VanillaICE: Hidden Markov Models for the Assessment of Chromosomal Alterations using High-throughput SNP Arrays

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1 Introduction

Chromosomal DNA is characterized by variation between individuals at the level of entire chromosomes (e.g. aneuploidy in which the chromosome copy number is altered), segmental changes (including insertions, deletions, inversions, and translocations), and changes to small genomic regions (including single nucleotide polymorphisms). A variety of alterations that occur in chromosomal DNA, many of which can be detected using high density single nucleotide polymorphism (SNP) microarrays, are linked to normal variation as well as disease and therefore of particular interest. These include changes in copy number (deletions and duplications) and genotype (e.g. the occurrence of regions of homozygosity). Hidden Markov models (HMM) are particularly useful for detecting such abnormalities, modeling the spatial dependence between neighboring SNPs. Here, we extend previous approaches that utilize HMM frameworks for inference in high throughput SNP arrays by integrating copy number, genotype calls, and the corresponding measures of uncertainty when available. Using simulated and real data, we demonstrate how confidence scores control smoothing in a probabilistic framework. The goal of this vignette is to provide a simple interface for fitting HMMs and plotting functions to help visualize the predicted states alongside the experimental data.

2 Simple Usage

2.1 Vanilla HMM

```
> library(VanillaICE)
```

Before fitting the HMM, the data must be organized into one of the following classes for high-throughput SNP data:

- `SnpCallSet` (genotype calls)
- `SnpCopyNumberSet` (copy number estimates)
- `oligoSnpSet` (genotype and copy number estimates)

When pre-processing Affymetrix SNP chips with the R package *oligo*, an object of one of the above classes is created and can be used directly with the HMMs described in this vignette. These classes can also be created from Illumina data as described in the IlluminaHowTo vignette. The simulated data provided with this package is an instance of class `oligoSnpSet`

```
> data(chromosome1)
> annotation(chromosome1)
```

```
[1] "pd.mapping50k.hind240,pd.mapping50k.xba240"
```

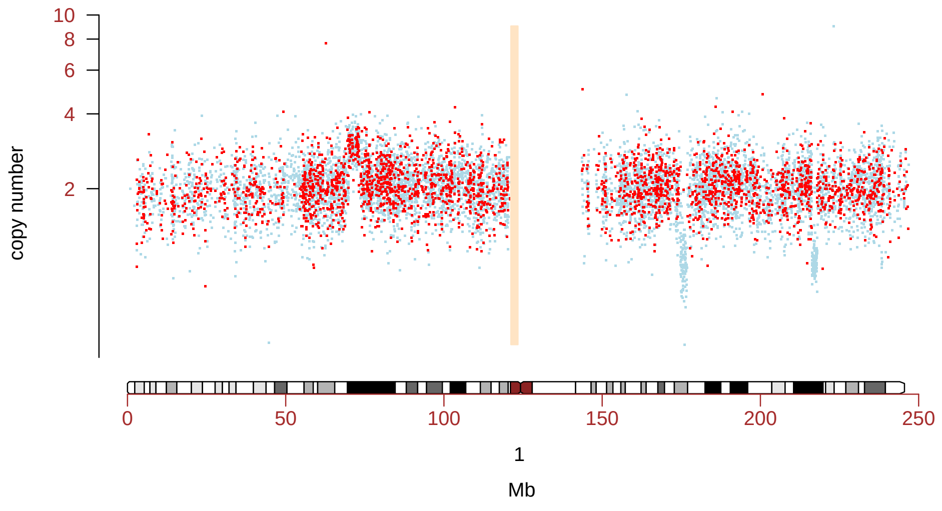
```
> chromosome1
```

```
oligoSnpSet (storageMode: lockedEnvironment)
assayData: 9165 features, 1 samples
  element names: calls, callsConfidence, cnConfidence, copyNumber
experimentData: use 'experimentData(object)'
Annotation: pd.mapping50k.hind240,pd.mapping50k.xba240
phenoData
An object of class "AnnotatedDataFrame"
  sampleNames: NA06993
  varLabels and varMetadata description:
    family: trio variable
    upd: uniparental isodisomy indicator
featureData
An object of class "AnnotatedDataFrame"
  rowNames: SNP_A-1677174, SNP_A-1718890, ..., SNP_A-1677548  (9165 total)
  varLabels and varMetadata description:
    dbsnp_rs_id: dbsnp_rs_id
    chromosome: chrom
    ...: ...
    enzyme: enzyme
    (8 total)
Annotation [1] "pd.mapping50k.hind240,pd.mapping50k.xba240"
```

Before deciding whether to fit a HMM, plot the data:

```
> gp <- new("ParSnpSet")
> gp <- getPar(gp, chromosome1)
> plotSnp(object = gp, snpset = chromosome1)
```

```
NULL
```



The HMM assumes that the copy number estimates, conditional on the hidden state, are approximately Gaussian. A log transformation is often helpful:

```
> copyNumber(chromosome1) <- log2(copyNumber(chromosome1))
```

See the documentation pages in the R package *VanillaICE* for more information about the `chromosome1` example dataset. Parameters for fitting the HMM are obtained by generating an instance of the class `HmmParameter`. By default, the HMM assumes the hidden states are deletion (D), normal (N), LOH (L), and amplification (A). See the HmmParameterClass vignette for more information. An instance of the class is created by the method new:

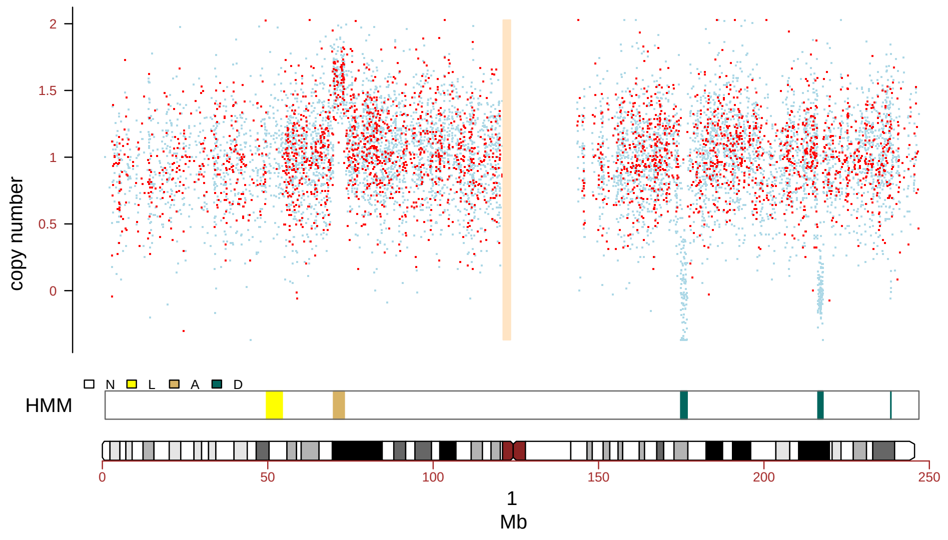
```
> params <- new("HmmParameter", chromosome1, states = c("D",
+     "N", "L", "A"), cn.location = log2(c(1, 2, 2, 3)))

[1] "Calculating emission probabilities for genotype calls.... "
[1] "Calculating emission probabilities for copy number estimates... "

> class(params)

[1] "HmmParameter"
attr(,"package")
[1] "VanillaICE"
```

We may then fit the HMM by

```
> fit <- hmm(params, chromosome1)
> fit

Instance of class HmmPredict
[1] "predictions:"
 num [1:9165, 1] 2 2 2 2 2 2 2 2 2 2 ...
 - attr(*, "dimnames")=List of 2
  ..$ : chr [1:9165] "SNP_A-1677174" "SNP_A-1718890" "SNP_A-1678466" "SNP_A-1676440" ...
  ..$ : chr "NA06993"

 breakpoints:
$NA06993
        id  chr state   size   N   start    last      prev      next
1  NA06993    1     N 48.708 997   0.837  49.545        NA  49.59781
2  NA06993    1     L  4.812 102  49.598  54.410  49.54504  54.40975
NA    <NA> <NA>  <NA>     NA  NA      NA      NA        NA        NA
10 NA06993    1     D  0.098   5 238.320 238.418 238.30267 238.41786
11 NA06993    1     N  8.431 160 238.430 246.861 238.41786 246.86099


 featureData
An object of class "AnnotatedDataFrame"
  rowNames: SNP_A-1677174, SNP_A-1718890, ..., SNP_A-1677548  (9165 total)
  varLabels and varMetadata description:
    chromosome: chromosome
    arm: chromosomal arm
    position: physical position
```

See [2] for a more complete description of the simulated dataset and the features detected by this HMM. We may plot the data along with the predictions as follows:

```
> fn <- featureNames(params)
> idx <- match(fn, featureNames(chromosome1))
> chromosome1 <- chromosome1[idx, ]
> gp <- new("ParHmmSnpSet")
> gp <- getPar(gp, snpset = chromosome1)
> plotSnp(object = gp, snpset = chromosome1, hmmPredict = fit)

[1] ""
```



2.2 Integrating Confidence Estimates (ICE)

In this section, we illustrate how one may fit an HMM that incorporates confidence esimates of the SNP-level summaries for genotype calls and copy number. Confidence scores (inverse of standard errors) are available for this object (see Section 2.3 for how confidence scores were derived). This information is incorporated into the HMM emission probabilities in the following way:

```
> params.ice <- new("HmmParameter", chromosome1, states = c("D",
+     "N", "L", "A"), cn.ICE = TRUE)

[1] "Calculating emission probabilities for genotype calls.... "
[1] "Calculating emission probabilities for copy number estimates... "
```

We may also incorporate the confidence scores for the genotype calls:

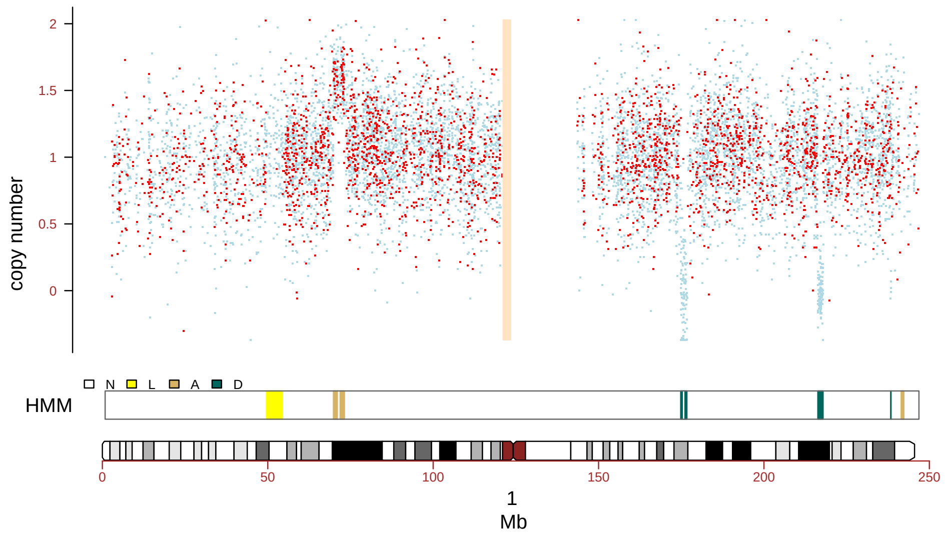
```
> params.ice <- new("HmmParameter", chromosome1, states = c("D",
+     "N", "L", "A"), cn.ICE = TRUE, gt.ICE = TRUE)
```

We fit the HMM in the usual way

```
> fit.ice <- hmm(params.ice, chromosome1)
```

and plot the results using the same graphical parameters as above:

```
> plotSnp(object = gp, snpset = chromosome1, hmmPredict = fit.ice)
[1] ""
```



Note that the ICE HMM correctly identifies the simulated normal segments in features B and C (the normal segments were simulated to have high confidence scores). Additionally, the ICE HMM detects the micro-amplification in region E (also simulated to have high confidence scores).

2.3 Confidence scores

Confidence scores for genotype calls We suggest using the CRLMM algorithm [1] for genotype calls. CRLMM (in the R package *oligo*) provides confidence scores ($S_{\widehat{GT}}$) of the genotype estimates ($\widehat{GT}$). From 269 HapMap samples assayed on the Affymetrix 50k Xba and Hind chips, we have a gold standard of the true genotype defined by the consensus of the HapMap centers. We use kernal based density estimates to obtain

$$f\left\{ S_{\widehat{HOM}} \mid \widehat{HOM}, HOM \right\},\ f\left\{ S_{\widehat{HOM}} \mid \widehat{HOM}, HET \right\},\ f\left\{ S_{\widehat{HET}} \mid \widehat{HET}, HOM \right\},\quad \text{and } f\left\{ S_{\widehat{HET}} \mid \widehat{HET}, HET \right\} \tag{1}$$

separately for the Xba and Hind 50k chips. The first term in (1), for example, denotes the density of the scores when the genotype is correctly called homozygous ($\widehat{HOM}$) and the true genotype is homozygous (HOM). See [2] for a more complete description of the methods. The data needed to estimate these densities is stored in the experiment data package *callsConfidence*. *callsConfidence* is available from the author's website.

Confidence scores for copy number estimates To illustrate how standard errors of the copy number estimate could be integrated in the HMM, the R object `chromosome1` contains standard errors simulated from a shifted Gamma: $\text{Gamma}(1, 2) + 0.3$, where 1 is the shape parameter and 2 is the rate parameter. To ascertain the effect of qualitatively high confidence scores on the ICE HMM, we scaled a robust estimate of the copy number standard deviation by $\frac{1}{2}$. Similarly, to simulate less precise $\widehat{CN}$ we scaled ϵ by 2. For more detailed information about how the data in the `chromosome1` was generated, see the documentation for this object in the R package *VanillaICE*.

3 The HmmParameter class

The minimal information required to create an instance of class `HmmParameter` is

1. an object inheriting from the class `SnpLevelSet`. For instance, an `oligoSnpSet`.
2. a vector of names for the hidden states. Only the name for the normal state, “N”, requires a controlled vocubulary.
3. optionally, one may define any of the following arguments when creating an instance of the class:
   - `SCALE`
   - `tau.scale`
   - `tau`
   - `pi`
   - `beta`
   - `cn.location`
   - `cn.scale`
   - `cn.ICE`
   - `gt.ICE`
   - `gt.gte`
   - `gt.state`
   - `gt.confidence`
   - `gt.confidence.states`
   - `notes`
   - `annotation`

An instance of the class is created by the method `new`:

```
> params <- new("HmmParameter", chromosome1, states = c("D",
+     "N", "L", "A"), cn.location = log2(c(1, 2, 2, 3)))
```

The object `params` contains all of the parameters needed for fitting the HMM, including the transition probabilities (tau), emission probabilities (beta), and initial state probabilities (pi). A summary of the parameters contained in the object `params` is obtained by

```
> params
```

```
Formal class 'HmmParameter' [package "VanillaICE"] with 7 slots
  ..@ hmmOptions :Formal class 'HmmOptions' [package "VanillaICE"] with 11 slots
  .. .. ..@ SnpClass            : atomic [1:1] oligoSnpSet
  .. .. .. ..- attr(*, "package")= chr "oligoClasses"
  .. .. ..@ states              : chr [1:4] "D" "N" "L" "A"
  .. .. ..@ cn.location         : num [1:4] 0.00 1.00 1.00 1.58
  .. .. ..@ cn.robustSE         : Named num 0.31
  .. .. .. ..- attr(*, "names")= chr "NA06993"
  .. .. ..@ cn.ICE              : logi FALSE
  .. .. ..@ gt.ICE              : logi FALSE
  .. .. ..@ gt.gte              : chr "not used if gt.ICE=FALSE"
  .. .. ..@ gte.state           : Named num [1:4] 0.999 0.75 0.999 0.75
  .. .. .. ..- attr(*, "names")= chr [1:4] "D" "N" "L" "A"
  .. .. ..@ gt.confidence.states: chr(0)
  .. .. ..@ annotation          : chr "pd.mapping50k.hind240,pd.mapping50k.xba240"
  .. .. ..@ notes               : chr ", gt.state is P(gte = HOM | state)"
  ..@ tau        : num [1:9164] 0.973 0.986 1.000 1.000 1.000 ...
  ..@ tau.scale  : num [1:4, 1:4] 1 1 0.75 0.75 1.5 1 1.5 1.5 0.75 1 ...
  .. ..- attr(*, "dimnames")=List of 2
  .. .. ..$ : chr [1:4] "D" "N" "L" "A"
  .. .. ..$ : chr [1:4] "D" "N" "L" "A"
  ..@ pi         : Named num [1:4] -8.006 -0.001 -8.006 -8.006
  .. ..- attr(*, "names")= chr [1:4] "D" "N" "L" "A"
  ..@ beta       : num [1:9165, 1:4, 1] -4.9494 -2.8644 -7.0156  0.0893 -1.0799 ...
  ..@ featureData:Formal class 'AnnotatedDataFrame' [package "Biobase"] with 4 slots
  .. .. ..@ varMetadata      :'data.frame':        3 obs. of  1 variable:
  .. .. .. ..$ labelDescription: chr [1:3] "chromosome" "chromosomal arm" "physical position"
  .. .. ..@ data             :'data.frame':        9165 obs. of  3 variables:
  .. .. .. ..$ chromosome: chr [1:9165] "1" "1" "1" "1" ...
  .. .. .. ..$ arm       : chr [1:9165] "p" "p" "p" "p" ...
  .. .. .. ..$ position  : int [1:9165] 836727 2224111 2915399 2926730 2941104 2941694 2963436 3084986
  .. .. ..@ dimLabels        : chr [1:2] "rowNames" "columnNames"
  .. .. ..@ .__classVersion__:Formal class 'Versions' [package "Biobase"] with 1 slots
  .. .. .. .. ..@ .Data:List of 1
  .. .. .. .. .. ..$ : int [1:3] 1 1 0
  ..@ notes      : chr(0)
```

The emission probabilities are stored as an array in the `params` object. The emission probability array has dimension R × S × C, where S is the number of hidden states, R is the number of rows (SNPs), and C is the number of samples. To obtain the emission probabilities for the *ith* SNP, one may use [to subset. For instance, the emission probabilities for the 5th SNP are:

```
> tmp <- params[5, 1]
> exp(Beta(tmp))

, , 1

          [,1]      [,2]      [,3]        [,4]
[1,] 0.3396269 0.2710860 0.3610865 0.002263227
```

FIXME: Note, currently the

```
> lapply(tmp[[1]]$tau, function(x) exp(round(x, 4)))
```

The probability of remaining in the same state, $P(S_t = S_{t+1})$ (the diagonal of the transition probability matrix) is a function of the distance between SNPs. The probability of transitioning to some other state is ϵ, where $\epsilon = 1 - P(S_t = S_{t+1})$. The ϵ is split among $S - 1$ states. By default, the probability of transitioning from an altered state back to the normal state is twice as likely as the probability of transitioning between two altered states. The weights for ϵ are provided in the `tau.scale` matrix in the R object `params`

```
> params@tau.scale
```

```
     D   N    L    A
D 1.00 1.5 0.75 0.75
N 1.00 1.0 1.00 1.00
L 0.75 1.5 1.00 0.75
A 0.75 1.5 0.75 1.00
```

and can be adjusted by the `SCALE` argument when creating an instance of the class. For instance, here we make the probability of transitioning from an altered state to a normal state 10 times as likely as the probability of transitioning between two altered states:

```
> params <- new("HmmParameter", chromosome1, states = c("D",
+     "N", "L", "A"), cn.location = log2(c(1, 2, 2, 3)),
+     SCALE = 10)

[1] "Calculating emission probabilities for genotype calls.... "
[1] "Calculating emission probabilities for copy number estimates... "
```

To retrieve the copy number and genotype for the 5th SNP, one should use `match`:

```
> i <- match(names(tmp), featureNames(chromosome1))
> c(copyNumber(chromosome1[i, ]), calls(chromosome1[i,
+     ]))

numeric(0)
```

4 The HmmPredict Class

The output from the HMM is an instance of the `HmmPredict` class and contains the predicted states as well as the breakpoints for the different states.

```
> fit

Instance of class HmmPredict
[1] "predictions:"
 num [1:9165, 1] 2 2 2 2 2 2 2 2 2 2 ...
 - attr(*, "dimnames")=List of 2
  ..$ : chr [1:9165] "SNP_A-1677174" "SNP_A-1718890" "SNP_A-1678466" "SNP_A-1676440" ...
  ..$ : chr "NA06993"

 breakpoints:
$NA06993
        id  chr state   size   N   start    last      prev      next
1  NA06993    1     N 48.708 997   0.837  49.545        NA  49.59781
2  NA06993    1     L  4.812 102  49.598  54.410  49.54504  54.40975
NA    <NA> <NA>  <NA>     NA  NA      NA      NA        NA        NA
10 NA06993    1     D  0.098   5 238.320 238.418 238.30267 238.41786
11 NA06993    1     N  8.431 160 238.430 246.861 238.41786 246.86099


 featureData
An object of class "AnnotatedDataFrame"
  rowNames: SNP_A-1677174, SNP_A-1718890, ..., SNP_A-1677548  (9165 total)
  varLabels and varMetadata description:
    chromosome: chromosome
    arm: chromosomal arm
    position: physical position
```

The breakpoints are provided as a list, whereby each element in the list corresponds to a sample:

```
> breaks <- breakpoints(fit)
> breaks <- breaks[[1]]
> breaks <- breaks[breaks[, "state"] != "N", ]
```

One may order the altered states from biggest to smallest for each chromosome as follows:

```
> breaks <- breaks[order(breaks[, "chr"], breaks[, "size"],
+     decreasing = TRUE), ]
```

5 HMMs for different classes of data

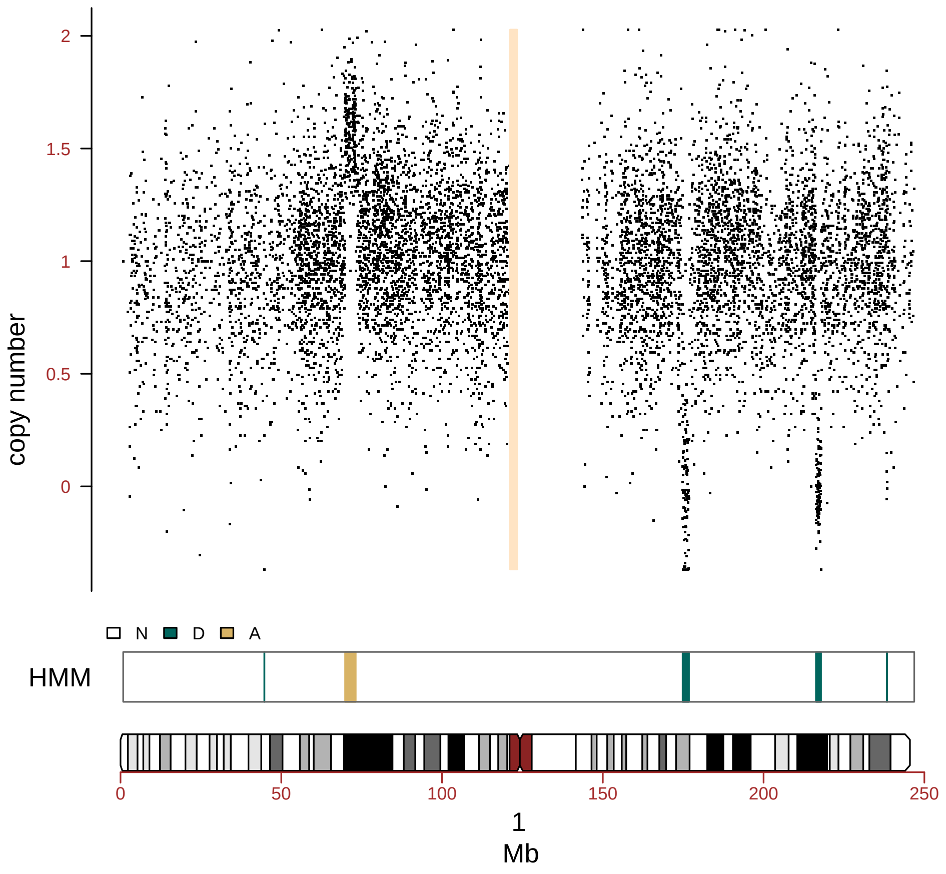
5.1 Copy number

The method `hmm` has a different set of underlying hidden states depending on whether copy number estimates, genotype calls, or both are available. When only copy number estimates are available, the hidden states (for autosomes) are hemizygous or homozygous deletion (one or fewer copies), normal (two copies), and amplification (three or more copies). The corresponding data class is SnpCopyNumberSet. To illustrate, we convert the `chromosome1` example to an object of this class and fit the HMM.

```
> chr1.cn <- as(chromosome1, "SnpCopyNumberSet")
> params.cn <- new("HmmParameter", snpset = chr1.cn, cn.location = log2(1:3),
+     states = c("D", "N", "A"))

[1] "Calculating emission probabilities for copy number estimates... "

> fit.cn <- hmm(params.cn, chr1.cn)
> breakpoints(fit.cn)

$NA06993
        id chr state       size    N      start      last      prev
1  NA06993   1     N  43.865725  877   0.836727  44.70245        NA
2  NA06993   1     D   0.040126    2  44.722116  44.76224  44.70245
3  NA06993   1     N  25.058717 1122  44.779351  69.83807  44.76224
4  NA06993   1     A   3.299434  202  69.854466  73.15390  69.83807
5  NA06993   1     N 101.640222 3797  73.174070 174.81429  73.15390
6  NA06993   1     D   1.985748  101 174.815096 176.80084 174.81429
7  NA06993   1     N  39.313361 1899 176.926556 216.23992 176.80084
8  NA06993   1     D   1.586808   99 216.286002 217.87281 216.23992
9  NA06993   1     N  20.410491  901 217.892177 238.30267 217.87281
10 NA06993   1     D   0.097921    5 238.319943 238.41786 238.30267
11 NA06993   1     N   8.431130  160 238.429864 246.86099 238.41786
        next
1   44.70245
2   44.76224
3   69.85447
```

```
4   73.17407
5  174.81429
6  176.80084
7  216.28600
8  217.89218
9  238.31994
10 238.41786
11 246.86099
```

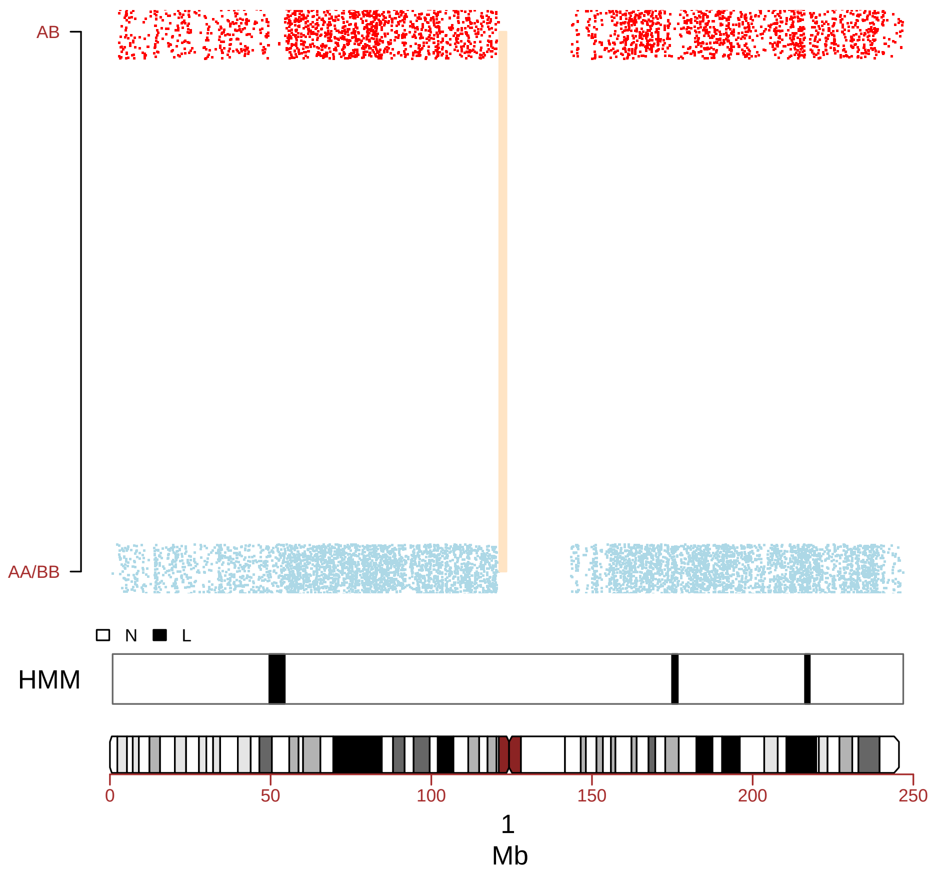
```
> graph.par <- new("ParHmmSnpCopyNumberSet")
> graph.par <- getPar(graph.par, chr1.cn)

> plotSnp(graph.par, chr1.cn, fit.cn)

[1] ""
```



5.2 Genotype calls

When only genotype calls are available, the hidden states are loss and retention (ret) of heterozygosity. We define *loss* to be a sequence of homozygous SNPs longer than what we would expect to observe by chance. Note that many long stretches of homozygosity may occur as a result of a population sharing a common underlying haplotype structure; loss predictions from an HMM fit to an indvidual do not necessarily reflect the 'loss' of an allele in that individual. For illustration, we convert the `chromosome1` example to an object of class `HmmSnpCallSet` and refit the HMM.

```
> chr1.calls <- as(chromosome1, "SnpCallSet")
> params.calls <- new("HmmParameter", snpset = chr1.calls,
+     states = c("L", "N"))

[1] "Calculating emission probabilities for genotype calls.... "

> fit.calls <- hmm(params.calls, chr1.calls)
> breakpoints(fit.calls)

$NA06993
       id chr state       size    N      start      last      prev
1 NA06993   1     N  48.708312  997   0.836727  49.54504        NA
2 NA06993   1     L   4.811945  102  49.597810  54.40975  49.54504
3 NA06993   1     N 120.350649 4907  54.498950 174.84960  54.40975
4 NA06993   1     L   1.788366   92 174.915701 176.70407 174.84960
5 NA06993   1     N  39.439518 1902 176.800399 216.23992 176.70407
6 NA06993   1     L   1.485826   97 216.286002 217.77183 216.23992
7 NA06993   1     N  28.988981 1068 217.872013 246.86099 217.77183
       next
1  49.59781
2  54.40975
3 174.91570
4 176.80040
5 216.28600
6 217.87201
7 246.86099

> gp <- new("ParHmmSnpCallSet")
> graph.par <- getPar(gp, chr1.calls)

> plotSnp(graph.par, chr1.calls, fit.calls)

[1] ""
```



5.3 Genotype calls and copy number

Section 2 illustrates how one may fit the HMM to objects of class `oligoSnpSet`.

More documentation about the classes can be found in the documentation for the R package *VanillaICE*.

6 Session Information

The version number of R and packages loaded for generating the vignette were:

- R version 2.7.0 Under development (unstable) (2008-01-28 r44219), `powerpc-apple-darwin8.11.0`
- Locale: `C`
- Base packages: base, datasets, grDevices, graphics, methods, stats, tools, utils
- Other packages: Biobase 1.17.13, RColorBrewer 1.0-1, SNPchip 1.3.13, VanillaICE 1.1.13, oligoClasses 1.1.13

References

[1] Benilton Carvalho, Henrik Bengtsson, Terence P Speed, and Rafael A Irizarry. Exploration, normalization, and genotype calls of high-density oligonucleotide SNP array data. *Biostatistics*, 8(2):485–499, Apr 2007.

[2] Robert B Scharpf, Giovanni Parmigiani, Jonathan Pevsner, and Ingo Ruczinski. A hidden Markov model for joint estimation of genotype and copy number in high-throughput SNP chips. Technical Report Working Paper 136, Johns Hopkins University, February 2007.