

Efficient genome searching with Biostrings and the BSgenome data packages

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1 The Biostrings-based genome data packages

The Bioconductor project provides data packages that contain the full genome sequences of a given organism. These packages are called *Biostrings-based genome data packages* because the sequences they contain are stored in some of the basic containers defined in the Biostrings package, like the *DNAString*, the *DNAStringSet* or the *MaskedDNAString* containers. Regardless of the particular sequence data that they contain, all the Biostrings-based genome data packages are very similar and can be manipulated in a consistent and easy way. They all require the BSgenome package in order to work properly. This package, unlike the Biostrings-based genome data packages, is a software package that provides the infrastructure needed to support them (this is why the Biostrings-based genome data packages are also called *BSgenome data packages*). The BSgenome package itself requires the Biostrings package.

See the man page for the `available.genomes` function (`?available.genomes`) for more information about how to get the list of all the BSgenome data packages currently available in your version of Bioconductor (you need an internet connection so that `available.genomes` can query the Bioconductor package repositories).

Note that the BSgenomeForge package provides tools that you can use to make your own BSgenome data package.

2 Finding an arbitrary nucleotide pattern in a chromosome

In this section we show how to find (or just count) the occurrences of some arbitrary nucleotide pattern in a chromosome. The basic tool for this is the `matchPattern` (or `countPattern`) function from the `Biostrings` package.

First we need to install and load the `BSgenome` data package for the organism that we want to look at. In our case, we want to search chromosome I of *Caenorhabditis elegans*.

UCSC provides several versions of the *C. elegans* genome: `ce1`, `ce2` and `ce4`. These versions correspond to different *releases* from WormBase, which are the WS100, WS120 and WS170 releases, respectively. See <http://genome.ucsc.edu/FAQ/FAQreleases#release1> for the list of all UCSC genome releases and for the correspondance between UCSC versions and release names.

The `BSgenome` data package for the `ce2` genome is `BSgenome.Celegans.UCSC.ce2`. Note that `ce1` and `ce4` are not available in Bioconductor but they could be added if there is demand for them.

See `?available.genomes` for how to install `BSgenome.Celegans.UCSC.ce2`. Then load the package and display the single object defined in it:

```
> library(BSgenome.Celegans.UCSC.ce2)
> ls("package:BSgenome.Celegans.UCSC.ce2")

[1] "BSgenome.Celegans.UCSC.ce2" "Celegans"

> genome <- BSgenome.Celegans.UCSC.ce2
> genome

| BSgenome object for Worm
| - organism: Caenorhabditis elegans
| - provider: UCSC
| - genome: ce2
| - release date: Mar. 2004
| - 7 sequence(s):
|   chrI   chrII  chrIII chrIV  chrV   chrX   chrM
|
| Tips: call 'seqnames()' on the object to get all the sequence names, call
| 'seqinfo()' to get the full sequence info, use the '$' or '[' operator to
| access a given sequence, see '?BSgenome' for more information.

genome is a BSgenome object:

> class(genome)

[1] "BSgenome"
attr(,"package")
[1] "BSgenome"
```

When displayed, some basic information about the origin of the genome is shown (organism, genome, provider, etc...) followed by the index of *single* sequences and eventually an additional index of *multiple* sequences. Methods (adequately called *accessor methods*) are defined for individual access to this information:

```
> metadata(genome)

$organism
[1] "Caenorhabditis elegans"

$common_name
```

```

[1] "Worm"

$provider
[1] "UCSC"

$genome
[1] "ce2"

$release_date
[1] "Mar. 2004"

$source_url
[1] "http://hgdownload.cse.ucsc.edu/goldenPath/ce2/bigZips/"

> seqnames(genome)

[1] "chrI"    "chrII"   "chrIII"  "chrIV"   "chrV"    "chrX"    "chrM"

> seqinfo(genome)

```

Seqinfo object with 7 sequences (1 circular) from ce2 genome:

seqnames	seqlengths	isCircular	genome
chrI	15080483	FALSE	ce2
chrII	15279308	FALSE	ce2
chrIII	13783313	FALSE	ce2
chrIV	17493791	FALSE	ce2
chrV	20922231	FALSE	ce2
chrX	17718849	FALSE	ce2
chrM	13794	TRUE	ce2

See the man page for the *BSgenome* class (`?BSgenome`) for a complete list of accessor methods and their descriptions.

Now we are ready to display chromosome I:

```

> genome$chrI

15080483-letter DNAString object
seq: GCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCCTAA...TTAGGCTTAGGCTTAGGCTTAGGTTTAGGCTTAGGC

```

Note that this chrI sequence corresponds to the *forward* strand (aka *direct* or *sense* or *positive* or *plus* strand) of chromosome I. UCSC, and genome providers in general, don't provide files containing the nucleotide sequence of the *reverse* strand (aka *indirect* or *antisense* or *negative* or *minus* or *opposite* strand) of the chromosomes because these sequences can be deduced from the *forward* sequences by taking their reverse complements. The BSgenome data packages are no exceptions: they only provide the *forward* strand sequence of every chromosome. See `?reverseComplement` for more details about the reverse complement of a *DNAString* object. It is important to remember that, in practice, the *reverse* strand sequence is almost never needed. The reason is that, in fact, a *reverse* strand analysis can (and should) always be transposed into a *forward* strand analysis. Therefore trying to compute the *reverse* strand sequence of an entire chromosome by applying `reverseComplement` to its *forward* strand sequence is almost always a bad idea. See the *Finding an arbitrary nucleotide pattern in an entire genome* section of this document for how to find arbitrary patterns in the *reverse* strand of a chromosome.

The number of bases in this sequence can be retrieved with:

```
> chrI <- genome$chrI
> length(chrI)
```

```
[1] 15080483
```

Some basic stats:

```
> afI <- alphabetFrequency(chrI)
> afI
```

	A	C	G	T	M	R	W	S	Y	K
4838561	2697177	2693544	4851201	0	0	0	0	0	0	0
	V	H	D	B	N	-	+	.		
	0	0	0	0	0	0	0	0		

```
> sum(afI) == length(chrI)
```

```
[1] TRUE
```

Count all *exact* matches of pattern "ACCCAGGGC":

```
> p1 <- "ACCCAGGGC"
> countPattern(p1, chrI)
```

```
[1] 0
```

Like most pattern matching functions in Biostrings, the `countPattern` and `matchPattern` functions support *inexact* matching. One form of inexact matching is to allow a few mismatching letters per match. Here we allow at most one:

```
> countPattern(p1, chrI, max.mismatch=1)
```

```
[1] 235
```

With the `matchPattern` function, the locations of the matches are stored in an *XStringViews* object:

```
> m1 <- matchPattern(p1, chrI, max.mismatch=1)
> m1[4:6]
```

Views on a 15080483-letter DNAString subject

subject: GCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCCT...AGGCTTAGGCTTAGGCTTAGGTTTAGGCTTAGGC

views:

	start	end	width	
[1]	187350	187358	9	[ACCCAAGGC]
[2]	213236	213244	9	[ACCCAGGGG]
[3]	424133	424141	9	[ACCCAGGAC]

```
> class(m1)
```

```
[1] "XStringViews"
attr(,"package")
[1] "Biostrings"
```

The `mismatch` function (new in Biostrings 2) returns the positions of the mismatching letters for each match:

```
> mismatch(p1, m1[4:6])
```

```
[[1]]
[1] 6
```

```
[[2]]
[1] 9
```

```
[[3]]
[1] 8
```

Note: The `mismatch` method is in fact a particular case of a (vectorized) *alignment* function where only “replacements” are allowed. Current implementation is slow but this will be addressed.

It may happen that a match is *out of limits* like in this example:

```
> p2 <- DNASTring("AAGCCTAAGCCTAAGCCTAA")
> m2 <- matchPattern(p2, chrI, max.mismatch=2)
> m2[1:4]
```

Views on a 15080483-letter DNASTring subject

subject: GCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCCT...AGGCTTAGGCTTAGGCTTAGGTTTAGGCTTAGGC

views:

	start	end	width	
[1]	-1	18	20	[GCCTAAGCCTAAGCCTAA]
[2]	5	24	20	[AAGCCTAAGCCTAAGCCTAA]
[3]	11	30	20	[AAGCCTAAGCCTAAGCCTAA]
[4]	17	36	20	[AAGCCTAAGCCTAAGCCTAA]

```
> p2 == m2[1:4]
```

```
[1] FALSE TRUE TRUE TRUE
```

```
> mismatch(p2, m2[1:4])
```

```
[[1]]
[1] 1 2
```

```
[[2]]
integer(0)
```

```
[[3]]
integer(0)
```

```
[[4]]
integer(0)
```

The list of exact matches and the list of inexact matches can both be obtained with:

```
> m2[p2 == m2]
> m2[p2 != m2]
```

Note that the length of `m2[p2 == m2]` should be equal to `countPattern(p2, chrI, max.mismatch=0)`.

3 Finding an arbitrary nucleotide pattern in an entire genome

Now we want to extend our analysis to the *forward* and *reverse* strands of all the *C. elegans* chromosomes. More precisely, here is the analysis we want to perform:

- The input dictionary: Our input is a dictionary of 50 patterns. Each pattern is a short nucleotide sequence of 15 to 25 bases (As, Cs, Gs and Ts only, no Ns). It is stored in a FASTA file called "ce2dict0.fa". See the *Finding all the patterns of a constant width dictionary in an entire genome* section of this document for a very efficient way to deal with the special case where all the patterns in the input dictionary have the same length.
- The target: Our target (or subject) is the *forward* and *reverse* strands of the seven *C. elegans* chromosomes (14 sequences in total). We want to find and report all occurrences (or hits) of every pattern in the target. Note that a given pattern can have 0, 1 or several hits in 0, 1 or 2 strands of 0, 1 or several chromosomes.
- Exact or inexact matching? We are interested in exact matches only (for now).
- The output: We want to put the results of this analysis in a file so we can send it to our collaborators for some post analysis work. Our collaborators are not necessarily familiar with R or Bioconductor so dumping a high-level R object (like a list or a data frame) into an .rda file is not an option. For maximum portability (one of our collaborators wants to use Microsoft Excel for the post analysis) we choose to put our results in a tabulated file where one line describes one hit. The columns (or fields) of this file will be (in this order):
 - seqname: the name of the chromosome where the hit occurs.
 - start: an integer giving the starting position of the hit.
 - end: an integer giving the ending position of the hit.
 - strand: a plus (+) for a hit in the positive strand or a minus (-) for a hit in the negative strand.
 - patternID: we use the unique ID provided for every pattern in the "ce2dict0.fa" file.

Let's start by loading the input dictionary with:

```
> ce2dict0_file <- system.file("extdata", "ce2dict0.fa", package="BSgenome")
> ce2dict0 <- readDNASTringSet(ce2dict0_file, "fasta")
> ce2dict0
```

DNASTringSet object of length 50:

	width	seq	names
[1]	18	GCGAACTAGGAGAGGCT	pattern01
[2]	25	CTGTTAGCTAATTTTAAAAATAAAT	pattern02
[3]	24	ACTACCACCCAAATTTAGATATTC	pattern03
[4]	24	AAATTTTTTTTGTGCAAATTTGA	pattern04
[5]	25	TCTTCTTGGCTTTGGTGGTACTTTT	pattern05
...
[46]	24	TTTTGAACAAAGCATGTCTAACTA	pattern46
[47]	20	TAAACGAATTTAGGATATAT	pattern47
[48]	19	AAGGACCAGGATTGGCACG	pattern48
[49]	24	AAATAACTGCGTAAAAACACAATA	pattern49
[50]	22	AAAATGCCGGAGCATTTTAAAG	pattern50

Here is how we can write the functions that will perform our analysis:

```

> writeHits <- function(seqname, matches, strand, file="", append=FALSE)
+ {
+   if (file.exists(file) && !append)
+     warning("existing file ", file, " will be overwritten with 'append=FALSE'")
+   if (!file.exists(file) && append)
+     warning("new file ", file, " will have no header with 'append=TRUE'")
+   hits <- data.frame(seqname=rep.int(seqname, length(matches)),
+                      start=start(matches),
+                      end=end(matches),
+                      strand=rep.int(strand, length(matches)),
+                      patternID=names(matches),
+                      check.names=FALSE)
+   write.table(hits, file=file, append=append, quote=FALSE, sep="\t",
+              row.names=FALSE, col.names=!append)
+ }
> runAnalysis1 <- function(dict0, outfile="")
+ {
+   library(BSgenome.Celegans.UCSC.ce2)
+   genome <- BSgenome.Celegans.UCSC.ce2
+   seqnames <- seqnames(genome)
+   seqnames_in1string <- paste(seqnames, collapse=", ")
+   cat("Target:", metadata(genome)$genome,
+       "chromosomes", seqnames_in1string, "\n")
+   append <- FALSE
+   for (seqname in seqnames) {
+     subject <- genome[[seqname]]
+     cat(">>> Finding all hits in chromosome", seqname, "... \n")
+     for (i in seq_len(length(dict0))) {
+       patternID <- names(dict0)[i]
+       pattern <- dict0[[i]]
+       plus_matches <- matchPattern(pattern, subject)
+       names(plus_matches) <- rep.int(patternID, length(plus_matches))
+       writeHits(seqname, plus_matches, "+", file=outfile, append=append)
+       append <- TRUE
+       rcpattern <- reverseComplement(pattern)
+       minus_matches <- matchPattern(rcpattern, subject)
+       names(minus_matches) <- rep.int(patternID, length(minus_matches))
+       writeHits(seqname, minus_matches, "-", file=outfile, append=append)
+     }
+     cat(">>> DONE \n")
+   }
+ }

```

Some important notes about the implementation of the `runAnalysis1` function:

- `subject <- genome[[seqname]]` is the code that actually loads a chromosome sequence into memory. Using only one sequence at a time is a good practice to avoid memory allocation problems on a machine with a limited amount of memory. For example, loading all the human chromosome sequences in memory would require more than 3GB of memory!
- We have 2 nested `for` loops: the outer loop walks thru the target (7 chromosomes) and the inner loop walks thru the set of patterns. Doing the other way around would be very inefficient, especially with a

bigger number of patterns because this would require to load each chromosome sequence into memory as many times as the number of patterns. `runAnalysis1` loads each sequence only once.

- We find the matches in the minus strand (`minus_matches`) by first taking the reverse complement of the current pattern (with `rcpattern <- reverseComplement(pattern)`) and NOT by taking the reverse complement of the current subject.

Now we are ready to run the analysis and put the results in the "ce2dict0_ana1.txt" file:

```
> runAnalysis1(ce2dict0, outfile="ce2dict0_ana1.txt")

Target: ce2 chromosomes chrI, chrII, chrIII, chrIV, chrV, chrX, chrM
>>> Finding all hits in chromosome chrI ...
>>> DONE
>>> Finding all hits in chromosome chrII ...
>>> DONE
>>> Finding all hits in chromosome chrIII ...
>>> DONE
>>> Finding all hits in chromosome chrIV ...
>>> DONE
>>> Finding all hits in chromosome chrV ...
>>> DONE
>>> Finding all hits in chromosome chrX ...
>>> DONE
>>> Finding all hits in chromosome chrM ...
>>> DONE
```

Here is some very simple example of post analysis:

- Get the total number of hits:

```
> hits1 <- read.table("ce2dict0_ana1.txt", header=TRUE)
> nrow(hits1)
```

```
[1] 79
```

- Get the number of hits per chromosome:

```
> table(hits1$seqname)
```

chrI	chrII	chrIII	chrIV	chrM	chrV	chrX
11	5	16	8	8	15	16

- Get the number of hits per pattern:

```
> hits1_table <- table(hits1$patternID)
> hits1_table
```

pattern01	pattern02	pattern03	pattern04	pattern06	pattern07	pattern08	pattern09
1	1	1	1	2	1	1	1
pattern10	pattern11	pattern12	pattern13	pattern14	pattern15	pattern16	pattern17
1	1	1	1	1	1	1	1
pattern18	pattern19	pattern20	pattern21	pattern22	pattern23	pattern24	pattern25
1	9	1	10	2	1	1	1
pattern26	pattern27	pattern28	pattern29	pattern30	pattern31	pattern32	pattern33


```

      1      1      1      1      1      1      1      1
pattern34 pattern35 pattern36 pattern37 pattern38 pattern39 pattern40 pattern41
      2      1      1      7      1      1      1      1
pattern42 pattern43 pattern44 pattern45 pattern46 pattern47 pattern48 pattern49
      1      5      1      1      1      1      1      1
pattern50
      1

```

- Get the pattern(s) with the higher number of hits:

```

> hits1_table[hits1_table == max(hits1_table)] # pattern(s) with more hits

pattern21
      10

```

- Get the pattern(s) with no hits:

```

> setdiff(names(ce2dict0), hits1$patternID) # pattern(s) with no hits

[1] "pattern05"

```

- And finally a function that can be used to plot the hits:

```

> plotGenomeHits <- function(bsgenome, seqnames, hits)
+ {
+   chrlengths <- seqlengths(bsgenome)[seqnames]
+   XMAX <- max(chrlengths)
+   YMAX <- length(seqnames)
+   plot.new()
+   plot.window(c(1, XMAX), c(0, YMAX))
+   axis(1)
+   axis(2, at=seq_len(length(seqnames)), labels=rev(seqnames), tick=FALSE, las=1)
+   ## Plot the chromosomes
+   for (i in seq_len(length(seqnames)))
+     lines(c(1, chrlengths[i]), c(YMAX + 1 - i, YMAX + 1 - i), type="l")
+   ## Plot the hits
+   for (i in seq_len(nrow(hits))) {
+     seqname <- hits$seqname[i]
+     y0 <- YMAX + 1 - match(seqname, seqnames)
+     if (hits$strand[i] == "+") {
+       y <- y0 + 0.05
+       col <- "red"
+     } else {
+       y <- y0 - 0.05
+       col <- "blue"
+     }
+     lines(c(hits$start[i], hits$end[i]), c(y, y), type="l", col=col, lwd=3)
+   }
+ }

```

Plot the hits found by runAnalysis1 with:

```

> plotGenomeHits(genome, seqnames(genome), hits1)

```

4 Some precautions when using *matchPattern*

Improper use of *matchPattern* (or *countPattern*) can affect performance.

If needed, the *matchPattern* and *countPattern* methods convert their first argument (the pattern) to an object of the same class than their second argument (the subject) before they pass it to the subroutine that actually implements the fast search algorithm.

So if you need to reuse the same pattern a high number of times, it's a good idea to convert it *before* to pass it to the *matchPattern* or *countPattern* method. This way the conversion is done only once:

```
> library(hgu95av2probe)
> tmpseq <- DNASTringSet(hgu95av2probe$sequence)
> someStats <- function(v)
+ {
+   GC <- DNASTring("GC")
+   CG <- DNASTring("CG")
+   sapply(seq_len(length(v)),
+     function(i) {
+       y <- v[[i]]
+       c(alphabetFrequency(y)[1:4],
+         GC=countPattern(GC, y),
+         CG=countPattern(CG, y))
+     }
+   )
+ }
> someStats(tmpseq[1:10])
```

	[,1]	[,2]	[,3]	[,4]	[,5]	[,6]	[,7]	[,8]	[,9]	[,10]
A	1	5	6	4	4	2	4	5	9	2
C	10	5	4	7	5	7	10	8	7	10
G	6	5	3	8	8	6	4	5	4	4
T	8	10	12	6	8	10	7	7	5	9
GC	2	1	1	4	3	2	2	2	1	1
CG	0	0	0	2	1	1	0	0	0	0

5 Masking the chromosome sequences

Starting with Bioconductor 2.2, the chromosome sequences in a *BSgenome* data package can have built-in masks. Starting with Bioconductor 2.3, there can be up to 4 built-in masks per sequence. These will always be (in this order): (1) the mask of assembly gaps, (2) the mask of intra-contig ambiguities, (3) the mask of repeat regions that were determined by the RepeatMasker software, and (4) the mask of repeat regions that were determined by the Tandem Repeats Finder software (where only repeats with period less than or equal to 12 were kept).

For a given package, all the sequences will always have the same number of masks.

```
> library(BSgenome.Hsapiens.UCSC.hg38.masked)
> genome <- BSgenome.Hsapiens.UCSC.hg38.masked
> chrY <- genome$chrY
> chrY
```

57227415-letter MaskedDNASTring object (# for masking)

```
seq: #####...#####
masks:
```

```

maskedwidth maskedratio active names desc
1 30812367 5.384197e-01 TRUE AGAPS assembly gaps
2 5 8.737071e-08 TRUE AMB intra-contig ambiguities
3 16525661 2.887718e-01 FALSE RM RepeatMasker
4 872171 1.524044e-02 FALSE TRF Tandem Repeats Finder [period<=12]
all masks together:
maskedwidth maskedratio
47464316 0.8293982
all active masks together:
maskedwidth maskedratio
30812372 0.5384198

> chrM <- genome$chrM
> chrM

16569-letter MaskedDNAString object (# for masking)
seq: GATCACAGGTCTATCACCCCTATTAACCACTCACGGG...AGCCCACACGTTCCCCTTAAATAAGACATCACGATG
masks:
maskedwidth maskedratio active names desc
1 0 0.000000e+00 TRUE AGAPS assembly gaps (empty)
2 1 6.035367e-05 TRUE AMB intra-contig ambiguities
3 418 2.522784e-02 FALSE RM RepeatMasker
4 0 0.000000e+00 FALSE TRF Tandem Repeats Finder [period<=12]
all masks together:
maskedwidth maskedratio
419 0.02528819
all active masks together:
maskedwidth maskedratio
1 6.035367e-05

```

The built-in masks are named consistently across all the BSgenome data packages available in Bioconductor:

Name	Active by default	Short description	Long description
AGAPS	yes	assembly gaps	Masks the big N-blocks that have been placed between the contigs
AMB	yes	intra-contig ambiguities	Masks any IUPAC ambiguity letter that was found in the contigs
RM	no	RepeatMasker	Masks the repeat regions determined by the RepeatMasker software
TRF	no	Tandem Repeats Finder	Masks the tandem repeat regions that were determined by the Tandem Repeats Finder

Table 1: The built-in masks provided by the BSgenome data packages.

When displaying a masked sequence (here a *MaskedDNAString* object), the *masked width* and *masked ratio* are reported for each individual mask, as well as for all the masks together, and for all the active masks together. The *masked width* is the total number of nucleotide positions that are masked and the *masked ratio* is the *masked width* divided by the length of the sequence.

To activate a mask, use the *active* replacement method in conjunction with the *masks* method. For example, to activate the RepeatMasker mask, do:

```

> active(masks(chrY))["RM"] <- TRUE
> chrY

57227415-letter MaskedDNAString object (# for masking)
seq: #####...#####

```


[5]	2132995	2137388	4394	[TGTCTGTGTATGTATATATAT...CCTCTCCCATCATCATCATCA]
...
[51]	21748372	21750013	1642	[GAAGTAAGCATTCTGTATTA...TCCAGCCAAGGTGACAGGGCA]
[52]	21750315	21789281	38967	[CTGTACTTTACAGTCTTGCTT...AGCAGCCAAATCTGCAGTCAT]
[53]	21805282	26673214	4867933	[AAGCTTTGGCTAATATATCTC...GAGTGGTGCAGAGTGAATTC]
[54]	56673215	56771509	98295	[GAATTCATTGGAATGGAAGGG...GATTGGAATGGAATGGAATTC]
[55]	56821510	57217415	395906	[GAATTC AACATTATTCTTGT...GGTGTGGTGTGTGGGTGTGGT]

This can be used in conjunction with the *gaps* method to see the gaps between the views i.e. the masked regions themselves:

```
> gaps(as(chrY, "XStringViews"))
```

To extract the sizes of the assembly gaps:

```
> width(gaps(as(chrY, "XStringViews")))
```

[1]	10000	50000	88475	75	183649	100	8260	50000
[9]	847	2052	50000	50000	50000	12393	1432	995
[17]	2486	2727	329	50000	25	808	534	2235
[25]	679	1523	1659	582	1078	899	908	1590
[33]	628	25	783	518	255	892	818	780
[41]	50000	267	38	1807	20	140	20	328
[49]	50000	1899	508	301	16000	30000000	50000	10000

Note that, if applied directly to `chrY`, `gaps` returns a *MaskedDNAString* object with a single mask masking the regions that are not masked in the original object:

```
> gaps(chrY)
```

57227415-letter MaskedDNAString object (# for masking)

[illegible]

masks:

	maskedwidth	maskedratio	active
1	26415048	0.4615803	TRUE

```
> alphabetFrequency(gaps(chrY))
```

A	C	G	T	M	R	W	S
O	O	O	O	O	O	O	O
Y	K	V	H	D	B	N	-
O	O	O	O	O	O	30812367	O
+	.						
O	O						

In fact, for any *MaskedDNAString* object, the following should always be **TRUE**, whatever the masks are:

```
> af0 <- alphabetFrequency(unmasked(chrY))
> af1 <- alphabetFrequency(chrY)
> af2 <- alphabetFrequency(gaps(chrY))
> all(af0 == af1 + af2)
```

```
[1] TRUE
```

With all chrY masks active:


```

      A      C      G      T  other
7886192 5285789 5286894 7956168      0

```

```
> countPattern(Ebox, chrY, fixed=FALSE)
```

```
[1] 141609
```

Note that some chromosomes can have Ns outside the assembly gaps:

```

> chr2 <- genome$chr2
> active(masks(chr2))[-2] <- FALSE
> alphabetFrequency(gaps(chr2))

```

```

  A   C   G   T   M   R   W   S   Y   K   V   H   D   B   N   -
0   0   0   0   0   0   0   0   0   0   0   0   0   0 2913  0
+   .
0   0

```

so it is recommended to always keep the AMB mask active (in addition to the AGAPS mask) whatever the sequence is.

Note that not all functions that work with an *XString* input are *mask aware* but more will be added in the near future. However, most of the times there is a alternate way to exclude some arbitrary regions from an analysis without having to use *mask aware* functions. This is described below in the *Hard masking* section.

6 Hard masking

coming soon...

7 Injecting known SNPs in the chromosome sequences

coming soon...

8 Finding all the patterns of a constant width dictionary in an entire genome

The `matchPDict` function can be used instead of `matchPattern` for the kind of analysis described in the *Finding an arbitrary nucleotide pattern in an entire genome* section but it will be much faster (between 100x and 10000x faster depending on the size of the input dictionary). Note that a current limitation of `matchPDict` is that it only works with a dictionary of DNA patterns where all the patterns have the same number of nucleotides (constant width dictionary). See `?matchPDict` for more information.

Here is how our `runAnalysis1` function can be modified in order to use `matchPDict` instead of `matchPattern`:

```

> runOneStrandAnalysis <- function(dict0, bsgenome, seqnames, strand,
+                                outfile="", append=FALSE)
+ {
+   cat("\nTarget: strand", strand, "of", metadata(bsgenome)$genome,
+       "chromosomes", paste(seqnames, collapse=", "), "\n")
+   if (strand == "-")
+     dict0 <- reverseComplement(dict0)
+   pdict <- PDict(dict0)

```

```

+   for (seqname in seqnames) {
+       subject <- bsgenome[[seqname]]
+       cat(">>> Finding all hits in strand", strand, "of chromosome", seqname, "...\\n")
+       mindex <- matchPDict(pdict, subject)
+       matches <- extractAllMatches(subject, mindex)
+       writeHits(seqname, matches, strand, file=outfile, append=append)
+       append <- TRUE
+       cat(">>> DONE\\n")
+   }
+ }
> runAnalysis2 <- function(dict0, outfile="")
+ {
+   library(BSgenome.Celegans.UCSC.ce2)
+   genome <- BSgenome.Celegans.UCSC.ce2
+   seqnames <- seqnames(genome)
+   runOneStrandAnalysis(dict0, genome, seqnames, "+", outfile=outfile, append=FALSE)
+   runOneStrandAnalysis(dict0, genome, seqnames, "-", outfile=outfile, append=TRUE)
+ }

```

Remember that `matchPDict` only works if all the patterns in the input dictionary have the same length so for this 2nd analysis, we will truncate the patterns in `ce2dict0` to 15 nucleotides:

```
> ce2dict0cw15 <- DNASTringSet(ce2dict0, end=15)
```

Now we can run this 2nd analysis and put the results in the "ce2dict0cw15_ana2.txt" file:

```
> runAnalysis2(ce2dict0cw15, outfile="ce2dict0cw15_ana2.txt")
```

Target: strand + of ce2 chromosomes chrI, chrII, chrIII, chrIV, chrV, chrX, chrM

```

>>> Finding all hits in strand + of chromosome chrI ...
>>> DONE
>>> Finding all hits in strand + of chromosome chrII ...
>>> DONE
>>> Finding all hits in strand + of chromosome chrIII ...
>>> DONE
>>> Finding all hits in strand + of chromosome chrIV ...
>>> DONE
>>> Finding all hits in strand + of chromosome chrV ...
>>> DONE
>>> Finding all hits in strand + of chromosome chrX ...
>>> DONE
>>> Finding all hits in strand + of chromosome chrM ...
>>> DONE

```

Target: strand - of ce2 chromosomes chrI, chrII, chrIII, chrIV, chrV, chrX, chrM

```

>>> Finding all hits in strand - of chromosome chrI ...
>>> DONE
>>> Finding all hits in strand - of chromosome chrII ...
>>> DONE
>>> Finding all hits in strand - of chromosome chrIII ...
>>> DONE
>>> Finding all hits in strand - of chromosome chrIV ...
>>> DONE

```



```
>>> Finding all hits in strand - of chromosome chrV ...
>>> DONE
>>> Finding all hits in strand - of chromosome chrX ...
>>> DONE
>>> Finding all hits in strand - of chromosome chrM ...
>>> DONE
```

9 Session info

```
> sessionInfo()
```

```
R version 4.3.2 (2023-10-31)
Platform: aarch64-apple-darwin20 (64-bit)
Running under: macOS Ventura 13.6.1
```

```
Matrix products: default
```

```
BLAS: /Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/lib/libRblas.0.dylib
```

```
LAPACK: /Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/lib/libRlapack.dylib; LAPACK ver
```

```
locale:
```

```
[1] C/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
```

```
time zone: America/New_York
```

```
tzcode source: internal
```

```
attached base packages:
```

```
[1] stats4      stats      graphics  grDevices  utils      datasets  methods
[8] base
```

```
other attached packages:
```

```
[1] BSgenome.Hsapiens.UCSC.hg38.masked_1.4.5
[2] BSgenome.Hsapiens.UCSC.hg38_1.4.5
[3] hgu95av2probe_2.18.0
[4] AnnotationDbi_1.64.1
[5] Biobase_2.62.0
[6] BSgenome.Celegans.UCSC.ce2_1.4.0
[7] BSgenome_1.70.2
[8] rtracklayer_1.62.0
[9] BiocIO_1.12.0
[10] GenomicRanges_1.54.1
[11] Biostrings_2.70.2
[12] GenomeInfoDb_1.38.6
[13] XVector_0.42.0
[14] IRanges_2.36.0
[15] S4Vectors_0.40.2
[16] BiocGenerics_0.48.1
```

```
loaded via a namespace (and not attached):
```

```
[1] SparseArray_1.2.4          bitops_1.0-7
[3] RSQLite_2.3.2              lattice_0.22-5
[5] grid_4.3.2                 fastmap_1.1.1
```

[7]	blob_1.2.4	Matrix_1.6-1.1
[9]	restfulr_0.0.15	DBI_1.1.3
[11]	httr_1.4.7	XML_3.99-0.15
[13]	codetools_0.2-19	abind_1.4-5
[15]	cli_3.6.1	rlang_1.1.1
[17]	crayon_1.5.2	bit64_4.0.5
[19]	cachem_1.0.8	DelayedArray_0.28.0
[21]	yaml_2.3.7	S4Arrays_1.2.0
[23]	tools_4.3.2	parallel_4.3.2
[25]	BiocParallel_1.36.0	memoise_2.0.1
[27]	GenomeInfoDbData_1.2.11	Rsamtools_2.18.0
[29]	SummarizedExperiment_1.32.0	vctrs_0.6.4
[31]	R6_2.5.1	png_0.1-8
[33]	matrixStats_1.0.0	zlibbioc_1.48.0
[35]	KEGGREST_1.42.0	bit_4.0.5
[37]	GenomicAlignments_1.38.2	MatrixGenerics_1.14.0
[39]	rjson_0.2.21	compiler_4.3.2
[41]	RCurl_1.98-1.13	