Package 'transformGamPoi'

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Type Package

Title Variance Stabilizing Transformation for Gamma-Poisson Models

Version 1.18.0

Description Variance-stabilizing transformations help with the analysis of heteroskedastic data (i.e., data where the variance is not constant, like count data). This package provide two types of variance stabilizing transformations: (1) methods based on the delta method (e.g., 'acosh', 'log(x+1)'), (2) model residual based (Pearson and randomized quantile residuals).

BugReports https://github.com/const-ae/transformGamPoi/issues

URL https://github.com/const-ae/transformGamPoi

License GPL-3

Encoding UTF-8

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Contents

.handle_data_parameter 2
acosh_transform 2
estimate_size_factors 5
residual_transform 5
transformGamPoi 9

Index **12**

`.handle_data_parameter` *Take any kind of data and extract the matrix*

Description

Adapted from glmGamPoi:::handle_data_parameter

Usage

```
.handle_data_parameter(data, on_disk, allow_sparse = TRUE)
```

Value

A matrix.

`acosh_transform` *Delta method-based variance stabilizing transformation*

Description

Delta method-based variance stabilizing transformation

Usage

```
acosh_transform(
  data,
  overdispersion = 0.05,
  size_factors = TRUE,
  ...,
  on_disk = NULL,
  verbose = FALSE
)

shifted_log_transform(
  data,
  overdispersion = 0.05,
```

```
  pseudo_count = 1/(4 * overdispersion),
  size_factors = TRUE,
  minimum_overdispersion = 0.001,
  ...,
  on_disk = NULL,
  verbose = FALSE
)
```

Arguments

data	any matrix-like object (e.g. matrix, dgCMatrix, DelayedArray, HDF5Matrix) with one column per sample and row per gene. It can also be an object of type `glmGamPoi`, in which case it is directly used to calculate the variance-stabilized values.
overdispersion	the simplest count model is the Poisson model. However, the Poisson model assumes that $variance = mean$. For many applications this is too rigid and the Gamma-Poisson allows a more flexible mean-variance relation ($variance = mean + mean^2 * overdispersion$). `overdispersion` can either be • a single boolean that indicates if an overdispersion is estimated for each gene. • a numeric vector of length `nrow(data)` fixing the overdispersion to those values. • the string `"global"` to indicate that one dispersion is fit across all genes. Note that `overdispersion = 0` and `overdispersion = FALSE` are equivalent and both reduce the Gamma-Poisson to the classical Poisson model. Default: `0.05` which is roughly the overdispersion observed on ostensibly homogeneous cell lines.
size_factors	in large scale experiments, each sample is typically of different size (for example different sequencing depths). A size factor is an internal mechanism of GLMs to correct for this effect. `size_factors` is either a numeric vector with positive entries that has the same lengths as columns in the data that specifies the size factors that are used. Or it can be a string that species the method that is used to estimate the size factors (one of `c("normed_sum", "deconvolution", "poscounts")`). Note that `"normed_sum"` and `"poscounts"` are fairly simple methods and can lead to sub-optimal results. For the best performance, I recommend to use `size_factors = "deconvolution"` which calls `scran::calculateSumFactors()`. However, you need to separately install the `scran` package from Bioconductor for this method to work. Also note that `size_factors = 1` and `size_factors = FALSE` are equivalent. If only a single gene is given, no size factor is estimated (ie. `size_factors = 1`). Default: `"normed_sum"`.
...	additional parameters for `glmGamPoi::glm_gp()` which is called in case `overdispersion = TRUE`.
on_disk	a boolean that indicates if the dataset is loaded into memory or if it is kept on disk to reduce the memory usage. Processing in memory can be significantly

faster than on disk. Default: `NULL` which means that the data is only processed in memory if `data` is an in-memory data structure.

`verbose` boolean that decides if information about the individual steps are printed. Default: `FALSE`

`pseudo_count` instead of specifying the overdispersion, the `shifted_log_transform` is commonly parameterized with a pseudo-count ($pseudo-count = 1/(4*overdispersion)$). If both the `pseudo-count` and `overdispersion` is specified, the `overdispersion` is ignored. Default: `1/(4 * overdispersion)`

`minimum_overdispersion`
the `acosh_transform` converges against $2 * sqrt(x)$ for `overdispersion == 0`. However, the `shifted_log_transform` would just become `0`, thus here we apply the `minimum_overdispersion` to avoid this behavior.

Value

a matrix (or a vector if the input is a vector) with the transformed values.

Functions

- `acosh_transform`: $1/sqrt(alpha)$ acosh(2 * alpha * x + 1)
- `shifted_log_transform`: $1/sqrt(alpha) log(4 * alpha * x + 1)$

References

Ahlmann-Eltze, Constantin, and Wolfgang Huber. "Transformation and Preprocessing of Single-Cell RNA-Seq Data." bioRxiv (2021).

Ahlmann-Eltze, Constantin, and Wolfgang Huber. "glmGamPoi: Fitting Gamma-Poisson Generalized Linear Models on Single Cell Count Data." Bioinformatics (2020)

Dunn, Peter K., and Gordon K. Smyth. "Randomized quantile residuals." Journal of Computational and Graphical Statistics 5.3 (1996): 236-244.

Hafemeister, Christoph, and Rahul Satija. "Normalization and variance stabilization of single-cell RNA-seq data using regularized negative binomial regression." Genome biology 20.1 (2019): 1-15.

Hafemeister, Christoph, and Rahul Satija. "Analyzing scRNA-seq data with the sctransform and offset models." (2020)

Lause, Jan, Philipp Berens, and Dmitry Kobak. "Analytic Pearson residuals for normalization of single-cell RNA-seq UMI data." Genome Biology (2021).

See Also

`acosh_transform`, `shifted_log_transform`, and `residual_transform`

Examples

```
# Load a single cell dataset
sce <- TENxPBMCData::TENxPBMCData("pbmc4k")
# Reduce size for this example
set.seed(1)
```

```
sce_red <- sce[sample(which(rowSums2(counts(sce)) > 0), 1000),
               sample(ncol(sce), 200)]

assay(sce_red, "acosh") <- acosh_transform(sce_red)
assay(sce_red, "shifted_log") <- shifted_log_transform(sce_red)
plot(rowMeans2(assay(sce_red, "acosh")), rowVars(assay(sce_red, "acosh")), log = "x")
points(rowMeans2(assay(sce_red, "shifted_log")), rowVars(assay(sce_red, "shifted_log")),
       col = "red")

# Sqrt transformation
sqrt_dat <- acosh_transform(sce_red, overdispersion = 0, size_factor = 1)
plot(2 * sqrt(assay(sce_red))[,1], sqrt_dat[,1]); abline(0,1)
```

---

estimate_size_factors *Estimate the Size Factors*

Description

Estimate the Size Factors

Usage

```
estimate_size_factors(Y, method, verbose = FALSE)
```

Arguments

Y	any matrix-like object (base::matrix(), DelayedArray, HDF5Matrix, Matrix::Matrix(), etc.)
method	one of c("normed_sum", "deconvolution", "poscounts")

Value

a vector with one size factor per column of Y

---

residual_transform *Residual-based Variance Stabilizing Transformation*

Description

Fit an intercept Gamma-Poisson model that corrects for sequencing depth and return the residuals as variance stabilized results for further downstream application, for which no proper count-based method exist or is performant enough (e.g., clustering, dimensionality reduction).

Usage

```
residual_transform(
  data,
  residual_type = c("randomized_quantile", "pearson", "analytic_pearson"),
  clipping = FALSE,
  overdispersion = 0.05,
  size_factors = TRUE,
  offset_model = TRUE,
  overdispersion_shrinkage = TRUE,
  ridge_penalty = 2,
  on_disk = NULL,
  return_fit = FALSE,
  verbose = FALSE,
  ...
)
```

Arguments

data	any matrix-like object (e.g. matrix, dgCMatrix, DelayedArray, HDF5Matrix) with one column per sample and row per gene. It can also be an object of type `glmGamPoi`, in which case it is directly used to calculate the variance-stabilized values.
residual_type	a string that specifies what kind of residual is returned as variance stabilized-value. `"randomized_quantile"` The discrete nature of count distribution stops simple transformations from obtaining a truly standard normal residuals. The trick of of quantile randomized residuals is to match the cumulative density function of the Gamma-Poisson and the Normal distribution. Due to the discrete nature of Gamma-Poisson distribution, a count does not correspond to a single quantile of the Normal distribution, but to a range of possible value. This is resolved by randomly choosing one of the mapping values from the Normal distribution as the residual. This ensures perfectly normal distributed residuals, for the cost of introducing randomness. More details are available in the documentation of `statmod::qresiduals()` and the corresponding publication by Dunn and Smyth (1996). `"pearson"` The Pearson residuals are defined as $res = (y - m)/sqrt(m + m^2 * theta)$. `"analytic_pearson"` Similar to the method above, however, instead of estimating m using a GLM model fit, m is approximated by $m_ij = (\sum_j y_{ij})(\sum_i y_{ij})/(\sum_{i,j} y_{ij})$. For all details, see Lause et al. (2021). Note that `overdispersion_shrinkage` and `ridge_penalty` are ignored when fitting analytic Pearson residuals. The two above options are the most common choices, however you can use any `residual_type` supported by `glmGamPoi::residuals.glmGamPoi()`. Default: `"randomized_quantile"`
clipping	a single boolean or numeric value specifying that all residuals are in the range `[-clipping, +clipping]`. If `clipping = TRUE`, we use the default of `clipping`

= sqrt(ncol(data)) which is the default behavior for sctransform. Default: FALSE, which means no clipping is applied.

overdispersion the simplest count model is the Poisson model. However, the Poisson model assumes that $variance = mean$. For many applications this is too rigid and the Gamma-Poisson allows a more flexible mean-variance relation ($variance = mean + mean^2 * overdispersion$).
overdispersion can either be

- a single boolean that indicates if an overdispersion is estimated for each gene.
- a numeric vector of length nrow(data) fixing the overdispersion to those values.
- the string "global" to indicate that one dispersion is fit across all genes.

Note that overdispersion = 0 and overdispersion = FALSE are equivalent and both reduce the Gamma-Poisson to the classical Poisson model. Default: 0.05 which is roughly the overdispersion observed on ostensibly homogeneous cell lines.

offset_model boolean to decide if β_1 in $y = \beta_0 + \beta_1 log(sf)$, is set to 1 (i.e., treating the log of the size factors as an offset) or is estimated per gene. From a theoretical point, it should be fine to treat β_1 as an offset, because a cell that is twice as big, should have twice as many counts per gene (without any gene-specific effects). However, sctransform suggested that it would be advantageous to nonetheless estimate β_0 as it may counter data artifacts. On the other side, Lause et al. (2020) demonstrated that the estimating β_0 and β_1 together can be difficult. If you still want to fit sctransform's model, you can set the ridge_penalty argument to a non-zero value, which shrinks β_1 towards 1 and resolves the degeneracy.
Default: TRUE.

overdispersion_shrinkage, size_factors
arguments that are passed to the underlying call to glmGamPoi::glm_gp(). Default for each: TRUE.

ridge_penalty another argument that is passed to glmGamPoi::glm_gp(). It is ignored if offset_model = TRUE. Default: 2.

on_disk a boolean that indicates if the dataset is loaded into memory or if it is kept on disk to reduce the memory usage. Processing in memory can be significantly faster than on disk. Default: NULL which means that the data is only processed in memory if data is an in-memory data structure.

return_fit boolean to decide if the matrix of residuals is returned directly (return_fit = FALSE) or if in addition the glmGamPoi-fit is returned (return_fit = TRUE) . Default: FALSE.

verbose boolean that decides if information about the individual steps are printed. Default: FALSE

... additional parameters passed to glmGamPoi::glm_gp().

Details

Internally, this method uses the glmGamPoi package. The function goes through the following steps

1. fit model using `glmGamPoi::glm_gp()`
2. plug in the trended overdispersion estimates
3. call `glmGamPoi::residuals.glmGamPoi()` to calculate the residuals.

Value

a matrix (or a vector if the input is a vector) with the transformed values. If `return_fit = TRUE`, a list is returned with two elements: `fit` and `Residuals`.

References

Ahlmann-Eltze, Constantin, and Wolfgang Huber. "glmGamPoi: Fitting Gamma-Poisson Generalized Linear Models on Single Cell Count Data." Bioinformatics (2020)

Dunn, Peter K., and Gordon K. Smyth. "Randomized quantile residuals." Journal of Computational and Graphical Statistics 5.3 (1996): 236-244.

Hafemeister, Christoph, and Rahul Satija. "Normalization and variance stabilization of single-cell RNA-seq data using regularized negative binomial regression." Genome biology 20.1 (2019): 1-15.

Hafemeister, Christoph, and Rahul Satija. "Analyzing scRNA-seq data with the sctransform and offset models." (2020)

Lause, Jan, Philipp Berens, and Dmitry Kobak. "Analytic Pearson residuals for normalization of single-cell RNA-seq UMI data." Genome Biology (2021).

See Also

`glmGamPoi::glm_gp()`, `glmGamPoi::residuals.glmGamPoi()`, `sctransform::vst()`, `statmod::qresiduals()`

Examples

```
# Load a single cell dataset
sce <- TENxPBMCData::TENxPBMCData("pbmc4k")
# Reduce size for this example
set.seed(1)
sce_red <- sce[sample(which(rowSums2(counts(sce)) > 0), 1000),
               sample(ncol(sce), 200)]
counts(sce_red) <- as.matrix(counts(sce_red))

# Residual Based Variance Stabilizing Transformation
rq <- residual_transform(sce_red, residual_type = "randomized_quantile",
                         verbose = TRUE)
pearson <- residual_transform(sce_red, residual_type = "pearson", verbose = TRUE)

# Plot first two principal components
pearson_pca <- prcomp(t(pearson), rank. = 2)
rq_pca <- prcomp(t(rq), rank. = 2)
plot(rq_pca$x, asp = 1)
points(pearson_pca$x, col = "red")
```

`transformGamPoi` *Variance Stabilizing Transformation for Gamma Poisson Data*

Description

Variance Stabilizing Transformation for Gamma Poisson Data

Usage

```
transformGamPoi(
  data,
  transformation = c("acosh", "shifted_log", "randomized_quantile_residuals",
    "pearson_residuals", "analytic_pearson_residuals"),
  overdispersion = 0.05,
  size_factors = TRUE,
  ...,
  on_disk = NULL,
  verbose = FALSE
)
```

Arguments

`data`	any matrix-like object (e.g. matrix, dgCMatrix, DelayedArray, HDF5Matrix) with one column per sample and row per gene. It can also be an object of type `glmGamPoi`, in which case it is directly used to calculate the variance-stabilized values.
`transformation`	one of `c("acosh", "shifted_log", "randomized_quantile_residuals", "pearson_residuals", "analytic_pearson_residuals")`. See `acosh_transform`, `shifted_log_transform`, or `residual_transform` for more information.
`overdispersion`	the simplest count model is the Poisson model. However, the Poisson model assumes that $variance = mean$. For many applications this is too rigid and the Gamma-Poisson allows a more flexible mean-variance relation ($variance = mean + mean^2 * overdispersion$). `overdispersion` can either be • a single boolean that indicates if an overdispersion is estimated for each gene. • a numeric vector of length `nrow(data)` fixing the overdispersion to those values. • the string `"global"` to indicate that one dispersion is fit across all genes. Note that `overdispersion = 0` and `overdispersion = FALSE` are equivalent and both reduce the Gamma-Poisson to the classical Poisson model. Default: `0.05` which is roughly the overdispersion observed on ostensibly homogeneous cell lines.

size_factors	in large scale experiments, each sample is typically of different size (for example different sequencing depths). A size factor is an internal mechanism of GLMs to correct for this effect. size_factors is either a numeric vector with positive entries that has the same lengths as columns in the data that specifies the size factors that are used. Or it can be a string that species the method that is used to estimate the size factors (one of c("normed_sum", "deconvolution", "poscounts")). Note that "normed_sum" and "poscounts" are fairly simple methods and can lead to suboptimal results. For the best performance, I recommend to use size_factors = "deconvolution" which calls scran::calculateSumFactors(). However, you need to separately install the scran package from Bioconductor for this method to work. Also note that size_factors = 1 and size_factors = FALSE are equivalent. If only a single gene is given, no size factor is estimated (ie. size_factors = 1). Default: "normed_sum".
...	additional parameters passed to acosh_transform, shifted_log_transform, or residual_transform
on_disk	a boolean that indicates if the dataset is loaded into memory or if it is kept on disk to reduce the memory usage. Processing in memory can be significantly faster than on disk. Default: NULL which means that the data is only processed in memory if data is an in-memory data structure.
verbose	boolean that decides if information about the individual steps are printed. Default: FALSE

Value

a matrix (or a vector if the input is a vector) with the transformed values.

References

Ahlmann-Eltze, Constantin, and Wolfgang Huber. "Transformation and Preprocessing of Single-Cell RNA-Seq Data." bioRxiv (2021).

Ahlmann-Eltze, Constantin, and Wolfgang Huber. "glmGamPoi: Fitting Gamma-Poisson Generalized Linear Models on Single Cell Count Data." Bioinformatics (2020)

Dunn, Peter K., and Gordon K. Smyth. "Randomized quantile residuals." Journal of Computational and Graphical Statistics 5.3 (1996): 236-244.

Hafemeister, Christoph, and Rahul Satija. "Normalization and variance stabilization of single-cell RNA-seq data using regularized negative binomial regression." Genome biology 20.1 (2019): 1-15.

Hafemeister, Christoph, and Rahul Satija. "Analyzing scRNA-seq data with the sctransform and offset models." (2020)

Lause, Jan, Philipp Berens, and Dmitry Kobak. "Analytic Pearson residuals for normalization of single-cell RNA-seq UMI data." Genome Biology (2021).

See Also

acosh_transform, shifted_log_transform, and residual_transform

Examples

```
# Load a single cell dataset
sce <- TENxPBMCData::TENxPBMCData("pbmc4k")
# Reduce size for this example
set.seed(1)
sce_red <- sce[sample(which(rowSums2(counts(sce)) > 0), 1000),
               sample(ncol(sce), 200)]

assay(sce_red, "acosh") <- transformGamPoi(sce_red, "acosh")
assay(sce_red, "shifted_log") <- transformGamPoi(sce_red, "shifted_log")

# Residual Based Variance Stabilizing Transformation
rq <- transformGamPoi(sce_red, transformation = "randomized_quantile", on_disk = FALSE,
                      verbose = TRUE)
pearson <- transformGamPoi(sce_red, transformation = "pearson", on_disk = FALSE, verbose = TRUE)

plot(rowMeans2(counts(sce_red)), rowVars(assay(sce_red, "acosh")), log = "x")
points(rowMeans2(counts(sce_red)), rowVars(assay(sce_red, "shifted_log")), col = "red")
points(rowMeans2(counts(sce_red)), rowVars(rq), col = "blue")


# Plot first two principal components
acosh_pca <- prcomp(t(assay(sce_red, "acosh")), rank. = 2)
rq_pca <- prcomp(t(rq), rank. = 2)
pearson_pca <- prcomp(t(pearson), rank. = 2)

plot(acosh_pca$x, asp = 1)
points(rq_pca$x, col = "blue")
points(pearson_pca$x, col = "green")
```

Index

∗ **internal**
.handle_data_parameter, 2
estimate_size_factors, 5
.handle_data_parameter, 2

acosh_transform, 2, *4*, *9, 10*

estimate_size_factors, 5

glmGamPoi::glm_gp(), *7, 8*
glmGamPoi::residuals.glmGamPoi(), *6*, *8*

residual_transform, *4*, 5, *9, 10*

shifted_log_transform, *4*, *9, 10*
shifted_log_transform
*(*acosh_transform*)*, 2
statmod::qresiduals(), *6*

transformGamPoi, 9