Introducing ShortRead

INTRODUCTION TO BIOCONDUCTOR IN R

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Plant genomes

- *Arabidopsis thaliana* is a small flowering plant
- First plant to have its genome sequenced
- Genome size 135 megabase pairs (Mbp)

Sequencing companies

illumina
Roche
life technologies
PACIFIC BIOSCIENCES
Helicos
BioSciences Corporation
ion torrent
Oxford NANOPORE Technologies

[1] Dan Koboldt massgenomics.org

fastq vs fasta

fastq

```
@ unique sequence identifier

raw sequence string

+ optional id

quality encoding per sequence letter
```

- fastq, fq

fasta

```
> unique sequence identifier

raw sequence string
```

- fasta, fa, seq

fasta

```r
library(ShortRead)
# read fasta
fasample <- readFasta(dirPath = "data/", pattern = "fasta")
# print fasample
print(fasample)
```

```
class: ShortRead
length: 500 reads; width: 50 cycles
```

```r
# methods accessors
methods(class = "ShortRead")
# Write a ShortRead object
writeFasta(fasample, file = "data/sample.fasta")
```

fastq

```r
library(ShortRead)
# read fastq
fqsample <- readFastq(dirPath = "data/", pattern = "fastq")
# print fqsample
fqsample
```

```
class: ShortReadQ
length: 500 reads; width: 50 cycles
```

```r
# methods accessors
methods(class = "ShortReadQ")
# Write a ShortRead object
writeFastq(fqsample, file = "data/sample.fastq.gz")
```

fastq sample

```r
library(ShortRead)
# set the seed to draw the same read sequences every time
set.seed(123)
# Subsample of 500 bases
sampler <- FastqSampler("data/SRR1971253.fastq", 500)
# save the yield of 500 read sequences
sample_small <- yield(sampler)
# Class ShortReadQ
class(sample_small)
# length 500 reads
length(sample_small)
```

You are ready!

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Sequence quality

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Quality scores - Phred table

Quality value	Chance it is wrong	Accuracy (%)
10	1 in 10	90
20	1 in 100	99
30	1 in 1000	99.9
40	1 in 10000	99.99
50	1 in 100000	99.999

Encoding - Phred +33

```
# quality encoding
encoding(quality(fqsample))
```

Encoding characters and their scores

```
 !  "  #  $  %  &  '  (  )  *  +  ,  -  .   #  encoding
 0  1  2  3  4  5  6  7  8  9 10 11 12 13   #  score

 /  0  1  2  3  4  5  6  7  8  9  :  ;  <   #  encoding
14 15 16 17 18 19 20 21 22 23 24 25 26 27   #  score

 =  >  ?  @  A  B  C  D  E  F  G  H  I      #  encoding
28 29 30 31 32 33 34 35 36 37 38 39 40      #  score
```

fastq quality

```
library(ShortRead)
quality(fqsample)
```

```
class: FastqQuality
A BStringSet instance

# Quality is represented with ASCII characters
[1]    40 ?@@DDDDDHDFDHE>AHFEGFIIEBGDBHH<3FEBEEEEG
[2]    40 BCCDFFFFHHHHHJJJJJJJJJJEHHGHIJJJJJJJJJJJ
[3]    40 BCCFFFFFHFHHHJJJJJJIIJJIIIIIGIIJJIJGIJII
[4]    40 CCCFFFFFHHHHHJJJJJJJJJJIJJJJJJJJJJJJJJJJ
```

```
library(ShortRead)
sread(fqsample)[1]
# Quality is represented with ASCII characters
quality(fqsample)[1]
```

```
50 GTCCCATTTACCTCTGACTCTTTTGATGCTGCAATTGCTGCTCATATACT
50 ?@@DDDDDHDFDHE>AHFEGFIIEBGDBHH<3FEBEEEEGGIGIIGHGHC
```

```
## PhredQuality instance
pq <- PhredQuality(quality(fqsample))
# transform encoding into scores
qs <- as(pq, "IntegerList")
qs # print scores
```

```
30 31 31 35 35 35 35 35 39 35 37 35 39 36 29 32 39 37 36 38 37 40 40 36 33 38 35 33 39 39 27 18 37 36 33 36
36 36 36 38 38 40 38 40 40 38 39 38 39 34
```

Quality assessment

```
library(ShortRead)
# Quality assessment
qaSummary <- qa(fqsample, lane = 1)    # optional lane
# class: ShortReadQQA(10)
# Names accessible with the quality assessment summary
names(qaSummary)
```

```
 [1] "readCounts"           "baseCalls"            "readQualityScore"          "baseQuality"
 [5] "alignQuality"         "frequentSequences"    "sequenceDistribution"      "perCycle"
 [9] "perTile"              "adapterContamination"
# QA elements are accessed with qa[["name"]]
```

```
# Get a HTML report
browseURL(report(qaSummary))
```

```r
library(ShortRead)
# sequences alphabet
alphabet(sread(fullSample))
```

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

```r
abc <- alphabetByCycle(sread(fullSample))
# Each observation is a letter and each variable is a cycle. First, select the 4 first rows nucleotides A, C, G, T
# Then transpose
nucByCycle <- t(abc[1:4,])
nucByCycle <- nucByCycle %>% as_tibble() %>% # convert to tibble
                      mutate(cycle = 1:50) # add cycle numbers
nucByCycle
```

```
    A     C     G     T cycle
16839 16335 16740 10878     1
13056 13327 12064 22389     2
13666 15617 13198 18355     3
14723 15439 14239 16435     4
```

Are you excited?

Match and filter

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Duplicate sequences

- Biological sequence duplicates occur in nature
- Amplification from the steps in library preparation (PCR)
- Sequencing the sample more than once

Remove duplicates or at least mark them

- Whole genome sequencing or exome sequencing

Mark duplicates using a threshold

- RNA-seq and ChIP-seq

srduplicated

```
library(ShortRead)
# Counting duplicates TRUE is the number of duplicates
table(srduplicated(dfqsample))
```

```
FALSE  TRUE
500   500
```

```
# Cleaning reads from duplicates x[fun(x)]
cleanReads <- mydReads[srduplicated(mydReads) == FALSE]
# Counting duplicates
table(srduplicated(cleanReads))
```

```
FALSE
500
```

Creating your own filters

`srFilter` to filter based on a condition `x[fun(x)]`

Filter example

```
library(ShortRead)
# Use a custom filter to remove reads from fqsample
# This filter to remove reads shorter than a min number of bases
readWidthCutOff <- srFilter(function(x) {width(x) >= minWidth},
                            name = "MinWidth")
minWidth <- 51
fqsample[readWidthCutOff(fqsample)]
```

nFilter

```
library(ShortRead)
# save your filter, .name is optional
myFilter <- nFilter(threshold = 10, .name = "cleanNFilter")
# use the filter at reading point
filtered <- readFastq(dirPath = "data",
                      pattern = ".fastq",
                       filter = myFilter)

# you will retrieve only those reads that have a maximum of 10 N's
filtered
```

idFilter and polynFilter

```r
library(ShortRead)
#id filter example
myFilterID <- idFilter(regex = ":3:1")
# will return only those ids that contain the regular expression

# optional parameters are .name, fixed and exclude
# use the filter at reading point
filtered <- readFastq(dirPath = "data", pattern = ".fastq",
                      filter = myFilterID)
# filter to remove poly-A regions
myFilterPolyA <- polynFilter(threshold = 10, nuc = c("A"))
# will return the sequences that have a maximun number of 10 consecutive A's
# use the filter for subsetting
filtered[myFilterPolyA(filtered)]
```

Let's practice using filters!

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Multiple and parallel sequence quality assessment

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Rqc

```
library(Rqc)
```

- Uses Bioconductor packages that you have already used:
  - `Biostrings` , `IRanges` , `methods` , `S4vectors`
- New packages to discover in the following Bioconductor courses:
  - `Rsamtools` , `GenomicAlignments` , `GenomicFiles` , `BiocParallel`
- CRAN packages:
  - `Knitr` , `dplyr` , `markdown` , `ggplot2` , `digest` , `shiny` , and `Rcpp`

rqcQA

```r
library(Rqc)
files <- # get the full path of the files you want to assess
qaRqc <- rqcQA(files)
# exploring qaRqc
class(qaRqc) # "list"
names(qaRqc) # name of the input files
# for each file
qaRqc[1]
# the class of the results is RqcResultSet
```

rqcQA arguments

```r
library(Rqc)

# get the path of the files you want to assess
files <- "data/seq1.fq" "data/seq2.fq" "data/seq3.fq" "data/se4.fq"

qaRqc <- rqcQA(files, workers = 4)
# sample of sequences
set.seed(1111)

qaRqc_sample <- rqcQA(files, workers = 4, sample = TRUE, n = 500))
# paired-end files
pfiles <- "data/seq_11.fq" "data/seq1_2.fq" "data/seq2_1.fq" "data/seq2_2.fq"

qaRqc_paired <- rqcQA(pfiles, workers = 4, pair = c(1, 1, 2, 2)))
```

rqcReport and rqcResultSet

```
# create a report
reportFile <- rqcReport(qaRqc, templateFile = "myReport.Rmd")
browseURL(reportFile)
#The class of qaRqc is rqcResultSet
methods(class = "RqcResultSet")
```

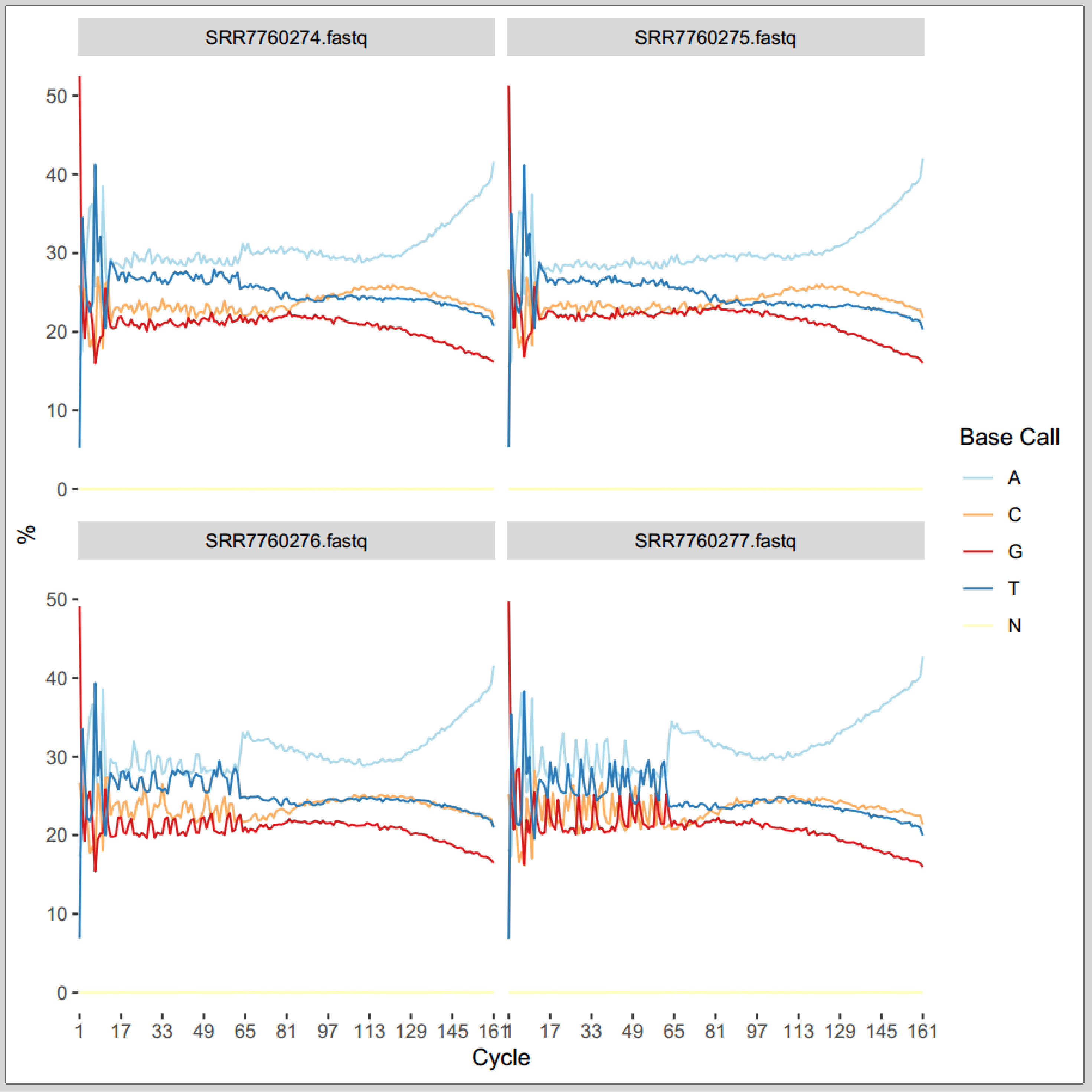
perFileInformation

```
qaRqc <- rqcQA(files, workers = 4))
perFileInformation(qaRqc)
```

```
filename          pair format  group  reads total.reads   path
SRR7760274.fastq  1    FASTQ    None  1e+06     2404795 ./data
SRR7760275.fastq  2    FASTQ    None  1e+06     1508139 ./data
SRR7760276.fastq  3    FASTQ    None  1e+06     1950463 ./data
SRR7760277.fastq  4    FASTQ    None  1e+06     2629588 ./data
```

Plot functions

rqc Plot functions	rqc Plot functions
`rqcCycleAverageQualityPcaPlot()`	`rqcGroupCycleAverageQualityPlot()`
`rqcCycleAverageQualityPlot()`	`rqcReadQualityBoxPlot()`
`rqcCycleBaseCallsLinePlot()`	`rqcReadQualityPlot()`
`rqcCycleBaseCallsPlot()`	`rqcReadWidthPlot()`
`rqcCycleGCPlot()`	`rqcReadFrequencyPlot()`
`rqcCycleQualityBoxPlot()`	`rqcCycleQualityPlot()`


SRR7760274.fastq
SRR7760275.fastq
SRR7760276.fastq
SRR7760277.fastq
%
50
40
30
20
10
0
Cycle
1
17
33
49
65
81
97
113
129
145
161
Base Call
A
C
G
T
N

KEEP
CALM
AND use
parallel quality
assessment

You are ready!

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Congratulations!

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You learned...

- Install packages from Bioconductor by using the `BiocManager` package.
- Techniques for reading, manipulating and filtering raw genomic data using `BioStrings` , `GenomicRanges` and `ShortRead` .
- To work with `BSgenome` and `TxDb` built-in datasets. Then used these to identify patterns by using matching functions.
- Check the quality of sequence files using `ShortRead` and `Rqc` .

You explored

Keep learning!

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