Overview of svcnvs package

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Introduction

This package is used to identify deletions and amplicons from Whole Genome Sequencing (WGS) data through a combination of changes in normalized coverage and read pairs with aberrant spacing or orientation.

Additional packages required for this vignette are the `svfilters.hg19` package that contains various sequence filters for structural variant analyses and the `svalignments` package that contains wrappers for extracting properly- and improperly-paired reads from a BAM file. We also employ functions from `RSamtools` and `Genomic Ranges`.

This package requires that normalized coverage is calculated for a set of bins in the package `svfilters.hg19` (or `svfilters.hg18`). See the `svpreprocess` vignette for comprehensive documentaion on generating normalized coverage. This process can take some time on large bam files so for the purpose of this tutorial we've distributed sample preprocessed coverage data from the `svpreprocess` package.

Preprocessing

Loading packages required for this analysis.

```
library(GenomicRanges)
library(Rsamtools)
library(svcnvs)
library(svfilters.hg19)
library(svalignments)
```

We first load an object from the `svpreprocess` package containing normalized and $\log_2$-transformed coverage estimates ($\log_2$ ratios) in non-overlapping 1kb bins along the genome (see `svpreprocess` to generate $\log_2$ ratios). The $\log_2$ ratios were multipled by 1000, rounded to the nearest integer, and saved as integers in a serialized R object to reduce the memory footpring. We append the $\log_2$ ratios to the `GRanges` object bins1kb from the `svfilters.hg19` package and remove chromosome Y from the analysis as this sample is of female origin. Note that in this instance we are using `svfilters.hg19` because we aligned our reads to the hg19 reference genome. There is an `svfilters.hg18` package for if your reads were aligned to hg18, and an `svfilters.hg38` package will be available in the future to support alignments to hg38.

```
ddir <- system.file("extdata", package="svpreprocess", mustWork=TRUE)
cov.file <- file.path(ddir, "preprocessed_coverage.rds")
log_ratio <- readRDS(cov.file)/1000
data(bins1kb, package="svfilters.hg19")
bins1kb <- dropSeqlevels(bins1kb, "chrY", pruning.mode = "coarse")
bins1kb$log_ratio <- log_ratio
```

Segmentation

Next, we segment the $\log_2$ ratios using the Circular Binary Segmentation algorithm to achieve estimated copy number states across the genome. To save time we load a pre-made `GRanges` object containing the segment

means.

```
path <- system.file("extdata", package="svcnvs")
data(segments, package="svcnvs")
```

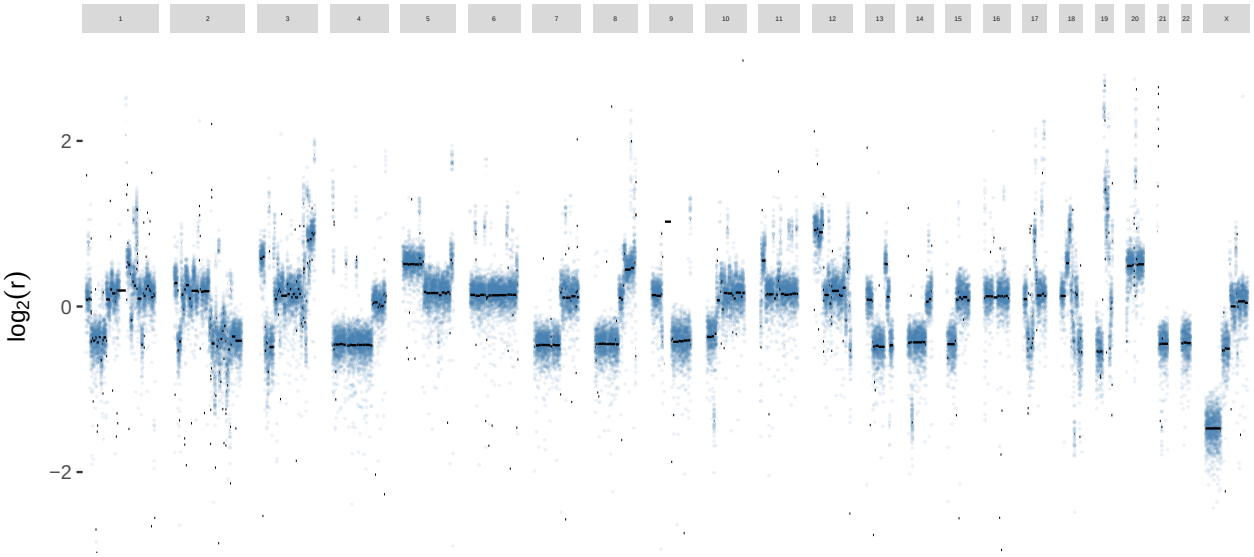
To generate this segments object on your own data use the `segmentBins` function.

```
segments <- segmentBins(bins1kb)
```

Plotting the genome

We routinely visualize the segmented $\log_2$ ratios of the entire genome to get an overview of the data. An example using the `ggplot2` library follows.

```
bins1kb <- sort(bins1kb)
lrr.df <- as.data.frame(bins1kb[seq(1, length(bins1kb), 50)])
seg.df <- as.data.frame(segments)
library(ggplot2)
chromlabels <- setNames(c(1:22, "X"), seqlevels(bins1kb))
ggplot(lrr.df, aes(start, log_ratio)) +
  geom_point(size=0.1, color="steelblue", alpha = 0.1) +
  geom_segment(data=seg.df,
               aes(x=start, xend=end, y=seg.mean, yend=seg.mean),
               color="black", inherit.aes=FALSE) +
  facet_grid(~seqnames, space="free", scales="free_x",
             labeller=as_labeller(chromlabels)) +
  theme(axis.text.x=element_blank(),
        panel.grid=element_blank(),
        panel.background=element_rect(fill="white"),
        axis.ticks.x=element_blank(),
        strip.text.x = element_text(size = 3)) +
  xlab("") +
  ylab(expression(log[2](r))) +
  ylim(c(-3, 3))
```



It is evident from visual inspection that this sample has numerous structural alterations. We will proceed to integrate read pair information with these segmented $\log_2$ ratios to identify true CNVs.

Deletions

In addtition to the segmented $\log_2$ ratios, the deletion analysis requires improperly paired reads. Below, we specify the complete file path to the BAM file used for this analysis that is provided by the `svbams` package. We extract improperly paired reads from the entire genome and an initial set of properly paired reads.

```
extdata <- system.file("extdata", package="svbams")
bam.file <- file.path(extdata, "cgov44t_revised.bam")
iparams <- improperAlignmentParams(what=c("flag", "mrnm", "mpos", "mapq"))
improper_rp <- getImproperAlignmentPairs(bam.file,
                                         param=iparams)
segs <- keepSeqlevels(segments, "chr15", pruning.mode="coarse")
del.gr <- reduce(segs[segs$seg.mean < hemizygousThr(DeletionParam())],
                 min.gapwidth=2000)
proper_rp <- properReadPairs(bam.file, gr=del.gr, DeletionParam())
read_pairs <- list(proper_del=proper_rp, improper=improper_rp)
```

Finally, we collect the bin-level summaries, the segmentation data, and the read pair data in a single list object:

```
pdata <- preprocessData(bam.file=bam.file, genome="hg19",
                        bins=bins1kb,
                        segments=segments, read_pairs=read_pairs)
```

Below, we call segments as homozygous deletion (homozygous), homozygous deletion supported by improperly paired reads (homozygous+), and hemizygous deletion supported by improperly paired reads. For the purpose of identifying somatic deletions without a matched normal, we exclude hemizygous deletions that are not supported by improperly paired reads. With this toy dataset, we identify two homozygous deletions and the calls are both `homozygous`.

```
deletions <- sv_deletions(preprocess=pdata)
```

```
## Revising junctions...
```

```
## Refining homozygous boundaries by spanning hemizygous+
```

```
variant(deletions)
```

```
## GRanges object with 16 ranges and 2 metadata columns:
##        seqnames              ranges strand |  seg.mean      sample
##           <Rle>           <IRanges>  <Rle> | <numeric> <character>
##    sv1     chr2 [95731001, 95736001]      * |   -7.8007     CGOV44T
##    sv2     chr4 [22602001, 22606001]      * |   -7.8356     CGOV44T
##    sv3     chr4 [26291001, 26294001]      * |   -8.8232     CGOV44T
##    sv4     chr4 [40377001, 40439001]      * |   -8.2143     CGOV44T
##    sv5     chr4 [92858001, 92946001]      * |   -8.4734     CGOV44T
##    ...      ...                 ...    ... .       ...         ...
##   sv12    chr15 [63201015, 63209243]      * |   -8.6549     CGOV44T
##   sv13    chr17 [29448001, 29705001]      * |   -8.5644     CGOV44T
##   sv14    chr20 [37009001, 37014001]      * |   -8.0983     CGOV44T
##   sv15    chr22 [24874001, 24885001]      * |   -8.6562     CGOV44T
##   sv16     chrX [56812001, 56862001]      * |   -3.4914     CGOV44T
##   -------
##   seqinfo: 23 sequences from hg19 genome
```

```
calls(deletions)
```

```
##  [1] "homozygous"  "homozygous"  "homozygous"  "homozygous"  "homozygous"
```

```
##  [6] "homozygous"  "homozygous"  "homozygous"  "homozygous"  "homozygous"
## [11] "homozygous"  "homozygous+" "homozygous"  "homozygous"  "homozygous"
## [16] "homozygous"
```

The improperly-paired reads supporting the `homozygous+` call can be extracted as a `GAlignmentPairs` object from the second element of the `StructuralVariant` object.

```
improper(deletions[2])
```

```
## GAlignmentPairs object with 0 pairs, strandMode=1, and 0 metadata columns:
##    seqnames strand :    ranges --    ranges
##       <Rle>  <Rle> : <IRanges> -- <IRanges>
##   -------
##   seqinfo: 23 sequences from an unspecified genome
```

Amplicons

Amplicons can be identified using the same list data structure for the preprocessed data.

```
params <- ampliconParams()
ag <- svcnvs:::sv_amplicons2(pdata, params=params)
```

Note the object returned by `sv_amplicons` is a graph where the nodes are the individual amplicons and the edges are links between amplicons given by improperly paired reads. By default, with 30x coverage we require at least 5 improperly paired reads to support an edge. See `?ampliconParams` for customing these settings.

Plotting deletions

We will use the *ggplot2* and *gridExtra* packages for plotting the deletions.

```
library(ggplot2)
suppressPackageStartupMessages(library(gridExtra))
library(scales)
```

In the following code chunk, we extract the genomic coordinates for the deletion stored in the *deletion* object. To view the deletion in the context of the surrounding region, we create a second *GRanges* object that includes 200kb of the flanking genome on each side of the deletion.

```
##
## region of interest (roi)
##
roi <- variant(deletion)
seqlevels(roi, pruning.mode="coarse") <- "chr15"
expand <- 200e3
roi2 <- GRanges(seqnames(roi), IRanges(start(roi)-expand,
                                       end(roi) + expand))
seqinfo(roi2) <- seqinfo(roi)
```

Next, we subset the views object to contain only the genomic bins in the region of interest defined above. In addition, we create a data.frame containing all the segments for this particular chromosome and sample and a data.frame containing the preprocessed coverage.

```
segs <- keepSeqlevels(segments, seqlevels(roi), pruning.mode="coarse")
segs.df <- as(segs, "data.frame")
hom.plus <- subsetByOverlaps(bins1kb, roi2)
```

```
df <- data.frame(logr=hom.plus$log_ratio,
                 start=start(hom.plus))
```

We restrict the y-axis limits to a suitable range for visualizing the log ratios, thresholding log ratios that are extreme. We highlight the region identified by the segmentation in the ggplot graphic (the boundaries for the deletion are subsequently revised by the improperly paired reads as described in the next section).

```
ylim <- c(-9, 2)
df$logr <- svpreprocess::threshold(df$logr, ylim)
brks <- pretty(df$start, n=8)
region <- subsetByOverlaps(segs, roi)
region <- region[region$seg.mean < -1]
region <- as.data.frame(region)
xlim <- c(start(roi2), end(roi2))

A <- ggplot(df, aes(start, logr)) +
  geom_point(size=1, color="gray50") +
  scale_x_continuous(expand=c(0,0), breaks=brks, labels=brks/1e6)+
  scale_y_continuous(expand=c(0,0)) +
  geom_segment(data=segs.df,
               aes(x=start, xend=end, y=seg.mean, yend=seg.mean),
               size=1) +
  coord_cartesian(xlim=xlim, ylim=ylim) +
  ylab(expression(log[2]~ratio)) +
  geom_rect(data=region,
            aes(xmin=start, xmax=end, ymin=-Inf, ymax=+Inf),
            fill="steelblue", color="transparent", alpha=0.3,
            inherit.aes=FALSE) +
  theme(axis.text=element_text(size=10),
        axis.text.x=element_blank()) + xlab("") +
  annotate("text", x=xlim[1] + 15e3, y=-8, label="chr15", size=3)
A1 <- ggplotGrob(A)
```

Plotting rearranged read pairs from a deletion object

In addition to the log ratios, we would like to visualize the rearranged read pairs (read pairs with aberrant spacing or orientation with respect to the reference genome) that support the deletion. The rearranged read pairs supporting the deletion are encapsulated in the *deletion* object that we already loaded. First, we pull read pairs flanking the candidate deletion that have normal spacing and orientation. Because there are typically a large number of the normal read pairs, we thin these using the function *thinReadPairs*. Next, we melt these reads into a *data.frame* useful for plotting.

```
rps <- thinReadPairs(deletion)
rps <- svcnvs:::meltReadPairs(rps)
```

We again use *ggplot* to plot the data. Note the vertical dashed lines depict the more precise boundaries of the deletion made possible by the improperly paired (rearranged) reads.

```
colors <- c("#0072B2", "#009E73")
p <- ggplot(rps, aes(ymin=readpair-0.2, ymax=readpair+0.2,
                xmin=start/1e6, xmax=end/1e6, color=read,
                fill=read, group=readpair)) +
  geom_rect() +
  xlim(c(min(rps$start), max(rps$end))/1e6) +
```

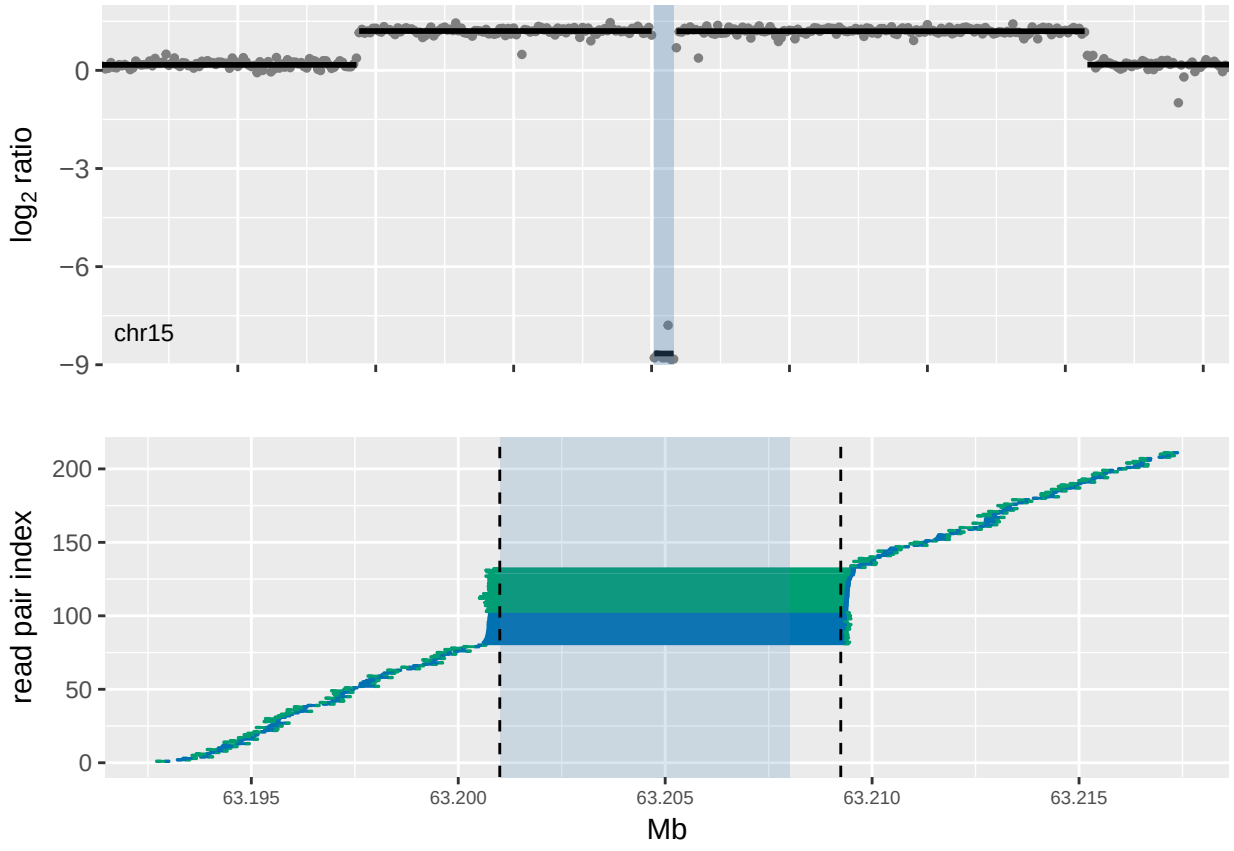
```
  geom_line(aes(x=start/1e6, y=readpair)) +
  ylab("read pair index") +
  scale_x_continuous(breaks=pretty_breaks(5)) +
  geom_rect(data=region,
            aes(xmin=start/1e6, xmax=end/1e6, ymin=-Inf, ymax=+Inf),
            fill="steelblue", color="transparent", alpha=0.2,
            inherit.aes=FALSE) +
  scale_color_manual(values=colors) +
  scale_fill_manual(values=colors) +
  xlab("Mb") +
  theme(axis.text.x=element_text(size=7)) +
  guides(fill=FALSE, color=FALSE) +
  geom_vline(xintercept=c(start(roi)/1e6, end(roi)/1e6), linetype="dashed")
```

```
## Scale for 'x' is already present. Adding another scale for 'x', which
## will replace the existing scale.
```

```
B <- ggplotGrob(p)
```

Finally, we make a composite graphic of the log ratios and rearranged reads. Note, the vertical dashed lines show the revised deletion boundaries using the improperly-paired reads that flank the new sequence junction formed as a result of the deletion.

```
grid.arrange(A1, B, ncol=1)
```



Plotting amplicon graphs

See the amplicons vignette.