MPFE

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April 15, 2025

Many of the known mechanisms driving gene regulation fall into the category of epigenomic modifications. DNA methylation is a common epigenomic modification, in which a cytosine (C) in the genomic DNA sequence can be altered by the addition of a methyl group. Methylation patterns can be are detected by treating DNA with bisulphite, which converts unmethylated cytosines to uracils while leaving methylated cytosines intact. This can be carried out at the whole genome level (whole-genome bisulfite sequencing) or at specific loci (PCR amplicons, capture or reduced representation bisulfite sequencing). The resulting reads can be mapped to a reference and methylation patterns inferred.

However, the bisulphite conversion is not 100% efficient, and this introduces errors in the observed distribution of methylation patterns. A second source of errors is the sequencing error. `MPFE` (for **M**ethylation **P**atterns **F**requency **E**stimation) [1] calculates the estimated distribution over methylation patterns based on an input of methylation pattern count data, an incomplete conversion rate and site-dependent read error rates.

The main component of the package is the function `estimatePatterns()`, which generates a table of estimates $\hat{\theta}_i$ of the distribution over methylation patterns, and a list of patterns identified as spurious. Input to the function is a data frame listing the methylation patterns in the first column followed by a number of columns of count data (one column per sample). Estimation will be performed on all columns by default unless specified by the variable `column`. The non-conversion and sequencing error rates are specified by the parameters `epsilon` and `eta` respectively. The parameter `eta` can be specified globally or as a site-dependent array with length equal to the number of CpG sites in sequence of interest. The boolean variable `fast` enables either a fast implementation (default) which ignores those patterns for which the observed read count is zero or a slow implementation. The parameters `steps` and `reltol` are passed to the function `constrOptim()` to control the accuracy of the determination of the maximum log-likelihood.

A second function in the package is `plotPatterns()`. Input to this function is a data frame, obtained from the output of `estimatePatterns()`. The output of `plotPatterns()` is a plot that compares the observed read distribution with the estimated distribution. The parameters `yLimit1` and `yLimit2` control the range of the y-axis on the plots produced.

In the following example, the input is the table of counts `patternsExample`. We analyse the second column. The parameter `epsilon` is 0.01, while the parameter `eta` is not specified and by default is 0.

```
> library(MPFE)
> data(patternsExample)
> patternsExample

   mPattern  k1   k2
1    m00000 629 2257
2    m00001  26   90
3    m00010  20   75
4    m00011   2    3
5    m00100  24   82
6    m00101   3    0
7    m00110   1   11
8    m00111   0    0
9    m01000  23   80
10   m01001   0    0
11   m01010   1    1
12   m01011   0    0
13   m01100   1    5
14   m01110   0    0
15   m10000  28   69
16   m10001   1    2
17   m10010   0    2
18   m10011   0    0
19   m10100   0    7
20   m11000   3    1
21   m11001   0    0

> estimatePatterns(patternsExample, epsilon=0.01, column=2)

[[1]]
   pattern coverage observedDistribution estimatedDistribution spurious
1    00000     2257         0.8405959032          0.8841394834    FALSE
2    00001       90         0.0335195531          0.0253622332    FALSE
3    00010       75         0.0279329609          0.0201750634    FALSE
4    00011        3         0.0011173184          0.0005735316    FALSE
5    00100       82         0.0305400372          0.0226431744    FALSE
6    00110       11         0.0040968343          0.0035802381    FALSE
7    01000       80         0.0297951583          0.0217187544    FALSE
8    01010        1         0.0003724395          0.0000000000     TRUE
```

```
9    01100        5         0.0018621974          0.0013301642    FALSE
10   10000       69         0.0256983240          0.0178634739    FALSE
11   10001        2         0.0007448790          0.0002242079    FALSE
12   10010        2         0.0007448790          0.0002760796    FALSE
13   10100        7         0.0026070764          0.0021135959    FALSE
14   11000        1         0.0003724395          0.0000000000     TRUE
```

Note that in this example two patterns have been identified as spurious; they are patterns 01010 and 11000.

The following example uses the same input table. The `column` variable is not specified, so the function `estimatePatterns()` by default applies to both columns of counts. The sequencing error rate `eta` is specified as a site-dependent array.

```
> estimates <- estimatePatterns(patternsExample,
+                               epsilon=0.01,
+                               eta=c(0.008, 0.01, 0.01, 0.01, 0.008))
> estimates

[[1]]
   pattern coverage observedDistribution estimatedDistribution spurious
1    00000      629          0.825459318          0.9088748331    FALSE
2    00001       26          0.034120735          0.0200854806    FALSE
3    00010       20          0.026246719          0.0099129217    FALSE
4    00011        2          0.002624672          0.0017646709    FALSE
5    00100       24          0.031496063          0.0155305960    FALSE
6    00101        3          0.003937008          0.0030002140    FALSE
7    00110        1          0.001312336          0.0004654103    FALSE
8    01000       23          0.030183727          0.0141238683    FALSE
9    01010        1          0.001312336          0.0004935455    FALSE
10   01100        1          0.001312336          0.0003809915    FALSE
11   10000       28          0.036745407          0.0221025625    FALSE
12   10001        1          0.001312336          0.0002793763    FALSE
13   11000        3          0.003937008          0.0029855293    FALSE

[[2]]
   pattern coverage observedDistribution estimatedDistribution spurious
1    00000     2257          0.8405959032          0.9257433597    FALSE
2    00001       90          0.0335195531          0.0183957879    FALSE
3    00010       75          0.0279329609          0.0115868249    FALSE
4    00011        3          0.0011173184          0.0002262905    FALSE
5    00100       82          0.0305400372          0.0141488595    FALSE
6    00110       11          0.0040968343          0.0032974071    FALSE
7    01000       80          0.0297951583          0.0127240011    FALSE
```

```
> plotPatterns(estimates[[2]])
```

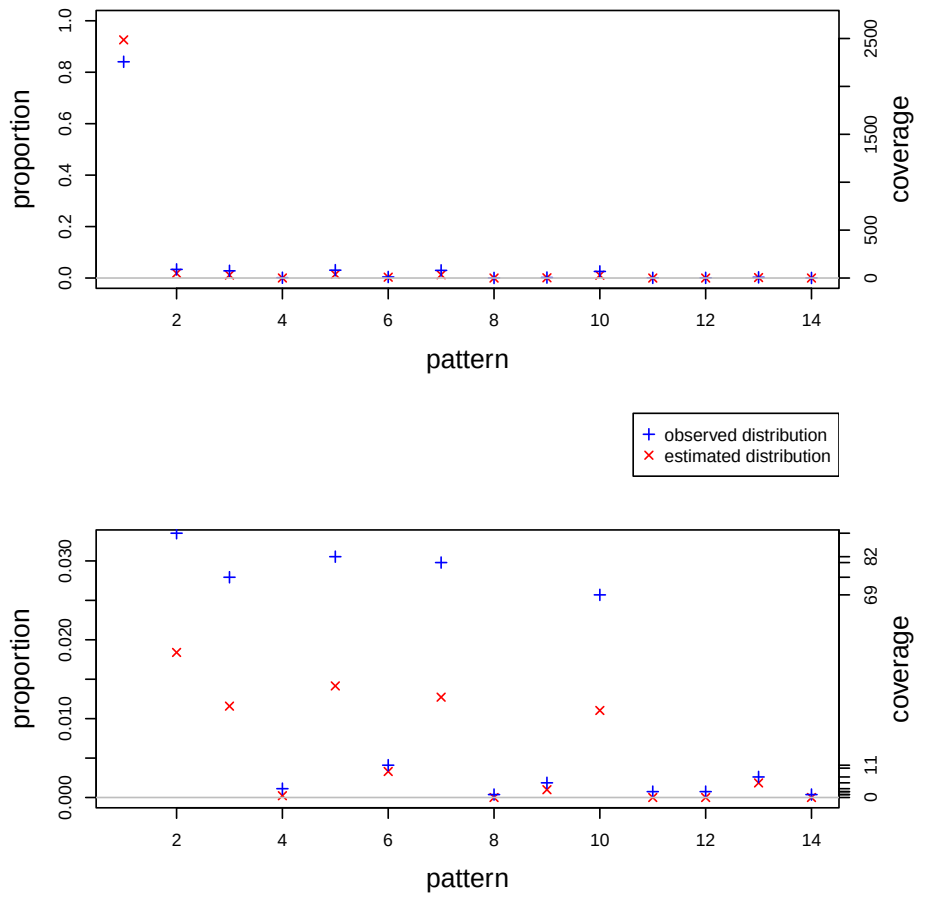

Figure 1: Plot of observed and estimated frequencies with `plotPatterns`

8	01010	1	0.0003724395	0.0000000000	TRUE
9	01100	5	0.0018621974	0.0009915809	FALSE
10	10000	69	0.0256983240	0.0110383332	FALSE
11	10001	2	0.0007448790	0.0000000000	TRUE
12	10010	2	0.0007448790	0.0000000000	TRUE
13	10100	7	0.0026070764	0.0018475551	FALSE
14	11000	1	0.0003724395	0.0000000000	TRUE

The ouput is a list of two data frames. We plot the observed and estimated pattern of the second data frame on figure 1. Two plots are produced: the lower plot is the expanded version of the upper plot.

We can also plot a map of the patterns and their frequencies. For instance, figure 2 illustrates different ways to plot all the patterns with frequency of 50% or less.

```
> par(mfrow=c(2, 2))
> patternMap(estimates[[1]],
+            maxFreq=0.5,
+            main='Estimated frequencies')
> patternMap(estimates[[1]],
+            estimatedDistribution=FALSE,
+            maxFreq=0.5,
+            topDown=FALSE,
+            main='Observed frequencies')
> patternMap(estimates[[1]],
+            maxFreq=0.5,
+            methCol=colorRampPalette(c('red', 'blue')),
+            unMethCol='lightgrey',
+            main='Estimated frequencies')
> patternMap(estimates[[1]],
+            estimatedDistribution=FALSE,
+            maxFreq=0.5,
+            methCol=c('bisque4', 'azure4'),
+            unMethCol=c('beige', 'azure'),
+            main='Observed frequencies')
```

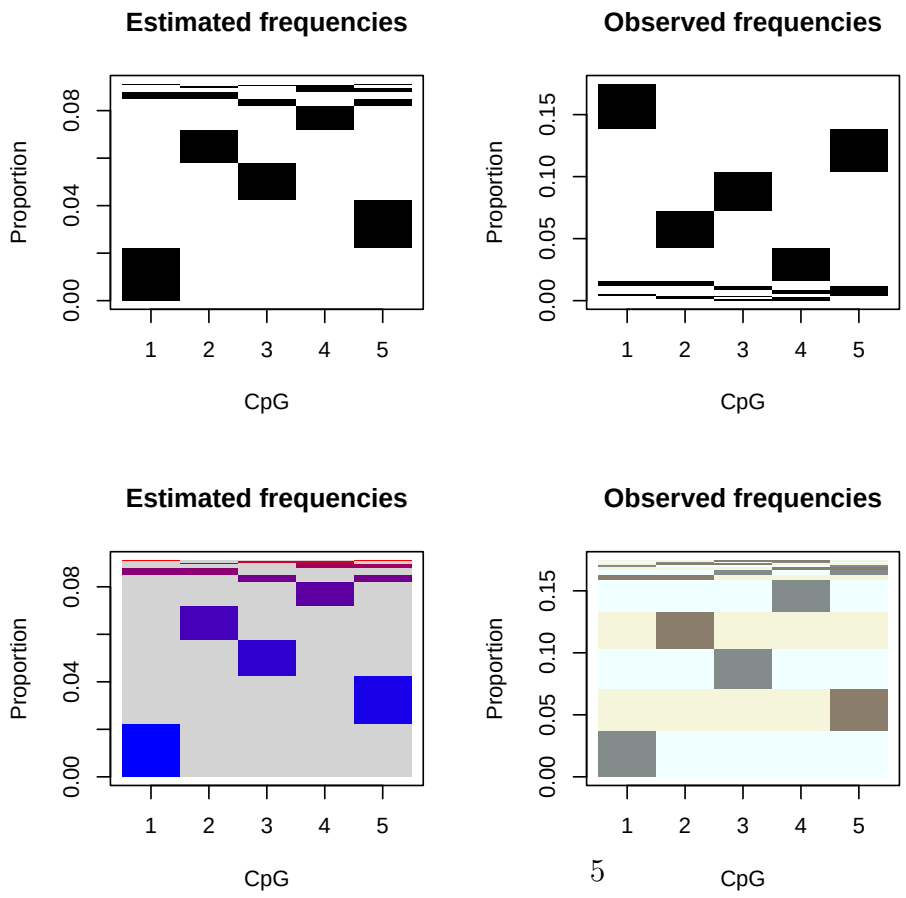

Figure 2: Different ways to plot the estimated frequencies with `patternMap`

References

[1] Lin, P., Forêt, S., Wilson, S.R. and Burden, C.J., *Estimation of the methylation pattern distribution from deep sequencing data.* BMC Bioinformatics 2015, 16:145 doi:10.1186/s12859-015-0600-6