# rnaSeqMap

# April 20, 2011

 ${\it addDataToReadset-adding\ one\ more\ sample\ in\ the\ SeqRead\ on\ R} \\ {\it level}$ 

### **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

```
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>
```

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```
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
```

#### 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 NucleotideDistrobjects.

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);
```

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#### **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

#### **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>
```

buildDESeq

buildDESeq - create CountDataSet

# **Description**

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

# 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)

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#### 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

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

# Usage

```
buildDGEList(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

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

# Author(s)

Michal Okoniewski, Anna Lesniewska

### See Also

buildDESeq

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

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 $\begin{tabular}{ll} find Regions As IR & \it{finding regions of high coverage using Lindell-Aumann algorithm.} \\ \end{tabular}$ 

# 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 ${\tt NucleotideDistr}$ 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 minimal length of the discovered region
exp	Sample (experiment) number

# Value

IRanges object with irreducible regions with high 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 <- findRegionsAsND(nd.cov, 10)
}</pre>
```

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 $\begin{tabular}{ll} find Regions As ND & \it{find Regions As ND} & \it{find Regi$ 

# 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 ${\tt NucleotideDistr}$ 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 minimal 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

```
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>
```

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```
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

# **Examples**

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

 ${\tt getCoverageFromRS} \ \ \textit{getCoverageFromRS} \ \textit{-} \ \textit{conversion to coverage object}$ 

# Description

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

### Usage

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

# Arguments

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

8 getFCFromND

#### 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
```

getFCFromND - calculating fold change of coverages

#### **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)
```

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#### **Arguments**

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

#### Value

NucleotideDistrobject 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)
```

#### **Arguments**

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

# Value

NucleotideDistrobject of type "FC" with a single vector of splicing index values

#### Author(s)

NDplots

### **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

# **Examples**

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

NDplots

Genomic plots based upon NucleotideDistr objects

# **Description**

Various plots of genomic coverage for data from NucleotideDistrobjects

# Usage

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

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# **Arguments**

nd	NucleotideDistrobject
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

### **Examples**

```
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>
```

normalizeBySum

Normalization of NucleotideDistr by global number of reads

#### **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 NucleotideDistrobject with raw read counts

vector of numbers. If there is no such parameter, a database procedure summarizing reads is run

### Value

NucleotideDistr object

# Author(s)

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#### See Also

getSumsExp

# **Examples**

```
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>
```

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

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```
plotGeneCoverage Genomic plots with rnaSeqMap
```

#### **Description**

Various plots of genomic coverage for experiments.

#### Usage

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

### **Arguments**

```
vectors of experiment numbers to plot
ex
                 experiment numbers for splicing index
exp1, exp2
gene_id
                 Ensembl gene ID
                 Ensembl exon ID
exon_id
chr
                 Chromosome
                 Start position of region on the chromosome
start
                 Start position of region on the chromosome
end
                 Strand
strand
db
                 uses database implementation
                 size of the bucket in histogram
skip
```

#### Author(s)

Michal Okoniewski, Anna Lesniewska

```
if (xmapConnected())
{
  plotGeneCoverage( "ENSG00000141510", 1:3) # plotting TP53 for experiments 1,2,3
  plotRegionCoverage( 17, 7565257, 7590856, -1, 1:3 ) # the same, using coordinates
}
```

reads	InRange

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

# **Examples**

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

regionBasedCoverage

regionBasedCoverage - transformation of the region coverage by the Lindell-Aumann regions

# 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.

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### 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

### **Examples**

```
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 the content of the con
```

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

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#### 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.

# Examples

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

setSpecies 17

# Usage

```
newSeqReads(chr, start, end, strand, datain=NULL)
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

# Value

Object of a class SeqReads

# Author(s)

Michal Okoniewski, Anna Lesniewska

# 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

```
setSpecies("mus_musculus")
```

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```
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)

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