# Package 'SignacX'

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CID.	ntropy Normalized Shannon entropy-based "unclassified" assignment

# Description

 ${\tt CID.entropy\ calculates\ the\ normalized\ Shannon\ entropy\ of\ labels\ for\ each\ cell\ among\ k-nearest\ neighbors\ less\ than\ four-degrees\ apart,\ and\ then\ sets\ cells\ with\ statistically\ significant\ large\ Shannon\ entropy\ to\ be\ "Unclassified."}$ 

### Usage

```
CID.entropy(ac, distM)
```

# Arguments

ac a character vector of cell type labels
distM the distance matrix, see ?CID.GetDistMat

# Value

A character vector like 'ac' but with cells type labels set to "Unclassified" if there was high normalized Shannon entropy.

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#### **Examples**

```
## Not run:
# load data classified previously (see \code{SignacFast})
P <- readRDS("celltypes.rds")
S <- readRDS("pbmcs.rds")

# get edges from default assay from Seurat object
default.assay <- Seurat::DefaultAssay(S)
edges = S@graphs[[which(grepl(paste0(default.assay, "_nn"), names(S@graphs)))]]

# get distance matrix
D = CID.GetDistMat(edges)

# entropy-based unclassified labels labels
entropy = CID.entropy(ac = P$L2, distM = D)

## End(Not run)</pre>
```

CID.GetDistMat

Computes distance matrix from edge list

# Description

CID.GetDistMat returns the distance matrix (i.e., adjacency matrix, second-degree adjacency matrix, ..., etc.) from an edge list. Here, edges is the edge list, and n is the order of the connetions.

#### Usage

```
CID.GetDistMat(edges, n = 4)
```

#### Arguments

edges a data frame with two columns; V1 and V2, specifying the cell-cell edge list for

the network

n maximum network distance to subtend (n neighbors)

#### Value

adjacency matrices for distances < n

```
## Not run:
# Loads edges
file.dir = "https://kleintools.hms.harvard.edu/tools/client_datasets/"
file = "CITESEQ_EXPLORATORY_CITESEQ_5K_PBMCS/FullDataset_v1_protein/edges.csv"
download.file(paste0(file.dir, file, "?raw=true"), destfile = "edges.csv")
# data.dir is your path to the "edges.csv" file
```

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```
edges = CID.LoadEdges(data.dir = ".")

# get distance matrix (adjacency matrices with up to fourth-order connections) from edge list
distance_matrices = CID.GetDistMat(edges)

## End(Not run)
```

CID.IsUnique

Extracts unique elements

# **Description**

CID. IsUnique returns a Boolean for the unique elements of a vector.

# Usage

```
CID. IsUnique(x)
```

#### **Arguments**

Х

A character vector

#### Value

boolean, unique elements are TRUE

#### **Examples**

```
# generate a dummy variable
dummy = c("A", "A", "B", "C")

# get only unique elements
logik = CID.IsUnique(dummy)
dummy[logik]
```

CID.LoadData

Load data file from directory

# Description

Loads 'matrix.mtx' and 'genes.txt' files from a directory.

## Usage

```
CID.LoadData(data.dir, mfn = "matrix.mtx")
```

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#### **Arguments**

data.dir Directory containing matrix.mtx and genes.txt.

mfn file name; default is 'matrix.mtx'

#### Value

A sparse matrix with rownames equivalent to the names in genes.txt

#### **Examples**

```
## Not run:
# Loads data from SPRING

# dir is your path to the "categorical_coloring_data.json" file
dir = "./FullDataset_v1"

# load expression data
E = CID.LoadData(data.dir = dir)

## End(Not run)
```

CID.LoadEdges

Load edges from edge list for single cell network

### **Description**

CID. LoadEdges loads edges, typically after running the SPRING pipeline.

#### Usage

```
CID.LoadEdges(data.dir)
```

### **Arguments**

data.dir A directory where "edges.csv" file is located

#### Value

The edge list in data frame format

```
## Not run:
# Loads edges
file.dir = "https://kleintools.hms.harvard.edu/tools/client_datasets/"
file = "CITESEQ_EXPLORATORY_CITESEQ_5K_PBMCS/FullDataset_v1_protein/edges.csv"
download.file(paste0(file.dir, file, "?raw=true"), destfile = "edges.csv")
# data.dir is your path to the "edges.csv" file
```

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```
edges = CID.LoadEdges(data.dir = ".")
## End(Not run)
```

CID.Louvain

Detects community substructure by Louvain community detection

# **Description**

CID. Louvain determines Louvain clusters from an edge list.

#### Usage

```
CID.Louvain(edges)
```

#### **Arguments**

edges

A data frame or matrix with edges

#### Value

A character vector with the community substructures of the graph corresponding to Louvain clusters.

```
## Not run:
# Loads edges
file.dir = "https://kleintools.hms.harvard.edu/tools/client_datasets/"
file = "CITESEQ_EXPLORATORY_CITESEQ_5K_PBMCS/FullDataset_v1_protein/edges.csv"
download.file(paste0(file.dir, file, "?raw=true"), destfile = "edges.csv")

# data.dir is your path to the "edges.csv" file
edges = CID.LoadEdges(data.dir = ".")

# get louvain clusters from edge list
clusters = CID.Louvain(edges)

## End(Not run)
```

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CID.Normalize

Library size normalize

# Description

CID. Normalize normalizes the expression matrix to the mean library size.

#### Usage

```
CID.Normalize(E)
```

# Arguments

Ε

Expression matrix

#### Value

Normalized expression matrix where each cell sums to the mean total counts

```
## Not run:
# download single cell data for classification
file.dir = "https://cf.10xgenomics.com/samples/cell-exp/3.0.0/pbmc_1k_v3/"
file = "pbmc_1k_v3_filtered_feature_bc_matrix.h5"
download.file(paste0(file.dir, file), "Ex.h5")
# load data, process with Seurat
library(Seurat)
E = Read10X_h5(filename = "Ex.h5")
# observe average total counts
mean(Matrix::colSums(E))
# run normalization
E_norm = CID.Normalize(E)
# check normalization
kmu = mean(Matrix::colSums(E_norm))
head(kmu)
## End(Not run)
```

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CID.smooth

Smoothing function

#### **Description**

CID. smooth uses k-nearest neighbors to identify cells which correspond to a different label than the majority of their first-degree neighbors. If so, those annotations are "smoothed."

#### Usage

```
CID.smooth(ac, dM)
```

#### **Arguments**

ac list containing a character vector where each element is a cell type or cell state

assignment.

dM distance matrix (see ?CID.GetDistMat).

#### Value

A character vector with smoothed labels

```
## Not run:
# load data classified previously (see SignacFast)
P <- readRDS("celltypes.rds")
S <- readRDS("pbmcs.rds")

# get edges from default assay from Seurat object
default.assay <- Seurat::DefaultAssay(S)
edges = S@graphs[[which(grepl(paste0(default.assay, "_nn"), names(S@graphs)))]]

# get distance matrix
D = CID.GetDistMat(edges)

# smooth labels
smoothed = CID.smooth(ac = P$CellTypes, dM = D[[1]])

## End(Not run)</pre>
```

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CID.writeJSON	Writes JSON file for SPRING integration	

# Description

CID.writeJSON is a SPRING-integrated function for writing a JSON file.

# Usage

```
CID.writeJSON(
    cr,
    json_new = "categorical_coloring_data.json",
    spring.dir,
    new_populations = NULL,
    new_colors = NULL
)
```

# Arguments

cr	output from GenerateLabels			
json_new	Filename where annotations will be saved for new SPRING color tracks. Default is "categorical_coloring_data_new.json".			
spring.dir	Directory where file 'categorical_coloring_data.json' is located. If set, will add Signac annotations to the file.			
new_populations				
	Character vector specifying any new cell types that Signac has learned. Default is NULL.			
new_colors	Character vector specifying the HEX color codes for new cell types. Default is NULL.			

#### Value

A categorical\_coloring\_data.json file with Signac annotations and Louvain clusters added.

# Description

GenerateLabels returns a list of cell type and cell state labels, as well as novel cellular phenotypes and unclassified cells.

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#### Usage

```
GenerateLabels(
   cr,
   E = NULL,
   smooth = TRUE,
   new_populations = NULL,
   new_categories = NULL,
   min.cells = 10,
   spring.dir = NULL,
   graph.used = "nn"
)
```

#### **Arguments**

cr list returned by Signac or by SignacFast.

E a sparse gene (rows) by cell (column) matrix, or a Seurat object. Rows are

HUGO symbols.

smooth if TRUE, smooths the cell type classifications. Default is TRUE.

new\_populations

Character vector specifying any new cell types that were learned by Signac.

Default is NULL.

new\_categories If new\_populations are set to a cell type, new\_category is a corresponding char-

acter vector indicating the population that the new population belongs to. De-

fault is NULL.

min.cells If desired, any cell population with equal to or less than N cells is set to "Un-

classified." Default is 10 cells.

spring.dir If using SPRING, directory to categorical\_coloring\_data.json. Default is NULL.

the graphs field of the Seurat object. Other options are "wnn" to use weighted

nearest neighbors, as well as "snn" to use shared nearest neighbors.

#### Value

A list of cell type labels for cell types, cell states and novel populations.

```
## Not run:
# download single cell data for classification
file.dir = "https://cf.10xgenomics.com/samples/cell-exp/3.0.0/pbmc_1k_v3/"
file = "pbmc_1k_v3_filtered_feature_bc_matrix.h5"
download.file(paste0(file.dir, file), "Ex.h5")

# load data, process with Seurat
library(Seurat)
E = Read10X_h5(filename = "Ex.h5")
pbmc <- CreateSeuratObject(counts = E, project = "pbmc")</pre>
```

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```
# run Seurat pipeline
pbmc <- SCTransform(pbmc, verbose = FALSE)
pbmc <- RunPCA(pbmc, verbose = FALSE)
pbmc <- FindNeighbors(pbmc, dims = 1:30, verbose = FALSE)
# classify cells
labels = SignacFast(E = pbmc)
celltypes = GenerateLabels(labels, E = pbmc)
## End(Not run)</pre>
```

Genes\_Of\_Interest

Genes of interest for drug discovery / disease biology research

# Description

3,304 genes curated from external sources

#### Usage

Genes\_Of\_Interest

#### **Format**

A data frame with five columns

Genes genes, identified by HUGO symbols

CellPhoneDB 0 if not in CellPhoneDB, 1 if gene is listed as a receptor in CellPhoneDB

**GWAS** 0 if not GWAS, 1 if GWAS in GWAS catalog

**PI\_drugs** 0 if not in Priority Index paper, 1 if gene is listed as a drug target in priority index paper **PI\_GWAS** 0 if not in Priority Index GWAS list, 1 if gene is listed as GWAS in priority index paper

...

GetModels\_HPCA

Loads neural network models from GitHub

#### **Description**

GetModels\_HPCA returns a list of neural network models that were trained with the HPCA training data. The HPCA training data has 18 classification tasks with bootstrapped training data. The training data are split into two distinct cellular phenotypes (i.e., immune and nonimmune). GetModels\_HPCA downloads pre-computed neural networks trained to classify cells for each task (n = 100 models trained for each tasks).

#### Usage

```
GetModels_HPCA()
```

#### Value

list of 1,800 neural network models stacked to solve 18 classification problems.

#### See Also

```
SignacFast, ModelGenerator
```

# **Examples**

```
## Not run:
P = GetModels()
## End(Not run)
```

GetTrainingData\_HPCA Loads bootstrapped HPCA training data from GitHub

# Description

GetTrainingData\_HPCA returns a list of bootstrapped HPCA training data. The HPCA training data has 18 classification tasks, each split into two distinct cellular phenotypes (i.e., immune and nonimmune). GetTrainingData\_HPCA downloads bootstrapped training data to use for model-building.

#### Usage

```
GetTrainingData_HPCA()
```

#### Value

A list with two elements; 'Reference' are the training data, and 'genes' – the union of all features present in the training data.

```
## Not run:
P = GetTrainingData_HPCA()
## End(Not run)
```

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KSoftImpute	KNN-based imputation	

#### **Description**

KSoftImpute is an ultra-fast method for imputing missing gene expression values in single cell data. KSoftImpute uses k-nearest neighbors to impute the expression of each gene by the weighted average of itself and it's first-degree neighbors. Weights for imputation are determined by the number of detected genes. This method works for large data sets (>100,000 cells) in under a minute.

#### Usage

```
KSoftImpute(E, dM = NULL, genes.to.use = NULL, verbose = FALSE)
```

#### **Arguments**

E A gene-by-sample count matrix (sparse matrix or matrix) with genes identified

by their HUGO symbols.

dM see ?CID.GetDistMat

genes.to.use a character vector of genes to impute. Default is NULL.

verbose If TRUE, code reports outputs. Default is FALSE.

#### Value

An expression matrix (sparse matrix) with imputed values.

#### See Also

```
Signac and SignacFast
```

```
## Not run:
# download single cell data for classification
file.dir = "https://cf.10xgenomics.com/samples/cell-exp/3.0.0/pbmc_1k_v3/"
file = "pbmc_1k_v3_filtered_feature_bc_matrix.h5"
download.file(paste0(file.dir, file), "Ex.h5")

# load data, process with Seurat
library(Seurat)
E = Read10X_h5(filename = "Ex.h5")
pbmc <- CreateSeuratObject(counts = E, project = "pbmc")

# run Seurat pipeline
pbmc <- SCTransform(pbmc, verbose = FALSE)
pbmc <- RunPCA(pbmc, verbose = FALSE)
pbmc <- RunUMAP(pbmc, dims = 1:30, verbose = FALSE)
pbmc <- FindNeighbors(pbmc, dims = 1:30, verbose = FALSE)</pre>
```

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```
# get edges from default assay from Seurat object
default.assay <- Seurat::DefaultAssay(pbmc)
edges = pbmc@graphs[[which(grepl(paste0(default.assay, "_nn"), names(pbmc@graphs)))]]
# get distance matrix
dM = CID.GetDistMat(edges)
# run imputation
Z = KSoftImpute(E = E, dM = dM, verbose = TRUE)
## End(Not run)</pre>
```

MASC

Mixed effect modeling

# **Description**

MASC was imported from https://github.com/immunogenomics/masc. Performs mixed-effect modeling.

# Usage

```
MASC(
   dataset,
   cluster,
   contrast,
   random_effects = NULL,
   fixed_effects = NULL,
   verbose = FALSE
)
```

#### Arguments

data frame of covariate, cell type, clustering or disease information

cluster celltypes returned by Signac or cluster identities

contrast Typically disease

random\_effects User specified random effect variables in dataset

fixed\_effects User specific fixed effects in dataset verbose If TRUE, algorithm reports outputs

#### Value

mixed effect model results

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#### **Examples**

```
## Not run:
# Load metadata
file.dir = "https://kleintools.hms.harvard.edu/tools/client_datasets/"
file = "AMP_Phase1_SLE_Apr2019/FullDataset_v1/categorical_coloring_data.json"
download.file(paste0(file.dir, file, "?raw=true"), destfile = "categorical_coloring_data.json")
d = rjson::fromJSON(file='categorical_coloring_data.json')
d = data.frame(sapply(d, function(x) x$label_list))

# run MASC
x = d$CellStates # optionally use clusters or cell types
d$Disease = factor(d$Disease) # the contrast term must be encoded as a factor
Q = MASC(d, cluster = x, contrast = 'Disease', random_effects = c( "Tissue", "Plate", "Sample"))
## End(Not run)
```

ModelGenerator

Generates an ensemble of neural network models.

#### **Description**

ModelGenerator generates an ensemble of neural network models each trained to classify cellular phenotypes using the reference data set.

#### Usage

```
ModelGenerator(
   R,
   N = 1,
   num.cores = 1,
   verbose = TRUE,
   hidden = 1,
   set.seed = TRUE,
   seed = "42"
)
```

#### **Arguments**

R	Reference data set returned by GetTrainingData_HPCA
N	Number of neural networks to train. Default is 1.
num.cores	Number of cores to use for parallel computing. Default is 1.
verbose	if TRUE, code will report outputs. Default is TRUE.
hidden	Number of hidden layers in the neural network. Default is 1.
set.seed	If TRUE, seed is set to ensure reproducibility of these results. Default is TRUE.
seed	if set.seed is TRUE, the seed can be set. Default is 42.

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#### Value

A list, each containing N neural network models

#### See Also

[SignacFast()] for a function that uses the models generated by this function.

#### **Examples**

```
## Not run:
# download training data set from GitHub
Ref = GetTrainingData_HPCA()

# train a stack of 1,800 neural network models
Models = ModelGenerator(R = Ref, N = 100, num.cores = 4)

# save models
save(Models, file = "models.rda")

## End(Not run)
```

SaveCountsToH5

Save count\_matrix.h5 files for SPRING integration

#### **Description**

To integrate with SPRING, SaveCountsToH5 saves expression matrices in a sparse ".h5" format to be read with SPRING notebooks in Jupyter.

#### Usage

```
SaveCountsToH5(D, data.dir, genome = "GRCh38")
```

### **Arguments**

D A list of count matrices

data.dir directory (will be created if it does not exist) where results are saved

genome default is GRCh38.

#### Value

matrix.h5 file, where each is background corrected

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#### **Examples**

```
## Not run:
# download single cell data for classification
file.dir = "https://cf.10xgenomics.com/samples/cell-exp/3.0.0/pbmc_1k_v3/"
file = "pbmc_1k_v3_filtered_feature_bc_matrix.h5"
download.file(paste0(file.dir, file), "Ex.h5")

# load data
library(Seurat)
E = Read10X_h5(filename = "Ex.h5")

# save counts to h5 files
SaveCountsToH5(E, data.dir = "counts_h5")

## End(Not run)
```

Signac

Classification of cellular phenotypes in single cell data

# Description

Signac trains and then uses an ensemble of neural networks to classify cellular phenotypes using an expression matrix or Seurat object. The neural networks are trained with the HPCA training data using only features that are present in both the single cell and HPCA training data set. Signac returns annotations at each level of the classification hierarchy, which are then converted into cell type labels using GenerateLabels. For a faster alternative, try SignacFast, which uses pre-computed neural network models.

#### Usage

```
Signac(
 Ε,
 R = "default",
  spring.dir = NULL,
 N = 100,
  num.cores = 1,
  threshold = 0,
  smooth = TRUE,
  impute = TRUE,
  verbose = TRUE,
  do.normalize = TRUE,
  return.probability = FALSE,
  hidden = 1,
  set.seed = TRUE,
  seed = "42",
  graph.used = "nn"
)
```

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#### **Arguments**

E a sparse gene (rows) by cell (column) matrix, or a Seurat object. Rows are

HUGO symbols.

R Reference data. If 'default', R is set to GetTrainingData\_HPCA().

spring.dir If using SPRING, directory to categorical\_coloring\_data.json. Default is NULL.

N Number of machine learning models to train (for nn and svm). Default is 100.

num. cores Number of cores to use. Default is 1.

threshold Probability threshold for assigning cells to "Unclassified." Default is 0.

smooth if TRUE, smooths the cell type classifications. Default is TRUE.

impute if TRUE, gene expression values are imputed prior to cell type classification (see

KSoftImpute). Default is TRUE.

verbose if TRUE, code will report outputs. Default is TRUE.

do.normalize if TRUE, cells are normalized to the mean library size. Default is TRUE.

return.probability

if TRUE, returns the probability associated with each cell type label. Default is

TRUE.

hidden Number of hidden layers in the neural network. Default is 1.

set.seed If true, seed is set to ensure reproducibility of these results. Default is TRUE.

seed if set.seed is TRUE, seed is set to 42.

graph.used If using Seurat object by default, Signac uses the nearest neighbor graph in

the graphs field of the Seurat object. Other options are "wnn" to use weighted

nearest neighbors, as well as "snn" to use shared nearest neighbors.

#### Value

A list of character vectors: cell type annotations (L1, L2, ...) at each level of the hierarchy as well as 'clusters' for the Louvain clustering results.

#### See Also

SignacFast, a faster alternative that only differs from Signac in nuanced T cell phenotypes.

```
## Not run:
# download single cell data for classification
file.dir = "https://cf.10xgenomics.com/samples/cell-exp/3.0.0/pbmc_1k_v3/"
file = "pbmc_1k_v3_filtered_feature_bc_matrix.h5"
download.file(paste0(file.dir, file), "Ex.h5")

# load data, process with Seurat
library(Seurat)
E = Read10X_h5(filename = "Ex.h5")
pbmc <- CreateSeuratObject(counts = E, project = "pbmc")</pre>
```

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```
# run Seurat pipeline
pbmc <- SCTransform(pbmc, verbose = FALSE)
pbmc <- RunPCA(pbmc, verbose = FALSE)
pbmc <- RunUMAP(pbmc, dims = 1:30, verbose = FALSE)
pbmc <- FindNeighbors(pbmc, dims = 1:30, verbose = FALSE)

# classify cells
labels = Signac(E = pbmc)
celltypes = GenerateLabels(labels, E = pbmc)

# add labels to Seurat object, visualize
pbmc <- Seurat::AddMetaData(pbmc, metadata=celltypes$CellTypes_novel, col.name = "immmune")
pbmc <- Seurat::SetIdent(pbmc, value='immmune')
DimPlot(pbmc)

# save results
saveRDS(pbmc, "example_pbmcs.rds")

## End(Not run)</pre>
```

SignacBoot

Generates bootstrapped single cell data

#### **Description**

SignacBoot uses a Seurat object or an expression matrix and performs feature selection, normalization and bootstrapping to generate a training data set to be used for cell type or cluster classification.

#### Usage

```
SignacBoot(
   E,
   L,
   labels,
   size = 1000,
   impute = TRUE,
   spring.dir = NULL,
   logfc.threshold = 0.25,
   p.val.adj = 0.05,
   verbose = TRUE
)
```

#### Arguments

E a gene (rows) by cell (column) matrix, sparse matrix or a Seurat object. Rows are HUGO symbols.

L cell type categories for learning.

labels cell type labels corresponding to the columns of E.

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Number of bootstrapped samples for machine learning. Default is 1,000. size if TRUE, performs imputation prior to bootstrapping (see KSoftImpute). Deimpute fault is TRUE. spring.dir if using SPRING, directory to categorical\_coloring\_data.json. Default is NULL. logfc.threshold Cutoff for feature selection. Default is 0.25.

p.val.adj Cutoff for feature selection. Default is 0.05. verbose if TRUE, code speaks. Default is TRUE.

#### Value

Training data set (data.frame) to be used for building new models=.

#### See Also

**ModelGenerator** 

#### **Examples**

```
## Not run:
# load Seurat object from SignacFast example
P <- readRDS("pbmcs.rds")</pre>
# run feature selection + bootstrapping to generate 2,000 bootstrapped cells
x = P@meta.data$celltypes
R_learned = SignacBoot(P, L = c("B.naive", "B.memory"), labels = x)
## End(Not run)
```

SignacFast

Fast classification of cellular phenotypes

#### **Description**

SignacFast uses pre-computed neural network models to classify cellular phenotypes in single cell data: these models were pre-trained with the HPCA training data. Any features that are present in the training data and absent in the single cell data are set to zero. This is a factor of ~5-10 speed improvement over Signac.

#### Usage

```
SignacFast(
 Ε,
 Models = "default",
  spring.dir = NULL,
  num.cores = 1,
  threshold = 0,
```

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```
smooth = TRUE,
impute = TRUE,
verbose = TRUE,
do.normalize = TRUE,
return.probability = FALSE,
graph.used = "nn"
)
```

#### **Arguments**

E a gene (rows) by cell (column) matrix, sparse matrix or a Seurat object. Rows

are HUGO symbols.

Models if 'default', as returned by GetModels\_HPCA. An ensemble of 1,800 neural net-

work models.

spring.dir If using SPRING, directory to categorical\_coloring\_data.json. Default is NULL.

num.cores number of cores to use for parallel computation. Default is 1.

threshold Probability threshold for assigning cells to "Unclassified." Default is 0.

smooth if TRUE, smooths the cell type classifications. Default is TRUE.

impute if TRUE, gene expression values are imputed prior to cell type classification (see

KSoftImpute). Default is TRUE.

verbose if TRUE, code will report outputs. Default is TRUE.

do.normalize if TRUE, cells are normalized to the mean library size. Default is TRUE.

return.probability

if TRUE, returns the probability associated with each cell type label. Default is

TRUE.

graph.used If using Seurat object by default, Signac uses the nearest neighbor graph in

the graphs field of the Seurat object. Other options are "wnn" to use weighted

nearest neighbors, as well as "snn" to use shared nearest neighbors.

#### Value

A list of character vectors: cell type annotations (L1, L2, ...) at each level of the hierarchy as well as 'clusters' for the Louvain clustering results.

#### See Also

```
Signac for another classification function.
```

Signac

```
Examples
```

```
## Not run:
# download single cell data for classification
file.dir = "https://cf.10xgenomics.com/samples/cell-exp/3.0.0/pbmc_1k_v3/"
file = "pbmc_1k_v3_filtered_feature_bc_matrix.h5"
download.file(paste0(file.dir, file), "Ex.h5")
```

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```
# load data, process with Seurat
library(Seurat)
E = Read10X_h5(filename = "Ex.h5")
pbmc <- CreateSeuratObject(counts = E, project = "pbmc")</pre>
pbmc <- SCTransform(pbmc)</pre>
pbmc <- RunPCA(pbmc, verbose = FALSE)</pre>
pbmc <- RunUMAP(pbmc, dims = 1:30, verbose = FALSE)</pre>
pbmc <- FindNeighbors(pbmc, dims = 1:30, verbose = FALSE)</pre>
# classify cells
labels = SignacFast(E = pbmc)
celltypes = GenerateLabels(labels, E = pbmc)
# add labels to Seurat object, visualize
lbls <- factor(celltypes$CellStates)</pre>
levels(lbls) <- sort(unique(lbls))</pre>
pbmc <- AddMetaData(pbmc, metadata=celltypes$CellStates, col.name = "celltypes")</pre>
pbmc <- SetIdent(pbmc, value='celltypes')</pre>
DimPlot(pbmc, label = T)
# save results
saveRDS(pbmc, "pbmcs.rds")
saveRDS(celltypes, "celltypes.rds")
## End(Not run)
```

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