

# MPFE

Conrad Burden, Sylvain Forêt, Peijie Lin

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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 parameter `steps` is 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	0.8839245649	FALSE
2	00001	90	0.0335195531	0.0254455613	FALSE
3	00010	75	0.0279329609	0.0202696843	FALSE
4	00011	3	0.0011173184	0.0005775861	FALSE
5	00100	82	0.0305400372	0.0226218133	FALSE
6	00110	11	0.0040968343	0.0035829509	FALSE
7	01000	80	0.0297951583	0.0217709103	FALSE
8	01010	1	0.0003724395	0.0000000000	TRUE

9	01100	5	0.0018621974	0.0013262456	FALSE
10	10000	69	0.0256983240	0.0179381554	FALSE
11	10001	2	0.0007448790	0.0002199047	FALSE
12	10010	2	0.0007448790	0.0002226042	FALSE
13	10100	7	0.0026070764	0.0021000190	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
```

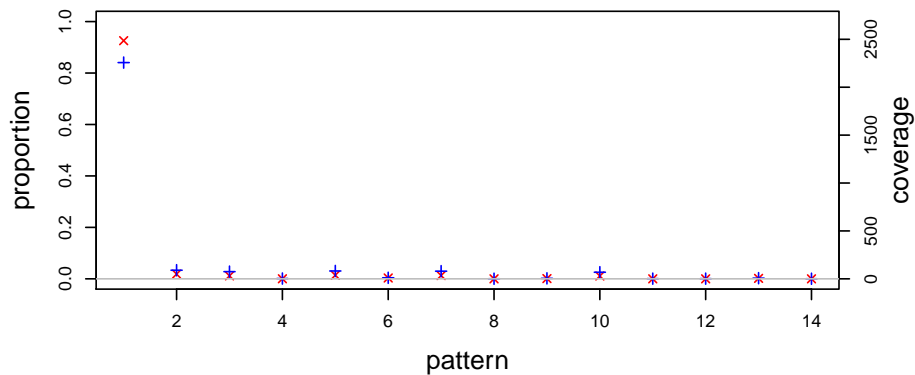
```
[[1]]
  pattern coverage observedDistribution estimatedDistribution spurious
1  00000      629      0.825459318      0.9086155891  FALSE
2  00001       26      0.034120735      0.0200619130  FALSE
3  00010       20      0.026246719      0.0100523143  FALSE
4  00011        2      0.002624672      0.0018028445  FALSE
5  00100       24      0.031496063      0.0155874563  FALSE
6  00101        3      0.003937008      0.0030043039  FALSE
7  00110        1      0.001312336      0.0004632978  FALSE
8  01000       23      0.030183727      0.0142452917  FALSE
9  01010        1      0.001312336      0.0004838187  FALSE
10 01100        1      0.001312336      0.0003786940  FALSE
11 10000       28      0.036745407      0.0220014017  FALSE
12 10001        1      0.001312336      0.0002860781  FALSE
13 11000        3      0.003937008      0.0030169970  FALSE
```

```
[[2]]
  pattern coverage observedDistribution estimatedDistribution spurious
1  00000     2257      0.8405959032      0.9257131882  FALSE
2  00001       90      0.0335195531      0.0183938459  FALSE
3  00010       75      0.0279329609      0.0115768498  FALSE
4  00011        3      0.0011173184      0.0002218223  FALSE
5  00100       82      0.0305400372      0.0141056682  FALSE
6  00110       11      0.0040968343      0.0033324517  FALSE
7  01000       80      0.0297951583      0.0127610644  FALSE
```

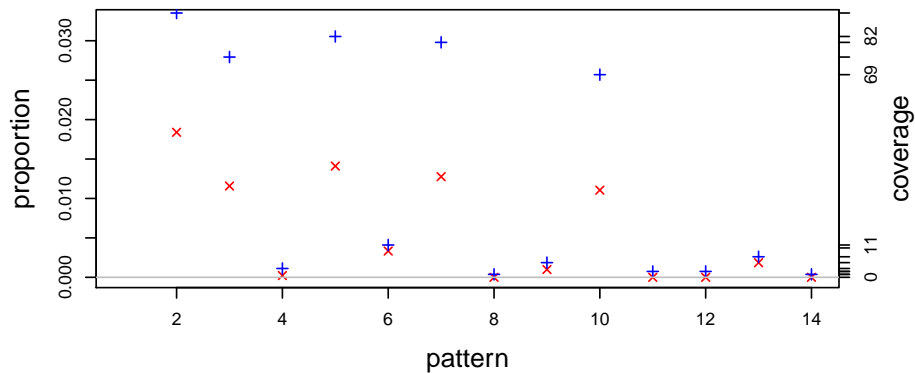
8	01010	1	0.0003724395	0.0000000000	TRUE
9	01100	5	0.0018621974	0.0009896189	FALSE
10	10000	69	0.0256983240	0.0110531371	FALSE
11	10001	2	0.0007448790	0.0000000000	TRUE
12	10010	2	0.0007448790	0.0000000000	TRUE
13	10100	7	0.0026070764	0.0018523535	FALSE
14	11000	1	0.0003724395	0.0000000000	TRUE

The output is a list of two data frames; now we plot the second data frame. Two plots are produced: the lower plot is the expanded version of the upper plot.

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



+ observed distribution  
x estimated distribution



## References

- [1] Lin, P., Forêt, S., Wilson, S.R. and Burden, C.J., *Estimation of the methylation pattern distribution from deep sequencing data.* (preprint)