

# Bpest

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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 specific loci in a genome by amplifying fragments of interest by PCR. These PCR products (called amplicons) 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. **Bpest** (for **B**isulphite **p**attern **e**stimator) [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 the amplicon. 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 `plotMethylationPatterns()`. Input to this function is a data frame, obtained from the output of `estimatePatterns()`. The output of `plotMethylationPatterns()` 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(Bpest)
> 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)
```

	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

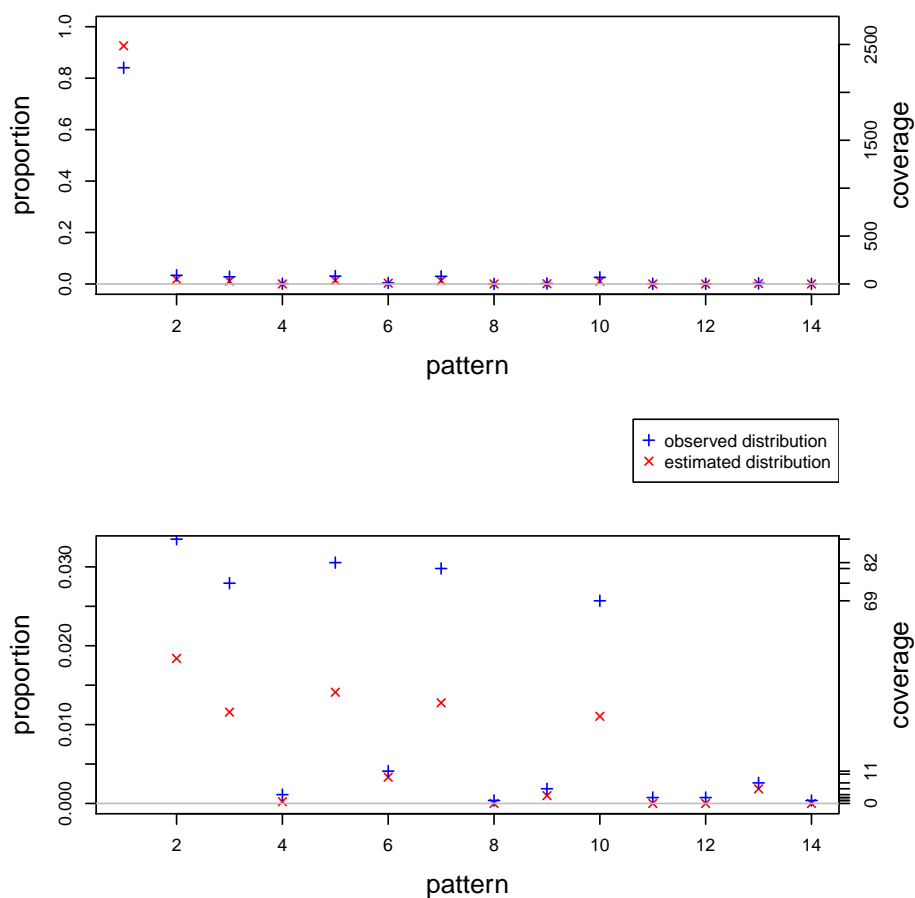
[[2]]

	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.

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



## References

- [1] Lin, P., Forêt, S., Wilson, S.R. and Burden, C.J., *Estimation of amplicon methylation patterns from bisulphite sequencing data* (preprint)