

# Package ‘seqCAT’

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**Title** High Throughput Sequencing Cell Authentication Toolkit

**Version** 1.29.0

**Description** The seqCAT package uses variant calling data (in the form of VCF files) from high throughput sequencing technologies to authenticate and validate the source, function and characteristics of biological samples used in scientific endeavours.

**Depends** R (>= 3.6), GenomicRanges (>= 1.26.4), VariantAnnotation(>= 1.20.3)

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---

calculate\_similarity *SNV profile similarity calculations*

---

### Description

Calculate the similarity statistics for SNV profile comparisons.

### Usage

```
calculate_similarity(data, similarity = NULL, a = 1, b = 5)
```

### Arguments

|            |                                         |
|------------|-----------------------------------------|
| data       | The input SNV data dataframe.           |
| similarity | Optional dataframe to add results to.   |
| a          | Similarity score parameter a (integer). |
| b          | Similarity score parameter b (integer). |

**Details**

This function calculates various summary statistics and sample similarities for a given profile comparison dataframe. It returns a small dataframe with the overall similarity score (whose parameters 'a' and 'b' can be adjusted in the function call), total SNV data, the concordance of the data and the sample names in question. This dataframe can also be given to the function, in which case it will simply add another row for the current samples, facilitating downstream aggregate analyses.

**Value**

A dataframe with summary statistics.

**Examples**

```
# Load test data
data(test_comparison)

# Calculate similarities
similarity <- calculate_similarity(test_comparison)

# Add another row of summary statistics
calculate_similarity(test_comparison, similarity = similarity)
```

---

 compare\_many

*Comparisons of many SNV profiles*


---

**Description**

Overlap and compare genotypes in many SNV profiles.

**Usage**

```
compare_many(many, one = NULL, a = 1, b = 5)
```

**Arguments**

|      |                                                       |
|------|-------------------------------------------------------|
| many | SNV profiles to be compared (list of dataframes).     |
| one  | SNV profile to be compared to all others (dataframe). |
| a    | Similarity score parameter a (integer).               |
| b    | Similarity score parameter b (integer).               |

**Details**

This is a function that compares all the combinations of the SNV profiles input to it, either in a one-to-many or many-to-many manner. It returns both a dataframe containing summary statistics for all unique combinations and a list of dataframes with all the performed comparisons, for easy re-use and downstream analyses of said comparisons.

**Value**

A list of summary statistics and comparisons.

**Examples**

```
# Load test data
data(test_profile_1)
data(test_profile_2)

# Perform many-to-many comparisons
profiles <- list(test_profile_1, test_profile_2)
comparisons <- compare_many(profiles)

# View aggregate similarities
## Not run: comparisons[[1]]

# View data of first comparison
## Not run: head(comparisons[[2]][[1]])
```

---

|                  |                                       |
|------------------|---------------------------------------|
| compare_profiles | <i>Binary SNV profile comparisons</i> |
|------------------|---------------------------------------|

---

**Description**

Overlap and compare genotypes in two SNV profiles.

**Usage**

```
compare_profiles(profile_1, profile_2, mode = "intersection")
```

**Arguments**

|           |                                                             |
|-----------|-------------------------------------------------------------|
| profile_1 | The first SNV profile (GRanges object).                     |
| profile_2 | The second SNV profile (GRanges object).                    |
| mode      | Merge profiles using "union" or "intersection" (character). |

**Details**

This is a function for finding overlapping variants in two different SNV profiles (stored as GenomicRanges objects), followed by comparing the genotypes of the overlapping variants. The "compare\_overlaps" function calls the "add\_metadata" function twice in succession in order to merge the metadata for the two profiles (supplied as GRanges objects), returns the results as a dataframe, compares the genotypes of the overlapping variants using the "compare\_genotypes" function and, finally, returns the final dataframe with all variant overlaps and their similarity.

**Value**

A dataframe.

## Examples

```
# Load test data
data(test_profile_1)
data(test_profile_2)

# Compare the two profiles
comparison <- compare_profiles(test_profile_1, test_profile_2)
```

---

|                |                             |
|----------------|-----------------------------|
| create_profile | <i>SNV profile creation</i> |
|----------------|-----------------------------|

---

## Description

Create an SNV profile from data in a VCF file.

## Usage

```
create_profile(vcf_file, sample, min_depth = 10, filter_vc = TRUE,
  filter_mt = TRUE, filter_ns = TRUE, filter_gd = TRUE,
  filter_pd = FALSE)
```

## Arguments

|           |                                                                        |
|-----------|------------------------------------------------------------------------|
| vcf_file  | The VCF file from which the profile will be created (path).            |
| sample    | The sample in the VCF for which a profile will be created (character). |
| min_depth | Filter variants below this sequencing depth (integer).                 |
| filter_vc | Filter variants failing variant caller criteria (boolean).             |
| filter_mt | Filter mitochondrial variants (boolean).                               |
| filter_ns | Filter non-standard chromosomes (boolean).                             |
| filter_gd | Filter duplicate variants at the gene-level (boolean).                 |
| filter_pd | Filter duplicate variants at the position-level (boolean).             |

## Details

This function creates a SNV profile from a given VCF file by extracting the variants that pass the filtering criterias. Profile creation is performed to facilitate and accelerate the cell authentication procedures, which is especially relevant when more than one pairwise comparison will be performed on the same sample.

## Value

A data frame.

**Examples**

```
# Path to the test VCF file
vcf_file = system.file("extdata", "test.vcf.gz", package = "seqCAT")

# Create SNV profiles
profile_1 <- create_profile(vcf_file, "sample1")
profile_1 <- create_profile(vcf_file, "sample1", min_depth = 15)
```

---

|                 |                             |
|-----------------|-----------------------------|
| create_profiles | <i>SNV profile creation</i> |
|-----------------|-----------------------------|

---

**Description**

Create SNV profiles from all VCF files in a directory

**Usage**

```
create_profiles(vcf_dir, min_depth = 10, filter_vc = TRUE,
  filter_mt = TRUE, filter_ns = TRUE, filter_gd = TRUE,
  filter_pd = FALSE, pattern = NULL, recursive = FALSE)
```

**Arguments**

|           |                                                                                       |
|-----------|---------------------------------------------------------------------------------------|
| vcf_dir   | The VCF directory from which the profiles will be created (path).                     |
| min_depth | Remove variants below this sequencing depth (integer).                                |
| filter_vc | Filter variants failing variant caller criteria (boolean).                            |
| filter_mt | Filter mitochondrial variants (boolean).                                              |
| filter_ns | Filter non-standard chromosomes (boolean).                                            |
| filter_gd | Filter duplicate variants at the gene-level (boolean).                                |
| filter_pd | Filter duplicate variants at the position-level (boolean).                            |
| pattern   | Only create profiles for a subset of files corresponding to this pattern (character). |
| recursive | Find VCF files recursively in sub-directories as well (boolean).                      |

**Details**

This functions is a convenience-wrapper for the ‘create\_profile’ function, which will create SNV profiles for each and every VCF file in the provided directory. The file naming scheme used is ‘<sample>.vcf’ and will dictate the each profile’s sample name.

**Value**

A list of data frames.

**Examples**

```
# Path to the test VCF directory
vcf_dir = system.file("extdata", package = "seqCAT")

# Create SNV profiles
profiles <- create_profiles(vcf_dir, pattern = "test", recursive = TRUE)
```

---

|                   |                               |
|-------------------|-------------------------------|
| filter_duplicates | <i>Variant de-duplication</i> |
|-------------------|-------------------------------|

---

**Description**

Filter duplicated variants.

**Usage**

```
filter_duplicates(data, filter_gd = TRUE, filter_pd = FALSE)
```

**Arguments**

|           |                                                            |
|-----------|------------------------------------------------------------|
| data      | The dataframe containing the variant data to be filtered.  |
| filter_gd | Filter duplicate variants at the gene-level (boolean).     |
| filter_pd | Filter duplicate variants at the position-level (boolean). |

**Details**

This is a function for filtering duplicated variants either on the gene-level or the position-level.

**Value**

A data frame containing the filtered variants.

**Examples**

```
# Load test comparisons
data(test_profile_1)

# Filter variants
filtered_gene <- filter_duplicates(test_profile_1)
filtered_position <- filter_duplicates(test_profile_1, filter_pd = TRUE)
```

---

|                 |                          |
|-----------------|--------------------------|
| filter_variants | <i>Variant filtering</i> |
|-----------------|--------------------------|

---

### Description

Filter variants on several criteria.

### Usage

```
filter_variants(data, min_depth = 10, filter_vc = FALSE,  
                filter_mt = FALSE, filter_ns = FALSE)
```

### Arguments

|           |                                                           |
|-----------|-----------------------------------------------------------|
| data      | The dataframe containing the variant data to be filtered. |
| min_depth | Threshold for variant depth (integer).                    |
| filter_vc | Filter variants not passing filtering criteria (boolean). |
| filter_mt | Filter mitochondrial variants (boolean).                  |
| filter_ns | Filter non-standard chromosomes (boolean).                |

### Details

This is a function for filtering SNV profiles on several criteria: sequencing depth, variant caller-specific filtering, mitochondrial variants and variants in non-standard chromosomes. Only filters by sequencing depth by default.

### Value

A data frame containing the filtered variants.

### Examples

```
# Load test comparisons  
data(test_profile_1)  
  
# Filter variants  
filtered <- filter_variants(test_profile_1, min_depth = 15)
```



---

|             |                                 |
|-------------|---------------------------------|
| list_cosmic | <i>List COSMIC sample names</i> |
|-------------|---------------------------------|

---

**Description**

List all available samples in the COSMIC database

**Usage**

```
list_cosmic(file_path)
```

**Arguments**

file\_path      The file containing COSMIC data (path).

**Details**

This function lists the available sample names in the provided COSMIC file (e.g. CosmicCLP\_MutantExport.tsv.gz), and takes about half the time it takes to read the full file with the read\_cosmic function, making it useful for just seeing if your particular sample is listed in COSMIC or not.

**Value**

A vector of sample names

**Examples**

```
file <- system.file("extdata",  
                    "subset_CosmicCLP_MutantExport.tsv.gz",  
                    package = "seqCAT")  
cosmic_samples <- list_cosmic(file)
```

---

|               |                            |
|---------------|----------------------------|
| list_variants | <i>List known variants</i> |
|---------------|----------------------------|

---

**Description**

List known variants present in SNV profiles

**Usage**

```
list_variants(profiles, known_variants)
```

**Arguments**

profiles      The SNV profiles to analyse (list)  
known\_variants      The known variants to look for (dataframe)

**Details**

This is a function for listing known variants present in SNV profiles. Input is a list of profiles and a dataframe of known variants, containing at least the genomic locations ("chr" and "pos"). Any additional columns will be retained.

**Value**

A dataframe containing the known variant genotypes in each profile.

**Examples**

```
# Load test data
data(test_profile_1)
data(test_profile_2)

# Create some variants to analyse
known_variants <- data.frame(chr = 1, pos = 16229, gene = "DDX11L1")

# List the known variants in each profile
profiles <- list(test_profile_1, test_profile_2)
known_variants <- list_variants(profiles, known_variants)
```

---

plot\_heatmap

*Plot similarity heatmap*


---

**Description**

Plot a heatmap of similarities from many-to-many SNV profile comparisons.

**Usage**

```
plot_heatmap(similarities, cluster = TRUE, annotate = TRUE,
  annotate_size = 9, legend = TRUE, legend_size = c(36, 8),
  limits = c(0, 50, 90, 100), text_size = 14, colour = "#1954A6")
```

**Arguments**

|               |                                                               |
|---------------|---------------------------------------------------------------|
| similarities  | The long-format dataframe containing the data.                |
| cluster       | Cluster the samples based on similarity (boolean).            |
| annotate      | Annotate each cell with the score (boolean).                  |
| annotate_size | Text size of the annotations (numeric).                       |
| legend        | Show a legend for the colour gradient (boolean).              |
| legend_size   | Height and width of the legend (vector of two integers).      |
| limits        | The limits for the colour gradient (vector of four integers). |
| text_size     | Text size for axes, labels and legend (numeric).              |
| colour        | The main colour to use for the gradient (character).          |

**Details**

This function creates publication-ready plots of heatmaps for many-to-many sample comparisons, taking a long-format dataframe containing the summary statistics of each comparison as input.

**Value**

A ggplot2 graphical object.

**Examples**

```
# Load test similarities
data(test_similarities)

# Plot a similarity heatmap
heatmap <- plot_heatmap(test_similarities)
```

---

|              |                                     |
|--------------|-------------------------------------|
| plot_impacts | <i>Plot SNV impact distribution</i> |
|--------------|-------------------------------------|

---

**Description**

Plot SNV impact distributions for a binary SNV profile comparison.

**Usage**

```
plot_impacts(comparison, legend = TRUE, annotate = TRUE,
  annotate_size = 9, text_size = 14, palette = c("#0D2D59",
  "#1954A6"))
```

**Arguments**

|               |                                                        |
|---------------|--------------------------------------------------------|
| comparison    | The SNV profile comparison to be plotted.              |
| legend        | Show the legend (boolean).                             |
| annotate      | Annotate each category (boolean).                      |
| annotate_size | Text size for annotations (numeric).                   |
| text_size     | Text size for axes, ticks and legend (numeric).        |
| palette       | Colour palette for filling of bars (character vector). |

**Details**

This function creates publication-ready plots of the impact distribution from a binary dataset comparison across the matched/mismatched SNVs.

**Value**

A ggplot2 graphical object.

## Examples

```
# Load test comparison data
data(test_comparison)

# Plot the impact distribution
impacts <- plot_impacts(test_comparison)
```

---

plot\_variant\_list      *Plot known variants list*

---

## Description

Plot a genotype grid from a list of known variants

## Usage

```
plot_variant_list(variant_list, legend = TRUE, legend_size = 22,
  text_size = 14, palette = c("#4e8ce4", "#a6c6f2", "#999999",
  "#cccccc"))
```

## Arguments

|              |                                                        |
|--------------|--------------------------------------------------------|
| variant_list | The data containing the variants (dataframe)           |
| legend       | Show a legend for the genotype colours (boolean)       |
| legend_size  | Size of the legend (numeric).                          |
| text_size    | Text size for axes and legend (numeric).               |
| palette      | Nucleotide colour palette (4-element character vector) |

## Details

This function creates publication-ready plots from lists of known variants, taking a dataframe containing all the genotypes (on "A1/A2" format) for each sample (columns) and variant (row names).

## Value

A ggplot2 graphical object.

## Examples

```
# Load test variant list
data(test_variant_list)

# Plot each variant's genotype per sample
genotype_grid <- plot_variant_list(test_variant_list)
```

---

|             |                         |
|-------------|-------------------------|
| read_cosmic | <i>Read COSMIC data</i> |
|-------------|-------------------------|

---

### Description

Read COSMIC sample-specific mutational data.

### Usage

```
read_cosmic(file_path, sample_name = NULL, primary_site = NULL)
```

### Arguments

|              |                                                     |
|--------------|-----------------------------------------------------|
| file_path    | The COSMIC data file path (path).                   |
| sample_name  | Subset the data on sample name (character).         |
| primary_site | Subset the data on primary tumour site (character). |

### Details

This function reads the COSMIC data files (e.g. "CosmicCLP\_MutantExport.tsv.gz") and returns a GRanges object with all the listed mutations for the specified sample, which can then be use in downstream profile comparisons. Only non-duplicated (gene-level) SNVs are included in COSMIC profiles.

### Value

A dataframe with COSMIC SNVs.

### Examples

```
# Path to COSMIC test data
file <- system.file("extdata",
                    "subset_CosmicCLP_MutantExport.tsv.gz",
                    package = "seqCAT")

# Read COSMIC test data for the HCT116 cell line
cosmic_hct116 <- read_cosmic(file, "HCT116")
```

---

|              |                         |
|--------------|-------------------------|
| read_profile | <i>Read SNV profile</i> |
|--------------|-------------------------|

---

**Description**

Read an SNV profile stored on disk.

**Usage**

```
read_profile(file, sample_name = NULL)
```

**Arguments**

|             |                                                                                     |
|-------------|-------------------------------------------------------------------------------------|
| file        | The SNV profile to be read (path).                                                  |
| sample_name | Sample name to be added; overrides profile sample if it already exists (character). |

**Details**

This is a function for reading SNV profiles created from VCF files that were stored on disk.

**Value**

A data frame.

**Examples**

```
# Path to test data
profile = system.file("extdata",
                      "test_1.profile.txt.gz",
                      package = "seqCAT")

# Read test profile
profile <- read_profile(profile)
```

---

|               |                          |
|---------------|--------------------------|
| read_profiles | <i>Read SNV profiles</i> |
|---------------|--------------------------|

---

**Description**

Read SNV profiles in a directory.

**Usage**

```
read_profiles(profile_dir, pattern = ".profile.txt",
              sample_names = FALSE)
```

**Arguments**

|              |                                                                                              |
|--------------|----------------------------------------------------------------------------------------------|
| profile_dir  | The directory containing the profiles to be read (path).                                     |
| pattern      | Pattern for file name or extension to be read (character).                                   |
| sample_names | Add sample name based on file name; overrides profile sample if it already exists (boolean). |

**Details**

This is a wrapper function for reading multiple SNV profiles present in a directory (and its sub-directories in recursive mode).

**Value**

A list of data frames.

**Examples**

```
# Path to test data
profile_dir = system.file("extdata", package = "seqCAT")

# Read test profiles
profile_list <- read_profiles(profile_dir, pattern = "profile.txt")
```

---

seqCAT

*seqCAT: High Throughput Sequencing Cell Authentication Toolkit*


---

**Description**

The *\*seqCAT\** package provides a number of functions for performing evaluation, characterisation and authentication of biological samples through analysis of high throughput sequencing data.

---

test\_comparison

*Overlapping and compared SNVs*


---

**Description**

Overlapping and compared variants from "sample1" and "sample2" originating from the example.vcf file included in the inst/extdata directory, for use in unit tests.

**Usage**

```
data(test_comparison)
```

**Format**

A dataframe with 51 rows and 39 columns:

**chr** chromosome

**pos** SNV position

**DP.sample\_1** total variant depth, sample 1

**AD1.sample\_1** allelic depth, allele 1, sample 1

**AD2.sample\_1** allelic depth, allele 2, sample 1

**A1.sample\_1** allele 1, sample 1

**A2.sample\_1** allele 2, sample 1

**warnings.sample\_1** warnings from variant calling, sample 1

**DP.sample\_2** total variant depth, sample 2

**AD1.sample\_2** allelic depth, allele 1, sample 2

**AD2.sample\_2** allelic depth, allele 2, sample 2

**A1.sample\_2** allele 1, sample 2

**A2.sample\_2** allele 2, sample 2

**warnings.sample\_2** warnings from variant calling, sample 2

**sample\_1** name, sample 1

**sample\_2** name, sample 2

**match** status of genotype comparison

**rsID** mutation ID

**gene** associated gene

**ENSGID** ensembl gene ID

**ENSTID** ensembl transcript ID

**REF** reference allele

**ALT** alternative allele

**impact** putative variant impact

**effect** variant effect

**feature** transcript feature

**biotype** transcript biotype



---

|                |                      |
|----------------|----------------------|
| test_profile_1 | <i>SNV profile 1</i> |
|----------------|----------------------|

---

### Description

SNV profile in GRanges format from "sample1", originating from the test\_profile\_1.txt in the inst/extdata directory, for use in unit tests.

### Usage

```
data(test_profile_1)
```

### Format

A GRanges object with 383 elements and 17 metadata columns:

**rsID** mutation ID, if available  
**gene** associated gene  
**ENSGID** ensembl gene ID  
**ENSTID** ensembl transcript ID  
**REF** reference allele  
**ALT** alternative allele  
**impact** putative variant impact  
**effect** variant effect  
**feature** transcript feature  
**biotype** transcript biotype  
**DP** total variant depth  
**AD1** allelic depth, allele 1  
**AD2** allelic depth, allele 2  
**A1** allele 1  
**A2** allele 2  
**warnings** warnings from variant calling  
**sample** sample name

---

|                |                      |
|----------------|----------------------|
| test_profile_2 | <i>SNV profile 2</i> |
|----------------|----------------------|

---

**Description**

SNV profile in GRanges format from "sample2", originating from the test\_profile\_2.txt in the inst/extdata directory, for use in unit tests.

**Usage**

```
data(test_profile_2)
```

**Format**

A GRanges object with 382 elements and 17 metadata columns:

**rsID** mutation ID, if available

**gene** associated gene

**ENSGID** ensembl gene ID

**ENSTID** ensembl transcript ID

**REF** reference allele

**ALT** alternative allele

**impact** putative variant impact

**effect** variant effect

**feature** transcript feature

**biotype** transcript biotype

**DP** total variant depth

**AD1** allelic depth, allele 1

**AD2** allelic depth, allele 2

**A1** allele 1

**A2** allele 2

**warnings** warnings from variant calling

**sample** sample name

---

|                |                      |
|----------------|----------------------|
| test_profile_3 | <i>SNV profile 3</i> |
|----------------|----------------------|

---

**Description**

SNV profile in GRanges format from "sample3", originating from the test\_profile\_3.txt in the inst/extdata directory, for use in unit tests.

**Usage**

```
data(test_profile_3)
```

**Format**

A GRanges object with 99 elements and 9 metadata columns:

**rsID** mutation ID, if available

**REF** reference allele

**ALT** alternative allele

**DP** total variant depth

**AD1** allelic depth, allele 1

**AD2** allelic depth, allele 2

**A1** allele 1

**A2** allele 2

**sample** sample name

---

|                   |                                     |
|-------------------|-------------------------------------|
| test_similarities | <i>Collated similarities object</i> |
|-------------------|-------------------------------------|

---

**Description**

Collated similarities of multiple sample comparisons from "sample1" and "sample" from the example.vcf file, for use in unit tests.

**Usage**

```
data(test_similarities)
```

**Format**

A dataframe with 3 rows and 6 columns:

**sample\_1** name of sample 1

**sample\_2** name of sample 2

**overlaps** the number of overlaps for the comparison

**matches** the number of matches for the comparison

**concordance** the concordance of the profiles

**similarity\_score** the similarity score of the profiles

---

|                   |                                     |
|-------------------|-------------------------------------|
| test_variant_list | <i>Modified variant list object</i> |
|-------------------|-------------------------------------|

---

**Description**

A variant list object from the 'list\_variants' function, where the row names have been defined as "chr: pos (gene)" and the corresponding columns removed, for use in plotting.

**Usage**

```
data(test_variant_list)
```

**Format**

A dataframe with 2 rows and 2 columns:

**sample1** the genotypes of sample1

**sample2** the genotypes of sample2

---

|               |                          |
|---------------|--------------------------|
| write_profile | <i>Write SNV profile</i> |
|---------------|--------------------------|

---

**Description**

Write an SNV profile to a file for later re-use.

**Usage**

```
write_profile(profile, file)
```

**Arguments**

profile            The SNV profile to be written (data frame).

file                The file to write to (path).

**Details**

This is a function for writing SNV profiles (created from VCF files) to disk for later re-use. Several formats are allowed, including BED, GTF, GFF and normal text files, which are automatically recognised based on the supplied filename.

**Value**

None; writes to disk only.

**Examples**

```
# Load test profile
data(test_profile_1)

# Write test profile to file
write_profile(test_profile_1, "test_profile_1.txt")
```

---

|                |                           |
|----------------|---------------------------|
| write_profiles | <i>Write SNV profiles</i> |
|----------------|---------------------------|

---

**Description**

Write several SNV profiles to file for later re-use.

**Usage**

```
write_profiles(profile_list, format = "TXT", directory = "./")
```

**Arguments**

|              |                                        |
|--------------|----------------------------------------|
| profile_list | The SNV profiles to be written (list). |
| format       | The desired file format (character).   |
| directory    | The directory to write to (path).      |

**Details**

This is a wrapper function for writing multiple SNV profiles present in a directory (and its sub-directories in recursive mode).

**Value**

None; writes to disk only.

**Examples**

```
# Load test profiles
data(test_profile_1)
data(test_profile_2)
profiles <- list(test_profile_1, test_profile_2)

# Write test profile to file
write_profiles(profiles, format = "TXT", directory = "./")
```

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