STAT718/BIOL703: Genomic Data Science
Quality Assessment
(Chapter 5 in Gondro's book)

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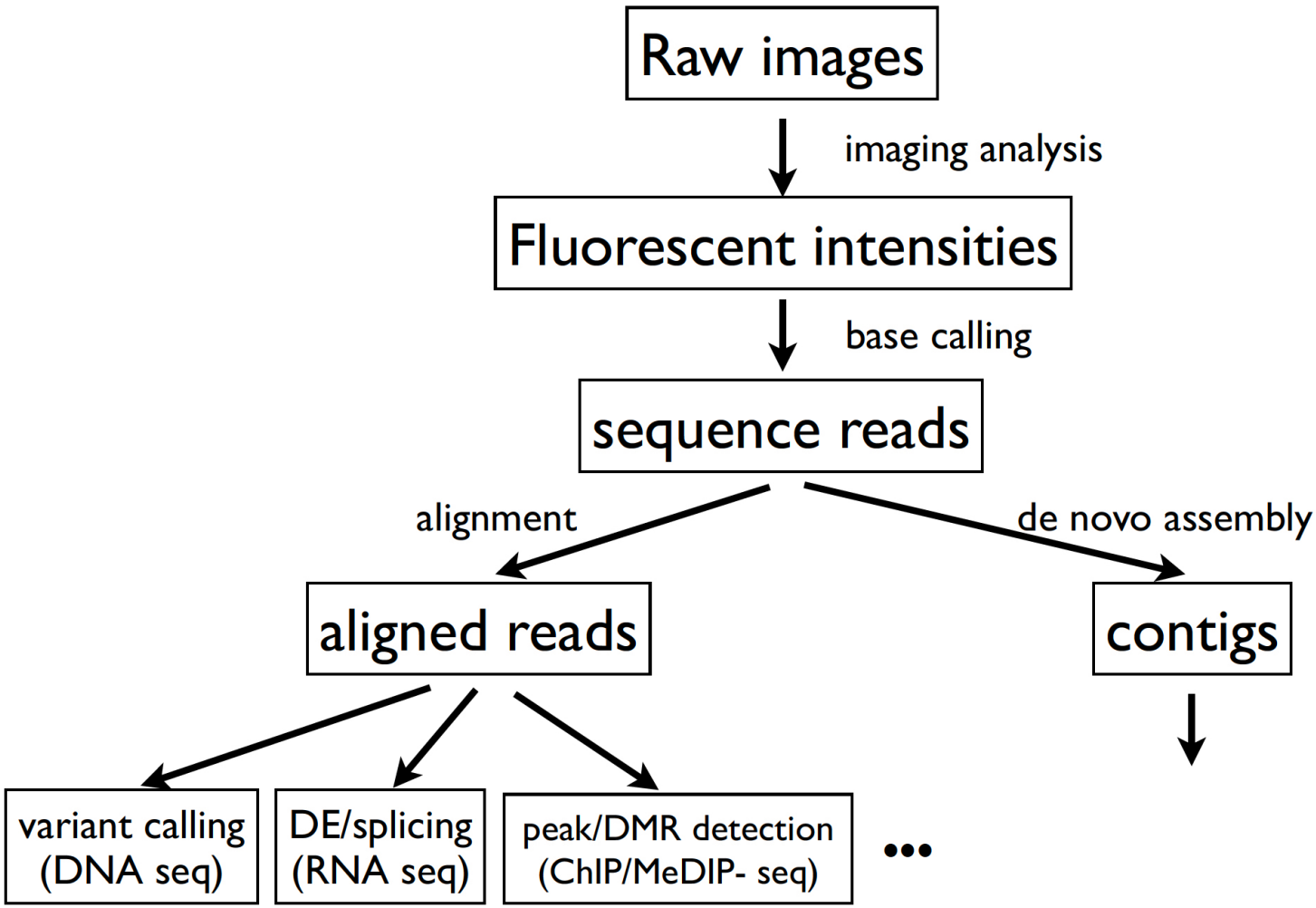
NGS Data Analysis Work Flow



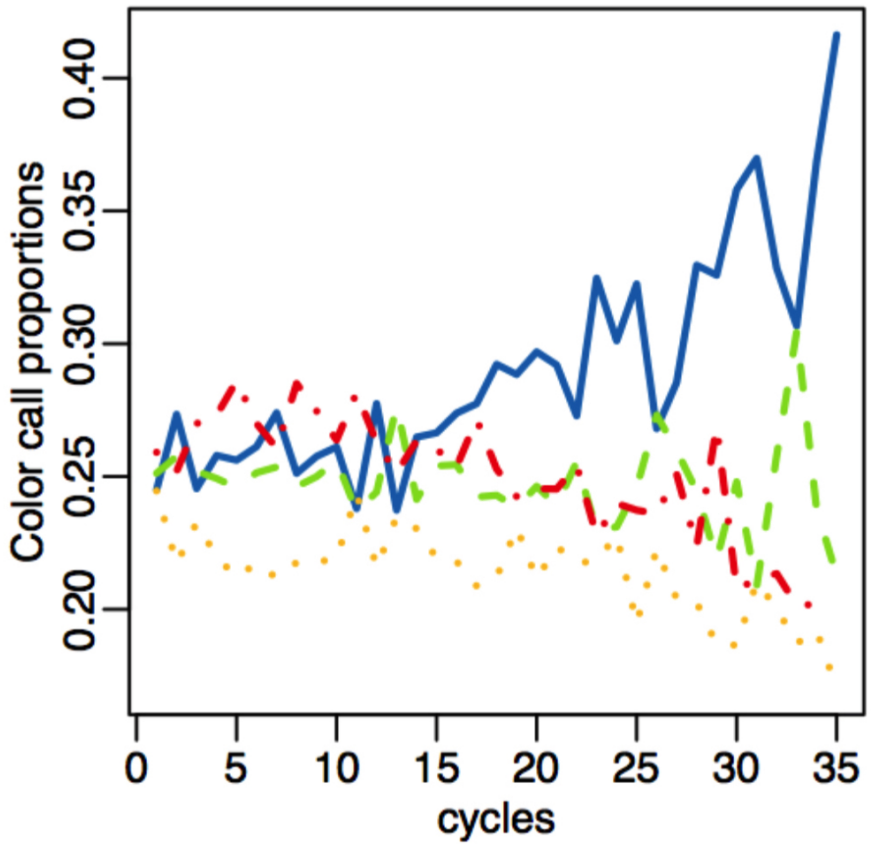
Base Calling

	Cycle1	Cycle2	Cycle3	Cycle4	Cycle5
A	0.05	0.08	0.31	0.41	0.14
C	0.47	0.12	0.28	0.40	0.30
G	0.05	0.43	0.17	0.01	0.39
T	0.42	0.37	0.24	0.18	0.16

CGAAG

Base Calling

Bases called are unbalanced toward the end of the reads.

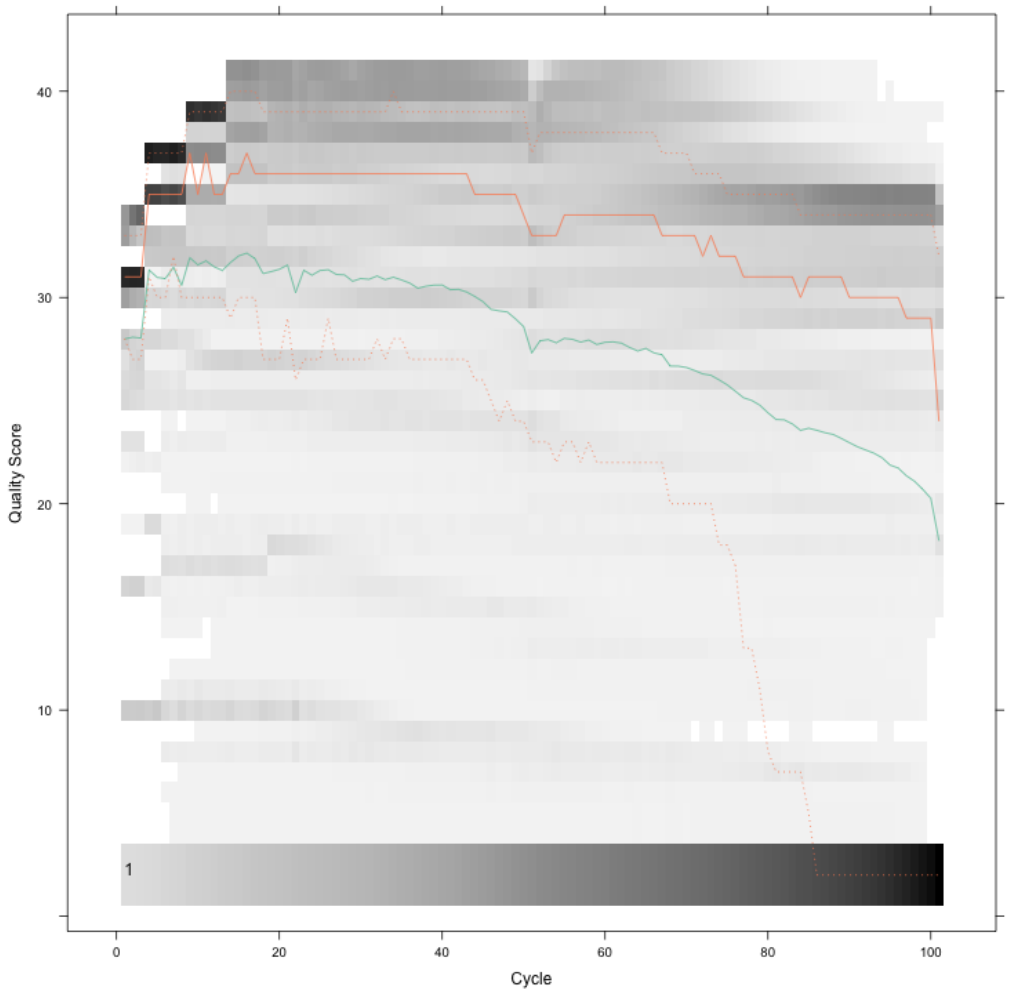


PHRED score

The quality of base called is measured on the PHRED scale, so if there is an estimated probability of an error of p, the PHRED based score is $10 \times -\log_{10} p$.

Phred quality score	Probability that the base is called wrong	Accuracy of the base call
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1,000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%

ShortRead: QCreport



Read quality starts to drop too low (score 30) after 80bp.

FASTA format

The raw sequence reads generated by the sequencer are stored in FASTA or FASTQ format. Format varies a little between platforms.

- first line starts with @ and sequence information
- second line are the raw sequence letters
- third line s usually just a plus symbol (+)
- forth (last) line contains the quality scores (PHRED or other scoring scheme) for each nucleotide in the second line

RNAseq for 1 sample usually requires around 2Gb storage space.

FASTA format

RNAseq.fastq

```
@DFM3XBQ1:197:D0F53ACXX:2:2305:15538:37703 2:N:0:TGTCAC
CCGCGAGCTACAGGCCCAGCTTCAGGAACAGCAGGTCCAGGTGGAGATGGACATGTCGAAACCAGACCTCACAGCTGCCCTCAGGGACATCCGTGCTCAGT
+
CCCFFDFFHHHHHHIGIIIIJJIJIJJGIIGJJJHIBFHGEI8BHEHHIJEGHIIJCEHHHFFFDDEDDDDDDDDDDDCCBBCDDDCBBBDCA?><<@?C:?4
@DFM3XBQ1:197:D0F53ACXX:2:2305:15528:37720 2:N:0:TGTCAC
TTCAAGTTCTGACCCACTTCAAGGTTGCATCTCCAAGGCAGGGTCTTTGATTGGGCTGCCATCAATAAACTGCAGCCATGAGTTCAGACCAGGAAATGGCT
+
@?@DDDBDDFFH>@FFHBDEGAHGEEDH>ABDFAE@@CFHEHJ0?DGIJB?FH@GEEEDFHIHIFIIGHEHACHCDFFBCAC>ACDCDDDDDDDBCDCCDA
@DFM3XBQ1:197:D0F53ACXX:2:2305:15822:37578 2:Y:0:TGTCAC
GTCACATTCGAGTGGCGATACGGGTGCATGACATTCCAGGTCACAATGCGGGCAGTTTTGTCTATGTCCATACGGGGACAAGGAAGTCCTAGACGATAACC
+
##########################################################################################################
@DFM3XBQ1:197:D0F53ACXX:2:2305:15961:37617 2:N:0:TGTCAC
AAAACATGAATCTTAAAAAAAACGAAAAACTGGCTTTCAGACTTTAAAATAAGCCTCCTCGTCCTTCACAGCTATCCTTCAAATATTTTAAGCAGAAAATT
+
CCCFFFFFHGGHHJJJJJJJJJJJJJIJJJJIIJJGIIJGGCEGHIGIJJGGEEHHGHHFFBBCDDDDDDDABCCDDDDCCDDDDDDECDDDDCDCCDDDD@
@DFM3XBQ1:197:D0F53ACXX:2:2305:16032:37567 2:N:0:TGTCAC
GTTTGACAAAGGCTTTTGCCGGGCCATGATCGAGGAAATTGGCCCAGAACTTGAAGGGCAAATCCCAGTGTCTAGAGGGTTACCCATCGTTAAGGTGTTCA
+
;B?:=ABDD:<A+<3C@GCCF3BFAFHIGC4:BBAGIGEHDB3=;FCAF3=C@ECEEF(5??3(6>6;@;;35(5;@2/,(,5@>53988?(<@3(:>@A#
@DFM3XBQ1:197:D0F53ACXX:2:2305:16110:37713 2:N:0:TGTCAC
CAAAAATCCTTGATGACATCTTTGCCTCTTTGGTACAAAATAAGACCTGCTCTGATTTATCAAAGAGGCCCCAAGGACTGACCATCAAGCCCAGCATTCTG
+
BCCFFFFFHGHHHJJIJJJJIJJJJJJJJJJJJJJFHHIIGIJJJJJIJJIIGIIJHIIJGJIIIIJJJJJJJJJGHHHFFFFEEEEEC@CABDDDB?CDDDD
@DFM3XBQ1:197:D0F53ACXX:2:2305:16370:37546 2:Y:0:TGTCAC
CGCACCCATTTCATCACTGCCTCAAATACTGCTTCTTCCTTTTCTACATTTAGGCTGTCATTGGAAATGACTGAGATCAGCTCCTGCGGAGACAGCTGCAT
+
?=1D:)ADFDHFCCC>+A3:CC3AEHFCC>+*?1:?9:<:**:*9?4**:*9BD@3?BB<DGCB<<F@@F78@@77C;DA######################
@DFM3XBQ1:197:D0F53ACXX:2:2305:16438:37550 2:Y:0:TGTCAC
CGAGAAGGTGCTGGCTGCTGTCTACAAGGCTCTGAGTGACCACCACATCTACCTGGAAGGCACCTTGCTGAAGCCCAATATGGTAACCCCAGGACACGCCT
+
?8?DDDFA+AFF<<C?BEHDF<H>DEA?;FB?CC>1C19BBBD@=88*0?/BFFB>@DE@@A;@7.7?;;;)7.;=AD<CC####################
```

Read name
Read sequence
Separator
Quality scores

Read fasta format file into R: ShortRead

```
>library(ShortRead)
>seq2<-readFastq("RNAseq.fastq")
>summary(seq2)
    Length      Class       Mode
    153557 ShortReadQ         S4
>slotNames(seq2)
[1] "quality" "sread"   "id"
>head(sread(seq2))
  A DNAStringSet instance of length 6
    width seq
[1]   101 CCGCGAGCTACAGGCCCAGCTT...CTCAGGGACATCCGTGCTCAGT
[2]   101 TTCAAGTTCTGACCCACTTCAA...GAGTTCAGACCAGGAAATGGCT
[3]   101 GTCACATTCGAGTGGCGATACG...AAGGAAGTCCTAGACGATAACC
[4]   101 AAAACATGAATCTTAAAAAAAA...CAAATATTTTAAGCAGAAAATT
[5]   101 GTTTGACAAAGGCTTTTGCCGG...TTACCCATCGTTAAGGTGTTCA
[6]   101 CAAAAATCCTTGATGACATCTT...GACCATCAAGCCCAGCATTCTG
```

ShortRead: QC Report

```
> head(quality(seq2),3)
class: FastqQuality
quality:
  A BStringSet instance of length 4
    width seq
[1]   101 CCCFFDFFHHHHHHIGIIIIJJIJI...BBCDDDCBBBDCA?><<@?C:?4
[2]   101 @?@DDDBDDFFH>@FFHBDEGAHG...BCAC>ACDCDDDDDDDBCDCCDA
[3]   101 ############################...########################
> head(id(seq2))
  A BStringSet instance of length 6
    width seq
[1]    54 DFM3XBQ1:197:D0F53ACXX:2...5538:37703 2:N:0:TGTCAC
[2]    54 DFM3XBQ1:197:D0F53ACXX:2...5528:37720 2:N:0:TGTCAC
[3]    54 DFM3XBQ1:197:D0F53ACXX:2...5822:37578 2:Y:0:TGTCAC
[4]    54 DFM3XBQ1:197:D0F53ACXX:2...5961:37617 2:N:0:TGTCAC
[5]    54 DFM3XBQ1:197:D0F53ACXX:2...6032:37567 2:N:0:TGTCAC
[6]    54 DFM3XBQ1:197:D0F53ACXX:2...6110:37713 2:N:0:TGTCAC
> encoding(quality(seq2))
 !  "  #  $  %  &  '  (  )  *  +  ,  -  .  /  0  1  2  3  4
 0  1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19
 5  6  7  8  9  :  ;  <  =  >  ?  @  A  B  C  D  E  F  G  H
20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39
 I  J
40 41
```

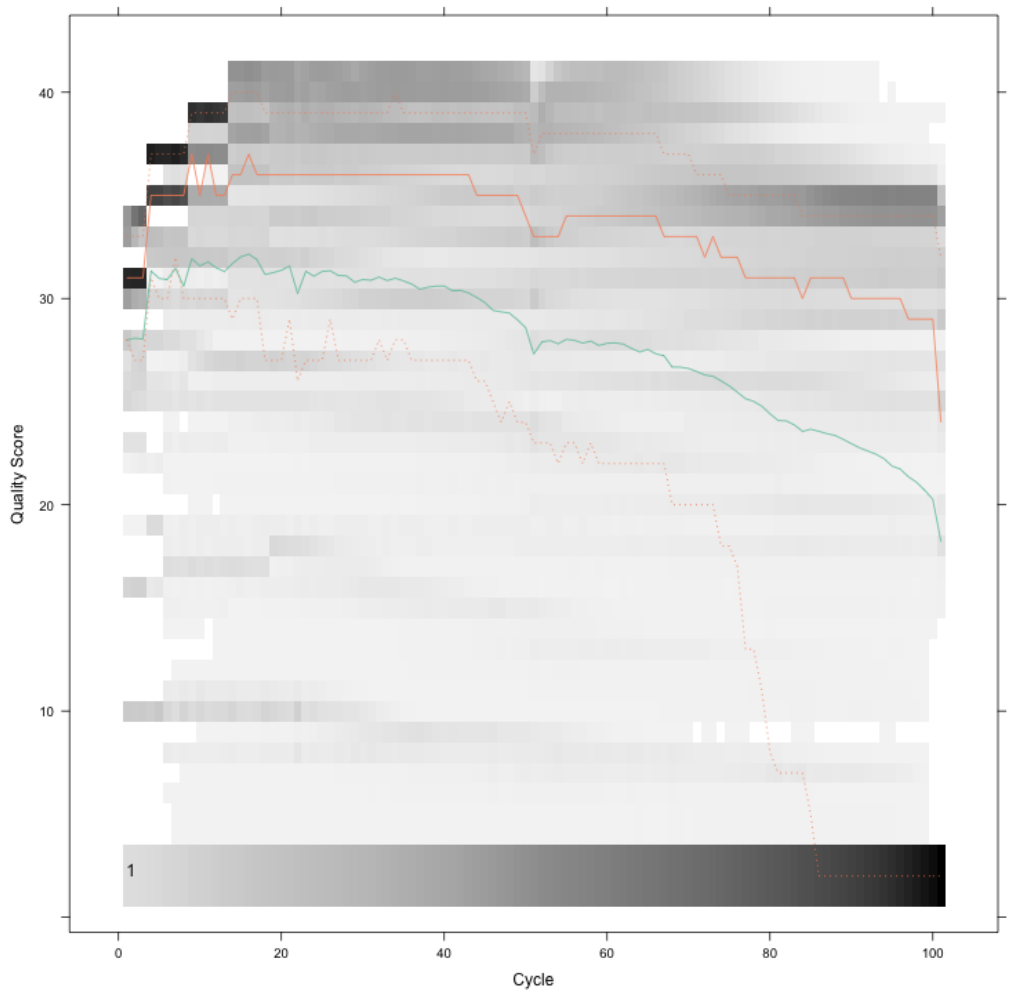
ShortRead: QC Report

```
> tbls<-tables(seq2)
> names(tbls)
[1] "top"          "distribution"
> tbls$top[1:5]
> tbls$distribution[1:3,]
  nOccurrences nReads
1            1 128065
2            2   4256
3            3   1244
> seq2
class: ShortReadQ
length: 153557 reads; width: 101 cycles
> sum(tbls$distribution[,1]*tbls$distribution[,2])
[1] 153557
```

ShortRead: QC Report

```
>seqQC<-qa("RNAseq.fastq")
> report(seqQC, dest="/Users/yen-yiho/Desktop/BIOL599/
Notes/LectureRNAseq1/index.html")
[1] "/Users/yen-yiho/Desktop/BIOL599/Notes/LectureRNAseq1/index.html/index.html
```

ShortRead: QCreport



Read quality starts to drop too low (score 30) after 80bp.

QuasR

The function **preprocessRead** in R package *QuasR* can be used to prepare the input sequences before alignment to the reference genome.

- **Truncate reads**: remove nucleotides from the start and/or end of each read.
- **Trim adapters**: remove nucleotide at the beginning and/or end of each read that match to a defined (adapter) sequence.
- **Filter out low quality reads**: filter out reads that contain more than *nBases* N bases, shorter than *minLength* or low complexity sequence.

Another popular command line tools is **Trimmomatic**.