Genome Assembly

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Feb 4, 2019

Lecture 3: Applied Comparative Genomics

Assignment 1: Chromosome Structures
Due Feb 6 @ 11:59pm

Assignment 1: Chromosome Structures

Assignment Date: Wednesday, Jan 30, 2019
Due Date: Wednesday, Feb. 6, 2019 @ 11:59pm

Assignment Overview

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

Question 1: Chromosome structures

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

1. Arabidopsis thaliana (TAIR10) - An important plant model species [info]
2. Corn (Zea mays B73v4)) - The most widely grown crop in the world [info]
3. E. coli (Escherichia coli K12) - One of the most commonly studied bacteria [info]
4. Fruit Fly (Drosophila melanogaster, dm6) - One of the most important model species for genetics [info]
5. Human (hg38) - us :) [info]
6. Rice (Oryza sativa, IRGSP-1.0) - One of the most important crops in the world [info]
7. Worm (Caenorhabditis elegans, ce10) - One of the most important animal model species [info]
8. Yeast (Saccharomyces cerevisiae, sacCer3) - an important eukaryotic model species, also good for bread and beer [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

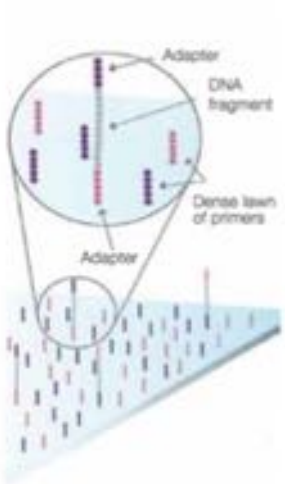
https://github.com/schatzlab/appliedgenomics2019

Part 1: Recap

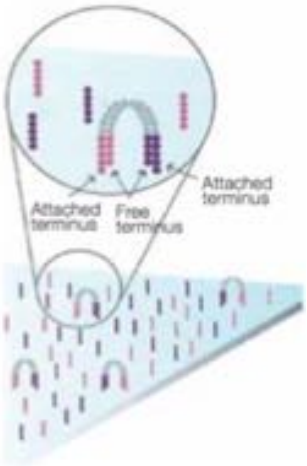
Second Generation Sequencing

Illumina HiSeq 2000
Sequencing by Synthesis

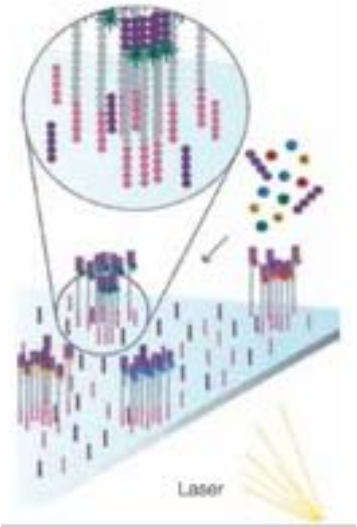
>60Gbp / day



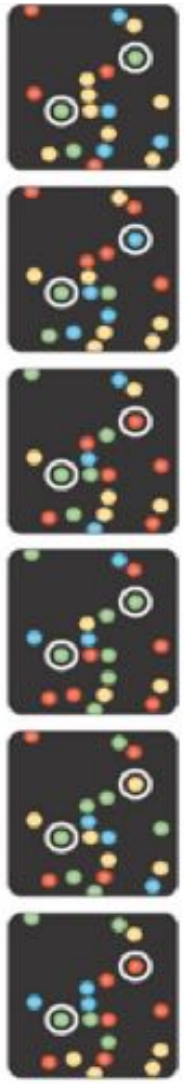
1. Attach



2. Amplify



3. Image

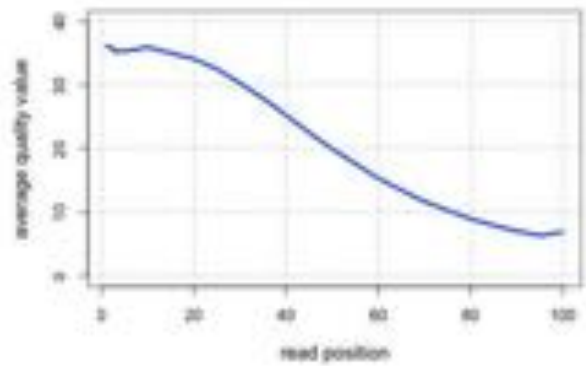


Metzker (2010) Nature Reviews Genetics 11:31-46
https://www.youtube.com/watch?v=fCd6B5HRaZ8

Illumina Quality

QV	p_{error}
40	1/10000
30	1/1000
20	1/100
10	1/10

$$Q_{\text{sanger}} = -10 \log_{10} p$$

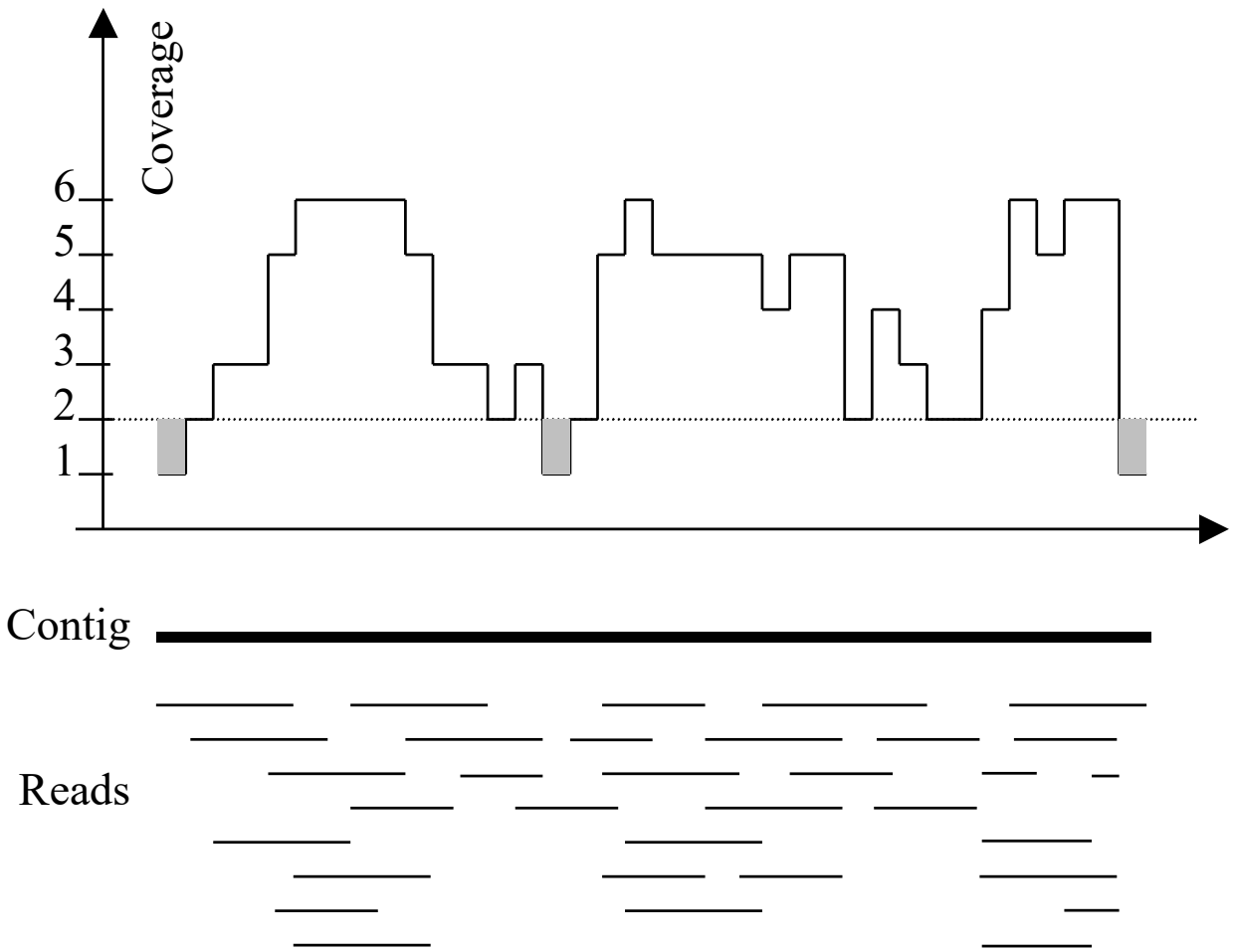



```
SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS.......................................................
.........................XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX.......................
...............................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII.......................
................................JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ.......................
LLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLL.......................................................
!"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
|                         |    |        |                              |                     |
33                        59   64       73                            104                   126

S - Sanger        Phred+33,  raw reads typically (0, 40)
X - Solexa        Solexa+64, raw reads typically (-5, 40)
I - Illumina 1.3+ Phred+64,  raw reads typically (0, 40)
J - Illumina 1.5+ Phred+64,  raw reads typically (3, 40)
   with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
   (Note: See discussion above).
L - Illumina 1.8+ Phred+33,  raw reads typically (0, 41)
```

http://en.wikipedia.org/wiki/FASTQ_format

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?

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.

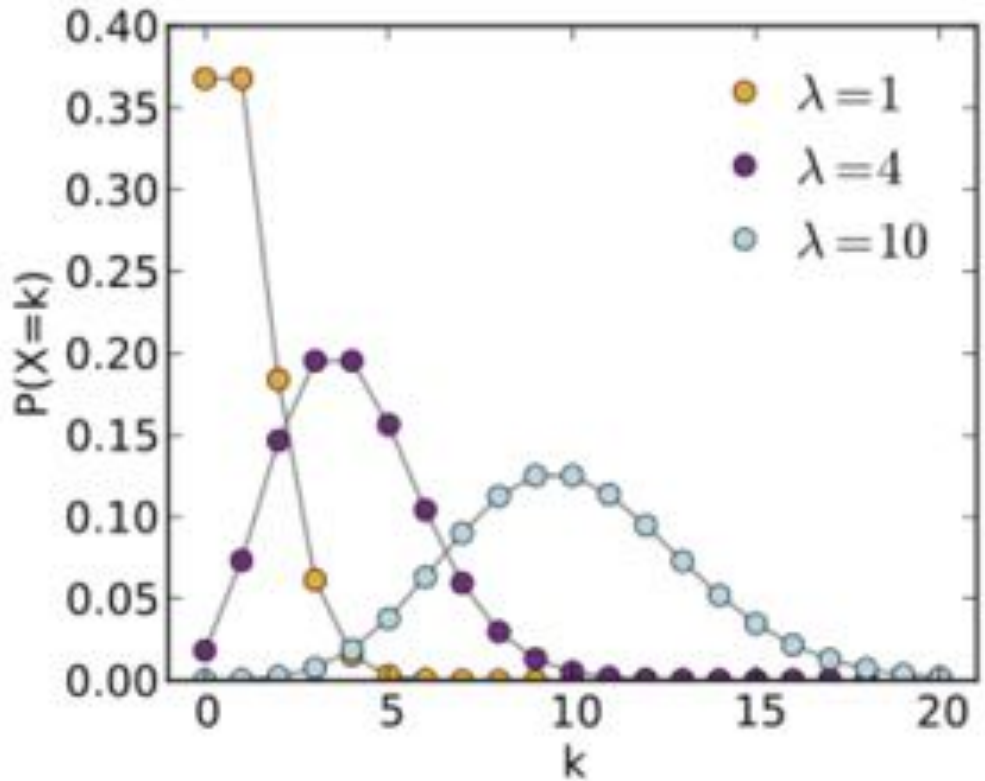
$$P(k) = \frac{\lambda^k}{k!} e^{-\lambda}$$

Formulation comes from the limit of the binomial equation

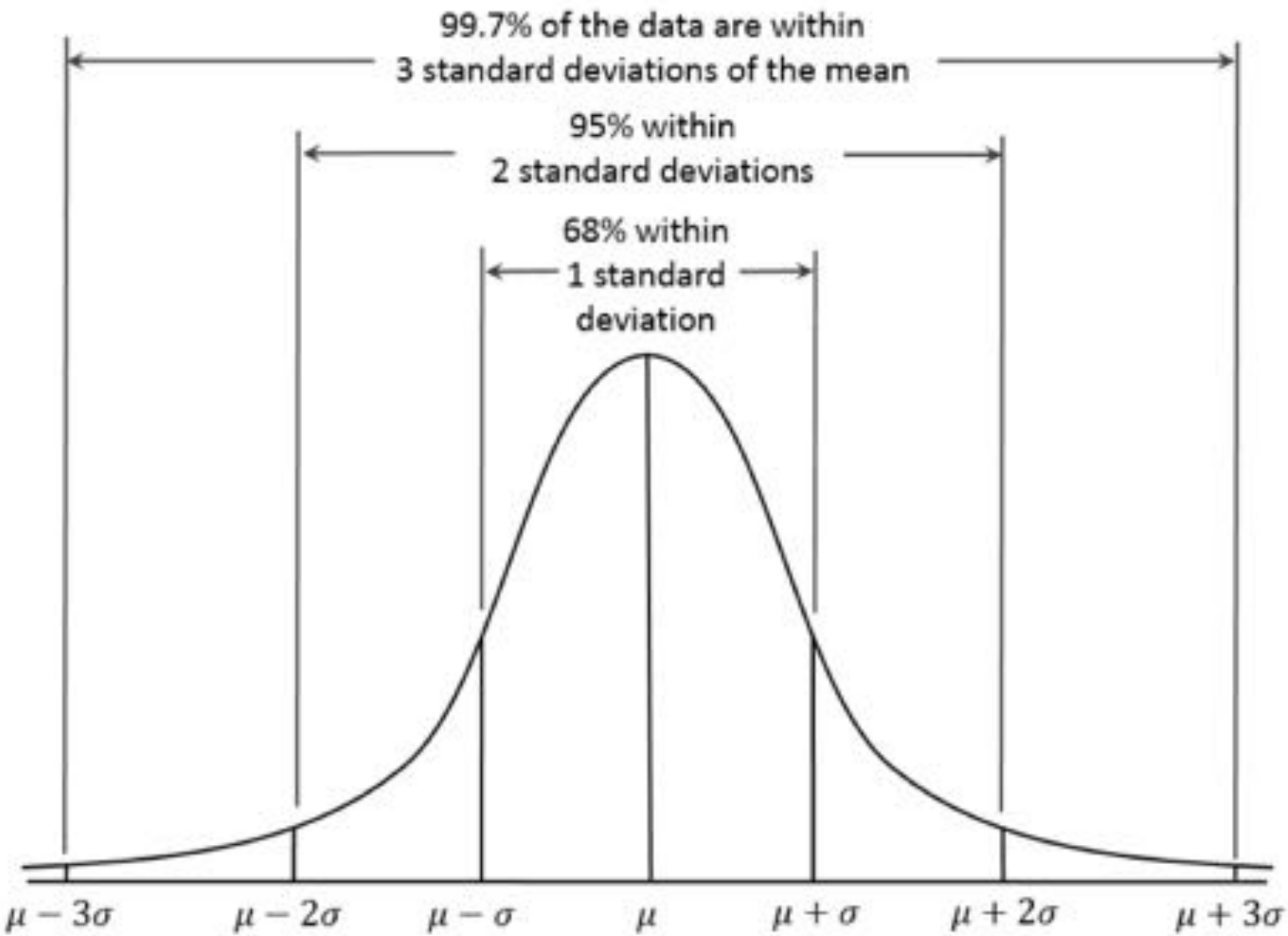
Resembles a normal distribution, but over the positive values, and with only a single parameter.

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

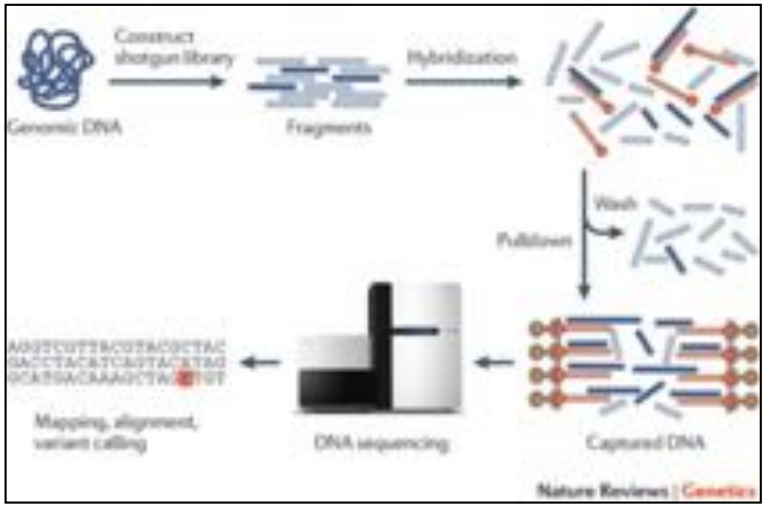
36-2*sqrt(36) = 24

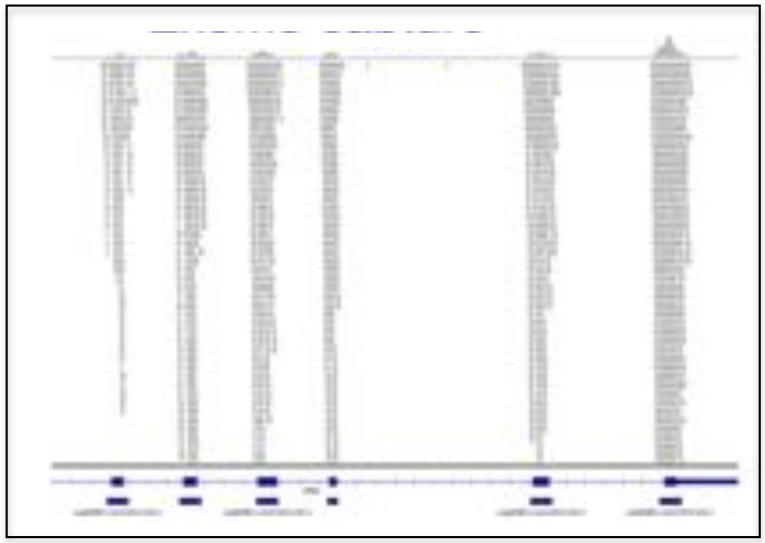
I need 10Mbp x 36x = 360Mbp of data
360Mbp / 120bp / read = 3M reads

Exome-Capture Sequencing

Exome-capture reduces the costs of sequencing

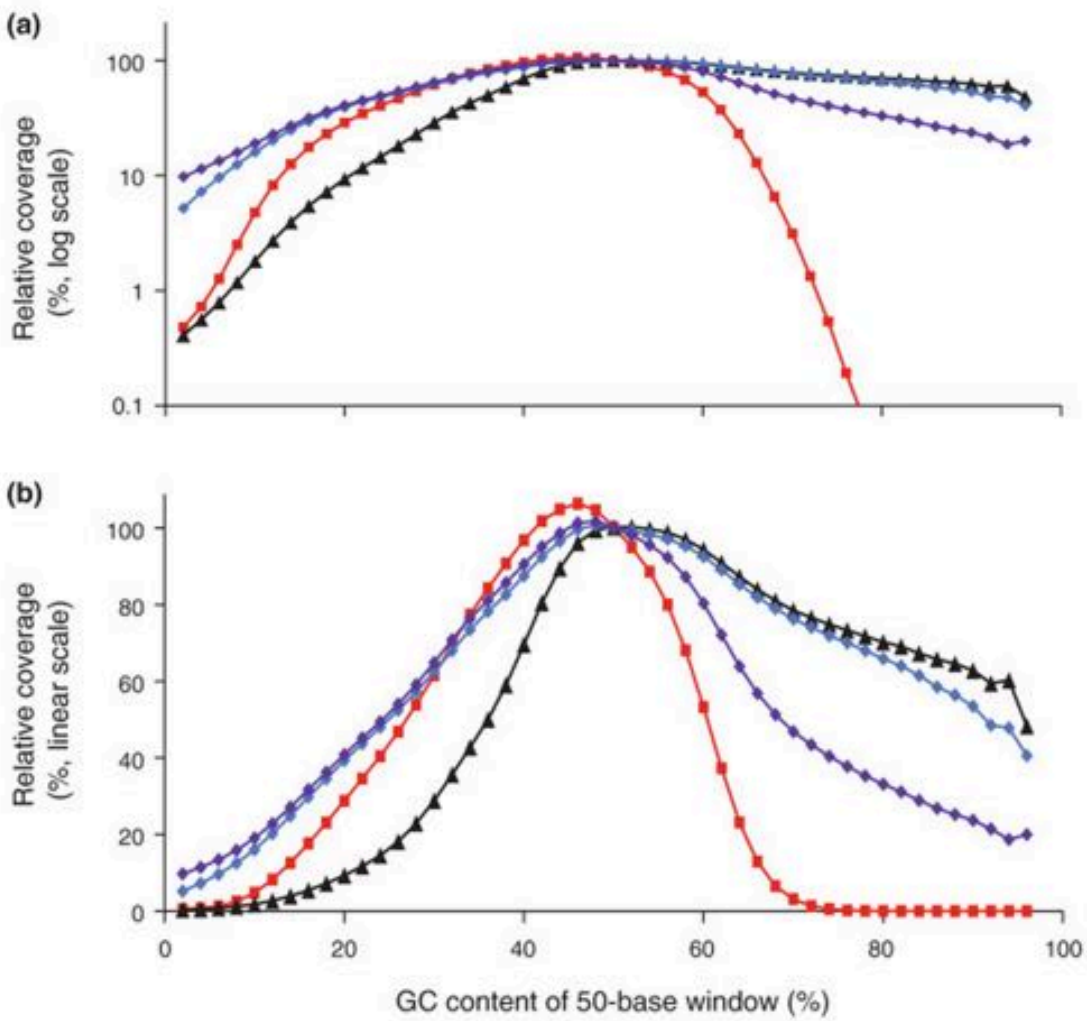
- Currently targets around 50Mbp of sequence: all exons plus flanking regions
- WGS currently costs ~$1000 per sample, while WES currently costs ~$250 per sample
- Coverage is highly localized around genes, although will get sparse coverage throughout rest of genome





Exome sequencing as a tool for Mendelian disease gene discovery
Bamshad et al. (2011) *Nature Reviews Genetics*. 12, 745-755

Beware of GC Biases

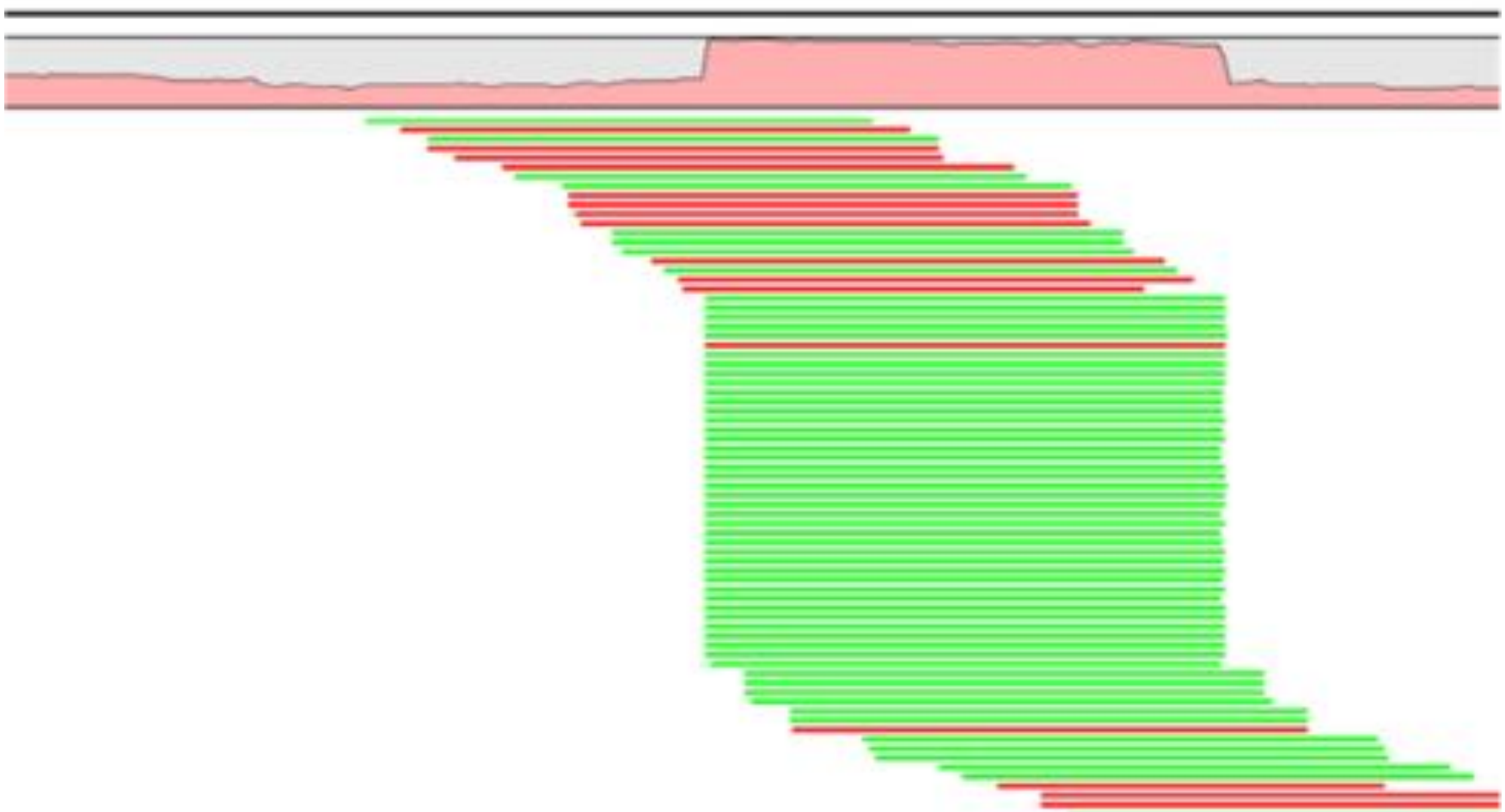


Illumina sequencing does not produce uniform coverage over the genome

- Coverage of extremely high or extremely low GC content will have reduced coverage in Illumina sequencing
- Biases primarily introduced during PCR; lower temperatures, slower heating, and fewer rounds minimize biases
- This makes it very difficult to identify variants (SNPs, CNVs, etc) in certain regions of the genome

Analyzing and minimizing PCR amplification bias in Illumina sequencing libraries.
Aird et al. (2011) *Genome Biology.* 12:R18.

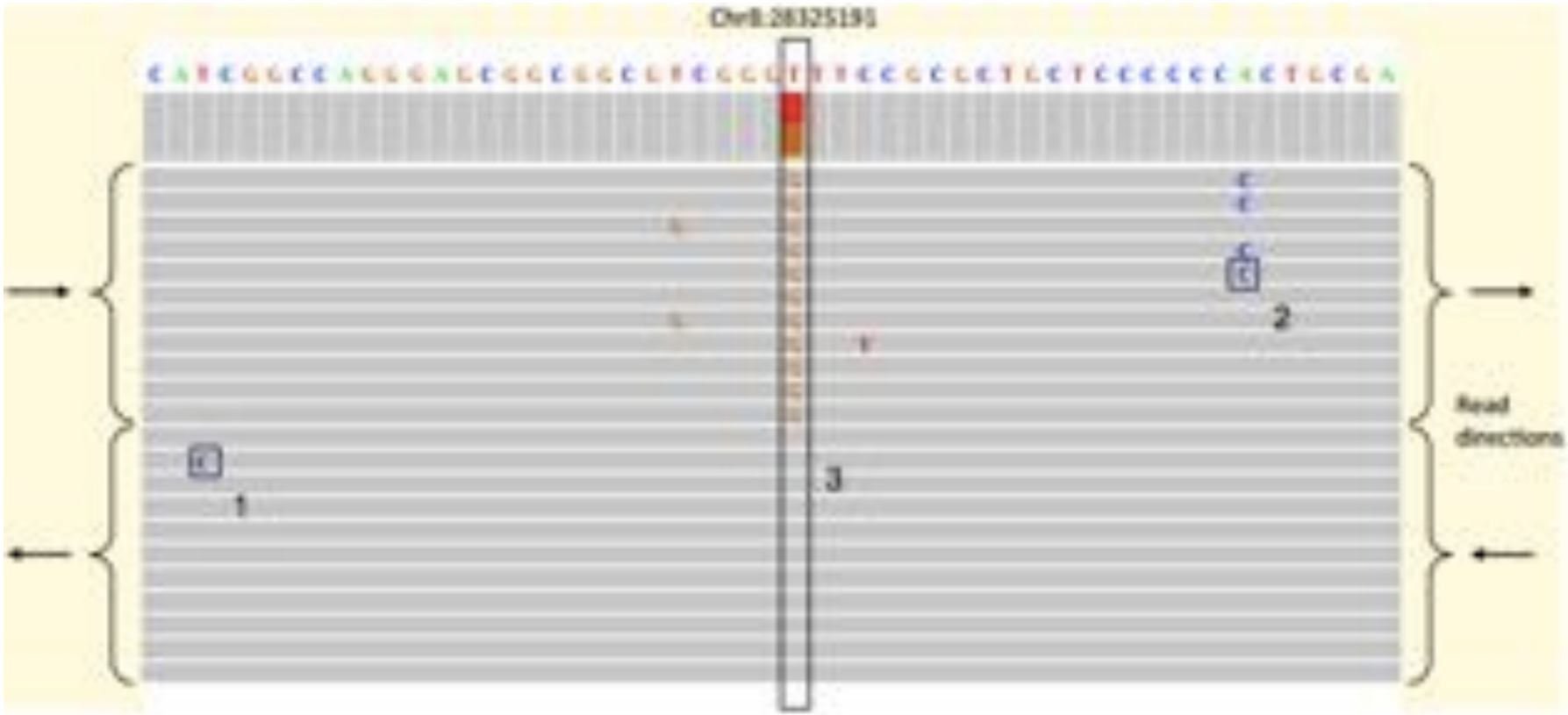
Beware of Duplicate Reads



The Sequence alignment/map (SAM) format and SAMtools.
Li et al. (2009) *Bioinformatics.* 25:2078-9

Picard: http://picard.sourceforge.net

Beware of (Systematic) Errors



Identification and correction of systematic error in high-throughput sequence data
Meacham et al. (2011) *BMC Bioinformatics.* 12:451

A closer look at RNA editing.
Lior Pachter (2012) *Nature Biotechnology.* 30:246-247

Illumina Sequencing Summary

Advantages:

- Best throughput, accuracy and read length for any 2nd gen. sequencer
- Fast & robust library preparation

Disadvantages:

- Inherent limits to read length (practically, 150bp)
- Some runs are error prone
- Requires amplification, sequences a population of molecules

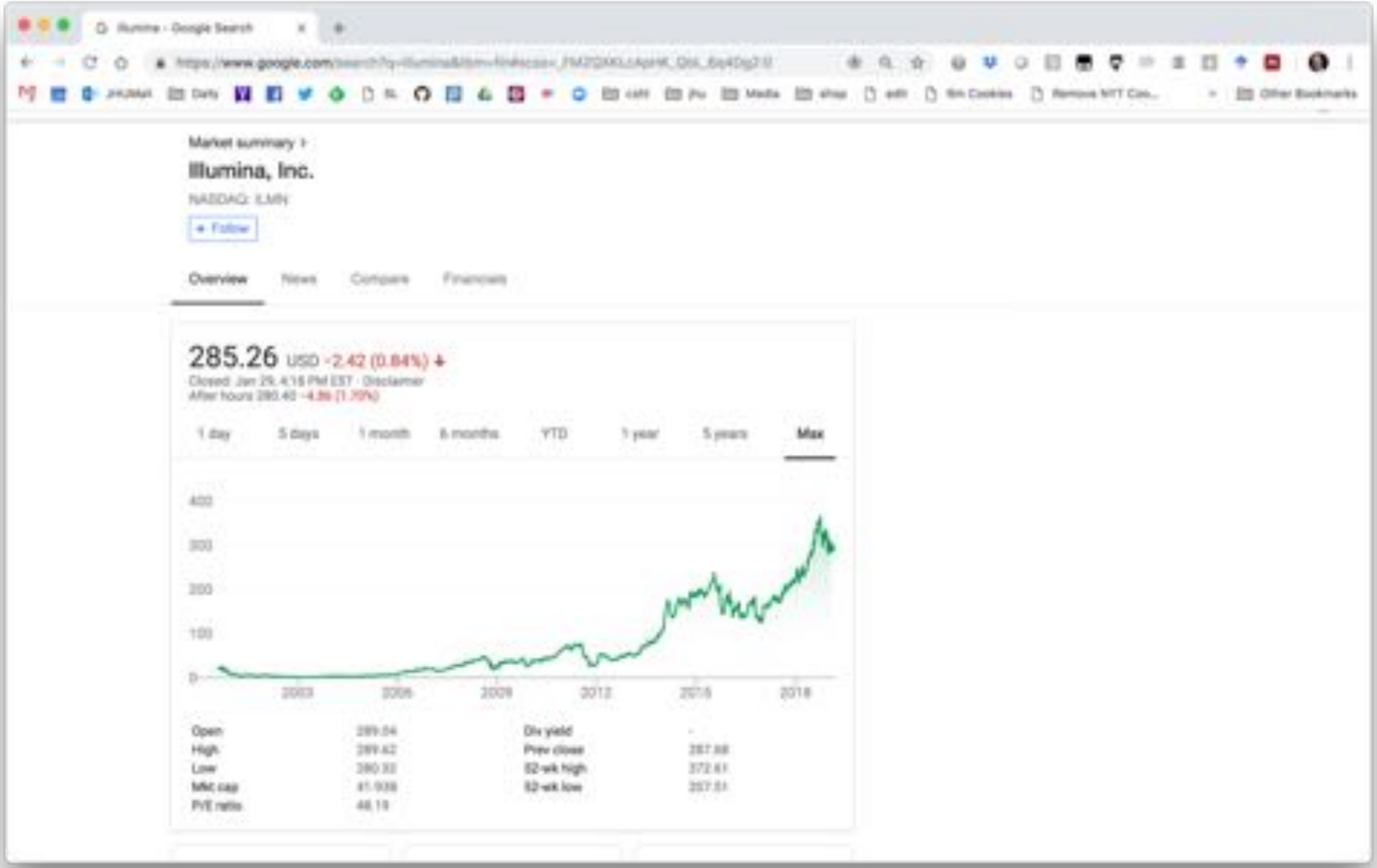
Illumina HiSeq
~3 billion paired 100bp reads
~600Gb, $10K, 8 days
(or "rapid run" ~90Gb in 1-2 days)

Illumina X Ten
~6 billion paired 150bp reads
1.8Tb, <3 days, ~1000 / genome($$)
(or "rapid run" ~90Gb in 1-2 days)

Illumina NovaSeq
Population-scale sequencing

Ira Hall

ILMN


Market summary >
Illumina, Inc.
NASDAQ: ILMN
+ Follow
Overview
News
Compare
Financials
285.26 USD -2.42 (0.84%)
1 day
5 days
1 month
6 months
YTD
1 year
5 years
Max
400
300
200
100
0
2003
2006
2009
2012
2015
2018
Open
High
Low
Mkt cap
P/E ratio
Div yield
Prev close
52-wk high
52-wk low

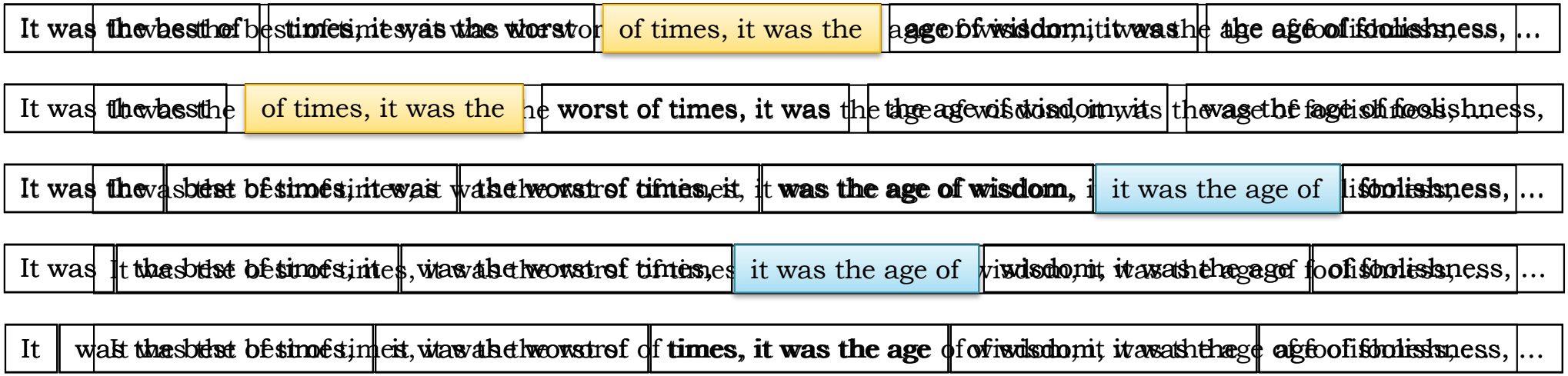
Part 2: De novo genome assembly

Outline

1. ***Assembly theory***
   - Assembly by analogy
2. ***Practical Issues***
   - Coverage, read length, errors, and repeats
3. ***Next-next-gen Assembly***
   - Canu: recommended for PacBio/ONT project
4. ***Whole Genome Alignment***
   - MUMmer recommended

Shredded Book Reconstruction

- Dickens accidentally shreds the first printing of A Tale of Two Cities
  - 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]

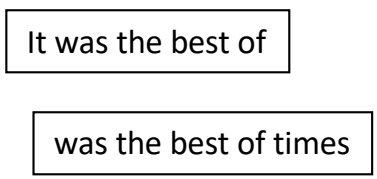
Model the assembly problem as a graph problem

How long will it take to compute the overlaps?

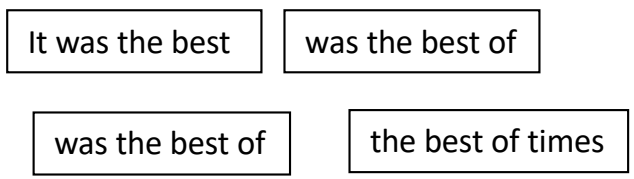
de Bruijn Graph Construction

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

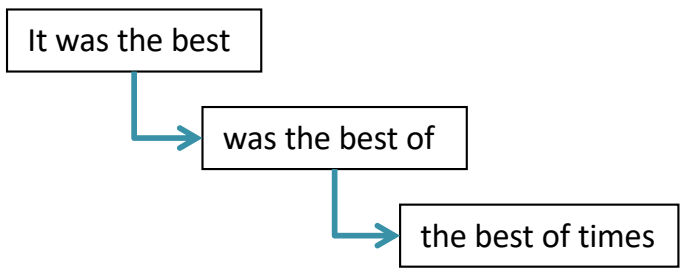
Fragments |f|=5



Sub-fragment *k*=4



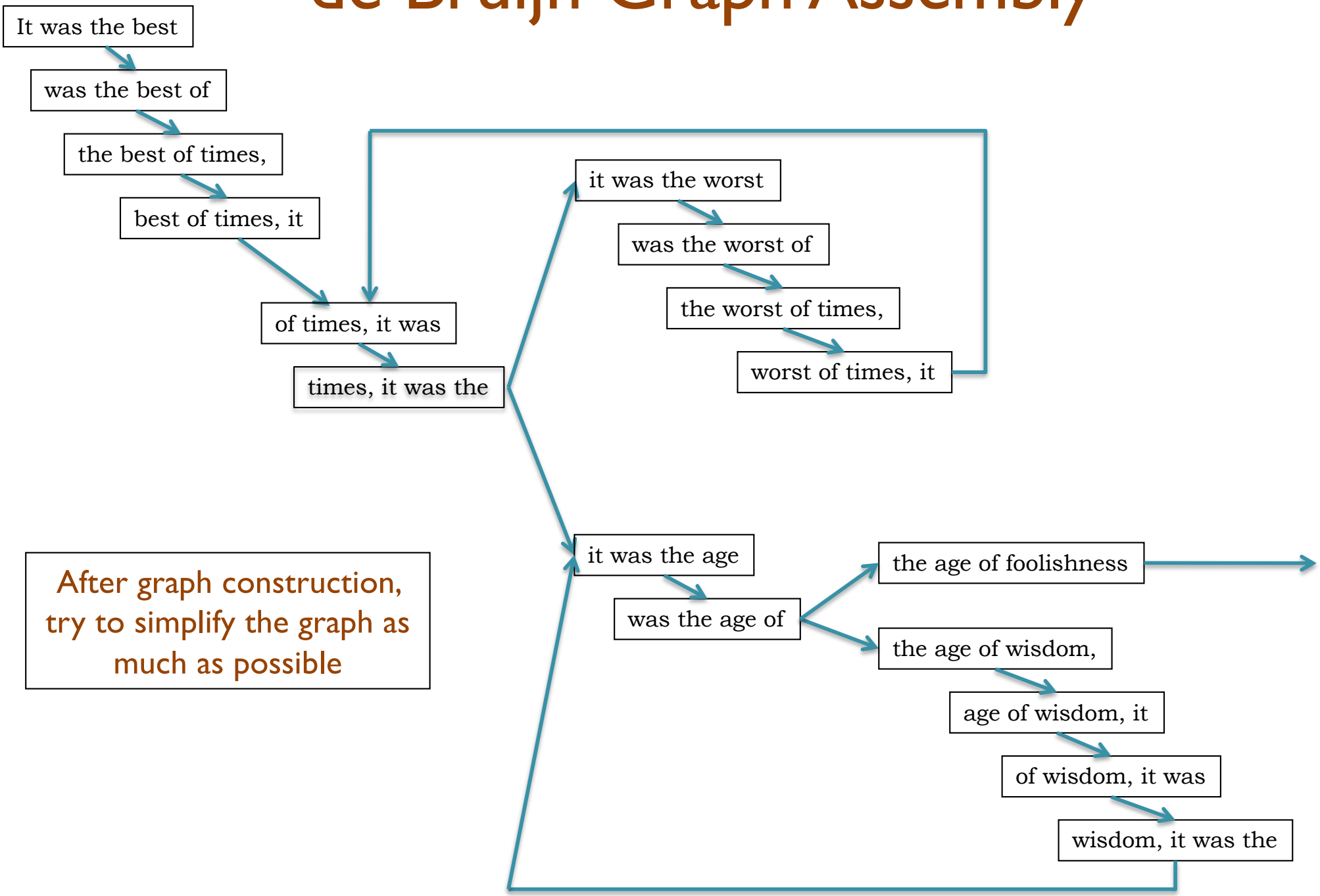
Directed edges (overlap by *k*-1)



– Overlaps between fragments are implicitly computed

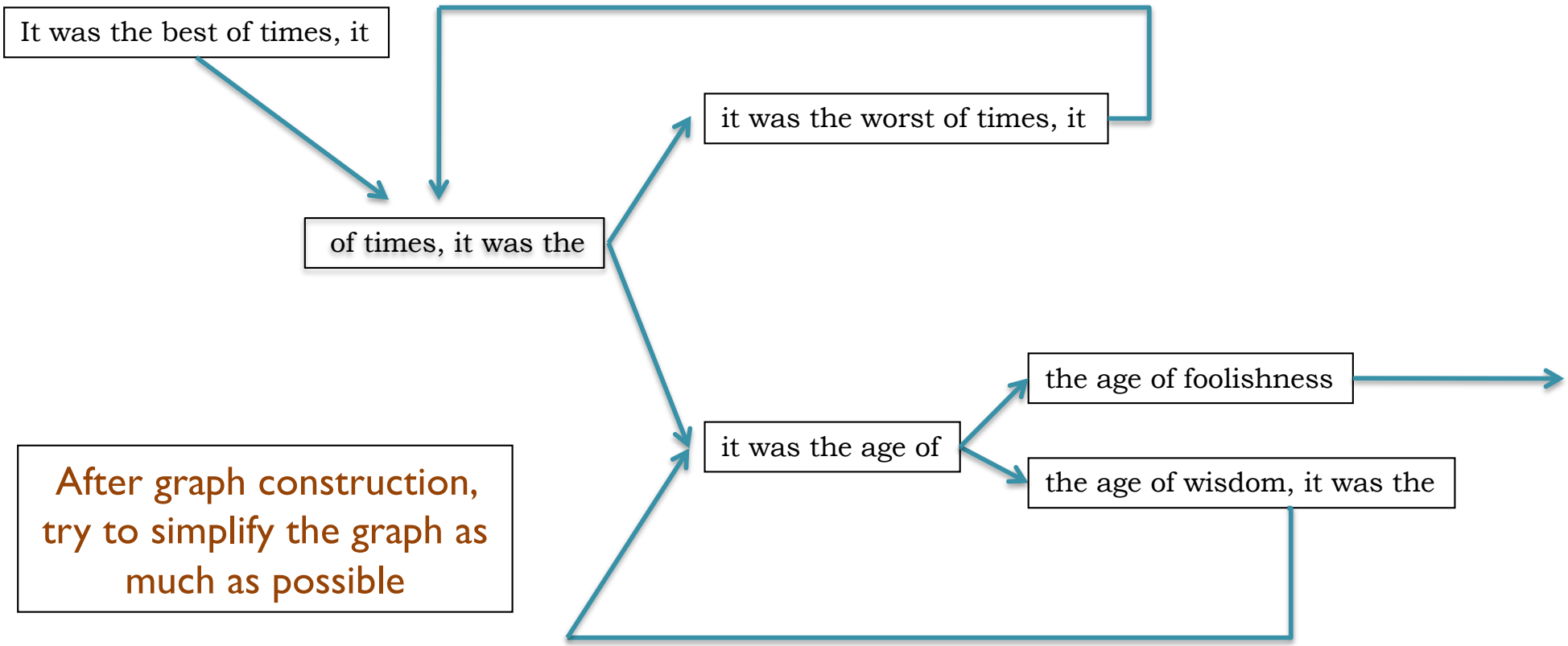
de Bruijn, 1946
Idury et al., 1995
Pevzner et al., 2001

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

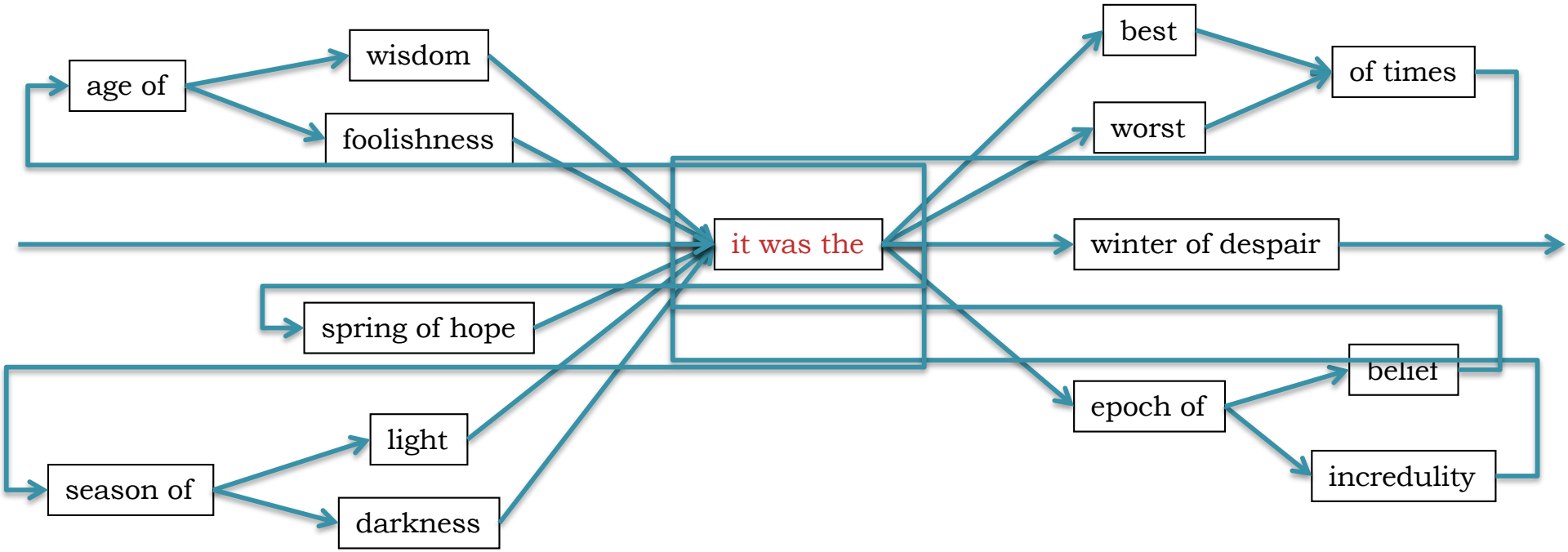
… it was the best of times it was the worst of times …

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

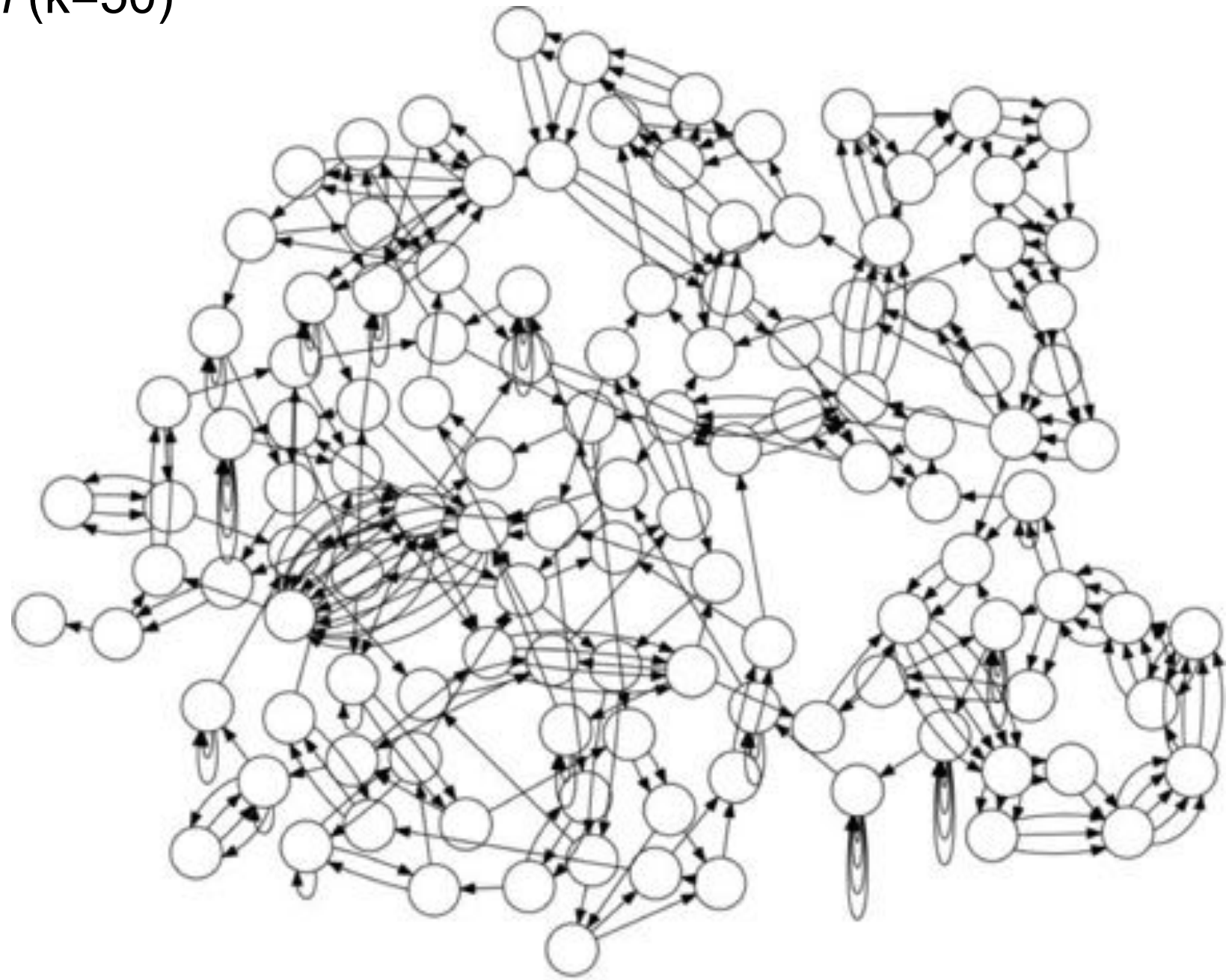
… it was the epoch of belief it was the epoch of incredulity …

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

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

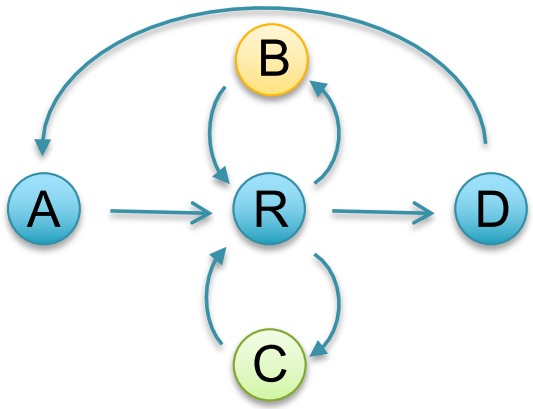


E. coli (k=50)



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

Counting Eulerian Cycles



ARBRCRD
or
ARCRBRD

Generally an exponential number of compatible sequences

– Value computed by application of the BEST theorem (Hutchinson, 1975)

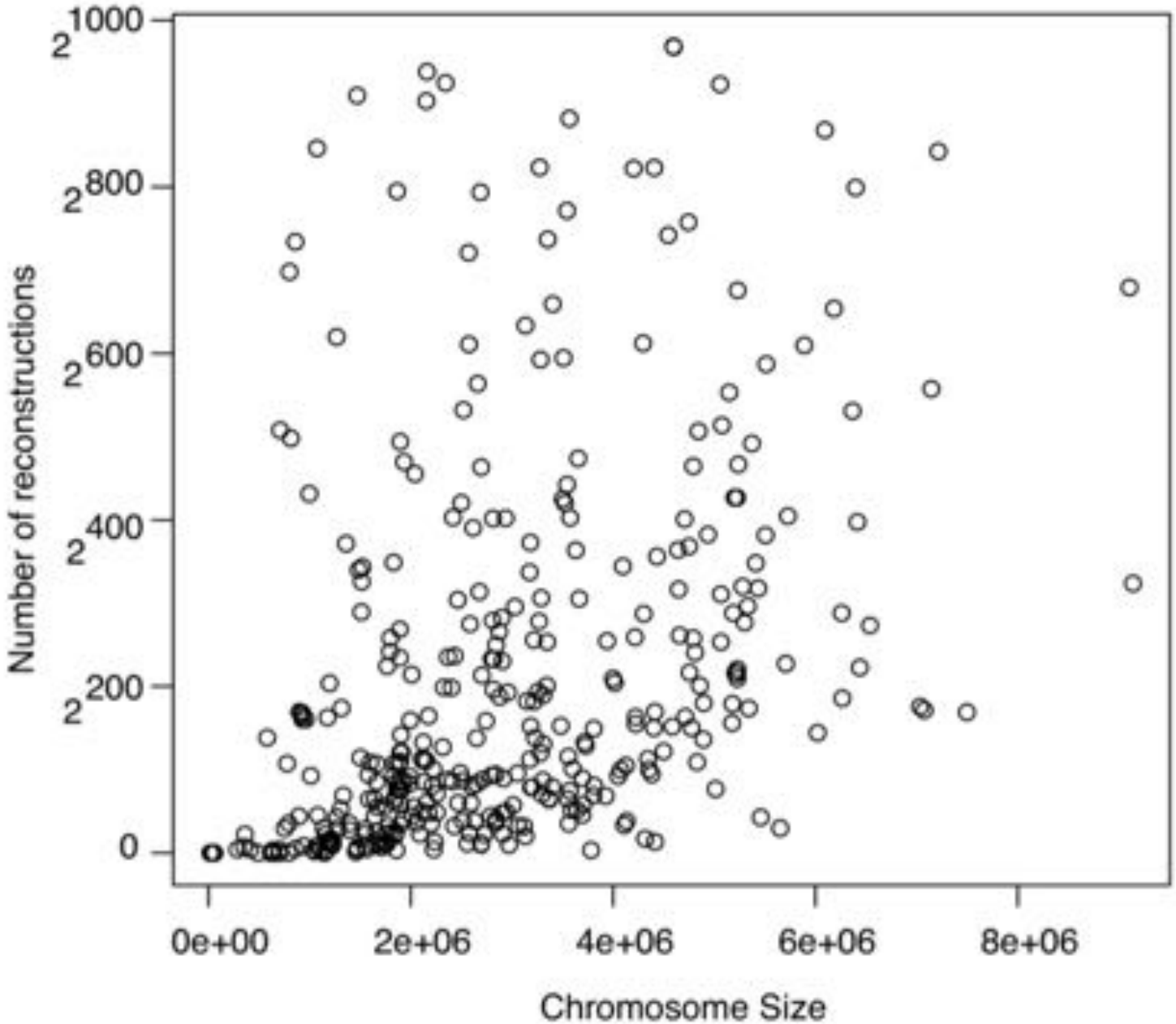
$$\mathcal{W}(G,t) = (\det L)\Big\{\prod_{u\in V}(r_u - 1)!\Big\}\Big\{\prod_{(u,v)\in E} a_{uv}!\Big\}^{-1}$$

L = n x n matrix with r_u-a_{uu} along the diagonal and -a_{uv} in entry uv

$r_u = d^+(u)+1$ if u=t, or $d^+(u)$ otherwise

a_{uv} = multiplicity of edge from u to v

Assembly Complexity of Prokaryotic Genomes using Short Reads.
Kingsford C, Schatz MC, Pop M (2010) *BMC Bioinformatics*.



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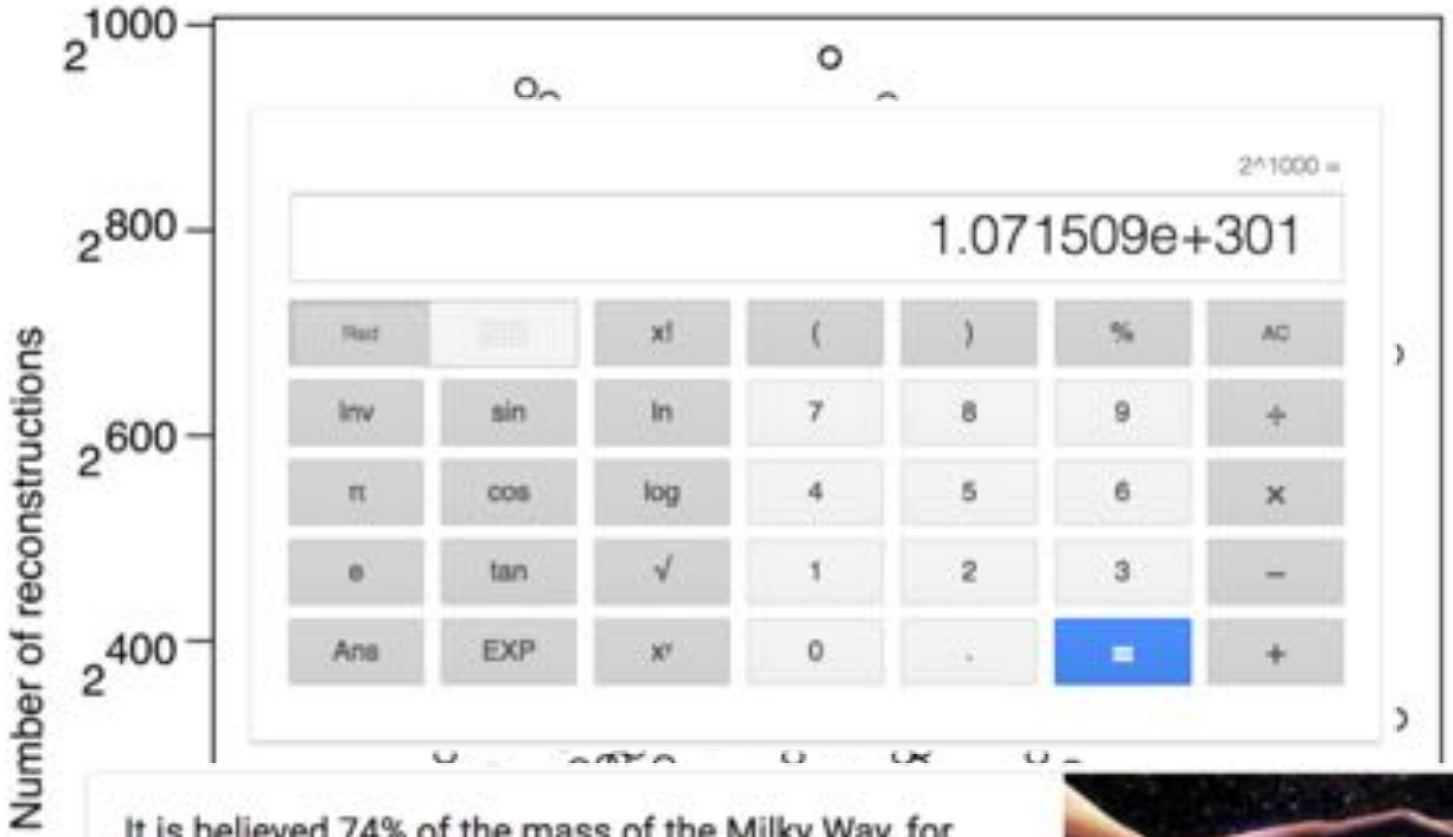

It is believed 74% of the mass of the Milky Way, for example, is in the form of hydrogen atoms. The Sun contains approximately **10^{57} atoms** of hydrogen. If you multiple the number of atoms per star (10^{57}) times the estimated number of stars in the universe (10^{23}), you get a value of **10^{80} atoms** in the known universe. Nov 5, 2017

How Many Atoms Are There in the Universe? - ThoughtCo
https://www.thoughtco.com/number-of-atoms-in-the-universe-603795

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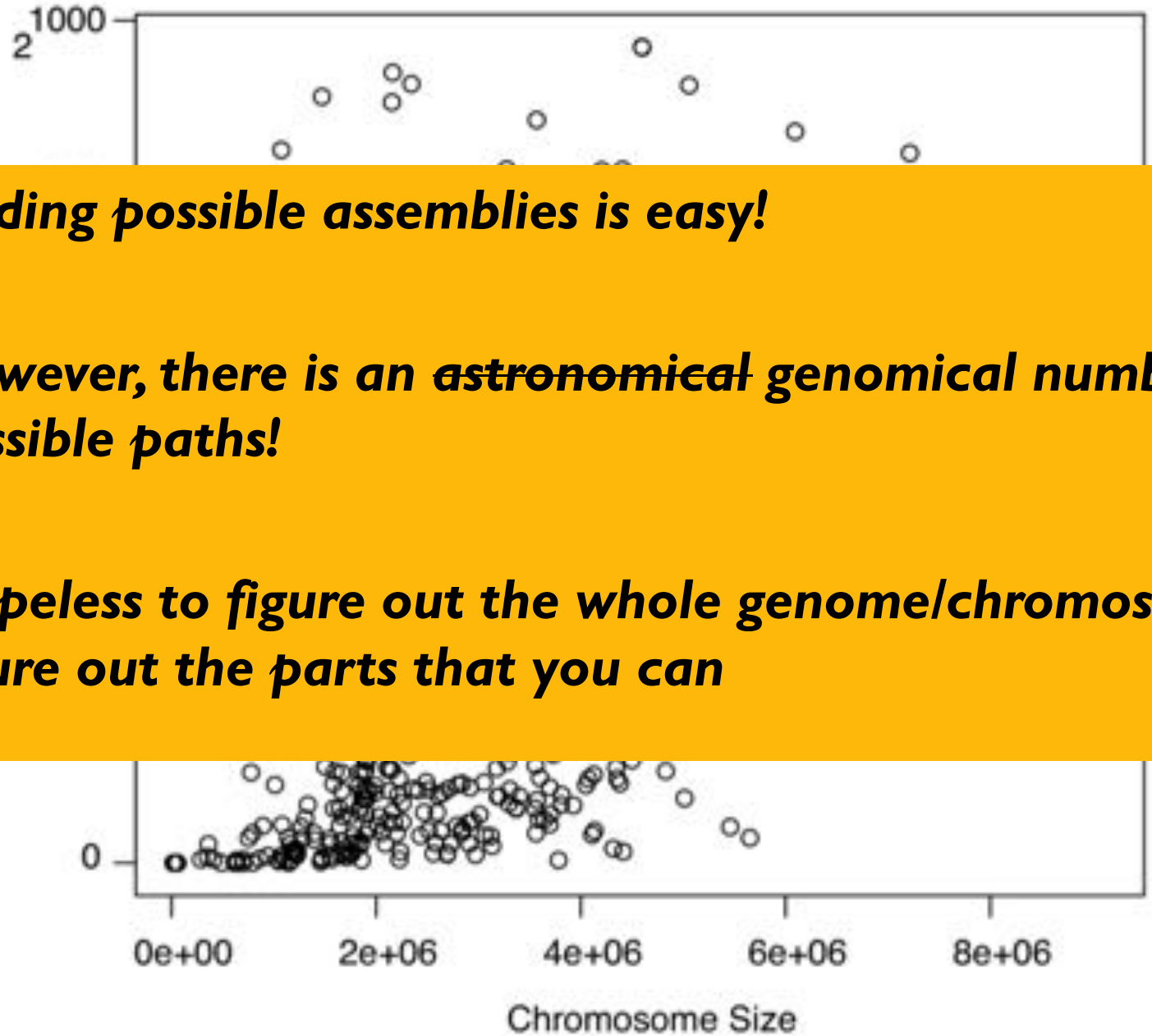
Kingsford C, Schatz MC, Pop M (2010) *BMC Bioinformatics*.



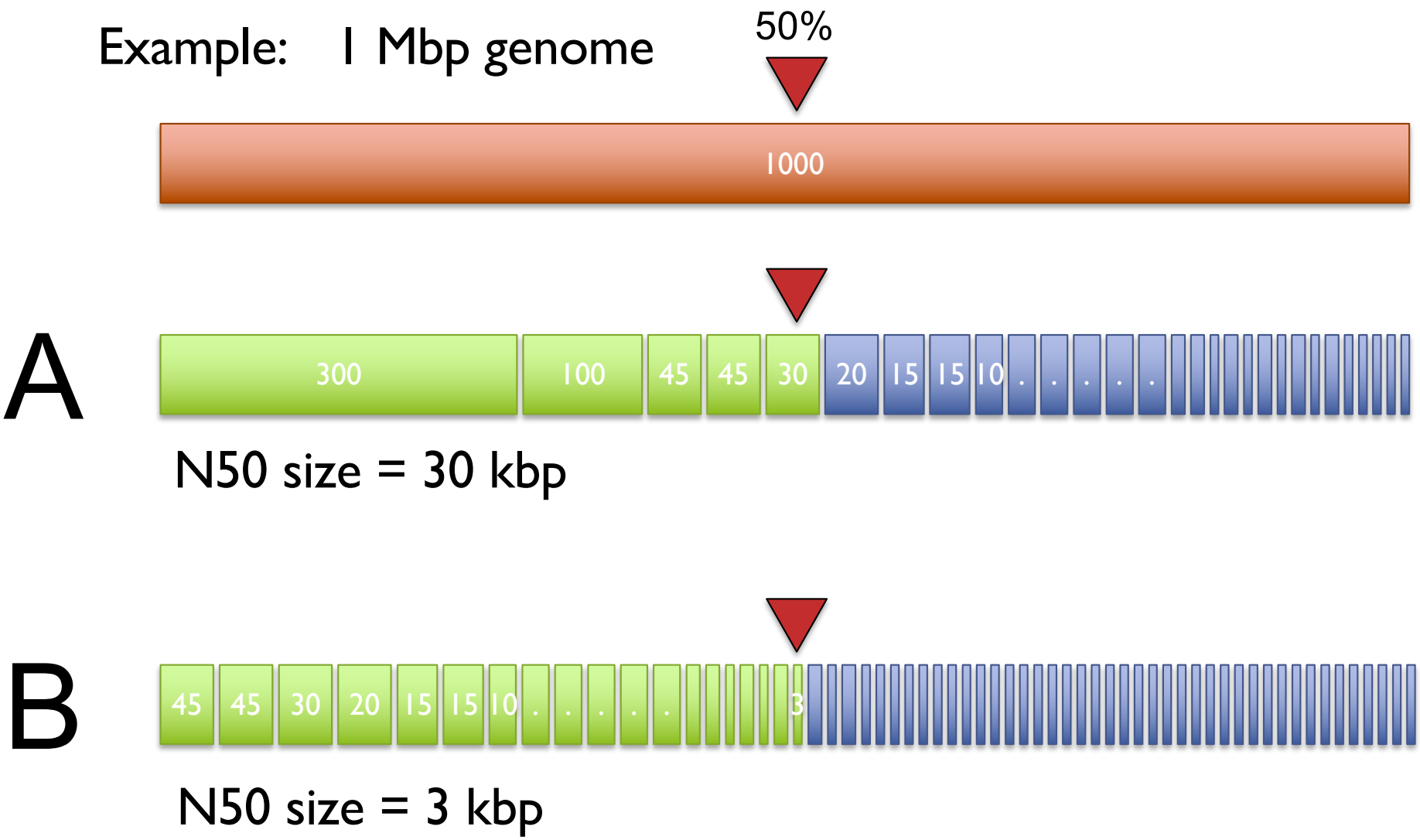
- ***Finding possible assemblies is easy!***
- ***However, there is an ~~astronomical~~ genomical number of possible paths!***
- ***Hopeless to figure out the whole genome/chromosome, figure out the parts that you can***

Assembly Complexity of Prokaryotic Genomes using Short Reads.
Kingsford C, Schatz MC, Pop M (2010) *BMC Bioinformatics*.

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):

```
ATTA
GATT
TACA
TTAC
```

Pop Quiz 1

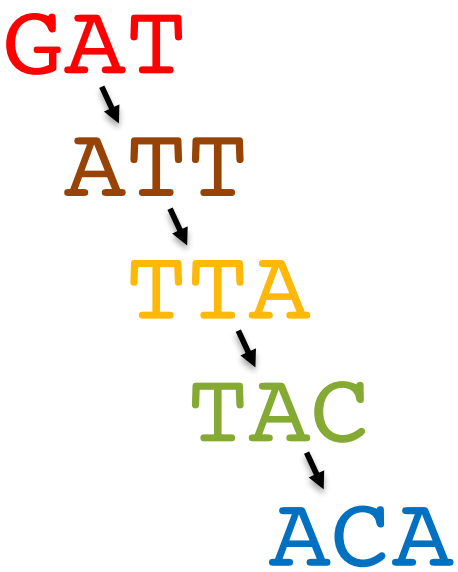
Assemble these reads using a de Bruijn graph approach (k=3):

```
ATTA: ATT -> TTA
GATT: GAT -> ATT
TACA: TAC -> ACA
TTAC: TTA -> TAC
```

Pop Quiz 1

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

```
ATTA:  ATT  ->  TTA
GATT:  GAT  ->  ATT
TACA:  TAC  ->  ACA
TTAC:  TTA  ->  TAC
```



GATTACA

Pop Quiz 2

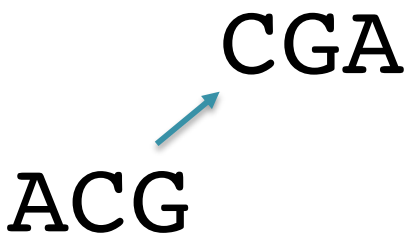
Assemble these reads using a de Bruijn graph approach (k=3):

```
ACGA
ACGT
ATAC
CGAC
CGTA
GACG
GTAT
TACG
```

Pop Quiz 2

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

~~ACGA~~
ACGT
ATAC
CGAC
CGTA
GACG
GTAT
TACG



Pop Quiz 2

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

~~ACGA~~

~~ACGT~~

ATAC

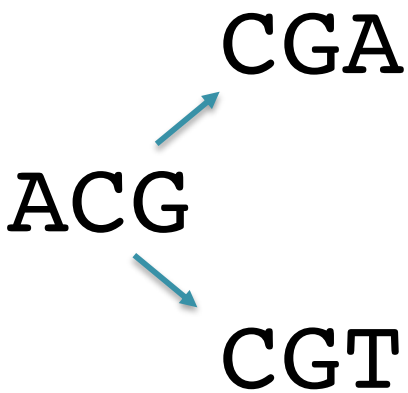
CGAC

CGTA

GACG

GTAT

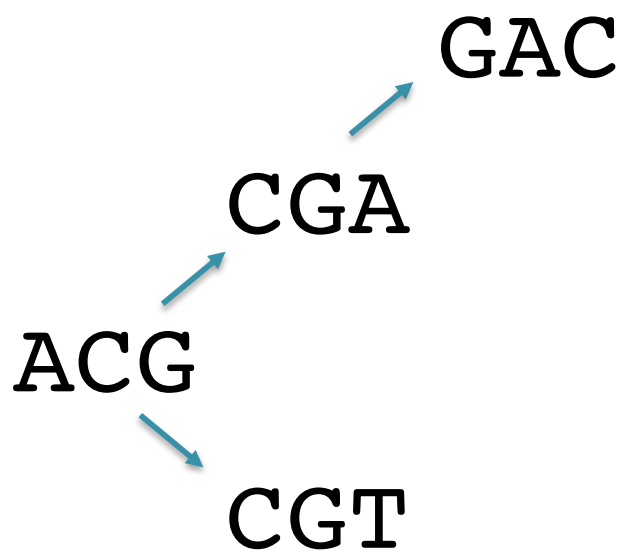
TACG



Pop Quiz 2

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

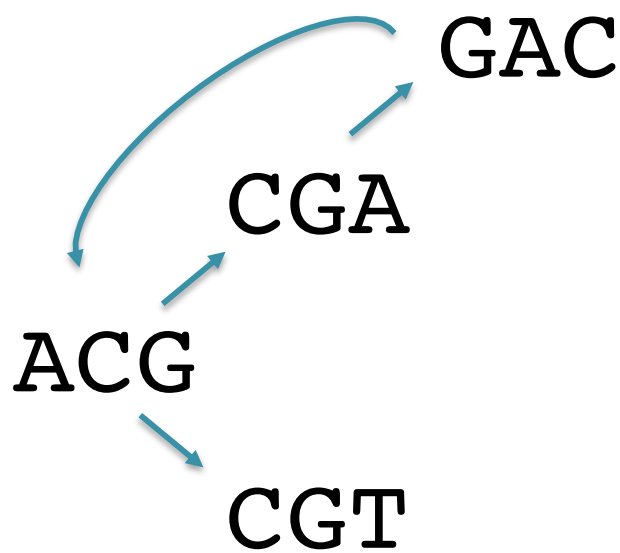
~~ACGA~~
~~ACGT~~
ATAC
~~CGAC~~
CGTA
GACG
GTAT
TACG



Pop Quiz 2

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

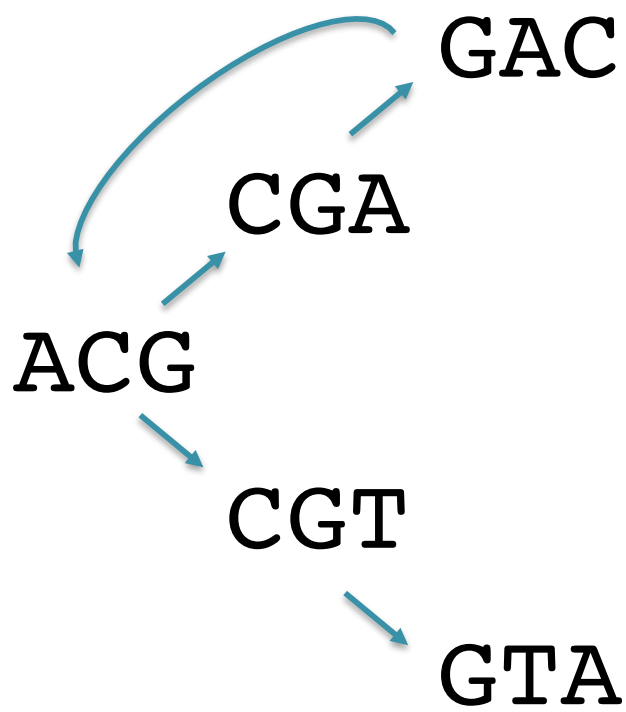
~~ACGA~~
~~ACGT~~
ATAC
~~CGAC~~
CGTA
~~GACG~~
GTAT
TACG



Pop Quiz 2

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

~~ACGA~~
~~ACGT~~
ATAC
~~CGAC~~
~~CGTA~~
~~GACG~~
GTAT
TACG



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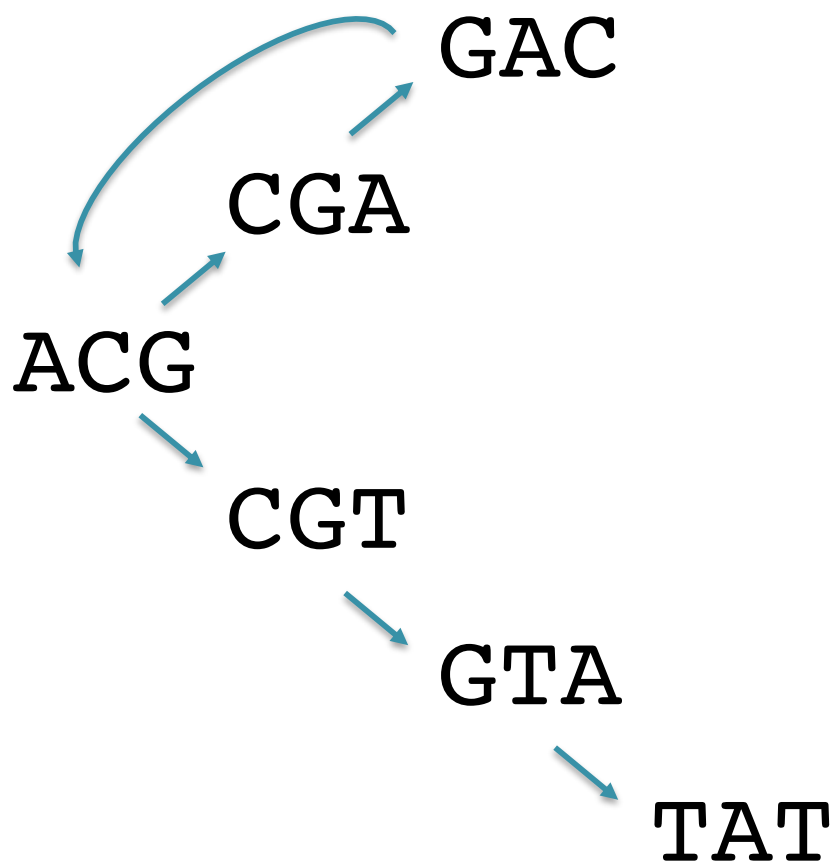

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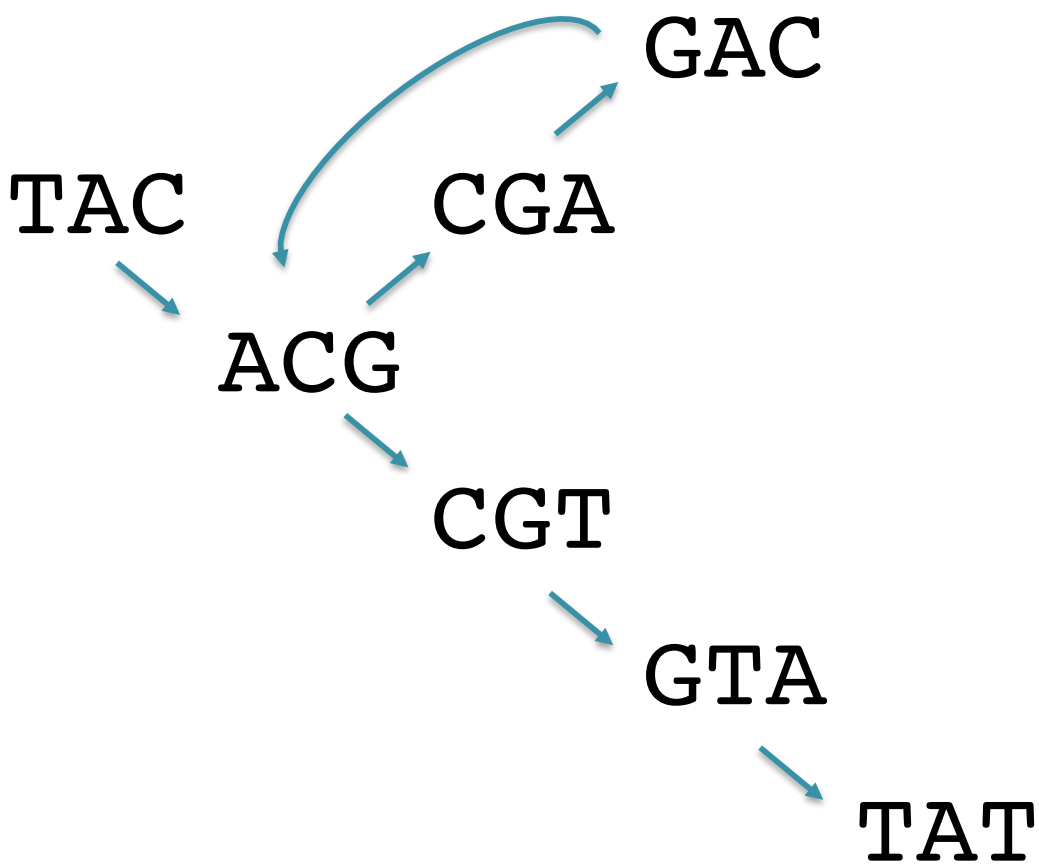

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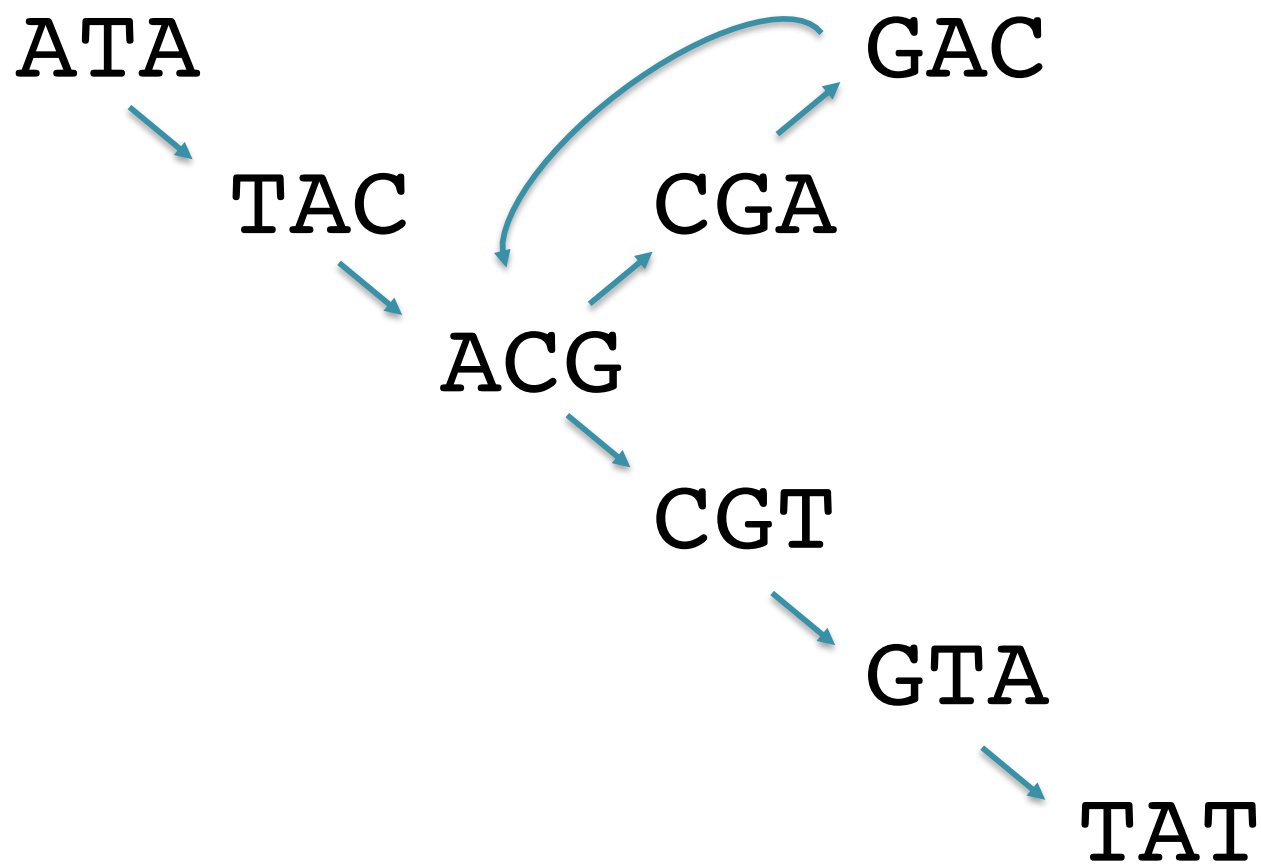

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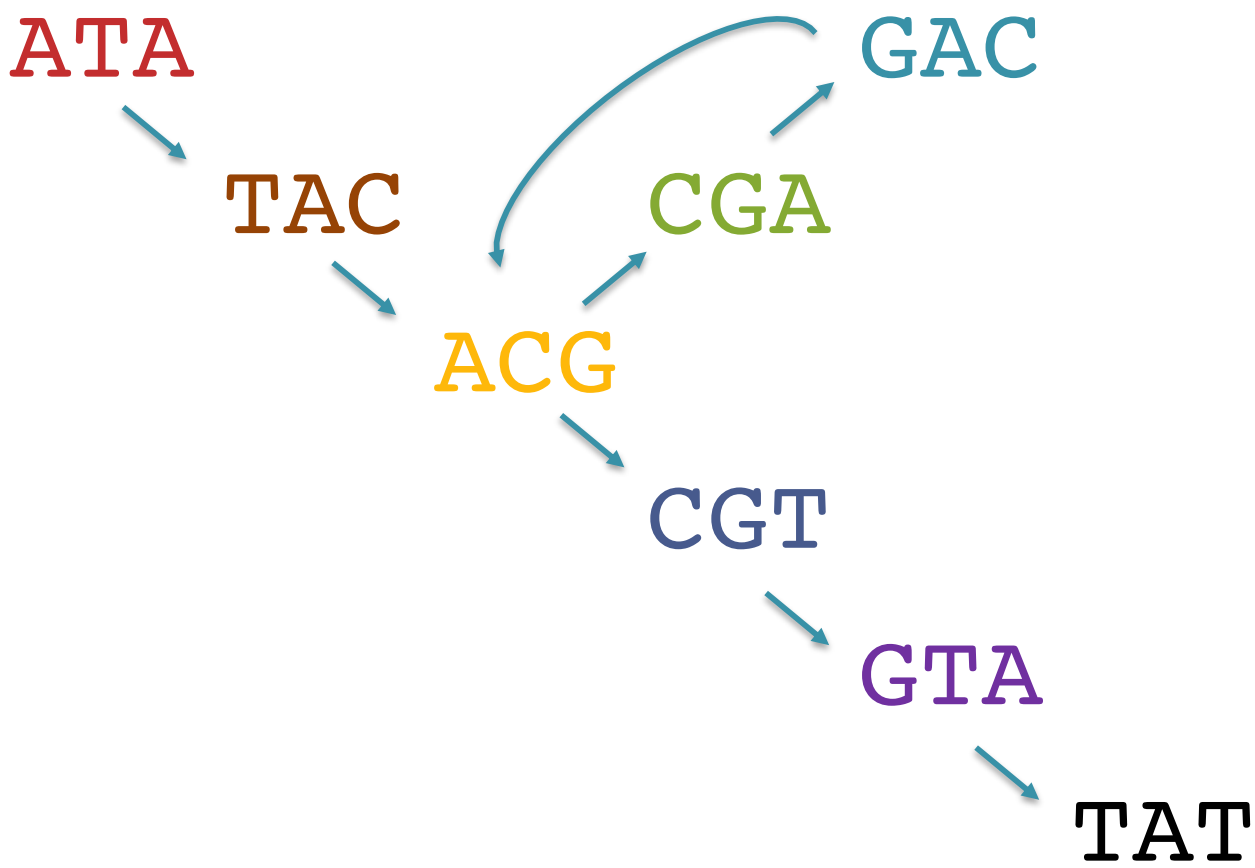

ATACGACGTAT

Pop Quiz 2

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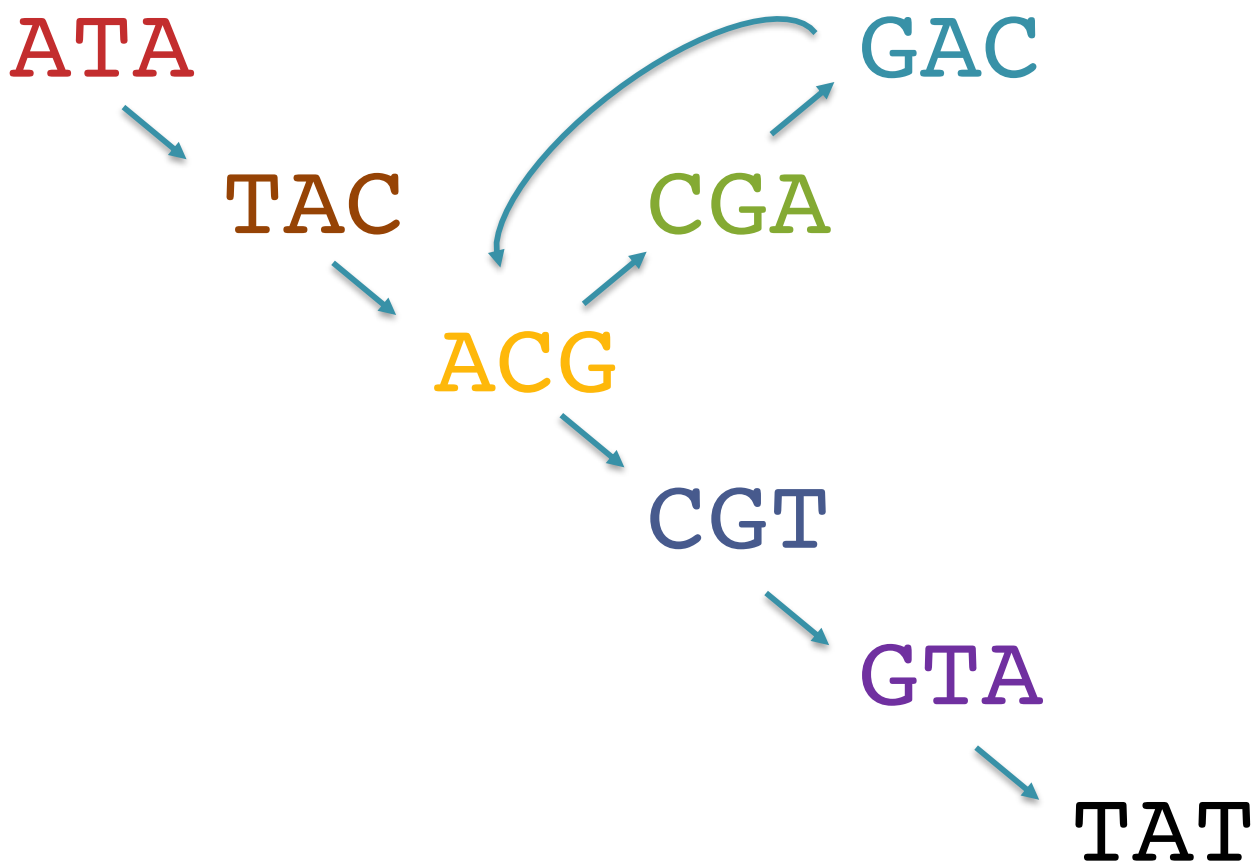
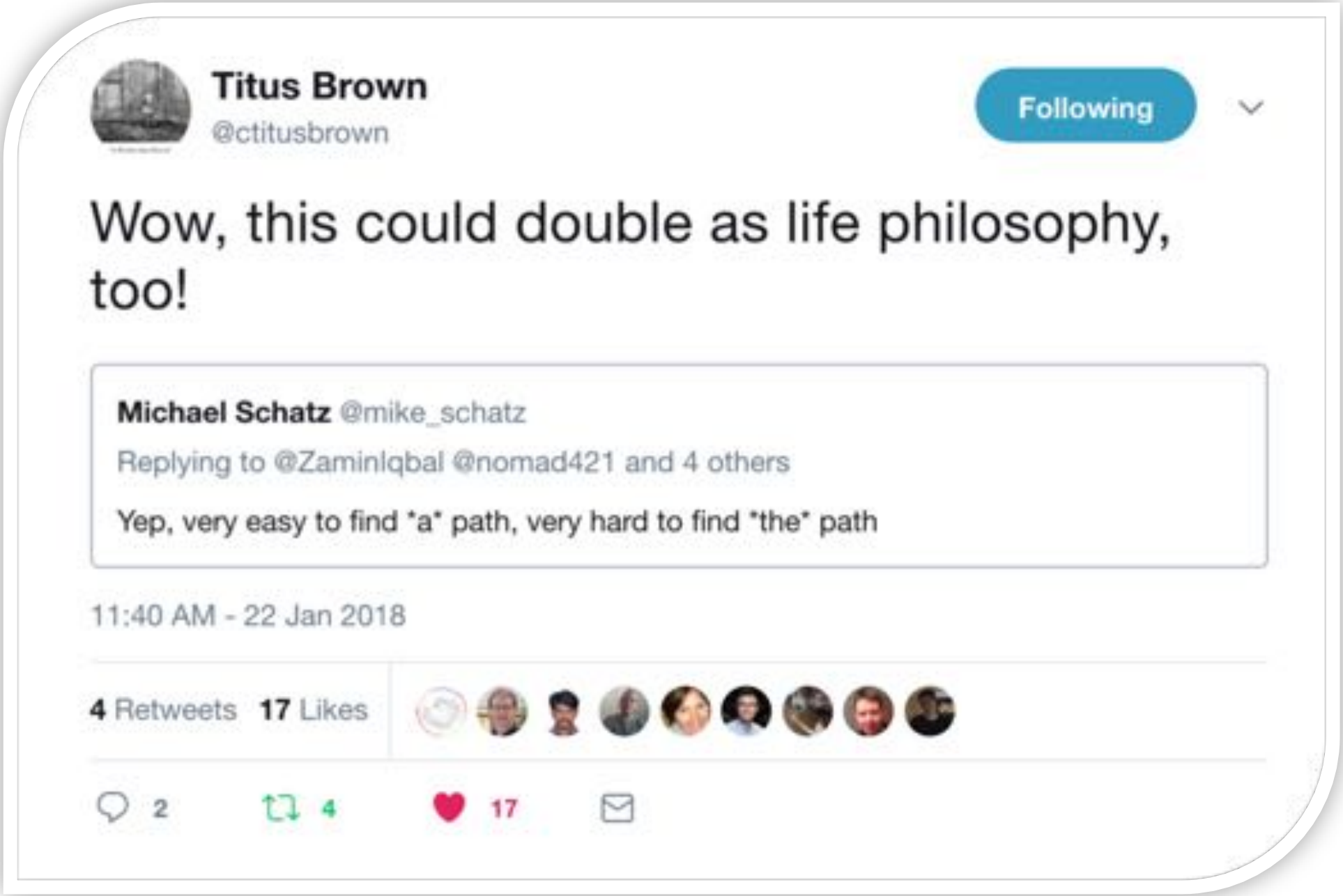


Whats another possible genome?

ATACGACGTAT


Titus Brown
@ctitusbrown
Following
Wow, this could double as life philosophy, too!
Michael Schatz @mike_schatz
Replying to @ZaminIqbal @nomad421 and 4 others
Yep, very easy to find "a" path, very hard to find "the" path
11:40 AM - 22 Jan 2018
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4
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