

An introduction to rSFFreader

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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:/tmp/RtmpNRHSSv/Rinst23de75f86787/rSFFreader/extdata/Small454Test.sff
```

```
reading file:/tmp/RtmpNRHSSv/Rinst23de75f86787/rSFFreader/extdata/Small454Test.sff
```

Accessing the read, quality, and header information:

```
> sread(sff)
```

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```

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
[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(sf)
```

```
class: FastqQuality
```

```
quality:
```

```

A BStringSet instance of length 1000
  width seq
[1]  422 !!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
[2]  157 !!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
[3]  376 !!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
[4]  243 !!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
[5]  727 !!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
...  ...  ...
[996] 652 !!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
[997] 756 !!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
[998] 574 !!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
[999] 693 !!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
[1000] 573 !!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!

```

```
> header(sf)
```

```
[[1]]
```

```
[[1]]$filename
```

```
[1] "/tmp/RtmpNRHSSv/Rinst23de75f86787/rSFFreader/extdata/Small454Test.sf"
```

```
[[1]]$magic_number
```

```
[1] 779314790
```

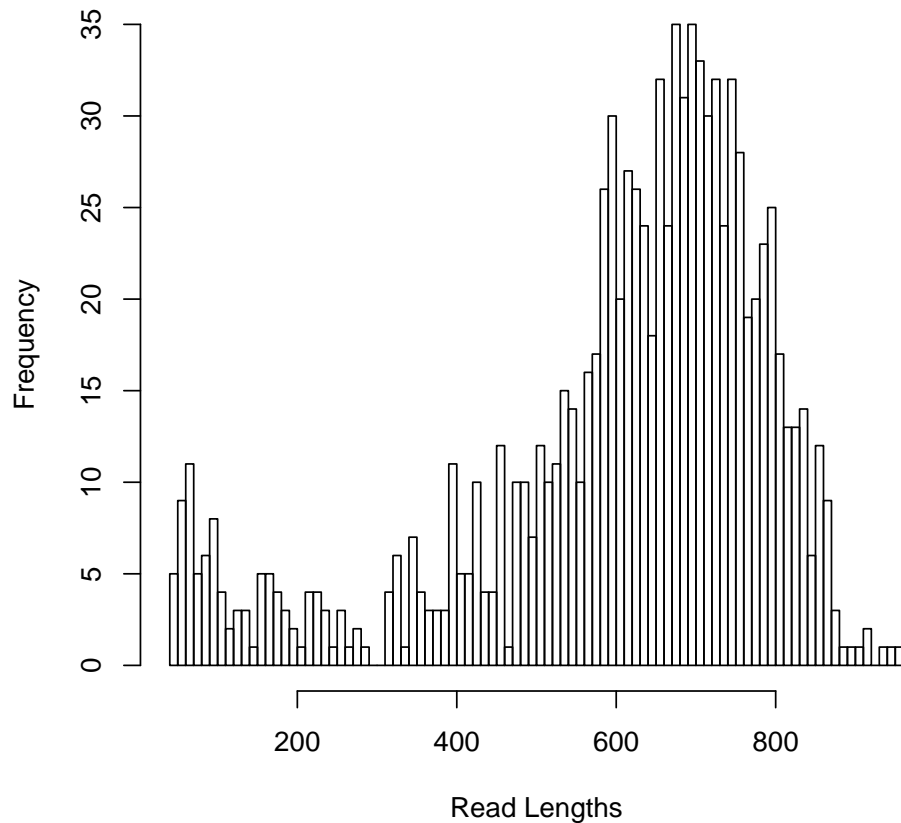
```
[[1]]$version
```

```
[1] ""
```

```
[[1]]$index_offset
```

```
[1] 6201592
```


Histogram of read lengths using full clip mode.



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"
```

```

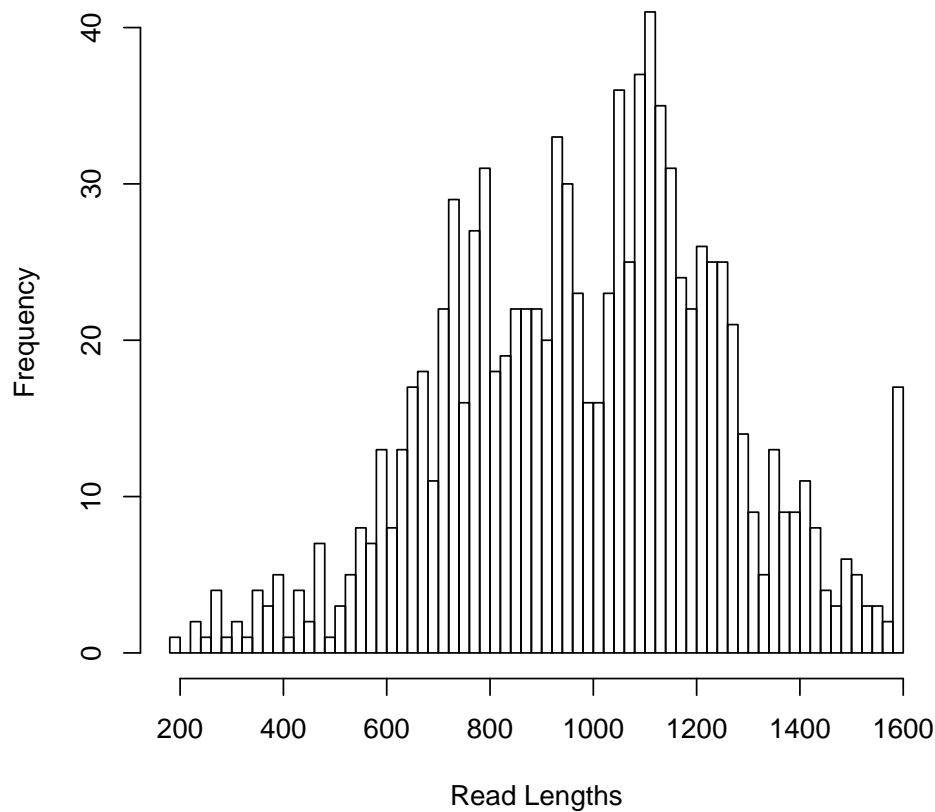
> clipMode(sff)

[1] "full"

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

```

Histogram of read lengths using raw clip mode.



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)))

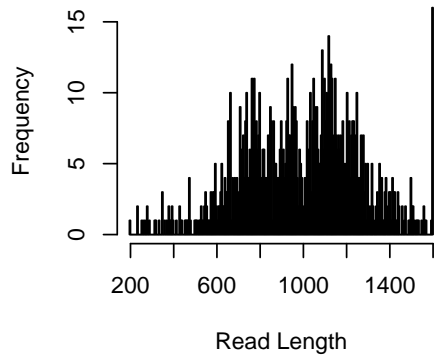
```

	counts
GACTACACGACGACT	284
GACTACACGTAGTAT	377
GACTACACTACTCGT	316

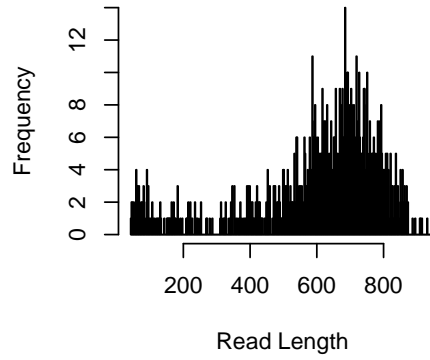
Finally, we can generate some useful QA plots and

```
> ## 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)
>
```

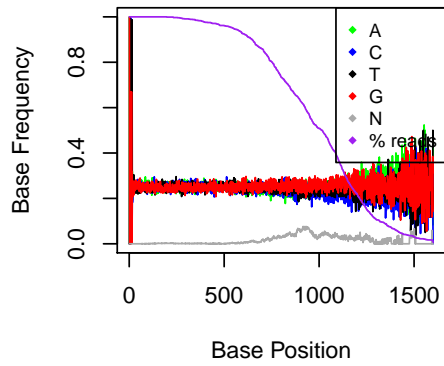
Raw Read Length



Clipped Read Length



Base by position



Base by position

