Quality assessment of NGS data

Ines de Santiago

CRUK Cambridge Research Institute

Ines.desantiago@cruk.cam.ac.uk

Quality control analysis

All sequencing platform have errors

Quality control

- It is important to check the quality of your sequenced reads!

- FASTQC: free program that reports quality profile of reads

- Pre-processing
  - Trim reads
  - exclude low quality reads
  - contaminations

```
Sequencing
    ↓
Quality control
    ↓
Data cleaning
(pre-processing)
    ↓
Quality control
    ↓
Mapping
```

Checking read quality with FASTQC

http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

1. Run FASQC

fastqc sample.fastq

2. Open output file

sample_fastq.html

Summary

- ✅ Basic Statistics
- ❌ Per base sequence quality
- ⚠️ Per tile sequence quality
- ✅ Per sequence quality scores
- ❌ Per base sequence content
- ✅ Per sequence GC content
- ✅ Per base N content
- ✅ Sequence Length Distribution
- ✅ Sequence Duplication Levels
- ❌ Overrepresented sequences
- ✅ Adapter Content
- ❌ Kmer Content

FASTQC: Report

1) Basic statistics
2) Per base sequence quality
3) Per tile sequence quality
4) Per sequence quality scores
5) Per base sequence content
6) Per sequence GC content
7) Per base N content
8) Sequence Length Distribution
9) Sequence duplication levels
10) Over-represented sequences
11) Adapter/Kmer content

Basic Statistics

Measure	Value
Filename	sample.fastq
File type	Conventional base calls
Encoding	Illumina 1.5
Total Sequences	9053
Sequences flagged as poor quality	0
Sequence length	36
%GC	50

(2) FASTQC: Per base sequence quality

- Poor quality at the end of reads

Quality scores across all bases

Position in read
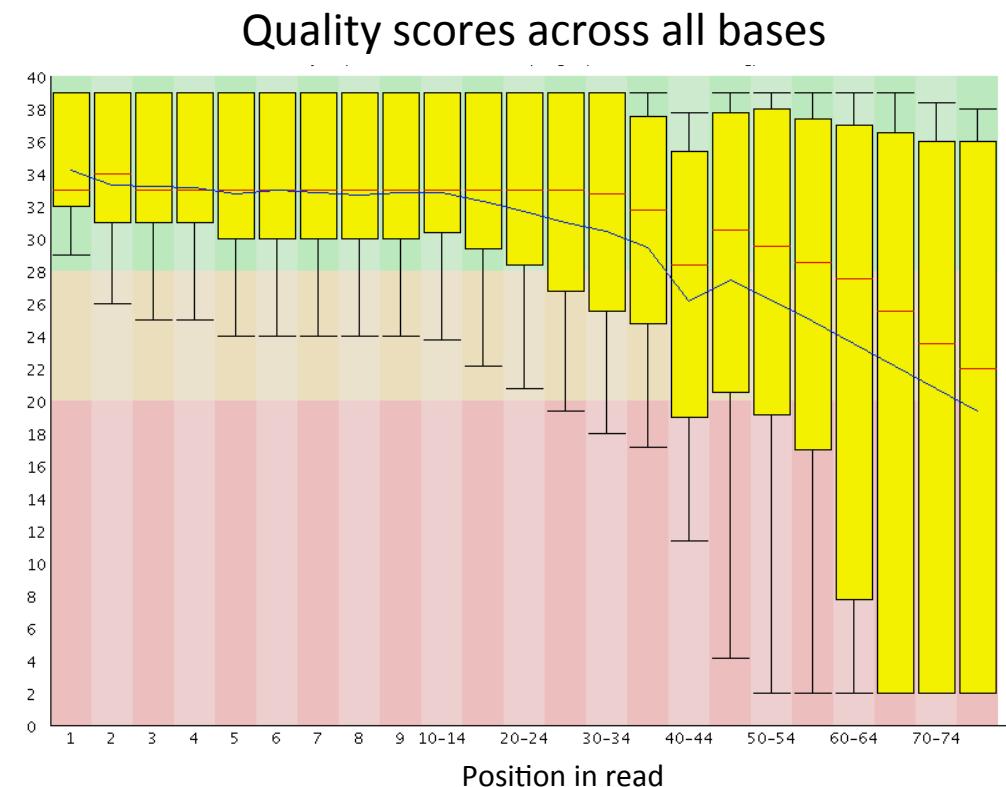
(2) FASTQC: Per base sequence quality
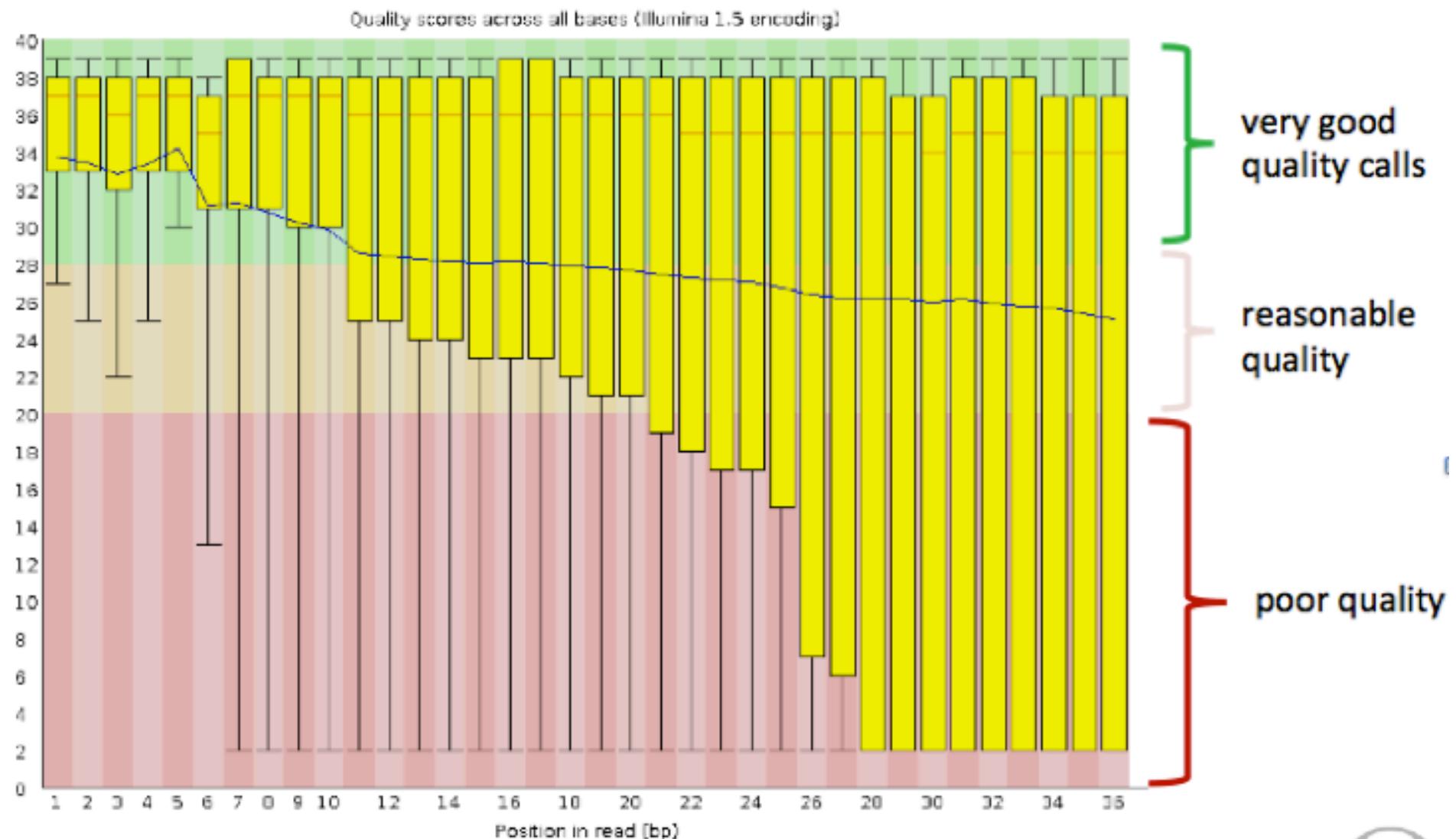
(2) FASTQC: Per base sequence quality

Good Illumina data:

(3) FASTQC: Per tile sequence quality

(3) FASTQC: Per tile sequence quality

Overclustering:

Quality per tile

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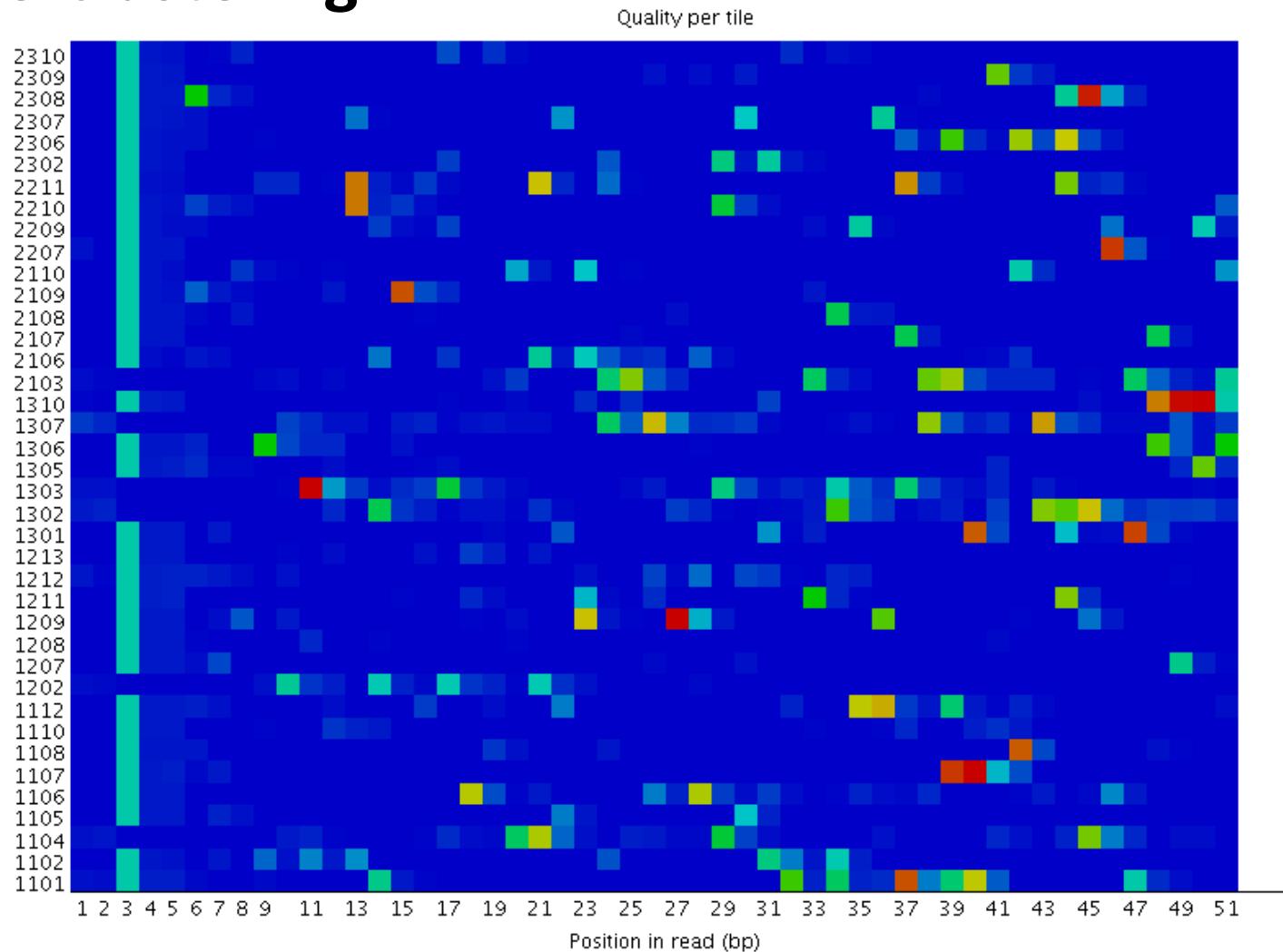
# (3) FASTQC: Per tile sequence quality

Tile fail:

SRR576938
anaerobic INPUT DNA
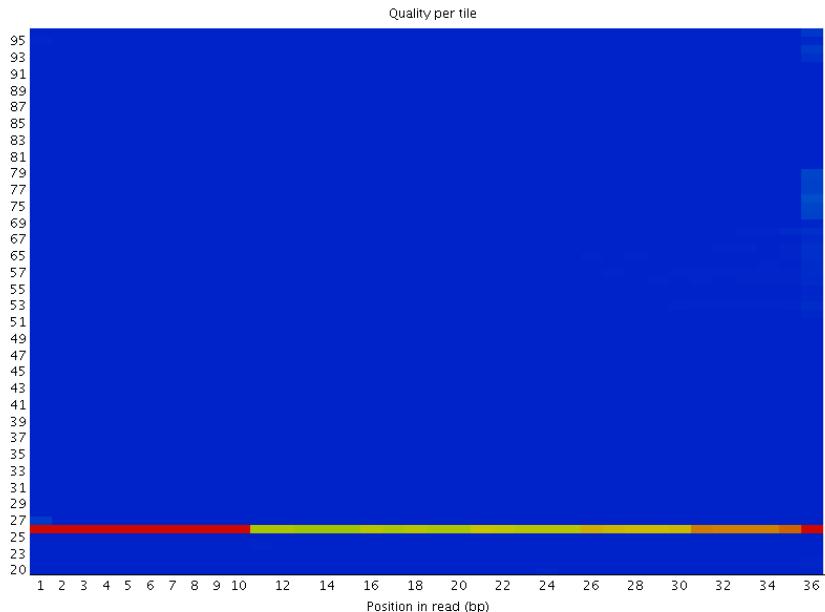
SRR576933
FNR IP ChIP-seq Anaerobic A
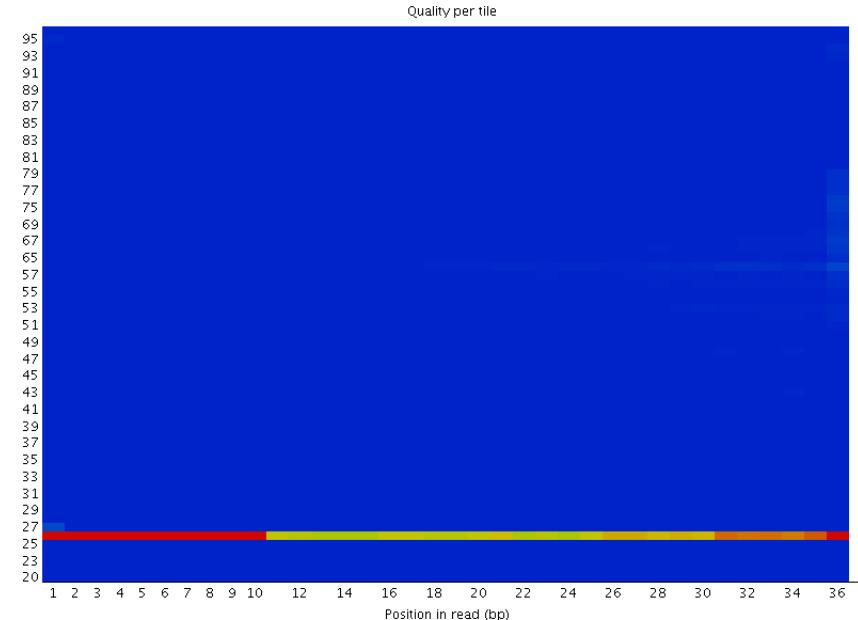
GSE41187: Genome-wide analysis of FNR and s70 in E. coli under aerobic and anaerobic growth conditions: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE41187

(4) FASTQC: Per sequence quality scores

http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
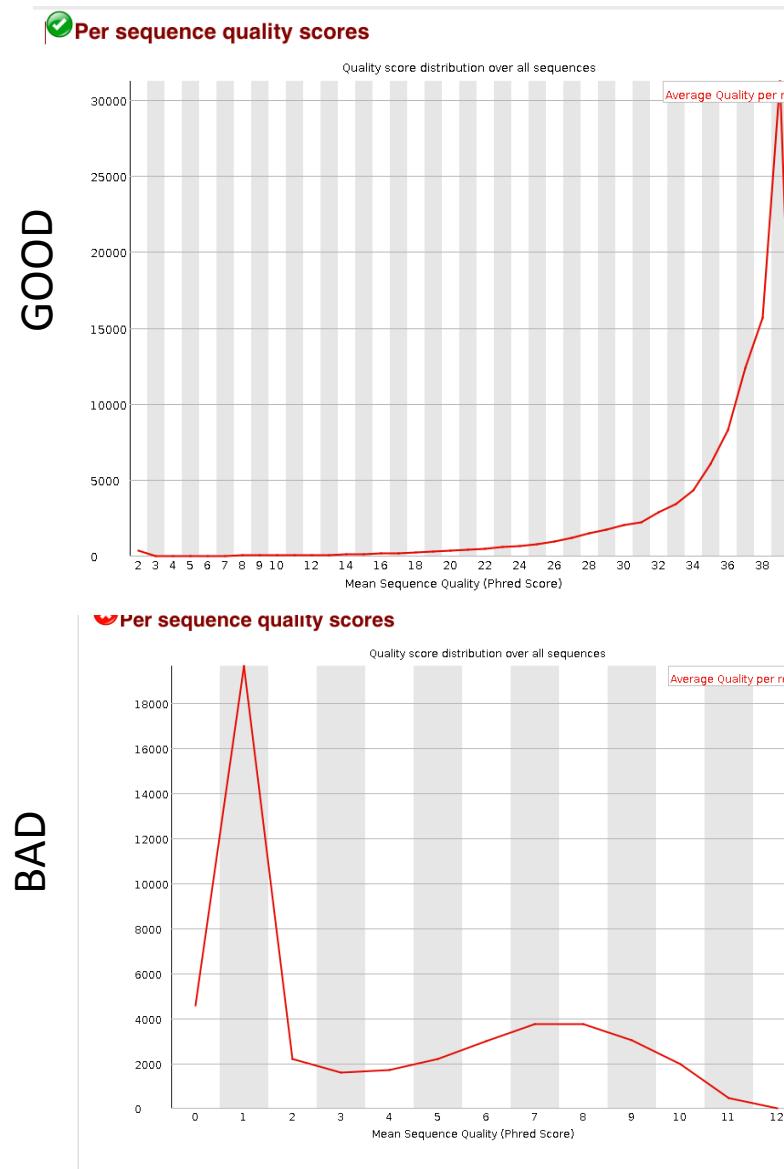
(5) FASTQC: Per base sequence content

http://bio-hpc.kisti.re.kr/MDS_03_normal_chr21.1.fq_fastqc/fastqc_report.html#M3

(5) FASTQC: Per base sequence content

Biased sequence composition (adapters?)
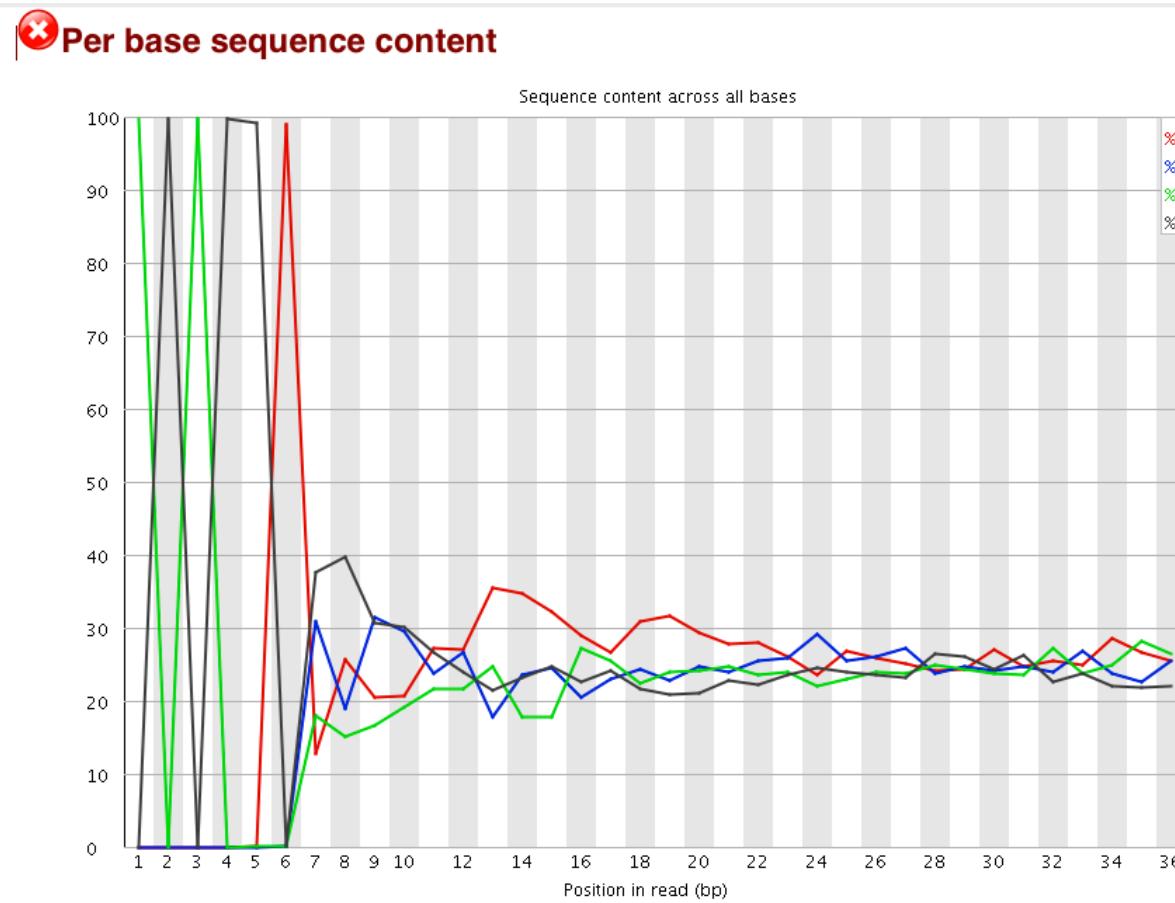
(5) FASTQC: Per base sequence content

Unavoidable – RNA-Seq
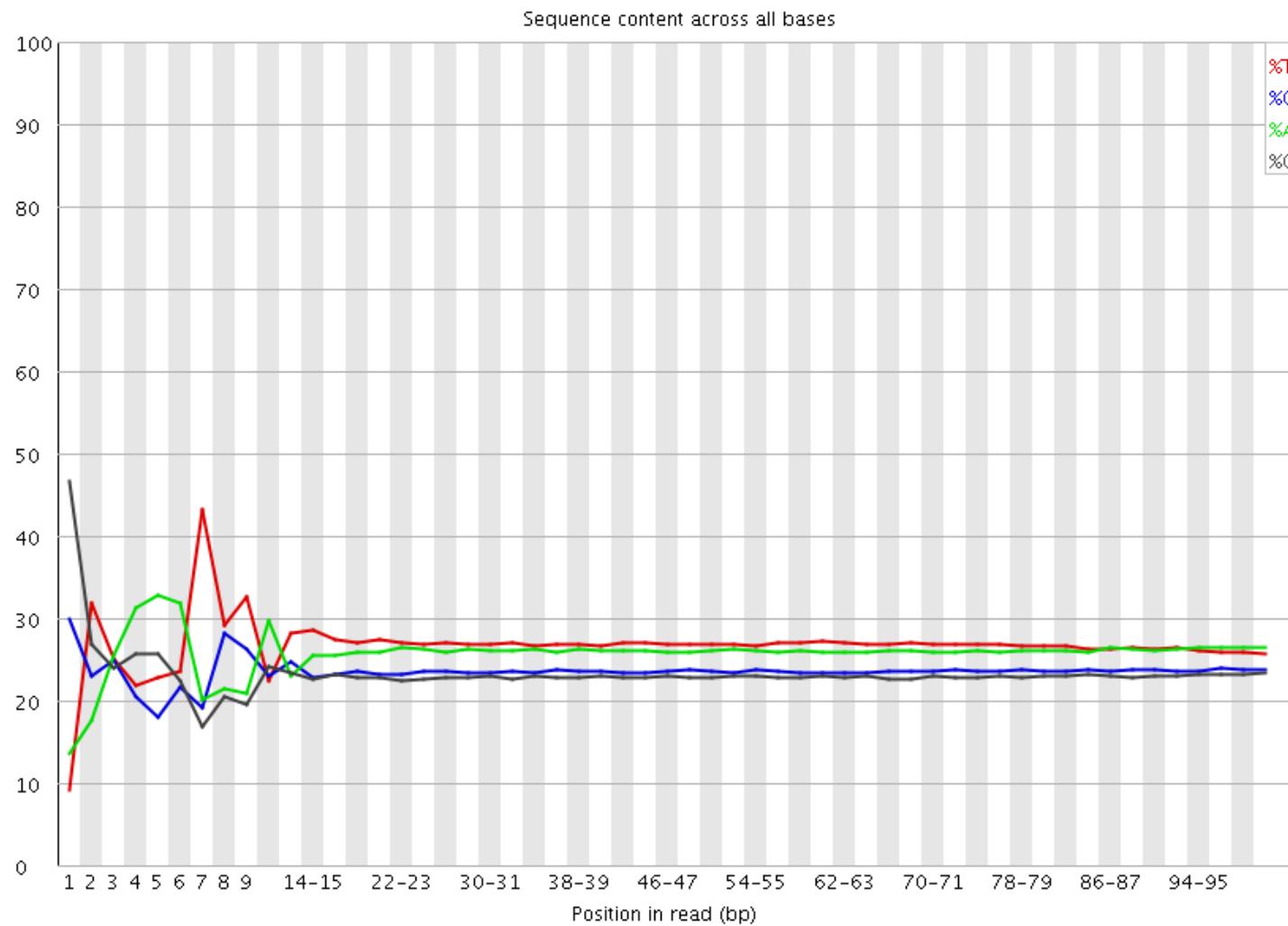
Simon Andrews

(5) FASTQC: Per base sequence content

Unavoidable – RRBS

Devoided of cytosines because the library was treated with sodium bisulphite (which will have converted most of the C to T)
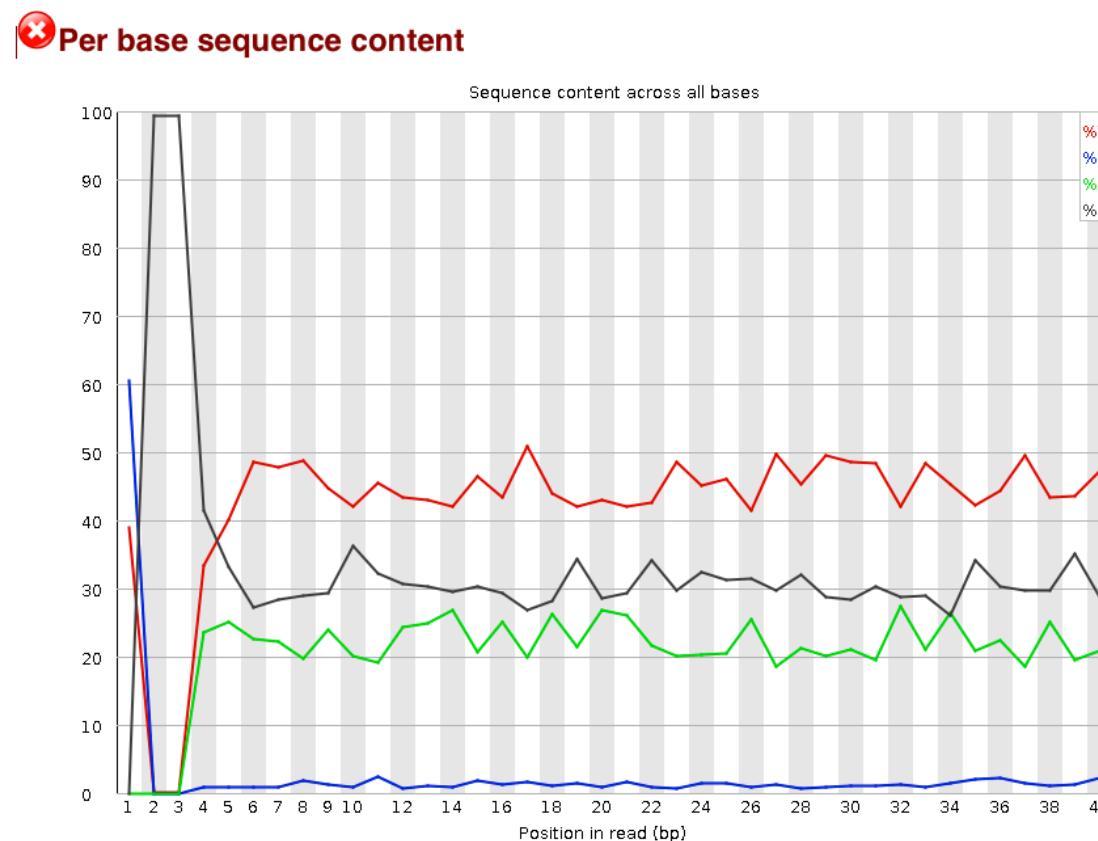
(6) FASTQC: Per sequence GC content

http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
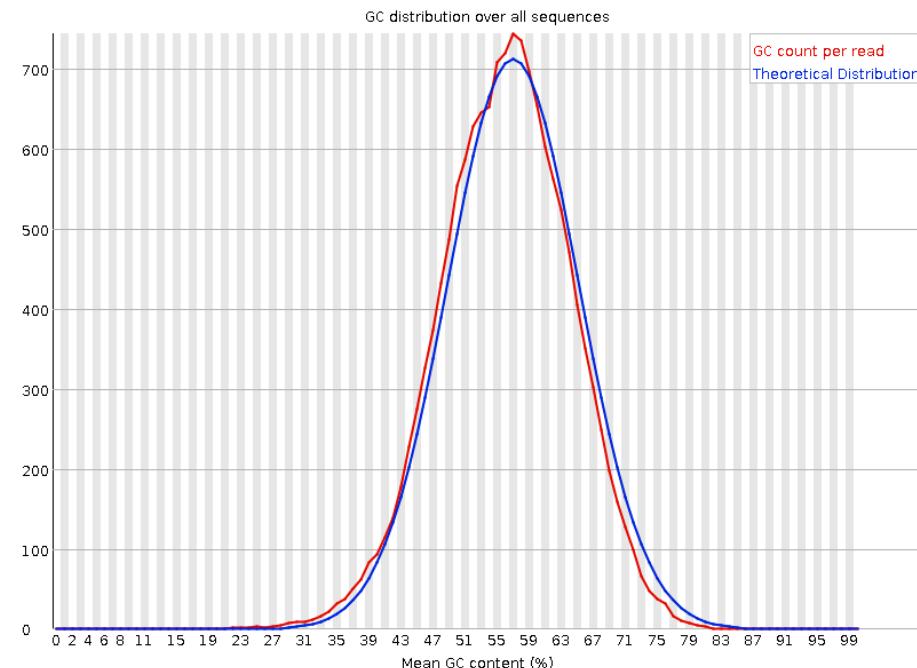
(7) FASTQC: Per base N content
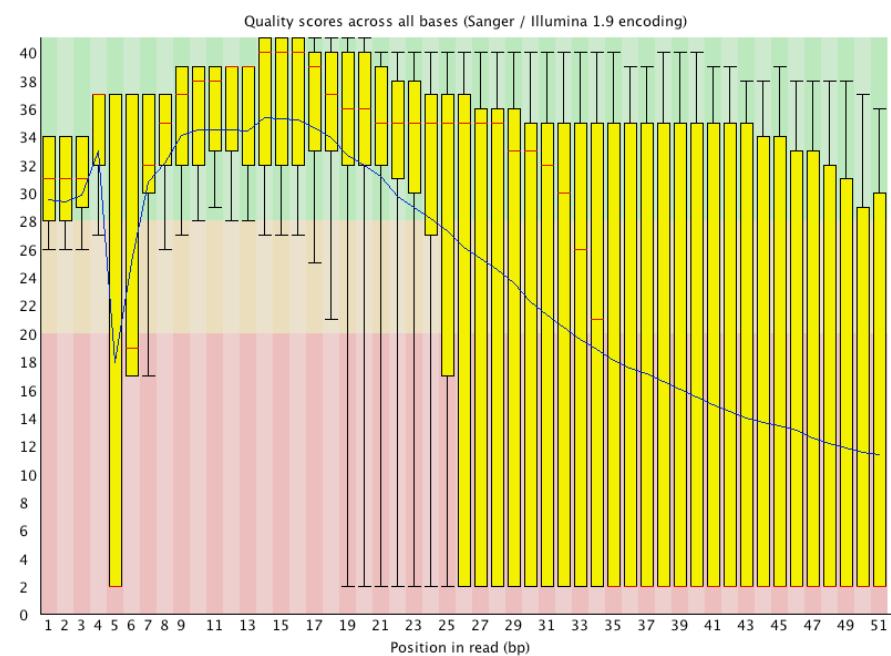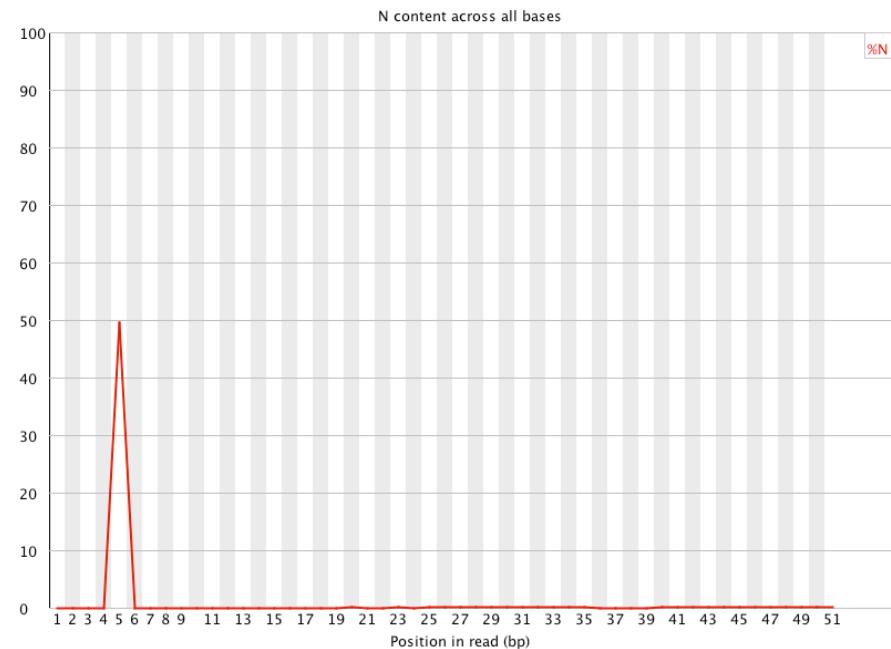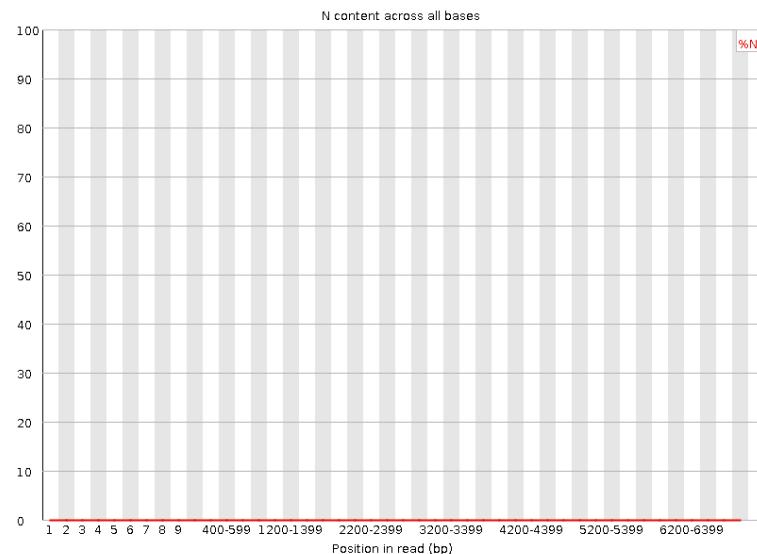
(8) FASTQC: Sequence Length Distribution

Summary

- ✅ Basic Statistics
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- ❌ Overrepresented sequences
- ❌ Kmer Content

Sequence fragments of uniform length (36bp)
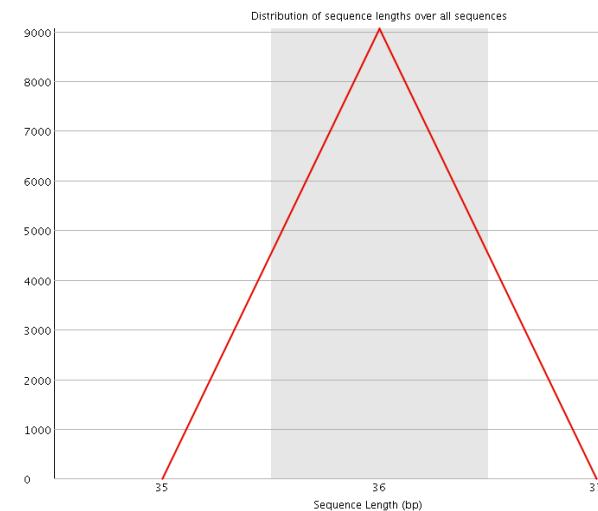
Reads of variable length:
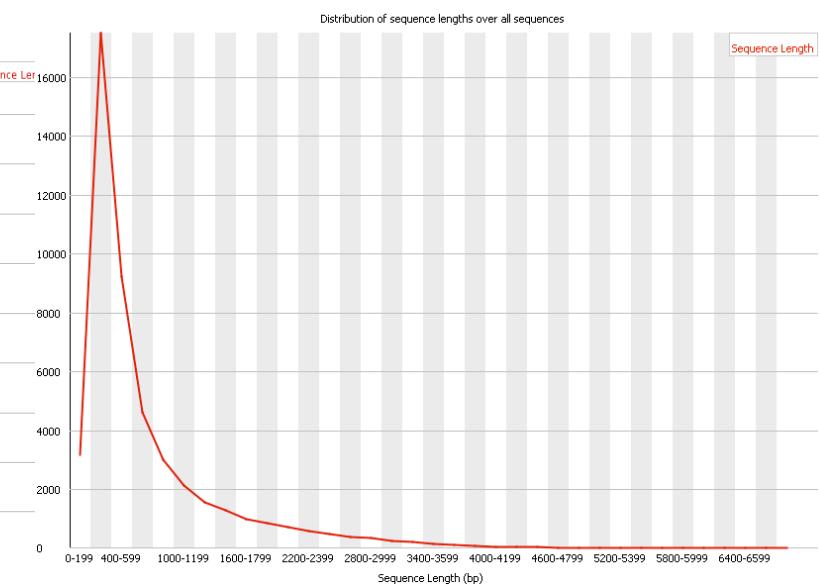
http://cbio.mskcc.org/~lianos/files/scott/2011-11-21/qc/Bcnr2_ATCACG_L001_R1_001_fastqc/fastqc_report.html#M2

(9) FASTQC: Sequence duplication levels

- PCR duplicates during sample preparation
- Optical duplicates: read the same cluster twice in the sequencer
- High duplication can lead to problems in downstream analysis (e.g. skew allele frequencies)
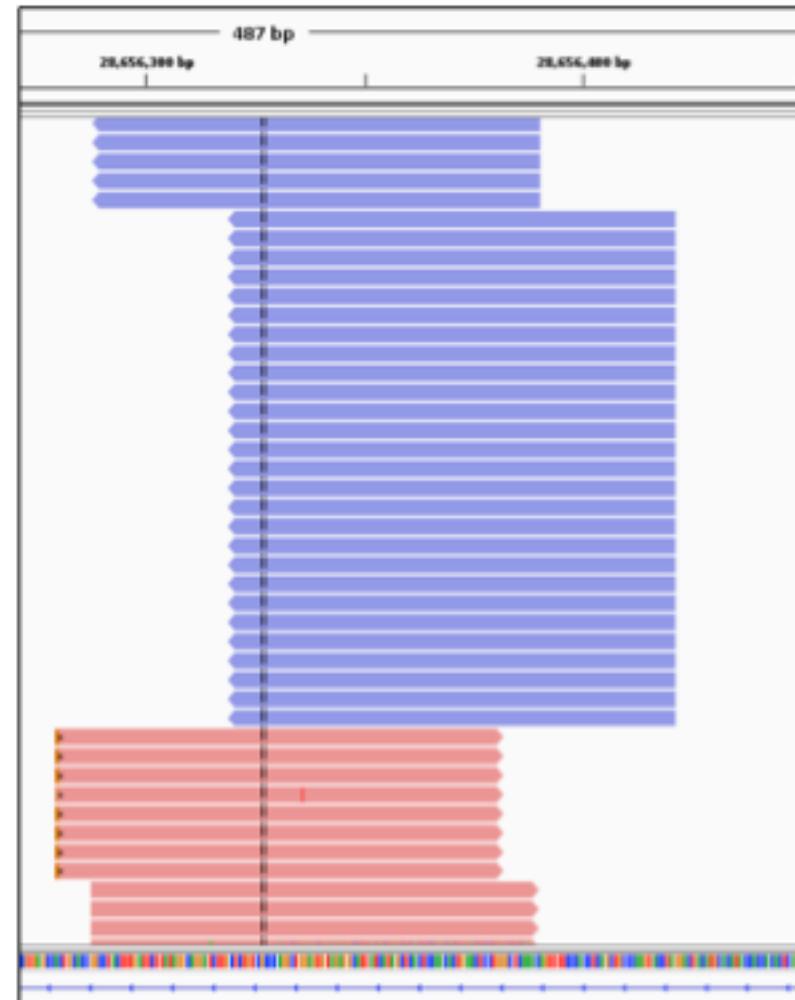
(9) FASTQC: Sequence duplication levels

Very diverse library
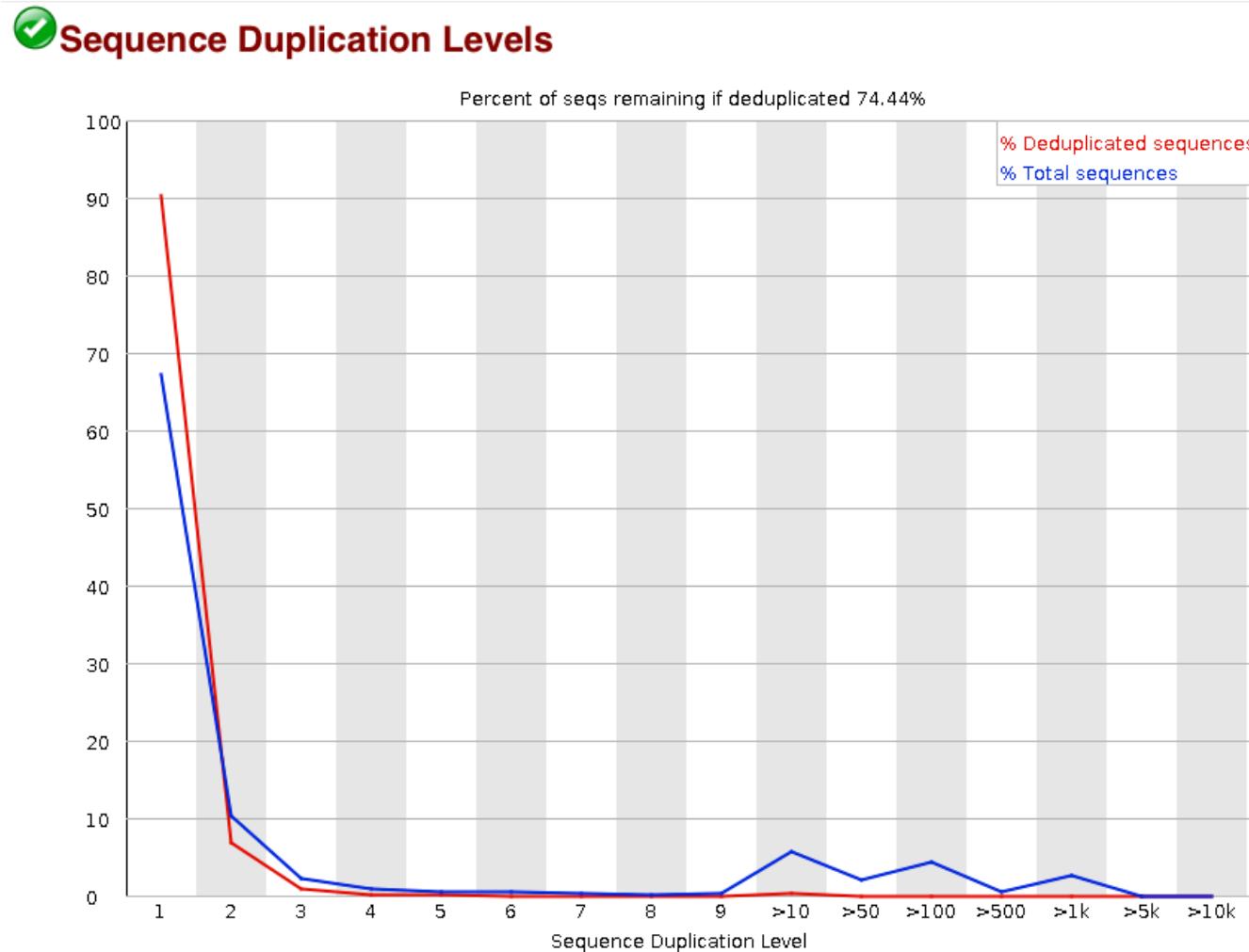
(9) FASTQC: Sequence duplication levels

A good RNA-Seq library (although dup levels > 50%)
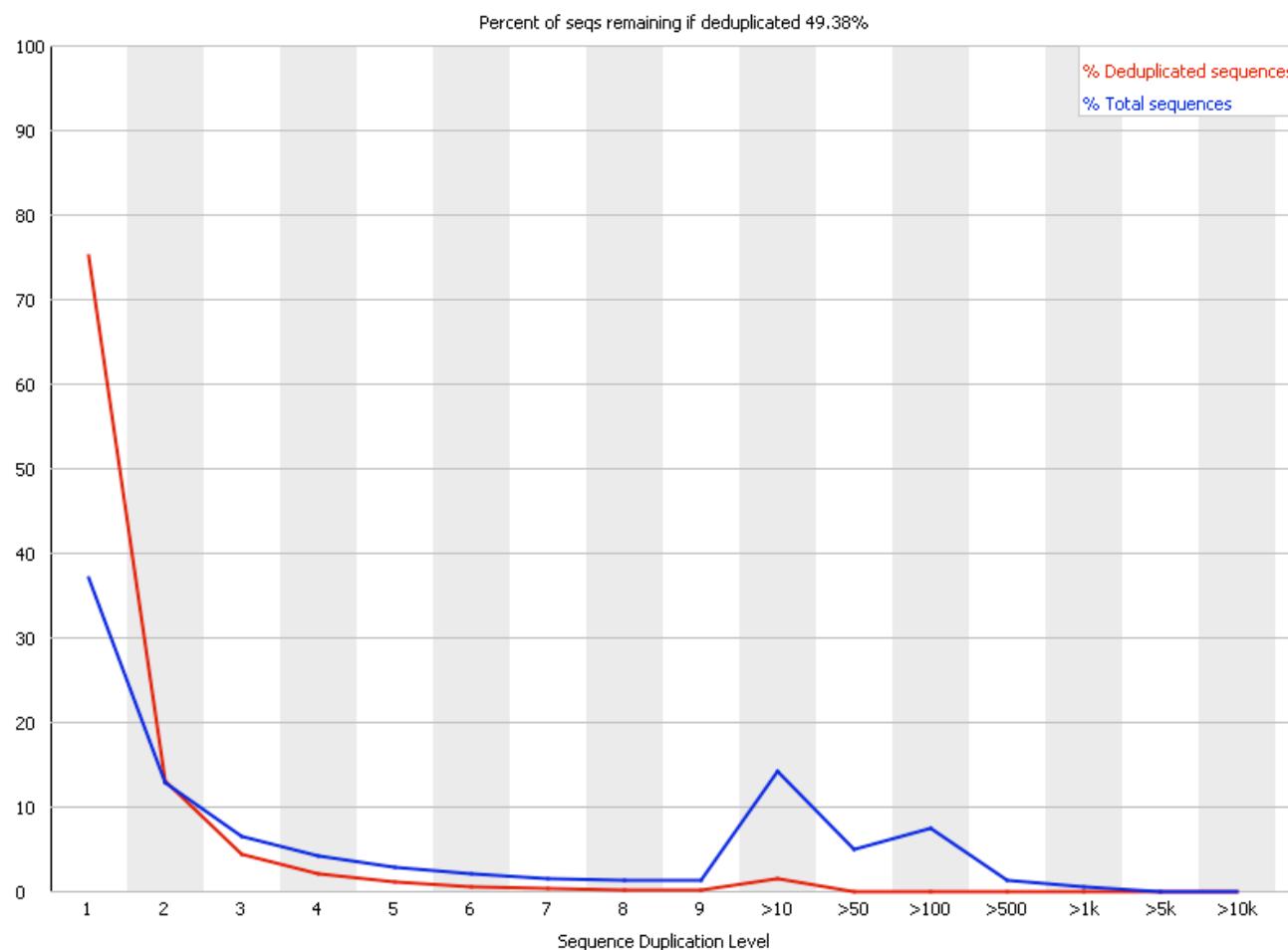
(9) FASTQC: Sequence duplication levels

PCR duplication
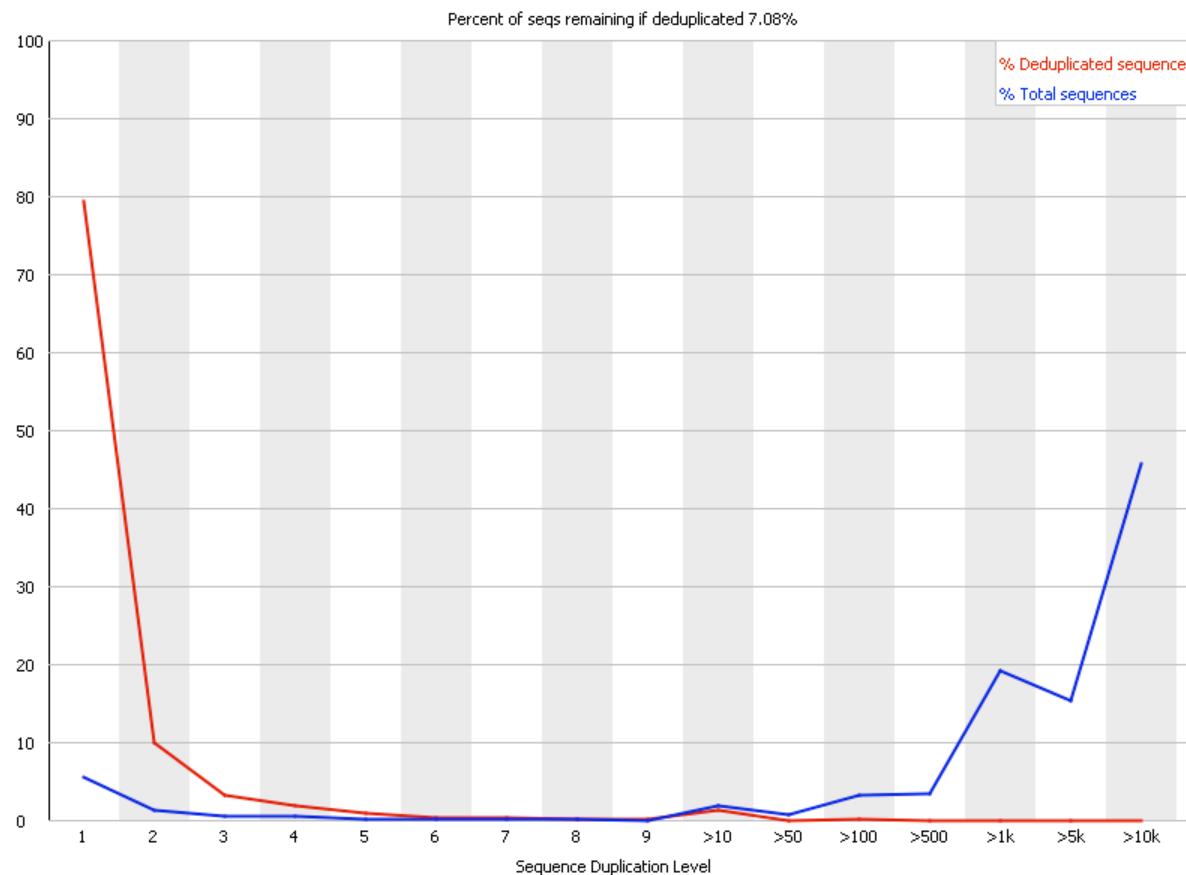
(10) FASTQC: Over-represented sequences

Good dataset

✅ Overrepresented sequences

No overrepresented sequences

Bad datasets:

## ⚠ Overrepresented sequences

Sequence	Count	Percentage	Possible Source
AGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGA	23247	0.13860048153338028	No Hit
AGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAG	19048	0.1135657062093099	No Hit
GAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGA	18343	0.10936243957357056	No Hit
AAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAG	17345	0.10341228339985724	No Hit

Back to summary

❌ Overrepresented sequences

Sequence	Count	Percentage	Possible Source
GATCGGAAGAGCACACGTCTGAACTCCAGTCACACA	28971	28.971000000000004	TruSeq Adapter, Index 5 (100% over 36bp)
GCTAACAAATACCCGACTAAATCAGTCAAGTAAATA	392	0.392	No Hit
GTTAGCTATTTACTTGACTGATTTAGTCGGGTATTT	356	0.356	No Hit
GATCGGAAGAGCACACGTCTGAACTCCAGTCACACC	108	0.108	TruSeq Adapter, Index 1 (97% over 36bp)
GATCGGAAGAGCACACGTCTGAACTCCAGTCACACG	107	0.107	TruSeq Adapter, Index 15 (97% over 36bp)

# (11) FASTQC: Kmer content

Good Illumina dataset:
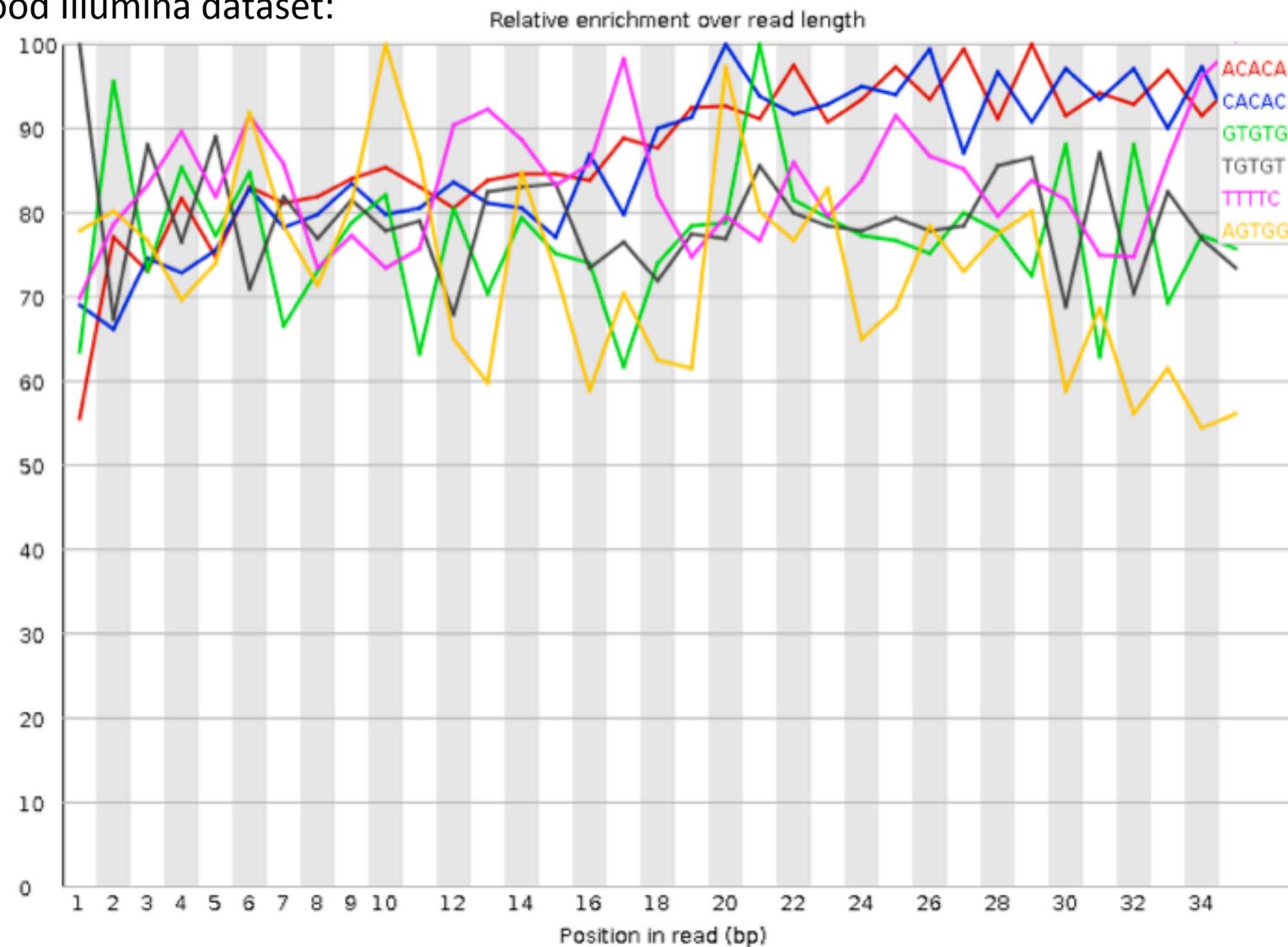
(11) FASTQC: Kmer content
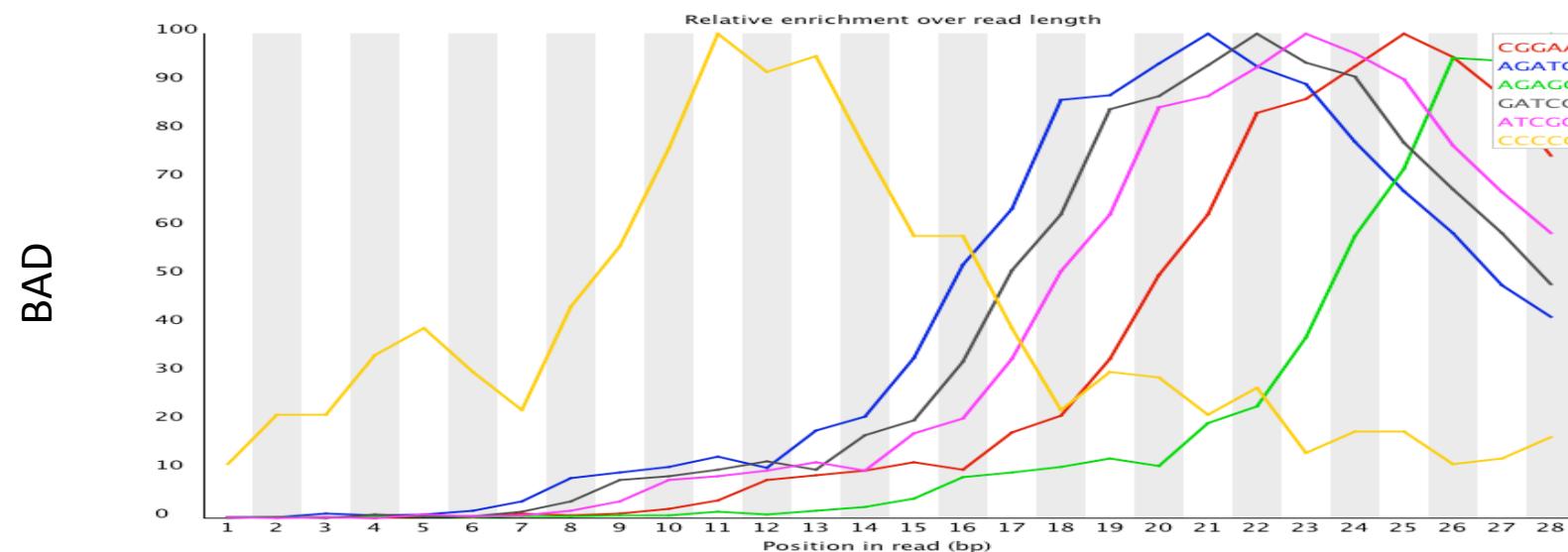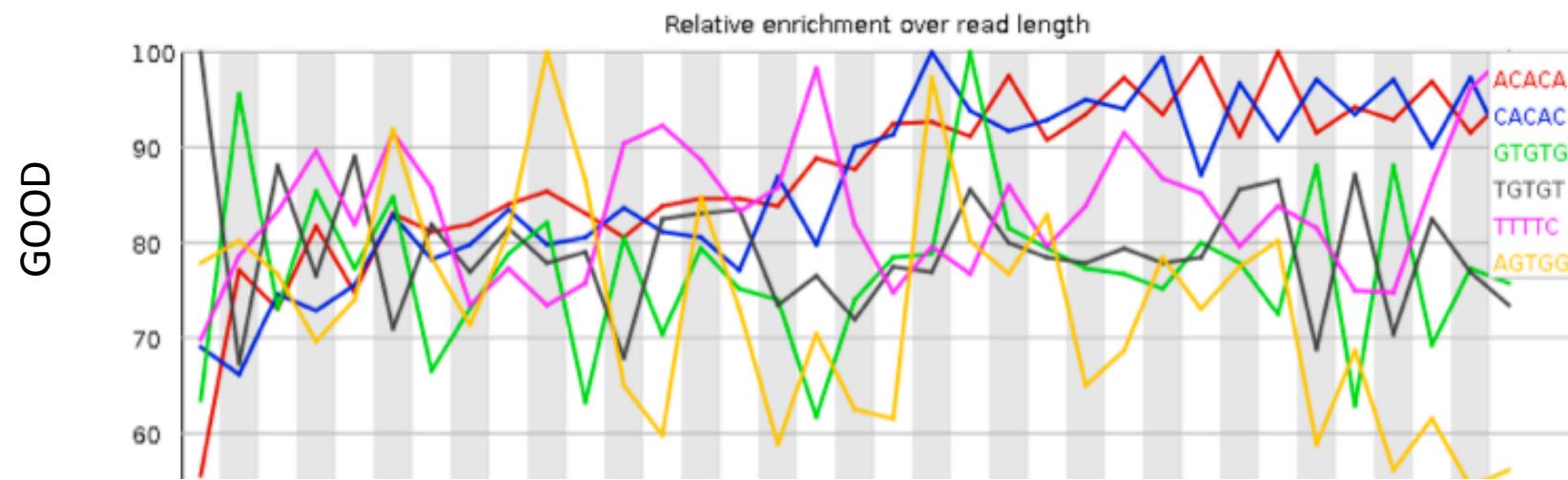
(11) FASTQC: Kmer content

AAAA k-mer that you're seeing at around 21 base pairs are arrested transcripts caused by cyclohexamide treatment.
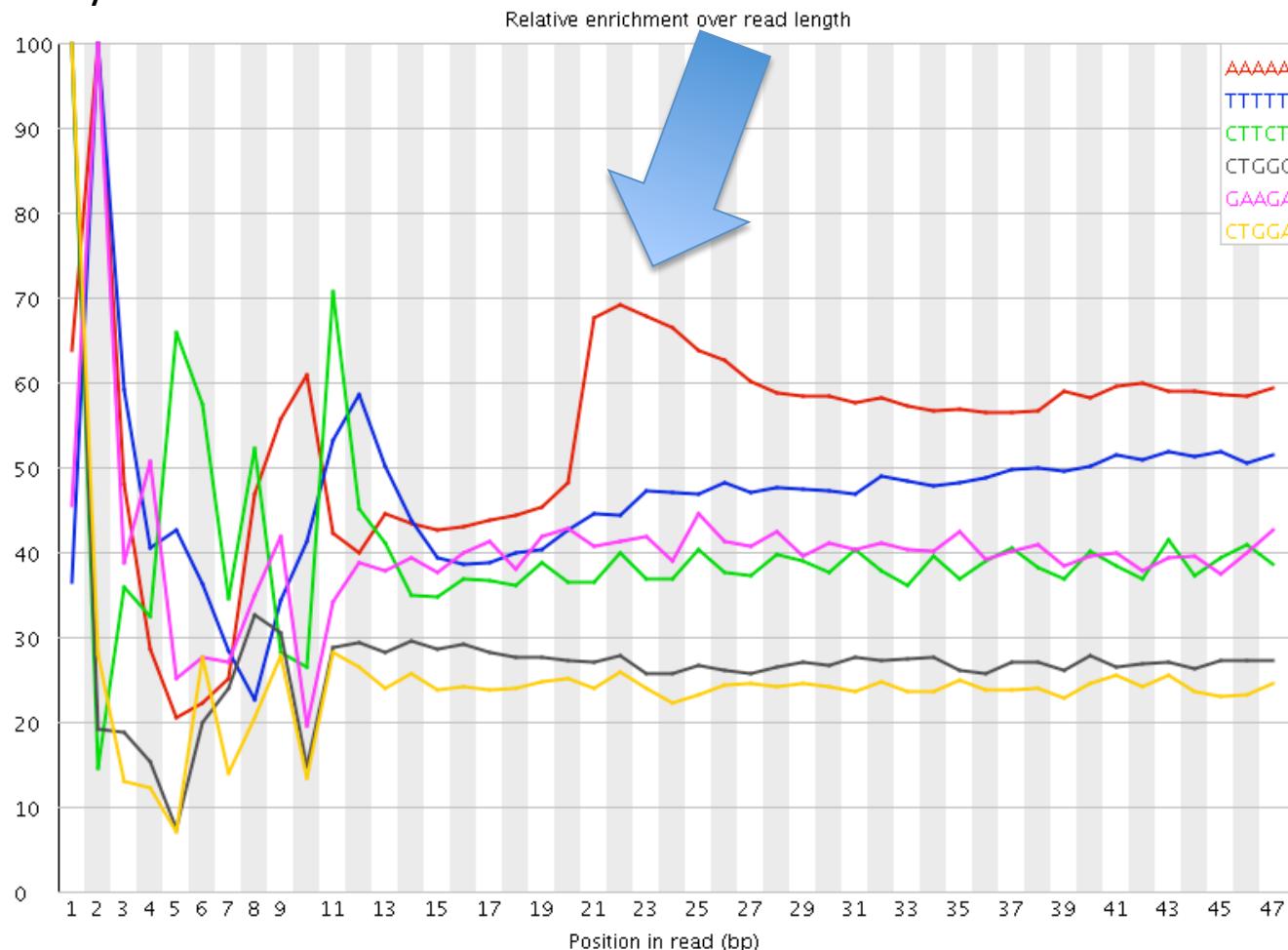
(11) FASTQC: Kmer content
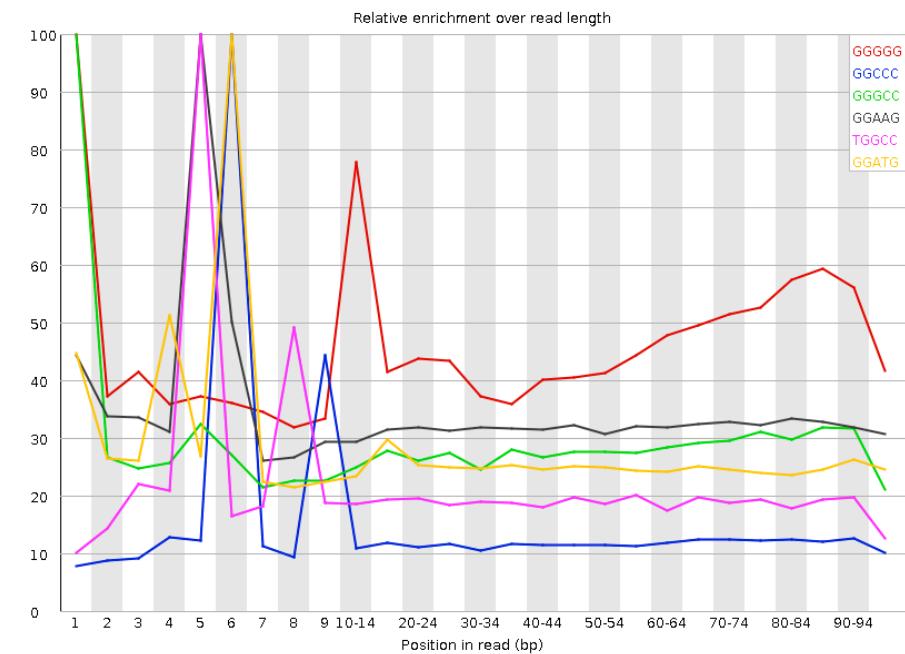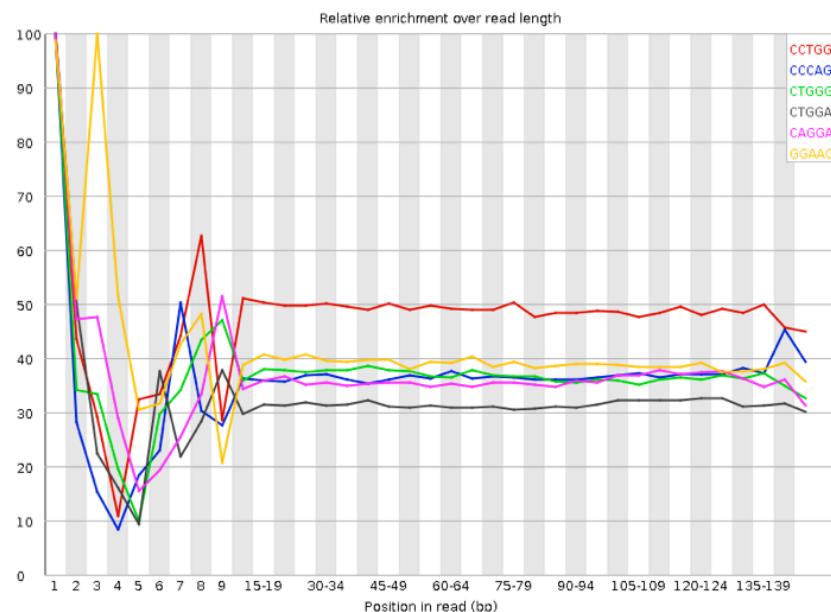
"Random" hexamer primer in RNA-seq libraries
(not that random after all)

(11) FASTQC: Kmer content

"Random" hexamer primer in RNA-seq libraries
(not that random afterall)
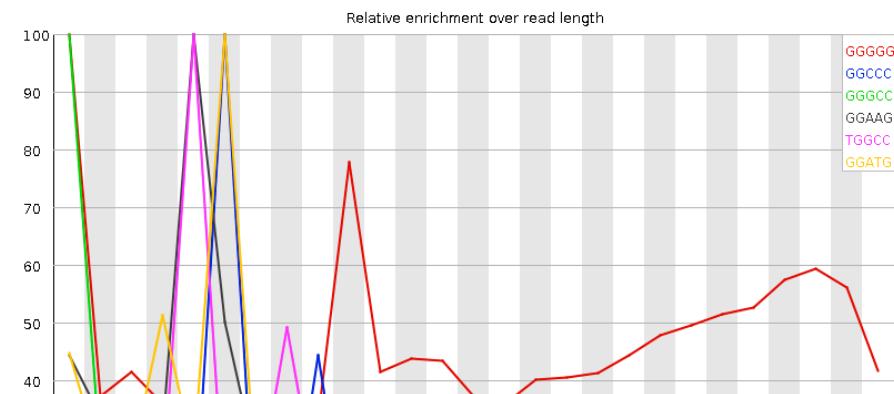
Biases in Illumina transcriptome sequencing caused by random hexamer priming

Kasper D. Hansen[1,*], Steven E. Brenner[2] and Sandrine Dudoit[1,3]

[1]Division of Biostatistics, School of Public Health, UC Berkeley, 101 Haviland Hall, Berkeley, CA 94720-7358, [2]Department of Plant and Microbial Biology, UC Berkeley, 461 Koshland Hall, Berkeley, CA 94720-3102 and [3]Department of Statistics, UC Berkeley, 367 Evans Hall, Berkeley, CA 94720-3860, USA

Hands on exercise:

Fastqc_sweave.pdf

Examples of FASTQC runs and preprocessing