Package 'infercnv'

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Type Package

Title Infer Copy Number Variation from Single-Cell RNA-Seq Data

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Description Using single-cell RNA-Seq expression to visualize CNV in cells.

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URL https://github.com/broadinstitute/inferCNV/wiki

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`infercnv-package` *infercnv: Infer Copy Number Variation from Single-Cell RNA-Seq Data*

Description

Using single-cell RNA-Seq expression to visualize CNV in cells.

Details

The main functions you will need to use are CreateInfercnvObject() and run(infercnv_object). For additional details on running the analysis step by step, please refer to the example vignette.

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See Also

Useful links:

- https://github.com/broadinstitute/inferCNV/wiki
- Report bugs at https://github.com/broadinstitute/inferCNV/issues

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`add_to_seurat` *add_to_seurat()*

Description

Add meta.data about CNAs to a Seurat object from an infercnv_obj

Usage

```
add_to_seurat(
  seurat_obj = NULL,
  assay_name = "RNA",
  infercnv_output_path,
  top_n = 10,
  bp_tolerance = 2e+06,
  column_prefix = NULL
)
```

Arguments

`seurat_obj`	Seurat object to add meta.data to (default: NULL)
`assay_name`	Name of the assay in the Seurat object if provided. (default: "RNA")
`infercnv_output_path`	Path to the output folder of the infercnv run to use
`top_n`	How many of the largest CNA (in number of genes) to get.
`bp_tolerance`	How many bp of tolerance to have around feature start/end positions for top_n largest CNVs.
`column_prefix`	String to add as a prefix to the Seurat metadata columns. Only applied to the seurat_obj, if supplied. Default is NULL

Value

seurat_obj

`apply_median_filtering` *apply_median_filtering*

Description

Apply a median filtering to the expression matrix within each tumor bounds

Usage

```
apply_median_filtering(
  infercnv_obj,
  window_size = 7,
  on_observations = TRUE,
  on_references = TRUE
)
```

Arguments

`infercnv_obj`	infercnv_object
`window_size`	Size of the window side centered on the data point to filter (default = 7).
`on_observations`	boolean (default=TRUE), run on observations data (tumor cells).
`on_references`	boolean (default=TRUE), run on references (normal cells).

Value

infercnv_obj with median filtering applied to observations

Examples

```
# data(infercnv_data_example)
# data(infercnv_annots_example)
# data(infercnv_genes_example)

# infercnv_object_example <- infercnv::CreateInfercnvObject(raw_counts_matrix=infercnv_data_example,
#                                                    gene_order_file=infercnv_genes_example,
#                                                  annotations_file=infercnv_annots_example,
#                                                              ref_group_names=c("normal"))

# infercnv_object_example <- infercnv::run(infercnv_object_example,
#                                          cutoff=1,
#                                          out_dir=tempfile(),
#                                          cluster_by_groups=TRUE,
#                                          denoise=TRUE,
#                                          HMM=FALSE,
#                                          num_threads=2,
#                                          no_plot=TRUE)
```

```
data(infercnv_object_example)

infercnv_object_example <- infercnv::apply_median_filtering(infercnv_object_example)
# plot result object
```

`color.palette` *Helper function allowing greater control over the steps in a color palette.*

Description

Helper function allowing greater control over the steps in a color palette. Source: http://menugget.blogspot.com/2011/11/ color-steps-for- colorramppalette.html#more

Usage

```
color.palette(steps, between = NULL, ...)
```

Arguments

`steps`	Vector of colors to change use in the palette
`between`	Steps where gradients change
`...`	Additional arguments of colorRampPalette

Value

Color palette

Examples

```
color.palette(c("darkblue", "white", "darkred"),
              c(2, 2))
```

`CreateInfercnvObject` *CreateInfercnvObject*

Description

Creation of an infercnv object. This requires the following inputs: A more detailed description of each input is provided below:

The raw_counts_matrix:

MGH54_P16_F12 MGH53_P5_C12 MGH54_P12_C10 MGH54_P16_F02 MGH54_P11_C11 ... DDX11L1 0.0000000 0.000000 0.000000 0.000000 0.0000000 WASH7P 0.0000000 2.231939 7.186235 5.284944 0.9650009 FAM138A 0.1709991 0.000000 0.000000 0.000000 0.0000000 OR4F5 0.0000000 0.000000 0.000000 0.000000 0.0000000 OR4F29 0.0000000 0.000000 0.000000 0.000000 0.0000000 ...

The gene_order_file, contains chromosome, start, and stop position for each gene, tab-delimited:

chr start stop DDX11L1 chr1 11869 14412 WASH7P chr1 14363 29806 FAM138A chr1 34554 36081 OR4F5 chr1 69091 70008 OR4F29 chr1 367640 368634 OR4F16 chr1 621059 622053 ...

The annotations_file, containing the cell name and the cell type classification, tab-delimited.

V1 V2 1 MGH54_P2_C12 Microglia/Macrophage 2 MGH36_P6_F03 Microglia/Macrophage 3 MGH53_P4_H08 Microglia/Macrophage 4 MGH53_P2_E09 Microglia/Macrophage 5 MGH36_P5_E12 Oligodendrocytes (non-malignant) 6 MGH54_P2_H07 Oligodendrocytes (non-malignant) ... 179 93_P9_H03 malignant 180 93_P10_D04 malignant 181 93_P8_G09 malignant 182 93_P10_B10 malignant 183 93_P9_C07 malignant 184 93_P8_A12 malignant ...

and the ref_group_names vector might look like so: c("Microglia/Macrophage","Oligodendrocytes (non-malignant)")

Usage

```
CreateInfercnvObject(
  raw_counts_matrix,
  gene_order_file,
  annotations_file,
  ref_group_names,
  delim = "\t",
  max_cells_per_group = NULL,
  min_max_counts_per_cell = c(100, +Inf),
  chr_exclude = c("chrX", "chrY", "chrM")
)
```

Arguments

`raw_counts_matrix`
: the matrix of genes (rows) vs. cells (columns) containing the raw counts If a filename is given, it'll be read via read.table() otherwise, if matrix or Matrix, will use the data directly.

`gene_order_file`
: data file containing the positions of each gene along each chromosome in the genome.

`annotations_file`
: a description of the cells, indicating the cell type classifications

`ref_group_names`
: a vector containing the classifications of the reference (normal) cells to use for infering cnv

`delim`
: delimiter used in the input files

`max_cells_per_group`
: maximun number of cells to use per group. Default=NULL, using all cells defined in the annotations_file. This option is useful for randomly subsetting the existing data for a quicker preview run, such as using 50 cells per group instead of hundreds.

`min_max_counts_per_cell`
: minimum and maximum counts allowed per cell. Any cells outside this range will be removed from the counts matrix. default=(100, +Inf) and uses all cells. If used, should be set as c(min_counts, max_counts)

`chr_exclude`
: list of chromosomes in the reference genome annotations that should be excluded from analysis. Default = c('chrX', 'chrY', 'chrM')

Value

infercnv

Examples

```
data(infercnv_data_example)
data(infercnv_annots_example)
data(infercnv_genes_example)

infercnv_object_example <- infercnv::CreateInfercnvObject(raw_counts_matrix=infercnv_data_example,
                                                          gene_order_file=infercnv_genes_example,
                                                          annotations_file=infercnv_annots_example,
                                                          ref_group_names=c("normal"))
```

---

filterHighPNormals — *filterHighPNormals: Filter the HMM identified CNV's by the CNV's posterior probability of belonging to a normal state.*

Description

The following function will filter the HMM identified CNV's by the CNV's posterior probability of belonging to a normal state identified by the function inferCNVBayesNet(). Will filter CNV's based on a user desired threshold probability. Any CNV with a probability of being normal above the threshold will be removed.

Usage

```
filterHighPNormals(MCMC_inferCNV_obj, HMM_states, BayesMaxPNormal, useRaster)
```

Arguments

MCMC_inferCNV_obj	MCMC infernCNV object.
HMM_states	InferCNV object with HMM states in expression data.
BayesMaxPNormal	Option to filter CNV or cell lines by some probability threshold.
useRaster	Option to use rasterization when plotting

Value

Returns a list of (MCMC_inferCNV_obj, HMM_states) With removed CNV's.

Examples

```
data(mcmc_obj)

mcmc_obj_hmm_states_list <- infercnv::filterHighPNormals( MCMC_inferCNV_obj = mcmc_obj,
                                          HMM_states        = HMM_states,
                                          BayesMaxPNormal   = 0.5)
```

HMM_states — *infercnv object result of the processing of run() in the HMM example, to be used for other examples.*

Description

infercnv object result of the processing of run() in the HMM example, to be used for other examples.

Usage

```
HMM_states
```

Format

An infercnv object containing HMM predictions

infercnv-class — *The infercnv Class*

Description

An infercnv object encapsulates the expression data and gene chromosome ordering information that is leveraged by infercnv for data exploration. The infercnv object is passed among the infercnv data processing and plotting routines.

Details

Slots in the infercnv object include:

Slots

`expr.data` <matrix> the count or expression data matrix, manipulated throughout infercnv ops

`count.data` <matrix> retains the original count data, but shrinks along with expr.data when genes are removed.

`gene_order` <data.frame> chromosomal gene order

`reference_grouped_cell_indices` <list> mapping [['group_name']] to c(cell column indices) for reference (normal) cells

`observation_grouped_cell_indices` <list> mapping [['group_name']] to c(cell column indices) for observation (tumor) cells

`tumor_subclusters` <list> stores subclustering of tumors if requested

`options` <list> stores the options relevant to the analysis in itself (in contrast with options relevant to plotting or paths)

`.hspike` a hidden infercnv object populated with simulated spiked-in data

`inferCNVBayesNet` *inferCNVBayesNet: Run Bayesian Network Mixture Model To Obtain Posterior Probabilities For HMM Predicted States*

Description

Uses Markov Chain Monte Carlo (MCMC) and Gibbs sampling to estimate the posterior probability of being in one of six Copy Number Variation states (states: 0, 0.5, 1, 1.5, 2, 3) for CNV's identified by inferCNV's HMM. Posterior probabilities are found for the entire CNV cluster and each individual cell line in the CNV.

Usage

```
inferCNVBayesNet(
  file_dir,
  infercnv_obj,
  HMM_states,
  out_dir,
  resume_file_token,
  model_file = NULL,
  CORES = 1,
  postMcmcMethod = NULL,
  plotingProbs = TRUE,
  quietly = TRUE,
  diagnostics = FALSE,
  HMM_type = HMM_type,
  k_obs_groups = k_obs_groups,
  cluster_by_groups = cluster_by_groups,
  reassignCNVs = TRUE,
  no_plot = no_plot,
  useRaster
)
```

Arguments

`file_dir`	Location of the directory of the inferCNV outputs.
`infercnv_obj`	InferCNV object.
`HMM_states`	InferCNV object with HMM states in expression data.
`out_dir`	(string) Path to where the output file should be saved to.
`resume_file_token`	(string) String token that contains some info on settings used to name files.
`model_file`	Path to the BUGS Model file.
`CORES`	Option to run parallel by specifying the number of cores to be used. (Default: 1)
`postMcmcMethod`	What actions to take after finishing the MCMC.
`plotingProbs`	Option for adding plots of Cell and CNV probabilities. (Default: TRUE)
`quietly`	Option to print descriptions along each step. (Default: TRUE)
`diagnostics`	Option to plot Diagnostic plots and tables. (Default: FALSE)

HMM_type	The type of HMM that was ra, either 'i3' or 'i6'. Determines how many state were predicted by the HMM.
k_obs_groups	Number of groups in which to break the observations. (default: 1)
cluster_by_groups	If observations are defined according to groups (ie. patients), each group of cells will be clustered separately. (default=FALSE, instead will use k_obs_groups setting)
reassignCNVs	(boolean) Given the CNV associated probability of belonging to each possible state, reassign the state assignments made by the HMM to the state that has the highest probability. (default: TRUE)
no_plot	(boolean) Option set by infercnv::run() for producing visualizations.
useRaster	Option to use rasterization when plotting

Value

Returns a MCMC_inferCNV_obj and posterior probability of being in one of six Copy Number Variation states (states: 0, 0.5, 1, 1.5, 2, 3) for CNV's identified by inferCNV's HMM.

Examples

```
data(infercnv_data_example)
data(infercnv_annots_example)
data(infercnv_genes_example)
data(HMM_states)

infercnv_object_example <- infercnv::CreateInfercnvObject(raw_counts_matrix=infercnv_data_example,
                                                    gene_order_file=infercnv_genes_example,
                                                  annotations_file=infercnv_annots_example,
                                                            ref_group_names=c("normal"))

out_dir = tempfile()
infercnv_object_example <- infercnv::run(infercnv_object_example,
                                         cutoff=1,
                                         out_dir=out_dir,
                                         cluster_by_groups=TRUE,
                                         analysis_mode="samples",
                                         denoise=TRUE,
                                         HMM=TRUE,
                                         num_threads=2,
                                         no_plot=TRUE)
mcmc_obj <- infercnv::inferCNVBayesNet(infercnv_obj      = infercnv_object_example,
                                       HMM_states        = HMM_states,
                                       file_dir          = out_dir,
                                       postMcmcMethod    = "removeCNV",
                                       out_dir           = out_dir,
                                       resume_file_token = "HMMi6.hmm_mode-samples",
                                       quietly           = TRUE,
                                       CORES             = 2,
                                       plotingProbs      = FALSE,
                                       diagnostics       = FALSE,
                                       HMM_type          = 'i6',
                                       k_obs_groups      = 1,
                                       cluster_by_groups = FALSE,
                                       reassignCNVs      = FALSE,
```

```
                                    no_plot           = TRUE)
```

`infercnv_annots_example` *Generated classification for 10 normal cells and 10 tumor cells.*

Description

Generated classification for 10 normal cells and 10 tumor cells.

Usage

```
infercnv_annots_example
```

Format

A data frame with 20 rows (cells) and 1 columns (classification)

`infercnv_data_example` *Generated SmartSeq2 expression data with 10 normal cells and 10 tumor cells. This is only to demonstrate how to use methods, not actual data to be used in an analysis.*

Description

Generated SmartSeq2 expression data with 10 normal cells and 10 tumor cells. This is only to demonstrate how to use methods, not actual data to be used in an analysis.

Usage

```
infercnv_data_example
```

Format

A data frame with 8252 rows (genes) and 20 columns (cells)

`infercnv_genes_example` *Downsampled gene coordinates file from GrCh37*

Description

Downsampled gene coordinates file from GrCh37

Usage

```
infercnv_genes_example
```

Format

A data frame with 10338 rows (genes) and 3 columns (chr, start, end)

infercnv_object_example

infercnv object result of the processing of run() in the example, to be used for other examples.

Description

infercnv object result of the processing of run() in the example, to be used for other examples.

Usage

```
infercnv_object_example
```

Format

An infercnv object

MCMC_inferCNV-class *MCMC_inferCNV class*

Description

Uses Markov Chain Monte Carlo (MCMC) and Gibbs sampling to estimate the posterior probability of being in one of six Copy Number Variation states (states: 0, 0.5, 1, 1.5, 2, 3) for CNV's identified by inferCNV's HMM. Posterior probabilities are found for the entire CNV cluster and each individual cell line in the CNV.

Slots

`bugs_model` BUGS model.

`sig` fitted values for cell lines, 1/standard deviation to be used for determining the distribution of each cell line

`mu` Mean values to be used for determining the distribution of each cell line

`group_id` ID's given to the cell clusters.

`cell_gene` List containing the Cells and Genes that make up each CNV.

`cnv_probabilities` Probabilities of each CNV belonging to a particular state from 0 (least likely)to 1 (most likely).

`cell_probabilities` Probabilities of each cell being in a particular state, from 0 (least likely)to 1 (most likely).

`args` Input arguments given by the user

`cnv_regions` ID for each CNV found by the HMM

mcmc_obj — *infercnv object result of the processing of inferCNVBayesNet in the example, to be used for other examples.*

Description

infercnv object result of the processing of inferCNVBayesNet in the example, to be used for other examples.

Usage

```
mcmc_obj
```

Format

An infercnv object containing posterior probability of CNV states

plot_cnv — *Plot the matrix as a heatmap, with cells as rows and genes as columns, ordered according to chromosome*

Description

Formats the data and sends it for plotting.

Usage

```
plot_cnv(
  infercnv_obj,
  out_dir = ".",
  title = "inferCNV",
  obs_title = "Observations (Cells)",
  ref_title = "References (Cells)",
  cluster_by_groups = TRUE,
  cluster_references = TRUE,
  plot_chr_scale = FALSE,
  chr_lengths = NULL,
  k_obs_groups = 1,
  contig_cex = 1,
  x.center = mean(infercnv_obj@expr.data),
  x.range = "auto",
  hclust_method = "ward.D",
  custom_color_pal = NULL,
  color_safe_pal = FALSE,
  output_filename = "infercnv",
  output_format = "png",
  png_res = 300,
  dynamic_resize = 0,
  ref_contig = NULL,
```

```
  write_expr_matrix = FALSE,
  write_phylo = FALSE,
  useRaster = TRUE
)
```

Arguments

infercnv_obj	infercnv object
out_dir	Directory in which to save pdf and other output.
title	Plot title.
obs_title	Title for the observations matrix.
ref_title	Title for the reference matrix.
cluster_by_groups	Whether to cluster observations by their annotations or not. Using this ignores k_obs_groups.
cluster_references	Whether to cluster references within their annotations or not. (dendrogram not displayed)
plot_chr_scale	Whether to scale the chromosme width on the heatmap based on their actual size rather than just the number of expressed genes.
chr_lengths	A named list of chromsomes lengths to use when plot_chr_scale=TRUE, or else chromosome size is assumed to be the last chromosome's stop position + 10k bp
k_obs_groups	Number of groups to break observation into.
contig_cex	Contig text size.
x.center	Value on which to center expression.
x.range	vector containing the extreme values in the heatmap (ie. c(-3,4))
hclust_method	Clustering method to use for hclust.
custom_color_pal	Specify a custom set of colors for the heatmap. Has to be in the shape color.palette(c("darkblue", "white", "darkred"), c(2, 2))
color_safe_pal	Logical indication of using a color blindness safe palette.
output_filename	Filename to save the figure to.
output_format	format for heatmap image file (default: 'png'), options('png', 'pdf', NA) If set to NA, will print graphics natively
png_res	Resolution for png output.
dynamic_resize	Factor (>= 0) by which to scale the dynamic resize of the observation heatmap and the overall plot based on how many cells there are. Default is 0, which disables the scaling. Try 1 first if you want to enable.
ref_contig	If given, will focus cluster on only genes in this contig.
write_expr_matrix	Includes writing a matrix file containing the expression data that is plotted in the heatmap.
write_phylo	Write newick strings of the dendrograms displayed on the left side of the heatmap to file.
useRaster	Whether to use rasterization for drawing heatmap. Only disable if it produces an error as it is much faster than not using it.

Value

A list of all relevent settings used for the plotting to be able to reuse them in another plot call while keeping consistant plotting settings, most importantly x.range.

Examples

```
# data(infercnv_data_example)
# data(infercnv_annots_example)
# data(infercnv_genes_example)

# infercnv_object_example <- infercnv::CreateInfercnvObject(raw_counts_matrix=infercnv_data_example,
#                                                 gene_order_file=infercnv_genes_example,
#                                               annotations_file=infercnv_annots_example,
#                                                           ref_group_names=c("normal"))

# infercnv_object_example <- infercnv::run(infercnv_object_example,
#                                          cutoff=1,
#                                          out_dir=tempfile(),
#                                          cluster_by_groups=TRUE,
#                                          denoise=TRUE,
#                                          HMM=FALSE,
#                                          num_threads=2,
#                                          no_plot=TRUE)

data(infercnv_object_example)

plot_cnv(infercnv_object_example,
         out_dir=tempfile(),
         obs_title="Observations (Cells)",
         ref_title="References (Cells)",
         cluster_by_groups=TRUE,
         x.center=1,
         x.range="auto",
         hclust_method='ward.D',
         color_safe_pal=FALSE,
         output_filename="infercnv",
         output_format="png",
         png_res=300,
         dynamic_resize=0
         )
```

`plot_per_group` *plot_per_group*

Description

Takes an infercnv object and subdivides it into one object per group of cells to allow plotting of each group on a seperate plot. If references are selected, they will appear on the observation heatmap area as it is larger.

Usage

```
plot_per_group(
  infercnv_obj,
  on_references = TRUE,
  on_observations = TRUE,
  sample = FALSE,
  n_cells = 1000,
  every_n = NULL,
  above_m = 1000,
  k_obs_groups = 1,
  base_filename = "infercnv_per_group",
  output_format = "png",
  write_expr_matrix = TRUE,
  save_objects = FALSE,
  png_res = 300,
  dynamic_resize = 0,
  useRaster = TRUE,
  out_dir
)
```

Arguments

infercnv_obj	infercnv_object
on_references	boolean (default=TRUE), plot references (normal cells).
on_observations	boolean (default=TRUE), plot observations data (tumor cells).
sample	Whether unique groups of cells should be sampled from or not. (see other parameters for how sampling is done) (Default: FALSE)
n_cells	Number of cells that should be sampled per group if sampling is enabled (default = 1000) .
every_n	Sample 1 cell every_n cells for each group that has above_m cells, if sampling is enabled. If subclusters are defined, this will make sure that at least one cell per subcluster is sampled. Requires above_m to be set to work, overriding n_cells parameter. (Default: NULL)
above_m	Sample only groups that have at least above_m cells if sampling is enabled. (default: 1000) Does not require every_n to be set.
k_obs_groups	Number of groups to break each group in with cutree (in the color bars on the left side of the plot only). (Default: 1)
base_filename	Base prefix for the output files names. Will be followed by OBS/REF to indidate the type of the group, and the group name. (Default: "infercnv_per_group")
output_format	Output format for the figure. Choose between "png", "pdf" and NA. NA means to only write the text outputs without generating the figure itself. (default: "png")
write_expr_matrix	Includes writing a matrix file containing the expression data that is plotted in the heatmap. (default: FALSE)
save_objects	Whether to save the infercnv objects generated for each group as RDS. (default: FALSE)
png_res	Resolution for png output. (Default: 300)

dynamic_resize	Factor (>= 0) by which to scale the dynamic resize of the observation heatmap and the overall plot based on how many cells there are. Default is 0, which disables the scaling. Try 1 first if you want to enable. (Default: 0)
useRaster	Whether to use rasterization for drawing heatmap. Only disable if it produces an error as it is much faster than not using it.
out_dir	Directory in which to save plots and other outputs.

Value

void

Examples

```
# data(infercnv_data_example)
# data(infercnv_annots_example)
# data(infercnv_genes_example)

# infercnv_object_example <- infercnv::CreateInfercnvObject(raw_counts_matrix=infercnv_data_example,
#                                                 gene_order_file=infercnv_genes_example,
#                                               annotations_file=infercnv_annots_example,
#                                                           ref_group_names=c("normal"))

# infercnv_object_example <- infercnv::run(infercnv_object_example,
#                                          cutoff=1,
#                                          out_dir=tempfile(),
#                                          cluster_by_groups=TRUE,
#                                          denoise=TRUE,
#                                          HMM=FALSE,
#                                          num_threads=2,
#                                          no_plot=TRUE)

data(infercnv_object_example)

infercnv::plot_per_group(infercnv_object_example, out_dir=tempfile())
```

---

plot_subclusters *Plot a heatmap of the data in the infercnv object with the subclusters being displayed as annotations.*

Description

Formats the data and sends it for plotting.

Usage

```
plot_subclusters(
  infercnv_obj,
  out_dir,
  output_filename = "subcluster_as_annotations"
)
```

Arguments

infercnv_obj	infercnv object
out_dir	Directory in which to output.
output_filename	Filename to save the figure to.

Value

infercnv_obj the modified infercnv object that was plotted where subclusters are assigned as annotation groups

Examples

```
# data(infercnv_data_example)
# data(infercnv_annots_example)
# data(infercnv_genes_example)

# infercnv_object_example <- infercnv::CreateInfercnvObject(raw_counts_matrix=infercnv_data_example,
#                                                 gene_order_file=infercnv_genes_example,
#                                               annotations_file=infercnv_annots_example,
#                                                           ref_group_names=c("normal"))

# infercnv_object_example <- infercnv::run(infercnv_object_example,
#                                          cutoff=1,
#                                          out_dir=tempfile(),
#                                          cluster_by_groups=TRUE,
#                                          denoise=TRUE,
#                                          HMM=FALSE,
#                                          num_threads=2,
#                                          no_plot=TRUE)

data(infercnv_object_example)

plot_subclusters(infercnv_object_example,
                 out_dir=tempfile(),
                 output_filename="subclusters_as_annotations"
                 )
```

---

run *run() : Invokes a routine inferCNV analysis to Infer CNV changes given a matrix of RNASeq counts.*

Description

Function doing the actual analysis before calling the plotting functions.

Usage

```
run(
  infercnv_obj,
  cutoff = 1,
```

```
  min_cells_per_gene = 3,
  out_dir = NULL,
  window_length = 101,
  smooth_method = c("pyramidinal", "runmeans", "coordinates"),
  num_ref_groups = NULL,
  ref_subtract_use_mean_bounds = TRUE,
  cluster_by_groups = TRUE,
  cluster_references = TRUE,
  k_obs_groups = 1,
  hclust_method = "ward.D2",
  max_centered_threshold = 3,
  scale_data = FALSE,
  HMM = FALSE,
  HMM_transition_prob = 1e-06,
  HMM_report_by = c("subcluster", "consensus", "cell"),
  HMM_type = c("i6", "i3"),
  HMM_i3_pval = 0.05,
  HMM_i3_use_KS = FALSE,
  BayesMaxPNormal = 0.5,
  sim_method = "meanvar",
  sim_foreground = FALSE,
  reassignCNVs = TRUE,
  analysis_mode = c("subclusters", "samples", "cells"),
 tumor_subcluster_partition_method = c("leiden", "random_trees", "qnorm", "pheight",
    "qgamma", "shc"),
  tumor_subcluster_pval = 0.1,
  k_nn = 20,
  leiden_method = c("PCA", "simple"),
  leiden_function = c("CPM", "modularity"),
  leiden_resolution = "auto",
  leiden_method_per_chr = c("simple", "PCA"),
  leiden_function_per_chr = c("modularity", "CPM"),
  leiden_resolution_per_chr = 1,
  per_chr_hmm_subclusters = FALSE,
  per_chr_hmm_subclusters_references = FALSE,
  z_score_filter = 0.8,
  denoise = FALSE,
  noise_filter = NA,
  sd_amplifier = 1.5,
  noise_logistic = FALSE,
  outlier_method_bound = "average_bound",
  outlier_lower_bound = NA,
  outlier_upper_bound = NA,
  final_scale_limits = NULL,
  final_center_val = NULL,
  debug = FALSE,
  num_threads = 4,
  plot_steps = FALSE,
  inspect_subclusters = TRUE,
  resume_mode = TRUE,
  png_res = 300,
  plot_probabilities = TRUE,
```

```
  save_rds = TRUE,
  save_final_rds = TRUE,
  diagnostics = FALSE,
  remove_genes_at_chr_ends = FALSE,
  prune_outliers = FALSE,
  mask_nonDE_genes = FALSE,
  mask_nonDE_pval = 0.05,
  test.use = "wilcoxon",
  require_DE_all_normals = "any",
  hspike_aggregate_normals = FALSE,
  no_plot = FALSE,
  no_prelim_plot = FALSE,
  write_expr_matrix = FALSE,
  write_phylo = FALSE,
  output_format = "png",
  plot_chr_scale = FALSE,
  chr_lengths = NULL,
  useRaster = TRUE,
  up_to_step = 100
)
```

Arguments

infercnv_obj	An infercnv object populated with raw count data
cutoff	Cut-off for the min average read counts per gene among reference cells. (default: 1)
min_cells_per_gene	minimum number of reference cells requiring expression measurements to include the corresponding gene. default: 3
out_dir	path to directory to deposit outputs (default: NULL, required to provide non NULL)
	## Smoothing params
window_length	Length of the window for the moving average (smoothing). Should be an odd integer. (default: 101)#'
smooth_method	Method to use for smoothing: c(runmeans,pyramidinal,coordinates) default: pyramidinal
	#####
num_ref_groups	The number of reference groups or a list of indices for each group of reference indices in relation to reference_obs. (default: NULL)
ref_subtract_use_mean_bounds	Determine means separately for each ref group, then remove intensities within bounds of means (default: TRUE) Otherwise, uses mean of the means across groups.
	##############################
cluster_by_groups	If observations are defined according to groups (ie. patients), each group of cells will be clustered separately. (default=FALSE, instead will use k_obs_groups setting)
cluster_references	Whether to cluster references within their annotations or not. (dendrogram not displayed) (default: TRUE)

k_obs_groups Number of groups in which to break the observations. (default: 1)

hclust_method Method used for hierarchical clustering of cells. Valid choices are: "ward.D", "ward.D2", "single", "complete", "average", "mcquitty", "median", "centroid". default("ward.D2")

max_centered_threshold
The maximum value a value can have after centering. Also sets a lower bound of -1 * this value. (default: 3), can set to a numeric value or "auto" to bound by the mean bounds across cells. Set to NA to turn off.

scale_data perform Z-scaling of logtransformed data (default: FALSE). This may be turned on if you have very different kinds of data for your normal and tumor samples. For example, you need to use GTEx representative normal expression profiles rather than being able to leverage normal single cell data that goes with your experiment.
Downstream Analyses (HMM or non-DE-masking) based on tumor subclusters

HMM when set to True, runs HMM to predict CNV level (default: FALSE)

HMM_transition_prob
transition probability in HMM (default: 1e-6)

HMM_report_by cell, consensus, subcluster (default: subcluster) Note, reporting is performed entirely separately from the HMM prediction. So, you can predict on subclusters, but get per-cell level reporting (more voluminous output).

HMM_type HMM model type. Options: (i6 or i3): i6: infercnv 6-state model (0, 0.5, 1, 1.5, 2, >2) where state emissions are calibrated based on simulated CNV levels. i3: infercnv 3-state model (del, neutral, amp) configured based on normal cells and HMM_i3_pval

HMM_i3_pval p-value for HMM i3 state overlap (default: 0.05)

HMM_i3_use_KS boolean: use the KS test statistic to estimate mean of amp/del distributions (ala HoneyBadger). (default=TRUE)
Filtering low-conf HMM preds via BayesNet P(Normal)

BayesMaxPNormal
maximum P(Normal) allowed for a CNV prediction according to BayesNet. (default=0.5, note zero turns it off)

sim_method method for calibrating CNV levels in the i6 HMM (default: 'meanvar')

sim_foreground don't use... for debugging, developer option.

reassignCNVs (boolean) Given the CNV associated probability of belonging to each possible state, reassign the state assignments made by the HMM to the state that has the highest probability. (default: TRUE)
###################### ## Tumor subclustering

analysis_mode options(samples|subclusters|cells), Grouping level for image filtering or HMM predictions. default: samples (fastest, but subclusters is ideal)

tumor_subcluster_partition_method
method for defining tumor subclusters. Options('leiden', 'random_trees', 'qnorm') leiden: Runs a nearest neighbor search, where communities are then partitionned with the Leiden algorithm. random_trees: Slow, uses permutation statistics w/ tree construction. qnorm: defines tree height based on the quantile defined by the tumor_subcluster_pval

tumor_subcluster_pval
: max p-value for defining a significant tumor subcluster (default: 0.1)

k_nn
: number k of nearest neighbors to search for when using the Leiden partition method for subclustering (default: 20)

leiden_method
: Method used to generate the graph on which the Leiden algorithm is applied, one of "PCA" or "simple". (default: "PCA")

leiden_function
: Whether to use the Constant Potts Model (CPM) or modularity in igraph. Must be either "CPM" or "modularity". (default: "CPM")

leiden_resolution
: resolution parameter for the Leiden algorithm using the CPM quality score (default: auto)

leiden_method_per_chr
: Method used to generate the graph on which the Leiden algorithm is applied for the per chromosome subclustering, one of "PCA" or "simple". (default: "simple")

leiden_function_per_chr
: Whether to use the Constant Potts Model (CPM) or modularity in igraph for the per chromosome subclustering. Must be either "CPM" or "modularity". (default: "modularity")

leiden_resolution_per_chr
: resolution parameter for the Leiden algorithm for the per chromosome subclustering (default: 1)

per_chr_hmm_subclusters
: Run subclustering per chromosome over all cells combined to run the HMM on those subclusters instead. Only applicable when using Leiden subclustering. This should provide enough definition in the predictions while avoiding subclusters that are too small thus providing less evidence to work with. (default: FALSE)

per_chr_hmm_subclusters_references
: Whether the per chromosome subclustering should also be done on references, which should not have as much variation as observations. (default = FALSE)

z_score_filter
: Z-score used as a treshold to filter genes used for subclustering. Applied based on reference genes to automatically ignore genes with high expression variability such as MHC genes. (default: 0.8)
############################## ## de-noising parameters ####

denoise
: If True, turns on denoising according to options below

noise_filter
: Values +- from the reference cell mean will be set to zero (whitening effect) default(NA, instead will use sd_amplifier below.

sd_amplifier
: Noise is defined as mean(reference_cells) +- sdev(reference_cells) * sd_amplifier default: 1.5

noise_logistic
: use the noise_filter or sd_amplifier based threshold (whichever is invoked) as the midpoint in a logistic model for downscaling values close to the mean. (default: FALSE)
################## ## Outlier pruning

outlier_method_bound
: Method to use for bounding outlier values. (default: "average_bound") Will preferentially use outlier_lower_bounda and outlier_upper_bound if set.

outlier_lower_bound
: Outliers below this lower bound will be set to this value.

outlier_upper_bound
: Outliers above this upper bound will be set to this value.
########################## ## Misc options

final_scale_limits
: The scale limits for the final heatmap output by the run() method. Default "auto". Alt, c(low,high)

final_center_val
: Center value for final heatmap output by the run() method.

debug
: If true, output debug level logging.

num_threads
: (int) number of threads for parallel steps (default: 4)

plot_steps
: If true, saves infercnv objects and plots data at the intermediate steps.

inspect_subclusters
: If true, plot subclusters as annotations after the subclustering step to easily see if the subclustering options are good. (default = TRUE)

resume_mode
: leverage pre-computed and stored infercnv objects where possible. (default=TRUE)

png_res
: Resolution for png output.

plot_probabilities
: option to plot posterior probabilities (default: TRUE)

save_rds
: Whether to save the current step object results as an .rds file (default: TRUE)

save_final_rds
: Whether to save the final object results as an .rds file (default: TRUE)

diagnostics
: option to create diagnostic plots after running the Bayesian model (default: FALSE)
####################### ## Experimental options

remove_genes_at_chr_ends
: experimental option: If true, removes the window_length/2 genes at both ends of the chromosome.

prune_outliers
: Define outliers loosely as those that exceed the mean boundaries among all cells. These are set to the bounds.
experimental opts involving DE analysis

mask_nonDE_genes
: If true, sets genes not significantly differentially expressed between tumor/normal to the mean value for the complete data set (default: 0.05)

mask_nonDE_pval
: p-value threshold for defining statistically significant DE genes between tumor/normal

test.use
: statistical test to use. (default: "wilcoxon") alternatives include 'perm' or 't'.'

require_DE_all_normals
: If mask_nonDE_genes is set, those genes will be masked only if they are are found as DE according to test.use and mask_nonDE_pval in each of the comparisons to normal cells options: "any", "most", "all" (default: "any")
other experimental opts

hspike_aggregate_normals
: instead of trying to model the different normal groupings individually, just merge them in the hspike.

no_plot	don't make any of the images. Instead, generate all non-image outputs as part of the run. (default: FALSE)
no_prelim_plot	don't make the preliminary infercnv image (default: FALSE)
write_expr_matrix	Whether to write text files with the content of matrices when generating plots (default: FALSE)
write_phylo	Whether to write newick strings of the dendrograms displayed on the left side of the heatmap to file (default: FALSE)
output_format	Output format for the figure. Choose between "png", "pdf" and NA. NA means to only write the text outputs without generating the figure itself. (default: "png")
plot_chr_scale	Whether to scale the chromosme width on the heatmap based on their actual size rather than just the number of expressed genes.
chr_lengths	A named list of chromsomes lengths to use when plot_chr_scale=TRUE, or else chromosome size is assumed to be the last chromosome's stop position + 10k bp
useRaster	Whether to use rasterization for drawing heatmap. Only disable if it produces an error as it is much faster than not using it. (default: TRUE)
up_to_step	run() only up to this exact step number (default: 100 » 23 steps currently in the process)

Value

infercnv_obj containing filtered and transformed data

Examples

```
data(infercnv_data_example)
data(infercnv_annots_example)
data(infercnv_genes_example)

infercnv_object_example <- infercnv::CreateInfercnvObject(raw_counts_matrix=infercnv_data_example,
                                                    gene_order_file=infercnv_genes_example,
                                                  annotations_file=infercnv_annots_example,
                                                            ref_group_names=c("normal"))

infercnv_object_example <- infercnv::run(infercnv_object_example,
                                         cutoff=1,
                                         out_dir=tempfile(),
                                         cluster_by_groups=TRUE,
                                         denoise=TRUE,
                                         HMM=FALSE,
                                         num_threads=2,
                                         analysis_mode="samples",
                                         no_plot=TRUE)
```

sample_object *sample_object*

Description

Apply sampling on an infercnv object to reduce the number of cells in it and allow faster plotting or have all groups take up the same height on the heatmap

Usage

```
sample_object(
  infercnv_obj,
  n_cells = 100,
  every_n = NULL,
  above_m = NULL,
  on_references = TRUE,
  on_observations = TRUE
)
```

Arguments

infercnv_obj	infercnv_object
n_cells	Number of cells that should be sampled per group (default = 100).
every_n	Sample 1 cell every_n cells for each group. If subclusters are defined, this will make sure that at least one cell per subcluster is sampled. Requires above_m to be set to work, overriding n_cells parameter.
above_m	Sample groups that have at least above_m cells. Requires every_n to be set to work, overriding n_cells parameter
on_references	boolean (default=TRUE), sample references (normal cells).
on_observations	boolean (default=TRUE), sample observations data (tumor cells).

Value

sampled infercnv_obj

Examples

```
# data(infercnv_data_example)
# data(infercnv_annots_example)
# data(infercnv_genes_example)

# infercnv_object_example <- infercnv::CreateInfercnvObject(raw_counts_matrix=infercnv_data_example,
#                                                    gene_order_file=infercnv_genes_example,
#                                                  annotations_file=infercnv_annots_example,
#                                                              ref_group_names=c("normal"))

# infercnv_object_example <- infercnv::run(infercnv_object_example,
#                                          cutoff=1,
#                                          out_dir=tempfile(),
#                                          cluster_by_groups=TRUE,
```

```
#                                          denoise=TRUE,
#                                          HMM=FALSE,
#                                          num_threads=2,
#                                          no_plot=TRUE)

data(infercnv_object_example)

infercnv_object_example <- infercnv::sample_object(infercnv_object_example, n_cells=5)
# plot result object
```

---

validate_infercnv_obj *validate_infercnv_obj()*

Description

validate an infercnv_obj ensures that order of genes in the @gene_order slot match up perfectly with the gene rows in the @expr.data matrix. Otherwise, throws an error and stops execution.

Usage

```
validate_infercnv_obj(infercnv_obj)
```

Arguments

infercnv_obj	infercnv_object

Value

none

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