Next Generation Sequencing data analysis

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From the very beginning

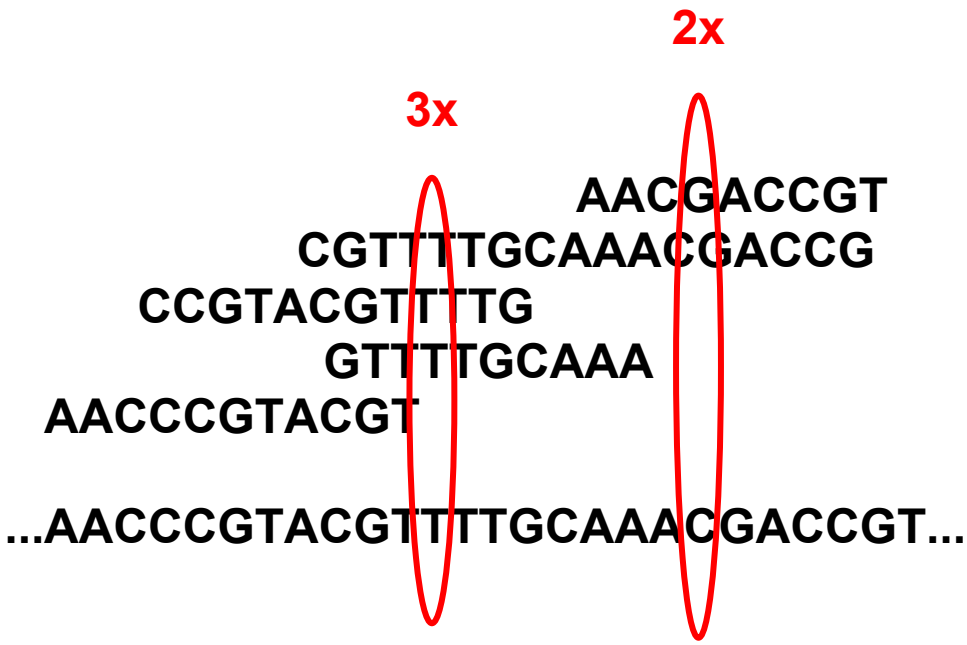
...AACCCGTACGTTTTGCAAACGACCGT...

From the very beginning

- Sequencing

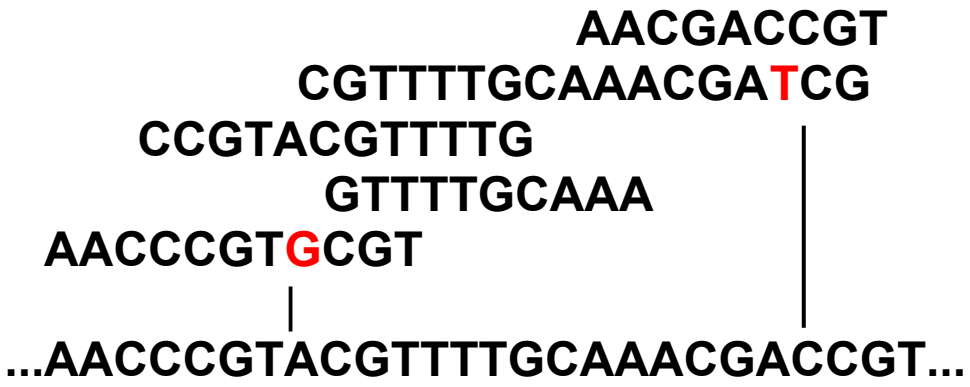
From the very beginning

- Sequencing
- Coverage



From the very beginning

- Sequencing
- Coverage
- Errors
  - Mismatches



From the very beginning

- Sequencing
- Coverage
- Errors
  - Mismatches
  - Indels

Early days

- Sanger sequencing
  - Long reads (~900 bp)
  - Low coverage (< 10x)
  - Extreme cost

- Human genome project
  - 3 Mbp
  - 3 billion USD
  - 10 years

NGS

- Shorter reads (25-500bp)
- High coverage (50-1000x)
- Huge amount of data
- Low cost
- **Required completely new algorithms**

NGS technologies

	illumina	Roche 454 SEQUENCING	ion torrent by life technologies	PACIFIC BIOSCIENCES
Read length, bp	25-250	400-1100	200-400	1000-5000
Error rate	0.01-1%	1%	1-2%	10-13%
Error type	Mismatches	Indels & Mismatches	Indels & Mismatches	Indels & Mismatches
Comments	Error rate grows to the end of the read	Problems with homopolymers	Problems with homopolymers	
Cost per 1 mbp, $	0.05 - 0.5	30	0.5 - 5	2

FASTA/FASTQ

- FASTA

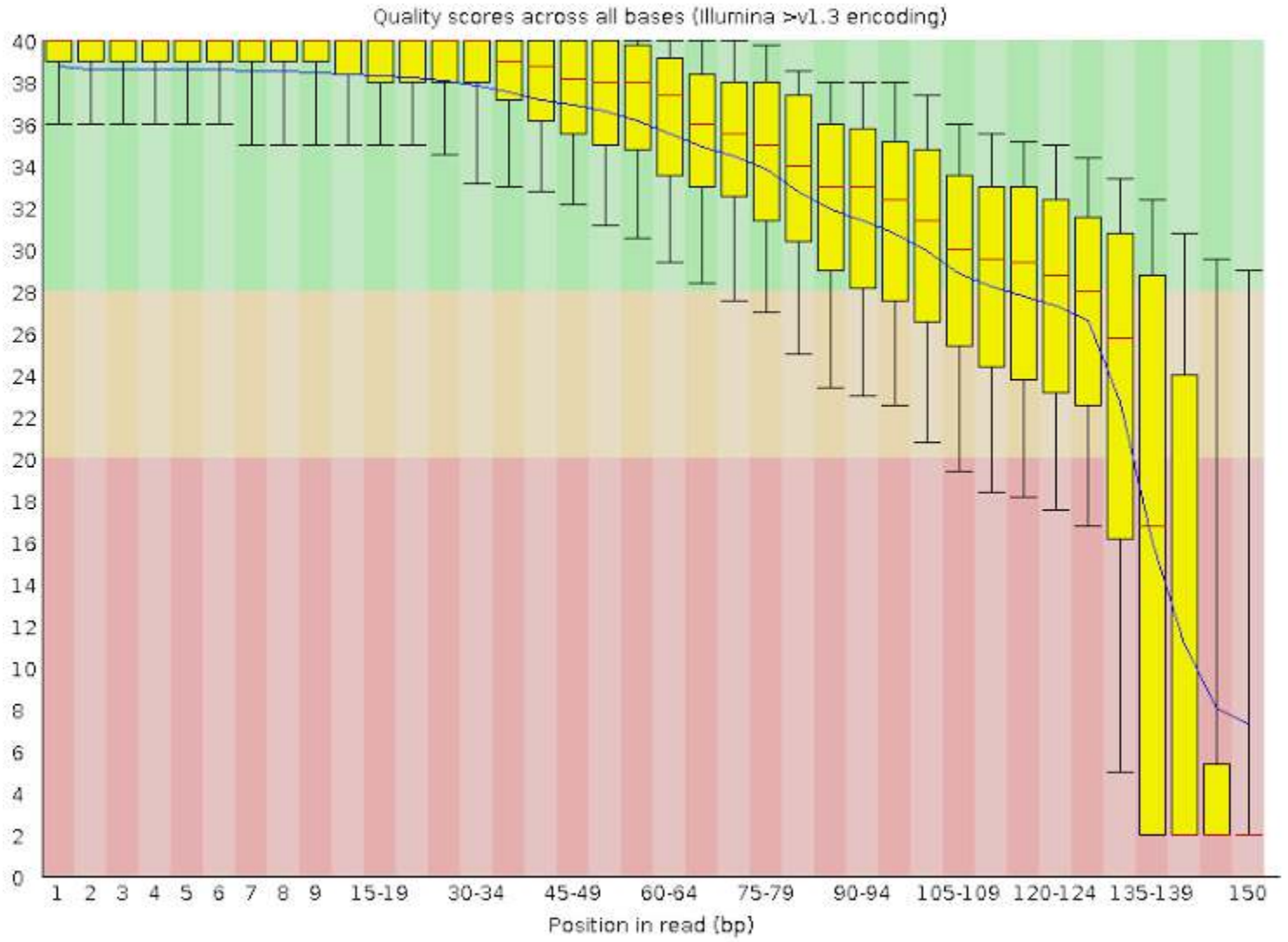
```
>EAS20_8_6_1_9_1972/1
ACCACCATTACCACCACCATCACCATTACCACAGGTAACGGTGCGGGCTGACGCG
T
>EAS20_8_6_1_163_1521/1
GCAGAAAACGTTCTGCATTTGCCACTGATGTACCGCCGAACTTCAACACTCGCAT
G
```

- FASTQ

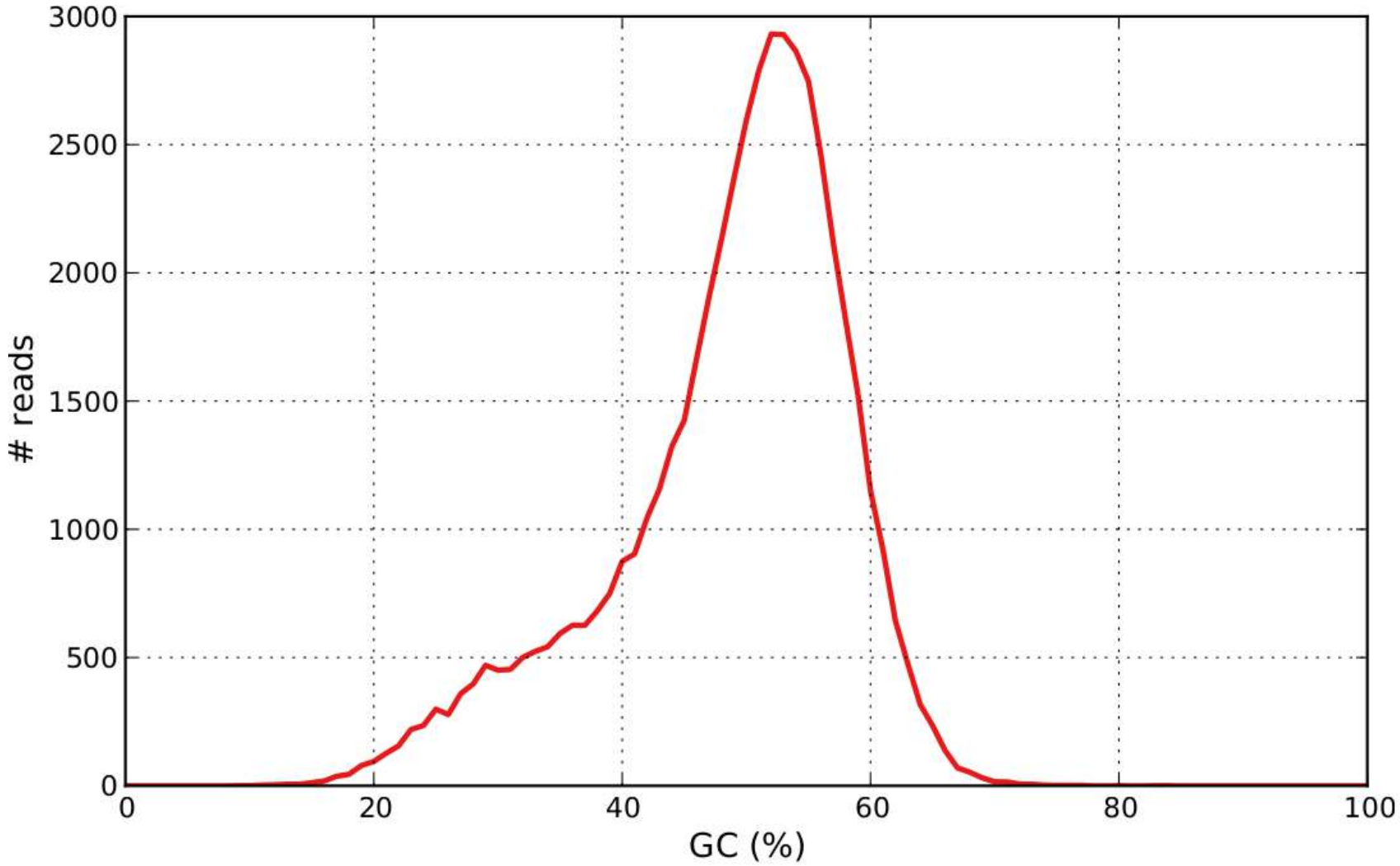
```
@EAS20_8_6_1_1477_92/1
ACCGTTACCTGTGGTAATGGTGATGGTGGTGGTAATGGTGGTGCTAATGCGTTTC
A
+EAS20_8_6_1_1477_92/1
HHGHFHHHHHHHHHGFFHHHBG?GGC8DD9GF??
=FFBCGBAF>FGCFHGHGGGD5
```

- Phred quality

Illumina error rate



GC content



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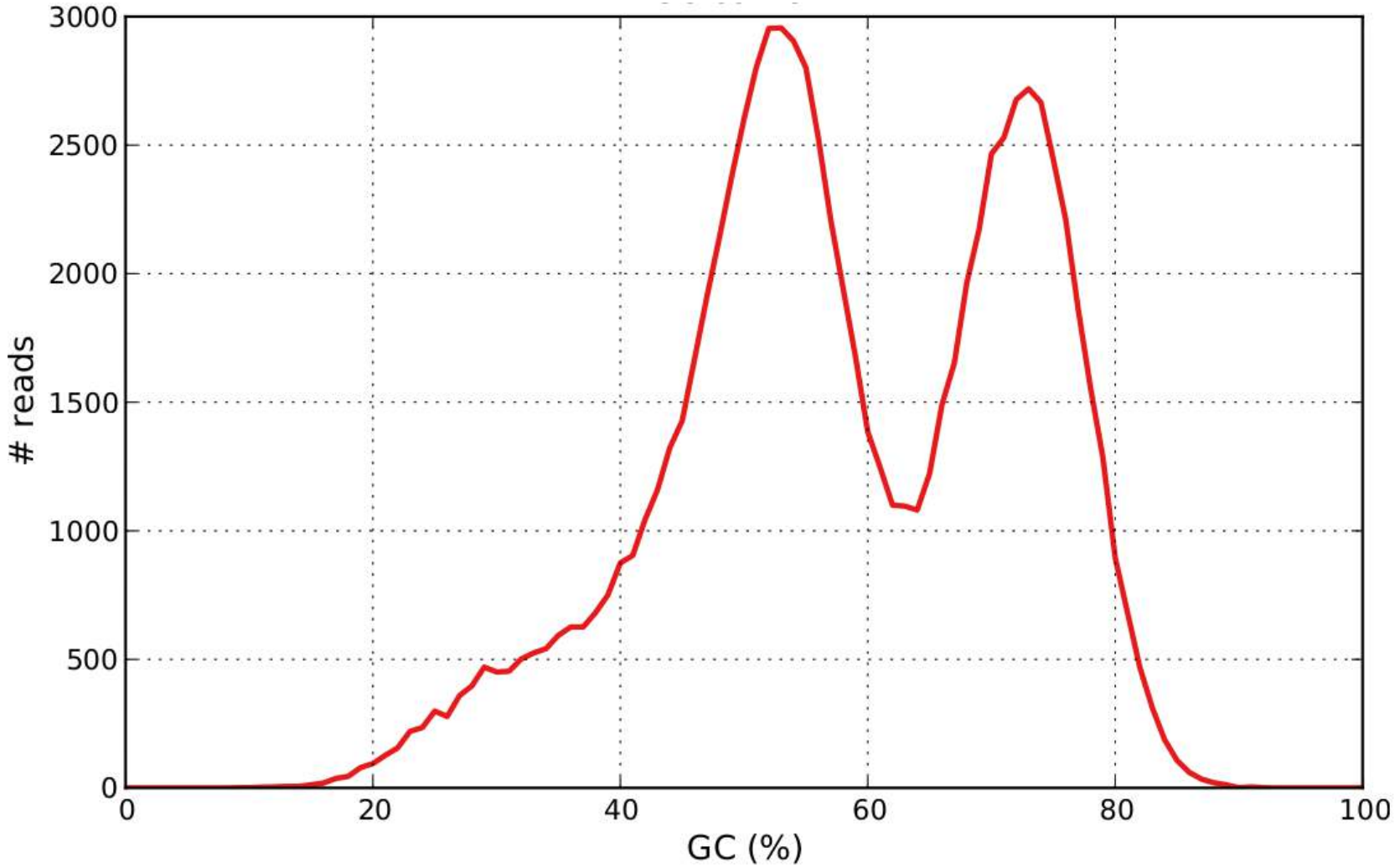
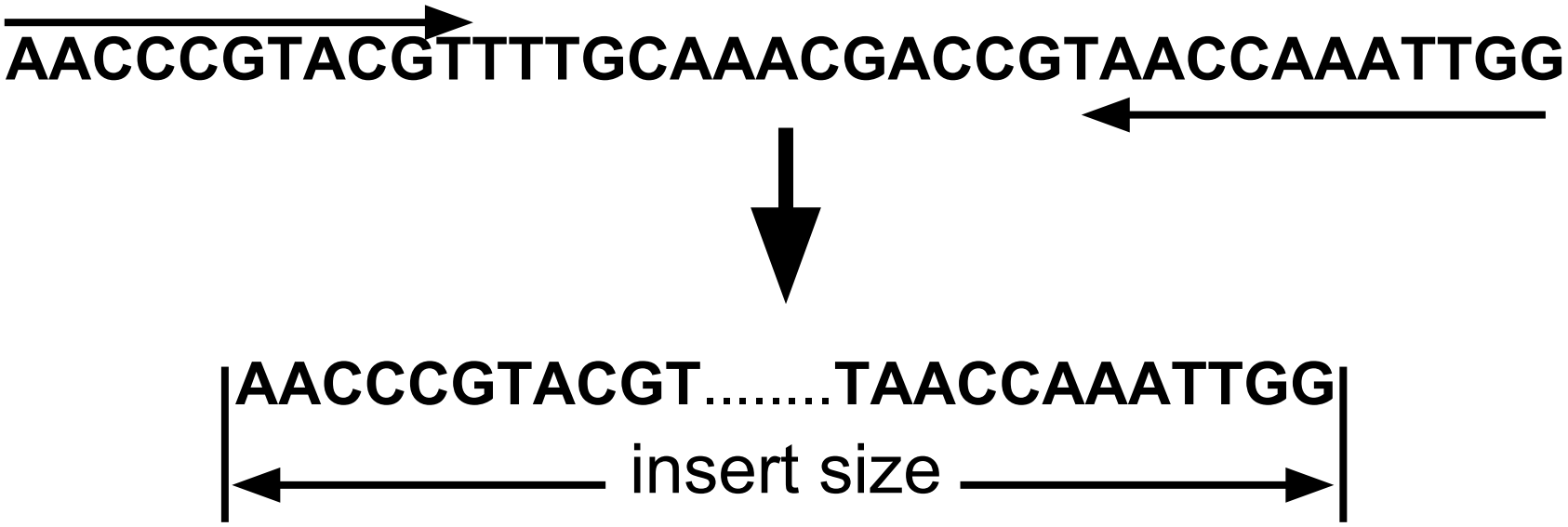


Paired reads



- Paired-end (< 1 kbp)
- Mate-pairs (1 - 20 kbp)

Short Read Archive

- http://www.ncbi.nlm.nih.gov/sra/
- SRA toolkit

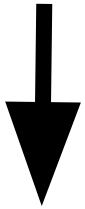
Alignment

AACGCTAACGGTAA
AACCGCGAACTAA

Alignment

AACGCTAACGGTAA

AACCGCGAACTAA



AAC - GCTAACGGTAA

AACCGCGAAC - - TAA

Short read alignment

- Challenges?

Short read alignment

- Challenges
  - Small length
  - Gigabytes of data
  - Sequencing errors
  - Genomic repeats

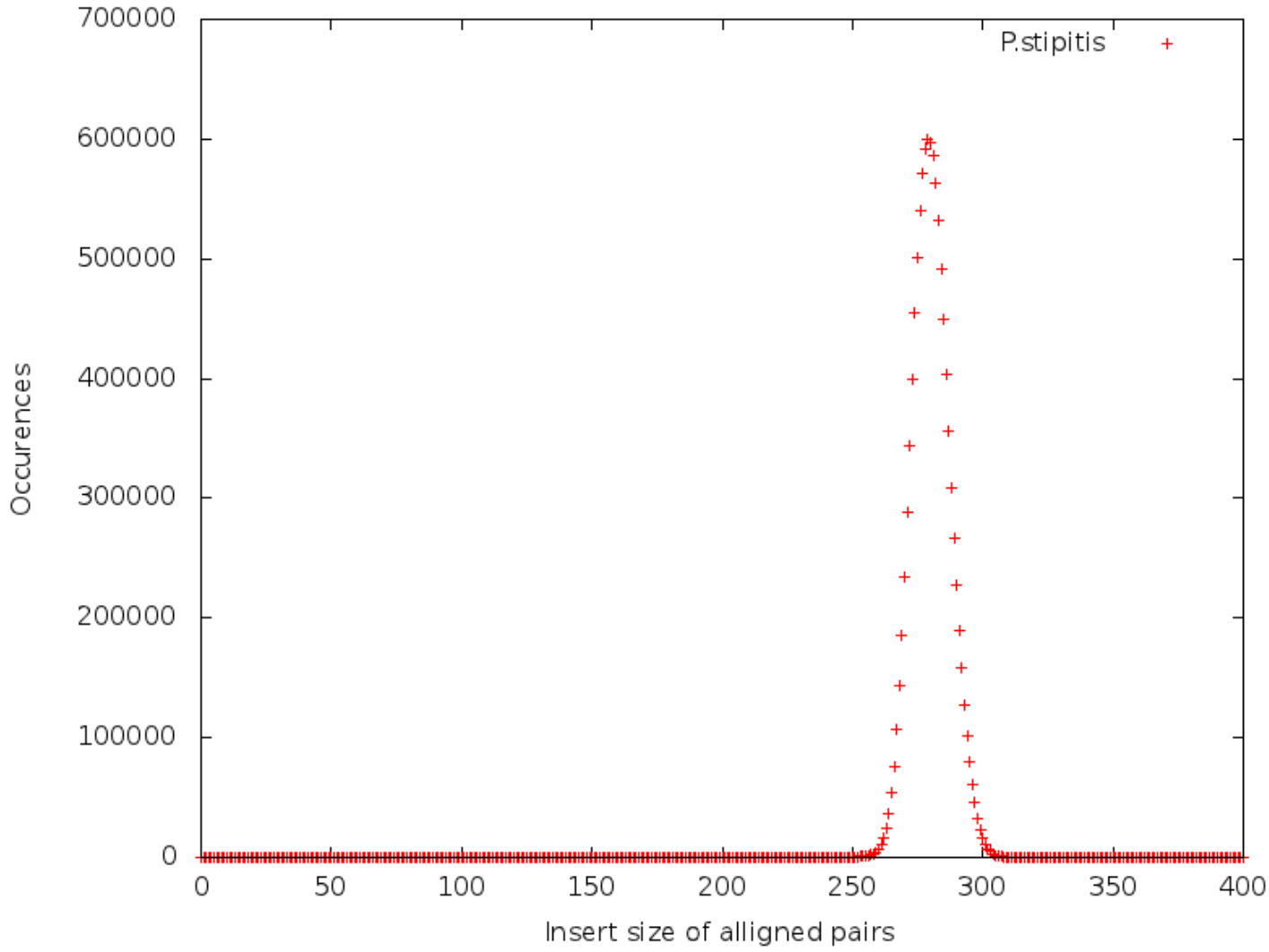
- Tools
  - Bowtie, BWA (Illumina)
  - BWA-SW (454, IonTorrent)
  - and many more

SAM/BAM

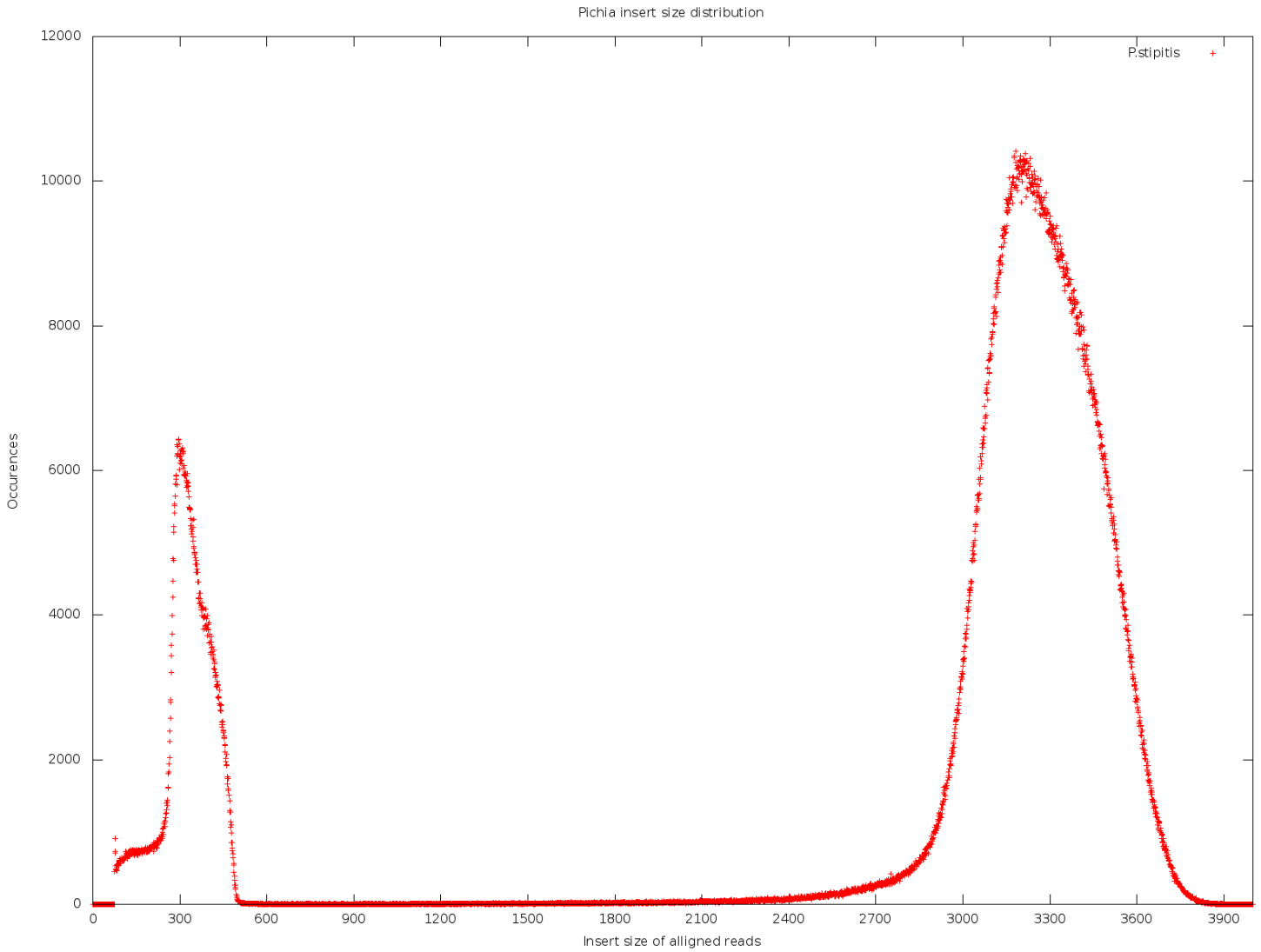
SAM/BAM

- Read ID (QNAME)
- Reference ID (RNAME)
- Mapping position (POS)
- Mate reference ID (RNEXT)
- Mate position (PNEXT)
- Observed insert length (TLEN)
- Read sequence (SEQ)
- Read quality (QUAL)
- CIGAR string
  - 34M 1I 4M 2D 1X 3M

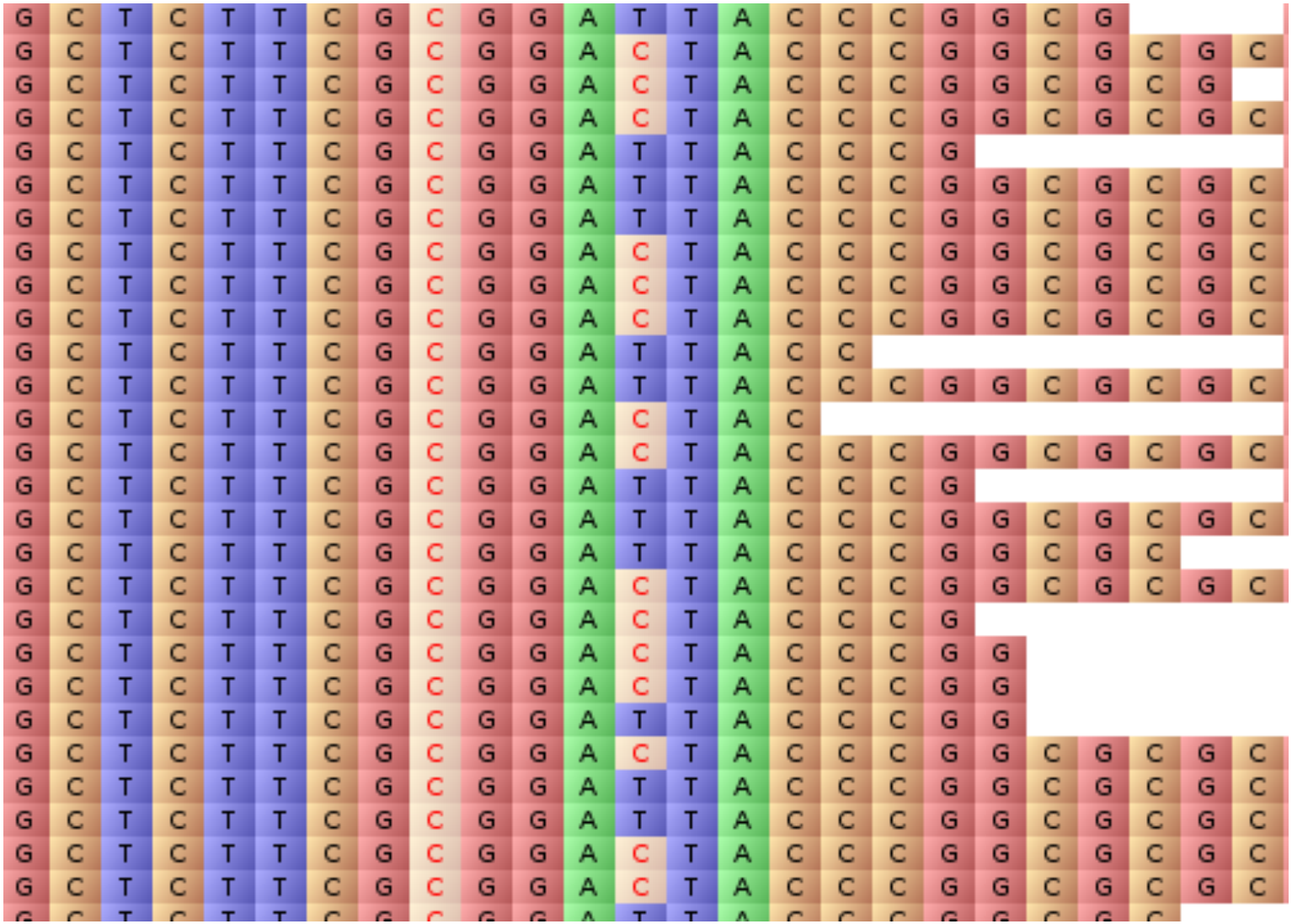
Insert size distribution



Insert size distribution



SNPs


GCTCTTCGCGCGGATTACCCCGGGCG
GCTCTTCGCGCGGACTACCCCGGGCGCGC
GCTCTTCGCGCGGACTACCCCGGGCGCG
GCTCTTCGCGCGGACTACCCCGGGCGCGC
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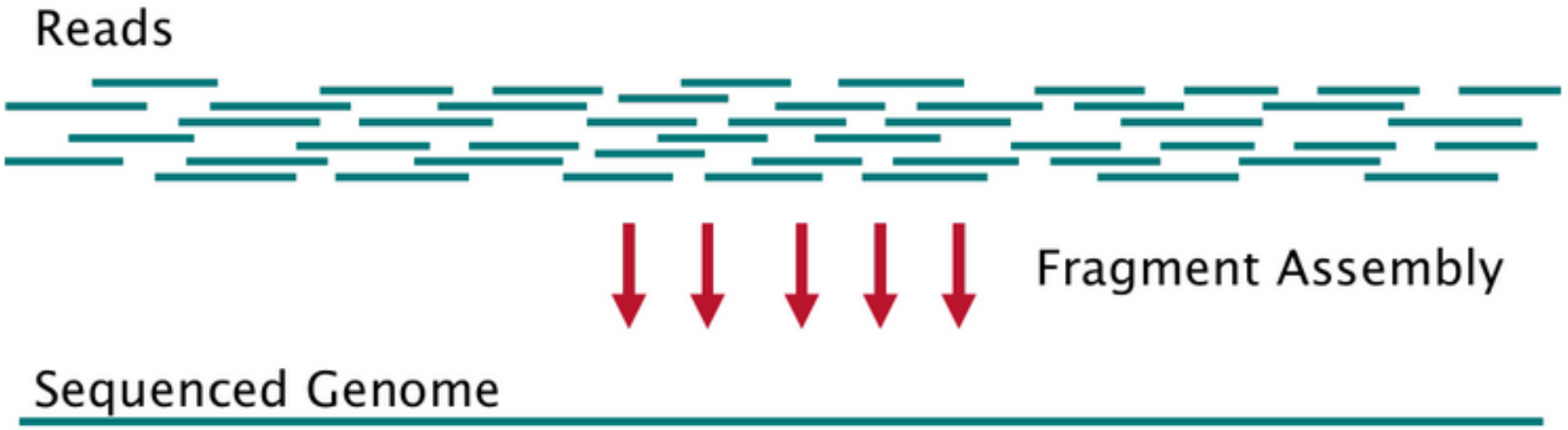
GCTCTTCGCGCGGATTACCCGGGCGCGC
GCTCTTCGCGCGGATTACCCGGGCGCGC
GCTCTTCGCGCGGACTACCCGGGCGCGC
GCTCTTCGCGCGGACTACCCGGGCGCGC
GCTCTTCGCGCGGACTACCCGGGCGCGC
GCTCTTCGCGCGGATTACC
GCTCTTCGCGCGGATTACCCGGGCGCGC
GCTCTTCGCGCGGACTAC
GCTCTTCGCGCGGACTACCCGGGCGCGC
GCTCTTCGCGCGGATTACCCG
GCTCTTCGCGCGGATTACCCGGGCGC
GCTCTTCGCGCGGATTACCCGGGCG
GCTCTTCGCGCGGACTACCCGGGCGCGC
GCTCTTCGCGCGGACTACCCG
GCTCTTCGCGCGGACTACCCGGG
GCTCTTCGCGCGGACTACCCGGG
GCTCTTCGCGCGGATTACCCGGG
GCTCTTCGCGCGGACTACCCGGGCGCGC
GCTCTTCGCGCGGATTACCCGGGCGCGC
GCTCTTCGCGCGGATTACCCGGGCGCGC
GCTCTTCGCGCGGACTACCCGGGCGCGC
GCTCTTCGCGCGGACTACCCGGGCGC

De novo whole genome assembly



Assembly pipeline

- Sequencing
- Artifacts & contaminants cleaning
- Draft assembly
  - Error correction
  - Assembly
  - Repeat resolution
  - Scaffolding
- Postprocessing
- Finishing
- Annotation

Why to assemble?

- NGS
  - Billions of short reads
  - Sequencing errors
  - Contaminants

Hard to perform analysis

- Assembly
  - Corrects sequencing errors
  - Much longer sequences
  - Each genomic region is presented only once
  - *May introduce errors*

Which assembler to use?

- ABySS
- ALLPATHS-LG
- CLC
- EULER
- IDBA-UD
- MaSuRCA
- Ray
- SOAPdenovo
- SPAdes
- Velvet
- and many more...

Which assembler to use?

- Assemblathon 1 & 2
  - Simulated and real datasets
  - More than 30 teams competing
- GAGE, GAGE-B papers
- Genome assembly evaluation tools
  - QUAST
  - GAGE

Genome Assembly Gold-Standard Evaluations

There is no best assembler.

Which assembler to use?

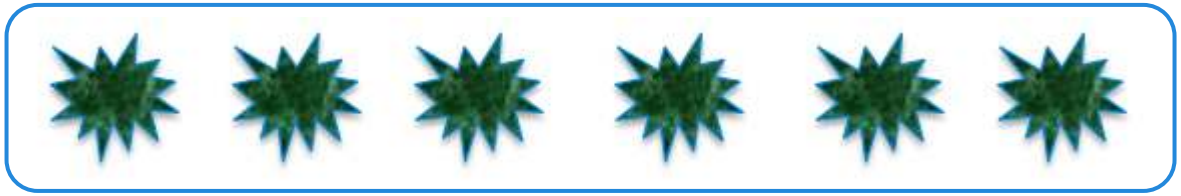
- Different technologies (Illumina, 454, IonTorrent, ...)
- Genome type and size (bacteria, insects, mammals, plants, ...)
- Type of prepared libraries (single reads, paired-end, mate-pairs, combinations)
- Type of data (multicell, metagenomic, single-cell)

Evaluating assemblies

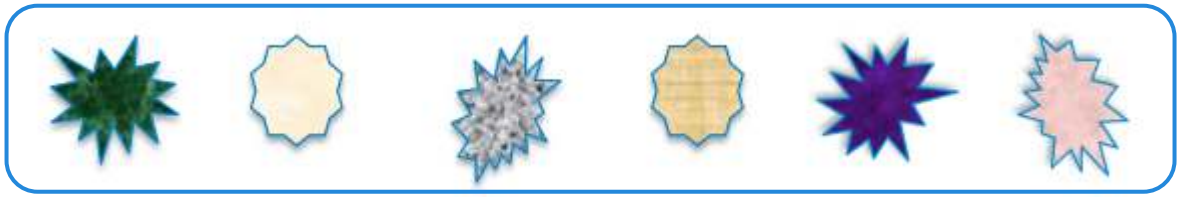
- BLAST
- Assembly statistics
  - Basic statistics (N50, Nx plots etc)
  - Genome fraction
  - Misassemblies
  - Mismatch/indel rates

Why to create new assembler?

- Conventional sequencing

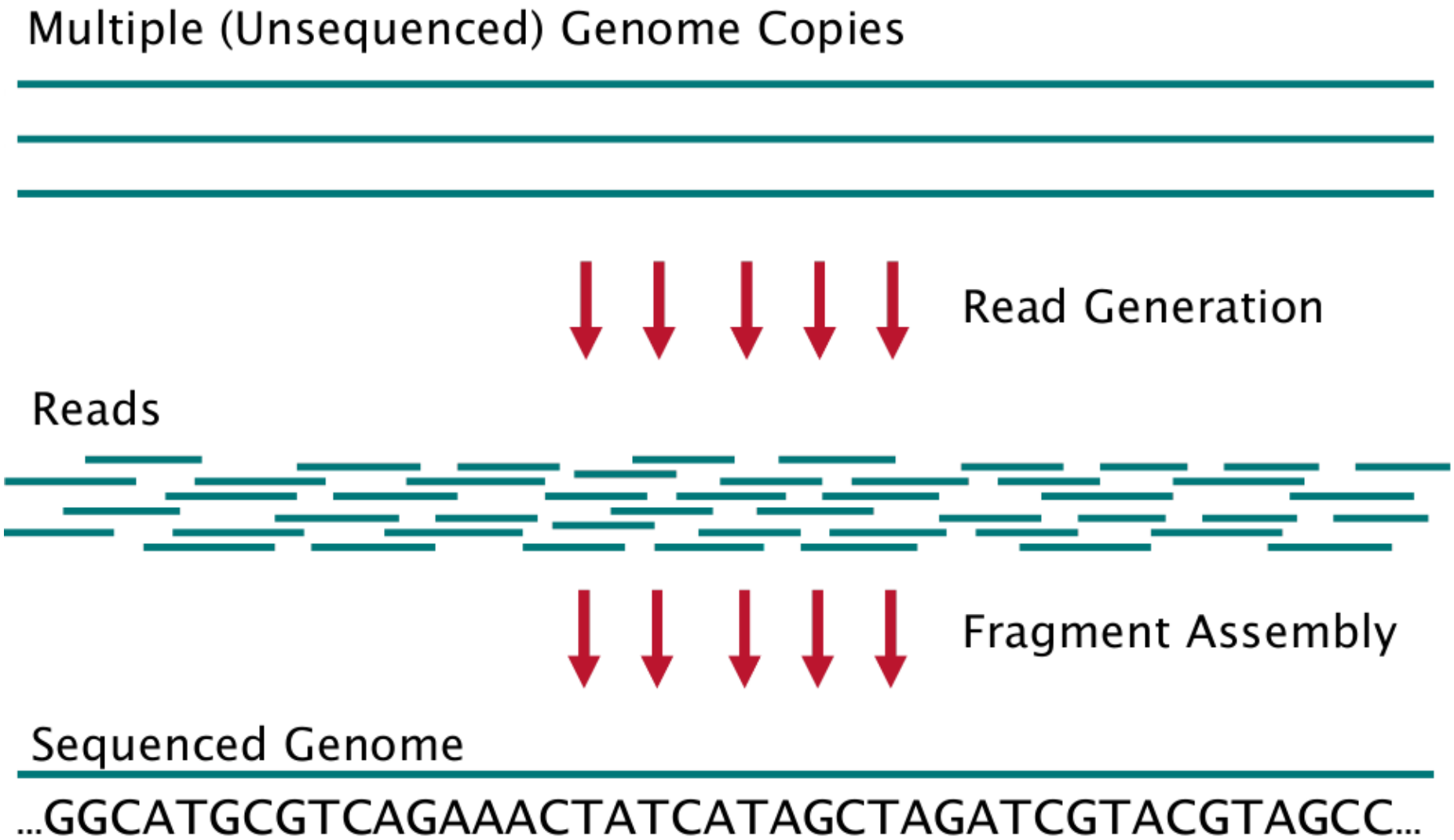


- Metagenomics



- Single cell

Conventional bacterial sequencing



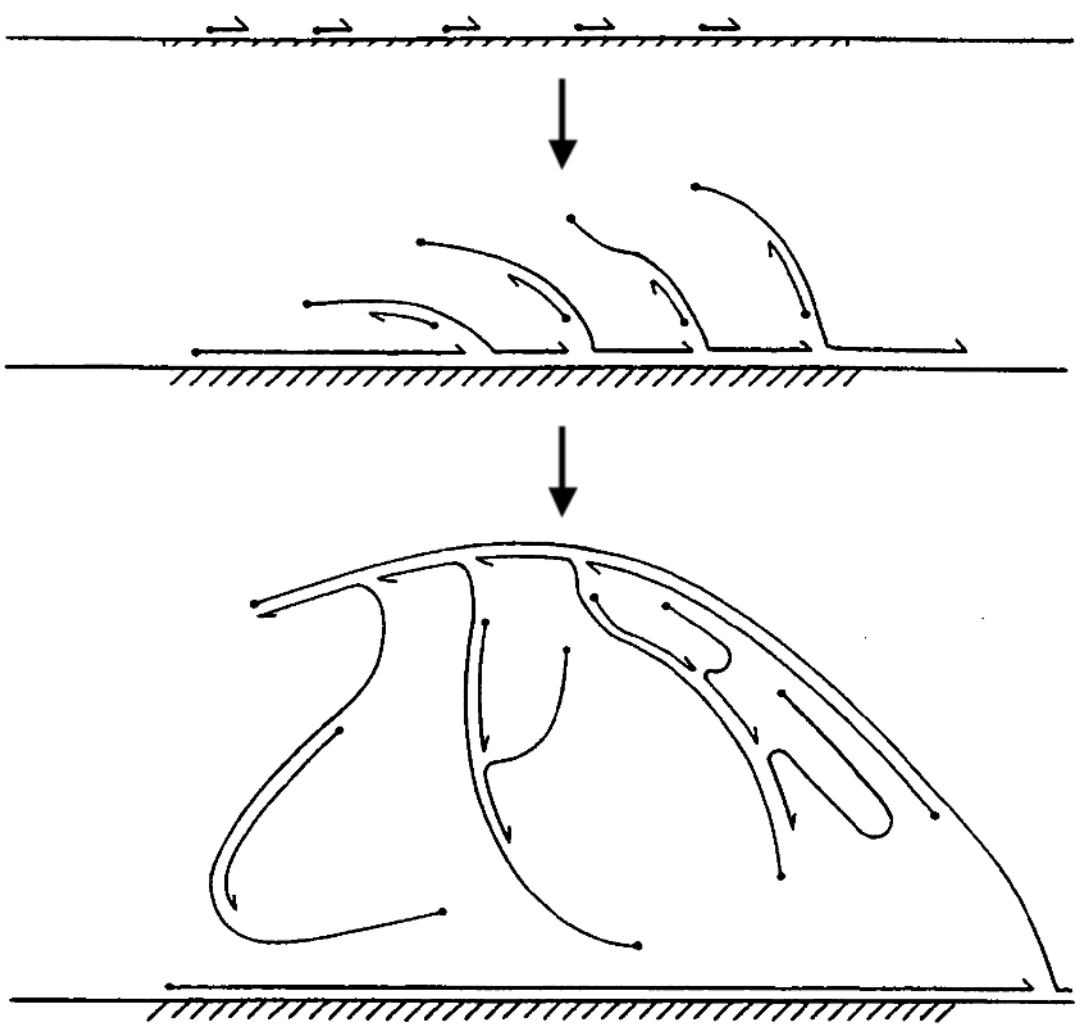
Metagenomics

- >99% bacteria cannot be cultured
- Metagenomics: sequencing of whole bacterial community
  - Reads from dozens of different genomes mixed in one data set
  - Different coverage for different bacteria
  - Presence of different strains
  - Conservative genomic regions
- Hard to assemble and classify resulting sequences
  - Usually allows to identify only a few genes

Single-cell sequencing via MDA

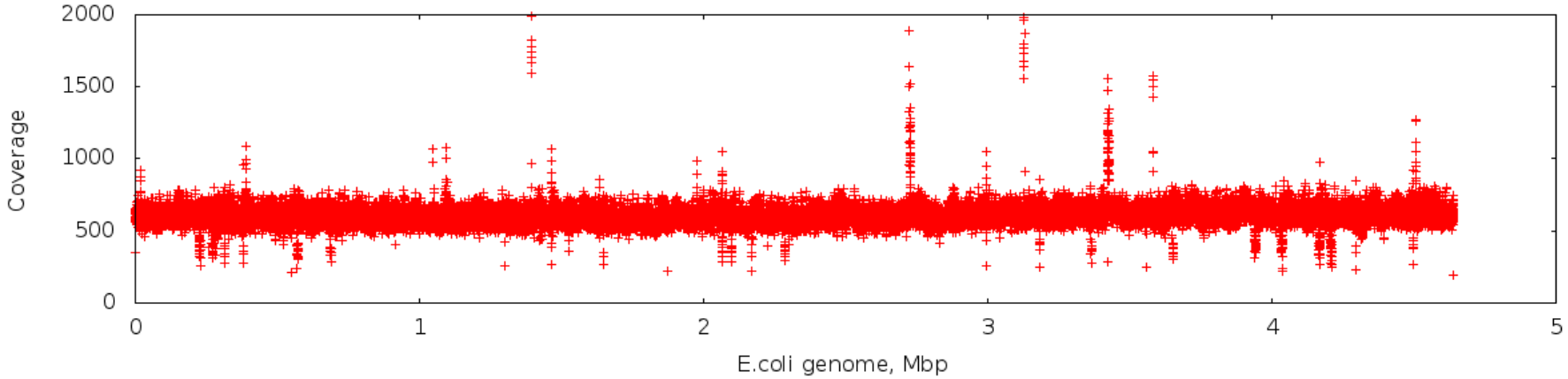
Multiple Displacement Amplification

- Random hexamer primers
- Phi29 DNA polymerase strand displacement

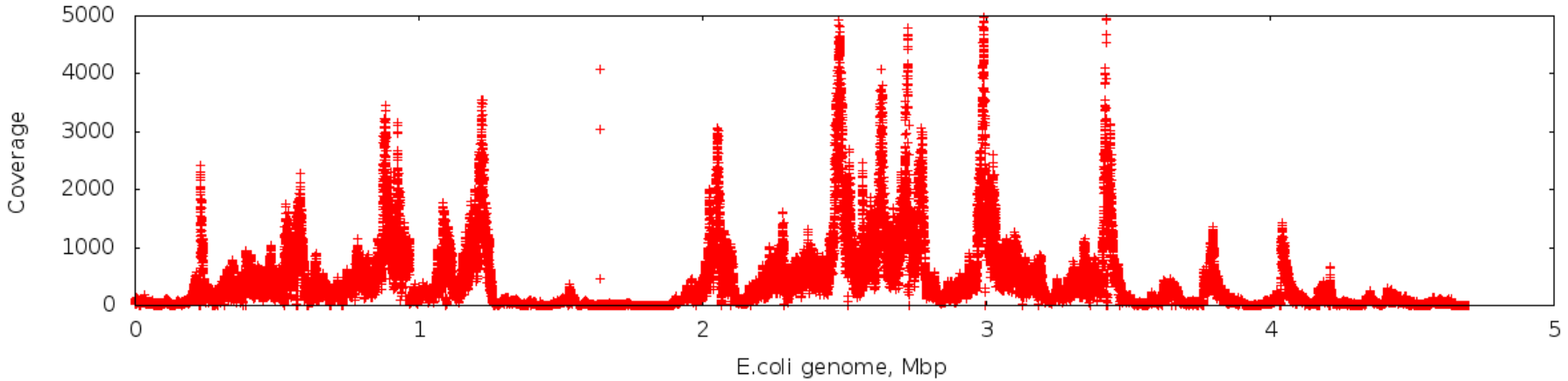


Challenges in single-cell assembly

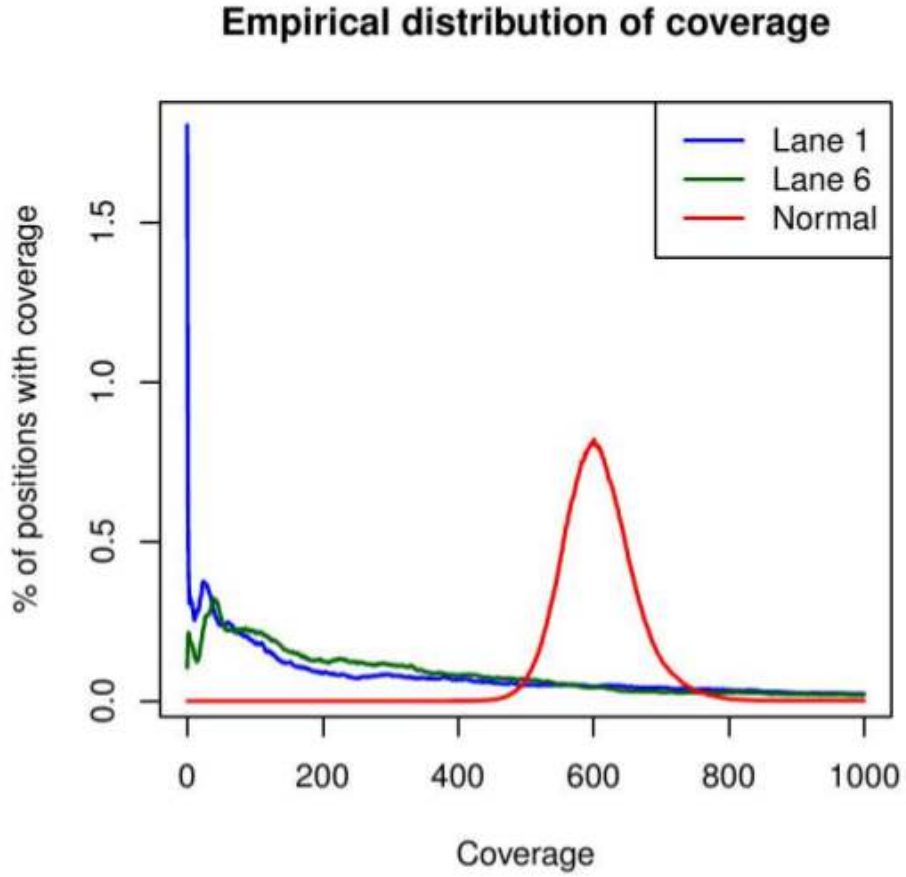
- *E. coli* isolate dataset



- *E.coli* single-cell dataset



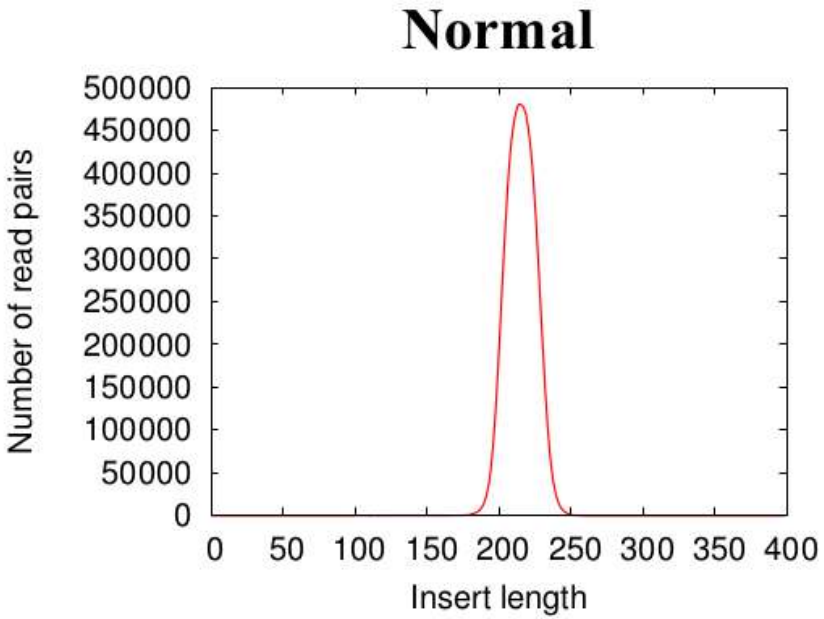
Challenges in single-cell assembly

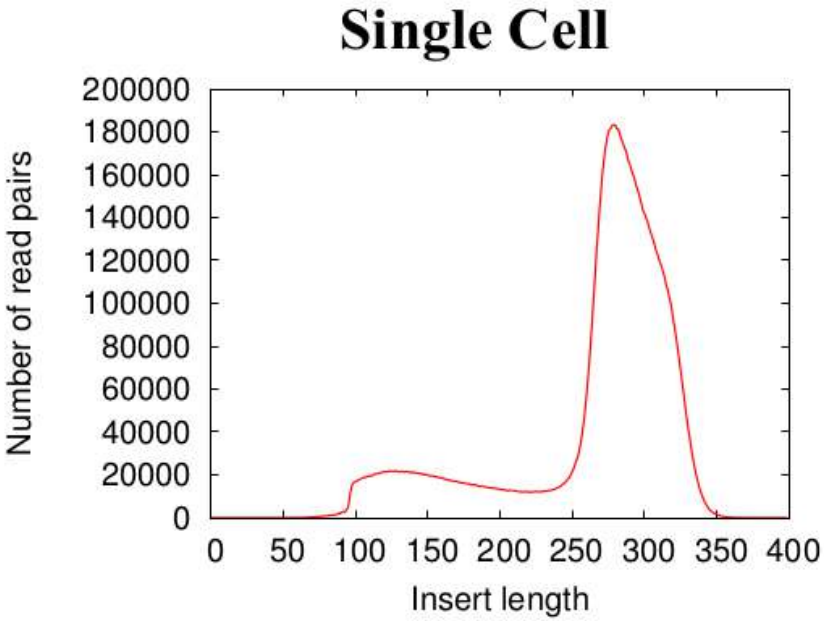


A cutoff threshold will eliminate about 25% of valid data in the single cell case, whereas it eliminates noise in the normal multicell case.

Challenges in single-cell assembly

- Insert size deviation





- Chimeric reads
  - Isolate dataset 0.01%
  - Single-cell dataset ~2%

Thank you!

Questions?