Package 'tLOH'

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Title Assessment of evidence for LOH in spatial transcriptomics pre-processed data using Bayes factor calculations

Description tLOH, or transcriptomicsLOH, assesses evidence for loss of heterozygosity (LOH) in pre-processed spatial transcriptomics data. This tool requires spatial transcriptomics cluster and allele count information at likely heterozygous single-nucleotide polymorphism (SNP) positions in VCF format. Bayes factors are calculated at each SNP to determine likelihood of potential loss of heterozygosity event. Two plotting functions are included to visualize allele fraction and aggregated Bayes factor per chromosome. Data generated with the 10X Genomics Visium Spatial Gene Expression platform must be pre-processed to obtain an individual sample VCF with columns for each cluster. Required fields are allele depth (AD) with counts for reference/alternative alleles and read depth (DP).

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URL https://github.com/USCDTG/tLOH

Encoding UTF-8

Suggests knitr, rmarkdown

Depends R (>= 4.2)

Imports scales, stats, utils, ggplot2, data.table, purrr, dplyr, VariantAnnotation, GenomicRanges, MatrixGenerics, bestNormalize, depmixS4, naniar, stringr

VignetteBuilder knitr

BugReports https://github.com/USCDTG/tLOH/issues

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aggregateCHRPlot *Visualization of data output from the tLOHCalc function, aggregated per chromosome*

Description

Output is a plot of the sum of Log10(1/K) values (K is a Bayes factor) per chromosome for each cluster. The dotted line at y=3 represents threshold for substantial evidence toward Model 2

Arguments

df	An input dataframe with merged cluster data output by tLOHCalc
sample	Name of sample for plot title

Value

Output is a plot where the y axis is sum of Log10(1/K) values (K is a Bayes factor) per chromosome and the x axis is chromosome

Author(s)

Michelle Webb

Examples

```
data('humanGBMsampleAC')
df <- tLOHCalc(humanGBMsampleAC)
aggregateCHRPlot(df,"Example")
```

alleleFrequencyPlot *Visualization of data output from the tLOHCalc function*

Description

Creates a plot with panels for each cluster. The x-axis is chromosome, y-axis is allele frequency. Point color is Log10(1/K) where K is a Bayes factor

Arguments

df	An input dataframe with merged cluster data
sample	Name of sample for plot title

Value

Output is a plot of allele frequency for each cluster. Can be assigned to object and visualized individually. For each panel, the y axis has a min of 0 and max of 1

Author(s)

Michelle Webb

Examples

```
data('humanGBMsampleAC')
df <- tLOHCalc(humanGBMsampleAC)
alleleFrequencyPlot(df,"Example")
```

---

documentErrorRegions *Identification of Errors*

Description

Generates dataframes equal to the length of original data containing NA if errors were found during the run HMM process. Important for final overview of results

Usage

```
documentErrorRegions(a,b)
```

Arguments

a	List of dataframes from prepareHMMdataframes
b	List of HMM state determination dataframes from the run_HMM series of functions

Value

Output is a list of dataframes to be used by the regionAnalysis function

Author(s)

Michelle Webb

Examples

```
estimatedStates <- data.frame(state = c(1,1,1), S1 = c(1,1,1), S2 = c(1,1,1))
sampleDataFrame <- data.frame(data = c(1,1,1))
list1 <- list(sampleDataFrame,sampleDataFrame)
list2 <- list(estimatedStates,estimatedStates)
documentErrorRegions(list1,list2)
```

hiddenMarkovAnalysis *Run Multi-Step HMM Analysis on tLOHCalcUpdate output*

Description

Applies the depmixS4 method of HMM Analysis on tLOHCalcUpdate output to obtain segments, noted by the output 'state' column

Usage

```
hiddenMarkovAnalysis(df, initProbs, trProbs)
```

Arguments

df	A dataframe output by tLOHCalcUpdate
initProbs	Dataframe containing 22 rows and two columns, initProb1 and initProb2. Each row represents a chromosome in sequential order, with initProb1 being the probability of state1 and initProb2 being the probability of state2.
trProbs	Matrix of transition start probabilities for HMM

Value

Output is a dataframe containing HMM analysis output and tLOHCalcUpdate output summary

Author(s)

Michelle Webb

Examples

```
data('humanGBMsampleAC')
data('initialStartProbabilities')
df <- tLOHCalcUpdate(humanGBMsampleAC,1.25,1.25,500,500,4)
trProbs <- cbind(c(0.8999,0.1001),c(0.1001,0.8999))
output <- hiddenMarkovAnalysis(df,initialStartProbabilities,trProbs)
```

humanGBMsampleAC *Imported dataset of a human glioblastoma spatial transcriptomics sample processed with tLOHImportData.*

Description

A dataset of a human glioblastoma sample containing the allele count (AC) information for 9 spatial transcriptomics clusters

Usage

```
data("humanGBMsampleAC")
```

Format

A data frame with 34601 rows and 7 variables:

rsID dbSNP rs identifier

CLUSTER cluster number

TOTAL total number of counts

REF counts for the reference allele

ALT counts for the alternative allele

CHR chromosome number

POS genomic position

Source

Craig Lab data repository

Examples

```
data("humanGBMsampleAC")
```

---

initialStartProbabilities

Imported dataset of sample start probabilities for hiddenMarkovAnalysis

---

Description

A dataset of initial start probabilities to use with the HMM analysis. Users may create their own dataset using the same format

Usage

```
data("initialStartProbabilities")
```

Format

A data frame with 22 rows and 2 variables:

initProb1 Initial Probability 1

initProb2 Initial Probability 2

Source

Craig Lab data repository

Examples

```
data("initialStartProbabilities")
```

`marginalLikelihoodM1` *Marginal M1 Calculation*

Description

Calculation of the marginal likelihood of Model 1, LOH

Arguments

x	Dataframe output by tLOHDataImport
a	Alpha value
b	Beta value

Details

The reference and total counts should come from a .csv output by the spatial LOH pre-processing pipeline. The recommended values for both Alpha1 and Beta1 is 1.25.

Value

The value returned from marginalLikelihoodM1 is numeric

Author(s)

Michelle Webb

Examples

```
test <- data.frame(REF=c(10,2,3,4,5,10),TOTAL=c(20,20,20,20,20,20))
apply(test, MARGIN = 1, FUN = marginalLikelihoodM1, a = 1.25, b = 1.25)
```

marginalLikelihoodM2 *Marginal M2 Calculation*

Description

Calculation of the marginal likelihood of Model 2, HET

Arguments

x	Dataframe output by tLOHDataImport
a	Alpha value
b	Beta value

Details

The reference and total counts should come from a .csv output by the spatial LOH pre-processing pipeline. The recommended values for both Alpha2 and Beta2 is 500.

Value

The value returned from marginalLikelihoodM1 is numeric

Author(s)

Michelle Webb

Examples

```
test <- data.frame(REF=c(10,2,3,4,5,10),TOTAL=c(20,20,20,20,20,20))
apply(test, MARGIN = 1, FUN = marginalLikelihoodM1, a = 500, b = 500)
```

marginalM1Calc *Calculate marginal of Model 1*

Description

This function takes the number of counts for a reference allele as x, and the number of total allele counts as y.

Arguments

x	Number of counts for the reference allele.
y	Number of counts total at this SNP position.

Details

The reference and total counts should come from a .csv output by the spatial LOH pre-processing pipeline.

Value

The value returned from marginalM1Calc is numeric

Author(s)

Michelle Webb

Examples

```
marginalM1Calc(10, 0.5)
```

---

marginalM2CalcBHET *Calculation of marginal M2 het*

Description

Calculation of marginal M2 het

Usage

```
marginalM2CalcBHET(x, a, b)
```

Arguments

x	Number of counts for the reference allele
a	Alpha value
b	Beta value

Value

The value returned from marginalM2CalcBHET is numeric

Author(s)

Michelle Webb

Examples

```
save <- data.frame(REF=c(10,2,3,4,5,10),TOTAL=c(20,20,20,20,20,20))
apply(save, MARGIN = 1, FUN = marginalM2CalcBHET, a = 10,b = 10)
```

marginalM2CalcBLOH *Marginal M2 Calculation*

Description

Calculation of the marginal for Model 2

Usage

```
marginalM2CalcBLOH(x, a, b)
```

Arguments

x	Counts for the reference allele
a	Alpha value
b	Beta value

Value

The value returned from marginalM2CalcBLOH is numeric

Author(s)

Michelle Webb

Examples

```
test <- data.frame(REF=c(10,2,3,4,5,10),TOTAL=c(20,20,20,20,20,20))
apply(test, MARGIN = 1, FUN = marginalM2CalcBLOH, a = 10,b = 10)
```

modePeakCalc *Calculation of mode peak*

Description

This function takes a set of numbers and outputs a mode peak value. To be used in a larger function that will be updated.

Arguments

x	List of allele fraction values

Details

List of values should be the allele fractions of SNPs with the top 25 percent of counts in a region. If only one value is input, that value is returned.

Value

The value returned is numeric

Author(s)

Michelle Webb

Examples

```
test <- c(1,2,3,4,5)
modePeakCalc(test)
```

`prepareHMMdataframes` *Prepare dataframes for HMM analysis*

Description

Split output from tLOHCalc or tLOHCalcUpdate into a list of cluster and chromosome separated dataframes. Applies an ordered quantile normalization on the bayes factor K values in each dataset.

Usage

```
prepareHMMdataframes(importedData)
```

Arguments

`importedData`	Input dataframe generated from the tLOHCalc or tLOHCalcUpdate function

Value

Output is a list of dataframes separated by chromosome and cluster

Author(s)

Michelle Webb

Examples

```
data('humanGBMsampleAC')
df <- tLOHCalcUpdate(humanGBMsampleAC,1.25,1.25,500,500,4)
output <- prepareHMMdataframes(df)
```

regionAnalysis *Summary of HMM Regions*

Description

Generates summary metrics for HMM regions

Usage

```
regionAnalysis(originalDF,dataframeList)
```

Arguments

originalDF	Original imported dataframe from tLOHCalcUpdated
dataframeList	List of HMM state determination dataframes from the run_HMM series of functions

Value

Output is a dataframe containing region metrics and data for each HMM segment

Author(s)

Michelle Webb

Examples

```
data('humanGBMsampleAC')
data('initialStartProbabilities')
df <- tLOHCalcUpdate(humanGBMsampleAC,1.25,1.25,500,500,4)
dataframeList <- prepareHMMdataframes(df)
trProbs <- cbind(c(0.8999,0.1001),c(0.1001,0.8999))
dataframeList2 <- runHMM_1(dataframeList, initialStartProbabilities, trProbs)
dataframeList3 <- runHMM_2(dataframeList2)
output <- runHMM_3(dataframeList3)
final <- regionAnalysis(dataframeList,output)
```

regionFinalize *Summary of HMM Regions*

Description

Final metrics and summary for regions

Usage

```
regionFinalize(finalList1)
```

Arguments

finalList1	List of dataframes output by the regionAnalysis function

Value

Output is a table containing all calculations from the bayes factor and HMM analysis

Author(s)

Michelle Webb

Examples

```
## Not run:
data('humanGBMsampleAC')
data('initialStartProbabilities')
df <- tLOHCalcUpdate(humanGBMsampleAC,1.25,1.25,500,500,4)
dataframeList <- prepareHMMdataframes(df)
trProbs <- cbind(c(0.8999,0.1001),c(0.1001,0.8999))
dataframeList2 <- runHMM_1(dataframeList, initialStartProbabilities, trProbs)
dataframeList3 <- runHMM_2(dataframeList2)
output <- runHMM_3(dataframeList3)
intermediate <- regionAnalysis(dataframeList,output)
final <- regionFinalize(intermediate)

## End(Not run)
```

---

removeOutlierFromCalc *Removes outliers*

Description

Take rows with a total count greater than 2000 and sets to NA

Arguments

dataframe	input dataframe
cols	which column
rows	which row
newValue	what to replace

Value

Dataframe returned

Author(s)

Michelle Webb

Examples

```
test <- data.frame(TOTAL=c(2000,20,20,20,20,20))
removeOutlierFromCalc(test,"TOTAL",test[test$TOTAL > 2000,],NA)
```

---

runHMM_1 *Step 1 of HMM process*

Description

Applies the depmixS4 method depmix on normalized K values.

Usage

```
runHMM_1(dataframeList, initProbs, trProbs)
```

Arguments

dataframeList	List of dataframes separated by cluster and chromosome from the prepareHMMdataframes function.
initProbs	Dataframe containing 22 rows and two columns, initProb1 and initProb2. Each row represents a chromosome in sequential order, with initProb1 being the probability of state1 and initProb2 being the probability of state2.
trProbs	Matrix of transition start probabilities for HMM

Value

Output is a list of depmixS4 depmix class objects for each input dataframe

Author(s)

Michelle Webb

Examples

```
data('humanGBMsampleAC')
data('initialStartProbabilities')
df <- tLOHCalcUpdate(humanGBMsampleAC,1.25,1.25,500,500,4)
dataframeList <- prepareHMMdataframes(df)
trProbs <- cbind(c(0.8999,0.1001),c(0.1001,0.8999))
output <- runHMM_1(dataframeList, initialStartProbabilities, trProbs)
```

runHMM_2 *Step 2 of HMM process*

Description

Applies the depmixS4 method fit on .

Usage

```
runHMM_2(dataframeList)
```

Arguments

dataframeList	List of depmixS4 depmix class objects generated from the runHMM_1 step

Value

Output is a list of depmixS4 depmix.fitted class output for each input dataframe

Author(s)

Michelle Webb

Examples

```
data('humanGBMsampleAC')
data('initialStartProbabilities')
df <- tLOHCalcUpdate(humanGBMsampleAC,1.25,1.25,500,500,4)
dataframeList <- prepareHMMdataframes(df)
trProbs <- cbind(c(0.8999,0.1001),c(0.1001,0.8999))
dataframeList2 <- runHMM_1(dataframeList, initialStartProbabilities, trProbs)
output <- runHMM_2(dataframeList2)
```

runHMM_3 *Step 3 of HMM process*

Description

Applies the depmixS4 method posterior on normalized K values.

Usage

```
runHMM_3(dataframeList)
```

Arguments

dataframeList	List of depmixS4 depmix.fitted class output generated from the runHMM_2 step

Value

Output is a list of depmixS4 posterior state classifications for each input dataframe

Author(s)

Michelle Webb

Examples

```
data('humanGBMsampleAC')
data('initialStartProbabilities')
df <- tLOHCalcUpdate(humanGBMsampleAC,1.25,1.25,500,500,4)
dataframeList <- prepareHMMdataframes(df)
trProbs <- cbind(c(0.8999,0.1001),c(0.1001,0.8999))
dataframeList2 <- runHMM_1(dataframeList, initialStartProbabilities, trProbs)
dataframeList3 <- runHMM_2(dataframeList2)
output <- runHMM_3(dataframeList3)
```

---

splitByChromosome *Split dataframe into individual chromosome dataframes*

Description

Creates individual chromosome dataframes

Usage

```
splitByChromosome(listOfDataframes,numberOfDataframes)
```

Arguments

listOfDataframes	Input dataframe generated from the tLOHDataImport function
numberOfDataframes	Number of dataframes in list

Value

Output is a list of dataframe separated by chromosome

Author(s)

Michelle Webb

Examples

```
data('humanGBMsampleAC')
df <- tLOHCalcUpdate(humanGBMsampleAC,1.25,1.25,500,500,4)
output <- splitByChromosome(list(df),1)
```

summarizeRegions1 *Step 1 of region summary*

Description

Function used by regionFinalize to group segments

Usage

```
summarizeRegions1(x)
```

Arguments

x	A list of dataframes containing calculated values and JMM state determinations

Value

Output is a list of dataframes

Author(s)

Michelle Webb

Examples

```
## Not run: data('humanGBMsampleAC')
data('initialStartProbabilities')
df <- tLOHCalcUpdate(humanGBMsampleAC,1.25,1.25,500,500,4)
dataframeList <- prepareHMMdataframes(df)
trProbs <- cbind(c(0.8999,0.1001),c(0.1001,0.8999))
dataframeList2 <- runHMM_1(dataframeList, initialStartProbabilities, trProbs)
dataframeList3 <- runHMM_2(dataframeList2)
output <- runHMM_3(dataframeList3)
intermediate <- regionAnalysis(dataframeList,output)
finalList1 <- purrr::map(intermediate,
~dplyr::mutate(.x, state = as.character(state)))
sampleValues <- as.data.frame(purrr::reduce(finalList1,full_join))
sampleData <- summarizeRegions1(finalList1)
## End(Not run)
```

summarizeRegions2 *Step 2 of region summary*

Description

Function used by regionFinalize to identify segment start and end positions

Usage

```
summarizeRegions2(finalTable)
```

Arguments

finalTable	A dataframe containing metrics from the Bayes Factor and HMM analysis

Value

Output is a dataframe

Author(s)

Michelle Webb

Examples

```
## Not run:
data('humanGBMsampleAC')
data('initialStartProbabilities')
df <- tLOHCalcUpdate(humanGBMsampleAC,1.25,1.25,500,500,4)
dataframeList <- prepareHMMdataframes(df)
trProbs <- cbind(c(0.8999,0.1001),c(0.1001,0.8999))
dataframeList2 <- runHMM_1(dataframeList, initialStartProbabilities, trProbs)
dataframeList3 <- runHMM_2(dataframeList2)
output <- runHMM_3(dataframeList3)
intermediate <- regionAnalysis(dataframeList,output)
finalList1 <- purrr::map(intermediate,
~dplyr::mutate(.x, state = as.character(state)))
sampleValues <- as.data.frame(purrr::reduce(finalList1,full_join))
sampleData <- summarizeRegions1(finalList1)
sampleData$lengthOfInterval <- sampleData$intervalEnd - sampleData$intervalStart
sampleDF <- summarizeRegions2(sampleValues)
## End(Not run)
```

tLOHCalc *Assement of evidence for LOH in clusters from spatial transcriptomics data*

Description

Calculates Bayes factors for allele fractions at each SNP position. Uses dataframe output by tLOHDataImport

Usage

```
tLOHCalc(forCalcDF)
```

Arguments

forCalcDF	Input dataframe generated from the tLOHDataImport function

Value

Output is a dataframe with values that can be visualized with alleleFrequencyPlot() or aggregateCHRPlot()

Author(s)

Michelle Webb

Examples

```
data('humanGBMsampleAC')
df <- tLOHCalc(humanGBMsampleAC)
head(df)
```

tLOHCalcUpdate *Assement of evidence for LOH in clusters from spatial transcriptomics allele count data*

Description

Calculates Bayes factors for allele fractions at each SNP position. Uses dataframe output by tLOHDataImport.

Usage

```
tLOHCalcUpdate(forCalcDF, alpha1, beta1,alpha2, beta2, countThreshold)
```

Arguments

forCalcDF	Input dataframe generated from the tLOHDataImport function
alpha1	Model 1 alpha value
beta1	Model 1 beta value
alpha2	Model 2 alpha value
beta2	Model 2 beta value
countThreshold	Threshold for minimum number of read counts

Value

Output is a dataframe with Bayes Factor values

Author(s)

Michelle Webb

Examples

```
data('humanGBMsampleAC')
df <- tLOHCalcUpdate(humanGBMsampleAC, 1.25,1.25,500,500,4)
head(df)
```

---

tLOHDataImport *Import VCF for tLOHCalc*

Description

Import a VCF with per-cluster allele count information at heterozygous SNP positions for the tLOHCalc calculation function.

Arguments

vcf	An input VCF file. Spatial transcriptomics clusters make up the sample columns. AD and DP fields are required. Each SNP should be annotated with dbSNP rsIDs.

Value

Output is a dataframe with required fields for tLOHCalc

Author(s)

Michelle Webb

Examples

```
    ## Not run:
    R.utils::gunzip("inst/extdata/Example.vcf.gz","inst/extdata/Example.vcf")
    exampleDF <- tLOHDataImport("inst/extdata/Example.vcf")
## End(Not run)
```

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