

Package ‘BASiCS’

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Type Package

Title Bayesian Analysis of Single-Cell Sequencing data

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Description Single-cell mRNA sequencing can uncover novel cell-to-cell heterogeneity in gene expression levels in seemingly homogeneous populations of cells. However, these experiments are prone to high levels of unexplained technical noise, creating new challenges for identifying genes that show genuine heterogeneous expression within the population of cells under study. BASiCS (Bayesian Analysis of Single-Cell Sequencing data) is an integrated Bayesian hierarchical model to perform statistical analyses of single-cell RNA sequencing datasets in the context of supervised experiments (where the groups of cells of interest are known a priori, e.g. experimental conditions or cell types). BASiCS performs built-in data normalisation (global scaling) and technical noise quantification (based on spike-in genes). BASiCS provides an intuitive detection criterion for highly (or lowly) variable genes within a single group of cells. Additionally, BASiCS can compare gene expression patterns between two or more pre-specified groups of cells. Unlike traditional differential expression tools, BASiCS quantifies changes in expression that lie beyond comparisons of means, also allowing the study of changes in cell-to-cell heterogeneity. The latter are quantified via a biological over-dispersion parameter that measures residual over-dispersion (with respect to Poisson sampling) after normalisation and the removal of technical variation.

License GPL (>= 2)

Depends R (>= 3.1.0), S4Vectors, SummarizedExperiment

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BASiCS_Chain-class	<i>The BASiCS_Chain class</i>
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Description

Container of an MCMC sample of the BASiCS' model parameters (see Vallejos et al, 2015) as generated by the function `BASiCS_MCMC`.

Slots

- `mu` MCMC chain for gene-specific expression levels $\mu[i]$, defined as true input molecules in case of technical genes (matrix with q columns, technical genes located at the end of the matrix, all elements must be positive numbers)
- `delta` MCMC chain for gene-specific biological cell-to-cell heterogeneity hyper-parameters $\delta[i]$, biological genes only (matrix with q.bio columns, all elements must be positive numbers)
- `phi` MCMC chain for cell-specific mRNA content normalising constants $\phi[j]$ (matrix with n columns, all elements must be positive numbers and the sum of its elements must be equal to n)
- `s` MCMC chain for cell-specific capture efficiency (or amplification biases if not using UMI based counts) normalising constants $s[j]$ (matrix with n columns, all elements must be positive numbers)
- `nu` MCMC chain for cell-specific random effects $\nu[j]$ (matrix with n columns, all elements must be positive numbers)
- `theta` MCMC chain for technical variability hyper-parameter(s) θ (matrix, all elements must be positive, each column represents 1 batch)

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl>

References

Vallejos, Marioni and Richardson (2015). Bayesian Analysis of Single-Cell Sequencing data. PLoS Computational Biology.

Examples

```
# A BASiCS_Chain object created by the BASiCS_MCMC function.
Data = makeExampleBASiCS_Data()
MCMC_Output <- BASiCS_MCMC(Data, N = 100, Thin = 2, Burn = 2)
```

BASiCS_Chain-methods *'show' method for BASiCS_Chain objects*

Description

'show' method for [BASiCS_Chain-class](#) objects.

Usage

```
## S4 method for signature 'BASiCS_Chain'
show(object)
```

Arguments

object A BASiCS_Chain object.

Value

Prints a summary of the properties of object.

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl>

References

Vallejos, Marioni and Richardson (2015). Bayesian Analysis of Single-Cell Sequencing data.

Examples

```
Data = makeExampleBASiCS_Data()
MCMC_Output <- BASiCS_MCMC(Data, N = 50, Thin = 2, Burn = 2)
```

BASiCS_DetectHVG *Detection method for highly and lowly variable genes*

Description

Functions to detect highly and lowly variable genes

Usage

```
BASiCS_DetectHVG(Chain, VarThreshold, ProbThreshold = NULL, EFDR = 0.05,
  OrderVariable = "Prob", Plot = FALSE, ...)
```

```
BASiCS_DetectLVG(Chain, VarThreshold, ProbThreshold = NULL, EFDR = 0.05,
  OrderVariable = "Prob", Plot = FALSE, ...)
```

Arguments

Chain	an object of class BASiCS_Chain-class
VarThreshold	Variance contribution threshold (must be a positive value, between 0 and 1)
ProbThreshold	Optional parameter. Posterior probability threshold (must be a positive value, between 0 and 1)
EFDR	Target for expected false discovery rate related to HVG/LVG detection (default = 0.05)
OrderVariable	Ordering variable for output. Must take values in <code>c("GeneIndex", "Mu", "Delta", "Sigma", "Prob")</code>
Plot	If <code>Plot = TRUE</code> a plot of the gene specific expression level against HVG or LVG is generated.
...	Graphical parameters (see par).

Details

See vignette

Value

BASiCS_DetectHVG returns a list of 4 elements:

Table Matrix whose columns contain

GeneIndex	Vector of length <code>q.bio</code> . Gene index as in the order present in the analysed SummarizedExperiment
GeneNames	Vector of length <code>q.bio</code> . Gene name as in the order present in the analysed SummarizedExperiment
Mu	Vector of length <code>q.bio</code> . For each biological gene, posterior median of gene-specific expression levels $\mu[i]$
Delta	Vector of length <code>q.bio</code> . For each biological gene, posterior median of gene-specific biological cell-to-cell heterogeneity hyper-parameter $\delta[i]$
Sigma	Vector of length <code>q.bio</code> . For each biological gene, proportion of the total variability that is due to a cell-to-cell biological heterogeneity component.
Prob	Vector of length <code>q.bio</code> . For each biological gene, probability of being highly variable according to the given thresholds.
HVG	Vector of length <code>q.bio</code> . For each biological gene, indicator of being detected as highly variable according to the given thresholds.

ProbThreshold Posterior probability threshold.

EFDR Expected false discovery rate for the given thresholds.

EFNR Expected false negative rate for the given thresholds.

BASiCS_DetectLVG produces a similar output, replacing the element HVG by LVG, an indicator of a gene being detected as lowly variable according to the given thresholds.

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl>

References

Vallejos, Marioni and Richardson (2015). Bayesian Analysis of Single-Cell Sequencing data. PLoS Computational Biology.

See Also[BASiCS_Chain-class](#)**Examples**

```
# See
help(BASiCS_MCMC)
```

BASiCS_D_TestDE

*Detection of genes with changes in expression.***Description**

Function to assess changes in expression (mean and over-dispersion). This function is no longer in use and will be removed in future releases. Please use [BASiCS_TestDE](#) instead.

Usage

```
BASiCS_D_TestDE(Data = NULL, object = NULL, GeneNames = NULL,
  EpsilonM = 0.1, EpsilonD = 0.1, EviThresholdM = NULL,
  EviThresholdD = NULL, OrderVariable = "ProbDiffExp",
  GroupLabelRef = "Ref", GroupLabelTest = "Test", Plot = FALSE,
  OffSet = FALSE, EFDR_M = 0.05, EFDR_D = 0.05, GenesSelect = NULL, ...)
```

Arguments

Data	an object of class BASiCS_D_Data-class (class no longer in use)
object	an object of class BASiCS_D_Data-class (class no longer in use)
GeneNames	Vector containing gene names to be used in results table (argument to be removed as 'GeneNames' will be an slot of 'BASiCS_D_Data' object)
EpsilonM	Minimum fold change tolerance threshold for detecting changes in overall expression (must be a positive real number)
EpsilonD	Minimum fold change tolerance threshold for detecting changes in cell-to-cell biological over dispersion (must be a positive real number)
EviThresholdM	Optional parameter. Evidence threshold for detecting changes in overall expression (must be a positive value, between 0 and 1)
EviThresholdD	Optional parameter. Evidence threshold for detecting changes in cell-to-cell biological over dispersion (must be a positive value, between 0 and 1)
OrderVariable	Ordering variable for output. Must take values in c("GeneIndex", "GeneNames", "ProbDiffExp",
GroupLabelRef	Label assigned to reference group. Default: GroupLabelRef = "Ref"
GroupLabelTest	Label assigned to reference group. Default: GroupLabelRef = "Test"
Plot	If Plot = T, error rates control rates and volcano plots are generated.
Offset	Optional argument to remove a fix offset effect (if not previously removed from the MCMC chains). This argument will be removed shortly, once offset removal is built as an internal step.

EFDR_M	Target for expected false discovery rate related to the comparison of means (default = 0.05)
EFDR_D	Target for expected false discovery rate related to the comparison of dispersions (default = 0.05)
GenesSelect	Optional argument to provide a user-defined list of genes to be considered for the comparison (default = NULL). When used, this argument must be a vector of 'TRUE' (include gene) / 'FALSE' (exclude gene) indicator, with the same length as the number of intrinsic genes and following the same order as how genes are displayed in the table of counts. This argument is necessary in order to have a meaningful EFDR calibration when the user decides to exclude some genes from the comparison.
...	Graphical parameters (see par).

Details

This function is no longer in use and will be removed in future releases.

Value

This function is no longer in use and will be removed in future releases. Please use [BASiCS_TestDE](#) instead.

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl>

Examples

```
# This function is no longer in use and will be removed in future releases.
```

BASiCS_Filter	<i>Filter for input datasets</i>
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Description

BASiCS_Filter indicates which transcripts and cells pass a pre-defined inclusion criteria. The output of this function can be combined with newBASiCS_Data to generate a the [SummarizedExperiment](#) object required to run BASiCS.

Usage

```
BASiCS_Filter(Counts, Tech, SpikeInput, BatchInfo = NULL,
  MinTotalCountsPerCell = 2, MinTotalCountsPerGene = 2,
  MinCellsWithExpression = 2, MinAvCountsPerCellsWithExpression = 2)
```

Arguments

Counts	Matrix of dimensions q times n whose elements corresponds to the simulated expression counts. First q.bio rows correspond to biological genes. Last q-q.bio rows correspond to technical spike-in genes.
Tech	Logical vector of length q. If Tech = F the gene is biological; otherwise the gene is spike-in.
SpikeInput	Vector of length q-q.bio whose elements indicate the simulated input concentrations for the spike-in genes.
BatchInfo	Vector of length n whose elements indicate batch information. Not required if a single batch is present on the data. Default value: BatchInfo = NULL.
MinTotalCountsPerCell	Minimum value of total expression counts required per cell (biological and technical). Default value: MinTotalCountsPerCell = 2.
MinTotalCountsPerGene	Minimum value of total expression counts required per transcript (biological and technical). Default value: MinTotalCountsPerGene = 2.
MinCellsWithExpression	Minimum number of cells where expression must be detected (positive count). Criteria applied to each transcript. Default value: MinCellsWithExpression = 2.
MinAvCountsPerCellsWithExpression	Minimum average number of counts per cells where expression is detected. Criteria applied to each transcript. Default value: MinAvCountsPerCellsWithExpression = 2.

Value

A list of 2 elements

Counts Filtered matrix of expression counts

Tech Filtered vector of spike-in indicators

SpikeInput Filtered vector of spike-in genes input molecules

BatchInfo Filtered vector of the 'BatchInfo' argument

IncludeGenes Inclusion indicators for transcripts

IncludeCells Inclusion indicators for cells

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl>

References

Vallejos, Marioni and Richardson (2015). Bayesian Analysis of Single-Cell Sequencing data. PLoS Computational Biology.

Vallejos, Marioni and Richardson (2016). Beyond comparisons of means: understanding changes in gene expression at the single-cell level. Genome Biology.

Examples

```

set.seed(1)
Counts = matrix(rpois(50*10, 2), ncol = 10)
rownames(Counts) <- c(paste0("Gene", 1:40), paste0("Spike", 1:10))
Tech = c(rep(FALSE,40),rep(TRUE,10))
set.seed(2)
SpikeInput = rgamma(10,1,1)
SpikeInfo <- data.frame("SpikeID" = paste0("Spike", 1:10), "SpikeInput" = SpikeInput)

Filter = BASiCS_Filter(Counts, Tech, SpikeInput,
                      MinTotalCountsPerCell = 2, MinTotalCountsPerGene = 2,
                      MinCellsWithExpression = 2, MinAvCountsPerCellsWithExpression = 2)
SpikeInfoFilter = SpikeInfo[SpikeInfo$SpikeID %in%
                             names(Filter$IncludeGenes)[Filter$IncludeGenes == TRUE],]
FilterData = newBASiCS_Data(Filter$Counts, Filter$Tech, SpikeInfoFilter)

```

BASiCS_LoadChain	<i>Loads pre-computed MCMC chains generated by the BASiCS_MCMC function</i>
------------------	---

Description

Loads pre-computed MCMC chains generated by the BASiCS_MCMC function, creating a [BASiCS_Chain-class](#) object

Usage

```
BASiCS_LoadChain(RunName, StoreDir = getwd(), WithSpikes = TRUE)
```

Arguments

RunName	String used to index ‘.Rds’ files storing the MCMC chains (produced by the BASiCS_MCMC function)
StoreDir	Directory where ‘.Rds’ files is stored. Default: StoreDir = getwd()
WithSpikes	TRUE/FALSE indicator of spike-ins usage. Default: WithSpikes = TRUE

Value

An object of class [BASiCS_Chain-class](#).

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl>

References

Vallejos, Marioni and Richardson (2015). Bayesian Analysis of Single-Cell Sequencing data.

See Also

[BASiCS_Chain-class](#)

Examples

```
Data = makeExampleBASiCS_Data()
Chain <- BASiCS_MCMC(Data, N = 50, Thin = 5, Burn = 5,
                     StoreChains = TRUE, StoreDir = tempdir(),
                     RunName = "Test")
ChainLoad <- BASiCS_LoadChain(RunName = "Test", StoreDir = tempdir())
```

BASiCS_MCMC

BASiCS MCMC sampler

Description

MCMC sampler to perform Bayesian inference for single-cell mRNA sequencing datasets using the model described in Vallejos et al (2015).

Usage

```
BASiCS_MCMC(Data, N, Thin, Burn, ...)
```

Arguments

Data	A SummarizedExperiment object. This MUST be formatted to include the spike-ins information. See vignette
N	Total number of iterations for the MCMC sampler. Use $N \geq \max(4, \text{Thin})$, N being a multiple of Thin.
Thin	Thinning period for the MCMC sampler. Use $\text{Thin} \geq 2$.
Burn	Burn-in period for the MCMC sampler. Use $\text{Burn} \geq 1$, $\text{Burn} < N$, Burn being a multiple of Thin.
...	Optional parameters.

PriorDelta Specifies the prior used for delta. Possible values are 'gamma' (Gamma(a.delta,b.delta) prior) and 'log-normal' (log-Normal(0,s2.delta) prior) .. Default value: PriorDelta = 'log-normal'.

PriorParam List of 7 elements, containing the hyper-parameter values required for the adopted prior (see Vallejos et al, 2015). All elements must be positive real numbers.

- s2.mu Scale hyper-parameter for the log-Normal(0,s2.mu) prior that is shared by all gene-specific expression rate parameters $\mu[i]$. Default: s2.mu = 0.5.
- s2.delta Only used when 'PriorDelta == 'log-normal''. Scale hyper-parameter for the log-Normal(0,s2.delta) prior that is shared by all gene-specific expression rate parameters $\delta[i]$. Default: s2.delta = 0.5.
- a.delta Only used when 'PriorDelta == 'gamma''. Shape hyper-parameter for the Gamma(a.delta,b.delta) prior that is shared by all gene-specific biological cell-to-cell heterogeneity hyper-parameters $\delta[i]$. Default: a.delta = 1.
- b.delta Only used when 'PriorDelta == 'gamma''. Rate hyper-parameter for the Gamma(a.delta,b.delta) prior that is shared by all gene-specific biological cell-to-cell heterogeneity hyper-parameters $\delta[i]$. Default: b.delta = 1.

- p.phi Dirichlet hyper-parameter for the joint of all (scaled by n) cell-specific mRNA content normalising constants $\phi[j]/n$. Default: p.phi = rep(1, n).
- a.s Shape hyper-parameter for the Gamma(a.s,b.s) prior that is shared by all cell-specific capture efficiency normalising constants $s[j]$. Default: a.s = 1.
- b.s Rate hyper-parameter for the Gamma(a.s,b.s) prior that is shared by all cell-specific capture efficiency normalising constants $s[j]$. Default: b.s = 1.
- a.theta Shape hyper-parameter for the Gamma(a.theta,b.theta) prior for technical noise hyper-parameter θ . Default: a.theta = 1.
- b.theta Rate hyper-parameter for the Gamma(a.theta,b.theta) prior for technical noise hyper-parameter θ . Default: b.theta = 1.
- AR Optimal acceptance rate for adaptive Metropolis Hastings updates. It must be a positive number between 0 and 1. Default (and recommended): AR = 0.44.
- StopAdapt Iteration at which adaptive proposals are not longer adapted. Use StopAdapt>=1. Default: StopAdapt = Burn.
- StoreChains If StoreChains = TRUE, the slots of the generated BASiCS_Chain object are stored in separate .txt files. Each row of the output file containing an iteration (RunName argument used to index file names). Default: StoreChains = FALSE.
- StoreAdapt If StoreAdapt = TRUE, trajectory of adaptive proposal variances (in log-scale) for each parameter are stored in separate .txt files. Each row of the output file containing an iteration (RunName argument used to index file names). Default: StoreAdapt = FALSE.
- StoreDir Directory where output files are stored. Only required if StoreChains = TRUE and/or StoreAdapt = TRUE). Default: StoreDir = getwd().
- RunName String used to index '.txt' files storing chains and/or adaptive proposal variances.
- PrintProgress If PrintProgress = FALSE, console-based progress report is suppressed.
- ls.phi0 Starting value for the adaptive concentration parameter of the Metropolis proposals for phi.
- Start In general, we do not advise to specify this argument. Default options have been tuned to facilitate convergence. It can be used to set user defined starting points for the MCMC algorithm. If used, it must be a list containing the following elements: mu0, delta0, phi0, s0, nu0, theta0, ls.mu0, ls.delta0, ls.phi0, ls.nu0, ls.theta0

Value

An object of class `BASiCS_Chain-class`.

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl> and Nils Eling

References

- Vallejos, Marioni and Richardson (2015). Bayesian Analysis of Single-Cell Sequencing data. PLoS Computational Biology.
- Vallejos, Marioni and Richardson (2016). Beyond comparisons of means: understanding changes in gene expression at the single-cell level. Genome Biology.

Examples

```
# Built-in simulated dataset
Data = makeExampleBASiCS_Data()
# To analyse real data, please refer to the instructions in:
# https://github.com/catavallejos/BASiCS/wiki/2.-Input-preparation

# Only a short run of the MCMC algorithm for illustration purposes
# Longer runs might be required to reach convergence
Chain <- BASiCS_MCMC(Data, N = 50, Thin = 2, Burn = 10, PrintProgress = FALSE)

# For illustration purposes we load a built-in 'BASiCS_Chain' object
# (obtained using the 'BASiCS_MCMC' function)
data(ChainSC)

# `displayChainBASiCS` can be used to extract information from this output. For example:
head(displayChainBASiCS(ChainSC, Param = "mu"))

# Traceplot (examples only)
plot(ChainSC, Param = "mu", Gene = 1)
plot(ChainSC, Param = "phi", Cell = 1)
plot(ChainSC, Param = "theta", Batch = 1)

# Calculating posterior medians and 95% HPD intervals
ChainSummary <- Summary(ChainSC)

# `displaySummaryBASiCS` can be used to extract information from this output. For example:
head(displaySummaryBASiCS(ChainSummary, Param = "mu"))

# Graphical display of posterior medians and 95% HPD intervals (examples only)
plot(ChainSummary, Param = "mu", main = "All genes")
plot(ChainSummary, Param = "mu", Genes = 1:10, main = "First 10 genes")
plot(ChainSummary, Param = "phi", main = "All cells")
plot(ChainSummary, Param = "phi", Cells = 1:5, main = "First 5 cells")
plot(ChainSummary, Param = "theta")

# To contrast posterior medians of cell-specific parameters (example only)
par(mfrow = c(1,2))
plot(ChainSummary, Param = "phi", Param2 = "s", SmoothPlot = FALSE)
# Recommended for large numbers of cells
plot(ChainSummary, Param = "phi", Param2 = "s", SmoothPlot = TRUE)

# To contrast posterior medians of gene-specific parameters
par(mfrow = c(1,2))
plot(ChainSummary, Param = "mu", Param2 = "delta", log = "x", SmoothPlot = FALSE)
# Recommended
plot(ChainSummary, Param = "mu", Param2 = "delta", log = "x", SmoothPlot = TRUE)

# Highly and lowly variable genes detection (within a single group of cells)
DetectHVG <- BASiCS_DetectHVG(ChainSC, VarThreshold = 0.60, EFDR = 0.10, Plot = TRUE)
DetectLVG <- BASiCS_DetectLVG(ChainSC, VarThreshold = 0.40, EFDR = 0.10, Plot = TRUE)

plot(ChainSummary, Param = "mu", Param2 = "delta", log = "x", col = 8)
with(DetectHVG$Table, points(Mu[HVG == TRUE], Delta[HVG == TRUE],
  pch = 16, col = "red", cex = 1))
with(DetectLVG$Table, points(Mu[LVG == TRUE], Delta[LVG == TRUE],
```

```

    pch = 16, col = "blue", cex = 1))

# If variance thresholds are not fixed
BASiCS_VarThresholdSearchHVG(ChainSC, VarThresholdsGrid = seq(0.55,0.65,by=0.01), EFDR = 0.10)
BASiCS_VarThresholdSearchLVG(ChainSC, VarThresholdsGrid = seq(0.35,0.45,by=0.01), EFDR = 0.10)

# For examples of differential analyses between 2 populations of cells:
?BASiCS_TestDE

```

BASiCS_Sim	<i>Simulates expression counts according to the model implemented in BASiCS</i>
------------	---

Description

BASiCS_Sim creates a simulated dataset from the model implemented in BASiCS. This function is used in order to illustrate the performance of the BASiCS library.

Usage

```
BASiCS_Sim(mu, mu_spikes, delta, phi, s, theta)
```

Arguments

mu	Gene-specific expression levels $\mu[i]$ for all biological genes (vector of length q.bio, all elements must be positive numbers)
mu_spikes	$\mu[i]$ for all technical genes defined as true input molecules (SpikeInfo) (vector of length q-q.bio, all elements must be positive numbers)
delta	Gene-specific biological cell-to-cell heterogeneity hyper-parameters $\delta[i]$, biological genes only (vector of length q.bio, all elements must be positive numbers)
phi	Cell-specific mRNA content normalising constants $\phi[j]$ (vector of length n, all elements must be positive numbers and the sum of its elements must be equal to n)
s	Cell-specific capture efficiency (or amplification biases if not using UMI based counts) normalising constants $s[j]$ (vector of length n, all elements must be positive numbers)
theta	Technical variability hyper-parameter θ (must be positive)

Value

An object of class [SummarizedExperiment](#), simulated from the model implemented in BASiCS.

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl>

References

Vallejos, Marioni and Richardson (2015). Bayesian Analysis of Single-Cell Sequencing data.

Examples

```
# Simulated parameter values for 10 genes
# (7 biological and 3 spike-in) measured in 5 cells
Mu = c(8.36, 10.65, 4.88, 6.29, 21.72, 12.93, 30.19)
Mu_spike = c(1010.72, 7.90, 31.59)
Delta = c(1.29, 0.88, 1.51, 1.49, 0.54, 0.40, 0.85)
Phi = c(1.00, 1.06, 1.09, 1.05, 0.80)
S = c(0.38, 0.40, 0.38, 0.39, 0.34)
Theta = 0.39

Data = BASiCS_Sim(Mu, Mu_spike, Delta, Phi, S, Theta)
head(assay(Data))
dim(assay(Data))
metadata(Data)$SpikeInput
rowData(Data)$Tech
```

BASiCS_Summary-class *The BASiCS_Summary class*

Description

Container of a summary of a [BASiCS_Chain-class](#) object. In each slot, first column contains posterior medians, second column contains the lower limits of an high posterior density interval and third column contains the upper limits of high posterior density intervals.

Slots

- mu Posterior medians (first column), lower (second column) and upper (third column) limits of gene-specific expression levels $\mu[i]$.
- delta Posterior medians (first column), lower (second column) and upper (third column) limits of gene-specific biological cell-to-cell heterogeneity hyper-parameters $\delta[i]$, biological genes only
- phi Posterior medians (first column), lower (second column) and upper (third column) limits of cell-specific mRNA content normalising constants $\phi[j]$
- s Posterior medians (first column), lower (second column) and upper (third column) limits of cell-specific capture efficiency (or amplification biases if not using UMI based counts) normalising constants $s[j]$
- nu Posterior medians (first column), lower (second column) and upper (third column) limits of cell-specific random effects $\nu[j]$
- theta Posterior median (first column), lower (second column) and upper (third column) limits of technical variability hyper-parameter θ (each row represents one batch)

Examples

```
# A BASiCS_Summary object created by the Summary method.
Data = makeExampleBASiCS_Data()
MCMC_Output <- BASiCS_MCMC(Data, N = 100, Thin = 2, Burn = 2)
MCMC_Summary <- Summary(MCMC_Output)
```

BASiCS_Summary-methods

'show' method for BASiCS_Summary objects

Description

'show' method for [BASiCS_Summary-class](#) objects.

Usage

```
## S4 method for signature 'BASiCS_Summary'
show(object)
```

Arguments

object A BASiCS_Summary object.

Value

Prints a summary of the properties of object.

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl>

References

Vallejos, Marioni and Richardson (2015). Bayesian Analysis of Single-Cell Sequencing data.

Examples

```
Data = makeExampleBASiCS_Data()
MCMC_Output <- BASiCS_MCMC(Data, N = 50, Thin = 2, Burn = 2)
Summary(MCMC_Output)
```

BASiCS_TestDE

Detection of genes with changes in expression

Description

Function to assess changes in expression (mean and over-dispersion)

Usage

```
BASiCS_TestDE(Chain1, Chain2, EpsilonM = log2(1.5), EpsilonD = log2(1.5),
  EviThresholdM = NULL, EviThresholdD = NULL, OrderVariable = "Prob",
  GroupLabel1 = "Group1", GroupLabel2 = "Group2", Plot = TRUE,
  PlotOffset = TRUE, OffSet = TRUE, EFDR_M = 0.05, EFDR_D = 0.05,
  GenesSelect = NULL, ...)
```

Arguments

Chain1	an object of class <code>BASiCS_Chain-class</code> containing parameter estimates for the first group of cells
Chain2	an object of class <code>BASiCS_Chain-class</code> containing parameter estimates for the second group of cells
EpsilonM	Minimum fold change tolerance threshold for detecting changes in overall expression (must be a positive real number). Default value: $\text{EpsilonM} = \log_2(1.5)$ (i.e. 50% increase).
EpsilonD	Minimum fold change tolerance threshold for detecting changes in cell-to-cell biological over dispersion (must be a positive real number). Default value: $\text{EpsilonM} = \log_2(1.5)$ (i.e. 50% increase).
EviThresholdM	Optional parameter. Evidence threshold for detecting changes in overall expression (must be a positive value, between 0 and 1)
EviThresholdD	Optional parameter. Evidence threshold for detecting changes in cell-to-cell biological over dispersion (must be a positive value, between 0 and 1)
OrderVariable	Ordering variable for output. Must take values in <code>c("GeneIndex", "GeneNames", "Prob")</code> .
GroupLabel1	Label assigned to reference group. Default: <code>GroupLabel1 = "Group1"</code>
GroupLabel2	Label assigned to reference group. Default: <code>GroupLabel2 = "Group2"</code>
Plot	If <code>Plot = TRUE</code> , MA and volcano plots are generated.
PlotOffset	If <code>Plot = TRUE</code> , the offset effect is visualised.
Offset	Optional argument to remove a fix offset effect (if not previously removed from the MCMC chains). This argument will be removed shortly, once offset removal is built as an internal step.
EFDR_M	Target for expected false discovery rate related to the comparison of means (default = 0.05)
EFDR_D	Target for expected false discovery rate related to the comparison of dispersions (default = 0.05)
GenesSelect	Optional argument to provide a user-defined list of genes to be considered for the comparison (default = <code>NULL</code>). When used, this argument must be a vector of 'TRUE' (include gene) / 'FALSE' (exclude gene) indicator, with the same length as the number of intrinsic genes and following the same order as how genes are displayed in the table of counts. This argument is necessary in order to have a meaningful EFDR calibration when the user decides to exclude some genes from the comparison.
...	Graphical parameters (see par).

Value

`BASiCS_TestDE` returns a list of 4 elements:

TableMean A `data.frame` containing the results of the differential mean test

GeneNames	Gene name
MeanOverall	For each gene, the estimated mean expression parameter $\mu[i]$ is averaged across both groups of cells (weighted by sample size).
Mean1	Estimated mean expression parameter $\mu[i]$ for each biological gene in the first group of cells.
Mean2	Estimated mean expression parameter $\mu[i]$ for each biological gene in the second group of cells.

MeanFC Fold change in mean expression parameters between the first and second groups of cells.

MeanLog2FC Log2-transformed fold change in mean expression between the first and second groups of cells.

ProbDiffMean Posterior probability for mean expression difference between the first and second groups of cells.

ResultDiffExp Indicator if a gene has a higher mean expression in the first or second groups of cells.

TableDisp A [data.frame](#) containing the results of the differential dispersion test (excludes genes for which the mean changes).

GeneNames Gene name

MeanOverall For each gene, the estimated mean expression parameter $\mu[i]$ is averaged across both groups of cells (weighted by sample size).

DispOverall For each gene, the estimated over-dispersion parameter $\delta[i]$ is averaged across both groups of cells (weighted by sample size).

Disp1 Estimated over-dispersion parameter $\delta[i]$ for each biological gene in the first group of cells.

Disp2 Estimated over-dispersion parameter $\delta[i]$ for each biological gene in the second group of cells.

DispFC Fold change in over-dispersion parameters between the between the first and second groups of cells.

DispLog2FC Log-transformed fold change in over-dispersion between the first and second groups of cells.

ProbDiffDisp Posterior probability for over-dispersion difference between the first and second groups of cells.

ResultDiffDisp Indicator if a gene has a higher over-dispersion in the first or second groups of cells.

DiffExpSummary A list containing the following information for the differential mean expression test:

EviThreshold Evidence thresholds.

EFDR Expected false discovery rate for the given thresholds.

EFNR Expected false negative rate for the given thresholds.

DiffOverDispSummary A list containing the following information for the differential over-dispersion test:

EviThreshold Evidence thresholds.

EFDR Expected false discovery rate for the given thresholds.

EFNR Expected false negative rate for the given thresholds.

Chain1_offset an [BASiCS_Chain-class](#) object: Chain1 after offset removal.

Chain2_offset an [BASiCS_Chain-class](#) object: Chain2 after offset removal (this is only provided for completeness; Chain2 is not affected by the offset).

OffsetChain MCMC chain calculated for the offset effect.

Offset Estimated offset (posterior median of OffsetChain). Default value set equal to 1 when offset correction is not performed.

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl> and Nils Eling

Examples

```
# Loading two 'BASiCS_Chain' objects (obtained using the 'BASiCS_MCMC' function)
data(ChainSC)
data(ChainRNA)

Test <- BASiCS_TestDE(Chain1 = ChainSC, Chain2 = ChainRNA,
                      GroupLabel1 = "SC", GroupLabel2 = "P&S",
                      EpsilonM = log2(1.5), EpsilonD = log2(1.5), Offset = TRUE)

# Results for the differential mean test
head(Test$TableMean)

# Results for the differential over-dispersion test
# This only includes genes marked as 'NoDiff' in Test$TableMean
head(Test$TableDisp)
```

BASiCS_VarianceDecomp *Decomposition of gene expression variability according to BASiCS*

Description

Function to decompose total variability of gene expression into biological and technical components.

Usage

```
BASiCS_VarianceDecomp(Chain, OrderVariable = "BioVarGlobal", Plot = TRUE,
  ...)
```

Arguments

Chain	an object of class BASiCS_Chain-class
OrderVariable	Ordering variable for output. Must take values in <code>c("GeneNames", "BioVarGlobal", "TechVarGlobal")</code>
Plot	If TRUE, a barplot of the variance decomposition (global and by batches, if any) is generated
...	Other arguments to be passed to barplot

Details

See vignette

Value

A [data.frame](#) whose first 4 columns correspond to

GeneName Gene name (as indicated by user)

BioVarGlobal Percentage of variance explained by a biological cell-to-cell heterogeneity component (overall across all cells)

TechVarGlobal Percentage of variance explained by the technical cell-to-cell heterogeneity component (overall across all cells)

ShotNoiseGlobal Percentage of variance explained by the shot noise component (baseline, overall across all cells)

If more than 1 batch of cells are being analysed, the remaining columns contain the corresponding variance decomposition calculated within each batch.

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl>

References

Vallejos, Marioni and Richardson (2015). Bayesian Analysis of Single-Cell Sequencing data.

See Also

[BASiCS_Chain-class](#)

Examples

```
# See
help(BASiCS_MCMC)
```

BASiCS_VarThresholdSearchHVG

Detection method for highly and lowly variable genes using a grid of variance contribution thresholds

Description

Detection method for highly and lowly variable genes using a grid of variance contribution thresholds

Usage

```
BASiCS_VarThresholdSearchHVG(Chain, VarThresholdsGrid, EFDR = 0.1,
  Progress = TRUE)
```

```
BASiCS_VarThresholdSearchLVG(Chain, VarThresholdsGrid, EFDR = 0.1,
  Progress = TRUE)
```

Arguments

Chain	an object of class BASiCS_Chain-class
VarThresholdsGrid	Grid of values for the variance contribution threshold (they must be contained in (0,1))
EFDR	Target for expected false discovery rate related to HVG/LVG detection (default = 0.10)
Progress	If Progress = TRUE, partial output is printed in the console.

Details

See vignette

Value

BASiCS_VarThresholdSearchHVG A table displaying the results of highly variable genes detecting for different variance contribution thresholds.

BASiCS_VarThresholdSearchLVG A table displaying the results of lowly variable genes detecting for different variance contribution thresholds.oo

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl>

See Also

[BASiCS_Chain-class](#)

Examples

```
# See  
help(BASiCS_MCMC)
```

ChainRNA

Extract from the chain Vallejos et al (2016): pool-and-split samples

Description

Small extract (100 MCMC iterations, 500 randomly selected genes) from the chain obtained in Vallejos et al (2016), related to pool-and-split samples (this corresponds to the RNA 2i samples in Grun et al, 2014).

Usage

ChainRNA

Format

[BASiCS_Chain-class](#) containing 10 MCMC iterations

References

Vallejos et al (2016) - Genome Biology Grun et al (2014) - Nature Methods

ChainSC

*Extract from the chain Vallejos et al (2016): single-cell samples***Description**

Small extract (100 MCMC iterations, 500 randomly selected genes) from the chain obtained in Vallejos et al (2016), related to single-cell samples (this corresponds to the SC 2i samples in Grun et al, 2014).

Usage

ChainSC

Format

BASiCS_Chain-class containing 10 MCMC iterations

References

Vallejos et al (2016) - Genome Biology Grun et al (2014) - Nature Methods

DenoisedCounts

*Calculates normalised and denoised expression counts***Description**

Calculates normalised and denoised expression counts, by removing the effect of technical variation.

Usage

BASiCS_DenoisedCounts(Data, Chain)

Arguments

Data an object of class [SummarizedExperiment](#)
Chain an object of class [BASiCS_Chain-class](#)

Details

See vignette

Value

A matrix of normalised and denoised expression counts.

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl>

See Also[BASiCS_Chain-class](#)**Examples**

```
# See
help(BASiCS_MCMC)
```

DenoisedRates	<i>Calculates normalised and denoised expression rates</i>
---------------	--

Description

Calculates normalised and denoised expression rates, by removing the effect of technical variation.

Usage

```
BASiCS_DenoisedRates(Data, Chain, PrintProgress = FALSE,
  Propensities = FALSE)
```

Arguments

Data	an object of class SummarizedExperiment
Chain	an object of class BASiCS_Chain-class
PrintProgress	If TRUE, partial progress information is printed in the console.
Propensities	If TRUE, returns underlying expression propensities $\rho[ij]$. Otherwise, denoised rates $\mu[i]\rho[ij]$ are returned.

Details

See vignette

Value

A matrix of normalised and denoised expression counts.

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl>

See Also[BASiCS_Chain-class](#)**Examples**

```
# See
help(BASiCS_MCMC)
```

`displayChainBASiCS-BASiCS_Chain-method`*Accessors for the slots of a BASiCS_Chain object*

Description

Accessors for the slots of a [BASiCS_Chain-class](#)

Usage

```
## S4 method for signature 'BASiCS_Chain'  
displayChainBASiCS(object, Param = "mu")
```

Arguments

object	an object of class BASiCS_Chain-class
Param	Name of the slot to be used for the accessed. Possible values: mu, delta, phi, s, nu, theta

Value

The requested slot of an object of class [BASiCS_Chain-class](#)

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl>

References

Vallejos, Marioni and Richardson (2015). Bayesian Analysis of Single-Cell Sequencing data.

See Also

[BASiCS_Chain-class](#)

Examples

```
# See  
help(BASiCS_MCMC)
```

displaySummaryBASiCS-BASiCS_Summary-method

Accessors for the slots of a BASiCS_Summary object

Description

Accessors for the slots of a [BASiCS_Summary-class](#)

Usage

```
## S4 method for signature 'BASiCS_Summary'  
displaySummaryBASiCS(object, Param = "mu")
```

Arguments

object	an object of class BASiCS_Summary-class
Param	Name of the slot to be used for the accessed. Possible values: mu, delta, phi, s, nu, theta

Value

The requested slot of an object of class [BASiCS_Summary-class](#)

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl>

References

Vallejos, Marioni and Richardson (2015). Bayesian Analysis of Single-Cell Sequencing data.

See Also

[BASiCS_Summary-class](#)

Examples

```
# See  
help(BASiCS_MCMC)
```

```
makeExampleBASiCS_Data
```

Create a simple example of a SummarizedExperiment object with random data

Description

A simple `SummarizedExperiment` object is generated by simulating a dataset from the model in BASiCS (Vallejos et al 2015). This is used to illustrate BASiCS in the package examples and in the vignette.

Usage

```
makeExampleBASiCS_Data(WithBatch = FALSE, WithSpikes = TRUE, Example = 1)
```

Arguments

<code>WithBatch</code>	If TRUE, 2 batches are generated (each of them containing 10 cells). Default value: <code>WithBatch = FALSE</code> .
<code>WithSpikes</code>	If TRUE, the simulated dataset contains 20 spike-in genes. Default value: <code>WithSpikes = TRUE</code> .
<code>Example</code>	Choice of example will be generated, defined by different parameter values (possible values = 1, 2). In both cases, expression counts associated to 50 biological genes are generated. This is used to generate two populations for differential testing. Default value <code>Example = 1</code> .

Value

An object of class `SummarizedExperiment`, simulated from the model implemented in BASiCS. If `WithSpikes = TRUE`, it contains 70 genes (50 biological and 20 spike-in) and 20 cells. Alternatively, it contains 50 biological genes and 20 cells.

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl> and Nils Eling

References

Vallejos, Marioni and Richardson (2015). Bayesian Analysis of Single-Cell Sequencing data. PLoS Computational Biology.

Vallejos, Marioni and Richardson (2016). Beyond comparisons of means: understanding changes in gene expression at the single-cell level. Genome Biology.

Examples

```
Data = makeExampleBASiCS_Data()
is(Data, "SummarizedExperiment")
```

newBASiCS_Chain	<i>Creates a BASiCS_Chain object from pre-computed MCMC chains</i>
-----------------	--

Description

BASiCS_Chain creates a [BASiCS_Chain-class](#) object from pre-computed MCMC chains.

Usage

```
newBASiCS_Chain(mu, delta, phi, s, nu, theta)
```

Arguments

mu	MCMC chain for gene-specific expression levels $\mu[i]$, defined as true input molecules in case of technical genes (matrix with q columns, technical genes located at the end of the matrix, all elements must be positive numbers)
delta	MCMC chain for gene-specific biological cell-to-cell heterogeneity hyper-parameters $\delta[i]$, biological genes only (matrix with q.bio columns, all elements must be positive numbers)
phi	MCMC chain for cell-specific mRNA content normalising constants $\phi[j]$ (matrix with n columns, all elements must be positive numbers and the sum of its elements must be equal to n)
s	MCMC chain for cell-specific capture efficiency (or amplification biases if not using UMI based counts) normalising constants $s[j]$ (matrix with n columns, all elements must be positive numbers)
nu	MCMC chain for cell-specific random effects $\nu[j]$ (matrix with n columns, all elements must be positive numbers)
theta	MCMC chain for technical variability hyper-parameter θ (vector, all elements must be positive)

Value

An object of class [BASiCS_Chain-class](#).

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl>

References

Vallejos, Marioni and Richardson (2015). Bayesian Analysis of Single-Cell Sequencing data.

See Also

[BASiCS_Chain-class](#)

Examples

```
# Data = makeExampleBASiCS_Data()
# MCMC_Output <- BASiCS_MCMC(Data, N = 50, Thin = 5, Burn = 5,
#                               StoreChains = TRUE, StoreDir = getwd(), RunName = "Test")

# ChainMu = as.matrix(read.table("chain_mu_Test.txt"))
# ChainDelta = as.matrix(read.table("chain_delta_Test.txt"))
# ChainPhi = as.matrix(read.table("chain_phi_Test.txt"))
# ChainS = as.matrix(read.table("chain_s_Test.txt"))
# ChainNu = as.matrix(read.table("chain_nu_Test.txt"))#
# ChainTheta = read.table("chain_theta_Test.txt")[,1]

# MCMC_Output_Load <- newBASiCS_Chain(mu = ChainMu, delta = ChainDelta,
#   phi = ChainPhi, s = ChainS, nu = ChainNu, theta = ChainTheta)
```

newBASiCS_Data	<i>Creates a SummarizedExperiment object from a matrix of expression counts and experimental information about spike-in genes</i>
----------------	---

Description

newBASiCS_Data creates a [SummarizedExperiment](#) object from a matrix of expression counts and experimental information about spike-in genes.

Usage

```
newBASiCS_Data(Counts, Tech, SpikeInfo, BatchInfo = NULL)
```

Arguments

Counts	Matrix of dimensions q times n whose elements contain the expression counts to be analyses (including biological and technical spike-in genes). Gene names must be stored as 'rownames(Counts)'.
Tech	Logical vector of length q. If Tech = FALSE the gene is biological; otherwise the gene is spike-in.
SpikeInfo	data.frame whose first and second columns contain the gene names assigned to the spike-in genes (they must match the ones in 'rownames(Counts)') and the associated input number of molecules, respectively.
BatchInfo	Vector of length n whose elements indicate batch information. Not required if a single batch is present on the data. Default value: BatchInfo = NULL.

Value

An object of class [SummarizedExperiment](#).

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl> and Nils Eling

References

Vallejos, Marioni and Richardson (2015). Bayesian Analysis of Single-Cell Sequencing data. PLoS Computational Biology.

Vallejos, Marioni and Richardson (2016). Beyond comparisons of means: understanding changes in gene expression at the single-cell level. Genome Biology.

See Also

[SummarizedExperiment](#)

Examples

```
# Expression counts
set.seed(1)
Counts = matrix(rpois(50*10, 2), ncol = 10)
rownames(Counts) <- c(paste0("Gene", 1:40), paste0("Spike", 1:10))

# Technical information
Tech = c(rep(FALSE,40),rep(TRUE,10))

# Spikes input number of molecules
set.seed(2)
SpikeInfo <- data.frame(gene=rownames(Counts)[Tech],amount=rgamma(10,1,1))

# Creating a BASiCS_Data object (no batch effect)
DataExample = newBASiCS_Data(Counts, Tech, SpikeInfo)

# Creating a BASiCS_Data object (with batch effect)
BatchInfo = c(rep(1, 5), rep(2, 5))
DataExample = newBASiCS_Data(Counts, Tech, SpikeInfo, BatchInfo)

# Thanks to Simon Andrews for reporting an issue in previous version of this documentation
```

plot-BASiCS_Chain-method

'plot' method for BASiCS_Chain objects

Description

'plot' method for BASiCS_Chain objects

Usage

```
## S4 method for signature 'BASiCS_Chain,ANY'
plot(x, Param = "mu", Gene = NULL,
     Cell = NULL, Batch = 1, ylab = "", xlab = "", ...)
```

Arguments

x	A BASiCS_Chain object.
Param	Name of the slot to be used for the plot. Possible values: mu, delta, phi, s, nu, theta
Gene	Specifies which gene is requested. Required only if Param = "mu" or "delta"
Cell	Specifies which cell is requested. Required only if Param = "phi", "s" or "nu"
Batch	Specifies which batch is requested. Required only if Param = "theta"
ylab	As in par .
xlab	As in par .
...	Other graphical parameters (see par).

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl>

References

Vallejos, Marioni and Richardson (2015). Bayesian Analysis of Single-Cell Sequencing data.

Examples

```
# See
help(BASiCS_MCMC)
```

plot-BASiCS_Summary-method

'plot' method for BASiCS_Summary objects

Description

'plot' method for BASiCS_Summary objects

Usage

```
## S4 method for signature 'BASiCS_Summary,ANY'
plot(x, Param = "mu", Param2 = NULL,
     Genes = NULL, Cells = NULL, Batches = NULL, xlab = "", ylab = "",
     xlim = "", ylim = "", pch = 16, col = "blue", bty = "n",
     SmoothPlot = TRUE, ...)
```

Arguments

<code>x</code>	A BASiCS_Summary object.
<code>Param</code>	Name of the slot to be used for the plot. Possible values: mu, delta, phi, s, nu, theta
<code>Param2</code>	Name of the second slot to be used for the plot. Possible values: mu, delta, phi, s, nu (combinations between gene-specific and cell-specific parameters are not admitted)
<code>Genes</code>	Specifies which genes are requested. Required only if <code>Param = "mu"</code> or <code>"delta"</code>
<code>Cells</code>	Specifies which cells are requested. Required only if <code>Param = "phi"</code> , <code>"s"</code> or <code>"nu"</code>
<code>Batches</code>	Specifies which batches are requested. Required only if <code>Param = "theta"</code>
<code>xlab</code>	As in par .
<code>ylab</code>	As in par .
<code>xlim</code>	As in par .
<code>ylim</code>	As in par .
<code>pch</code>	As in par .
<code>col</code>	As in par .
<code>bty</code>	As in par .
<code>SmoothPlot</code>	Logical parameter. If TRUE, transparency will be added to the color of the dots.
<code>...</code>	Other graphical parameters (see par).

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl>

References

Vallejos, Marioni and Richardson (2015). Bayesian Analysis of Single-Cell Sequencing data.

Examples

```
# See
help(BASiCS_MCMC)
```

Summary

'Summary' method for BASiCS_Chain objects

Description

For each of the BASiCS parameters (see Vallejos et al 2015), `Summary` returns the corresponding posterior medians and limits of the high posterior density interval with probability equal to `prob`.

Usage

```
## S4 method for signature 'BASiCS_Chain'
Summary(x, prob = 0.95)
```

Arguments

x	A BASiCS_Chain object.
prob	prob argument for HPDinterval function.

Value

An object of class [BASiCS_Summary-class](#).

Author(s)

Catalina A. Vallejos <cnvallej@uc.cl>

References

Vallejos, Marioni and Richardson (2015). Bayesian Analysis of Single-Cell Sequencing data.

Examples

```
Data = makeExampleBASiCS_Data()
MCMC_Output <- BASiCS_MCMC(Data, N = 50, Thin = 2, Burn = 2)
MCMC_Summary <- Summary(MCMC_Output)

# See documentation of function BASiCS_MCMC
```

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