normalize	Which SE assay to do normalization on
output_assay_name	name for the resulting normalized assay

Value

the original SE object with normalized assay appended

Examples

```
library(scran)
se <- mockSCE()
se_CPM_normalized <- BatchQC::normalize_SE(se, method = "CPM",
  log_bool = FALSE,
  assay_to_normalize = "counts",
  output_assay_name =
    "CPM_normalized_counts")
se_DESeq_normalized <- BatchQC::normalize_SE(se, method = "DESeq",
  log_bool = FALSE,
  assay_to_normalize = "counts",
  output_assay_name =
    "DESeq_normalized_counts")

se_CPM_normalized
se_DESeq_normalized
```

PCA_plotter

*This function allows you to plot PCA***Description**

This function allows you to plot PCA

Usage

```
PCA_plotter(se, nfeature, color, shape, assays, xaxisPC, yaxisPC,
log_option = FALSE)
```

Arguments

se	SummarizedExperiment object
nfeature	number of features
color	choose a color
shape	choose a shape
assays	array of assay names from se
xaxisPC	the PC to plot as the x axis
yaxisPC	the PC to plot as the y axis
log_option	TRUE if data should be logged before plotting (recommended for sequencing counts), FALSE if data should not be logged (for instance, data is already logged); FALSE by default

Value

List containing PCA info, PCA variance and PCA plot

Examples

```
library(scran)
se <- mockSCE()
se_object_ComBat_Seq <- BatchQC::batch_correct(se, method = "ComBat-Seq",
                                             assay_to_normalize = "counts",
                                             batch = "Mutation_Status",
                                             covar = "Treatment",
                                             output_assay_name =
                                             "ComBat_Seq_Corrected")
pca_plot <- BatchQC::PCA_plotter(se = se_object_ComBat_Seq,
                                nfeature = 2, color = "Mutation_Status",
                                shape = "Treatment",
                                assays = c("counts", "ComBat_Seq_Corrected"),
                                xaxisPC = 1, yaxisPC = 2, log_option = FALSE)

pca_plot$plot
pca_plot$var_explained
```

plot_data	<i>This function formats the PCA plot using ggplot</i>
-----------	--

Description

This function formats the PCA plot using ggplot

Usage

```
plot_data(pca_plot_data, color, shape, xaxisPC, yaxisPC)
```

Arguments

pca_plot_data	Data for all assays to plot
color	variable that will be plotted as color
shape	variable that will be plotted as shape
xaxisPC	the PC to plot as the x axis
yaxisPC	the PC to plot as the y axis

Value

PCA plot

preprocess	<i>Preprocess assay data</i>
------------	------------------------------

Description

Preprocess assay data

Usage

```
preprocess(se, assay, nfeature, log_option)
```

Arguments

se	Summarized Experiment object
assay	Assay from SummarizedExperiment object
nfeature	Number of variable features to use
log_option	"True" if data should be logged, "False" otherwise

Value

Returns processed data

process_dendrogram	<i>Process Dendrogram</i>
--------------------	---------------------------

Description

This function processes count data for dendrogram plotting

Usage

```
process_dendrogram(se, assay)
```

Arguments

se	SummarizedExperiment object
assay	assay to plot

Value

named list of dendrogram data
dendrogram_segments is data representing segments of the dendrogram
dendrogram_ends is data representing ends of the dendrogram

Examples

```
library(scran)  
se <- mockSCE()  
process_dendro <- BatchQC::process_dendrogram(se, "counts")  
process_dendro
```

protein_data	<i>Protein data with 39 protein expression levels</i>
--------------	---

Description

This data consists of two batches and two conditions corresponding to case and control. The columns are case/control samples, and the rows represent 39 different proteins.

Usage

```
data(protein_data)
```

Format

A data frame with 39 rows and 24 variables

protein_sample_info	<i>Batch and Condition indicator for protein expression data</i>
---------------------	--

Description

This data consists of two batches and two conditions corresponding to case and control for the protein expression data

Usage

```
data(protein_sample_info)
```

Format

A data frame with 24 rows and 2 variables:

batch Batch Indicator

category Condition (Case vs Control) Indicator

pval_plotter	<i>P-value Plotter This function allows you to plot p-values of explained variation</i>
--------------	---

Description

P-value Plotter This function allows you to plot p-values of explained variation

Usage

```
pval_plotter(DE_results)
```

Arguments

DE_results Differential Expression analysis result (a named list of dataframes corresponding to each analysis completed with a "pvalue" column)

Value

boxplots of pvalues for each condition

Examples

```
library(scran)
se <- mockSCE()
differential_expression <- BatchQC::DE_analyze(se = se,
                                              method = "DESeq2",
                                              batch = "Treatment",
                                              conditions = c(
                                                "Mutation_Status"),
                                              assay_to_analyze = "counts")

pval_summary(differential_expression)
pval_plotter(differential_expression)
```

pval_summary	Returns summary table for p-values of explained variation
--------------	---

Description

Returns summary table for p-values of explained variation

Usage

```
pval_summary(res_list)
```

Arguments

res_list	Differential Expression analysis result (a named list of dataframes corresponding to each analysis completed with a "pvalue" column)
----------	--

Value

summary table for p-values of explained variation for each analysis

Examples

```
library(scran)
se <- mockSCE()
differential_expression <- BatchQC::DE_analyze(se = se,
                                             method = "DESeq2",
                                             batch = "Treatment",
                                             conditions = c(
                                               "Mutation_Status"),
                                             assay_to_analyze = "counts")

pval_summary(differential_expression)
```

ratio_plotter	This function allows you to plot ratios of explained variation
---------------	--

Description

This function allows you to plot ratios of explained variation

Usage

```
ratio_plotter(ev_ratio)
```

Arguments

ev_ratio	table of ratios from variation_ratios()
----------	---

Value

boxplot of ratios

Examples

```

library(scran)
se <- mockSCE()
se$Mutation_Status <- as.factor(se$Mutation_Status)
se$Treatment <- as.factor(se$Treatment)
expl_var_result <- batchqc_explained_variation(se, batch = "Mutation_Status",
                                             condition = "Treatment", assay_name = "counts")
ratios_results <- variation_ratios(expl_var_result[[1]],
                                   batch = "Mutation_Status")
ratio_boxplot <- BatchQC::ratio_plotter(ratios_results)
ratio_boxplot

```

signature_data

*Signature data with 1600 gene expression levels***Description**

This data consists of three batches and ten conditions. The columns are samples, and the rows represent 1600 different genes.

Usage

```
data(signature_data)
```

Format

A data frame with 1600 rows and 89 variables

std_pearson_corr_coef *Calculate a standardized Pearson correlation coefficient*

Description

Calculate a standardized Pearson correlation coefficient

Usage

```
std_pearson_corr_coef(bd)
```

Arguments

bd batch design

Value

standardized Pearson correlation coefficient

Examples

```
library(scran)
se <- mockSCE()
batch_design_tibble <- batch_design(se, batch = "Mutation_Status",
                                   covariate = "Treatment")
pearson_cor_result <- BatchQC::std_pearson_corr_coef(batch_design_tibble)
pearson_cor_result
```

summarized_experiment *This function creates a summarized experiment object from count and metadata files uploaded by the user*

Description

This function creates a summarized experiment object from count and metadata files uploaded by the user

Usage

```
summarized_experiment(counts, columndata)
```

Arguments

counts	counts dataframe
columndata	metadata dataframe

Value

a summarized experiment object

Examples

```
data(protein_data)
data(protein_sample_info)
se_object <- summarized_experiment(protein_data, protein_sample_info)
```

variation_ratios *Creates Ratios of batch to variable variation statistic*

Description

Creates Ratios of batch to variable variation statistic

Usage

```
variation_ratios(ex_variation_table, batch)
```



```
                                "Cell_Cycle"),
                                assay_to_analyze = "counts")
value <- round((max(abs(
  differential_expression[[length(differential_expression)]][, 1]))
+ min(abs(
  differential_expression[[length(differential_expression)]][, 1])) / 2)

volcano_plot(differential_expression[[1]], pslider = 0.05, fcslider = value)
```

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