Genome Assembly

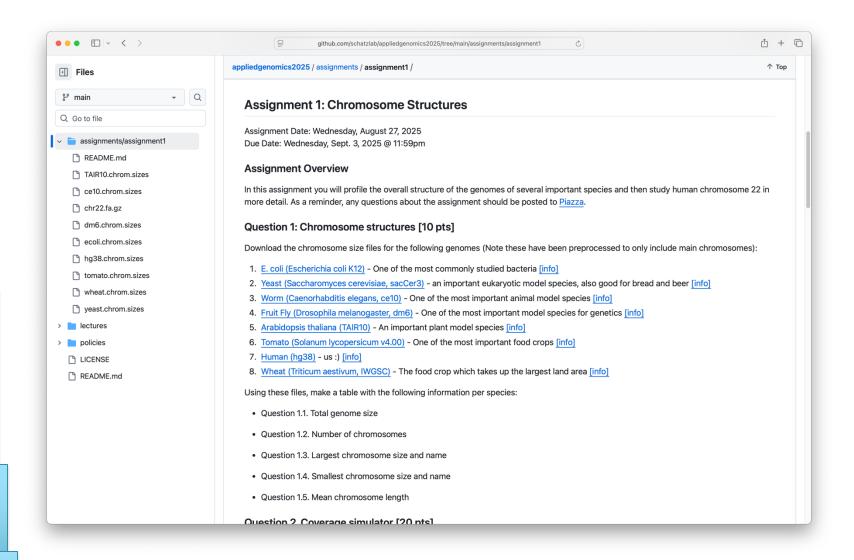
Michael Schatz

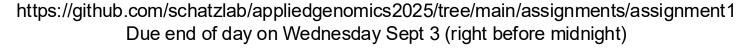
Sept 3, 2025

Lecture 3: Applied Comparative Genomics

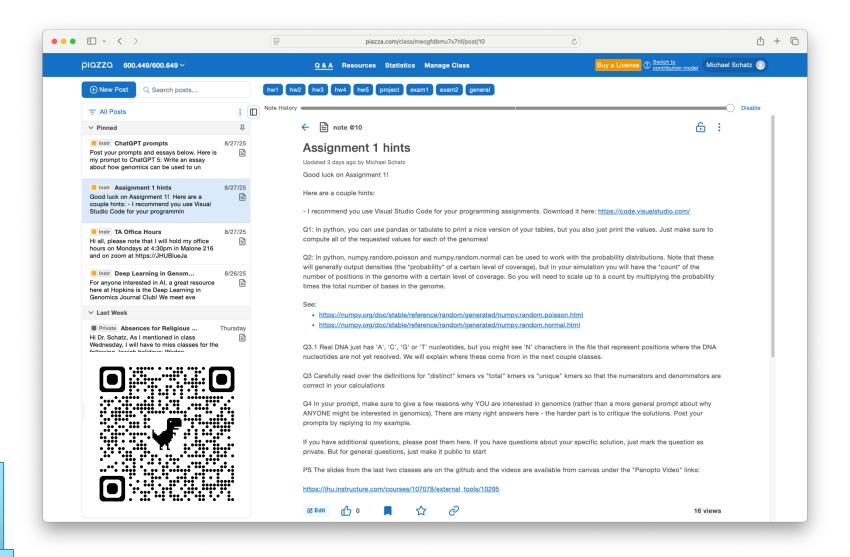


Assignment I









TA: Mahler Revsine



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

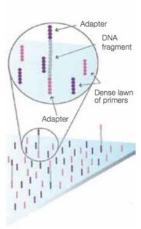
Part I: Recap and Illumina Sequencing

Second Generation Sequencing

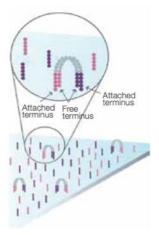


Illumina NovaSeq 6000 Sequencing by Synthesis

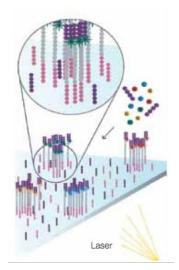
>3Tbp / day (JHU has 4 of these!)



1. Attach



2. Amplify



3. Image







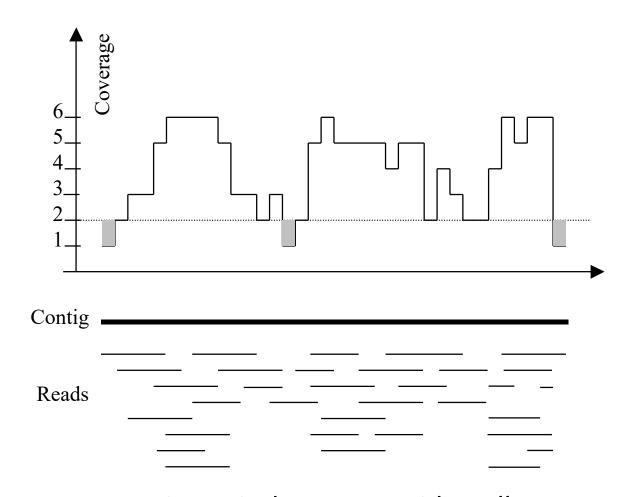






Metzker (2010) Nature Reviews Genetics 11:31-46 https://www.youtube.com/watch?v=fCd6B5HRaZ8

Typical sequencing coverage



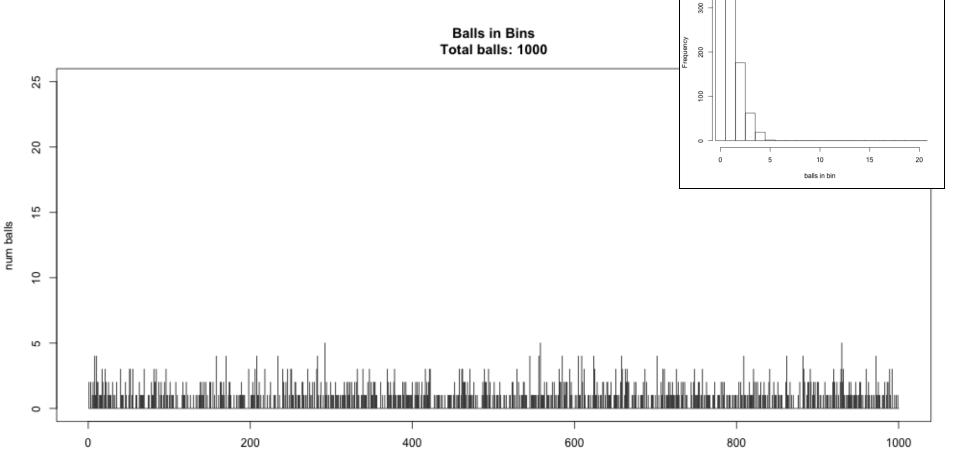
Imagine raindrops on a sidewalk
We want to cover the entire sidewalk but each drop costs \$1

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

Ix sequencing

Histogram of balls in each bin Total balls: 1000 Empty bins: 361

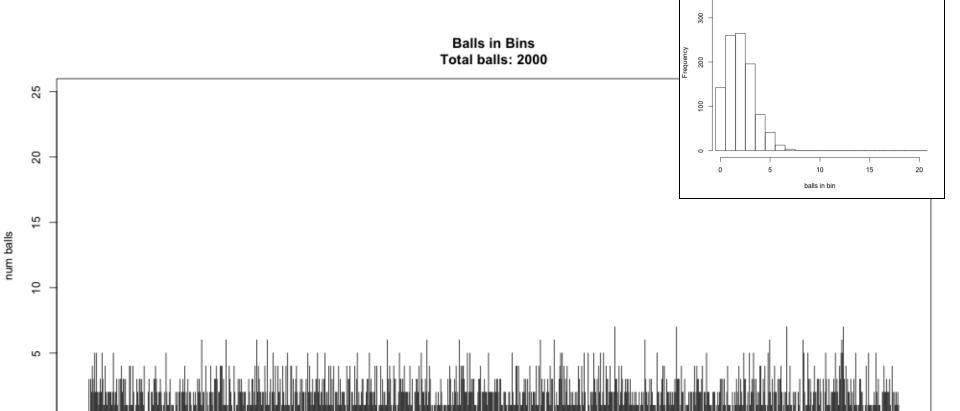
400



bin id

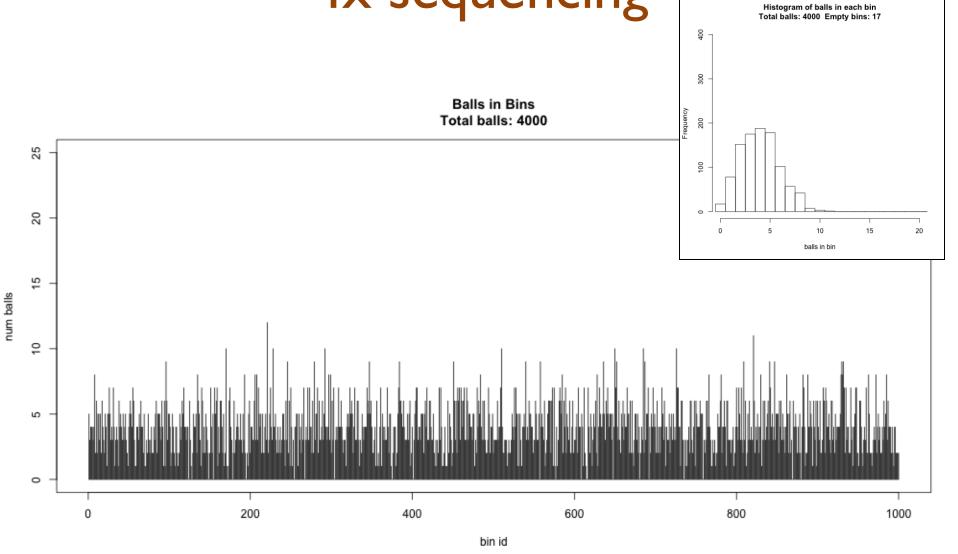
2x sequencing

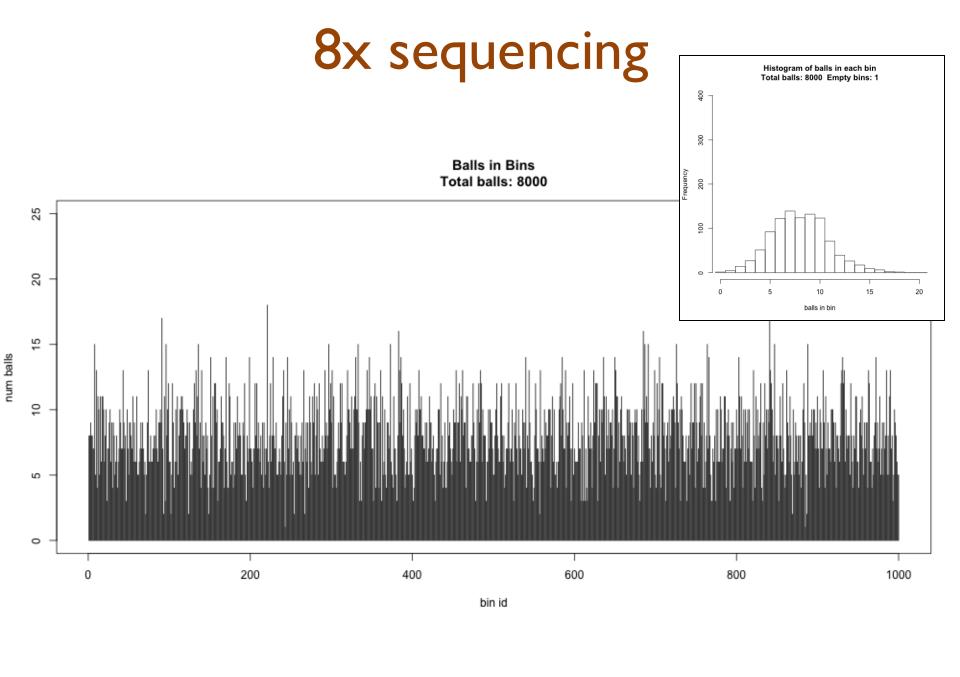
Histogram of balls in each bin Total balls: 2000 Empty bins: 142



bin id

4x 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.

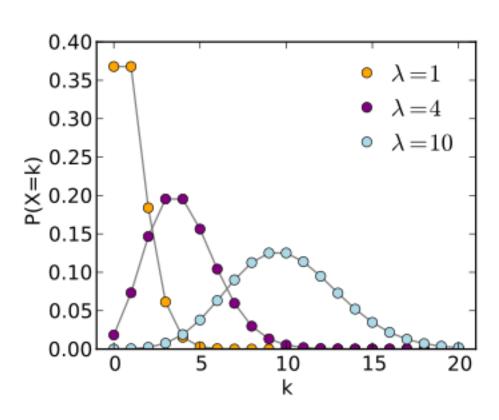
Formulation comes from the limit of the binomial equation

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

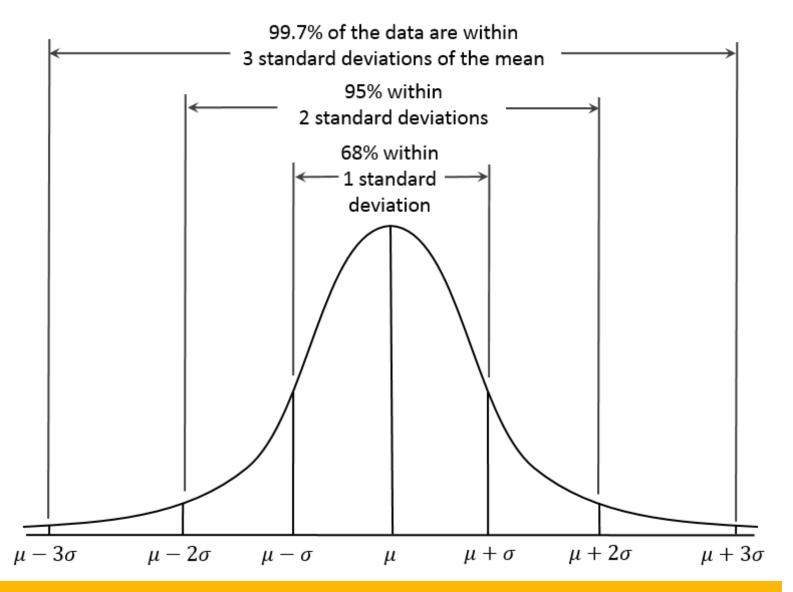
Key properties:

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

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



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** 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 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

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

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

```
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

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

How long should k be?

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

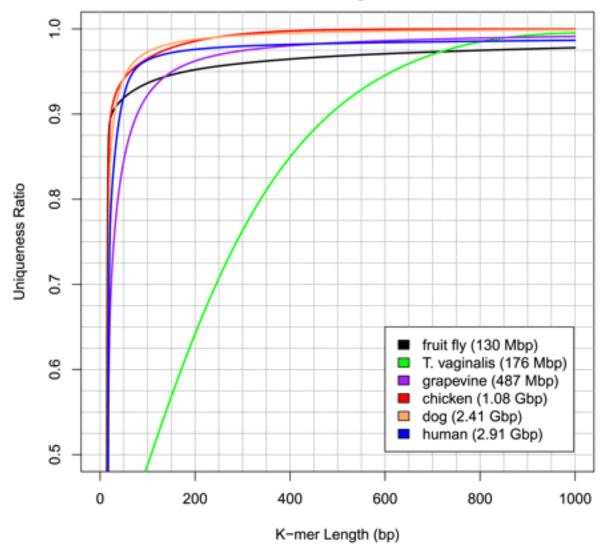
How long should k be?

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 = log₄ (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

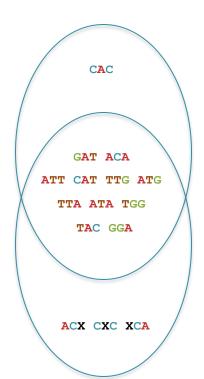
$$J(A,B) = rac{|A \cap B|}{|A \cup B|} = rac{18}{18} = 100\%$$

GATTACATAC A CATTGGATG

GATTACATAC X CATTGGATG

GATTACAT ACACA TTGGATG
GAT ACA ACA ATT GAT
ATT CAT CAC TTG ATG
TTA ATA ACA TGG
TAC TAC CAT GGA

GATTACAT ACXCA TTGGATG
GAT ACA ACX A TT GAT
ATT CAT CXC TTG ATG
TTA ATA XCA TGG
TAC TAC CAT GGA



GATTACAT ACACA TTGGATG 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

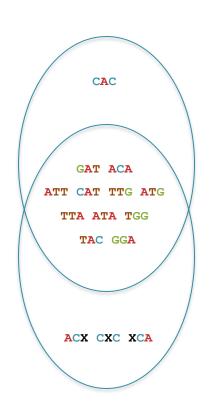


Jaccard Coefficient

$$J(A,B) = rac{|A\cap B|}{|A\cup B|} = rac{11}{15} = 73.3\%$$

GATTACAT ACACATTGGATG 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

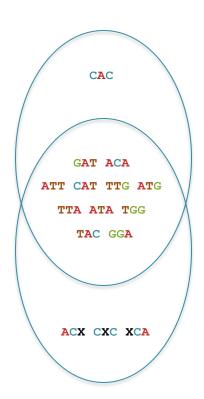


$$J(A,B) = rac{|A \cap B|}{|A \cup B|} = rac{11}{15} = 73.3\%$$

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

GATTACAT ACACA ITGGATG GAT ACA ACA AIT GAT ATT CAT CAC ITG ATG TTA ATA ACA TGG TAC TAC CAI GGA

GATTACAT ACXCA TTGGATG
GAT ACA ACX A TT GAT
ATT CAT CXC TTG ATG
TTA ATA XCA TGG
TAC TAC CAT GGA

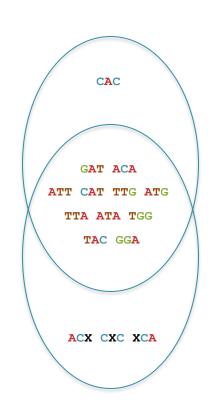


From Jaccard to Average Nucleotide Identity

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

GATTACAT ACACA TTGGATG AT ACA ACA ATT GAT ATT CAT CAC TTG ATG TTA ATA ACA TGG TAC TAC CAT GGA

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



From Jaccard to Average Nucleotide Identity

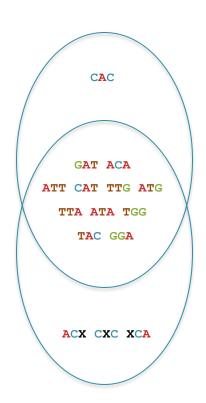
$$J \approx \frac{p}{2-p} \implies p = \frac{2J}{1+J}$$

$$p = I^k$$

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

GATTACAT ACACA TTGGATG
GAT ACA ACA ATT GAT
ATT CAT CAC TTG ATG
TTA ATA ACA TGG
TAC TAC CAT GGA

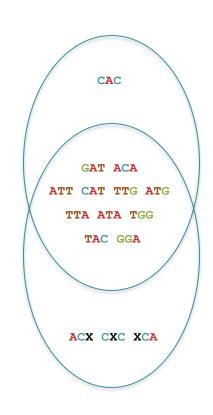
GATTACAT ACXCA TTGGATG
GAT ACA ACX A TT GAT
ATT CAT CXC TTG ATG
TTA ATA XCA TGG
TAC TAC CAT GGA



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

GATTACAT ACACA ITGGATG GAT ACA ACA AIT GAT ATT CAT CAC ITG 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



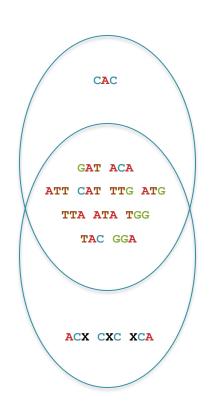
Identity for small differences (d): $p=I^k=(1-d)^kpprox e^{-kd}$

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

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

GATTACAT ACACA TTGGATG GAT ACA ACA ATT GAT ATT CAT CAC TTG ATG TTA ATA ACA TGG TAC TAC CAT GGA

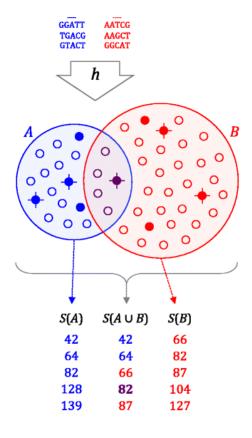
GATTACAT ACX CATTGGATG
GAT ACA ACX ATT GAT
ATT CAT CXC TTG ATG
TTA ATA XCA TGG
TAC TAC CAT GGA



$$J(A,B) = rac{|A \cap B|}{|A \cup B|} = rac{11}{15} = 73.3\%$$

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

ANI = 94.4%



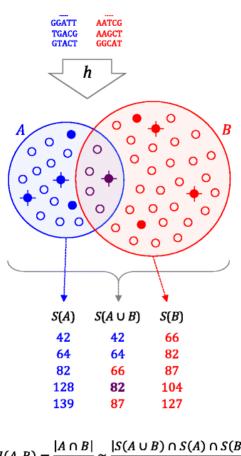
$$J(A,B) = \frac{|A \cap B|}{|A \cup B|} \approx \frac{|S(A \cup B) \cap S(A) \cap S(B)|}{|S(A \cup B)|}$$

Mash: fast genome and metagenome distance estimation using MinHash

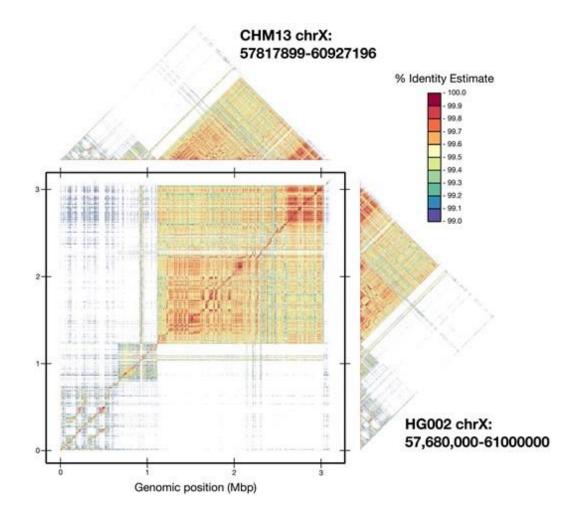
Ondov et al (2016) Genome Biology. https://doi.org/10.1186/s13059-016-0997-x

ModDotPlot—rapid and interactive visualization of tandem repeats

Sweeten, Schatz, Phillippy (2024) Bioinformatics. https://doi.org/10.1093/bioinformatics/btae493



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



Mash: fast genome and metagenome distance estimation using MinHash

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ModDotPlot—rapid and interactive visualization of tandem repeats

Sweeten, Schatz, Phillippy (2024) Bioinformatics. https://doi.org/10.1093/bioinformatics/btae493

Part 2: De novo genome assembly



Outline

I. Assembly theory

Assembly by analogy

2. Practical Issues

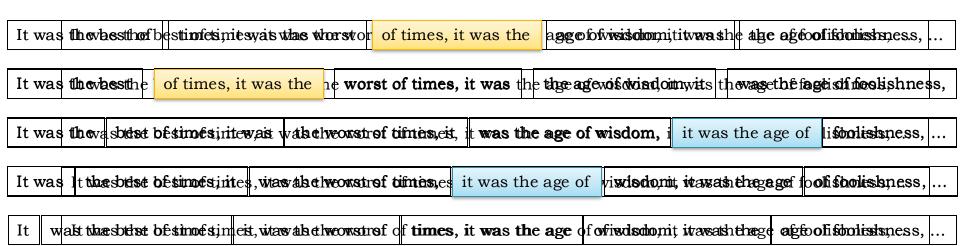
Coverage, read length, errors, and repeats

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

```
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
```

Greedy Reconstruction

```
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

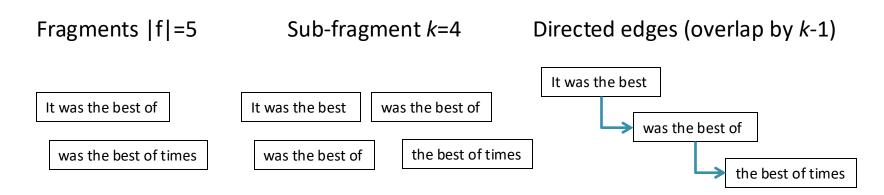
• It was the best of times, it was the [worst/age]

Model the assembly problem as a graph problem

How long will it take to compute the overlaps?

de Bruijn Graph Construction

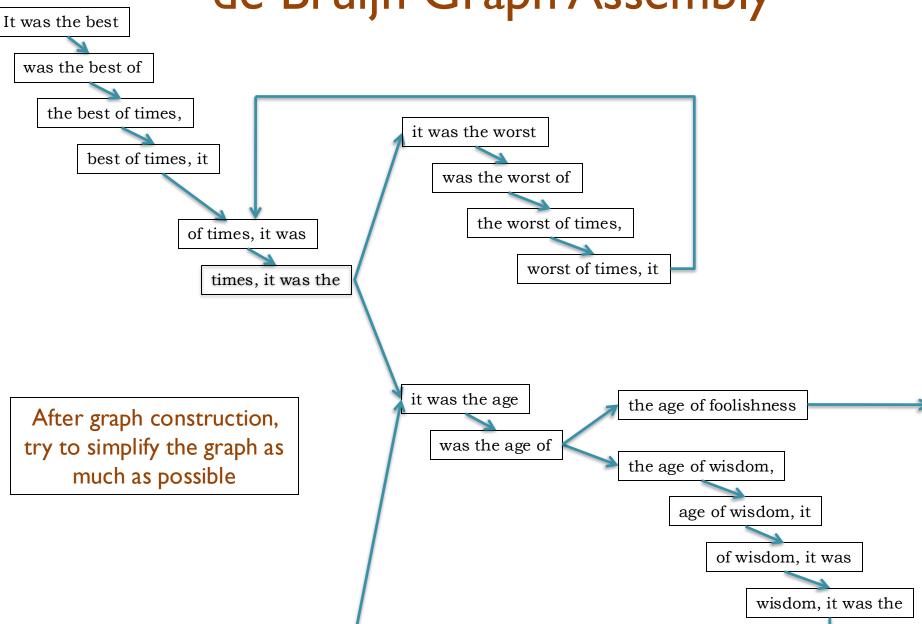
- $G_k = (V,E)$
 - V = Length-k sub-fragments
 - E = Directed edges between consecutive sub-fragments
 - Sub-fragments overlap by k-1 words



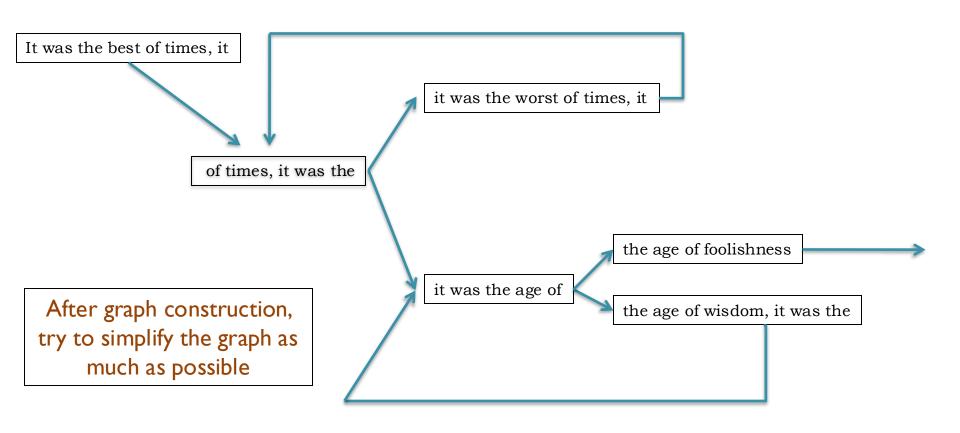
- Overlaps between fragments are implicitly computed

How to pronounce:

de Bruijn Graph Assembly

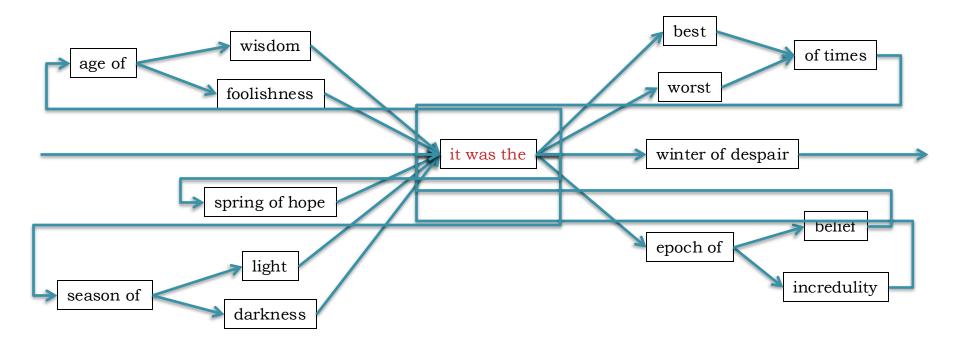


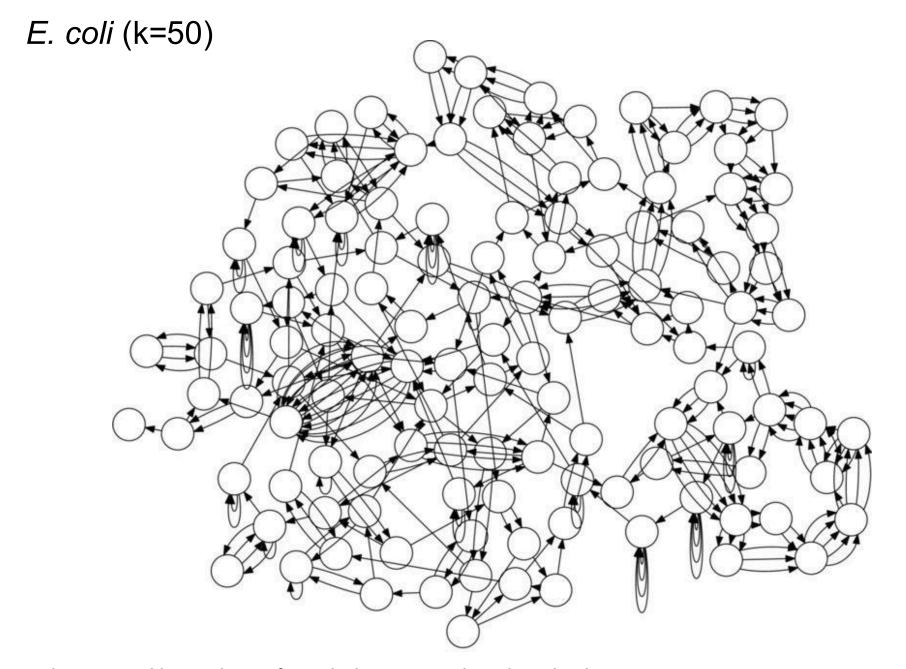
de Bruijn Graph Assembly



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 ...





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

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

ACACG

ATTAC

GATTA

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

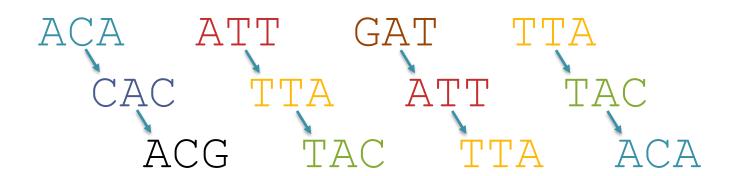
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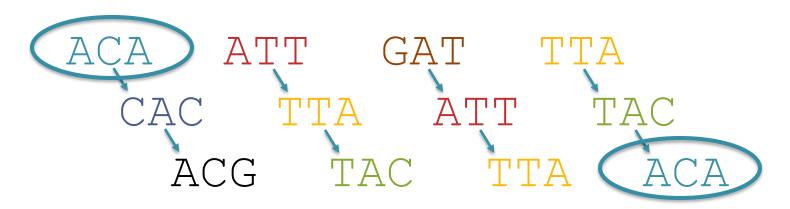


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GATTA

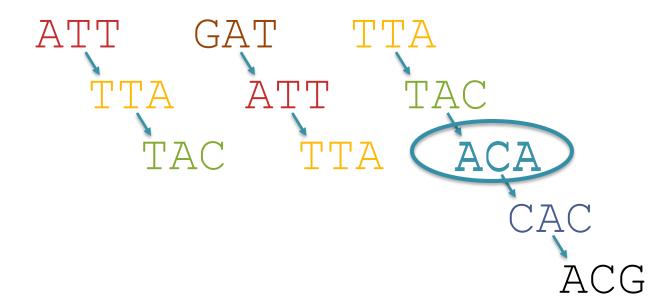


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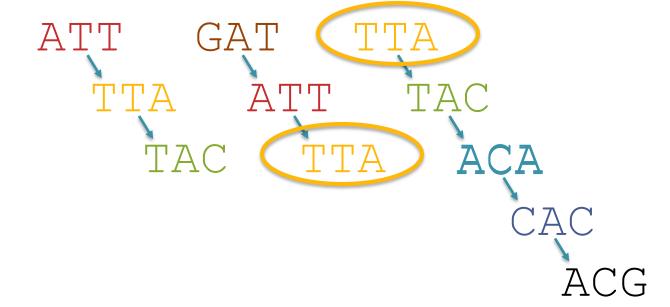


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ACACG

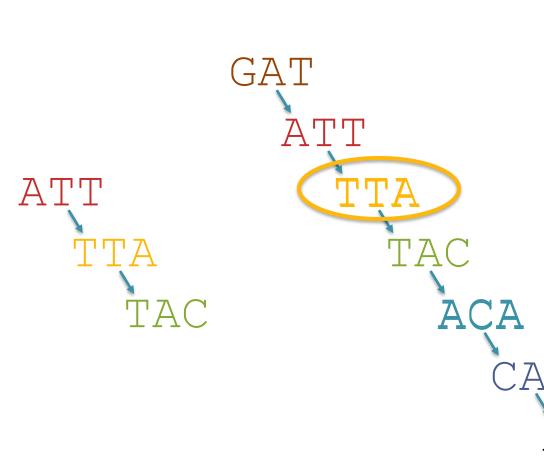
ATTAC

GATTA



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

ACACG ATTAC GATTA TTACA

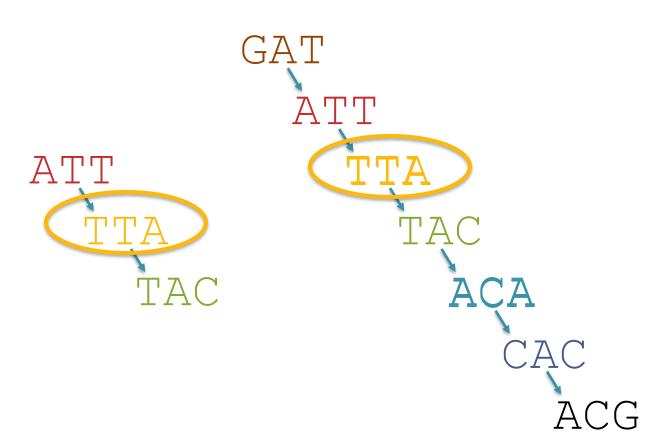


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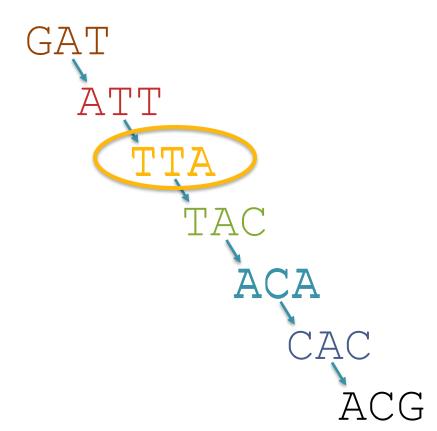


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ACACG

ATTAC

GATTA

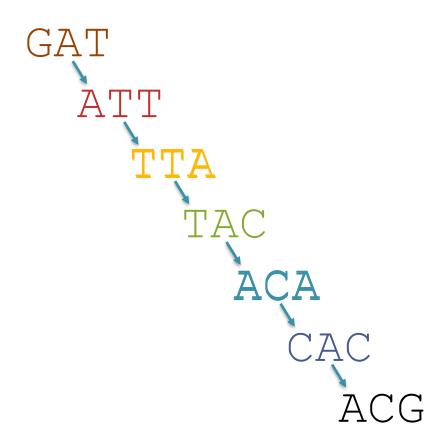


Assemble these reads using a de Bruijn graph approach (k=3):

ACACG

ATTAC

GATTA



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

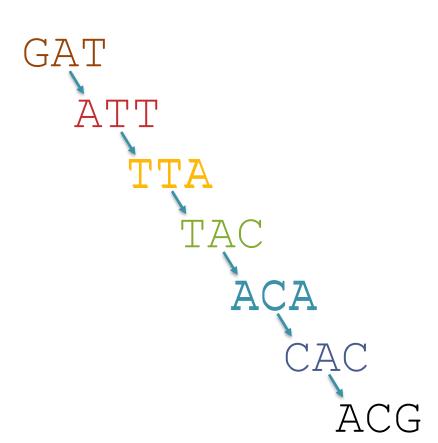
ACACG

ATTAC

GATTA

TTACA

GATTACACG



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

ACACG

ATTAC

GATTA

TTACA

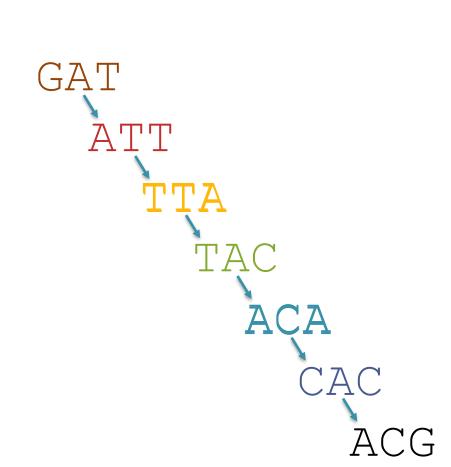
GATTACACG

GATTA

ATTAC

TTACA

ACACG



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

ACGA

ACGT

ATAC

CGAC

CGTA

GACG

GTAT

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

ACGA

ACGT

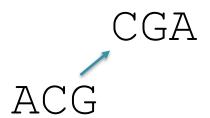
ATAC

CGAC

CGTA

GACG

GTAT



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

ACGA

ACGT

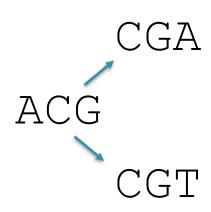
ATAC

CGAC

CGTA

GACG

GTAT



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

ACGA

ACGT

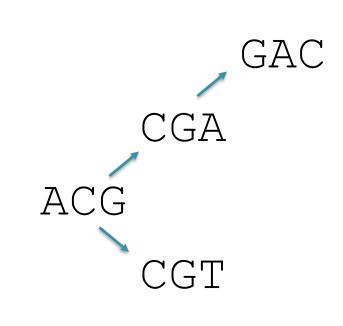
ATAC

CGAC

CGTA

GACG

GTAT



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ACGA

ACGT

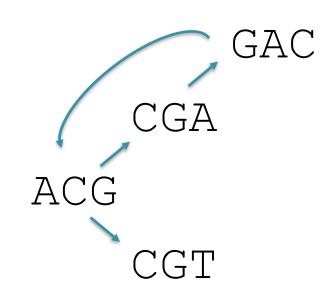
ATAC

CGAC

CGTA

 GACG

GTAT



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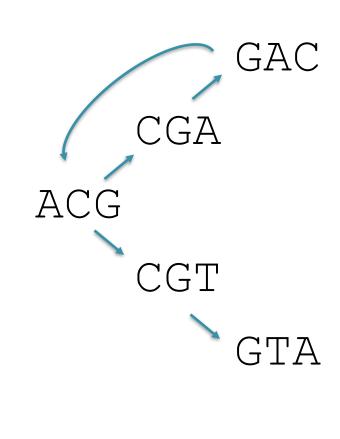
ATAC

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GTAT



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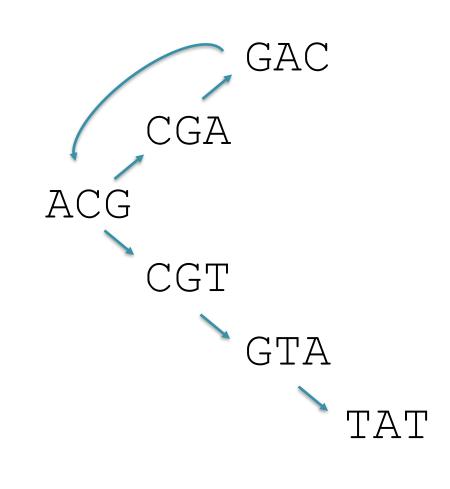
ATAC

CGAC

CGTA

GACG

GTAT



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ACGA

ACGT

ATAC

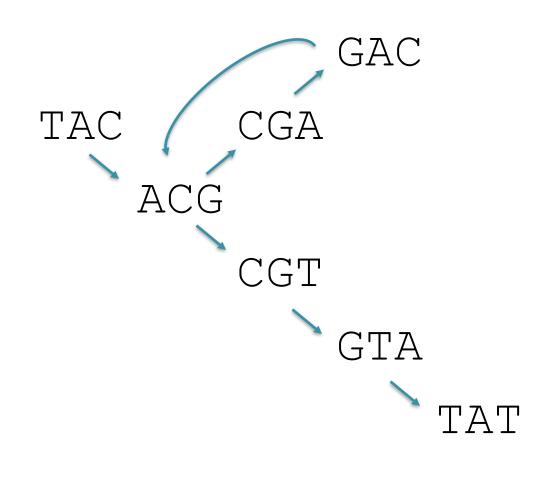
CGAC

CGTA

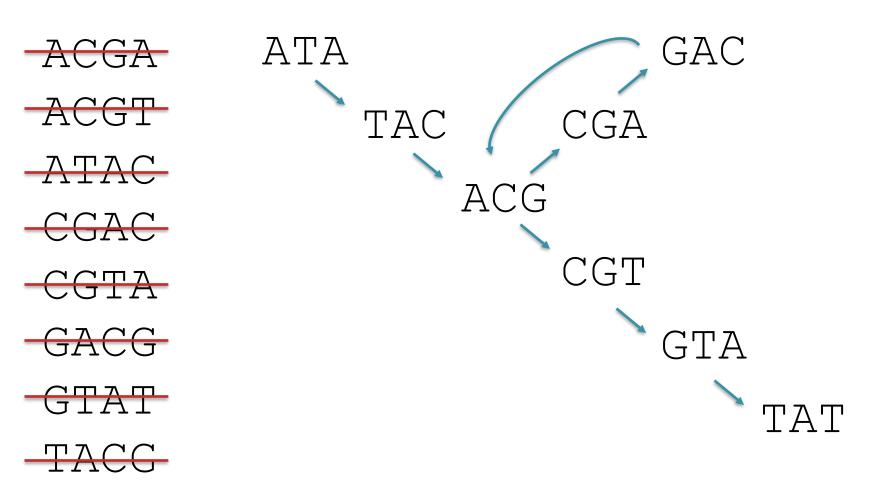
GACG

GTAT

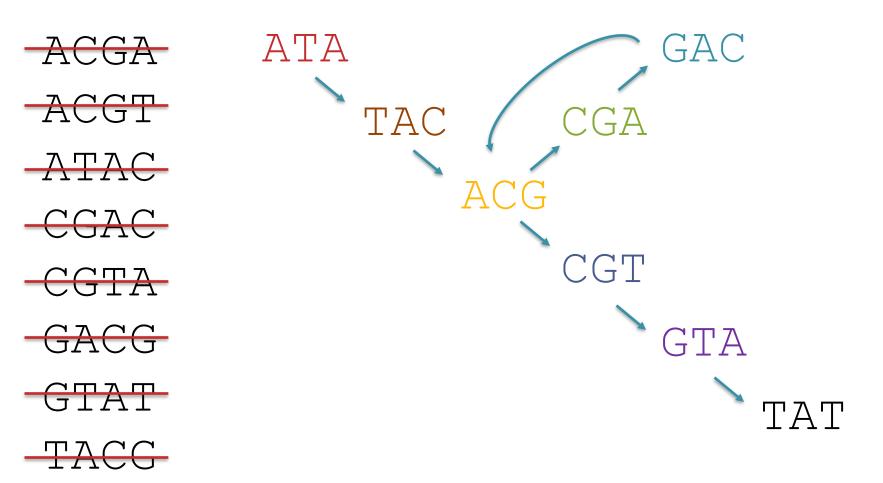
TACC



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



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



ATACGACGTAT

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

