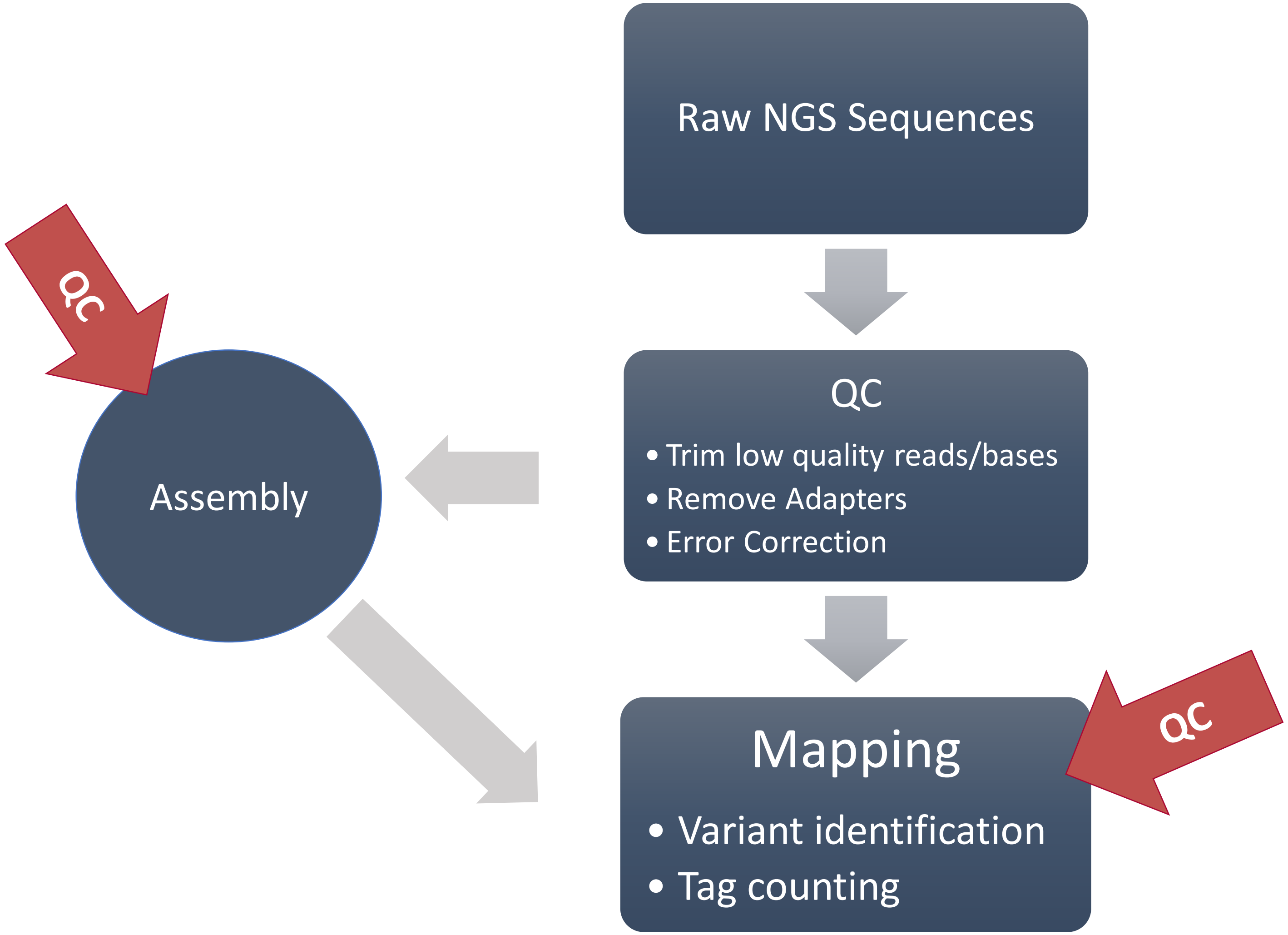
# NGS QC

An introduction

# Outline

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

# The Big Picture



Raw NGS Sequences

QC

- Trim low quality reads/bases
- Remove Adapters
- Error Correction

Assembly

Mapping

- Variant identification
- Tag counting

# 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

# Common Sequence Formats

## FASTA

- Simple
- Nucleotide or amino acid strings
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- 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

# The FASTA format

```
>sequence 1
CATCGATCGCATGCTACTGACTG
CATGCTCGCGCCCCCCCGATG
>sequence 2
ACTGACTCGCGCGCGGGGG
GAGCTGATGTG
>sequence 3
CATCGATCGCATGCTACTGACTG
CATGCTCGCGCCCCCCCGATG
ACTGACTCGCGCGCGGGGG
GAGCTGATGTG
```

# The FASTA format

```
>sequence 1
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ACTGACTCGCGCGCGGGGG
GAGCTGATGTG
```

“*interleaved*”

# The FASTA format

```
>sequence 1
CATCGATCGCATGCTACTGACTG
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>sequence 2
ACTGACTCGCGCGCGGGGG
GAGCTGATGTG
>sequence 3
CATCGATCGCATGCTACTGACTG
CATGCTCGCGCCCCCCCGATG
ACTGACTCGCGCGCGGGGG
GAGCTGATGTG
```

# The FASTA format

“*non-interleaved*”

```
>sequence 1
CATCGATCGCATGCTACTGACTGCATGCTCGCGCCCCCCCCGATG……
>sequence 2
ACTGACTCGCGCGCGGGGGGAGCTGATGTG
>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
hhhhhhhhhhhghhhhhhehhhedhhhhfhhhhhh
```

# The FASTQ format

Sequence ID

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

# The FASTQ format

**Sequence**

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

# 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
hhhhhhhhhhghhhhhhhhehhhedhhhhfhhhhhh
```

# The FASTQ format

+ description (or empty)

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

# 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
hhhhhhhhhhhghhhhhhhehhhedhhhhfhhhhhh
```

# Illumina Sequence ID Lines: A Decoder

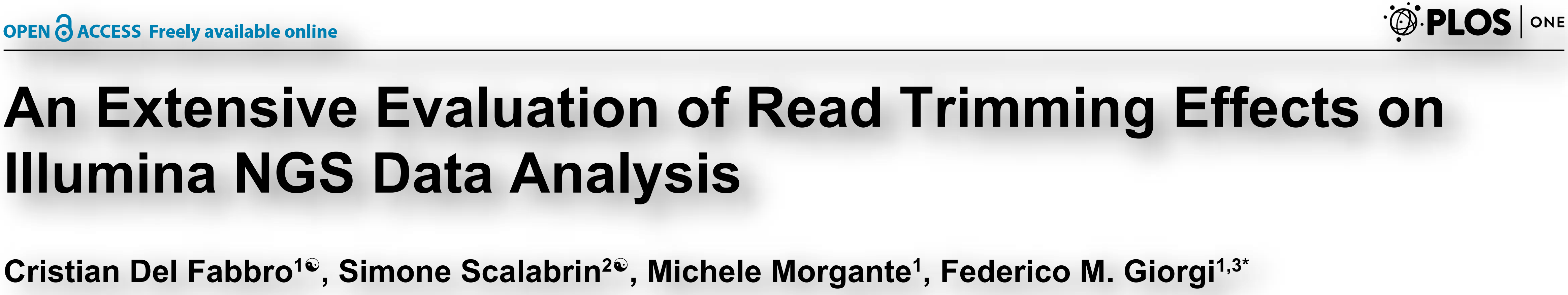
| **@M01137:30:000000000-AA299:1:1101:10929:1966** | |
|---|---|
| M01137 | the unique instrument name |
| 30 | the run id |
| 000000000-AA29 | the flowcell id |
| 1 | flowcell lane |
| 1101 | tile number within the flowcell lane |
| 10929 | 'x'-coordinate of the cluster within the tile |
| 1966 | 'y'-coordinate 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)

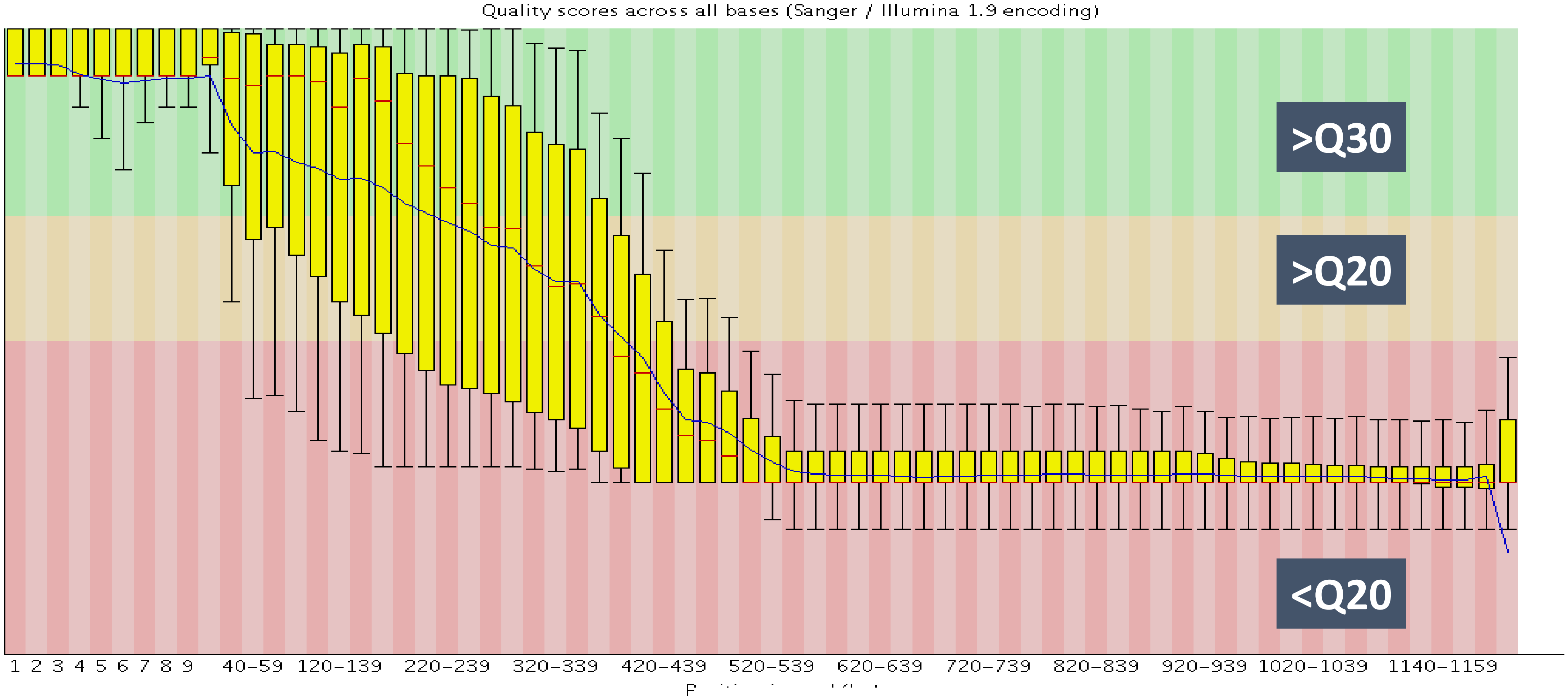
### Complexity

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

### Contamination

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

# Low Quality Sequences Before Trimming (Puma 454 sequences)



Q

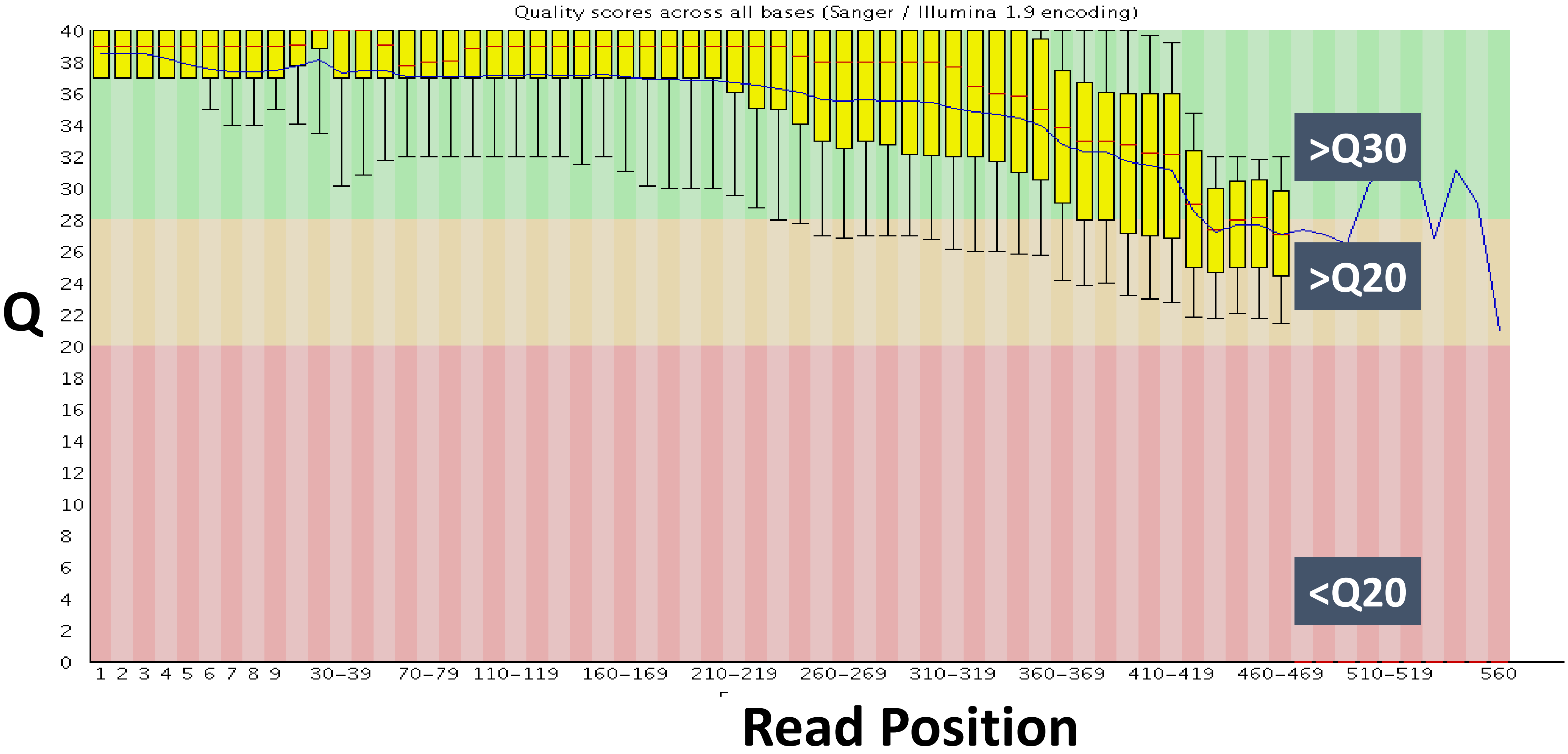
>Q30

>Q20

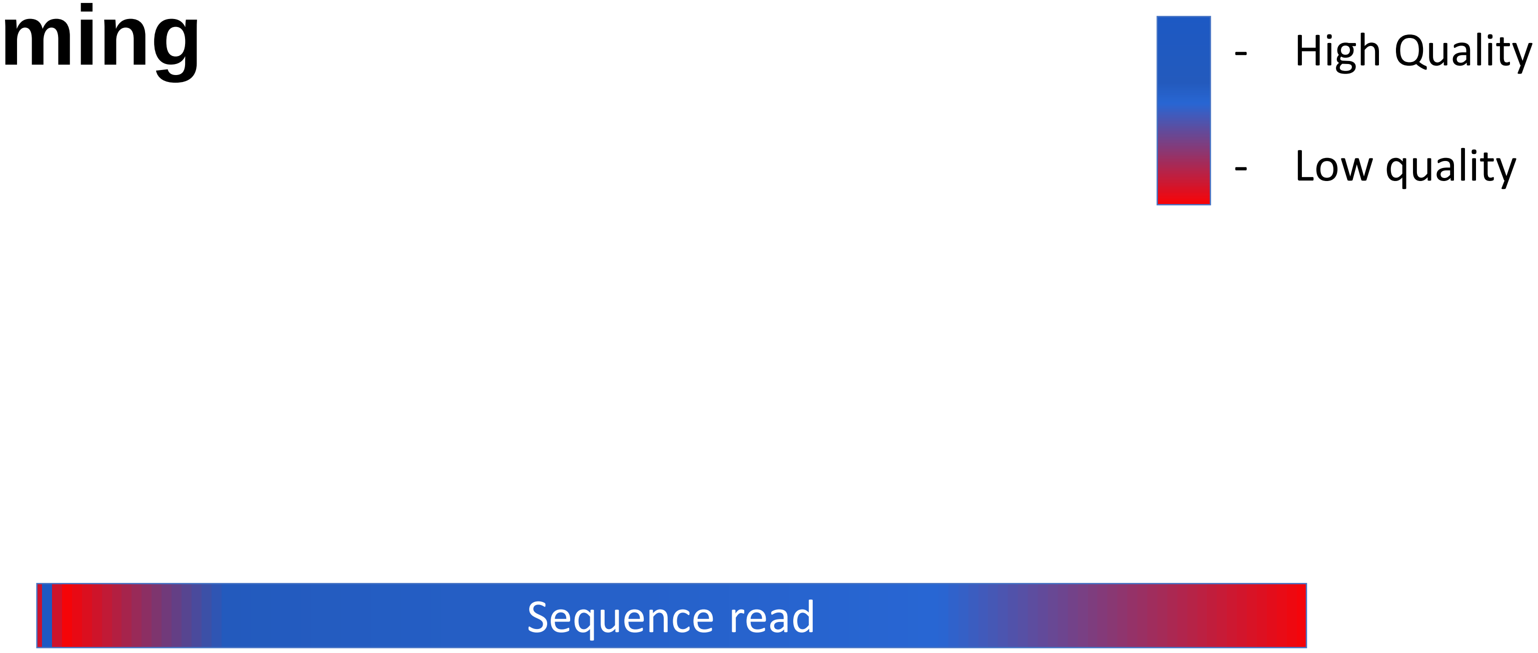
<Q20

Read Position

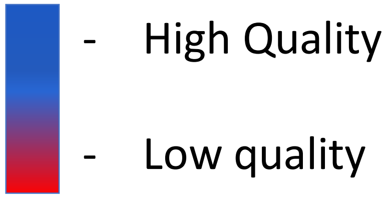
# Same Sequences After Trimming (Puma 454 sequences)

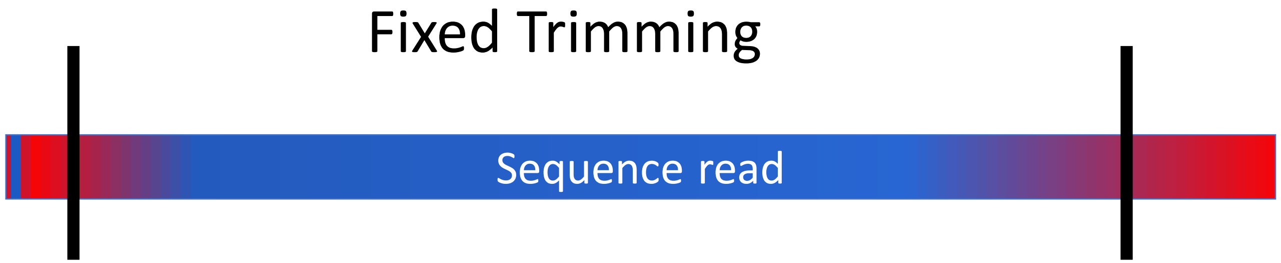


# Types of Trimming



# Types of Trimming

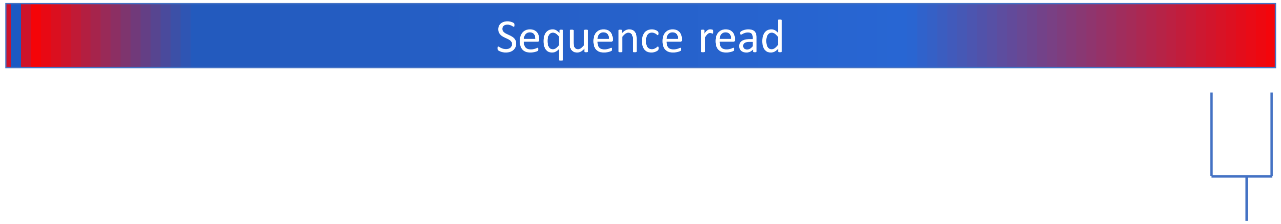




# Types of Trimming

- High Quality
- Low quality

Sliding Window Trimming

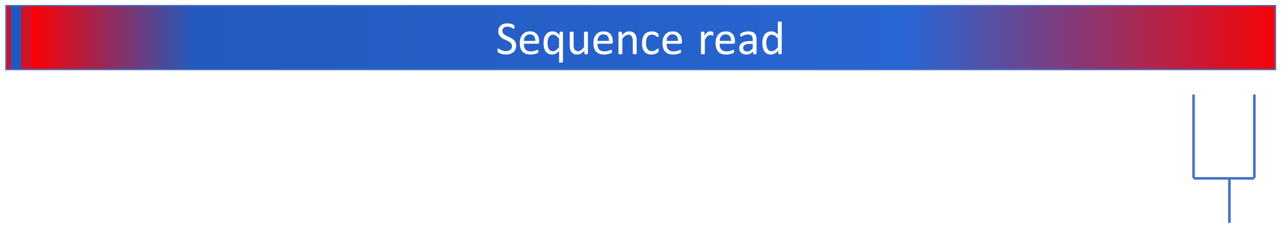

Sequence read

# Types of Trimming

🟦 - High Quality

🟥 - Low quality

## Sliding Window Trimming

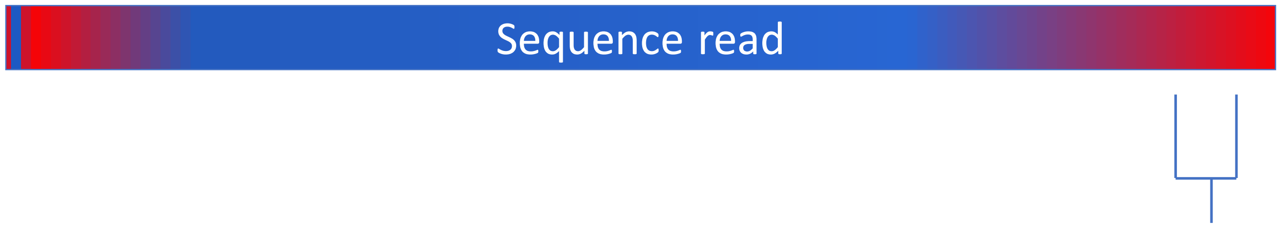


# Types of Trimming

- High Quality

- Low quality

## Sliding Window Trimming

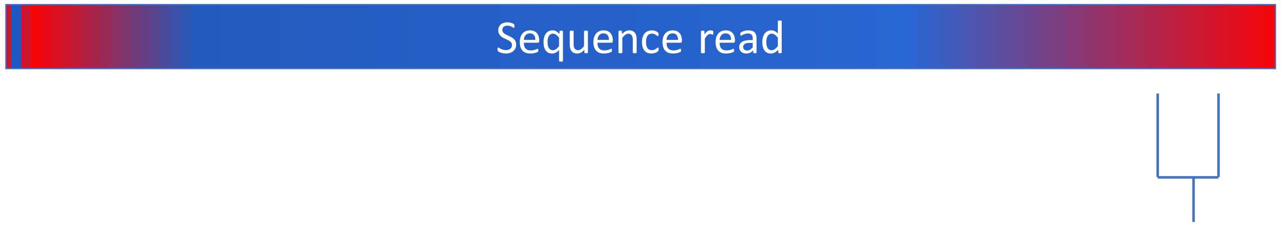


# Types of Trimming

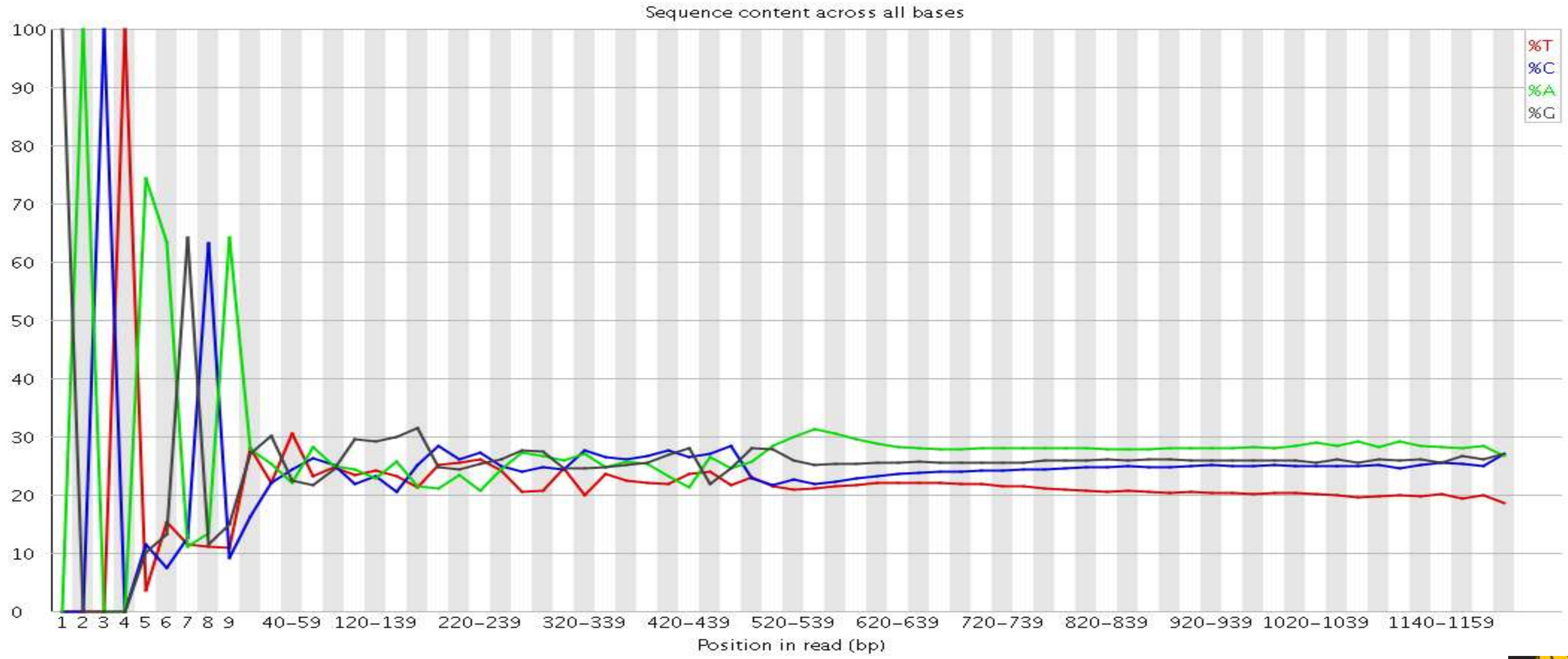
- High Quality

- Low quality

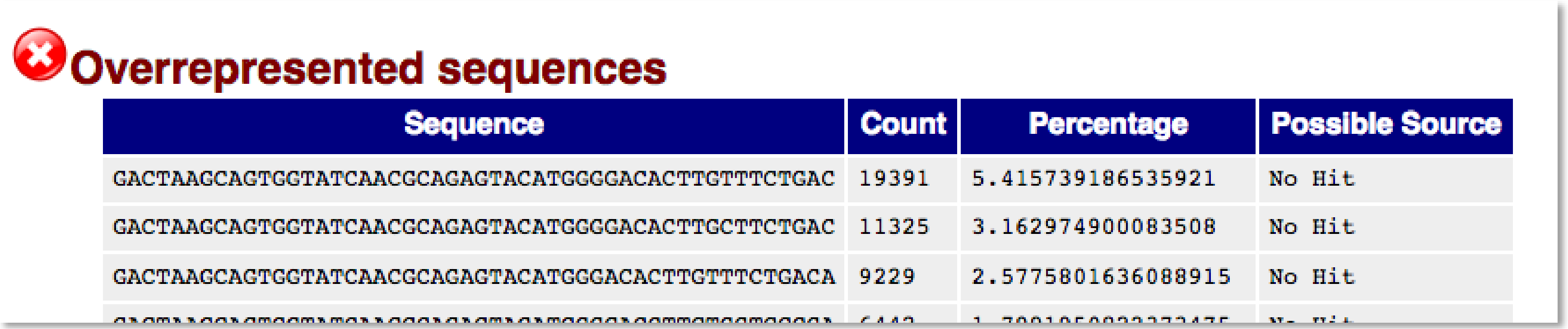
Sliding Window Trimming



# Adapter Contamination

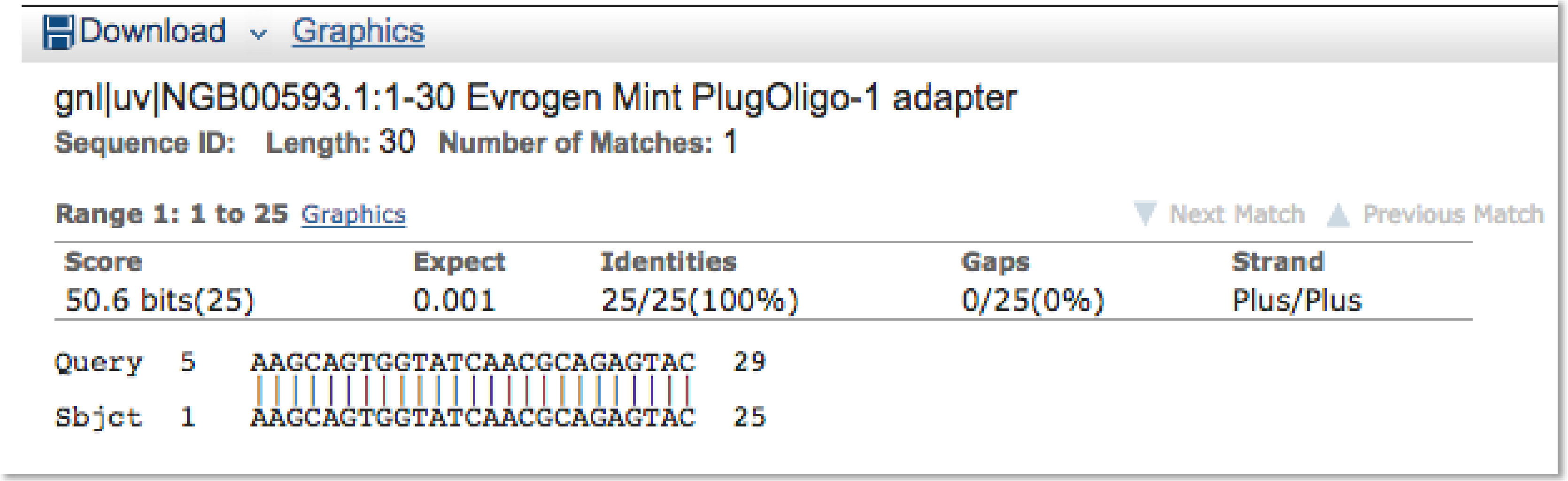


# Adapter Contamination



## 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 |
| [illegible] | 6442 | [illegible] | No Hit |



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

| 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)

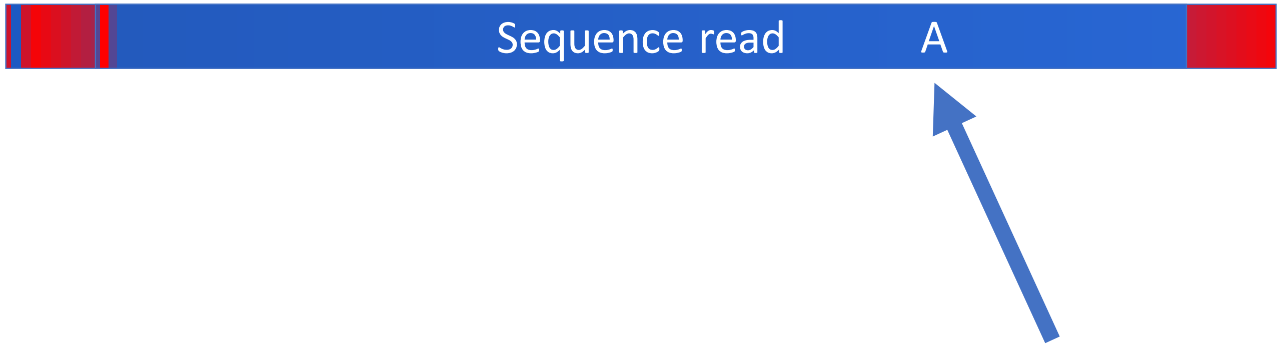
GAGE: A critical evaluation of genome assemblies and assembly algorithms

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

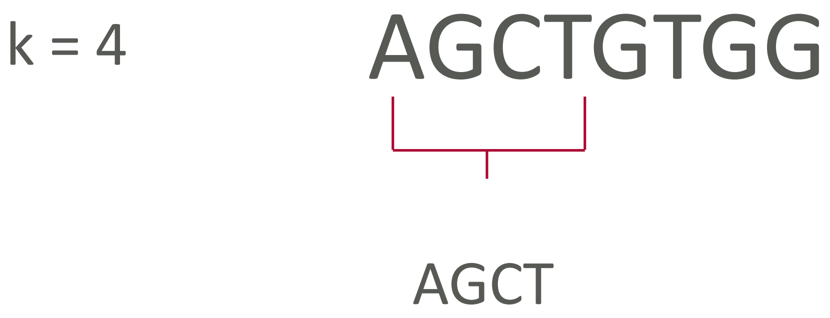


High quality base, but an Error

# Error Correction: *K*-mer Counting

k = 4 AGCTGTGG

# Error Correction: *K*-mer Counting



k = 4

AGCTGTGG

AGCT

# Error Correction: *K*-mer Counting

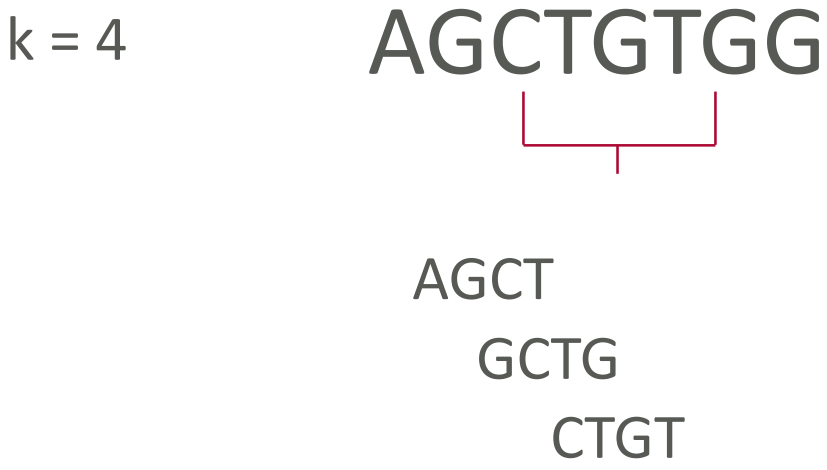
k = 4

AGCTGTGG

AGCT

GCTG

# Error Correction: *K*-mer Counting



k = 4

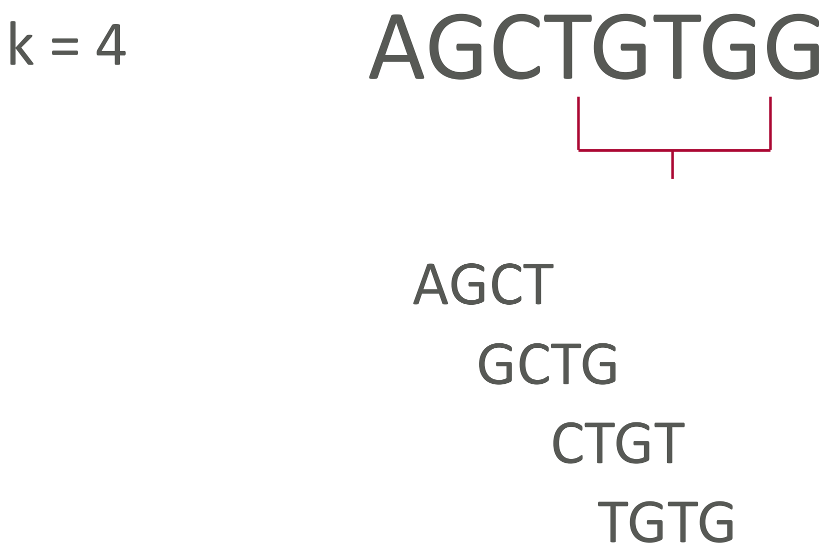
AGCTGTGG

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

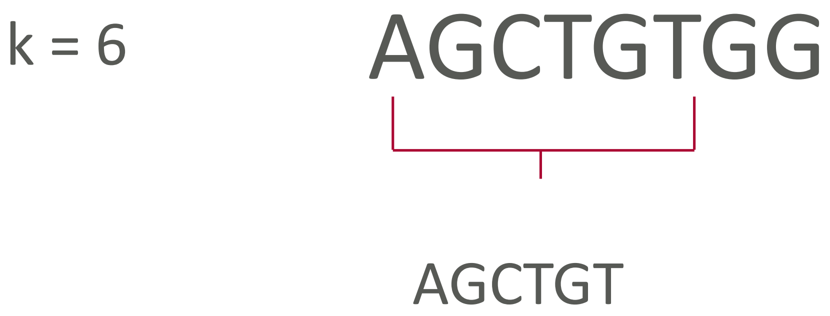
GTGG

# Error Correction: *K*-mer Counting

k = 6

AGCTGTGG

# Error Correction: *K*-mer Counting

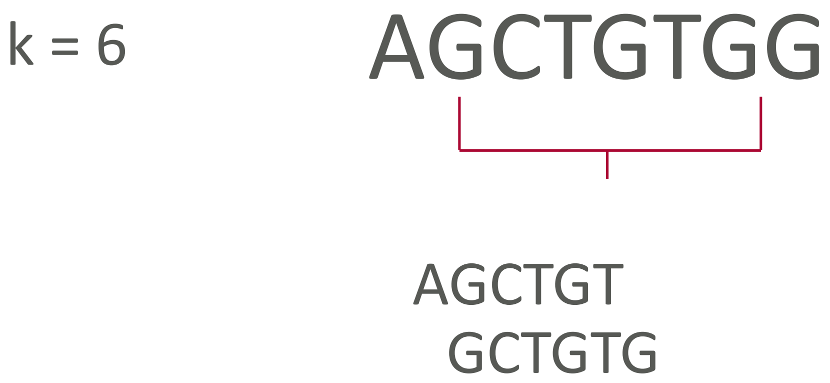


k = 6

AGCTGTGG

AGCTGT

# Error Correction: *K*-mer Counting

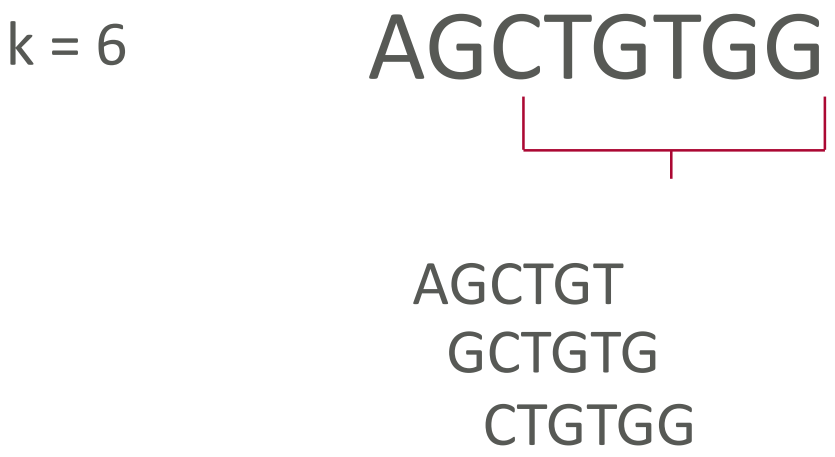


k = 6

AGCTGTGG

AGCTGT
GCTGTG

# Error Correction: *K*-mer Counting



k = 6

AGCTGTGG

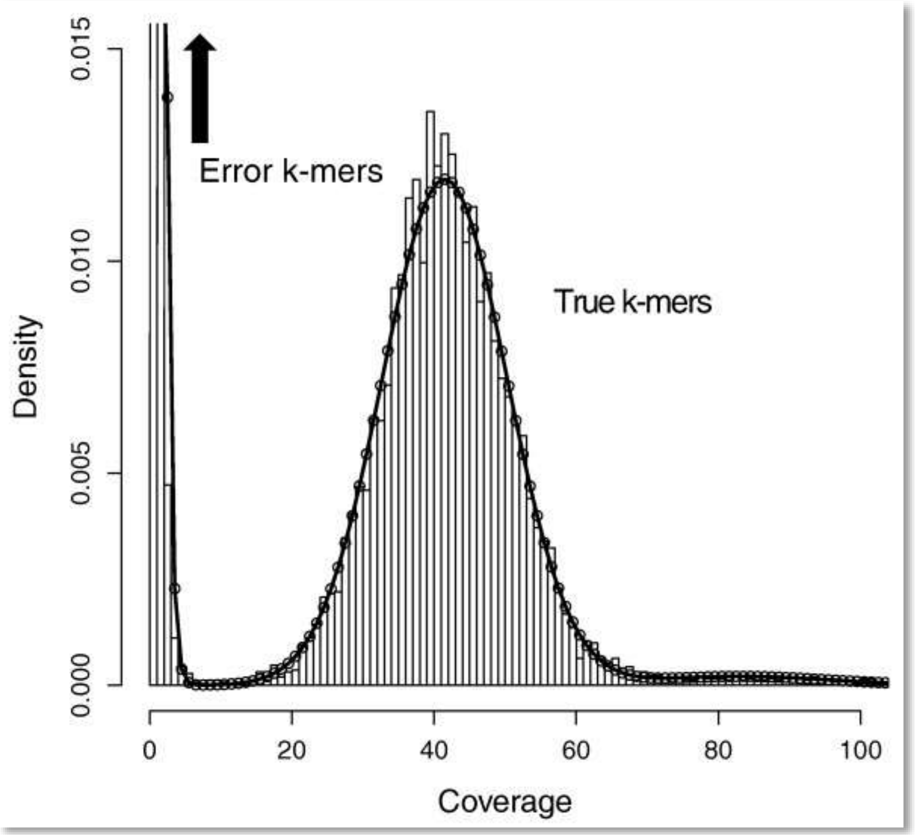
AGCTGT

GCTGTG

CTGTGG

# Error Correction: *K*-mer Counting

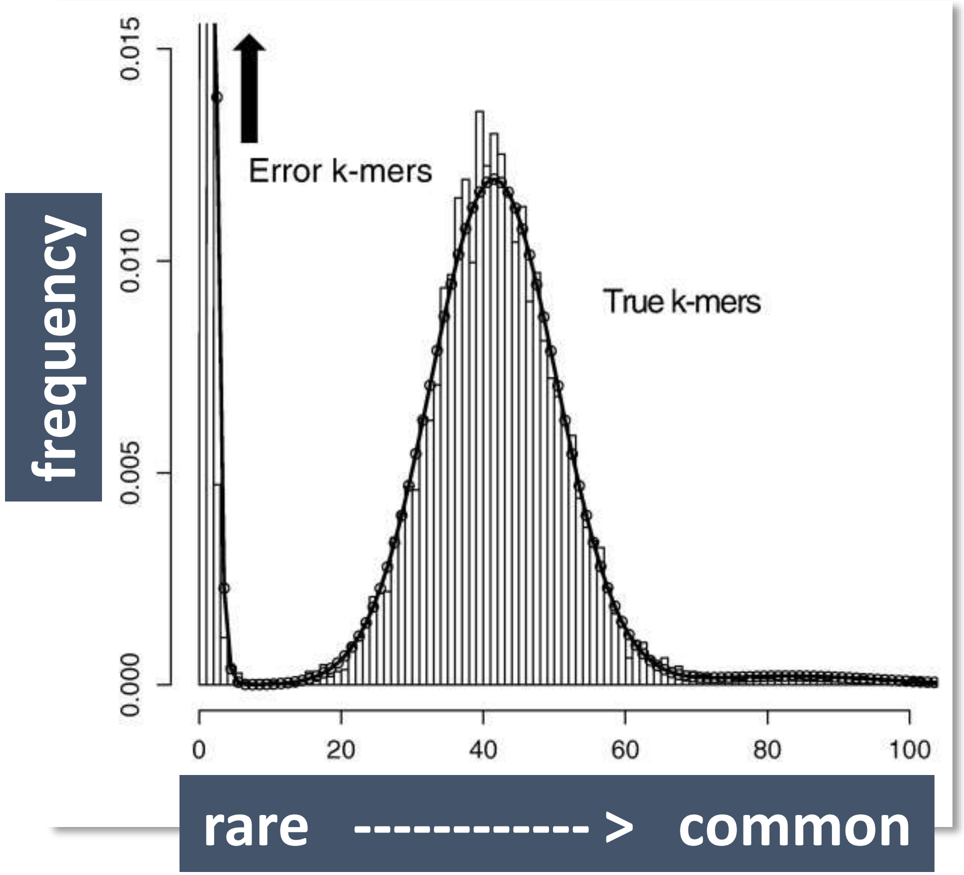
- Expected Distribution of k-mer frequency



DSK; Rizk et al. 2013

# Error Correction: *K*-mer Counting

- Expected Distribution of k-mer frequency

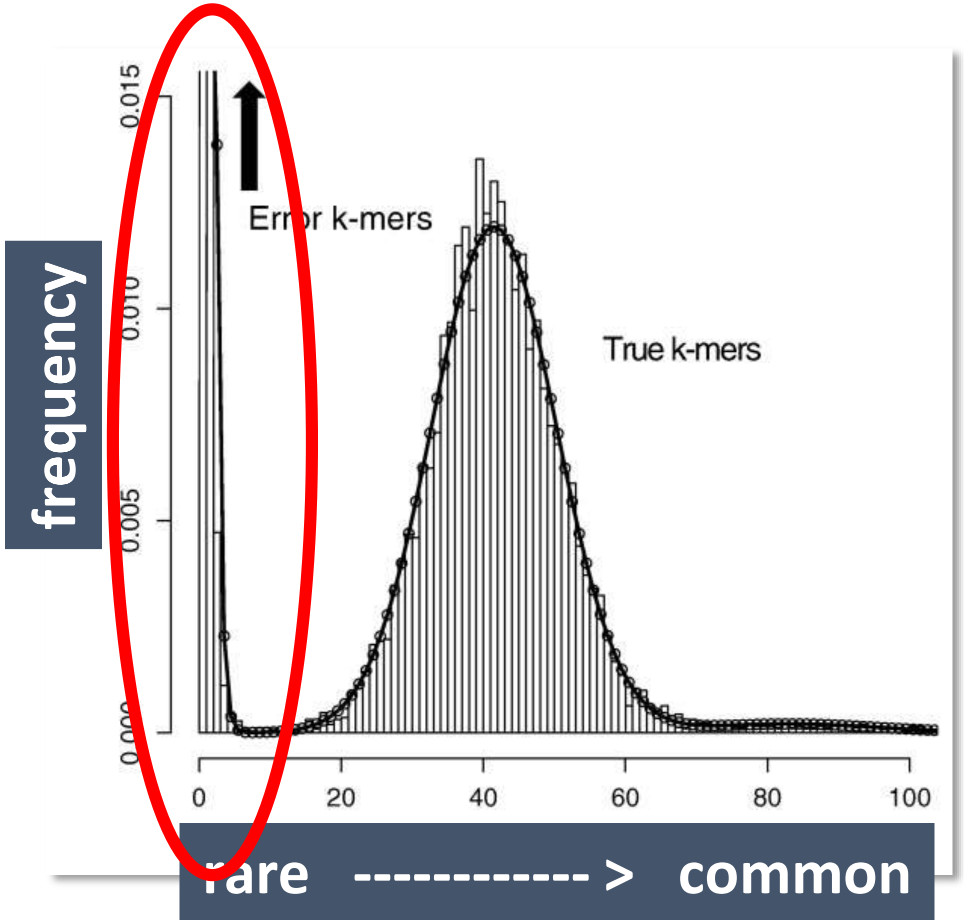


DSK; Rizk et al. 2013

# Error Correction: *K*-mer Counting

- Expected Distribution of k-mer frequency

Corrected

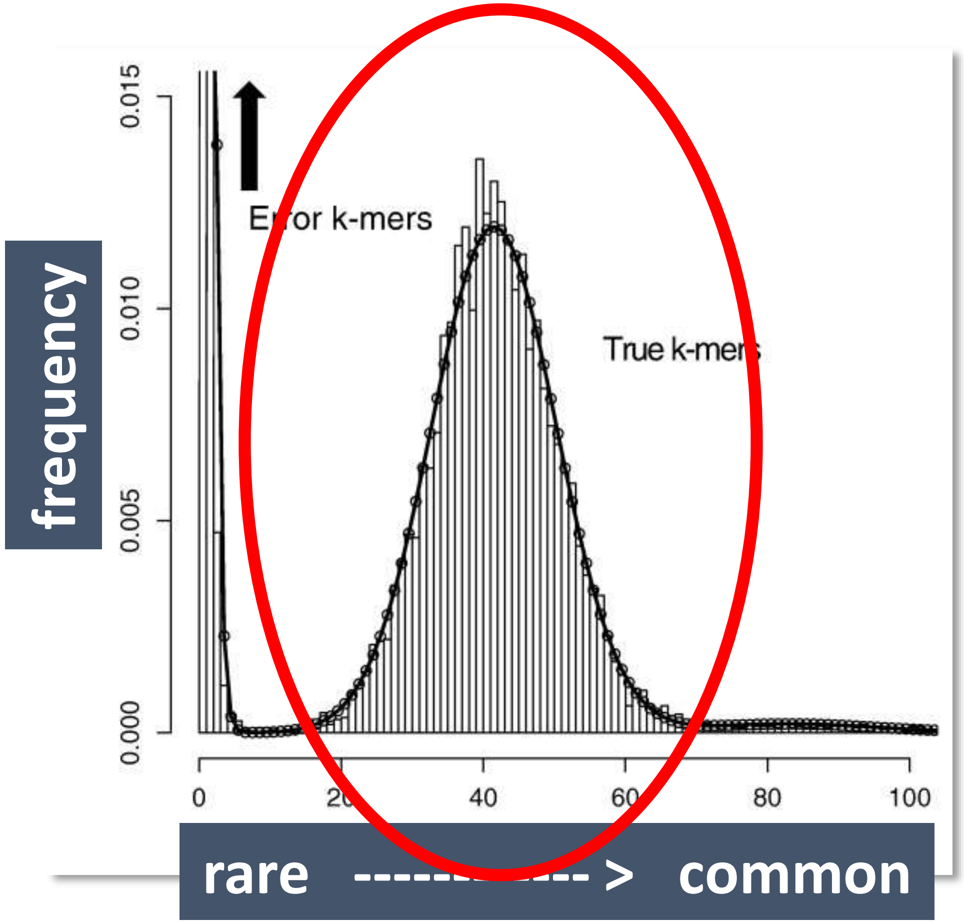


DSK; Rizk et al. 2013

# Error Correction: *K*-mer Counting

- Expected Distribution of k-mer frequency

**Estimate genome size**

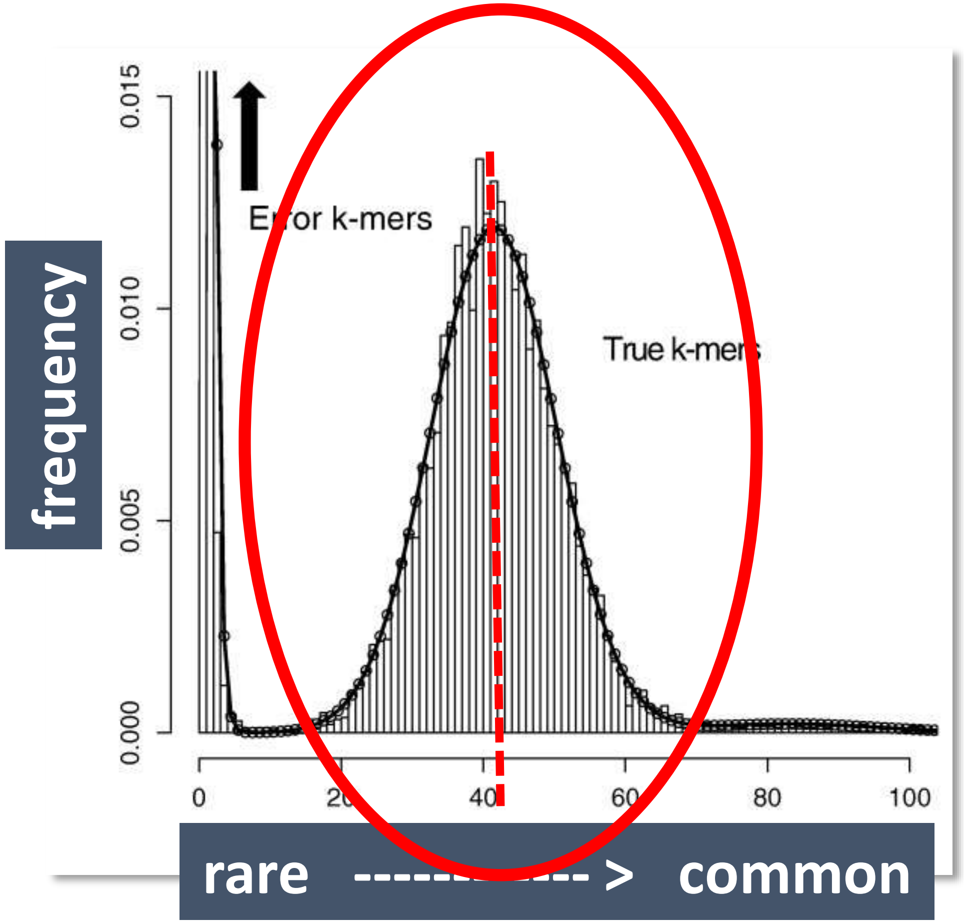


DSK; Rizk et al. 2013

# Error Correction: *K*-mer Counting

- Expected Distribution of k-mer frequency

Estimate genome size



DSK; Rizk et al. 2013

G = C / P

G = genome size

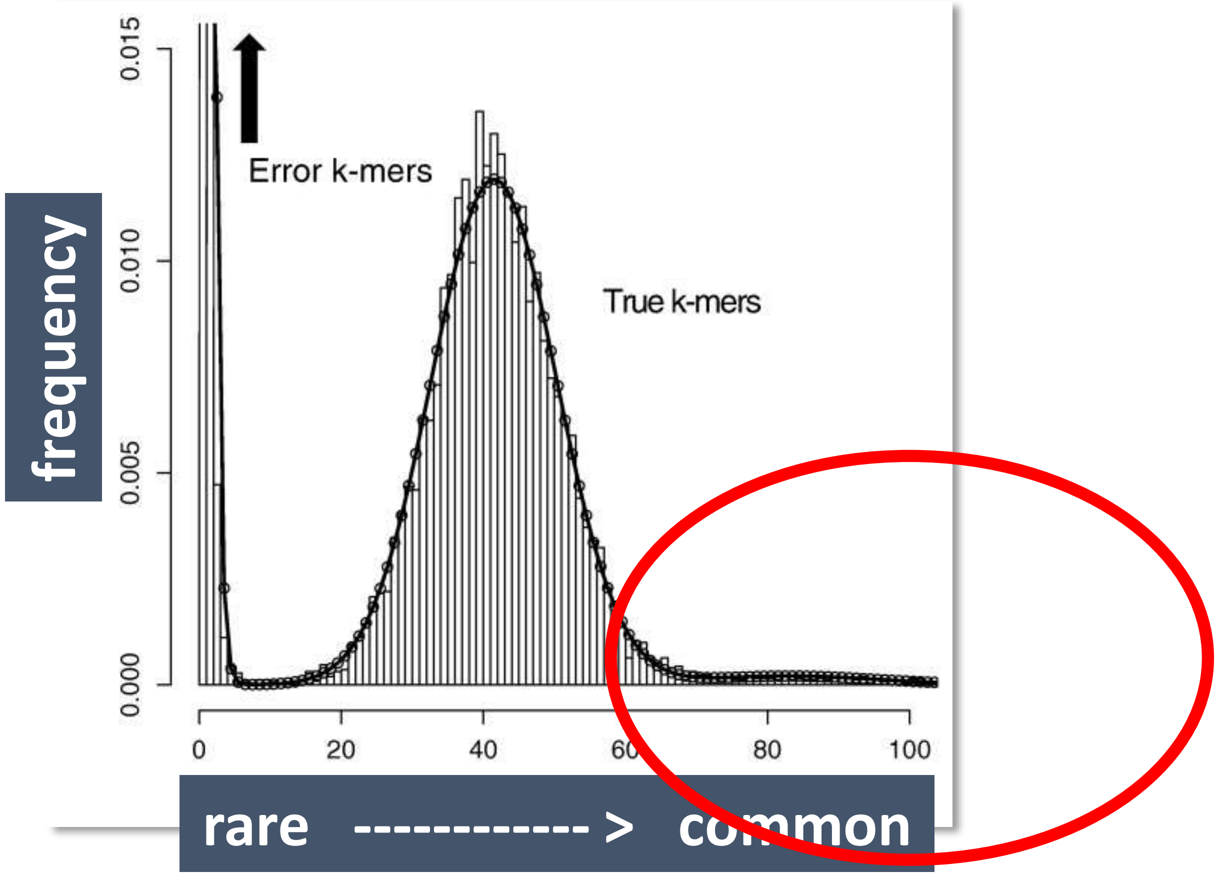
C = total count of true k-mers

P = peak coverage

# Error Correction: *K*-mer Counting

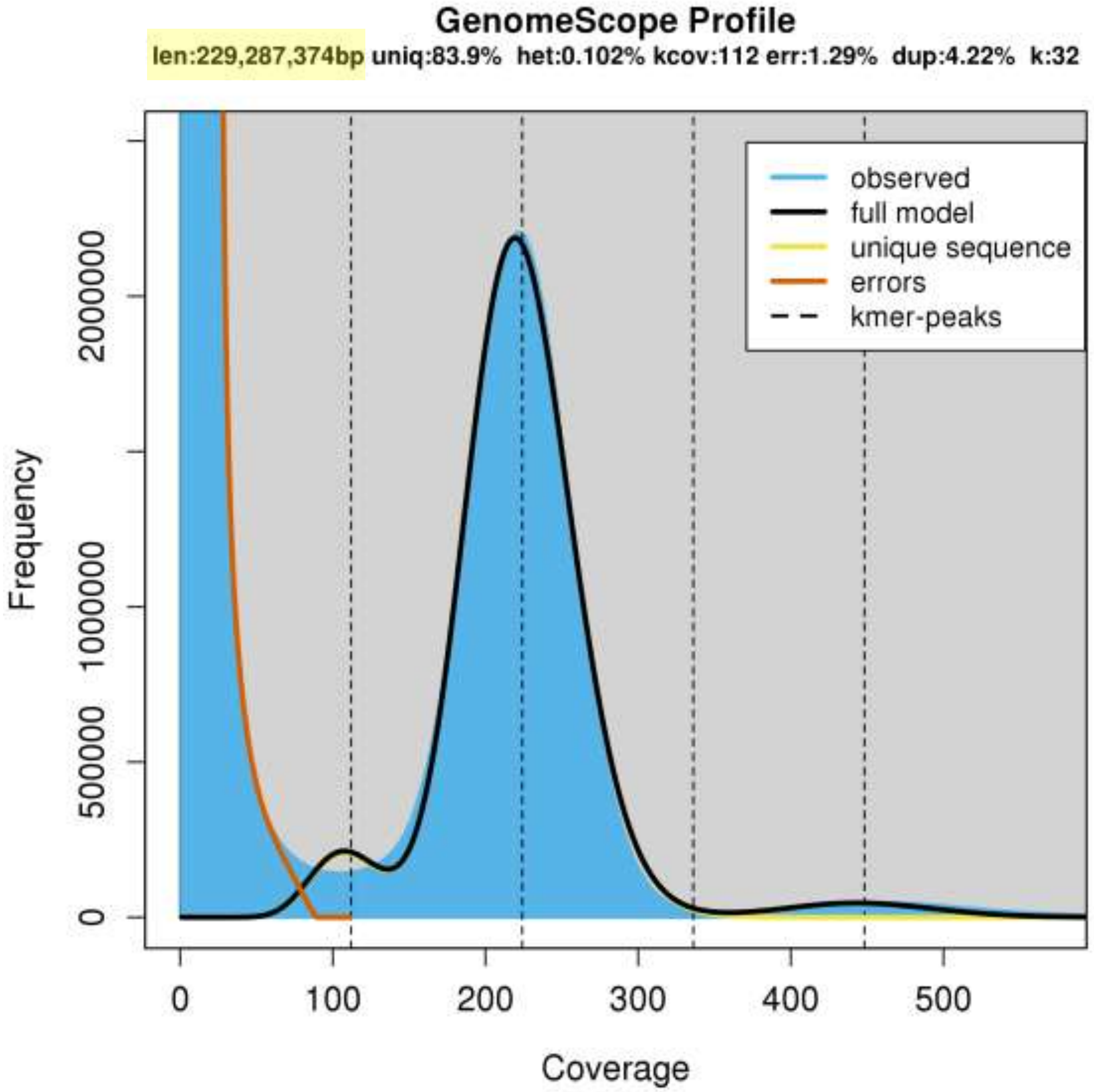
- Expected Distribution of k-mer frequency

Estimate repetitive content



DSK; Rizk et al. 2013

# *K*-mer Profiling Example: *Raillietiella orientalis*



# 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!