# Package 'demuxSNP'

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Title scRNAseq demultiplexing using cell hashing and SNPs

Version 1.9.0

#### Description

This package assists in demultiplexing scRNAseq data using both cell hashing and SNPs data. The SNP profile of each group os learned using high confidence assignments from the cell hashing data.

Cells which cannot be assigned with high confidence from the cell hashing data are assigned to their most similar group based on their SNPs.

We also provide some helper function to optimise SNP selection, create training data and merge SNP data into the SingleCellExperiment framework.

URL https://github.com/michaelplynch/demuxSNP

BugReports https://github.com/michaelplynch/demuxSNP/issues

**License** GPL-3 **Encoding** UTF-8

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2 add\_snps

# **Contents**

add_snps														. 2
commonvariants_1kgenomes_	subs	set								 				. 3
common_genes										 				3
high_conf_calls														4
multiplexed_scrnaseq_sce .										 				5
reassign						 				 				5
reassign_balanced						 				 				6
reassign_centroid						 				 				7
reassign_jaccard						 				 				8
subset_vcf						 				 				9
vartrix consensus snps			 			 				 				10

add\_snps

Add SNPs to SingleCellExperiment object

11

# **Description**

Add SNPs to SingleCellExperiment object

#### Usage

Index

```
add\_snps(sce, mat, thresh = 0.8)
```

# **Arguments**

sce object of class SingleCellExperiment

mat object of class matrix, output from VarTrix in 'consensus' mode (default)

thresh threshold presence of SNP, defaults to 0.8

#### Value

Updated SingleCellExperiment object with snps in altExp slot

#### **Examples**

```
data(multiplexed_scrnaseq_sce, vartrix_consensus_snps)
multiplexed_scrnaseq_sce <- add_snps(sce = multiplexed_scrnaseq_sce,
mat = vartrix_consensus_snps,
thresh = 0.8)</pre>
```

```
common variants\_1 kgenomes\_subset \\ Sample \ vcf \ file
```

#### **Description**

VCF file containing SNPs from a subset of the 1k Genomes common variants HG38 genome build.

#### Usage

```
data(commonvariants_1kgenomes_subset)
```

#### **Format**

An object of class CollapsedVCF with 2609 rows and 0 columns.

# Value

```
commonvariants_1kgenomes_subset:
An object of class CollapsedVcf
```

#### **Source**

https://cellsnp-lite.readthedocs.io/en/latest/snp\_list.html

common_genes	Return a character vector of top n most frequent genes from a Single-
	CellExperiment object.

# Description

Returns a character vector of the top n most frequently expressed genes from the counts of the SingleCellExperiment object. Expression is based on having a count > 0 in a given cell.

```
common\_genes(sce, n = 100)
```

high\_conf\_calls

#### **Arguments**

sce a SingleCellExperiment object

n number of genes to be returned. Defaults to n=100.

#### Value

character vector of n most frequently expressed genes.

# **Examples**

```
data(multiplexed_scrnaseq_sce)
multiplexed_scrnaseq_sce <- common_genes(multiplexed_scrnaseq_sce)</pre>
```

high\_conf\_calls

Run demuxmix to determine high-confidence calls

# Description

Run demuxmix to determine high-confidence calls

# Usage

```
high_conf_calls(sce, assay = "HTO", pacpt = 0.95)
```

# **Arguments**

sce Object of class SingleCellExperiment with HTO (or similar) altExp assay

assay Name of altExp for cell hashing counts to be retrieved from

pacpt acceptance probability for demuxmix model

#### Value

Updated SingleCellExperiment object with logical vector indicating training data, data to be classified (all cells) and assigned labels for all cells.

# **Examples**

```
data(multiplexed_scrnaseq_sce)
multiplexed_scrnaseq_sce <- high_conf_calls(multiplexed_scrnaseq_sce)</pre>
```

```
multiplexed_scrnaseq_sce
```

SingleCellExperiment object containing multiplexed RNA and HTO data from six biological smamples

# **Description**

Example SingleCellExperiment object containing demultiplexed scRNAseq data from six donors, used throughout and built upon in demuxSNP workflow.

# Usage

```
data(multiplexed_scrnaseq_sce)
```

#### **Format**

An object of class SingleCellExperiment with 259 rows and 2000 columns.

# Value

```
multiplexed_scrnaseq_sce:
An object of class SingleCellExperiment
```

reassign

Reassign cells using knn

# Description

k-nearest neighbour classification of cells. Training data is intended to be labels of cells confidently called using cell hashing based methods and their corresponding SNPs. Prediction data can be remaining cells but can also include the training data. Doublets are simulated by randomly combining 'd' SNP profiles from each grouping combination.

```
reassign(
   sce,
   k = 10,
   d = 10,
   train_cells = sce$train,
   predict_cells = sce$predict
)
```

6 reassign\_balanced

# Arguments

sce	object of class SingleCellExperiment
k	number of neighbours used in knn, defaults to 10
d	number of doublets per group combination to simulate, defaults to 10
train_cells	logical vector specifying which cells to use to train classifier
predict_cells	logical vector specifying which cells to classify

#### Value

A SingleCellExperiment with updated group assignments called 'knn'

# **Examples**

```
data(multiplexed_scrnaseq_sce, vartrix_consensus_snps)
multiplexed_scrnaseq_sce <- high_conf_calls(multiplexed_scrnaseq_sce)
multiplexed_scrnaseq_sce <- add_snps(sce = multiplexed_scrnaseq_sce,
mat = vartrix_consensus_snps,
thresh = 0.8)
multiplexed_scrnaseq_sce <- reassign(sce = multiplexed_scrnaseq_sce, k = 10)</pre>
```

reassign\_balanced

Reassign cells using balanced knn with jaccard distance

# **Description**

k-nearest neighbour classification of cells. Training data is intended to be labels of cells confidently called using cell hashing based methods and their corresponding SNPs. Prediction data can be remaining cells but can also include the training data. Doublets are simulated by randomly combining 'd' SNP profiles from each grouping combination.

```
reassign_balanced(
    sce,
    k = 20,
    d_prop = 0.5,
    train_cells = sce$train,
    predict_cells = sce$predict,
    nmin = 50,
    n = NULL
)
```

reassign\_centroid 7

# **Arguments**

sce	object of class SingleCellExperiment
k	number of neighbours used in knn, defaults to 10
d_prop	determines number of doublets simulatted d, as a proportions of n (specified or calculated)
train_cells	logical vector specifying which cells to use to train classifier
predict_cells	logical vector specifying which cells to classify
nmin	min n per class (where available)
n	number of cells per group (otherwise will be calculated from data)

#### Value

A SingleCellExperiment with updated group assignments called 'knn\_balanced'

# **Examples**

```
data(multiplexed_scrnaseq_sce, vartrix_consensus_snps)
multiplexed_scrnaseq_sce <- high_conf_calls(multiplexed_scrnaseq_sce)
multiplexed_scrnaseq_sce <- add_snps(sce = multiplexed_scrnaseq_sce,
mat = vartrix_consensus_snps,
thresh = 0.8)
multiplexed_scrnaseq_sce <- reassign_balanced(sce = multiplexed_scrnaseq_sce, k = 10, d=0.5)</pre>
```

reassign\_centroid

Reassign cells based on SNPs

# **Description**

Reassign cells based on SNPs

```
reassign_centroid(
   sce,
   train_cells = sce$train,
   predict_cells = sce$predict,
   labels = sce$labels,
   min_cells = 30,
   key = "Hashtag"
)
```

8 reassign\_jaccard

#### **Arguments**

sce SingleCellExperiment object
train\_cells logical, cells to be used for training
predict\_cells logical, cells to be used for prediction

labels provisional cell labels

min\_cells minimum coverage (number of cells with read at SNP location) for SNP to be

used for classification.

key unique key in naming of singlet groups used with grep to remove doublet/negative/uncertain

labels

#### Value

character vector containing reassignments

# **Examples**

```
data(multiplexed_scrnaseq_sce, vartrix_consensus_snps)
multiplexed_scrnaseq_sce <- high_conf_calls(multiplexed_scrnaseq_sce)
multiplexed_scrnaseq_sce <- add_snps(sce = multiplexed_scrnaseq_sce,
mat = vartrix_consensus_snps,
thresh = 0.8)
multiplexed_scrnaseq_sce<-reassign_centroid(multiplexed_scrnaseq_sce)</pre>
```

reassign\_jaccard

Reassign cells using knn with jaccard distance

#### **Description**

k-nearest neighbour classification of cells. Training data is intended to be labels of cells confidently called using cell hashing based methods and their corresponding SNPs. Prediction data can be remaining cells but can also include the training data. Doublets are simulated by randomly combining 'd' SNP profiles from each grouping combination.

```
reassign_jaccard(
   sce,
   k = 10,
   d = 10,
   train_cells = sce$train,
   predict_cells = sce$predict
)
```

subset\_vcf 9

# **Arguments**

sce	object of class	SingleCellExperiment

k number of neighbours used in knn, defaults to 10

d number of doublets per group combination to simulate, defaults to 10

train\_cells logical vector specifying which cells to use to train classifier

predict\_cells logical vector specifying which cells to classify

#### Value

A SingleCellExperiment with updated group assignments called 'knn\_jaccard'

# **Examples**

```
data(multiplexed_scrnaseq_sce, vartrix_consensus_snps)
multiplexed_scrnaseq_sce <- high_conf_calls(multiplexed_scrnaseq_sce)
multiplexed_scrnaseq_sce <- add_snps(sce = multiplexed_scrnaseq_sce,
mat = vartrix_consensus_snps,
thresh = 0.8)
multiplexed_scrnaseq_sce <- reassign(sce = multiplexed_scrnaseq_sce, k = 10)</pre>
```

subset\_vcf

Subset common variants vcf file to only SNPs seen in 'top\_genes'

#### **Description**

Subset common variants vcf file to only SNPs seen in 'top\_genes'

#### Usage

```
subset_vcf(vcf, top_genes, ensdb)
```

#### **Arguments**

vcf object of class CollapsedVCF

top\_genes output from 'common\_genes' function, alternatively character vector containing

custom gene names.

ensdb object of class EnsDb corresponding to organism, genome of data

#### Value

object of class CollapsedVCF containing subset of SNPs from supplied vcf seen in commonly expressed genes

# **Examples**

```
data(multiplexed_scrnaseq_sce, commonvariants_1kgenomes_subset)
top_genes <- common_genes(multiplexed_scrnaseq_sce)
ensdb <- EnsDb.Hsapiens.v86::EnsDb.Hsapiens.v86
small_vcf <- subset_vcf(commonvariants_1kgenomes_subset, top_genes, ensdb)</pre>
```

vartrix\_consensus\_snps

Sample VarTrix output

# **Description**

A sample output from VarTrix corresponding to the sce SingleCellExperiment objec for a subset of SNPs located in well observed genes.

# Usage

```
data(vartrix_consensus_snps)
```

#### **Format**

An object of class matrix (inherits from array) with 2542 rows and 2000 columns.

#### Value

vartrix\_consensus\_snps:
An object of class matrix

# **Index**