

MPFE

Conrad Burden, Sylvain Forêt, Peijie Lin

October 27, 2023

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 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 `reitol` 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	0.8841384462	FALSE
2	00001	90	0.0335195531	0.0253622343	FALSE
3	00010	75	0.0279329609	0.0201750701	FALSE
4	00011	3	0.0011173184	0.0005735323	FALSE
5	00100	82	0.0305400372	0.0226431758	FALSE
6	00110	11	0.0040968343	0.0035802255	FALSE
7	01000	80	0.0297951583	0.0217188279	FALSE
8	01010	1	0.0003724395	0.0000000000	TRUE

9	01100	5	0.0018621974	0.0013301704	FALSE
10	10000	69	0.0256983240	0.0178635373	FALSE
11	10001	2	0.0007448790	0.0002245734	FALSE
12	10010	2	0.0007448790	0.0002766459	FALSE
13	10100	7	0.0026070764	0.0021135609	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	0.9257447551	FALSE
2	00001	90	0.0335195531	0.0183974743	FALSE
3	00010	75	0.0279329609	0.0115890973	FALSE
4	00011	3	0.0011173184	0.0002261950	FALSE
5	00100	82	0.0305400372	0.0141439446	FALSE
6	00110	11	0.0040968343	0.0032949972	FALSE
7	01000	80	0.0297951583	0.0127250752	FALSE

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

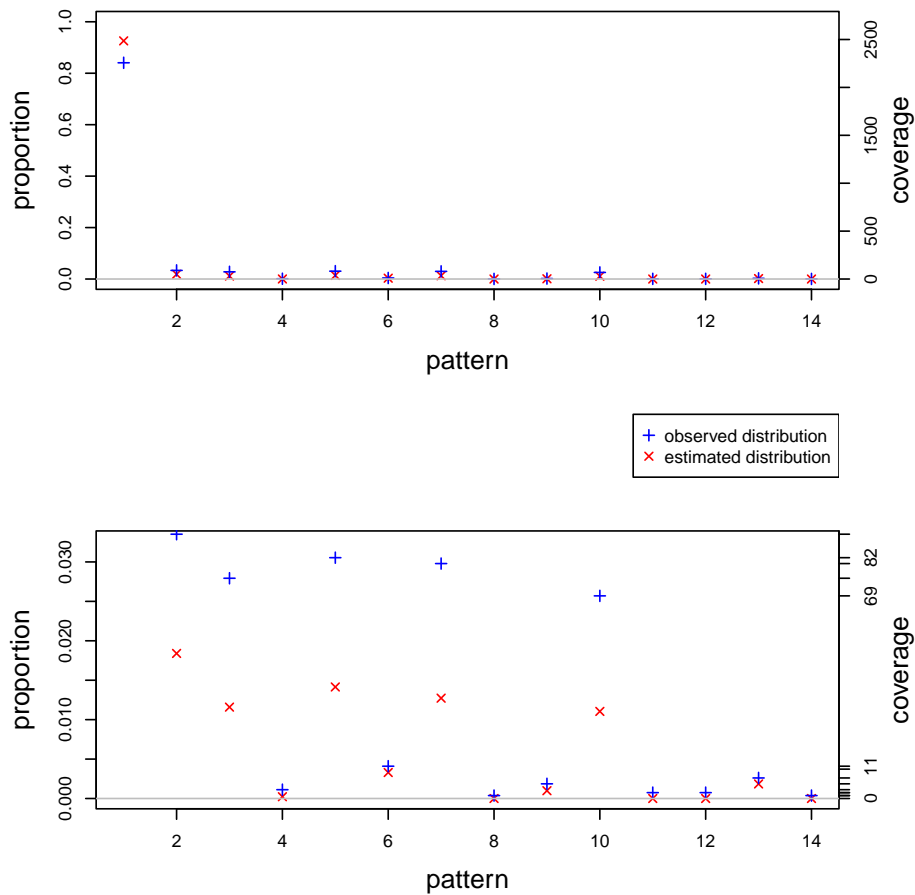


Figure 1: Plot of observed and estimated frequencies with `plotPatterns`

8	01010	1	0.0003724395	0.0000000000	TRUE
9	01100	5	0.0018621974	0.0009886546	FALSE
10	10000	69	0.0256983240	0.0110445253	FALSE
11	10001	2	0.0007448790	0.0000000000	TRUE
12	10010	2	0.0007448790	0.0000000000	TRUE
13	10100	7	0.0026070764	0.0018452813	FALSE
14	11000	1	0.0003724395	0.0000000000	TRUE

The output 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')

```

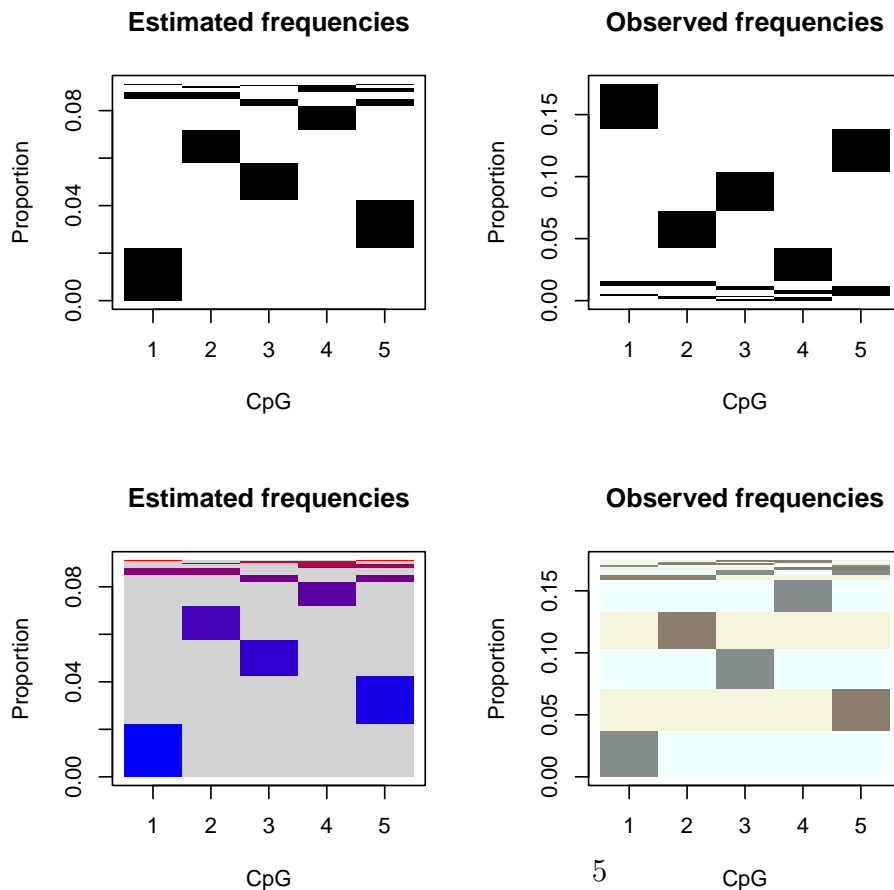


Figure 2: Different ways to plot the estimated frequencies with `patternMap`

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