Using spliceSites package

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1 Introduction

The data structures and algorithms in this package work on align-gaps which are found in alignments of RNA-seq data. The analysis starts by reading BAM-files [2], so *spliceSites* assumes that the sequenced RNA is already aligned by external alignment software (e.g. tophat [3]). *spliceSites* technically builds upon CRAN package *rbamtools* which performs the reading and data collecting part and CRAN package *refGenome* from which processed annotation data is imported.

Splice-site information focuses on align-gaps (which are identified by the **N** CIGAR tag) here in this package. Gapped alignments are highly informative because they are calculated by specialized alignment recognition algorithms. Ungapped alignments are only globally counted but not further traced here. This cuts out a relative small but specific part of the alignment information. By doing this possibly valuable information but also many uncertainties are removed from the calculated models.

Align gaps are assumed to arise from splice-sites. This package technically deals with align-gaps but heavily relies on the fact that align-gaps represent splice-sites. That's why the descriptions contain many switches between the align-view and the splice-site view. An important detail at this point is that the inner align frontiers represent exon-intron boundaries. Their positions, read-counts and the surrounding DNA-sequence are the central objectives of the contained algorithms. In contrast, the outer align-boundaries are merely considered as technical artifacts.

Nomenclature. Align-gaps denote gaps in individual aligns. Each align-gap corresponds to a single **N** CIGAR-item. A gap-site is the unique genomic range where align-gaps are placed. Typically, there are many align-gaps which share one gap-site. A gap-site is described by the framing two genomic ranges: The **left** range denotes the one with the lower genomic coordinates which is on the left side of the gap in genome-browser views. The corresponding **right** range denotes the one with the higher genomic coordinates. The **left**-**right** nomenclature is independent of strand orientation.

Each range is described by a start (left) and an and (right) position. All position values are 1-based, which means that the leftmost character in a sequence is addressed by 1. Start and stop positions denote the 1-based position of the first and

last nucleotide, respectively, which are contained in the range.

The content of one BAM-file is associated here with one biological probe.

2 Formal concepts

2.1 Quantification of gap-site align numbers

Quantification of align numbers for gap-sites differs from the widely used **FPKM** method in that gap sites are not associated with some kind of genomic extend which is addressed by the **K** (kilobase of transcript). Instead number of aligns which contain a specified gap-site (defined by a unique left-end and right-start value) are counted and normalized by a somehow global align number. The spliceSite package provides two quantification indexes.

GPTM gptm abbreviates "Gapped Per Ten Million reads". The value represents the relative amount of aligns for a specific splice-site in relation to ten million aligned reads per probe. The definition of gptm is:

$$gptm = \frac{\texttt{Number of aligns per gap-site}}{\texttt{Total number of aligns}} \cdot 10^7.$$

RPMG RPMG abbreviates "Reads Per Million Gapped". The value represents the relative amount of aligns for a specific splice-site in relation to one million gapped reads per probe. The value is calculated as

$$rpmg = \frac{\texttt{Number of aligns per splice site}}{\texttt{Number of gapped aligns in probe}} \cdot 10^6.$$

Both values are influenced by the size of the underlying align pool. During merge operations, the site-specific and total align numbers are summed and the gptm and rpmg values are recalculated. The resulting values are weighted by the align numbers in each component and differs from the mean. Both values are given as rounded values.

3 Technical description of data structures

Like most software products, this package uses data containers and associated functions. In the following technical part, each container type will be described. The associated functions will be specified direct subsequently for each class.

spliceSites data container The data-structures in this package can be divided in two lineages of data containers and some additional classes which are used for specialized tasks. The two lineages are unilateral container (derived from `cRanges`) and bilateral container (derived from `gapSites`):

- cRanges (centered ranges) focus on exon-intron boundaries on one side of the gap-sites. They contain coordinates of genomic ranges (refid, start, end) and additionally a position inside. The position points to where the gap-boundary

(exon-intron boundary) lies inside the range. The derived classes `cdRanges` and `caRanges` additionally contain DNA and amino acid (AA) sequences.

- `gapSites` (Gapped ranges) focus on two genomic ranges which together surround an gap-sites. The ranges represent two (connected) exons with an interposed intron. The derived classes `annGapSites`, `dnaGapSites` and `aaGapSites` additionally contain annotation data, DNA-sequence and amino-acid sequence respectively.

Additionally there is a class `keyProfiler` which is used to cumulate values for gapSites in multiple Probes (BAM-files) for probe subgroups (e.g. gender specific) and a class `maxEntScore` from which maxEntScores can be calculated [4].

3.1 `gapSites` **class lineage**

From the base class `gapSites` the lineage derives three child classes: `dnaGapSites`, `aaGapSites` which additionally contain a sequence slot and the class `annGapSites` in which annotation data is shifted into the main table.

gapSites is the central container of the spliceSite package. gapSites objects are intended to organize information about gap-sites. Typically the collected sites arise from analysis of multiple probes (BAM-files). The underlying concept is to accumulate information about the biological existance of gap-sites over the whole experiment.
The gapSites class contains the following slots:

Name	Type	Content
dt	data.frame	Table containing the main (gap-site) data
nAligns	numeric	Total number of aligns counted in the data source
nAlignGaps	numeric	Total number of align gaps counted in the data source
annotation	data.frame	Annotation data
profile	data.frame	Table describing the probes in the data source

The nAlignGaps value counts the number of **N** CIGAR-items in the data source. Therefore aligns with two or more align-gaps genereate multiple counts. It is possible albeit uncommon to have more nAlignGaps than nAligns.

gapSites keep the gap-sites data in the dt slot inside a `data.frame`. Each gap-site is represented as one record (line). The table keeps 12 columns which are organized as follows:

Name	Type	Content
id	numeric	Row identifier
seqid	factor	Sequence id (usually chromosome name)
lstart	numeric	Start position of left framing range
lend	numeric	End position left framing range
rstart	numeric	Start position of right framing range
rend	numeric	End position of right framing range
gaplen	numeric	Number of nucleotides in gap
strand	numeric	"+" or "-" or "*"
nAligns	numeric	Number of aligns
nProbes	numeric	Number of probes (BAM-files)
gptm	numeric	Expression quantifier
rpmg	numeric	Expression quantifier

The gptm and rpmg quantifier contain the previously described quantification scores. To be precise the lend value contains the 1-based position of the last exon nucleotide. rstart denotes the 1-based position of the first exon nucleotide.

dnaGapSites and aaGapSites Both classes derive from `gapSites` and additionally contain a seq slot which contains a `DNAStringSet` or `AAStringSet` object respectively.

annGapSites `annGapSites` derives from `gapSites` and additionally keeps information about number of probes and annotation data (which is produced by overlapping). `annGapSites` are returned by the member function `annotation` for class `gapSites`.

Creation of gapSites objects is done by directly reading gap-site data from BAM files.

3.2 Reading align data from BAM-files

The spliceSite package contains four different functions for reading gap-sites from BAM-files. All of them return `gapSites` objects. `getGapSites` and `alignGapList` read from single BAM files via bamReader. `readMergedBamGaps` and `readTabled-BamGaps` receive names of BAM-files and return multi-probe merged gap-site data:

Function	Argument	Read range	Profile
getGapSites	bamReader	Range within BAM-file	no
alignGapList	bamReader	BAM-file	no
readMergedBamGaps	filenames	BAM-files	no
readTabledBamGaps	filenames	BAM-files	yes

Existing BAM-indices are an important prerequisite for reading aligns. Either must a given `bamReader` contain an intitialized index or a name of BAM-index files must be provided. By default, BAM-index files are expected to be named as the BAM-files with a ".bai" suffix.

getGapSites   reads gap-sites for a given seqid from a single BAM-file (provided as bamReader). The seqid argument is given as numeric 1-based index.

```
> library(spliceSites)
> bam<-character(2)
> bam[1]<-system.file("extdata","rna_fem.bam",package="spliceSites")
> bam[2]<-system.file("extdata","rna_mal.bam",package="spliceSites")
> reader<-bamReader(bam[1],idx=TRUE)
> gs<-getGapSites(reader,seqid=1)
> gs

Object of class gapSites with 32 rows and 12 columns.
nAligns:  2,216          nAlignGaps:  2,297
  id seqid lstart  lend rstart  rend gaplen nAligns strand        gptm
1  1  chr1  14730 14829  14970 15052    140     553      * 2495487.37
2  2  chr1  14944 15038  15796 15888    757     201      *  907039.71
3  3  chr1  15909 15947  16607 16702    659      29      *  130866.43
4  4  chr1  15953 16027  16607 16669    579       4      *   18050.54
5  5  chr1  16730 16765  16854 16941     88       5      *   22563.18
6  6  chr1  16682 16765  16858 16957     92      34      *  153429.60
        rpmg nProbes
1 240748.803       1
2  87505.442       1
3  12625.163       1
4   1741.402       1
5   2176.752       1
6  14801.916       1
```

alignGapList   also works on a given bamReader but reads gap-sites from the entire file and internally calls bamGapList.

```
> ga<-alignGapList(reader)
> ga

Object of class gapSites with 46 rows and 16 columns.
nAligns:  3,107          nAlignGaps:  3,368
  id seqid lstart  lend rstart  rend gaplen nAligns nProbes nlstart qsm nmcl
0  1  chr1  14730 14829  14970 15052    140     553       1       8 200    8
1  2  chr1  14944 15038  15796 15888    757     201       1       8 181    8
2  3  chr1  15909 15947  16607 16702    659      29       1       8 115    8
3  4  chr1  15953 16027  16607 16669    579       4       1       4 138    4
4  5  chr1  16730 16765  16854 16941     88       5       1       5  95    5
5  6  chr1  16682 16765  16858 16957     92      34       1       8 172    8
    gqs strand       gptm       rpmg
0 1000      * 1779851.95 164192.399
1  905      *  646926.30  59679.335
2  575      *   93337.62   8610.451
3  345      *   12874.16   1187.648
4  296      *   16092.69   1484.561
5  860      *  109430.32  10095.012
```

Both functions test the given reader for file-open status (via `isOpen`) and for initialized index.

readMergedBamGaps takes a vector of BAM-file names (plus optionally names of the corresponding BAM-index files) and reads gap-site data from each BAM-file (via rbamtools bamGapList). gap-site data is merged into a gapSites object. The number of files in which each align-gap site is identified is counted in the value *nProbes*.

```
> mbg<-readMergedBamGaps(bam)
> mbg

Object of class gapSites with 71 rows and 16 columns.
nAligns:  7,171          nAlignGaps:  7,665
  id seqid lstart  lend rstart  rend gaplen strand nAligns nProbes        gptm
0  1  chr1 14713 14734  14970 15038    235      *       1       1    1394.506
1  2  chr1 14730 14829  14970 15052    140      *    1126       2 1570213.359
2  3  chr1 14970 15038  15311 15361    272      *       7       1    9761.540
3  4  chr1 14944 15038  15796 15888    757      *     373       2  520150.607
4  5  chr1 15909 15947  16607 16702    659      *      71       2   99009.901
5  6  chr1 15933 16027  16607 16669    579      *       9       2   12550.551
        rpmg nlstart qsm nmcl  gqs
0    130.463       1  22    1   13
1 146901.500       8 200    8 1000
2    913.242       2 103    7  128
3  48662.753       8 182    8  910
4   9262.883       8 121    8  605
5   1174.168       8 170    8  850
```

readTableBamGaps takes a vector of BAM-file names (plus optionally names of the corresponding BAM-index files) and a profile table. The profile tables describes the probe profile for each BAM-file (number of BAM-files = number of rows in profile). Every column describes a categorial partition of the BAM-files. For each category, the number of probes (=files), number of aligns and optionally gptm-values are separately calculated. `readTabledBamGaps` collects gap-site data (as `readMergedBamGaps` and adds profile columns. The returned gapSites object also contains a profile table which can be retrieved via `getProfile`.

```
> prof<-data.frame(gender=c("f","m"))
> rtbg<-readTabledBamGaps(bam,prof=prof,rpmg=TRUE)
> rtbg

Object of class gapSites with 71 rows and 22 columns.
nAligns:  7,171          nAlignGaps:  7,665
  id seqid lstart  lend rstart  rend gaplen strand nAligns nProbes nlstart qsm
1  1  chr1 14713 14734  14970 15038    235      *       1       1       1  22
2  2  chr1 14730 14829  14970 15052    140      *    1126       2       8 200
3  3  chr1 14970 15038  15311 15361    272      *       7       1       2 103
4  4  chr1 14944 15038  15796 15888    757      *     373       2       8 182
5  5  chr1 15909 15947  16607 16702    659      *      71       2       8 121
6  6  chr1 15933 16027  16607 16669    579      *       9       2       8 170
```

```
   nmcl  gqs         gptm         rpmg c.gender.f c.gender.m aln.gender.f
1    1   13     1394.506      130.463          0          1            0
2    8 1000 1570213.359   146901.500          1          1          553
3    7  128     9761.540      913.242          0          1            0
4    8  910  520150.607    48662.753          1          1          201
5    8  605   99009.901     9262.883          1          1           29
6    8  850   12550.551     1174.168          1          1            4
  aln.gender.m rpmg.gender.f rpmg.gender.m
1            1         0.000       232.721
2          573    164192.399    133348.848
3            7         0.000      1629.044
4          172     59679.335     40027.926
5           42      8610.451      9774.261
6            5      1187.648      1163.603

> getProfile(rtbg)

  gender nAligns nAlignGaps nSites cSites
1      f    3107       3368     46     46
2      m    4064       4297     64     71
                                                       infile
1 /tmp/RtmpFVRTOs/Rinst136d3b66dec3/spliceSites/extdata/rna_fem.bam
2 /tmp/RtmpFVRTOs/Rinst136d3b66dec3/spliceSites/extdata/rna_mal.bam
```

3.3 cRanges class lineage

cRanges represent genomic ranges which contain a point of interest inside. In the present setting ranges lie around (left or right) gap-site borders. The class is intended to manage sequence data which crosses exon-intron boundaries. position is defined as the 1-based position of the last exon nucleotide. For '+' strand, position=4 means, that usually the 5th and 6th nucleotide are 'GT'.

From the base class cRanges the lineage derives two child classes: cdRanges and caRanges which additionally contain a sequence slot. Sequence information is important for validation splice-sites because required intronic sequence is not contained in the BAM-align structures an must be included from reference sequence.

cRanges contains a data.frame in a single slot. Each centered range is represented as one record (line). The table keeps 7 columns which are organized as follows:

Name	Type	Content
seqid	factor	Sequence id (usually chromosome name)
start	numeric	Start position of range
end	numeric	End position range
strand	numeric	'+' or '-' or '*'
position	numeric	
id	numeric	Row identifier
nAligns	numeric	Number of aligns

3.4 Extracting gap-site boundary ranges

xJunc functions extract ranges from `gapSites` objects. `lJunc` and `rJunc` objects extract ranges around one gap-site boundaries and return `cRanges` objects. The position values point to the gap-site (exon-intron) boundary.

`rlJunc` objects extract ranges from both sides of the gap-site and return `gapSites` objects. Inside the returned object the contained data.frame has two additional columns (lfeatlen and rfeatlen) which mark the boundary position.

The ranges are intended to be padded with DNA-sequence. Therefore the given strand value is used:

```
> ljp<-lJunc(ga,featlen=6,gaplen=6,strand='+')
> ljp

Object of class cRanges with 46 rows and 7 columns.
  seqid start   end strand position id nAligns
1  chr1 14824 14835      +        6  1     553
2  chr1 15033 15044      +        6  2     201
3  chr1 15942 15953      +        6  3      29
4  chr1 16022 16033      +        6  4       4
5  chr1 16760 16771      +        6  5       5
6  chr1 16760 16771      +        6  6      34

> ljm<-lJunc(ga,featlen=6,gaplen=6,strand='-')
> ljm

Object of class cRanges with 46 rows and 7 columns.
  seqid start   end strand position id nAligns
1  chr1 14824 14835      -        6  1     553
2  chr1 15033 15044      -        6  2     201
3  chr1 15942 15953      -        6  3      29
4  chr1 16022 16033      -        6  4       4
5  chr1 16760 16771      -        6  5       5
6  chr1 16760 16771      -        6  6      34

> rjp<-rJunc(ga,featlen=6,gaplen=6,strand='+')
> rjp
```

```
Object of class cRanges with 46 rows and 7 columns.
  seqid start   end strand position id nAligns
1  chr1 14964 14975      +        6  1     553
2  chr1 15790 15801      +        6  2     201
3  chr1 16601 16612      +        6  3      29
4  chr1 16601 16612      +        6  4       4
5  chr1 16848 16859      +        6  5       5
6  chr1 16852 16863      +        6  6      34

> rjm<-rJunc(ga,featlen=6,gaplen=6,strand='-')
> rjm

Object of class cRanges with 46 rows and 7 columns.
  seqid start   end strand position id nAligns
1  chr1 14964 14975      -        6  1     553
2  chr1 15790 15801      -        6  2     201
3  chr1 16601 16612      -        6  3      29
4  chr1 16601 16612      -        6  4       4
5  chr1 16848 16859      -        6  5       5
6  chr1 16852 16863      -        6  6      34

> lrjp<-lrJunc(ga,lfeatlen=6,rfeatlen=6,strand='+')
> lrjp

Object of class gapSites with 46 rows and 14 columns.
nAligns:  3,107         nAlignGaps:  3,368
  id seqid lstart  lend rstart  rend gaplen strand nAligns        gptm
1  1  chr1  14824 14829  14970 14975    140      +     553 1779851.95
2  2  chr1  15033 15038  15796 15801    757      +     201  646926.30
3  3  chr1  15942 15947  16607 16612    659      +      29   93337.62
4  4  chr1  16022 16027  16607 16612    579      +       4   12874.16
5  5  chr1  16760 16765  16854 16859     88      +       5   16092.69
6  6  chr1  16760 16765  16858 16863     92      +      34  109430.32
        rpmg nProbes lfeatlen rfeatlen
1 164192.399       1        6        6
2  59679.335       1        6        6
3   8610.451       1        6        6
4   1187.648       1        6        6
5   1484.561       1        6        6
6  10095.012       1        6        6

> lrjm<-lrJunc(ga,lfeatlen=6,rfeatlen=6,strand='-')
> lrjm

Object of class gapSites with 46 rows and 14 columns.
nAligns:  3,107         nAlignGaps:  3,368
  id seqid lstart  lend rstart  rend gaplen strand nAligns        gptm
1  1  chr1  14824 14829  14970 14975    140      -     553 1779851.95
2  2  chr1  15033 15038  15796 15801    757      -     201  646926.30
3  3  chr1  15942 15947  16607 16612    659      -      29   93337.62
4  4  chr1  16022 16027  16607 16612    579      -       4   12874.16
```

```
5  5  chr1  16760 16765  16854 16859      88     -       5    16092.69
6  6  chr1  16760 16765  16858 16863      92     -      34   109430.32
          rpmg nProbes lfeatlen rfeatlen
1 164192.399       1        6        6
2  59679.335       1        6        6
3   8610.451       1        6        6
4   1187.648       1        6        6
5   1484.561       1        6        6
6  10095.012       1        6        6
```

xCodons functions. lCodons and rCodons both take and return cRanges objects. The functions provide two tasks:

- Shift start position for sequence extraction according to reading frame.

- Truncate range end to full codon size.

Both operations act unilateral on ranges and so strand information must be provided in order to decide which side of the range (left or right) represents start and end. lCodons and rCodons take a numeric frame argument and a logical keepStrand argument besides the cRanges object. For frame = 2 or 3, the start position is shifted 1 or 2 nucleotides respectively. The sequence length is then truncated to the largest contained multiple of 3. The functions then correct the position-values in order to keep the positions pointer on the same nucleotide. When keepStrand is FALSE' (the default), the lCodons function sets all strand entries to "+" and the rCodons sets all Strand entries to "-".

The lCodons function should be used for "+"-strand and the rCodons function should be used for "-"-strand.

```
> ljp1<-lCodons(ljp,frame=1)
> ljp1

Object of class cRanges with 46 rows and 8 columns.
  seqid start   end strand position id nAligns frame
1  chr1 14824 14835      +        6  1     553     1
2  chr1 15033 15044      +        6  2     201     1
3  chr1 15942 15953      +        6  3      29     1
4  chr1 16022 16033      +        6  4       4     1
5  chr1 16760 16771      +        6  5       5     1
6  chr1 16760 16771      +        6  6      34     1

> ljp2<-lCodons(ljp,frame=2)
> rjm1<-rCodons(ljm,frame=1)
> rjm2<-rCodons(ljm,frame=2)
```

The xCodons functions provide a preparative step which allows translation of subsequently added DNA-sequence into AA-sequence. The strand information is therein used to determine the fraction of DNA-sequence must be reverseComplement'ed.

The lrCodons function works similar as the xCodons functions but does the same on the two gap-site enframing ranges simultaneously. lrCodons takes and returns gapAligns objects. The strand value can be manually set (default: '*') for later use by dnaGapAligns

```
> lr1<-lrCodons(lrjp,frame=1,strand='+')
> lr2<-lrCodons(lrjp,frame=2,strand='+')
> lr3<-lrCodons(lrjp,frame=3,strand='+')
```

The c-Operator for cRanges and gapSites is used to combine objects made for different frame and strand together to one cRanges or gapSites for joint subsequent analysis:
In the next step we transform these ranges into codons in both directions and all three frames.

```
> ljpc<-c(ljp1,ljp2)
> rjmc<-c(rjm1,rjm2)
> lrj<-c(lr1,lr2,lr3)
```

In order to provide better readable tables there is an optional function for sorting the combined cRanges and gapSites:

```
> ljpc<-sortTable(ljpc)
> rjmc<-sortTable(rjmc)
> lrj<-sortTable(lrj)
```

Trim and resize functions provide upper size limits or fixed size for boundary ranges in gapSites objects. trim_left works on left boundary ranges (i.e. lstart and rend values) and trim_right works on right boundary ranges (i.e. rstart and rend).

All four functions leave the inner boundaries (lend and rstart) unchanged.

```
> trim_left(lrj,3)
> trim_right(lrj,3)
> resize_left(lrj,8)
> resize_right(lrj,8)
```

3.5 Provide additional information

Genuine gapSites and cRanges objects contain numeric coordinate and count data but no sequence or gene association. The conceptual idea is to add gene-annotations and sequence data to primed coordinate containers.

Gene annotation data can be added with the annotate and annotation<- functions. Therefore a refGenome object (ucscGenome or ensemblGenome from package refGenome) must be provided.

```
> ucf<-system.file("extdata","uc_small.RData",package="spliceSites")
> uc<-loadGenome(ucf)
> juc <- getSpliceTable(uc)
> annotation(ga)<-annotate(ga, juc)
```

Adding annotation data internally works by calling the `overlapJuncs` functions (refGenome package). The same mechanism also works for `gapSites` objects which arise from `readTabledBamGaps`:

Strand information can be deduced from gene annotation. The `getAnnStrand` function looks at the annotation derived strand information on both sides. When the strand information is equal, the function takes the value as strand for the gap-site. Otherwise the strand will be set to '*'. The strand information can be integrated into the internal data.frame by using the `strand` function.

```
> strand(ga)<-getAnnStrand(ga)
```

addGeneAligns adds information about distribution of aligns over different gap-sites for each gene.

```
> gap<-addGeneAligns(ga)
> gap
```

```
Object of class gapSites with 46 rows and 17 columns.
nAligns:  3,107         nAlignGaps:  3,368
  id seqid lstart  lend rstart  rend gaplen nAligns nProbes nlstart qsm nmcl
1  1  chr1  14730 14829  14970 15052    140     553       1       8 200    8
2  2  chr1  14944 15038  15796 15888    757     201       1       8 181    8
3  3  chr1  15909 15947  16607 16702    659      29       1       8 115    8
4  4  chr1  15953 16027  16607 16669    579       4       1       4 138    4
5  5  chr1  16730 16765  16854 16941     88       5       1       5  95    5
6  6  chr1  16682 16765  16858 16957     92      34       1       8 172    8
   gqs strand        gptm       rpmg gene_aligns    gene_id gene_name
1 1000      - 1779851.95 164192.399         639 uc001aac.4    WASH7P
2  905      -  646926.30  59679.335         201 uc009vis.3    WASH7P
3  575      -   93337.62   8610.451          34 uc009vix.2    WASH7P
4  345      -   12874.16   1187.648          34 uc009vix.2    WASH7P
5  296      -   16092.69   1484.561         610 uc009viz.2    WASH7P
6  860      -  109430.32  10095.012          34 uc009viv.2    WASH7P
```

DNA-sequence can be added from a `DNAStringSet` (Biostrings) object via the `dnaRanges` function. `dnaRanges` function takes a `cRanges` object and a `DNAStringSet` object and adds the corresponding DNA-sequence to each contained range. The function returns an object of class `cdRanges`.
We first load an example `DNAStringSet` which contains the reference sequence. The sample object *dna_small* contains a small extract of UCSC human reference sequence.
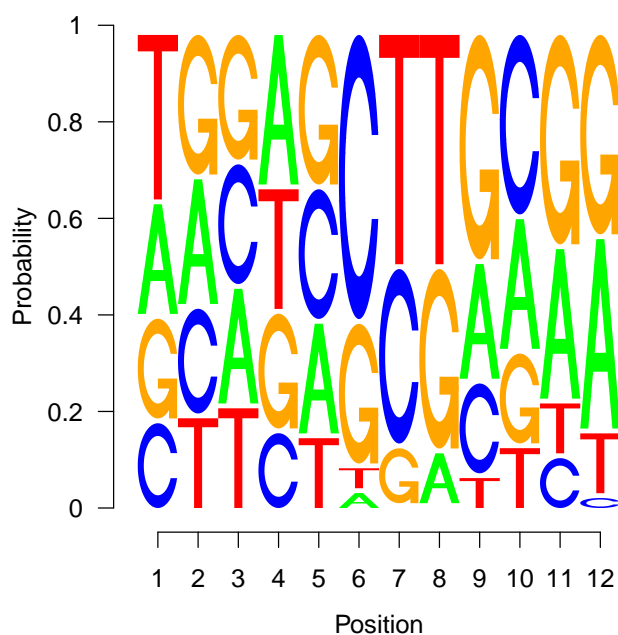
```
> dnafile<-system.file("extdata","dna_small.RData",package="spliceSites")
> load(dnafile)
> dna_small
```

```
  A DNAStringSet instance of length 4
      width seq                                            names
[1]  104000 NNNNNNNNNNNNNNNNNNNNNNN...ACTCCGAGGCCATGGACGTGTT chr16
[2]   30000 NNNNNNNNNNNNNNNNNNNNNNN...AGAAGCAGTGGTCGCCAGGAAT chr1
```

```
[3]   360000 NNNNNNNNNNNNNNNNNNNNNNNN...TGGAGCTCAGGGGCACCCACCA chr6
[4]  2851000 NNNNNNNNNNNNNNNNNNNNNNNN...ATCCTCCTGCCTCAGCCTACGC chrY
```

Then we create a `cdRanges` instance dnaRanges and then produce a seqlogo:

```
> ljpcd<-dnaRanges(ljpc,dna_small)
> seqlogo(ljpcd)
```


Amino-Acid sequences can be obtained after the previous preparatory steps are done. The `translate` function converts a `cdRanges` object into a `caRanges` object by translating the contained DNA-sequences. The function internally uses the Bioconductor Biostrings `translate` function.

```
> ljpca<-translate(ljpcd)
```

Extracting subsets from `cRanges` and `gapSites` (and derived classes) can be done with `extractX` functions. They come in two flavours: `extractRange` and `extractByGeneName`. Main application for these function is visual inspection of the results.

`extractRange` takes a `cRanges` or `gapSites` object and a triple of genomic coordinates (seqid, start, end). From the given `cRanges` object, the stored ranges which are contained in the range defined by the coordinates is extracted and returned as `cRanges` object.

```
> # A) For gapSites
> extractRange(ga,seqid="chr1",start=14000,end=30000)
```

```
Object of class gapSites with 32 rows and 16 columns.
nAligns:  3,107          nAlignGaps:  3,368
  id seqid lstart  lend rstart  rend gaplen nAligns nProbes nlstart qsm nmcl
1  1 chr1  14730 14829  14970 15052    140     553       1       8 200    8
2  2 chr1  14944 15038  15796 15888    757     201       1       8 181    8
3  3 chr1  15909 15947  16607 16702    659      29       1       8 115    8
4  4 chr1  15953 16027  16607 16669    579       4       1       4 138    4
5  5 chr1  16730 16765  16854 16941     88       5       1       5  95    5
6  6 chr1  16682 16765  16858 16957     92      34       1       8 172    8
    gqs strand       gptm       rpmg     gene_id gene_name
1 1000      - 1779851.95 164192.399 uc001aac.4    WASH7P
2  905      -  646926.30  59679.335 uc009vis.3    WASH7P
3  575      -   93337.62   8610.451 uc009vix.2    WASH7P
4  345      -   12874.16   1187.648 uc009vix.2    WASH7P
5  296      -   16092.69   1484.561 uc009viz.2    WASH7P
6  860      -  109430.32  10095.012 uc009viv.2    WASH7P
> # B) For cRanges
> lj<-lJunc(ga,featlen=3,gaplen=6,strand='+')
> extractRange(lj,seqid="chr1",start=14000,end=30000)

Object of class cRanges with 32 rows and 7 columns.
  seqid start   end strand position id nAligns
1  chr1 14827 14835      +        3  1     553
2  chr1 15036 15044      +        3  2     201
3  chr1 15945 15953      +        3  3      29
4  chr1 16025 16033      +        3  4       4
5  chr1 16763 16771      +        3  5       5
6  chr1 16763 16771      +        3  6      34
```

extractByGeneName also takes a cRanges or gapSites object but instead of a
numeric range, a vector of gene-names and a refGenome object. The refGenome
object first calculates a set of numerical coordinates from the given gene-names and
then calls extractRange for each set of coordinates. The resulting objects are then
concatenated.

```
> lj<-lJunc(ga,featlen=6,gaplen=3,strand='+')
> ljw<-extractByGeneName(ljp,geneNames="POLR3K",src=uc)
> ljw

Object of class cRanges with 2 rows and 7 columns.
   seqid  start    end strand position id nAligns
33 chr16  97552  97563      +        6 33     168
34 chr16 101640 101651      +        6 34     152

> ljw<-extractByGeneName(ljpcd,geneNames="POLR3K",src=uc)
> ljw

Object of class cdRanges with 4 rows and 8 columns.
   seqid  start    end strand position id nAligns frame          seq
33 chr16  97552  97563      +        6 33     168     1 ACGACTCTGGCA
79 chr16  97553  97561      +        5 33     168     2   CGACTCTGG
34 chr16 101640 101651      +        6 34     152     1 TGTTACCTAACA
80 chr16 101641 101649      +        5 34     152     2   GTTACCTAA
```

3.6 Working with amino-acid (AA) sequences

For approaching AA based views whe first prepare longer DNA sequences for different frames and add DNA-sequence:

```
> l<-12
> lj<-lJunc(mbg,featlen=l,gaplen=l,strand='+')
> ljc<-c(lCodons(lj,1),lCodons(lj,2),lCodons(lj,3))
> lrj<-lrJunc(mbg,lfeatlen=l,rfeatlen=l,strand='+')
> lrjc<-c(lrCodons(lrj,1),lrCodons(lrj,2),lrCodons(lrj,3))
> jlrd<-dnaRanges(ljc,dna_small)
```

Translation of DNA sequences can simply be obtained by using `translate`:

```
> jlrt<-translate(jlrd)
> jlrt
```

```
Object of class caRanges with 213 rows and 8 columns.
    seqid start   end strand position id nAligns frame       seq
1    chr1 14723 14746      +        5  1       1     1  LCLWLLRW
72   chr1 14724 14744      +        4  1       1     2   SACGCCG
143  chr1 14725 14745      +        4  1       1     3   LPVAAAV
2    chr1 14818 14841      +        5  2    1126     1  SQRCLEGK
73   chr1 14819 14839      +        4  2    1126     2   PRDAWRE
144  chr1 14820 14840      +        4  2    1126     3   PEMPGGK
```

truncateSeq function addresses stop codons '*' which may be identified in the amino acid sequence. The function truncates the sequence when the stop-codon appears behind (right-hand of) the position and removes rows where the stop-codon appears before (left-hand of) the position. This removes data-sets where the exon-intron boundary lies downstream of a stop-codon.

```
> jlrtt<-truncateSeq(jlrt)
> jlrtt
```

```
Object of class caRanges with 195 rows and 9 columns.
    seqid start   end strand position id nAligns frame lseq      seq
1    chr1 14723 14746      +        5  1       1     1    8 LCLWLLRW
72   chr1 14724 14744      +        4  1       1     2    7  SACGCCG
143  chr1 14725 14745      +        4  1       1     3    7  LPVAAAV
2    chr1 14818 14841      +        5  2    1126     1    8 SQRCLEGK
73   chr1 14819 14839      +        4  2    1126     2    7  PRDAWRE
144  chr1 14820 14840      +        4  2    1126     3    7  PEMPGGK
```

trypsinCleave function performs in silico trypsinisation on the provided AA sequences. Trypsin sites are identified by the regex rule "[RK](?!P)" which implements the "Keil"-rule. From the sequence fragments, the one which contains the position marked gap-site (exon-intron) boundary is returned.

```
> jtry<-trypsinCleave(jlrtt)
> jtry<-sortTable(jtry)
```

3.7 Writing output tables

Write functions provide functionality for exporting generated tables into '.csv' files.

3.7.1 write.annDNA.tables

writes content of `gapSites` objects together with DNA-sequence segments.

```
> annotation(rtbg)<-annotate(rtbg, juc)
> write.annDNA.tables(rtbg, dna_small, "gapSites.csv", featlen=3, gaplen=8)
```

Additional columns output are added by `write.annDNA.tables` to the existing tables in `gapSites` objects (shown in Table 1).

Table 1: Additional columns by write.annDNA.tables

Column	Content
leftseq	Sequence of Exon-Intron boundary on left side
rightseq	Sequence of Exon-Intron boundary on right side

The Sequences are obtained using the `dnaRanges` function called with parameters given in table 2. DNA sequences are *reverseComplement*ed for '-'-Strand.

Table 2: Arguments passed to dnaRanges

Class	Content
cRanges	lJuncStrand (rJuncStrand) using featlen and gaplen arguments
dnaset	DNAStringSet (Reference sequence)
useStrand	TRUE
removeUnknownStrand	FALSE

Note the output of `write.annDNA.tables`:
The sequence from which the `lhbond` is calculated will only coincide with the `leftseq` column in rows with strand='+'. The sequence from which the `rhbond` is calculated will only coincide with the `rightseq` column in rows with strand='-'. Otherwise the Hbond is calculated from the *reverseComplement*'ed sequence.

3.7.2 write.files

The `write.files` function works on `caRanges` objects and produces two output files: One '.csv' file which contains a copy of the data and a '.fa' file which contains sequence in fasta format.

```
> write.files(jtry,path=getwd(),filename="proteomic",quote=FALSE)
```

The fasta headers contain two tags which are separated by a vertical bar '|'. The first item is the `id` of the corresponding table row and the second item is the prefix of the filename.

3.8 Working with alternative splice-sites

Alternative splice-sites are sites where one exon-intron boundary corresponds to multiple counterparts. In gap-site tables, alternative sites are characterized by multiple occuring entries of `lend` or `rstart`. The `alt_left_ranks` function work by putting gap-sites which share the same `rstart` values in a group which is identified by the same `alt_id` value. Gap-sites which don't share their `rstart` value with any other gap-site have the `alt_id` value 0.

alt_left_ranks   looks for multiple entries of `rstart` values and returns a table with 6 numeric columns:

Name	Content
id	Row identifier (from `gapSites` object
alt_id	Group number. Identifies group of gap-sites which share same ; 0 for sigular entries
diff_ranks	Rank of gaplen inside same alt_id group
gap_diff	Difference in gaplen to the next greater gaplen value in group
nr_alt	Number of rows with same rstart value

So the rank numbering increases with gaplen (from inside to outide) and the `gap_diff` values are always ≥ 0 because the row with the smallest gaplen gets the rank 1 and gap_diff 0.

When the option `extensive=TRUE` is given, four more columns are contained in the result table: seq (seqid), group (=rstart, by which the input table is grouped), alt (lend) and len (gaplen).
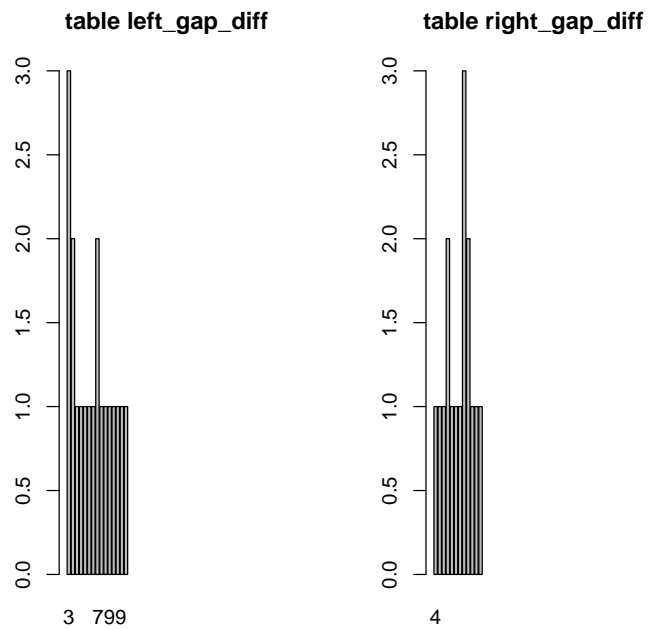
```
> al<-alt_left_ranks(ga)
```

The alt_ranks   function combines the results of `alt_left_ranks` and `alt_right_ranks` into one table. There should be characteristic peaks at multiples of 3:

```
> ar<-alt_ranks(ga)
```
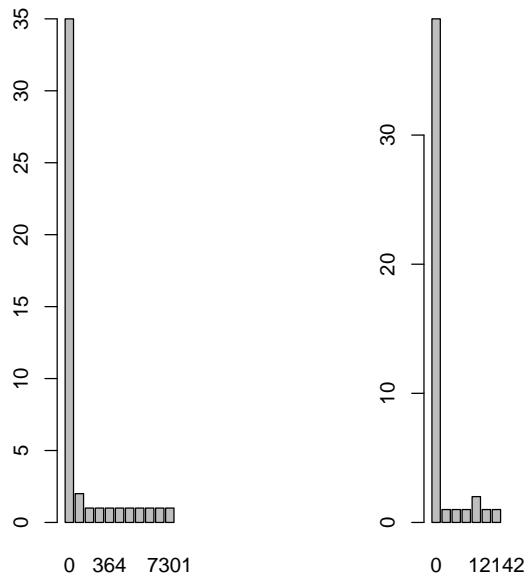
The tabled `gap_diff` values can be plotted with:

```
> plot_diff_ranks(ga)
```

plot_diff gives a visual overview about the congruence between gap-site positions and the associated annotations. Differences of zero say that a gap-site boundary exactly meets the annotated position.

```
> aga<-annotation(ga)
> plot_diff(aga)
```

Annotation left–gap_site distan Annotation right–gap site distan

MaxEntScores Maximum Entropy (MaxEnt) scores were developed by Gene Yeo and Christopher Burge [4] and are widely accepted as method for quantification of splice-site strength for a given sequence motif from the exon-intron boundary. MaxEnt scores can be calculated for the 5' and the 3' side of the splice-site. MaxEnt scores can be calculated for vectors of sequences for a given position (1-based position of last exon nucleotide) using the score3 and score5 functions.
For sliding window calculations on longer DNA-sequences the scoreSeq5 and score-Seq3 functions can be used. The addMaxEnt function is used for adding MaxEnt scores to a gapSites object.

```
> mes<-load.maxEnt()
> score5(mes,"CCGGGTAAGAA",4)

[1] 9.844127

> score3(mes,"CTCTACTACTATCTATCTAGATC",pos=20)

[1] 6.706947

> sq5<-scoreSeq5(mes,seq="ACGGTAAGTCAGGTAAGT")
> sq3<-scoreSeq3(mes,seq="TTTATTTTTCTCACTTTTAGAGACTTCATTCTTTCTCAAATAGGTT")
> gae<-addMaxEnt(ga,dna_small,mes)
> gae

Object of class gapSites with 46 rows and 23 columns.
nAligns:  3,107        nAlignGaps:  3,368
  id seqid lstart  lend rstart  rend gaplen nAligns nProbes nlstart qsm nmcl
```

19

```
0  1  chr1  14730 14829  14970 15052      140      553         1        8 200     8
1  2  chr1  14944 15038  15796 15888      757      201         1        8 181     8
2  3  chr1  15909 15947  16607 16702      659       29         1        8 115     8
3  4  chr1  15953 16027  16607 16669      579        4         1        4 138     4
4  5  chr1  16730 16765  16854 16941       88        5         1        5  95     5
5  6  chr1  16682 16765  16858 16957       92       34         1        8 172     8
   gqs strand        gptm       rpmg mxe_ps5 mxe_ps3 mxe_ms5 mxe_ms3 s5strand
0 1000      - 1779851.95 164192.399   -30.3    -5.3     6.5     8.3        -
1  905      -  646926.30  59679.335   -21.4   -14.3     9.6     9.2        -
2  575      -   93337.62   8610.451   -26.1   -13.7     6.7     6.5        -
3  345      -   12874.16   1187.648   -17.4   -13.7     6.7     5.8        -
4  296      -   16092.69   1484.561   -19.4   -15.8     8.0     8.7        -
5  860      -  109430.32  10095.012   -19.4   -15.6    10.3     8.7        -
  s3strand meStrand
0        -        -
1        -        -
2        -        -
3        -        -
4        -        -
5        -        -

> table(getMeStrand(gae))

 +  -  *
11 35  0

> sae<-setMeStrand(gae)
> sae

Object of class gapSites with 46 rows and 23 columns.
nAligns:  0         nAlignGaps:  0
  id seqid lstart  lend rstart  rend gaplen nAligns nProbes nlstart qsm nmcl
0  1  chr1  14730 14829  14970 15052      140      553         1        8 200     8
1  2  chr1  14944 15038  15796 15888      757      201         1        8 181     8
2  3  chr1  15909 15947  16607 16702      659       29         1        8 115     8
3  4  chr1  15953 16027  16607 16669      579        4         1        4 138     4
4  5  chr1  16730 16765  16854 16941       88        5         1        5  95     5
5  6  chr1  16682 16765  16858 16957       92       34         1        8 172     8
   gqs strand        gptm       rpmg mxe_ps5 mxe_ps3 mxe_ms5 mxe_ms3 s5strand
0 1000      - 1779851.95 164192.399   -30.3    -5.3     6.5     8.3        -
1  905      -  646926.30  59679.335   -21.4   -14.3     9.6     9.2        -
2  575      -   93337.62   8610.451   -26.1   -13.7     6.7     6.5        -
3  345      -   12874.16   1187.648   -17.4   -13.7     6.7     5.8        -
4  296      -   16092.69   1484.561   -19.4   -15.8     8.0     8.7        -
5  860      -  109430.32  10095.012   -19.4   -15.6    10.3     8.7        -
  s3strand meStrand
0        -        -
1        -        -
2        -        -
3        -        -
4        -        -
5        -        -
```

HBond scores The HBond score provides a measure for the capability of a 5'
splice-site to form H-bonds with the U1 snRNA [1]. The HBond score can be
calculated for a vector of sequences

```
> #
> hb<-load.hbond()
> seq<-c("CAGGTGAGTTC", "ATGCTGGAGAA", "AGGGTGCGGGC", "AAGGTAACGTC", "AAGGTGAGTTC")
> hbond(hb,seq,3)

[1] 19.4  0.0  8.3 14.1 17.7
```

or can be added to `gapSites` and `cdRanges` objects:

```
> gab<-addHbond(ga,dna_small)
> # D) cdRanges
> lj<-lJunc(ga, featlen=3, gaplen=8, strand='+')
> ljd<-dnaRanges(lj,dna_small)
> ljdh<-addHbond(ljd)
```

The `addHbond` function algorithm is made up of three steps (left side shown, right
side analog):

- Call to `lJunc` using parameters featlen=3, gaplen=8 and strand='+'.

- Call to `dnaRanges` function with useStrand=TRUE

- C-call to `hbond_score`.

Because the Hbond score is only valid for the splice donor (5' junction) site and the
`addHbond` function always assumes '+'-strand on the left gap-site border and '-'-
strand on the right gap-site border. Therefore the DNA sequence is always passed
as is on the left side and *reverseComplement*'ed on the right side irrespective of
strand value in the given gap-sites object.

The Hbond score is 0 when no 'GT' is present in the first two intron positions. Usu-
ally, `lhbond` >0 and `rhbond` =0 when strand='+' and `lhbond`=0 and `hbond`>0
when strand='-'.

Note the output of `write.annDNA.tables`:
The sequence from which the `lhbond` is calculated will only coincide with the `left-
seq` column in rows with strand='+'. The sequence from which the `rhbond` is
calculated will only coincide with the `rightseq` column in rows with strand='-'.
Otherwise the Hbond is calculated from the *reverseComplement*'ed sequence.

3.9 Creating ExpressionSet objects containing gap-site align-count values.

In order to provide technical requirements for analyzing expression data inside the
standard Bioconductor framework, there is a `readExpSet` function which produces
`ExpressionSet` objects with `rpmg` (default) and `gptm` expression values.

readExpSet reads gap-site aligns abundance from a given list of BAM file names into ExpressionSet

```
> prof<-data.frame(gender=c("f", "m"))
> rtbg<-readTabledBamGaps(bam, prof=prof, rpmg=TRUE)
> getProfile(rtbg)

  gender nAligns nAlignGaps nSites cSites
1      f    3107       3368     46     46
2      m    4064       4297     64     71
                                                            infile
1 /tmp/RtmpFVRTOs/Rinst136d3b66dec3/spliceSites/extdata/rna_fem.bam
2 /tmp/RtmpFVRTOs/Rinst136d3b66dec3/spliceSites/extdata/rna_mal.bam

> meta<-data.frame(labelDescription=names(prof),row.names=names(prof))
> pd<-new("AnnotatedDataFrame",data=prof,varMetadata=meta)
> es<-readExpSet(bam,phenoData=pd)
```

There are two annotation functions for ExpressionSets which are created by read-ExpSet: annotate and uniqueJuncAnn. Annotate finds overlaps to a given refJunctions object. uniqueJuncAnn finds exact matches with known splice-sites:

```
> ann<-annotate(es, juc)
> ucj<-getSpliceTable(uc)
> uja<-uniqueJuncAnn(es, ucj)
```

From ExpressionSets, the alignment counts can directly be used as input for differential expression analysis with the DESeq2 package:

```
> library(DESeq2)
> cds <- DESeqDataSetFromMatrix(exprs(es), colData=prof, design=~gender)
> des <- DESeq(cds)
> binom.res<-results(des)
> br <- na.omit(binom.res)
> bro <- br[order(br$padj, decreasing=TRUE),]
```

readCuffGeneFpkm reads FPKM values from all given cufflinks files and collects the values into an ExpressionSet. In order to get unique gene identifier, the contained values are grouped and for each gene the maximum FPKM values is selected.

```
> n <- 10
> cuff <- system.file("extdata","cuff_files",
+              paste(1:n, "genes", "fpkm_tracking", sep="."),
+              package="spliceSites")
> gr <- system.file("extdata", "cuff_files", "groups.csv", package="spliceSites")
> groups <- read.table(gr, sep="\t", header=TRUE)
> meta <- data.frame(labelDescription=c("gender", "age-group", "location"),
+                row.names=c("gen", "agg", "loc"))
> phenoData <- new("AnnotatedDataFrame", data=groups, varMetadata=meta)
> exset <- readCuffGeneFpkm(cuff, phenoData)
```
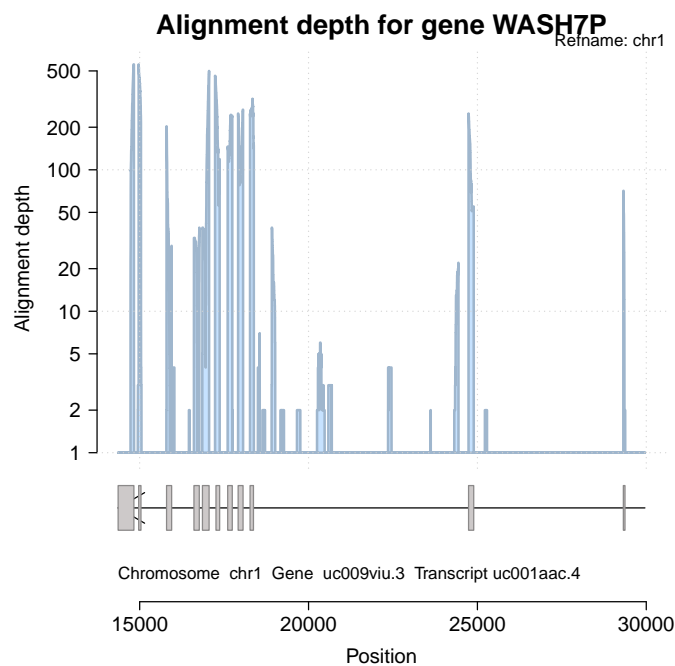
4 Appendix

4.1 Plot read alignment depth

The function *plotGeneAlignDepth* draws read alignment depth based on data from a *bamReader* and a *refGenome* object and a given gene name and a transcript name:

```
> bam <- system.file("extdata","rna_fem.bam",package="spliceSites")
> reader <- bamReader(bam, idx=TRUE)
> # Load annotation data
> ucf <- system.file("extdata", "uc_small.RData", package="spliceSites")
> uc <- loadGenome(ucf)
```

An example is shown in the following plot:

```
> plotGeneAlignDepth(reader, uc, gene="WASH7P", transcript="uc001aac.4",
+                    col="slategray3", fill="slategray1",
+                    box.col="snow3", box.border="snow4")
```


4.2 The keytable class

The `keytable` class is designed to count align data and for computation of expression values for experimental groups separately. The class is only internally used by the `readTabledBamGaps` function.

Group assignment of probes (BAM-files) is done by providing a profile table. Align count values then can subsequently be added to the created object. After data-collection a result table can be retrieved.

For description of functionality first artificial data is created. A profile-table defines the group association of all analyzed probes (usually each probe is one BAM-file).

```
> prof<-data.frame(gen=factor(c("w","m","w","w")),levels=c("m","w")),
+                  loc=factor(c("thx","thx","abd","abd")),levels=c("thx","abd")),
+                  ag =factor(c("y","y","m","o")),levels=c("y","m","o")))
> prof

  gen loc ag
1   w thx  y
2   m thx  y
3   w abd  m
4   w abd  o
```

We the create artificial align-count data for several some gap-sites. The input table key allows for multipe entries for the same site. For the output the sites are merged (and align numbers summed) into `ku`.

```
> key1<-data.frame(id=1:5,
+                  seqid=c(1,1,2,2,3),
+                  lend=c(10,20,10,30,10),
+                  rstart=c(20,30,20,40,20),
+                  nAligns=c(11,21,31,41,51))
> key2<-data.frame(id=1:5,
+                  seqid=c(1,1,2,2,4),
+                  lend=c(10,20,10,30,50),
+                  rstart=c(20,30,20,40,70),
+                  nAligns=c(21,22,23,24,25))
> key3<-data.frame(id=1:5,
+                  seqid=c(1,2,4,5,5),
+                  lend=c(10,10,60,10,20),
+                  rstart=c(20,20,80,20,30),
+                  nAligns=c(31,32,33,34,35))
> key<-rbind(key1,key2,key3)
> # Group positions
> ku<-aggregate(data.frame(nAligns=key$nAligns),
+               by=list(seqid=key$seqid,lend=key$lend,rstart=key$rstart),
+               FUN=sum)
```

The next steps comes in two versions: one version where only number of probes are counted for each site and the second version where aligns are counted for each site. The first example shows the probe-counting procedure: The `keyProfiler` object is created from the first probe data and subsequently data for two probes is added (via `addKeyTable`).

```
> # Count probes
> kpc<-new("keyProfiler",keyTable=key1[,c("seqid","lend","rstart")],prof=prof)
> addKeyTable(kpc,keyTable=key2[,c("seqid","lend","rstart")],index=2)
> addKeyTable(kpc,keyTable=key3[,c("seqid","lend","rstart")],index=4)
>
```

The result is then appended to the grouped input table:

```
> cp<-appendKeyTable(kpc,ku,prefix="c.")
> cp

  seqid lend rstart nAligns c.gen.m c.gen.w c.loc.thx c.loc.abd c.ag.y c.ag.m
1     1   10     20      63       1       2         2         1      2      0
2     1   20     30      43       1       1         2         0      2      0
3     2   10     20      86       1       2         2         1      2      0
4     2   30     40      65       1       1         2         0      2      0
5     3   10     20      51       0       1         1         0      1      0
6     4   50     70      25       1       0         1         0      1      0
7     4   60     80      33       0       1         0         1      0      0
8     5   10     20      34       0       1         0         1      0      0
9     5   20     30      35       0       1         0         1      0      0
  c.ag.o
1      1
2      0
3      1
4      0
5      0
6      0
7      1
8      1
9      1
```

The second version counts align numbers over probes:

```
> # Count aligns
> kpa<-new("keyProfiler",keyTable=key1[,c("seqid","lend","rstart")],prof=prof,values=key1$
> kpa@ev$dtb

  seqid lend rstart gen.m gen.w loc.thx loc.abd ag.y ag.m ag.o
1     1   10     20     0    11      11       0   11    0    0
2     1   20     30     0    21      21       0   21    0    0
3     2   10     20     0    31      31       0   31    0    0
4     2   30     40     0    41      41       0   41    0    0
5     3   10     20     0    51      51       0   51    0    0

> addKeyTable(kpa,keyTable=key2[,c("seqid","lend","rstart")],index=2,values=key2$nAligns)
> addKeyTable(kpa,keyTable=key3[,c("seqid","lend","rstart")],index=4,values=key3$nAligns)
> ca<-appendKeyTable(kpa,ku,prefix="aln.")
> ca

  seqid lend rstart nAligns aln.gen.m aln.gen.w aln.loc.thx aln.loc.abd
1     1   10     20      63        21        42          32          31
2     1   20     30      43        22        21          43           0
3     2   10     20      86        23        63          54          32
4     2   30     40      65        24        41          65           0
5     3   10     20      51         0        51          51           0
6     4   50     70      25        25         0          25           0
7     4   60     80      33         0        33           0          33
8     5   10     20      34         0        34           0          34
```

```
9       5      20       30       35           0          35           0          35
   aln.ag.y aln.ag.m aln.ag.o
1         32        0       31
2         43        0        0
3         54        0       32
4         65        0        0
5         51        0        0
6         25        0        0
7          0        0       33
8          0        0       34
9          0        0       35
```

The `readTableBamGaps` function uses both versions simultaneously: Two `keyPro-filer` objects keep probe and align data separately. The two tables are then appended to the key table subsequently.

References

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[3] D Kim, C Pertea, C Trapnell, H Pimentel, R Kelley, and SL Salzberg. Tophat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fucions. *Genome Biology*, 14:R36, 2013. `http://tophat.cbcb.umd.edu/`.

[4] G Yeo and CB Burge. Maximum entropy modeling of short sequence motifs with applications to rna splicing signals. *J Comput Biol*, 11:377–394, 2004. `http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html`.