

Package ‘gkmSVM’

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Description

Imports the 'gkmSVM' v2.0 functionalities into R <<https://www.beerlab.org/gkmsvm/>>
It also uses the 'kernlab' library (separate R package by different authors) for various SVM algorithms.
Users should note that the suggested packages 'rtracklayer', 'GenomicRanges', 'BSgenome', 'BiocGenerics', 'Biostrings', 'GenomeInfoDb', 'IRanges', and 'S4Vectors' are all BioConductor packages <<https://bioconductor.org>>.

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Imports kernlab, seqinr, utils, ROCR, Rcpp, grDevices, graphics, stats

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gkmSVM-package	<i>Gapped-Kmer Support Vector Machine</i>
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Description

Imports the 'gkmSVM' v2.0 functionalities into R <<http://www.beerlab.org/gkmsvm/>> . It also uses the 'kernlab' library (separate R package by different authors) for various SVM algorithms.

Details

The gkm-SVM provides implementation of a new SVM kernel method using gapped k-mers as features for DNA or Protein sequences.

There are three main functions in the gkmSVM package:

gkmsvm_kernel: computes the kernel matrix

gkmsvm_train: computes the SVM coefficients

gkmsvm_classify: scores new sequences using the SVM model

Tutorial

=====

We introduce the users to the basic workflow of our gkmSVM step-by-step. Please refer to help messages for more detailed information of each function.

1) making a kernel matrix

First of all, we should calculate a full kernel matrix before training SVM classifiers. In this tutorial, we are going to use test_positives.fa as a positive set, and test_negatives.fa as a negative set.

#Input file names:

posfn= 'test_positives.fa' #positive set (FASTA format)

negfn= 'test_negatives.fa' #negative set (FASTA format)

testfn= 'test_testset.fa' #test set (FASTA format)

Alternatively if the negative set is not available, and positive set is provided as a bed file, gen-NullSeqs function could be used to generate the negative set and positive set sequences.

#Output file names:

kernelfn= 'test_kernel.txt' #kernel matrix

svmfprfx= 'test_svmtrain' #SVM files

outfn = 'output.txt' #output scores for sequences in the test set

gkmsvm_kernel(posfn, negfn, kernelfn); #computes kernel

2) training SVM

We can now train a SVM classifier using the kernel matrix generated above. For that we use `gkmsvm_train` function. It takes four arguments; kernel file, positive sequences file, negative sequences file, and prefix of output file names for the svm model.

```
gkmsvm_train(kernelfn, posfn, negfn, svmfnprfx); #trains SVM
```

It will generate two files, `test_svmtrain_svalpha.out` and `test_svmtrain_svseq.fa`, which will then be used for classification/scoring of test sequences as described below.

3) classification using SVM

`gkmsvm_classify` can be used to score any set of sequences. Here, we will score the test sequences which are given in `test_testset.fa`. Note that the same set of parameters used in the `gkmsvm_kernel` should always be specified for optimal classification (here we used default parameters).

```
gkmsvm_classify(testfn, svmfnprfx, outfn); #scores test sequences
```

In a more advanced example, we set the word length `L=18`, and the number of non-gapped positions `K=7`, and maximum number of mismatches `maxnmm=4`:

```
gkmsvm_kernel(posfn, negfn, kernelfn, L=18, K=7, maxnmm=4); #computes kernel
```

```
gkmsvm_train(kernelfn, posfn, negfn, svmfnprfx); #trains SVM
```

```
gkmsvm_classify(testfn, svmfnprfx, outfn, L=18, K=7, maxnmm=4); #scores test sequences
```

In another example, we run a 5-fold cross validation to plot the ROC curves:

```
gkmsvm_kernel(posfn, negfn, kernelfn); #computes kernel
```

```
cvres = gkmsvm_trainCV(kernelfn, posfn, negfn, svmfnprfx, outputPDFfn='ROC.pdf', outputCVpredfn='cvpred.out');
#trains SVM, plots ROC and PRC curves, and outputs model predictions.
```

Author(s)

Mahmoud Ghandi

Maintainer: Mike Beer <mbeer@jhu.edu>

References

Ghandi M, Lee D, Mohammad-Noori M, Beer MA. 2014. Enhanced Regulatory Sequence Prediction Using Gapped k-mer Features. *PLoS Comput Biol* 10: e1003711.

Ghandi M, Mohammad-Noori M, Ghareghani N, Lee D, Garraway LA, and Beer MA. 2016. `gkmSVM` an R package for gapped-kmer SVM, *Bioinformatics* 32 (14), 2205-2207.

Examples

```
#Input file names:
posfn= 'test_positives.fa' #positive set (FASTA format)
negfn= 'test_negatives.fa' #negative set (FASTA format)
testfn= 'test_testset.fa' #test set (FASTA format)

#Output file names:
kernelfn= 'test_kernel.txt' #kernel matrix
svmfnprfx= 'test_svmtrain' #SVM files
outfn = 'output.txt' #output scores for sequences in the test set
```

```

# gkmsvm_kernel(posfn, negfn, kernelfn);           #computes kernel
# gkmsvm_train(kernelfn, posfn, negfn, svmfnprfx); #trains SVM
# gkmsvm_classify(testfn, svmfnprfx, outfn);       #scores test sequences

# using L=18, K=7, maxnmm=4

# gkmsvm_kernel(posfn, negfn, kernelfn, L=18, K=7, maxnmm=4); #computes kernel
# gkmsvm_train(kernelfn, posfn, negfn, svmfnprfx);           #trains SVM
# gkmsvm_classify(testfn, svmfnprfx, outfn, L=18, K=7, maxnmm=4); #scores test sequences

```

genNullSeqs	<i>Generating GC/repeat matched randomly selected genomic sequences for the negative set</i>
-------------	--

Description

Generates null sequences (negative set) with matching repeat and GC content as the input bed file for positive set regions.

Usage

```

genNullSeqs(
  inputBedFN,
  genomeVersion='hg19',
  outputBedFN = 'negSet.bed',
  outputPosFastaFN = 'posSet.fa',
  outputNegFastaFN = 'negSet.fa',
  xfold = 1,
  repeat_match_tol = 0.02,
  GC_match_tol = 0.02,
  length_match_tol = 0.02,
  batchsize = 5000,
  nMaxTrials = 20,
  genome = NULL)

```

Arguments

inputBedFN	positive set regions
genomeVersion	genome version: 'hg19' and 'hg18' are supported. Default='hg19'. For other genomes, provide the BSgenome object using parameter 'genome'
outputBedFN	output file name for the null sequences genomic regions. Default='negSet.bed'
outputPosFastaFN	output file name for the positive set sequences. Default='posSet.fa'
outputNegFastaFN	output file name for the negative set sequences. Default='negSet.fa'

xfold	controls the desired number of sequences in the negative set. Default=1 (same number as in positive set)
repeat_match_tol	tolerance for difference in repeat ratio. Default=0.02 (repeat content difference of 0.02 or less is acceptable)
GC_match_tol	tolerance for difference in GC content. Default=0.02
length_match_tol	tolerance for difference in relative sequence length. Default=0.02
batchsize	number of candidate random sequences tested in each trial. Default=5000
nMaxTrials	maximum number of trials. Default=20.
genome	BSgenome object. Default=NULL. If this parameter is used, parameter genomeVersion is ignored.

Value

Writes the null sequences to files with the provided filenames. Outputs the filename for the output negative sequences file.

Author(s)

Mahmoud Ghandi

Examples

```
# Example 1:
# genNullSeqs('ctcfpos.bed' );

#Example 2:
# genNullSeqs('ctcfpos.bed', nMaxTrials=3, xfold=2, genomeVersion = 'hg18' );

#Example 3:
# genNullSeqs('ctcfpos.bed', xfold=2, genomeVersion = 'hg18', outputBedFN = 'ctcf_negSet.bed',
# outputPosFastaFN = 'ctcf_posSet.fa', outputNegFastaFN = 'ctcf_negSet.fa' );

#Example 4:
# Input file names:

posBedFN = 'test_positives.bed' # positive set genomic ranges (bed format)
genomeVer = 'hg19' #genome version
testfn= 'test_testset.fa' #test set (FASTA format)

# output file names:
posfn= 'test_positives.fa' #positive set (FASTA format)
negfn= 'test_negatives.fa' #negative set (FASTA format)
kernelfn= 'test_kernel.txt' #kernel matrix
svmfprfx= 'test_svmtrain' #SVM files
outfn = 'output.txt' #output scores for sequences in the test set

# genNullSeqs(posBedFN, genomeVersion = genomeVer,
# outputPosFastaFN = posfn, outputNegFastaFN = negfn );
```

```

# gkmsvm_kernel(posfn, negfn, kernelfn);           #computes kernel
# gkmsvm_train(kernelfn, posfn, negfn, svmfnprfx); #trains SVM
# gkmsvm_classify(testfn, svmfnprfx, outfn);      #scores test sequences

# using L=18, K=7, maxnmm=4

# gkmsvm_kernel(posfn, negfn, kernelfn, L=18, K=7, maxnmm=4); #computes kernel
# gkmsvm_train(kernelfn, posfn, negfn, svmfnprfx);           #trains SVM
# gkmsvm_classify(testfn, svmfnprfx, outfn, L=18, K=7, maxnmm=4); #scores test sequences

```

gkmsvm_classify *Classifying(/scoring) new sequences using the gkmSVM model*

Description

Given support vectors SVs and corresponding coefficients alphas and a set of sequences, calculates the SVM scores for the sequences.

Usage

```

gkmsvm_classify(seqfile, svmfnprfx, outfile, L=10, K=6, maxnmm=3,
maxseqlen=10000, maxnumseq=1000000, useTgkm=1, alg=0, addRC=TRUE, usePseudocnt=FALSE,
batchSize=100000, wildcardLambda=1.0, wildcardMismatchM=2, alphabetFN="NULL",
svseqfile=NA, alphafile=NA)

```

Arguments

seqfile	input sequences file name (FASTA format)
svmfnprfx	SVM model file name prefix
outfile	output file name
L	word length, default=10
K	number of informative columns, default=6
maxnmm	maximum number of mismatches to consider, default=3
maxseqlen	maximum sequence length in the sequence files, default=10000
maxnumseq	maximum number of sequences in the sequence files, default=1000000
useTgkm	filter type: 0(use full filter), 1(use truncated filter: this gaurantees non-negative counts for all L-mers), 2(use h[m], gkm count vector), 3(wildcard), 4(mismatch), default=1
alg	algorithm type: 0(auto), 1(XOR Hashtable), 2(tree), default=0
addRC	adds reverse complement sequences, default=TRUE
usePseudocnt	adds a constant to count estimates, default=FALSE

```

batchSize      number of sequences to compute scores for in batch, default=100000
wildcardLambda lambda for wildcard kernel, default=0.9
wildcardMismatchM
                max mismatch for Mismatch kernel or wildcard kernel, default=2
alphabetFN     alphabets file name, if not specified, it is assumed the inputs are DNA sequences
svseqfile     SVM support vectors sequence file name (not needed if svfnprfx is provided)
alphafile     SVM support vectors weights file name (not needed if svfnprfx is provided)

```

Details

classification using SVM: gkmsvm_classify can be used to score any set of sequences. Note that the same set of parameters (L, K, maxnm) used in the gkmsvm_kernel should be specified for optimal classification.

```
gkmsvm_classify(testfn, svfnprfx, outfn); #scores test sequences
```

Author(s)

Mahmoud Ghandi

Examples

```

#Input file names:
posfn= 'test_positives.fa'  #positive set (FASTA format)
negfn= 'test_negatives.fa'  #negative set (FASTA format)
testfn= 'test_testset.fa'   #test set (FASTA format)

#Output file names:
kernelfn= 'test_kernel.txt' #kernel matrix
svfnprfx= 'test_svmtrain'   #SVM files
outfn = 'output.txt'        #output scores for sequences in the test set

# gkmsvm_kernel(posfn, negfn, kernelfn);           #computes kernel
# gkmsvm_train(kernelfn, posfn, negfn, svfnprfx);  #trains SVM
# gkmsvm_classify(testfn, svfnprfx, outfn);        #scores test sequences

```

gkmsvm_delta

Calculating deltaSVM scores

Description

Given support vectors SVs and corresponding coefficients alphas and a pair of file test sequence files (one for reference allele, and one for alternate allele), calculates the deltaSVM scores for the sequences.

Usage

```
gkmsvm_delta(seqfile_allele1, seqfile_allele2, svmfprfx, outfile, L = 10,
K = 6, maxnmm = 3, maxseqlen = 10000, maxnumseq = 1e+06, useTgkm = 1, alg = 2,
addRC = TRUE, usePseudocnt = FALSE, batchSize = 1e+05, wildcardLambda = 1,
wildcardMismatchM = 2, alphabetFN = "NULL", svseqfile = NA, alphafile = NA,
outfile_allele1 = NA, outfile_allele2 = NA)
```

Arguments

seqfile_allele1	fasta file containing the test sequences (reference allele)
seqfile_allele2	fasta file containing the test sequences (alternate allele). The sequences in this file should be in the exact same order as in seqfile_allele1.
svmfprfx	SVM model file name prefix
outfile	output file name
L	word length, default=10
K	number of informative columns, default=6
maxnmm	maximum number of mismatches to consider, default=3
maxseqlen	maximum sequence length in the sequence files, default=10000
maxnumseq	maximum number of sequences in the sequence files, default=1000000
useTgkm	filter type: 0(use full filter), 1(use truncated filter: this gaurantees non-negative counts for all L-mers), 2(use h[m], gkm count vector), 3(wildcard), 4(mismatch), default=1
alg	algorithm type: 0(auto), 1(XOR Hashtable), 2(tree), default=0
addRC	adds reverse complement sequences, default=TRUE
usePseudocnt	adds a constant to count estimates, default=FALSE
batchSize	number of sequences to compute scores for in batch, default=100000
wildcardLambda	lambda for wildcard kernel, defaul=0.9
wildcardMismatchM	max mismatch for Mismatch kernel or wildcard kernel, default=2
alphabetFN	alphabets file name, if not specified, it is assumed the inputs are DNA sequences
svseqfile	SVM support vectors sequence file name (not needed if svmfprfx is provided)
alphafile	SVM support vectors weights file name (not needed if svmfprfx is provided)
outfile_allele1	output filename for gkmSVM scores for the reference sequences (optional)
outfile_allele2	output filename for gkmSVM scores for the alternate sequences (optional)

Details

predicting the effect of variants using gkmSVM model: gkmsvm_delta can be used to predict the effect of sequence variants. The sequences corresponding to reference allele and alternate alleles are given in two separate files. gkmSVM is used to score each set of sequences, and the difference in the gkmSVM score for the reference and alternate allele is reported. Note that the same set of parameters (L, K, maxnmm) used in the gkmsvm_kernel should be specified for optimal scoring.

```
gkmsvm_kernel(seqfile_allele1, seqfile_allele2, svmfnprefix, outfn); #scores test sequences
```

Value

deltaSVM scores

Author(s)

Mahmoud Ghandi

References

Ghandi M, Mohammad-Noori M, Ghareghani N, Lee D, Garraway LA, and Beer MA. gkmSVM: an R package for gapped-kmer SVM, Bioinformatics 2016.

Ghandi M, Lee D, Mohammad-Noori M, Beer MA. 2014. Enhanced Regulatory Sequence Prediction Using Gapped k-mer Features. PLoS Comput Biol 10: e1003711.

Lee D, Gorkin DU, Baker M, Strober BJ, Asoni AL, McCallion AS, and Beer MA. A method to predict the impact of regulatory variants from DNA sequence. Nature Genetics 2015.

Examples

```
#Input file names:
posfn= 'test_positives.fa' #positive set (FASTA format)
negfn= 'test_negatives.fa' #negative set (FASTA format)
testfn_ref= 'test_testsetRef.fa' #test set (reference allele) (FASTA format)
testfn_alt= 'test_testsetAlt.fa' #test set (alternate allele) (FASTA format)

#Output file names:
kernelfn= 'test_kernel.txt' #kernel matrix
svmfnprefix= 'test_svmtrain' #SVM files
outfn = 'output.txt' #output delta svm scores for sequences in the test set

# gkmsvm_kernel(posfn, negfn, kernelfn); #computes kernel
# gkmsvm_train(kernelfn, posfn, negfn, svmfnprefix); #trains SVM
# gkmsvm_delta(testfn_ref, testfn_alt, svmfnprefix, outfn); #scores test sequences
```

gkmsvm_kernel *Computing the kernel matrix*

Description

Generates a lower triangle of kernel matrix (i.e. pairwise similarities) between the sequences.

Usage

```
gkmsvm_kernel(posfile, negfile, outfile, L=10, K=6, maxnmm=3, maxseqlen=10000,
maxnumseq=1000000, useTgkm=1, alg=0, addRC=TRUE, usePseudocnt=FALSE, wildcardLambda=1.0,
wildcardMismatchM=2, alphabetFN="NULL")
```

Arguments

posfile	positive sequences file name (FASTA format)
negfile	negative sequences file name (FASTA format)
outfile	output file name
L	word length, default=10
K	number of informative columns, default=6
maxnmm	maximum number of mismatches to consider, default=3
maxseqlen	maximum sequence length in the sequence files, default=10000
maxnumseq	maximum number of sequences in the sequence files, default=1000000
useTgkm	filter type: 0(use full filter), 1(use truncated filter: this gaurantees non-negative counts for all L-mers), 2(use h[m], gkm count vector), 3(wildcard), 4(mismatch), default=1
alg	algorithm type: 0(auto), 1(XOR Hashtable), 2(tree), default=0
addRC	adds reverse complement sequences, default=TRUE
usePseudocnt	adds a constant to count estimates, default=FALSE
wildcardLambda	lambda for wildcard kernel, defaul=0.9
wildcardMismatchM	max mismatch for Mismatch kernel or wildcard kernel, default=2
alphabetFN	alphabets file name, if not specified, it is assumed the inputs are DNA sequences

Details

It calculates the full kernel matrix that can be then used to train an SVM classifier. `gkmsvm_kernel(posfn, negfn, kernelfn)`;

Author(s)

Mahmoud Ghandi

Examples

```

#Input file names:
posfn= 'test_positives.fa' #positive set (FASTA format)
negfn= 'test_negatives.fa' #negative set (FASTA format)
testfn= 'test_testset.fa' #test set (FASTA format)

#Output file names:
kernelfn= 'test_kernel.txt' #kernel matrix
svmfnprefix= 'test_svmtrain' #SVM files
outfn = 'output.txt' #output scores for sequences in the test set

# gkmsvm_kernel(posfn, negfn, kernelfn); #computes kernel
# gkmsvm_train(kernelfn,posfn, negfn, svmfnprefix); #trains SVM
# gkmsvm_classify(testfn, svmfnprefix, outfn); #scores test sequences

```

gkmsvm_train	<i>Training the SVM model</i>
--------------	-------------------------------

Description

Using the kernel matrix created by 'gkmsvm_kernel', this function trains the SVM classifier. Here we rely on the 'kernlab' package, and merely provide a wrapper function.

Usage

```
gkmsvm_train(kernelfn, posfn, negfn, svmfnprefix, Type="C-svc", C=1, shrinking=FALSE, ...)
```

Arguments

kernelfn	kernel matrix file name
posfn	positive sequences file name
negfn	negative sequences file name
svmfnprefix	output SVM model file name prefix
Type	optional: SVM type (default='C-svc'), see 'kernlab' documentation for more details.
C	optional: SVM parameter C (default=1), see 'kernlab' documentation for more details.
shrinking	optional: shrinking parameter for kernlab (default=FALSE), see 'kernlab' documentation for more details.
...	optional: additional SVM parameters, see 'kernlab' documentation for more details.

Details

Trains SVM classifier and generates two files: [svmfnprefix]_svalpha.out for SVM alphas and the other for the corresponding SV sequences ([svmfnprefix]_svseq.fa)

Author(s)

Mahmoud Ghandi

Examples

```

#Input file names:
posfn= 'test_positives.fa'  #positive set (FASTA format)
negfn= 'test_negatives.fa'  #negative set (FASTA format)
testfn= 'test_testset.fa'   #test set (FASTA format)

#Output file names:
kernelfn= 'test_kernel.txt' #kernel matrix
svmfnprefix= 'test_svmtrain' #SVM files
outfn = 'output.txt'        #output scores for sequences in the test set

# gkmsvm_kernel(posfn, negfn, kernelfn);           #computes kernel
# gkmsvm_train(kernelfn, posfn, negfn, svmfnprefix); #trains SVM
# gkmsvm_classify(testfn, svmfnprefix, outfn);     #scores test sequences

```

gkmsvm_trainCV	<i>Training the SVM model, using repeated CV to tune parameter C and plot ROC curves</i>
----------------	--

Description

Using the kernel matrix created by 'gkmsvm_kernel', this function trains the SVM classifier. It uses repeated CV to find optimum SVM parameter C. Also generates ROC and PRC curves.

Usage

```

gkmsvm_trainCV(kernelfn, posfn, negfn, svmfnprefix=NA,
  nCV=5, nrepeat=1, cv=NA, Type="C-svc", C=1, shrinking=FALSE,
  showPlots=TRUE, outputPDFfn=NA, outputCVpredfn=NA, outputROCFn=NA, ...)

```

Arguments

kernelfn	kernel matrix file name
posfn	positive sequences file name
negfn	negative sequences file name
svmfnprefix	(optional) output SVM model file name prefix
nCV	(optional) number of CV folds
nrepeat	(optional) number of repeated CVs
cv	(optional) CV group label. An array of length (npos+nneg), containing CV group number (between 1 and nCV) for each sequence
Type	(optional) SVM type (default='C-svc'), see 'kernlab' documentation for more details.

C	(optional)a vector of all values of C (SVM parameter) to be tested. (default=1), see 'kernlab' documentation for more details.
shrinking	optional: shrinking parameter for kernlab (default=FALSE), see 'kernlab' documentation for more details.
showPlots	generate plots (default==TRUE)
outputPDFfn	filename for output PDF, default=NA (no PDF output)
outputCVpredfn	filename for output cvpred (predicted CV values), default=NA (no output)
outputROCFn	filename for output auROC (Area Under an ROC Curve) and auPRC (Area Under the Precision Recall Curve) values, default=NA (no output)
...	optional: additional SVM parameters, see 'kernlab' documentation for more details.

Details

Trains SVM classifier and generates two files: [svmfnprefix]_svalpha.out for SVM alphas and the other for the corresponding SV sequences ([svmfnprefix]_svseq.fa)

Author(s)

Mahmoud Ghandi

Examples

```
#Input file names:
posfn= 'test_positives.fa' #positive set (FASTA format)
negfn= 'test_negatives.fa' #negative set (FASTA format)
testfn= 'test_testset.fa' #test set (FASTA format)

#Output file names:
kernelfn= 'test_kernel.txt' #kernel matrix
svmfnprefix= 'test_svmtrain' #SVM files
outfn = 'output.txt' #output scores for sequences in the test set

# gkmsvm_kernel(posfn, negfn, kernelfn); #computes kernel
# cvres = gkmsvm_trainCV(kernelfn,posfn, negfn, svmfnprefix,
#   outputPDFfn='ROC.pdf', outputCVpredfn='cvpred.out');
# #trains SVM, plots ROC and PRC curves, and outputs model predictions.
# gkmsvm_classify(testfn, svmfnprefix, outfn); #scores test sequences
```

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