

# The cleanUpdTSeq user's guide

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## 1 Introduction

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3' ends of transcripts have generally been poorly annotated. With the advent of deep sequencing, many methods have been developed to identify 3' ends. The majority of these methods use an oligo-dT primer, which can bind to internal adenine-rich sequences, and lead to artifactual identification of polyadenylation sites. Heuristic filtering methods rely on a certain number of adenines in the genomic sequence downstream of a putative polyadenylation site to remove internal priming events. We introduce a package to provide a robust method to classify putative polyadenylation sites. cleanUpdTSeq uses a naïve Bayes classifier, implemented through the *e1071* [1], and sequence features surrounding the putative polyadenylation sites for classification.

The package includes a training dataset constructed from 6 different Zebrafish sequencing dataset, and functions for fetching surrounding sequences using BSgenome [2], building feature vectors

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and classifying whether the putative polyadenylations site is a true polyadenylation site or a mis-primed false site.

A paper has been submitted to Bioinformatics and currently under revision [3].

## 2 step-by-step guide

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Here is a step-by-step guide on using `cleanUpdTSeq` to classify a list of putative polyadenylation sites

### 2.1 Step 1. Load the package `cleanUpdTSeq`, read in the test dataset and then use the function `BED2GRangesSeq` to convert it to `GRanges`.

```
> library(cleanUpdTSeq)
> testFile <- system.file("extdata", "test.bed", package="cleanUpdTSeq")
> testSet <- read.table(testFile, sep="\t", header=TRUE)
> peaks <- BED2GRangesSeq(testSet, withSeq=FALSE)
```

If test dataset contains sequence information already, then use the following command instead.

```
> peaks <- BED2GRangesSeq(testSet, upstream.seq.ind=7,
+                          downstream.seq.ind=8, withSeq=TRUE)
```

To work with your own test dataset, please set `testFile` to the file path that contains the putative sites.

Here is how the test dataset look like.

```
> head(testSet)
      chr  start  stop  name score strand
1 chr10 2965327 2965327 6hpas-22249    1    -
2 chr10 2966558 2966558 6hpas-22250    1    -
3 chr10 2974251 2974251 6hpas-22251    2    -
4 chr10 2978441 2978441 6hpas-22252    1    -
5 chr11 16772291 16772291 6hpas-33204    1    -
6 chr11 16777848 16777848 6hpas-33205    1    -
      upstream                                 downstream
1 TCTTCATCATGGTCATCTCGCACCAGAGAGTGTGCCAGGG CAGGAAGTTTTACCTGTCTGTCATTATCGT
2 ACCCTGGTGAGGGTATAGAGCTGGTCCAGTGTGCCACGGC AAAGAGGAAAACAGCATTGTTCCCTCCTGGA
3 TGATTTGTTTGTAAGTGAATTTATCTTTTAATAAAAAAAGA AAAAAAGAAAGTCAAGCCAAGAGGCAAATAC
4 GGAGCGCGACCGCATCAACAAAATCTTGCAGGATTATCAG  AAGAAAAAGATGGTGAGTTATTATCATTCA
```

```

5 AGGGAAATAAATACAAAAGAATAAAAAATATGATTCATTGT AAGAAAAACACTTTAGCTACAAAAGTCCTT
6 ATTTAGTTGGGTATTATTTCAAATAAAGAGAGAGAGAGAC ACAAANAACACTACATCAAATTTGAGGACAAAA

```

## 2.2 Step2. Build feature vectors for the classifier using the function `buildFeatureVector`.

The zebrafish genome from BSgenome is used in this example for obtaining surrounding sequences. For a list of other genomes available through BSgenome, please refer to the BSgenome package documentation [2].

```

> testSet.NaiveBayes <- buildFeatureVector(peaks, BSgenomeName=Drerio,
+                                       upstream=40, downstream=30,
+                                       wordSize=6, alphabet=c("ACGT"),
+                                       sampleType="unknown",
+                                       replaceNAdistance=30,
+                                       method="NaiveBayes",
+                                       ZeroBasedIndex=1, fetchSeq=TRUE)

```

If sequences are present in the test dataset already, then set `fetchSeq=FALSE`.

## 2.3 Step 3. Load the training dataset and classify putative polyadenylation sites.

The output file is a tab-delimited file containing the name of the putative polyadenylation sites, the probability that the putative polyadenylation site is false/oligodT internally primed, the probability the putative polyadenylation site is true, the predicted class based on the assignment cutoff and the sequence surrounding the putative polyadenylation site.

```

> data(data.NaiveBayes)
> if(interactive()){
+   predictTestSet(data.NaiveBayes$Negative, data.NaiveBayes$Positive,
+                 testSet.NaiveBayes=testSet.NaiveBayes,
+                 outputFile="test-predNaiveBayes.tsv",
+                 assignmentCutoff=0.5)
+ }

```

Alternatively, instead of passing in a positive and a negative training dataset, set the parameter classifier to a pre-built `PASclassifier` to speed up the process. To build a `PASclassifier` using the training dataset, please use function `buildClassifier`. A `PASclassifier` named as `classifier` is included in the package which is generated using the included training dataset with `upstream=40`, `downstream=30`, and `wordSize=6`. Please note that in order to use this pre-built classier, you need

to build feature vector using `buildFeatureVector` from your test dataset with the same setting, i.e., `upstream=40`, `downstream=30`, and `wordSize=6`.

```
> data(classifier)
> testResults <- predictTestSet(testSet.NaiveBayes=testSet.NaiveBayes,
+                               classifier=classifier,
+                               outputFile=NULL,
+                               assignmentCutoff=0.5)
> head(testResults)
```

	PeakName	prob	False/oligo	dT internally	primed	prob	True	pred.class
1	6hpas-78439				9.999997e-01	3.137403e-07		0
2	6hpas-78440				1.000000e+00	7.431591e-14		0
3	6hpas-78441				4.474675e-06	9.999955e-01		1
4	6hpas-78442				3.085156e-07	9.999997e-01		1
5	6hpas-22249				9.899582e-01	1.004181e-02		0
6	6hpas-22250				1.000000e+00	1.636415e-09		0

```
                                UpstreamSeq                                DownstreamSeq
1 GGTCATTGTCCTGCAAAAATGGACTACTTAACCGAACTGGA GAAGTATAAGAAGTAAGTACATTAAGCTAC
2 TGGATTTAAATAACAAACAAGTTAAATAAAACGATTTGTA AAAAAATAAAACAACACTGAAGAAGAAAATGAA
3 ATCTGCTTCAAAAATGGATGCTCTGTTGAATCCTGAGCTCA GGTAATCTTTCAAGTGCTGCTATTGAGCCAA
4 AAATGCTTGCACATAATAAATGTAGGCTTAAAAGATTTCA AAACGTTTGTGAGAGACGGATTTTACTTTGC
5 TCTTCATCATGGTCATCTCGCACCAGAGAGTGTGCCAGGG CAGGAAGTTTTACCTGTCTGTCATTATCGTC
6 ACCCTGGTGAGGGTATAGAGCTGGTCCAGTGTGCCACGGC AAAGAGGAAAACAGCATTGTTCCCTCCTGGAT
```

### 3 References

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1. Meyer, D., et al., e1071: Misc Functions of the Department of Statistics (e1071), TU Wien. 2012.
2. Pages, H., BSgenome: Infrastructure for Biostrings-based genome data packages.
3. Sarah Sheppard, Nathan D. Lawson, and Lihua Julie Zhu. 2013. Accurate identification of polyadenylation sites from 3' end deep sequencing using a naïve Bayes classifier. Bioinformatics. Under revision

### 4 Session Info

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

- R version 3.2.0 (2015-04-16), 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=en\_US.UTF-8, 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, parallel, stats, stats4, utils
- Other packages: BSgenome 1.36.0, BSgenome.Drerio.UCSC.danRer7 1.4.0, BiocGenerics 0.14.0, Biostrings 2.36.1, GenomInfoDb 1.4.0, GenomicRanges 1.20.3, IRanges 2.2.1, S4Vectors 0.6.0, XVector 0.8.0, ade4 1.7-2, cleanUpdTSeq 1.6.1, e1071 1.6-4, rtracklayer 1.28.2, seqinr 3.1-3
- Loaded via a namespace (and not attached): BiocParallel 1.2.1, BiocStyle 1.6.0, GenomicAlignments 1.4.1, RCurl 1.95-4.6, Rsamtools 1.20.2, XML 3.98-1.1, bitops 1.0-6, class 7.3-12, futile.logger 1.4.1, futile.options 1.0.0, lambda.r 1.1.7, tools 3.2.0, zlibbioc 1.14.0