

Package ‘ASCAT’

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Type Package

Title Allele-Specific Copy Number Analysis of Tumors

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Description R package of ASCAT as published in <http://www.ncbi.nlm.nih.gov/pubmed/20837533>

Depends R (>= 2.13.0),

RColorBrewer,
grDevices,
graphics,
stats,
splines,
data.table,
GenomicRanges,
IRanges,
parallel,
doParallel,
foreach,
utils

License GPL-3

LazyLoad yes

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R topics documented:

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ascat.asmultipcf	<i>Allele-specific segmentation of multiple samples</i>
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Description

This segmentation function should only be used if part of the breakpoints are expected to be shared between samples, e.g. due to a common ancestry.

Usage

```
ascat.asmultipcf(
  ASCATobj,
  ascat.gg = NULL,
  penalty = 70,
  wsample = NULL,
  selectAlg = "exact",
  refine = TRUE
)
```

Arguments

ASCATobj	an ASCAT object
ascat.gg	germline genotypes (NULL if germline data is available)
penalty	penalty of introducing an additional ASPCF breakpoint (expert parameter, don't adapt unless you know what you are doing)
wsample	Vector of length length(ASCATobj\$samples). Can be used to assign different weights to samples, for example to account for differences in sequencing quality. (Default = NULL)
selectAlg	Set to "exact" to run the exact algorithm, or "fast" to run the heuristic algorithm. (Default = "exact")
refine	Logical. Should breakpoints be refined on a per sample base? Otherwise each breakpoint is assumed to be present in each sample. (Default = TRUE)

Details

This function saves the results in in [sample].LogR.PCFed.txt and [sample].BAF.PCFed.txt

Value

output: ascat data structure containing:

1. Tumor_LogR data matrix
2. Tumor_BAF data matrix
3. Tumor_LogR_segmented: matrix of LogR segmented values
4. Tumor_BAF_segmented: list of BAF segmented values; each element in the list is a matrix containing the segmented values for one sample (only for probes that are germline homozygous)
5. Germline_LogR data matrix
6. Germline_BAF data matrix
7. SNPpos: position of all SNPs
8. ch: a list containing vectors with the indices for each chromosome (e.g. Tumor_LogR[ch[[13]],] will output the Tumor_LogR data of chromosome 13)
9. chr: a list containing vectors with the indices for each distinct part that can be segmented separately (e.g. chromosome arm, stretch of DNA between gaps in the array design)

ascat.aspcf	<i>ascat.aspcf</i>
-------------	--------------------

Description

run ASPCF segmentation

Usage

```
ascat.aspcf(
  ASCATobj,
  selectsamples = 1:length(ASCATobj$samples),
  ascat.gg = NULL,
  penalty = 70,
  out.dir = ".",
  out.prefix = ""
)
```

Arguments

ASCATobj	an ASCAT object
selectsamples	a vector containing the sample number(s) to PCF. Default = all
ascat.gg	germline genotypes (NULL if germline data is available)
penalty	penalty of introducing an additional ASPCF breakpoint (expert parameter, don't adapt unless you know what you're doing)
out.dir	directory in which output files will be written
out.prefix	prefix for output file names

Details

This function can be easily parallelised by controlling the selectsamples parameter it saves the results in LogR_PCFed[sample]_[segment].txt and BAF_PCFed[sample]_[segment].txt

Value

output: ascat data structure containing:

1. Tumor_LogR data matrix
2. Tumor_BAF data matrix
3. Tumor_LogR_segmented: matrix of LogR segmented values
4. Tumor_BAF_segmented: list of BAF segmented values; each element in the list is a matrix containing the segmented values for one sample (only for probes that are not germline homozygous)
5. Germline_LogR data matrix
6. Germline_BAF data matrix
7. SNPpos: position of all SNPs
8. ch: a list containing vectors with the indices for each chromosome (e.g. Tumor_LogR[ch[[13]],] will output the Tumor_LogR data of chromosome 13)
9. chr: a list containing vectors with the indices for each distinct part that can be segmented separately (e.g. chromosome arm, stretch of DNA between gaps in the array design)

ascat.correctLogR	<i>ascat.correctLogR</i>
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Description

Corrects logR of the tumour sample(s) with genomic GC content (replication timing is optional)

Usage

```
ascat.correctLogR(ASCATobj, GCcontentfile = NULL, replictimingfile = NULL)
```

Arguments

ASCATobj	an ASCAT object
GCcontentfile	File containing the GC content around every SNP for increasing window sizes
replictimingfile	File containing replication timing at every SNP for various cell lines (optional)

Details

Note that probes not present in the GC content file will be lost from the results

Value

ASCAT object with corrected tumour logR

ascat.GCcorrect	<i>ascat.GCcorrect</i>
-----------------	------------------------

Description

Function kept for backward compatibility, please use ascat.correctLogR instead

Usage

```
ascat.GCcorrect(ASCATobj, GCcontentfile = NULL)
```

Arguments

ASCATobj	an ASCAT object
GCcontentfile	File containing the GC content around every SNP for increasing window sizes

ascat.getAlleleCounts	<i>Obtain allele counts for a given set of loci through external program alleleCounter.</i>
-----------------------	---

Description

Obtain allele counts for a given set of loci through external program alleleCounter.

Usage

```
ascat.getAlleleCounts(
  seq.file,
  output.file,
  loci.file,
  min.base.qual = 20,
  min.map.qual = 35,
  allelecounter.exe = "alleleCounter",
  ref.fasta = NA
)
```

Arguments

seq.file	A BAM/CRAM alignment file on which the counter should be run.
output.file	The file where output should go.
loci.file	A file with SNP loci.
min.base.qual	The minimum base quality required for it to be counted (optional, default=20).
min.map.qual	The minimum mapping quality required for it to be counted (optional, default=35).
allelecounter.exe	A pointer to where the alleleCounter executable can be found (optional, default points to \$PATH).
ref.fasta	A FASTA file for CRAM processing (optional).

Author(s)

sd11, tl

ascat.getBAFsAndLogRs *Obtain BAF and LogR from the allele counts.*

Description

Obtain BAF and LogR from the allele counts.

Usage

```
ascat.getBAFsAndLogRs(
  samplename,
  tumourAlleleCountsFile.prefix,
  normalAlleleCountsFile.prefix,
  tumourLogR_file,
  tumourBAF_file,
  normalLogR_file,
  normalBAF_file,
  alleles.prefix,
  gender,
  genomeVersion,
  chrom_names = c(1:22, "X"),
  minCounts = 20,
  BED_file = NA,
  probloci_file = NA,
  seed = as.integer(Sys.time())
)
```

Arguments

samplename	String, name of the sample.
tumourAlleleCountsFile.prefix	Prefix of the allele counts files for the tumour (e.g. "Tumour_alleleFrequencies_chr").
normalAlleleCountsFile.prefix	Prefix of the allele counts files for the normal (e.g. "Normal_alleleFrequencies_chr").
tumourLogR_file	File where LogR from the tumour will be written.
tumourBAF_file	File where BAF from the tumour will be written.
normalLogR_file	File where LogR from the normal will be written.
normalBAF_file	File where BAF from the normal will be written.
alleles.prefix	Prefix path to the allele data (e.g. "G1000_alleles_chr")
gender	Gender information, either 'XX' (=female) or 'XY' (=male).
genomeVersion	Genome version, either 'hg19' or 'hg38'.
chrom_names	A vector with allowed chromosome names (optional, default=c(1:22,'X')).
minCounts	Minimum depth, in normal, required for a SNP to be considered (optional, default=20).
BED_file	A BED file for only looking at SNPs within specific intervals (optional, default=NA).

probleci_file A file (chromosome <tab> position; no header) containing specific loci to ignore (optional, default=NA).

seed A seed to be set for when randomising the alleles (optional, default=as.integer(Sys.time())).

Author(s)

dw9, sd11, tl

ascat.loadData	<i>ascat.loadData</i>
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Description

Function to read in SNP array data

Usage

```
ascat.loadData(
  Tumor_LogR_file,
  Tumor_BAF_file,
  Germline_LogR_file = NULL,
  Germline_BAF_file = NULL,
  chrs = c(1:22, "X", "Y"),
  gender = NULL,
  sexchromosomes = c("X", "Y"),
  genomeVersion = NULL
)
```

Arguments

Tumor_LogR_file file containing logR of tumour sample(s)

Tumor_BAF_file file containing BAF of tumour sample(s)

Germline_LogR_file file containing logR of germline sample(s), NULL

Germline_BAF_file file containing BAF of germline sample(s), NULL

chrs a vector containing the names for the chromosomes (e.g. c(1:22,"X"))

gender a vector of gender for each cases ("XX" or "XY"). Default = all female ("XX")

sexchromosomes a vector containing the names for the sex chromosomes. Default = c("X","Y")

genomeVersion a string (either 'hg19' or 'hg38') so nonPAR coordinates on X can be stored, NULL

Details

germline data files can be NULL - in that case these are not read in

Value

ascat data structure containing:

1. Tumor_LogR data matrix
2. Tumor_BAF data matrix
3. Tumor_LogR_segmented: placeholder, NULL
4. Tumor_BAF_segmented: placeholder, NULL
5. Germline_LogR data matrix
6. Germline_BAF data matrix
7. SNPpos: position of all SNPs
8. ch: a list containing vectors with the indices for each chromosome (e.g. Tumor_LogR[ch[[13]],] will output the Tumor_LogR data of chromosome 13)
9. chr: a list containing vectors with the indices for each distinct part that can be segmented separately (e.g. chromosome arm, stretch of DNA between gaps in the array design)
10. chrs: a vector containing chromosome names
11. samples: a vector containing sample name(s)
12. gender: a vector of gender for each cases ("XX" or "XY"). Default = NULL: all female ("XX")
13. sexchromosomes: a vector containing names of sex chromosomes
14. X_nonPAR: a vector of two values (start and stop) to define where the nonPAR region is on X
15. failedarrays: placeholder, NULL

ascat.metrics

Function to extract different metrics from ASCAT profiles.

Description

Function to extract different metrics from ASCAT profiles.

Usage

```
ascat.metrics(ASCAT_input_object, ASCAT_output_object)
```

Arguments

ASCAT_input_object

R object generated by the ascat.aspcf function and given to the ascat.runAscat function.

ASCAT_output_object

R object generated by the ascat.runAscat function.

Value

A dataframe (one sample per line) with the following metrics (as columns):

1. sex - Sex information as provided.
2. tumour_mapd - Median Absolute Pairwise Difference (MAPD) in tumour logR track.
3. normal_mapd - Median Absolute Pairwise Difference (MAPD) in normal logR track (should be NA without matched normals and 0 for sequencing data).
4. GC_correction_before - logR/GC correlation before correction.
5. GC_correction_after - logR/GC correlation after correction.
6. RT_correction_before - logR/RT correlation before correction.
7. RT_correction_after - logR/RT correlation after correction.

8. n_het_SNP - Number of heterozygous SNPs.
9. n_segs_logR - Number of segments in the logR track.
10. n_segs_BAF - Number of segments in the BAF track.
11. n_segs_logRBAF_diff - Difference between number of segments in the logR versus BAF track.
12. frac_homo - Fraction of homozygous (<0.1 | >0.9) probes in tumour.
13. purity - Purity estimate.
14. ploidy - Ploidy estimate.
15. goodness_of_fit - Goodness of fit.
16. n_segs - Number of copy-number segments.
17. segs_size - Total size of all segments.
18. n_segs_1kSNP - Number of segments per 1k heterozygous SNPs.
19. homdel_segs - Number of segments with homozygous deletion.
20. homdel_largest - largest segment with homozygous deletion.
21. homdel_size - Total size of segments with homozygous deletion.
22. homdel_fraction - Fraction of the genome with homozygous deletion.
23. LOH - Fraction of the genome with LOH (ignoring sex chromosomes).
24. mode_minA - Mode of the minor allele (ignoring sex chromosomes).
25. mode_majA - Mode of the major allele (ignoring sex chromosomes).
26. WGD - Whole genome doubling event (ignoring sex chromosomes).
27. GI - Genomic instability score (ignoring sex chromosomes).

Author(s)

tl

ascat.plotAdjustedAscatProfile
ascat.plotAdjustedAscatProfile

Description

Function plotting the "adjusted" (with realistic chromosome sizes) rounded/unrounded ASCAT profiles over all chromosomes.

Usage

```
ascat.plotAdjustedAscatProfile(
  ASCAT_output_object,
  REF,
  y_limit = 5,
  plot_unrounded = F,
  png_prefix = ""
)
```

Arguments

ASCAT_output_object	R object generated by the ascat.runAscat function.
REF	Can be either "hg19" or "hg38" for standard human genome or a data.frame with three columns: chrom, start and end.

y_limit	Optional parameter determining the size of the y axis in the profile (default=5).
plot_unrounded	Optional parameter to define whether rounded (default) or unrounded profile (set to TRUE) should be plotted.
png_prefix	Optional parameter to add a prefix to png name (can be also used to set a path).

Value

Plot showing the adjusted (rounded/unrounded) ASCAT profile of the sample

```
ascat.plotAscatProfile
```

ascat.plotAscatProfile

Description

Function plotting the rounded ASCAT profiles over all chromosomes

Usage

```
ascat.plotAscatProfile(
  n1all,
  n2all,
  heteroprobes,
  ploidy,
  rho,
  goodnessOfFit,
  nonaberrant,
  y_limit = 5,
  ch,
  lrr,
  bafsegmented,
  chrs
)
```

Arguments

n1all	copy number major allele
n2all	copy number minor allele
heteroprobes	probes with heterozygous germline
ploidy	ploidy of the sample
rho	purity of the sample
goodnessOfFit	estimated goodness of fit
nonaberrant	boolean flag denoting non-aberrated samples
y_limit	Optional parameter determining the size of the y axis in the nonrounded plot and ASCAT profile. Default=5
ch	a list containing c vectors, where c is the number of chromosomes and every vector contains all probe numbers per chromosome
lrr	(unsegmented) log R, in genomic sequence (all probes), with probe IDs
bafsegmented	B Allele Frequency, segmented, in genomic sequence (only probes heterozygous in germline), with probe IDs
chrs	a vector containing the names for the chromosomes (e.g. c(1:22,"X"))

Value

plot showing the ASCAT profile of the sample

ascat.plotGenotypes	<i>ascat.plotGenotypes</i>
---------------------	----------------------------

Description

ascat.plotGenotypes

Usage

```
ascat.plotGenotypes(ASCATobj, title, Tumor_BAF_noNA, Hom, ch_noNA)
```

Arguments

ASCATobj	an ASCAT object
title	main title of the plot
Tumor_BAF_noNA	B-allele frequencies of the tumour sample with removed NA values
Hom	Boolean vector denoting homozygous SNPs
ch_noNA	vector of probes per chromosome (NA values excluded)

Value

plot showing classified BAF per sample, with unused SNPs in green, germline homozygous SNPs in blue and all others in red

ascat.plotNonRounded	<i>ascat.plotNonRounded</i>
----------------------	-----------------------------

Description

Function plotting the unrounded ASCAT copy number over all chromosomes

Usage

```
ascat.plotNonRounded(
  ploidy,
  rho,
  goodnessOfFit,
  nonaberrant,
  nAfull,
  nBfull,
  y_limit = 5,
  bafsegmented,
  ch,
  lrr,
  chrs
)
```

Arguments

ploidy	ploidy of the sample
rho	purity of the sample
goodnessOfFit	estimated goodness of fit
nonaberrant	boolean flag denoting non-aberrated samples
nAfull	copy number major allele
nBfull	copy number minor allele
y_limit	Optional parameter determining the size of the y axis in the nonrounded plot and ASCAT profile. Default=5
bafsegmented	B Allele Frequency, segmented, in genomic sequence (only probes heterozygous in germline), with probe IDs
ch	a list containing c vectors, where c is the number of chromosomes and every vector contains all probe numbers per chromosome
lrr	(unsegmented) log R, in genomic sequence (all probes), with probe IDs
chrs	a vector containing the names for the chromosomes (e.g. c(1:22,"X"))

Value

plot showing the nonrounded copy number profile, using base plotting function

ascat.plotRawData	<i>ascat.plotRawData</i>
-------------------	--------------------------

Description

Plots SNP array data

Usage

```
ascat.plotRawData(
  ASCATobj,
  img.dir = ".",
  img.prefix = "",
  logr.y_values = c(-2, 2)
)
```

Arguments

ASCATobj	an ASCAT object (e.g. data structure from ascat.loadData)
img.dir	directory in which figures will be written
img.prefix	prefix for figure names
logr.y_values	define Y min and max values for logR track (optional; default: c(-2,2))

Value

Produces png files showing the logR and BAF values for tumour and germline samples

```
ascat.plotSegmentedData
      ascat.plotSegmentedData
```

Description

plots the SNP array data before and after segmentation

Usage

```
ascat.plotSegmentedData(
  ASCATobj,
  img.dir = ".",
  img.prefix = "",
  logr.y_values = c(-2, 2)
)
```

Arguments

ASCATobj	an ASCAT object (e.g. from ascat.aspcf)
img.dir	directory in which figures will be written
img.prefix	prefix for figure names
logr.y_values	define Y min and max values for logR track (optional; default: c(-2,2))

Value

png files showing raw and segmented tumour logR and BAF

```
ascat.plotSunrise      ascat.plotSunrise
```

Description

ascat.plotSunrise

Usage

```
ascat.plotSunrise(d, psi_opt1, rho_opt1, minim = T)
```

Arguments

d	distance matrix for a range of ploidy and tumour percentage values
psi_opt1	optimal ploidy
rho_opt1	optimal purity
minim	when set to true, optimal regions in the sunrise plot are depicted in blue; if set to false, colours are inverted and red corresponds to optimal values (default: TRUE)

Value

plot visualising range of ploidy and tumour percentage values

```
ascat.predictGermlineGenotypes
      ascat.predictGermlineGenotypes
```

Description

predicts the germline genotypes of samples for which no matched germline sample is available

Usage

```
ascat.predictGermlineGenotypes(
  ASCATobj,
  platform = "AffySNP6",
  img.dir = ".",
  img.prefix = ""
)
```

Arguments

ASCATobj	an ASCAT object
platform	used array platform
img.dir	directory in which figures will be written
img.prefix	prefix for figure names

Details

Currently possible values for platform:

```
AffySNP6 (default)
Custom10k
IlluminaASA
IlluminaGSAv3
Illumina109k
IlluminaCytoSNP
IlluminaCytoSNP850k
Illumina610k
Illumina660k
Illumina700k
Illumina1M
Illumina2.5M
IlluminaOmni5
Affy10k
Affy100k
Affy250k_sty
Affy250k_nsp
AffyOncoScan
AffyCytoScanHD
HumanCNV370quad
HumanCore12
HumanCoreExome24
HumanOmniExpress12
```

IlluminaOmniExpressExome

Value

predicted germline genotypes

ascat.prepareHTS	<i>Extract both logR and BAF values from sequencing data</i>
------------------	--

Description

Method derived from the Battenberg package (<https://github.com/Wedge-lab/battenberg>).

Usage

```
ascat.prepareHTS(
  tumourseqfile,
  normalseqfile,
  tumourname,
  normalname,
  allelecounter_exe,
  alleles.prefix,
  loci.prefix,
  gender,
  genomeVersion,
  nthreads = 1,
  tumourLogR_file = NA,
  tumourBAF_file = NA,
  normalLogR_file = NA,
  normalBAF_file = NA,
  minCounts = 10,
  BED_file = NA,
  probloci_file = NA,
  chrom_names = c(1:22, "X"),
  min_base_qual = 20,
  min_map_qual = 35,
  ref.fasta = NA,
  skip_allele_counting_tumour = F,
  skip_allele_counting_normal = F
)
```

Arguments

tumourseqfile	Full path to the tumour BAM/CRAM file.
normalseqfile	Full path to the normal BAM/CRAM file.
tumourname	Identifier to be used for tumour output files.
normalname	Identifier to be used for normal output files.
allelecounter_exe	Path to the allele counter executable.

alleles.prefix	Prefix path to the allele data (e.g. "G1000_alleles_chr").
loci.prefix	Prefix path to the loci data (e.g. "G1000_loci_chr").
gender	Gender information, either 'XX' (=female) or 'XY' (=male).
genomeVersion	Genome version, either 'hg19' or 'hg38'.
nthreads	The number of parallel processes for getting allele counts (optional, default=1).
tumourLogR_file	Path to the tumour logR output (optional, paste0(tumourname,"_tumourLogR.txt")).
tumourBAF_file	Path to the tumour BAF output (optional, paste0(tumourname,"_tumourBAF.txt")).
normalLogR_file	Path to the normal logR output (optional, paste0(tumourname,"_normalLogR.txt")).
normalBAF_file	Path to the normal BAF output (optional, paste0(tumourname,"_normalBAF.txt")).
minCounts	Minimum depth required in the normal for a SNP to be considered (optional, default=10).
BED_file	A BED file for only looking at SNPs within specific intervals (optional, default=NA).
probloci_file	A file (chromosome <tab> position; no header) containing specific loci to ignore (optional, default=NA).
chrom_names	A vector containing the names of chromosomes to be considered (optional, default=c(1:22,'X')).
min_base_qual	Minimum base quality required for a read to be counted (optional, default=20).
min_map_qual	Minimum mapping quality required for a read to be counted (optional, default=35).
ref.fasta	FASTA file used for generating CRAMs (optional, default=NA).
skip_allele_counting_tumour	Flag, set to TRUE if tumour allele counting is already complete (files are expected in the working directory on disk; optional, default=FALSE).
skip_allele_counting_normal	Flag, set to TRUE if normal allele counting is already complete (files are expected in the working directory on disk; optional, default=FALSE).

Author(s)

sd11, tl

ascat.runAscat

*ascat.runAscat***Description**

ASCAT main function, calculating the allele-specific copy numbers

Usage

```
ascat.runAscat(
  ASCATobj,
  gamma = 0.55,
  pdfPlot = F,
  y_limit = 5,
  circos = NA,
  min_ploidy = 1.5,
  max_ploidy = 5.5,
  rho_manual = NA,
  psi_manual = NA,
  img.dir = ".",
  img.prefix = ""
)
```

Arguments

ASCATobj	an ASCAT object from ascat.aspcf
gamma	technology parameter, compaction of Log R profiles (expected decrease in case of deletion in diploid sample, 100% aberrant cells; 1 in ideal case, 0.55 of Illumina 109K arrays)
pdfPlot	Optional flag if nonrounded plots and ASCAT profile in pdf format are desired. Default=F
y_limit	Optional parameter determining the size of the y axis in the nonrounded plot and ASCAT profile. Default=5
circos	Optional file to output the non-rounded values in Circos track format. Default=NA
min_ploidy	optional numerical parameter determining the minimum boundary of the ploidy solution search space. Default=1.5
max_ploidy	optional numerical parameter determining the maximum boundary of the ploidy solution search space. Default=5.5
rho_manual	optional argument to override ASCAT optimization and supply rho parameter (not recommended)
psi_manual	optional argument to override ASCAT optimization and supply psi parameter (not recommended)
img.dir	directory in which figures will be written
img.prefix	prefix for figure names

Details

Note: for copy number only probes, nA contains the copy number value and nB = 0.

Value

an ASCAT output object, containing:

1. nA: copy number of the A allele
2. nB: copy number of the B allele
3. purity: the tumour purity of all arrays
4. aberrantcellfraction: the aberrant cell fraction (=tumour purity) of all arrays

- 5. ploidy: the ploidy of all arrays
- 6. failedarrays: arrays on which ASCAT analysis failed
- 7. nonaberrantarrays: arrays on which ASCAT analysis indicates that they show virtually no aberrations
- 8. segments: an array containing the copy number segments of each sample (not including failed arrays)
- 9. segments_raw: an array containing the copy number segments of each sample without any rounding applied
- 10. distance_matrix: distances for a range of ploidy and tumor percentage values

```
ascat.synchroniseFiles
```

Synchronise SNPs across files

Description

Synchronise SNPs across files

Usage

```
ascat.synchroniseFiles(
  samplename,
  tumourLogR_file,
  tumourBAF_file,
  normalLogR_file,
  normalBAF_file
)
```

Arguments

samplename	String, name of the sample.
tumourLogR_file	File where LogR from the tumour will be read and overwritten.
tumourBAF_file	File where BAF from the tumour will be read and overwritten.
normalLogR_file	File where LogR from the normal will be read and overwritten.
normalBAF_file	File where BAF from the normal will be read and overwritten.

Author(s)

tl

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