Best practices in the analysis of RNA-seq and ChIP-seq data

27th – 31st, July 2015

University of Cambridge, Cambridge, UK

Quality assessment of NGS data

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Quality control analysis

All sequencing platform have errors

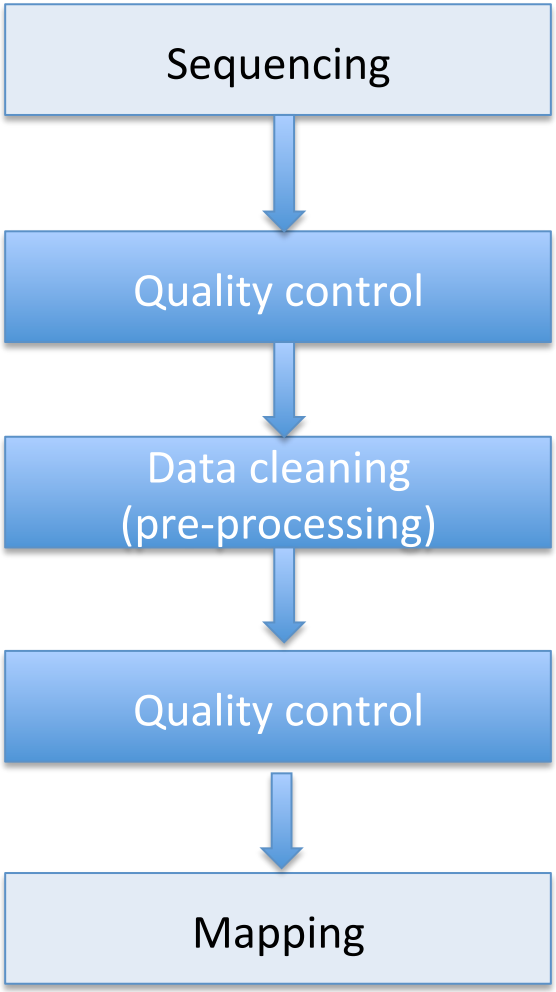
http://rnaseq.agbioinfo.utk.edu/index.php/

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



Checking read quality with FASTQC

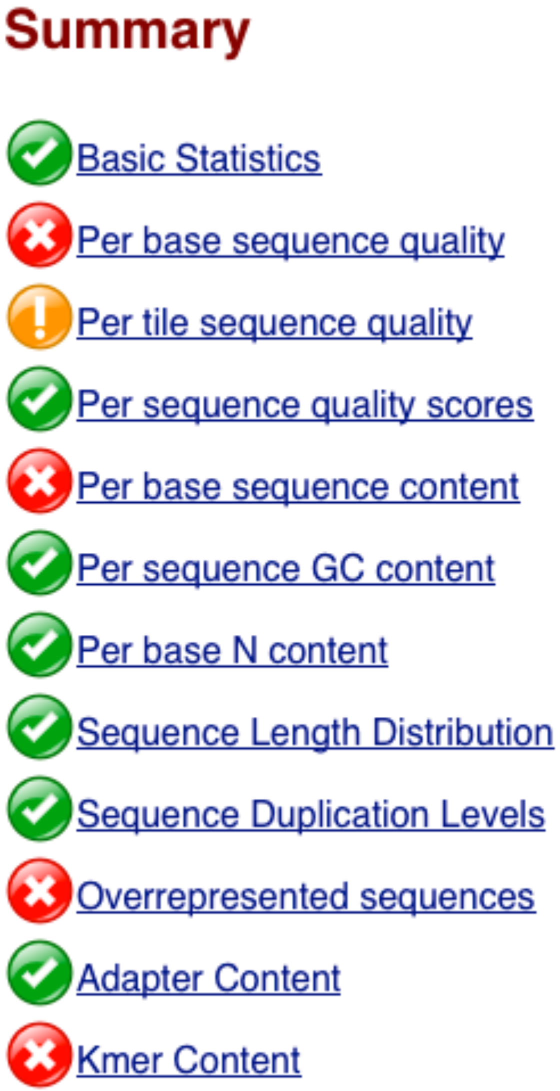
http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

1. Run FASQC

```
fastqc sample.fastq
```

2. Open output file

sample_fastq.html

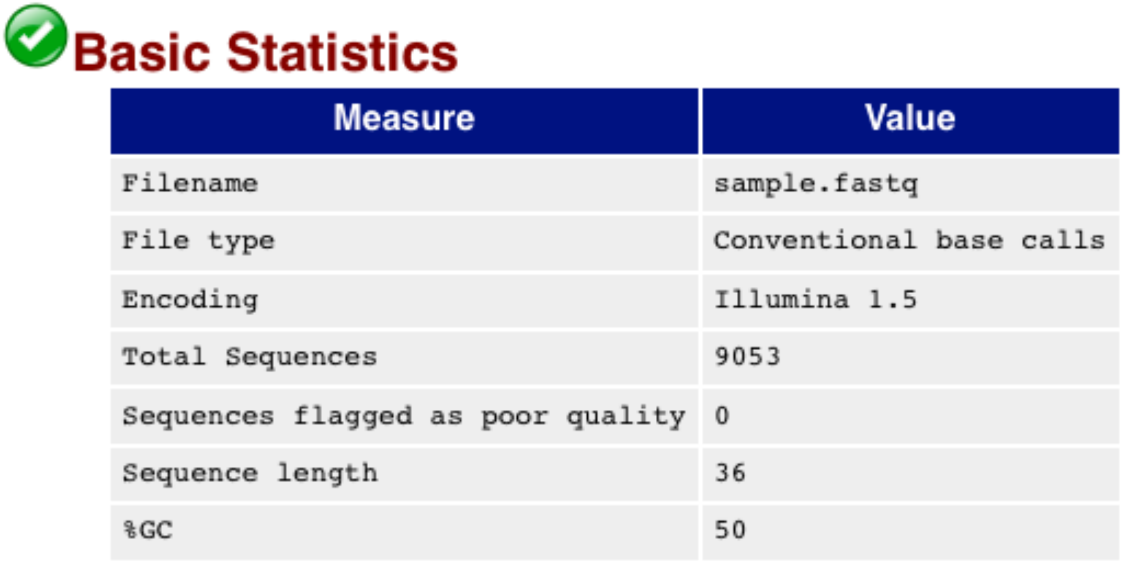


Summary

- ✓ 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

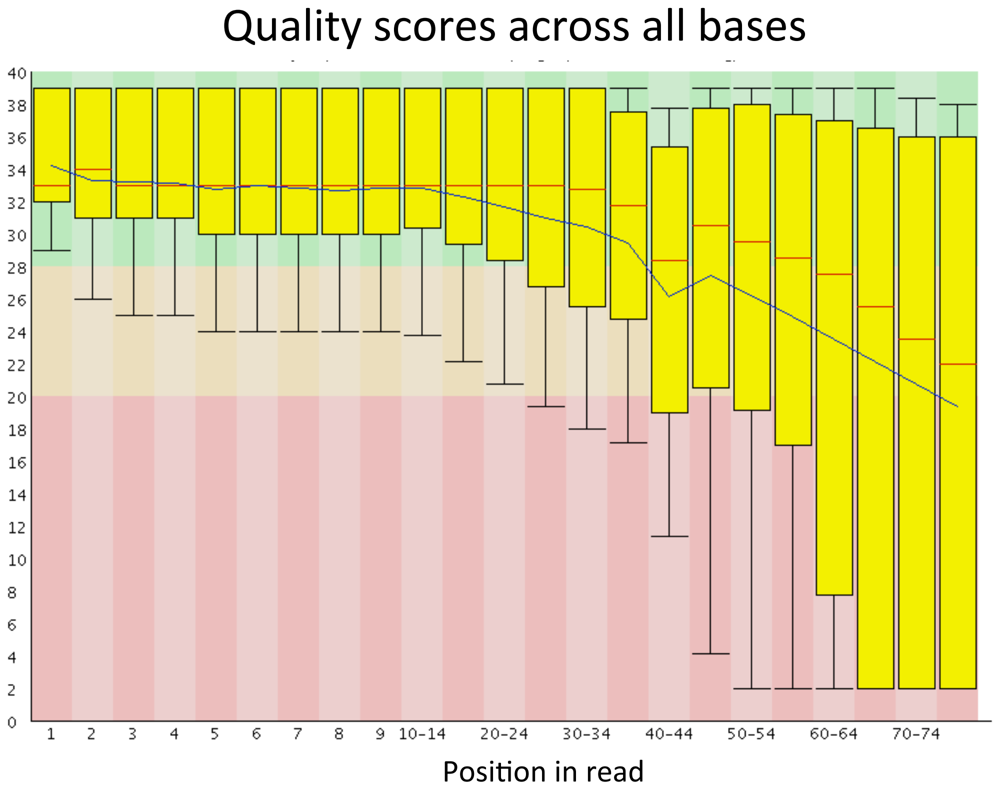


✓ **Basic Statistics**

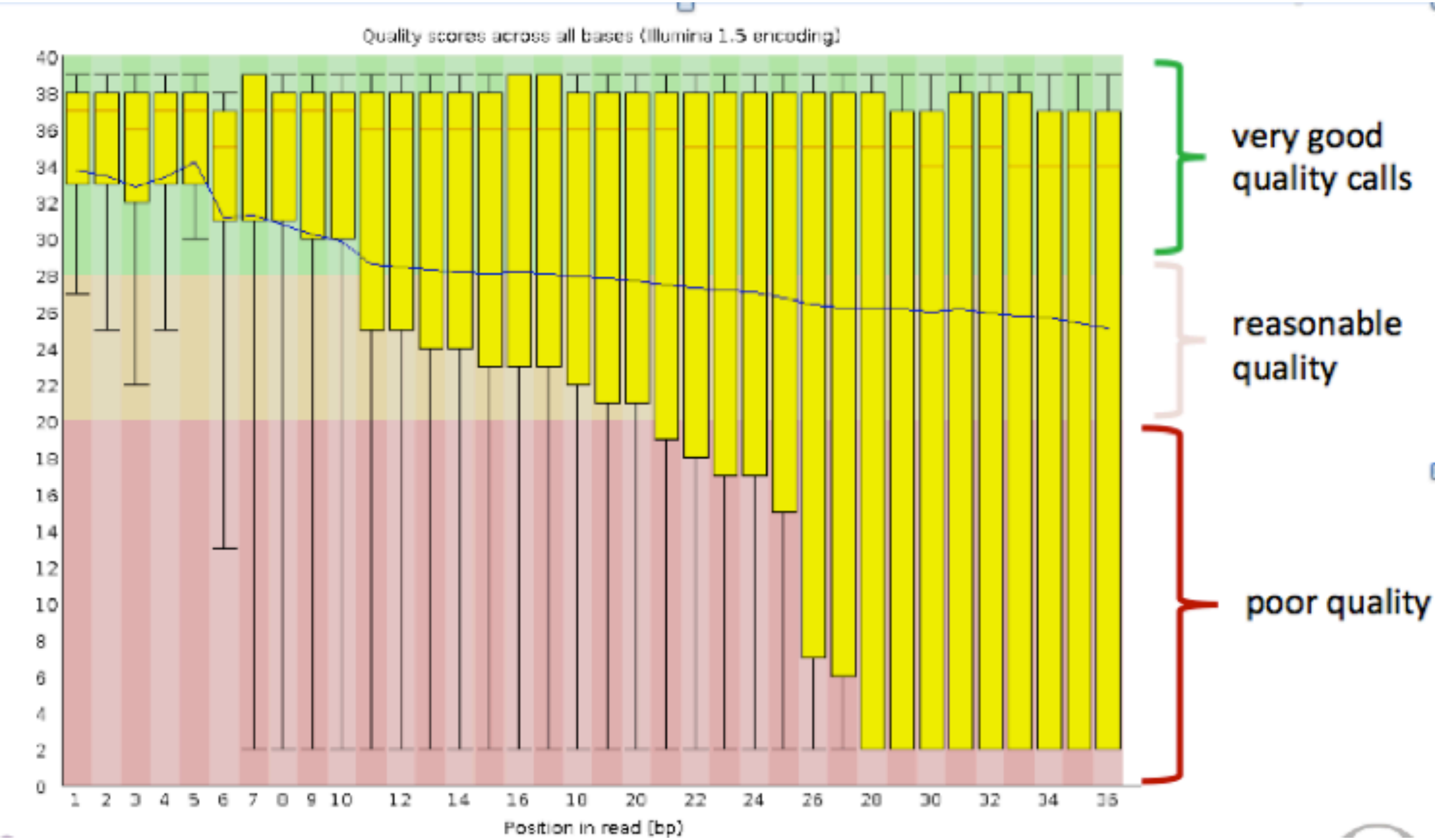
Measure	Value
Filename	sample.fastq
File type	Conventional base calls
Encoding	Illumina 1.5
Total Sequences	9053
Sequences flagged as poor quality	0
Sequence length	36
%GC	50

(2) FASTQC: Per base sequence quality

- Poor quality at the end of reads



(2) FASTQC: Per base sequence quality



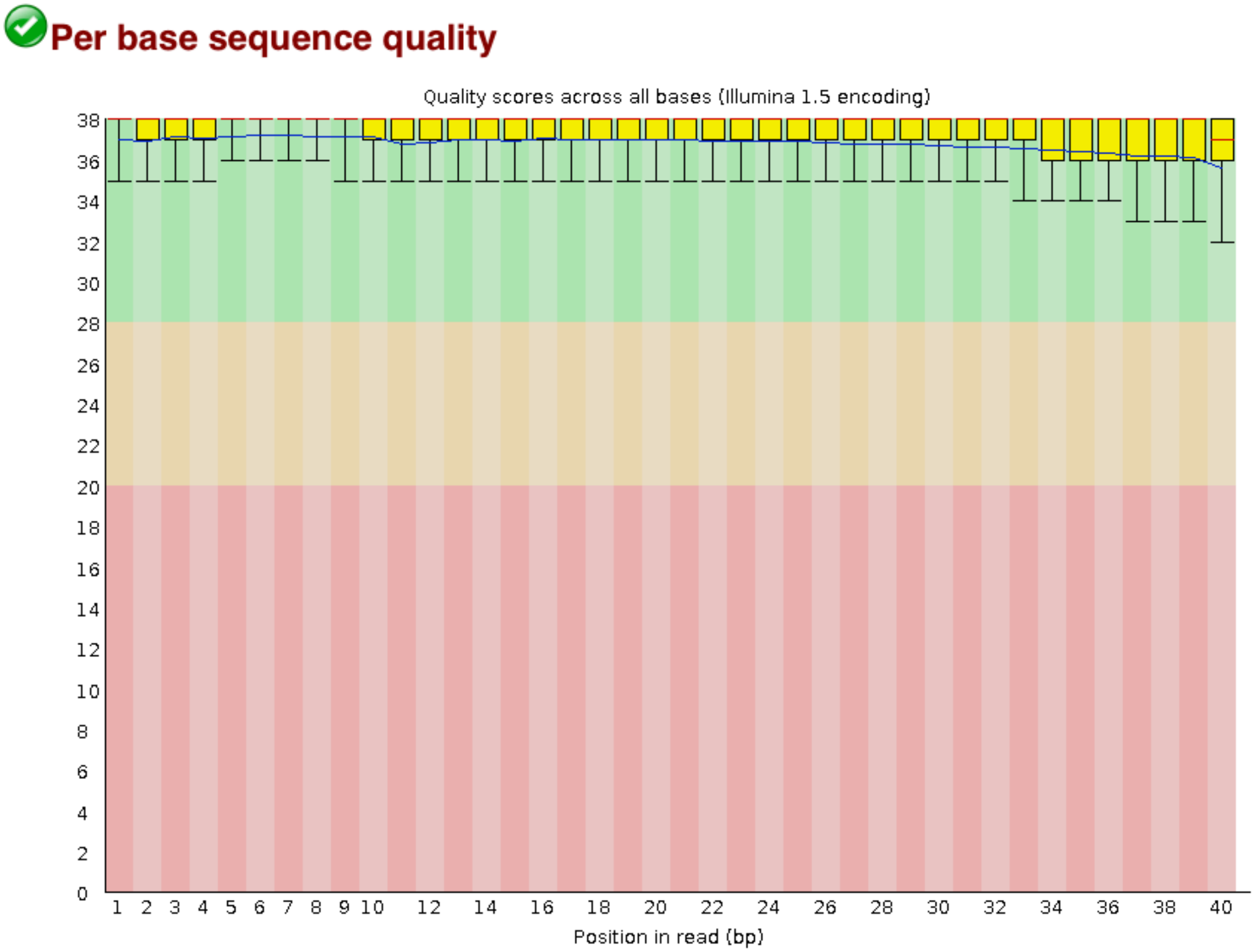
very good quality calls

reasonable quality

poor quality

(2) FASTQC: Per base sequence quality

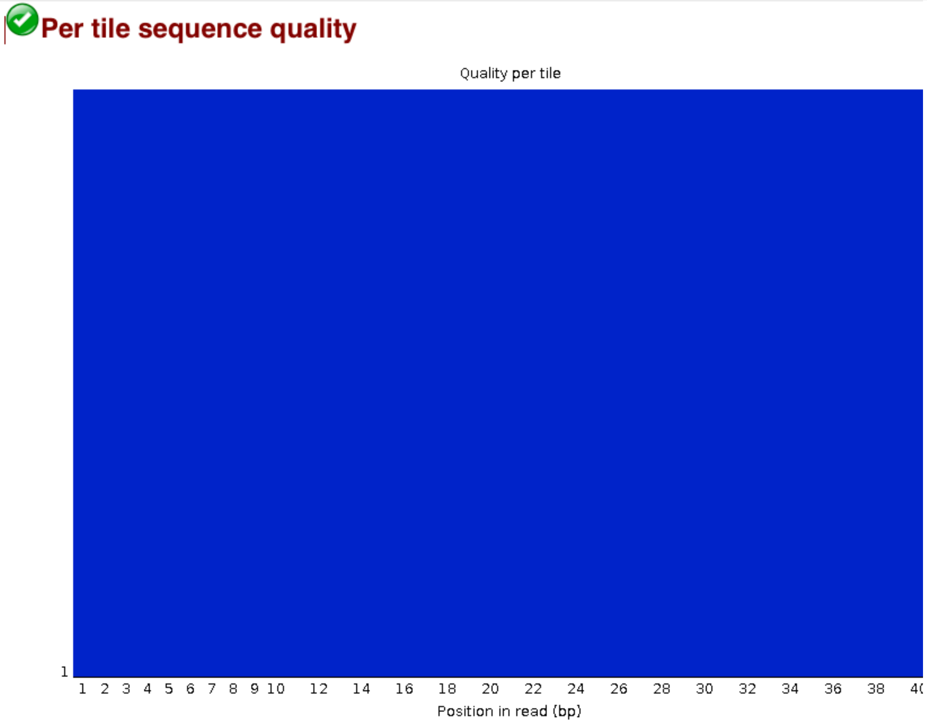
Good Illumina data:



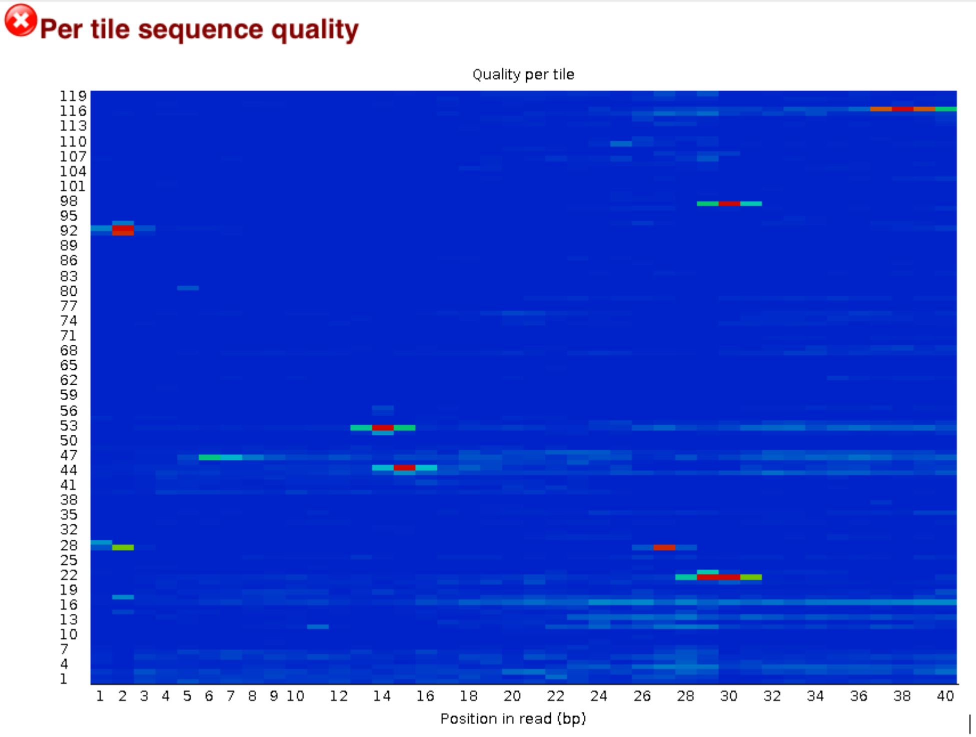
✓ Per base sequence quality

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

(3) FASTQC: Per tile sequence quality



✓ Per tile sequence quality

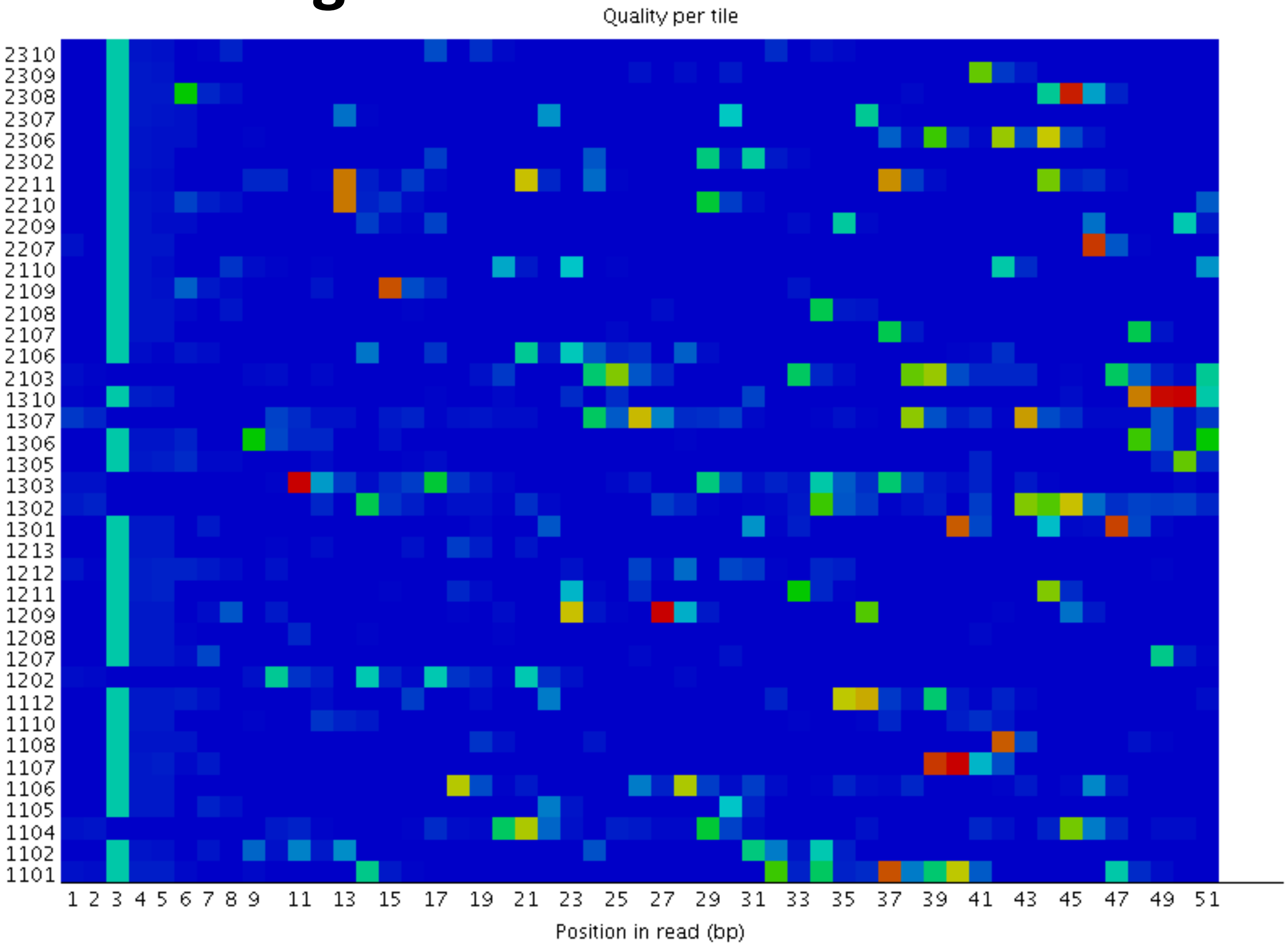


✗ Per tile sequence quality

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

(3) FASTQC: Per tile sequence quality

Overclustering:



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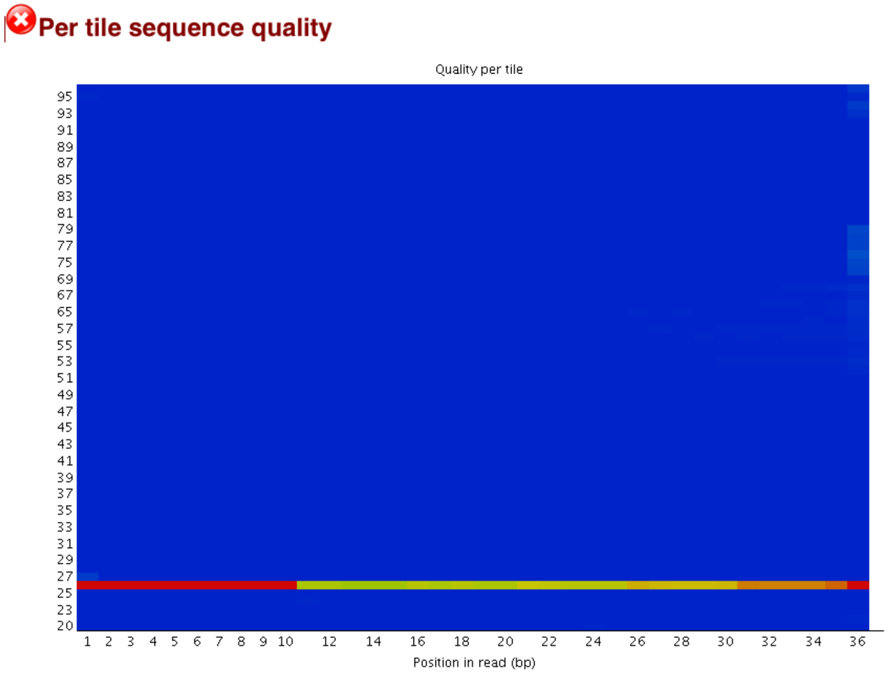
(3) FASTQC: Per tile sequence quality

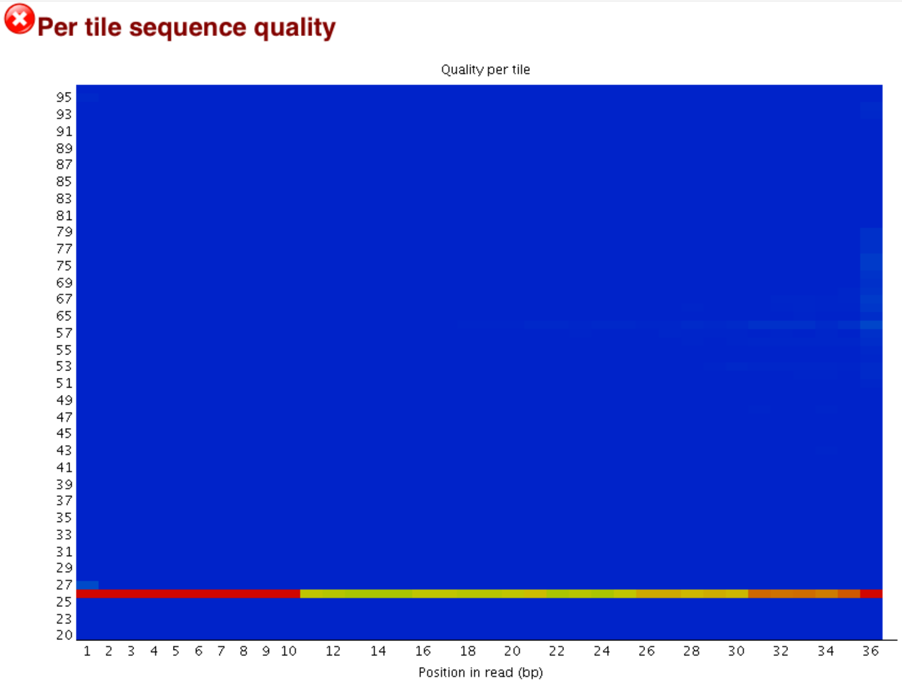
Tile fail:

SRR576938
anaerobic INPUT DNA



Per tile sequence quality

SRR576933
FNR IP ChIP-seq Anaerobic A

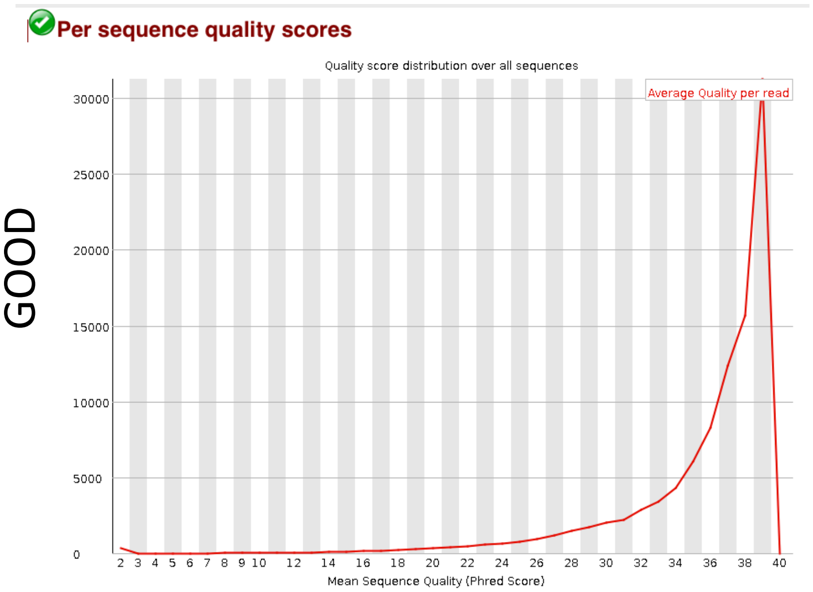


Per tile sequence quality

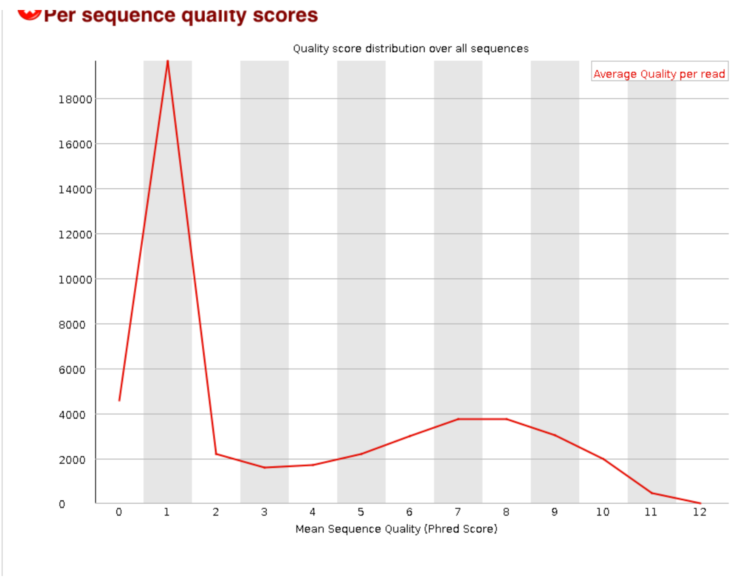
GSE41187: Genome-wide analysis of FNR and s70 in E. coli under aerobic and anaerobic growth conditions: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE41187

(4) FASTQC: Per sequence quality scores

GOOD

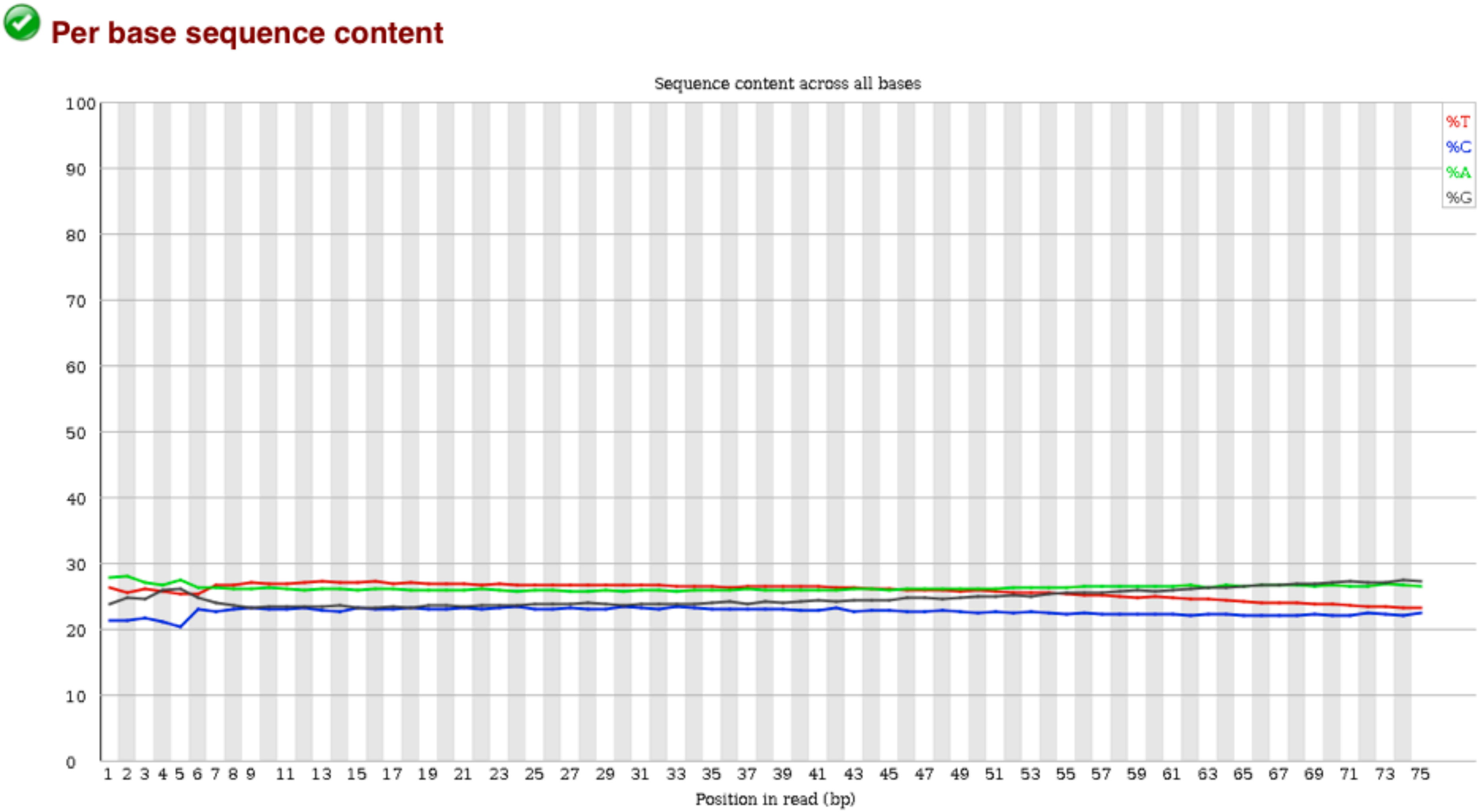


BAD



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

(5) FASTQC: Per base sequence content

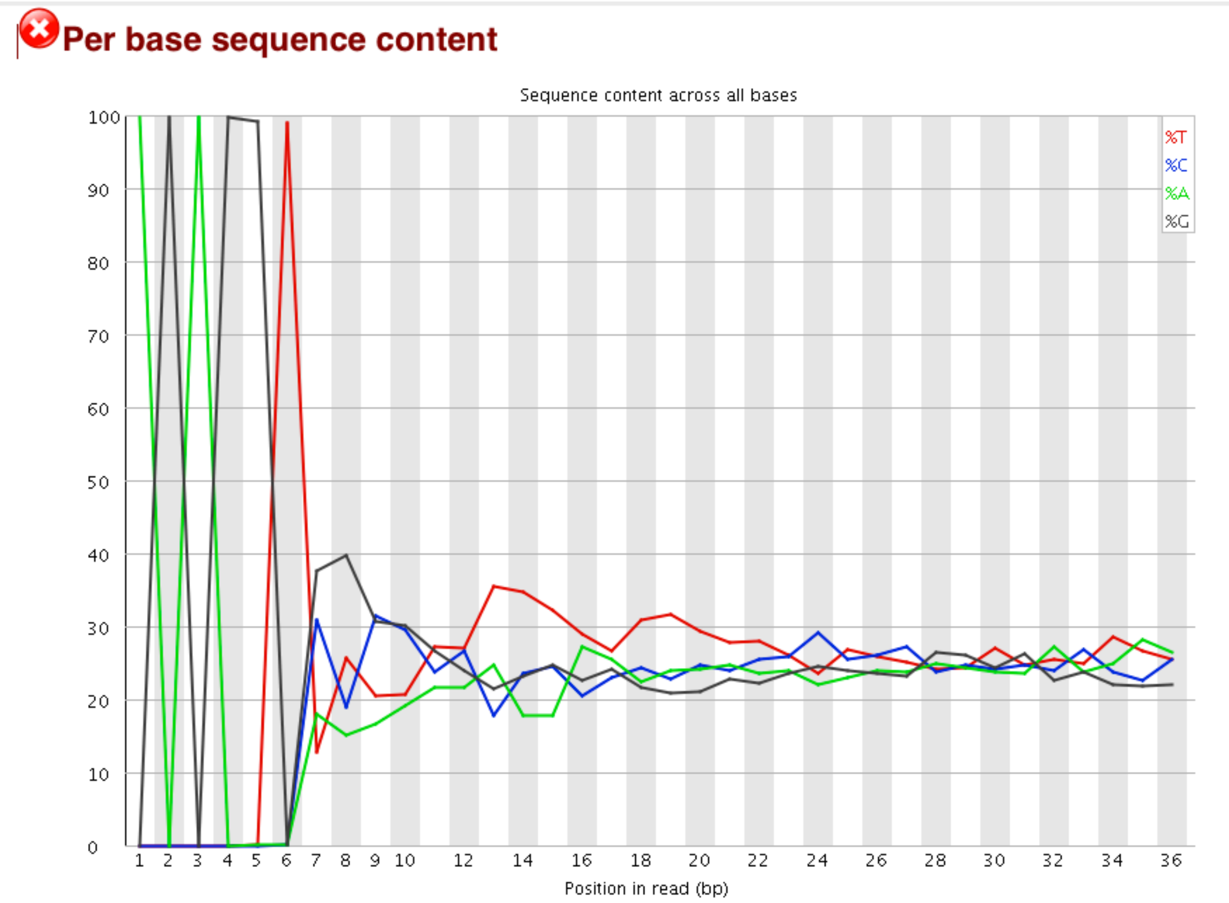


✓ Per base sequence content

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

(5) FASTQC: Per base sequence content

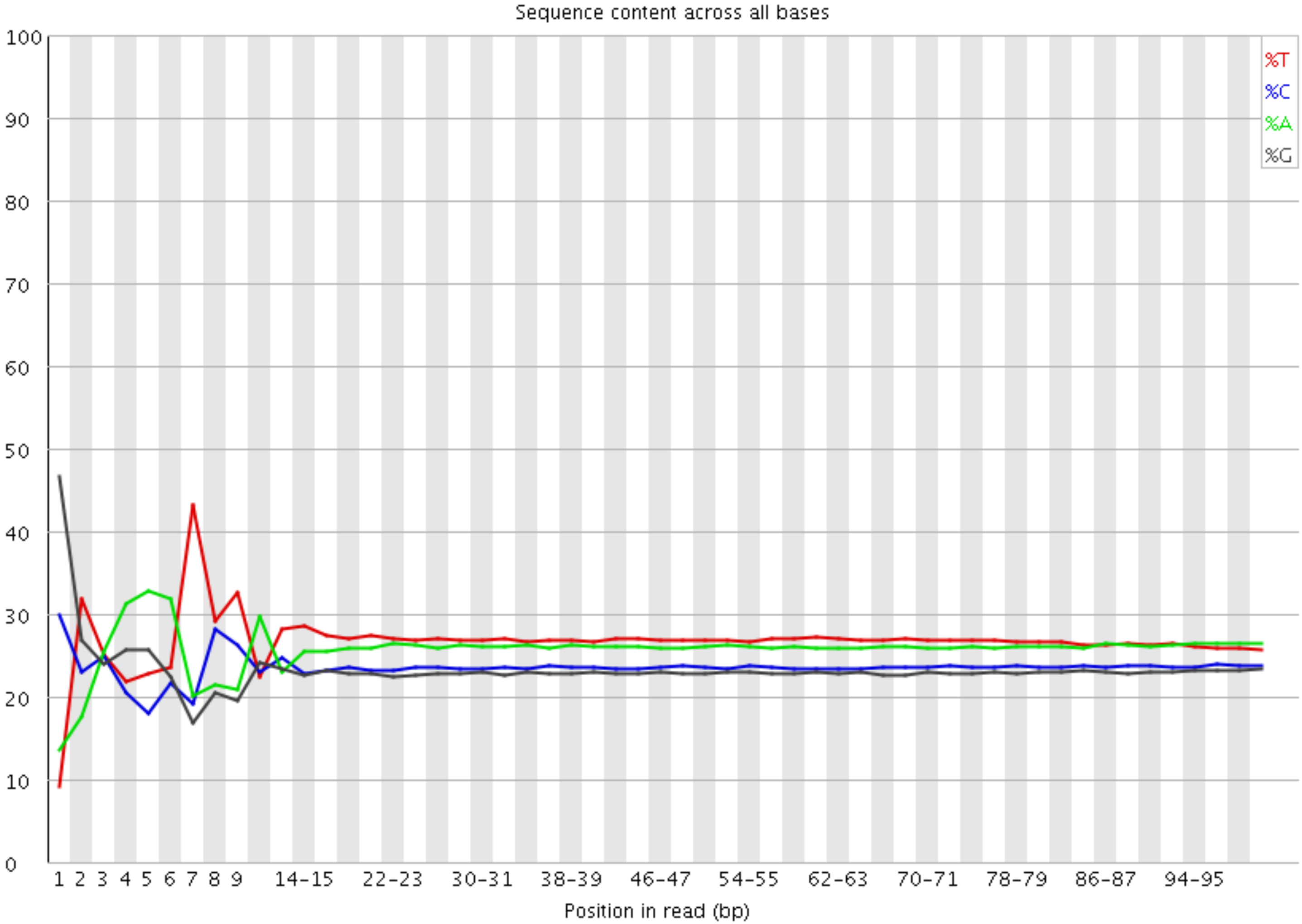
Biased sequence composition (adapters?)



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

(5) FASTQC: Per base sequence content

Unavoidable – RNA-Seq



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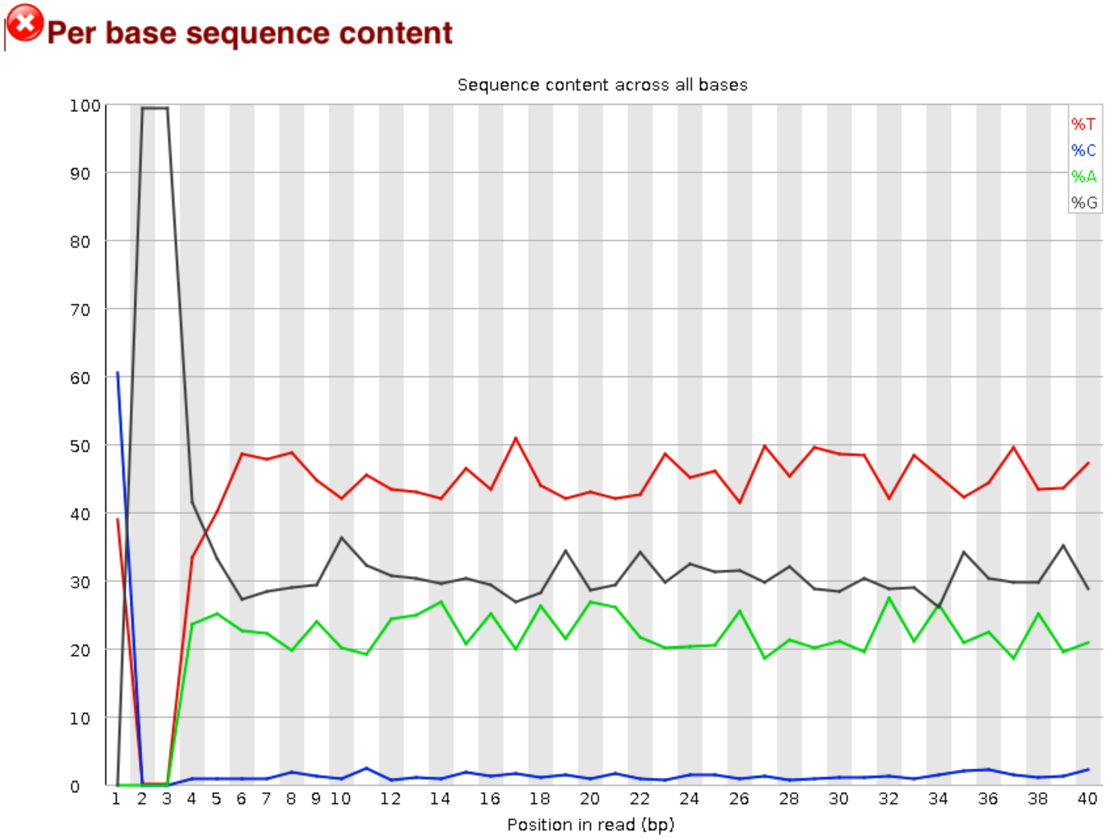
(5) FASTQC: Per base sequence content

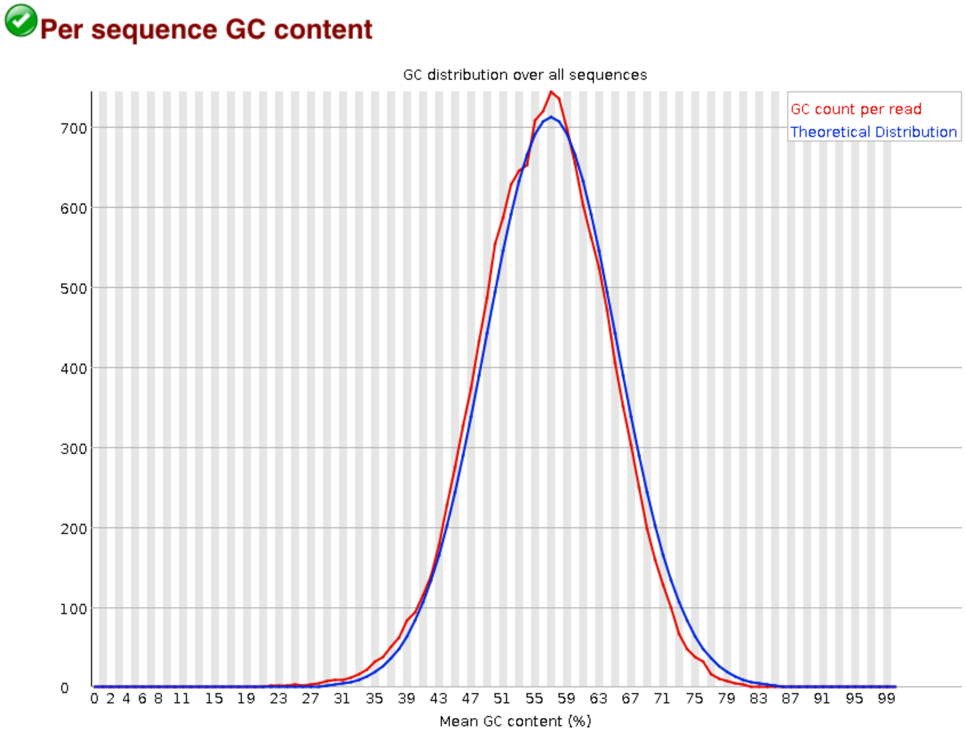
Unavoidable – RRBS

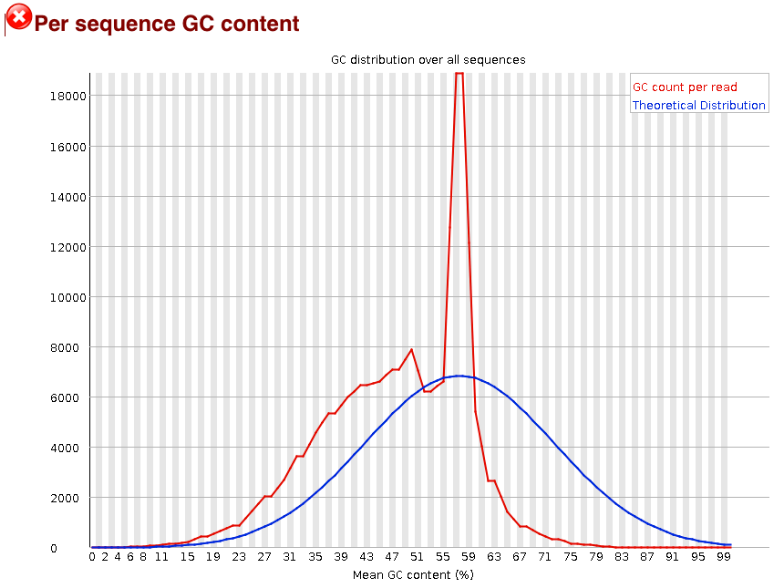
Devoided of cytosines because the library was treated with sodium bisulphite (which will have converted most of the C to T)

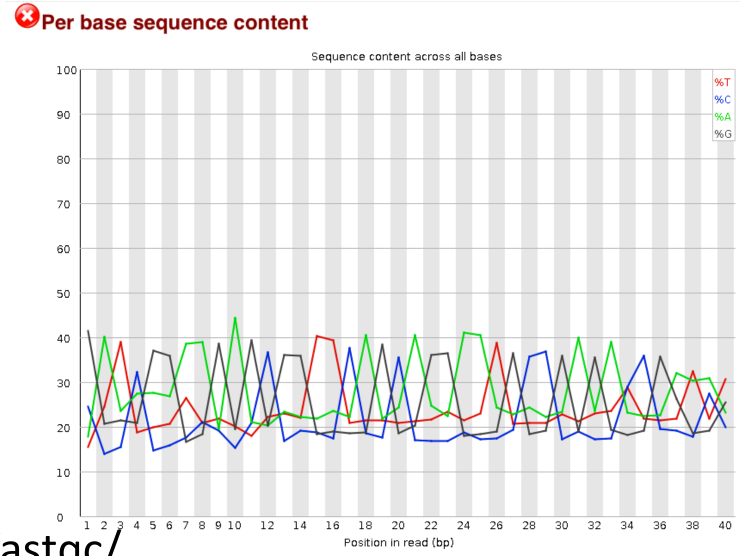


http://www.bioinformatics.babraham.ac.uk/projects/fastqc/RRBS_fastqc.html#M4

(6) FASTQC: Per sequence GC content

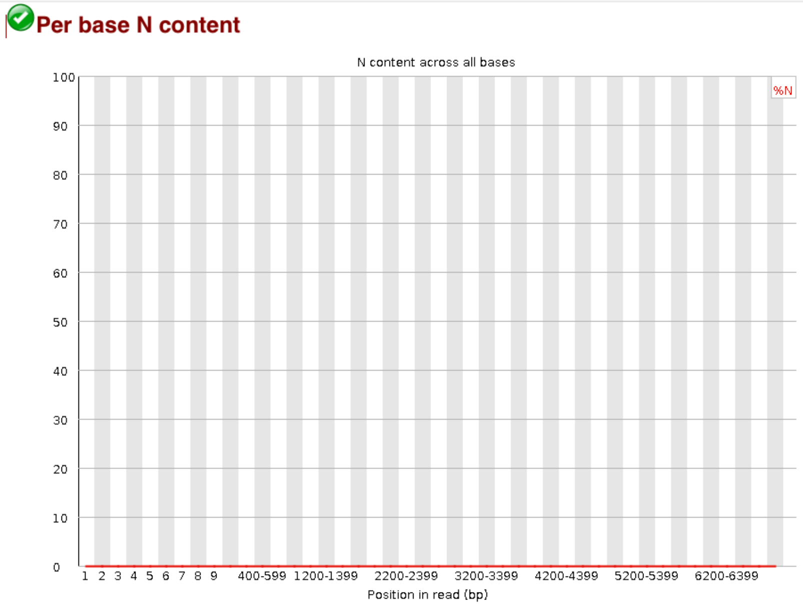




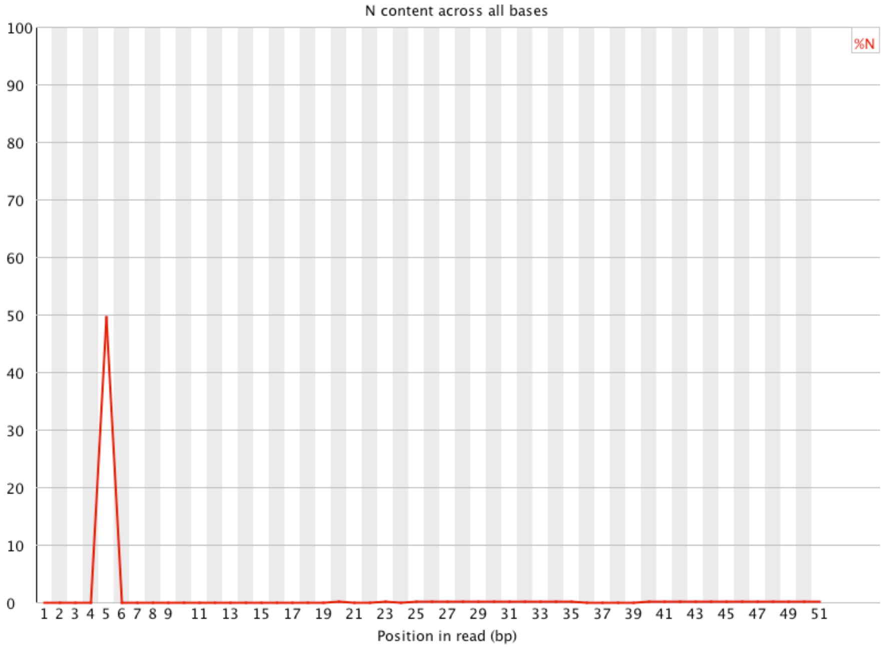


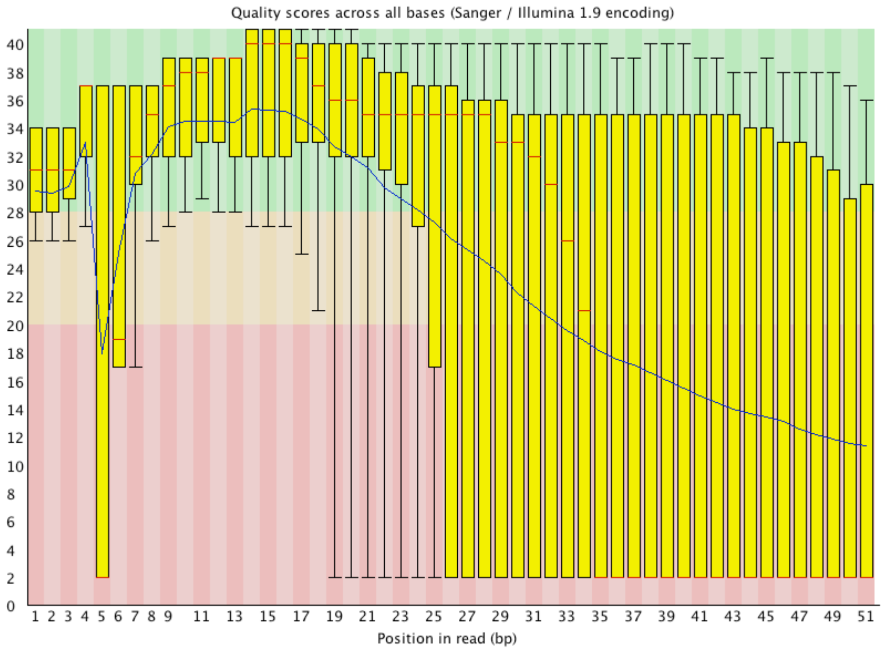
http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

(7) FASTQC: Per base N content



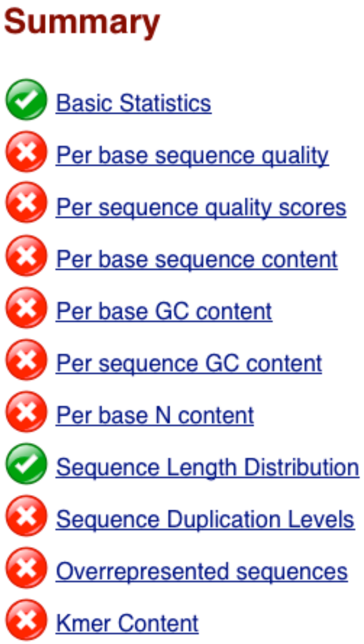
Per base N content





http://cbio.mskcc.org/~lianos/files/scott/2011-11-21/qc/

(8) FASTQC: Sequence Length Distribution



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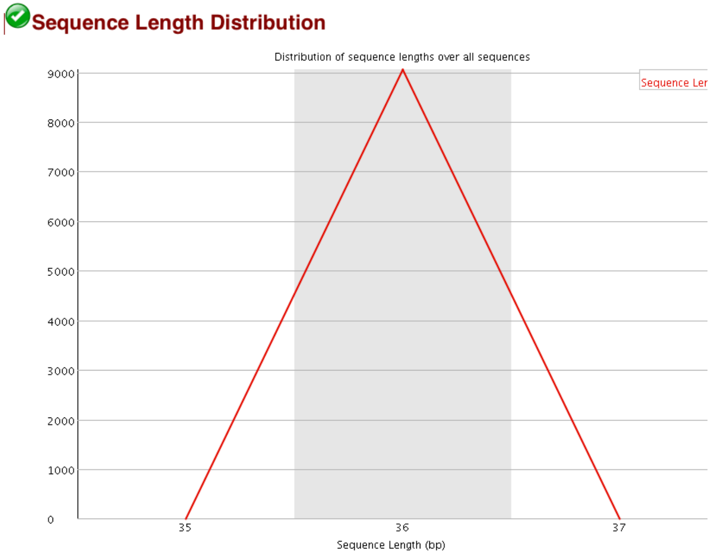
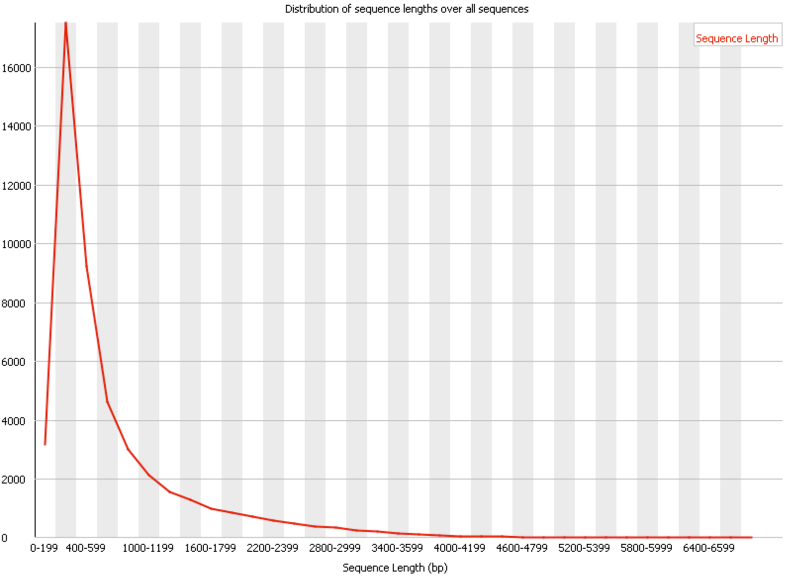
- ✗ Sequence Duplication Levels
- ✗ Overrepresented sequences
- ✗ Kmer Content

Sequence fragments of uniform length (36bp)



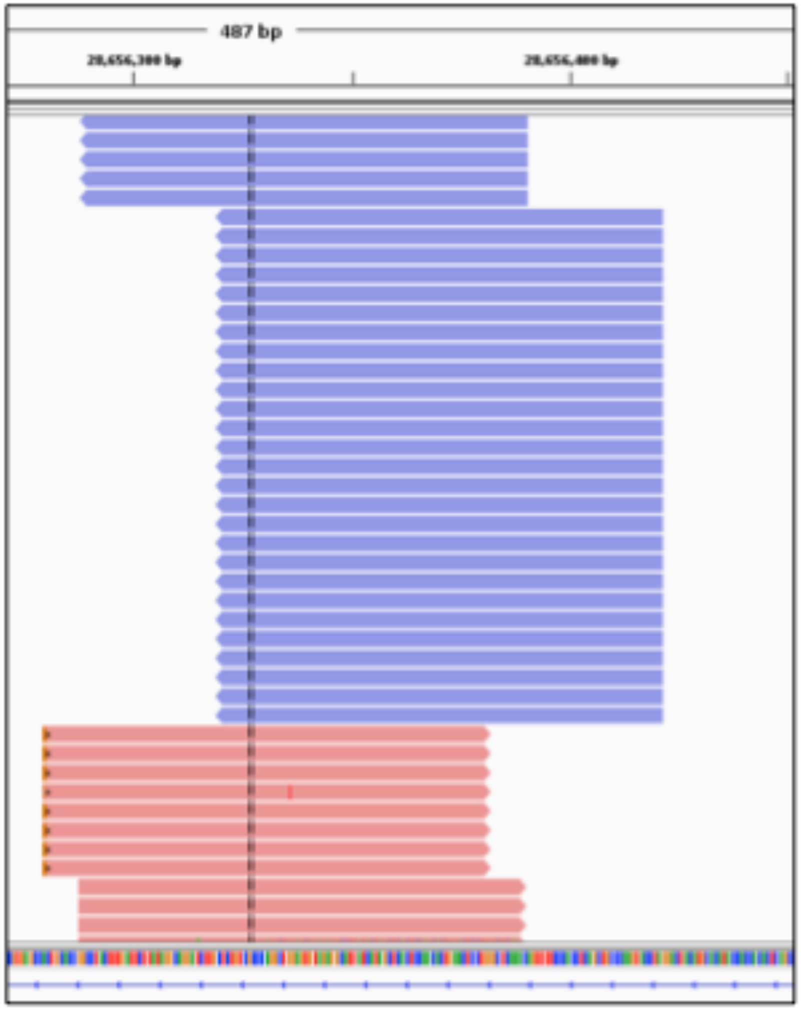
Reads of variable length:



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

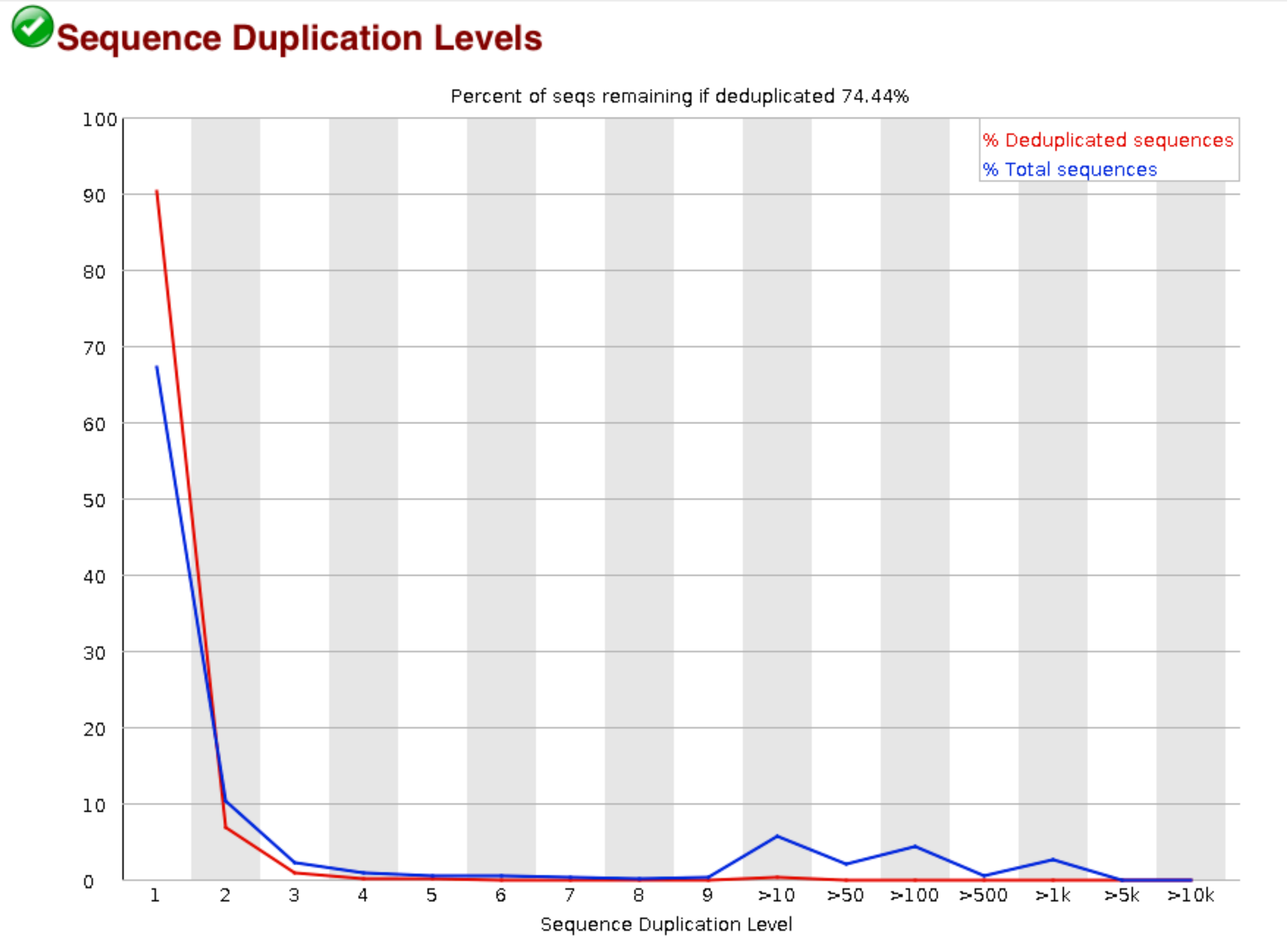
- PCR duplicates during sample preparation
- Optical duplicates: read the same cluster twice in the sequencer
- High duplication can lead to problems in downstream analysis (e.g. skew allele frequencies)



http://bioinformatics.org.au/ws14/wp-content/uploads/ws14/sites/5/2014/07/Felicity-Newell_presentation.pdf

(9) FASTQC: Sequence duplication levels

Very diverse library

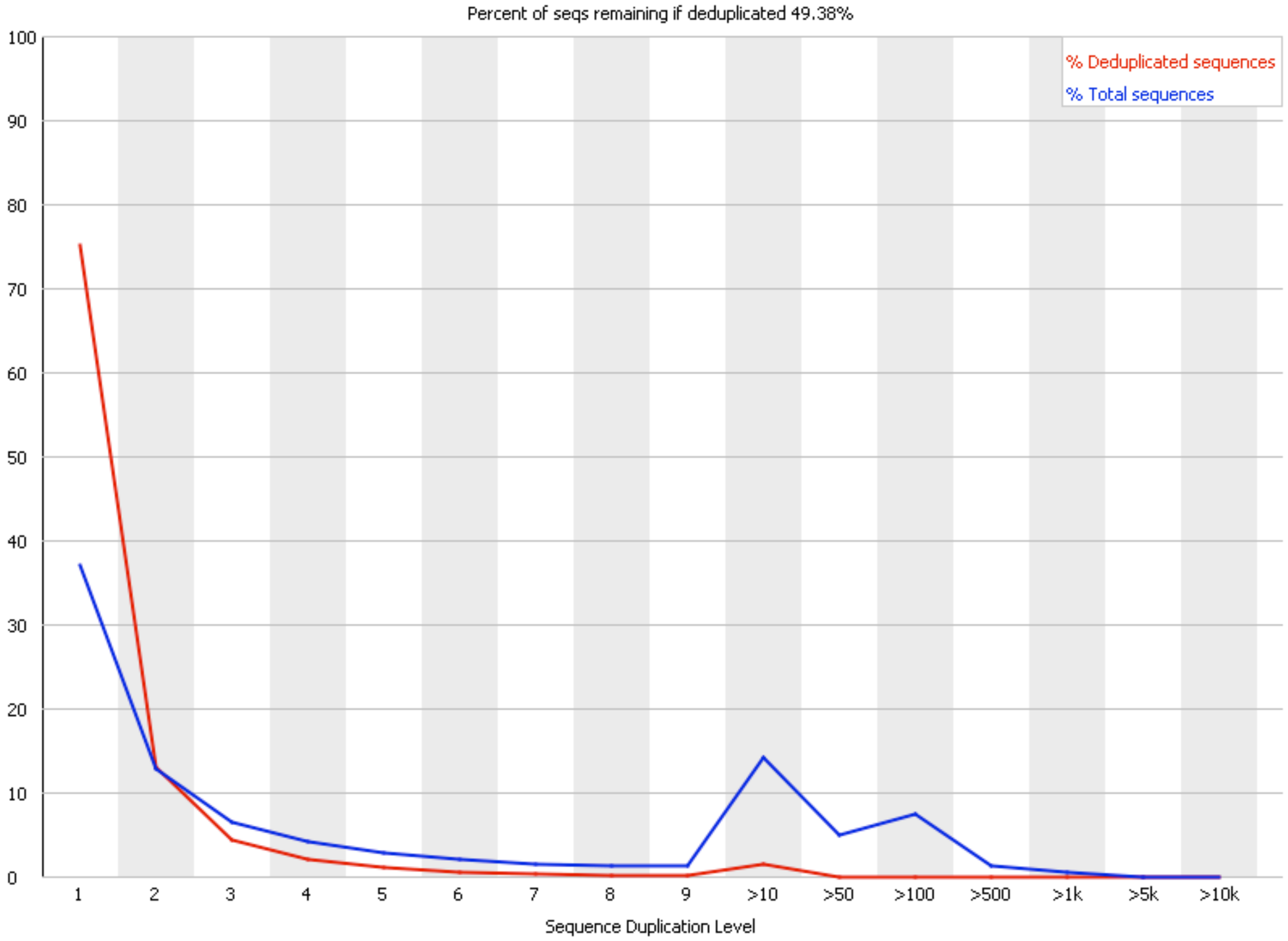


✓ Sequence Duplication Levels

http://proteo.me.uk/2013/09/a-new-way-to-look-at-duplication-in-fastqc-v0-11/

(9) FASTQC: Sequence duplication levels

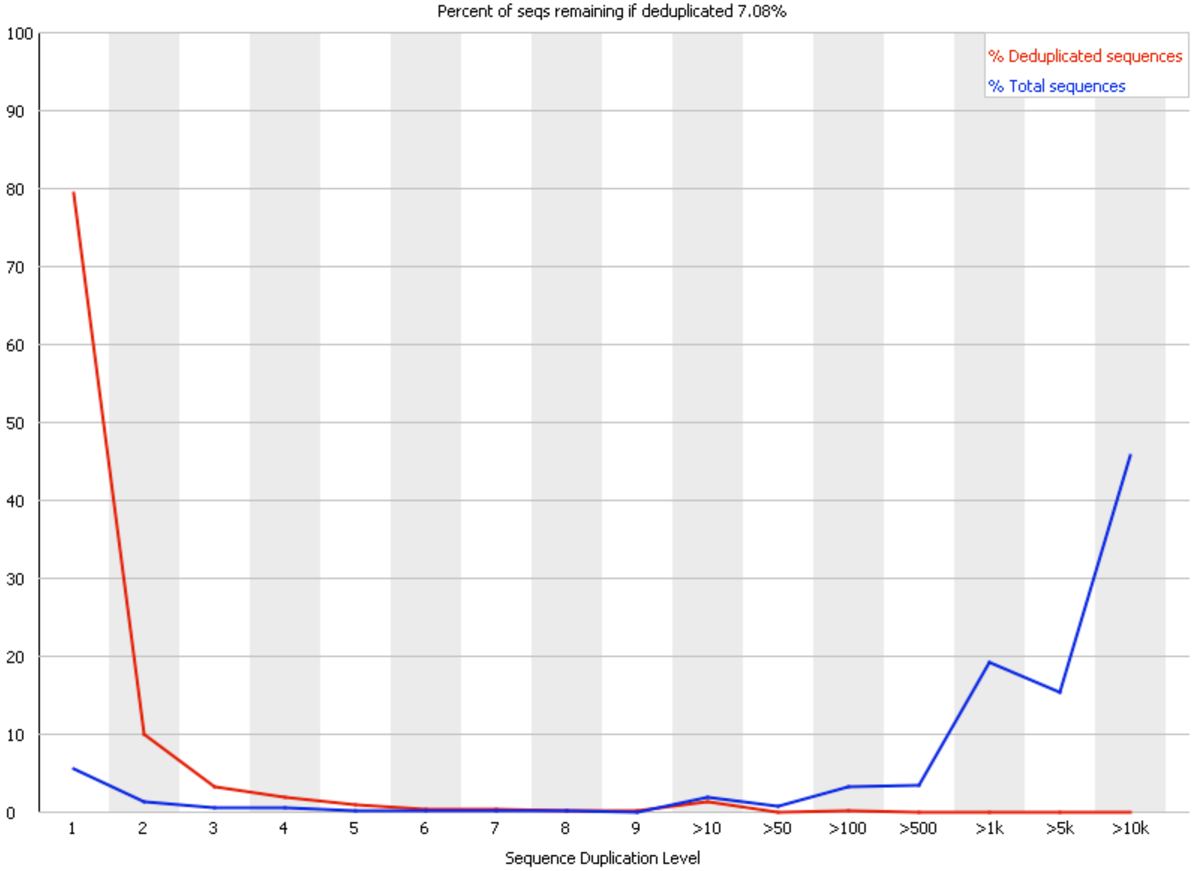
A good RNA-Seq library (although dup levels > 50%)



http://proteo.me.uk/2013/09/a-new-way-to-look-at-duplication-in-fastqc-v0-11/

(9) FASTQC: Sequence duplication levels

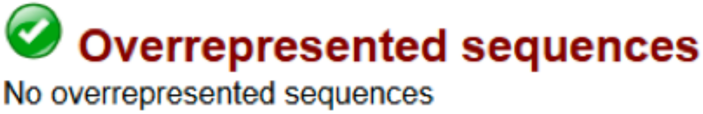
PCR duplication



http://proteo.me.uk/2013/09/a-new-way-to-look-at-duplication-in-fastqc-v0-11/

(10) FASTQC: Over-represented sequences

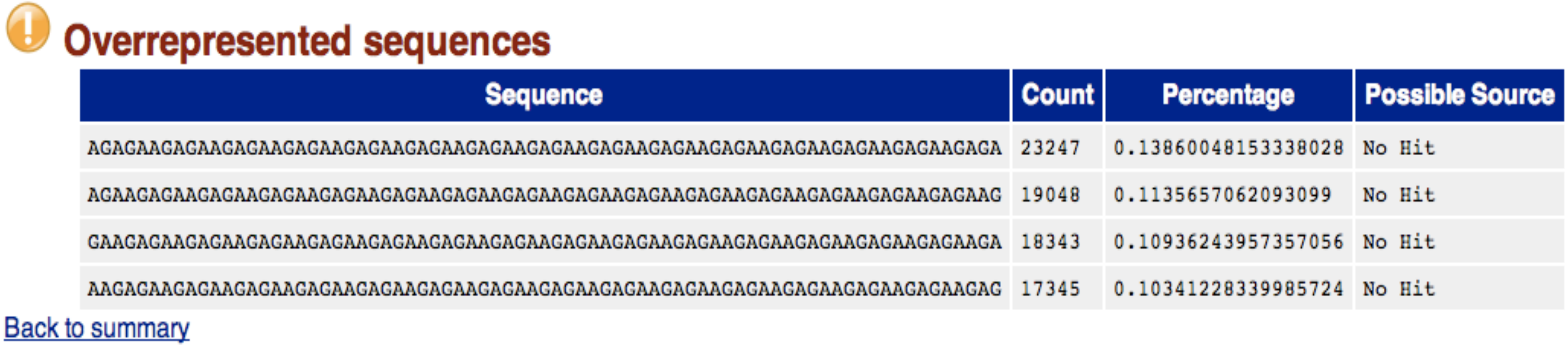
Good dataset



✓ Overrepresented sequences

No overrepresented sequences

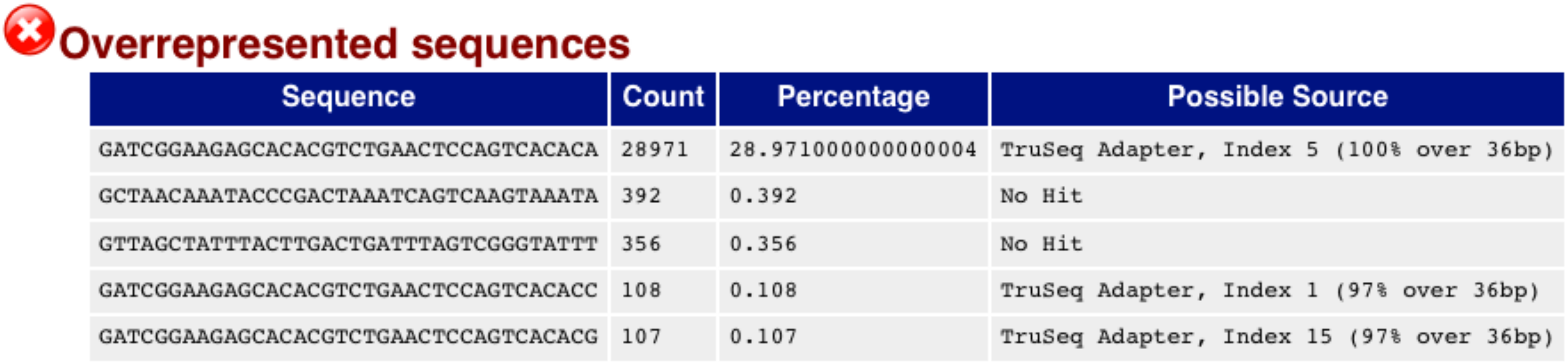
Bad datasets:



! Overrepresented sequences

Sequence	Count	Percentage	Possible Source
AGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGA	23247	0.13860048153338028	No Hit
AGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAG	19048	0.1135657062093099	No Hit
GAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGA	18343	0.10936243957357056	No Hit
AAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAG	17345	0.10341228339985724	No Hit

Back to summary

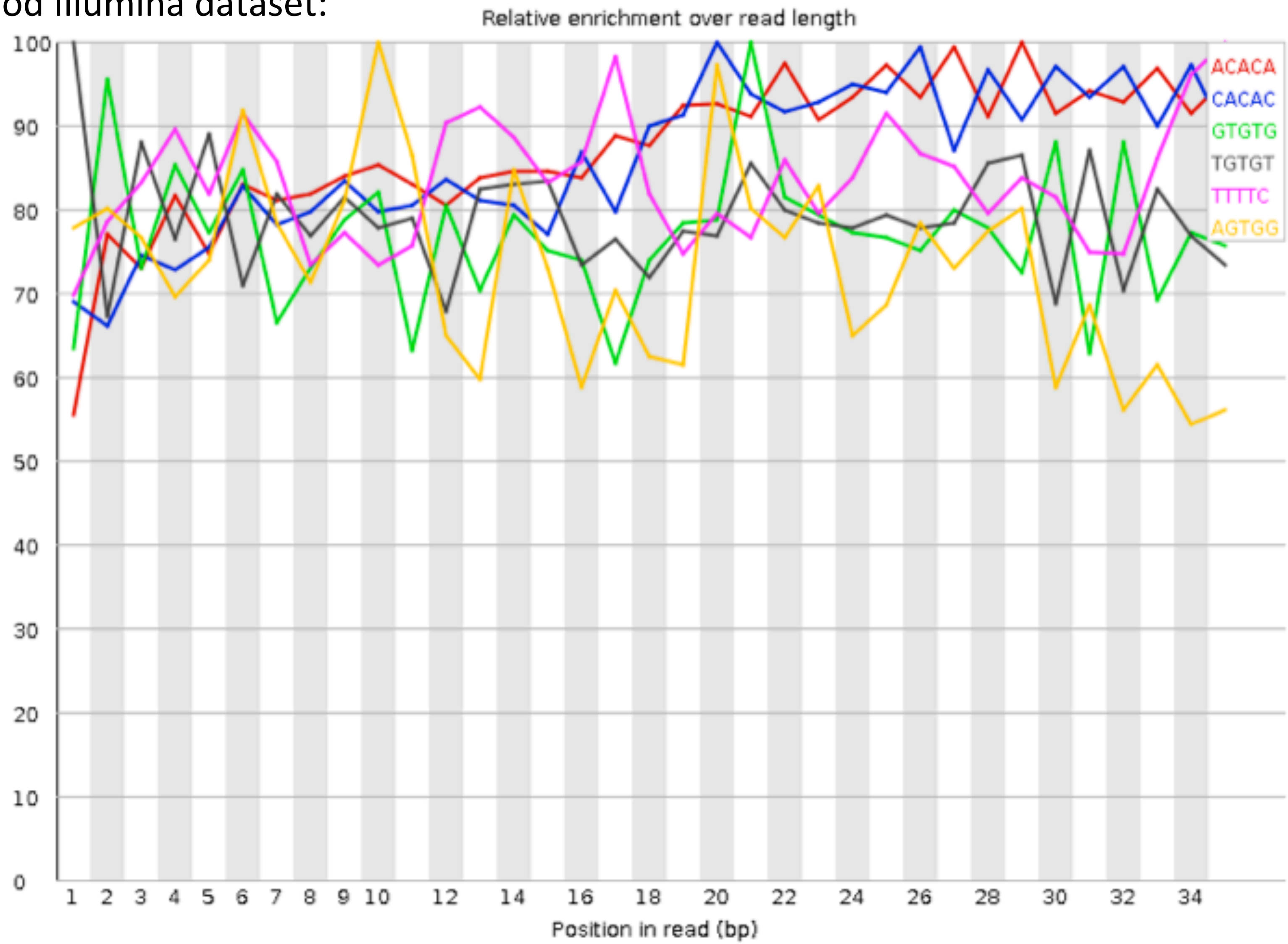


✗ Overrepresented sequences

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

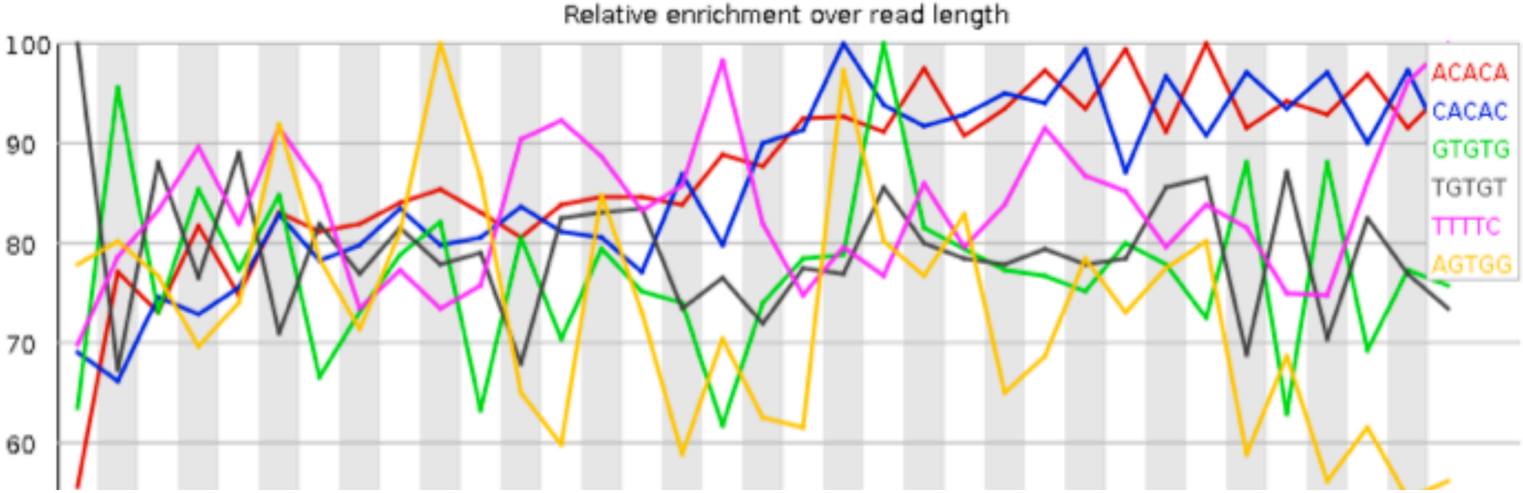
Good Illumina dataset:



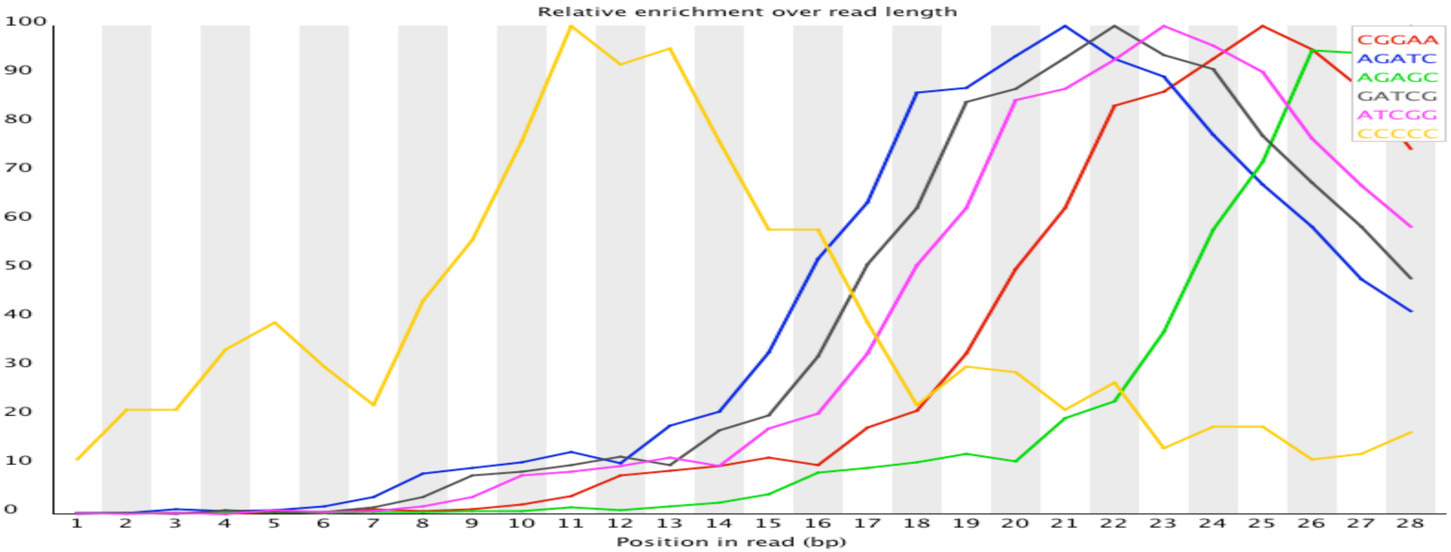
http://www.slideshare.net/suryasaha/sequencing-quality-filtering?related=1

(11) FASTQC: Kmer content

GOOD



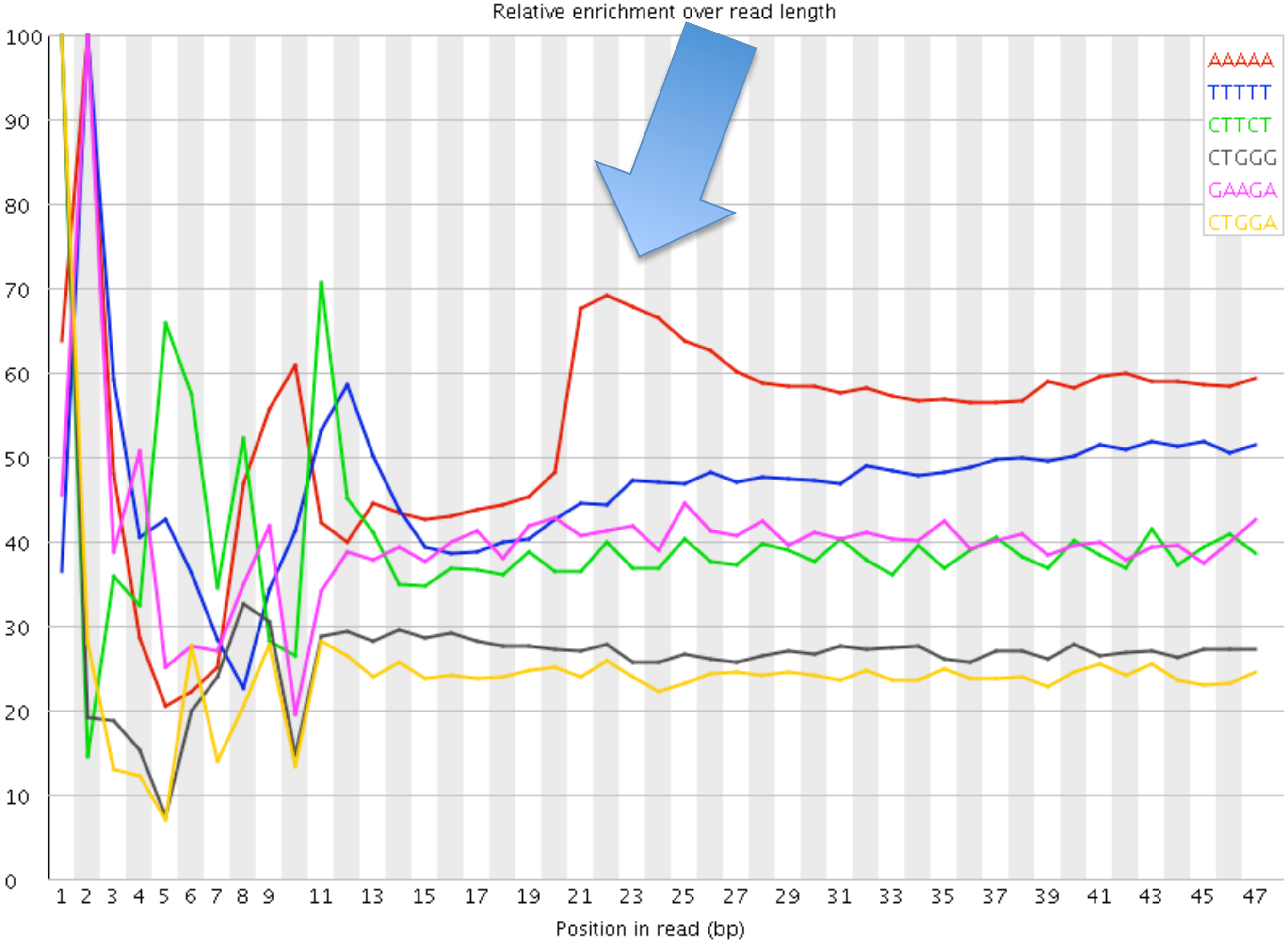
BAD



http://www.slideshare.net/suryasaha/sequencing-quality-filtering?related=1

(11) FASTQC: Kmer content

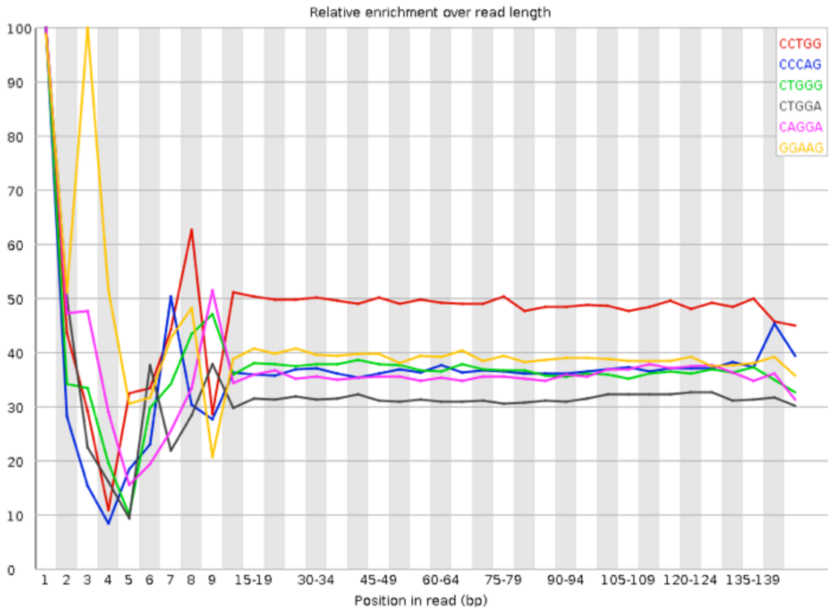
AAAA k-mer that you're seeing at around 21 base pairs are arrested transcripts caused by cyclohexamide treatment.

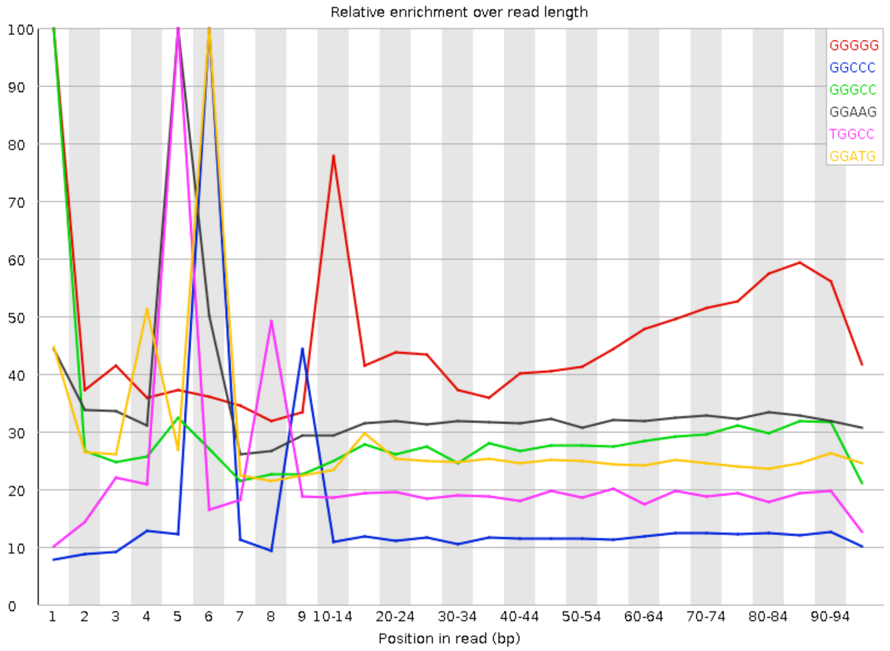


http://seqanswers.com/forums/showthread.php?t=18447

(11) FASTQC: Kmer content

"Random" hexamer primer in RNA-seq libraries
(not that random after all)

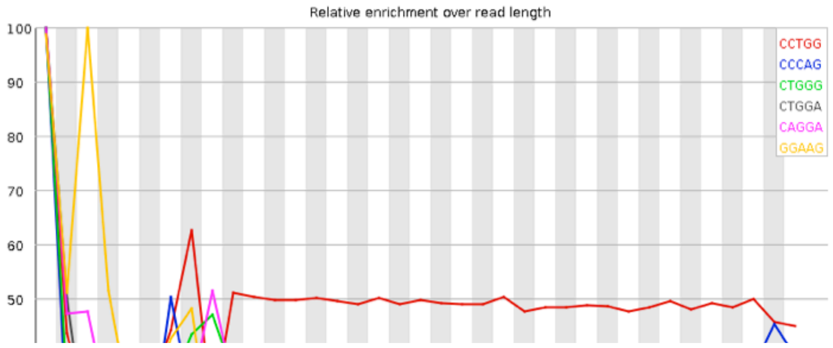


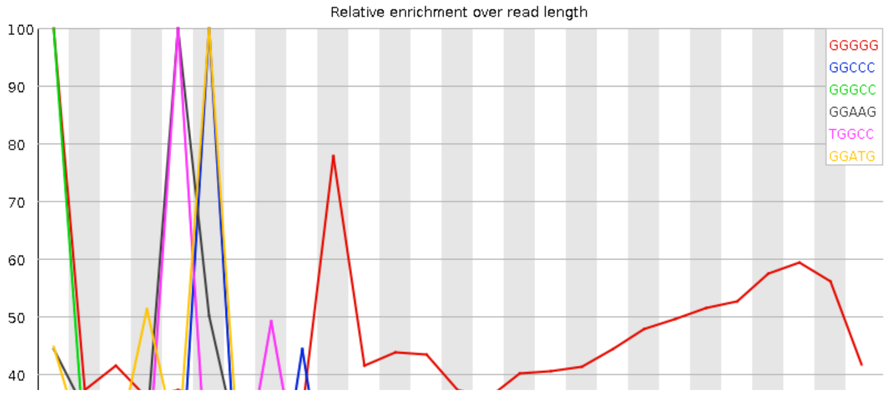


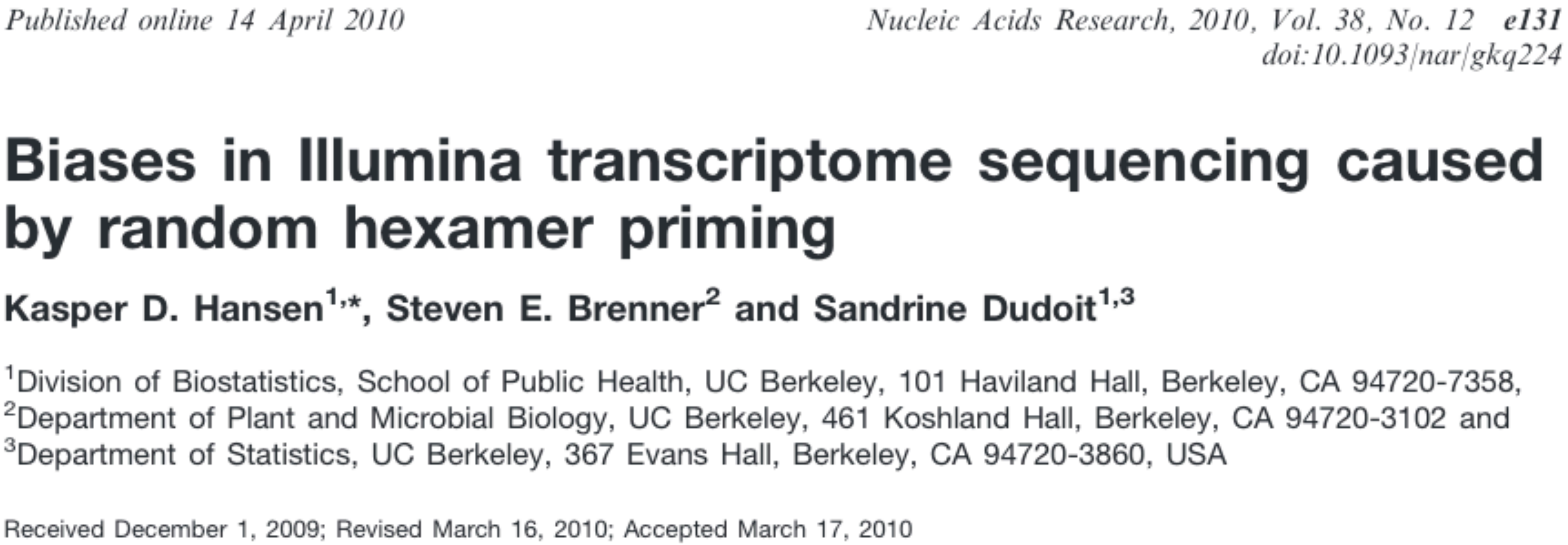
http://seqanswers.com/forums/showthread.php?t=44770&highlight=kmer+fastq
http://seqanswers.com/forums/showthread.php?t=16669

(11) FASTQC: Kmer content

“Random” hexamer primer in RNA-seq libraries
(not that random afterall)







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doi:10.1093/nar/gkq224

Biases in Illumina transcriptome sequencing caused by random hexamer priming

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Received December 1, 2009; Revised March 16, 2010; Accepted March 17, 2010

Useful resources

https://seqqc.wordpress.com/

https://sequencing.qcfail.com/

Hands on exercise:

Fastqc_sweave.pdf

Examples of FASTQC runs and preprocessing