An introduction to rSFFreader

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```
> library("rSFFreader")
> library("xtable")
```

1 Introduction

The SFF file format has been adopted by both Roche 454 and Ion Torrent next generation sequencing platforms. *rSFFreader* provides functionality for loading sequence, quality scores, and flowgram information from these files. This package has been modeled after the excellent *(*ShortRead) package released by Martin Morgan. It aims to maintain compatibility with that package while enabling direct processing of SFF files.

2 A simple workflow

Read in an SFF file:

```
> sff <- readSff(system.file("extdata","Small454Test.sff",package="rSFFreader"))
```

```
Total number of reads to be read: 1000
reading header for sff file:/private/tmp/RtmpTgc7h7/Rinst4dac4ab37a/rSFFreader/extdata/Small454Test.sff
reading file:/private/tmp/RtmpTgc7h7/Rinst4dac4ab37a/rSFFreader/extdata/Small454Test.sff
```

Accessing the read, quality, and header information:

```
> sread(sff)
```

```
  A DNAStringSet instance of length 1000
      width seq                         names
   [1]   422 ACACGACGACTT...GGCGCTCGCTC HRWLTHE02G15D7
   [2]   157 ACACTACTCGTG...CTGGTGCCGGC HRWLTHE02H2PCX
   [3]   376 ACACGACGACTG...CGTTACAAATC HRWLTHE02HB23L
   [4]   243 ACACGACGACTC...GAGAAGATCAT HRWLTHE02IYLA2
```

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```
   [5]   727 ACACTACTCGTG...GGTCTCCGTTA HRWLTHE02F3E10
   ...   ... ...
 [996]   652 ACACGACGACTC...CGCCTTCCTGC HRWLTHE02JSWSM
 [997]   756 ACACGACGACTG...CCCGGTCACCG HRWLTHE02FJUSH
 [998]   574 ACACGACGACTT...ACGAGGGGGGT HRWLTHE02GCJZT
 [999]   693 ACACTACTCGTC...TACCGGCAGCA HRWLTHE02IFUFC
[1000]   573 ACACTACTCGTC...TTGTGAATACG HRWLTHE02GF2BA
```

```
> quality(sff)
```

```
class: FastqQuality
quality:
  A BStringSet instance of length 1000
      width seq
   [1]   422 IIIIIIIIIHIHHFFFFFFFF>...55577;;997977777878787
   [2]   157 IIHHIHAAADFHDAACAA>A>@...>>???FFD@?B@??=;4/...7
   [3]   376 >>FHHHHFFFFFDCA@@=?=77...777778<;;0002999:74444
   [4]   243 IIIIIIIIIEEEFHHFHHHFII...887==<;;;87555641.144/
   [5]   727 IIIIIIIIIIHHIIIIFHHHHH...22--/,/+++------+++--/
   ...   ... ...
 [996]   652 IIIIIIIIIHHHGGDD>>>FII...0225231111.10////,,,/,
 [997]   756 IIIIIIIIIIIIIIIHHHHIII...100---)))++001001*10-+
 [998]   574 IIIIIIIIHHFFFFA<444>EC...++++),.111111.,+++++++
 [999]   693 IIIIIIIIIIIIIIIIIIIII...011111101010-----,//-+
[1000]   573 IIIIIIIIIIIIIIIIIIII...33355322500--)+.10-+++
```

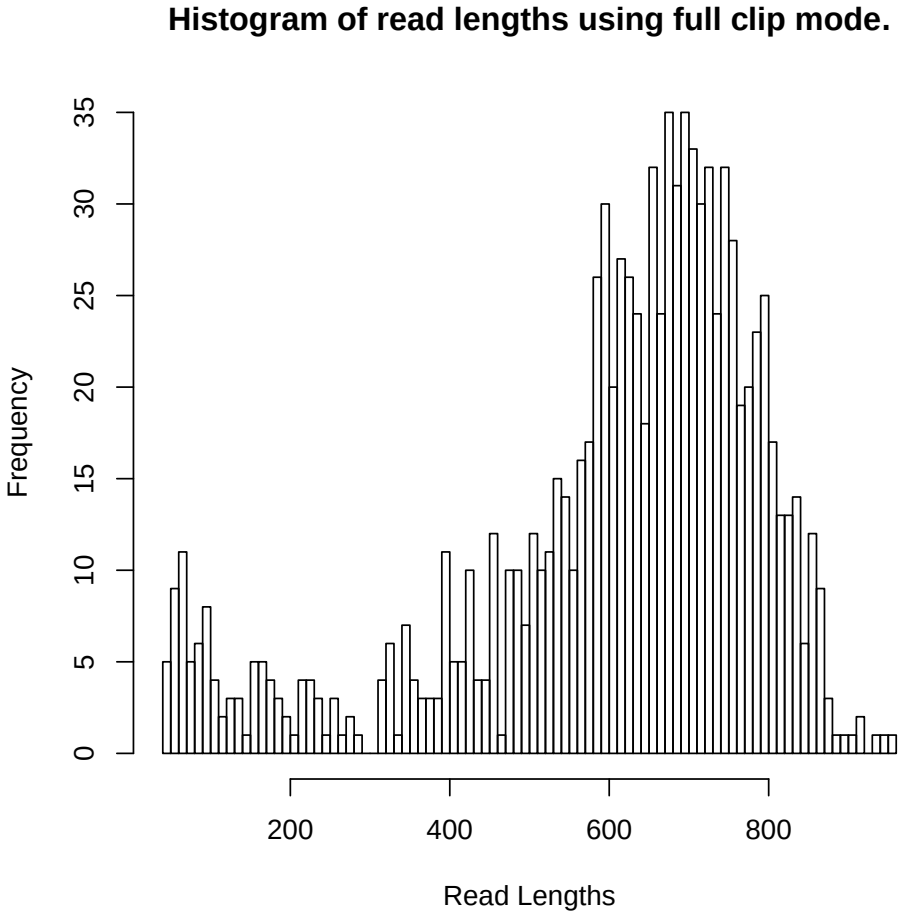
```
> header(sff)
```

```
[[1]]
[[1]]$filename
[1] "/private/tmp/RtmpTgc7h7/Rinst4dac4ab37a/rSFFreader/extdata/Small454Test.sff"

[[1]]$magic_number
[1] 779314790

[[1]]$version
[1] ""

[[1]]$index_offset
[1] 6201592

[[1]]$index_length
[1] 20728

[[1]]$number_of_reads
[1] 1000
```

```
[[1]]$header_length
[1] 1640

[[1]]$key_length
[1] 4

[[1]]$number_of_flows_per_read
[1] 1600

[[1]]$flowgram_format_code
[1] 1

[[1]]$flow_chars
[1] "TACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTAC

[[1]]$key_sequence
[1] "GACT"
```

Plot histogram of read lengths:

```
> hist(width(sff), xlab="Read Lengths",
+      main=paste("Histogram of read lengths using", clipMode(sff), "clip mode."),
+      breaks=100)
```



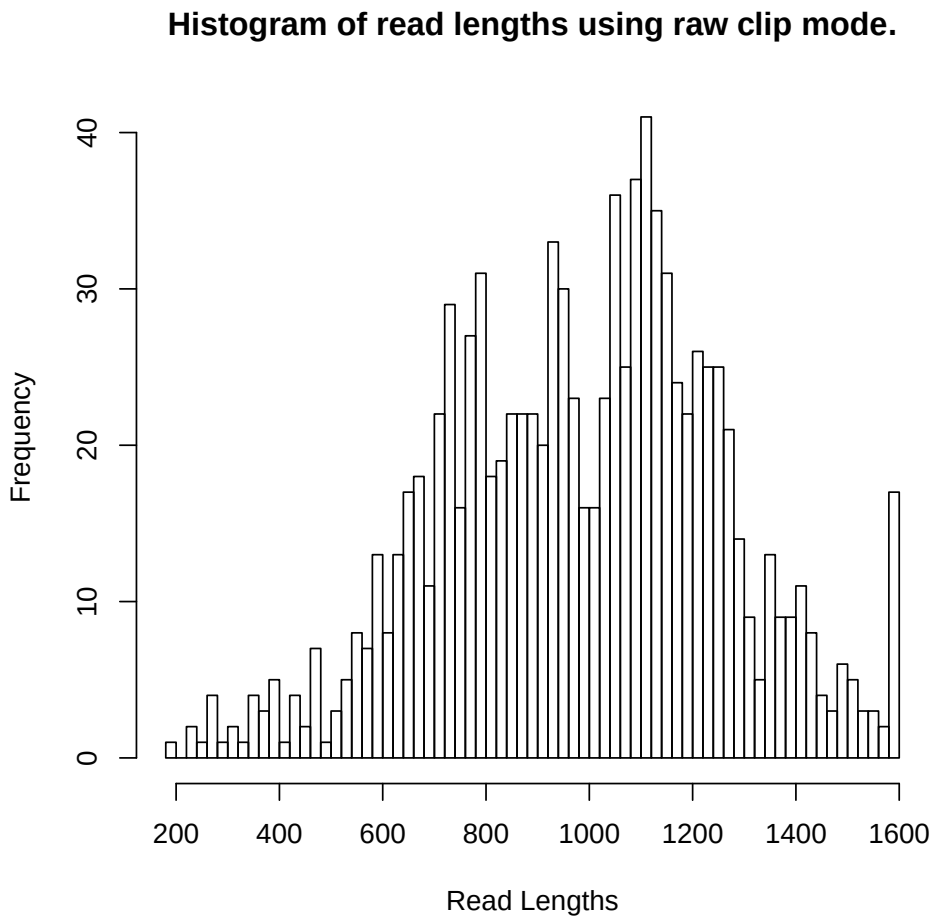
Setting the `clipMode` will change the read lengths that are reported by `width` and plotted by `hist`. Currently the following modes are supported:

- `adapter` : defined in the SFF file, and meant to remove adapter sequence
- `quality` : defined in the SFF file, and meant to remove low-quality regions of the sequence
- `full` : uses the "interior" of quality and adapter and is the most conservative
- `raw` : no clipping is applied and full length reads are returned
- `custom` : clip points set by the user as an *IRanges* object.

```
> availableClipModes(sff)
```

```
[1] "full"    "quality" "adapter" "raw"
```

```
> clipMode(sff)

[1] "full"

> clipMode(sff) <- "raw"
> hist(width(sff), xlab="Read Lengths",
+      main=paste("Histogram of read lengths using", clipMode(sff), "clip mode."),
+      breaks=100)
```



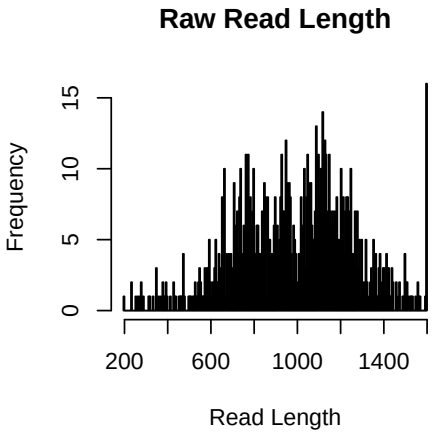
Custom clip points can be set using *IRanges*. For example, it is sometimes useful to look for barcodes (MID tags) in the first 15 bases of a set of reads.

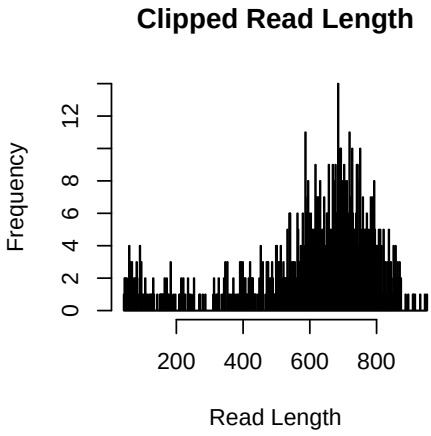
```
> customClip(sff) <- IRanges(start = 1, end = 15)
> clipMode(sff) <- "custom"
> t = table(counts=as.character(sread(sff)))
```

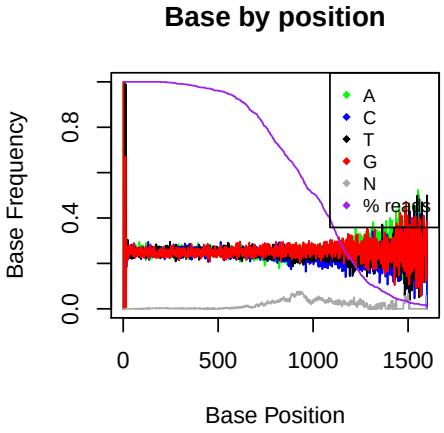
	counts
GACTACACGACGACT	284
GACTACACGTAGTAT	377
GACTACACTACTCGT	316

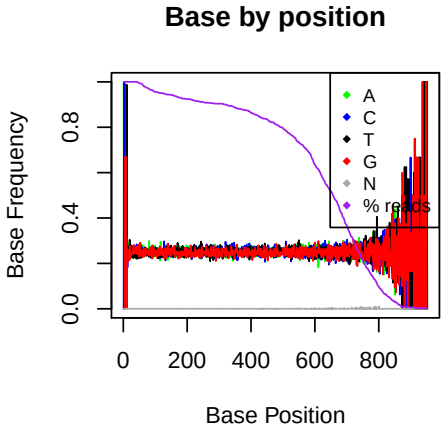
Finally, we can generate some useful QA plots and

```
> ## Generate some QA plots:
> ## Read length histograms:
> par(mfrow=c(2,2))
> clipMode(sff) <- "raw"
> hist(width(sff),breaks=500,col="grey",xlab="Read Length",main="Raw Read Length")
> clipMode(sff) <- "full"
> hist(width(sff),breaks=500,col="grey",xlab="Read Length",main="Clipped Read Length")
> ## Base by position plots:
> clipMode(sff) <- "raw"
> ac <- alphabetByCycle(sread(sff),alphabet=c("A","C","T","G","N"))
> ac.reads <- apply(ac,2,sum)
> acf <- sweep(ac,MARGIN=2,FUN="/",STATS=apply(ac,2,sum))
> matplot(cbind(t(acf),ac.reads/ac.reads[1]),col=c("green","blue","black","red","darkgrey","purple"),
+           type="l",lty=1,xlab="Base Position",ylab="Base Frequency",
+           main="Base by position")
> cols <- c("green","blue","black","red","darkgrey","purple")
> leg <-  c("A","C","T","G","N","% reads")
> legend("topright", col=cols, legend=leg, pch=18, cex=.8)
> clipMode(sff) <- "full"
> ac <- alphabetByCycle(sread(sff),alphabet=c("A","C","T","G","N"))
> ac.reads <- apply(ac,2,sum)
> acf <- sweep(ac,MARGIN=2,FUN="/",STATS=apply(ac,2,sum))
> matplot(cbind(t(acf),ac.reads/ac.reads[1]),col=c("green","blue","black","red","darkgrey","purple"),
+           type="l",lty=1,xlab="Base Position",ylab="Base Frequency",
+           main="Base by position")
> legend("topright", col=cols, legend=leg, pch=18, cex=.8)
>
```


Raw Read Length
Frequency
0
5
10
15
200
600
1000
1400
Read Length


Clipped Read Length
Frequency
0
2
4
6
8
12
200
400
600
800
Read Length


Base by position
Base Frequency
0.0
0.4
0.8
0
500
1000
1500
Base Position
A
C
T
G
N
% reads


Base by position
Base Frequency
0.0
0.4
0.8
0
200
400
600
800
Base Position
A
C
T
G
N
% reads