

# Package: SlideCNA (via r-universe)

February 23, 2025

**Title** Calls Copy Number Alterations from Slide-Seq Data

**Version** 0.1.0

**Description** This takes spatial single-cell-type RNA-seq data (specifically designed for Slide-seq v2) that calls copy number alterations (CNAs) using pseudo-spatial binning, clusters cellular units (e.g. beads) based on CNA profile, and visualizes spatial CNA patterns. Documentation about 'SlideCNA' is included in the the pre-print by Zhang et al. (2022, <[doi:10.1101/2022.11.25.517982](https://doi.org/10.1101/2022.11.25.517982)>). The package 'enrichR' (>= 3.0), conditionally used to annotate SlideCNA-determined clusters with gene ontology terms, can be installed at <<https://github.com/wjawaid/enrichR>> or with `install_github("`wjawaid/enrichR")`.

**Imports** data.table, reshape2, dplyr, ggplot2, scales, pheatmap, cluster, factoextra, dendextend, Seurat, tidyselect, stringr, magrittr, tibble, futile.logger, mltools, utils

**License** GPL (>= 3)

**Encoding** UTF-8

**RoxygenNote** 7.3.2

**Suggests** testthat (>= 3.0.0), enrichR (>= 3.0)

**Config/testthat/edition** 3

**NeedsCompilation** no

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**Date/Publication** 2025-01-23 17:40:05 UTC

**Config/pak/sysreqs** cmake libglpk-dev make libicu-dev libpng-dev libxml2-dev libssl-dev python3 zlib1g-dev

**Repository** <https://dzhang777.r-universe.dev>

**RemoteUrl** <https://github.com/cran/SlideCNA>

**RemoteRef** HEAD

**RemoteSha** fdea2a492e8474f60b4dd58168977ca7fba5021a

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bin

*Subfunction of bin\_metadata() for expression/positional binning*

---

### Description

This function computes a pseudospacial distance between beads that combines spatial distance and distance from the expression space, then using the silhouette score and hierarchical clustering, segregates beads into bins

**Usage**

```
bin(
  dat,
  md,
  k,
  pos = TRUE,
  pos_k = 55,
  ex_k = 1,
  hc_function = "ward.D2",
  plot_directory
)
```

**Arguments**

dat	data.table of smoothed relative expression intensities
md	data.table of metadata of each bead
k	number of malignant bins to set
pos	TRUE if doing spatial and expressional binning, FALSE if just expressional binning
pos_k	positional weight
ex_k	expressional weight
hc_function	hierarchical clustering function
plot_directory	output plot directory path

**Value**

A data.table of bead metadata combined with bin designations

---

bin_metadata	<i>Spatio-molecular binning of relative expression intensities</i>
--------------	--

---

**Description**

This function combines metadata with binned relative expression intensities

**Usage**

```
bin_metadata(
  md,
  dat,
  avg_bead_per_bin = 12,
  pos = TRUE,
  pos_k = 55,
  ex_k = 1,
  hc_function = "ward.D2",
  plot_directory
)
```

**Arguments**

md	data.table of metadata of each bead
dat	data.table of smoothed relative expression intensities
avg_bead_per_bin	integer of average number of beads there should be per bin
pos	TRUE if doing spatial and expressional binning, FALSE if just expressional binning
pos_k	positional weight
ex_k	expressional weight
hc_function	hierarchical clustering function
plot_directory	output plot directory path

**Value**

A data.table of bead metadata combined with binned expression intensities for all genes for all beads

---

center_rm	<i>Center expression intensities</i>
-----------	--------------------------------------

---

**Description**

Take in a data.table of genomic positions and smoothed expression intensities counts and center by subtracting average intensity across all beads for each gene

**Usage**

```
center_rm(rm)
```

**Arguments**

rm	data.table of smoothed expression intensities counts
----	--

**Value**

centered\_rm data.table of smoothed, centered expression intensities

---

clone_so	<i>Add clone information to meta data of seurat object and bin the beads</i>
----------	--

---

**Description**

This function adds another column for cluster designation to a seurat object's meta data and bins beads

**Usage**

```
clone_so(so, hcl_sub, md, mal = FALSE)
```

**Arguments**

so	Seurat object of beads and their meta data
hcl_sub	hierarchical clustering object of cluster assignemnt as outputted from SlideCNA::plot_clones()
md	data.table of metadata of each bead
mal	TRUE if only using malignant beads

**Value**

A seurat object updated with clone information

---

cnv_heatmap	<i>Plot CNV scores on a heat map</i>
-------------	--------------------------------------

---

**Description**

This function prepares data for plotting and makes a heat map of CNV scores per bead across all genes

**Usage**

```
cnv_heatmap(  
  cnv_data,  
  md,  
  chrom_colors,  
  hc_function = "ward.D2",  
  plot_directory  
)
```

**Arguments**

cnv_data	list object of cnv data from SlideCNA::prep_cnv_dat()
md	data.table of metadata of each bead
chrom_colors	vector of colors labeled by which chromosome they correspond to
hc_function	character for which hierarchical clustering function to use
plot_directory	output plot directory path

**Value**

None

---

dat_to_long	<i>Convert data to long format and add in metadata</i>
-------------	--

---

**Description**

This function will create rows for each bead and gene combination, adding in new metadata with bin designations

**Usage**

```
dat_to_long(dat, md)
```

**Arguments**

dat	data.table of smoothed relative expression intensities
md	data.table of metadata per bead

**Value**

A data.table of bead expression intensities per gene with metadata in long format

---

find\_cluster\_markers *Find and plot top n DEGs per cluster*

---

### Description

This function uses Seurat's marker finding capability to find DEGs of each cluster

### Usage

```
find_cluster_markers(
  so_clone,
  type,
  logfc.threshold = 0.2,
  min.pct = 0,
  only.pos = TRUE,
  n_markers = 5,
  value = "log2_expr",
  text_size = 16,
  title_size = 18,
  legend_size_pt = 4,
  p_val_thresh = 0.05,
  bin = TRUE,
  plot_directory = None
)
```

### Arguments

so_clone	seurat object with 'clone' (SlideCNA-designated cluster) and bin annotations
type	character string that is 'all' if using malignant and normal clusters and 'malig' if just using malignant clusters
logfc.threshold	numeric float that is seurat parameter, representing the minimum log2 fold change for DEGs to be significant
min.pct	numeric Seurat function parameter
only.pos	TRUE if only using DEGs with positive log2 fold change
n_markers	integer of number of top DEGs to plot/use
value	expression value of DEGs; one of ("log2_expr", "avg_expr", and "avg_log2FC") for log2-normalized average expression, average expression, or log2 fold change
text_size	Ggplot2 text size
title_size	Ggplot2 title size
legend_size_pt	Ggplot2 legend_size_pt
p_val_thresh	value for p value cutoff for DEGs
bin	TRUE if using binned beads
plot_directory	output plot directory path

**Value**

A list object with cluster marker information  
 markers\_clone = data.table of all cluster markers  
 top\_markers\_clone = data.table of just top cluster markers  
 top\_clone\_vis = data.frame formatted for plot visualization of top cluster markers

---

find_go_terms	<i>Find and plot top n GO-enriched terms per cluster</i>
---------------	--

---

**Description**

This function utilizes cluster-specific DEGs to identify cluster-specific GO biological processes and plots these if they occur

**Usage**

```
find_go_terms(
  cluster_markers_obj,
  type,
  n_terms = 5,
  text_size,
  title_size,
  plot_directory
)
```

**Arguments**

cluster_markers_obj	list object with cluster marker information
type	character string that is 'all' if using malignant and normal clusters and 'malign' if just using malignant clusters
n_terms	integer of number of top DEGs to plot/use
text_size	integer of text size for ggplot
title_size	integer of title size for ggplot
plot_directory	output plot directory path

**Value**

A list object with cluster GO term information  
 en\_clone = data.table of cluster GO terms  
 top\_en\_clone = data.table of just top cluster GO terms



---

get_num_clust	<i>Find optimal number of clusters</i>
---------------	--

---

### Description

This function uses the Silhouette Method applied to CNV scores to determine the best number of clusters to divide the binned beads into

### Usage

```
get_num_clust(
  data,
  hc_func = "ward.D2",
  max_k = 10,
  plot = TRUE,
  malig = FALSE,
  k = NA,
  plot_directory
)
```

### Arguments

data	cnv_data list object of cnv data from SlideCNA::prep_cnv_dat()
hc_func	character string for which hierarchical clustering function to use
max_k	integer of number max number of clusters to evaluate (2:max_k)
plot	TRUE if plotting silhouette scores per cluster
malig	TRUE if only using malignant bins and FALSE if using all bins
k	integer of optimal number of clusters, if known, and NA if not known
plot_directory	output plot directory path

### Value

An integer representing the number of clusters that optimizes the silhouette score

---

long_to_bin	<i>Convert to wide bin x genes + metadata format</i>
-------------	--

---

### Description

This function will combine beads into bins, taking the average expression intensities, average positions, most common cluster seurat cluster, and most common cluster/tissue type of constituent beads

**Usage**

```
long_to_bin(dat_long, plot_directory, spatial = TRUE)
```

**Arguments**

dat\_long            data.table of bead expression intensities per gene with metadata in long format  
 plot\_directory    output plot directory path  
 spatial            True if using spatial information

**Value**

data.table of expression intensities at aggregated bin level

---

make_seurat_annot	<i>Creation of Seurat object</i>
-------------------	----------------------------------

---

**Description**

This function takes in raw counts (and potentially meta data) to make a Seurat object and process it

**Usage**

```
make_seurat_annot(  
  cb,  
  md = NULL,  
  seed_FindClusters = 0,  
  seed_RunTSNE = 1,  
  seed_RunUMAP = 42  
)
```

**Arguments**

cb                    sparse counts matrix (genes x cells/beads)  
 md                   data.frame of meta data for cells/beads if specific annotations known  
 seed\_FindClusters    seed number for FindClusters  
 seed\_RunTSNE        seed number for RunTSNE  
 seed\_RunUMAP        seed number for RunUMAP

**Value**

A Seurat object with specific Seurat features run

---

make_so_bin	<i>Make a binned version of a Seurat object</i>
-------------	---

---

### Description

Aggregate Seurat object counts by bin to create a new Seurat object with binned beads as units instead of beads

### Usage

```
make_so_bin(so, md, hcl_sub, mal = FALSE)
```

### Arguments

so	Seurat object of beads and their meta data
md	data.frame of metadata for Seurat object
hcl_sub	hierarchical clustering object of cluster assignemnt as outputted from SlideCNA::plot_clones()
mal	TRUE if using malignant beads only

### Value

A Seurat object with binned beads as units and corresponding binned metadata

---

mean_cnv_plot	<i>Plot mean CNV scores per bin and per chromosome</i>
---------------	--

---

### Description

This function colors and plots each bin by its mean CNV score on spatial coordinates for each chromosome

### Usage

```
mean_cnv_plot(
  cnv_data,
  text_size,
  title_size,
  legend_height_bar,
  plot_directory
)
```

**Arguments**

cnv\_data            list object of cnv data from SlideCNA::prep\_cnv\_dat()  
 text\_size           integer of text size for ggplot  
 title\_size          integer of title size for ggplot  
 legend\_height\_bar   integer of bar height of legend for ggplot  
 plot\_directory    output plot directory path

**Value**

None

---

mode	<i>Subfunction of long_to_bin() that finds mode of vector/column</i>
------	--

---

**Description**

This function finds the mode of a vector

**Usage**

```
mode(x)
```

**Arguments**

x                    vector (column in data.table) to calculate the mode from

**Value**

mode of the vector

---

plot_clones	<i>Plot cluster/clone information</i>
-------------	---------------------------------------

---

**Description**

This function plots cluster dendrograms, spatial assignment, and the CNV heat map

**Usage**

```
plot_clones(  
  cnv_data,  
  md,  
  k,  
  type,  
  chrom_colors,  
  text_size,  
  title_size,  
  legend_size_pt,  
  legend_height_bar,  
  hc_function = "ward.D2",  
  plot_directory,  
  spatial = TRUE  
)
```

**Arguments**

cnv_data	list object of cnv data from SlideCNA::prep_cnv_dat()
md	data.table of metadata of each bead
k	integer of number of clusters/clones
type	character string, being "all" if using all binned beads, or "malig" if just malignant binned beads
chrom_colors	vector of colors labeled by which chromosome they correspond to
text_size	Ggplot2 text size
title_size	Ggplot2 title size
legend_size_pt	Ggplot2 legend_size_pt
legend_height_bar	Ggplot2 legend_height_bar
hc_function	character string for which hierarchical clustering function to use
plot_directory	output plot directory path
spatial	TRUE if using spatial information

**Value**

A hierarchical clustering object of the clusters

---

```
prep
```

*Infercnv-based preparation of relative gene expression intensities*

---

### Description

This function takes in a data table of raw counts and a vector of reference/normal beads to normalize counts and adjust for reference expression.

### Usage

```
prep(so, normal_beads, gene_pos, chrom_ord, logTPM = FALSE)
```

### Arguments

so	Seurat object of Slide-seq data with raw counts
normal_beads	vector of names of normal beads
gene_pos	data.table with columns for GENE, chr, start, end, rel_gene_pos (1 : # of genes on chromosome)
chrom_ord	vector of the names of chromosomes in order
logTPM	TRUE if performing adjustment with logTPM

### Value

A data.table of normalized, capped, and ref-adjusted counts with genomic position info

---

```
prep_cnv_dat
```

*Prepare data for CNV heat map*

---

### Description

This function caps CNV scores, adds annotation columns for plotting, performs hierarchical clustering of bins based on similar CNV score, and plots nUMI per bin

### Usage

```
prep_cnv_dat(
  dat_bin,
  lower = 0.6,
  upper = 1.4,
  hc_function = "ward.D2",
  plot_directory
)
```

**Arguments**

dat\_bin            data.table of CNV scores per bin  
 lower             numeric float to represent the lower cap for CNV scores  
 upper             numeric float to represent the upper cap for CNV scores  
 hc\_function      character for which hierarchical clustering function to use  
 plot\_directory    output plot directory path

**Value**

A list object for downstream cnv plotting and analysis all = data.table of CNV scores of all bins x (metadata + genes) malig = data.table of CNV scores of just malignant bins x (metadata + genes) all\_wide = data.frame in wide format of CNV scores of all bins x (metadata + genes) malig\_wide = data.frame in wide format of CNV scores of just malignant bins x (metadata + genes) hcl = hclust object that describes the hierarchical clustering for malignant bins hcl\_all = hclust object that describes the hierarchical clustering for all bins

---

quantile_plot	<i>Plot CNV score quantiles per bin and per chromosome</i>
---------------	--

---

**Description**

This function colors and plots each bin by its CNV score quantiles (min, 1st quartile, median, 3rd quartile, max) on spatial coordinates for each chromosome

**Usage**

```

quantile_plot(
  cnv_data,
  cluster_label = "seurat_clusters",
  text_size,
  title_size,
  legend_height_bar,
  plot_directory
)

```

**Arguments**

cnv\_data            list object of cnv data from SlideCNA::prep\_cnv\_dat()  
 cluster\_label      character string of which column name to keep  
 text\_size          integer of text size for ggplot  
 title\_size         integer of title size for ggplot  
 legend\_height\_bar            integer of bar height of legend for ggplot  
 plot\_directory    output plot directory path

**Value**

None

---

ref_adj	<i>Adjust for Reference (Normal) Beads</i>
---------	--

---

**Description**

Take in a data.table of genomic positions and smoothed, centered expression intensities counts and adjust for reference beads by subtracting average intensities of reference beads for each gene. This is the second reference adjustment.

**Usage**

```
ref_adj(centered_rm, normal_beads)
```

**Arguments**

centered_rm	data.table of smoothed, centered expression intensities counts
normal_beads	vector of names of normal beads

**Value**

rm\_adj data.table of smoothed relative expression intensities

---

run_enrichr	<i>Subfunction to get significantly enriched GO terms given a set of significant beads and genes</i>
-------------	--

---

**Description**

This function finds the GO biological processes associated with the top n genes using enrichR

**Usage**

```
run_enrichr(genes, n_genes)
```

**Arguments**

genes	vector of differentially expressed genes
n_genes	number of the most significantly enriched DEGs to base gene enrichment from

**Value**

A data.table of the most significant GO terms and their meta data



---

run_slide_cna	<i>Run SlideCNA workflow</i>
---------------	------------------------------

---

## Description

Take a raw expression counts, cell type annotations, and positional coordinates to identify CNA patterns across space and CNA-based clustering patterns

## Usage

```
run_slide_cna(
  counts,
  beads_df,
  gene_pos,
  output_directory,
  plot_directory,
  spatial = TRUE,
  roll_mean_window = 101,
  avg_bead_per_bin = 12,
  pos = TRUE,
  pos_k = 55,
  ex_k = 1,
  hc_function_bin = "ward.D2",
  spatial_vars_to_plot = c("seurat_clusters", "bin_all", "N_bin", "umi_bin",
    "cluster_type"),
  scale_bin_thresh_hard = TRUE,
  lower_bound_cnv = 0.6,
  upper_bound_cnv = 1.4,
  hc_function_cnv = "ward.D2",
  hc_function_cnv_heatmap = "ward.D2",
  quantile_plot_cluster_label = "seurat_clusters",
  hc_function_silhouette = "ward.D2",
  max_k_silhouette = 10,
  plot_silhouette = TRUE,
  hc_function_plot_clones = "ward.D2",
  use_GO_terms = TRUE,
  chrom_ord = c("chr1", "chr2", "chr3", "chr4", "chr5", "chr6", "chr7", "chr8", "chr9",
    "chr10", "chr11", "chr12", "chr13", "chr14", "chr15", "chr16", "chr17", "chr18",
    "chr19", "chr20", "chr21", "chr22", "chr23", "chrX", "chrY", "chrM"),
  chrom_colors = c(chr1 = "#8DD3C7", chr2 = "#FFFFB3", chr3 = "#BEBADA", chr4 =
    "#FB8072", chr5 = "#80B1D3", chr6 = "#FDB462", chr7 = "#B3DE69", chr8 = "#FCCDE5",
    chr9 = "#D9D9D9", chr10 = "#BC80BD", chr11 = "#CCEBC5", chr12 = "#FFED6F", chr13 =
    "#1B9E77", chr14 = "#D95F02", chr15 = "#7570B3", chr16 = "#E7298A", chr17 =
    "#66A61E", chr18 = "#E6AB02", chr19 = "#A6761D", chr20 = "#666666", chr21 =
    "#A6CEE3", chr22 = "#1F78B4", chrX = "#B2DF8A"),
  text_size = 16,
  title_size = 18,
```

```

    legend_size_pt = 4,
    legend_height_bar = 1.5
)

```

### Arguments

counts	data.frame of raw counts (genes x beads)
beads_df	data.frame of annotation of each bead (beads x annotations); contains columns 'bc' for bead names, 'cluster_type' for annotations of 'Normal' or 'Malignant', 'pos_x' for x-coordinate bead positions, and 'pos_y' for y-coordinate bead positions
gene_pos	data.frame with columns for GENE, chr, start, end, rel_gene_pos (1 : # of genes on chromosome)
output_directory	output directory path
plot_directory	output plot directory path
spatial	TRUE if using spatial information FALSE if not
roll_mean_window	integer number of adjacent genes for which to average over in pyramidal weighting scheme
avg_bead_per_bin	integer of average number of beads there should be per bin
pos	TRUE if doing spatial and expressional binning, FALSE if just expressional binning
pos_k	positional weight
ex_k	expressional weight
hc_function_bin	hierarchical clustering function for binning; to feed hclust's method argument, one of "ward.D", "ward.D2", "single", "complete", "average", "mcquitty", "median" or "centroid"
spatial_vars_to_plot	character vector of features to plot/columns of metadata
scale_bin_thresh_hard	TRUE if using strict thresholds for expression thresholds and FALSE if adjusting thresholds based on 1 + or - the mean of absolute min and max vlaues
lower_bound_cnv	numeric float to represent the lower cap for CNV scores
upper_bound_cnv	numeric float to represent the upper cap for CNV scores
hc_function_cnv	character for which hierarchical clustering function to use for CNV-calling; to feed hclust's method argument, one of "ward.D", "ward.D2", "single", "complete", "average", "mcquitty", "median" or "centroid"

hc_function_cnv_heatmap	character for which hierarchical clustering function to use for visualizing CNV heat map; to feed hclust's method argument, one of "ward.D", "ward.D2", "single", "complete", "average", "mcquitty", "median" or "centroid"
quantile_plot_cluster_label	character string of which column name to keep in quantile plot
hc_function_silhouette	character string for which hierarchical clustering function to use for the Silhouette method; to feed hclust's method argument, one of "ward.D", "ward.D2", "single", "complete", "average", "mcquitty", "median" or "centroid"
max_k_silhouette	integer of number max number of clusters to evaluate (2:max_k_silhouette) . in Silhouette method
plot_silhouette	TRUE if plotting silhouette scores for clustering
hc_function_plot_clones	character string for which hierarchical clustering function to use in plotting clones
use_GO_terms	TRUE if using enrichR to get Gene Ontology terms for SlideCNA-defined clusters
chrom_ord	character vector of order and names of chromosomes
chrom_colors	character vector of which colors each chromosome should be in heat map
text_size	integer of size of text in some ggplots
title_size	integer of size of title in some ggplots
legend_size_pt	integer of size of legend text size in some ggplots
legend_height_bar	integer of height of legend bar in some ggplots

**Value**

None

---

scalefit	<i>Subfunction for scale_nUMI that normalizes a given bin for UMI count and centers the mean CNV score at 1</i>
----------	---

---

**Description**

This function re-scales expression intensities to be in a smaller range, normalizes for nUMI per bin, and centers the CNV scores to have a mean of 1

**Usage**

```
scalefit(obj, nbin, start, end)
```

**Arguments**

obj	data.table of relative expression intensities per bin
nbin	nUMIs in that specific bin
start	lower bound of CNV scores
end	upper bound of CNV scores

**Value**

vector of adjusted CNV scores for that bins with nbin number of nUMIs within the range (inclusive) of start to end

---

scale_nUMI	<i>Scale for nUMI (UMI Count) to generate CNV scores</i>
------------	--

---

**Description**

This function re-scales expression intensities to be in a smaller range, normalizes for nUMI per bin, and subtracts reference bead signal

**Usage**

```
scale_nUMI(dat_bin, thresh_hard = FALSE)
```

**Arguments**

dat_bin	data.table of relative expression intensities per bin
thresh_hard	TRUE if using strict thresholds for expression thresholds and FALSE if adjusting thresholds based on 1 + or - the mean of absolute min and max values

**Value**

data.table of CNV scores per bin

---

SpatialPlot	<i>Spatial plots of meta data</i>
-------------	-----------------------------------

---

**Description**

This function will plot information about beads and bins on x and y coordinates

**Usage**

```
SpatialPlot(
  dat_long,
  vars = NULL,
  text_size,
  title_size,
  legend_size_pt,
  legend_height_bar,
  plot_directory
)
```

**Arguments**

dat_long	data.table of bead expression intensities per gene with metadata in long format
vars	character vector of features to plot/columns of metadata
text_size	Ggplot2 text size
title_size	Ggplot2 title size
legend_size_pt	Ggplot2 legend_size_pt
legend_height_bar	Ggplot2 legend_height_bar
plot_directory	output plot directory path

**Value**

None

---

weight_rollmean	<i>Expressional smoothing along a chromosome using a weighted pyramidal moving average</i>
-----------------	--

---

**Description**

Take in a data.table of genomic positions and bead normalized/modified counts and apply pyramidal weighting with a window size k to create smoothed expression intensities

**Usage**

```
weight_rollmean(dat, k = 101)
```

**Arguments**

dat	data.table of normalized/adjusted counts
k	size of window for weighting

**Value**

A data.table of expression intensities

---

weight\_rollmean\_sub    *Subfunction of weight\_rollmean*

---

**Description**

Take in a counts matrix and apply pyramidal weighting with a window size k to create smoothed expression intensities

**Usage**

```
weight_rollmean_sub(mat, k)
```

**Arguments**

mat	matrix of normalized/adjusted counts
k	size of window for weighting

**Value**

A matrix of smoothed counts

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