Genome Assembly

Michael Schatz

Sept 3, 2025

Lecture 3: Applied Comparative Genomics

Assignment 1

github.com/schatzlab/appliedgenomics2025/tree/main/assignments/assignment1

Files

main

Go to file

- assignments/assignment1
  - README.md
  - TAIR10.chrom.sizes
  - ce10.chrom.sizes
  - chr22.fa.gz
  - dm6.chrom.sizes
  - ecoli.chrom.sizes
  - hg38.chrom.sizes
  - tomato.chrom.sizes
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Assignment 1: Chromosome Structures

Assignment Date: Wednesday, August 27, 2025
Due Date: Wednesday, Sept. 3, 2025 @ 11:59pm

Assignment Overview

In this assignment you will profile the overall structure of the genomes of several important species and then study human chromosome 22 in more detail. As a reminder, any questions about the assignment should be posted to Piazza.

Question 1: Chromosome structures [10 pts]

Download the chromosome size files for the following genomes (Note these have been preprocessed to only include main chromosomes):

1. E. coli (Escherichia coli K12) - One of the most commonly studied bacteria [info]
2. Yeast (Saccharomyces cerevisiae, sacCer3) - an important eukaryotic model species, also good for bread and beer [info]
3. Worm (Caenorhabditis elegans, ce10) - One of the most important animal model species [info]
4. Fruit Fly (Drosophila melanogaster, dm6) - One of the most important model species for genetics [info]
5. Arabidopsis thaliana (TAIR10) - An important plant model species [info]
6. Tomato (Solanum lycopersicum v4.00) - One of the most important food crops [info]
7. Human (hg38) - us :) [info]
8. Wheat (Triticum aestivum, IWGSC) - The food crop which takes up the largest land area [info]

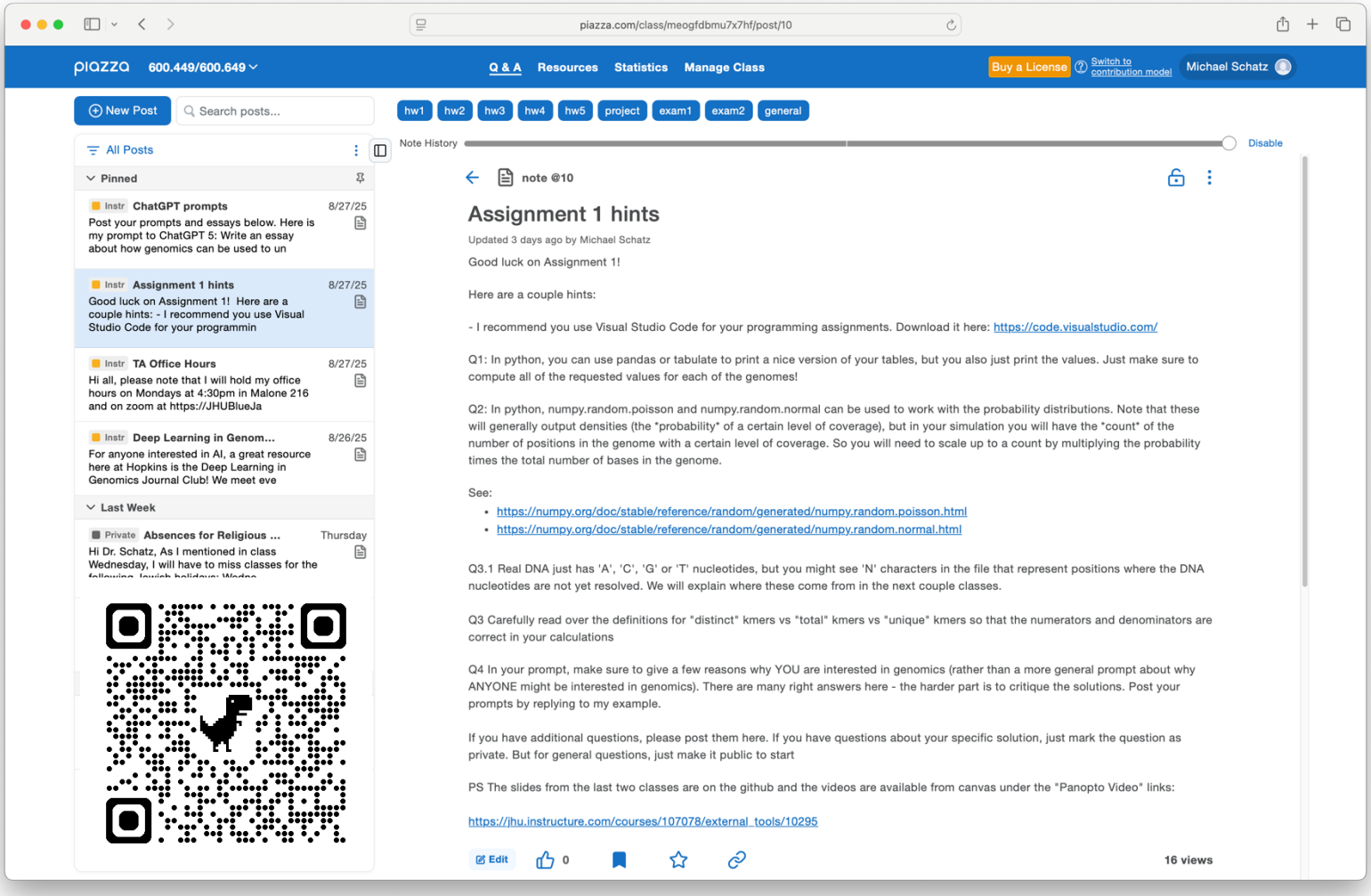
Using these files, make a table with the following information per species:

- Question 1.1. Total genome size
- Question 1.2. Number of chromosomes
- Question 1.3. Largest chromosome size and name
- Question 1.4. Smallest chromosome size and name
- Question 1.5. Mean chromosome length

Question 2. Coverage simulator [20 pts]

https://github.com/schatzlab/appliedgenomics2025/tree/main/assignments/assignment1
Due end of day on Wednesday Sept 3 (right before midnight)

Piazza



https://piazza.com/jhu/fall2025/600449600649

TA: Mahler Revsine

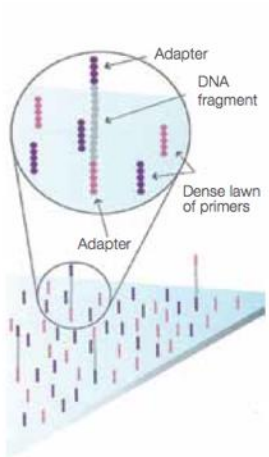
Office Hours: Mondays at 4:30pm
@ Malone 216 and Zoom

Part 1: Recap and Illumina Sequencing

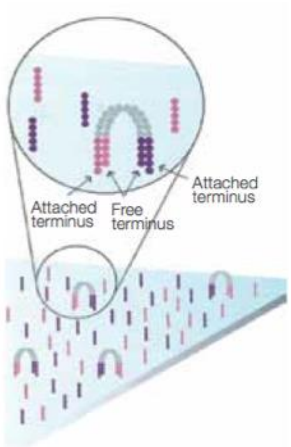
Second Generation Sequencing

Illumina NovaSeq 6000
Sequencing by Synthesis

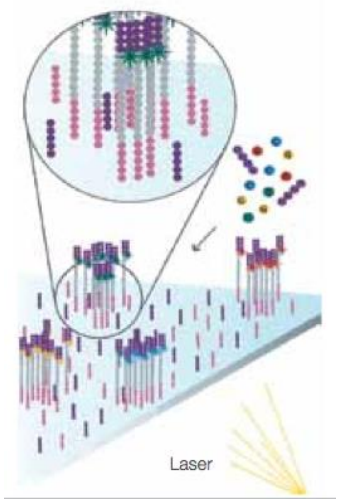
>3Tbp / day
(JHU has 4 of these!)



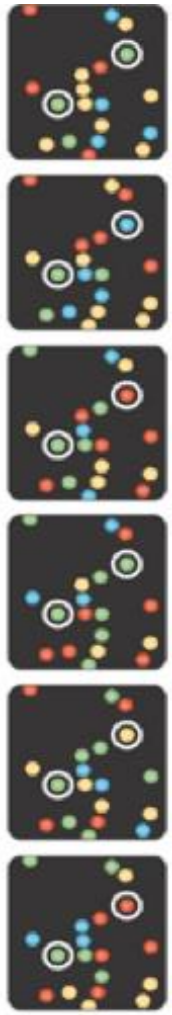
1. Attach



2. Amplify

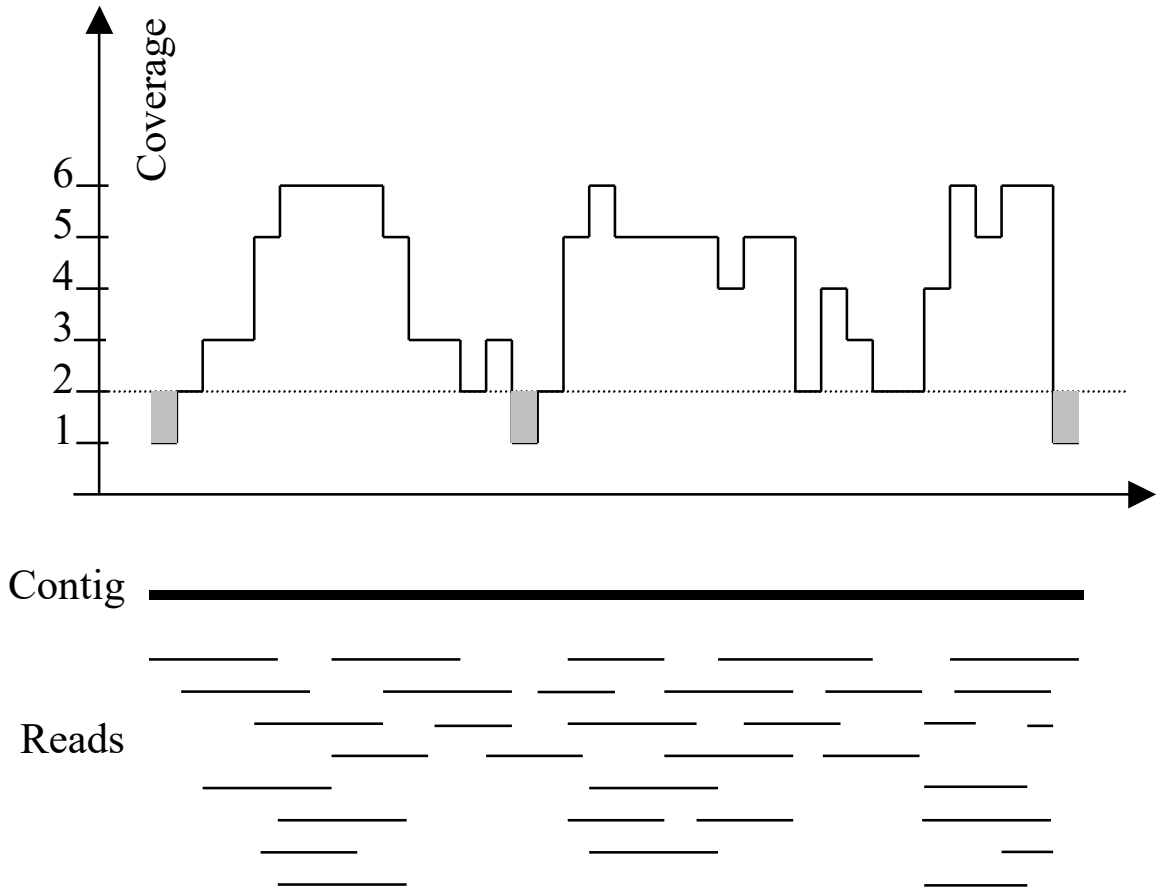


3. Image



Metzker (2010) Nature Reviews Genetics 11:31-46
https://www.youtube.com/watch?v=fCd6B5HRaZ8

Typical sequencing coverage

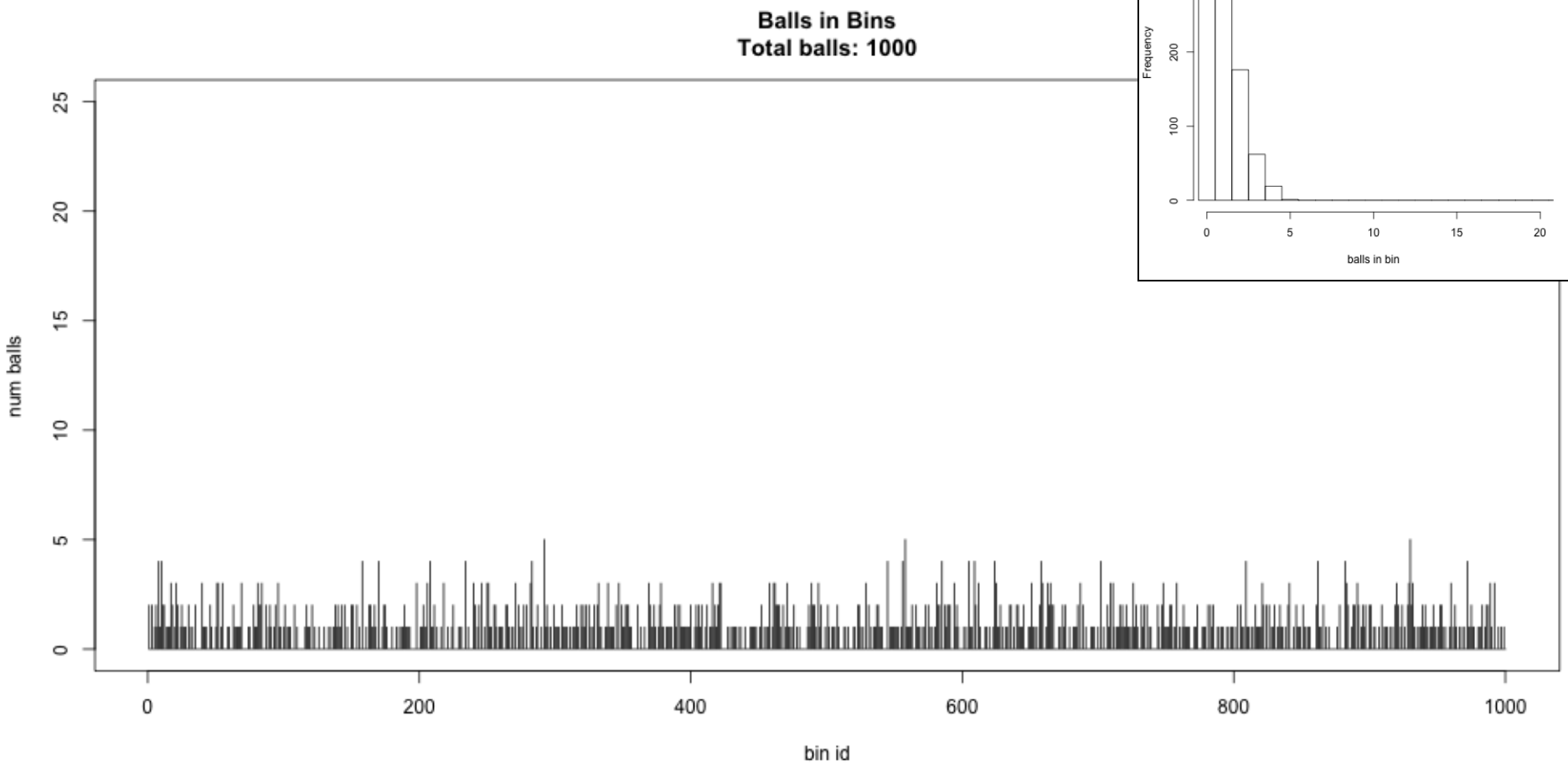


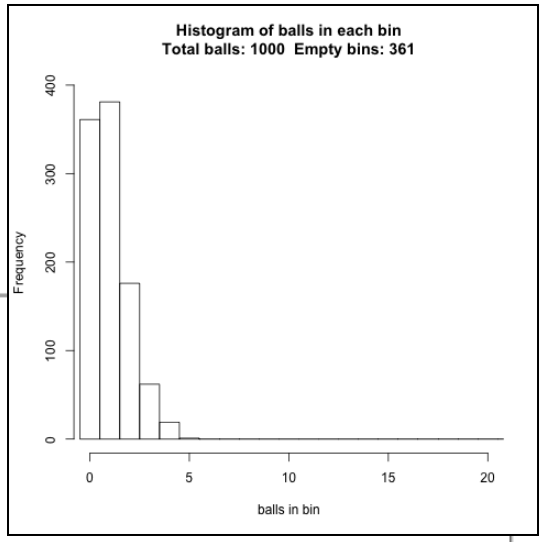
Imagine raindrops on a sidewalk

We want to cover the entire sidewalk but each drop costs $1

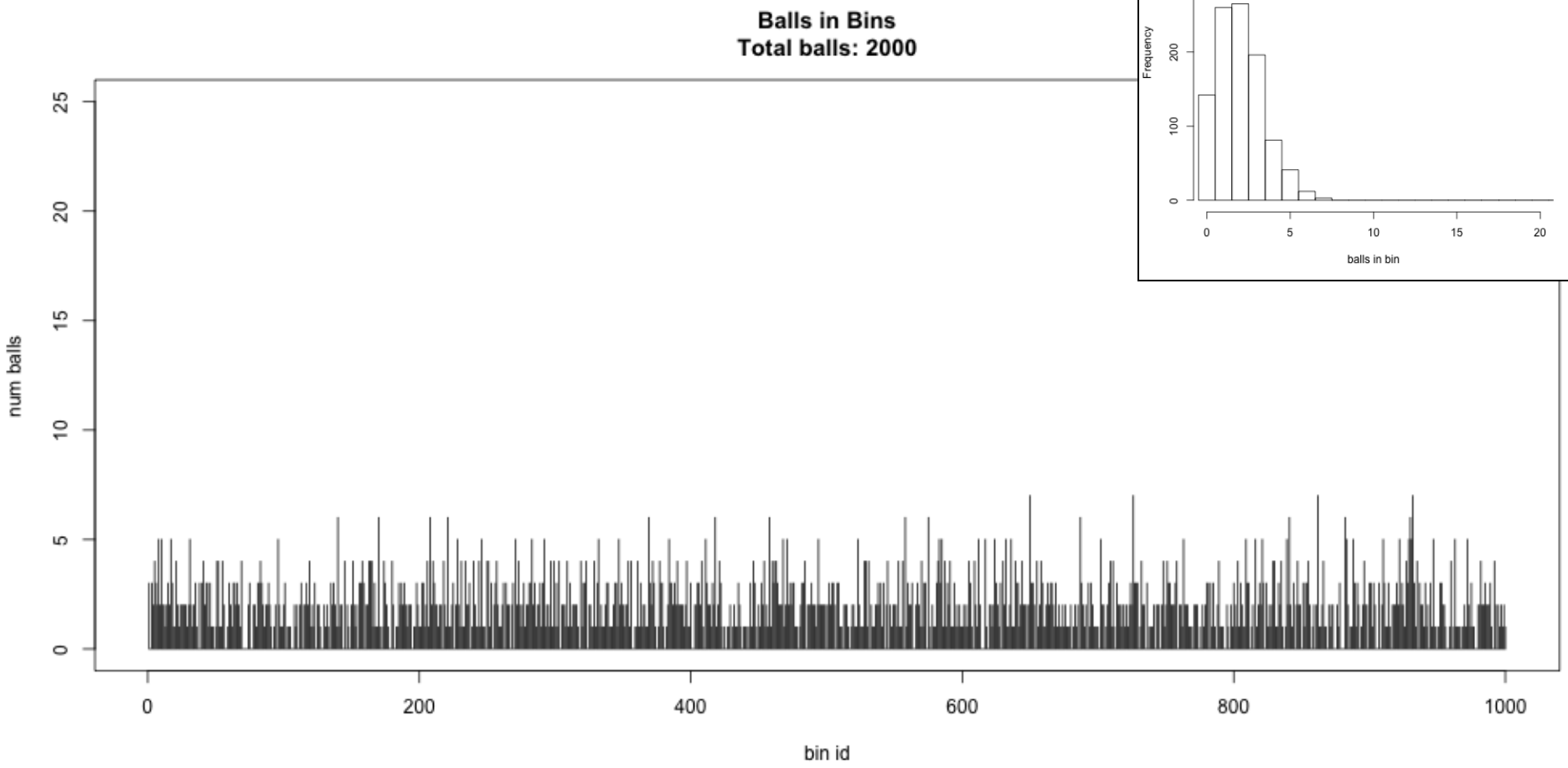
If the genome is 10 Mbp, should we sequence 100k 100bp reads?

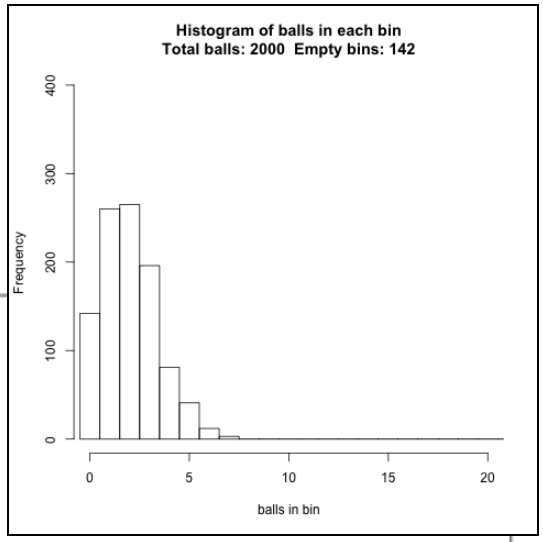
1x sequencing



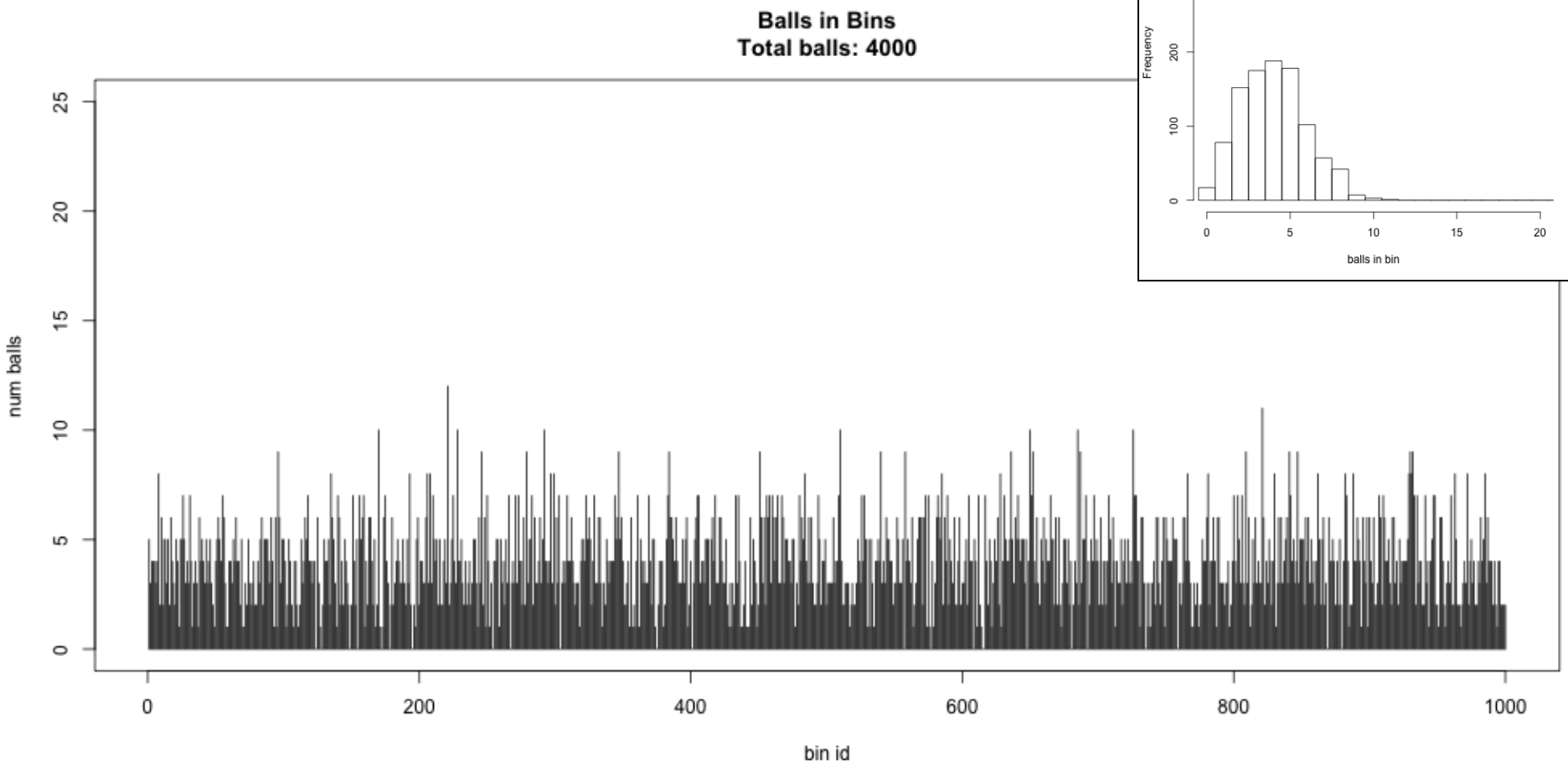


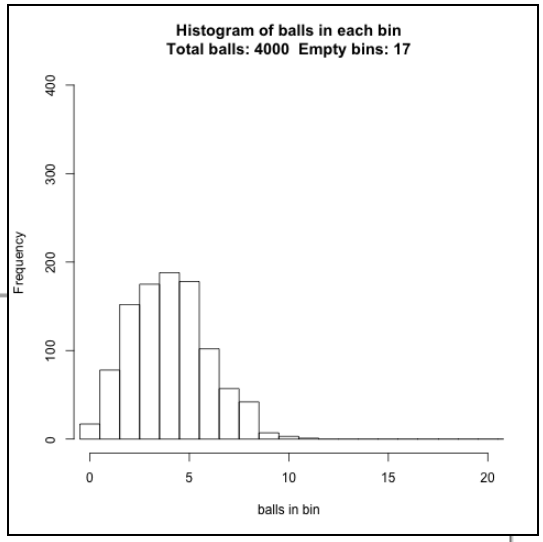
2x sequencing



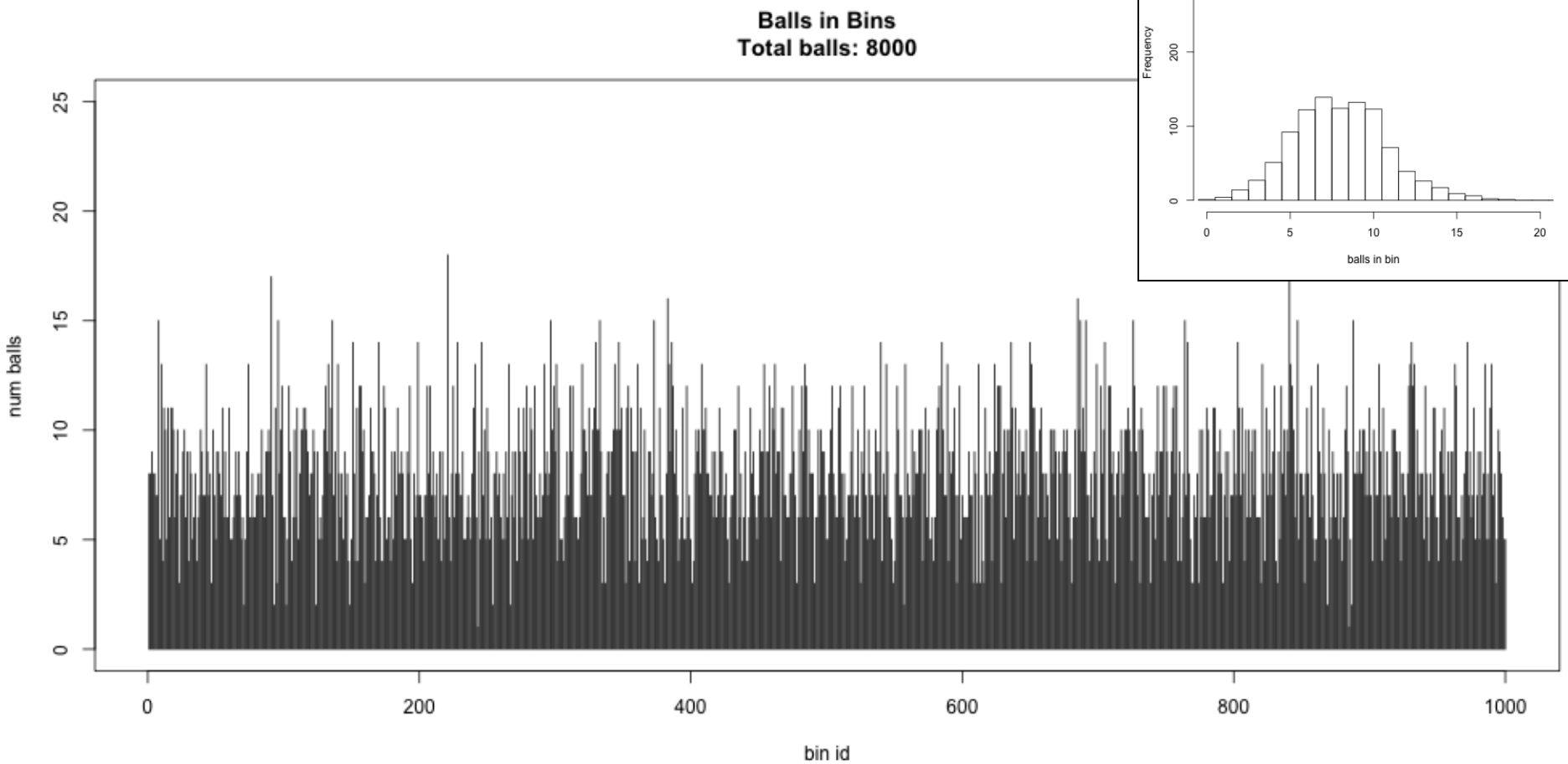


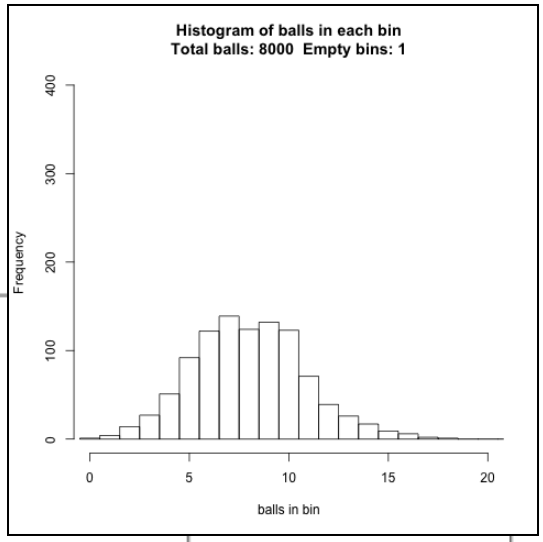
4x sequencing





8x sequencing





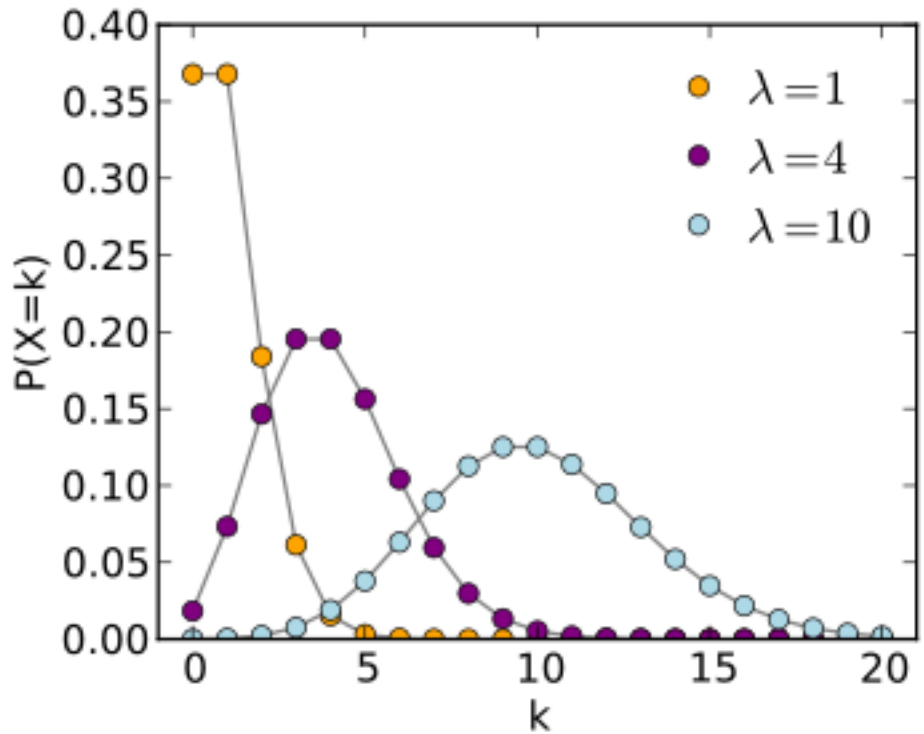
Poisson Distribution

The probability of a given number of events occurring in a fixed interval of time and/or space if these events occur with a known average rate and independently of the time since the last event.

$$P(k) = \frac{\lambda^k}{k!} e^{-\lambda}$$

Formulation comes from the limit of the binomial equation

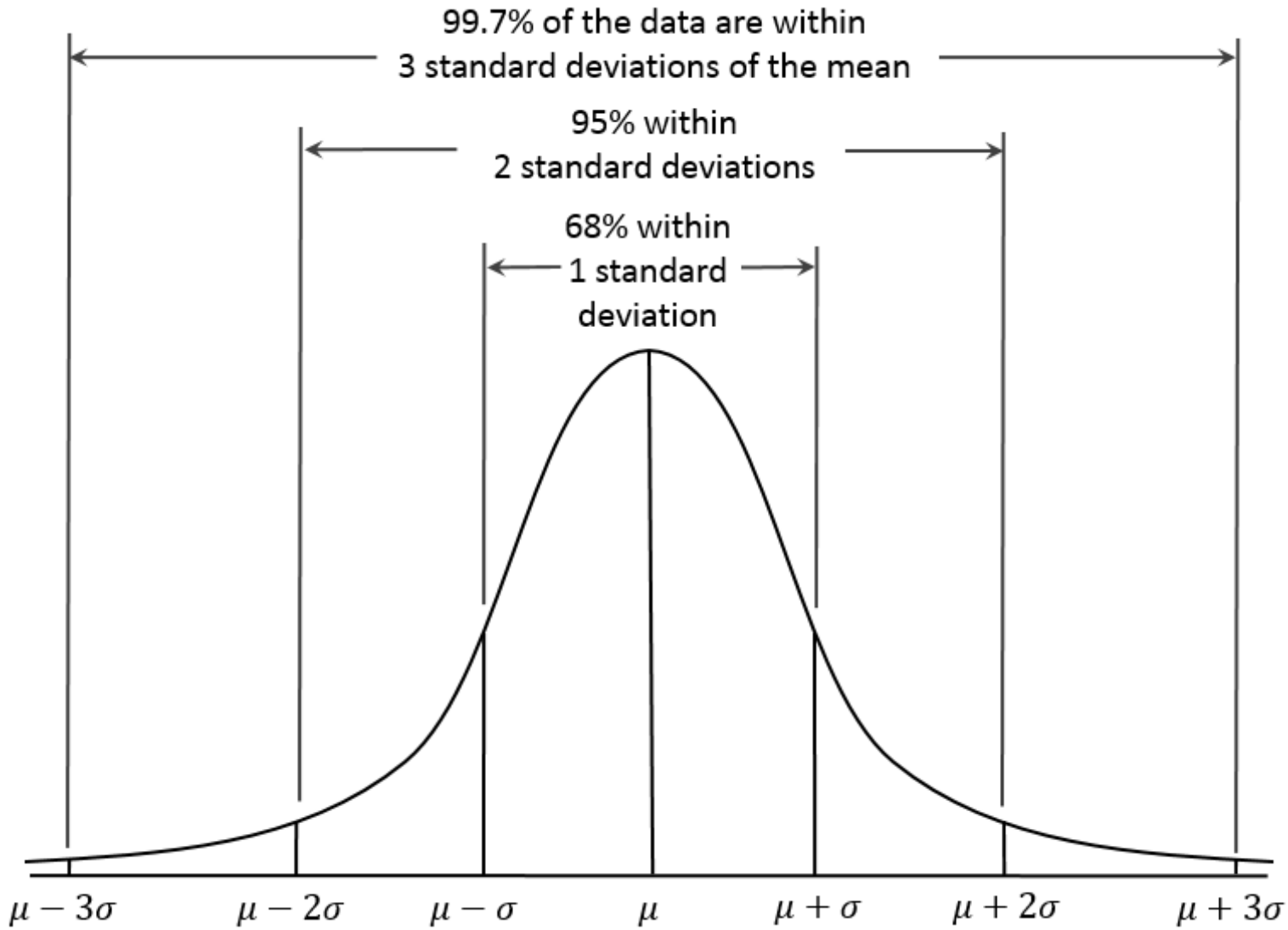
Resembles a normal distribution, but over the positive values, and with only a single parameter.



Key properties:

- ***The standard deviation is the square root of the mean.***
- ***For mean > 5, well approximated by a normal distribution***

Normal Approximation



Can estimate Poisson distribution as a normal distribution when $\lambda > 10$

Pop Quiz!

I want to sequence a 10Mbp genome to 24x coverage.
How many 120bp reads do I need?

I need 10Mbp x 24x = 240Mbp of data
240Mbp / 120bp / read = 2M reads

I want to sequence a 10Mbp genome so that
>97.5% of the genome has at least 24x coverage.
How many 120bp reads do I need?

Find X such that X-2*sqrt(X) = 24

36-2*sqrt(36) = 24

I need 10Mbp x 36x = 360Mbp of data
360Mbp / 120bp / read = 3M reads (50% more $$$)

K-mers and K-mer counting

GATTACATACACATTGGATG

K-mers and K-mer counting

GATTACATACACATTGGATG

GAT ACA ACA ATT GAT

ATT CAT CAC TTG ATG

TTA ATA ACA TGG

TAC TAC CAT GGA

Kmers:

- Divide a string into substrings of length k
- Notice every position is covered k times
- Notice there are G – k + 1 kmers from a string of length G

K-mers and K-mer counting

GATTACATACACATTGGATG

GAT ACA ACA ATT GAT

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TTA ATA ACA TGG

TAC TAC CAT GGA

Kmers:
- Divide a string into substrings of length k
- Notice every position is covered k times
- Notice there are G – k + 1 kmers from a string of length G

Computation: Very easy to compute, exact matches, represent 32mers in 64 bits
Biological: The “atomic unit” of a sequence, creates a fingerprint of a genome/read

K-mers and K-mer counting

GATTACATACACATTGGATG

GAT ACA ACA ATT GAT

ATT CAT CAC TTG ATG

TTA ATA ACA TGG

TAC TAC CAT GGA

GAT:2 CAT:2 ATG:1 TGG:1

ACA:3 CAC:1 TTA:1 TAC:2

ATT:2 TTG:1 ATA:1 GGA:1

K-mers and K-mer counting

GATTACATACACATTGGATG

GAT:2	CAT:2	ATG:1	TGG:1
ACA:3	CAC:1	TTA:1	TAC:2
ATT:2	TTG:1	ATA:1	GGA:1

```
1:  7  (ATG,  TGG,  …)
2:  4  (GAT,  CAT,  ATT,  TAC)
3:  1  (ACA)
```

See HW1

K-mers and K-mer counting

GATTACATACACATTGGATG

```
1:  7  (ATG,  TGG,  …)
2:  4  (GAT,  CAT,  ATT,  TAC)
3:  1  (ACA)
```

How long should k be?

K-mers and K-mer counting

GATTACATACACATTGGATG

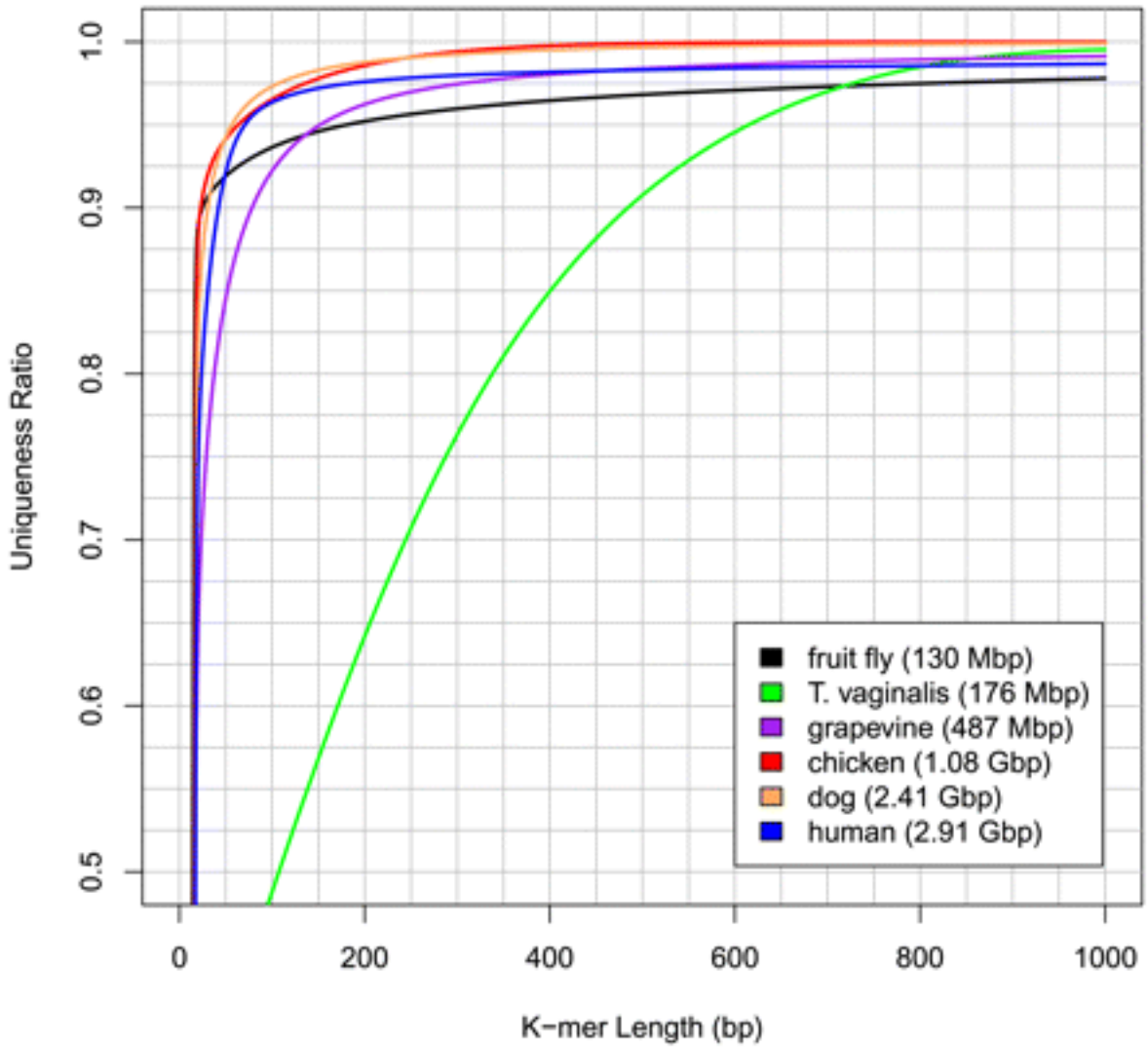
```
1:  7  (ATG,  TGG,  …)
2:  4  (GAT,  CAT,  ATT,  TAC)
3:  1  (ACA)
```

How long should k be?

K=1 : Too short, every base is present
K=2 : Too short, every pair of bases will be present

Pick k so that $G/(4^k) << 1$
$k = \log_4 (G)$
At least 15 for human, often a bit longer
But not too long or could loose resolution

K-mer Uniqueness



Assembly of large genomes using second-generation sequencing
Schatz et al. (2010) Genome Research. doi: 10.1101/gr.101360.109

GATTACATACACATTGGATG

GATTACATACACATTGGATG

GATTACATACACATTGGATG

GAT ACA ACA ATT GAT

ATT CAT CAC TTG ATG

TTA ATA ACA TGG

TAC TAC CAT GGA

GAT ACA ACA ATT GAT

ATT CAT CAC TTG ATG

TTA ATA ACA TGG

TAC TAC CAT GGA

=

GATTACATACACATTGGATG

GAT ACA ACA ATT GAT

ATT CAT CAC TTG ATG

TTA ATA ACA TGG

TAC TAC CAT GGA

GAT ACA ACA ATT GAT

ATT CAT CAC TTG ATG

TTA ATA ACA TGG

TAC TAC CAT GGA

GATTACATACACATTGGATG

GAT ACA ACA ATT GAT

ATT CAT CAC TTG ATG

TTA ATA ACA TGG

TAC TAC CAT GGA

GAT ACA ACA ATT GAT

ATT CAT CAC TTG ATG

TTA ATA ACA TGG

TAC TAC CAT GGA

=

GATTACATACACATTGGATG

GAT ACA ACA ATT GAT

ATT CAT CAC TTG ATG

TTA ATA ACA TGG

TAC TAC CAT GGA

GAT ACA ACA ATT GAT

ATT CAT CAC TTG ATG

TTA ATA ACA TGG

TAC TAC CAT GGA

Jaccard Coefficient

$$J(A,B) = \frac{|A \cap B|}{|A \cup B|} = \frac{18}{18} = 100\%$$

GATTACATACACATTGGATG

GATTACATACXCATTGGATG

GATTACATACACATTGGATG

GAT ACA ACA ATT GAT

ATT CAT CAC TTG ATG

TTA ATA ACA TGG

TAC TAC CAT GGA

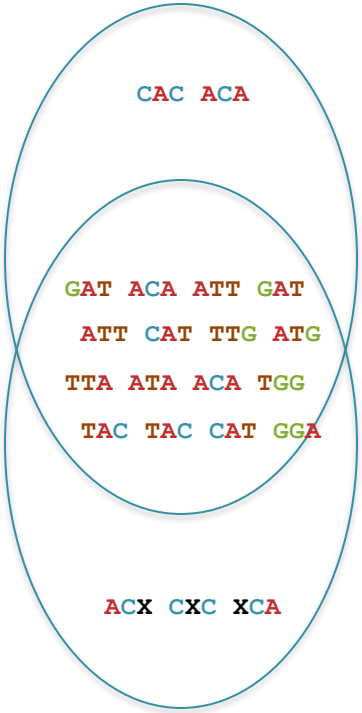
GATTACATACXCATTGGATG

GAT ACA ACX ATT GAT

ATT CAT CXC TTG ATG

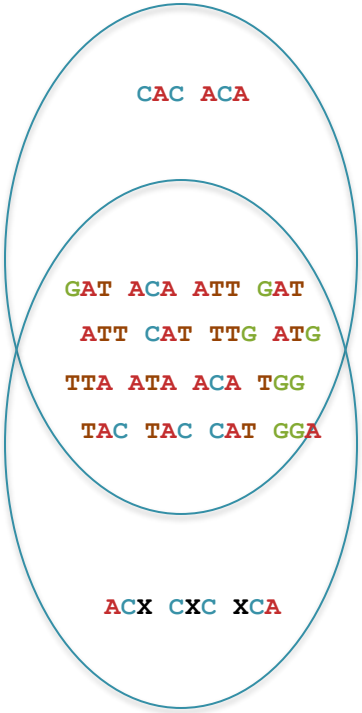
TTA ATA XCA TGG

TAC TAC CAT GGA



```
GATTACATACACATTGGATG
GAT ACA ACA ATT GAT
 ATT CAT CAC TTG ATG
  TTA ATA ACA TGG
   TAC TAC CAT GGA
```

```
GATTACATACXCATTGGATG
GAT ACA ACX ATT GAT
 ATT CAT CXC TTG ATG
  TTA ATA XCA TGG
   TAC TAC CAT GGA
```

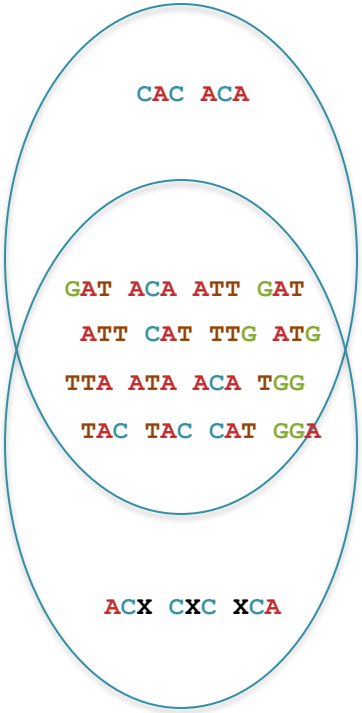


Jaccard Coefficient

$$J(A,B) = \frac{|A \cap B|}{|A \cup B|} = \frac{16}{21} = 76\%$$

```
GATTACATACACATTGGATG
GAT ACA ACA ATT GAT
 ATT CAT CAC TTG ATG
  TTA ATA ACA TGG
   TAC TAC CAT GGA
```

```
GATTACATACXCATTGGATG
GAT ACA ACX ATT GAT
 ATT CAT CXC TTG ATG
  TTA ATA XCA TGG
   TAC TAC CAT GGA
```



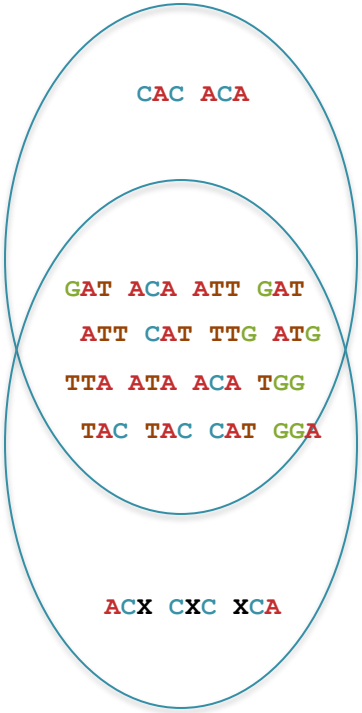
$$J(A,B) = \frac{|A \cap B|}{|A \cup B|} = \frac{16}{21} = 76\%$$

$$J \approx \frac{p}{2-p}$$

p = prob(kmer shared)

```
GATTACATACACATTGGATG
GAT ACA ACA ATT GAT
 ATT CAT CAC TTG ATG
  TTA ATA ACA TGG
   TAC TAC CAT GGA
```

```
GATTACATACXCATTGGATG
GAT ACA ACX ATT GAT
 ATT CAT CXC TTG ATG
  TTA ATA XCA TGG
   TAC TAC CAT GGA
```

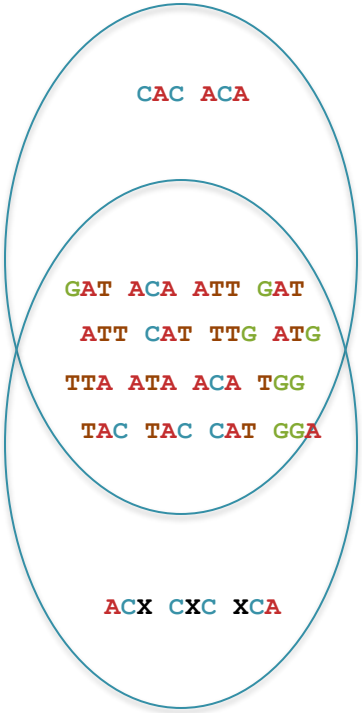


From Jaccard to Average Nucleotide Identity

$$J \approx \frac{p}{2-p} \quad \Longrightarrow \quad p = \frac{2J}{1+J}$$

```
GATTACATACACATTGGATG
GAT ACA ACA ATT GAT
 ATT CAT CAC TTG ATG
  TTA ATA ACA TGG
   TAC TAC CAT GGA
```

```
GATTACATACXCATTGGATG
GAT ACA ACX ATT GAT
 ATT CAT CXC TTG ATG
  TTA ATA XCA TGG
   TAC TAC CAT GGA
```



From Jaccard to Average Nucleotide Identity

$$J \approx \frac{p}{2-p} \quad \Longrightarrow \quad p = \frac{2J}{1+J}$$

$$p = I^k$$

$$I = \text{ANI} = p^{1/k} = \left(\frac{2J}{1+J}\right)^{1/k}$$

```
GATTACATACACATTGGATG
GAT ACA ACA ATT GAT
 ATT CAT CAC TTG ATG
  TTA ATA ACA TGG
   TAC TAC CAT GGA

GATTACATACXCATTGGATG
GAT ACA ACX ATT GAT
 ATT CAT CXC TTG ATG
  TTA ATA XCA TGG
   TAC TAC CAT GGA
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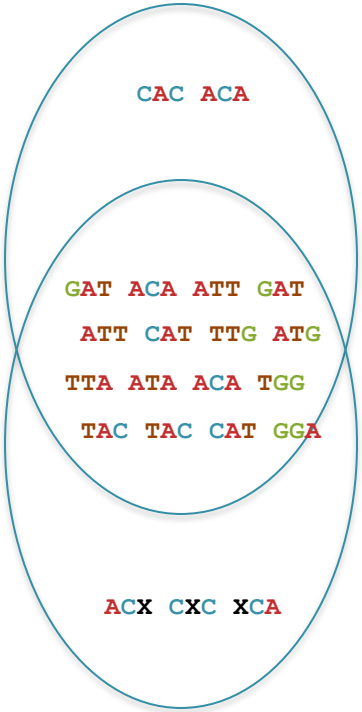
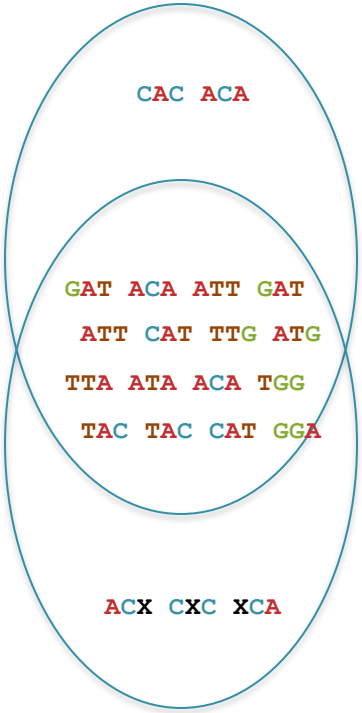


Identity for small differences (d): $p = I^k = (1-d)^k \approx e^{-kd}$

```
GATTACATACACATTGGATG
GAT ACA ACA ATT GAT
 ATT CAT CAC TTG ATG
  TTA ATA ACA TGG
   TAC TAC CAT GGA
```

```
GATTACATACXCATTGGATG
GAT ACA ACX ATT GAT
 ATT CAT CXC TTG ATG
  TTA ATA XCA TGG
   TAC TAC CAT GGA
```



Identity for small differences (d): $p = I^k = (1-d)^k \approx e^{-kd}$

$$p \approx e^{-kd} \quad \Rightarrow \quad d \approx -\frac{1}{k}\ln p \quad \Rightarrow \quad \mathrm{ANI} = 1 - d \approx 1 + \frac{1}{k}\ln p.$$

$$\mathrm{ANI} \approx 1 + \frac{1}{k}\ln\left(\frac{2J}{1+J}\right)$$

```
GATTACATACACATTGGATG
GAT ACA ACA ATT GAT
 ATT CAT CAC TTG ATG
  TTA ATA ACA TGG
   TAC TAC CAT GGA
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```
GATTACATACXCATTGGATG
GAT ACA ACX ATT GAT
 ATT CAT CXC TTG ATG
  TTA ATA XCA TGG
   TAC TAC CAT GGA
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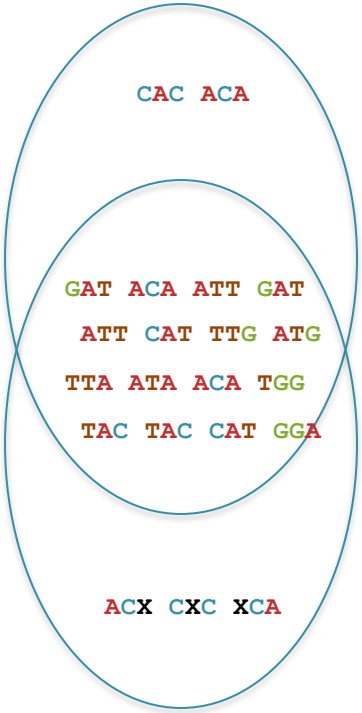
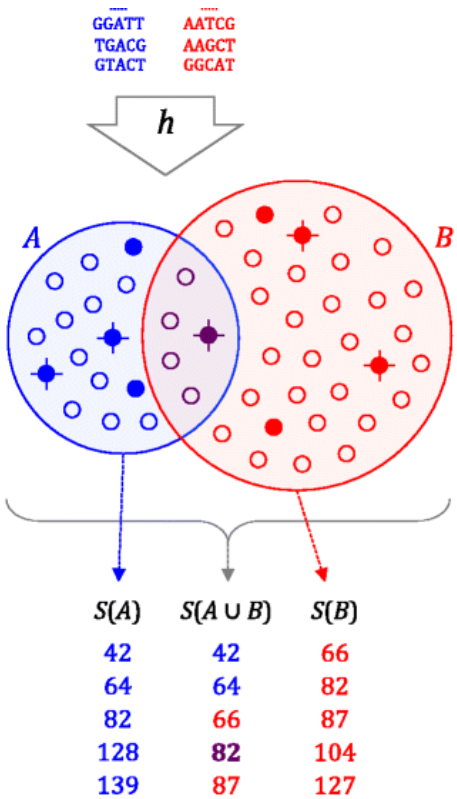


$$J(A,B) = \frac{|A \cap B|}{|A \cup B|} = \frac{16}{21} = 76\%$$

$$\text{ANI} \approx 1 + \frac{1}{k}\ln\left(\frac{2J}{1+J}\right)$$

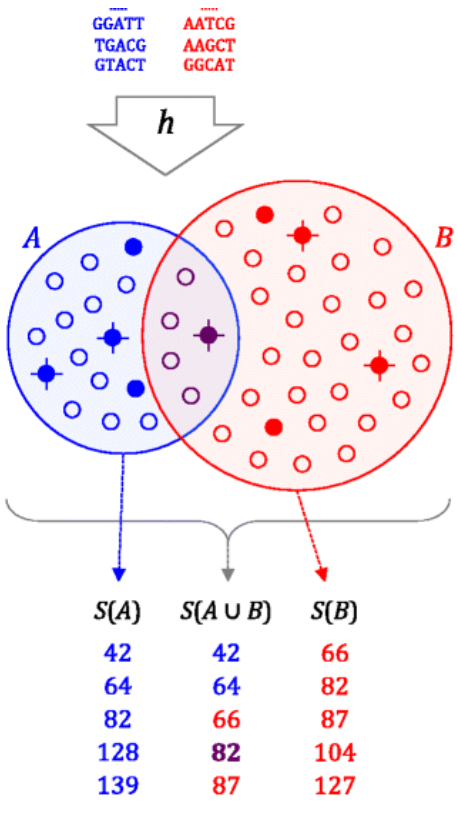
ANI = 95.1%



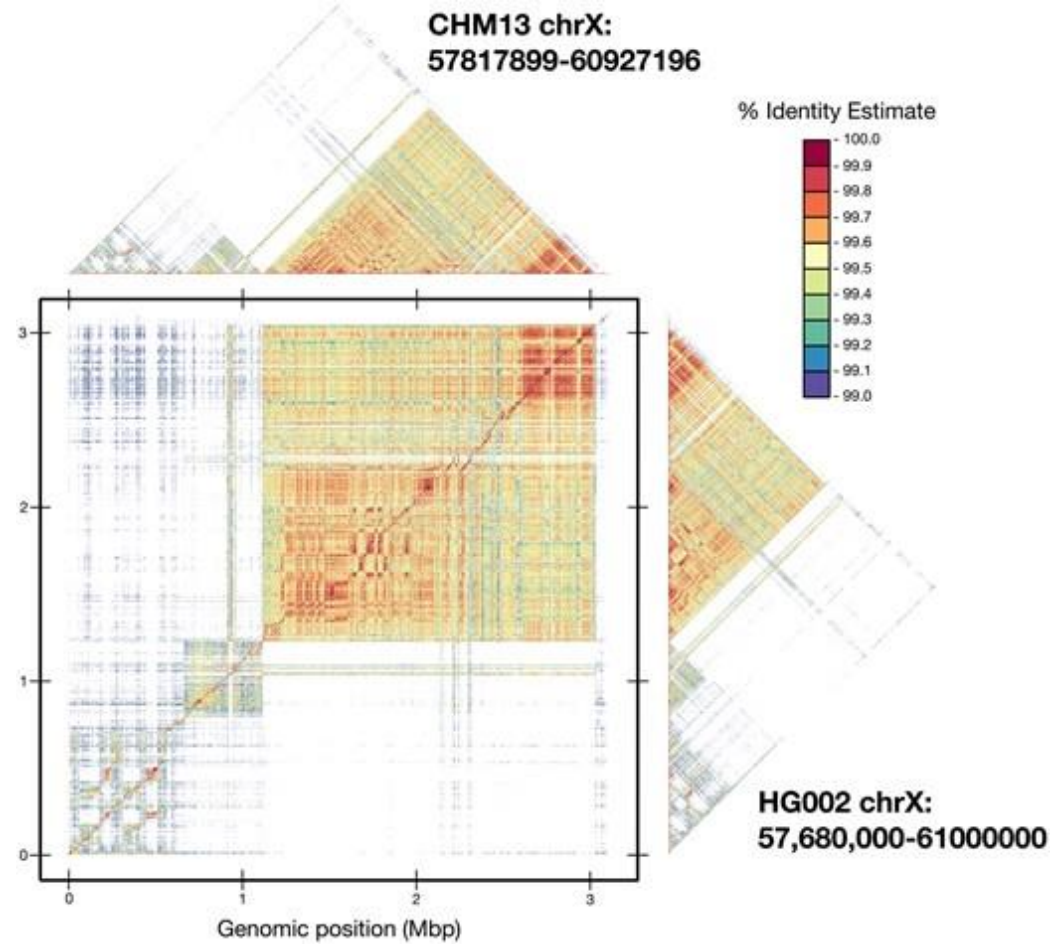
$$J(A,B) = \frac{|A \cap B|}{|A \cup B|} \approx \frac{|S(A \cup B) \cap S(A) \cap S(B)|}{|S(A \cup B)|}$$

Mash: fast genome and metagenome distance estimation using MinHash
Ondov et al (2016) Genome Biology. https://doi.org/10.1186/s13059-016-0997-x

ModDotPlot—rapid and interactive visualization of tandem repeats
Sweeten, Schatz, Phillippy (2024) Bioinformatics. https://doi.org/10.1093/bioinformatics/btae493



$$J(A,B) = \frac{|A \cap B|}{|A \cup B|} \approx \frac{|S(A \cup B) \cap S(A) \cap S(B)|}{|S(A \cup B)|}$$



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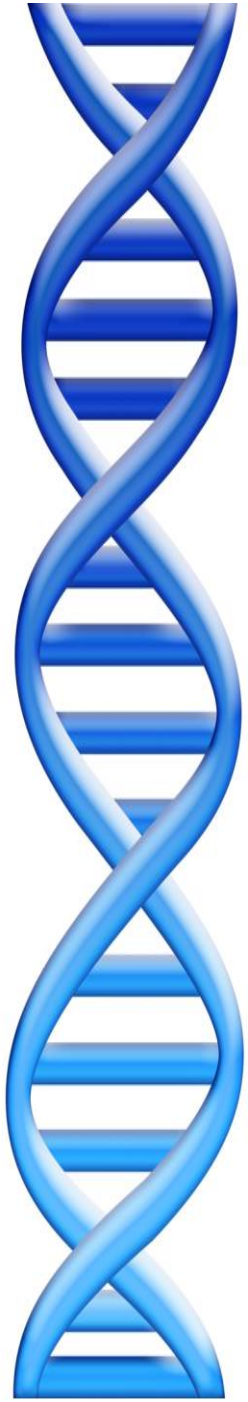
Part 2: De novo genome assembly

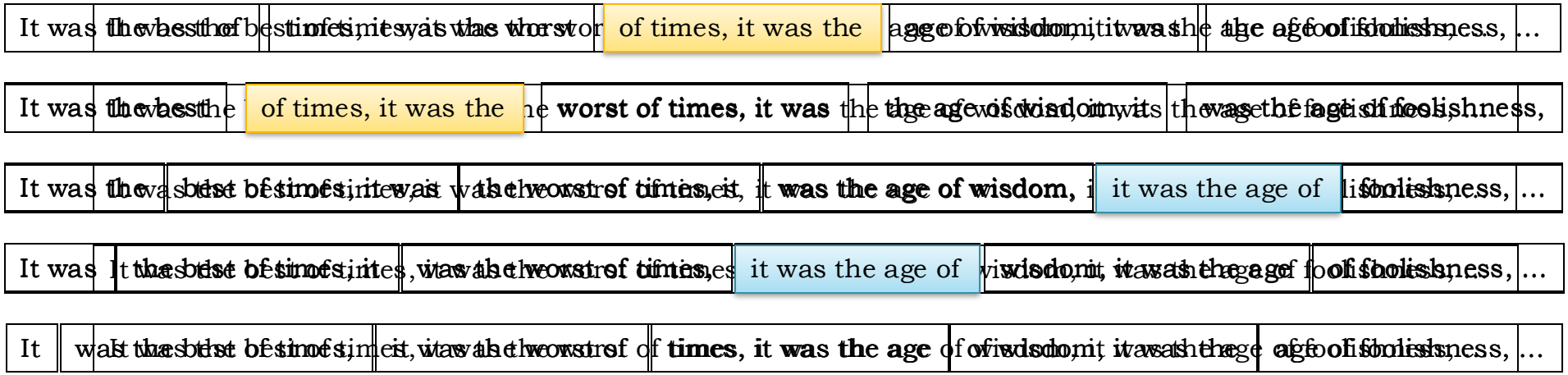


Outline

1. ***Assembly theory***
   - Assembly by analogy
2. ***Practical Issues***
   - Coverage, read length, errors, and repeats
3. ***Whole Genome Alignment***
   - MUMmer recommended

Shredded Book Reconstruction

- Dickens accidentally shreds the first printing of <u>A Tale of Two Cities</u>
  - Text printed on 5 long spools



- How can he reconstruct the text?
  - 5 copies x 138, 656 words / 5 words per fragment = 138k fragments
  - The short fragments from every copy are mixed together
  - Some fragments are identical

Greedy Reconstruction

It was the best of
age of wisdom, it was
best of times, it was
it was the age of
it was the age of
it was the worst of
of times, it was the
of times, it was the
of wisdom, it was the
the age of wisdom, it
the best of times, it
the worst of times, it
times, it was the age
times, it was the worst
was the age of wisdom,
was the age of foolishness,
was the best of times,
was the worst of times,
wisdom, it was the age
worst of times, it was

It was the best of
was the best of times,
the best of times, it
best of times, it was
of times, it was the
of times, it was the
times, it was the worst
times, it was the age

The repeated sequence make the correct reconstruction ambiguous

- It was the best of times, it was the [worst/age]

Model the assembly problem as a graph problem

How long will it take to compute the overlaps?

de Bruijn Graph Construction

- $G_k = (V,E)$
  - V = Length-*k* sub-fragments
  - E = Directed edges between consecutive sub-fragments
    - Sub-fragments overlap by *k*-1 words

Fragments |f|=5

It was the best of

was the best of times

Sub-fragment *k*=4

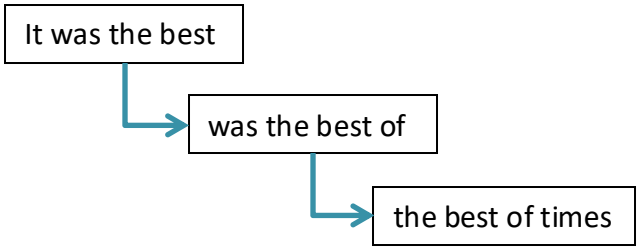
It was the best

was the best of

was the best of

the best of times

Directed edges (overlap by *k*-1)

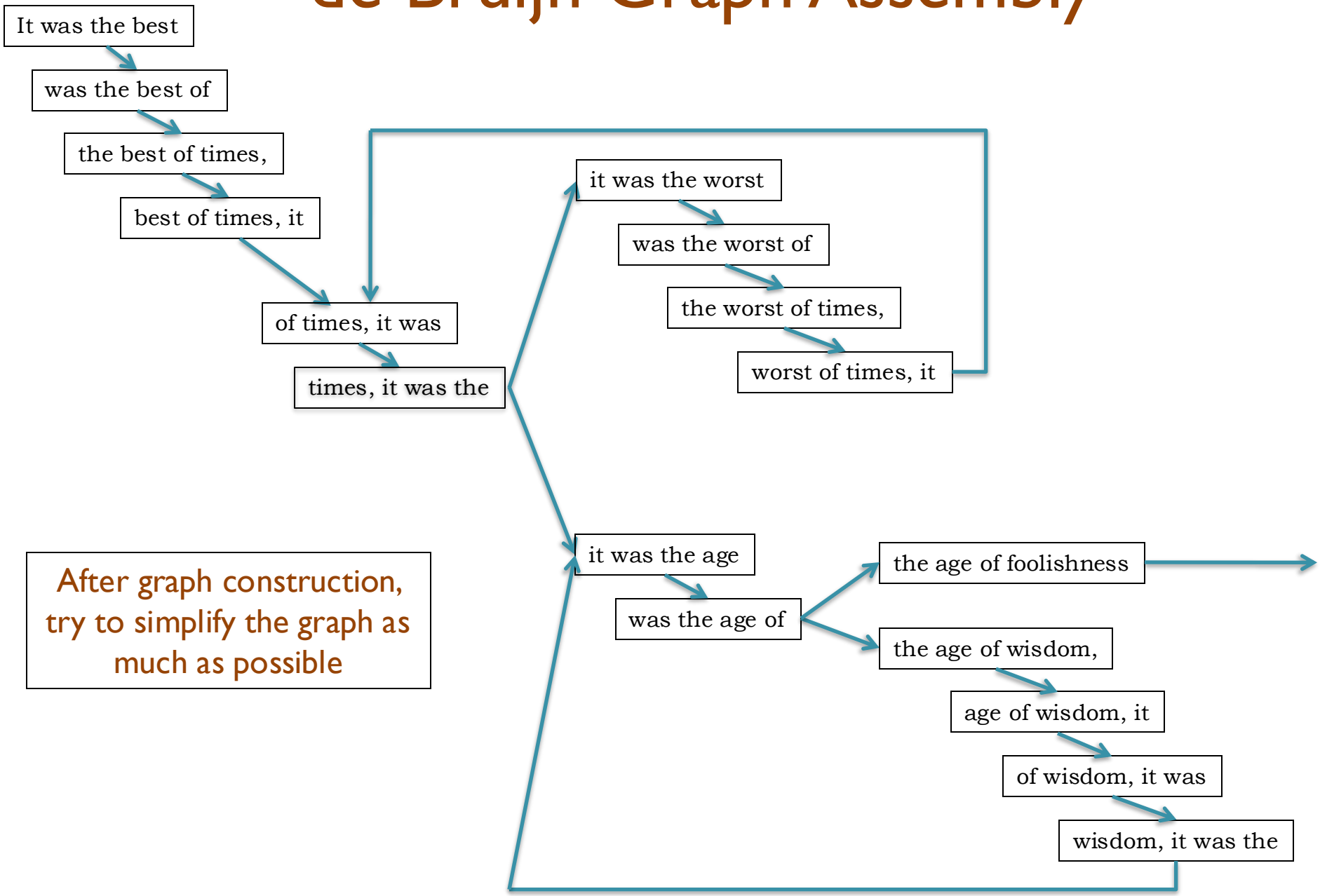


– Overlaps between fragments are implicitly computed

How to pronounce:
https://forvo.com/word/de_bruijn/

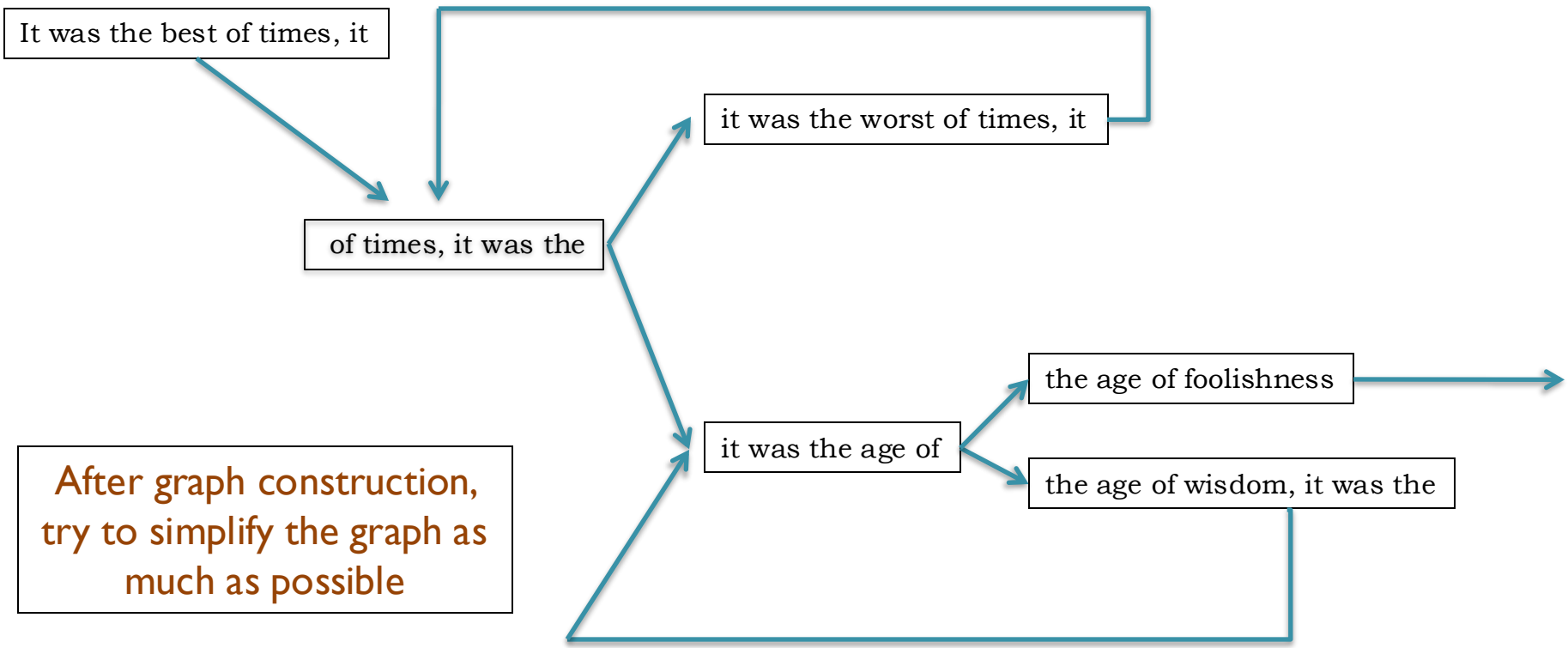
de Bruijn, 1946
Idury et al., 1995
Pevzner et al., 2001

de Bruijn Graph Assembly



After graph construction, try to simplify the graph as much as possible

de Bruijn Graph Assembly



After graph construction, try to simplify the graph as much as possible

The full tale

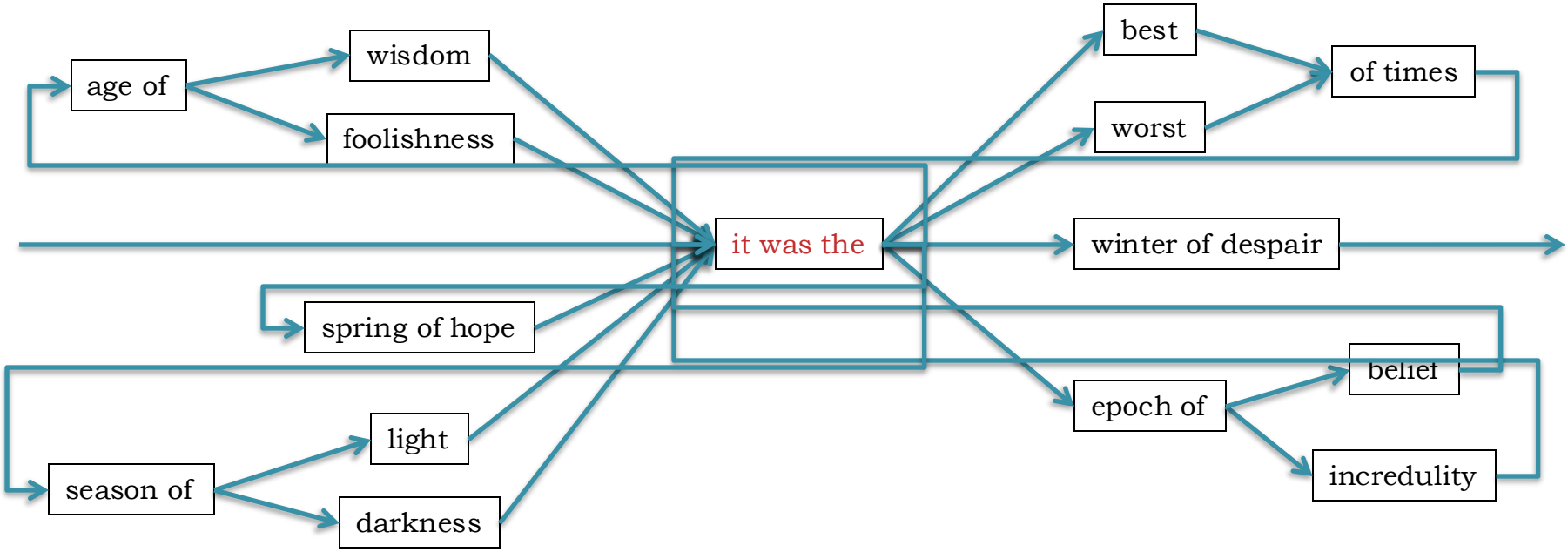
… it was the best of times it was the worst of times …

… it was the age of wisdom it was the age of foolishness …

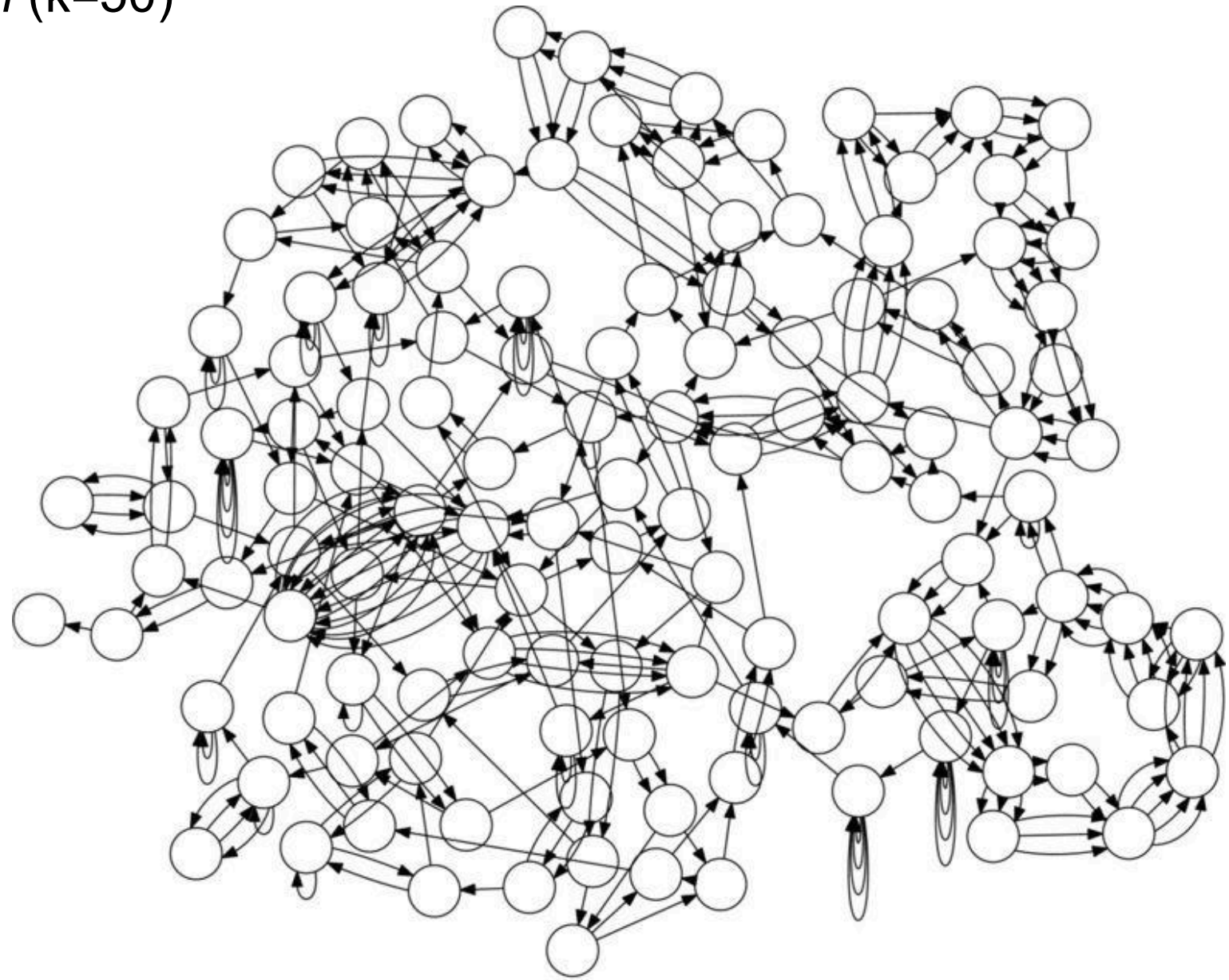
… it was the epoch of belief it was the epoch of incredulity …

… it was the season of light it was the season of darkness …

… it was the spring of hope it was the winder of despair …



E. coli (k=50)

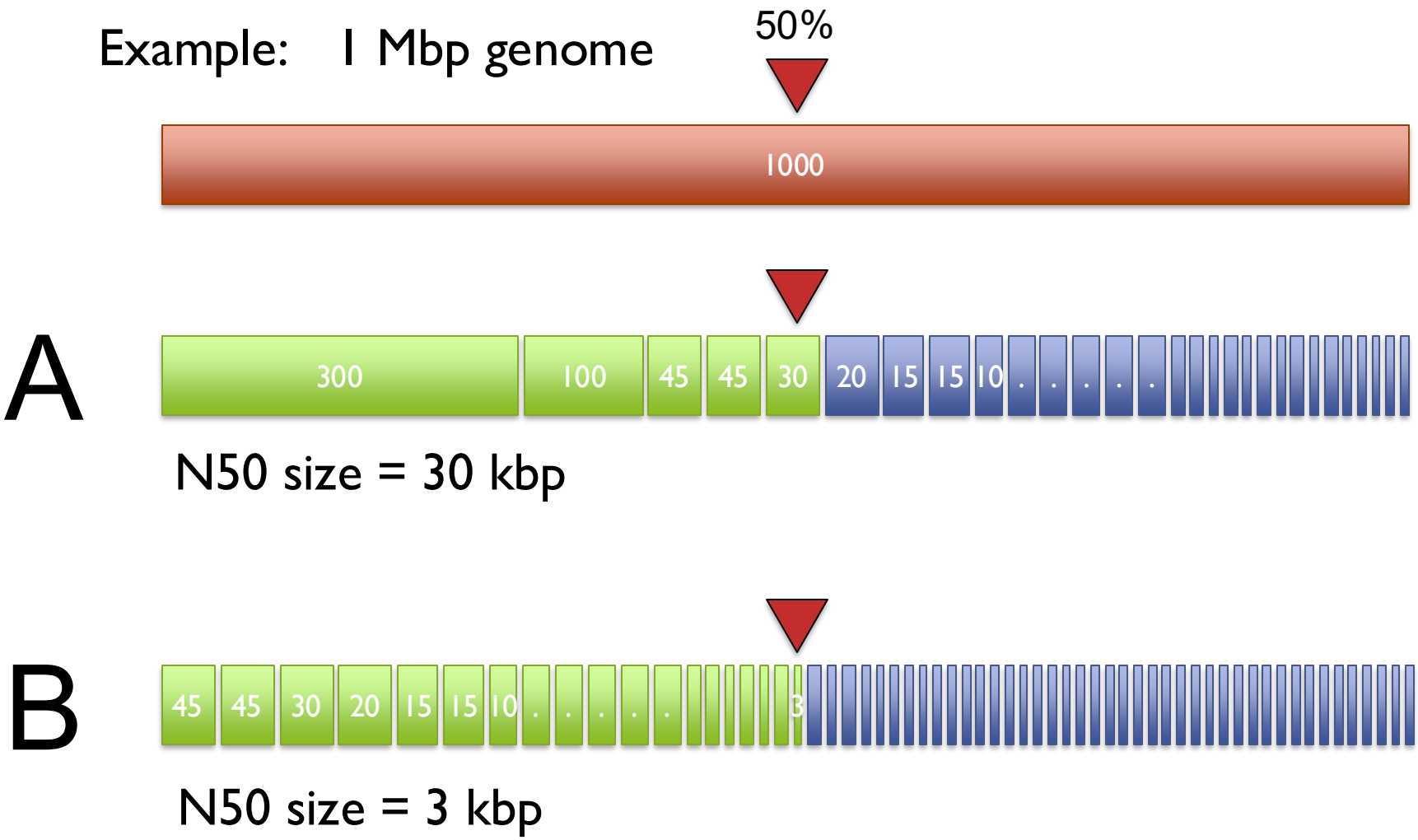


Reducing assembly complexity of microbial genomes with single-molecule sequencing
Koren et al (2013) Genome Biology. **14**:R101 https://doi.org/10.1186/gb-2013-14-9-r101

Contig N50

Def: 50% of the genome is in contigs as large as the N50 value

Example: 1 Mbp genome



Contig N50

Def: 50% of the genome is in contigs as large as the N50 value

Better N50s improves the analysis in every dimension

- Better resolution of genes and flanking regulatory regions
- Better resolution of transposons and other complex sequences
- Better resolution of chromosome organization
- Better sequence for all downstream analysis

Just be careful of N50 inflation!

- *A very very very bad assembler in 1 line of bash:*
- *cat *.reads.fa > genome.fa*

N50 size = 3 kbp

Pop Quiz 1

Assemble these reads using a de Bruijn graph approach (k=3):

ACACG

ATTAC

GATTA

TTACA

Pop Quiz 1

Assemble these reads using a de Bruijn graph approach (k=3):

```
ACACG:  ACA -> CAC -> ACG
ATTAC:  ATT -> TTA -> TAC
GATTA:  GAT -> ATT -> TTA
TTACA:  TTA -> TAC -> ACA
```

Pop Quiz 1

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ACACG:  ACA -> CAC -> ACG
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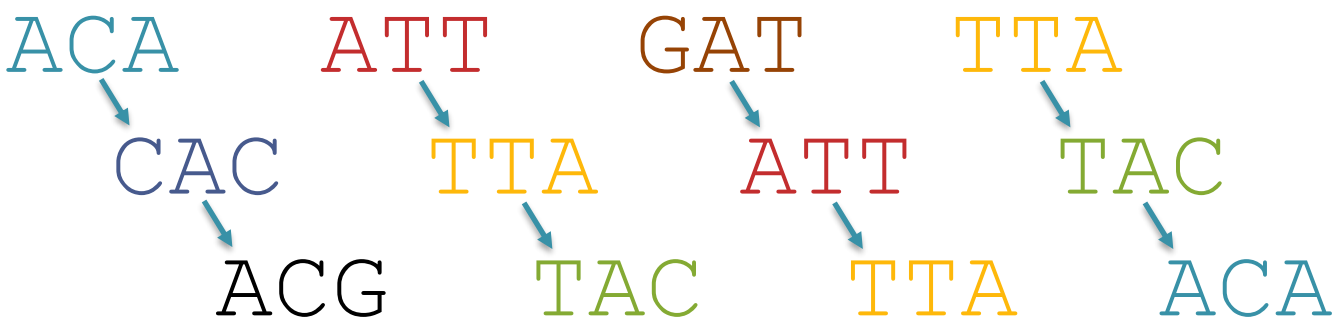
```



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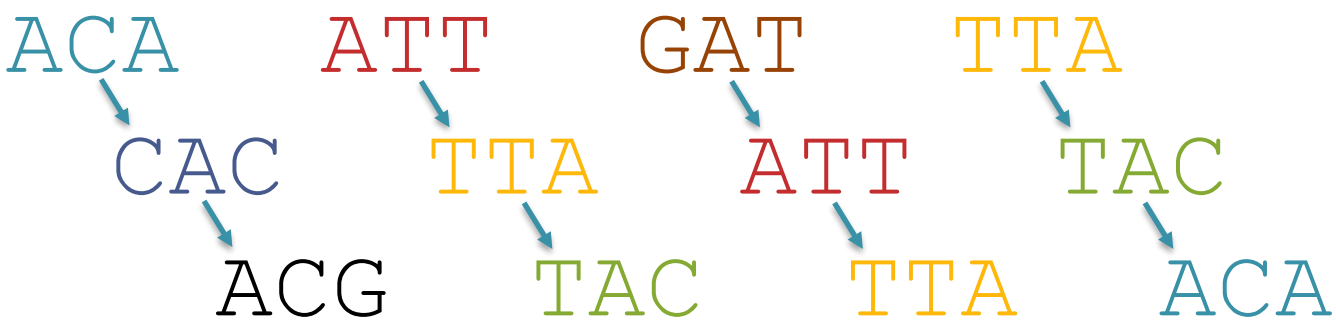

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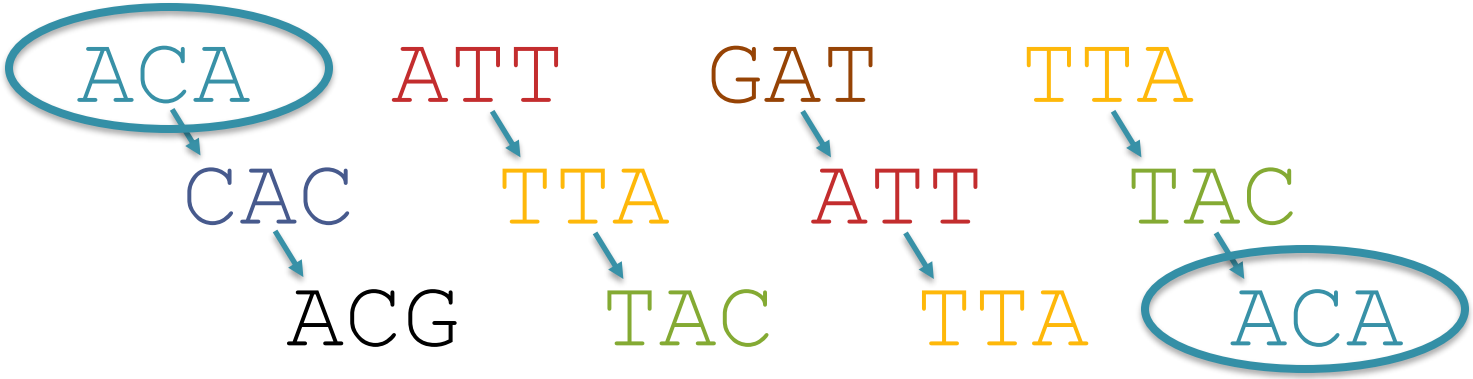

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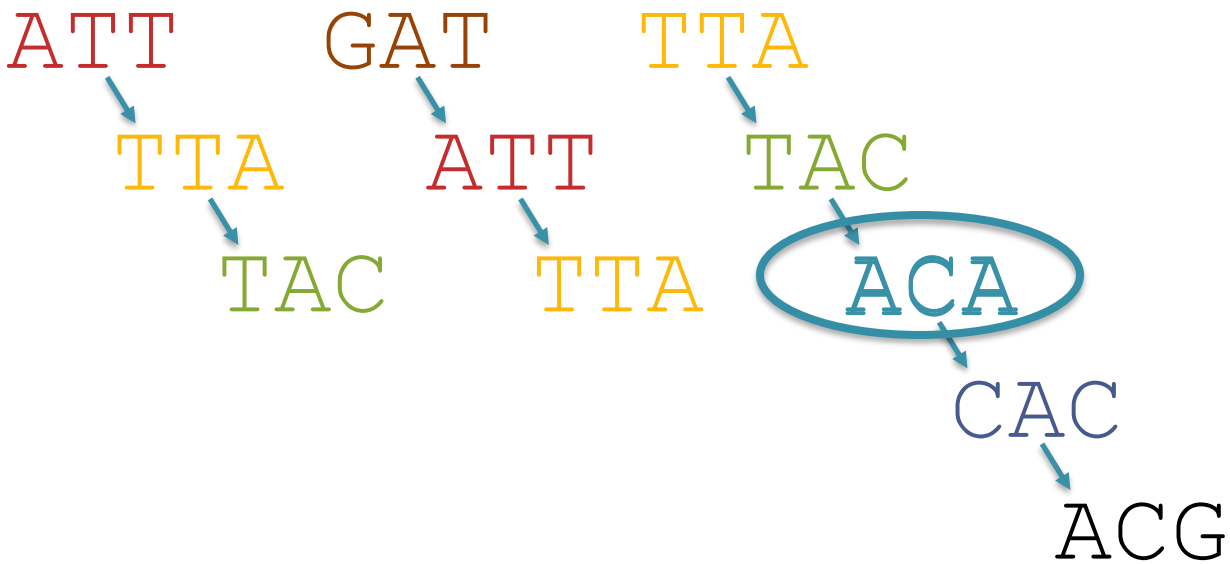

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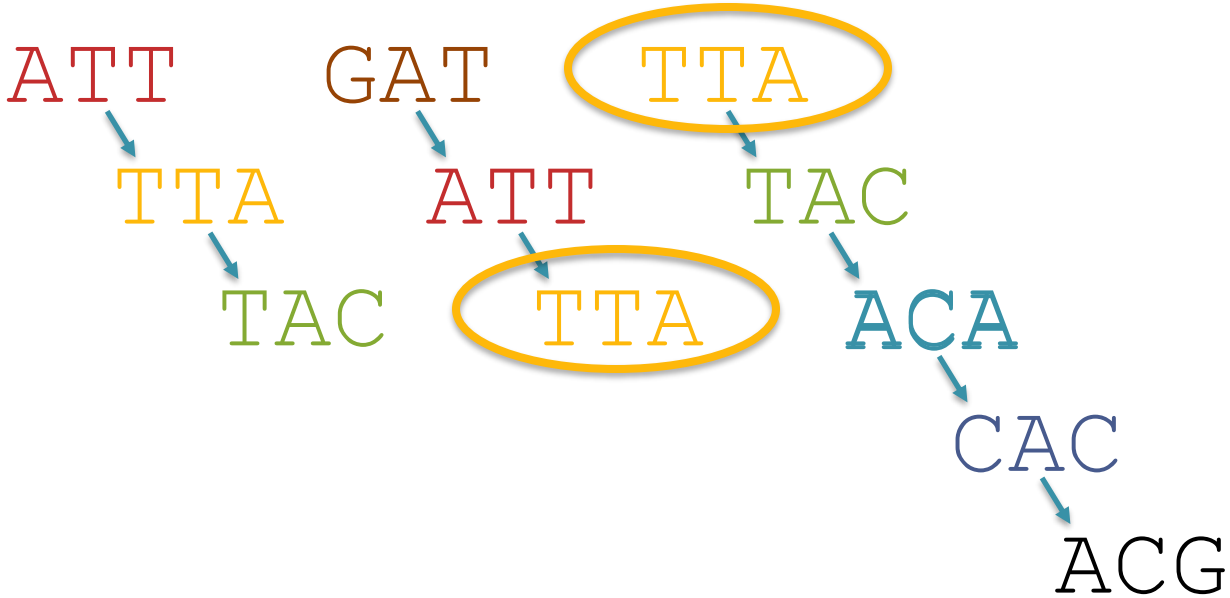

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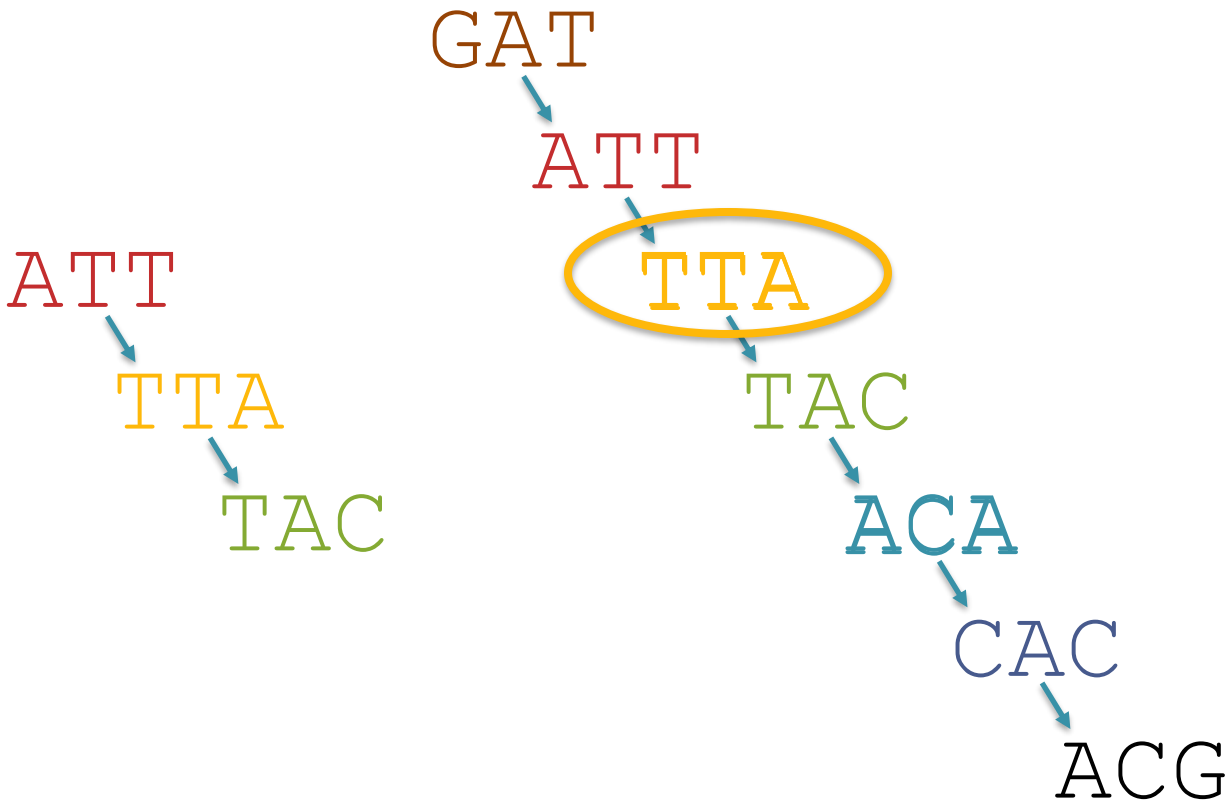

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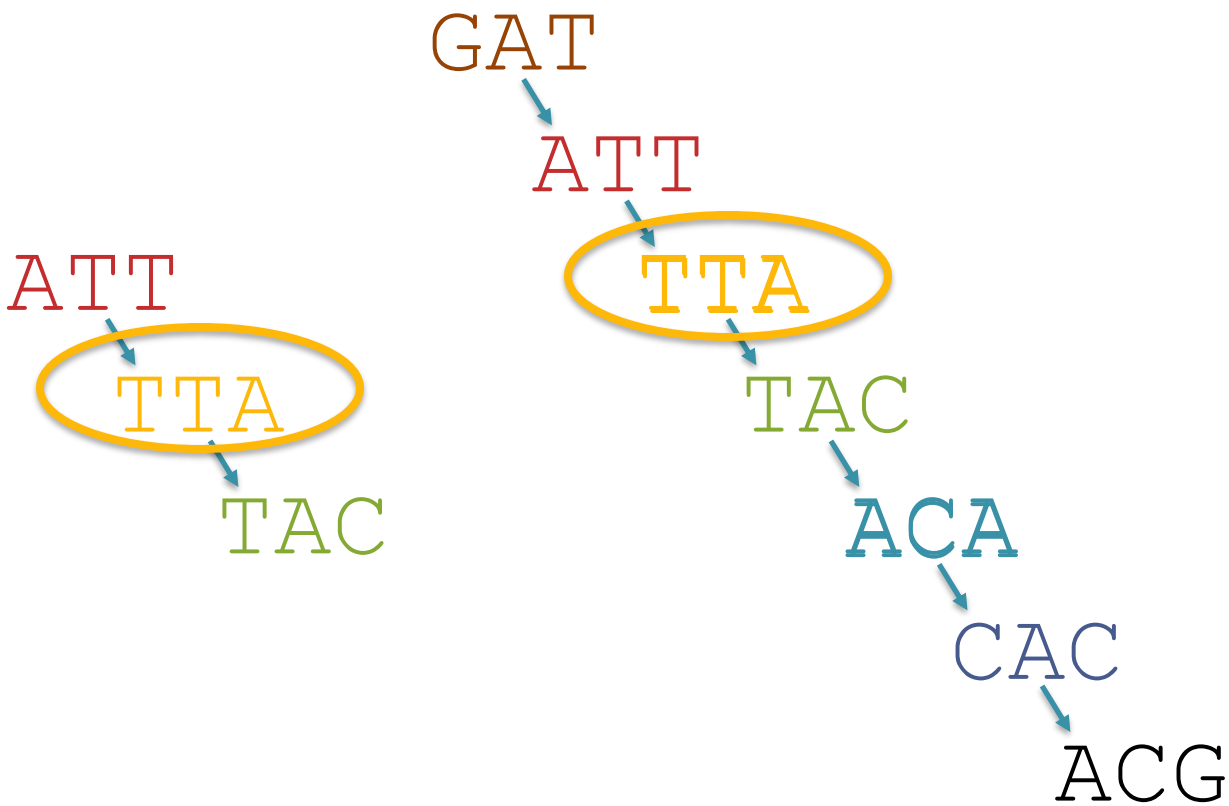

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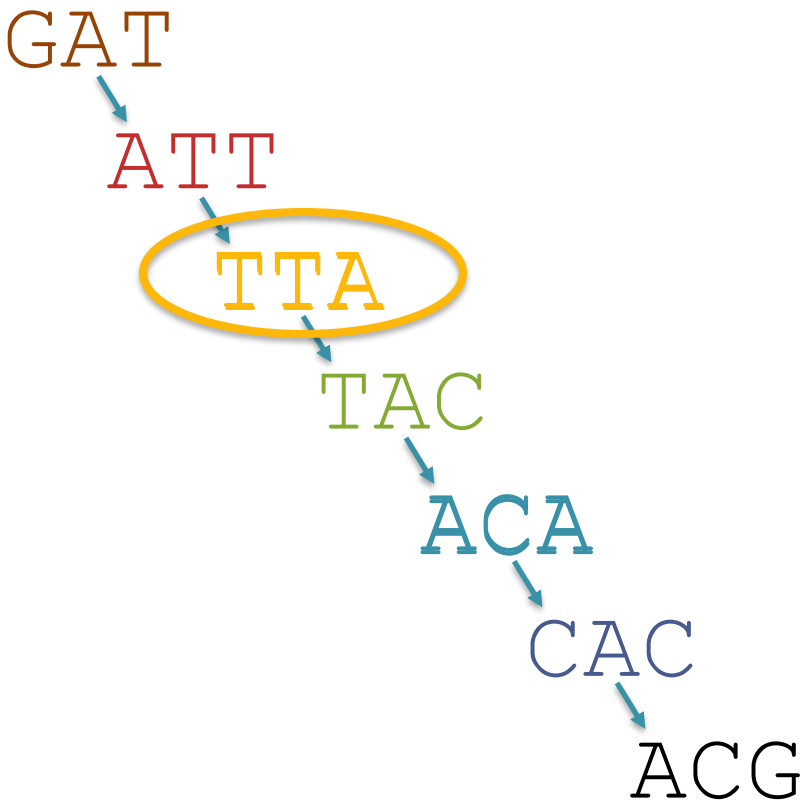

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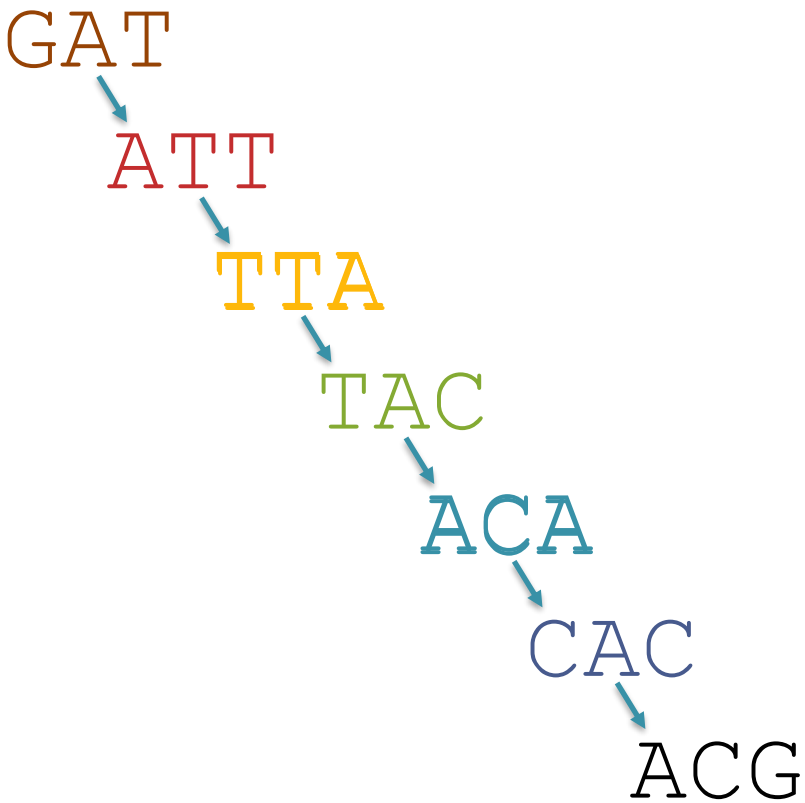

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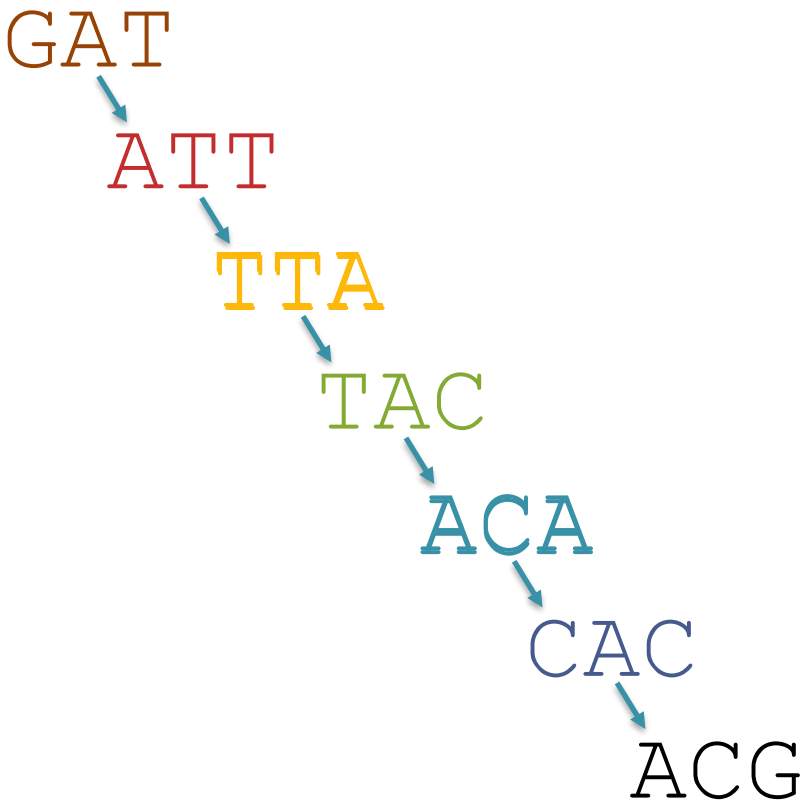

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```

```
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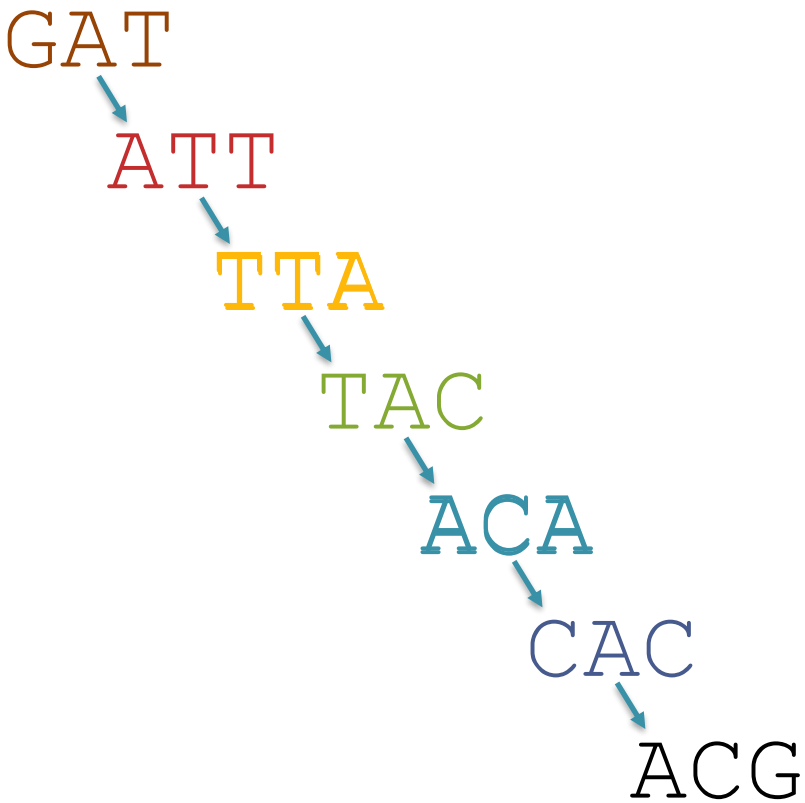
 ATTAC
  TTACA
    ACACG
```



Pop Quiz 2

Assemble these reads using a de Bruijn graph approach (k=3):

ACGA

ACGT

ATAC

CGAC

CGTA

GACG

GTAT

TACG

Pop Quiz 2

Assemble these reads using a de Bruijn graph approach (k=3):

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ATAC

CGAC

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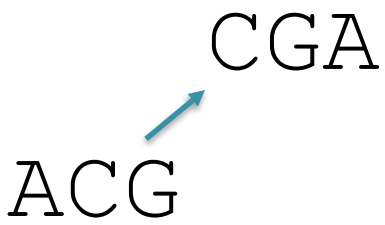
GTAT

TACG



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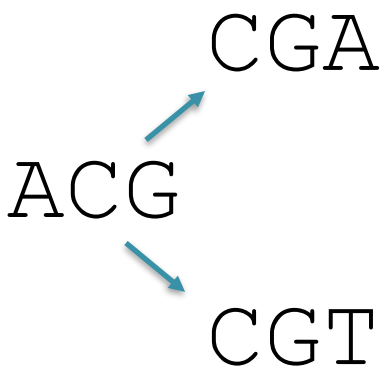

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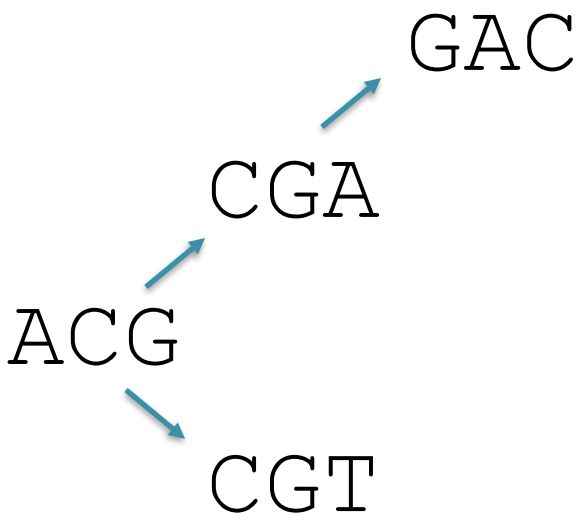

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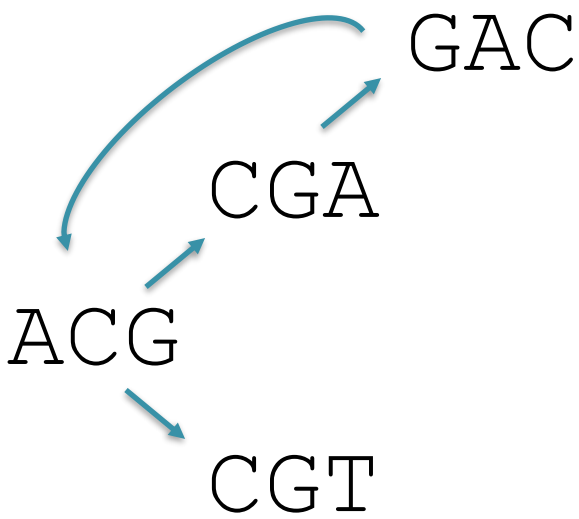

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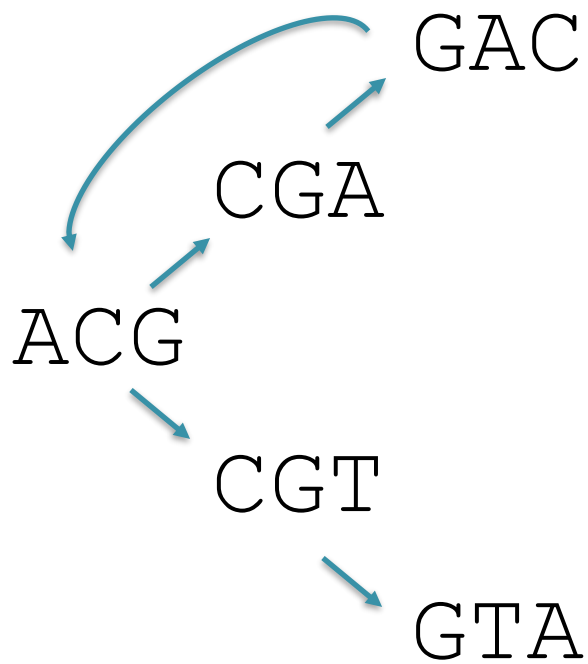

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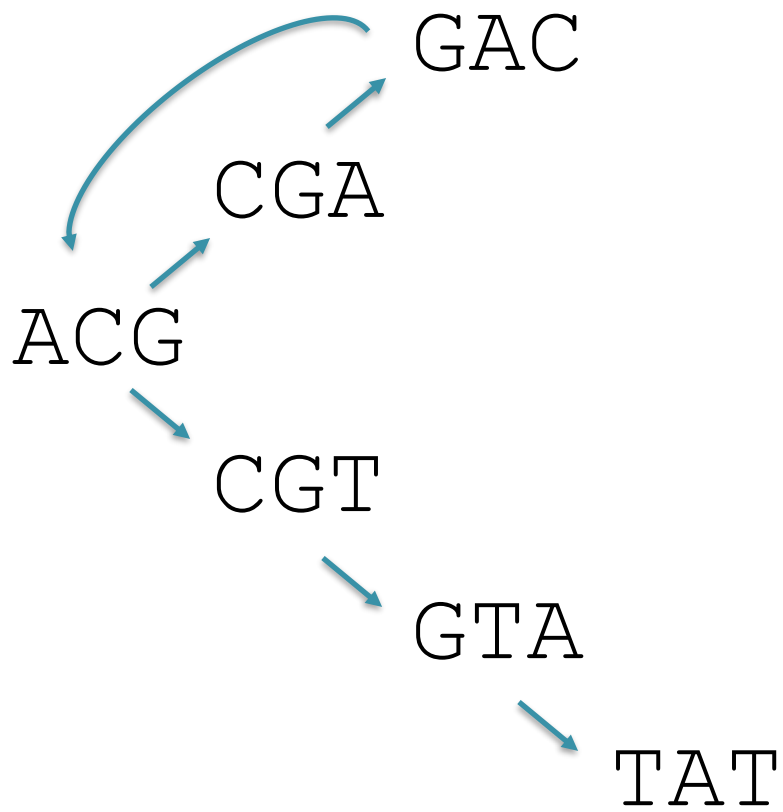

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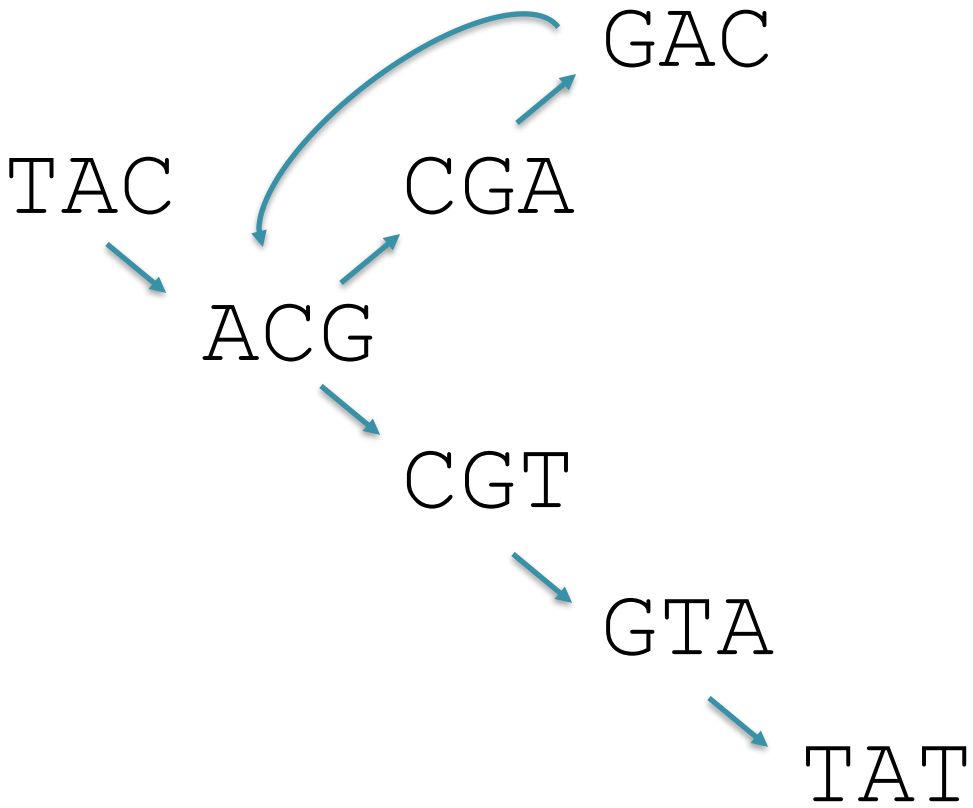

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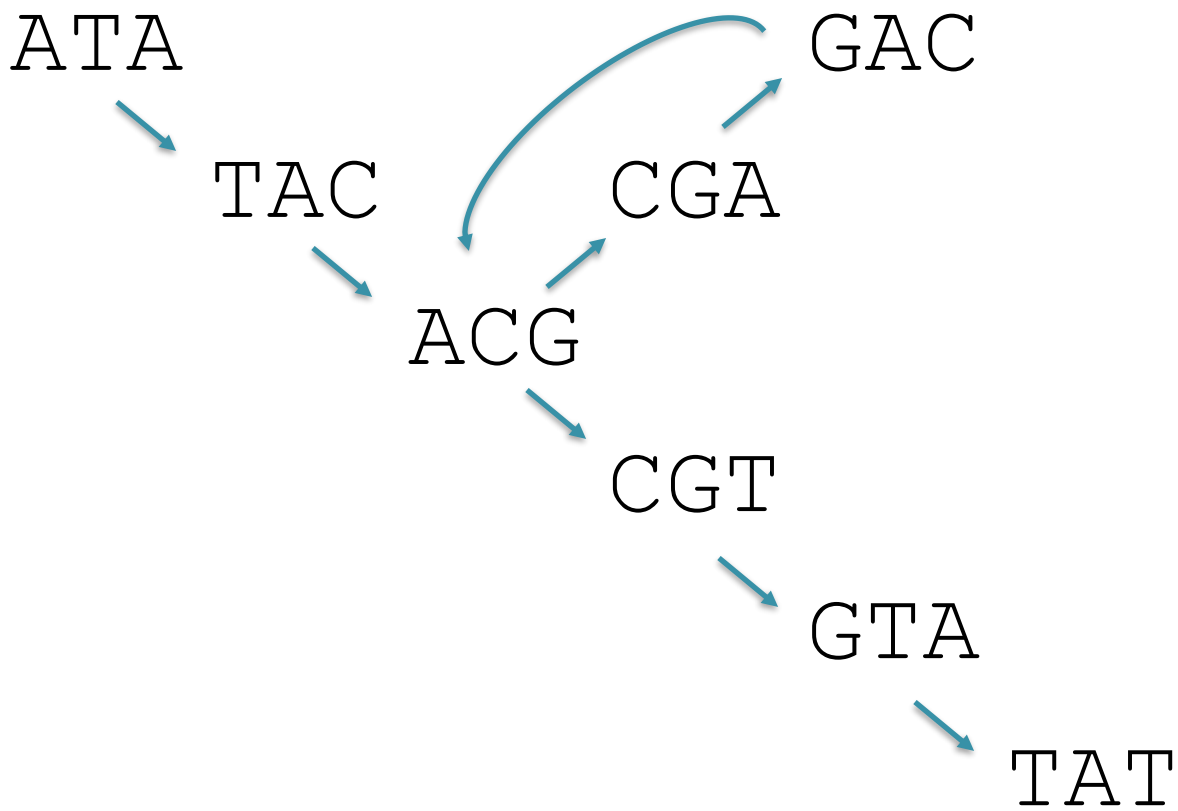

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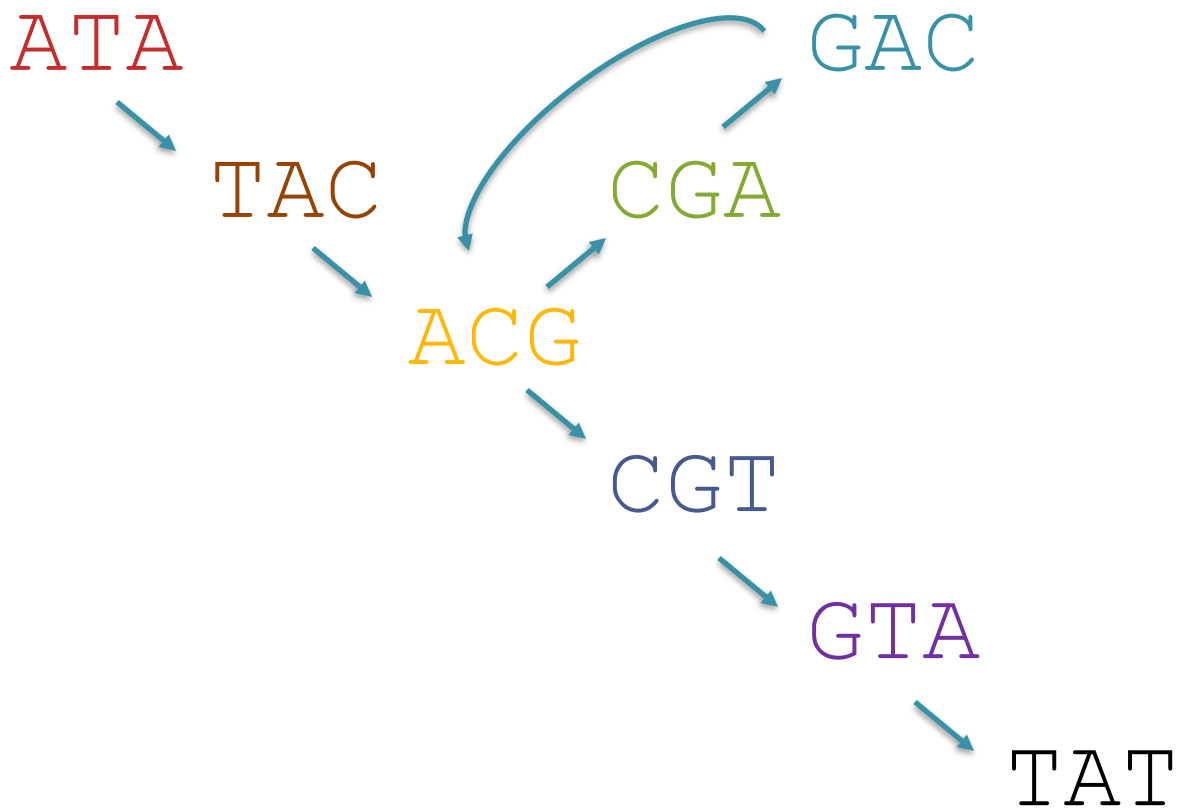

ATACGACGTAT

Pop Quiz 2

Assemble these reads using a de Bruijn graph approach (k=3):

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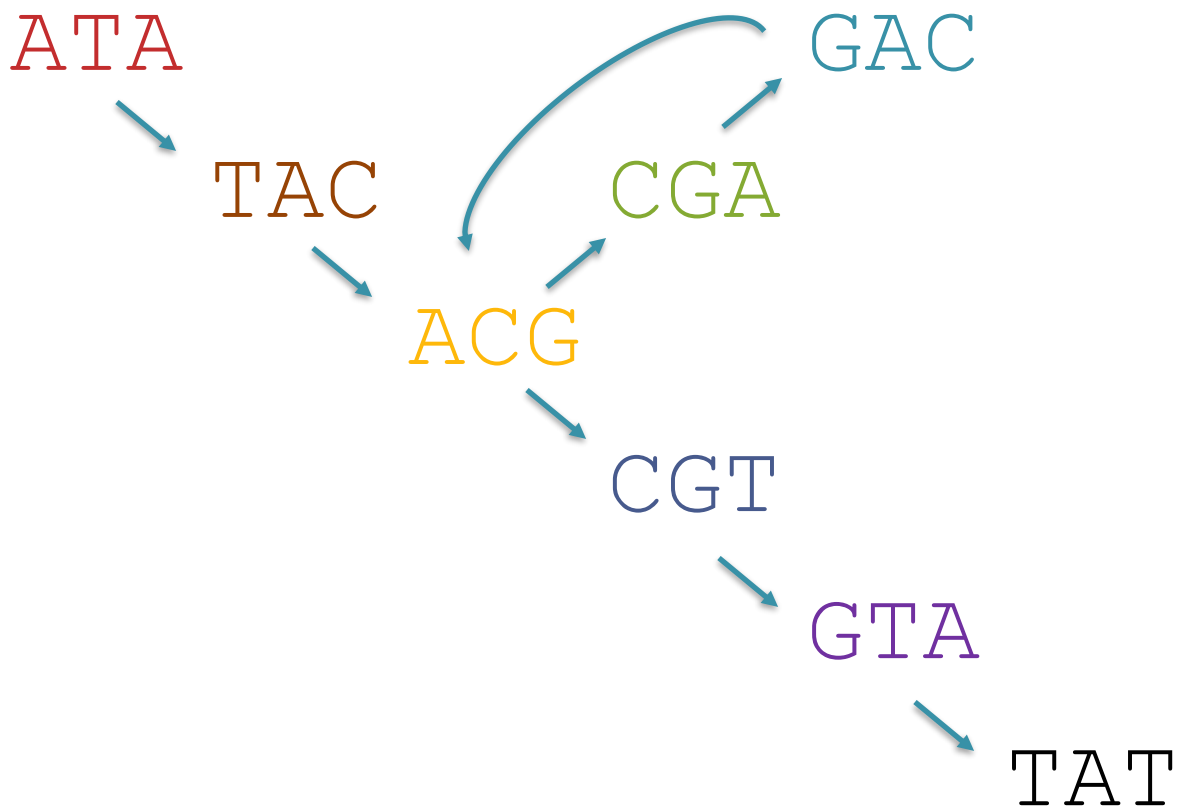

Whats another possible genome?

ATACGACGTAT

Pop Quiz 2

Assemble these reads using a de Bruijn graph approach (k=3):

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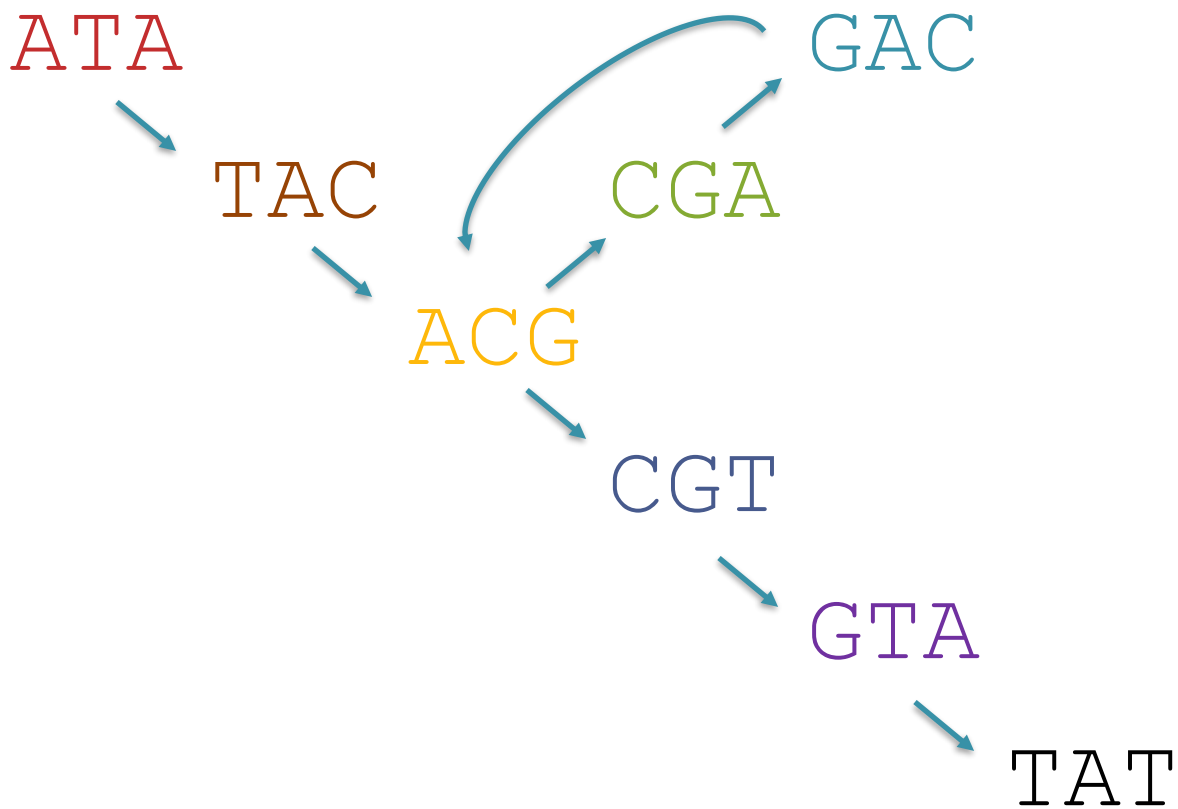
~~GACG~~
~~GTAT~~
~~TACG~~



Should we add the edge TAT -> ATA?

ATACGACGTAT