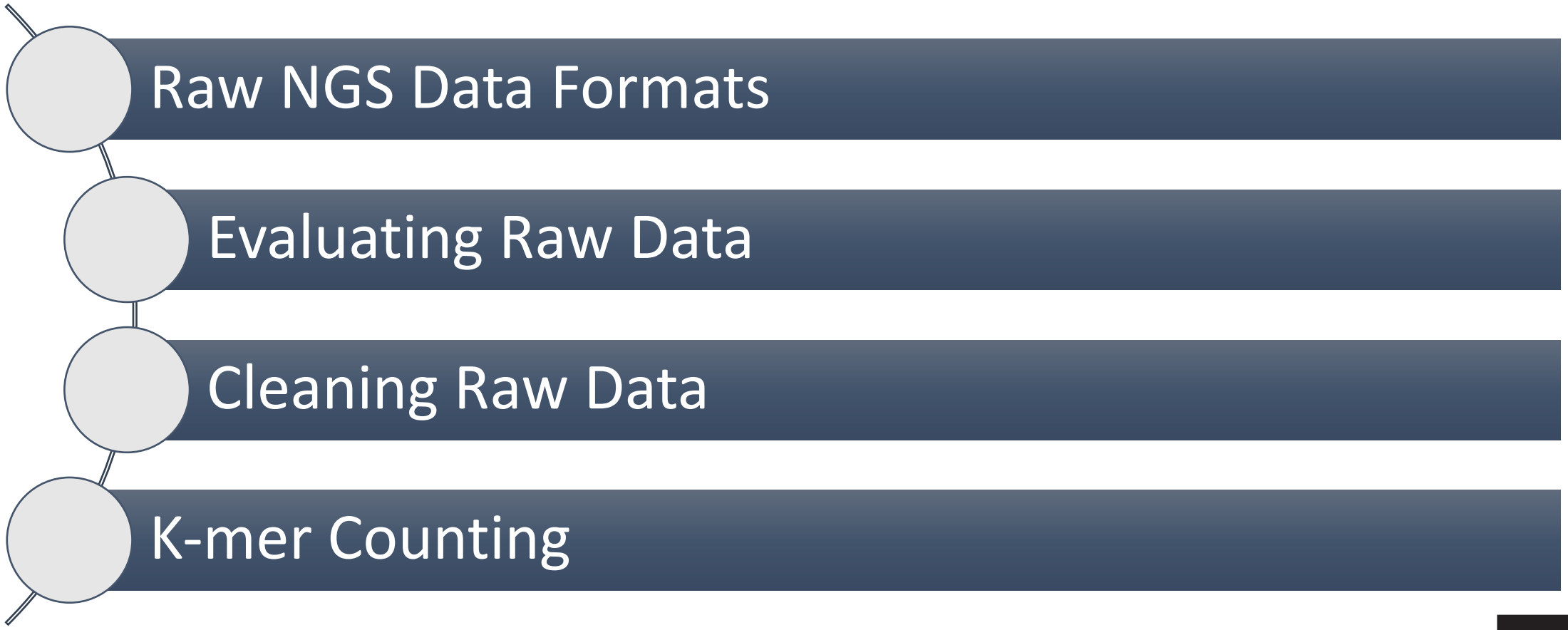
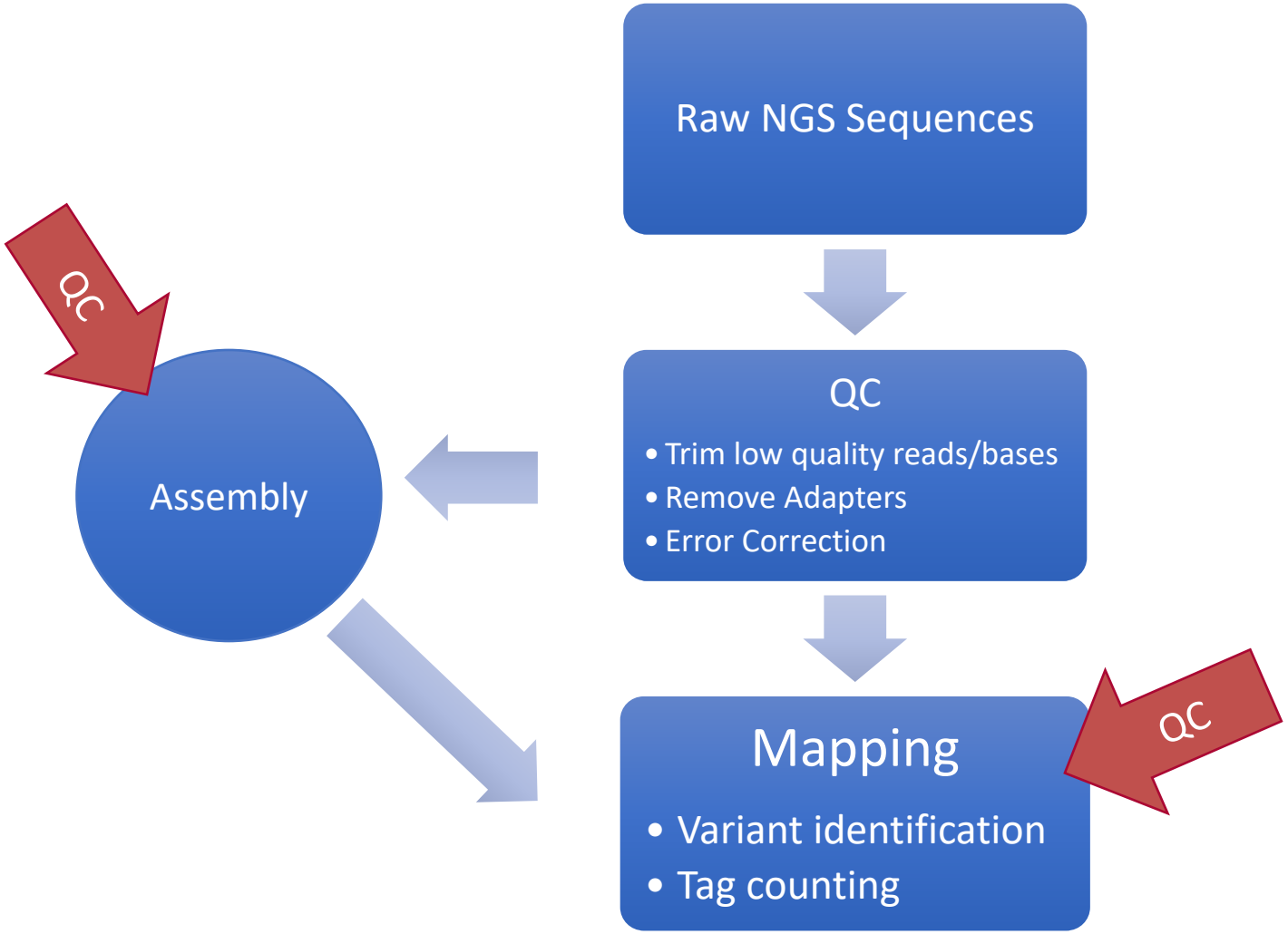
# NGS QC

An introduction

# Outline


Raw NGS Data Formats
Evaluating Raw Data
Cleaning Raw Data
K-mer Counting

# The Big Picture



# Common Sequence Formats

## FASTA

- Simple
- Nucleotide or amino acid strings
- No quality info
- Compressible (.gz)

## FASTQ

- Mildly complex
- Nucleotide strings (not AA)
- Quality information included
- Compressible (.gz)

## FAST5

- Complex (HDF5)
- Nanopore Data
- Nucleotide strings (not AA)
- Raw ***squiggles***
- Natively compressed

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## FAST5

- Complex (HDF5)
- Nanopore Data
- Nucleotide strings (not AA)
- Raw ***squiggles***
- Natively compressed

# The FASTA format

>sequence 1
CATCGATCGCATGCTACTGACTG
CATGCTCGCGCCCCCCCCGATG
>sequence 2
ACTGACTCGCGCGCGCGGGGG
GAGCTGATGTG
>sequence 3
CATCGATCGCATGCTACTGACTG
CATGCTCGCGCCCCCCCCGATG
ACTGACTCGCGCGCGCGGGGG
GAGCTGATGTG

# The FASTA format

>sequence 1
CATCGATCGCATGCTACTGACTGCATGCTCGCGCCCCCCCCGATG……
>sequence 2
ACTGACTCGCGCGCGGGGGGGAGCTGATGTG
>sequence 3
CATCGATCGCATGCTACTGACTGCATGCTCGCGCCCCCCCCGATGAC…

# The FASTQ format

@ILLUMINA-F6C19_0048_FC:5:1:12440:1460#0/1
GTAGAACTGGTACGGACAAGGGGAATCTGACTGTAG
+ILLUMINA-F6C19_0048_FC:5:1:12440:1460#0/1
hhhhhhhhhhhghhhhhhhhehhhedhhhhfhhhhhh

# The FASTQ format

Sequence ID

@ILLUMINA-F6C19_0048_FC:5:1:12440:1460#0/1
GTAGAACTGGTACGGACAAGGGGAATCTGACTGTAG
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hhhhhhhhhhhghhhhhhhhehhhedhhhhfhhhhhh

# The FASTQ format

+ description (or empty)

@ILLUMINA-F6C19_0048_FC:5:1:12440:1460#0/1
GTAGAACTGGTACGGACAAGGGGAATCTGACTGTAG
+ILLUMINA-F6C19_0048_FC:5:1:12440:1460#0/1
hhhhhhhhhhhghhhhhhhhehhhedhhhhfhhhhhh

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@ILLUMINA-F6C19_0048_FC:5:1:12440:1460#0/1
GTAGAACTGGTACGGACAAGGGGAATCTGACTGTAG
+
hhhhhhhhhhhghhhhhhhhehhhedhhhhfhhhhhh

# The FASTQ format

Quality score of each base

@ILLUMINA-F6C19_0048_FC:5:1:12440:1460#0/1
GTAGAACTGGTACGGACAAGGGGAATCTGACTGTAG
+ILLUMINA-F6C19_0048_FC:5:1:12440:1460#0/1
hhhhhhhhhhhghhhhhhhhehhhedhhhhfhhhhhh

# Illumina Sequence ID Lines: A Decoder

| @M01137:30:000000000-AA299:1:1101:10929:1966 | |
|---|---|
| M01137 | the unique instrument name |
| 30 | the run id |
| 000000000-AA29 | the flowcell id |
| 1 | flowcell lane |
| 1101 | tile number within the flowcell lane |
| 10929 | 'x'-coordinate of the cluster within the tile |
| 1966 | 'y'-coordinate of the cluster within the tile |
| 1 or 2 (not shown, optional) | the member of a pair, 1 or 2 *(paired-end or mate-pair reads only)* |
| ATCACG (not shown, optional) | index sequence |

# Quality Scores

- Phred Score
- $Q = -10*\log_{10}P$ P = probability the base call is incorrect
- ASCII (character) - 33

| Phred Quality Score | Probability of incorrect base call | Base call accuracy |
|---|---|---|
| 0 | 1 | 0 % |
| 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 % |
| 93 | 1 in 2000000000 | 99.9999995 % |

# Why QC NGS Data?

OPEN ACCESS Freely available online

PLOS ONE

## An Extensive Evaluation of Read Trimming Effects on Illumina NGS Data Analysis

Cristian Del Fabbro[1☯], Simone Scalabrin[2☯], Michele Morgante[1], Federico M. Giorgi[1,3*]

"Trimming is shown to increase the quality and reliability of the analysis, with concurrent gains in terms of execution time and computational resources needed"

# Types of Trimming

## Quality

- remove low quality bases and reads
  - Q20 (1% error) and Q30 (0.1% error) are standard
- Remove too short reads
- Too many ‘N’ (uncalled bases)

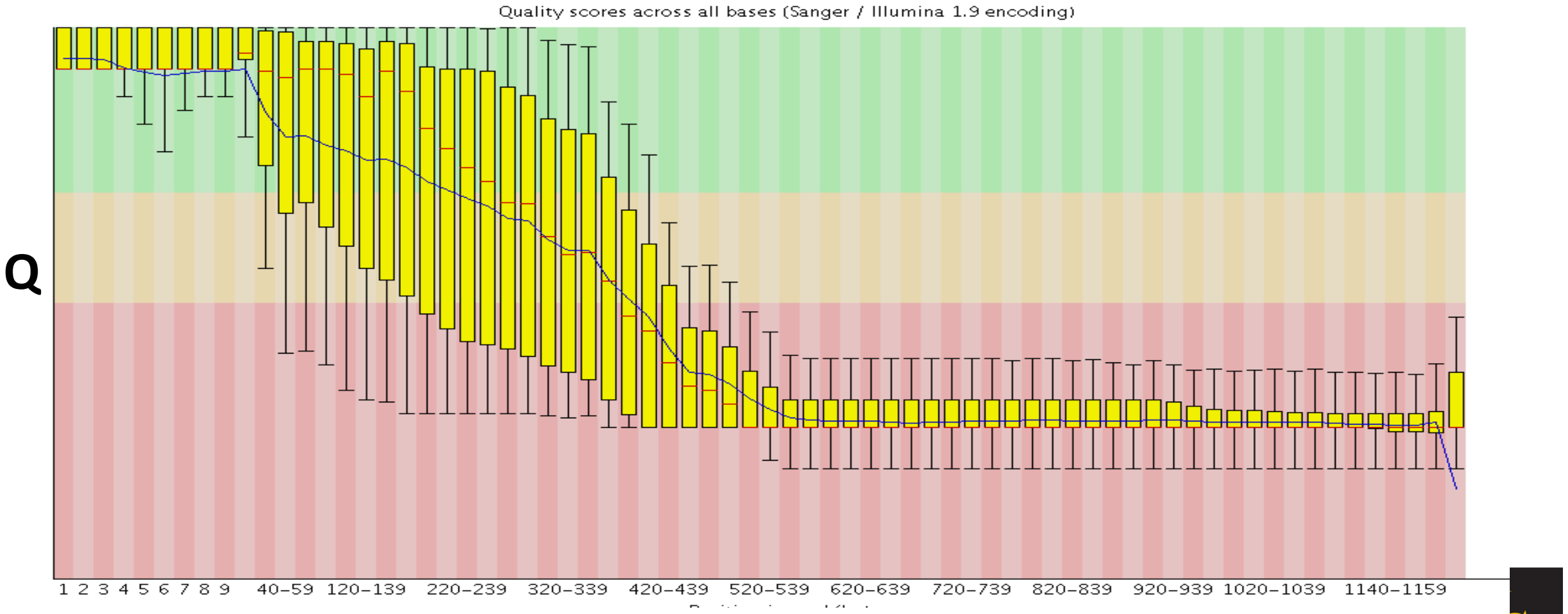
## Complexity

- simple repeats (e.g. TGTGTGTGTGTG)
- Homopolymers (e.g., AAAAAAAA)

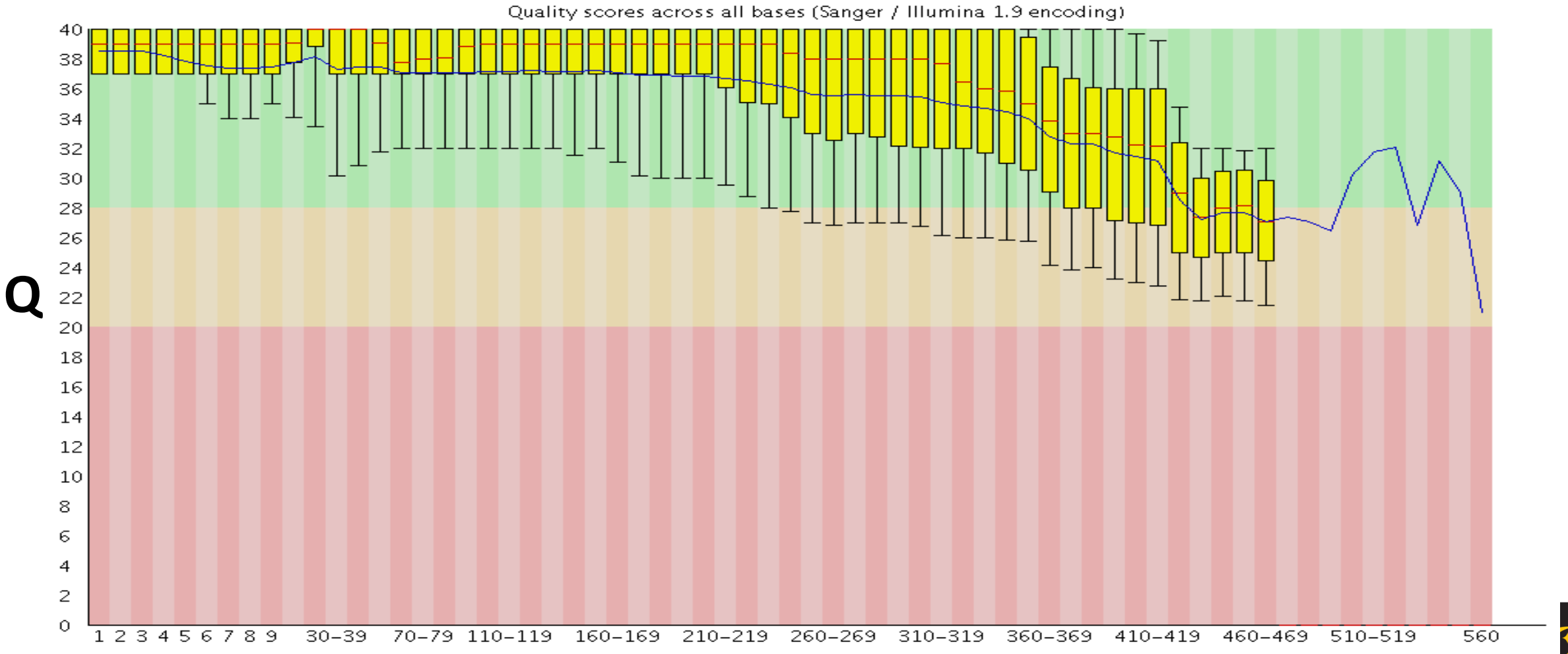
## Contamination

- Sequencing adapters!!!!!!
- lab contamination (human, bacteria)
- environmental

# Low Quality Sequences Before Trimming (Puma 454 sequences)



# Same Sequences After Trimming (Puma 454 sequences)



# Types of Trimming

- High Quality
- Low quality

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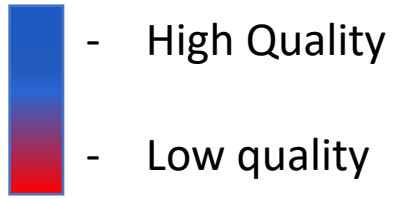
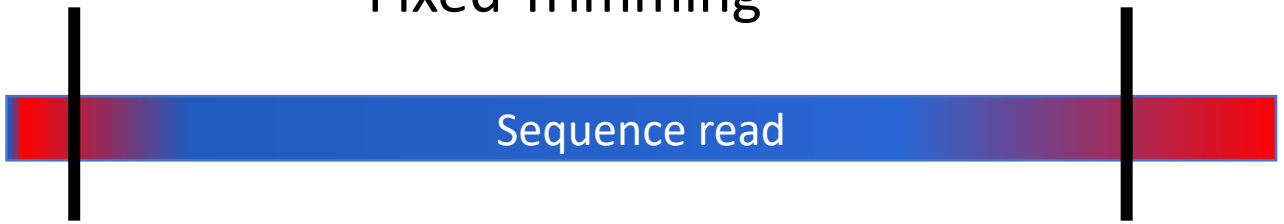


- High Quality
- Low quality

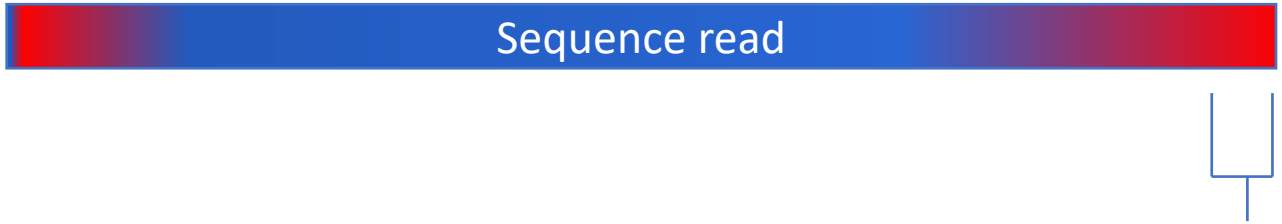
Fixed Trimming



# Types of Trimming

- High Quality
- Low quality

Sliding Window Trimming



# Types of Trimming

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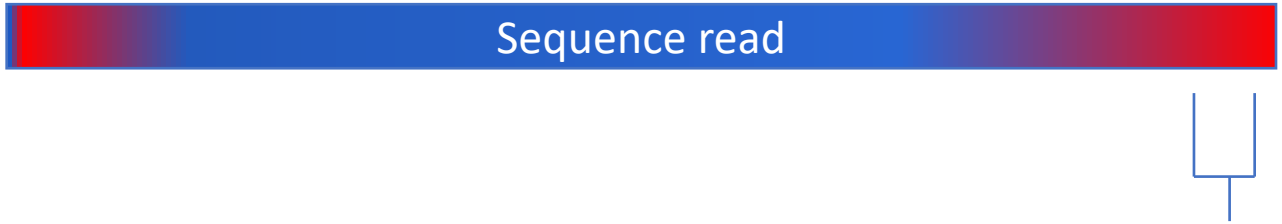
Sliding Window Trimming



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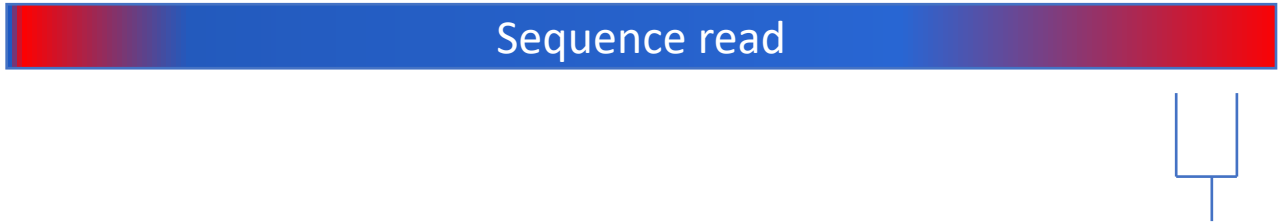
Sliding Window Trimming



# Types of Trimming

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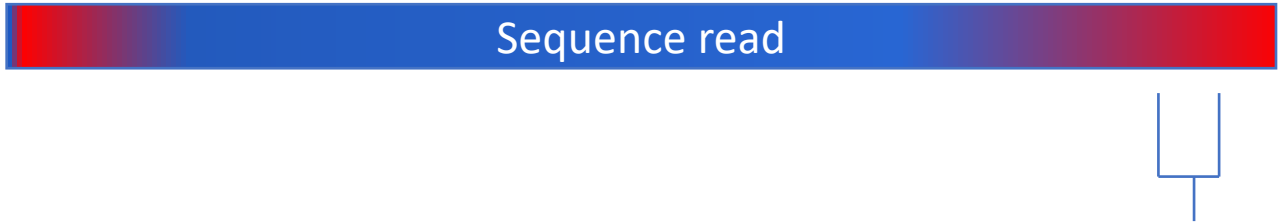
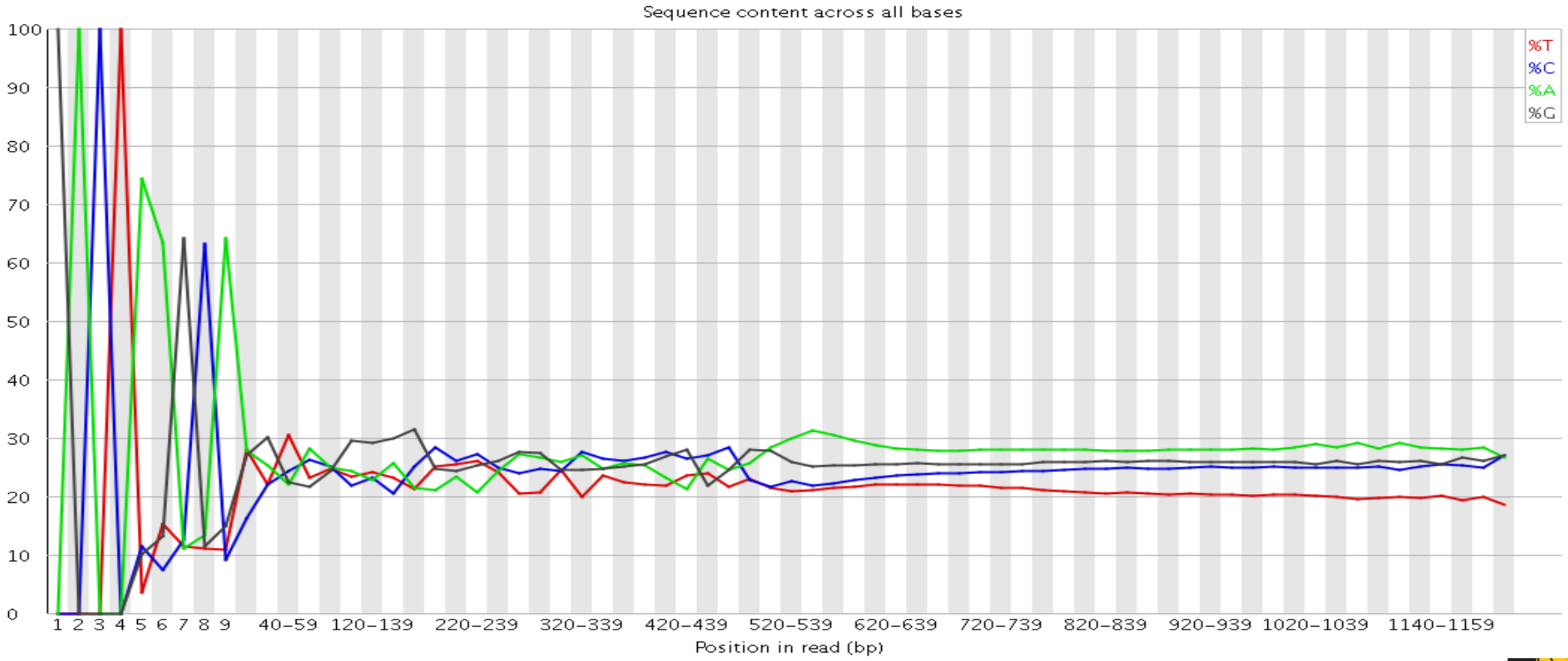
Sliding Window Trimming



# Adapter Contamination



# Adapter Contamination

## Overrepresented sequences

| Sequence | Count | Percentage | Possible Source |
|---|---|---|---|
| GACTAAGCAGTGGTATCAACGCAGAGTACATGGGGACACTTGTTTCTGAC | 19391 | 5.415739186535921 | No Hit |
| GACTAAGCAGTGGTATCAACGCAGAGTACATGGGGACACTTGCTTCTGAC | 11325 | 3.162974900083508 | No Hit |
| GACTAAGCAGTGGTATCAACGCAGAGTACATGGGACACTTGTTTCTGACA | 9229 | 2.5775801636088915 | No Hit |

Download Graphics

gnl|uv|NGB00593.1:1-30 Evrogen Mint PlugOligo-1 adapter

**Sequence ID:** **Length:** 30 **Number of Matches:** 1

**Range 1: 1 to 25** Graphics Next Match Previous Match

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 50.6 bits(25) | 0.001 | 25/25(100%) | 0/25(0%) | Plus/Plus |

```
Query  5   AAGCAGTGGTATCAACGCAGAGTAC  29
           |||||||||||||||||||||||||
Sbjct  1   AAGCAGTGGTATCAACGCAGAGTAC  25
```

# Error Correction (Illumina data)

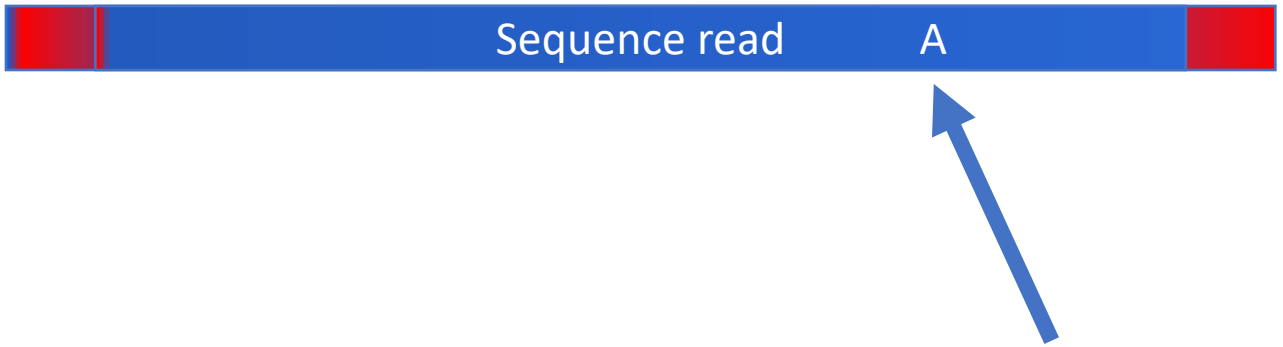
## GAGE: A critical evaluation of genome assemblies and assembly algorithms

Steven L. Salzberg,[1,7] Adam M. Phillippy,[2] Aleksey Zimin,[3] Daniela Puiu,[1] Tanja Magoc,[1] Sergey Koren,[2,4] Todd J. Treangen,[1] Michael C. Schatz,[5] Arthur L. Delcher,[6] Michael Roberts,[3] Guillaume Marçais,[3] Mihai Pop,[4] and James A. Yorke[3]

"For all four genomes and for all eight assemblers used in GAGE, the best assemblies were created from reads that had been processed through extensive error correction routines"

Illumina Sequencing Errors: ~0.1 - 1%,  Substitution errors

# Error Correction




High quality base, but an Error

# Error Correction: *K*-mer Counting

k = 4

AGCTGTGG

# Error Correction: *K*-mer Counting

k = 4

AGCTGTGG

AGCT

# Error Correction: *K*-mer Counting

k = 4

AGCTGTGG

AGCT

GCTG

# Error Correction: *K*-mer Counting

k = 4

AGCTGTGG

AGCT

GCTG

CTGT

# Error Correction: *K*-mer Counting

k = 4

AGCTGTGG

AGCT

GCTG

CTGT

TGTG

# Error Correction: *K*-mer Counting

k = 4

AGCTGTGG

AGCT

GCTG

CTGT

TGTG

GTGG

# Error Correction: *K*-mer Counting

k = 6

AGCTGTGG

# Error Correction: *K*-mer Counting

k = 6

AGCTGTGG

AGCTGT

# Error Correction: *K*-mer Counting

k = 6

AGCTGTGG

AGCTGT
GCTGTG

# Error Correction: *K*-mer Counting

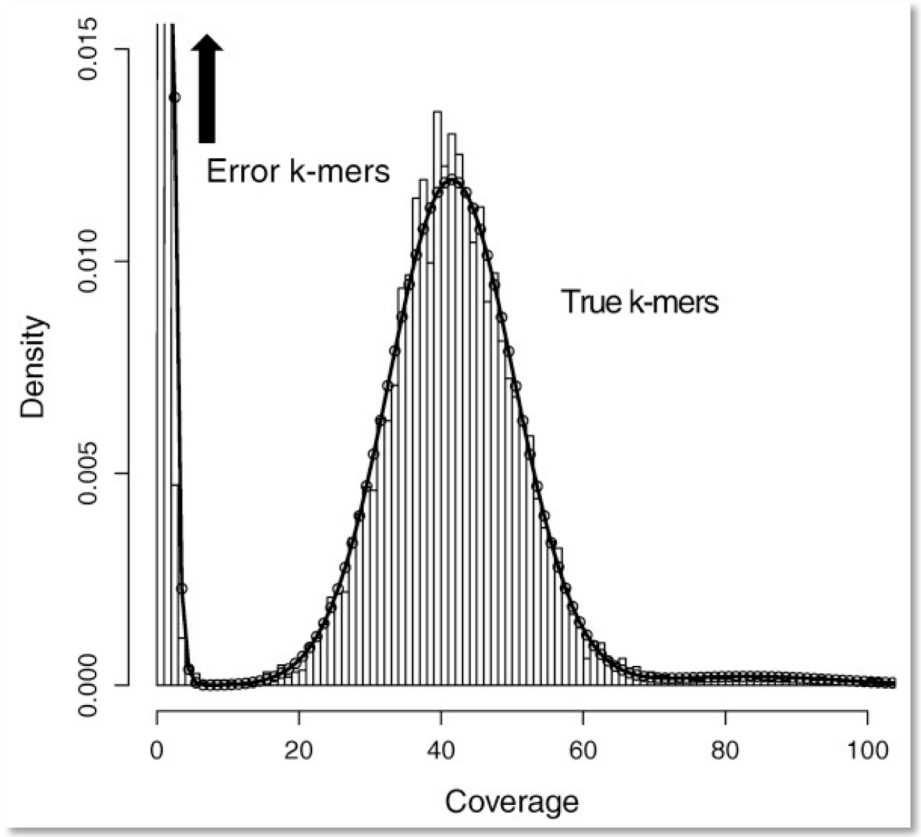
k = 6

AGCTGTGG

AGCTGT
GCTGTG
CTGTGG

# Error Correction: *K*-mer Counting

- Expected Distribution of k-mer frequency

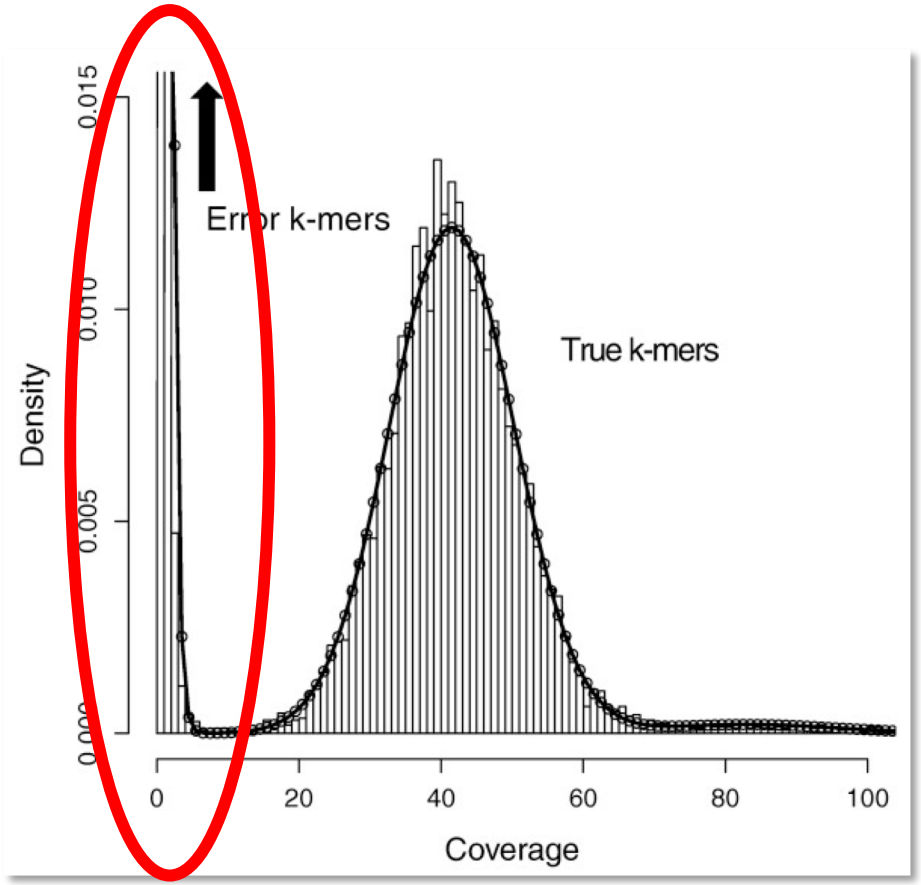



DSK; Rizk et al. 2013

# Error Correction: *K*-mer Counting

- Expected Distribution of k-mer frequency

Corrected

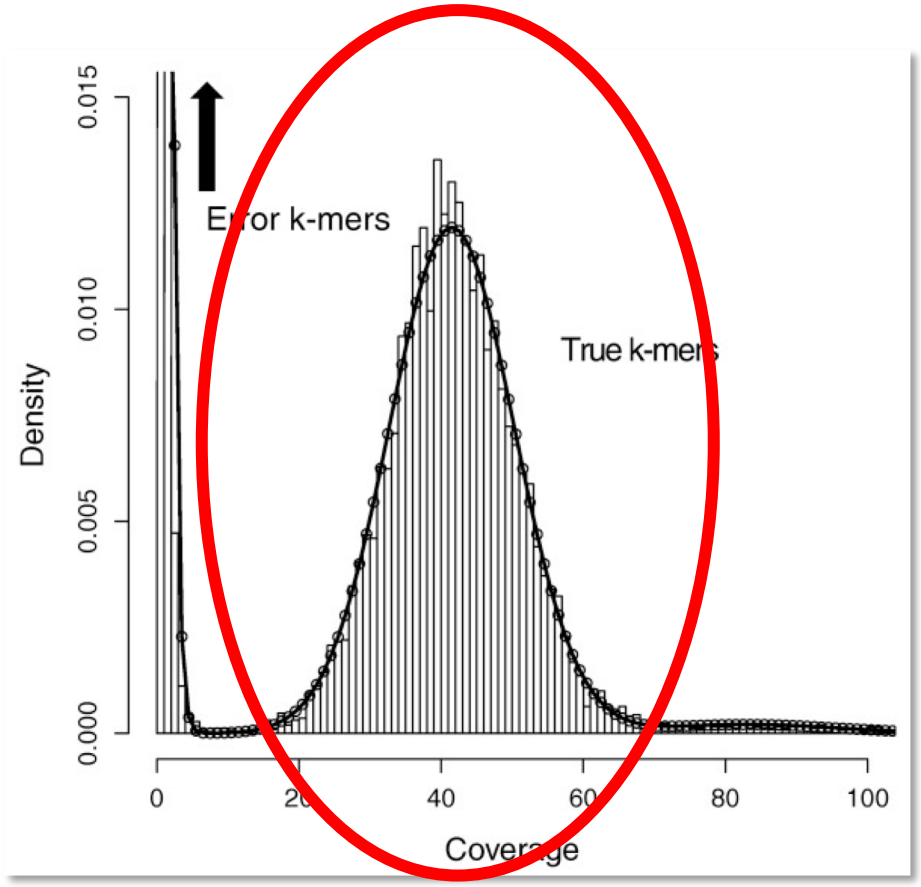



DSK; Rizk et al. 2013

# Error Correction: *K*-mer Counting

- Expected Distribution of k-mer frequency

Estimate genome size




DSK; Rizk et al. 2013

# Error Correction: *K*-mer Counting

- Expected Distribution of k-mer frequency

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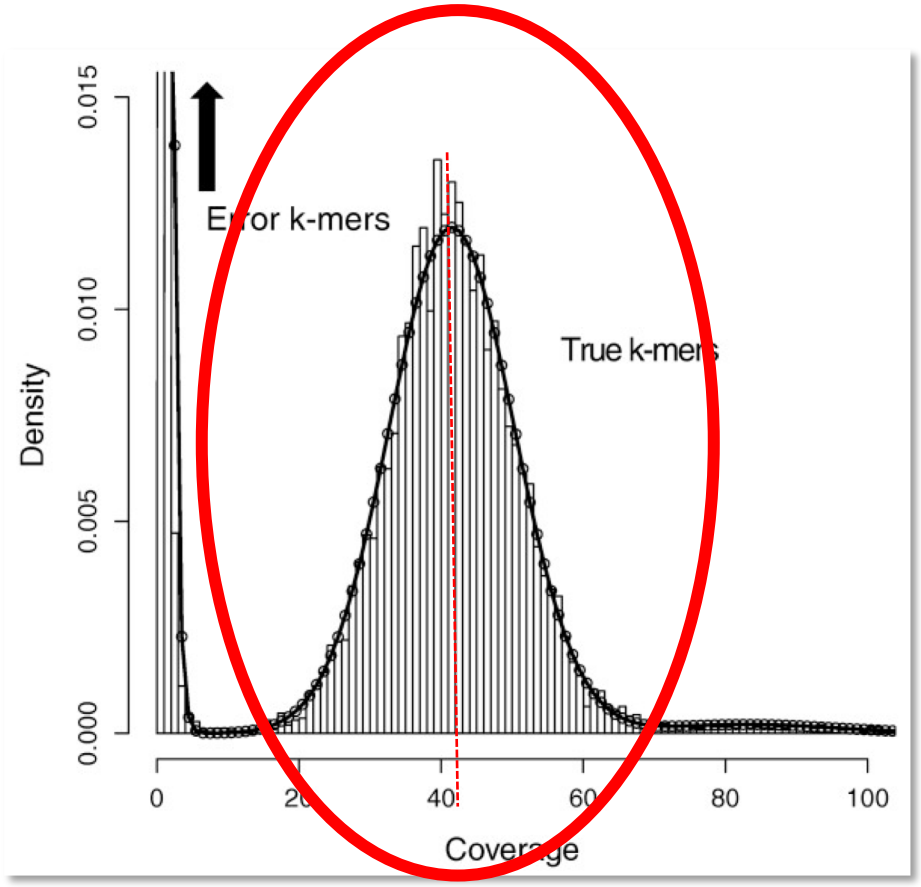


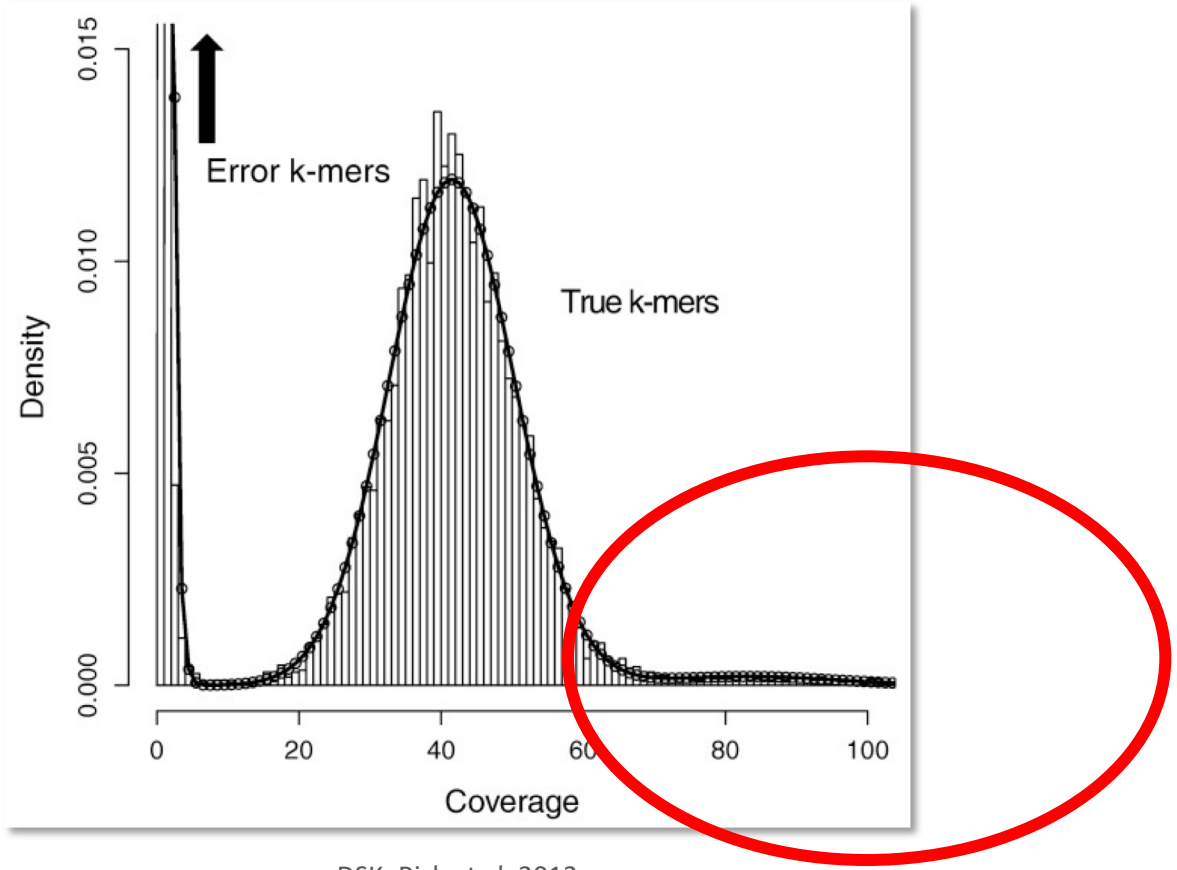

DSK; Rizk et al. 2013

G = C / P
G = genome size
C = total count of true k-mers
P = peak coverage

# Error Correction: *K*-mer Counting

- Expected Distribution of k-mer frequency

Estimate repetitive content




DSK; Rizk et al. 2013

# Recap: NGS QC

- Remove low quality bases and reads
- Identify and remove adapter contamination
- Optional: Correct substitution sequencing errors
- Optional: De-duplication

# To Your Terminals!