

Tools for high throughput SNP chip data

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Introduction

SNPchip defines classes and methods useful for organizing high throughput genomic data. The classes defined here extend the `eSet` class in *Biobase*, utilizing the existing Bioconductor infrastructure for organizing high dimensional genomic data. This provides a foundation upon which statistical and visualization tools can be further developed.

1 Simple Usage

We illustrate the structure of the class `AnnotatedSnpSet` with a small dataset provided with the package.

```
> library(SNPchip)
> data(annSnpset)
> annSnpset
```

```
AnnotatedSnpSet (storageMode: lockedEnvironment)
assayData: 5896 features, 5 samples
  element names: calls, callsConfidence, cnConfidence, copyNumber
phenoData
  sampleNames: NA17101_X_hAF_A1_4000091.CEL, NA17102_X_hAF_A2_4000091.CEL, ..., NA17105_
  varLabels and varMetadata: none
featureData
  rowNames: SNP_A-1507972, SNP_A-1641761, ..., SNP_A-1759046 (5896 total)
  varLabels and varMetadata:
    dbsnp_rs_id: dbsnp_rs_id
    chrom: chrom
    ...: ...
    enzyme: enzyme
    (8 total)
experimentData: use 'experimentData(object)'
```

```
Annotation [1] "pd.mapping50k.xba240"
```

```
chromosomeAnnotation
  centromereStart centromereEnd chromosomeSize
1      121147476    123387476    245522847
2      91748045     94748045    243018229
3      90587544     93487544    199505740
4      49501045     52501045    191411218
5      46441398     49441398    180857866
6      58938125     61938125    170975699
7      57864988     60864988    158628139
8      43958052     46958052    146274826
9      46035928     49035928    138429268
10     39244941     41624941    135413628
11     51450781     54450781    134452384
12     34747961     36142961    132449811
13     16000000     17868000    114142980
14     15070000     18070000    106368585
15     15260000     18260000    100338915
16     35143302     36943302     88827254
17     22187133     22287133     78774742
18     15400898     16764896     76117153
19     26923622     29923622     63811651
20     26267569     28033230     62435964
21     10260000     13260000     46944323
22     11330000     14330000     49554710
X      58465033     61465033    154824264
Y      11237315     12237315     57701691
```

`annSnpset` is an instance of the `AnnotatedSnpSet` class. Here, the `assayData` slot in `AnnotatedSnpSet` contains 5896 SNPs with estimates of copy number and genotype calls, as well as a corresponding confidence score. Typically, such an object would contain 100,000 - 500,000 estimates of genotype calls and copy number. We illustrate in Section 3 how to create an instance of `AnnotatedSnpSet` from probe-level summaries of SNP chip data. In addition to estimates of genotype call and copy number, the `annSnpset` contains both chromosome-level annotation, as well as SNP-level annotation. The chromosome-level annotation includes the centromere start and stop sites and chromosome size (in number of base pairs), and could be extended to include location of cytobands, or any other feature of a chromosome.

```
> data(chromosomeAnnotation)
> chromosomeAnnotation[1:5, ]
```

	centromereStart	centromereEnd	chromosomeSize
1	121147476	123387476	245522847
2	91748045	94748045	243018229
3	90587544	93487544	199505740
4	49501045	52501045	191411218
5	46441398	49441398	180857866

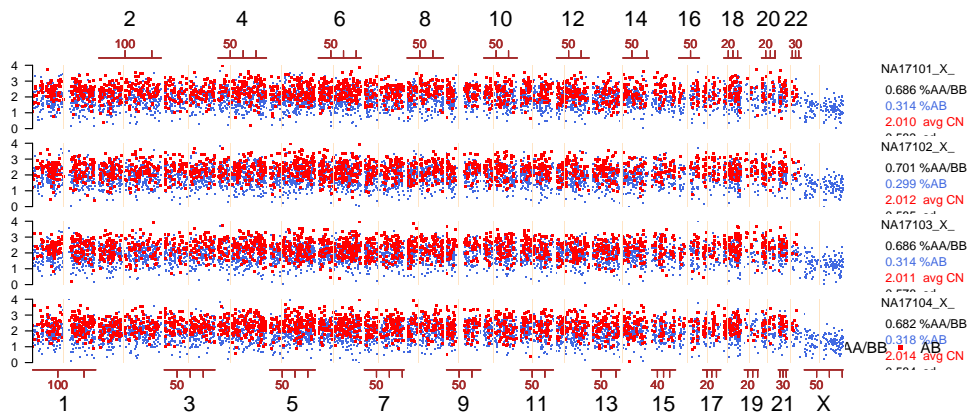
The method `getSnpAnnotation` retrieves SNP-level annotation from annotation packages maintained at Bioconductor. A more rich annotation is in development. The appropriate `pd.mapping` library needs to be loaded for this method to work.

```
> annotation(annSnpset)
> library("pd.mapping50k.xba240")

> featureData(annSnpset) <- getSnpAnnotation(annSnpset)
```

A genome-wide view of copy number and genotype calls versus physical position can be made using `plotSnp`. Here, we plot chromosomes 1-22 and X (the integer 23 is used to represent X) of samples 1 - 4 in the object `annSnpset`:

```
> plotSnp(annSnpset, chromosomes = c(1:22, "X"), samples = 1:4,
+         width.right = 10, cex.axis = 1, lab = c(3, 3, 5), cex.lab = 1.2,
+         cex.legend = c(1, 1.2))
```

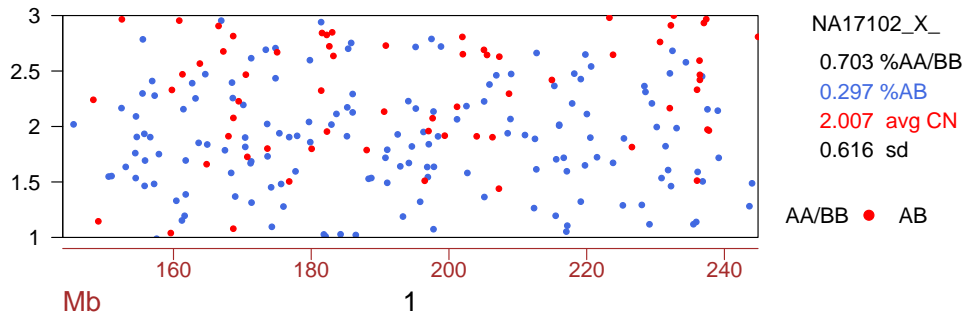


The copy number estimates have been centered to have mean zero – a centered copy number of 0 corresponds to a copy number of two. The default plot layout generally works well, but can be adjusted through the arguments `mar`, `oma`, and `width.right` in `plotSnp`. The latter argument specifies how much room to allow for the summary panel relative to the size of the smallest chromosome plotted. For instance, if plotting chromosomes 1-22 and X, `width.right` set to 15 allows a plotting region for the summary panel that is 15 times larger than chromosome 21. A more focused view of chromosomes 1, 7, 16, 19, and X of sample 2 could be obtained by

```

> plotSnp(annSnpset, c(1, 7, 16, 19, "X"), c(2, 5), cex = c(1,
+ 1, 1), pch = c(20, 21, 20), bg = c("royalblue", "white",
+ "royalblue"), bty = "o", width.right = 1.5, cex.axis = 1.2,
+ cex.lab = 1.5, cex.legend = c(1.2, 1.2), xaxs = "r")

```

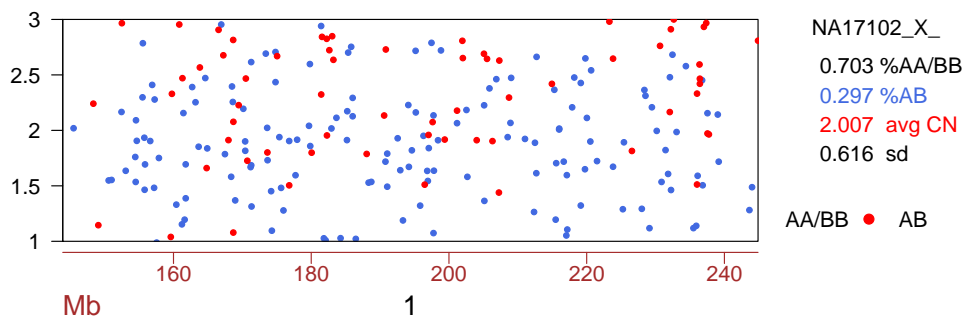


A plot of just the p-arm in sample 2 of chromosome 1:

```

> chr1 <- annSnpset[chromosome(annSnpset) == "1", ]
> start <- min(position(chr1)[position(chr1) > chromosomeAnnotation["1",
+ 1]], na.rm = TRUE)
> plotSnp(chr1[position(chr1) > start, ], 1, 2, xlim = range(position(chr1)[position(c
+ start], na.rm = TRUE), cex = c(1, 1, 1), pch = c(20, 20,
+ 20), bg = c("royalblue", "red", "royalblue"), bty = "o",
+ width.right = 0.3, cex.axis = 1.2, cex.lab = 1.5, cex.legend = c(1.2,
+ 1.2))

```



Cytobands can be added to graphs as follows:

```

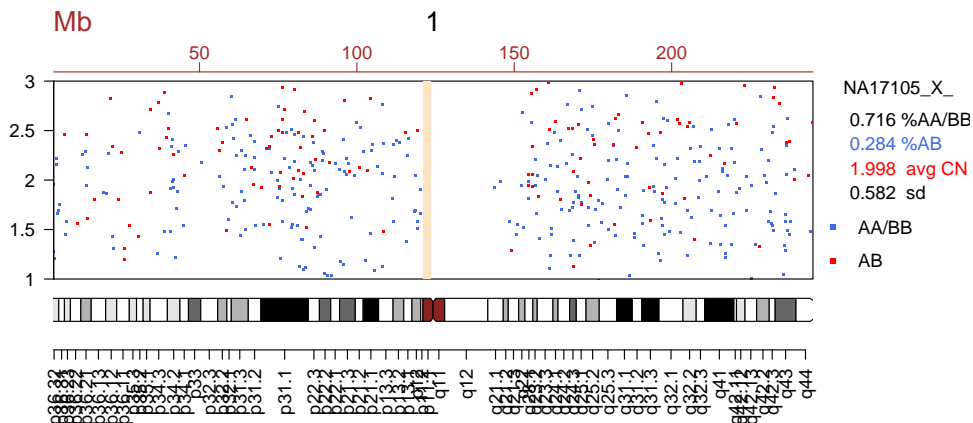
> data(annSnpset)
> data(cytoband)

```

```

> chr1 <- annSnpset[chromosome(annSnpset) == "1", ]
> plotSnp(chr1, 1, 5, oma = c(6, 4, 0, 3), cex = c(3, 3, 3), cex.axis = 1.2,
+       cex.legend = c(1.2, 1.2), addCytoband = TRUE, legend.location = c("topleft",
+       "bottomleft"), height.cytoband = 0.2, width.right = 0.2,
+       bty = "o", cex.lab = 1.5, ncol = 1, adj = 0)

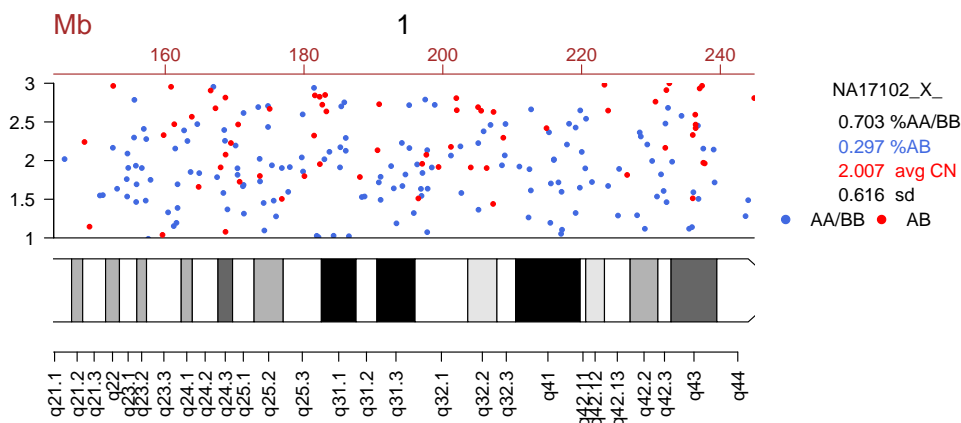
```



```

> plotSnp(chr1[position(chr1) > start, ], 1, 2, xlim = range(position(chr1)[position(c
+   start], na.rm = TRUE), cex = c(1, 1, 1), pch = c(20, 20,
+   20), bg = c("royalblue", "red", "royalblue"), bty = "l",
+   width.right = 0.3, cex.axis = 1.2, cex.lab = 1.5, cex.legend = c(1.2,
+   1.2), addCytoband = TRUE)

```



2 Available annotation

Bioconductor annotation packages for high throughput SNP platforms are under development. Column headers for the annotation that is currently available for each SNP is here:

```

> colnames(fData(annSnpset))

```

We store SNP-level attributes in the featureData slot. The command

```
> featureData(obj) <- getSnpAnnotation(annSnpset)
```

automatically loads the appropriate annotation package according to the annotation slot. The Bioconductor annotation packages must first be downloaded.

Alternatively, one may obtain the NetAffx annotation saved as an R object here:

```
> try(load(url("http://biostat.jhsph.edu/~iruczins/publications/sm/2006.scharpf.bioinf"))
> colnames(mapping10k$annotation)
```

For more detailed annotation on specific SNPs, see the R package RSNPper available at Bioconductor.

3 High throughput SNP classes

All that is needed to create an instance of `AnnotatedSnpCallSet` or `AnnotatedSnpCopyNumberSet` is a matrix of genotype calls and copy number, respectively, and their corresponding confidence scores. If estimates of both copy number and genotype calls are available, we can create an `AnnotatedSnpSet` that inherits methods from both `AnnotatedSnpCopyNumberSet` and `AnnotatedSnpCallSet`. In this way, *SNPchip* is completely independent of the pre-processing method used to produce probe-level summaries. To illustrate, the following code chunk loads a list of matrices obtained from normal subjects in the Hapmap project and pre-processed by CRLMM (B. Carvalho *et al.*, *Biostatistics*, in press). Only every 10th SNP from the Xba 50k chip is included in the matrices.

```
> data(hapmap)
> str(hapmap)
```

List of 3

```
$ calls          : int [1:5896, 1:5] 2 2 2 3 3 3 1 1 3 3 ...
..- attr(*, "dimnames")=List of 2
.. ..$ : chr [1:5896] "SNP_A-1507972" "SNP_A-1641761" "SNP_A-1641781" "SNP_A-1641805"
.. ..$ : chr [1:5] "NA17101_X_hAF_A1_4000091.CEL" "NA17102_X_hAF_A2_4000091.CEL" "NA17
$ callsConfidence: num [1:5896, 1:5] 566 326 202 668 674 ...
..- attr(*, "dimnames")=List of 2
.. ..$ : chr [1:5896] "SNP_A-1507972" "SNP_A-1641761" "SNP_A-1641781" "SNP_A-1641805"
.. ..$ : chr [1:5] "NA17101_X_hAF_A1_4000091.CEL" "NA17102_X_hAF_A2_4000091.CEL" "NA17
$ copyNumber     : num [1:5896, 1:5] 2.67 1.77 2.18 2.08 2.02 ...
..- attr(*, "dimnames")=List of 2
.. ..$ : chr [1:5896] "SNP_A-1507972" "SNP_A-1641761" "SNP_A-1641781" "SNP_A-1641805"
.. ..$ : chr [1:5] "NA17101_X_hAF_A1_4000091.CEL" "NA17102_X_hAF_A2_4000091.CEL" "NA17
```

Each matrix in the list contains probeset summaries (rows) by column (samples). Currently, we only provide annotation for the Affymetrix SNP chips and so the rownames of the matrices should be Affymetrix probeset id's. For purposes of visualization, an identifier from any technology could be used so long as the SNP-level annotation stored in the featureData slot is a data.frame with columns `Physical.Position` and `chromosome`.

```
> rownames(hapmap$calls)[1:5]

[1] "SNP_A-1507972" "SNP_A-1641761" "SNP_A-1641781" "SNP_A-1641805"
[5] "SNP_A-1641825"

> rowSds <- function(x) apply(x, 1, "sd")
> colSds <- function(x) apply(x, 2, "sd")
> nr <- nrow(hapmap$copyNumber)
> nc <- ncol(hapmap$copyNumber)
> snpset <- new("AnnotatedSnpSet", calls = hapmap$calls, callsConfidence = hapmap$call
+   copyNumber = hapmap$copyNumber, cnConfidence = matrix(NA,
+   nr, nc), annotation = "pd.mapping50k.xba240", chromosomeAnnotation = chromos
> library("pd.mapping50k.xba240")
> annSnpset <- getSnpAnnotation(snpset)
```

This may take several minutes depending on your internet connection. To do this manually using NetAffx annotation files, the annotation files can be downloaded from <http://biostat.jhsph.edu/>. This object should be converted to an object of class `AnnotatedDataFrame` with SNPs in the same order as in the `AnnotatedSnpSet` object. To download a static data.frame of the NetAffx annotation for the 50k Xba SNP chip, execute the following command:

```
> try(load(url("http://biostat.jhsph.edu/~iruczins/publications/sm/2006.scharpf.bioinf
data.frames for the 50k Hind and the 250k Nsp and Sty chips are also available:
```

```
> try(load(url("http://biostat.jhsph.edu/~iruczins/publications/sm/2006.scharpf.bioinf
> try(load(url("http://biostat.jhsph.edu/~iruczins/publications/sm/2006.scharpf.bioinf
> try(load(url("http://biostat.jhsph.edu/~iruczins/publications/sm/2006.scharpf.bioinf
```

Below, we illustrate how one might convert output from Affymetrix CNAT software to an object of class `AnnotatedSnpSet`. For instance, any one of the .txt files for the CEPH trios provided at the Affymetrix website can be converted as follows

```
> cnat <- read.table("100k_trios.Hind.1.txt", as.is = TRUE, sep = "\t",
+   header = TRUE, row.names = 1, skip = 0)
> cn <- as.matrix(cnat[, grep("SPA_CN", colnames(x))])
> calls <- cnat[, grep("_Call", colnames(x))]
```

```

> calls[calls == "AA"] <- 1
> calls[calls == "AB"] <- 2
> calls[calls == "BB"] <- 3
> calls[calls == "NoCall"] <- 4
> calls <- matrix(as.integer(as.matrix(calls)), nc = dim(calls)[2],
+   byrow = FALSE)
> cnConfidence <- as.matrix(cnat[, grep("SPA_pVal", colnames(cnat))])
> callsConfidence <- as.matrix(cnat[, grep("LOH", colnames(cnat))])
> rownames(calls) <- rownames(cn) <- rownames(cnConfidence) <- rownames(callsConfidence)
> colnames(cn) <- colnames(calls) <- colnames(callsConfidence) <- colnames(cnConfidence)
+   1, 7)
> trios <- new("AnnotatedSnpSet", calls = calls, copyNumber = copyNumber,
+   callsConfidence = callsConfidence, cnConfidence = cnConfidence,
+   annotation = "pd.mapping50k.hind240", chromosomeAnnotation = chromosomeAnnotation)
> library("pd.mapping50k.hind240")
> featureData(trios) <- getSnpAnnotation(trios)

```

The SNP-level annotation for the trios data can be retrieved as described previously.

```

> cnset <- as(annSnpset, "AnnotatedSnpCopyNumberSet")
> plotSnp(object = cnset, chromosomes = 1:10, samples = 1:3, cex = 5,
+   pch = ".", width.right = 3, cex.axis = 1, cex.legend = c(1.2,
+   1.2), legend = c(TRUE, FALSE))

```

4 Descriptive and statistical summaries

Descriptive statistics for copy number and genotype calls are provided with the `summary` method. For each chromosome in the `AnnotatedSnpSet`, `summary` calculates the average and standard deviation of the copy number estimates, as well as the % homozygous and heterozygous calls. In addition, `summary` calculates the average copy number, standard deviation, % homozygous and heterozygous across all autosomes in the `AnnotatedSnpSet`. The dimensions of the four matrices are $S \times C + 1$, where S is the number of samples and C is the number of chromosomes in the `AnnotatedSnpSet`.

```

> x <- summary(annSnpset, digits = 1)
> str(x)

```

List of 5

```

$ avgCN   : num [1:6, 1:23] 2 2 2 2 2 2 2 2 2 2 ...
  ..- attr(*, "dimnames")=List of 2
  .. ..$ : chr [1:6] "NA17101_X_hAF_A1_4000091.CEL" "NA17102_X_hAF_A2_4000091.CEL" "NA17103_X_hAF_A3_4000091.CEL" "NA17104_X_hAF_A4_4000091.CEL" "NA17105_X_hAF_A5_4000091.CEL" "NA17106_X_hAF_A6_4000091.CEL"
  .. ..$ : chr [1:23] "1" "2" "3" "4" ...
$ sdCN    : num [1:6, 1:23] 0.6 0.6 0.6 0.6 0.6 0.6 0.6 0.6 0.6 0.6 ...

```



```

..- attr(*, "dimnames")=List of 2
.. ..$ : chr [1:6] "NA17101_X_hAF_A1_4000091.CEL" "NA17102_X_hAF_A2_4000091.CEL" "NA17
.. ..$ : chr [1:23] "1" "2" "3" "4" ...
$ %NoCalls: num [1:6, 1:23] 0 0 0 0 0 0 0 0 0 0 0 0 ...
..- attr(*, "dimnames")=List of 2
.. ..$ : chr [1:6] "NA17101_X_hAF_A1_4000091.CEL" "NA17102_X_hAF_A2_4000091.CEL" "NA17
.. ..$ : chr [1:23] "1" "2" "3" "4" ...
$ %Hom      : num [1:6, 1:23] 0.7 0.7 0.7 0.7 0.7 0.7 0.7 0.7 0.7 0.7 0.7 0.7 ...
..- attr(*, "dimnames")=List of 2
.. ..$ : chr [1:6] "NA17101_X_hAF_A1_4000091.CEL" "NA17102_X_hAF_A2_4000091.CEL" "NA17
.. ..$ : chr [1:23] "1" "2" "3" "4" ...
$ %Het      : num [1:6, 1:23] 0.3 0.3 0.3 0.3 0.3 0.3 0.3 0.3 0.3 0.3 0.3 0.3 ...
..- attr(*, "dimnames")=List of 2
.. ..$ : chr [1:6] "NA17101_X_hAF_A1_4000091.CEL" "NA17102_X_hAF_A2_4000091.CEL" "NA17
.. ..$ : chr [1:23] "1" "2" "3" "4" ...

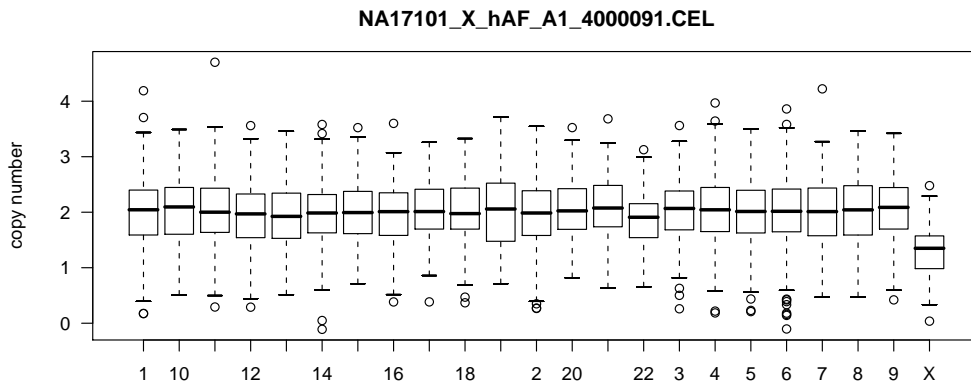
```

Boxplot by chromosome:

```

> par(mfrow = c(1, 1), mar = c(4, 4, 3, 1), las = 1)
> boxplot(split(copyNumber(annSnpset[, 1]), chromosome(annSnpset)),
+         ylab = "copy number", main = sampleNames(annSnpset)[1])

```



Smoothing example

The basic unit for all of the above visualization tools and summary methods is an `AnnotatedSnpSet` of a single chromosome. For instance, `plotSnp` converts the `AnnotatedSnpSet` to a list of `AnnotatedSnpSet`, where each element in the list is an `AnnotatedSnpSet` of a single chromosome. In the code below, we are interested in a quick method for smoothing copy number estimates for each chromosome and apply a loess smoother to each chromosome. The following code chunk first assigns heterozygous calls to the integer 1 and homozygous calls to the integer zero. In this way, regions of deletions will have homozygous calls of zero.

We simulated a deletion of 50 consecutive SNPs and then converted the `AnnotatedSnpSet` to a list where each element in the list is an `AnnotatedSnpSet` for a particular chromosome.

```
> sim1 <- annSnpset[chromosome(annSnpset) %in% 1:5, 1:3]
> sim1
```

```
AnnotatedSnpSet (storageMode: lockedEnvironment)
assayData: 2212 features, 3 samples
  element names: calls, callsConfidence, cnConfidence, copyNumber
phenoData
  rowNames: NA17101_X_hAF_A1_4000091.CEL, NA17102_X_hAF_A2_4000091.CEL, NA17103_X_hAF_A3
  varLabels and varMetadata: none
featureData
  rowNames: SNP_A-1507972, SNP_A-1641781, ..., SNP_A-1759046 (2212 total)
  varLabels and varMetadata:
    dbsnp_rs_id: dbsnp_rs_id
    chrom: chrom
    ...: ...
    enzyme: enzyme
    (8 total)
experimentData: use 'experimentData(object)'
Annotation [1] "pd.mapping50k.xba240"
```

```
chromosomeAnnotation
```

	centromereStart	centromereEnd	chromosomeSize
1	121147476	123387476	245522847
2	91748045	94748045	243018229
3	90587544	93487544	199505740
4	49501045	52501045	191411218
5	46441398	49441398	180857866
6	58938125	61938125	170975699
7	57864988	60864988	158628139
8	43958052	46958052	146274826
9	46035928	49035928	138429268
10	39244941	41624941	135413628
11	51450781	54450781	134452384
12	34747961	36142961	132449811
13	16000000	17868000	114142980
14	15070000	18070000	106368585
15	15260000	18260000	100338915
16	35143302	36943302	88827254
17	22187133	22287133	78774742
18	15400898	16764896	76117153

19	26923622	29923622	63811651
20	26267569	28033230	62435964
21	10260000	13260000	46944323
22	11330000	14330000	49554710
X	58465033	61465033	154824264
Y	11237315	12237315	57701691

```

> tmp <- sim1[chromosome(sim1) == "1", ]
> tmp <- tmp[order(position(tmp)), ]
> snps <- featureNames(tmp)[101:150]
> ids <- match(snps, featureNames(sim1))
> copyNumber(sim1)[ids, 1] <- copyNumber(sim1)[ids, 1] - 1
> calls(sim1)[ids, 1] <- 1

> sim2 <- sim1
> calls(sim2)[calls(sim2) == 1 | calls(sim2) == 3] <- 0
> calls(sim2)[calls(sim2) == 2] <- 1
> sim.list <- split(sim2, chromosome(sim2))
> sim.list[[1]]

```

```

AnnotatedSnpsSet (storageMode: lockedEnvironment)
assayData: 469 features, 3 samples
  element names: calls, callsConfidence, cnConfidence, copyNumber
phenoData
  rowNames: NA17101_X_hAF_A1_4000091.CEL, NA17102_X_hAF_A2_4000091.CEL, NA17103_X_hAF_A3
  varLabels and varMetadata: none
featureData
  rowNames: SNP_A-1642387, SNP_A-1643189, ..., SNP_A-1759036 (469 total)
  varLabels and varMetadata:
    dbsnp_rs_id: dbsnp_rs_id
    chrom: chrom
    ...: ...
    enzyme: enzyme
    (8 total)
experimentData: use 'experimentData(object)'
Annotation [1] "pd.mapping50k.xba240"

```

```

chromosomeAnnotation
  centromereStart centromereEnd chromosomeSize
1      121147476      123387476      245522847
2      91748045      94748045      243018229
3      90587544      93487544      199505740
4      49501045      52501045      191411218

```

5	46441398	49441398	180857866
6	58938125	61938125	170975699
7	57864988	60864988	158628139
8	43958052	46958052	146274826
9	46035928	49035928	138429268
10	39244941	41624941	135413628
11	51450781	54450781	134452384
12	34747961	36142961	132449811
13	16000000	17868000	114142980
14	15070000	18070000	106368585
15	15260000	18260000	100338915
16	35143302	36943302	88827254
17	22187133	22287133	78774742
18	15400898	16764896	76117153
19	26923622	29923622	63811651
20	26267569	28033230	62435964
21	10260000	13260000	46944323
22	11330000	14330000	49554710
X	58465033	61465033	154824264
Y	11237315	12237315	57701691

```

> smoothChromosome <- function(obj, span) {
+   loessX <- function(X, location, span) {
+     fit <- loess(X ~ location, span = span)$fitted
+     return(fit)
+   }
+   obj <- obj[order(position(obj)), ]
+   cn.smooth <- apply(copyNumber(obj), 2, loessX, position(obj),
+     span = span)
+   rownames(cn.smooth) <- featureNames(obj)
+   call.smooth <- apply(calls(obj), 2, loessX, location = position(obj),
+     span = span)
+   rownames(call.smooth) <- featureNames(obj)
+   copyNumber(obj) <- cn.smooth
+   calls(obj) <- call.smooth
+   obj
+ }
> smoothList <- lapply(sim.list, smoothChromosome, span = 1/10)
> smoothSet <- unsplitS4(smoothList, featureData(sim2))

```

Or equivalently,

```

> smoothSet2 <- smoothSnp(sim1, 1:5, 1:3, span = 1/10)
> identical(copyNumber(smoothSet2), copyNumber(smoothSet))

```

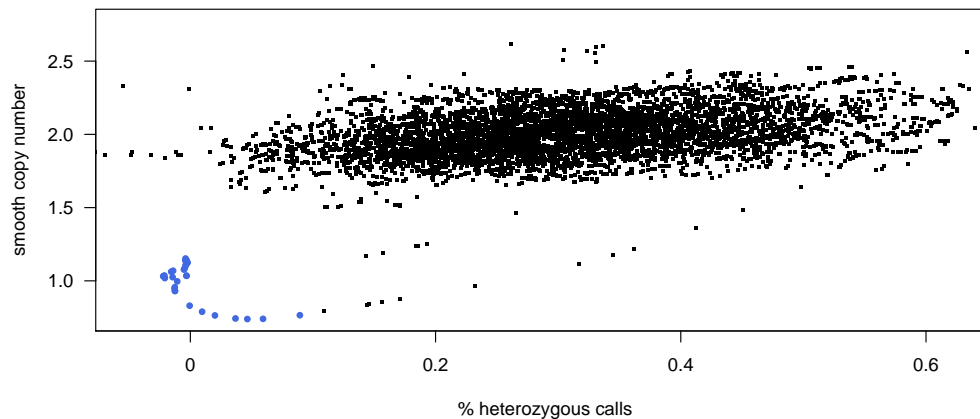
```
[1] TRUE
```

```
> identical(calls(smoothSet2), calls(smoothSet))
```

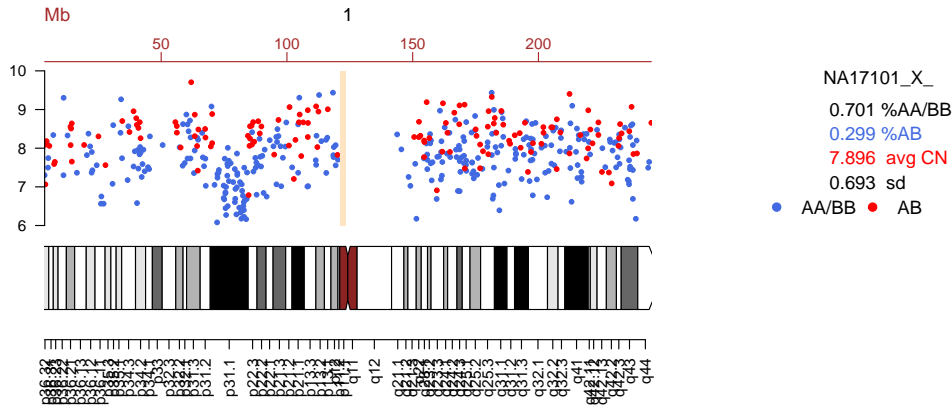
```
[1] TRUE
```

A plot of the smoothed calls versus copynumber can be used to visualize the deletion and deciding on a threshold for calling deletions.

```
> par(las = 1, mar = c(5, 4, 0.5, 0.5), oma = rep(0, 4))
> plot(calls(smoothSet2), copyNumber(smoothSet2), ylim = range(copyNumber(smoothSet2))
+     pch = ".", cex = 3, xlab = "% heterozygous calls", ylab = "smooth copy number",
+     xaxt = "n", xlim = c(-0.05, 30/70 + 0.2))
> axis(1, at = pretty(calls(smoothSet2)), labels = pretty(calls(smoothSet2)))
> highlight <- calls(smoothSet2) <= 0.1 & copyNumber(smoothSet2) <=
+     1.5
> points(calls(smoothSet2)[highlight], copyNumber(smoothSet2)[highlight],
+     pch = 20, col = "royalblue", bg = "white")
```



```
> copyNumber(sim1) <- copyNumber(sim1) + 2
> plotSnp(sim1, 1, 1, log = "", width.right = 0.5, cex.legend = c(1.2,
+     1.2), legend.panel = c(TRUE, FALSE), cex = c(1, 1, 1), pch = rep(20,
+     3), legend.location = c("topright", "bottomright"), addCytoband = TRUE)
```



5 Integration with other Bioconductor packages

To retrieve additional annotation on the known SNP's in the region of this simulated deletion, we could use the *RSNPper*. The installation instructions for *RSNPper* is available at Bioconductor.

```
> library(RSNPper)
> (dbId <- dbSnpId(annSnpset)[snps[2] == featureNames(annSnpset)])
> dbId <- strsplit(dbId, "rs")[[1]][2]
> print(SNPinfo(dbId))
```