# rnaSeqMap

October 25, 2011

NDplots

Genomic plots based upon NucleotideDistr objects

# Description

Various plots of genomic coverage for data from NucleotideDistr objects

# Usage

```
distrCOVPlot(nd, exps)
distrSIPlot(nd, ex1, ex2, mi,minsup=5)
```

# Arguments

nd	NucleotideDistr object
exps	vectors of experiment numbers to plot
ex1,ex2	experiment numbers to plot
mi	threshold in the region mining algorithm
minsup	minimal support - minimal length of the irreducible region found

## Author(s)

Michal Okoniewski, Anna Lesniewska

```
data(sample_data_rnaSeqMap)
rs <- rs.list[[1]]
if (xmapConnected())
{
   nd.cov <- getCoverageFromRS(rs,1:6)
   distrSIPlot(nd.cov, 1,3, mi=5, minsup=10)
}</pre>
```

NucleotideDistr-class

Numeric distributions by nucleotide - class

## Description

An S4 class that inherits from eSet and holds all the numeric distributions of functions defined over the genome. The values may include coverage, splicing, fold change, etc. for a region defined by genomic coordinates.

#### **Slots/List Components**

Objects of this class contain (at least) the following list components:

chr: numeric matrix containing the read counts.

start: data.frame containing the library size and group labels.

end: data.frame containing the library size and group labels.

strand: data.frame containing the library size and group labels.

start: data.frame containing the library size and group labels.

# Methods

distribs gives the matrix of distributions from assayData

getDistr gives a single distributions from assayData as a vector

```
newNuctleotideDistr (distribs, chr, start, end, strand, type="UNKNOWN", phenoData=NULL, featureData=NULL) constructor from a matrix of data and chromosome coordinates.
```

#### Author(s)

Anna Lesniewska, Michal Okoniewski

#### See Also

SeqReads, NDtransforms

RleList2matrix RleList2matrix

## Description

Function transforms list of Rle objects to matrix.

# Usage

RleList2matrix(list);

#### SeqReads

#### Arguments

list list of Rle objects.

# Value

Produces the full, unpacked coverage matrix from a list of Rle objects. Used to re-format the coverage data.

## Author(s)

Michal Okoniewski, Anna Lesniewska

#### Examples

```
if (xmapConnected())
{
    rs <- newSeqReads(1,1,20000,1)
    rs <- getBamData(rs,1:3)
    nd.cov <- getCoverageFromRS(rs,1:3)
    RleList2matrix(nd.cov@data)
}</pre>
```

SeqReads

SeqReads - a container for RNAseq reads

#### Description

SeqReads objects keep the reads information in the form of a list, containing one matrix of reads per experiment. Matrices of dimension n x 2 should come from a mapping to the regions defined by genome coordinates (chromosome, start, end, strand) in the SeqReads object.

The object may be filled in from the database or from list with read data. It is recommended to create one SeqReads object per gene or intergenic region. The object are used then ot create object of class NucleotideDistr

## Usage

```
newSeqReads(chr, start, end, strand, datain=NULL, phenoData=NULL, featureData=NU
newSeqReadsFromGene(g)
```

#### Arguments

chr	Chromosome
start	Start of the region on a chromosome
end	End of the region on a chromosome
strand	Genome strand: 1 or -1
datain	If supplied, it must be a list of matrices of reads start and stop
g	Ensembl identifier of a gene
phenoData	
featureData	
covdesc	Filename for experiment description

## Value

Object of a class SeqReads

#### Author(s)

Michal Okoniewski, Anna Lesniewska

addBamData

addBamData - getting sample data from BAM file.

## Description

Add data from experimental samples stored in BAM file.

## Usage

addBamData(rs, file, exp, phenoDesc=NULL)

# Arguments

rs	SeqReads object to modify
file	BAM file to read
exp	Numbers of sample slot in the object
phenoDesc	A vector to add to phenoData

## Value

SeqReads object with samples added from the BAM files. List of BAM files comes from the covdesc. The covdesc content becomes phenoData of the object.

#### Author(s)

Michal Okoniewski, Anna Lesniewska

```
if (xmapConnected())
{
    rs <- newSeqReads(1,1,20000,1)
    rs <- addBamData(rs,1:3)
}</pre>
```

addDataToReadset addDataToReadset - adding one more sample in the SeqRead on R level

# Description

Add another reads matrix to the readset. No control of region consistency, the matrix needs just 2 columns: starts and ends.

#### Usage

```
addDataToReadset(rs, datain, spl)
```

#### Arguments

rs datain spl Number or name of the experimental sample

## Value

SeqReads object with one more sample added.

#### Author(s)

Michal Okoniewski, Anna Lesniewska

## Examples

```
rs <- newSeqReads(1,1,20000,1)
my.data1 <- rbind(c(1,50), c(3,53), c(11,60))
rs <- addDataToReadset(rs, my.data1, 1)</pre>
```

addExperimentsToReadset

addExperimentsToReadset - getting sample data from the database.

#### Description

Add data from experimental samples in the xXMAP database to the readset. Connection to the database required.

#### Usage

addExperimentsToReadset(rs, exps)

#### Arguments

rs	SeqReads object to modify
exps	Vector of numbers of experimental samples in xXMAP

#### averageND

## Value

SeqReads object with samples added from the database.

## Author(s)

Michal Okoniewski, Anna Lesniewska

## Examples

```
if (xmapConnected())
{
    rs <- newSeqReads(1,1,20000,1)
    rs <- addExperimentsToReadset(rs,1:3)
}</pre>
```

averageND

averageND, sumND, combineNS, log2ND - operations on distributions

#### Description

Set of functions to operate on NucleotideDistr objects.

averageND calculates the mean for samples, sumND adds up selected samples' distributions, combineND adds two objects with the same size of distribution matrix, log2ND transforms all numeric data in the object into log space.

## Usage

```
averageND(nd, exps);
sumND(nd, exps);
combineND(nd1, nd2);
log2ND(nd);
```

#### Arguments

nd, nd1, nd2 NucleotideDistrobjects exps a pair of numbers of samples in the experiment

# Value

NucleotideDistr object of the same type as input objects

#### Author(s)

Michal Okoniewski, Anna Lesniewska

#### bam2sig

## Examples

```
if (xmapConnected())
{
    rs <- newSeqReads(1,1,20000,1)
    nd.cov <- getCoverageFromRS(rs,1:3)
    nd.avg <- averageND(nd.cov,c(1,3))
    nd.sum <- averageND(nd.cov,c(1,3))
    nd.sum <- combineND(nd.cov,nd.cov)
    nd.log <- log2ND(nd.cov)
}</pre>
```

bam2sig

bam2sig - encapsulated pipeline of finding significant expression

## Description

Reads BAM files according to annotation and produces output table processed with DESeq negative binomial test.

## Usage

```
bam2sig(annot, covdesc="covdesc", species="homo_sapiens")
```

#### Arguments

annot	Character table or data frame with colums: chr, start, end, strand, name
covdesc	Name of the file that includes BAM files (experiment description file)
species	Species name - needed for .chr.convert function - to match BAM and annotation chromosome names

## Value

Output table with significant expression results, as from DESeq

## Author(s)

Michal Okoniewski, Anna Lesniewska

```
if (xmapConnected())
{
    all.g <- all.genes(as.vector=F)
    ss <- sample(1:20000, 10)
    genes <- as.data.frame(all.g[ss,])

    genes <- cbind(as.vector(genes[,"stable_id"]), as.vector(genes[,"space"]), as.vector(g
    colnames(genes) <- c("name", "chr", "start", "end", "strand")

    deseqRes <- bam2sig()
    deseqRes[1:10,]
}</pre>
```

buildDESeq

## Description

 $Creates \verb|CountDataSet| from the data in the database using the list of genes supplied - for further analysis with DESeq$ 

# Usage

buildDESeq(genes, exps, conds=NULL)

#### Arguments

genes	vector of Ensembl gene IDs
exps	vector of experiments
conds	Vector of experimental condition descriptions for the samples

## Value

CountDataSet object filled with the data of gene-level counts of reads

## Author(s)

Michal Okoniewski, Anna Lesniewska

## See Also

buildDGEList

## Examples

```
if (xmapConnected())
{
    data(sample_data_rnaSeqMap)
    gg <- names(rs.list)
    cds <- buildDESeq(gg,1:6, c("a","b","b","a","a","b"))
}</pre>
```

buildDGEList buildDGEList - create DGEList (edgeR)

#### Description

 $Creates \, {\tt DGEList} \ from \ the \ data \ in \ the \ data base \ using \ the \ list \ of \ genes \ supplied \ - \ for \ further \ analysis \ with \ edgeR$ 

# Usage

```
buildDGEList(genes,exps,conds=NULL)
```

#### findRegionsAsIR

#### Arguments

genes	vector of Ensembl gene IDs
exps	vector of experiments
conds	Vector of experimental condition descriptions for the samples

# Value

DGEList object filled with the data of gene-level counts of reads

# Author(s)

Michal Okoniewski, Anna Lesniewska

#### See Also

buildDESeq

## Examples

```
if (xmapConnected())
{
    data(sample_data_rnaSeqMap)
    gg <- names(rs.list)
    cds <- buildDGEList(gg,1:6, c("a","b","b","a","a","b"))
}</pre>
```

findRegionsAsIR findRegionsAsIR - finding regions of high coverage using Lindell-Aumann

#### Description

The function is running Lindell-Aumann algorithm to find regions of irreducible expression on the coverage data in the NucleotideDistr object. The function may be used to find the location and boundaries of significant expression of exons and small RNA.

#### Usage

```
findRegionsAsIR(nd, mi, minsup=5, exp)
```

## Arguments

nd	An object of $\ensuremath{\texttt{NucleotideDistr}}$ class that has coverage values for a given region
mi	The threshold of coverage that makes the region significant
minsup	Minimal support of the numeric association rule - namely, in this case, the min- inmal length of the discovered region
exp	Sample (experiment) number

IRanges object with irreducible regions with high coverage.

#### Author(s)

Michal Okoniewski, Anna Lesniewska

# Examples

```
if (xmapConnected())
{
   rs <- newSeqReads(1,1,20000,1)
   rs <- addExperimentsToReadset(rs,1:3)
   nd.cov <- getCoverageFromRS(rs,1:3)
   nd.regs <- findRegionsAsND(nd.cov, 10)
}</pre>
```

findRegionsAsND	findRegionsAsND - finding regions of high coverage using Lindell-
	Aumann

## Description

The function is running Lindell-Aumann algorithm to find regions of irreducible expression on the coverage data in the NucleotideDistr object. The function may be used to find the location and boundaries of significant expression of exons and small RNA.

#### Usage

findRegionsAsND(nd, mi, minsup=5)

#### Arguments

nd	An object of NucleotideDistr class that has coverage values for a given region
mi	The threshold of coverage that makes the region significant
minsup	Minimal support of the numeric association rule - namely, in this case, the min- inmal length of the discovered region

## Value

NucleotideDistr object that includes a matrix with zeros where no region was found and the value of mi for all the nucleotides included in the region. The type fo the object is "REG".

## Author(s)

Michal Okoniewski, Anna Lesniewska

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#### geneInChromosome

# Examples

```
if (xmapConnected())
{
    rs <- newSeqReads(1,1,20000,1)
    rs <- addExperimentsToReadset(rs,1:3)
    nd.cov <- getCoverageFromRS(rs,1:3)
    nd.regs <- findRegionsAsND(nd.cov, 10)
}</pre>
```

geneInChromosome geneInChromosome

## Description

Finds all the genes in the given chromosome regions

## Usage

geneInChromosome(chr, start, end, strand)

# Arguments

chr	Chromosome
start	Start of the region on a chromosome
end	End of the region on a chromosome
strand	Genome strand: 1 or -1

## Value

table of the genes in a given regions, produced with stored procedure

## Author(s)

Michal Okoniewski, Anna Lesniewska

```
if (xmapConnected())
{
   geneInChromosome(1, 1, 80000, 1)
}
```

getBamData

## Description

Add data from experimental samples stored in BAM file.

## Usage

getBamData(rs, exps=NULL, files=NULL, unstranded=FALSE, covdesc="covdesc")

## Arguments

rs	SeqReads object to modify
exps	Vector of numbers of experimental samples
files	Vector of BAM files to read
unstranded	Flag which type of data are using (with distinguishing strand or not)
covdesc	Alternatively, the experiment description file

# Value

SeqReads object with samples added from the BAM files. List of BAM files comes from the covdesc. The covdesc content becomes phenoData of the object.

## Author(s)

Michal Okoniewski, Anna Lesniewska

#### Examples

```
if (xmapConnected())
{
    rs <- newSeqReads(1,1,20000,1)
    rs <- getBamData(rs,1:3)
}</pre>
```

getCoverageFromRS getCoverageFromRS - conversion to coverage object

# Description

Calculates the coverage function for the reads encapsulated in the SeqReads object.

# Usage

```
getCoverageFromRS(rs, exps)
```

#### getExpDescription

#### Arguments

rs	SeqReads object to modify
exps	Vector of numbers of experimental samples in xXMAP

# Value

NucleotideDistr object with coverage matrix in assayData slot and type "COV".

## Author(s)

Michal Okoniewski, Anna Lesniewska

## Examples

```
if (xmapConnected())
{
    rs <- newSeqReads(1,1,20000,1)
    rs <- addExperimentsToReadset(rs,1:6)
    nd.cov <- getCoverageFromRS(rs,1:3)
}</pre>
```

getExpDescription getExpDescription

# Description

Gets the bio\_sample table from the database. May be used as phenoData.

## Usage

```
getExpDescription()
```

## Value

Table of experimental factors assigned to numbers of samples.

#### Author(s)

Michal Okoniewski, Anna Lesniewska

getFCFromND

## Description

This function calculates the fold change of two sample coverages from a NucleotideDistr objects. The coverages are assumed to be after logarithmic transformation, so the function basically subtracts the value and generates new NucleotideDistr object with a single vector of fold changes.

## Usage

```
getFCFromND(nd, exps)
```

#### Arguments

nd	NucleotideDistr object with coverages
exps	a pair of numbers of samples in the experiment

## Value

NucleotideDistr object of type "FC" with a single vector of fold changes

#### Author(s)

Michal Okoniewski, Anna Lesniewska

#### Examples

```
if (xmapConnected())
{
    rs <- newSeqReads(1,1,20000,1)
    rs <- addExperimentsToReadset(rs,1:3)
    nd.cov <- getCoverageFromRS(rs,1:3)
    nd.fc <- getFCFromND(nd.cov,c(1,3))
}</pre>
```

getSIFromND getSIFromND - calculating splicing index of two coverages

## Description

This function calculates the splicing index value of two sample coverages from a NucleotideDistr object. It is assumed that the region in the NucleotideDistr is a single gene. Splicing index is calculated in similar way to the implementation for exon Affy microarrays (see Gardina et al, Genome Biology, 2007 for details), but it is run for each nucleotide in the region and instead of gene-level average expression values, it uses sums of reads for both samples.

# Usage

```
getSIFromND(nd, exps)
```

#### getSumsExp

## Arguments

nd	NucleotideDistr object with coverages
exps	a pair of numbers of samples in the experiment

## Value

NucleotideDistr object of type "FC" with a single vector of splicing index values

## Author(s)

Michal Okoniewski, Anna Lesniewska

## Examples

```
if (xmapConnected())
{
    rs <- newSeqReads(1,1,20000,1)
    nd.cov <- getCoverageFromRS(rs,1:3)
    nd.fc <- getSIFromND(nd.cov,c(1,3))
}</pre>
```

getSumsExp getSumsExp

## Description

Gets the sum of reads in all the samples present in the database in the seq\_read table

# Usage

```
getSumsExp()
```

# Value

Vector of sums

## Author(s)

Michal Okoniewski, Anna Lesniewska

```
if (xmapConnected())
{
   sums <- getSumsExp()
   nsums
}</pre>
```

```
normalizeBySum
```

#### Description

normalizeBySum function normalizes the coverage values in NucleotideDistr by dividing all the numbers for all samples by the sum of reads for each sample. The number of reads from each sample may be taken from the database by the function getSumsExp, which is a wrapper for an appropriate SQL procedure. Alternatively, it is passed directly as a vector of numeric values of the same length as the number of samples analyzed. Such simple normalization allows comparisons of the coverage values for samples with different number of reads

# Usage

```
normalizeBySum(nd, r=NULL)
```

## Arguments

nd	NucleotideDistr object with raw read counts
r	Vector of numbers. If there is no such parameter, a database procedure summa- rizing reads is run

#### Value

NucleotideDistr object

#### Author(s)

Michal Okoniewski, Anna Lesniewska

#### See Also

getSumsExp

```
if (xmapConnected())
{
    rs <- newSeqReads(1,10000,20000,1)
    nd.cov <- getCoverageFromRS(rs,1:3)
    nd.norm <- normalizeBySum(nd.cov)
    nd.norm <- normalizeBySum(nd.cov, r=c(100, 200, 1000))
}</pre>
```

parseGff3

#### Description

Parses gff3 file into genes, transcripts and exons.

## Usage

```
parseGff3(filegff, fileg="genes.txt", filet="transcripts.txt", filee="exons.txt"
```

#### Arguments

filegff	Input file in GFF3 format
fileg	Filename for output: genes
filet	Filename for output: transcripts
filee	Filename for output: exons
nofiles	Flag: just optput list, no files

## Value

List with elements "genes", "transcripts", "exons" with appropriate tables.

#### Author(s)

Michal Okoniewski, Anna Lesniewska

## Examples

```
if (xmapConnected())
{
    parseGff3("Athaliana.gff3")
}
```

plotGeneCoverage Genomic plots with rnaSeqMap

# Description

Various plots of genomic coverage for experiments.

# Usage

```
plotGeneCoverage(gene_id, ex)
plotRegionCoverage(chr, start, end, strand, ex)
plotExonCoverage (exon_id,ex)
plotCoverageHistogram (chr,start,end,strand,ex, skip)
plotGeneExonCoverage(gene_id, ex)
plotSI(chr,start,end,strand, exp1, exp2 )
```

## Arguments

1 I	
exp1, exp2 experiment numbers for splicing index	
gene_id Ensembl gene ID	
exon_id Ensembl exon ID	
chr Chromosome	
start Start position of region on the chromosom	e
end Start position of region on the chromosom	e
strand Strand	
skip size of the bucket in histogram	

#### Author(s)

Michal Okoniewski, Anna Lesniewska

## Examples

```
if (xmapConnected())
{
    plotGeneCoverage( "ENSG00000144567", 1:3) # plotting FAM134A for experiments 1,2,3
    plotRegionCoverage( 2, 220040947, 220050201, 1, 1:3 ) # the same, using coordinates
}
```

readsInRange readsInRange

# Description

Finds all the reads for a genomic range

## Usage

readsInRange(chr, start, end, strand, ex)

## Arguments

chr	Chromosome
start	Start of the region on a chromosome
end	End of the region on a chromosome
strand	Genome strand: 1 or -1
ex	experiment

#### Value

table of reads, as in the database

# Author(s)

Michal Okoniewski, Anna Lesniewska

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#### regionBasedCoverage

#### Examples

```
if (xmapConnected())
{
   tmp <- readsInRange( 1, 10000, 20000, 1,3)
}</pre>
```

#### regionBasedCoverage

regionBasedCoverage - transformation of the region coverage by the

## Description

The function builds a NucleotideDistr object from another object of coverage, using sequential call of Lindell-Aumann algorithm on the same data with a sequence of mi-levels. Each nucleotide is assigned the maximum mi-value of a region that covers it.

The output NucleotideDistr object has the distribution without peaks and small drops of coverage, but the thade-off is that the level of coverage are discrete: seq\\*maxexp.

#### Usage

```
regionBasedCoverage(nd, seqq=1:10, maxexp=20, minsup=5)
```

#### Arguments

nd	An object of NucleotideDistr class that has coverage values for a given region
seqq	Vector of numbers used to divide the range of coverage for subsequent mi-levels
maxexp	The maximal mi-level for coverage
minsup	Minimal support of the numeric association rule - namely, in this case, the min- inmal length of the discovered region

#### Value

NucleotideDistr object that includes a matrix with zeros where no region was found and a maximum of mi-levels used for the sequential region searched. The distributions are similar to coverage, but have removed outliers of coverage peaks and short drops of coverage.

## Author(s)

Michal Okoniewski, Anna Lesniewska

```
if (xmapConnected())
{
    rs <- newSeqReads(1,1,20000,1)
    rs <- addExperimentsToReadset(rs,1:3)
    nd.cov <- getCoverageFromRS(rs,1:3)
    nd.regs <- regionBasedCoverage(nd.cov, 1:10, 100)
    #runs the Lindell-Aumann algorithm at 100, 90, ... and picks maximal mi-level, where th
}</pre>
```

regionCoverage regionCoverage

# Description

Finds all the reads for a genomic range

# Usage

```
regionCoverage(chr, start, end, strand, ex, db = "FALSE" )
```

#### Arguments

chr	Chromosome
start	Start of the region on a chromosome
end	End of the region on a chromosome
strand	Genome strand: 1 or -1
ex	experiment
db	Use database (SQL) implementation of the algorithm

## Value

coverage vector, independent from NucleotideDistr

#### Author(s)

Michal Okoniewski, Anna Lesniewska

# Examples

```
if (xmapConnected())
{
  tmp <- regionCoverage( 1, 10000, 20000, 1,3)
}</pre>
```

rs.list

```
Example of sequencing data for rnaSeqMap library
```

#### Description

A fragment of sequencing data from 6 samples - human.

# Usage

```
data(sample_data_rnaSeqMap)
```

#### Format

A list with 17 SeqReads objects, each with sequencing reads from 6 samples sequenced with ABI SOLID machine.

#### setSpecies

#### Examples

```
data(sample_data_rnaSeqMap)
length(rs.list)
gene1rs <- rs.list[[1]]</pre>
```

setSpecies setSpecies

#### Description

Sets the species name for chromosomes X, Y and MT translation into consecutive numbers. If you use xmap.connect, no need to call setSpecies. Both set the internal variable of xmapcore.

#### Usage

```
setSpecies(name=NULL)
```

# Arguments

name Species name

## Author(s)

Michal Okoniewski, Anna Lesniewska

#### Examples

setSpecies("mus\_musculus")

spaceInChromosome spaceInChromosome

## Description

Finds all the intergenic spaces in the given chromosome region

# Usage

```
spaceInChromosome(chr, start, end, strand)
```

## Arguments

chr	Chromosome
start	Start of the region on a chromosome
end	End of the region on a chromosome
strand	Genome strand: 1 or -1

# Value

table of the intergenic spaces in a given regions, produced with stored procedure

# Author(s)

Michal Okoniewski, Anna Lesniewska

## Examples

```
if (xmapConnected())
{
   spaceInChromosome(1, 1, 80000, 1)
}
```

xmapConnected xmapConnected

# Description

Checks if the connection to the xmap database has been already done. If not, use xmap.connect.

# Usage

xmapConnected()

# Author(s)

Michal Okoniewski, Anna Lesniewska

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