Rolexa: Probabilistic Base Calling of Solexa Sequencing Data

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1 Introduction

This package provides an alternative base calling algorithm using model-based clustering (*mclust*) and probability theory to identify ambiguous bases and code them with IUPAC symbols. We also select optimal sub-tags using a score based on information content to remove uncertain bases towards the ends of the reads. There are also a few diagnostic plots functionalities. Details of the algorithms were published in [1].

2 Environment variables

The *Rolexa* package uses a `RolexaRun` object to store the various parameters of the run, and uses the *ShortRead* for manipulating data, in particular many *Rolexa* functions take a `SolexaPath` object as argument.

We load the library and create a configuration with default parameters except for the `idsep` variable:

```
> library(Rolexa)

by using mclust, invoked on its own or through another package,
you accept the license agreement in the mclust LICENSE file
and at http://www.stat.washington.edu/mclust/license.txt

> rolenv = SetModel(idsep = "_")
> GetModel(rolenv)

$MinimumTagLength
[1] 15

$SequencingLength
[1] 36

$Barcode
[1] 0

$HThresholds
[1] 0.5849625 1.3219281 1.8073549

$IThresholds
 [1] 2.058894 2.115477 2.169925 2.222392 2.273018 2.321928 2.369234 2.415037
 [9] 2.459432 2.502500 2.544321 2.584963 2.624491 2.662965 2.700440 2.736966
[17] 2.772590 2.807355 2.841302 2.874469 2.906891 2.938599 2.969626 3.000000
[25] 3.029747 3.058894 3.087463 3.115477 3.142958 3.169925 3.196397 3.222392
[33] 3.247928 3.273018 3.297681 3.321928

$PET
[1] FALSE

$fit
[1] FALSE

$normal
[1] TRUE

$decorrelate
[1] "both"

$verbose
[1] 0

$colors
 [1] "black"      "green"      "blue"       "chocolate3" "red"
 [6] "#007F7F"    "#66B20E"    "#7F7F00"    "#66338E"    "#7F007F"
[11] "#E6330E"    "#7F464E"    "#7F6035"    "#6C5649"    "#685F4C"
[16] "gray"

$idsep
[1] "_"
```

The meaning of these parameters is as follows:

MinimumTagLength tags shorter than this will not be saved

SequencingLength number of sequencing cycles, used to calculate the number of columns in files

Barcode number of bases used as barcode at the beginning of the tag

HThresholds entropy thresholds between 1 and 2-base ambiguities, 2 and 3-base ambiguities and 3-base ambiguity or undecided (the default is $\log_2(c(1.5, 2.5, 3.5)))$

IThresholds total entropy thresholds, as a function of tag length (the default is $\log_2(4 + 1 : 36/6))$

PET paired-end sequencing run

fit use full EM convergence instead of only one-step optimization if `TRUE`

normal use tile-level normalization before base-calling if `TRUE`

decorrelate use 'cycle'-level decorrelation procedure, 'channel'-level, 'both' or 'none'

idsep character separating coordinate fields in sequence headers (default is ":")

verbose print debug information if > 0

3 Loading data

Loading data is done using the *ShortRead* utilities (in particular the `SolexaPath` class) with two additional wrappers `CombineReads` and `CombineFastQ`:

```
> path = SolexaPath(system.file("extdata", package = "ShortRead"))
```

Then use the loading functions to read a selection of those files:

```
> (int = readIntensities(path, pattern = "s_1_0001", withVariability = FALSE))

class: SolexaIntensity
dim: 256 4 36
readInfo: SolexaIntensityInfo
intensity: ArrayIntensity
measurementError: not available

> (seq = CombineReads(run = rolenv, path = path, pattern = "s_1_0001_seq*"))
```

```
class: ShortRead
length: 256 reads; width: 36 cycles

> (seq_fastq = readFastq(path))

class: ShortReadQ
length: 256 reads; width: 36 cycles
```

4 Data transforms

Before going into the base calling itself, we can perform several data transformations to remove some of the systematic biases:

1. Reduce cross-talk between color channels

   ```
   > (theta = OptimizeAngle(int = int))[1:10, ]

              [,1]     [,2]      [,3]     [,4]
    [1,] 0.7767119 1.375080 0.4721182 1.557188
    [2,] 0.7653824 1.377907 0.5618510 1.570796
    [3,] 0.7276859 1.367992 0.5290140 1.570796
    [4,] 0.7551378 1.384266 0.6453509 1.570796
    [5,] 0.7349694 1.377229 0.6220983 1.570796
    [6,] 0.7377151 1.383378 0.6556697 1.564773
    [7,] 0.7213154 1.377866 0.6412864 1.570796
    [8,] 0.7685749 1.384597 0.6472642 1.570796
    [9,] 0.7681729 1.387350 0.5537521 1.570796
   [10,] 0.7710965 1.379977 0.6961033 1.570796

   > int = DeCorrelateChannels(int = int, theta = theta)
   ```

2. Reduce dephasing along cycles

   ```
   > (rate = OptimizeRate(int = int))

   [1] 0.01760222

   > int = DeCorrelateCycles(int = int, rate = rate)
   ```

3. Reduce position-dependent bias within each tile

   ```
   > int2 = TileNormalize(run = rolenv, int = int)
   ```

5 Base calling

The base calling algorithm fits a gaussian mixture model to the four-dimensional intenity values from each cycle. Sequences from a previous base calling, if available, are used to seed the algorithm:

```
> (res = SeqScore(run = rolenv, int = int, seqInit = seq, cycles = 1:36))$sread

  A DNAStringSet instance of length 256
      width seq
  [1]    36 TTGTTTTCATGTGATTTTAAAAATGTATTTGTTTGT
  [2]    36 TCCAAACTGGTAGACAATACAAACATTCTCAAATCT
  [3]    36 TGCACCTGATAGGGTCTCTGCTCTGAGAGAGDAAGK
  [4]    36 TATGAGAGTAGCYAATGCCACAAAGWSGRKGTGKBY
  [5]    36 TAGTAGGTGTCCTATTCTGATGCYCAGCACGCCAAG
  [6]    36 GAGAGAACTGAAAATCACAGAATATGAGAAATAGAC
  [7]    36 GCAGAGACCCACAASCCAGCCAAGCGGCTCCWGACC
  [8]    36 GAGATATTTATTGAACACTAACACTCTGTCATGCAA
  [9]    36 GGTGGAAAWAGGAAGCAYCCCSYTYTCYGCTTAYAT
  ...   ... ...
[248]    36 TGGGGAGMYGKGGGMYMTGGCKGGMYRTHHRWVVDK
[249]    36 GTGGAGGCTAGCACCTGTTTGTGGCBTTGTGARGBA
[250]    36 GATTTTCAAAGTTAAGGGTAAAAATGTTATCACCCG
[251]    36 GAAAATGAGAAACATACAATTGACGACTTGAAAAAT
[252]    36 GGYATTTTCCTTTTGTTTTATTTMRCTTTGKWGBDH
[253]    36 GGTAGGRAGAGCTCGTGKGCCGTCTTCTGCTTRRAW
[254]    36 GAAAAACGWGAAAAATGAGAAWTGCACACTGTAGRA
[255]    36 GATTCCTTATGTGGTAATGGAAAATAATATTTCATC
[256]    36 GGATGAGAAGAATAGTATATTACATCTCTAGCCACA
```

6 Filtering and saving

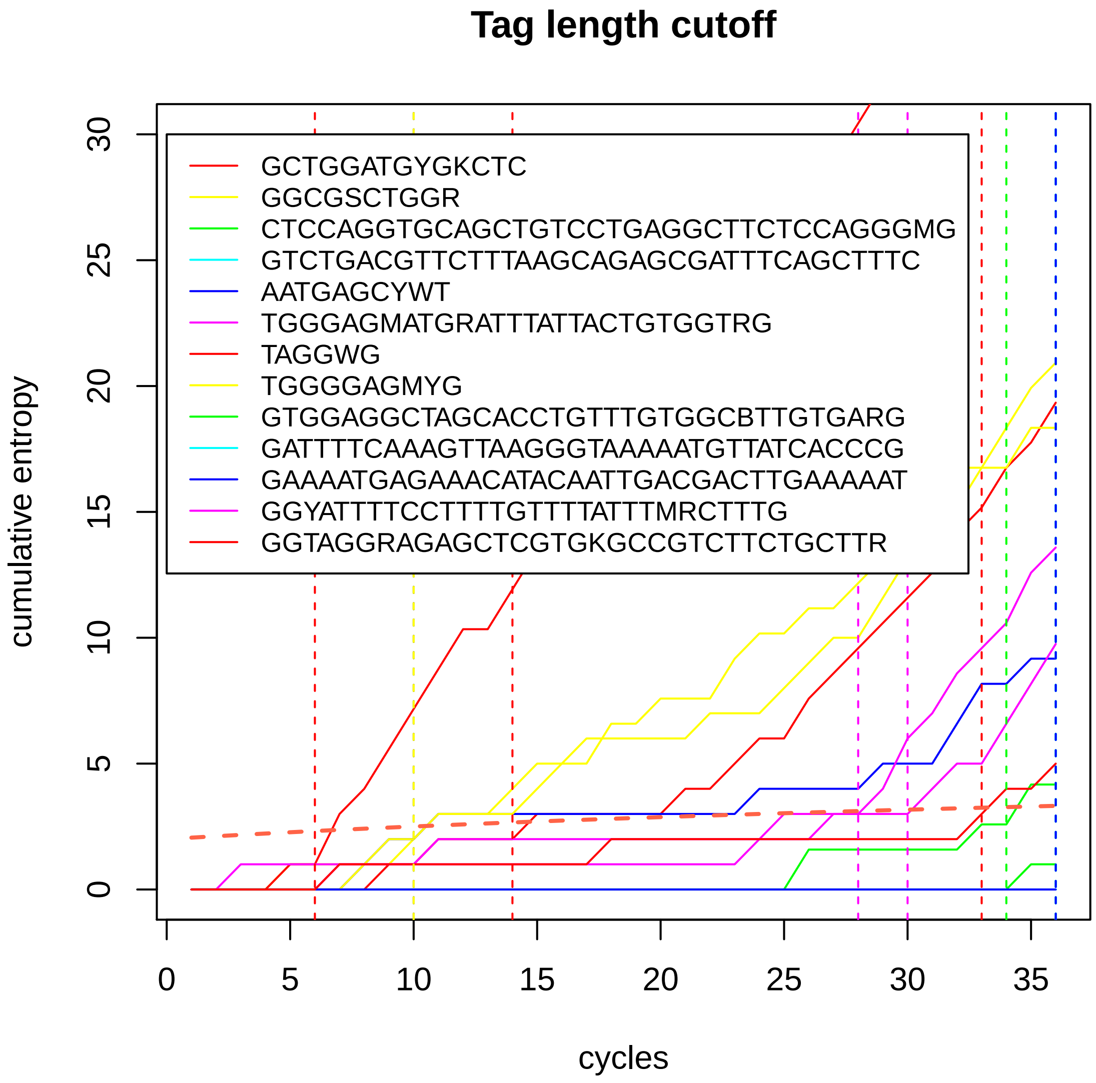
The base calling results consist of a full-length tag with base quality entropy scores, which can then be filtered to extract the best sequence tag for each colony. This is where the parameters IThresholds comes into play:

```
> rolenv@MinimumTagLength = as.integer(1)
> (res2 = FilterResults(run = rolenv, results = res))$sread

  A DNAStringSet instance of length 256
      width seq
  [1]    36 TTGTTTTCATGTGATTTTAAAAATGTATTTGTTTGT
  [2]    36 TCCAAACTGGTAGACAATACAAACATTCTCAAATCT
  [3]    36 TGCACCTGATAGGGTCTCTGCTCTGAGAGAGDAAGK
  [4]    28 TATGAGAGTAGCYAATGCCACAAAGWSG
  [5]    36 TAGTAGGTGTCCTATTCTGATGCYCAGCACGCCAAG
  [6]    36 GAGAGAACTGAAAATCACAGAATATGAGAAATAGAC
  [7]    36 GCAGAGACCCACAASCCAGCCAAGCGGCTCCWGACC
  [8]    36 GAGATATTTATTGAACACTAACACTCTGTCATGCAA
  [9]    21 GGTGGAAAWAGGAAGCAYCCC
  ...   ... ...
[248]    10 TGGGGAGMYG
[249]    34 GTGGAGGCTAGCACCTGTTTGTGGCBTTGTGARG
```

```
[250]    36 GATTTTCAAAGTTAAGGGTAAAAATGTTATCACCCG
[251]    36 GAAAATGAGAAACATACAATTGACGACTTGAAAAAT
[252]    30 GGYATTTTCCTTTTGTTTTATTTMRCTTTG
[253]    33 GGTAGGRAGAGCTCGTGKGCCGTCTTCTGCTTR
[254]    36 GAAAAACGWGAAAAATGAGAAWTGCACACTGTAGRA
[255]    36 GATTCCTTATGTGGTAATGGAAAATAATATTTCATC
[256]    36 GGATGAGAAGAATAGTATATTACATCTCTAGCCACA
```

```
> str = as.matrix(res$sread[241:253])
> nt = DNA_ALPHABET
> post.entropy = matrix(0, nrow = nrow(str), ncol = 36)
> post.entropy[which(str %in% nt[5:10])] = 1
> post.entropy[which(str %in% nt[11:14])] = log2(3)
> post.entropy[which(str == "N")] = 2
> matplot(1:36, y = apply(post.entropy, 1, cumsum), t = "l", lty = 1,
+     col = rainbow(6), ylim = c(0, 30), xlim = c(1, 36), xlab = "cycles",
+     ylab = "cumulative entropy", main = "Tag length cutoff")
> lines(1:36, rolenv@IThresholds, t = "l", lty = 2, lwd = 2, col = "tomato")
> abline(v = nchar(res2$sread[241:253]), col = rainbow(6), lty = 2)
> legend(x = 0, y = 30, res2$sread[241:253], col = rainbow(6),
+     lty = 1, bg = "white", cex = 0.8)
```



The final step is to save results:

```
> SaveResults(run = rolenv, results = res2, outpath = "./")
```

7 Batch execution

The whole sequence of operations needed to load, analyse, filter and save a sequencing run can be performed in parallel (using calls to the *fork* package) via the function `ForkBatch`:

```
> library(fork)
> ForkBatch(run = rolenv, path = path, outpath = "./", prefix = "rs_",
+     nthreads = 2, nfiles = 5, lane = 1, tiles = 1, idsep = "_")
```

Each of the `nthreads` threads will execute a call to

```
> OneBatch(run = rolenv, path = path, lane = 1, tiles = tiles[n:m],
+     outpath = "./", prefix = "rs_")
```

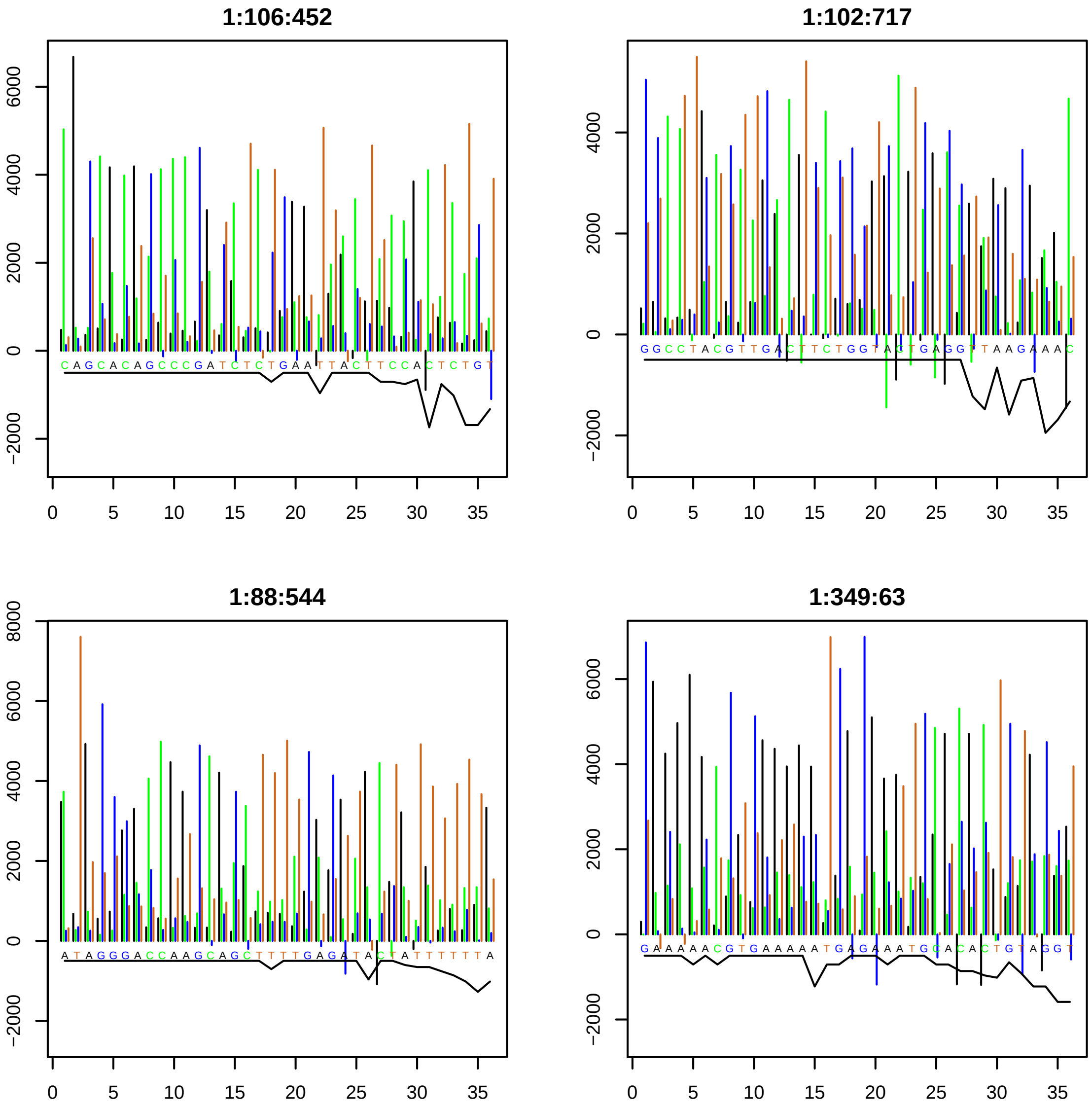
This function can be used in a loop on single-processor systems or in independent jobs distributed on a computing cluster.

8 Diagnostic plots

There are multiple possibilities for evaluating the quality of the base calling, at the level of each sequence, tile or lane.

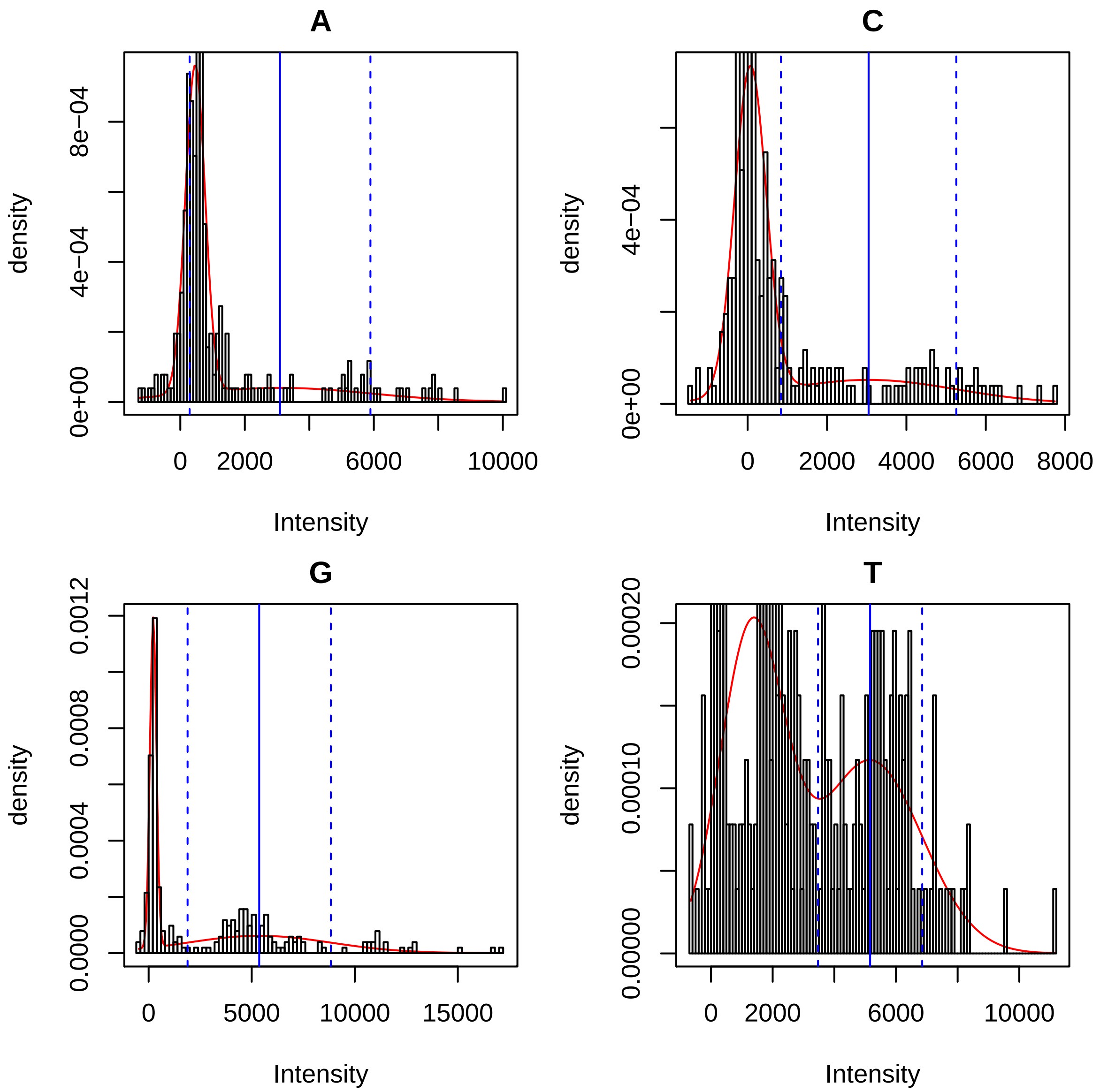
Given a sequence tag, the corresponding raw intensities and a base quality score, we can use `CombinedPlot`:

```
> CombinedPlot(run = rolenv, int = int, seq = seq, scores = as(quality(seq_fastq),
+     "matrix"), colonies = sample(1:nrow(int), 4), par = list(mfrow = c(2,
+     2), cex = 0.6, mar = c(4, 4, 2, 1) + 0.1))
```

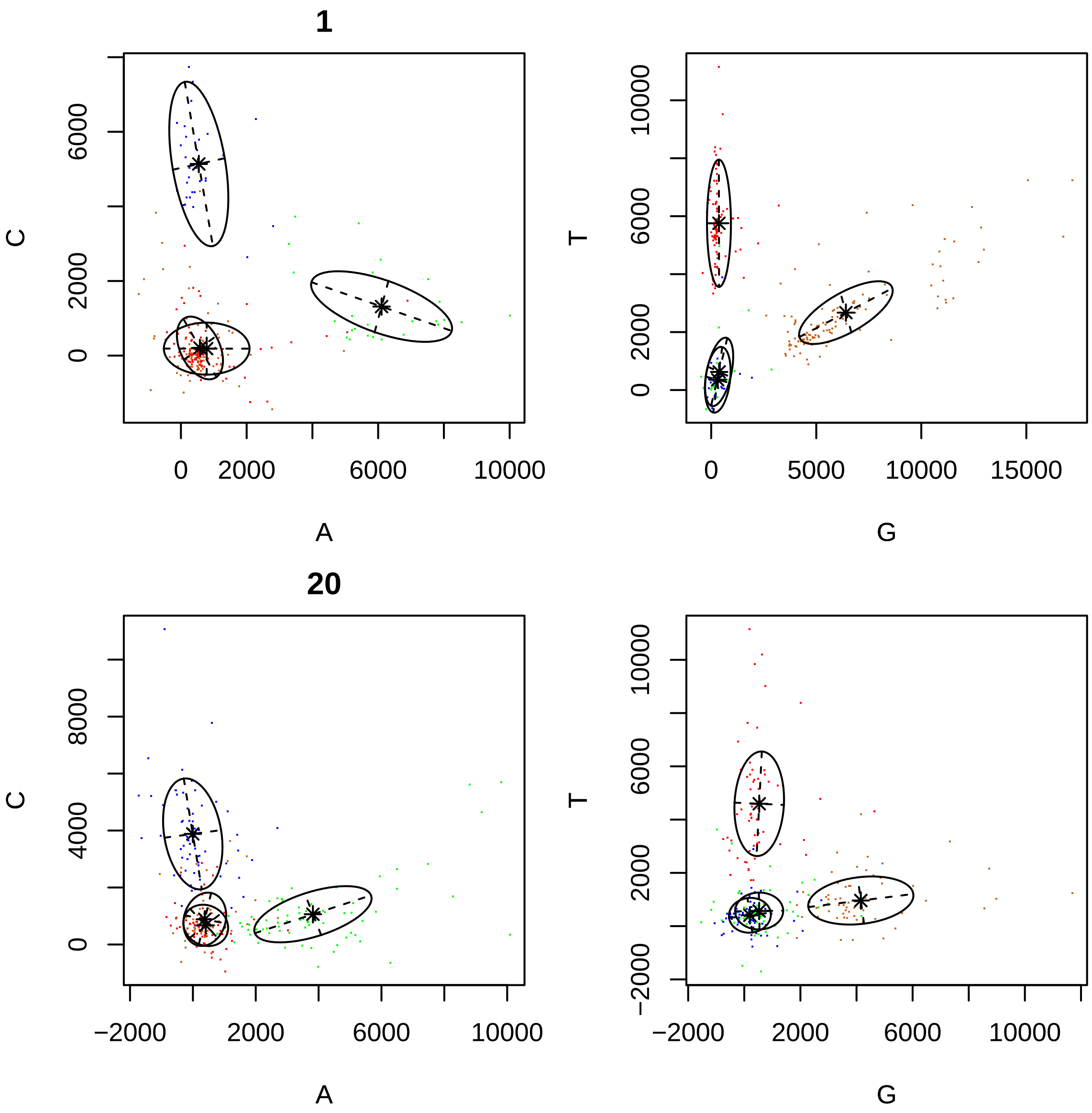


we can also evaluate the distribution of intensity values at selected cycles via 1- and 2-dimensional projections:

```
> ChannelHistogram(int = int, cycles = 1, par = list(mfrow = c(2,
+     2), mar = c(4, 4, 2, 1) + 0.1))
```

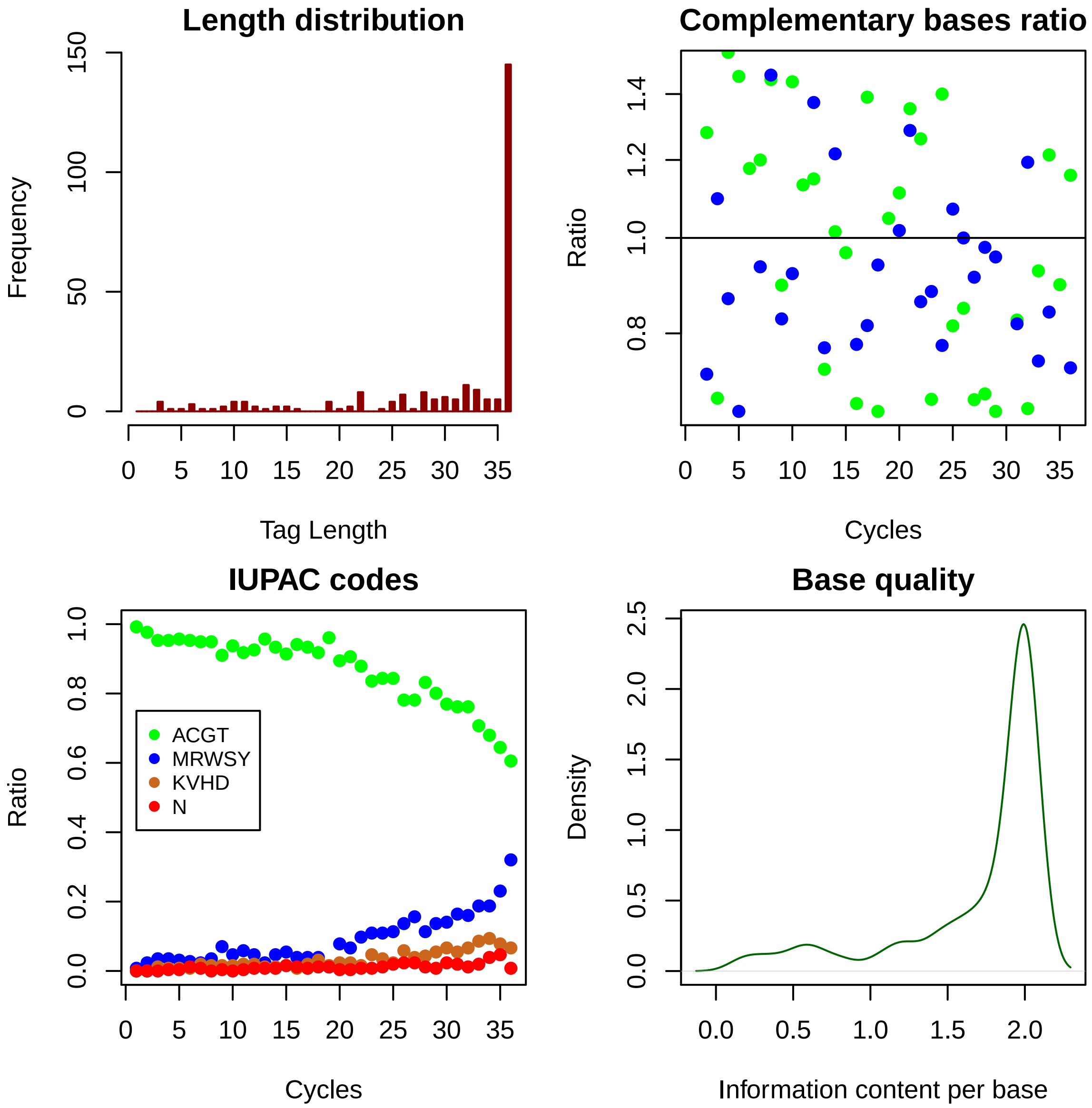


```
> par(mfrow = c(2, 2), mar = c(4, 4, 2, 1) + 0.1)
> PlotCycles(run = rolenv, int = int, seq = seq, cycles = c(1,
+     20))
```



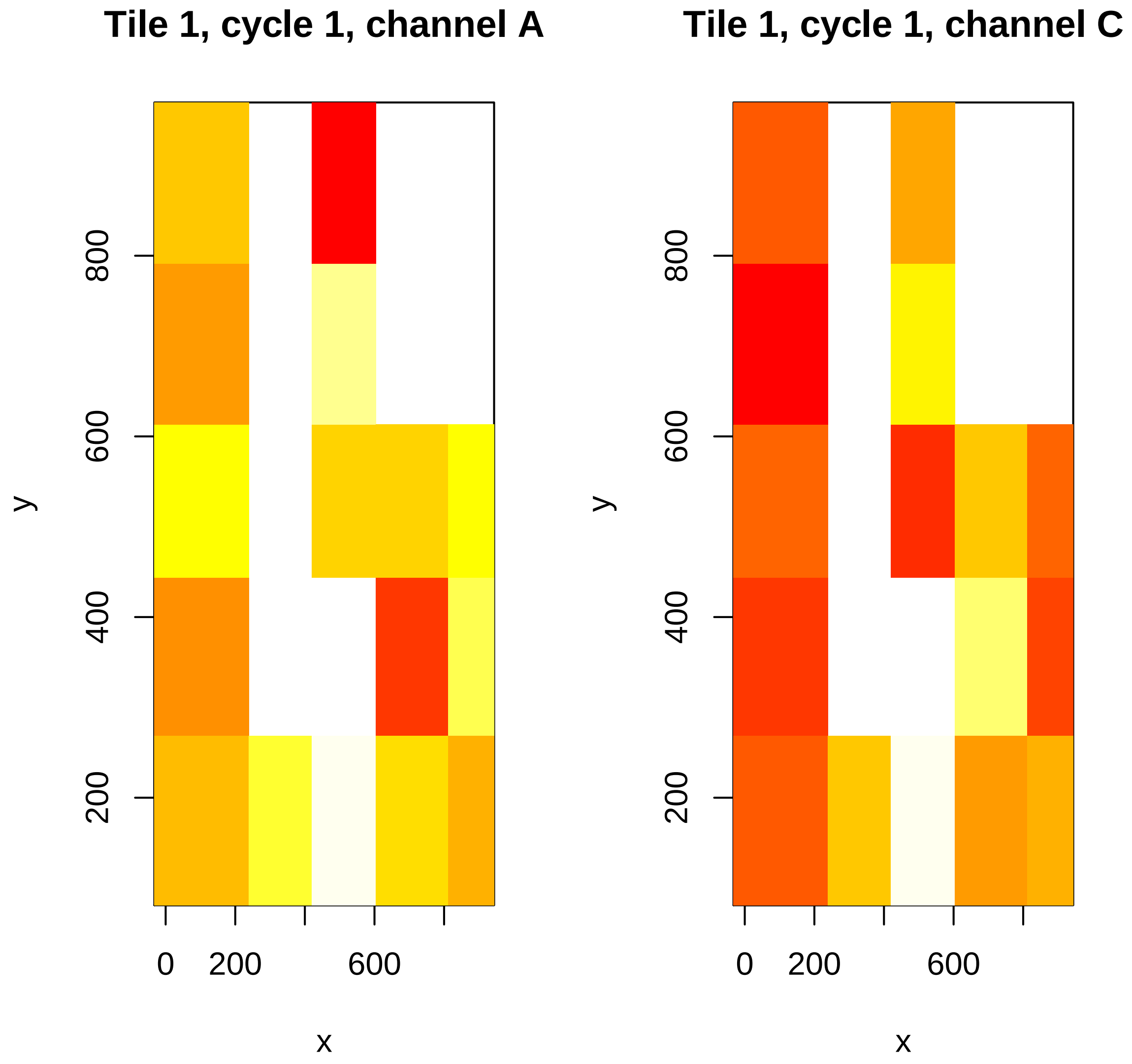
and look at global statistics of a base-calling:

```
> par(mfrow = c(2, 2), cex = 0.8, mar = c(4, 4, 2, 1) + 0.1)
> BatchAnalysis(run = rolenv, seq = res2$sread, scores = res2$entropy,
+     what = "length", main = "Length distribution")
> BatchAnalysis(run = rolenv, seq = res$sread, scores = res$entropy,
+     what = "ratio", main = "Complementary bases ratio")
> BatchAnalysis(run = rolenv, seq = res$sread, scores = res$entropy,
+     what = "iupac", main = "IUPAC codes")
> BatchAnalysis(run = rolenv, seq = res2$sread, scores = res2$entropy,
+     what = "information", main = "Base quality")
```



and visualize the positional bias over a tile by

```
> par(mfrow = c(1, 2))
> TileImage(int = int, cycle = 1, tile = readInfo(int)$tile[1],
+     ncell = 5, channel = "A")
> TileImage(int = int, cycle = 1, tile = readInfo(int)$tile[1],
+     ncell = 5, channel = "C")
```



9 Session Information

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

```
> toLatex(sessionInfo())
```

- R version 2.13.0 (2011-04-13), `x86_64-unknown-linux-gnu`
- Locale: `LC_CTYPE=en_US.UTF-8`, `LC_NUMERIC=C`, `LC_TIME=en_US.UTF-8`, `LC_COLLATE=C`, `LC_MONETARY=C`, `LC_MESSAGES=en_US.UTF-8`, `LC_PAPER=en_US.UTF-8`, `LC_NAME=C`, `LC_ADDRESS=C`, `LC_TELEPHONE=C`, `LC_MEASUREMENT=en_US.UTF-8`, `LC_IDENTIFICATION=C`
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils

- Other packages: Biostrings 2.20.0, GenomicRanges 1.4.0, IRanges 1.10.0, Rolexa 1.8.0, Rsamtools 1.4.0, ShortRead 1.10.0, lattice 0.19-23, mclust 3.4.8
- Loaded via a namespace (and not attached): Biobase 2.12.0, grid 2.13.0, hwriter 1.3, tools 2.13.0

References

[1] Jacques Rougemont, Arnaud Amzallag, Christian Iseli, Laurent Farinelli, Ioannis Xenarios, and Felix Naef. Probabilistic base calling of Solexa sequencing data. *BMC Bioinformatics*, **9**:431, 2008.