StructuralVariantUtil

Table of Contents

Introduction

StructuralVariantUtil is a R package providing utility functions for structural variant (SV) analyses, including estimating SV calling performance, parsing SV VCF, integrating results from different SV callers and cohort-level spectrum analysis. This user guide details how to install and use *StructuralVariantUtil*.

Installation

Download the latest release of *StructuralVariantUtil* by running the following command in R:

```
# Download StructuralVariantUtil
devtools::install_github("tgong1/StructuralVariantUtil")
# Load the package
library(StructuralVariantUtil)
```

Third Party Software

Some functions of *StructuralVariantUtil* require *Shiny-SoSV* [1] and *bedtools* [2].

Download the latest release of *Shiny-SoSV* by cloning the repository:

```
git clone https://github.com/tgong1/Shiny-SoSV.git
```

Download and install bedtools (https://bedtools.readthedocs.io/en/latest/content/installation.html). Here shows one way to download from bedtools GitHub:

```
wget https://github.com/arq5x/bedtools2/releases/download/vx.xx.x/bedtools-
x.xx.x.tar.gz
tar -zxvf bedtools-x.xx.x.tar.gz
cd bedtools2
make
```

Main Utilities

SV calling performance estimation

StructuralVariantUtil estimates somatic SV calling performance including precision, sensitivity and F1 score, from whole genome sequencing data, based on prediction models developed in *ShinySoSV* [1]. Input parameters required include any candidate caller(s) (*Manta*, *Lumpy*, *GRIDSS*, *Delly*, *SvABA*) and variables impacting somatic SV calling, including variant

allele frequency (VAF), normal coverage, tumour coverage and tolerance of breakpoint precision.

We require the following input parameters from your study to be stored in a data.frame.

- sampleID (character): unique identifier of sample(s). The following example demonstrates the SV calling performance prediction for a cohort of 100 samples.
- VAF (numeric): variant allele frequency or estimated tumour purity
- N_coverage (numeric): depth of coverage of normal sample
- T_coverage (numeric): depth of coverage of tumour sample
- BND_threshold (numeric): tolerance of breakpoint precision. The predicted sensitivity and precision will be higher if you have higher tolerance of breakpoint precision, i.e. higher *BND_threshold.*

Other input parameters required include performance measurement(s) to estimate (*sensitivity*, *precision*, *F1_score*) for any call set(s) (*individual*, *union*, *intersection*). We demonstrate SV calling performance estimation here using a simulated data set as input, stored in data.frame *newdata*. Then we predict F1 score of five SV callers' calling performance based on the input variables.

```
set.seed(1)
newdata <- data.frame(sampleID = paste0("sample_",c(1:100)), VAF =
round(rnorm(100, mean=0.5, sd=0.1),digits = 2), N_coverage = round(rnorm(100,
mean=30, sd=10),digits = 2), T_coverage = round(rnorm(100, mean=60,
sd=10),digits = 2), BND_threshold = 100)
head(newdata)
  sampleID  VAF N_coverage T_coverage BND_threshold
1 sample_1 0.44      23.80      64.09           100
2 sample_2 0.52      30.42      76.89           100
3 sample_3 0.42      20.89      75.87           100
4 sample_4 0.66      31.58      56.69           100
5 sample_5 0.53      23.45      37.15           100
6 sample_6 0.42      47.67      84.98           100
performance <- "F1_score"
callset <- "individual"
candidate_callers <- c("Manta","Lumpy","GRIDSS","Delly","SvABA")
```

Once all input data has been loaded, we proceed to run the function *ShinySoSV_prediction*, which outputs data.frame with value of predicted performance (e.g. F1 score in this example) for all selected caller(s) and/or their pairwise union and intersection sets. Please note the *Shiny-SoSV* tool must be downloaded in the same directory when running *ShinySoSV_prediction* in R.

```
df_prediction <- ShinySoSV_prediction(Candidate_callers, newdata, performance,
callset)
head(df_prediction)
  sampleID  VAF N_coverage T_coverage BND_threshold fit_F1_score_Manta
fit_F1_score_Lumpy fit_F1_score_GRIDSS
1 sample_1 0.44      23.80      64.09           100          0.9019996
0.8416341           0.8575614
2 sample_2 0.52      30.42      76.89           100          0.8850921
0.8359865           0.8291313
3 sample_3 0.42      20.89      75.87           100          0.9056688
0.8452554           0.8640465
4 sample_4 0.66      31.58      56.69           100          0.8953312
0.8383520           0.8328662
5 sample_5 0.53      23.45      37.15           100          0.8712206
0.8140931           0.8192697
6 sample_6 0.42      47.67      84.98           100          0.9185124
0.8469072           0.8561267
  fit_F1_score_Delly fit_F1_score_SvABA
1          0.7229912          0.7674706
2          0.7320435          0.7206198
3          0.7244832          0.7801245
4          0.7614952          0.7334944
5          0.7138015          0.7284613
6          0.7279539          0.7773021
```

Convert VCF format to a R data frame

The function *vcf_to_dataframe* can be used to convert SVs in VCF format to a R data frame. This function requires input as the file path to a VCF file.

The following example shows the use of function *vcf_to_dataframe* on VCF output from Manta (v1.4.0) ran on a pair of simulated tumour and normal BAM [1]. This function is expected to work for VCF format v4.1 or above, and has been tested on VCF output files from Manta, GRIDSS, Lumpy, Delly and SvABA.

This function outputs a data.frame with the following variables:

- CHROM: chromosome of the first breakpoint; CHROM field in VCF
- POS: genomic location of the first breakpoint; POS field in VCF
- ID_caller: ID field in VCF
- REF: reference allele; REF field in VCF
- ALT: alternate allele; ALT field in VCF
- QUAL: quality score; QUAL field in VCF
- FILTER: FILTER field in VCF

- INFO_END: END in INFO field in VCF
- INFO_SVTYPE: SVTYPE in INFO field in VCF
- INFO_SVLEN: SVLEN in INFO field
- INFO_STRANDS: STRANDS in INFO field
- INFO_CT: CT in INFO field
- INFO_INV5: INV5 in INFO field
- INFO_INV3: INV5 in INFO field
- INFO_MATEID_caller: MATEID in INFO field in VCF reported by caller

All other fields in the VCF file are ignored.

```
vcf_file <- system.file("extdata", "manta_sample1.vcf",package =
"StructuralVariantUtil")

df <- vcf_to_dataframe(vcf_file = vcf_file)
head(df)
CHROM       POS                 ID_caller REF
1  chr1  55008308    MantaINS:469:0:0:0:0:0   C
2  chr1  61988078    MantaINV:533:0:0:1:1:0   G
3  chr2  68529703   MantaINS:2496:0:0:0:0:0   C
4  chr2 119653368 MantaBND:2900:0:1:0:0:0:1   A
5  chr2 199398206   MantaINV:3544:0:0:1:3:0   A
6  chr2 212506915 MantaBND:1725:0:1:0:0:0:0   C

ALT QUAL FILTER
1
CATGGGGCAGGATGGCCATATTGGCCGGGGTGATGTGGAGGGCTTCCTAGAGGAACAGACATTGGAGCCGAGGCCTGAGG
TCAAGTTTATAACTTTCCTCT   NA   PASS
2
<INV>   NA   PASS
3
CTTTCTTATCAACTCCAAACTTACAGGGTGAAGTTAGCCATCTCTTTCAGT   NA   PASS
4
A[CHR3:58161076[   NA   PASS
5
<INV>   NA   PASS
6
C[CHR1:224467224[   NA   PASS
   INFO_END INFO_SVTYPE INFO_SVLEN INFO_STRANDS INFO_CT INFO_INV5 INFO_INV3
INFO_MATEID_caller
1  55008308         INS        100           NA      NA     FALSE     FALSE
<NA>
2  61988179         INV        101           NA      NA      TRUE     FALSE
<NA>
3  68529703         INS         50           NA      NA     FALSE     FALSE
<NA>
4        NA         BND         NA           NA      NA     FALSE     FALSE
MantaBND:2900:0:1:0:0:0:0
5 199398307         INV        101           NA      NA      TRUE     FALSE
<NA>
6        NA         BND         NA           NA      NA     FALSE     FALSE
MantaBND:1725:0:1:0:0:0:1
```

Simple SV type classification

The function *simple_SVTYPE_classification* converts SVs in VCF format into pairs of BNDs at each fusion junction, and classifies them into one of five simple SV types, including deletion (DEL), duplication (DUP), insertion (INS), inversion (INV) and inter-chromosomal translocation (TRA).

The only required input parameter to this function is either the file path to a VCF file or SV VCF loaded as a R data frame. For the latter, the data frame must have at least the following variables (see section ***Convert VCF format to data.frame in R***):

- CHROM: chromosome of the first breakpoint; CHROM field in VCF
- POS: genomic location of the first breakpoint; POS field in VCF
- ALT: alternate allele; ALT field in VCF reported by caller following the format described in VCF specification (Version 4.1 above) section 5.4

This function is expected to work for VCF format v4.1 or above, and has been tested on VCF output files from Manta, GRIDSS, Lumpy, Delly and SvABA.

Here, we demonstrated the use of this function using a VCF output file from Manta (v1.4.0).

```
vcf_file <- system.file("extdata", "manta_sample1.vcf",package =
"StructuralVariantUtil")
bedpe <- simple_SVTYPE_classification(SV_data = vcf_file, caller_name="manta")
head(bedpe)
  chrom1    start1      end1 chrom2    start2      end2 SVTYPE strand1 strand2
ID     ID_mate
1   chr1  55008307  55008308   chr1  55008307  55008308    INS    <NA>    <NA>
manta_1_1_1 manta_1_2_1
2   chr1  61988077  61988078   chr1  61988178  61988179    INV       -       -
manta_2_1_2 manta_2_2_2
3   chr2  68529702  68529703   chr2  68529702  68529703    INS    <NA>    <NA>
manta_3_1_3 manta_3_2_3
4   chr2 119653367 119653368   chr3  58161075  58161076    TRA       +       -
manta_4_1_4 manta_4_2_4
5   chr2 199398205 199398206   chr2 199398306 199398307    INV       -       -
manta_5_1_5 manta_5_2_5
6   chr2 212506914 212506915   chr1 224467223 224467224    TRA       +       -
manta_6_1_6 manta_6_2_6

ALT                 ID_caller REF QUAL
1
CATGGGGCAGGATGGCCATATTGGCCGGGGTGATGTGGAGGGCTTCCTAGAGGAACAGACATTGGAGCCGAGGCCTGAGG
TCAAGTTTATAACTTTCCTCT    MantaINS:469:0:0:0:0:0   C   NA
```

```
2
<INV>    MantaINV:533:0:0:1:1:0   G   NA
3
CTTTCTTATCAACTCCAAACTTACAGGGTGAAGTTAGCCATCTCTTTCAGT   MantaINS:2496:0:0:0:0:0
C   NA
4
A[CHR3:58161076[ MantaBND:2900:0:1:0:0:0:1   A   NA
5
<INV>   MantaINV:3544:0:0:1:3:0   A   NA
6
C[CHR1:224467224[ MantaBND:1725:0:1:0:0:0:0   C   NA
  FILTER INFO_SVTYPE INFO_SVLEN INFO_STRANDS INFO_CT INFO_INV5 INFO_INV3
INFO_MATEID_caller
1   PASS         INS        100           NA      NA     FALSE     FALSE
<NA>
2   PASS         INV        101           NA      NA      TRUE     FALSE
<NA>
3   PASS         INS         50           NA      NA     FALSE     FALSE
<NA>
4   PASS         BND         NA           NA      NA     FALSE     FALSE
MantaBND:2900:0:1:0:0:0:0
5   PASS         INV        101           NA      NA      TRUE     FALSE
<NA>
6   PASS         BND         NA           NA      NA     FALSE     FALSE
MantaBND:1725:0:1:0:0:0:1
```

The output SV calls are in BEDPE format with variables as described in **Table 1** for each SV event. Additional variables (except CHROM and POS) in the input data frame are appended to the output unchanged. SVTYPE in the output is identical to SVTYPE in INFO field of the input VCF if the values were already DEL, DUP, INS, INV and TRA. If input SVTYPE is BND, simple SV types are derived from ALT filed in VCF (**Table 2**). If the ALT field of the input VCF does not follow BND format but contain an inserted sequence or has the <INS> designation, INS is assigned as the simple SV type.

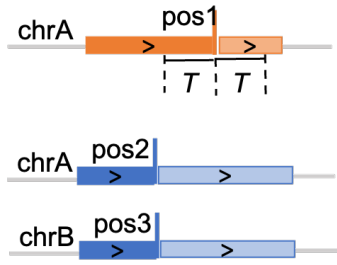
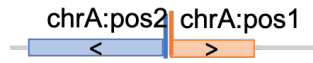
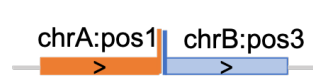
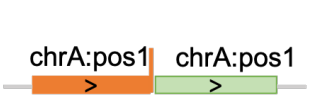
Table 1. The BEDPE format of SV fusion junctions

Variables	VCF fields[1]	Note
chrom1	#CHROM	The name of the chromosome of first breakend
start1	POS	The zero-based starting position of first breakend on chrom1
end1	POS	The one-based ending position of first breakend on chrom1
chrom2	#CHROM or ALT	chromosome of second breakend

start2	ALT or INFO: END	The zero-based starting position of second breakend on chrom2
end2	ALT or INFO: END	The one-based starting position of second breakend on chrom2
Strand1	INFO: STRANDS (e.g. Lumpy); CT=3to3 or 5to5 (e.g. Delly); INV5 or INV3 (e.g. Manta) or ALT[2]	strand of first breakend
Strand2		strand of second breakend
SVTYPE	INFO: SVTYPE or ALT[2]	Simple SV types, based on strands
ID[3]	NA	Unique identifier of first breakend
ID_mate[3]	NA	Unique identifier of second breakend

[1]Information source from input VCF.
[2]see Table 2.
3Unique identifiers assigned by *StructuralVariantUtil*.

Table 2. Strands and SV type derivation in reference to ALT field in VCF

Reference	sample	ALT field in VCF	Simple SV type identified	Strands identified
		t[p[	DEL	+-
		]p]t	INS	-+
		t]p]	INV	++
		[p[t		--
		Any ALT	Inter-chromosomal TRA	Any strands
			INS	

The output data frame of *simple_SVTYPE_classification* contains all required information for presenting the SVs in a CIRCOS plot using the *circlize* R package (demonstrated below, **Figure 1**). The function *prepare_SV_for_circos* can be used to prepare the output from simple_SVTYPE_classification for generating CIRCOS plots with R/circlize.

Here we demonstrate the CIRCOS plot drawing for this example SV call set (**Figure 1**).

```
SVdata_for_circos <- prepare_SV_for_circos(bedpe)

SVColours <- c(RColorBrewer::brewer.pal(n = 5, name = 'Set1'))
names(SVColours) <- c("DEL", "DUP", "INS", "INV", "TRA")

circlize::circos.initializeWithIdeogram(species = "hg38", plotType =
c("ideogram", "labels"))
circlize::circos.genomicLink(SVdata_for_circos[[1]], SVdata_for_circos[[2]],
                             col =
SVColours[match(SVdata_for_circos[[1]]$SVTYPE, names(SVColours))],h=0.2)
circlize::circos.genomicLink(SVdata_for_circos[[3]],SVdata_for_circos[[4]],
                             col =
SVColours[match(SVdata_for_circos[[3]]$SVTYPE, names(SVColours))])
title(main = "sample_1")

lgd_links = ComplexHeatmap::Legend(at = c("DEL", "DUP", "INS", "INV", "TRA"),
legend_gp = grid::gpar(fill=SVColours), type ="grid",by_row = TRUE,ncol=5,
                                   title_position = "topleft", title = "SV")
ComplexHeatmap::draw(lgd_links,
                     y = ggplot2::unit(ComplexHeatmap:::height(lgd_links),
"mm"))
circlize::circos.clear()
```

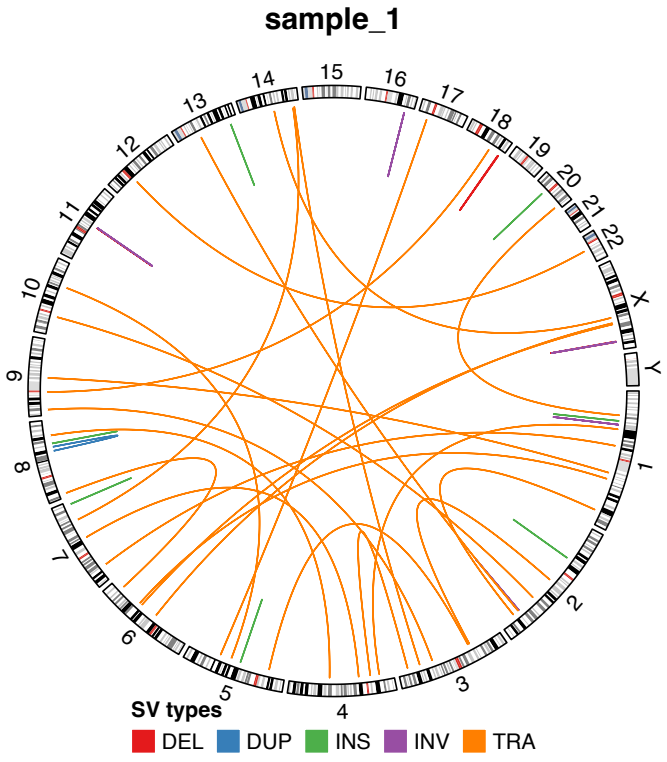


Figure 1. CIRCOS plot of the example SV call set

SV caller Integration filtration

Function *SV_integration* allows users to integrate SV call sets, provided in VCF format (e.g. results from different SV callers). The function does this by first standardising BND pairs in simple SV types then merging SVs based on BND positions. The function outputs integrated SV calls as a R data frame in bedpe format.

Input parameters required to run *SV_integration* include:

- vcf_files: list of VCF files in the same order as SVCaller_name
- SVCaller_names: vector of names of SV callers or unique identifier of the VCFs
- sampleID: unique identifier for the sample. Default as “sample_1”.
- bkpt_T_callers: threshold of breakpoint position difference between two calls to be concordant. Default is 100bp.
- PASS_filter: filtering based on FILTER field of two calls in VCF: “both” to require both two calls with “PASS”, “one” to require one of the two calls with “PASS”, “none” to ignore this filtering. Default is “both”.
- SVTYPE_ignore: whether to consider same SV type for SV concordance. TRUE or FALSE.
- bedtools_dir: path of bedtools. Specify your path here or add its path to system PATH.

```
# two example VCFs from two different SV callers (Manta & GRIDSS)
vcf_files<- c(system.file("extdata",
"manta_SVEngine_TumorSV2.60x_NormalSV1.60x_0.5.T.PASS.recode.vcf", package =
"StructuralVariantUtil"),
              system.file("extdata",
"GRIDSS_SVEngine_TumorSV2.60x_NormalSV1.60x_0.5_somatic_PASS_annotated.vcf",
package = "StructuralVariantUtil"))

# optional sample name
sampleID <- "sample_1"

# mandatory character vector of SV call names
SVCaller_names <- c("manta","gridss")

# SV integration
integrated_bedpe <- SV_integration(vcf_files, SVCaller_names, sampleID)
head(integrated_bedpe)
  chrom1  start1    end1 chrom2   start2     end2 SVTYPE strand1 strand2
ID   ID_mate
1   chr1 1615683 1615684   chr1  1615683  1615684    INS    <NA>    <NA>
all_1_1_1 all_1_2_1
```

```
2   chr1 1615683 1615684  chr19 16196780 16196781    TRA       +       -
all_2_1_2 all_2_2_2
4   chr1 6170587 6170588  chr22 23815023 23815024    TRA       -       +
all_4_1_4 all_4_2_4
5   chr1 6170587 6170588   chr1  6170687  6170688    DEL       +       -
all_5_1_5 all_5_2_5
7   chr1 7572789 7572790   chr1  8572789  8572790    DEL       +       -
all_7_1_7 all_7_2_7
8   chr1 8065641 8065642  chr16 62560615 62560616    TRA       +       -
all_8_1_8 all_8_2_8

ALT               ID_caller
1
TAAAATTAGCTAGGTGTGGTGGCACATGCCTGTAATCCCAGCCACTTGAGAGGCTGACACACAAGAGAATCACTTGAACC
CAGGAGGCAGAGGTTGCAGTG    MantaINS:4:0:0:0:0:0
2
T[CHR19:16196781[  MantaBND:4:0:1:0:0:0:1
4
]CHR22:23815024]T MantaBND:30:0:1:0:0:0:1
5
T   MantaDEL:30:0:0:0:1:0
7
<DEL>   MantaDEL:41:0:1:0:0:0
8
A[CHR16:62560616[ MantaBND:45:0:1:0:0:0:0

REF QUAL FILTER INFO_SVTYPE INFO_SVLEN INFO_STRANDS
1
T   NA   PASS         INS        100           NA
2
T   NA   PASS         BND         NA           NA
4
T   NA   PASS         BND         NA           NA
5
TGCTTGGGGCTCCCACACAGGGAGGGCACCCTGTGGAGGGCTAGGGCACACAGGGGAGCCAGCAGCAAGGGCCGCCCCAG
GTGGGTTTATGTGGGTGAGGC   NA   PASS         DEL       -100           NA
7
A   NA   PASS         DEL   -1000000           NA
8
A   NA   PASS         BND         NA           NA
  INFO_CT INFO_INV5 INFO_INV3      INFO_MATEID_caller                manta_ID
gridss_ID
1      NA     FALSE     FALSE                    <NA>             manta_1_1_1
<NA>
2      NA     FALSE     FALSE  MantaBND:4:0:1:0:0:0:0 manta_2_1_2,manta_3_1_3
gridss_1_1_1,gridss_2_1_2
4      NA     FALSE     FALSE MantaBND:30:0:1:0:0:0:0 manta_4_1_4,manta_6_1_6
gridss_4_1_4,gridss_5_1_5
5      NA     FALSE     FALSE                    <NA>             manta_5_1_5
gridss_3_1_3
7      NA     FALSE     FALSE                    <NA>             manta_7_1_7
gridss_6_1_6
8      NA     FALSE     FALSE MantaBND:45:0:1:0:0:0:1             manta_8_1_8
gridss_7_1_7
```

Integrated SV output from *SV_integration* can be used as input to the R/ VennDiagram package to generate a Venn diagram of SV calls from the input SV callsets, as shown in **Figure 2**.

```
union_ID <- integrated_bedpe[, (ncol(integrated_bedpe)-
length(SVCaller_names)+1):ncol(integrated_bedpe)]
for(i in c(1: length(SVCaller_names))){
  index <- rowSums(!is.na(union_ID)) != 1 & (!is.na(union_ID[,i]))
  union_ID[index,i] <- integrated_bedpe[index,]$ID
  assign(SVCaller_names[i], union_ID[,i][!is.na(union_ID[,i])])
}
x <- do.call("list", lapply(SVCaller_name,function(s) eval(parse(text=s))))
g <- VennDiagram::venn.diagram(
  x = x,
  category.names = SVCaller_name,
  # Circles
  lwd = 2,
  fill = RColorBrewer::brewer.pal(3, "Pastel2")[1:length(SVCaller_name)],

  # Numbers
  cex = 1,
  fontface = "bold",
  filename = NULL,
  output=FALSE
)
grid::grid.newpage()
grid::grid.draw(g)
```

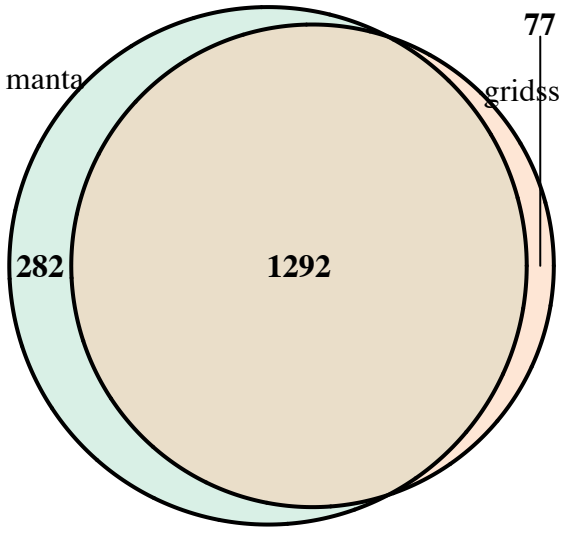


Figure 2. Venn diagram of the integrated SV call set

Spectrum of SV types for large cohort

The function *spectrum_SV_type* can be used to summarize the spectrum of SV types at a cohort level using function *spectrum_SV_type*. For a large cancer cohort (recommended n > 30), the function can also identify hyper-SV mutated tumour samples.

Input parameters required for *spectrum_SV_type* include a character vector of sample ID and a list of SV data, which can be a list of VCF file paths or a list of data frames of SV callsets, for all samples in a cohort. To use a list of SV data frames as input, see sections of ***Convert VCF format to data.frame in R*** and ***Simple SV type classification***. Function *spectrum_SV_type* outputs a data.frame summarising the number of each SV type for each input sample.

```
# create character vector of three sample identifiers
All_sampleID <- paste0("sample",c(1,2,3))
# identifiy three example VCFs
vcf_files <- c(system.file("extdata", "manta_sample1.vcf", package =
"StructuralVariantUtil"),
            system.file("extdata", "manta_sample2.vcf", package =
"StructuralVariantUtil"),
            system.file("extdata", "manta_sample3.vcf", package =
"StructuralVariantUtil"))

# summarise SV types
Spectrum_SVTYPE <- spectrum_SV_type(All_sampleID, All_SV_data = vcf_files)
Spectrum_SVTYPE
  sampleID INS INV TRA DUP DEL
1  sample1   7   5  24   1   3
2  sample2   2   2  30   5  11
3  sample3  11  17  53   5  13
```

To estimate hyper-SV analysis, set the argument *identify_hyperSV_tumour* to TRUE. Additionally, provide the optional arguments:

- identify_hyperSV_tumour: TRUE or FALSE. Whether to identify hyper-SV mutated tumour samples for large cancer cohort. Default as FALSE.
- threshold_total: threshold of minimum total count of SVs per sample. The default value is the average total SV count in the cohort.
- threshold_relative_freq: the threshold of minimum relative frequency of one SV type per sample. The default value is 50%.

Setting *identify_hyperSV_tumour* to TRUE will cause *spectrum_SV_type* to output a list of two data frames. The first will be a data frame of counts of SV types (as for *identify_hyperSV_tumour* = FALSE) and a second data frame with variables:

- sampleID: ID of sample with hyper-SV identified.
- count: count of SVs in this type
- relative_freq: relative frequency of the particular SV type per sample.

- total_count: total count of SVs in this sample.
- HYPER_SVTYPE: which type of hyper-SV mutation.

In the example below, we load the test input SV callsets, in a data frame, for 100 samples, with breakpoints simulated randomely based on Hg38 genomic positions, excluding gap, centromere and telomere regions.

```
data(list)
All_sampleID <- paste0("sample_",c(1:100))

results <- spectrum_SV_type(All_sampleID, All_SV_data =list,
identify_hyperSV_tumour = TRUE)

# First output list object: counts of each SV type
Spectrum_SVTYPE <- results[[1]]
head(Spectrum_SVTYPE)
sampleID DEL DUP INS INV TRA
1 sample_1 167 300  33 164 248
2 sample_2 129  49   4  99 198
3 sample_3 299 242  53 163 204
4 sample_4 270 246  86 148 167
5 sample_5 187 247  89 150 289
6 sample_6  85 239  24 121 244

# Second output list object: hyper-SV results
Hyper_SV_sample <- results[[2]]
Hyper_SV_sample
      sampleID count relative_freq total_count HYPER_SVTYPE
96   sample_96   982     0.6323245        1553    hyper-DEL
97   sample_97   826     0.5772187        1431    hyper-DEL
98   sample_98   609     0.6017787        1012    hyper-DEL
99   sample_99   854     0.5685752        1502    hyper-DEL
100 sample_100   806     0.6494762        1241    hyper-DEL
```

Spectrum of SV breakpoints in genomic bins

In a cohort analysis, genomic regions recurrently impacted by SVs or specific SV types may be of interest, such genome-wide pattern of SV hotspots is sometimes known as the spectrum of SVs. The function *spectrum_SV_breakpoint* can be used for identifying and visualising the spectrum of SVs, by summing the number of SV breakpoints within 1 Mb non-overlapping bin across the genome.

Input parameters required by *spectrum_SV_breakpoint* include a character vector of sample ID and SV data, which can be a list of VCF file paths or a list of data frames of SV callsets, one

for each sample in the cohort. The list of callsets can be provided as a list of VCF file paths or a list of SV data frames for all samples in the cohort, similar to the input required for *summary_SV_type*. SV hotspots are defined as genomic regions most frequently ($> Q_3 + k \times (Q_3 - Q_1)$) affected by SV breakpoints, either in the same genome or recurrent across genomes.

The required input thresholds to define SV hotspots are:

- *threshold_count_breakpoint*: the k value in Tukey's fences approach to find outliers in breakpoint count. The default is set as 1.5. Considering clustered SV breakpoints such as chromothripsis can be attained in a single tumour, it can be more stringent on this threshold by setting this threshold to be higher (for example, as 3).
- *threshold_count_sample*: the k value in Tukey's fences approach to define outliers for sample count. The default is set as 1.5.

Function *spectrum_SV_breakpoint* outputs a data.frame with the following variables.

- chrom_bin_labels (character): unique identifier of genomic bin
- bin_labels (character): ID of bin for each chromosome
- bin (character): genomic region of bin
- breaks (numeric): start of genomic bin
- sampleID (character): sample name or ID
- chrom (character): chromosome of the breakpoint in the genomic bin
- pos (numeric): genomic location of the breakpoint in the genomic bin
- count_breakpoints (numeric): the count of SV breakpoint in the genomic bin
- count_sample (numeric): the number of samples with at least one SV breakpoint in the genomic bin
- is_hotspot_breakpoint (TRUE or FALSE): whether defined as hotspot based on count of breakpoints ($> Q_3 + \text{threshold\_count\_breakpoint} \times (Q_3 - Q_1)$)
- is_hotspot_sample (TRUE or FALSE): whether defined as hotspot based on count of samples ($> Q_3 + \text{threshold\_count\_sample} \times (Q_3 - Q_1)$)
- is_hotspot (TRUE or FALSE): whether defined as hotspot either in the same genome or recurrent across genomes

```
All_sampleID <- paste0("sample",c(1,2,3))
```

```
vcf_files <- c(system.file("extdata", "manta_sample1.vcf", package =
"StructuralVariantUtil"),
             system.file("extdata", "manta_sample2.vcf", package =
"StructuralVariantUtil"),
             system.file("extdata", "manta_sample3.vcf", package =
"StructuralVariantUtil"))


df_bin_all_hotspots <- spectrum_SV_breakpoint(All_sampleID, All_SV_data =
vcf_files)
head(df_bin_all_hotspots)
   chrom_bin_labels bin_labels                 bin   breaks sampleID chrom
pos                          ID
1           chr1_56         56   (5.5e+07,5.6e+07] 5.50e+07  sample1  chr1
55008308 StructuralVariantUtil_1_1_1
2           chr1_62         62   (6.1e+07,6.2e+07] 6.10e+07  sample1  chr1
61988078 StructuralVariantUtil_2_1_2
41          chr1_56         56   (5.5e+07,5.6e+07] 5.50e+07  sample1  chr1
55008308 StructuralVariantUtil_1_2_1
42          chr1_62         62   (6.1e+07,6.2e+07] 6.10e+07  sample1  chr1
61988179 StructuralVariantUtil_2_2_2
46         chr1_225        225 (2.24e+08,2.25e+08] 2.24e+08  sample1  chr1
224467224 StructuralVariantUtil_6_2_6
49          chr1_71         71     (7e+07,7.1e+07] 7.00e+07  sample1  chr1
70505412 StructuralVariantUtil_9_2_9
   count_breakpoints count_sample is_hotspot_breakpoint is_hotspot_sample
is_hotspot
1                  2            1                 FALSE             FALSE
FALSE
2                  2            1                 FALSE             FALSE
FALSE
41                 2            1                 FALSE             FALSE
FALSE
42                 2            1                 FALSE             FALSE
FALSE
46                 1            1                 FALSE             FALSE
FALSE
49                 1            1                 FALSE             FALSE
FALSE
```

Additionally the function *spectrum_SV_breakpoint* produces two figures, one showing the number of breakpoints at each genomic sites and another showing the number of samples in the cohort harbouring a breakpoin at each genomic site (**Figure 3** and **4**). In both figures, SV hotspots are highlighted in green.

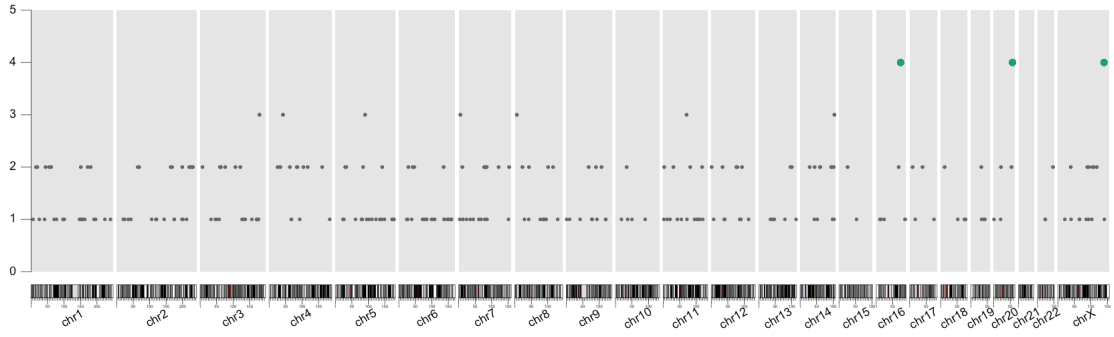


Figure 3. SV frequency genome-wide for all samples, showing the number of breakpoints. Hotspot regions are highlighted as green.

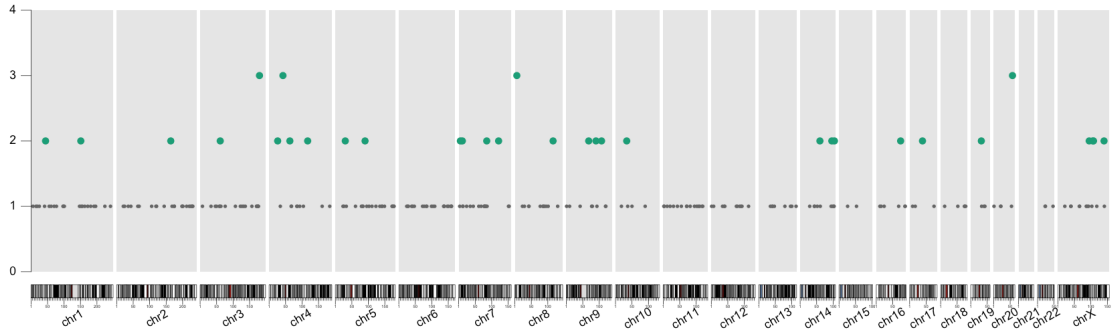


Figure 4. SV frequency genome-wide for all samples, showing the number of samples. Hotspot regions are highlighted as green.

Copy number changes of deletion and duplication events

DEL and DUP SV types can result in copy number loss and gains respectively. However, the actual number of DNA fragment copies in a DEL or DUP event is not available from SV calling results. CNV callers are developed with a focus on inferring and reporting copy number changes, while SV callers infer SV breakpoint and type and rarely attempt to infer copy number changes resulting by SV events. To better understand the DELs and DUPs, *StructuralVariantUtil* facilitates integrating DEL and DUP with copy number segments and categorise DEL and DUP based on copy number values with function *SV_CNV_integration*.

The input parameters required by function *SV_CNV_integration* include SV and CNV data in data.frame. Prepare your SV data.frame using function *simple_SVTYPE_classification* or prepare the input in bedpe format with at least the following variables:

- chrom1(character): chromosome of the first breakpoint
- start1(numeric): The zero-based starting position of first breakend on chrom1
- end1(numeric): The one-based starting position of first breakend on chrom1
- chrom2(character): chromosome of the second breakpoint
- start2(numeric): The zero-based starting position of second breakend on chrom2
- end2(numeric): The one-based starting position of second breakend on chrom2
- ID(character): ID of SV first breakpoint
- ID_mate(character): ID of SV second breakpoint
- SVTYPE(character): SV types

```
vcf_file = system.file("extdata", "manta_sample4.vcf", package =
"StructuralVariantUtil")
SV_data <- simple_SVTYPE_classification(vcf_file, caller_name = "manta")
head(SV_data)
```

Load the test input CNV data as following. The input CNV data is required to be stored in data.frame with at least the following columns:

- chrom(character): chromocome
- start (numeric): start position for the CN segment
- end(numeric) end position for the CN segment
- cn (numeric): integer total copy number (e.g. 2 for copy number neutral/unaltered region)

```
data(CNV_bed)
head(CNV_bed)
  chromosome   start     end cn
1       chr1   10000  905503  2
2       chr1  905503 1374792  2
3       chr1 1374792 1943930  2
4       chr1 1943930 2423204  2
5       chr1 2423204 2796280  2
6       chr1 2796280 3446159  2
```

Other input parameters include *sample_ID* (sample name or ID) and *overlap_f* (the fraction of minimum overlap required of CNV segment as a fraction of SV, default as 0.5). In addition, define parameter of *bedtools_dir* to provide path of bedtools by specifying your path or adding its path to system PATH.

Once the input data and parameters have been loaded, we proceed to run the function *SV_CNV_integration*, which results a data.frame with deletions and duplications and their overlapping discrete copy number segments.

- sampleID: Unique identifier of sample
- SV_chrom: chromosome of SV
- SV_start: start position of DEL or DUP region
- SV_end: end position of DEL or DUP region
- SV_ID: ID of SV
- SV_type: type of SV
- CNV_chrom: CNV chromosome
- CNV_start: CNV start position
- CNV_end: CNV end position
- CNV_cn: copy number value
- overlap: the number of bases overlapping between SV region and CNV region.

```
sampleID <- "sample_4"
SV_CNV_integrated <- SV_CNV_integration(sampleID, SV_data, CNV_data = CNV_bed,
bedtools_dir = "/opt/homebrew/bin/bedtools")
head(SV_CNV_integrated)
  sampleID SV_chrom  SV_start    SV_end           SV_ID SV_type CNV_chrom
CNV_start   CNV_end CNV_cn overlap
1 sample_4     chr2 190055341 190534896  Manta_105_1_93     DUP      chr2
190052119 190552133      2  479555
2 sample_4     chr3  83218893  90328953 Manta_135_1_117     DEL      chr3
83222254  90772459      1 7106699
3 sample_4     chr3 140607229 140793386 Manta_172_1_153     DUP      chr3
140605567 140795567      3  186157
4 sample_4     chr3 161840785 167251478 Manta_173_1_154     DEL      chr3
162905564 166285563      2 3379999
5 sample_4     chr4  29153967  29368058 Manta_187_1_166     DEL      chr4
29149724  29369689      1  214091
6 sample_4     chr4  61828504  61869283 Manta_201_1_179     DEL      chr4
61831419  61871420      1   37864
```

SV breakpoint gene annotation

StructuralVariantUtil facilitates SV breakpoints annotation based on gene regions using function *SV_gene_annotation*. In addition, it summarized all gene fusion pairs resulted from SVs and provides a table of gene fusion frequency in the sample or cohort. A SV breakpoint is annotated as interrupting a gene if it is within the gene region.

Firstly, provide the input vcf file or use *simple_SVTYPE_classification* to format VCF to SV BND pairs in bedpe format with at least following variables:

- chrom1(character): chromosome of the first breakpoint
- start1(numeric): The zero-based starting position of first breakend on chrom1
- end1(numeric): The one-based starting position of first breakend on chrom1
- chrom2(character): chromosome of the second breakpoint
- start2(numeric): The zero-based starting position of second breakend on chrom2
- end2(numeric): The one-based starting position of second breakend on chrom2
- ID(character): ID of SV first breakpoint
- ID_mate(character): ID of SV second breakpoint

```
vcf_file <- system.file("extdata", "manta_sample1.vcf", package =
"StructuralVariantUtil")
```

The provided bed file of gene regions (ensemble_release99_gene.bed) was derived based on ENSEMBL gene annotation release version 99 for GCRh38. User can use their preferred gene database and prepare it in data.frame with at least following variables:

- **chrom**: Reference sequence chromosome of the gene
- **start**: gene or transcription start position
- **end**: gene or transcription end position
- **gene_name**: name or unique identifier of gene

```
gene_file = system.file("extdata", "ensembl_release99_gene.bed", package =
"StructuralVariantUtil")
gene_bed <- read.table(gene_file, header=TRUE)
```

Make sure you have also defined parameter of *bedtools_dir* to provide path of bedtools by specifying your path or adding its path to system PATH. Once the input data have been loaded, we proceed to run the function *SV_gene_annotation*, which outputs a data.frame with interrupted genes added to the input SV data.frame:

- **pos1_overlap_gene**: name of gene interrupted by first breakpoint (chr1 and pos1 in input data.frame)
- **pos2_overlap_gene**: name of gene interrupted by second position (chr2 and pos2 in input data.frame)

```
results <- SV_gene_annotation(SV_data = vcf_file, gene_bed)
SV_geneAnnotated <- results[[1]]
```

```
  chrom1    start1      end1 chrom2    start2      end2 SVTYPE strand1 strand2
1   chr1  55008307  55008308   chr1  55008307  55008308    INS    <NA>    <NA>
2   chr1  61988077  61988078   chr1  61988178  61988179    INV       -       -
3   chr2  68529702  68529703   chr2  68529702  68529703    INS    <NA>    <NA>
4   chr2 119653367 119653368   chr3  58161075  58161076    TRA       +       -
5   chr2 199398205 199398206   chr2 199398306 199398307    INV       -       -
6   chr2 212506914 212506915   chr1 224467223 224467224    TRA       +       -
           ID     ID_mate
1 manta_1_1_1 manta_1_2_1
2 manta_2_1_2 manta_2_2_2
3 manta_3_1_3 manta_3_2_3
4 manta_4_1_4 manta_4_2_4
5 manta_5_1_5 manta_5_2_5
6 manta_6_1_6 manta_6_2_6

ALT
1
CATGGGGCAGGATGGCCATATTGGCCGGGGTGATGTGGAGGGCTTCCTAGAGGAACAGACATTGGAGCCGAGGCCTGAGG
TCAAGTTTATAACTTTCCTCT
2
<INV>
3
CTTTCTTATCAACTCCAAACTTACAGGGTGAAGTTAGCCATCTCTTTCAGT
4
A[CHR3:58161076[
5
<INV>
6
C[CHR1:224467224[
                  ID_caller REF QUAL FILTER INFO_SVTYPE INFO_SVLEN INFO_STRANDS
1    MantaINS:469:0:0:0:0:0   C   NA   PASS         INS        100           NA
2    MantaINV:533:0:0:1:1:0   G   NA   PASS         INV        101           NA
3   MantaINS:2496:0:0:0:0:0   C   NA   PASS         INS         50           NA
4 MantaBND:2900:0:1:0:0:0:1   A   NA   PASS         BND         NA           NA
5   MantaINV:3544:0:0:1:3:0   A   NA   PASS         INV        101           NA
6 MantaBND:1725:0:1:0:0:0:0   C   NA   PASS         BND         NA           NA
  INFO_CT INFO_INV5 INFO_INV3        INFO_MATEID_caller pos1_overlap_gene
1      NA     FALSE     FALSE                     <NA>              BSND
2      NA      TRUE     FALSE                     <NA>              PATJ
3      NA     FALSE     FALSE                     <NA>              APLF
4      NA     FALSE     FALSE MantaBND:2900:0:1:0:0:0:0           CFAP221
5      NA      TRUE     FALSE                     <NA>             SATB2
6      NA     FALSE     FALSE MantaBND:1725:0:1:0:0:0:1             ERBB4
  pos2_overlap_gene
1              BSND
2              PATJ
3              APLF
4              FLNB
5             SATB2
6             CNIH3
```

In addition, function *SV_gene_annotation* output a table of summarised gene fusions resulted from SV, with the following variables:

- **pos1_overlap_gene**: name of gene interrupted by pos1
- **pos2_overlap_gene**: name of gene interrupted by pos2

- **gene_fusions**: name of gene fusions
- **breakpoint_count**: count of fusion events due to SVs
- **sample_count**: count of samples having this gene fusion

```
df_summary_gene_fusions <- results[[2]]
head(df_summary_gene_fusions)
  pos1_overlap_gene pos2_overlap_gene          gene_fusions breakpoint_count
1             ABCD2           SPECC1L         ABCD2;SPECC1L                1
2             ABCD2   SPECC1L-ADORA2A ABCD2;SPECC1L-ADORA2A                1
3           CFAP221              FLNB          CFAP221;FLNB                1
4            COL4A6             HACE1          COL4A6;HACE1                1
5             ERBB4             CNIH3           ERBB4;CNIH3                1
6              ESR1              XKR5             ESR1;XKR5                1
  sample_count
1            1
2            1
3            1
4            1
5            1
6            1
```

REFERENCES

1. Gong T, Hayes VM, Chan EKF: **Shiny-SoSV: A web-based performance calculator for somatic structural variant detection.** *PLOS ONE* 2020, **15**:e0238108.
2. Quinlan AR, Hall IM: **BEDTools: a flexible suite of utilities for comparing genomic features.** *Bioinformatics* 2010, **26**:841-842.