

# How to use the ASEB Package

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## 1 Introduction

Lysine acetylation is a well-studied posttranslational modification on kinds of proteins. About four thousand lysine acetylation sites and over 20 lysine (K)-acetyl-transferases (KATs) have been identified. However, which KAT is responsible for a given protein or lysine site acetylation is mostly unknown. In our previous study, we found that different KAT families acetylate lysine sites with different sequence features (Li and et al., 2011). Based on these differences, we developed a computer program, Acetylation Set Enrichment Based (ASEB) method to predict which KAT-families are responsible for acetylation of a given protein or lysine site.

## 2 Getting started

To load the *ASEB* package, type `library(ASEB)`. Total six methods are presented in this package. They are `readSequence`, `asebSites`, `asebProteins`, `drawStat` and `drawEScurve`.

## 3 Methods

In this package, we use a GSEA-like method to make predictions. Gene Set Enrichment Analysis (GSEA) method was developed and successfully used to detect coordinated expression changes (Subramanian and et al., 2005; Mootha and et al., 2003) and find the putative functions of the long non-coding RNAs (Guttman and et al., 2009). In our study (Li and et al., 2011), we treated the (acetylated) lysine sites and their surrounding amino acids (8 on each side) as (acetylated) peptide sequences. We first define all the validated acetylated peptide sequences from one KAT family as a KAT-specific set, and define 10000 random selected peptide sequences from the whole proteome as a background set. When given a new query peptide sequence, similarity scores are calculated according to the BLOSUM62 matrix between this query peptide and peptides in the KAT-specific set and background set. A list is then created by ranking the scores. Similar with GSEA method, a running sum score (enrichment score) was calculated by walking down the list. To estimate the significance of the enrichment score for a query peptide, at first, a certain number of peptide sets with the same size as KAT-specific set was randomly generated. Secondly, treating each randomly generated set as a pseudo predefined KAT-specific set, an enrichment score can be calculated for each randomly generated set. At last, rank all the enrichment scores (from high to low) and a nominal P-value could be calculated. The nominal P-value is defined as the rank of enrichment score for the KAT-specific set divided by the total number of random selected sets. The whole process is similar with the GSEA method (permuting gene sets). Please see Li and et al. (2011) for details.

## 4 Data

We provide the KAT-specific set for CBP/P300 and GCN5/PCAF family in this package. The file `predefined_sites.fa` under `extdata` contains the KAT-specific set for CBP/P300 family (total 267 sites). While the file `predefined_sites2.fa` contains the KAT-specific set for GCN5/PCAF family (total 82 sites). The two sets were generated by searching the PubMed literature. The file `background_sites.fa` contains 10000 randomly selected sites and the KAT-specific set for CBP/P300 family (total 10000+267 sites). While the file `background_sites2.fa` contains 10000 randomly selected sites and the KAT-specific set for GCN5/PCAF family (total 10000+82 sites).

## 5 Examples

### 5.1 Example for `readSequence`

This function return an object of `SequenceInfo` that contains sequences and identifiers from FASTA format input file.

```

> library(ASEB)
> ff <- system.file("extdata", "background_sites.fa", package="ASEB")
> readSequence(ff)

object of SequenceInfo
total 10267 sequences
first 10 sequences
Slot "ids":
 [1] "O60313_K698" "Q96H12_K26"  "P35227_K233" "Q9BX69_K747" "Q5T619_K231"
 [6] "Q9NYD6_K324" "Q08043_K227" "Q9Y305_K346" "Q5XPI4_K813" "Q9Y3Q7_K370"
Slot "sequences":
 [1] "KEHDDIFDKLKEAVKEE" "SILLALVEKYKYVLECK" "KYRVQPACKRLTLATVP"
 [4] "GNFNHVSLKASWVMGRP" "LAKARNSRKVQNPAGRR" "QNRMKLKKMNRENRI"
 [7] "NTAFEVAEKYLDIPKML" "AVDDIMFQKPVEVGSLL" "KSQKVFSEKLDHLSRRL"
[10] "HDYRYFVSKFETKCLQK"

```

## 5.2 Example for asebSites

This function is used to predict lysine sites that can be acetylated by a specific KAT-family.

```

> backgroundSites <- readSequence(system.file("extdata", "background_sites.fa", package="ASEB"))
> predefinedSites <- readSequence(system.file("extdata", "predefined_sites.fa", package="ASEB"))
> testSites <- readSequence(system.file("extdata", "sites_to_test.fa", package="ASEB"))
> resultList <- asebSites(backgroundSites, predefinedSites, testSites, permutationTimes=100)

```

Please wait patiently!

```

background sites: /tmp/RtmpSUA15S/backgroundSitesFile
predefined sites: /tmp/RtmpSUA15S/predefinedSitesFile
sites to test: /tmp/RtmpSUA15S/testSitesFile
output: /tmp/RtmpSUA15S/outputFile
Permutation times: 100

```

processed 2 sites

```

> resultList$results[1:2,]

      site      ES p-value
1 AOAVK6_K550 0.004100    0.95
2 AOAVK6_K555 0.111549    0.01

```

This method can also process FASTA format files directly without loading all the sequences to the workspace of R. In this case, it can process huge number of lysine sites each time.

```

> backgroundSitesFile <- system.file("extdata", "background_sites.fa", package="ASEB")
> predefinedSitesFile <- system.file("extdata", "predefined_sites.fa", package="ASEB")
> testSitesFile <- system.file("extdata", "sites_to_test.fa", package="ASEB")
> asebSites(backgroundSitesFile, predefinedSitesFile, testSitesFile, permutationTimes=100)

```

Please wait patiently!

background sites: /tmp/RtmpcD1qyA/Rinst23570c5709d/ASEB/extdata/background\_sites.fa

predefined sites: /tmp/RtmpcD1qyA/Rinst23570c5709d/ASEB/extdata/predefined\_sites.fa

sites to test: /tmp/RtmpcD1qyA/Rinst23570c5709d/ASEB/extdata/sites\_to\_test.fa

output: /tmp/RtmpSUA15S/outputFile

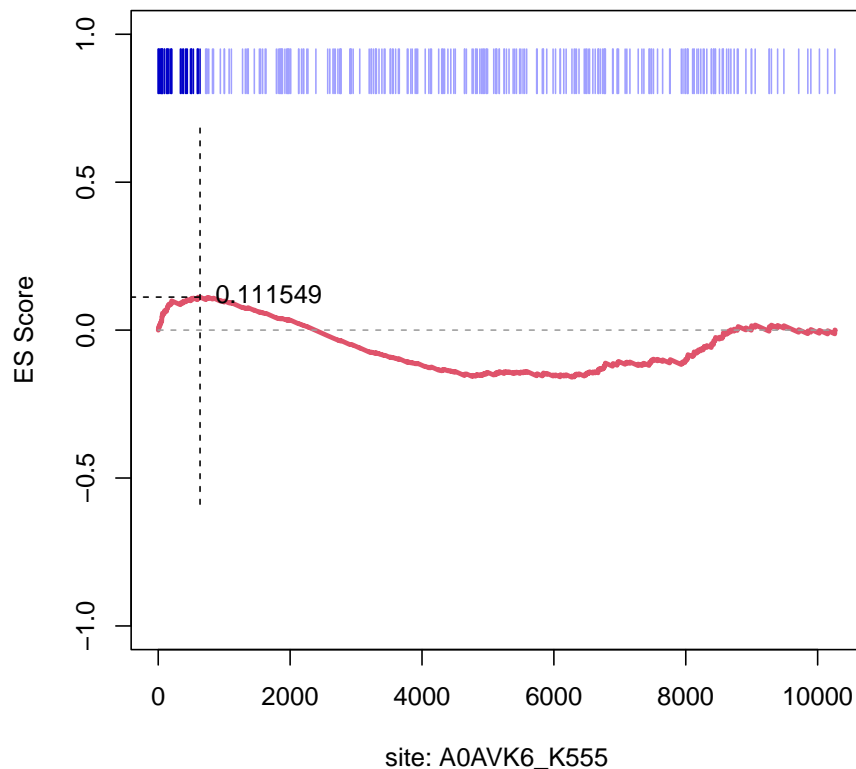
Permutation times: 100

processed 2 sites

### 5.3 Example for drawEScurve

This method can be used to draw enrichment score curve for a specific site. Please see Li and et al. (2011) for details about enrichment score curve.

```
> drawEScurve(resultList$curveInfo, max_p_value=0.1, min_es=0.1)
```



These curves show running-sum process for calculating enrichment score. Users can find detail algorithm from Li and et al. (2011). The data.frame object contains curve information is given by `asebSites`, or `asebProteins`.

## 5.4 Example for asebProteins

This function is used to predict all lysine sites on a specific protein that can be acetylated by a specific KAT-family.

```
> backgroundSites <- readSequence(system.file("extdata", "background_sites.fa", package="ASEB"))
> predefinedSites <- readSequence(system.file("extdata", "predefined_sites.fa", package="ASEB"))
> testProteins <- readSequence(system.file("extdata", "proteins_to_test.fa", package="ASEB"))
> resultList <- asebProteins(backgroundSites, predefinedSites, testProteins, permutationTimes=1000)
> resultList$results[1:2,]
```

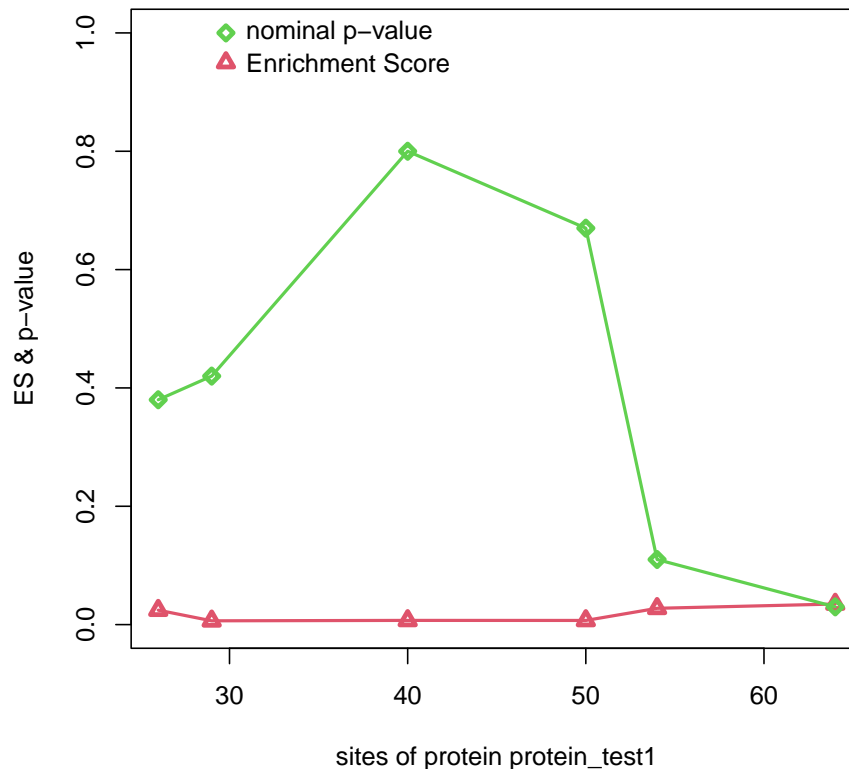
For processing huge number of lysine sites on proteins, this method can be used as below.

```
> backgroundSitesFile <- system.file("extdata", "background_sites.fa", package="ASEB")
> predefinedSitesFile <- system.file("extdata", "predefined_sites.fa", package="ASEB")
> testProteinsFile <- system.file("extdata", "sites_to_test.fa", package="ASEB")
> asebProteins(backgroundSitesFile, predefinedSitesFile, testProteinsFile, permutationTimes=1000)
```

## 5.5 Example for drawStat

This function is used to show P-values and enrichment scores for all lysine sites on a specific protein. The X-axis shows positions of all lysine sites on a specific protein, and Y-axis shows the enrichment scores (0 1) and P-values (0 1) for each lysine site.

```
> drawStat(curveInfoDataFrame=resultList$curveInfo);
```



The sites with less P-values are more significant. For sites that have similar P-values, the ones with higher enrichment scores are more likely to be acetylated. These P-values are nominal (Subramanian and et al., 2005; Mootha and et al., 2003), hence it is hard to give an threshold to predict significant sites. However, users can order all the sites and pay more attention to the ones with relatively less P-values. Please see Li and et al. (2011) for details.

## References

- Guttman, M. and et al. (2009). Chromatin signature reveals over a thousand highly conserved large non-coding rnas in mammals. *Nature*, 458:223–227.
- Li, T. and et al. (2011). Characterization and prediction of lysine (k)-acetyl-transferase (kat) specific acetylation sites. *Mol Cell Proteomics*, in press.
- Mootha, V. and et al. (2003). PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet*, 34:267–273.
- Subramanian, A. and et al. (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A*, 102:15545–15550.