بنام خدا

DNA Sequencing

سید ابوالفضل مطهری

Sequencing Problem

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$$S = X_1 X_2 X_3 X_4 \ldots X_{G-1} X_G$$

Sequencing Problem

$$S = X_1 X_2 X_3 X_4 \ldots X_{G-1} X_G$$

- finding the sequence

Sequencing Problem

$$S = X_1 X_2 X_3 X_4 \ldots X_{G-1} X_G$$

- finding the sequence
- or finding some parts of the sequence

Sequencing Problem

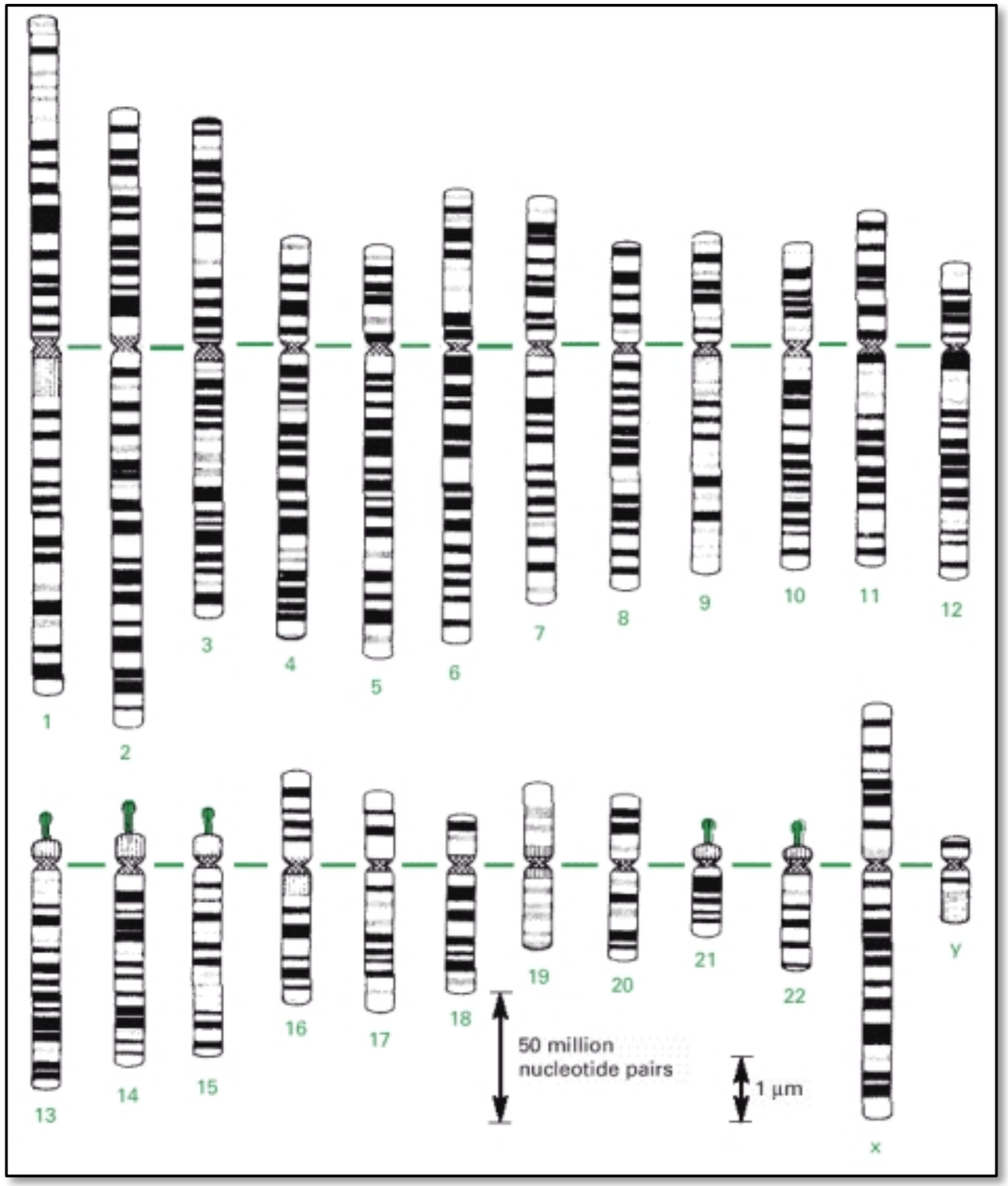
$$S = X_1 X_2 X_3 X_4 \ldots X_{G-1} X_G$$

- finding the sequence
- or finding some parts of the sequence

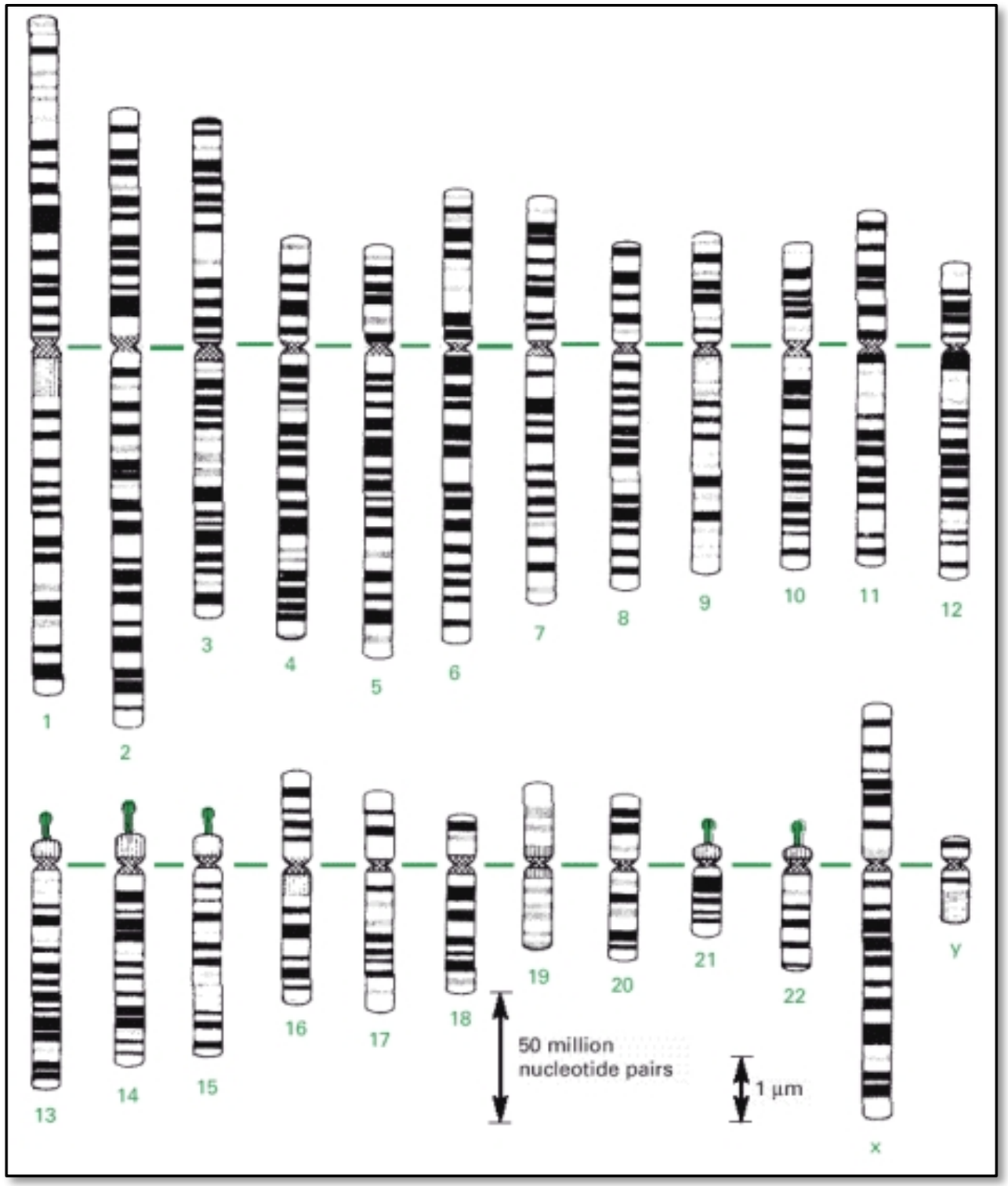
DNA Sequencing

sequence is the genome

Genome

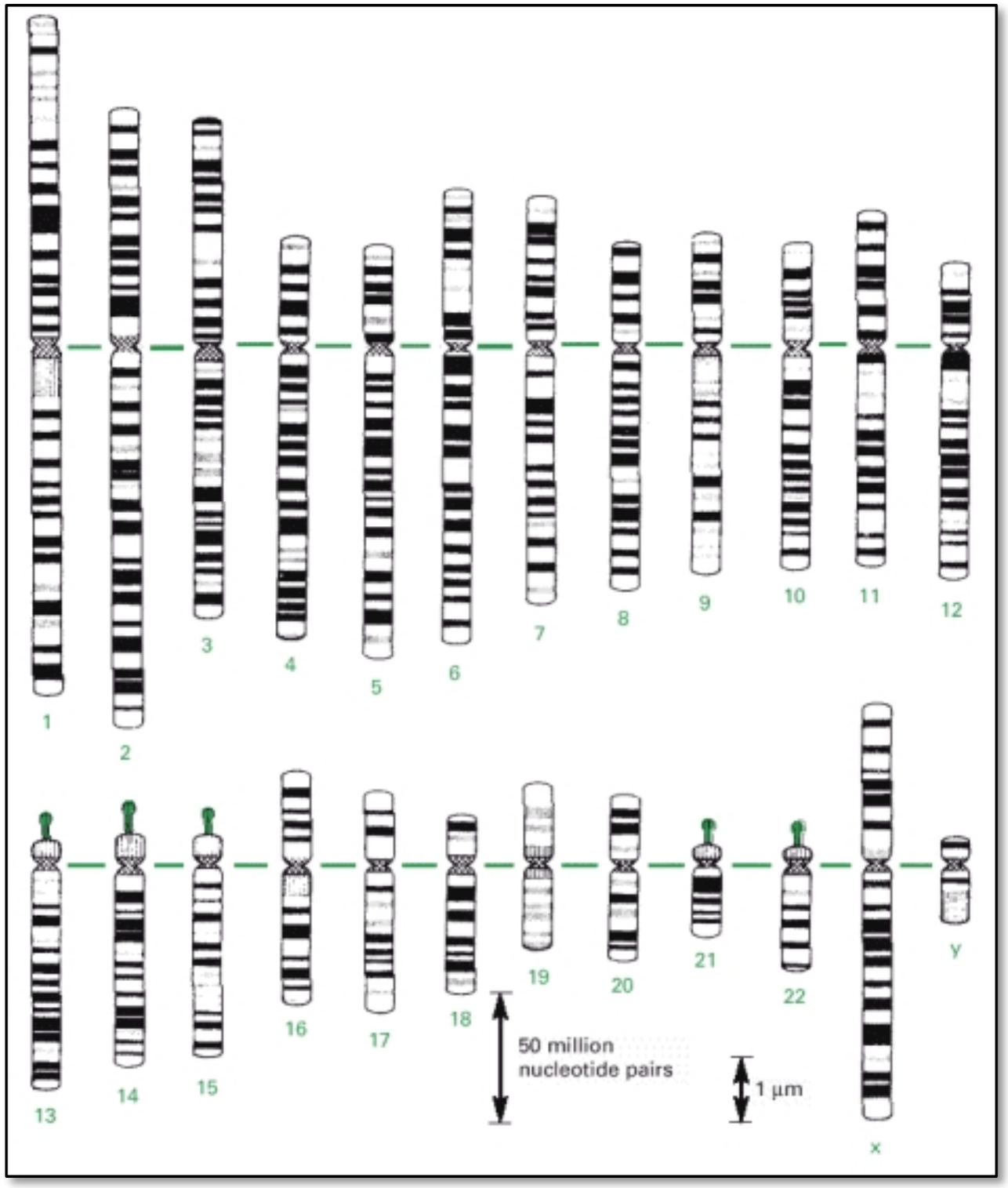

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
x
y
50 million
nucleotide pairs
1 µm

Genome



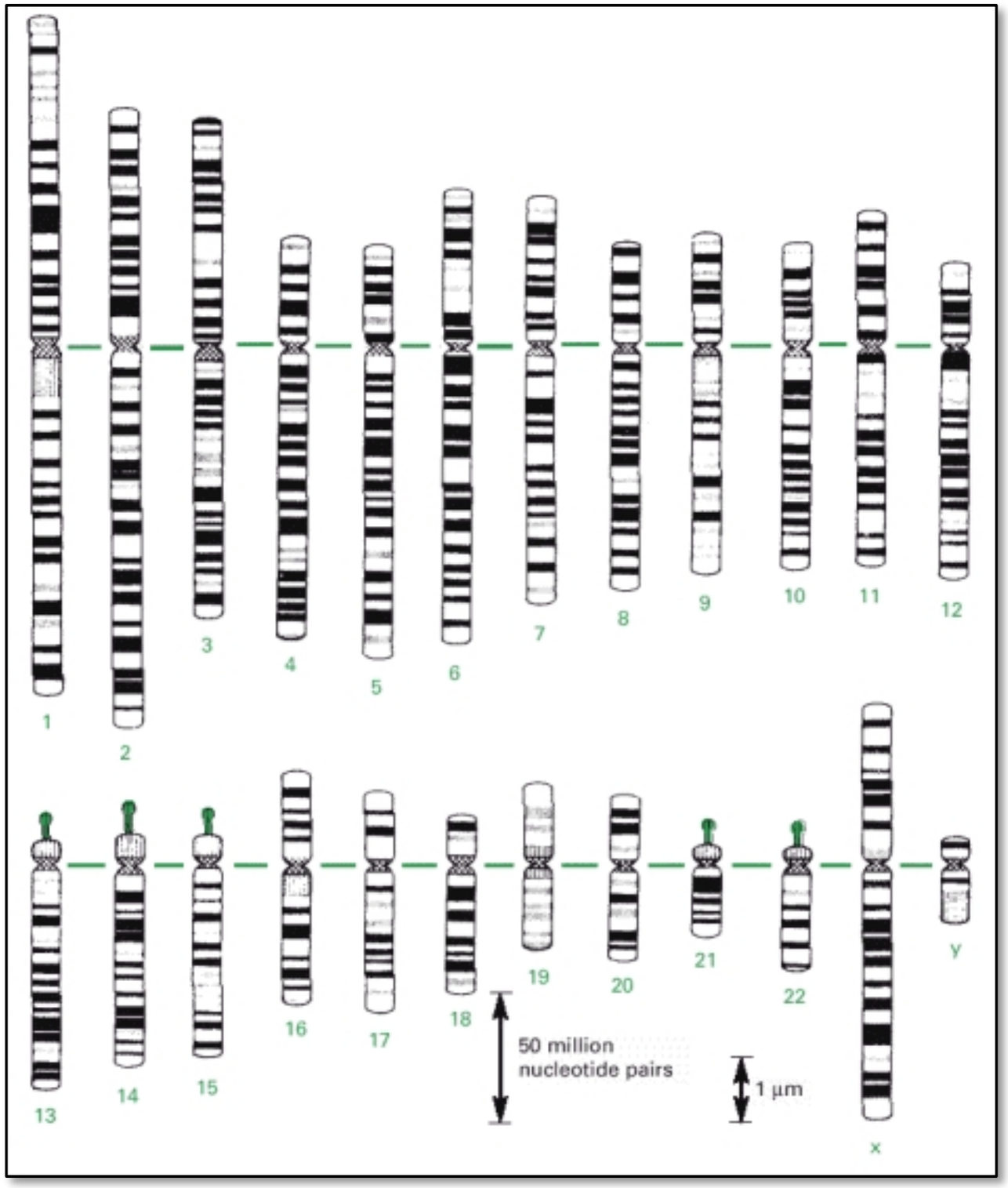
- Human Genome consists of 23 chromosomes

Genome



- Human Genome consists of 23 chromosomes
- Each chromosome contains a DNA sequence

Genome



- Human Genome consists of 23 chromosomes
- Each chromosome contains a DNA sequence
- The total length is

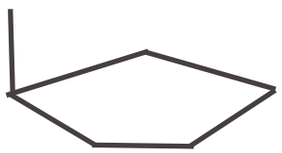
$$G = 3.2 \times 10^9$$

Genome

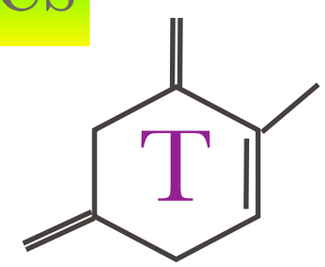
DexoyriboNucleic Acid (DNA)

Phosphate

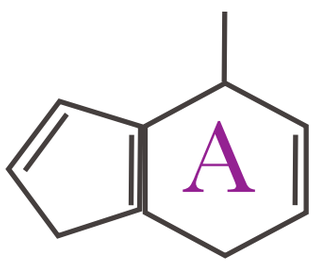
Sugar



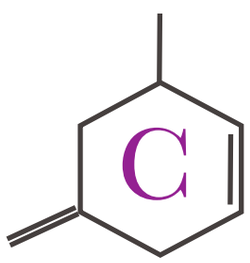
Bases



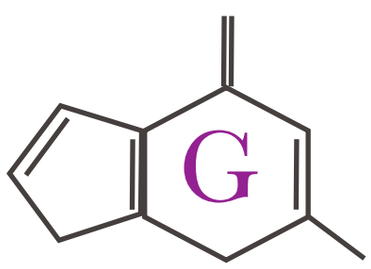
Thymine



Adenine



Cytosine

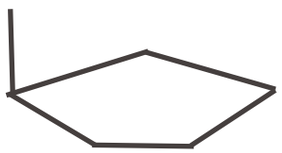


Guanine

DexoyriboNucleic Acid (DNA)

Phosphate

Sugar



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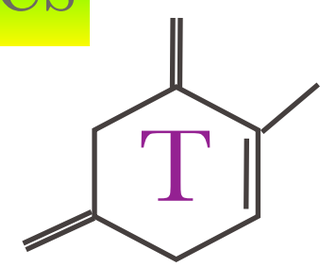

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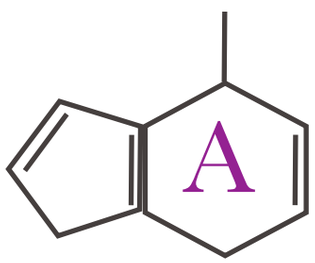

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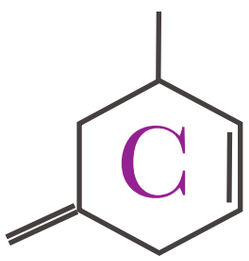
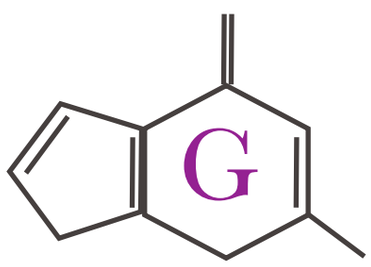
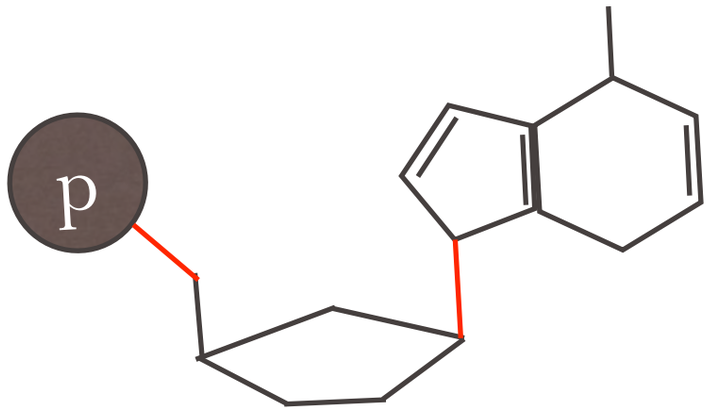


Cytosine



Guanine

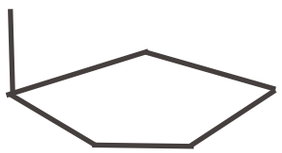
Covalent



DexoyriboNucleic Acid (DNA)

Phosphate

Sugar



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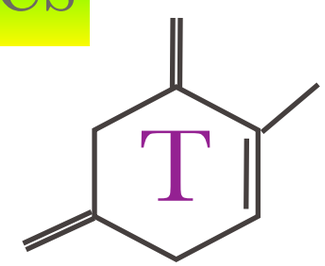
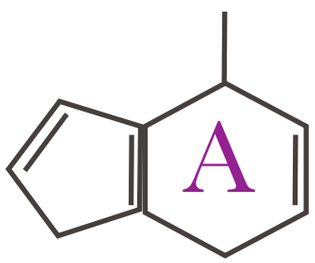
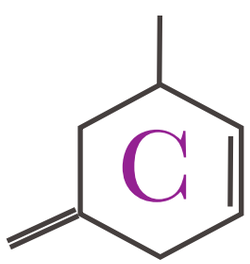


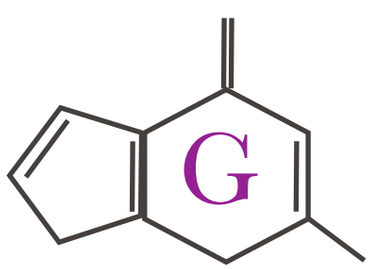
Thymine



Adenine



Cytosine



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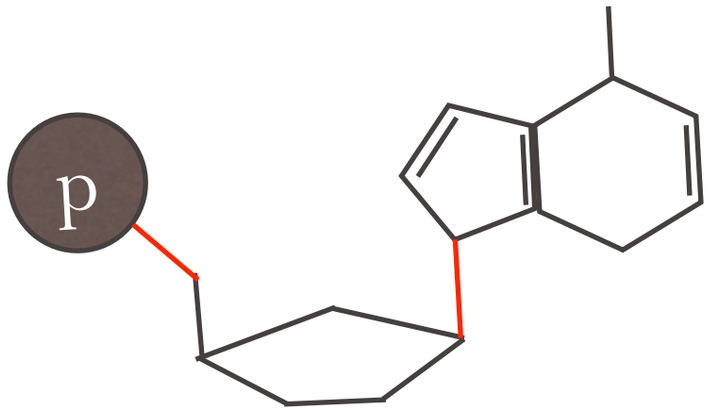
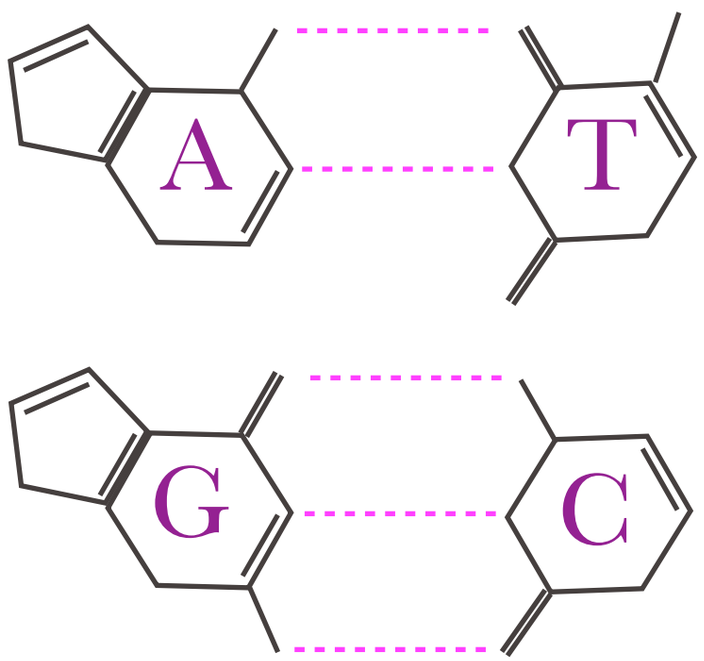
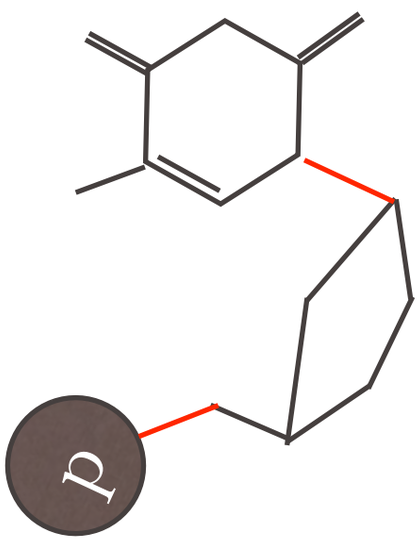


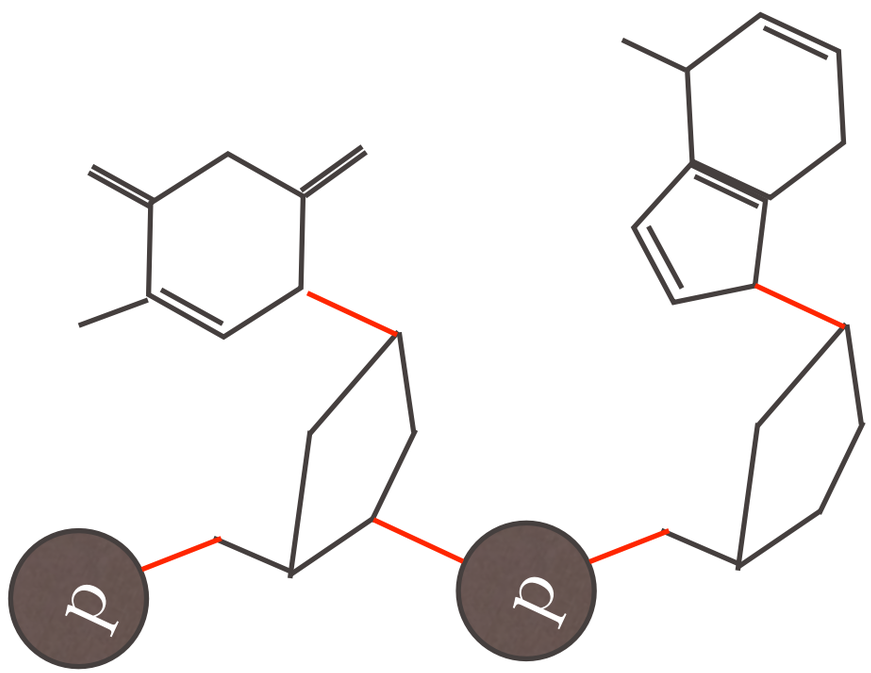
Hydrogen



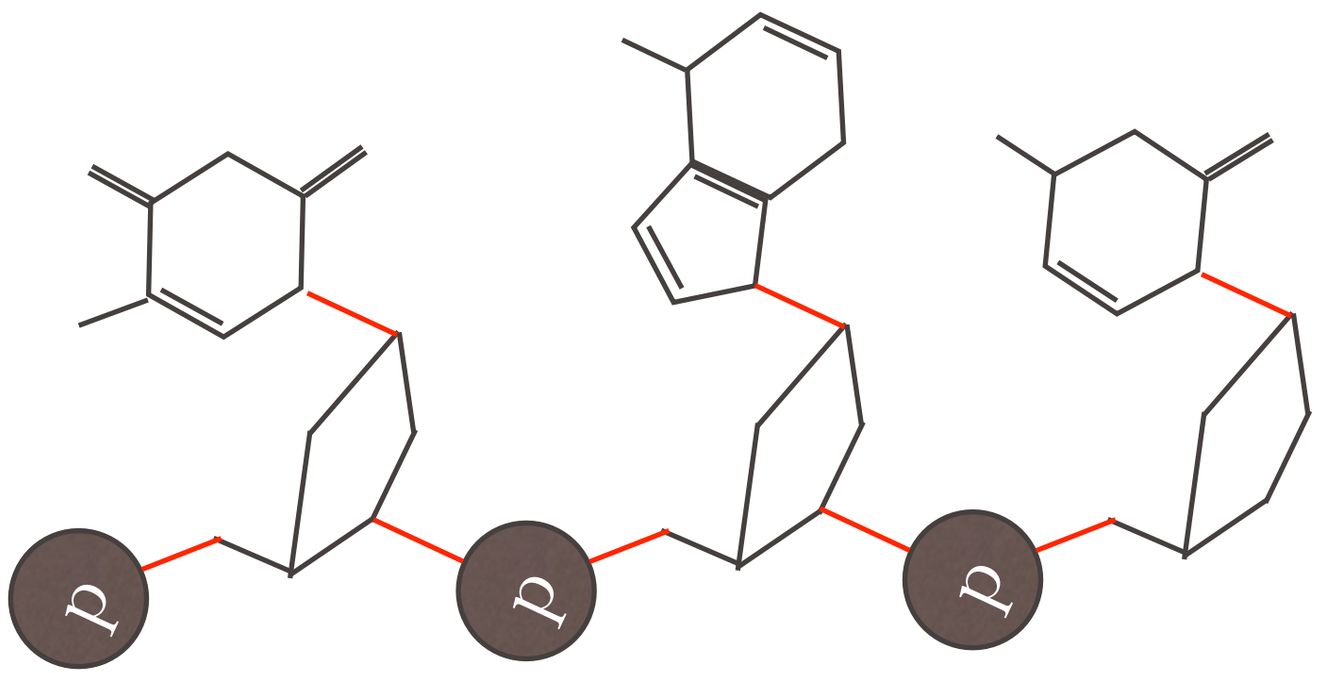
Single Stranded DNA



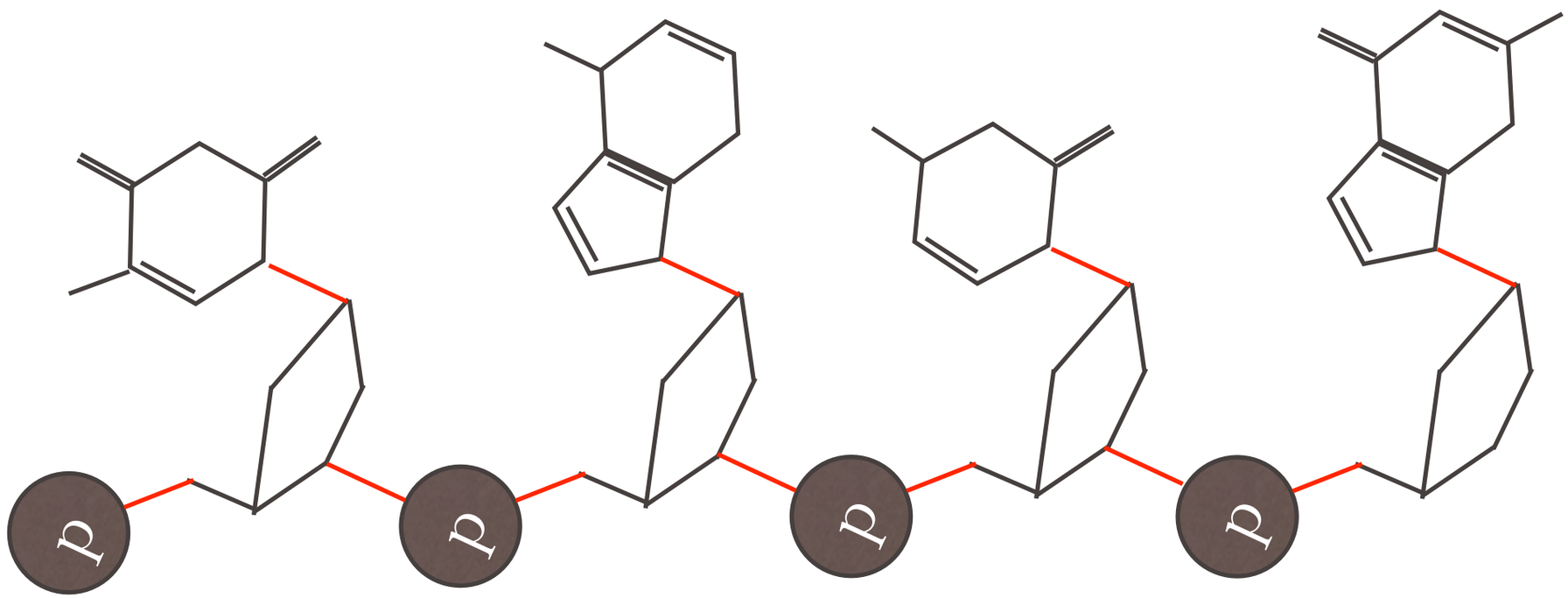
Single Stranded DNA



Single Stranded DNA



Single Stranded DNA



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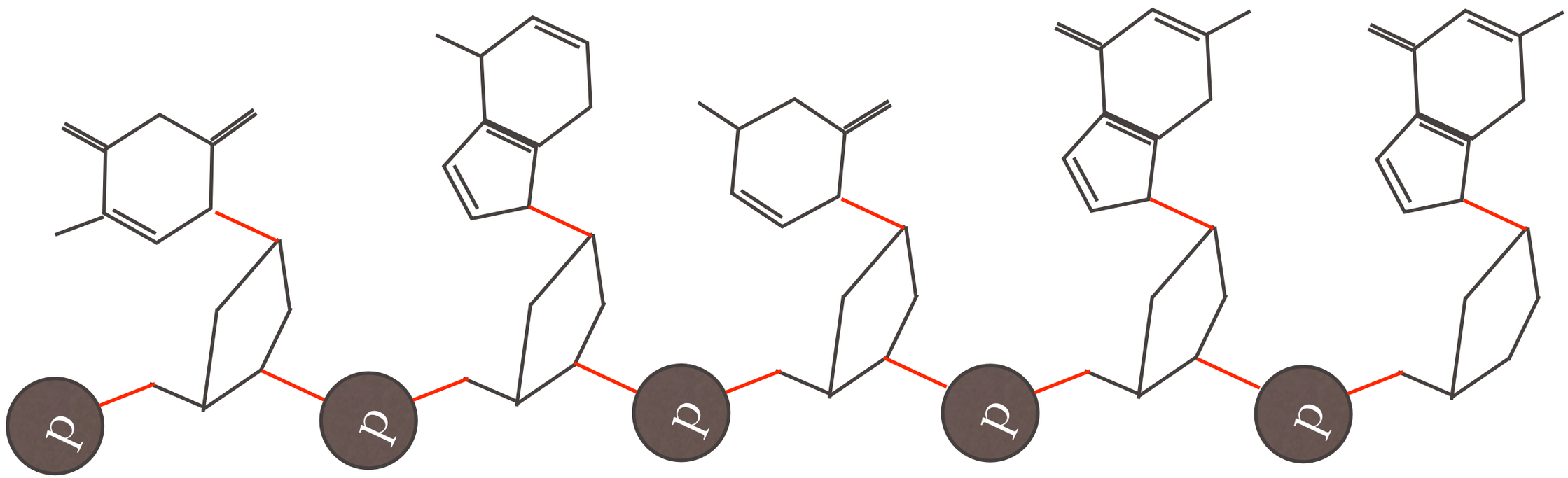

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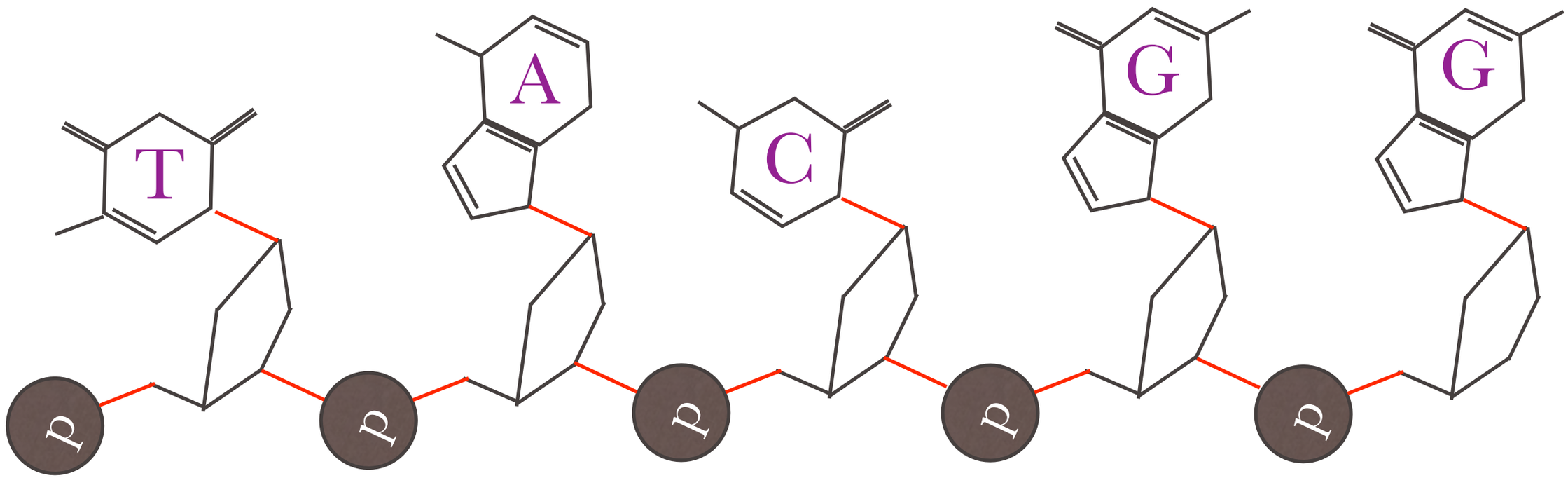
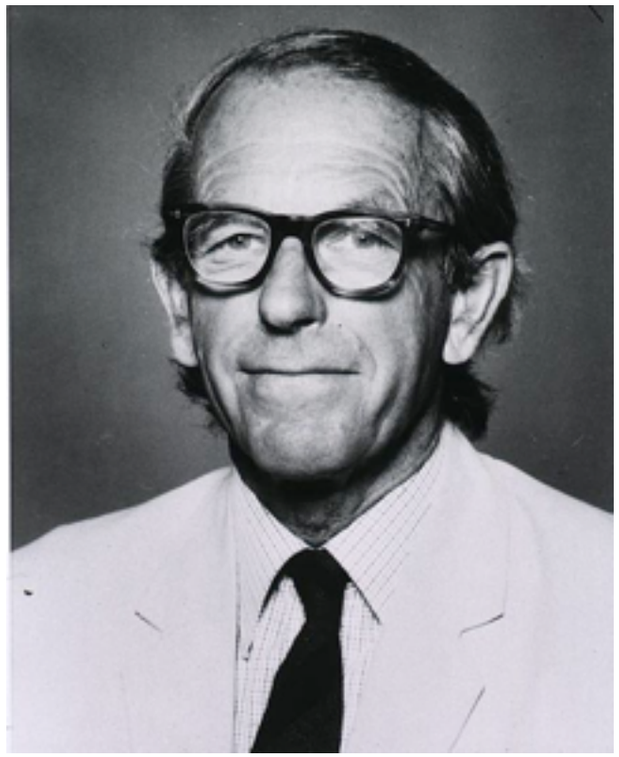


TACGG.....

Fred Sanger

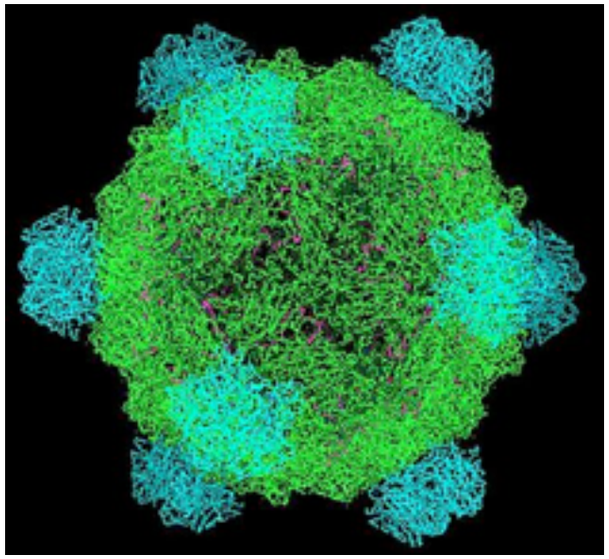
- Won the Nobel Prize for Chemistry twice
  Sequencing Insulin
  DNA Sequencing



1918-2013

Phi X 174 Bactriophage

- First Genome to be Sequenced
- Genome Size: 5386 bp
- Fred Sanger (1977)



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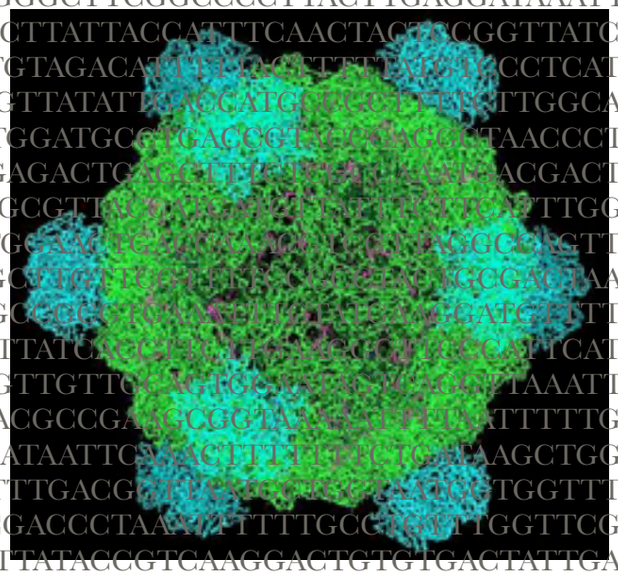

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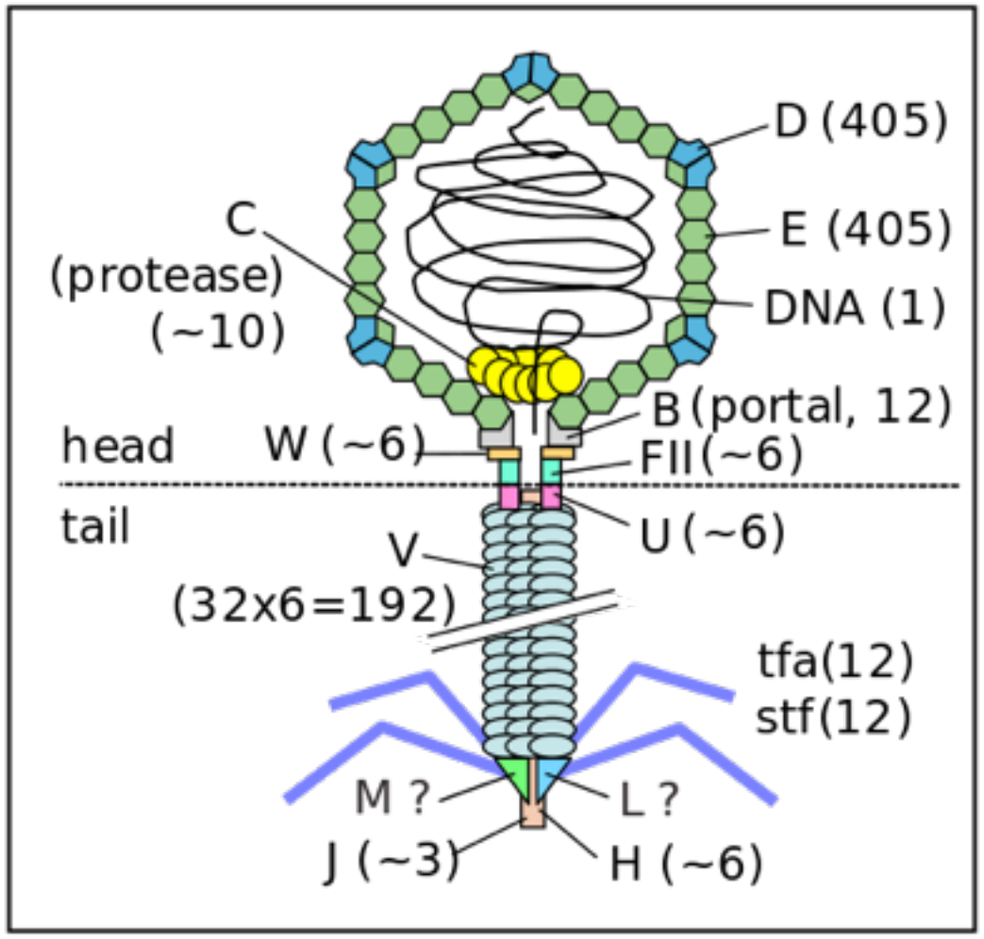
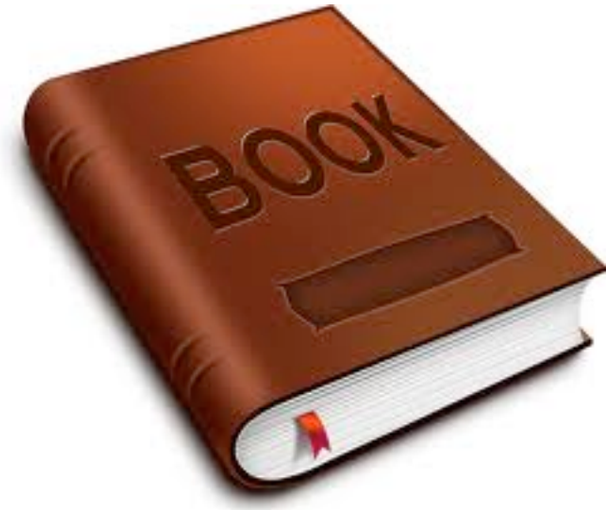
Lambda Phage

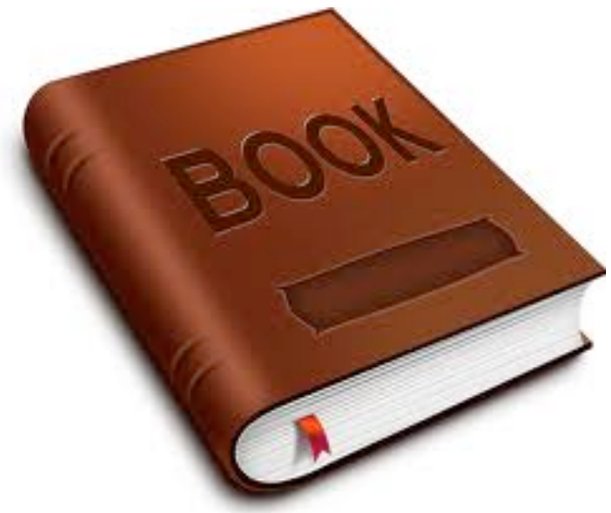
- Genome Size: 48,502 bp
- Fred Sanger, et al (1982)
- Method: Shotgun Sequencing



Shotgun Sequencing

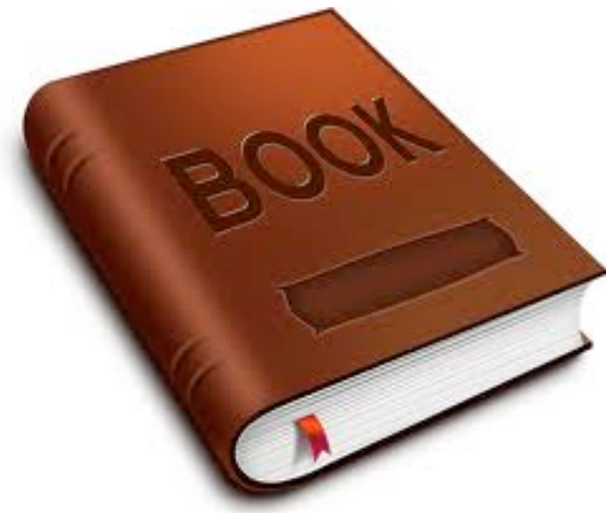


Shotgun Sequencing



Reads

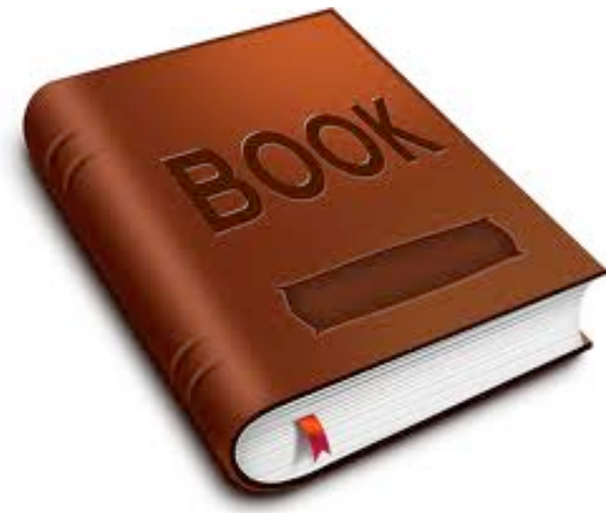
Shotgun Sequencing



Reads

Hearken to the reed-flute

Shotgun Sequencing



Reads

banishment from its home

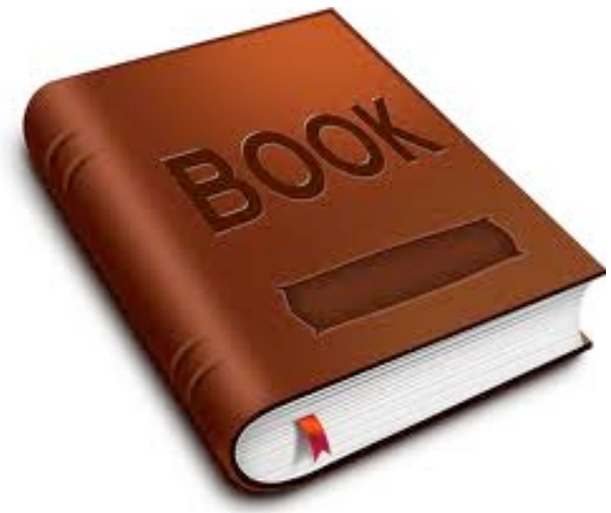
Lamenting its banishment from

it complains, Lamenting its

Hearken to the reed-flute

reed-flute, how it complains,

Shotgun Sequencing



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banishment from its home

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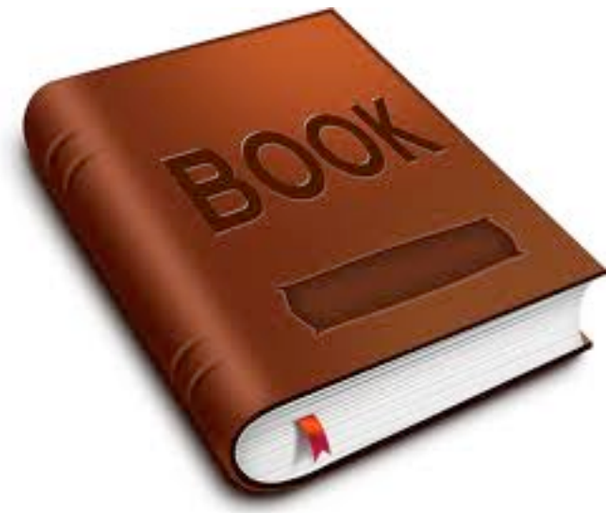
reed-flute, how it complains,

Hearken to the reed-flute

Shotgun Sequencing



Reads

banishment from its home

Lamenting its banishment from

it complains, Lamenting its

Hearken to the reed-flute, how it complains,

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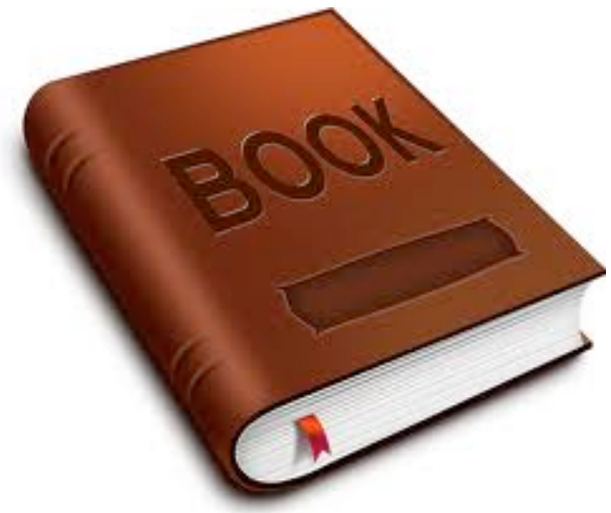

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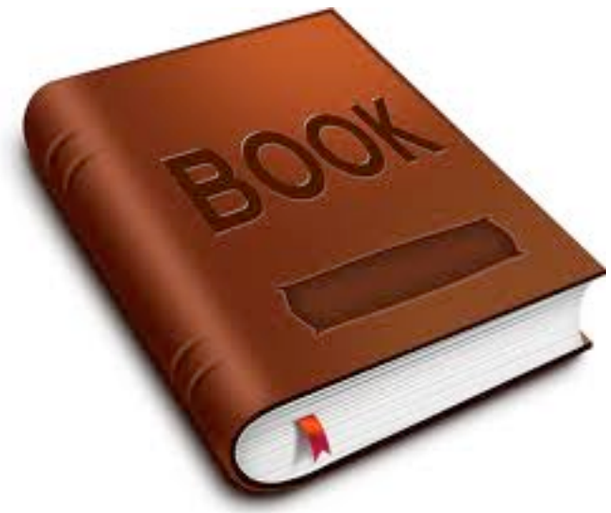

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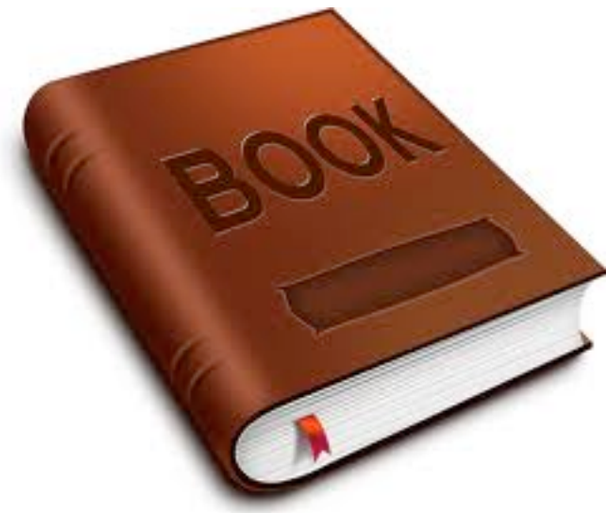

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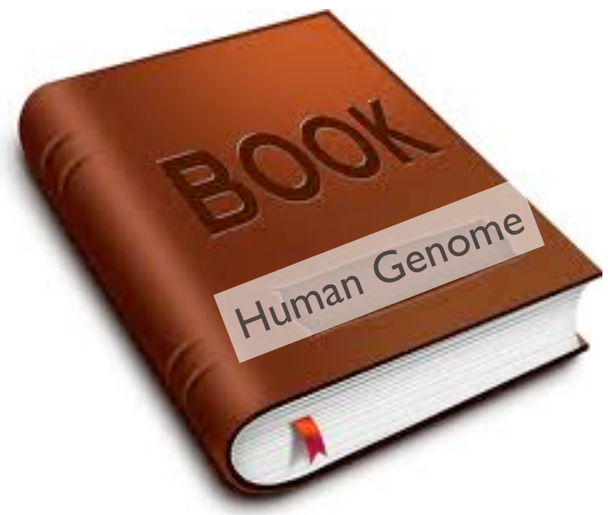
DNA Shotgun Sequencing



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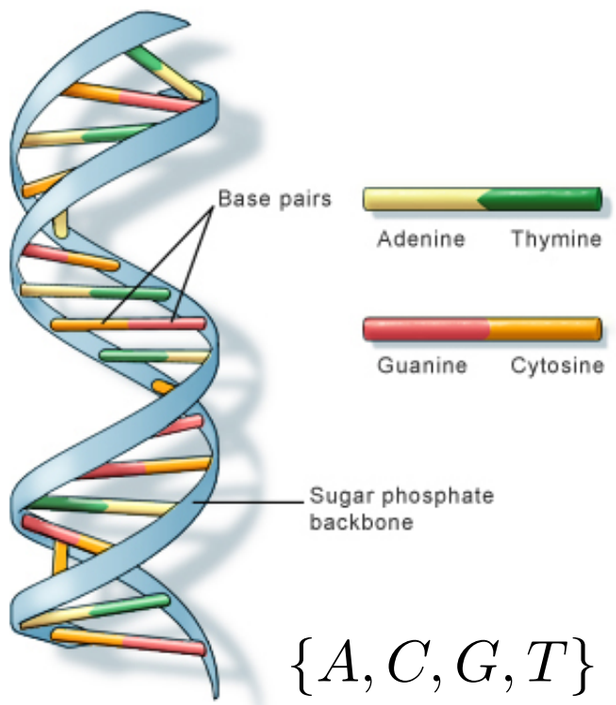
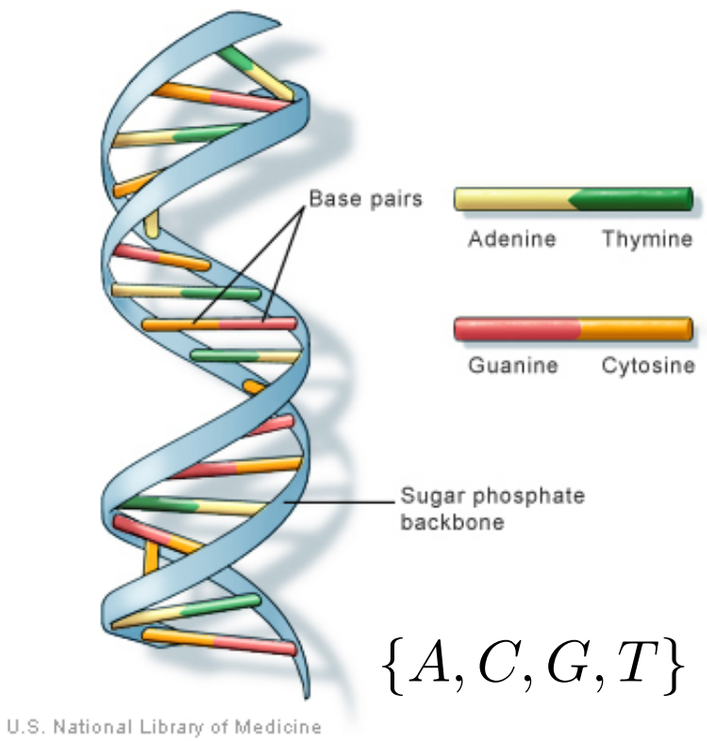


U.S. National Library of Medicine

$$\{A, C, G, T\}$$

DNA Shotgun Sequencing



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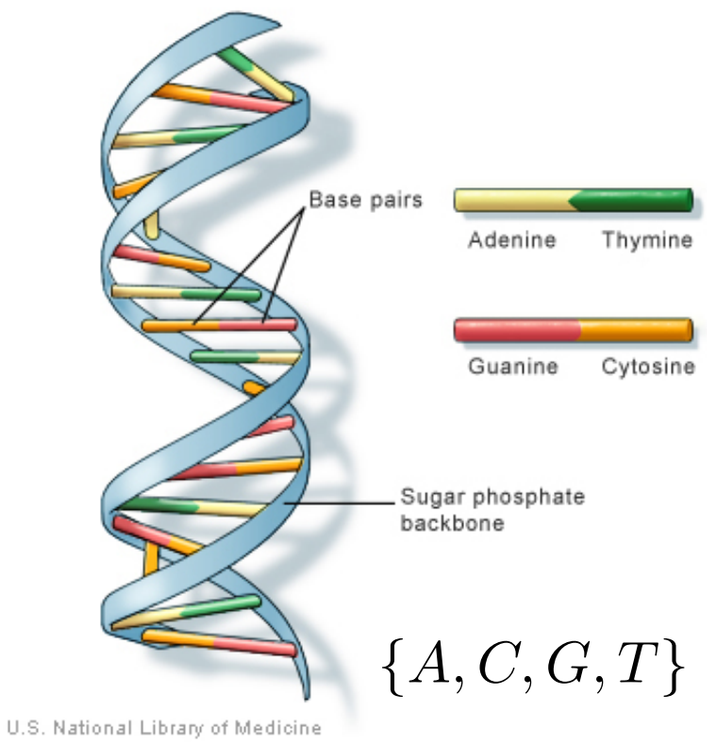
Reads

DNA Shotgun Sequencing

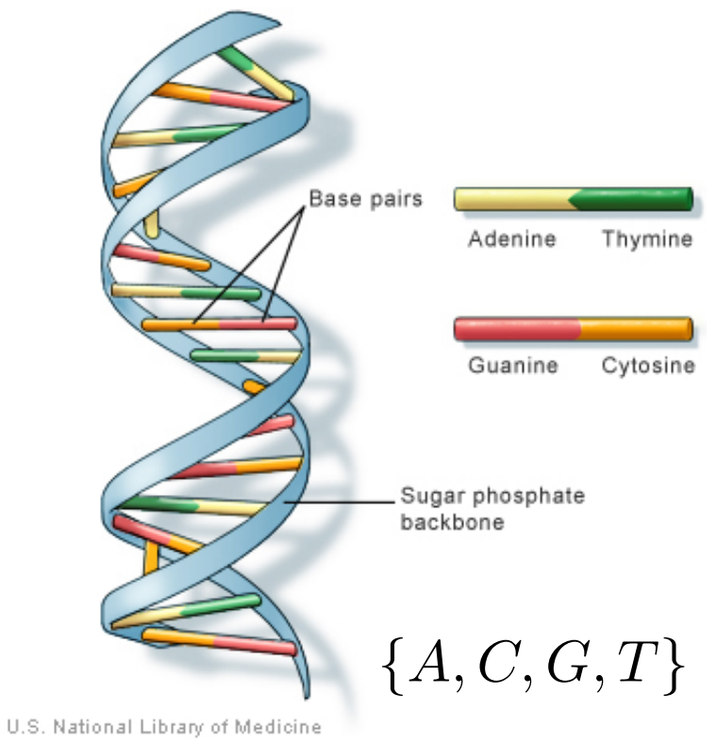


$\{A, C, G, T\}$

π

Reads

DNA Shotgun Sequencing



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ACCGTCCTTAAGG

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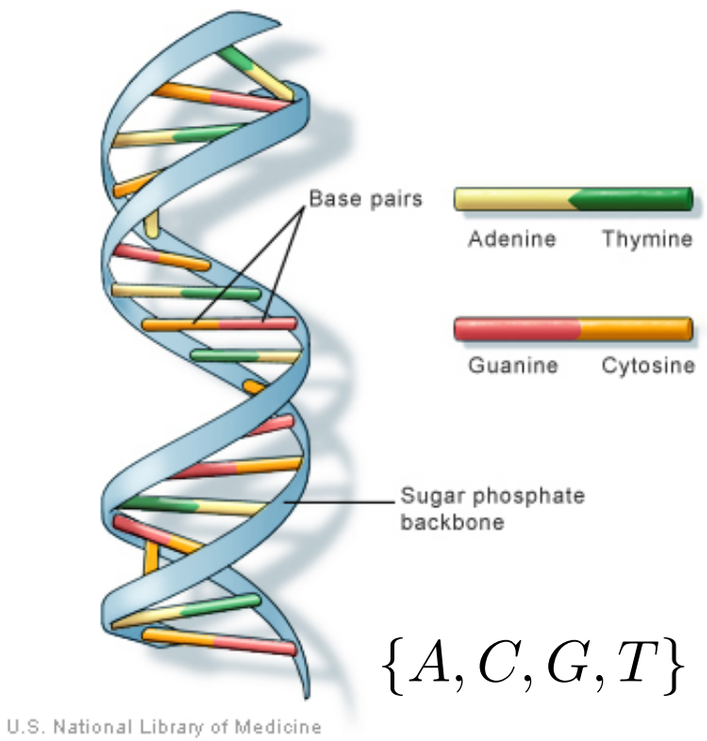
Reads

DNA Shotgun Sequencing



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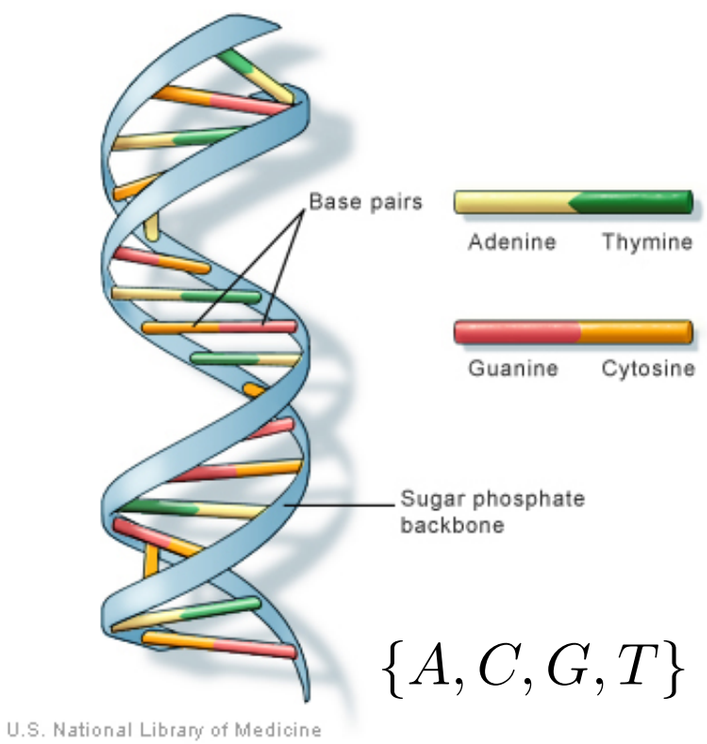

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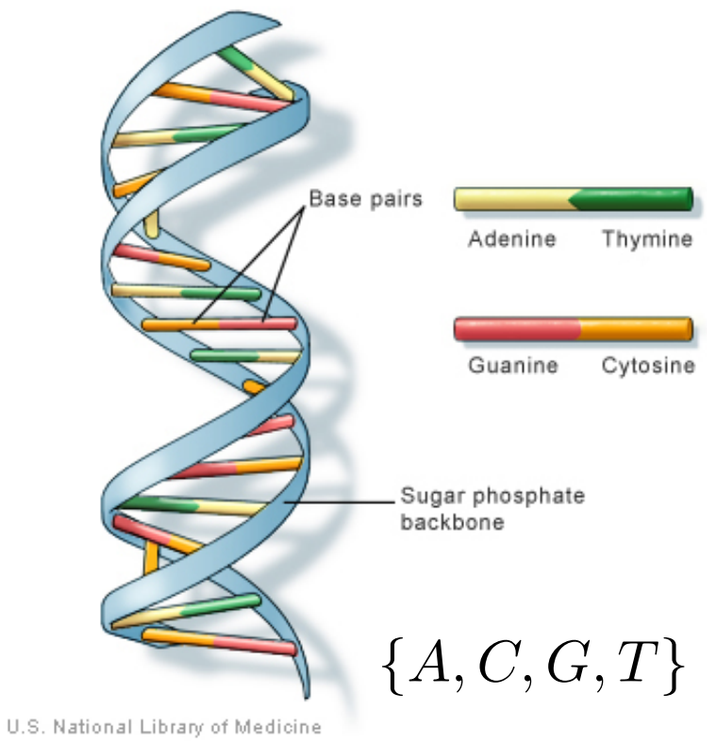

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Reads

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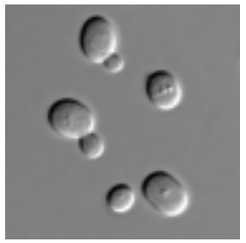
Starting Big Projects

Starting Big Projects

1989

- Genome of s. cerevisiae
- Size of Genome: 12 Mb
- 16 Chromosomes
- Finished 1996



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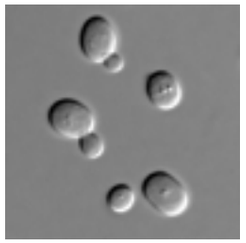

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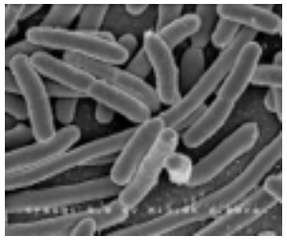
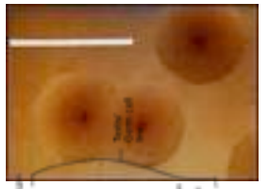
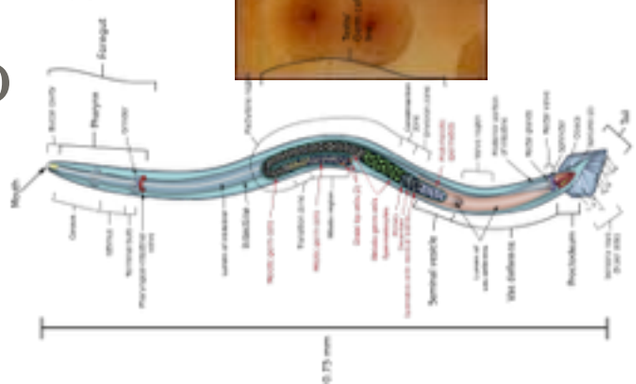


- E. Coli: 4.6 Mb
- M. Capricolum: 1 Mb
- C. elegans: 100 Mb





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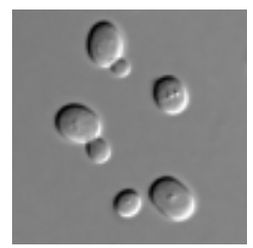
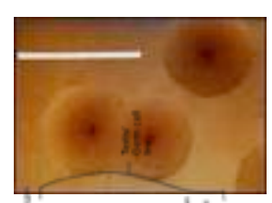


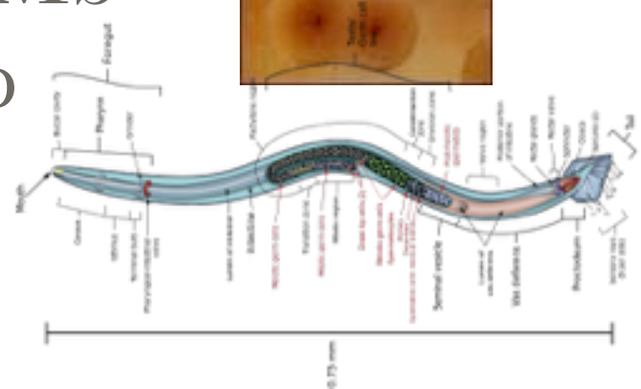
1990

- Human Genome Project (HGP)
- Size of Genome: 3.2 Gb
- 23 Chromosomes
- Finished 2003

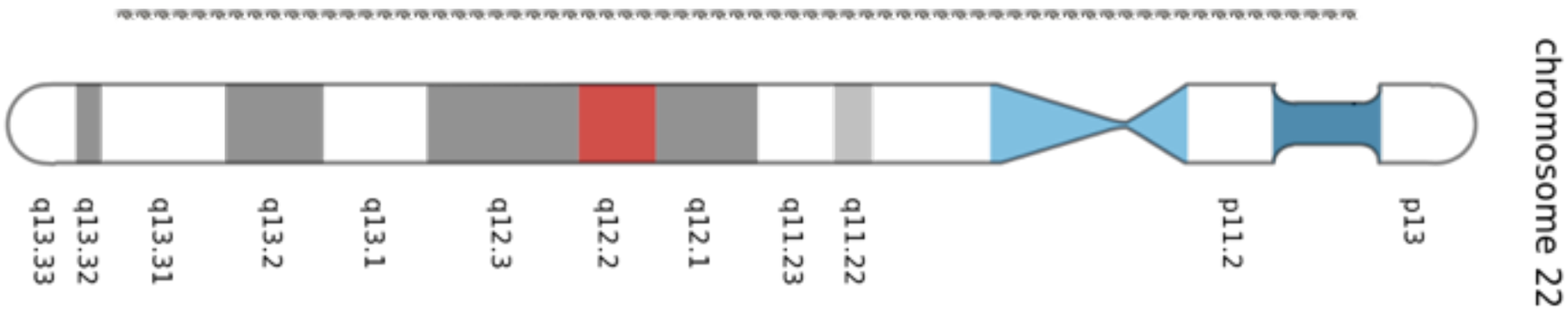
1990

- E. Coli: 4.6 Mb
- M. Capricolum: 1 Mb
- C. elegans: 100 Mb





Strategy: Hierarchical SG



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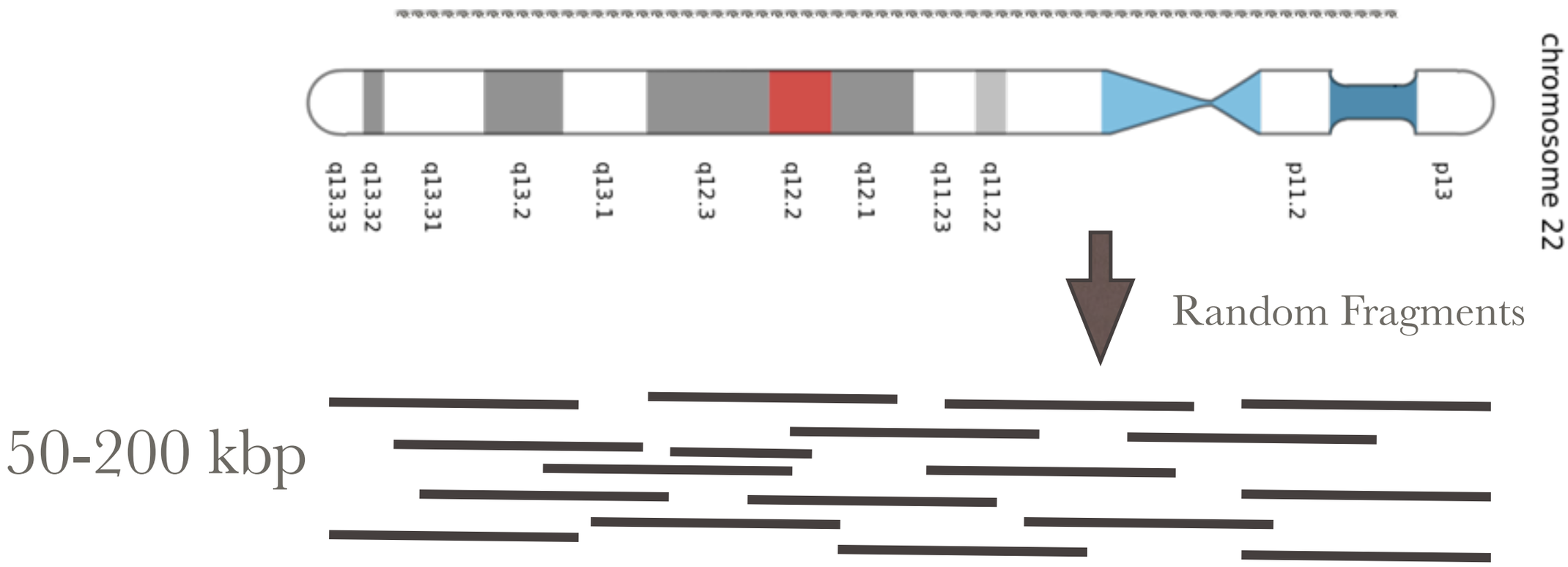

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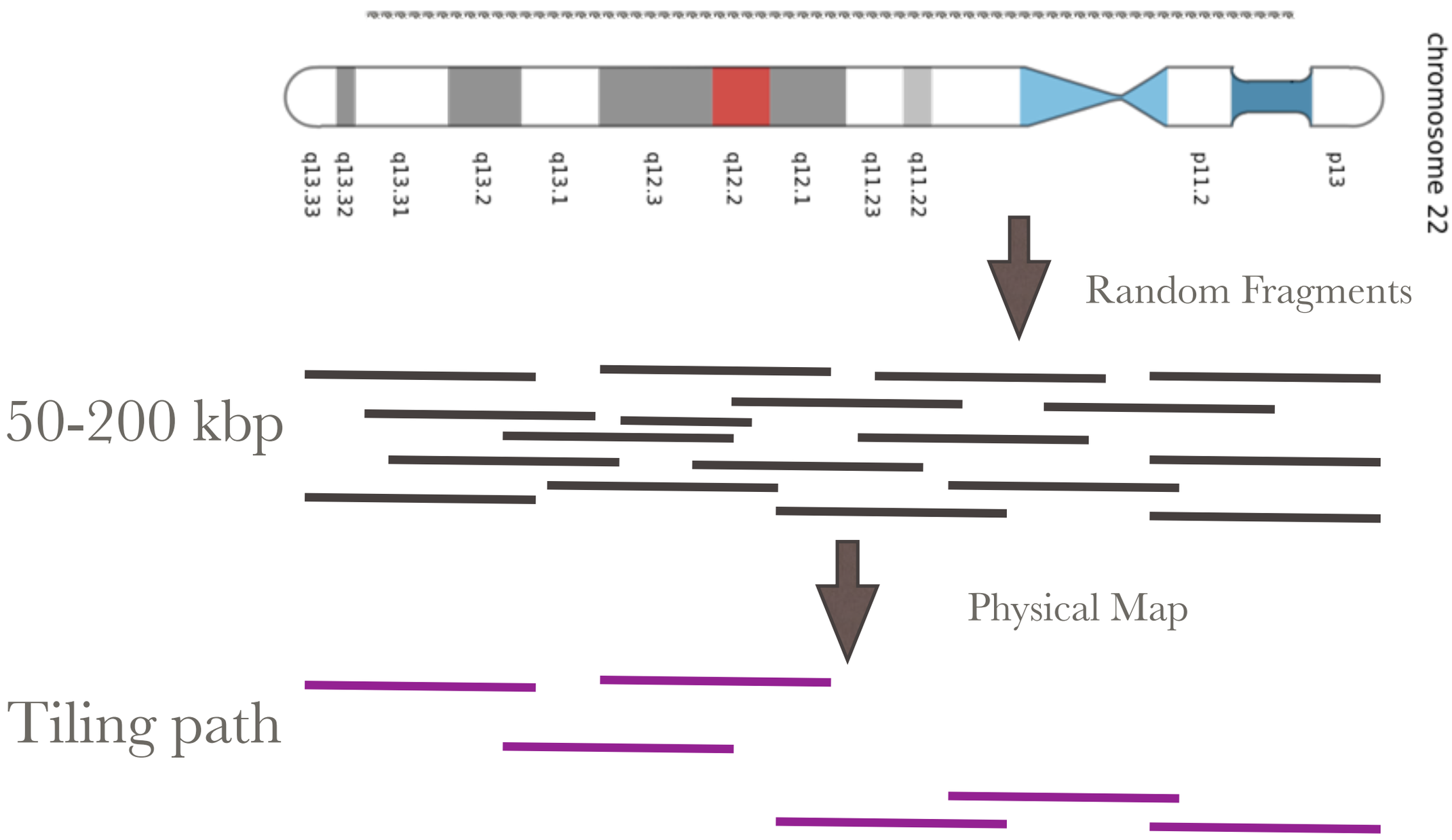

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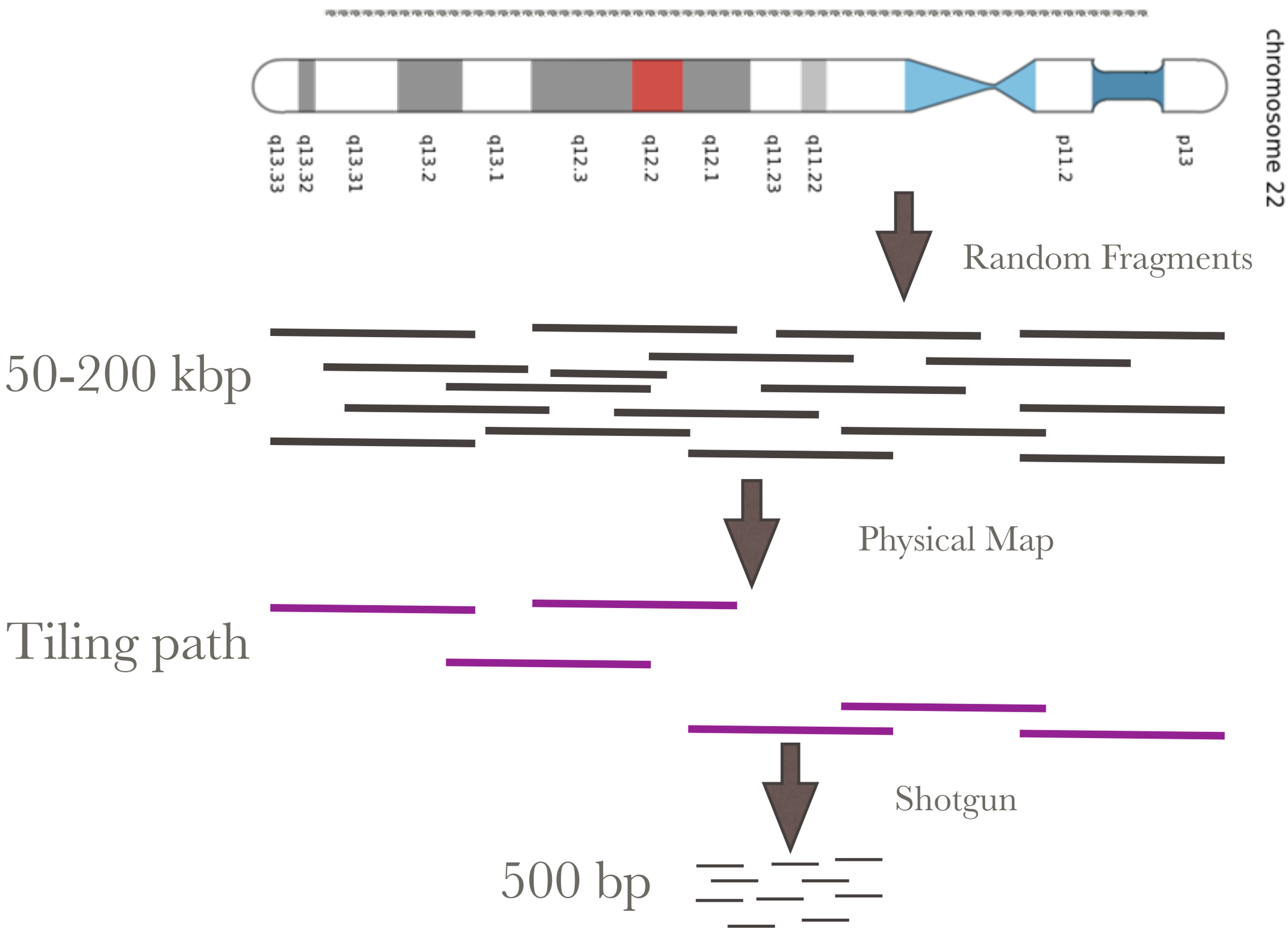
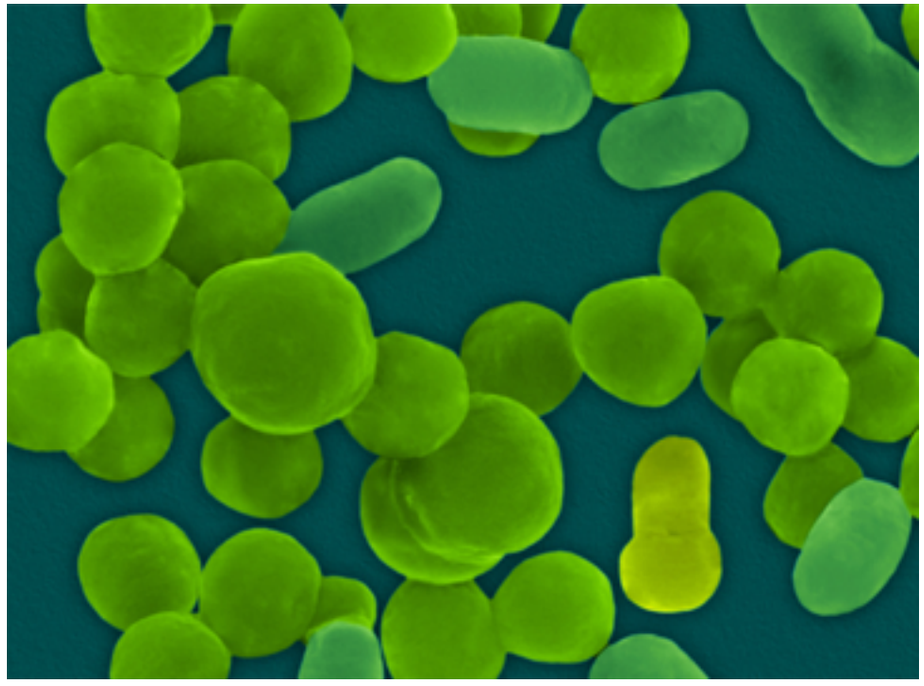
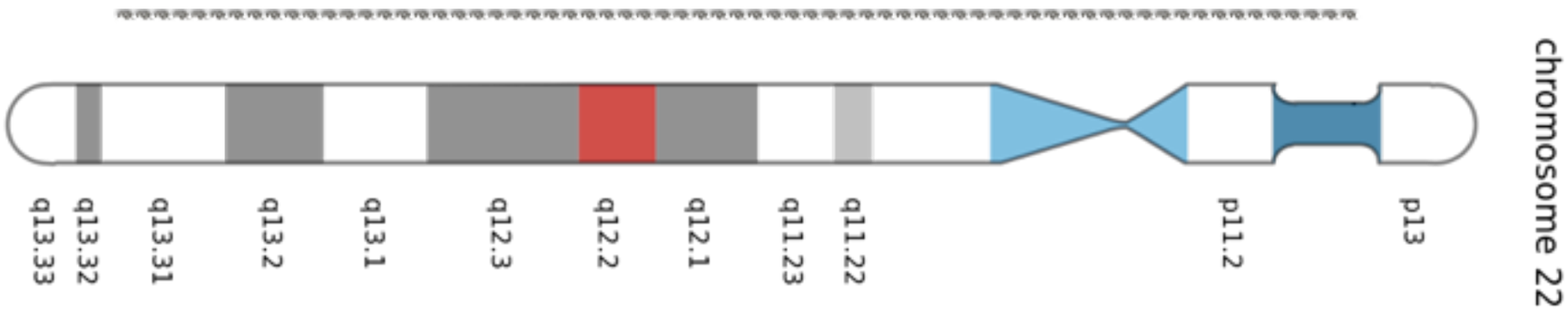


H Influenzae

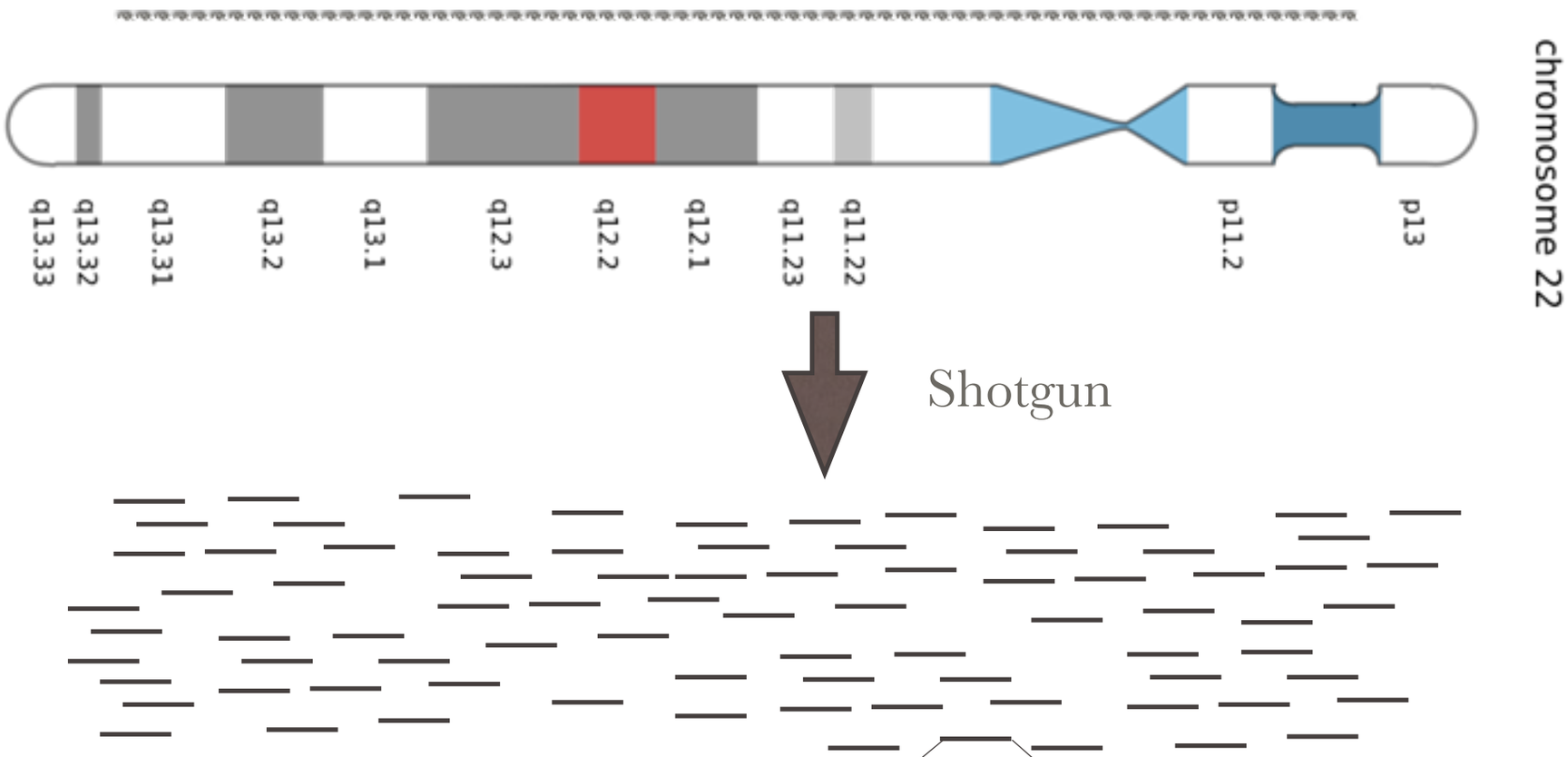
- The first sequenced free-living organism
- Genome Size: 1.8 Mb
- Strategy: Whole Genome Shotgun Sequencing
- The Institute for Genomic Research TIGR
- Finished 1995
- Done in 13 months
- Cost: 50 cents per base



Whole Genome Shotgun Sequencing



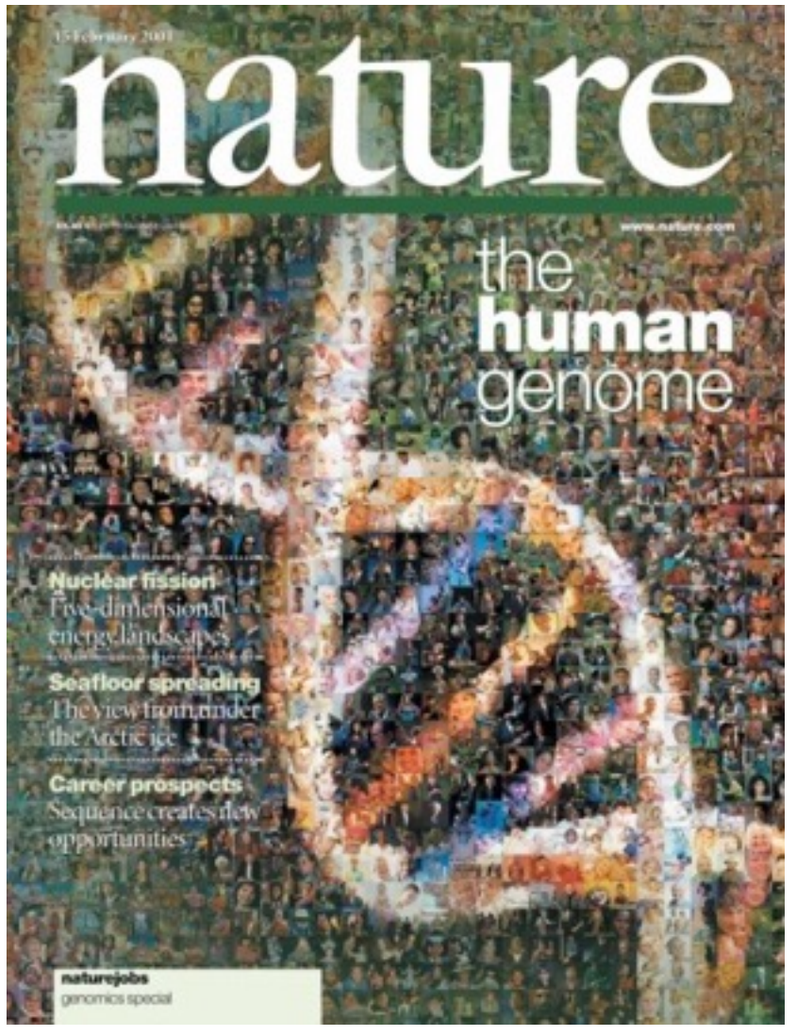
Whole Genome Shotgun Sequencing



Number of Reads: N
Read Length: L

ATCCGGTACGGATCGTACA

HGP FINISHED



There is Never Enough Sequencing

courtesy: Batzoglou

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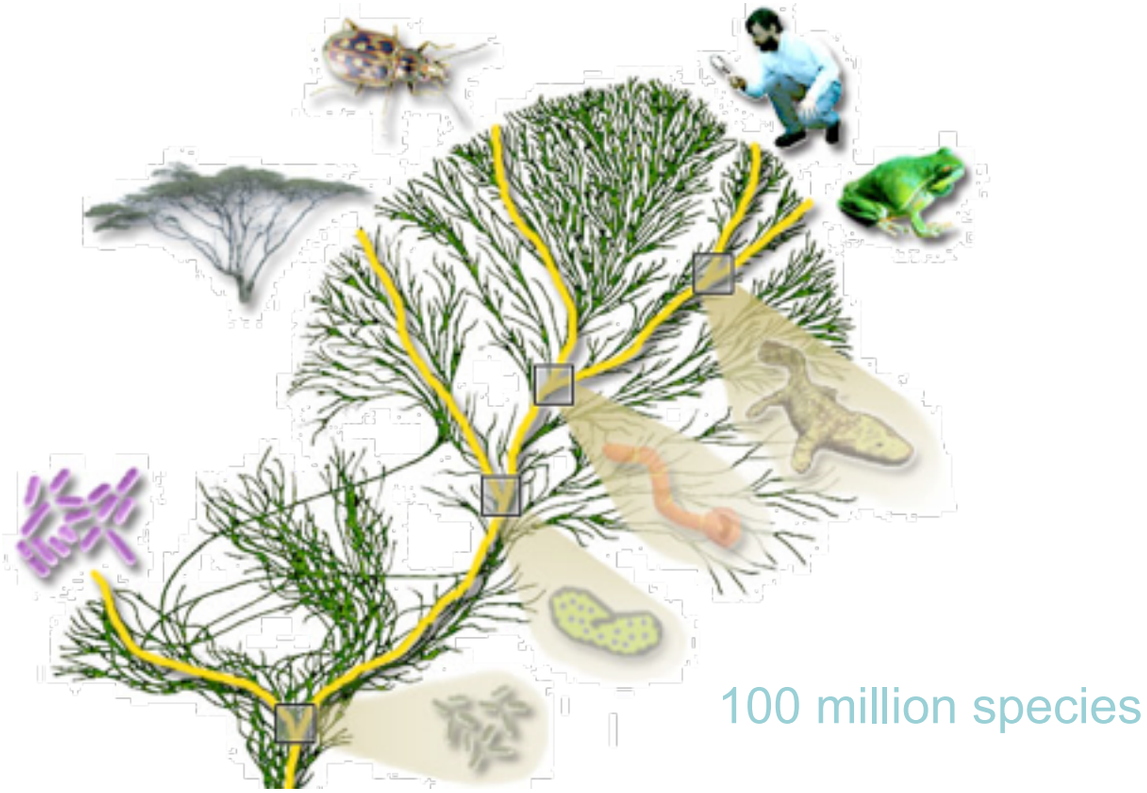

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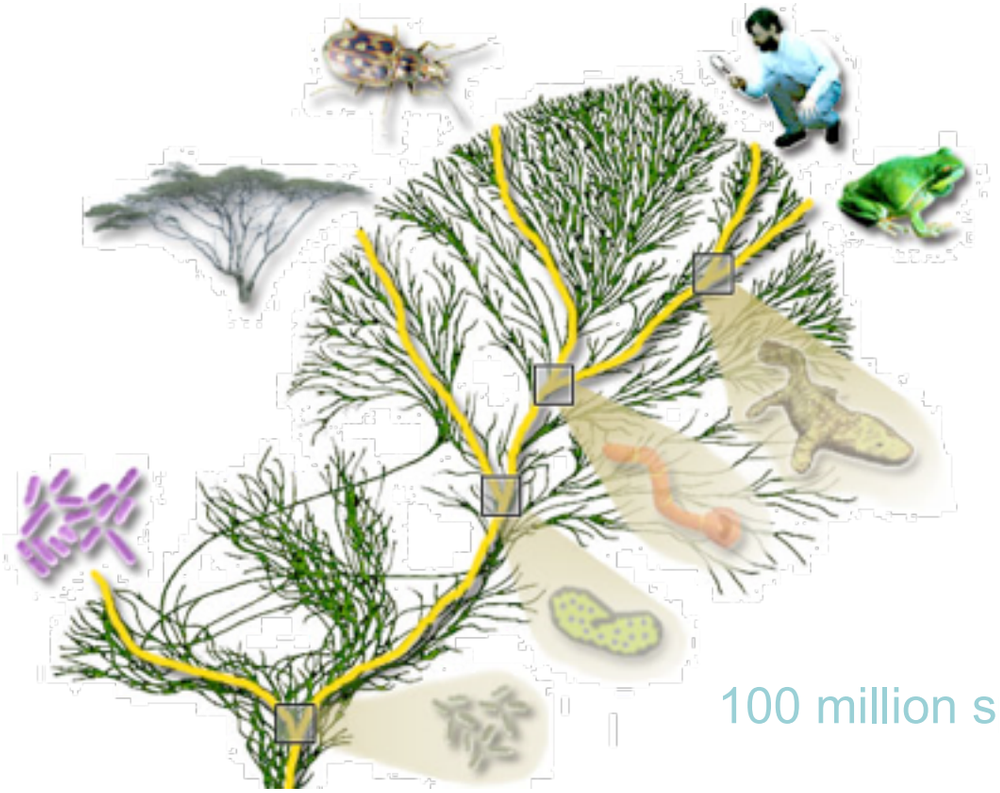

100 million species

7 billion individuals

courtesy: Batzoglou

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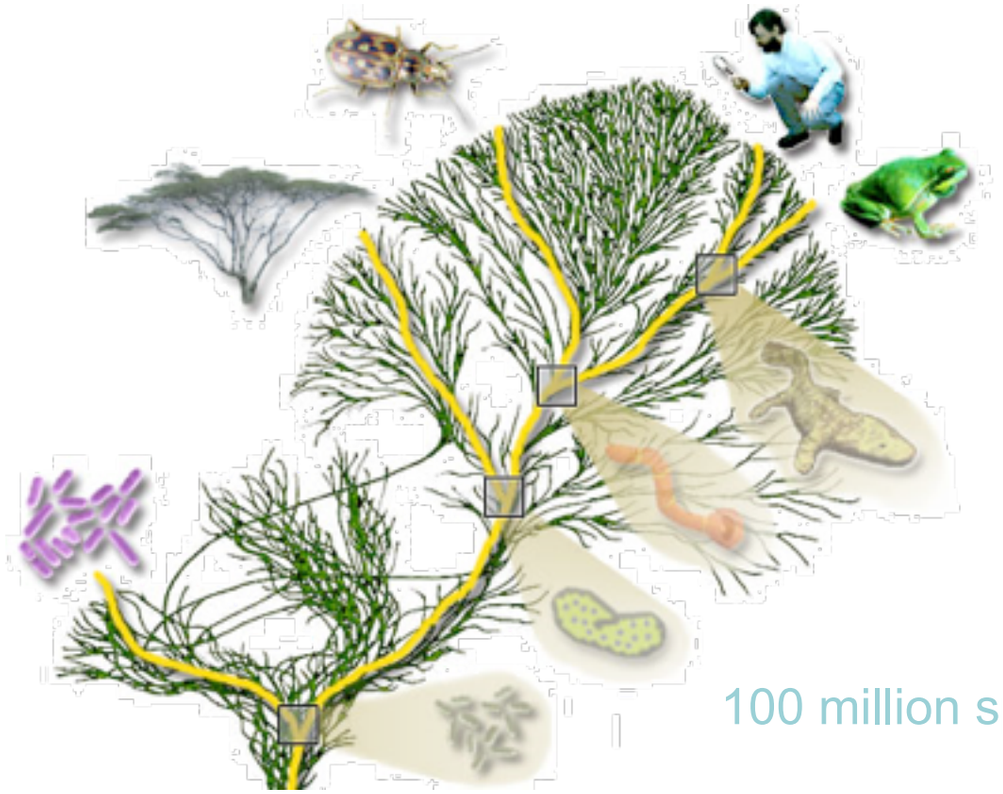

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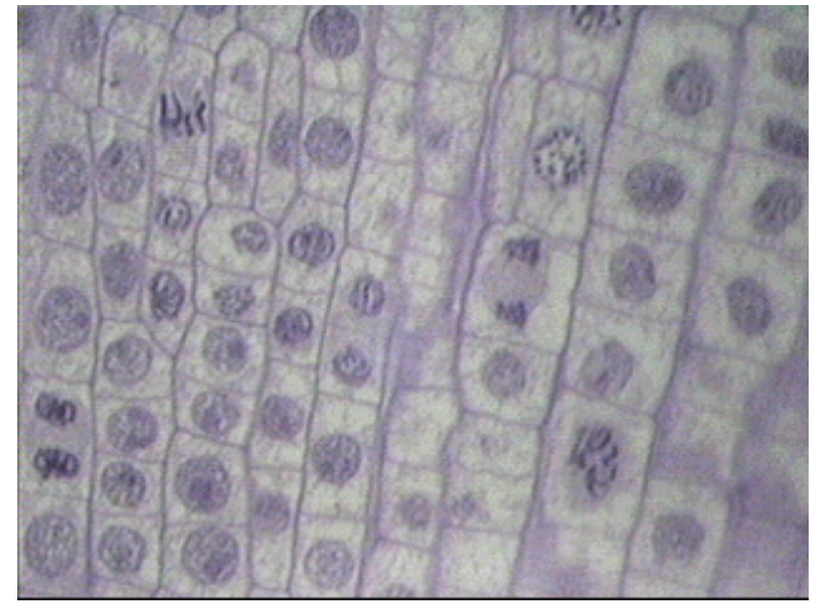


Somatic mutations
(e.g., HIV, cancer)

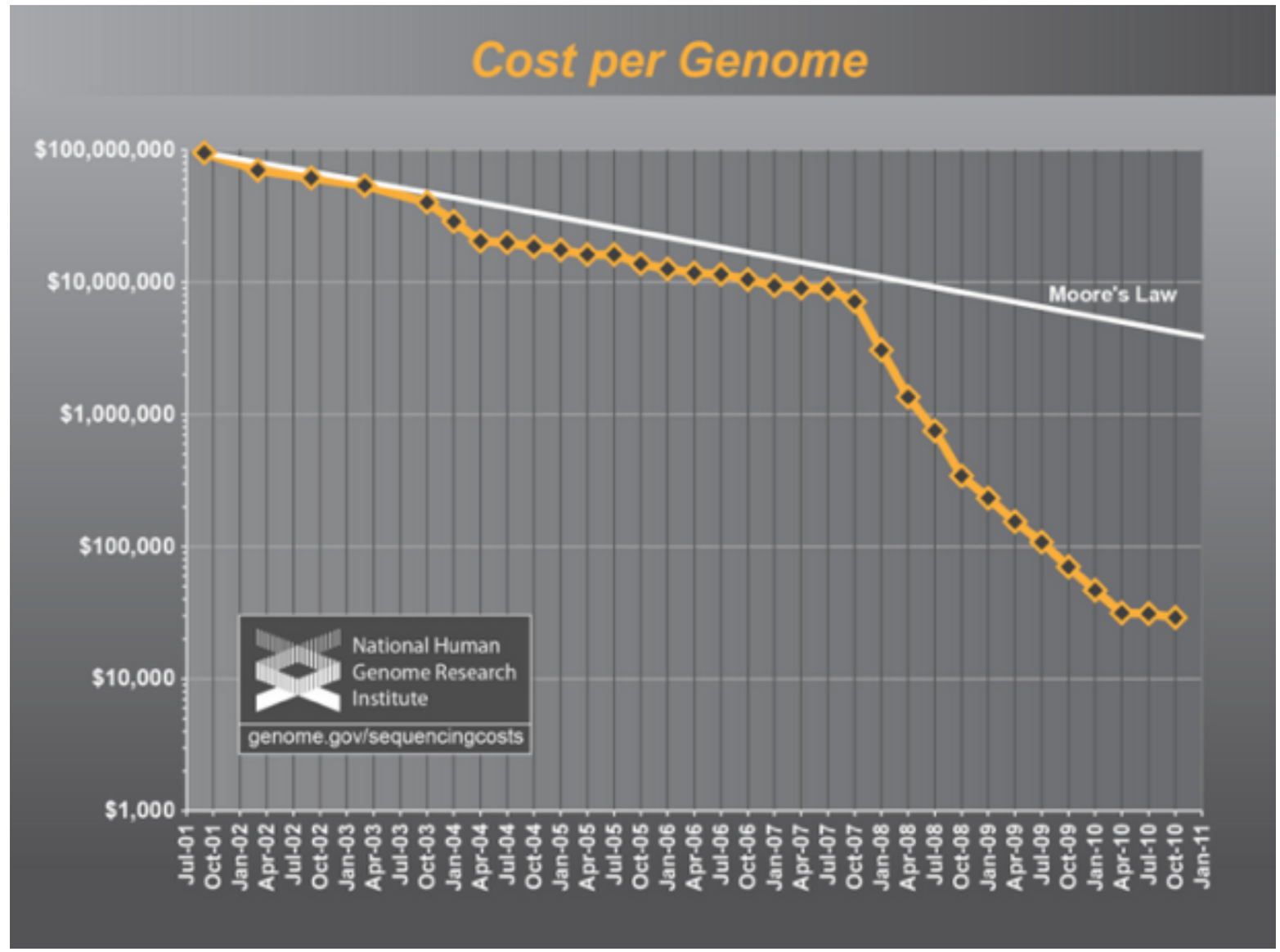
7 billion individuals

courtesy: Batzoglou

Sequencing Growth

<u>Cost of one human genome</u>

- 2004: $30,000,000
- 2008: $100,000
- 2010: $10,000
- **2013: (today) $4,000**
- ???: $300



courtesy: Batzoglou

Next Generation Sequencing

- Very High Throughput
- Higher error rate
- Shorter Length
- Very Cheap

Computational Challenges

courtesy: Batzoglou

Computational Challenges

- How to assemble the reads?

courtesy: Batzoglou

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- How to assemble the reads?
- For given genome size G, what are the requirements

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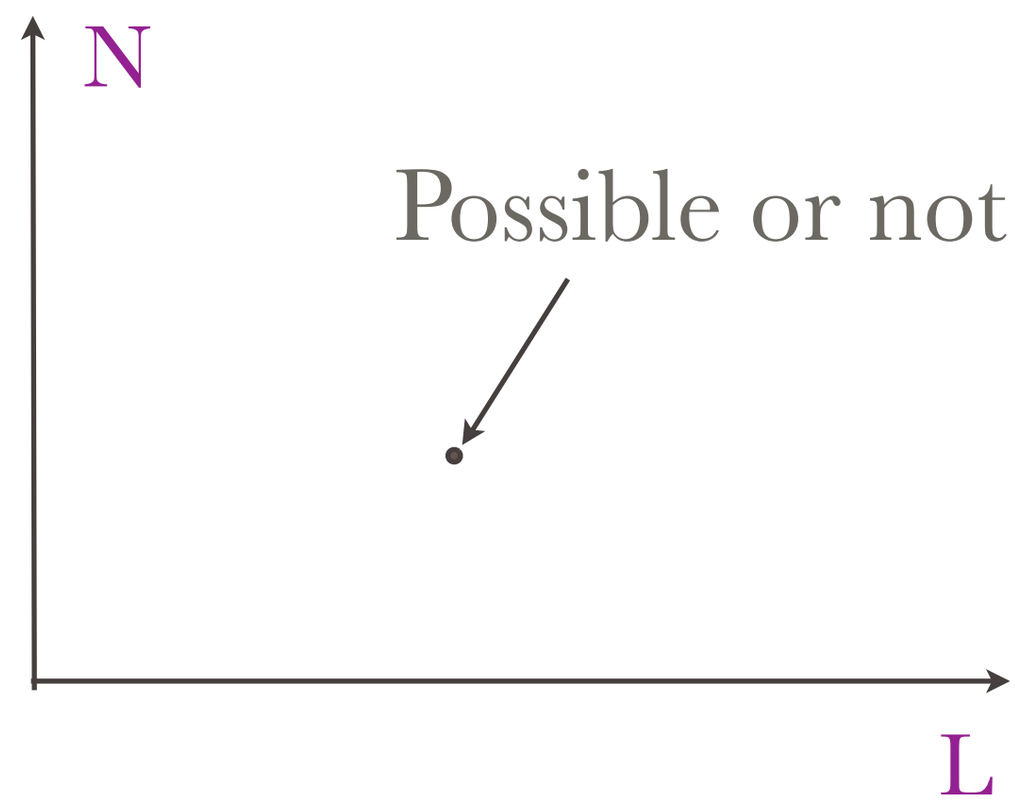

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Coverage Condition

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N_{cov} → is the minimum number of reads required to cover the sequence

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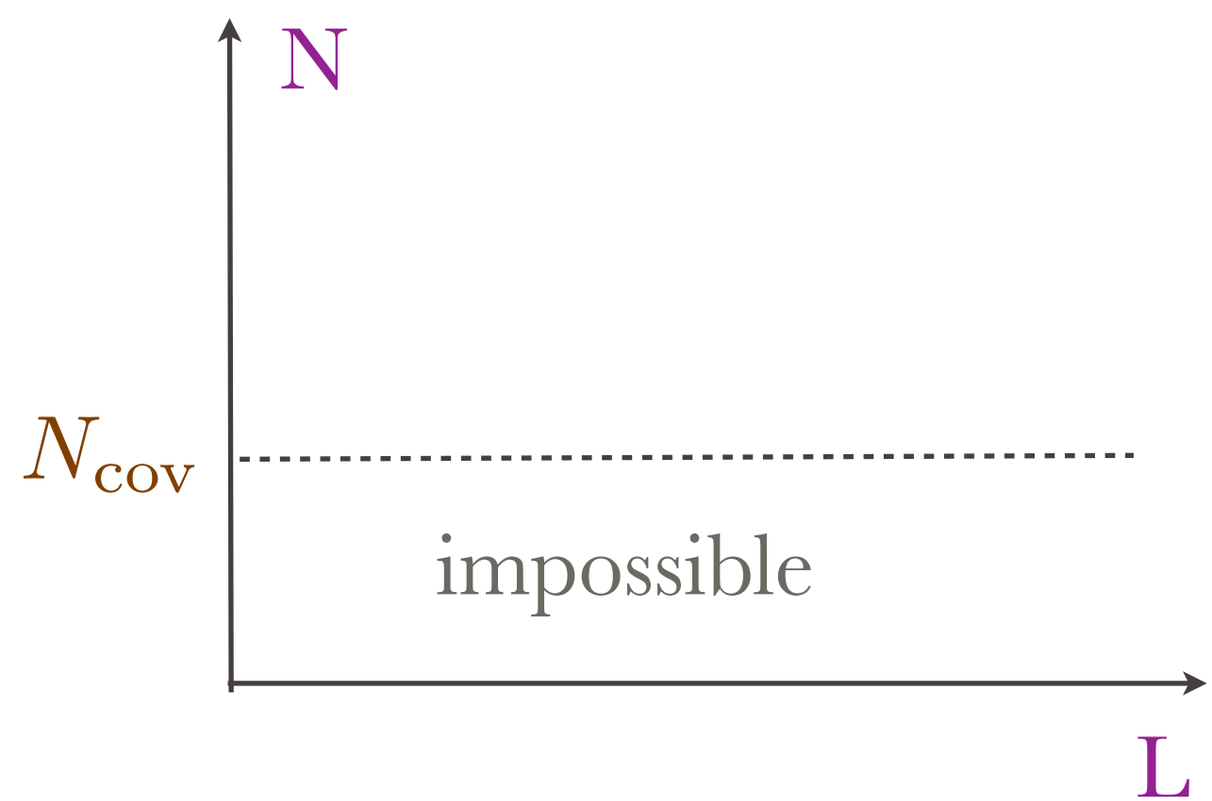

Repeat Condition

courtesy: Tse

Repeat Condition

- L-spectrum is given

Repeat Condition

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ATAGCCTAGCGAT

Repeat Condition

- L-spectrum is given

ATAGCCTAGCGAT
ATAGC
TAGCC
AGCCT
GCCTA
CCTAG
CTAGC
TAGCG
AGCGA
GCGAT

Repeat Condition

de Bruijn Graph

- Add a node for each (L-1)- mer in a read
- Add edges for adjacent (L-1)-mers

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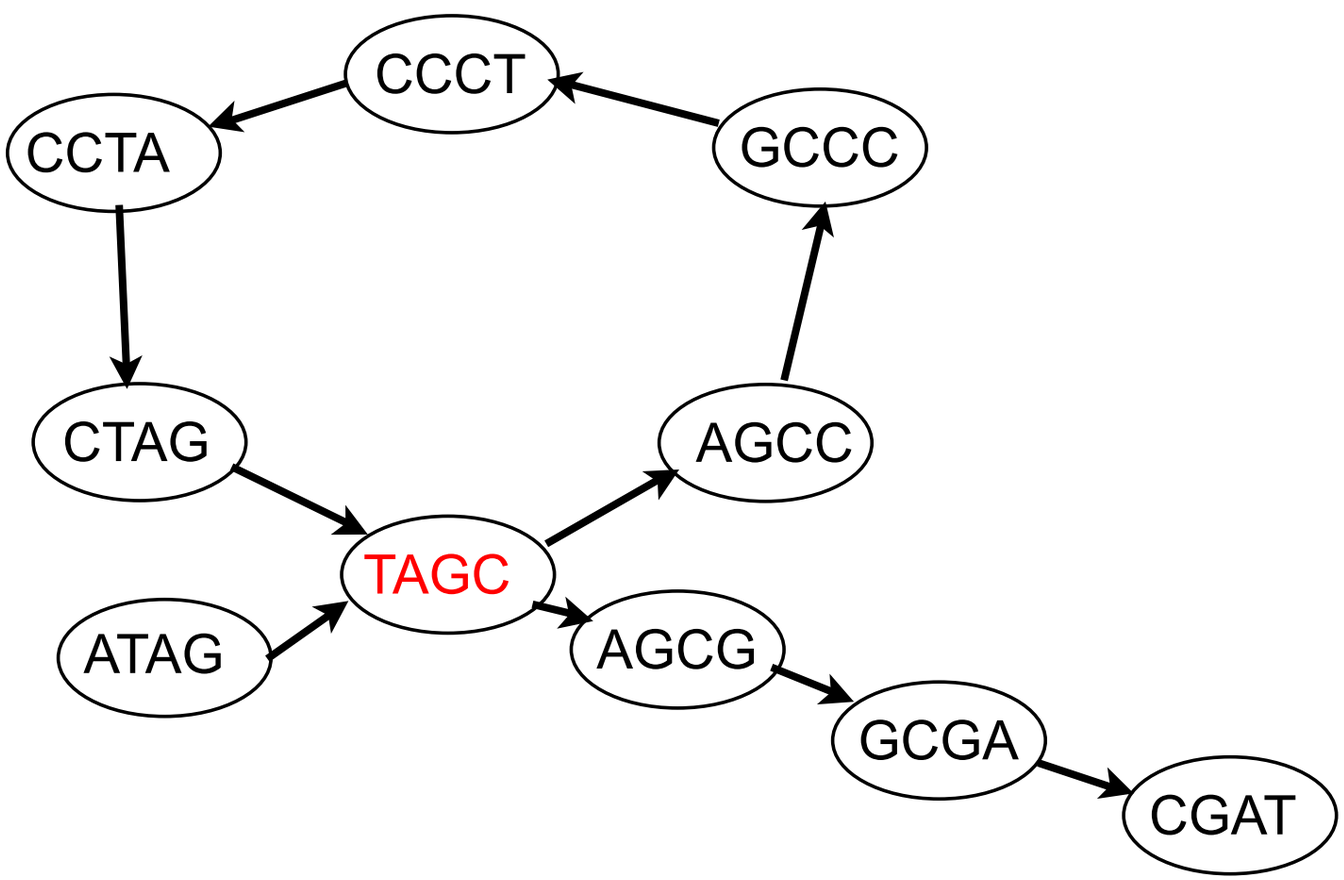

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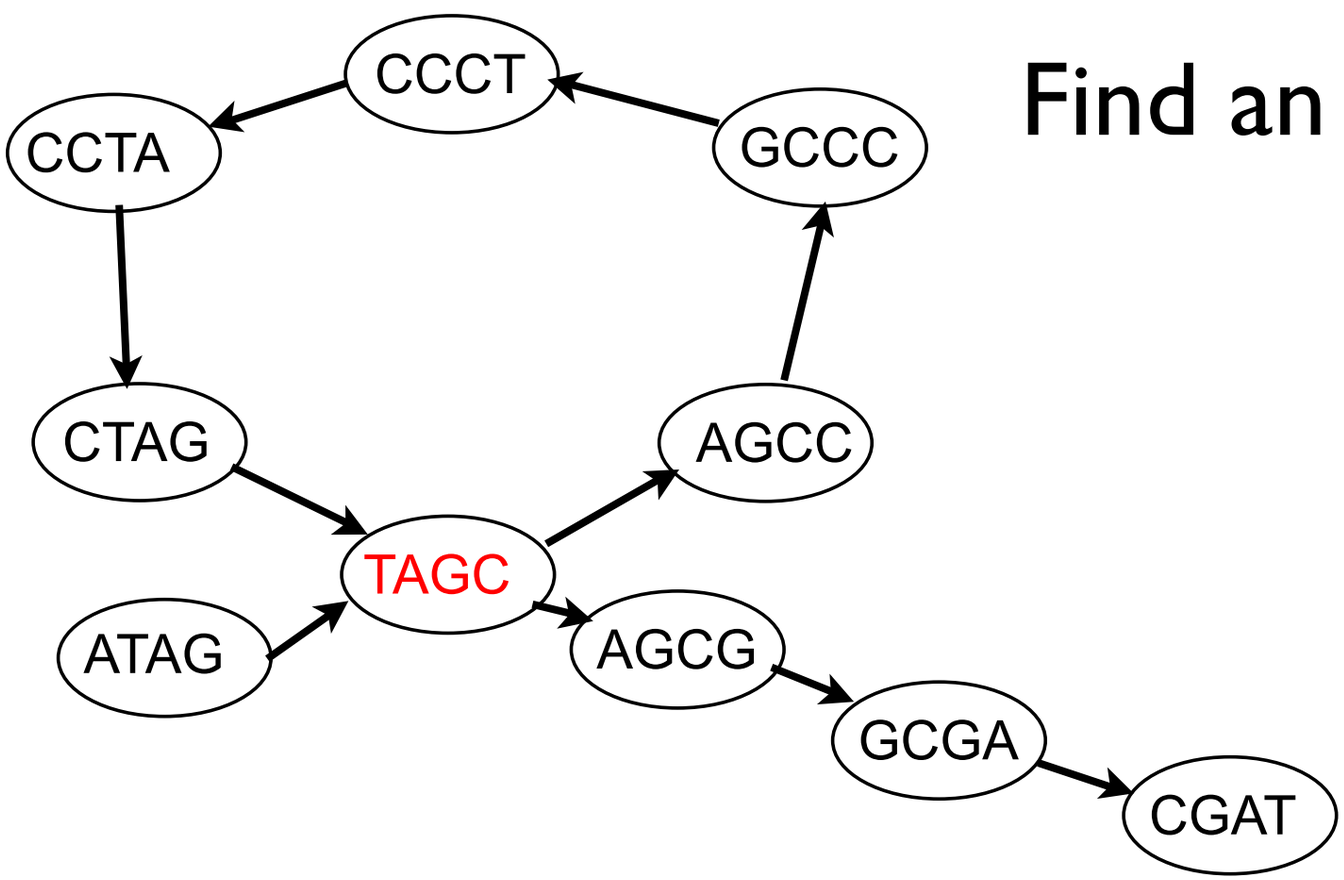

Find an Eulerian Path

Repeat Condition

de Bruijn Graph

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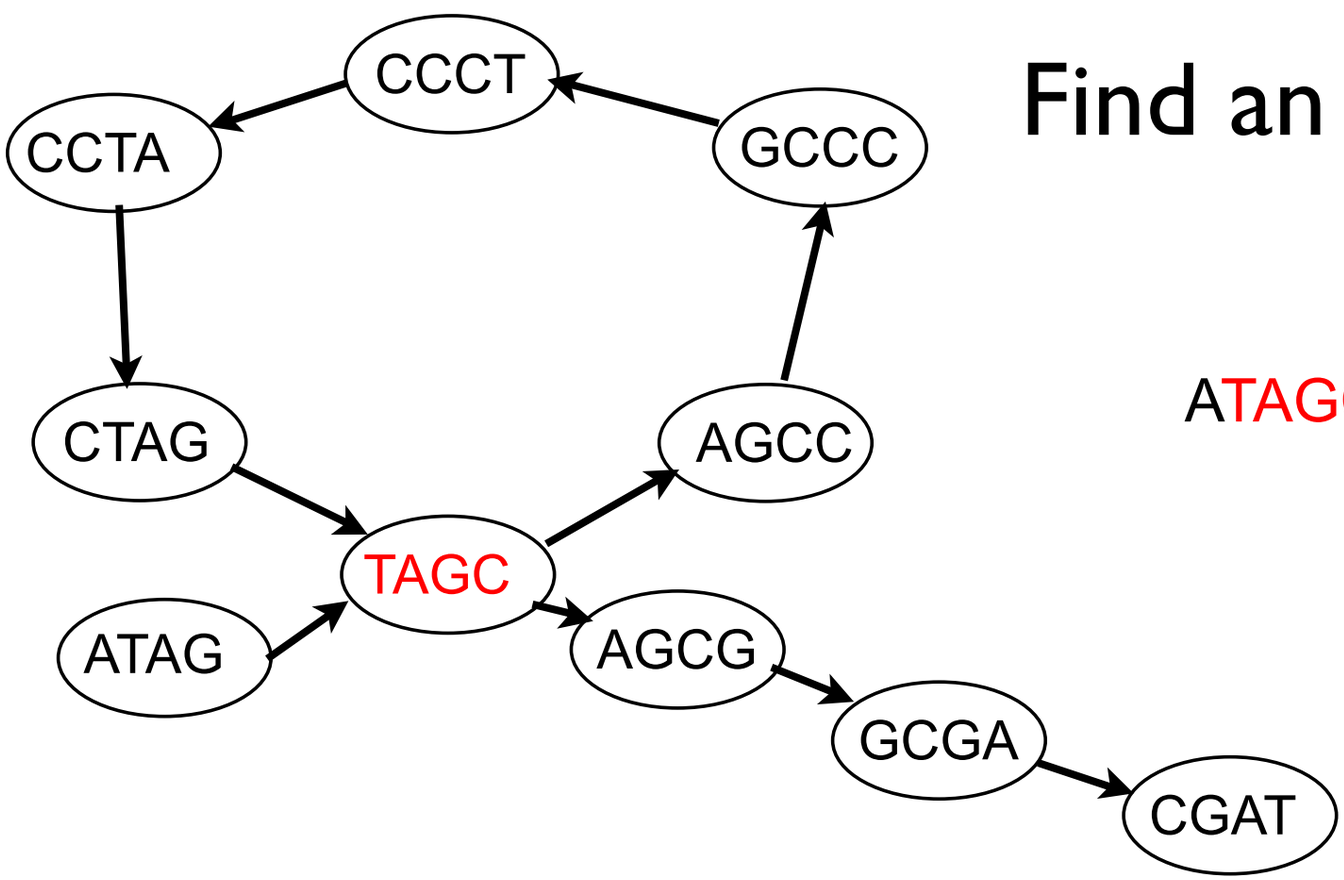
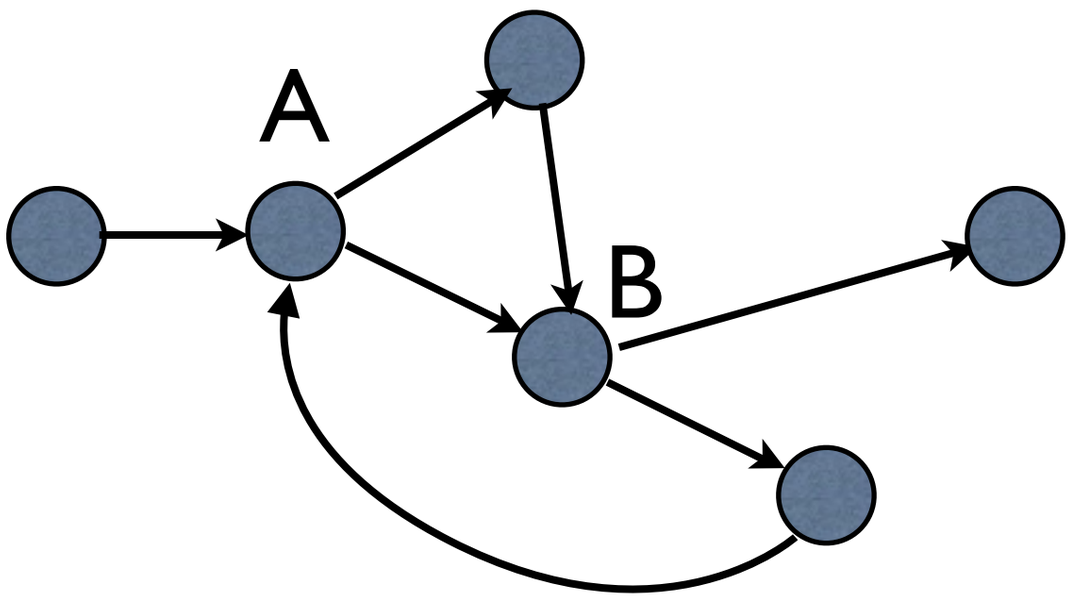
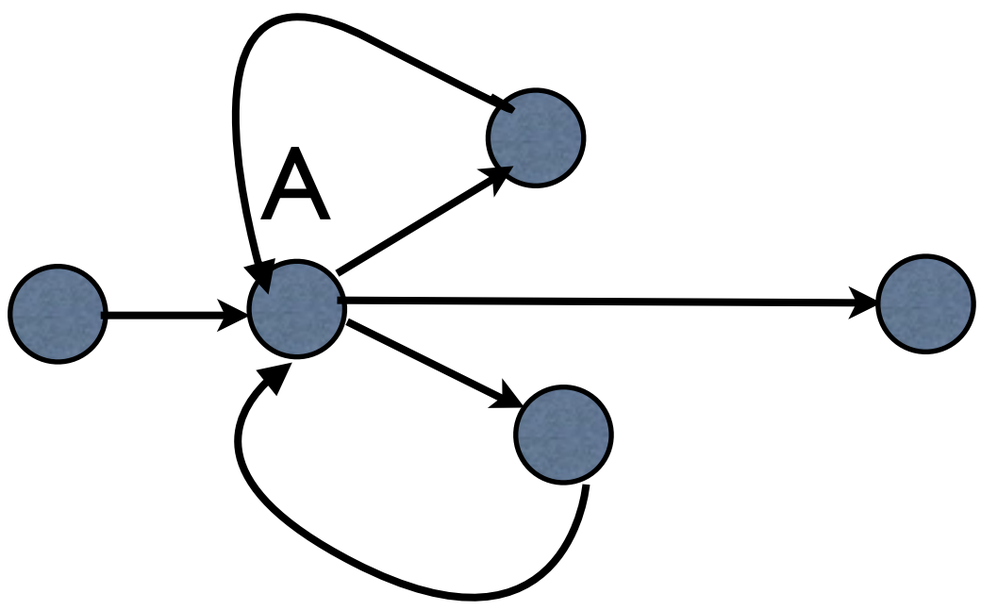


Find an Eulerian Path

ATAGCCCTAGCGAT

Repeat Condition





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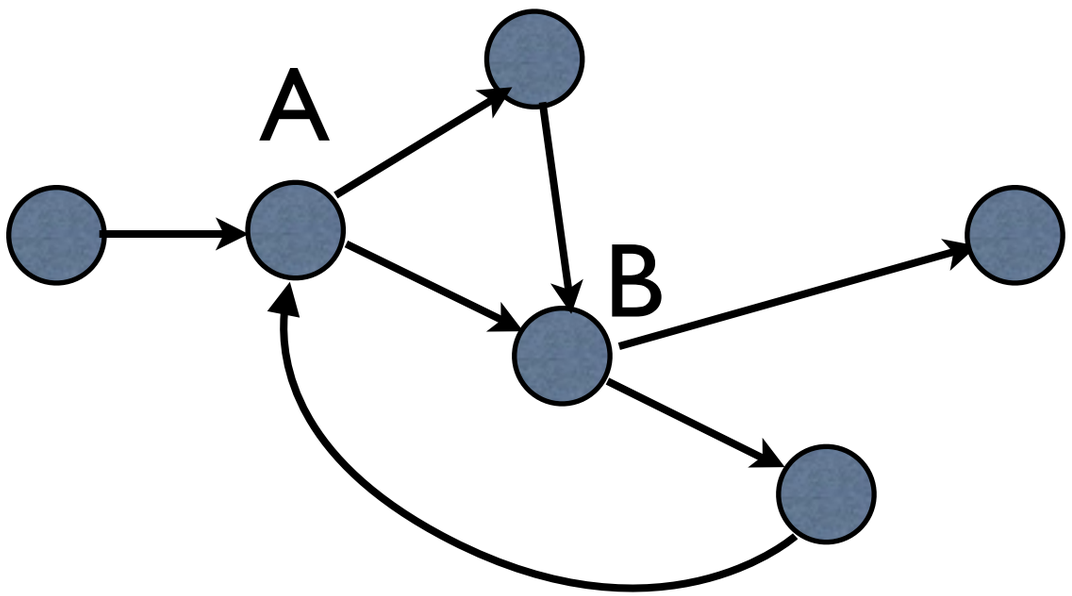
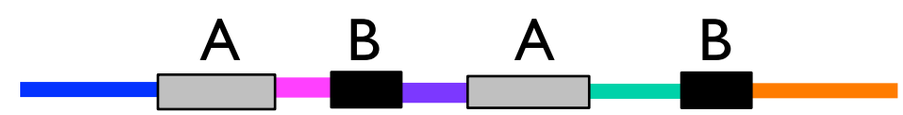
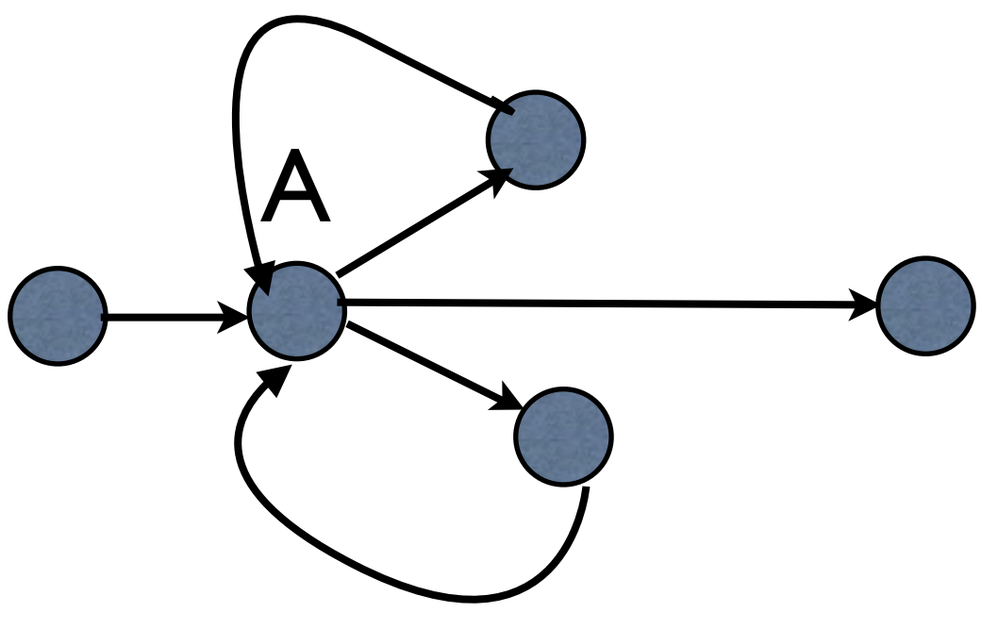


Interleaved repeats





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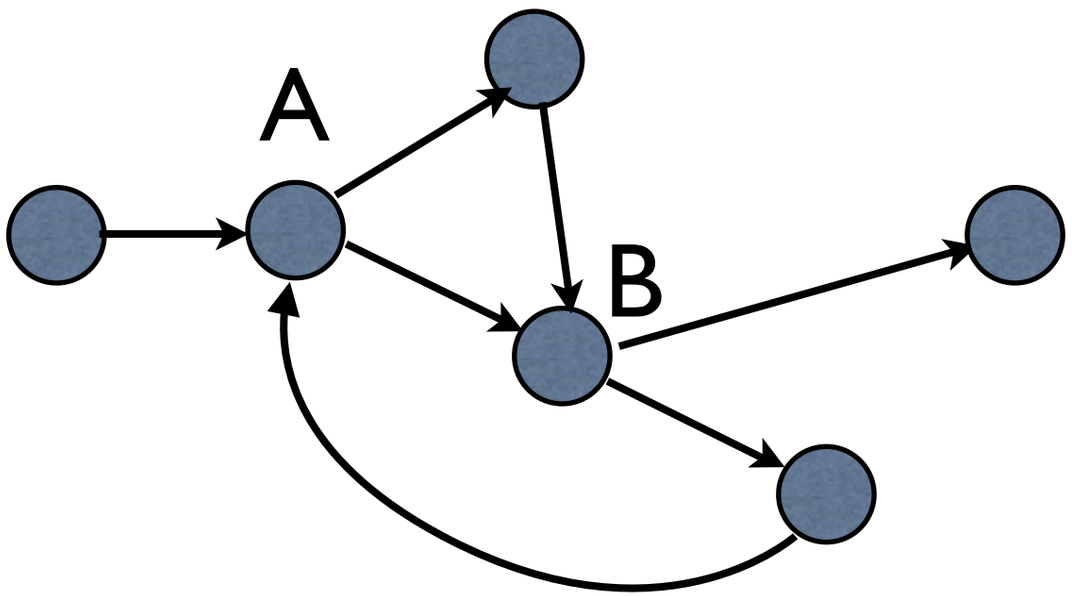

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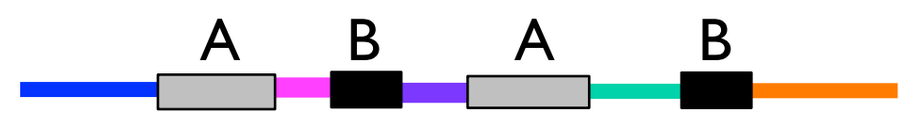
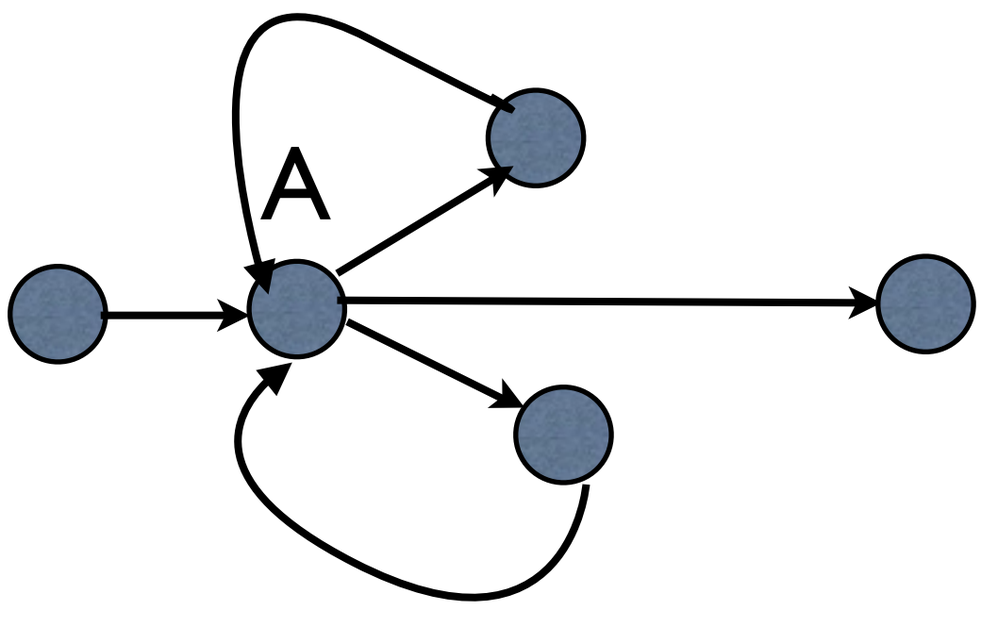
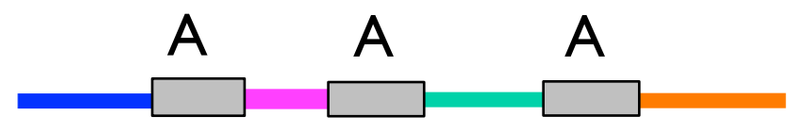




Triple repeat



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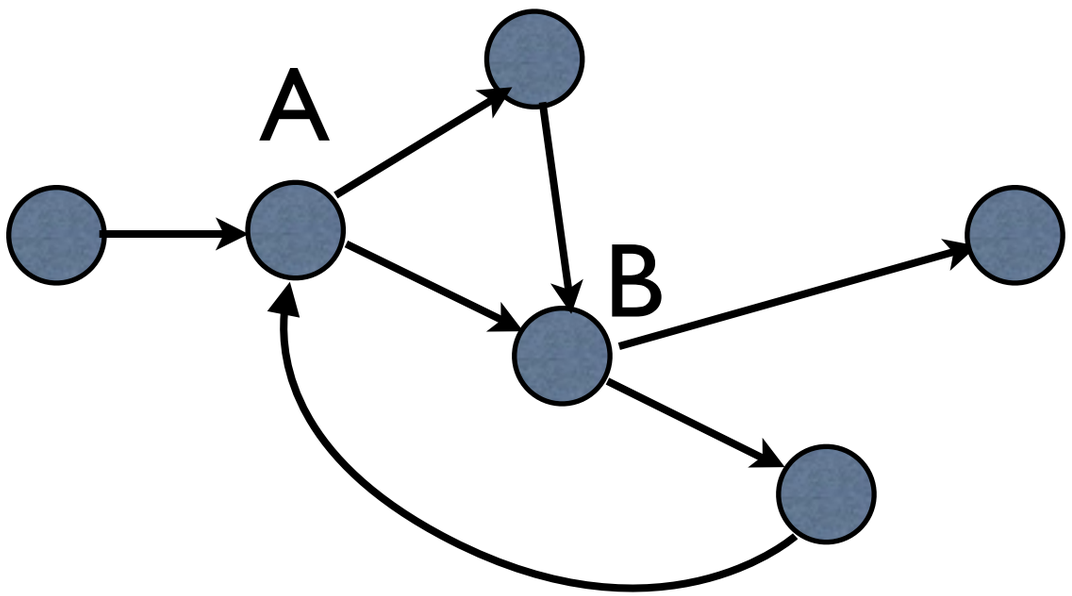

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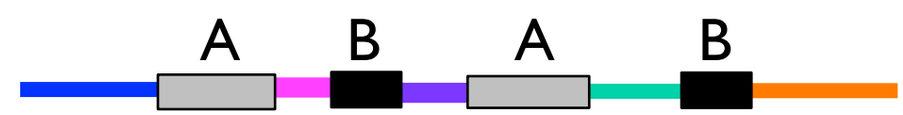
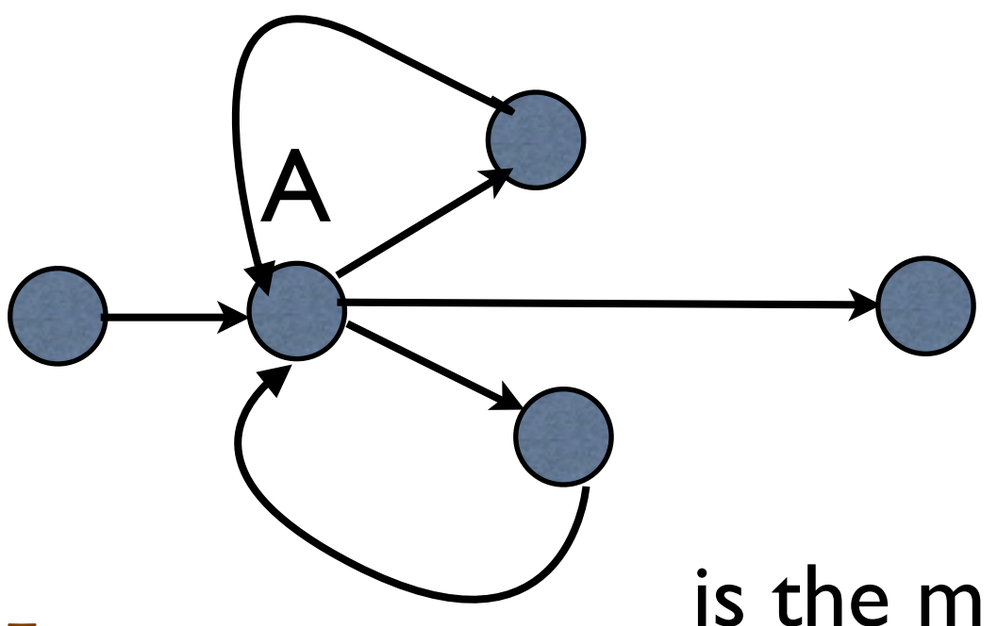
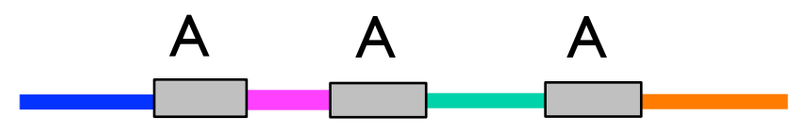




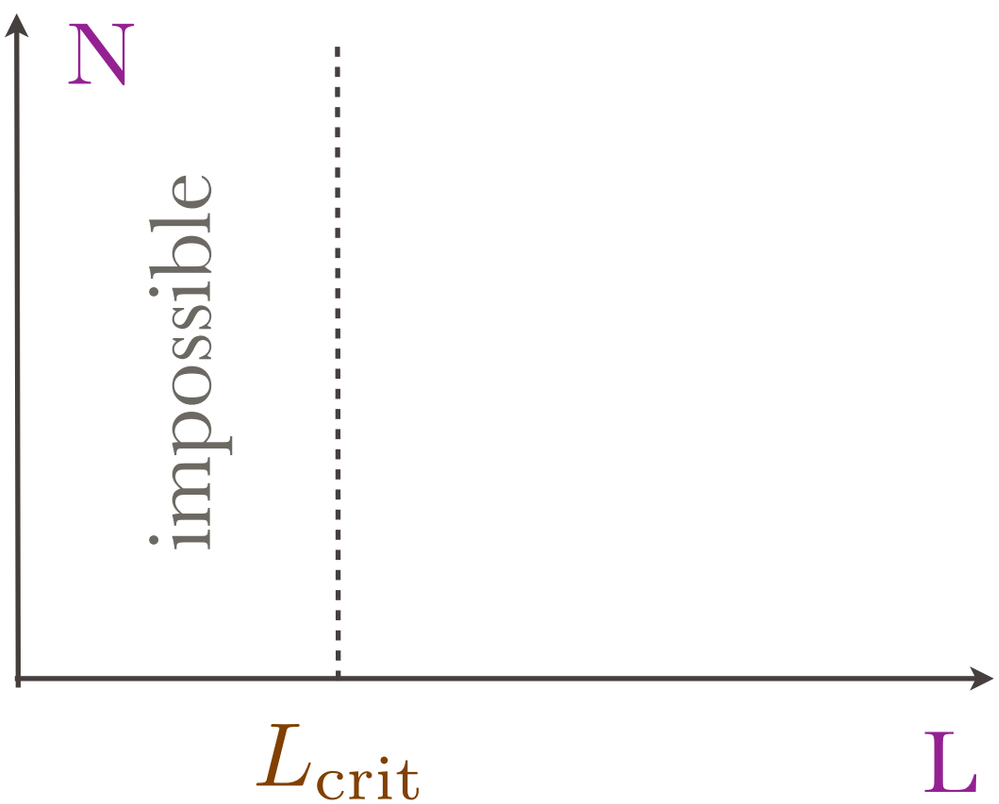
Triple repeat



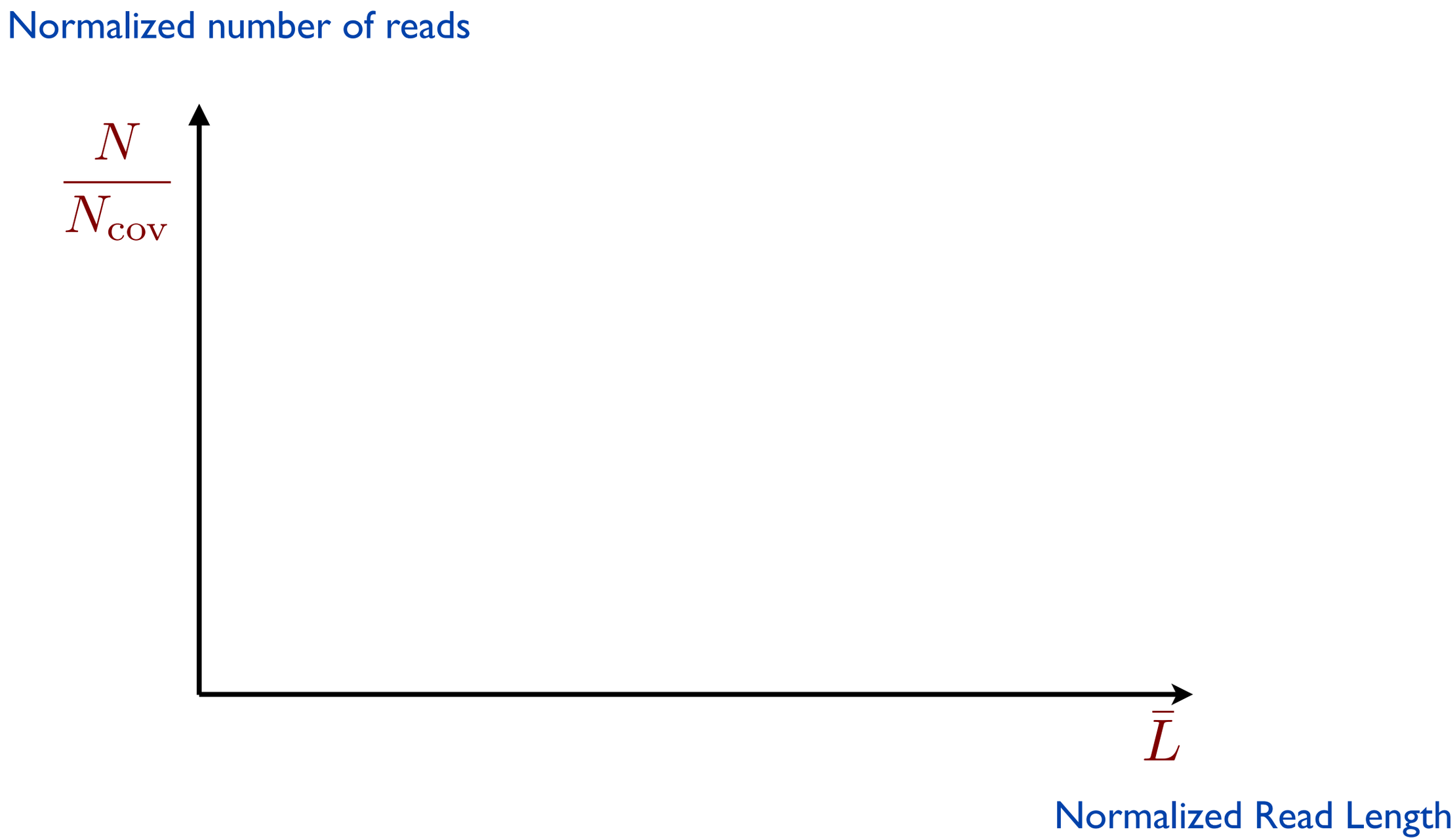
L_{crit} is the minimum read length where there exists a unique solution for the problem

Repeat Condition

$$L > L_{\text{crit}}$$



Putting together



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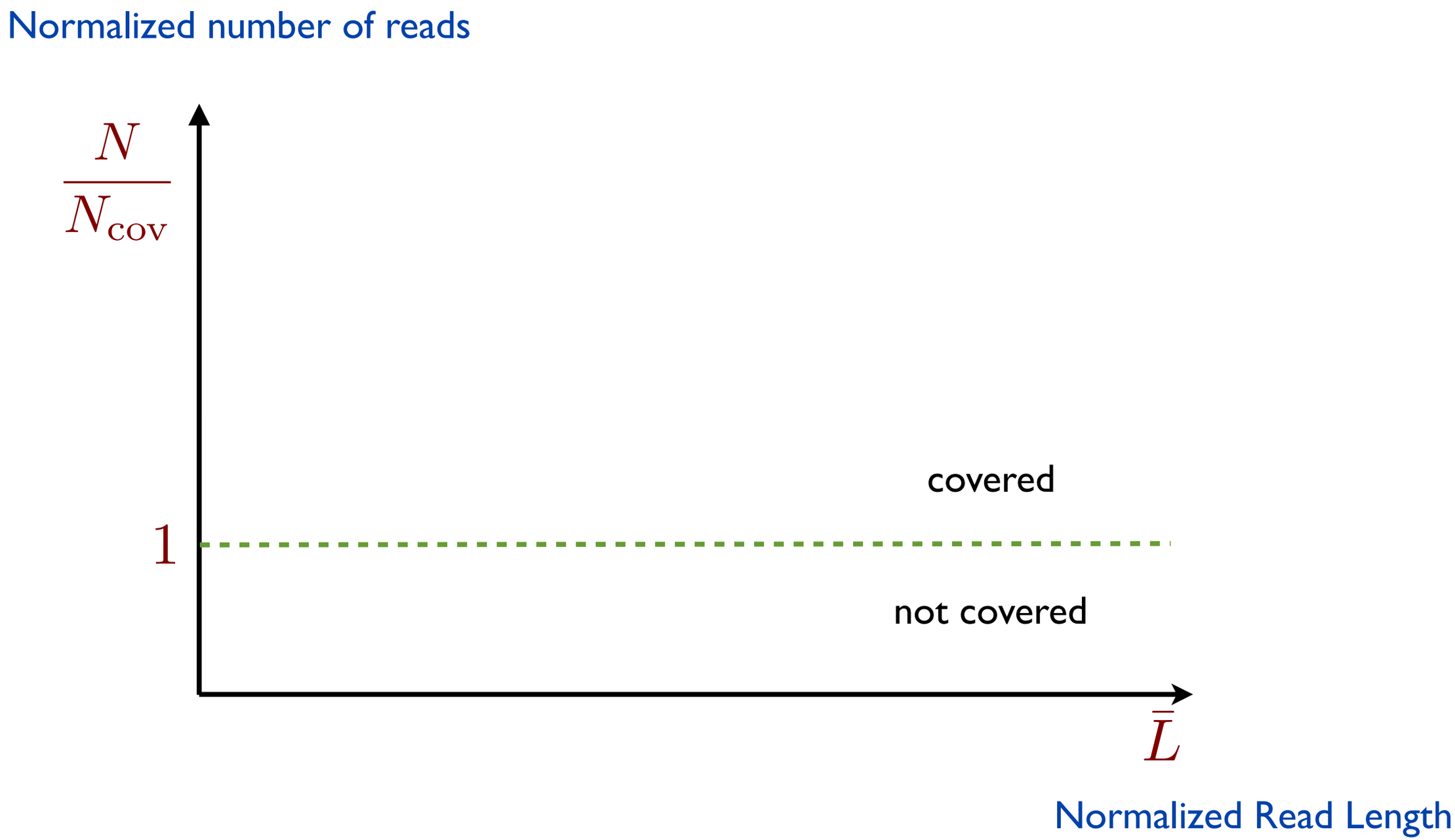

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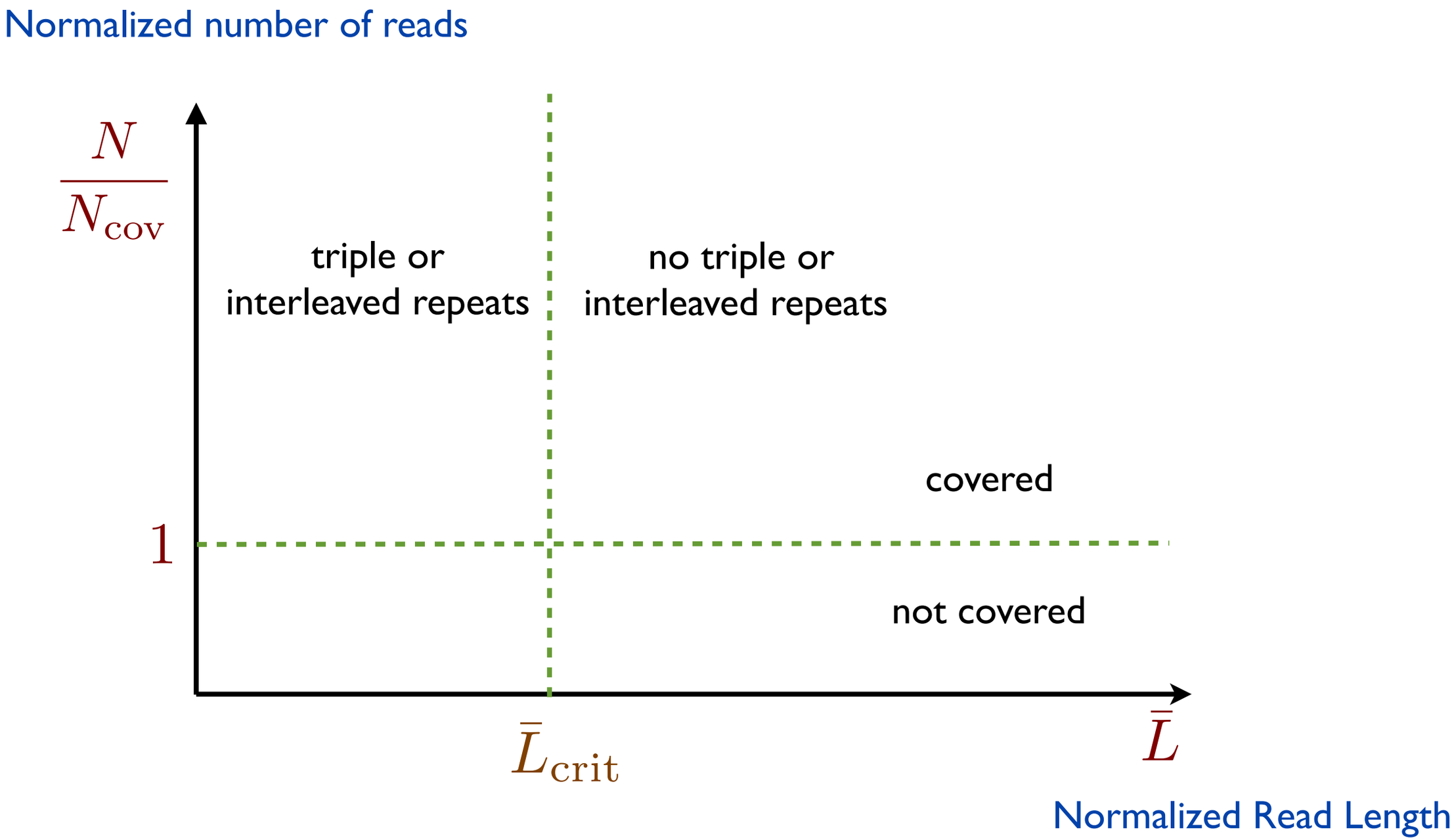

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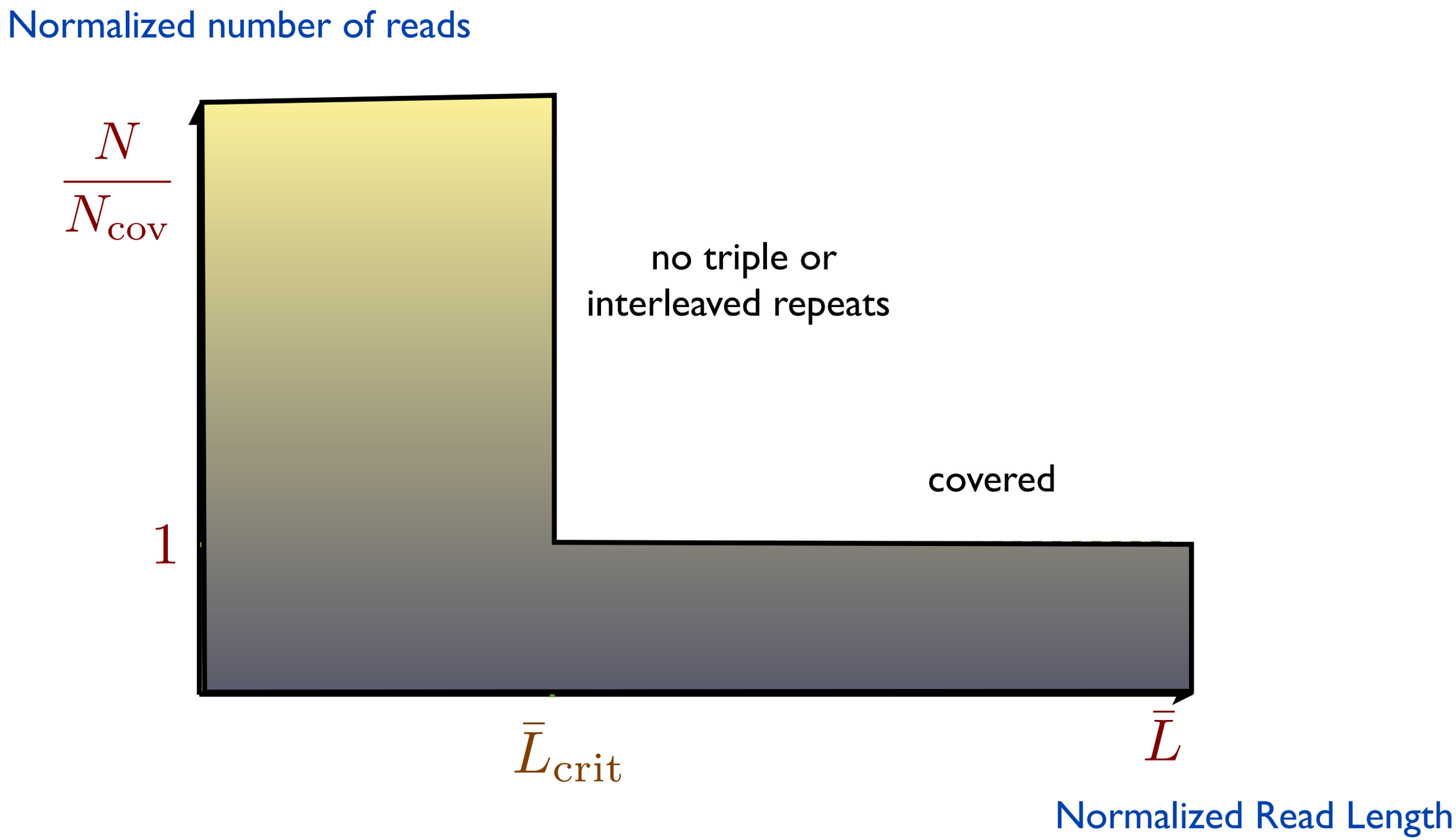
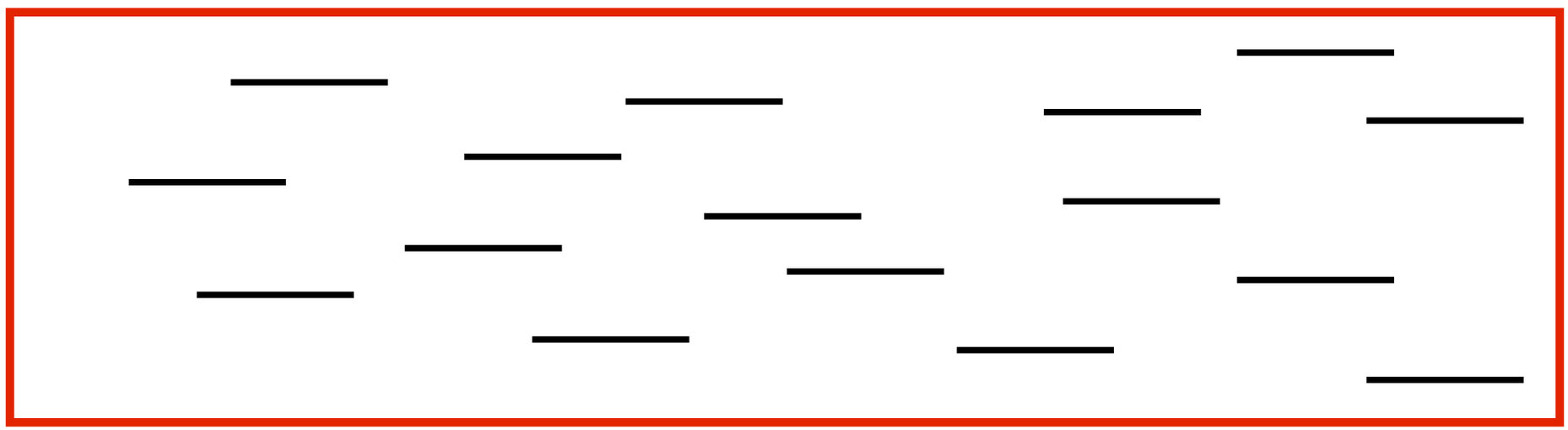


what about the rest?

The Greedy Algorithm



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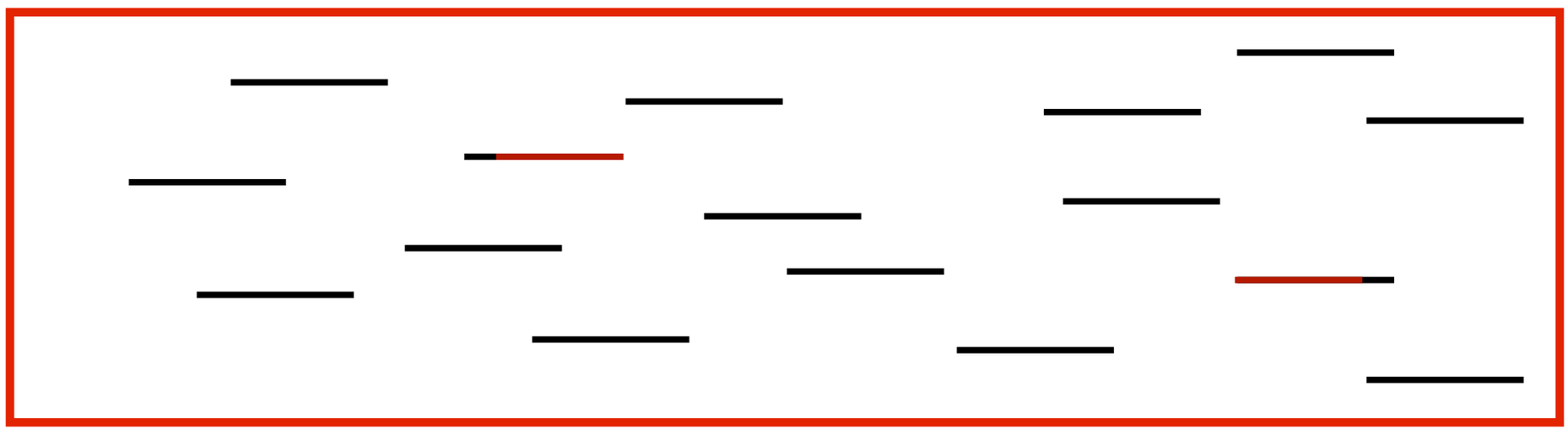

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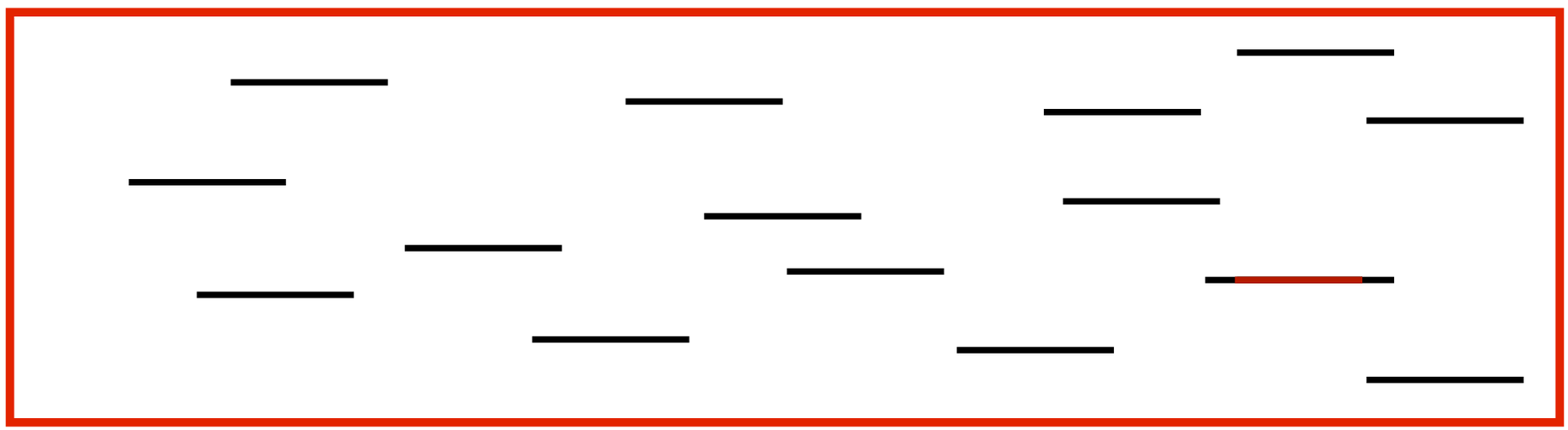

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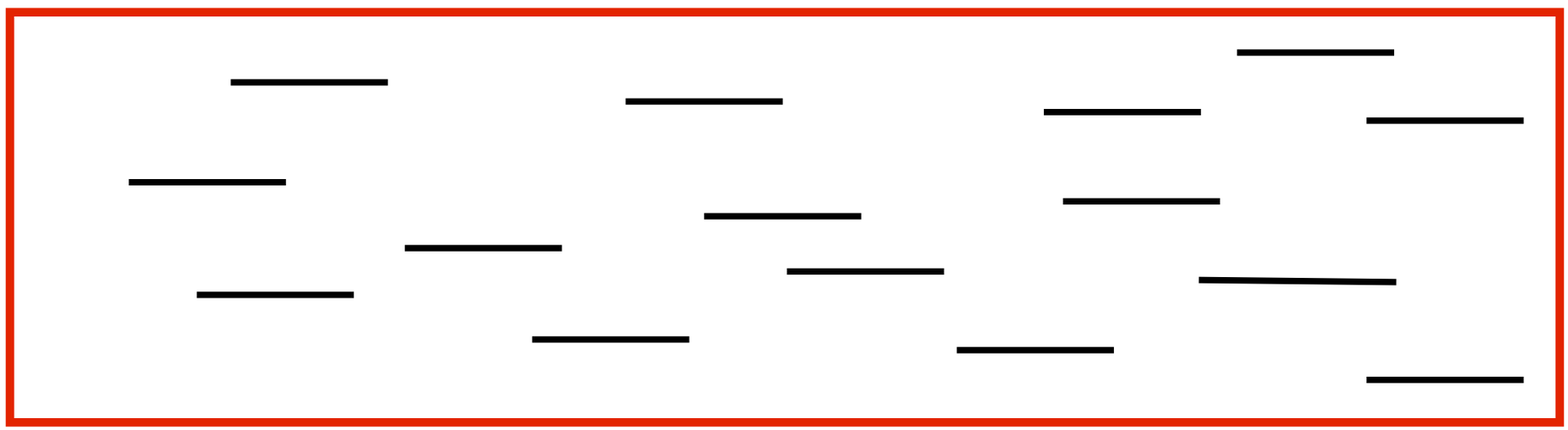

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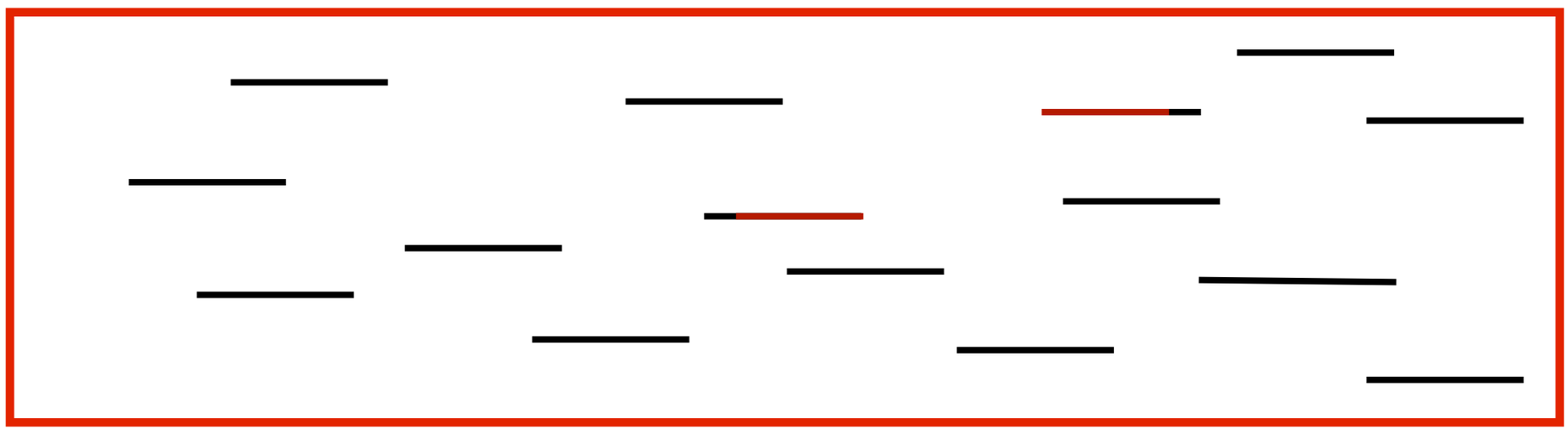

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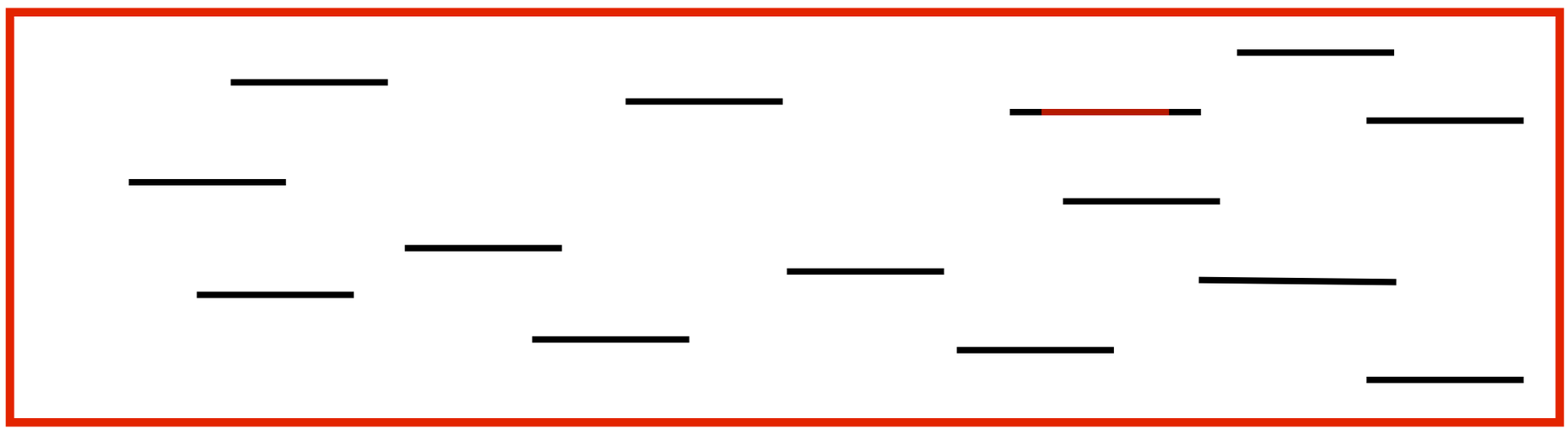

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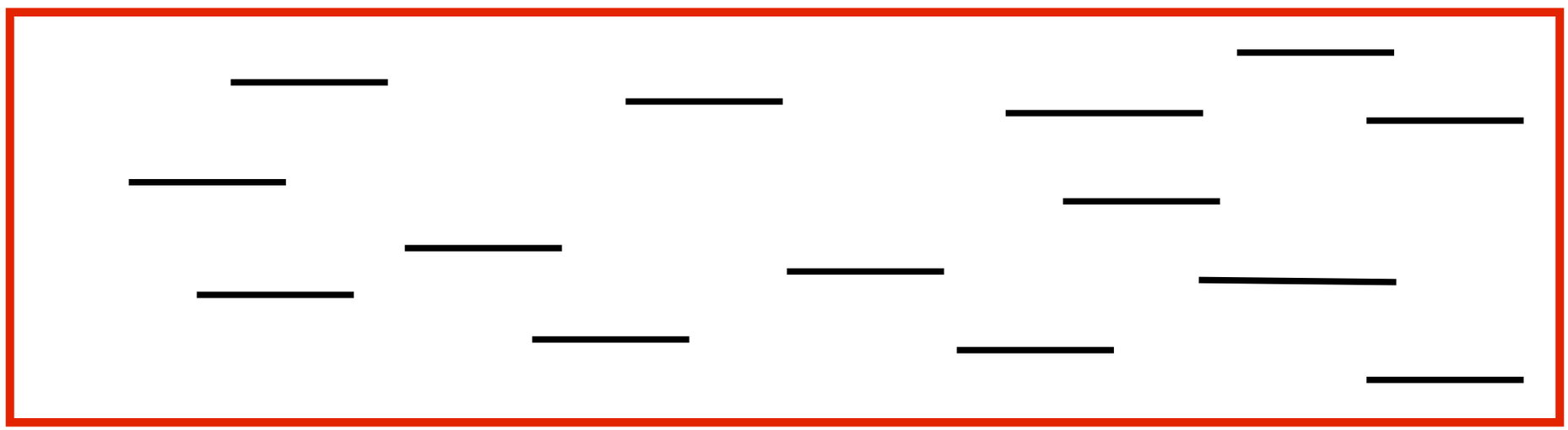

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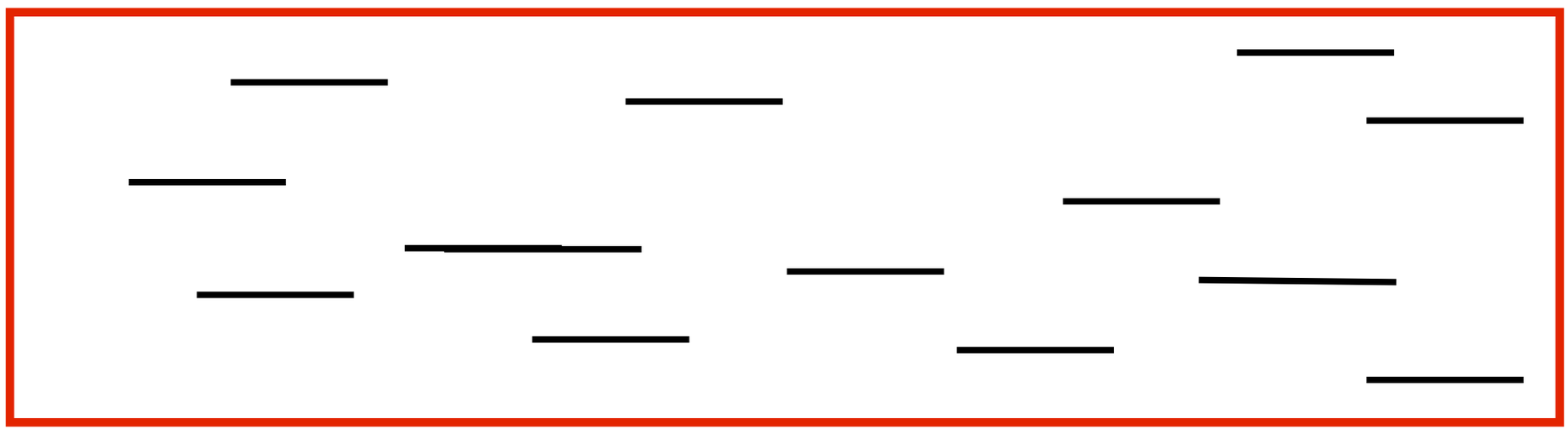

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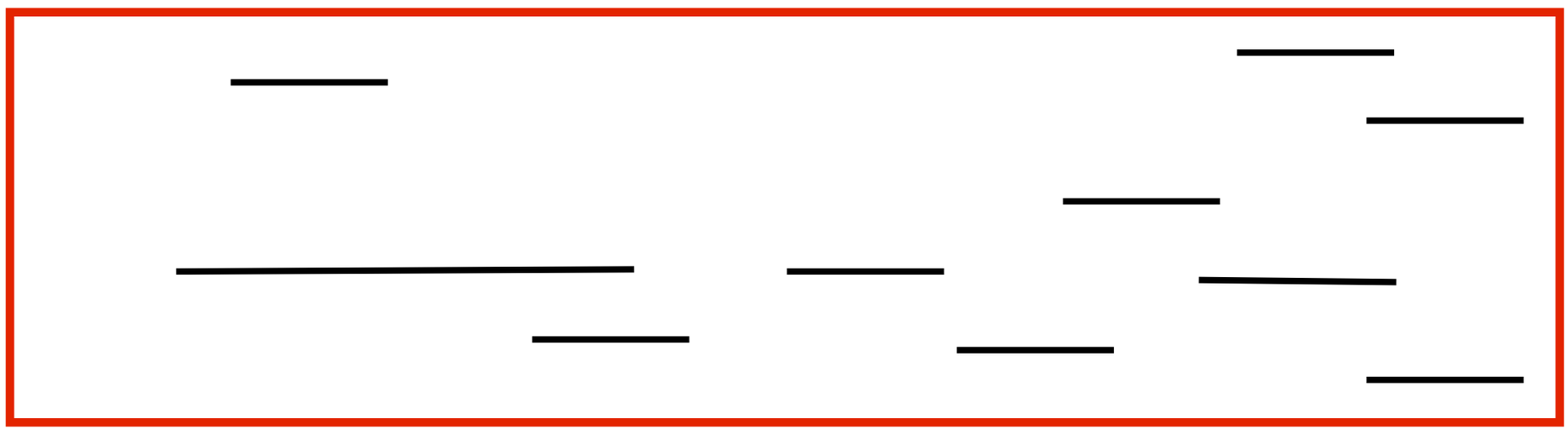

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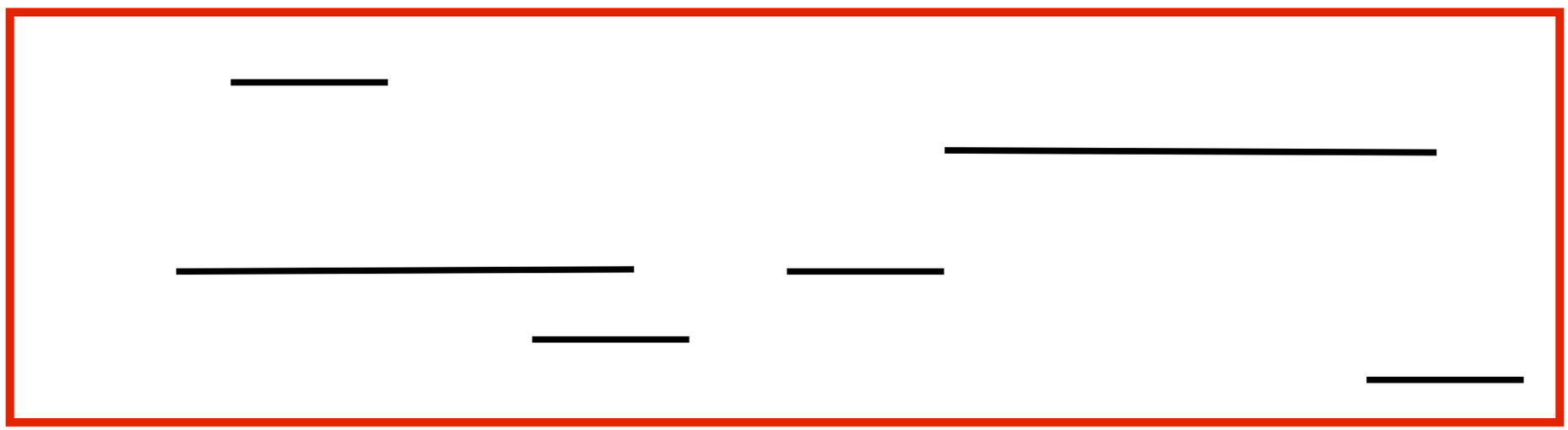

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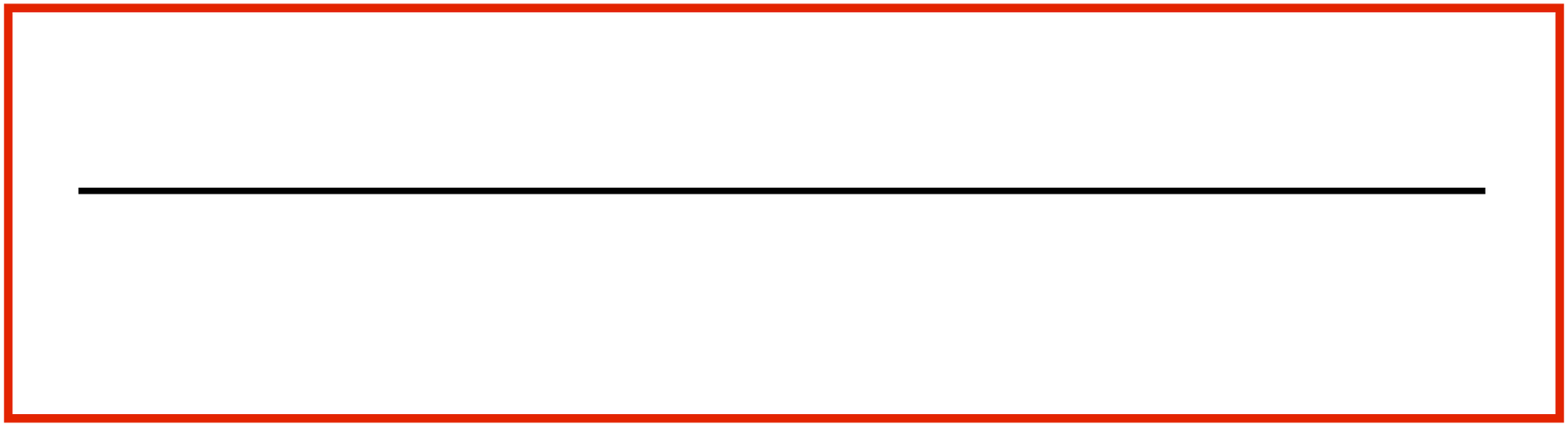

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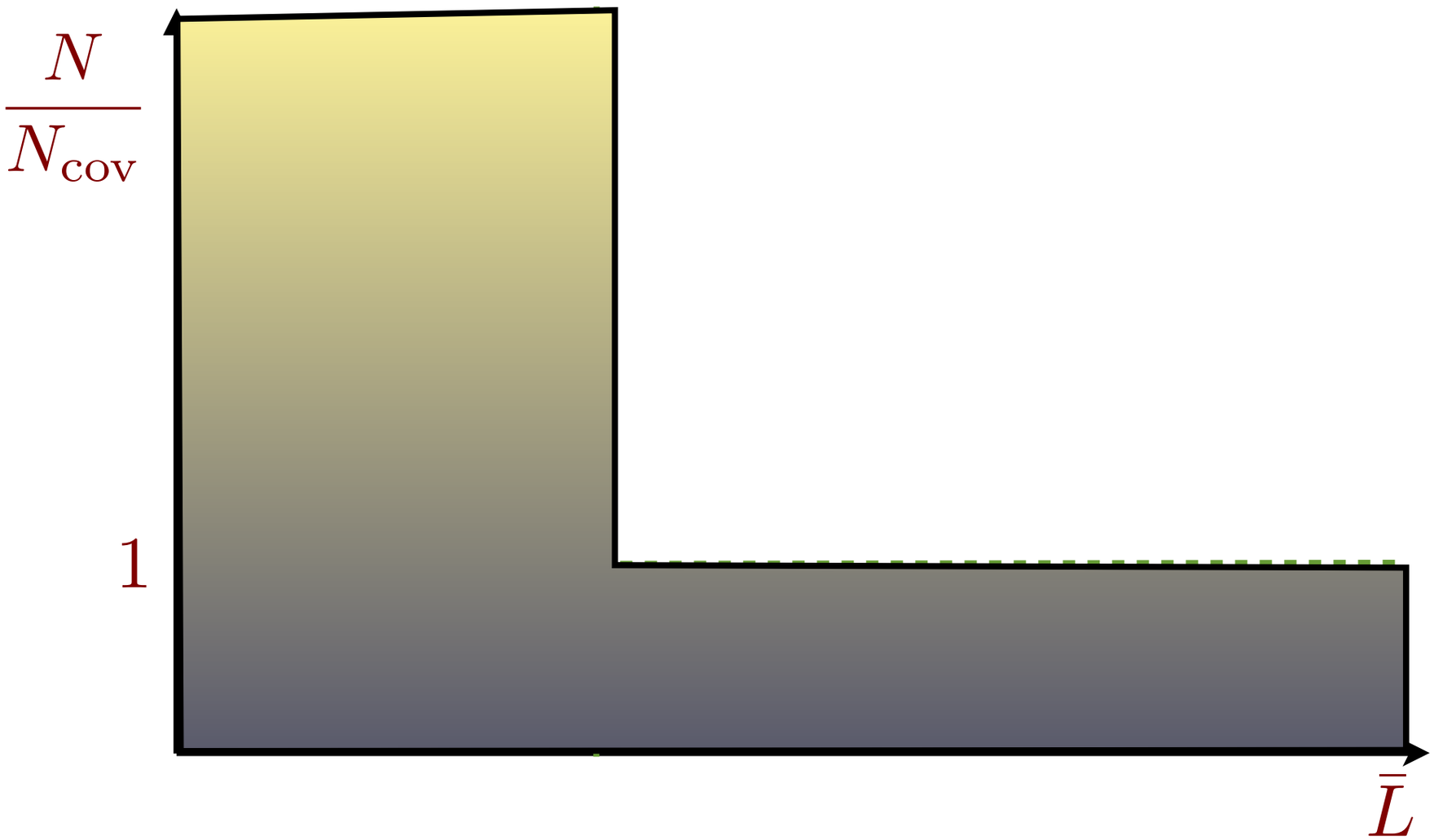
RANDOM Sequence



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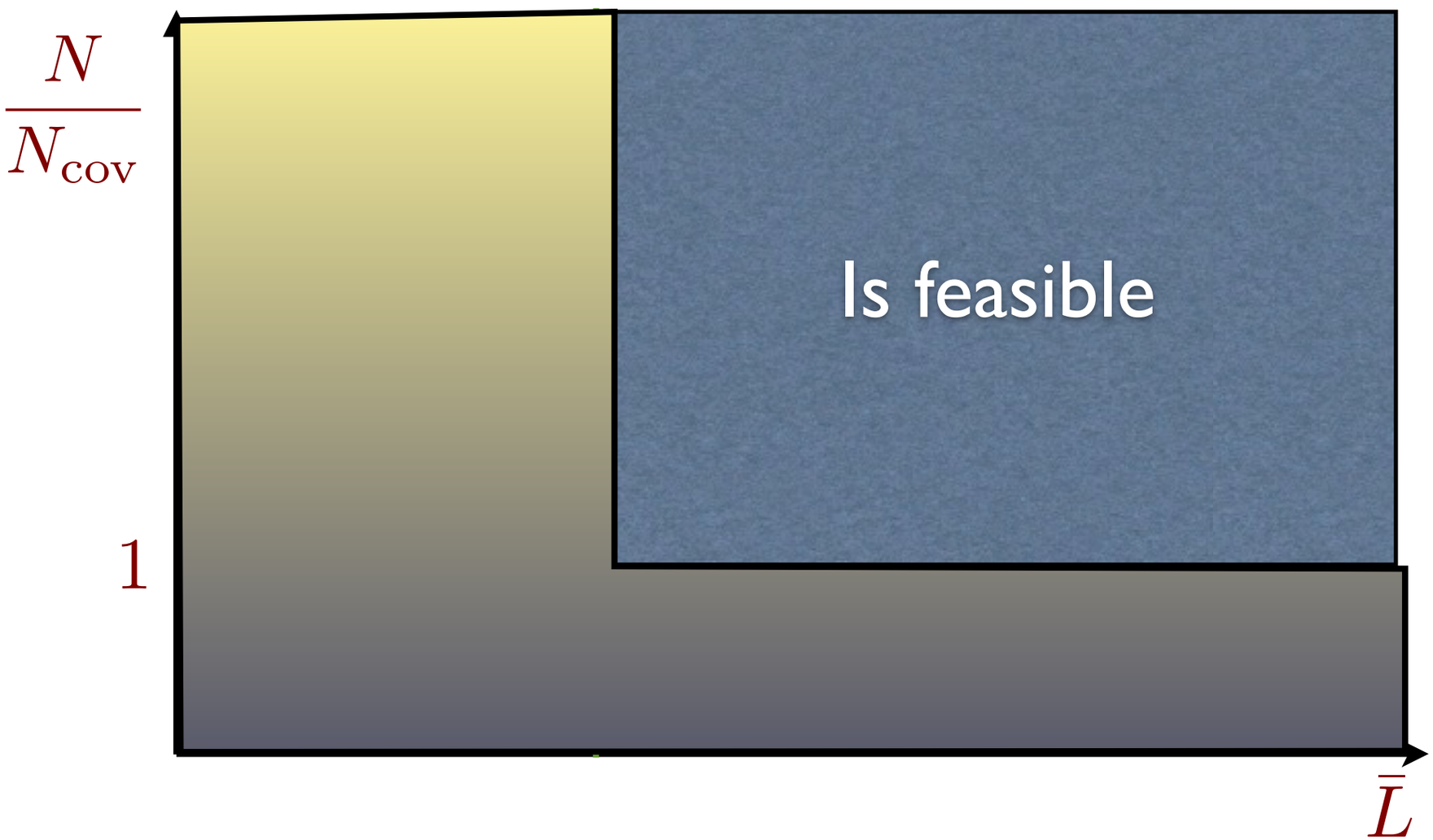
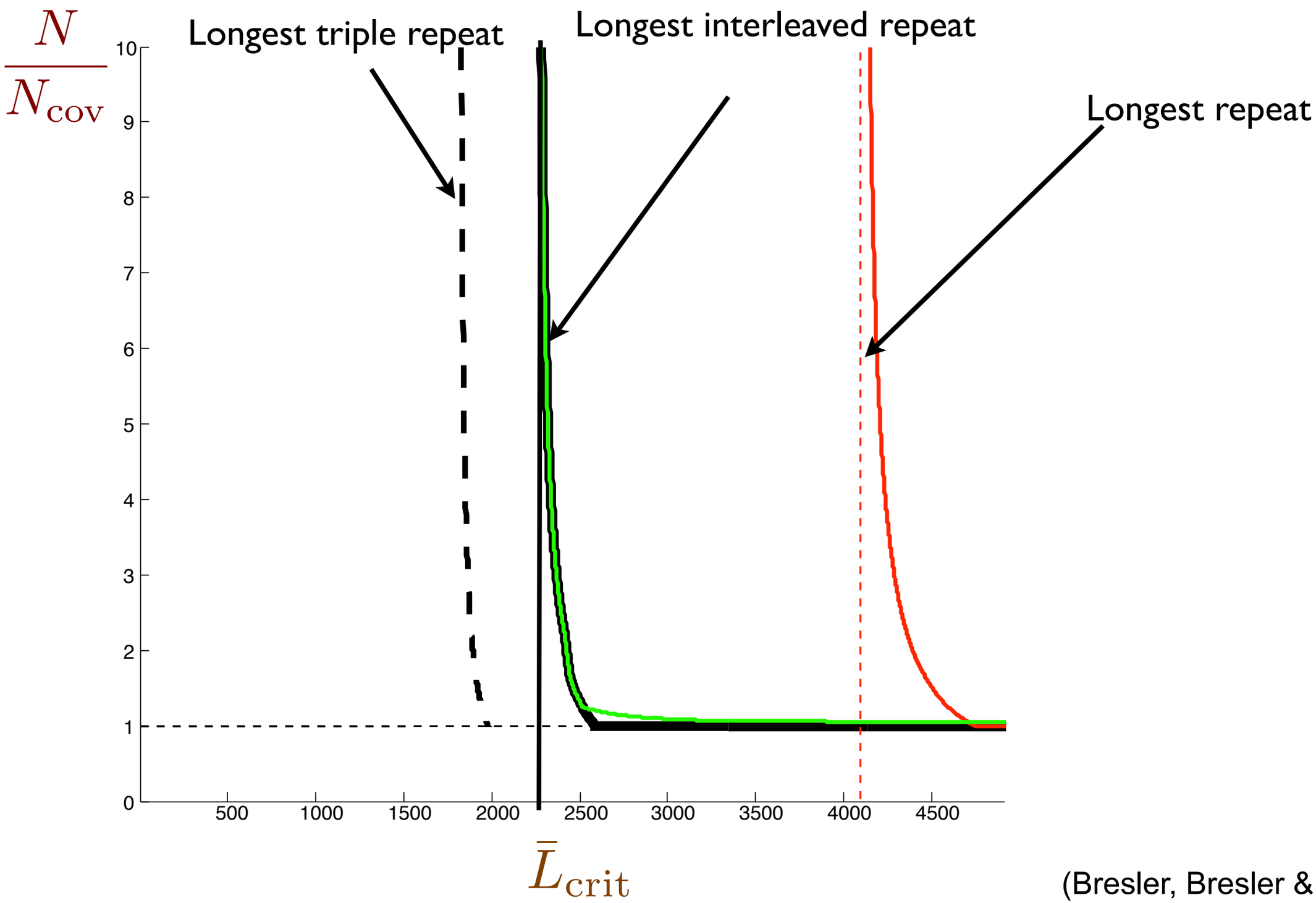


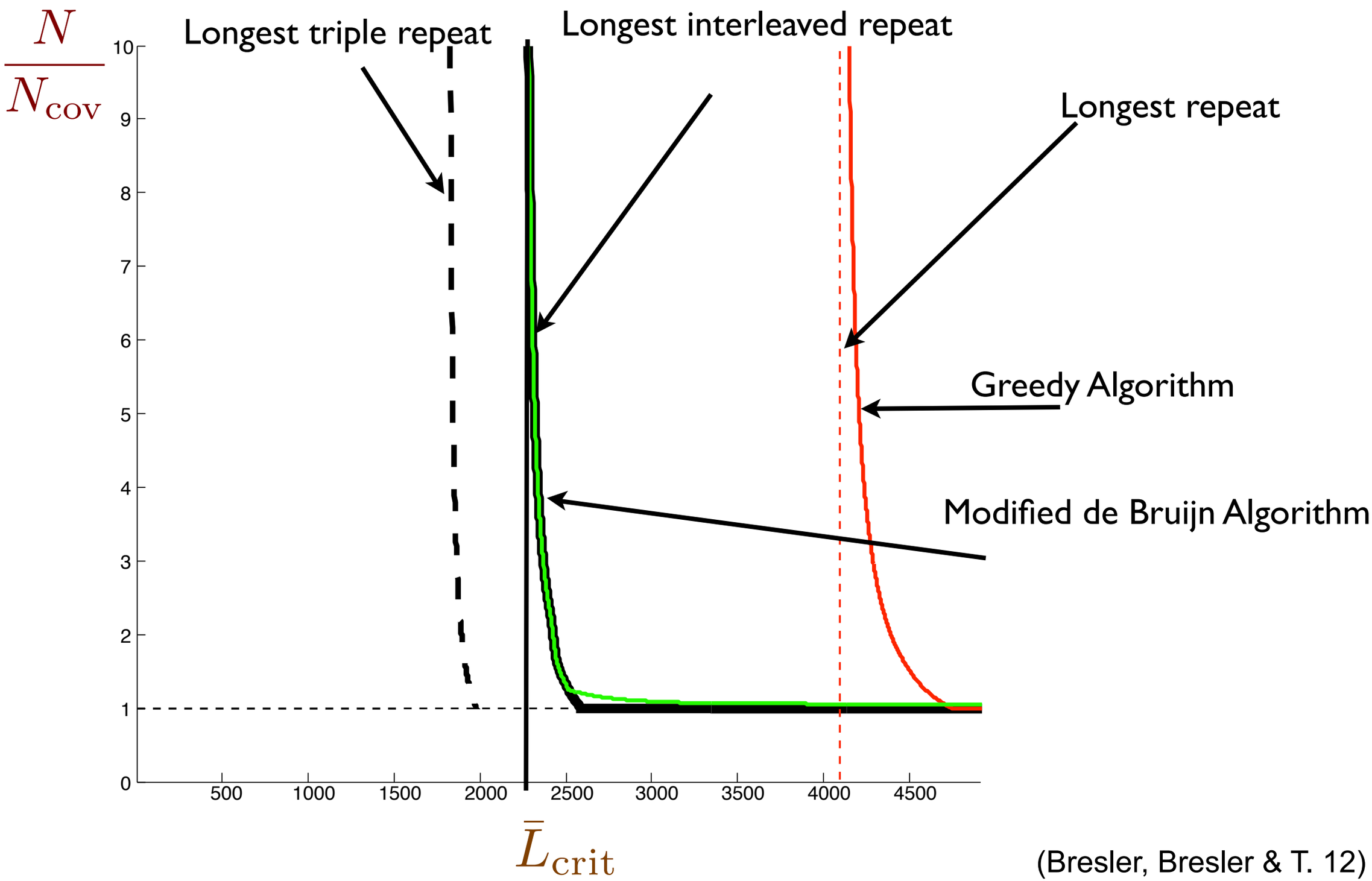
Motahari, Bresler, Tse (2012)

Human Chromosome 19



(Bresler, Bresler & T. 12)

Human Chromosome 19

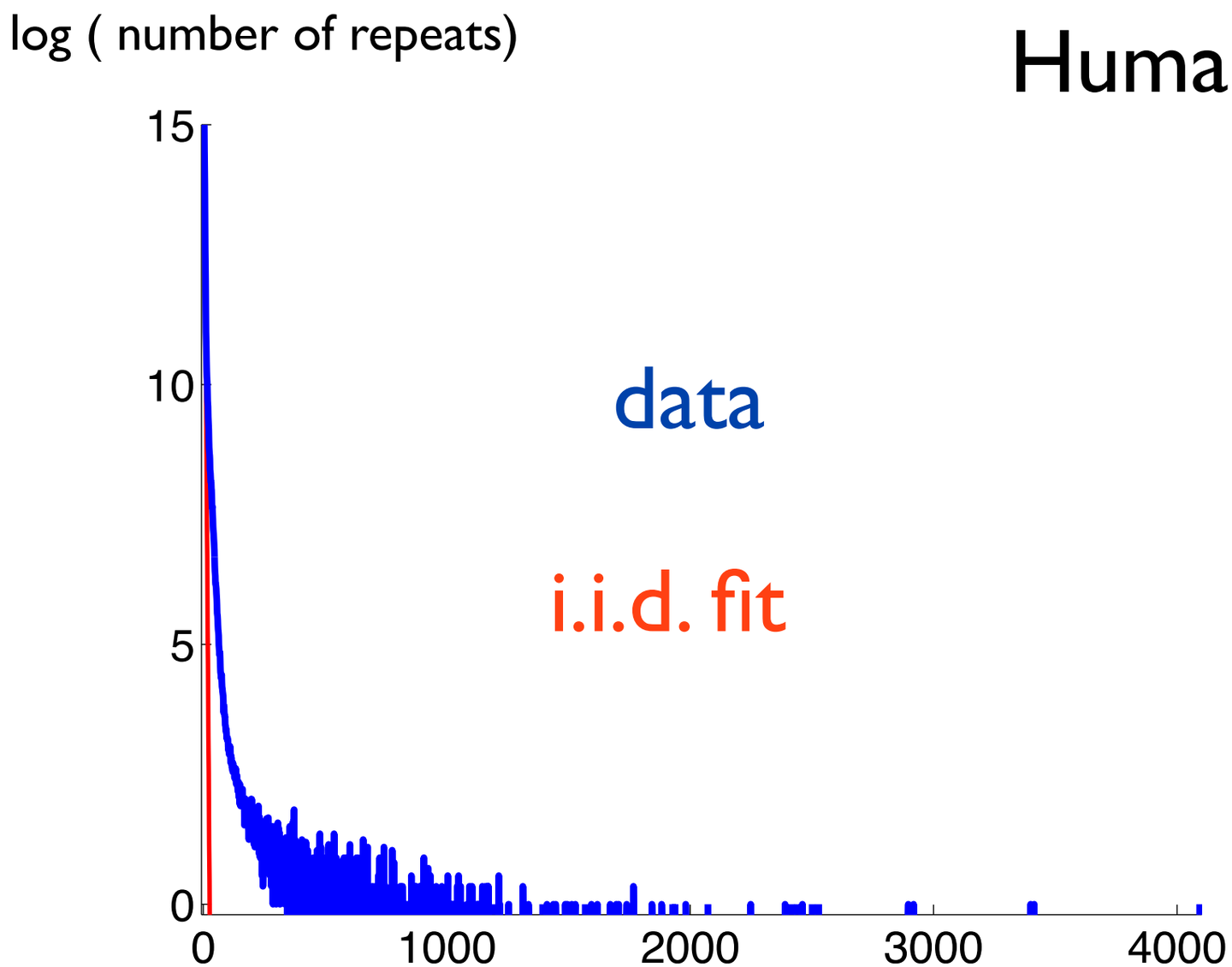


(Bresler, Bresler & T. 12)

WHY?

(Bresler, Bresler & T. 12)

WHY?



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