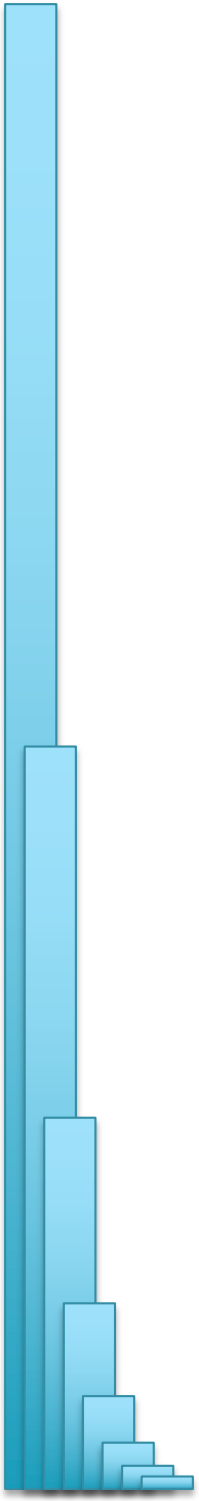
Genome Assembly

Michael Schatz

September 11, 2020

CMDB Quantitative Biology

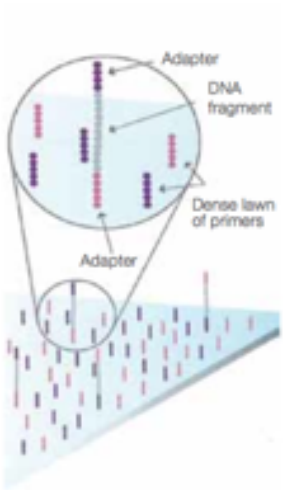


Part 1: Recap & Coverage Analysis

Second Generation Sequencing

Illumina NovoSeq
Sequencing by Synthesis

>1Tbp / day



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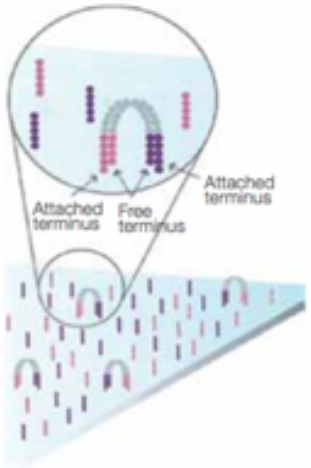

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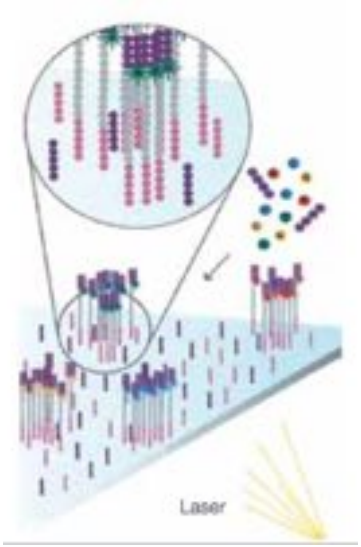
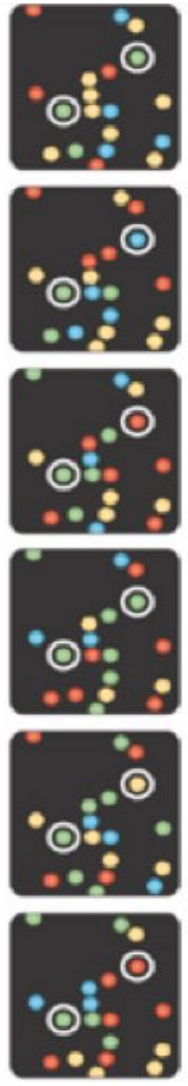


3. Image



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

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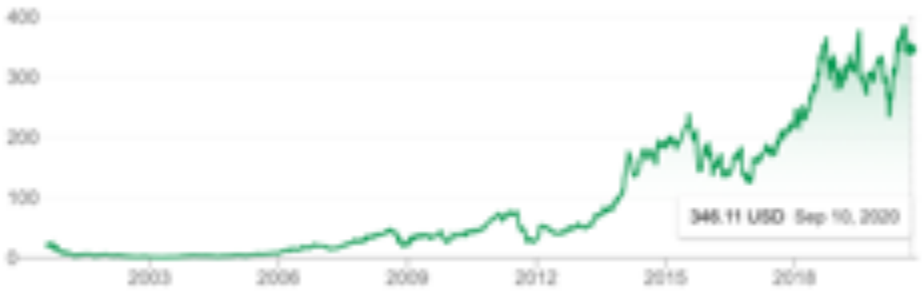
Market Summary > **Illumina, Inc.**
NASDAQ: ILMN

346.11 USD −0.89 (0.26%)
Closed: Sep 10, 4:35 PM EDT · Disclaimer
After hours 346.11 0.00 (0.00%)

1 day 5 days 1 month 6 months YTD 1 year 5 years Max



Open	348.83	Div yield	-
High	353.68	Prev close	347.00
Low	343.56	52-wk high	404.20
Mkt cap	50.53B	52-wk low	196.78
PE ratio	74.09		

More about Illumina, Inc.

Top stories

Zacks.com featured highlights include: ILMN, PFSW, CCJ and

Illumina

Biotechnology company

illumina

illumina.com

Illumina, Inc. is an American company. Incorporated in April 1998, Illumina develops, manufactures, and markets integrated systems for the analysis of genetic variation and biological function.
Wikipedia

Customer service: 1 (800) 809-4566

CEO: Francis deSouza (2016–)

Headquarters: San Diego, CA

Revenue: 3.33 billion USD (2018)

Subsidiaries: Bluebee Holding B.V., BlueGnome Ltd, MORE

Founders: Larry Bock, John R. Stuelpnagel, Anthony Czarnik, David R. Walt Ph.D., Mark Chee

Disclaimer

Profiles

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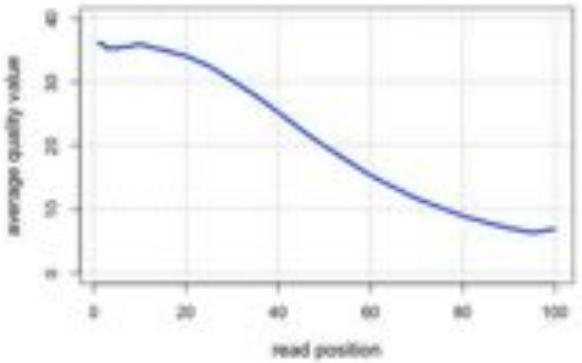
Illumina Quality

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

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

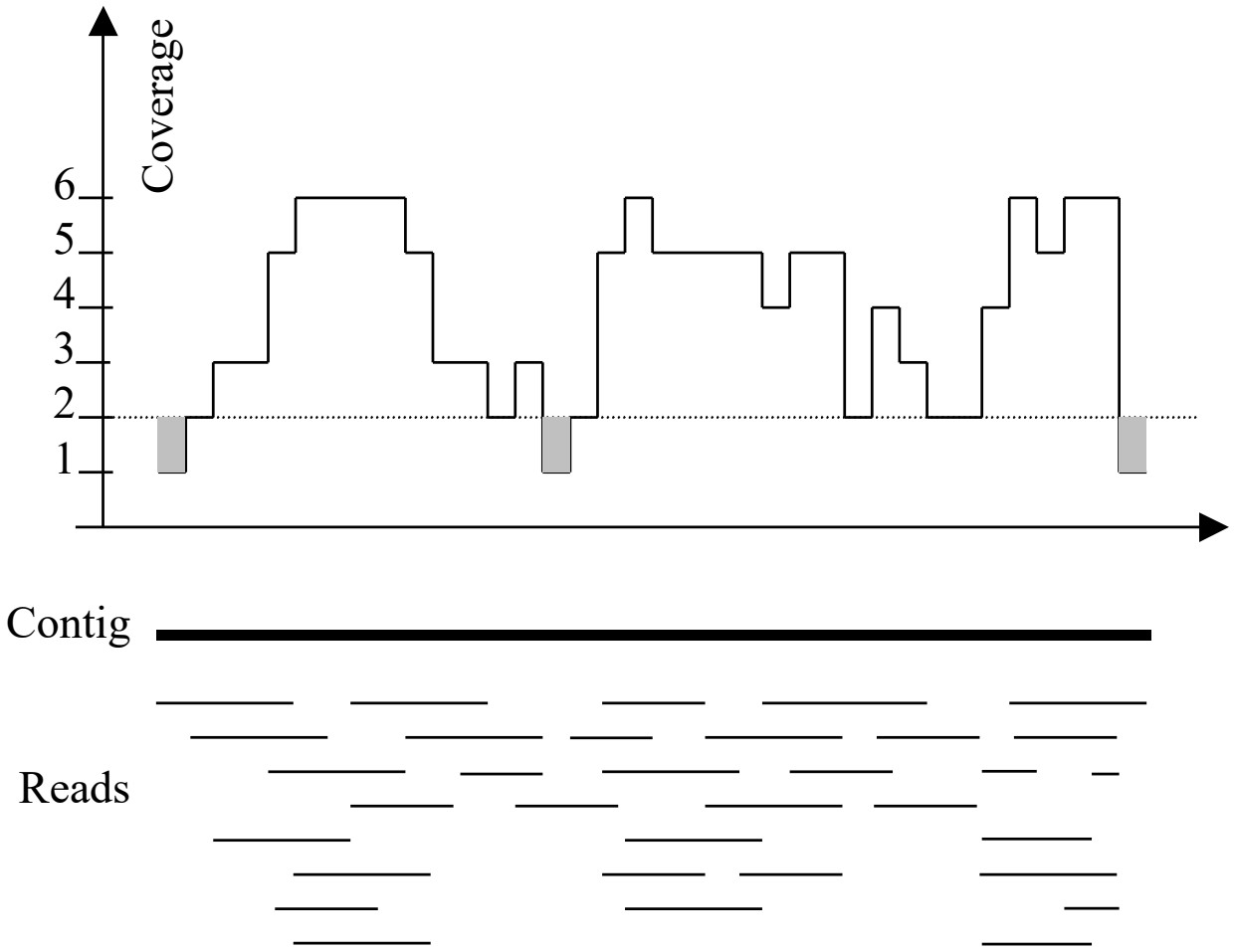



```
  SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS.....................................................
  ........................XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX.....................
  ....................................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII.....................
  ....................................JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ.....................
  LLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLL.....................................................
  !"#$%&'()*+,-./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

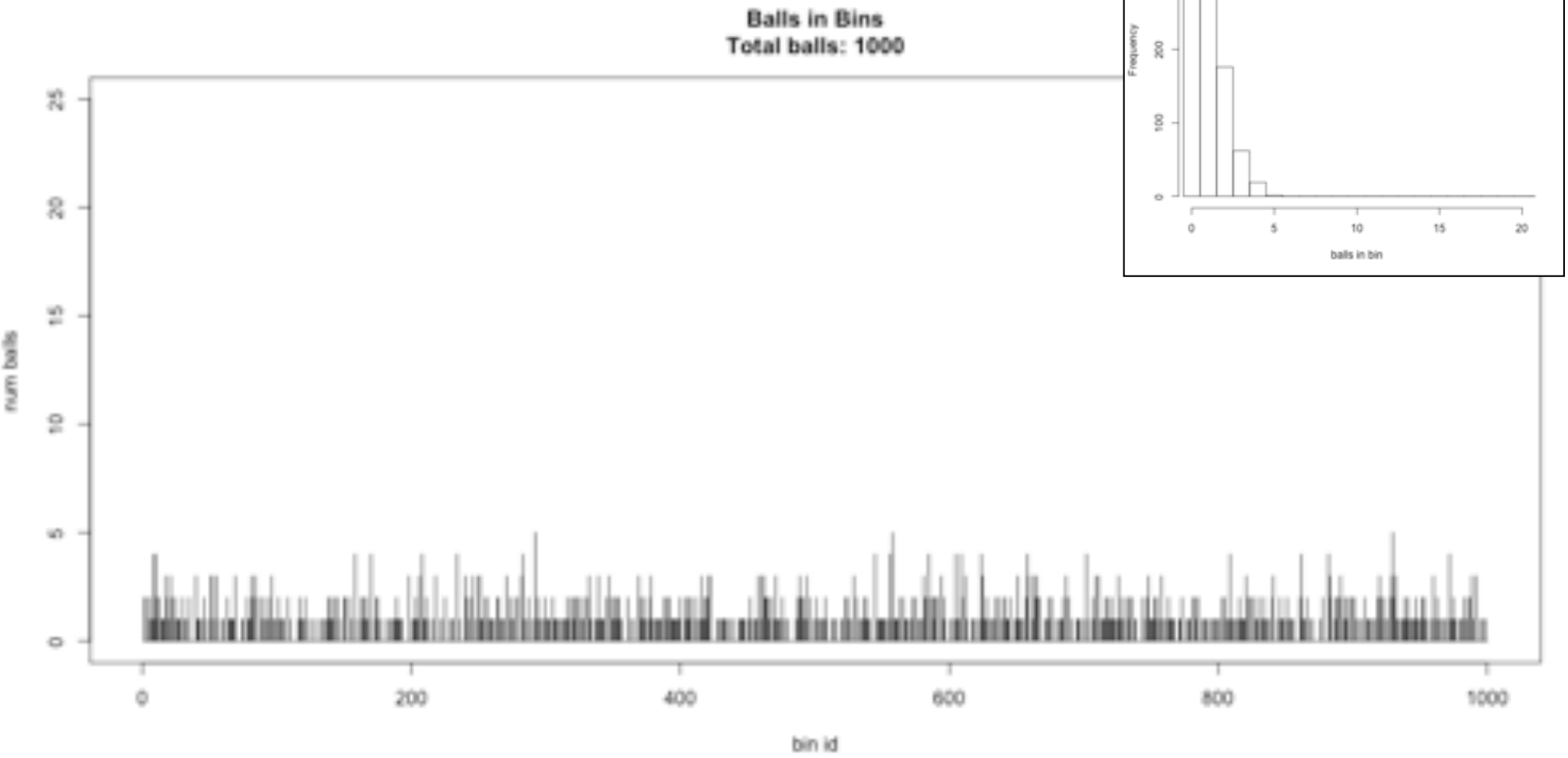


Imagine raindrops on a sidewalk

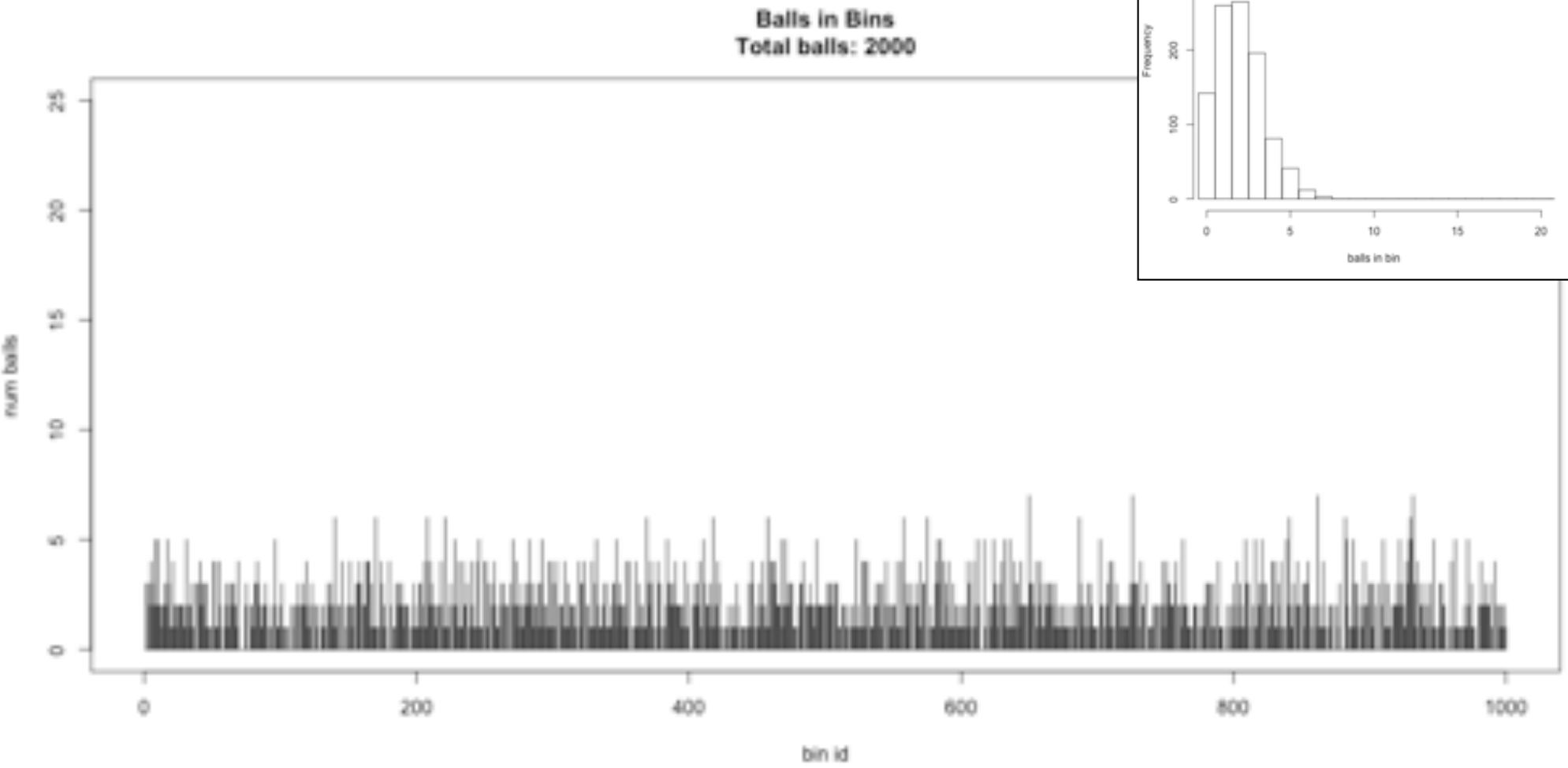
We want to cover the entire sidewalk but each drop costs $1

If the genome is 100 Mbp, should we sequence 1M 100bp reads?

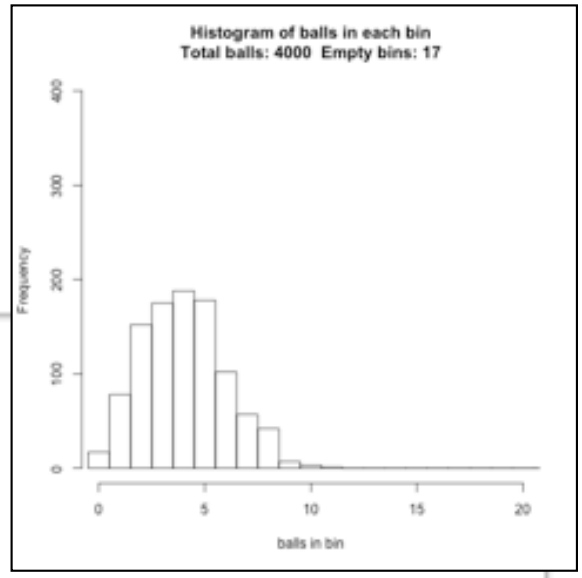
1x sequencing



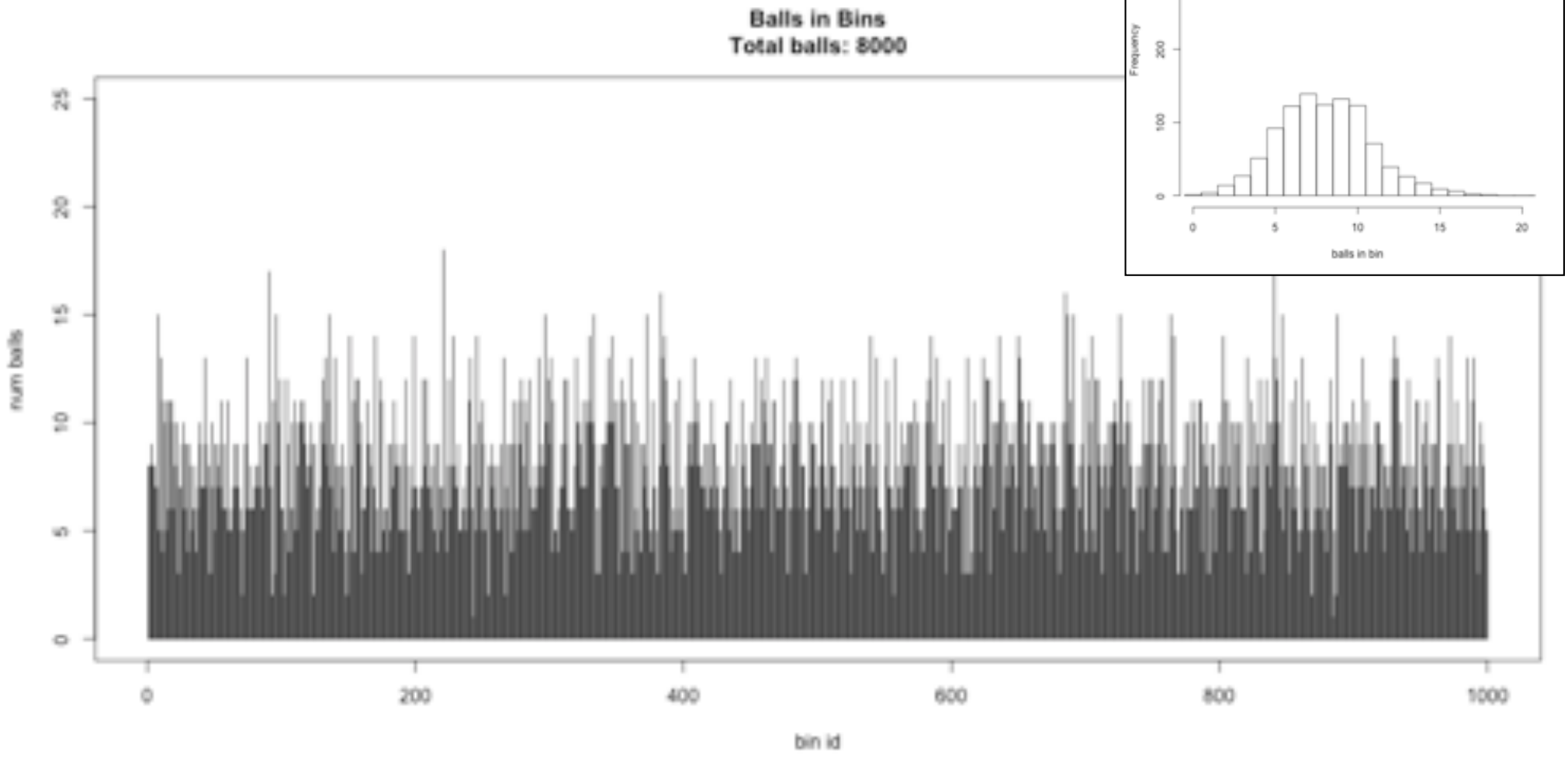
2x sequencing



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.

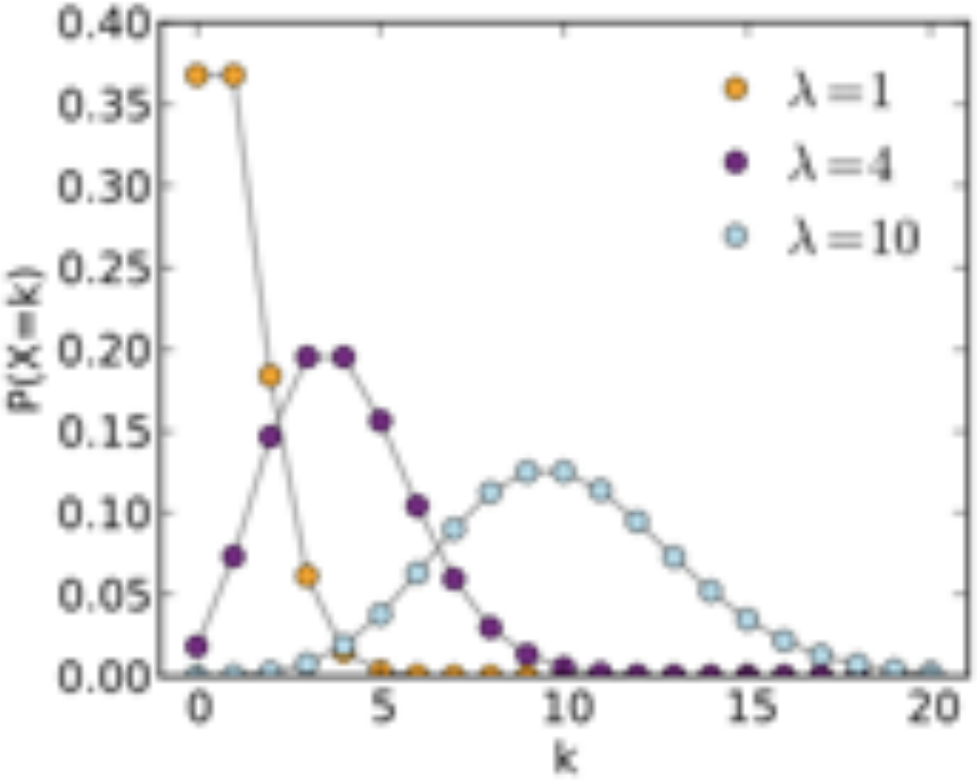
$$P(k) = \frac{\lambda^k}{k!} e^{-\lambda}$$

Formulation comes from the limit of the binomial equation

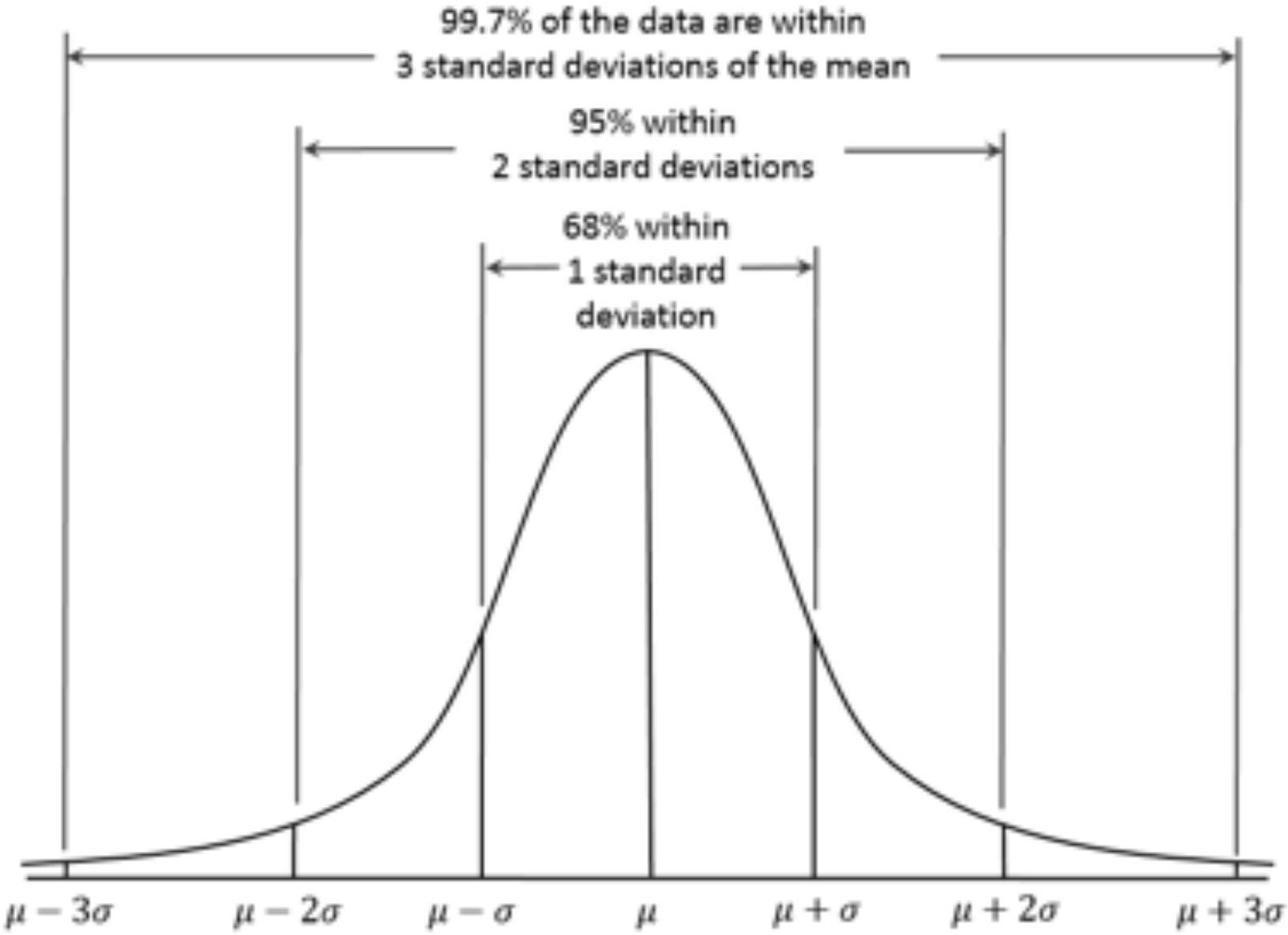
Resembles a normal distribution, but over the positive values, and with only a single parameter.

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

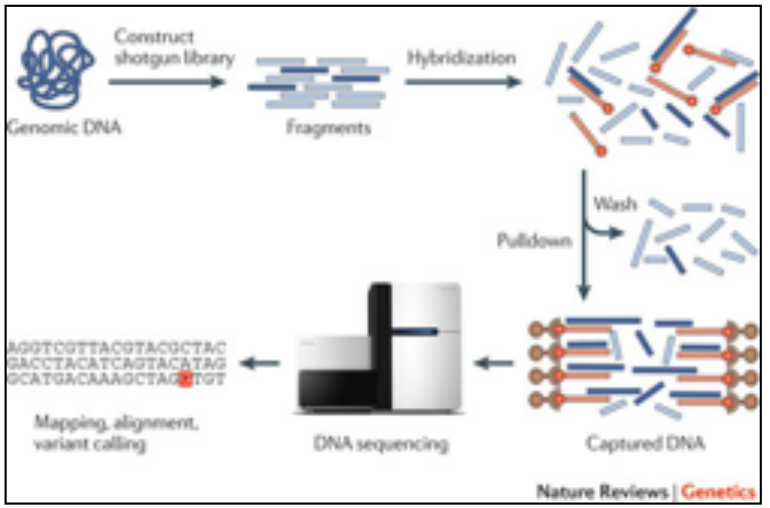
36-2*sqrt(36) = 24

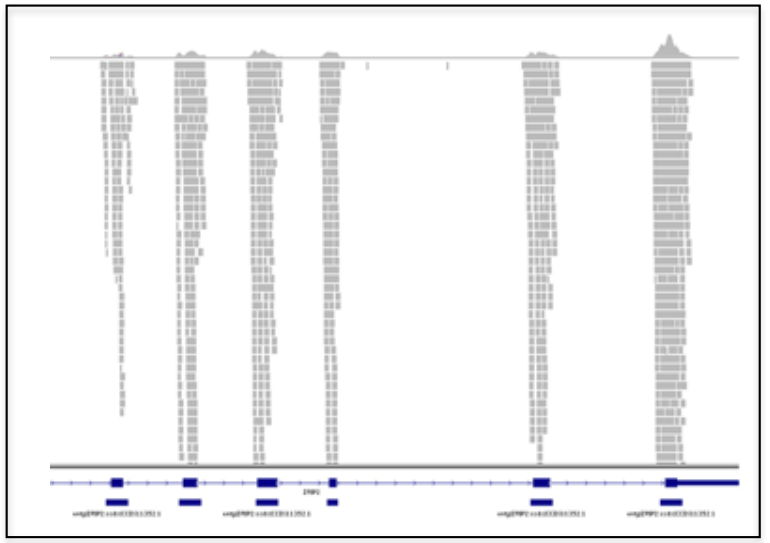
I need 10Mbp x 36x = 360Mbp of data
360Mbp / 120bp / read = 3M reads

Exome-Capture Sequencing

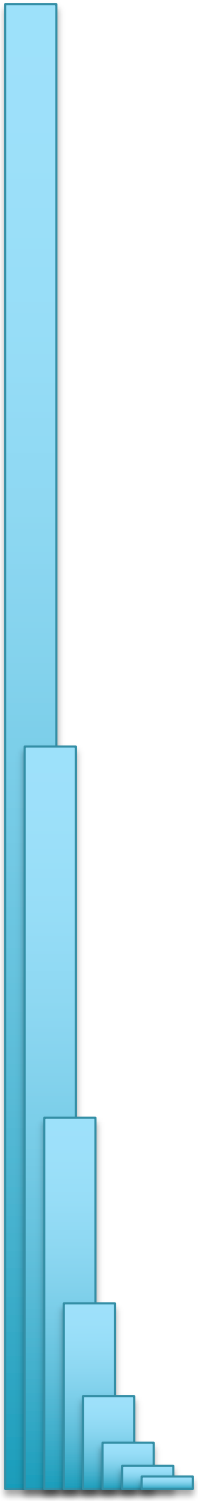
Exome-capture reduces the costs of sequencing

- 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



Part 2: De novo genome assembly

Outline

1. ***Assembly theory***
   - Assembly by analogy

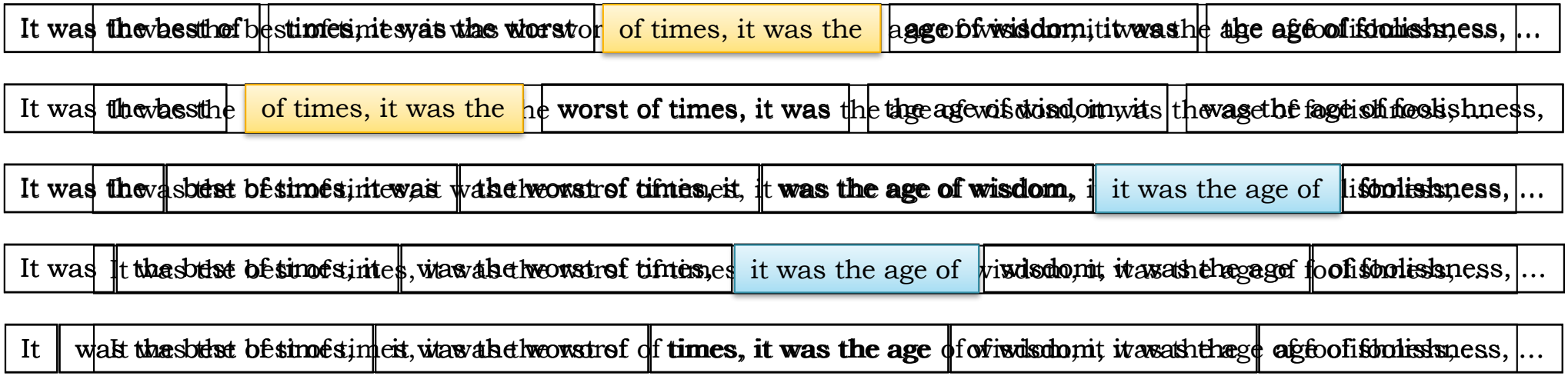
2. ***Practical Issues***
   - Coverage, read length, errors, and repeats

3. ***Whole Genome Alignment***
   - MUMmer recommended

4. ***Next-next-gen Assembly***
   - Canu: recommended for PacBio/ONT project

Shredded Book Reconstruction

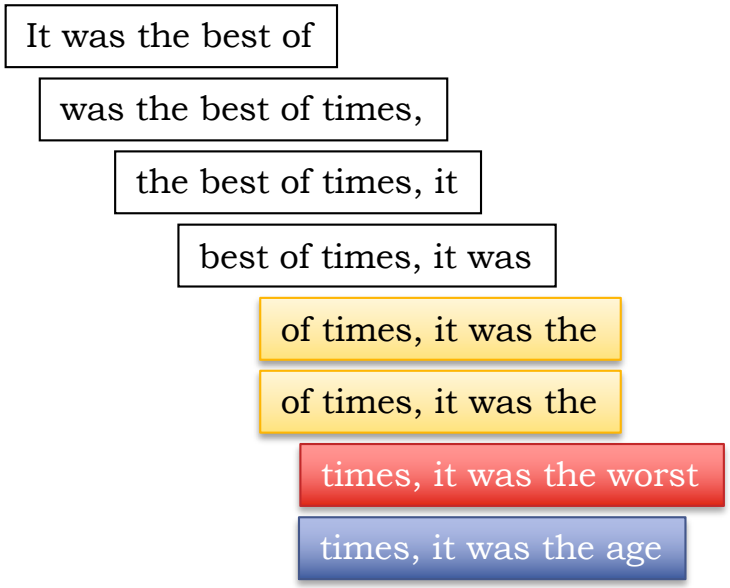
- Dickens accidentally shreds the first printing of A Tale of Two Cities
  - Text printed on 5 long spools



- 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



The repeated sequence make the correct reconstruction ambiguous

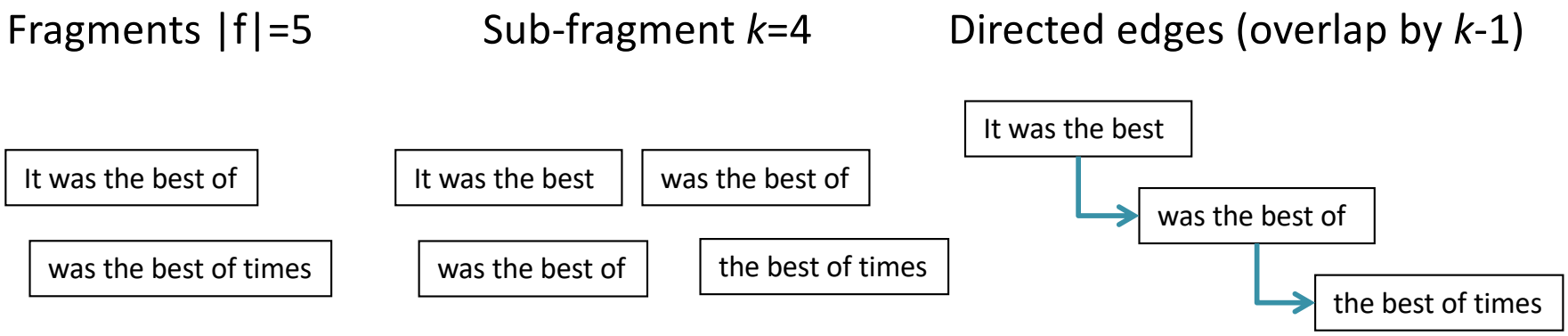
- It was the best of times, it was the [worst/age]

Model the assembly problem as a graph problem

How long will it take to compute the overlaps?

de Bruijn Graph Construction

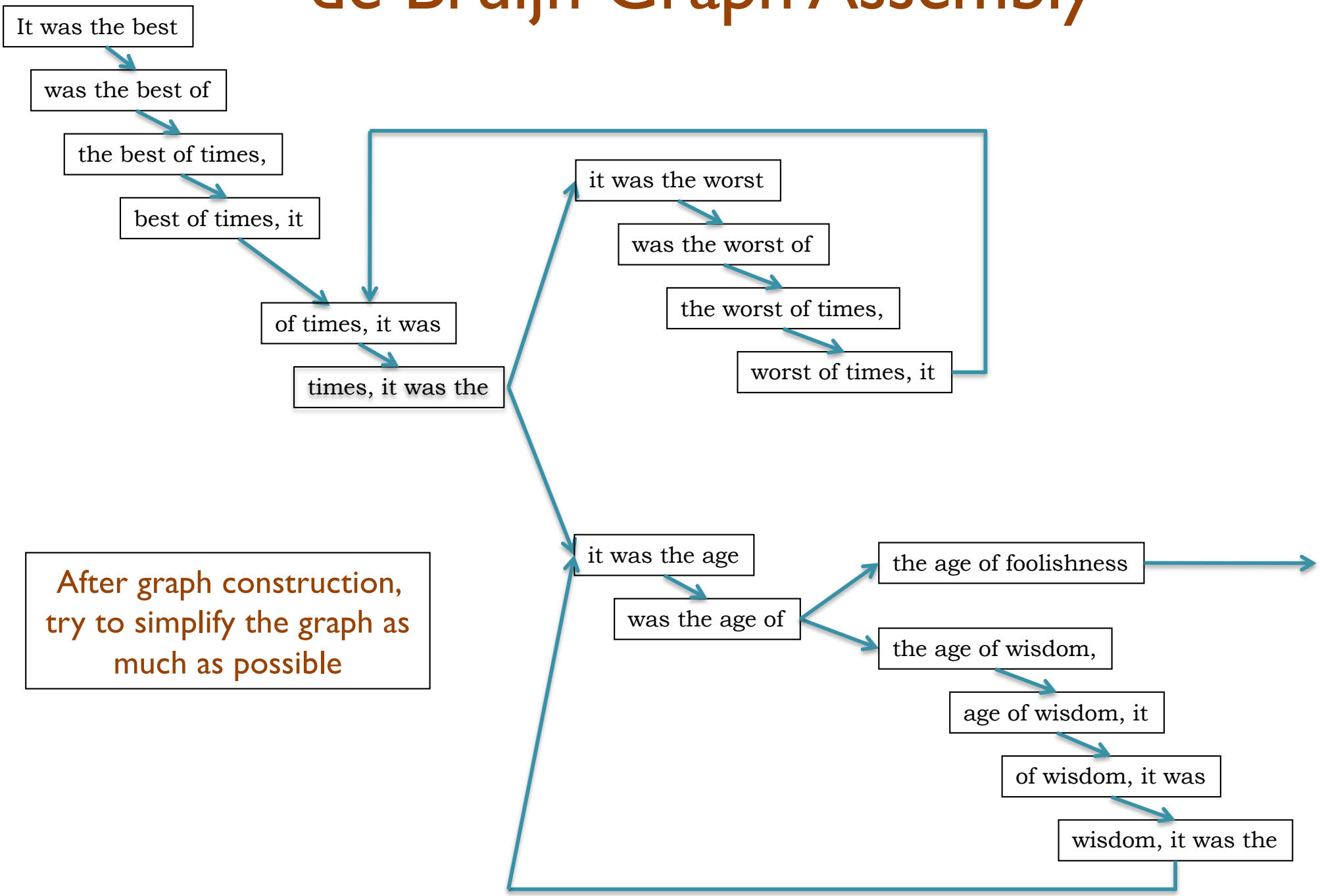
- $G_k = (V,E)$
  - V = Length-*k* sub-fragments
  - E = Directed edges between consecutive sub-fragments
    - Sub-fragments overlap by *k*-1 words



– Overlaps between fragments are implicitly computed

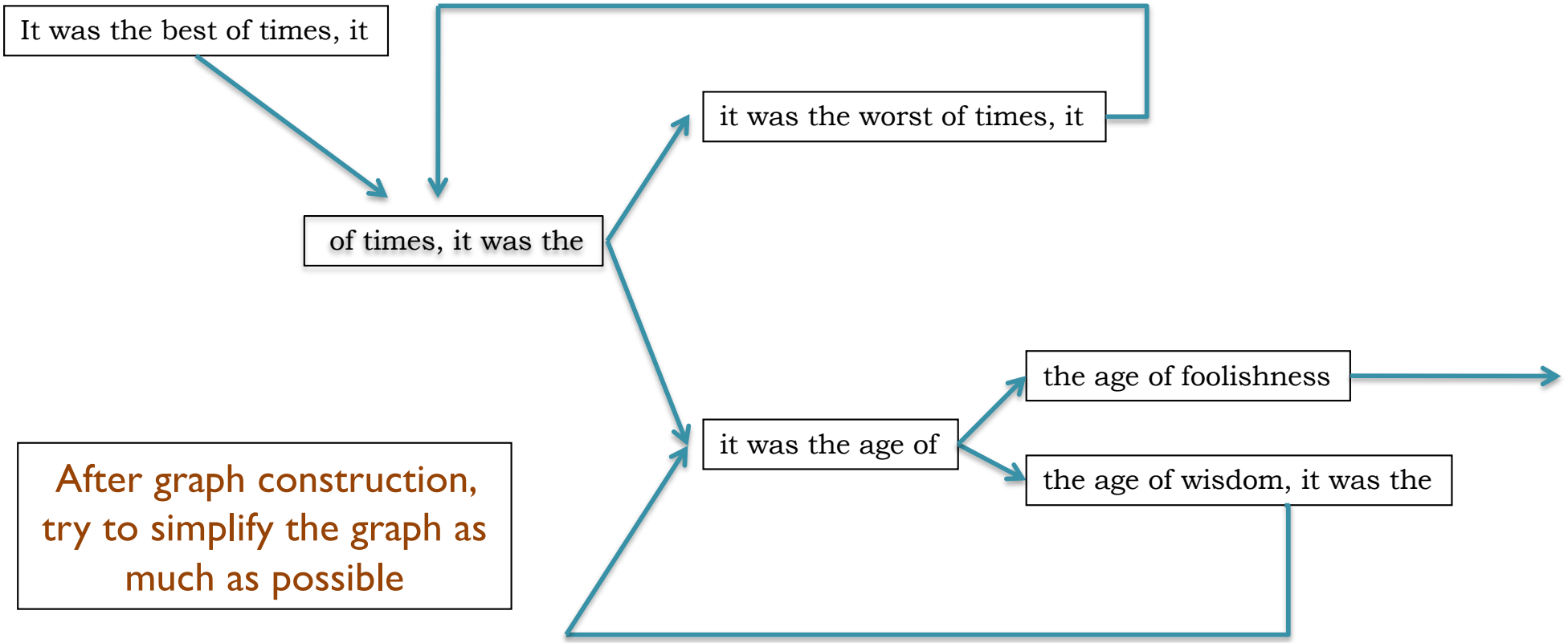
de Bruijn, 1946
Idury et al., 1995
Pevzner et al., 2001

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

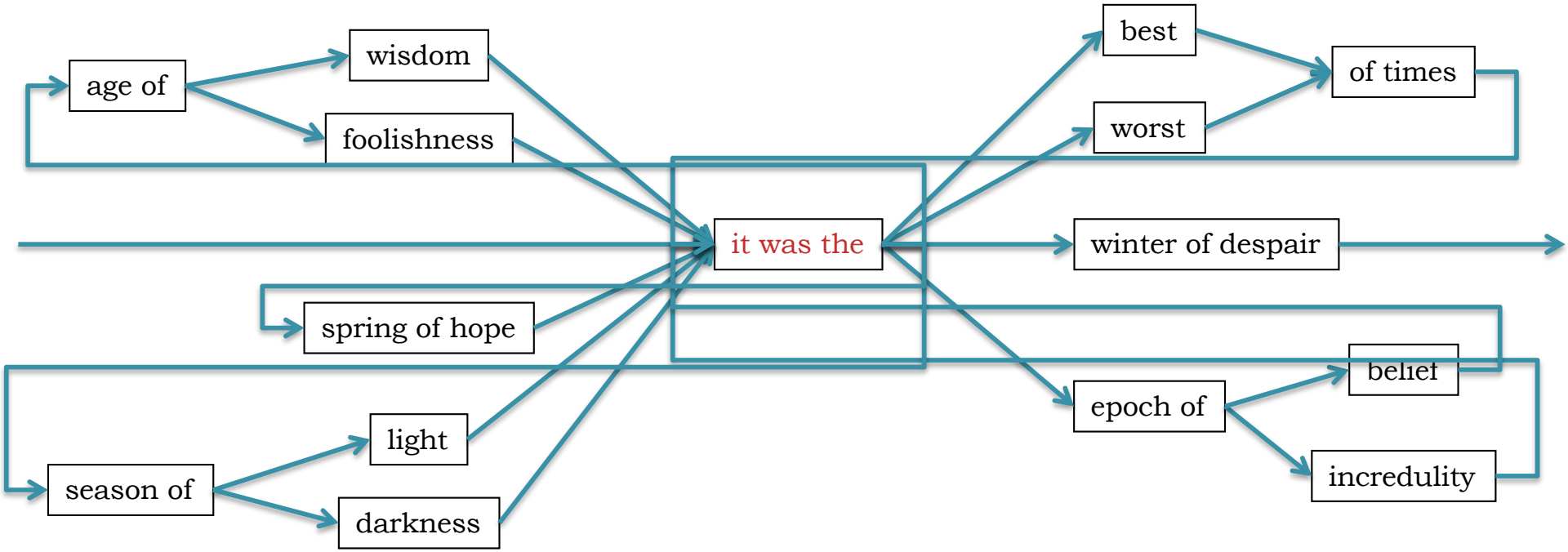
… it was the best of times it was the worst of times …

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

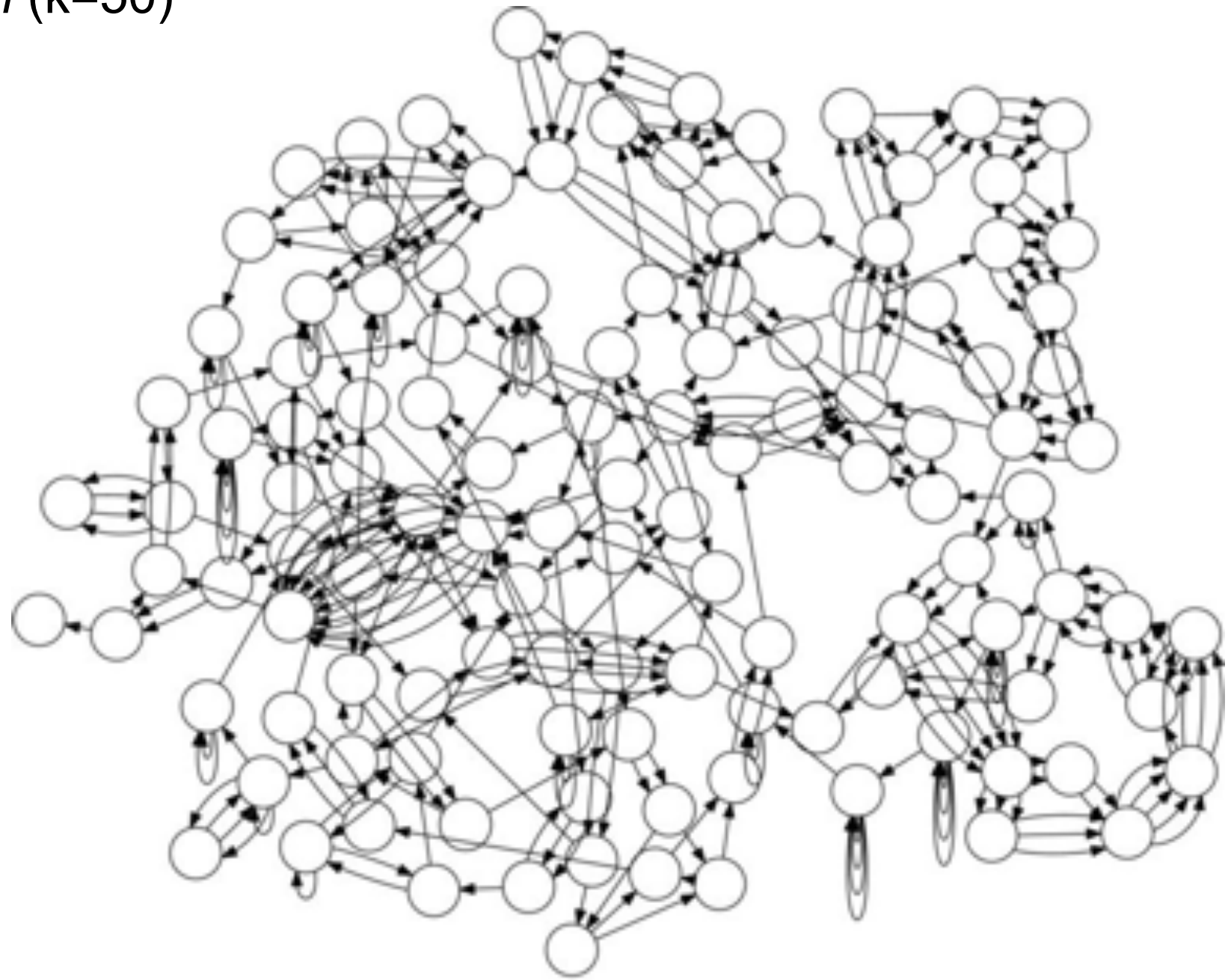
… it was the epoch of belief it was the epoch of incredulity …

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

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

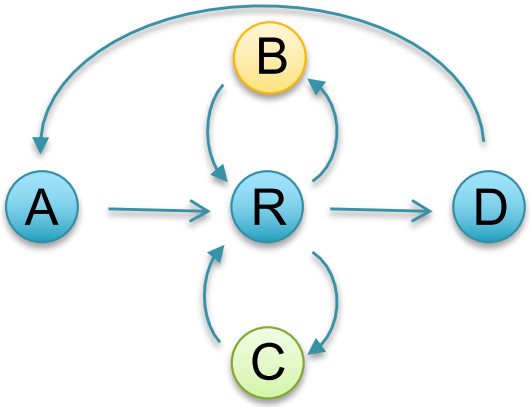


E. coli (k=50)



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)

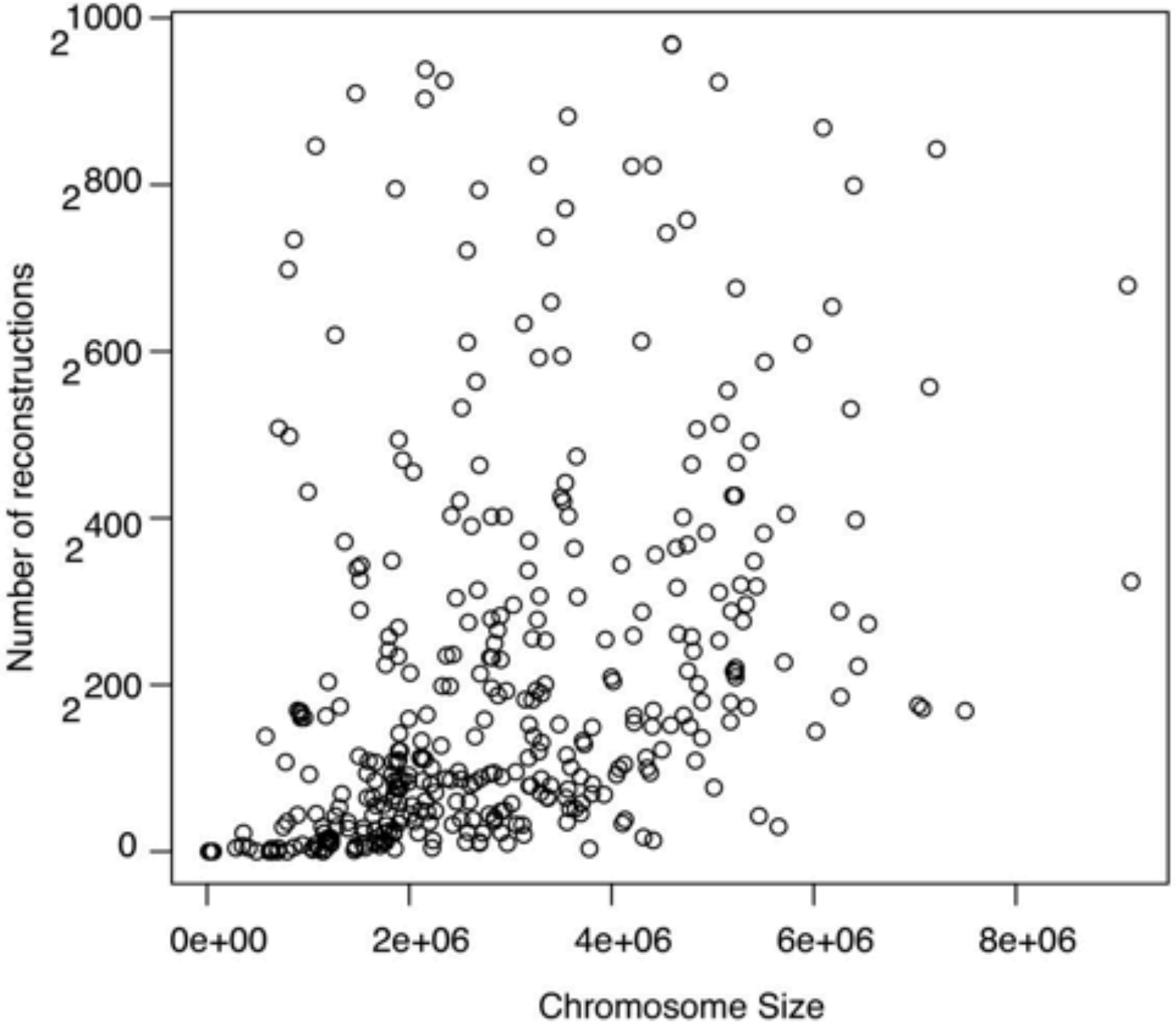
$$\mathcal{W}(G,t) = (\det L)\left\{\prod_{u\in V}(r_u-1)!\right\}\left\{\prod_{(u,v)\in E} a_{uv}!\right\}^{-1}$$

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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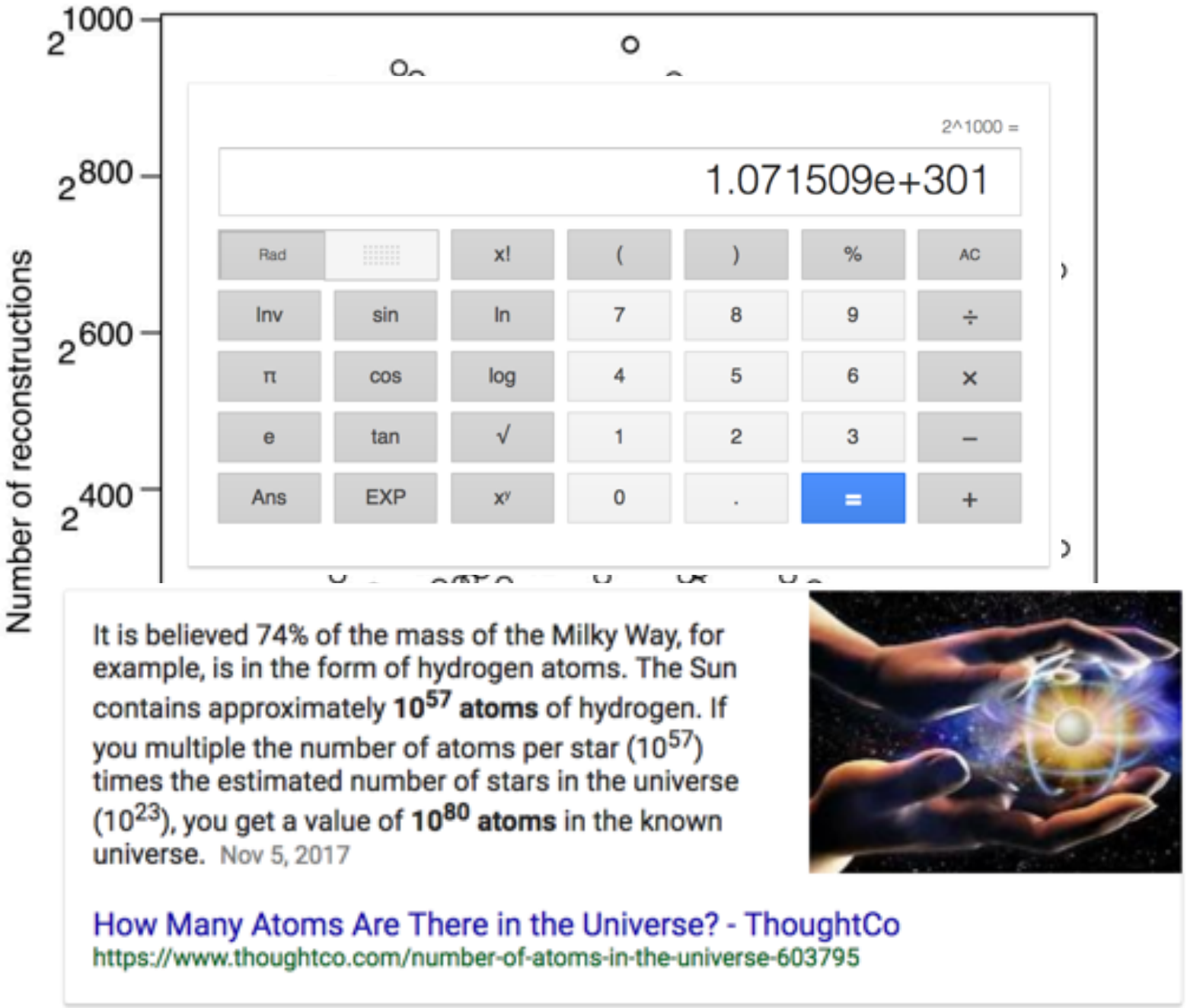

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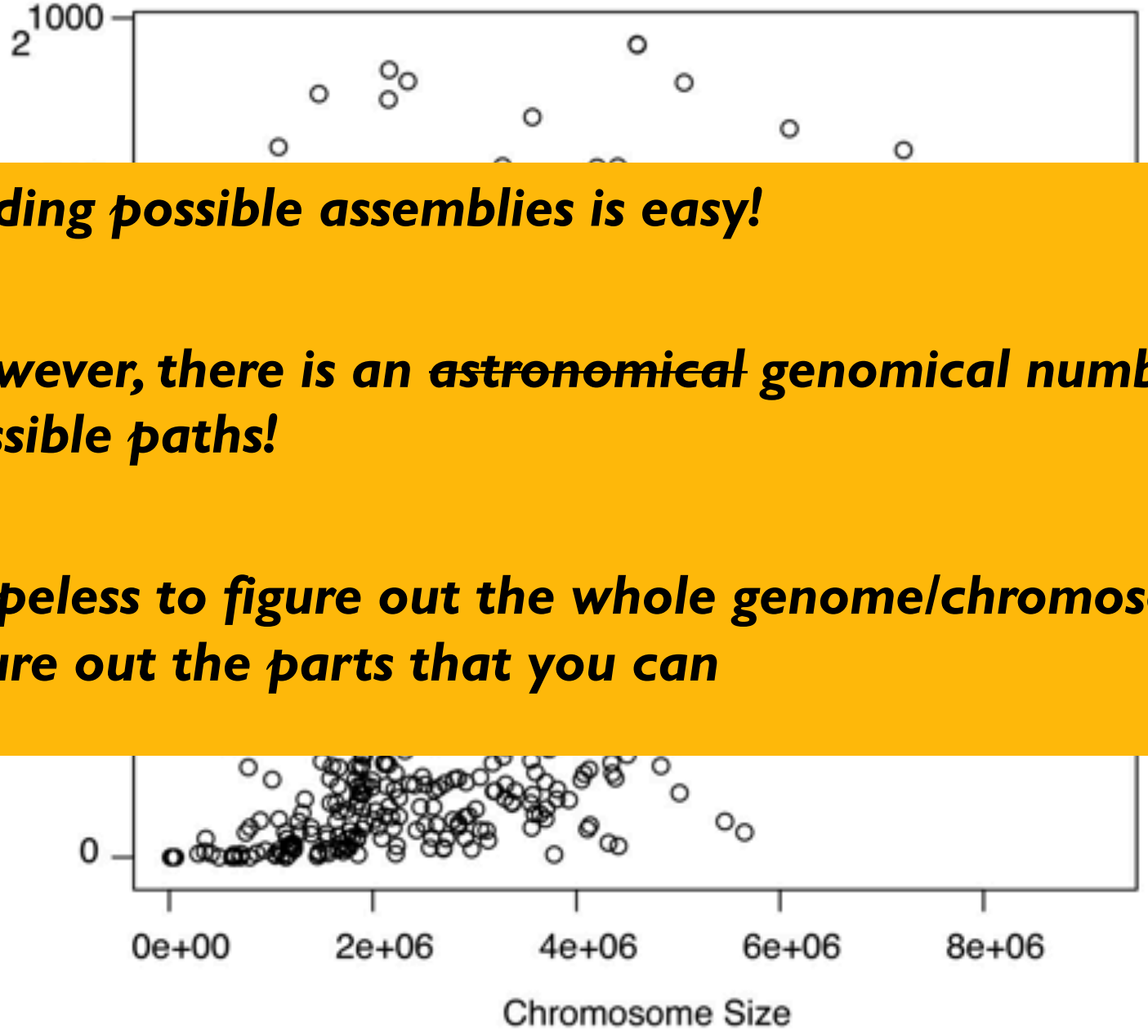


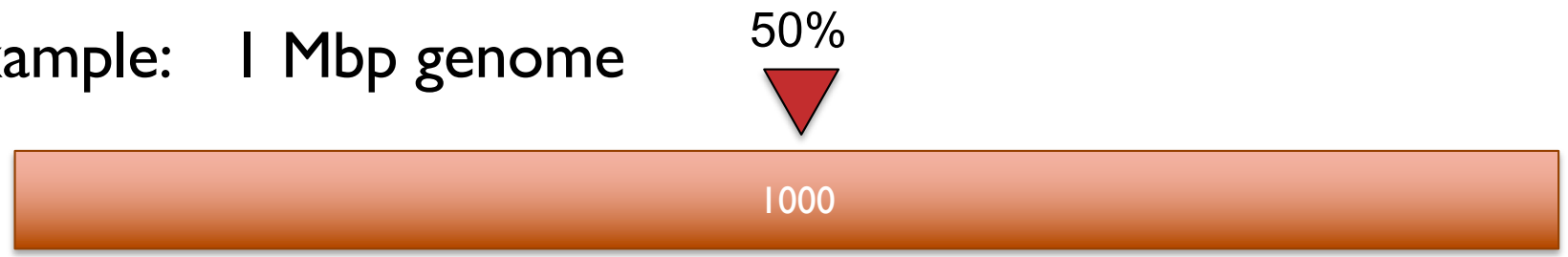
- ***Finding possible assemblies is easy!***
- ***However, there is an ~~astronomical~~ genomical number of possible paths!***
- ***Hopeless to figure out the whole genome/chromosome, figure out the parts that you can***

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

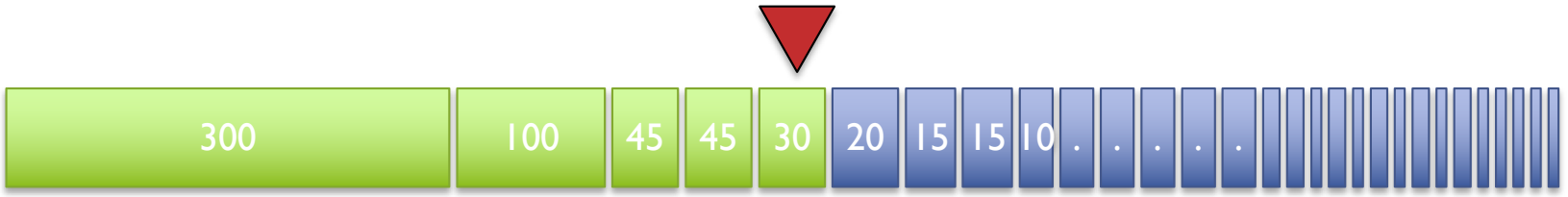
Contig N50

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

Example: 1 Mbp genome

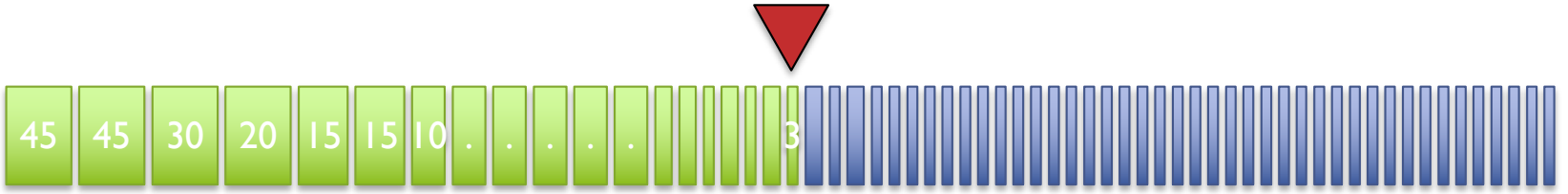


A



N50 size = 30 kbp

B



N50 size = 3 kbp

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

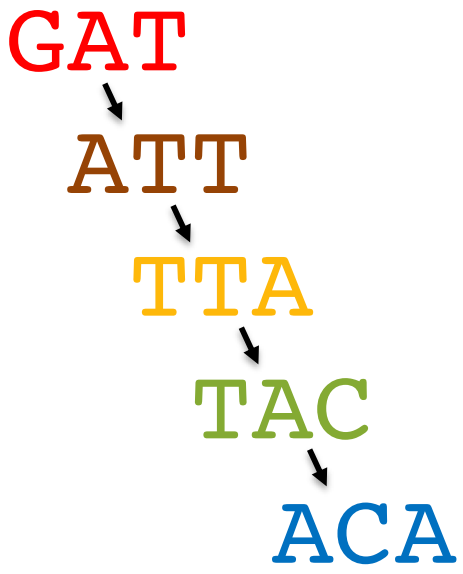
Assemble these reads using a de Bruijn graph approach (k=3):

```
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

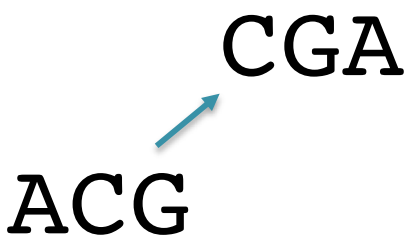
CGAC

CGTA

GACG

GTAT

TACG



Pop Quiz 2

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

~~ACGA~~

~~ACGT~~

ATAC

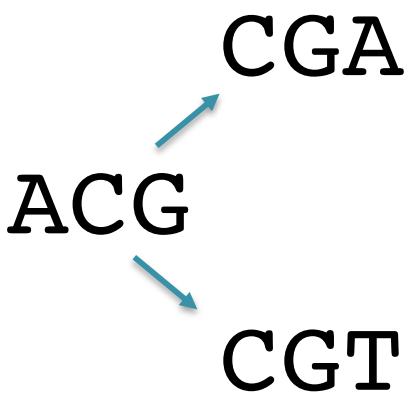
CGAC

CGTA

GACG

GTAT

TACG



Pop Quiz 2

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

~~ACGA~~

~~ACGT~~

ATAC

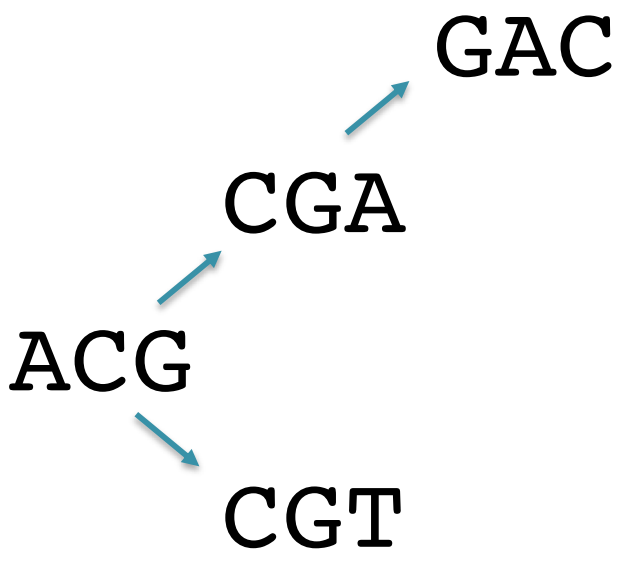
~~CGAC~~

CGTA

GACG

GTAT

TACG



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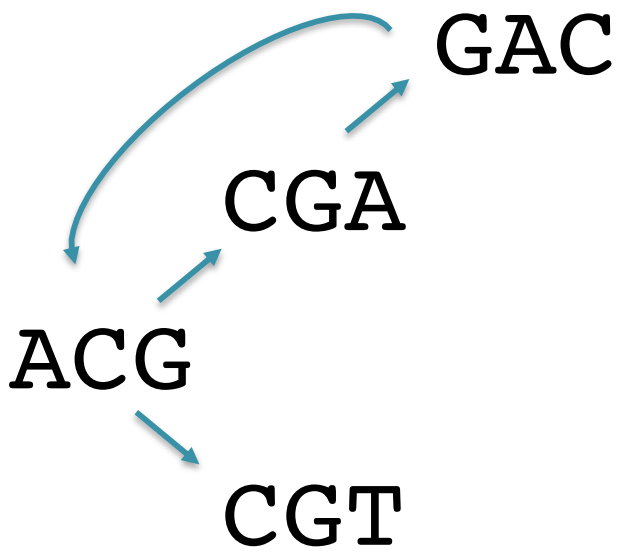

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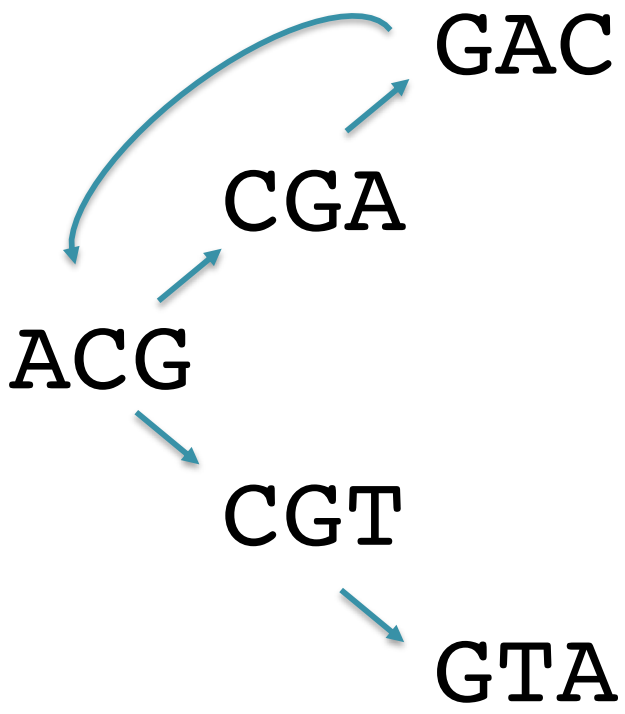

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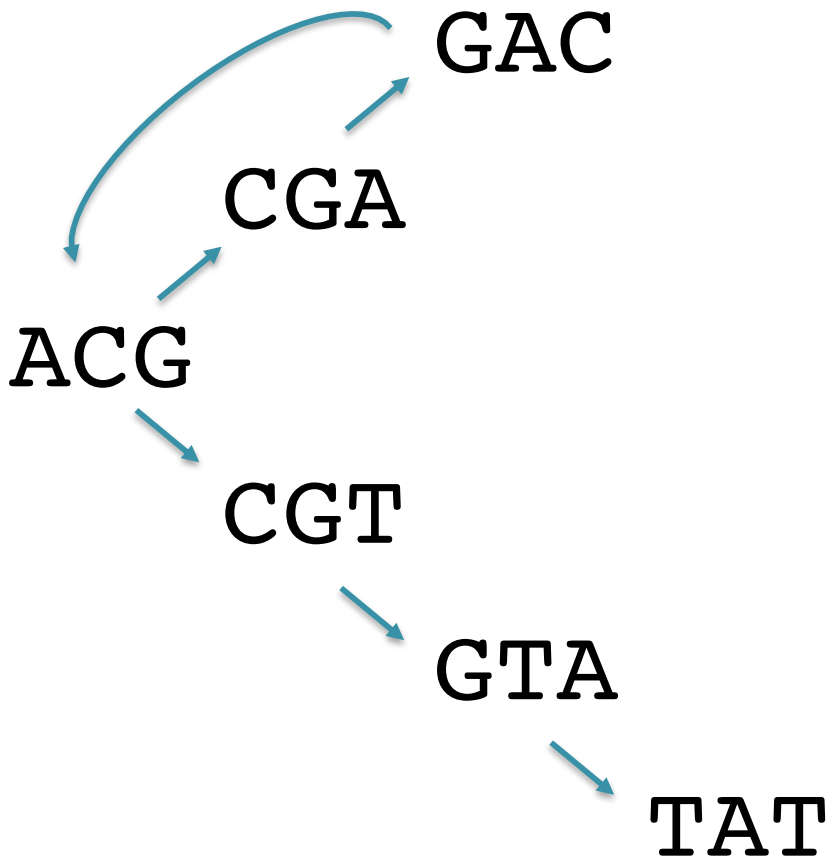
~~GACG~~

~~GTAT~~

TACG



Pop Quiz 2

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

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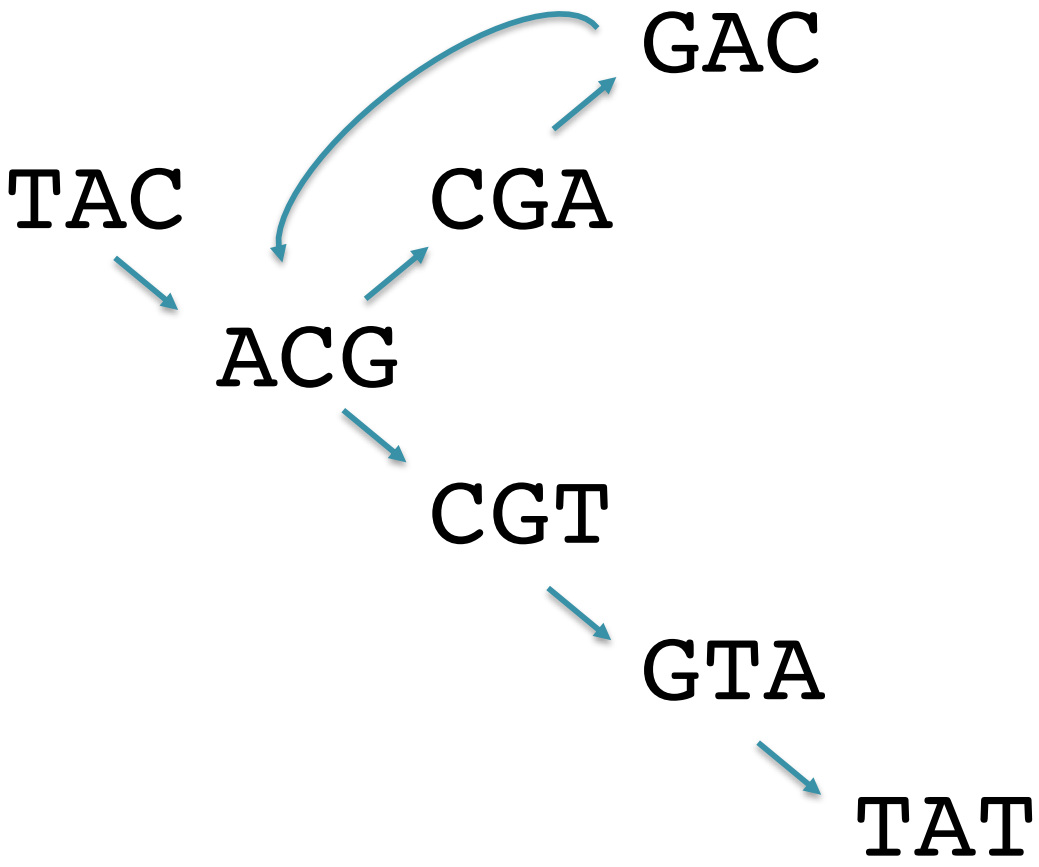
~~GACG~~
~~GTAT~~
~~TACG~~



Pop Quiz 2

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

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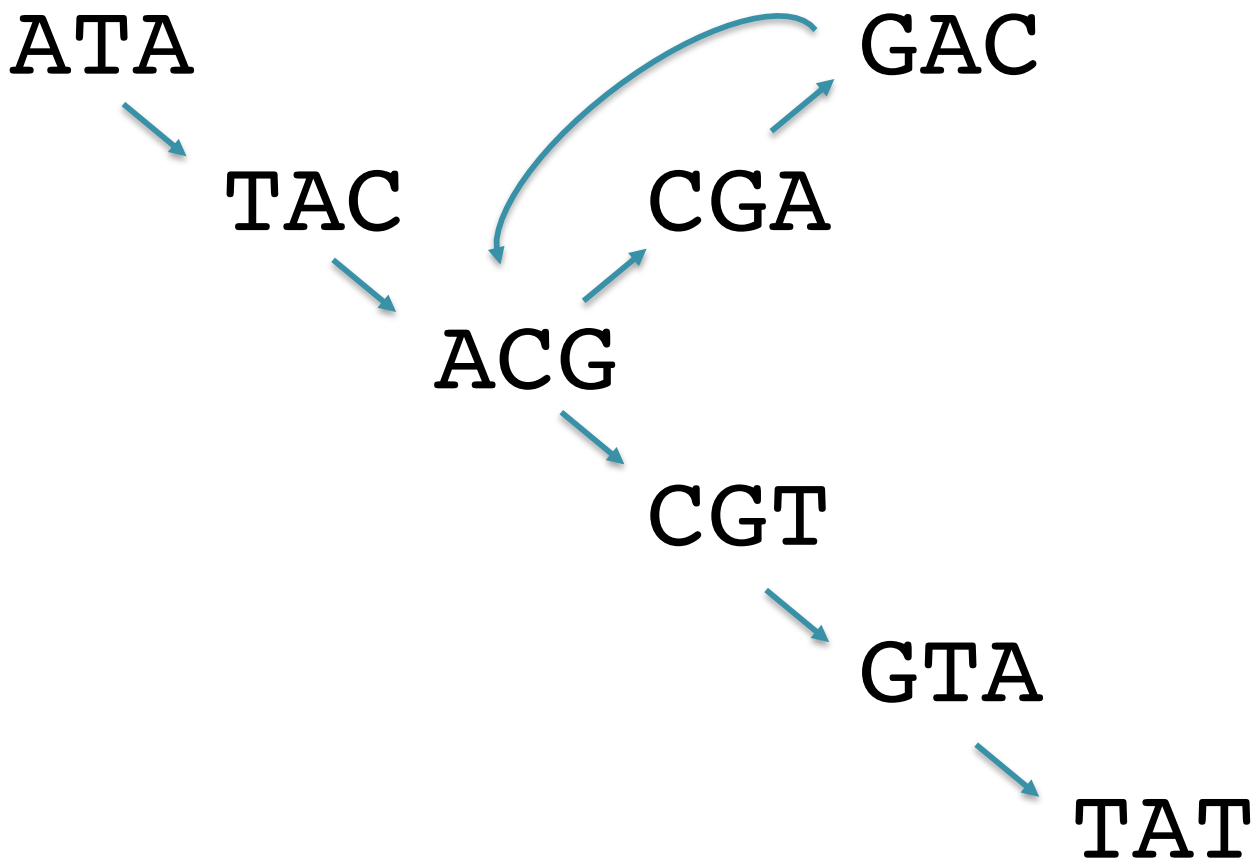
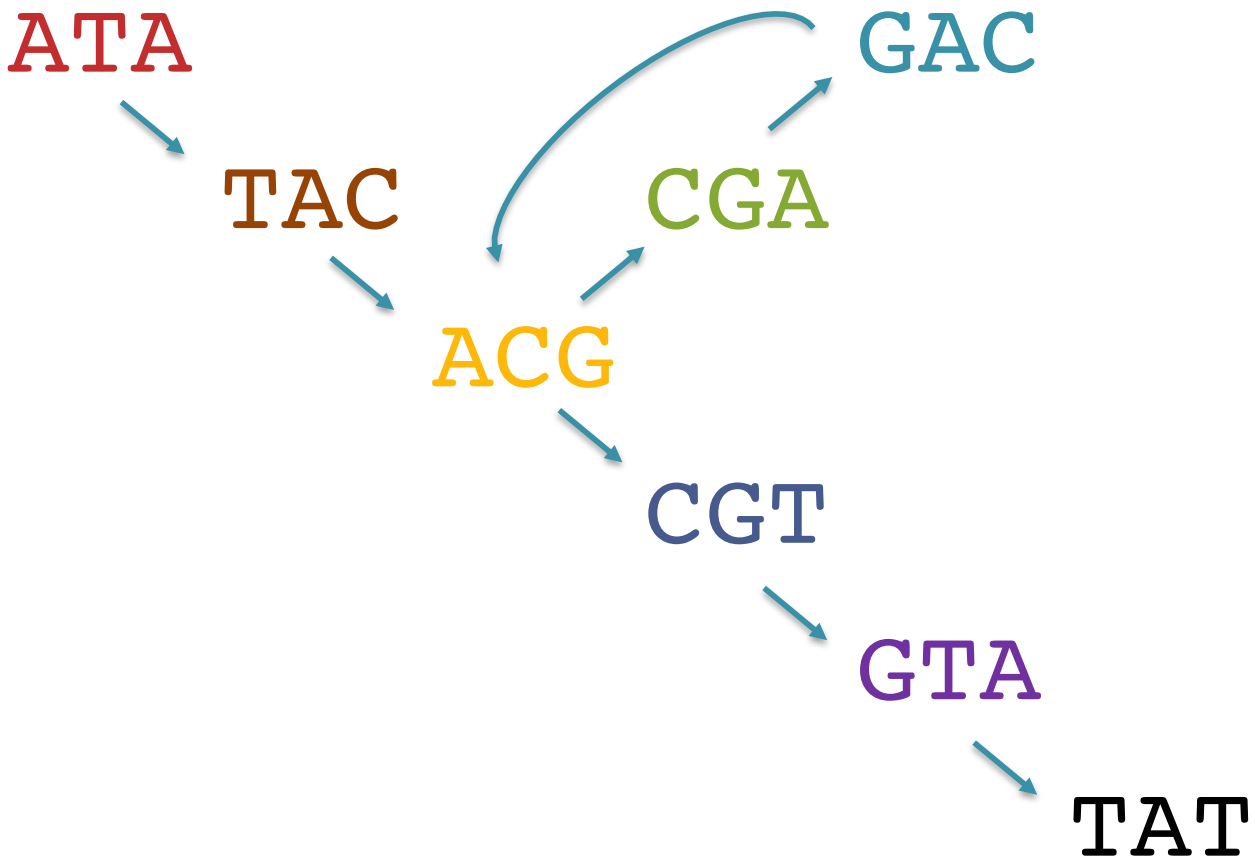
~~GTAT~~
~~TACG~~



Pop Quiz 2

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

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

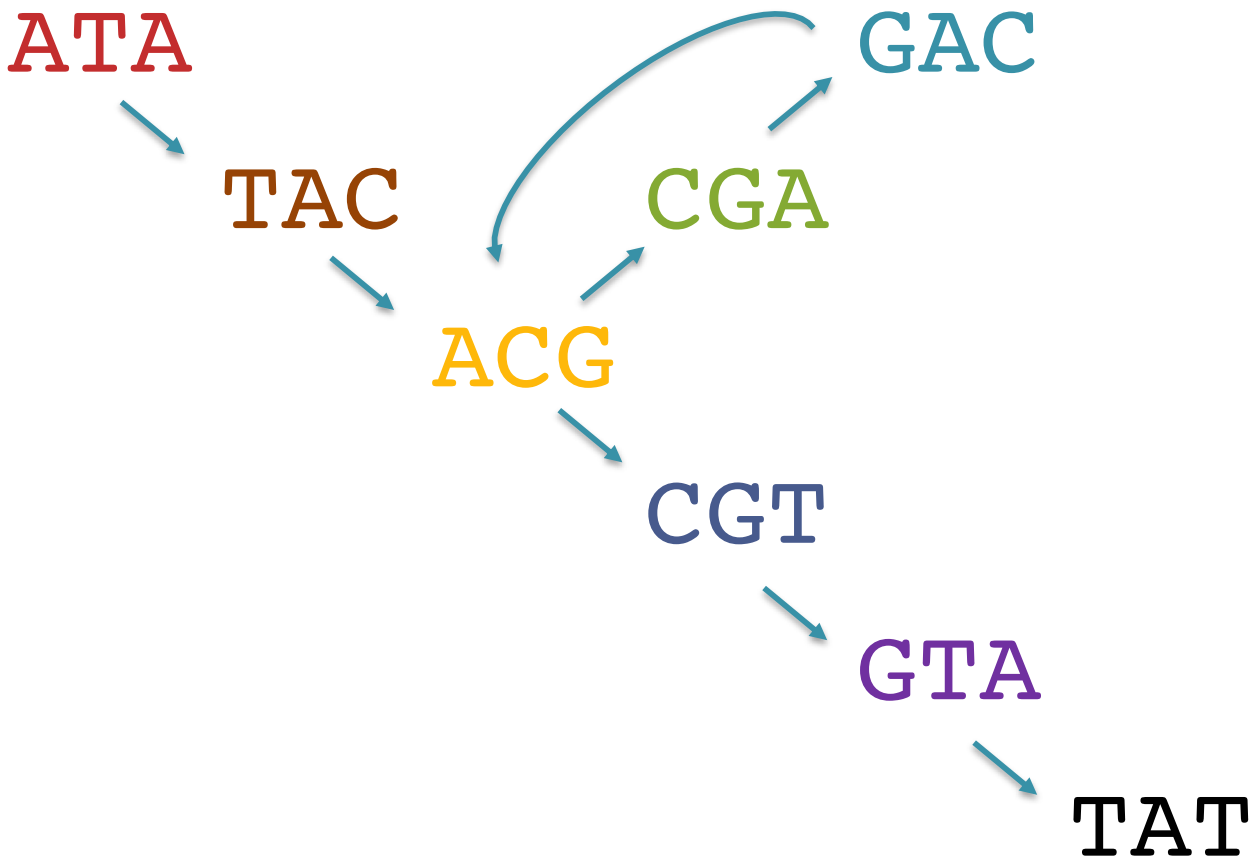


ATACGACGTAT

Pop Quiz 2

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

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



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

4 Retweets **17** Likes

2 4 17

Outline

1. ***Assembly theory***
   - Assembly by analogy

2. ***Practical Issues***
   - Coverage, read length, errors, and repeats

3. ***Whole Genome Alignment***
   - MUMmer recommended

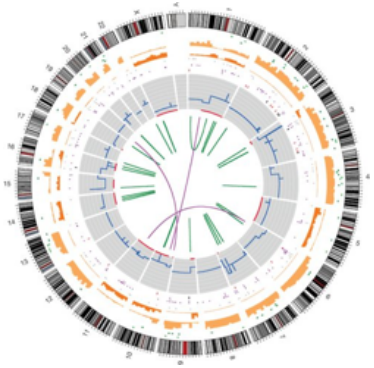
4. ***Next-next-gen Assembly***
   - Canu: recommended for PacBio/ONT project

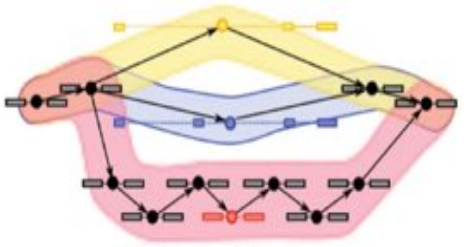
Assembly Applications

- Novel genomes

- Metagenomes

- Sequencing assays
  - Structural variations
  - Transcript assembly
  - …





Why are genomes hard to assemble?

1. ***Biological***:
   - (Very) High ploidy, heterozygosity, repeat content

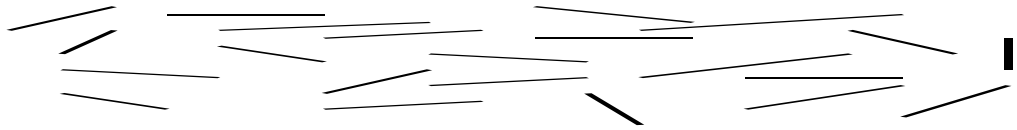
2. ***Sequencing***:
   - (Very) large genomes, imperfect sequencing

3. ***Computational***:
   - (Very) Large genomes, complex structure

4. ***Accuracy***:
   - (Very) Hard to assess correctness

Assembling a Genome

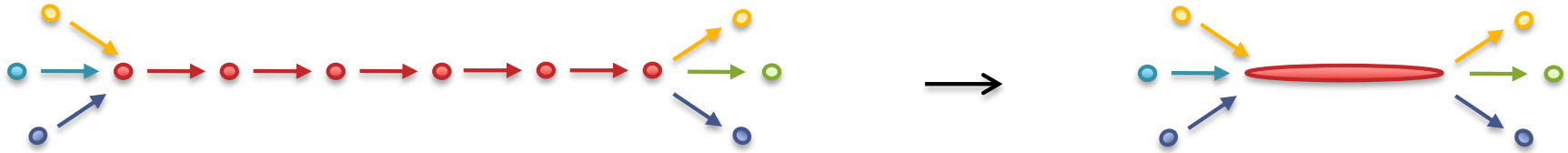
1. Shear & Sequence DNA



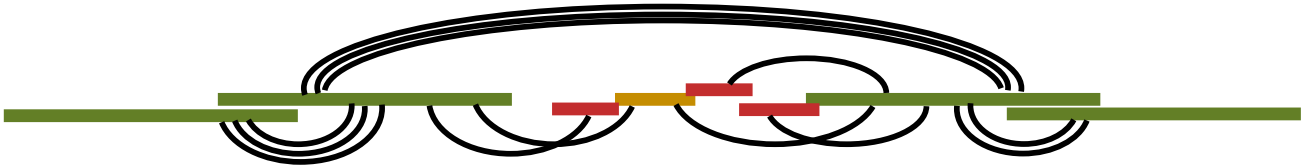
2. Construct assembly graph from reads (de Bruijn / overlap graph)

...AGCCTAGGGATGCGCGACACGT
GGATGCGCGACACGTCGCATATCCGGTTTGGTCAACCTCGGACGGAC
CAACCTCGGACGGACCTCAGCGAA...

3. Simplify assembly graph

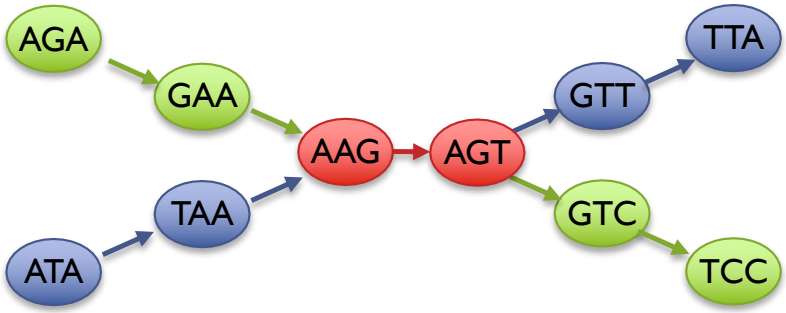


4. Detangle graph with long reads, mates, and other links



Two Paradigms for Assembly

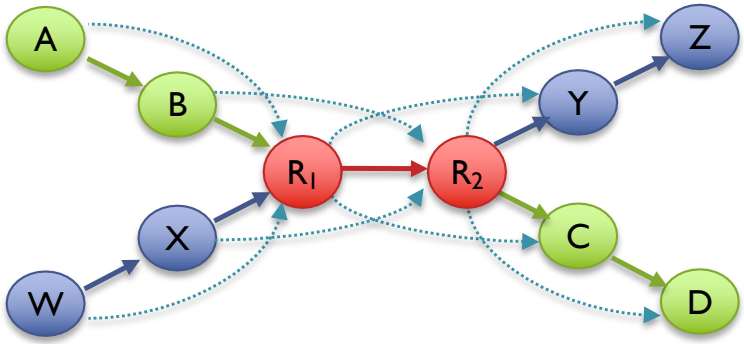
de Bruijn Graph



Short read assemblers

- Repeats depends on word length
- Read coherency, placements lost
- Robust to high coverage

Overlap Graph



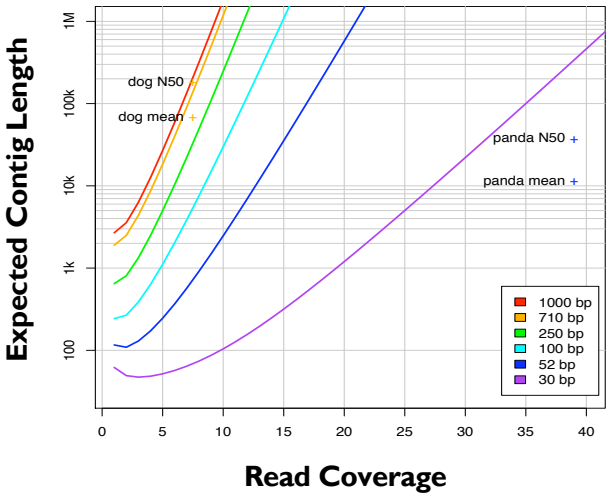
Long read assemblers

- Repeats depends on read length
- Read coherency, placements kept
- Tangled by high coverage

Assembly of Large Genomes using Second Generation Sequencing
Schatz MC, Delcher AL, Salzberg SL (2010) *Genome Research*. 20:1165-1173.

Ingredients for a good assembly

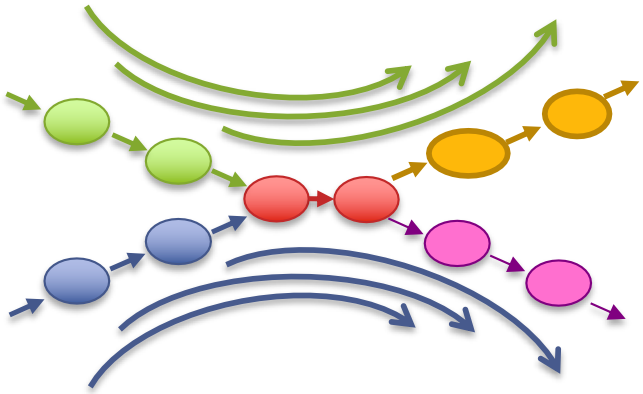
Coverage



High coverage is required

- Oversample the genome to ensure every base is sequenced with long overlaps between reads
- Biased coverage will also fragment assembly

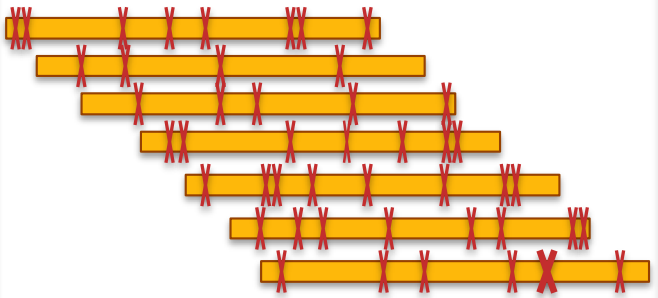
Read Length



Reads & mates must be longer than the repeats

- Short reads will have ***false overlaps*** forming hairball assembly graphs
- With long enough reads, assemble entire chromosomes into contigs

Quality



Errors obscure overlaps

- Reads are assembled by finding kmers shared in pair of reads
- High error rate requires very short seeds, increasing complexity and forming assembly hairballs

Current challenges in *de novo* plant genome sequencing and assembly
Schatz MC, Witkowski, McCombie, WR (2012) *Genome Biology*. 12:243

Coverage Statistics

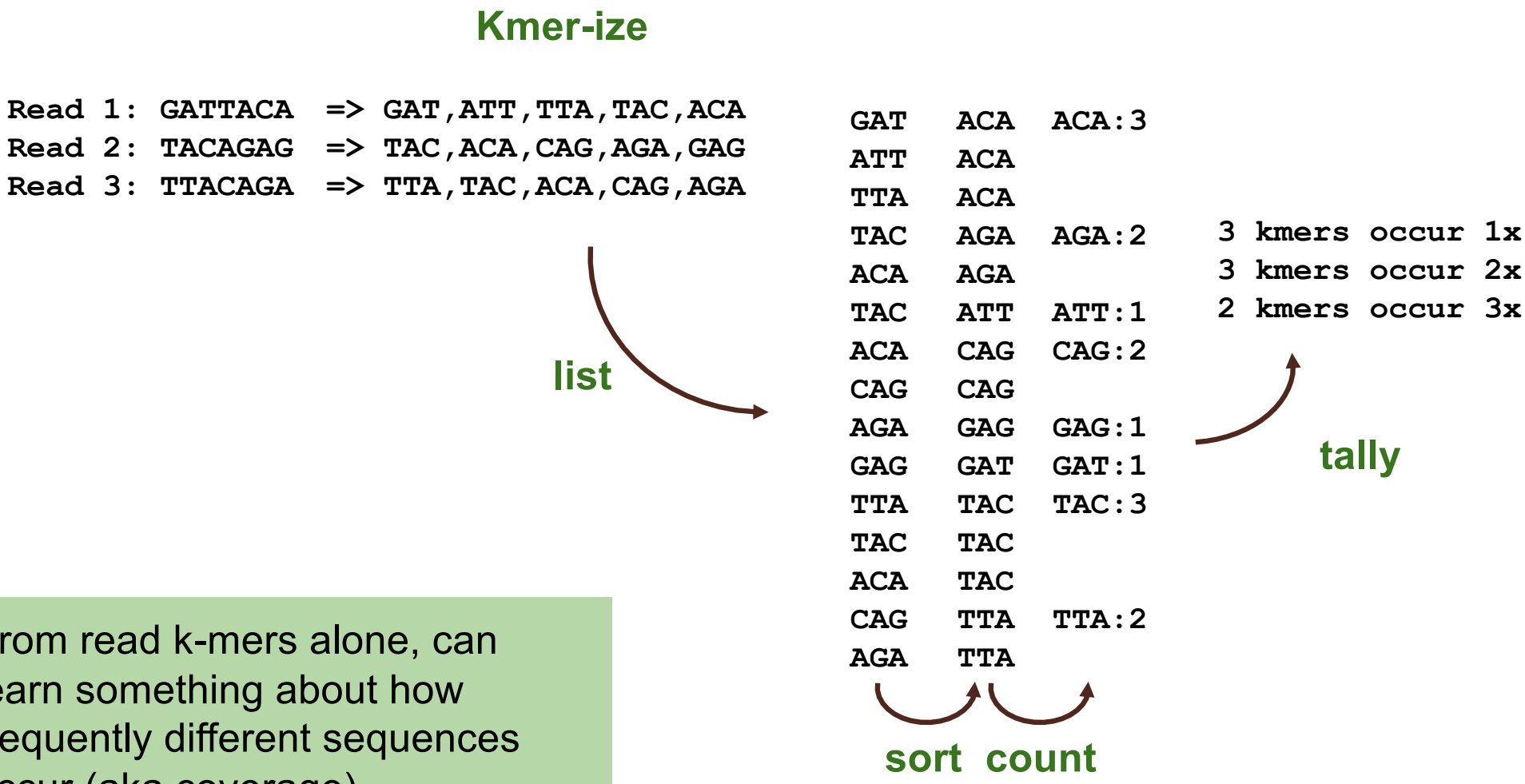
$$\text{sequencing\_coverage} = \frac{\text{total\_bases\_sequenced}}{\text{genome\_size}}$$

$$\text{genome\_size} = \frac{\text{total\_bases\_sequenced}}{\text{sequencing\_coverage}}$$

$$\text{genome\_size} = \frac{100\text{Gb}}{50\text{x}} = 2\text{Gb}$$

But how can you figure out
the coverage without a genome?

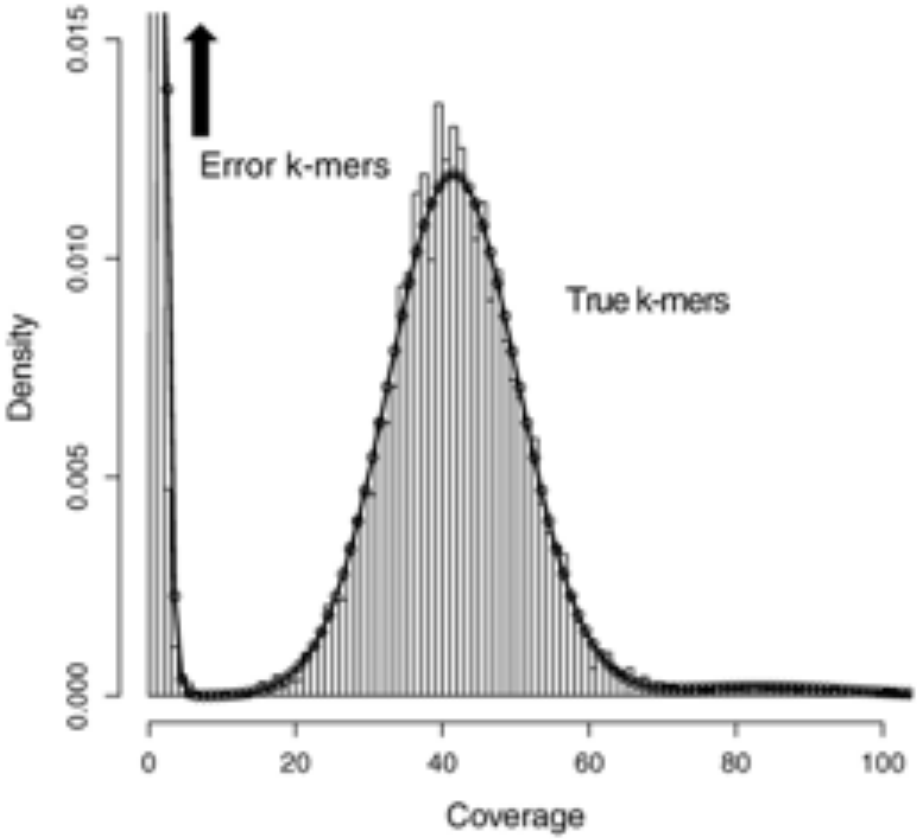
K-mer counting



From read k-mers alone, can learn something about how frequently different sequences occur (aka coverage)

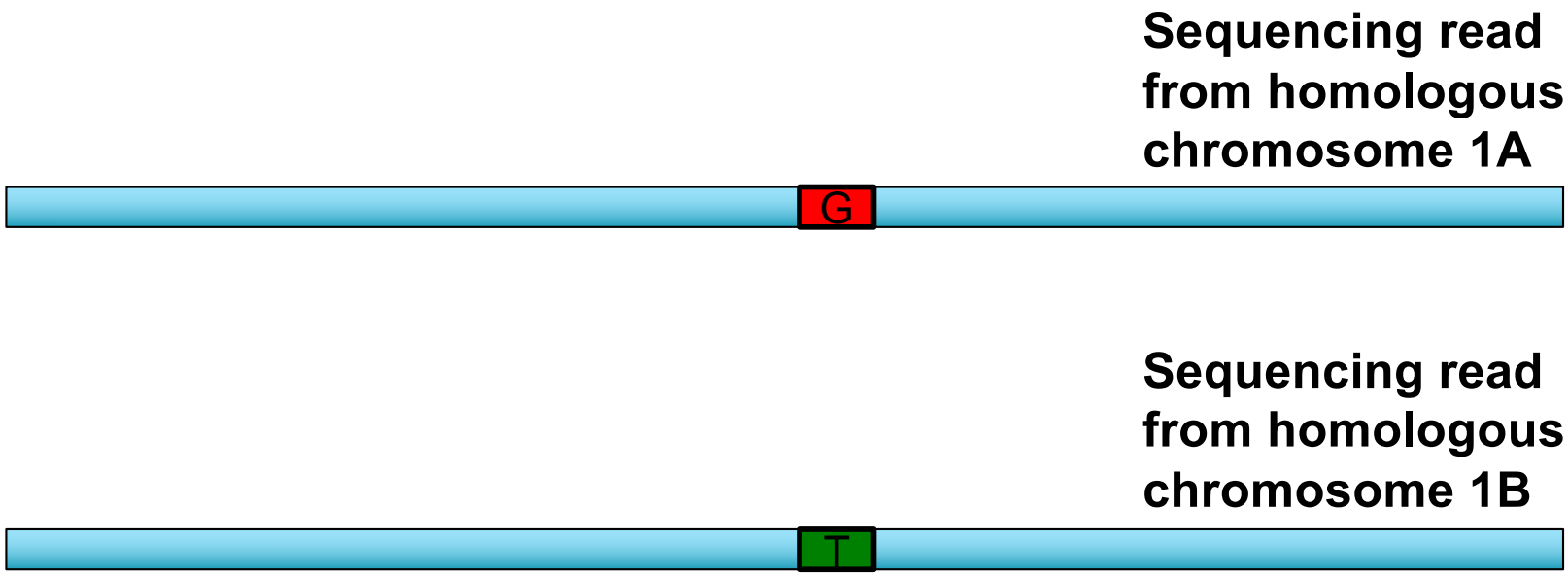
Fast to compute even over huge datasets

K-mer counting in real genomes

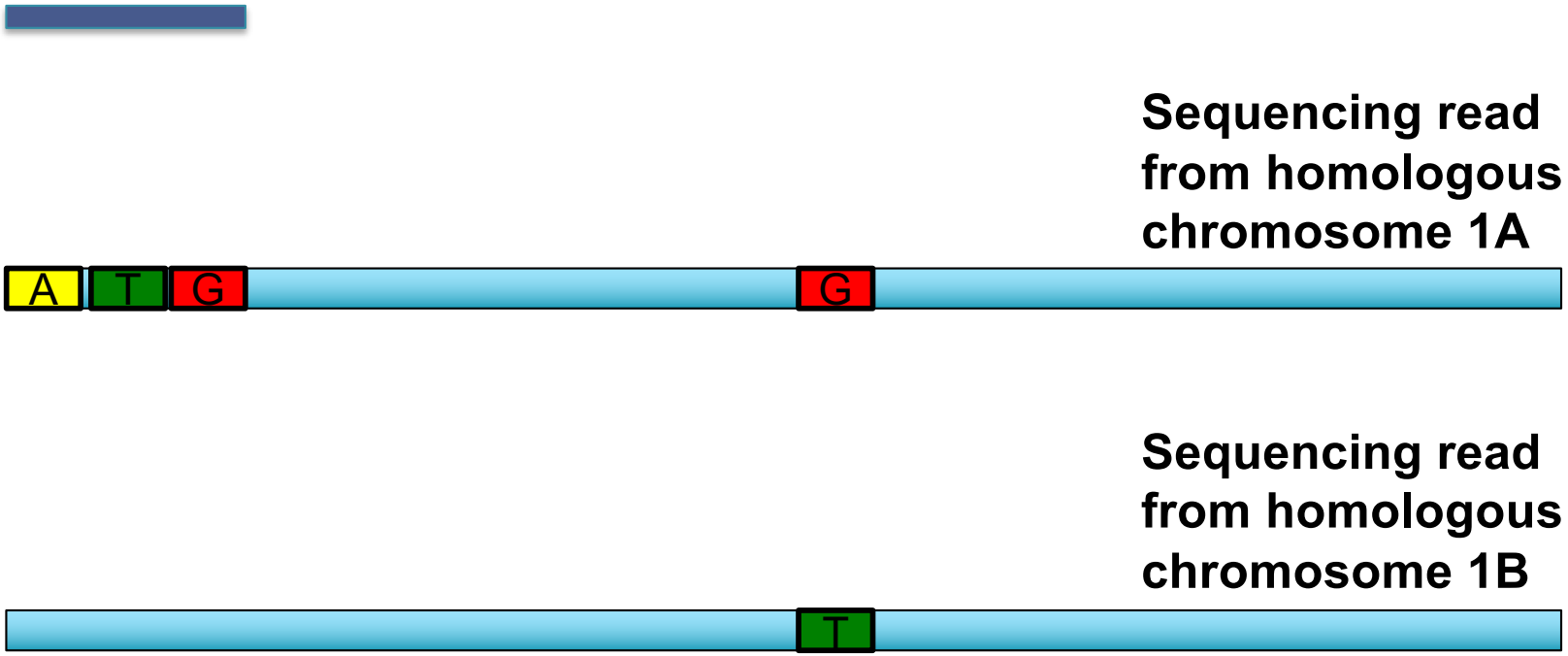


- The tally of k-mer counts in real genomes reveals the coverage distribution.
- Here we sequenced 120Gb of reads from a female human (haploid human genome size is 3Gb), and indeed we see a clear peak centered at 40x coverage
- There are also many kmers that only occur <5 times. These are from errors in the reads
- There are also kmers that occur many times (>>70 times). These are repeats in the genome

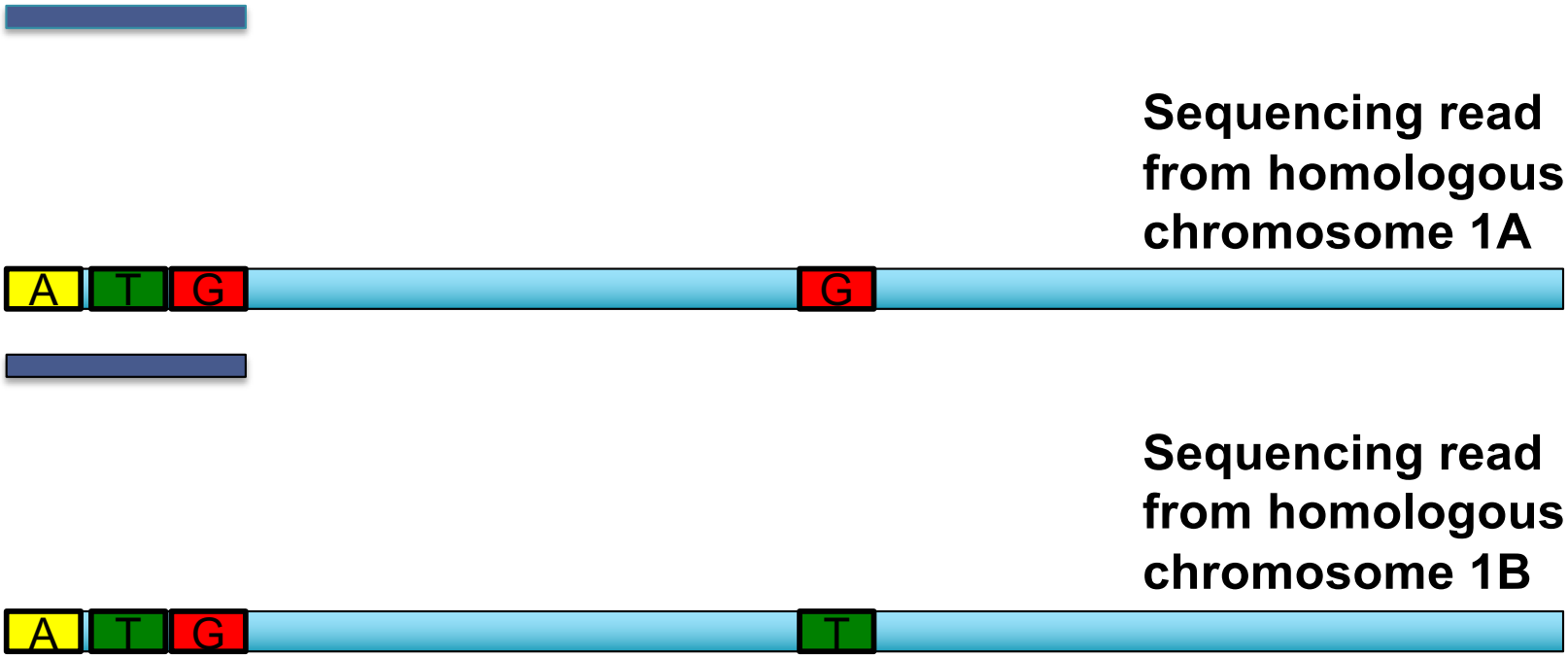
K-mer counting in heterozygous genomes



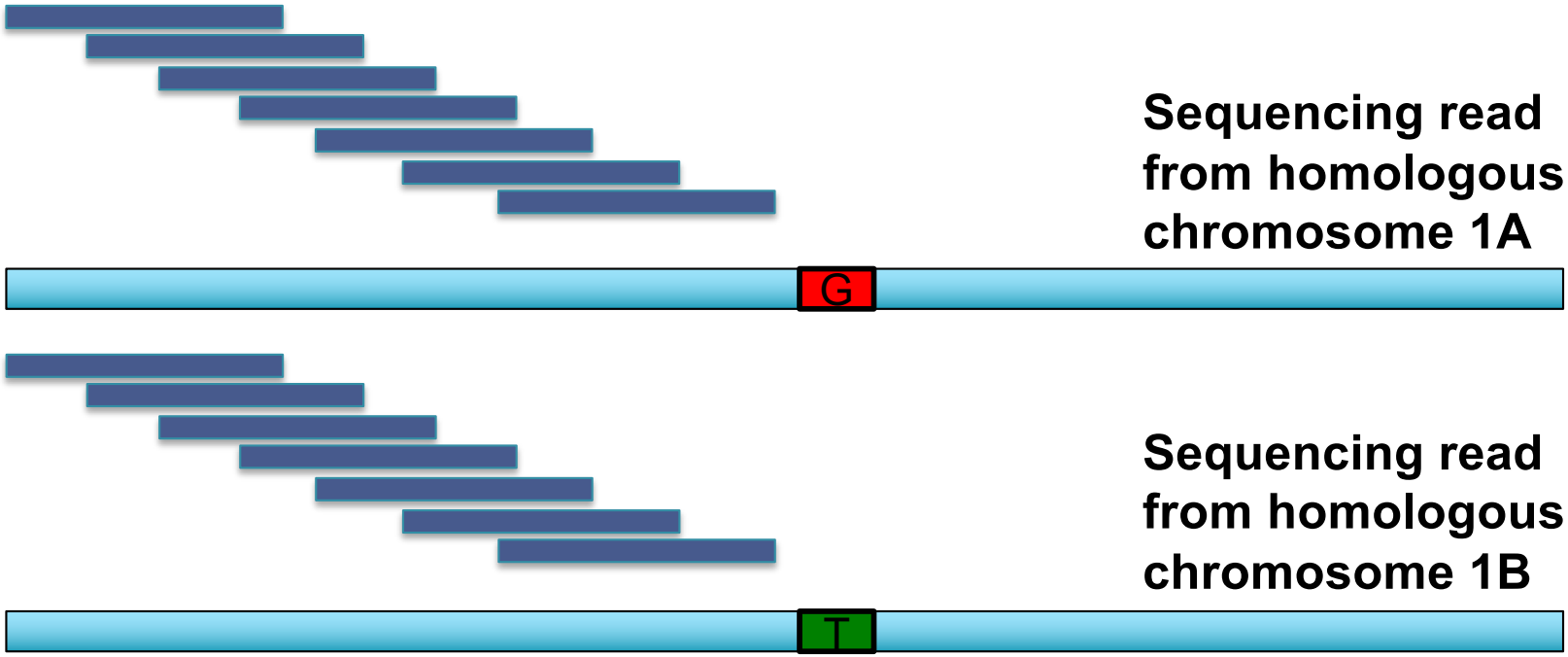
K-mer counting in heterozygous genomes



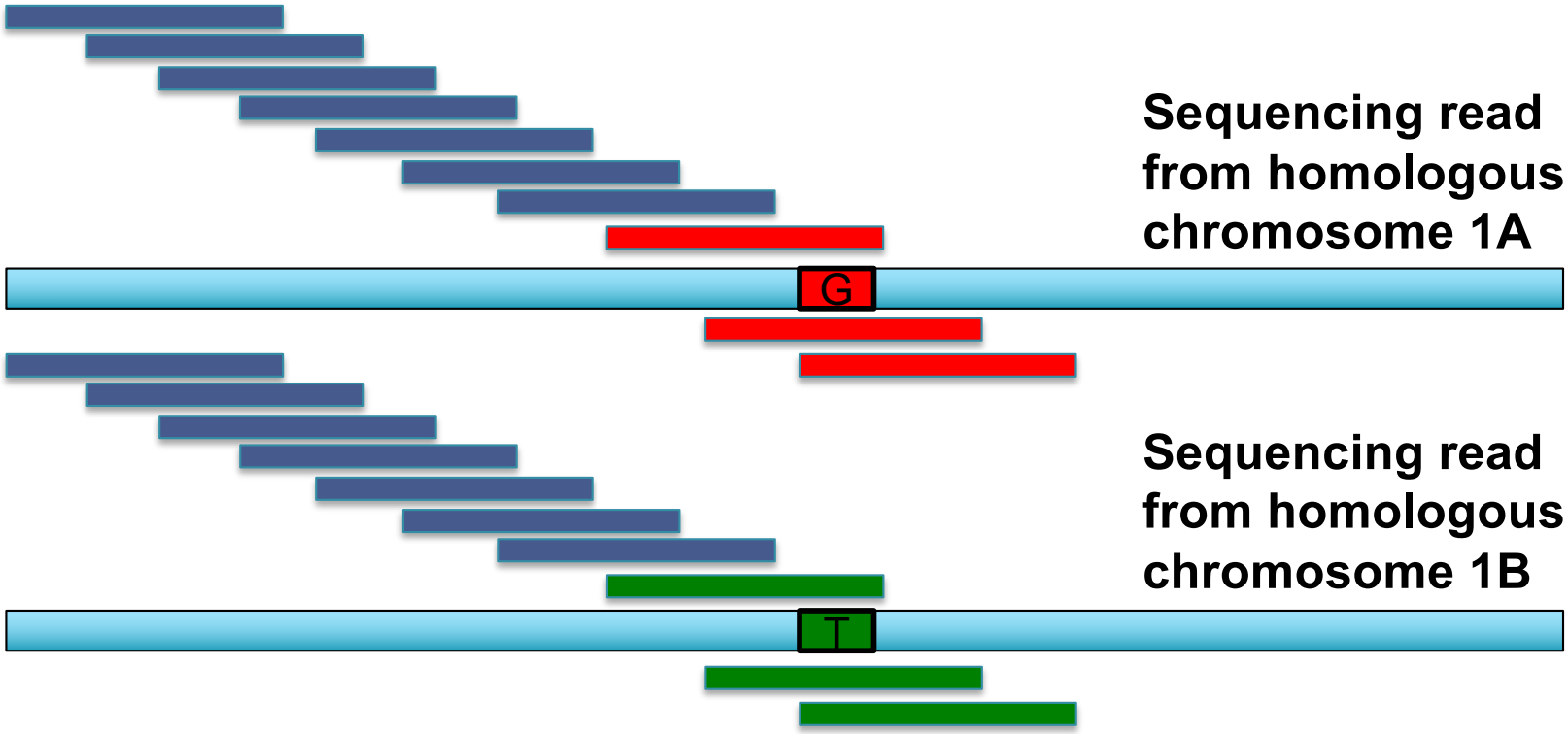
K-mer counting in heterozygous genomes



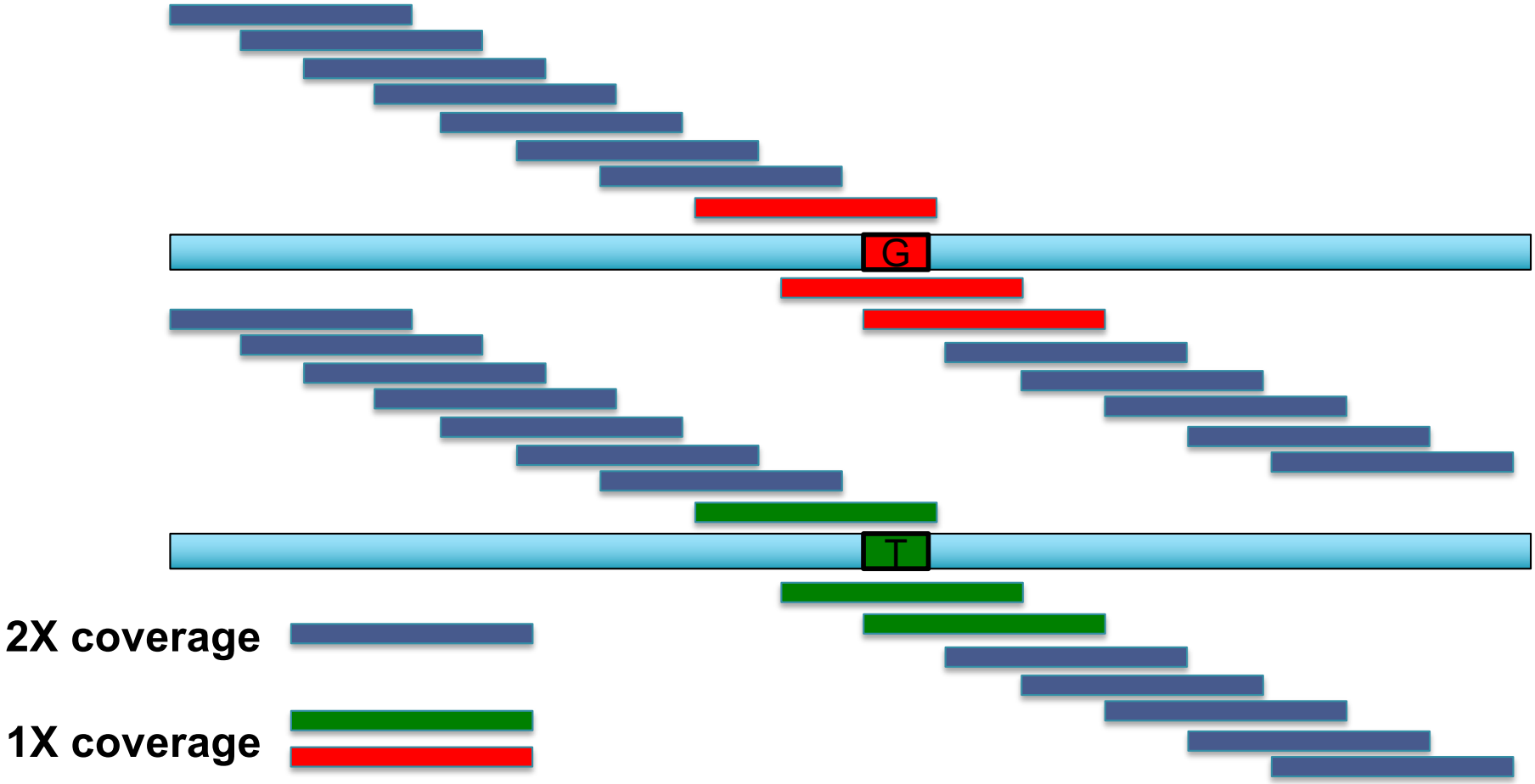
K-mer counting in heterozygous genomes



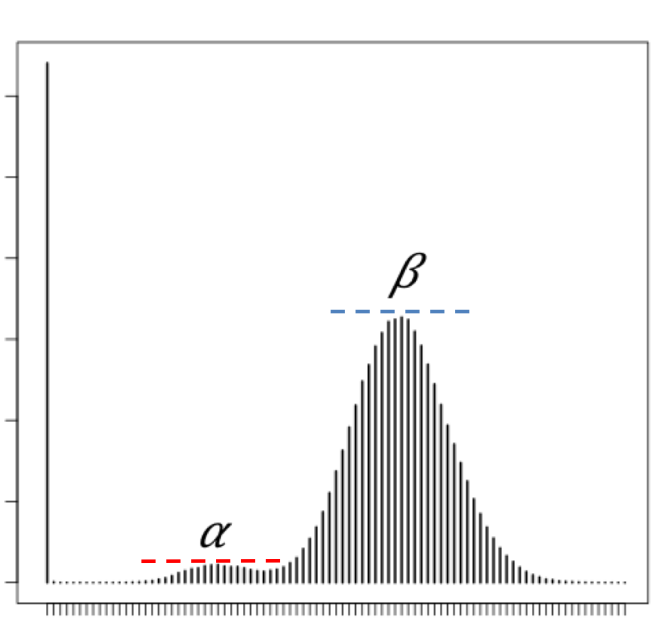
K-mer counting in heterozygous genomes



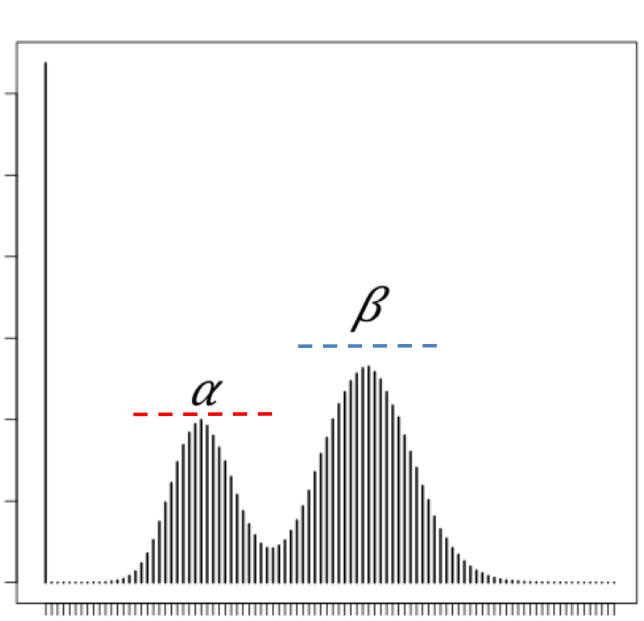
K-mer counting in heterozygous genomes



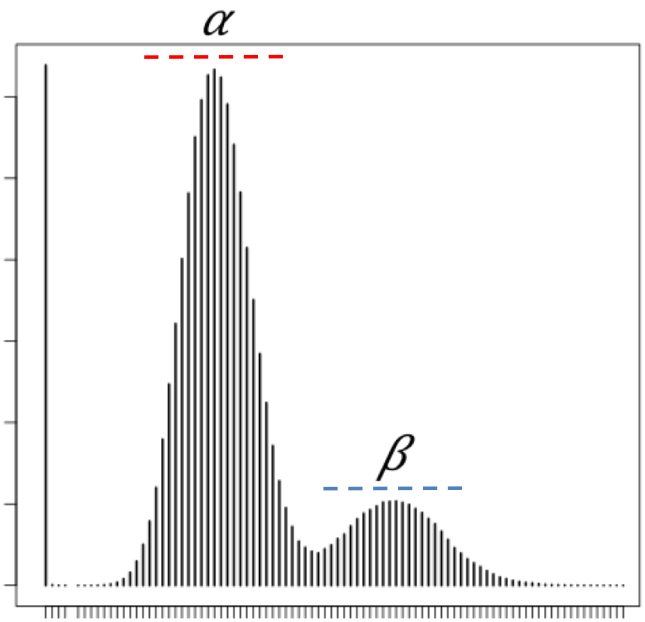
Heterozygous Kmer Profiles



0.1% heterozygosity



1% heterozygosity



5% heterozygosity

- ***Heterozygosity creates a characteristic "double-peak" in the Kmer profile***
  - Second peak at twice k-mer coverage as the first: heterozygous kmers average 50x coverage, homozygous kmers average 100x coverage

- ***Relative heights of the peaks is directly proportional to the heterozygosity rate***
  - The peaks are balanced at around 1.25% because each heterozygous SNP creates 2*k heterozygous kmers (typically k = 21)

GenomeScope Model

$$f(x) = G\Big\{\alpha NB(x, \lambda, \lambda/\rho) + \beta NB(x, 2\lambda, 2\lambda/\rho) + \gamma NB(x, 3\lambda, 3\lambda/\rho) + \delta NB(x, 4\lambda, 4\lambda/\rho)\Big\}$$

Analyze k-mer profiles using a mixture model of 4 negative binominal components

- Components centered at 1,2,3,4 * λ
- Four components capture heterozygous and homozygous unique (α,β) and 2 copy repeats (γ,δ). Higher order repeats do not contribute a significant number of kmers
- Negative binomial instead of Poisson to account for over dispersion observed in real data (especially PCR duplicates); variance modeled by ρ

$$\alpha = 2(1-d)(1-(1-r)^k) + 2d(1-(1-r)^k)^2 + 2d((1-r)^k)(1-(1-r)^k)$$

$$\beta = (1-d)((1-r)^k) + d(1-(1-r)^k)^2$$

$$\gamma = 2d((1-r)^k)(1-(1-r)^k)$$

$$\delta = d(1-r)^{2k}$$

k is the *k-mer* length

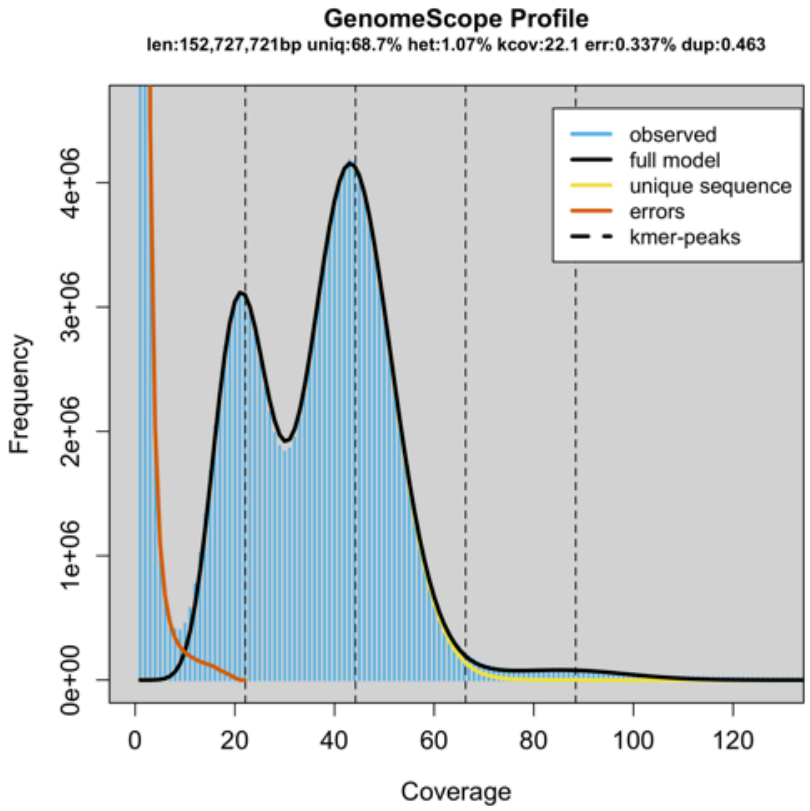
r is the rate of heterozygosity

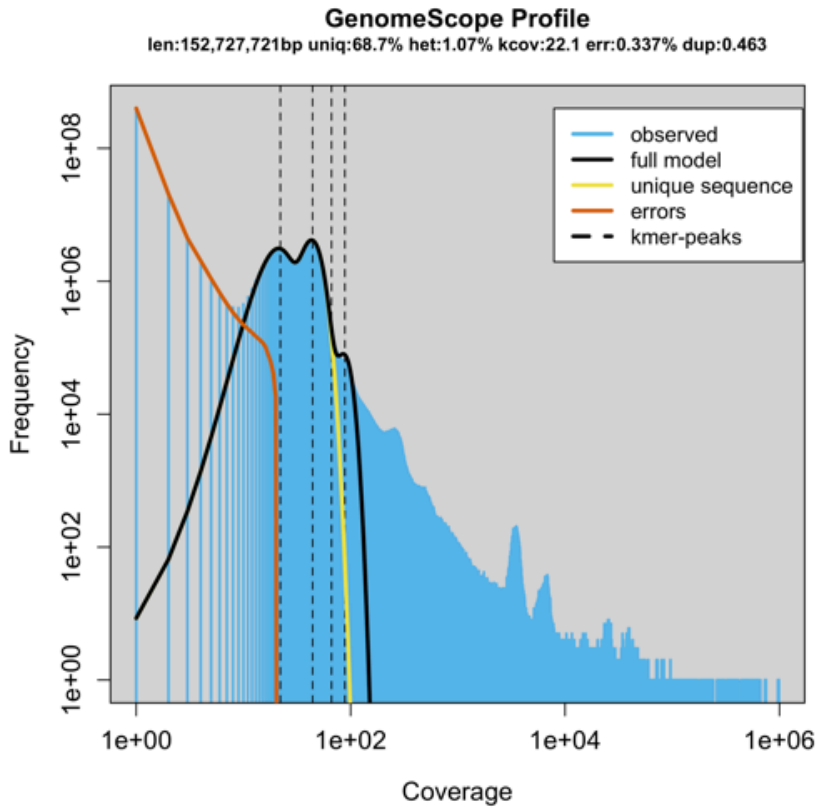
d represents the percentage of the genome that is two-copy repeat

Fit model with nls, infer rate of heterozygosity, genome size, unique/repetitive content, sequencing error rate, rate of PCR duplicates

GenomeScope: Fast genome analysis from short reads

http://genomescope.org





- Theoretical model agrees well with published results:
  - Rate of heterozygosity is higher than reported by other approaches but likely correct.
  - Genome size of plants inflated by organelle sequences (exclude very high freq. kmers)

Vurture, GW*, Sedlazeck FJ*, et al. (2017) *Bioinformatics*
Ranallo-Benavidez, TR. *et al.* (2029) *Nature Communication*

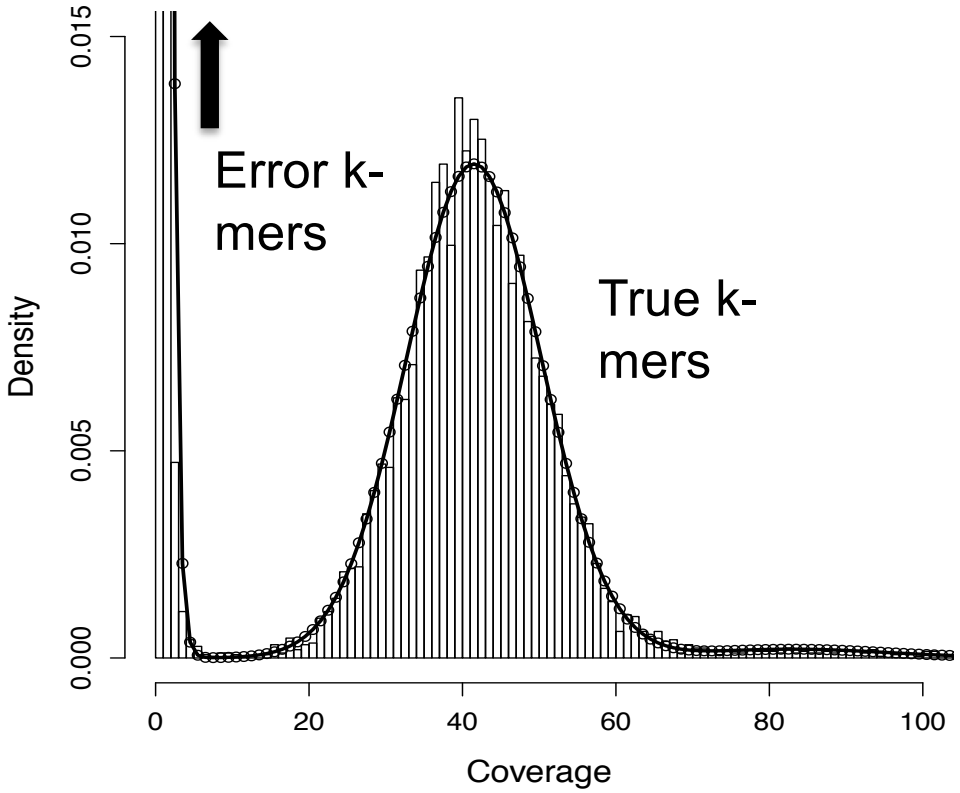
Error Correction with Quake

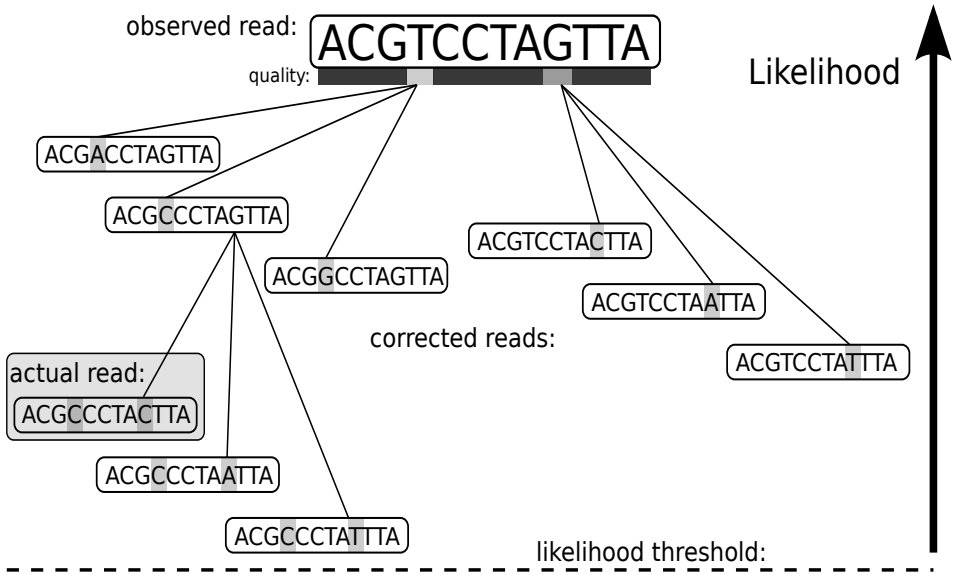
1. Count all "Q-mers" in reads

- Fit coverage distribution to mixture model of errors and regular coverage
- Automatically determines threshold for trusted k-mers

2. Correction Algorithm

- Considers editing erroneous kmers into trusted kmers in decreasing likelihood
- Includes quality values, nucleotide/nucleotide substitution rate



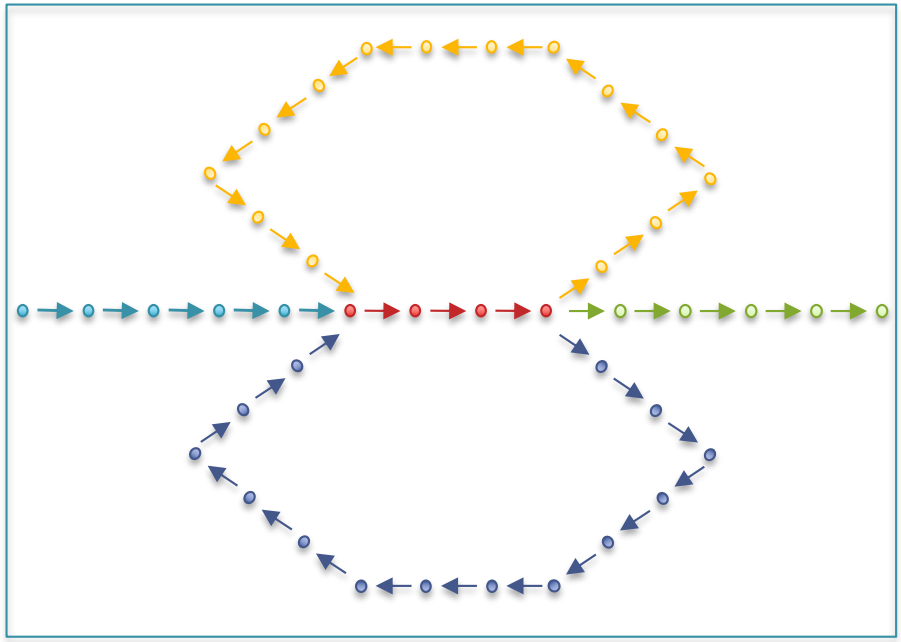


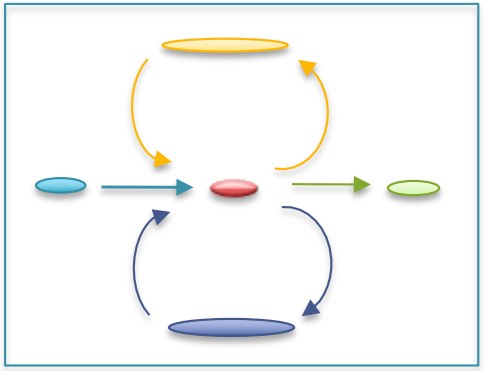
Quake: quality-aware detection and correction of sequencing reads.
Kelley, DR, Schatz, MC, Salzberg SL (2010) *Genome Biology*. 11:R116

Errors

Unitigging / Unipathing

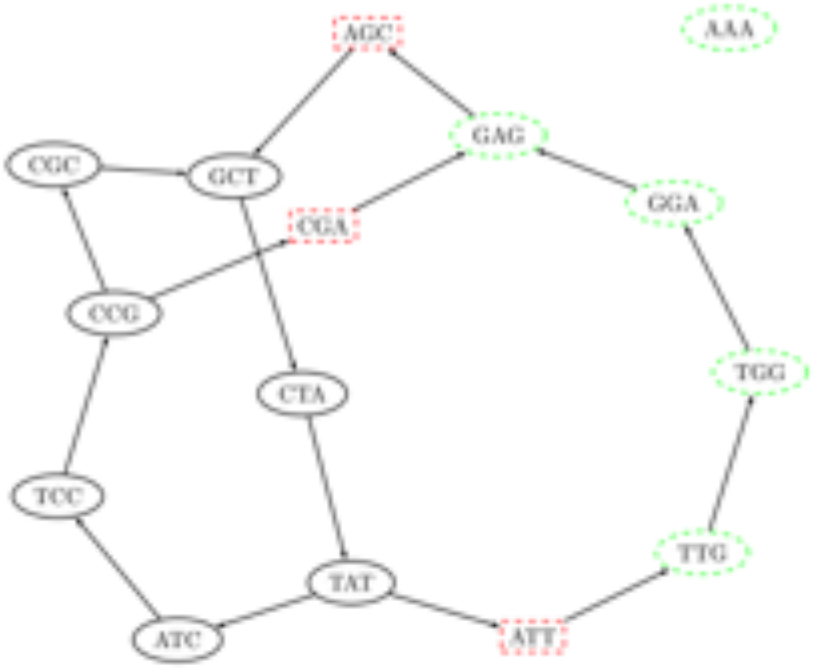
- After simplification and correction, compress graph down to its non-branching initial contigs
  - Aka "unitigs", "unipaths"



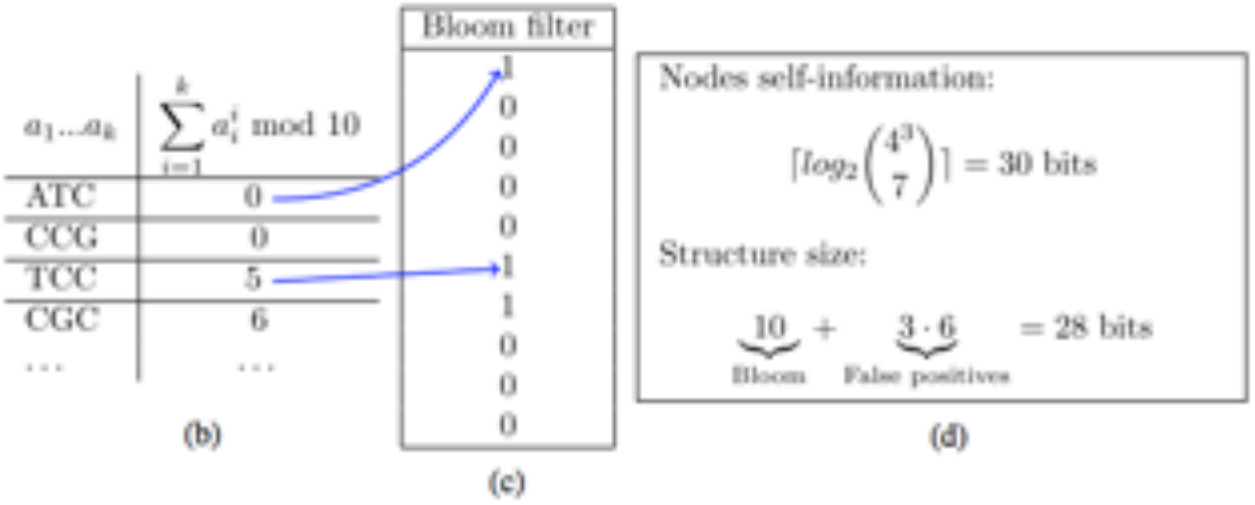


Why do contigs end?

(1) End of chromosome! ☺, (2) lack of coverage, (3) errors,
(4) heterozygosity and (5) repeats



(a)



(b) (c) (d)

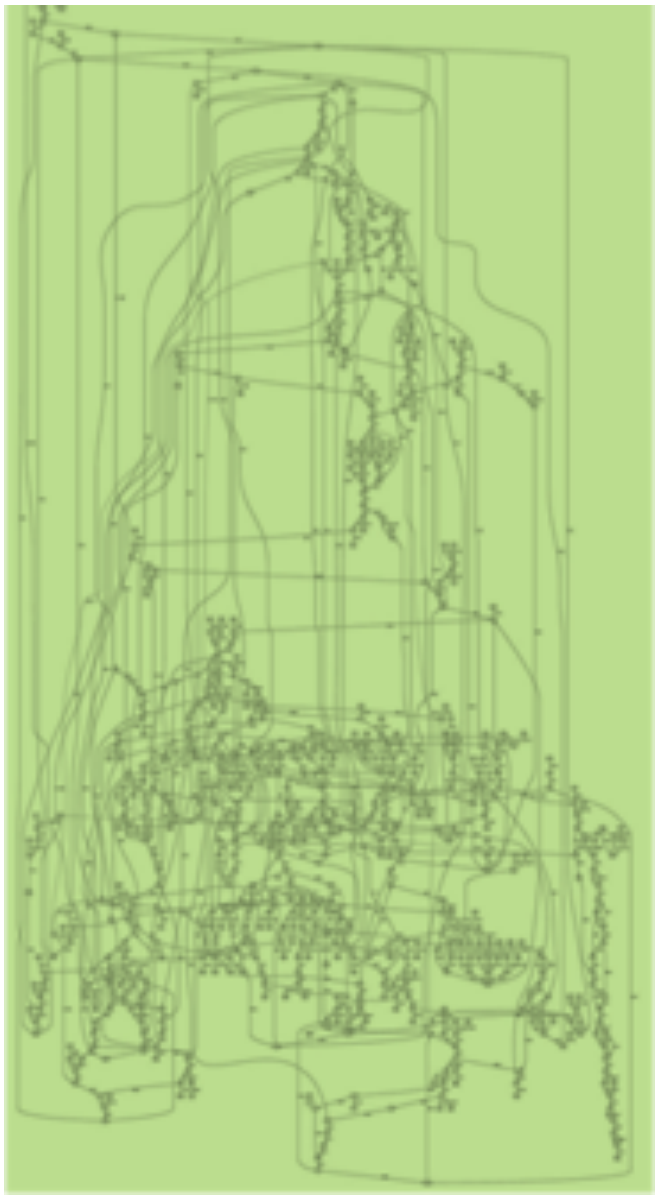
Table 2 de novo human genome (NA18507) assemblies

Method	Minia	C. & B.	ABySS	SOAPdenovo
Value of *k* chosen	27	27	27	25
Number of contigs (M)	3.49	7.69	4.35	-
Longest contig (kbp)	18.6	22.0	15.9	-
Contig N50 (bp)	1156	250	870	886
Sum (Gbp)	2.09	1.72	2.10	2.08
Nb of nodes/cores	1/1	1/8	21/168	1/16
Time (wall-clock, h)	23	50	15	33
Memory (sum of nodes, GB)	**5.7**	**32**	**336**	**140**

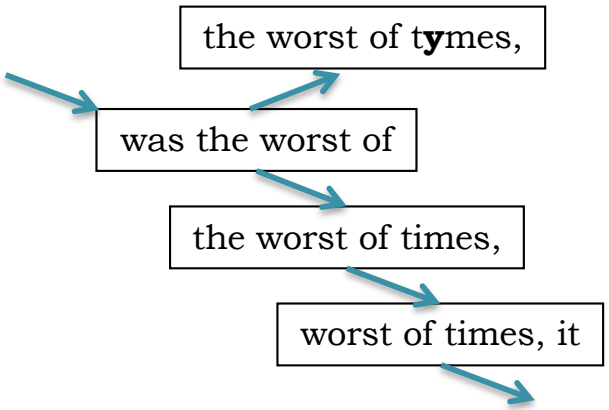
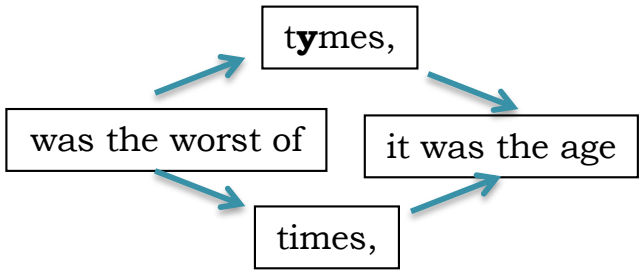
de novo human genome (NA18507) assemblies reported by our assembler (Minia), Conway and Bromage assembler [9], ABySS [8], and SOAPdenovo [7]. Contigs shorter than 100 bp were discarded. Assemblies were made without any pairing information.

Space-efficient and exact de Bruijn graph representation based on a Bloom filter
Chikhi and Rizk (2013) *Algorithms for Molecular Biology*. 8:22

Errors in the graph



(Chaisson, 2009)

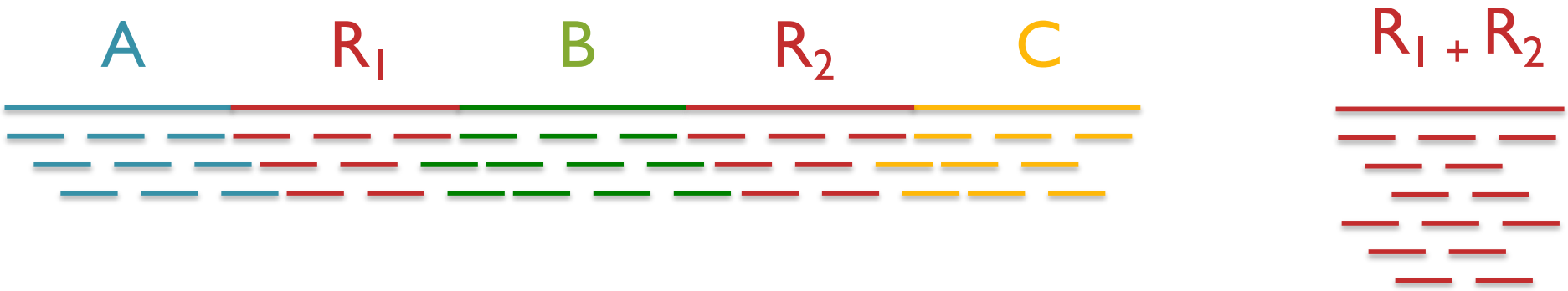
Clip Tips	Pop Bubbles
was the worst of times, was the worst of t**y**mes, the worst of times, it	was the worst of times, was the worst of t**y**mes, times, it was the age t**y**mes, it was the age
	

Repetitive regions

Repeat Type	Definition / Example	Prevalence
Low-complexity DNA / Microsatellites	$(b_1b_2\ldots b_k)^N$ where $1 \leq k \leq 6$ CACACACACACACACACACA	2%
SINEs (Short Interspersed Nuclear Elements)	*Alu* sequence (~280 bp) Mariner elements (~80 bp)	13%
LINEs (Long Interspersed Nuclear Elements)	~500 – 5,000 bp	21%
LTR (long terminal repeat) retrotransposons	Ty1-copia, Ty3-gypsy, Pao-BEL (~100 – 5,000 bp)	8%
Other DNA transposons		3%
Gene families & segmental duplications		4%

- Over 50% of mammalian genomes are repetitive
  - Large plant genomes tend to be even worse
  - Wheat: 16 Gbp; Pine: 24 Gbp

Repeats and Coverage Statistics



- If *n* reads are a uniform random sample of the genome of length *G*, we expect $k=n\Delta/G$ reads to start in a region of length Δ.
  - If we see many more reads than k (if the arrival rate is > A) , it is likely to be a collapsed repeat

$$\Pr(X-copy)=\binom{n}{k}\left(\frac{X\Delta}{G}\right)^{k}\left(\frac{G-X\Delta}{G}\right)^{n-k}$$

$$A(\Delta,k)=\ln\left(\frac{\Pr(1-copy)}{\Pr(2-copy)}\right)=\ln\left(\frac{\frac{(\Delta n/G)^{k}}{k!}e^{\frac{-\Delta n}{G}}}{\frac{(2\Delta n/G)^{k}}{k!}e^{\frac{-2\Delta n}{G}}}\right)=\frac{n\Delta}{G}-k\ln 2$$

The fragment assembly string graph

Myers, EW (2005) Bioinformatics. 21(suppl 2): ii79-85.

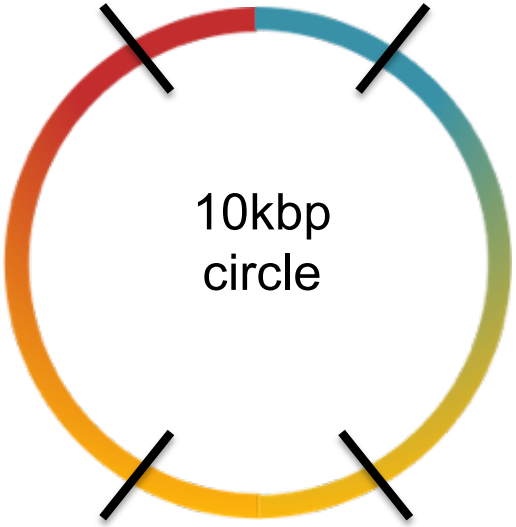
Paired-end and Mate-pairs

Paired-end sequencing

- Read one end of the molecule, flip, and read the other end
- Generate pair of reads separated by up to 500bp with inward orientation

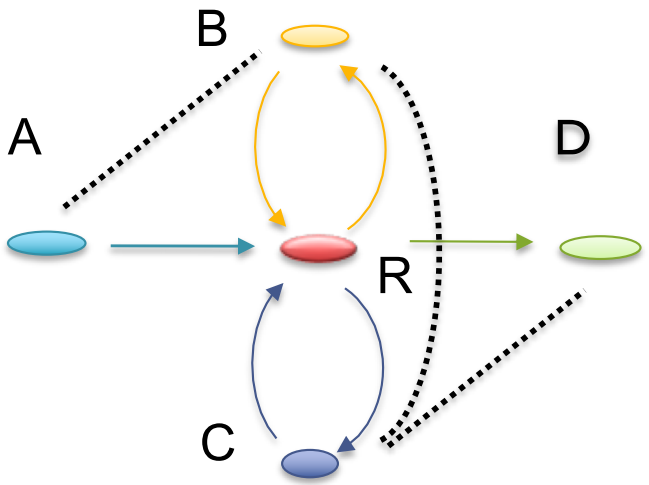
Mate-pair sequencing

- Circularize long molecules (1-10kbp), shear into fragments, & sequence
- Mate failures create short paired-end reads

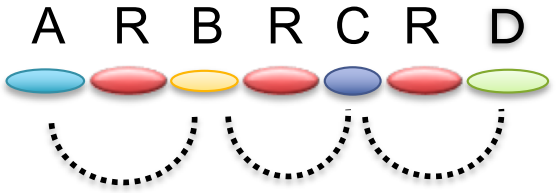


Scaffolding

- Initial contigs (*aka* unipaths, unitigs) terminate at
  - *Coverage gaps*: especially extreme GC
  - *Conflicts*: errors, repeat boundaries



- Use mate-pairs to resolve correct order through assembly graph
  - Place sequence to satisfy the mate constraints
  - Mates through repeat nodes are tangled



- Final scaffold may have internal gaps called sequencing gaps
  - We know the order, orientation, and spacing, but just not the bases. Fill with Ns instead

Why do scaffolds end?

Assemblathon Results

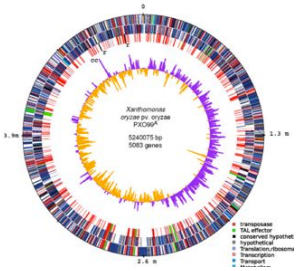
ID	Overall	CPNG50	SPNG50	Struct.	CC50	Subs.	Copy. Num.	Cov. Tot.	Cov. CDS
BGI	36	★					☆	★	☆
Broad	37	☆	★	★	★				
WTSI-S	46		★	☆	★	★			
CSHL	52	★							☆
BCCGSC	53							☆	★
DOEJGI	56		☆	★	☆	★			
RHUL	58								

- SOAPdenovo and ALLPATHS came out neck-and-neck followed closely behind by SGA, Celera Assembler, ABySS
- My recommendation for "typical" short read assembly is to use ALLPATHS or Spades

Assemblathon 1: A competitive assessment of de novo short read assembly methods

Earl et al. (2011) Genome Research. 21: 2224-2241

Assembly Summary



Assembly quality depends on

1. ***Coverage***: low coverage is mathematically hopeless
2. ***Repeat composition***: high repeat content is challenging
3. ***Read length***: longer reads help resolve repeats
4. ***Error rate***: errors reduce coverage, obscure true overlaps

- Assembly is a hierarchical, starting from individual reads, build high confidence contigs/unitigs, incorporate the mates to build scaffolds
  - Extensive error correction is the key to getting the best assembly possible from a given data set

- Watch out for collapsed repeats & other misassemblies
  - Globally/Locally reassemble data from scratch with better parameters & stitch the 2 assemblies together

Outline

1. ***Assembly theory***
   - Assembly by analogy

2. ***Practical Issues***
   - Coverage, read length, errors, and repeats

3. ***Whole Genome Alignment***
   - MUMmer recommended

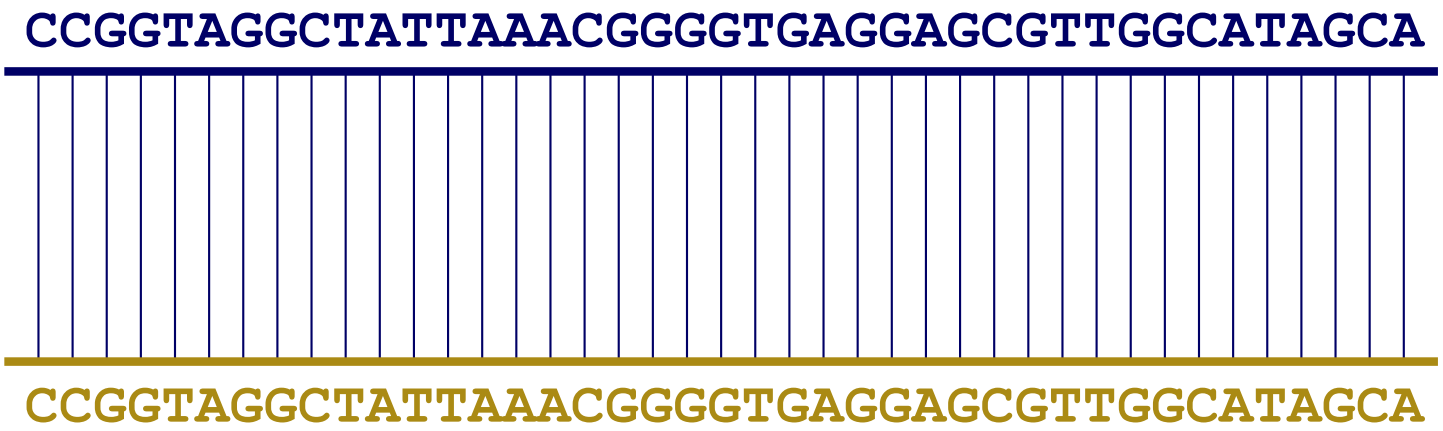
4. ***Next-next-gen Assembly***
   - Canu: recommended for PacBio/ONT project

Whole Genome Alignment with MUMmer

Slides Courtesy of Adam M. Phillippy
NHGRI

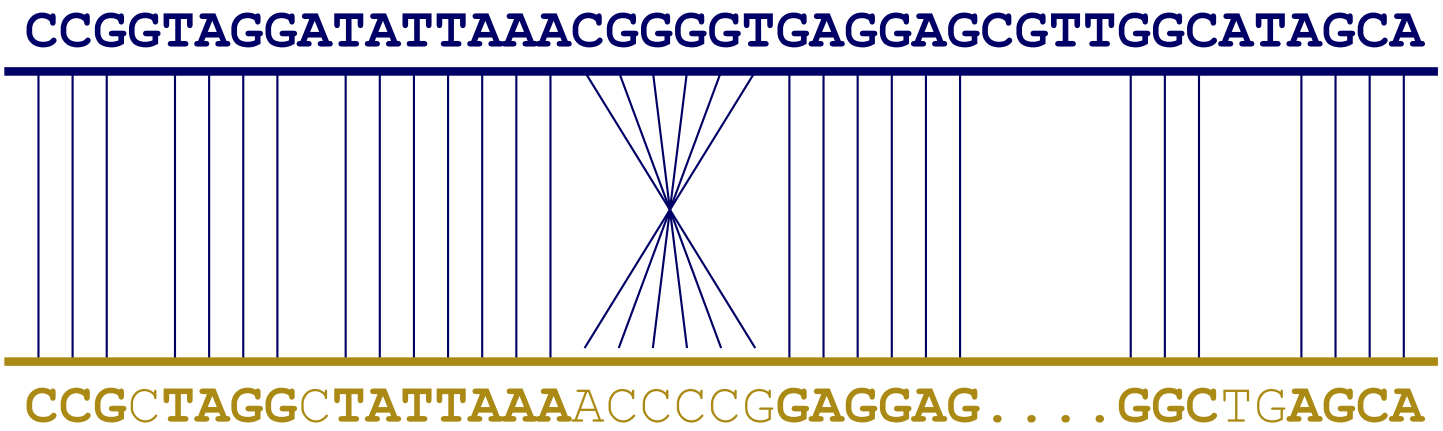
Goal of WGA

- For two genomes, *A* and *B*, find a mapping from each position in *A* to its corresponding position in *B*



Not so fast...

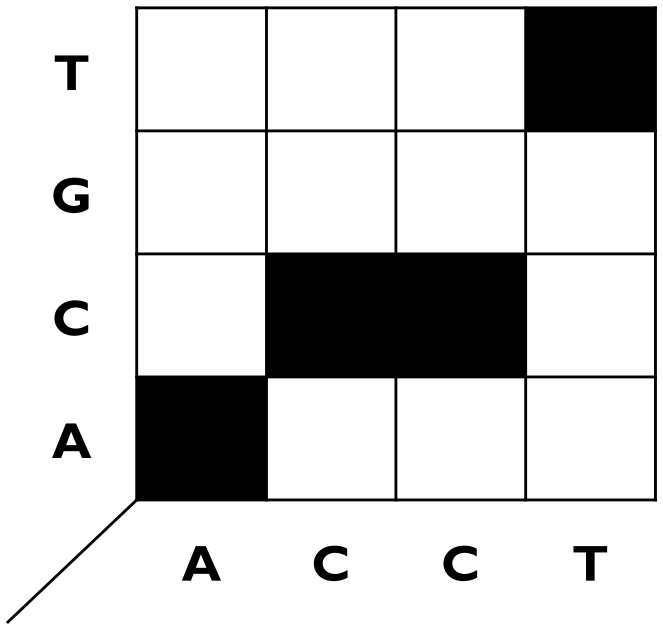
- Genome *A* may have insertions, deletions, translocations, inversions, duplications or SNPs with respect to *B* (sometimes all of the above)



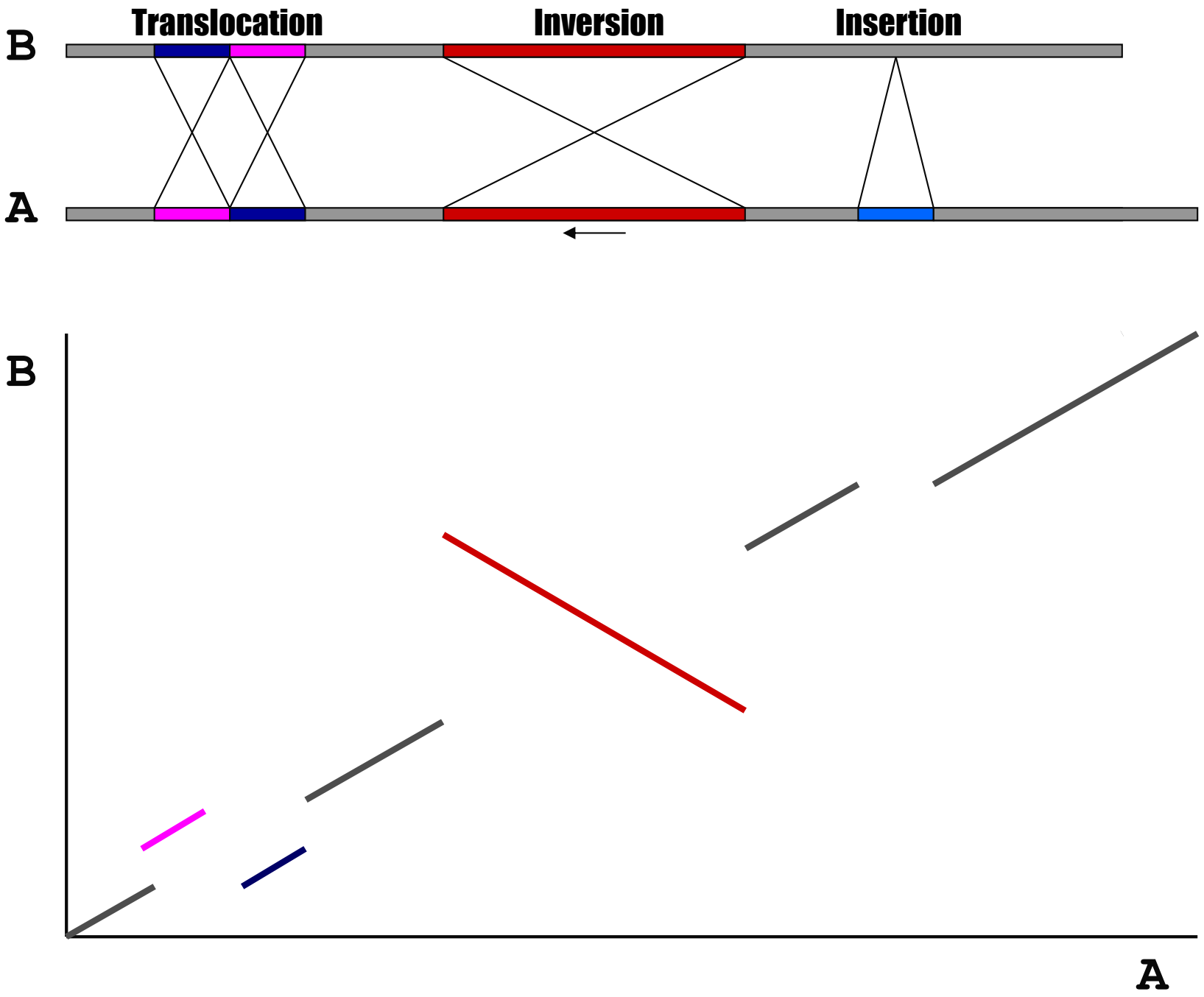
WGA visualization

- How can we visualize *whole* genome alignments?

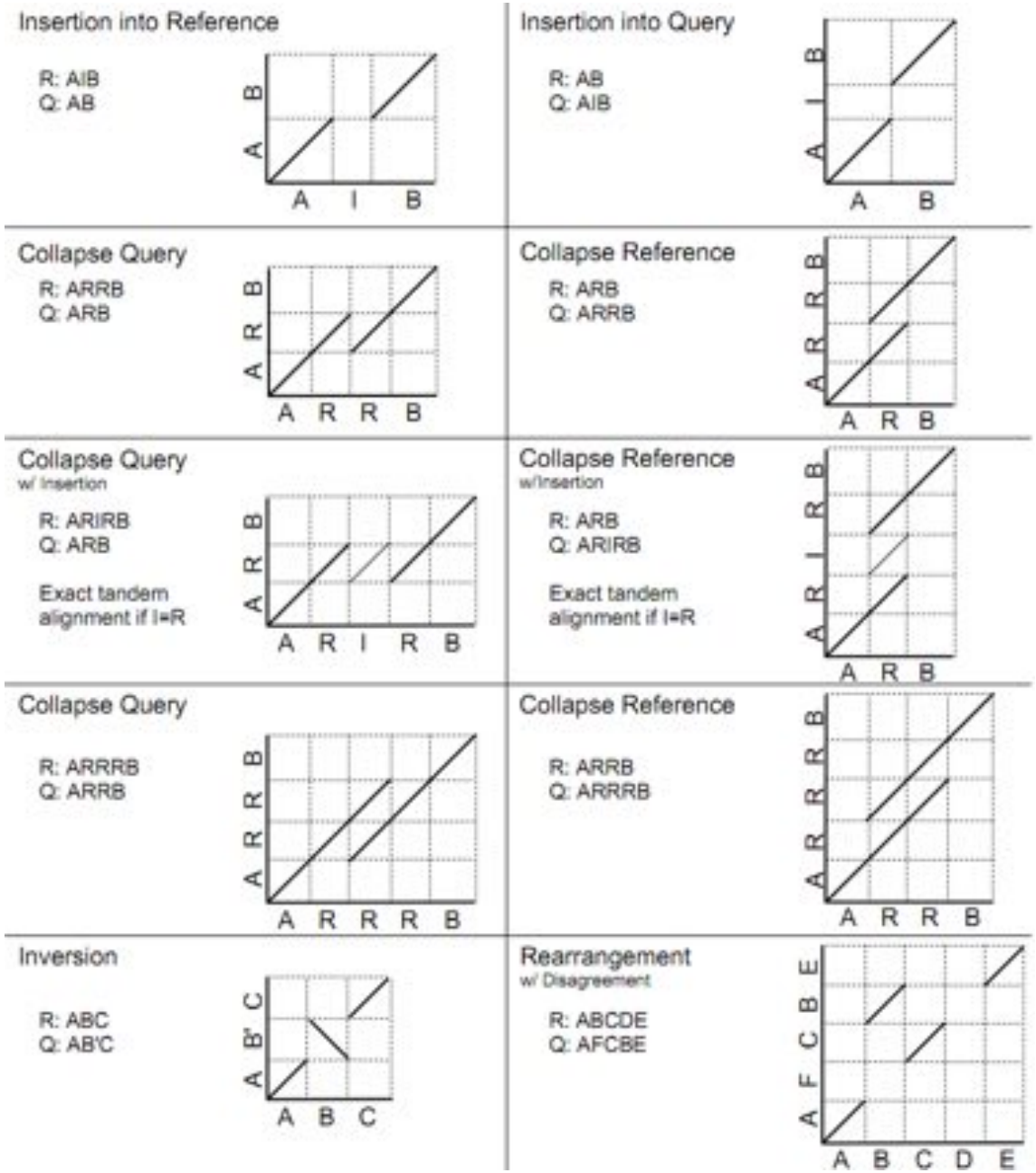
- With an alignment dot plot
  - *N* x *M* matrix
    - Let *i* = position in genome *A*
    - Let *j* = position in genome *B*
    - Fill cell *(i,j)* if A_i shows similarity to B_j



  - A perfect alignment between *A* and *B* would completely fill the positive diagonal

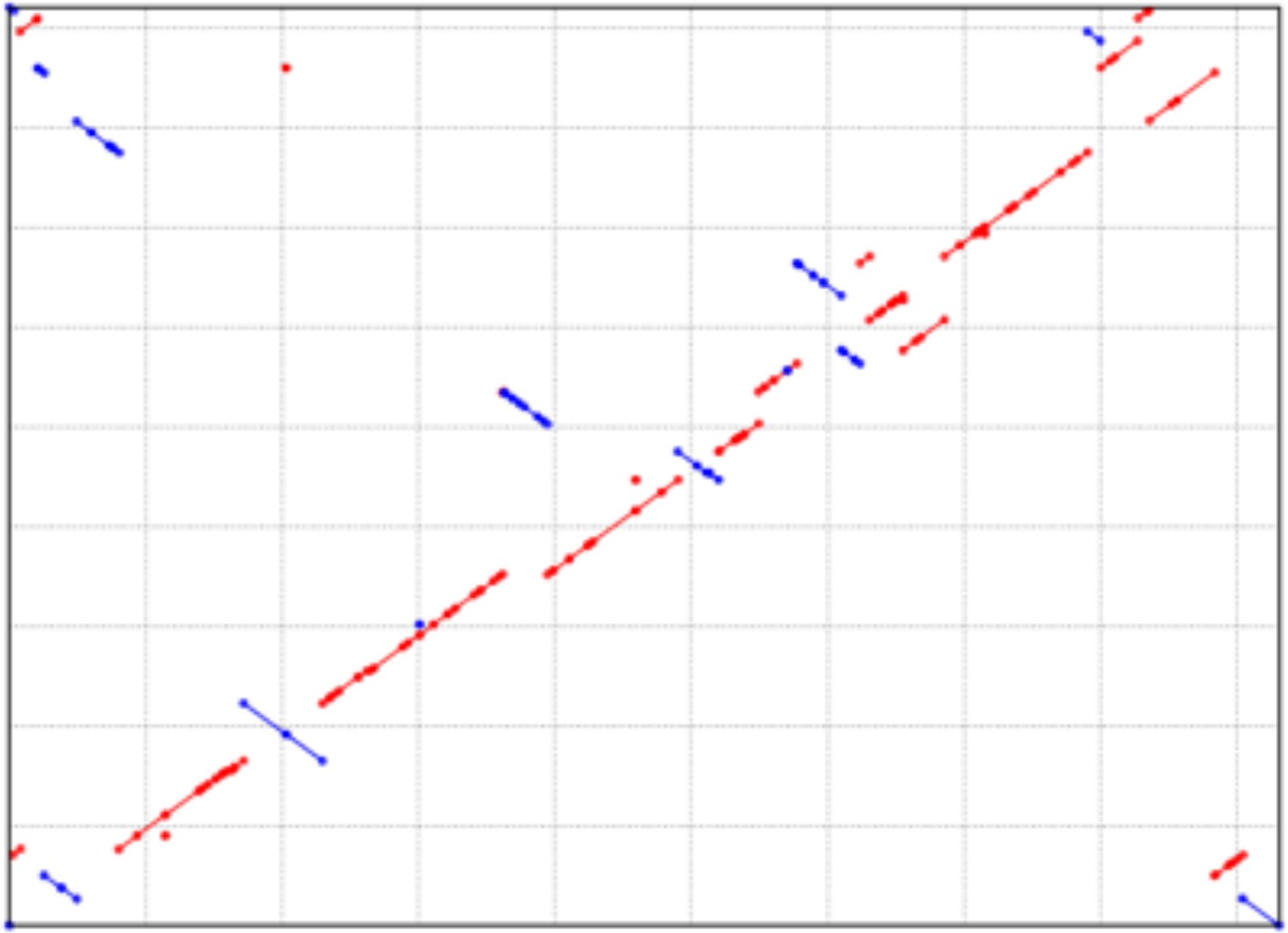

Translocation
Inversion
Insertion
B
A
B
A

SV Types



- Different structural variation types / misassemblies will be apparent by their pattern of breakpoints
- Most breakpoints will be at or near repeats
- Things quickly get complicated in real genomes

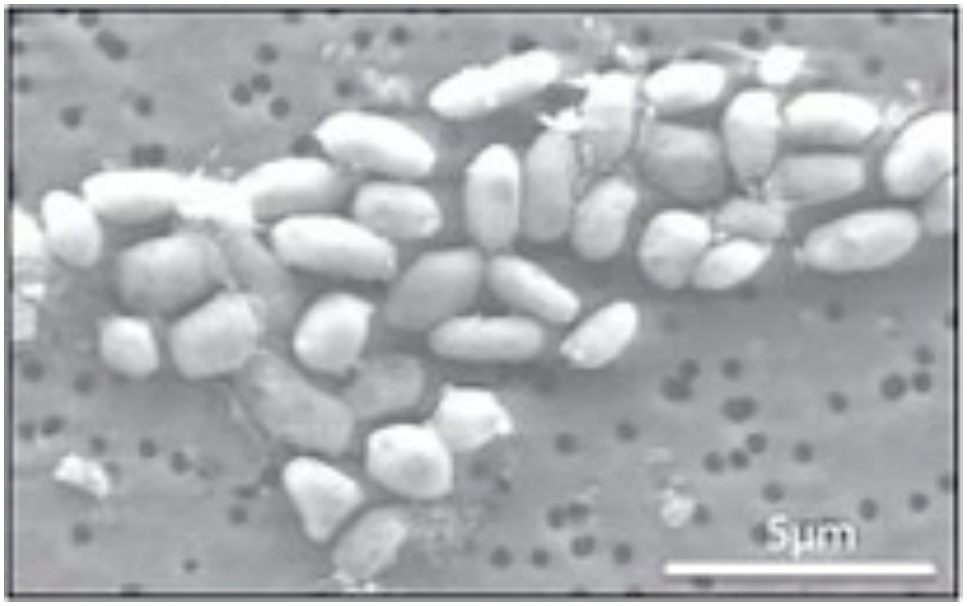
http://mummer.sf.net/manual/AlignmentTypes.pdf



Alignment of 2 strains of *Y. pestis*

http://mummer.sourceforge.net/manual/

Halomonas sp. GFAJ-1



Library 1: Fragment

Avg Read length: 100bp

Insert length: 180bp

Library 2: Short jump

Avg Read length: 50bp

Insert length: 2000bp

A Bacterium That Can Grow by Using Arsenic Instead of Phosphorus
Wolfe-Simon et al (2010) *Science. 332(6034)1163-1166.*

Digital Information Storage

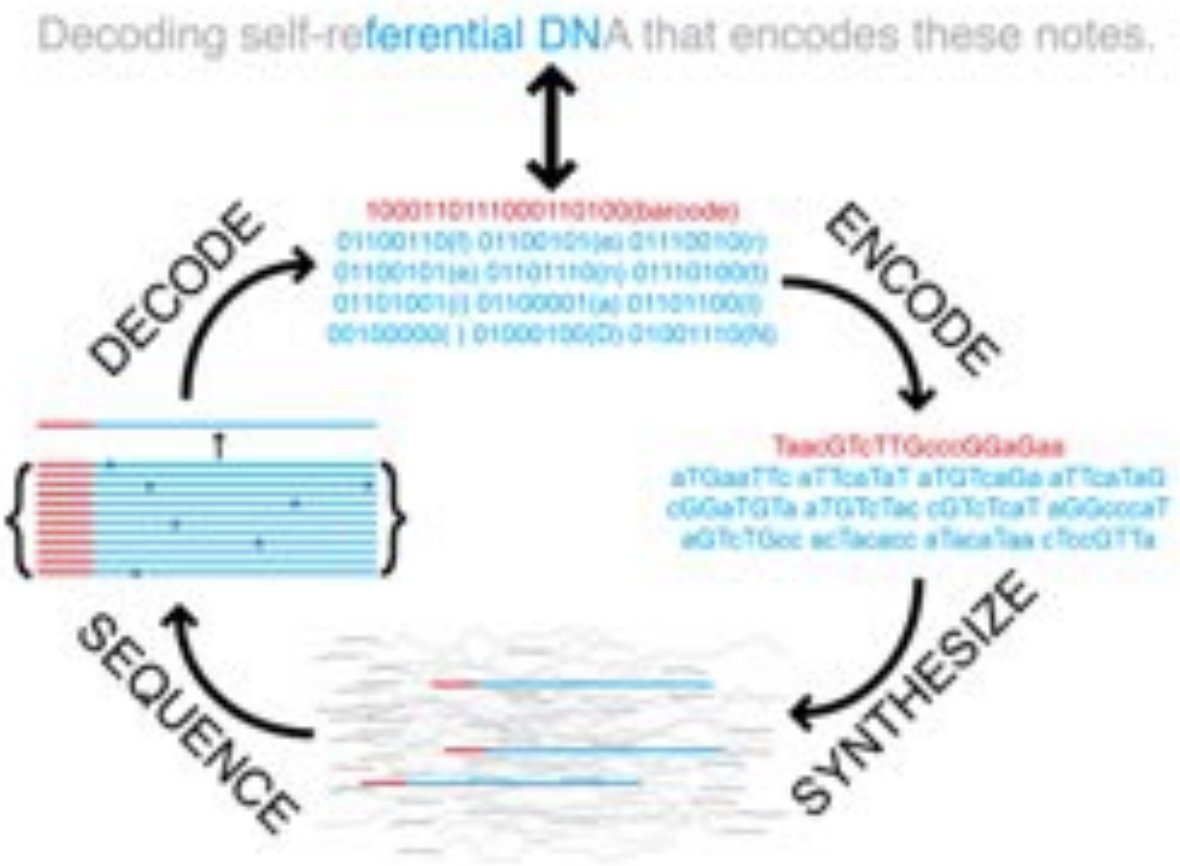


Fig. S1. Schematic of DNA information storage.

Encoding/decoding algorithm implemented in dna-encode.pl from David Dooling.

Next-generation Digital Information Storage in DNA
Church et al (2010) *Science*. 337(6102)1628

Genome Assembly Lab
Due September 18 @ 11:59pm

1. ***Initialize Tools***
2. ***Download Reference Genome & Reads***
3. ***Decode the secret message***
    1. *Estimate coverage, check read quality*
    2. *Check kmer distribution*
    3. *Assemble the reads with spades*
    4. *Align to reference with MUMmer*
    5. *Extract foreign sequence*
    6. *dna-decode.py -d*

http://bxlab.github.io/cmdb-lab/2020/

Find and decode

```
nucmer -maxmatch ref.fasta \
   default/ASSEMBLIES/test/final.contigs.fasta
   -maxmatch     Find maximal exact matches (MEMs) without repeat filtering
   -p refctg     Set the output prefix for delta file

show-coords -rclo out.delta
   -r            Sort alignments by reference position
   -c            Show percent coverage
   -l            Show sequence lengths
   -o            Annotate each alignment with BEGIN/END/CONTAINS

samtools faidx default/ASSEMBLIES/test/final.contigs.fasta
   Index the fasta file

samtools faidx default/ASSEMBLIES/test/final.contigs.fasta \
    contig_XXX:YYY-ZZZ > msg.fa

./dna-decode.py -d -input msg.fa
```

***** Note you may need to reverse complement the extracted sequence depending on the orientation of the alignments *****

See manual at http://mummer.sourceforge.net/manual

Next Steps

1. Reflect on the magic and power of DNA ☺
2. Check out the course webpage
3. Work on Assembly Assignment
    1. Set up Dropbox for yourself!
    2. Get comfortable on the command line