Genome Sequencing & Assembly

Michael Schatz

Feb 24, 2016
Fundamentals of Genome Informatics

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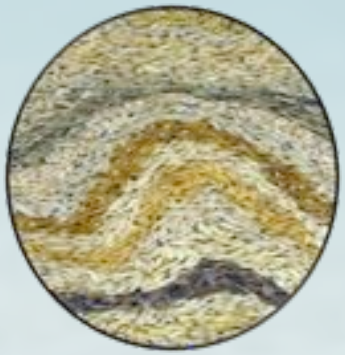
Schatzlab Overview

Human Genetics

Role of mutations in disease

Narzisi *et al.* (2015)
Iossifov *et al.* (2014)



Plant Biology

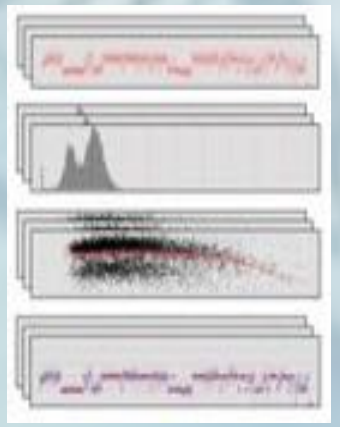
Genomes & Transcriptomes

Ming *et al.* (2015)
Schatz *et al.* (2014)

Algorithmics & Systems Research

Ultra-large scale biocomputing

Stevens *et al.* (2015)
Marcus *et al.* (2014)



Single Cell & Single Molecule

CNVs, SVs, & Cell Phylogenetics

Garvin *et al.* (2015)
Goodwin *et al.* (2015)

Genomics Across the Tree of Life

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

Outline

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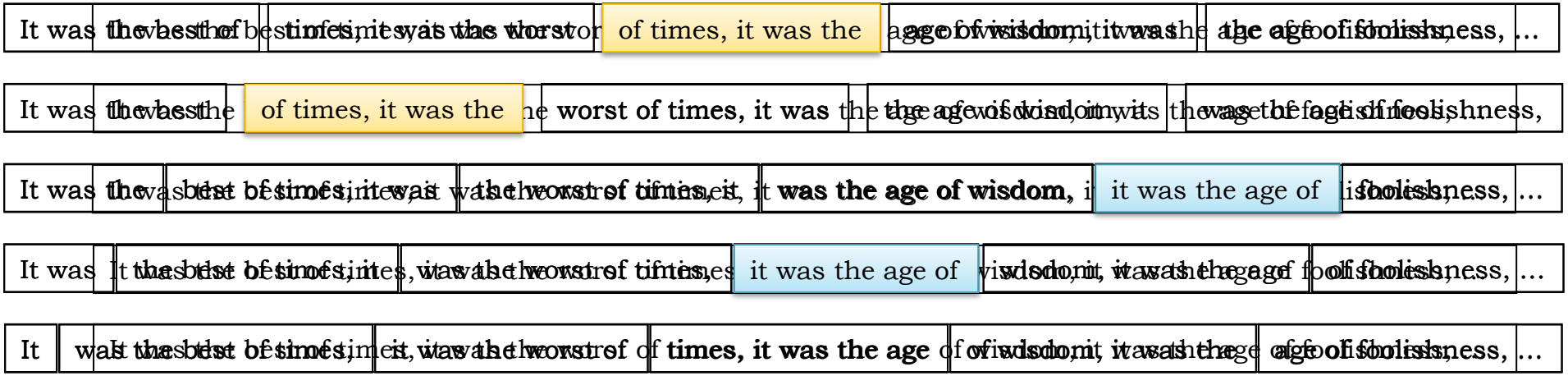
Shredded Book Reconstruction

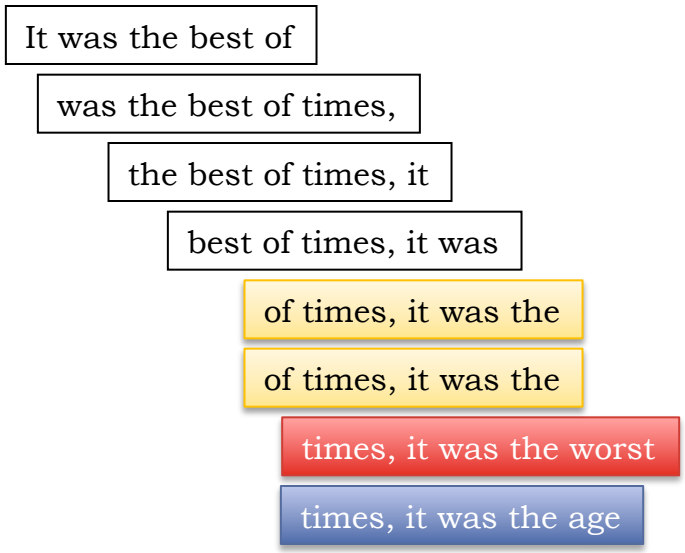
- Dickens accidentally shreds the first printing of A Tale of Two Cities
  - Text printed on 5 long spools



- 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



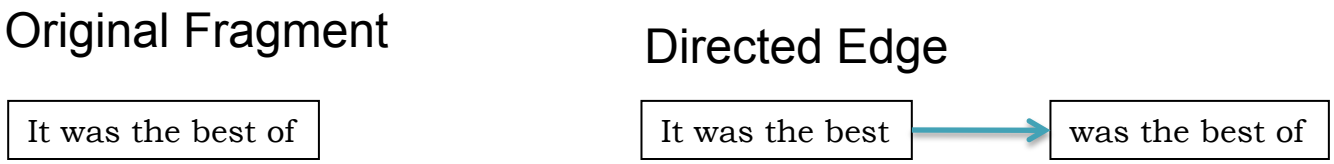
The repeated sequence make the correct reconstruction ambiguous

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

Model the assembly problem as a graph problem

de Bruijn Graph Construction

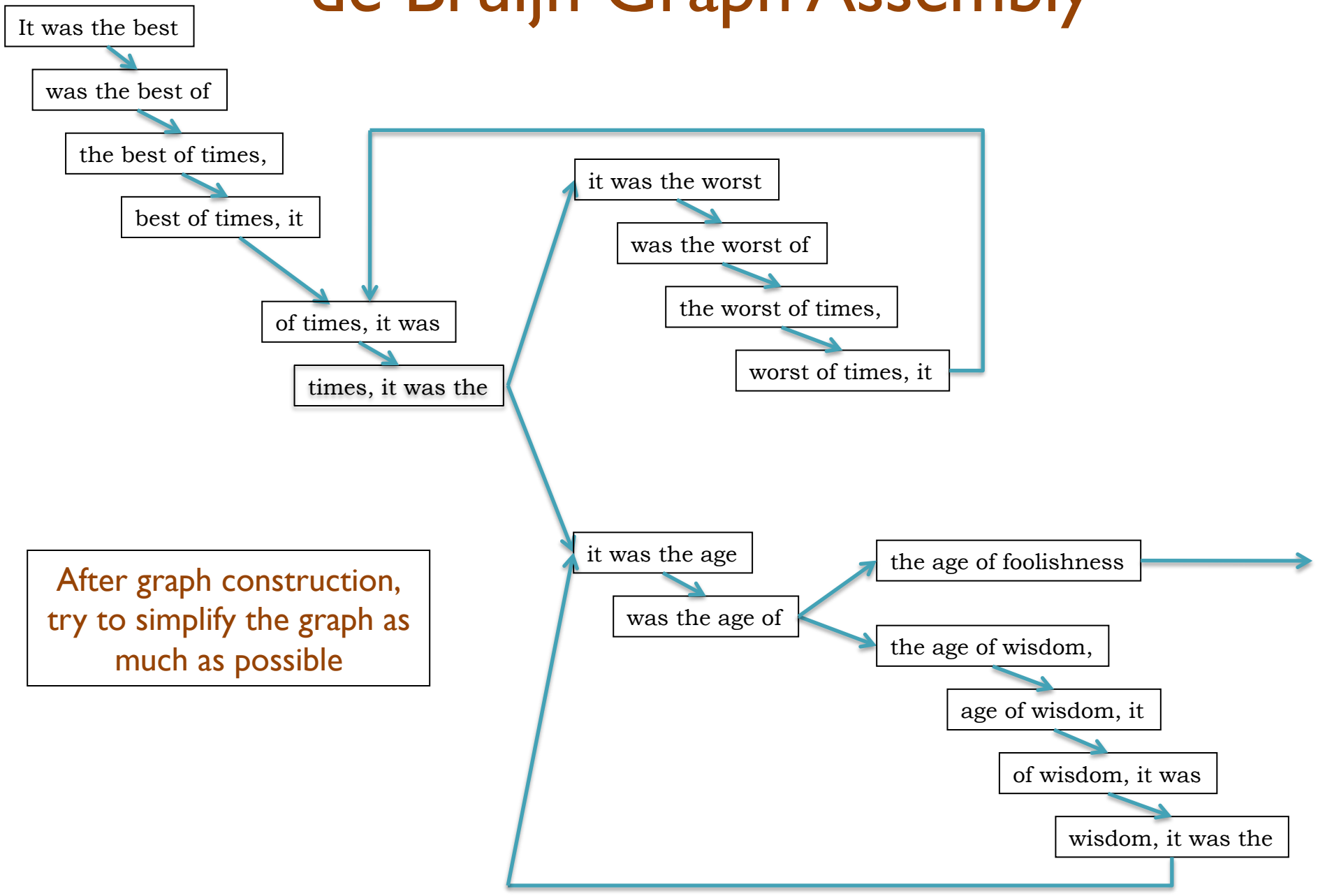
- $D_k = (V,E)$
  - V = All length-k subfragments (k < l)
  - E = Directed edges between consecutive subfragments
    - Nodes overlap by k-1 words



- Locally constructed graph reveals the global sequence structure
  - Overlaps between sequences implicitly computed

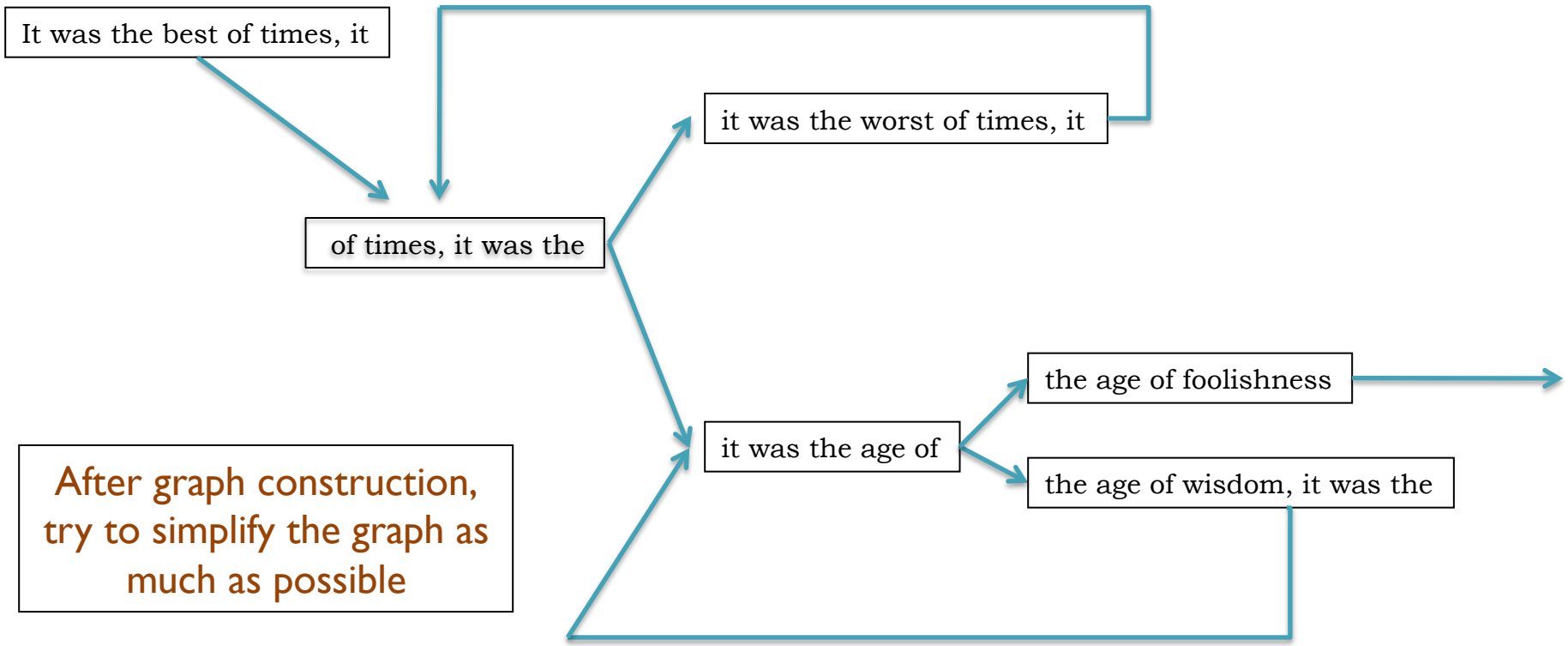
de Bruijn, 1946
Idury and Waterman, 1995
Pevzner, Tang, Waterman, 2001

de Bruijn Graph Assembly



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

de Bruijn Graph Assembly

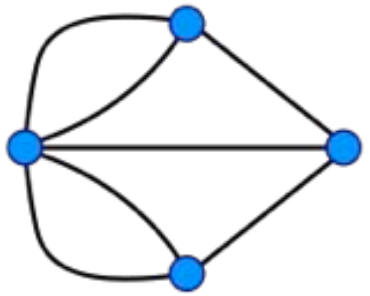


After graph construction, try to simplify the graph as much as possible

Eulerian Cycle Problem

- **Seven Bridges of Königsberg**
  - Find a cycle that visits every ***edge*** exactly once

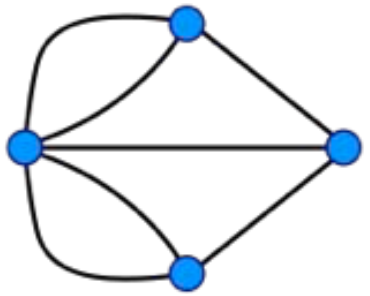
[Can you find the cycle?]



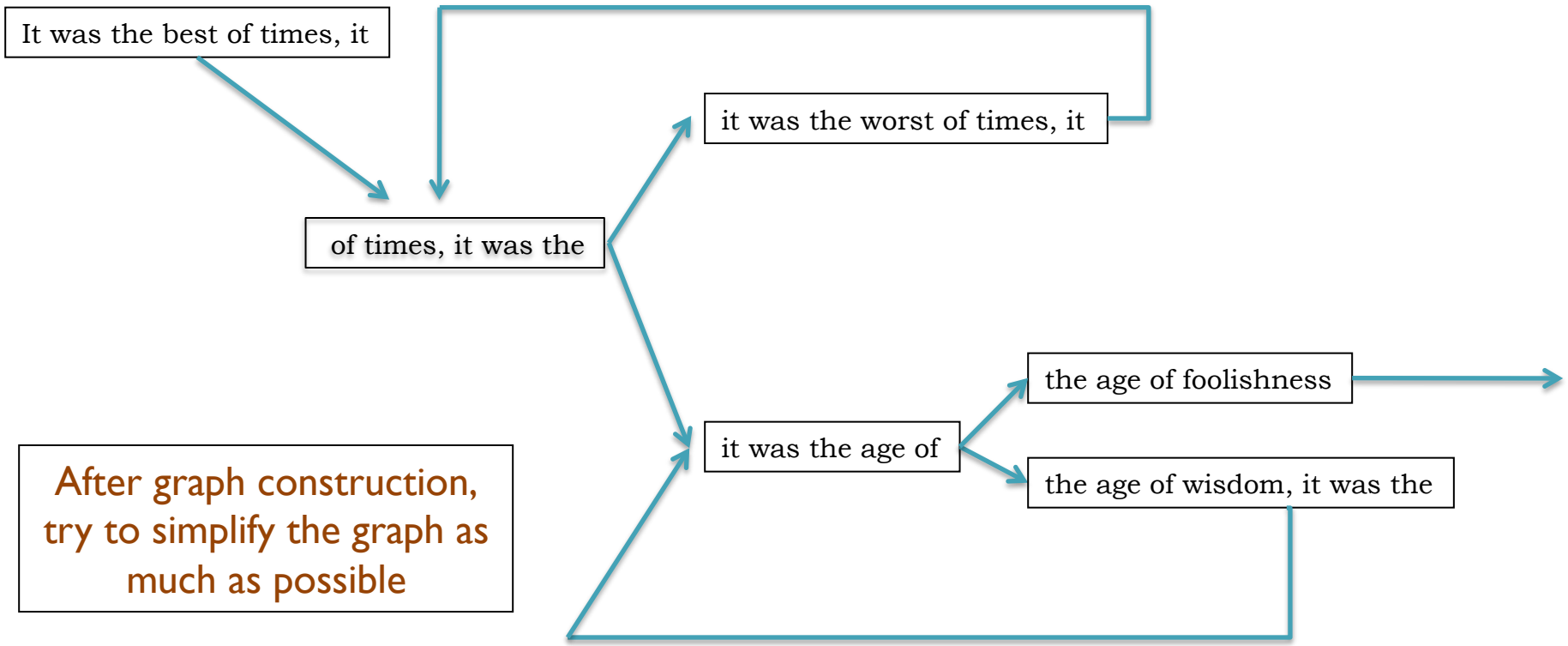
Eulerian Cycle Problem

- **Seven Bridges of Königsberg**
  - Find a cycle that visits every ***edge*** exactly once
  - ***Easy test***: every node has same number of incoming and outgoing edges (directed graph) or even degree (undirected graph)
  - ***Easy search***: arbitrarily walk to find first cycle, then walk again from an unmarked edge

[Can you find the cycle?]



de Bruijn Graph Assembly



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

The full tale

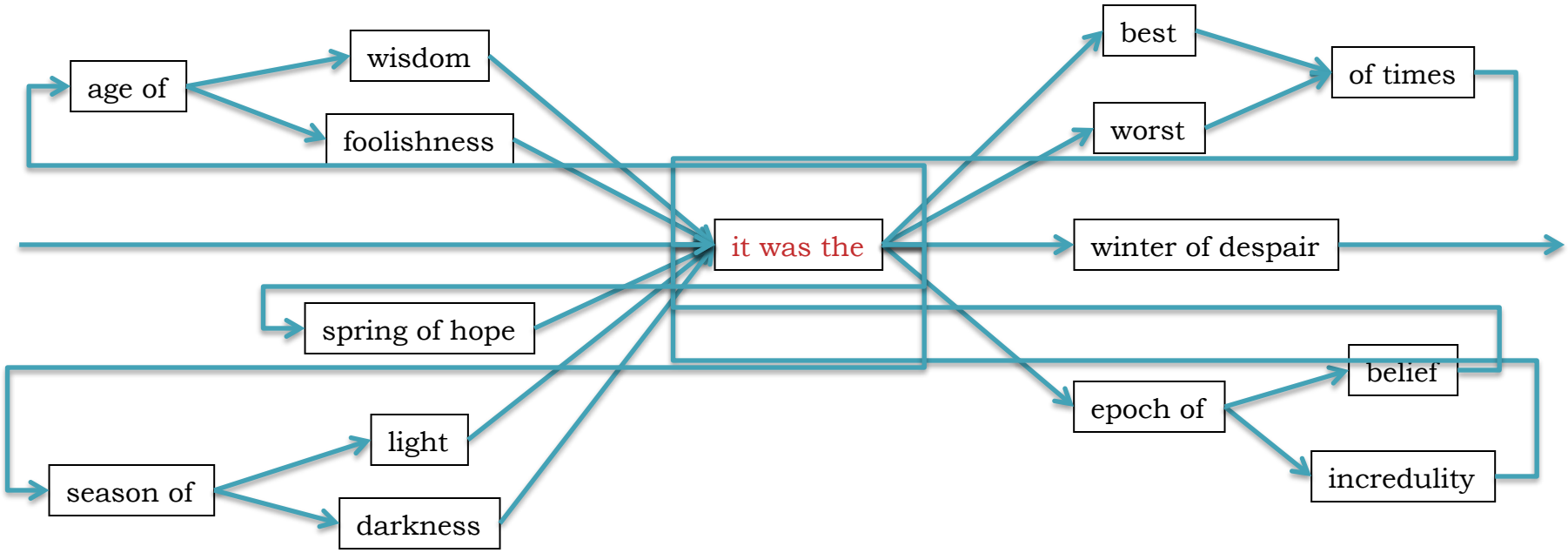
… it was the best of times it was the worst of times …

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

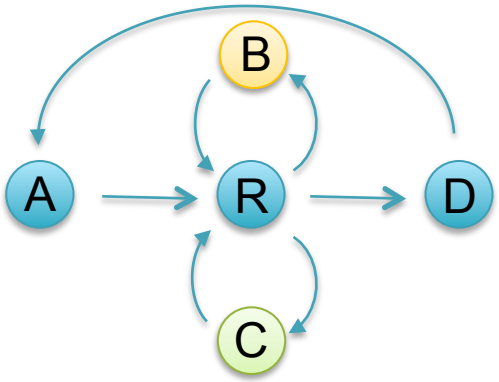
… it was the epoch of belief it was the epoch of incredulity …

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

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



Counting Eulerian Cycles



ARBRCRD
or
ARCRBRD

Generally an exponential number of compatible sequences

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

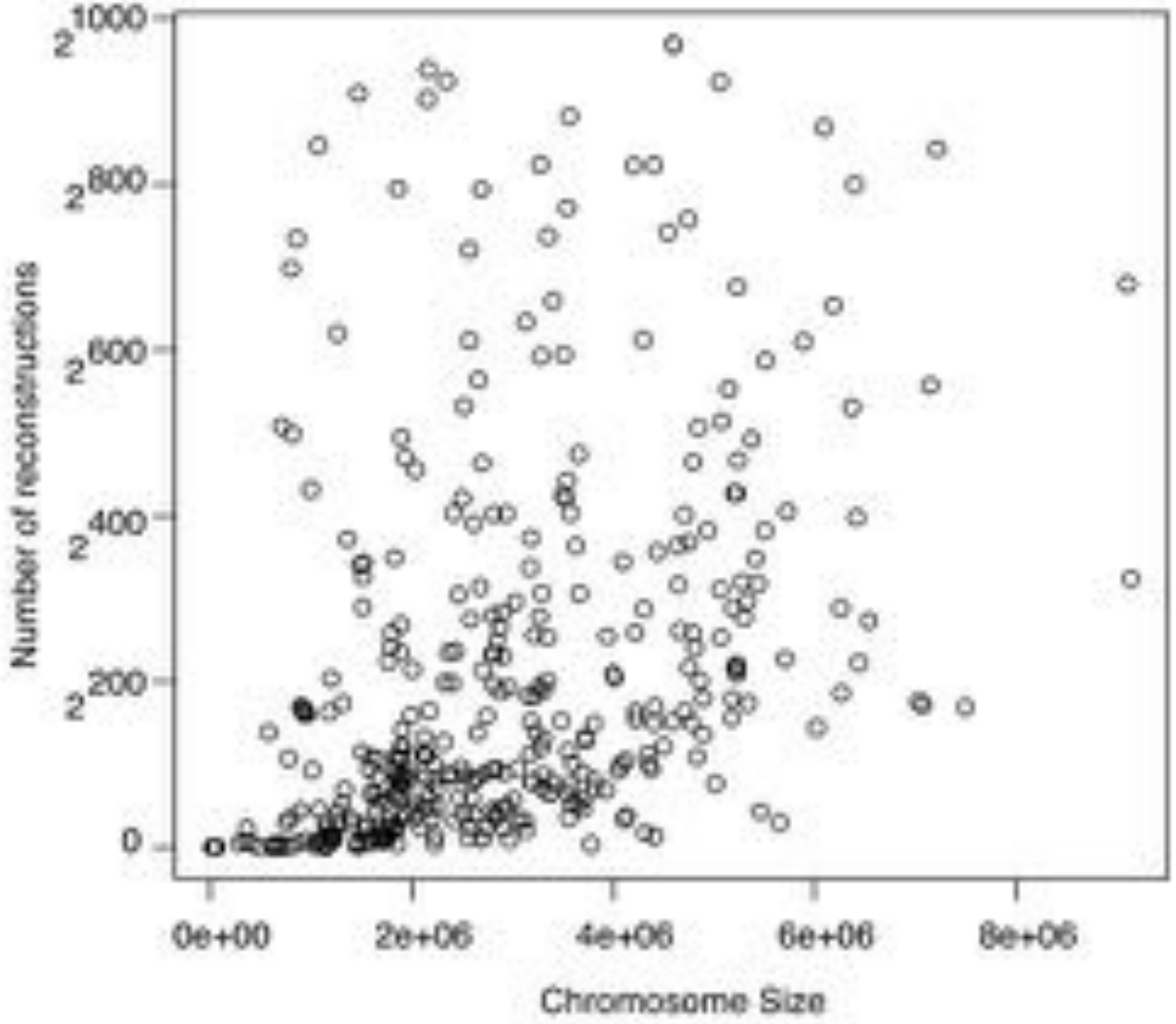
$$\mathcal{W}(G,t) = (\det L)\left\{\prod_{u\in V}(r_u-1)!\right\}\left\{\prod_{(u,v)\in E} a_{uv}!\right\}^{-1}$$

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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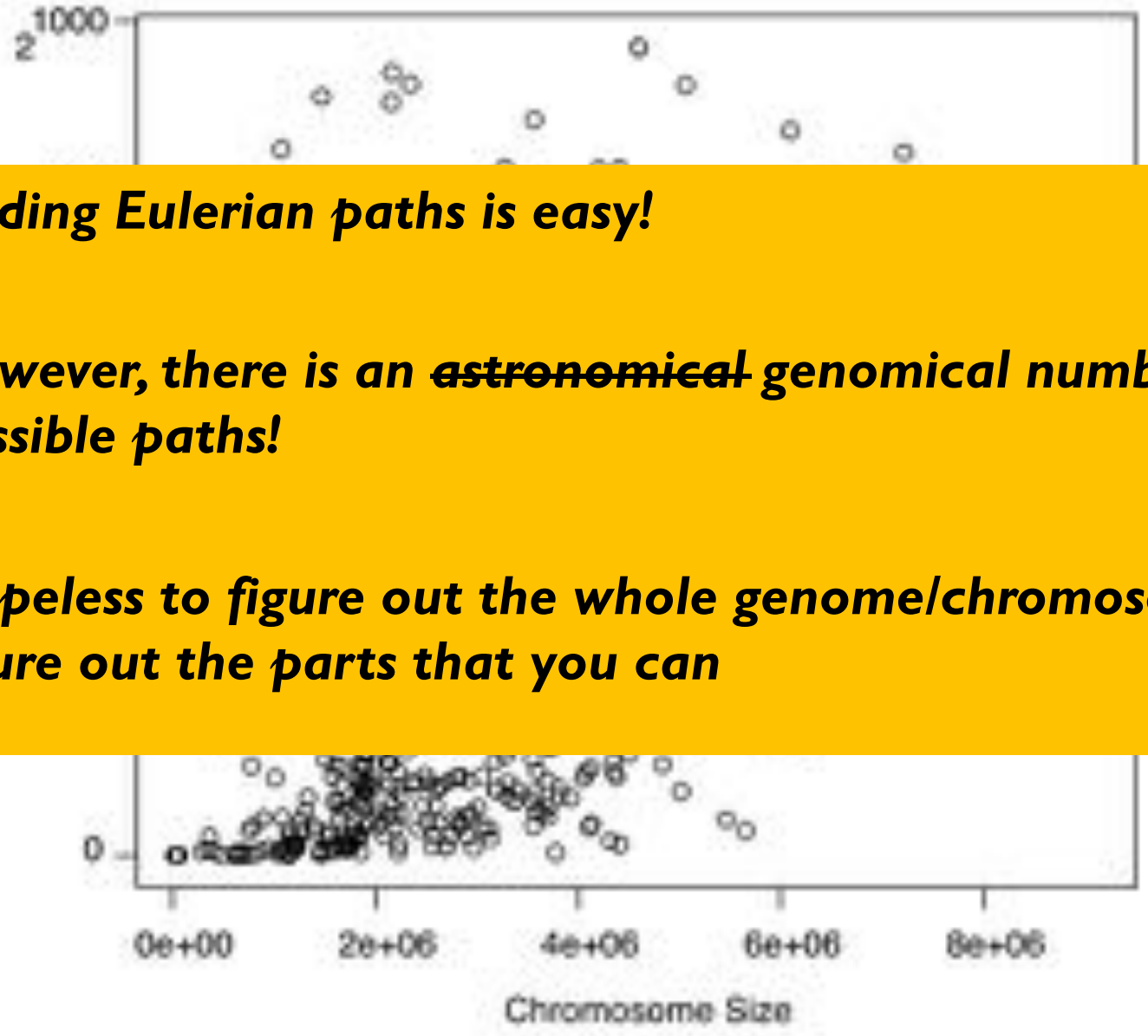
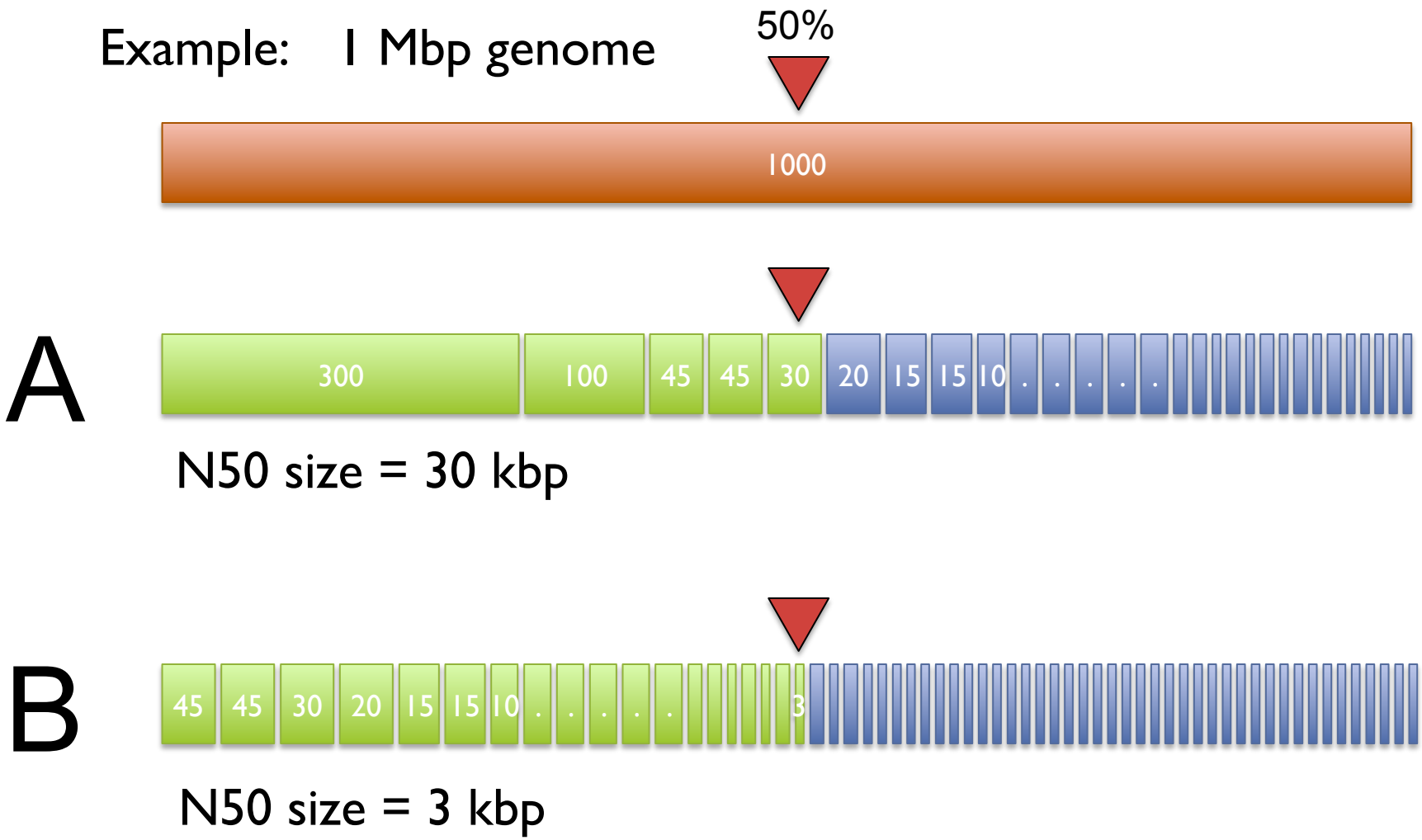


- ***Finding Eulerian paths is easy!***
- ***However, there is an ~~astronomical~~ genomical number of possible paths!***
- ***Hopeless to figure out the whole genome/chromosome, figure out the parts that you can***

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



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

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

Milestones in Genome Assembly


articles

Nucleotide sequence of bacteriophage ΦX174 DNA

1977. Sanger *et al.*
1st Complete Organism
5375 bp

1995. Fleischmann *et al.*
1st Free Living Organism
TIGR Assembler. 1.8Mbp

1998. C.elegans SC
1st Multicellular Organism
BAC-by-BAC Phrap. 97Mbp

2000. Myers *et al.*
1st Large WGS Assembly.
Celera Assembler. 116 Mbp

2001. Venter *et al.*, IHGSC
Human Genome
Celera Assembler/GigaAssembler. 2.9 Gbp

2010. Li *et al.*
1st Large SGS Assembly.
SOAPdenovo 2.2 Gbp

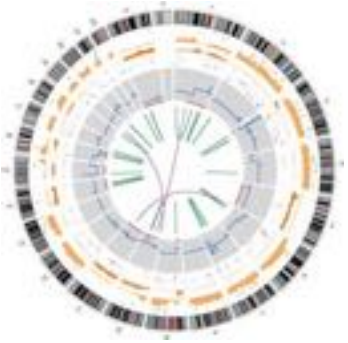
Like Dickens, we must computationally reconstruct a genome from short fragments

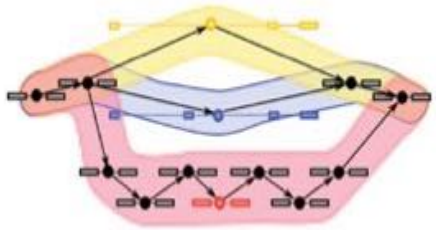
Assembly Applications

- Novel genomes

- Metagenomes

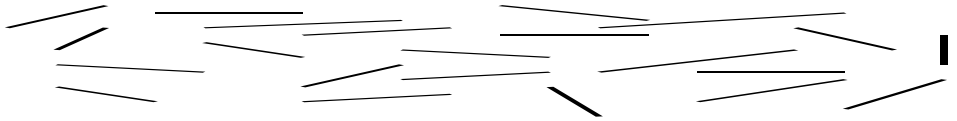
- Sequencing assays
  - Structural variations
  - Transcript assembly
  - …





Assembling a Genome

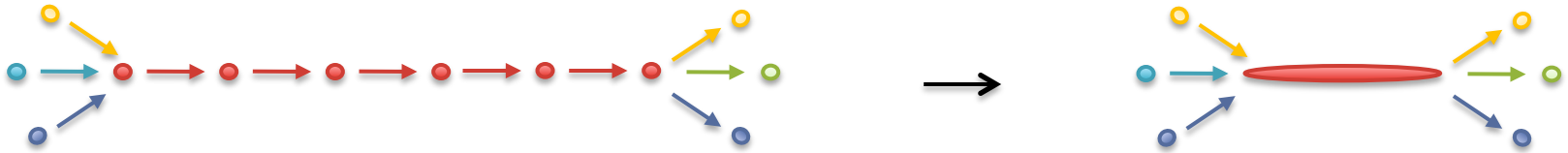
1. Shear & Sequence DNA



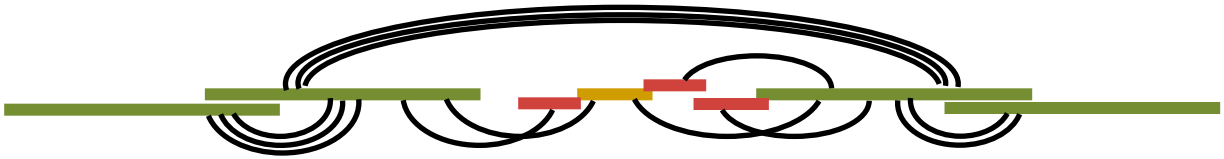
2. Construct assembly graph from reads (de Bruijn / overlap graph)

...AGCCTAGGGATGCGCGACACGT
GGATGCGCGACACGTCGCATATCCGGTTTGGTCAACCTCGGACGGAC
CAACCTCGGACGGACCTCAGCGAA...

3. Simplify assembly graph



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



Why are genomes hard to assemble?

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

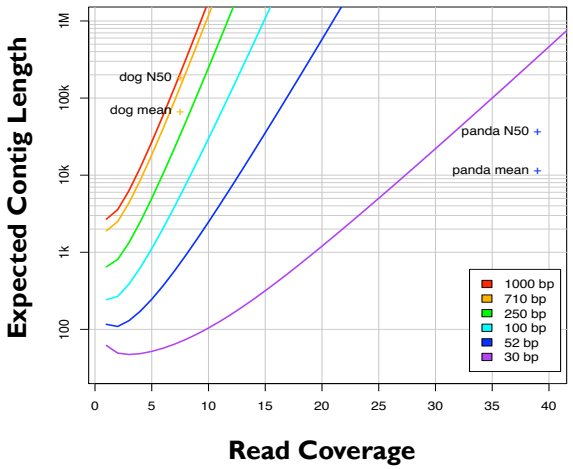
2. ***Sequencing***:
   - (Very) large genomes, imperfect sequencing

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

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

Ingredients for a good assembly

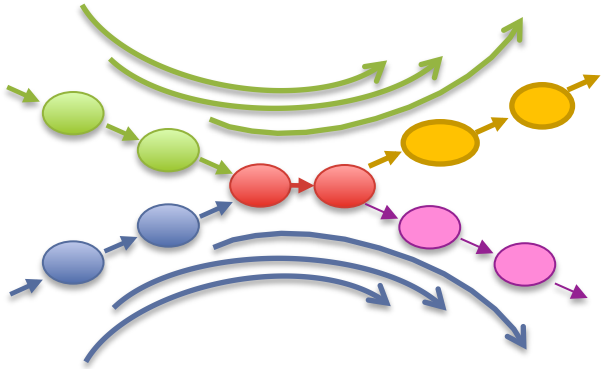
Coverage



High coverage is required

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

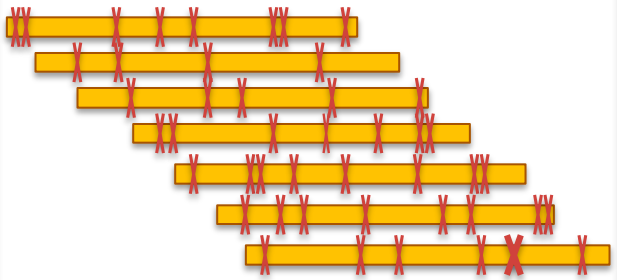
Read Length



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

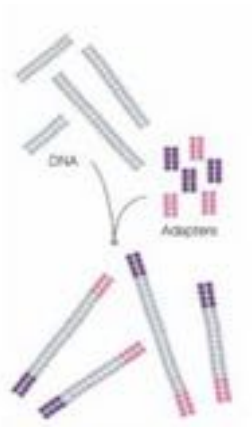


Errors obscure overlaps

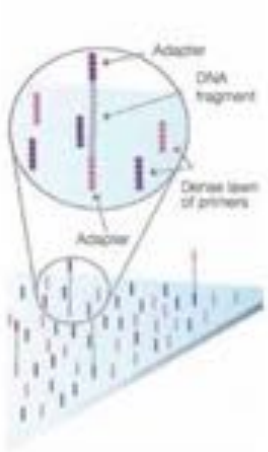
- Reads are assembled by finding kmers shared in pair of reads
- High error rate requires very short seeds, increasing complexity and forming assembly hairballs

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

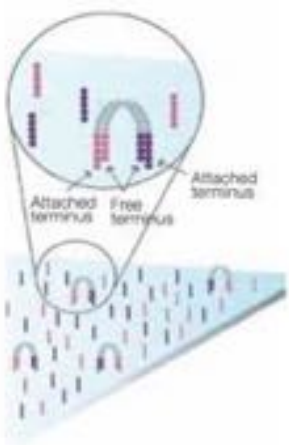
Illumina Sequencing by Synthesis



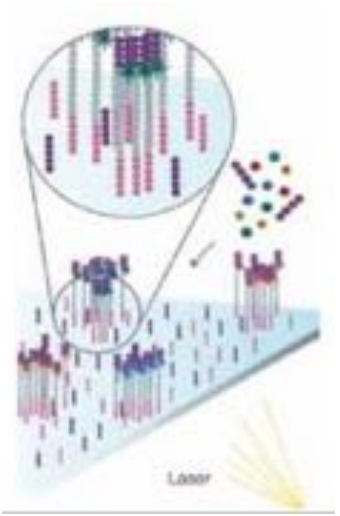
1. Prepare



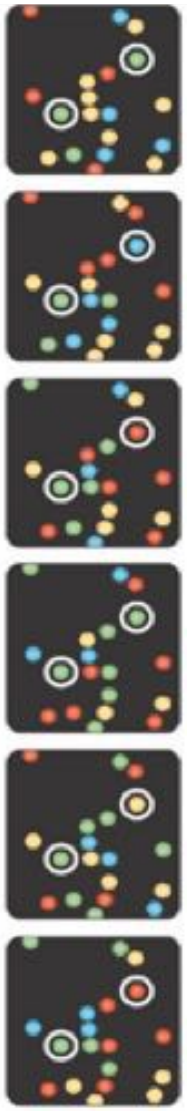
2. Attach



3. Amplify



4. Image

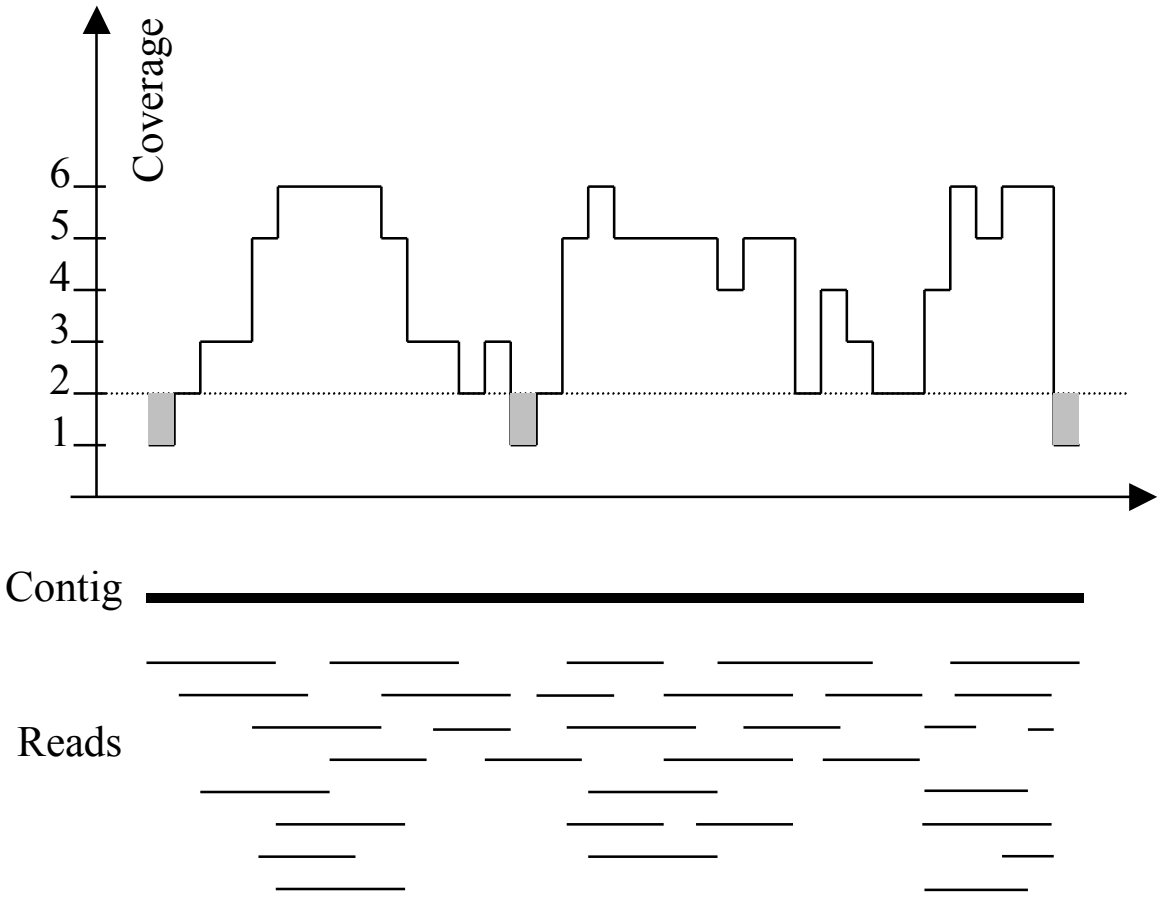


5. Basecall

Metzker (2010) Nature Reviews Genetics 11:31-46

http://www.youtube.com/watch?v=l99aKKHcxC4

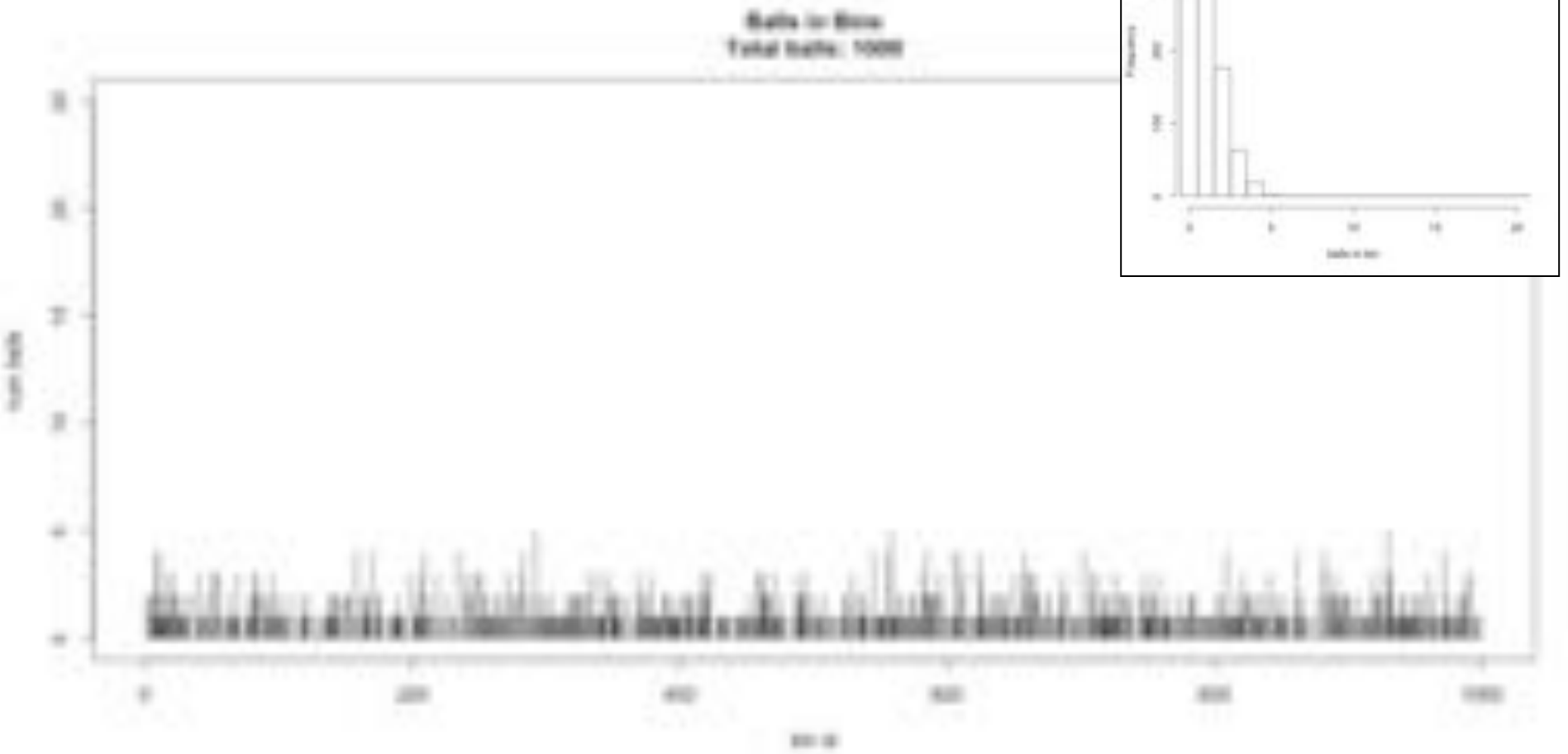
Typical sequencing coverage



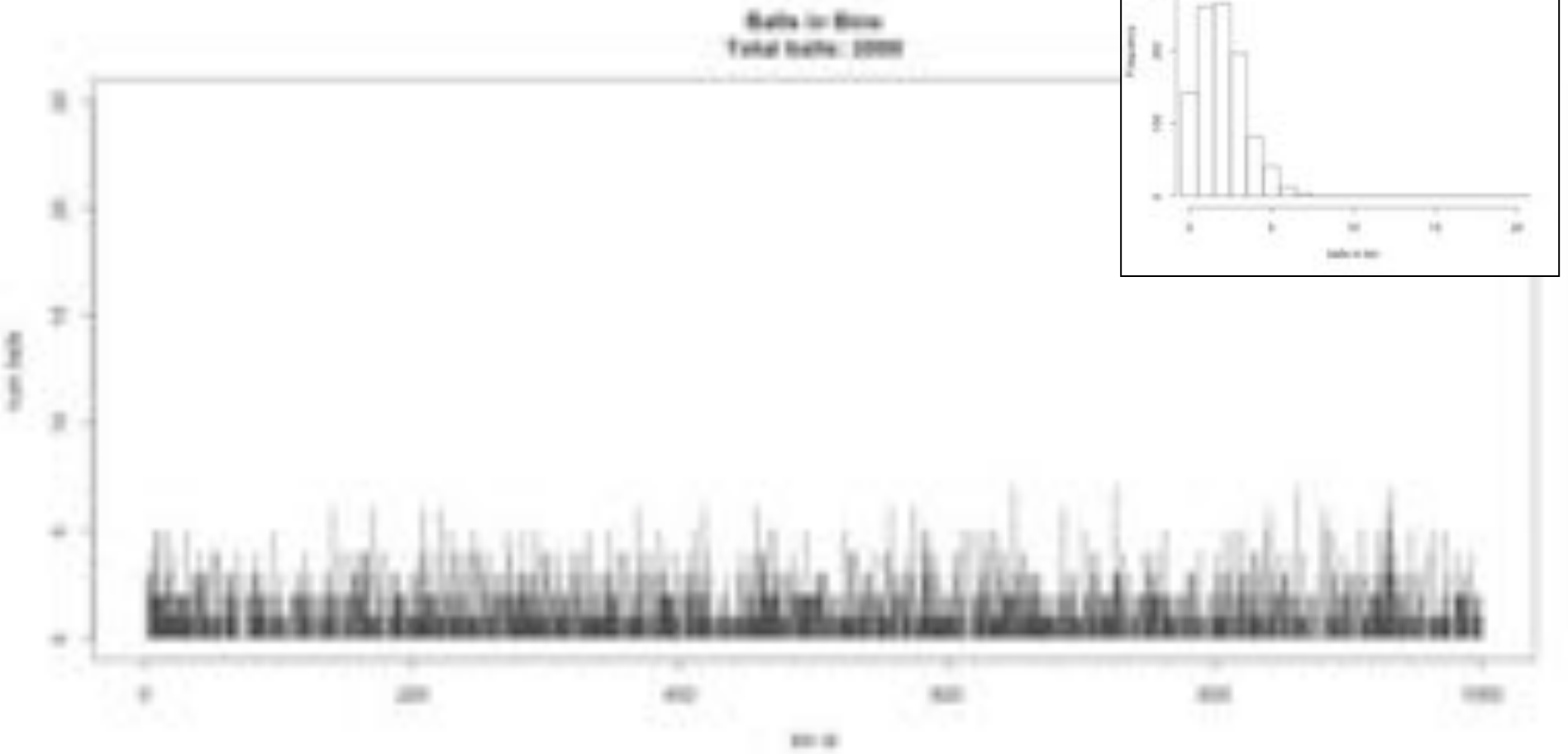
Imagine raindrops on a sidewalk

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

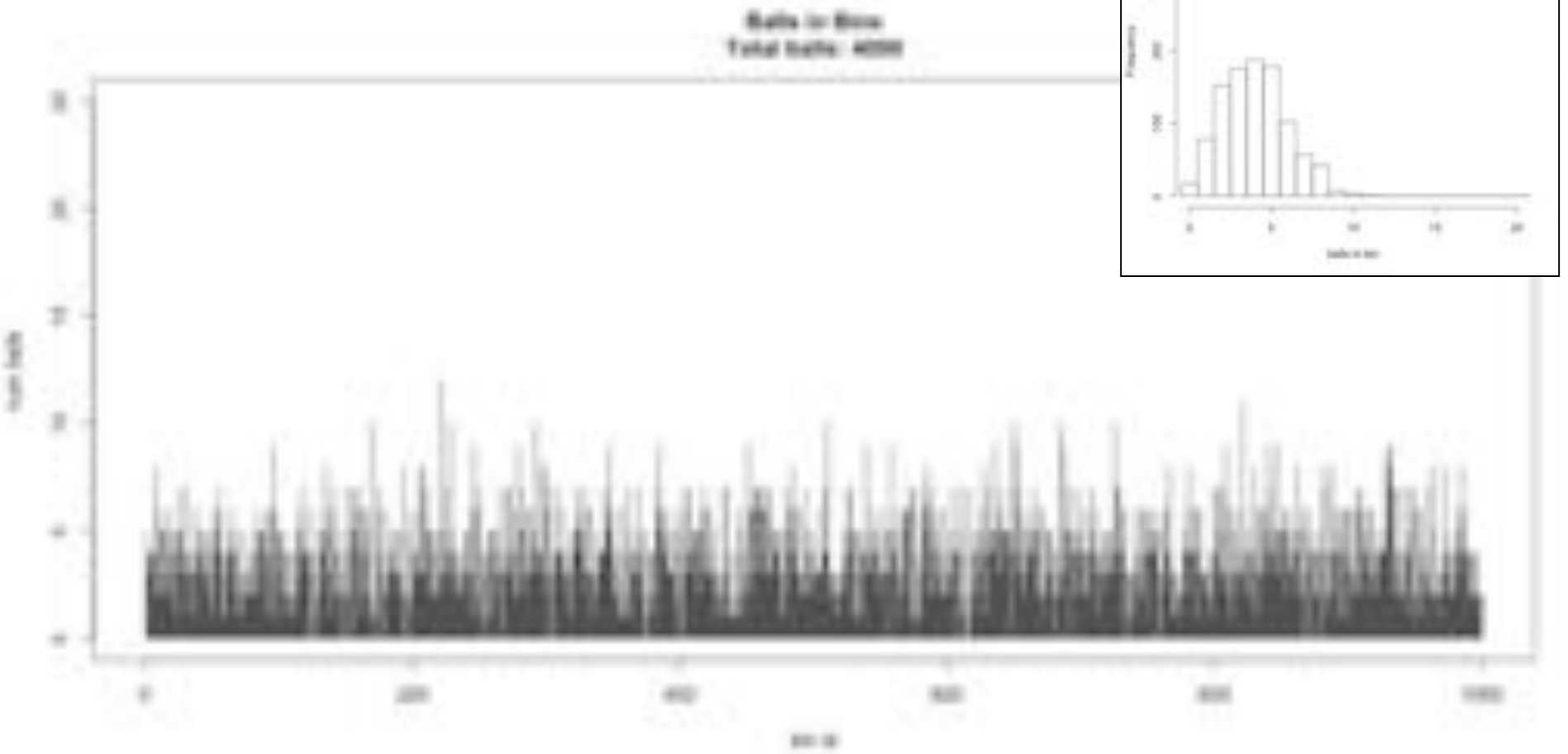
1x sequencing



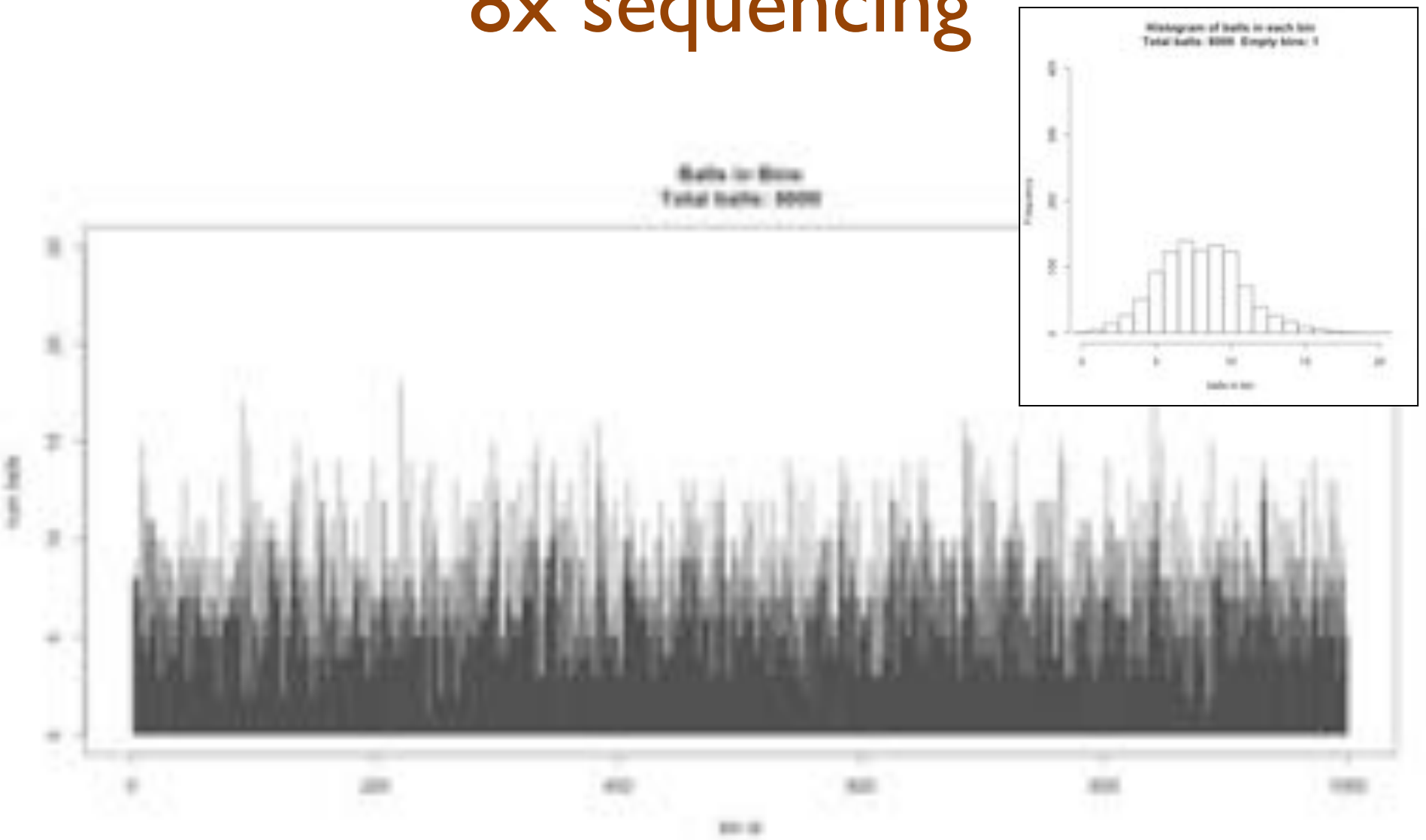
2x sequencing



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.

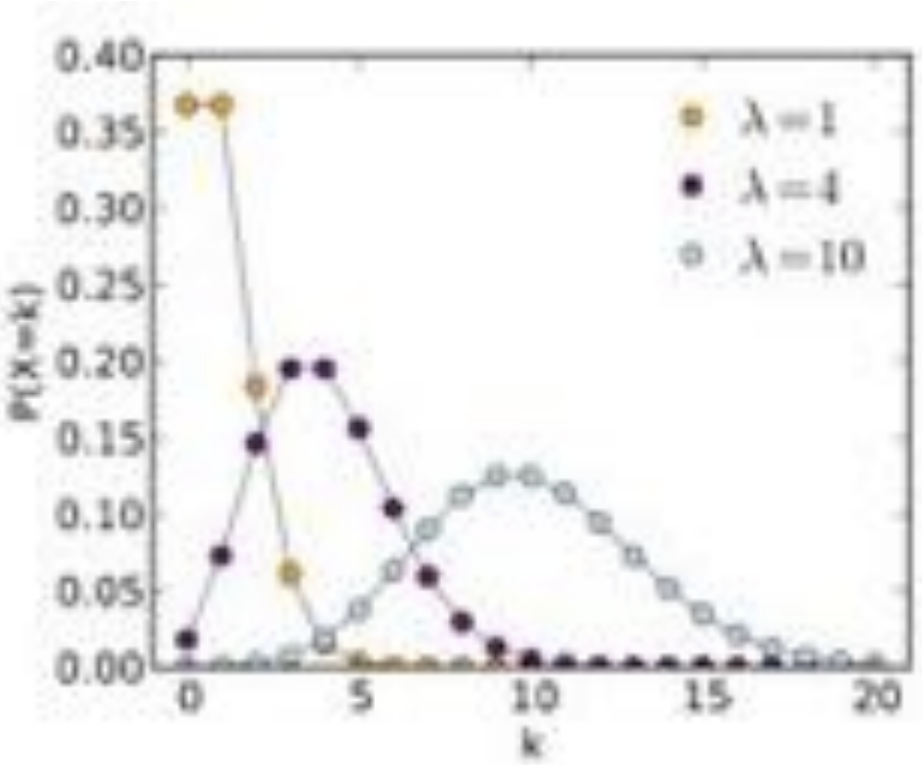
Formulation comes from the limit of the binomial equation

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

Key property:

- ***The standard deviation is the square root of the mean.***

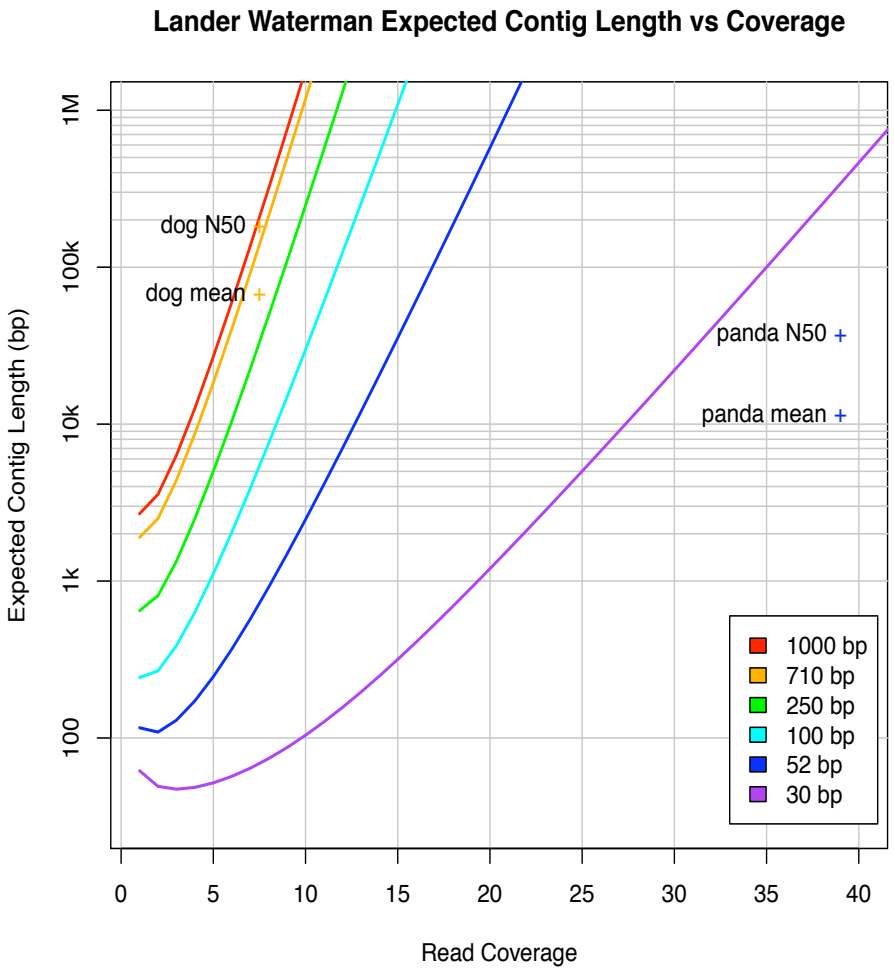
$$P(k) = \frac{\lambda^k}{k!} e^{-\lambda}$$



Coverage and Read Length

Idealized Lander-Waterman model

- Reads start at perfectly random positions
- Contig length is a function of coverage and read length
  - Short reads require much higher coverage to reach same expected contig length
- Need even high coverage for higher ploidy, sequencing errors, sequencing biases
  - Recommend 100x coverage

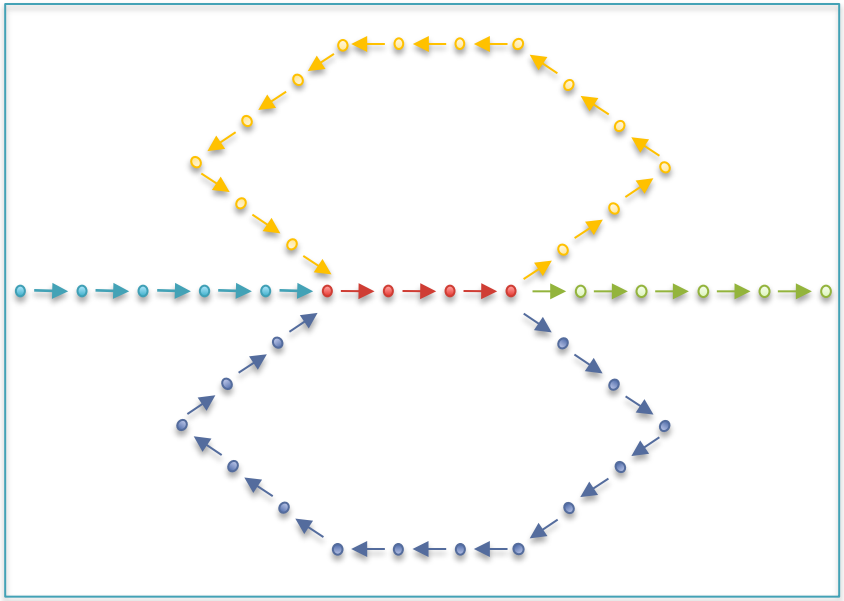


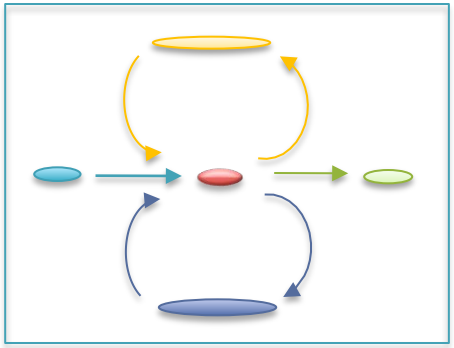
Assembly of Large Genomes using Second Generation Sequencing
Schatz MC, Delcher AL, Salzberg SL (2010) *Genome Research.* 20:1165-1173.

Errors

Unitigging / Unipathing

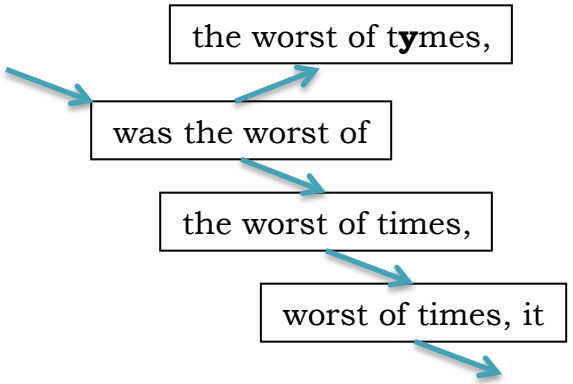
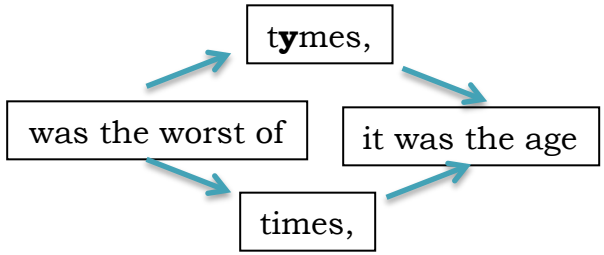
- After simplification and correction, compress graph down to its non-branching initial contigs
  - Aka "unitigs", "unipaths"
  - Unitigs end because of (1) lack of coverage, (2) errors, (3) heterozygosity and (4) repeats





Errors in the graph

(Chaisson, 2009)

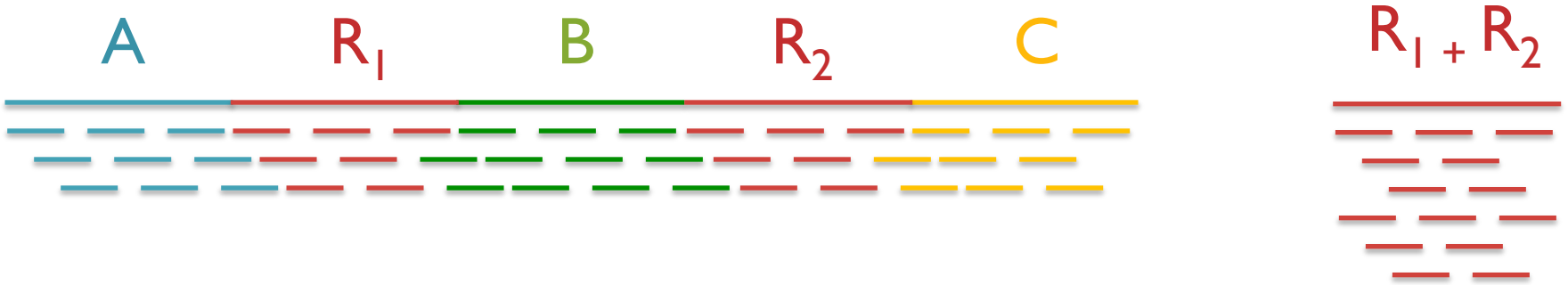
Clip Tips	Pop Bubbles
was the worst of times, was the worst of t**y**mes, the worst of times, it	was the worst of times, was the worst of t**y**mes, times, it was the age t**y**mes, it was the age
	

Repetitive regions

Repeat Type	Definition / Example	Prevalence
Low-complexity DNA / Microsatellites	$(b_1 b_2 \ldots b_k)^N$ 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.

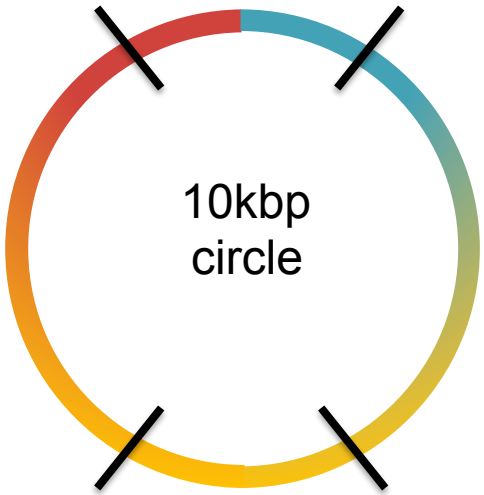
Paired-end and Mate-pairs

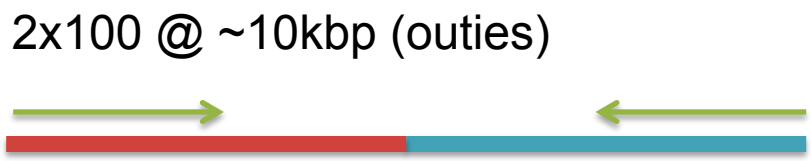
Paired-end sequencing

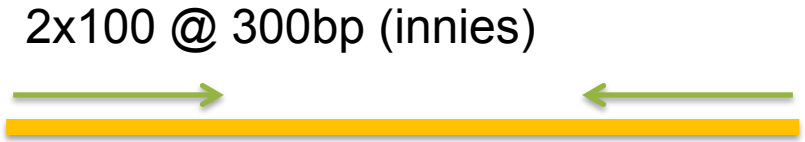
- Read one end of the molecule, flip, and read the other end
- Generate pair of reads separated by up to 500bp with inward orientation

Mate-pair sequencing

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

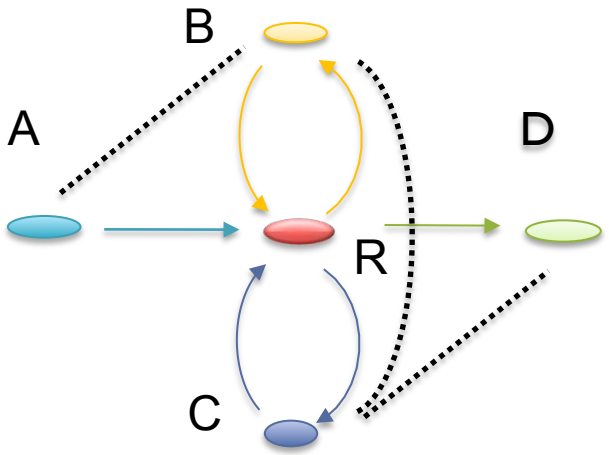




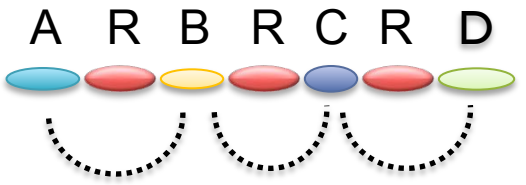


Scaffolding

- 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

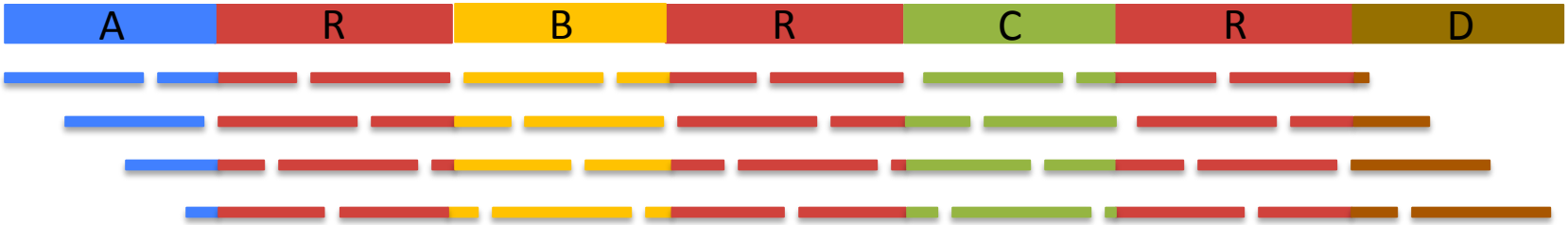
Outline

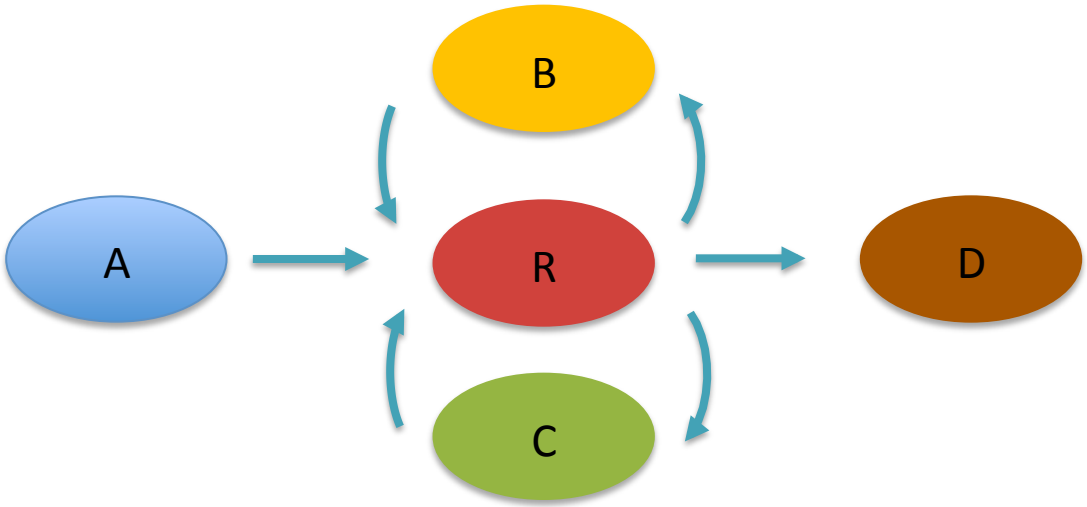
1. *Assembly theory*
   - Assembly by analogy

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

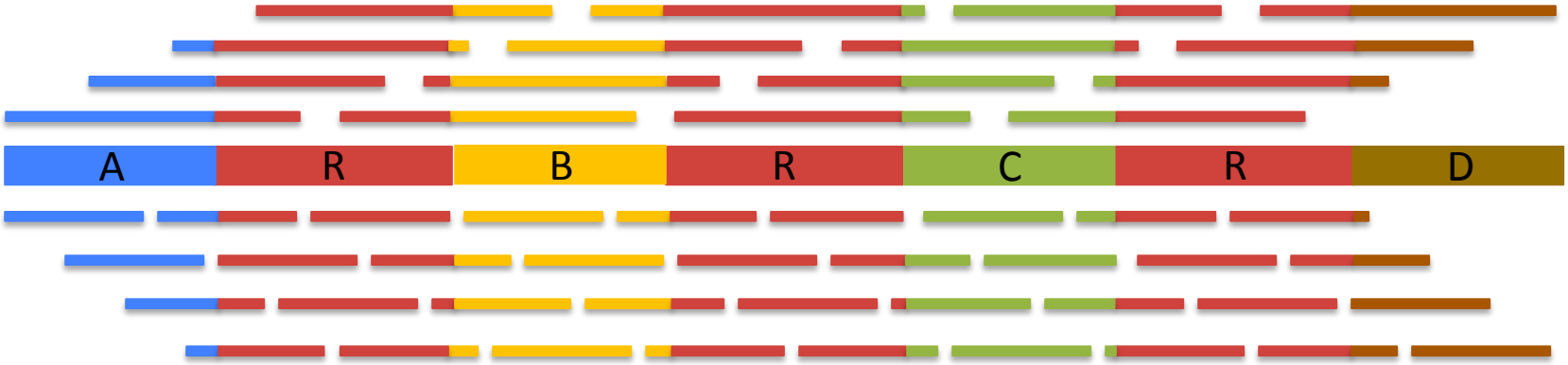
3. ***Next-next-gen Assembly***
   - Canu: recommended for PacBio/ONT project

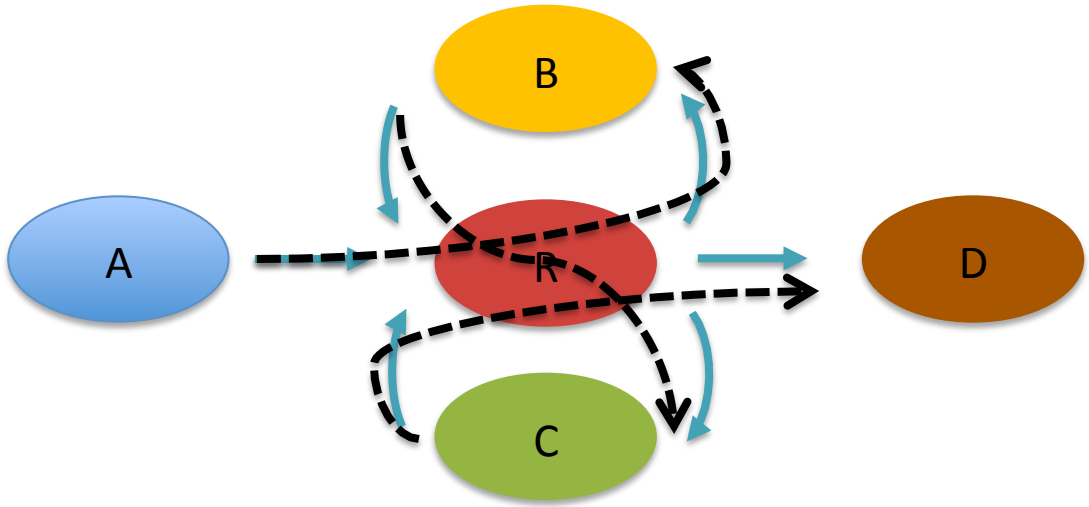
Assembly Complexity



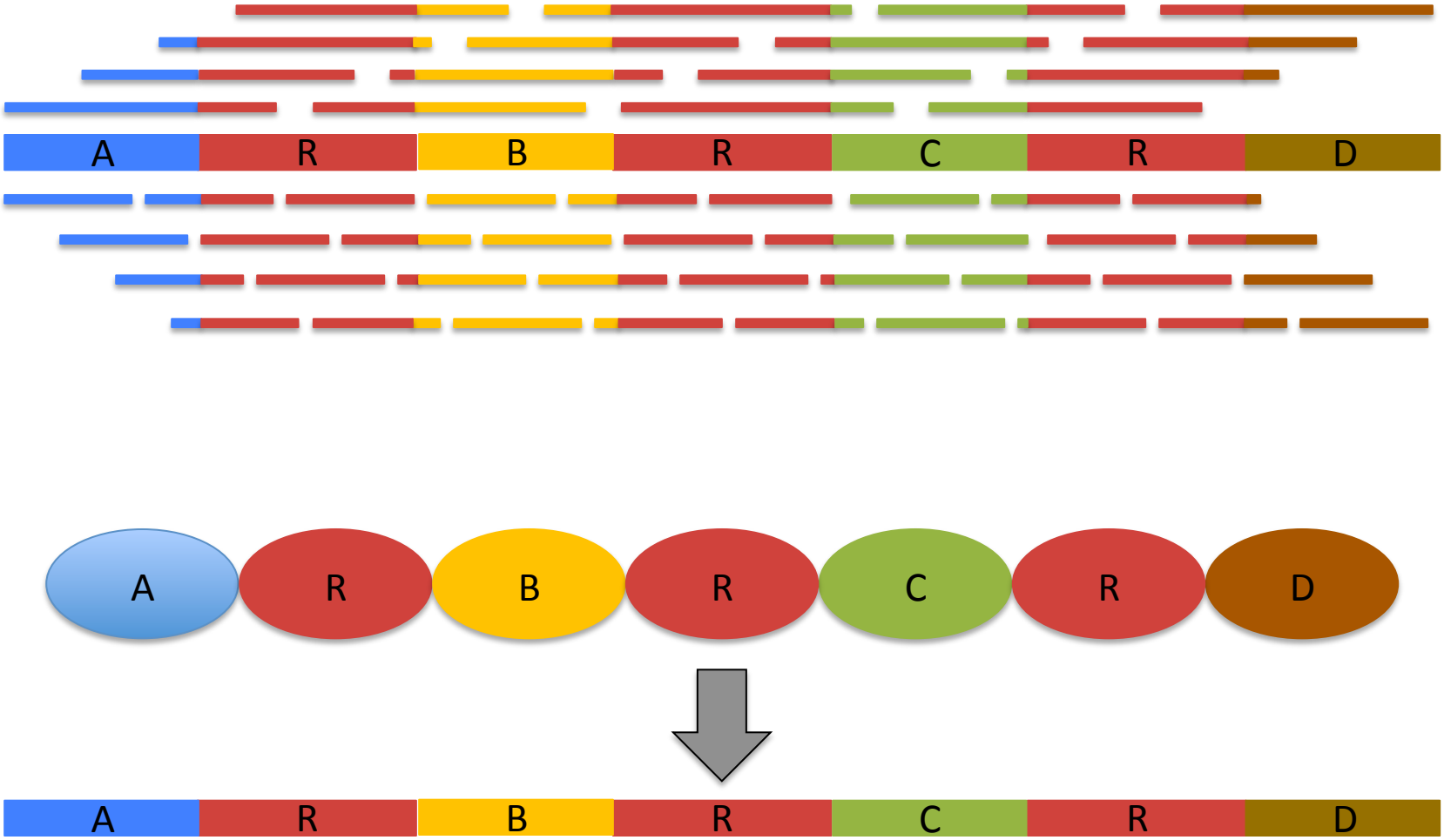


Assembly Complexity





Assembly Complexity



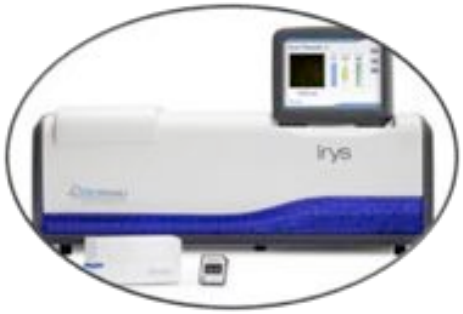
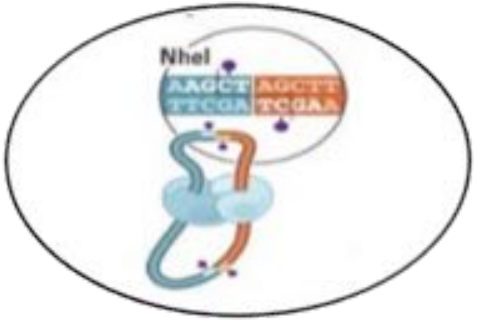
The advantages of SMRT sequencing
Roberts, RJ, Carneiro, MO, Schatz, MC (2013) *Genome Biology.* 14:405

Genomics Arsenal in the Year 2016

Long Read Sequencing: De novo assembly, SV analysis, phasing

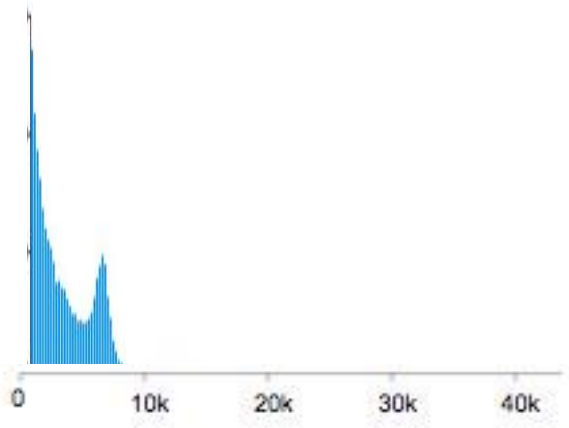
Illumina/Moleculo	*Pacific Biosciences*	*Oxford Nanopore*
		
(Kuleshov et al. 2014)	(Berlin et al, 2014)	(Quick et al, 2014)

Long Span Sequencing: Chromosome Scaffolding, SV analysis, phasing

Molecular Barcoding	*Optical Mapping*	*Chromatin Assays*
		
(10Xgenomics.com)	(Cao et al, 2014)	(Putnam et al, 2015)

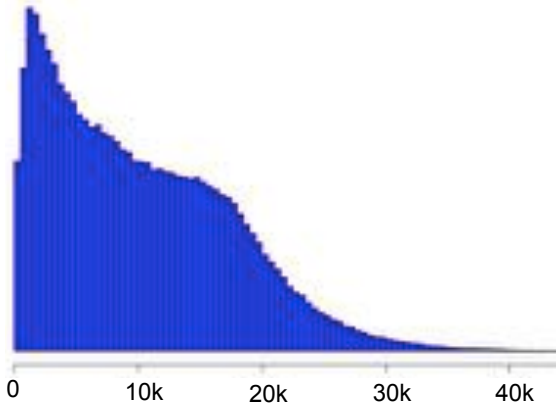
Long Read Sequencing Technology

Moleculo



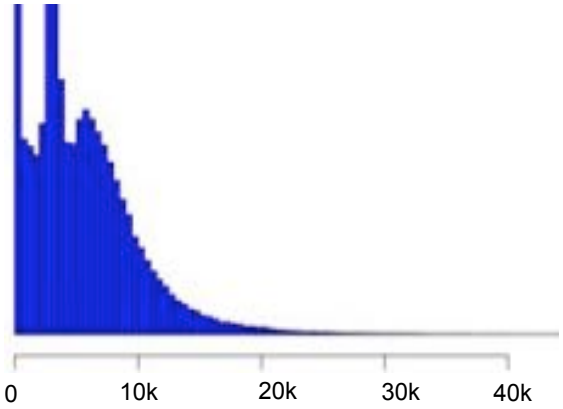
(Voskoboynik et al. 2013)

PacBio RS II



CSHL/PacBio

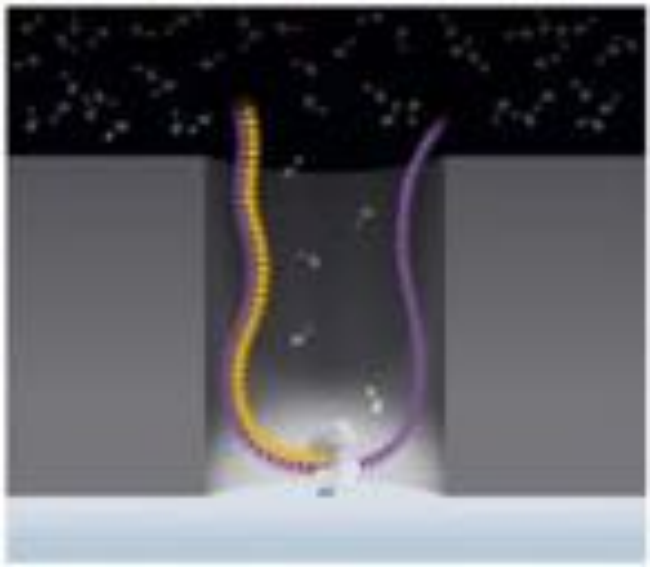
Oxford Nanopore

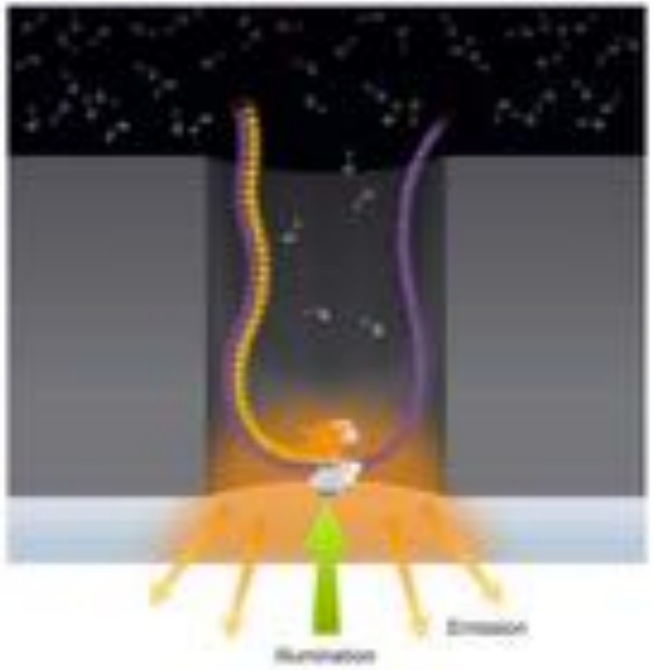


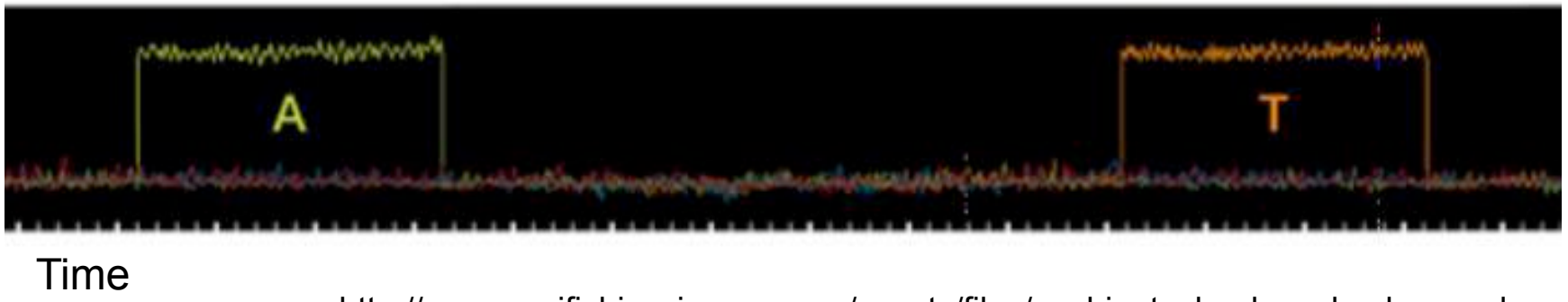
CSHL/ONT

PacBio SMRT Sequencing

Imaging of fluorescently phospholinked labeled nucleotides as they are incorporated by a polymerase anchored to a Zero-Mode Waveguide (ZMW).

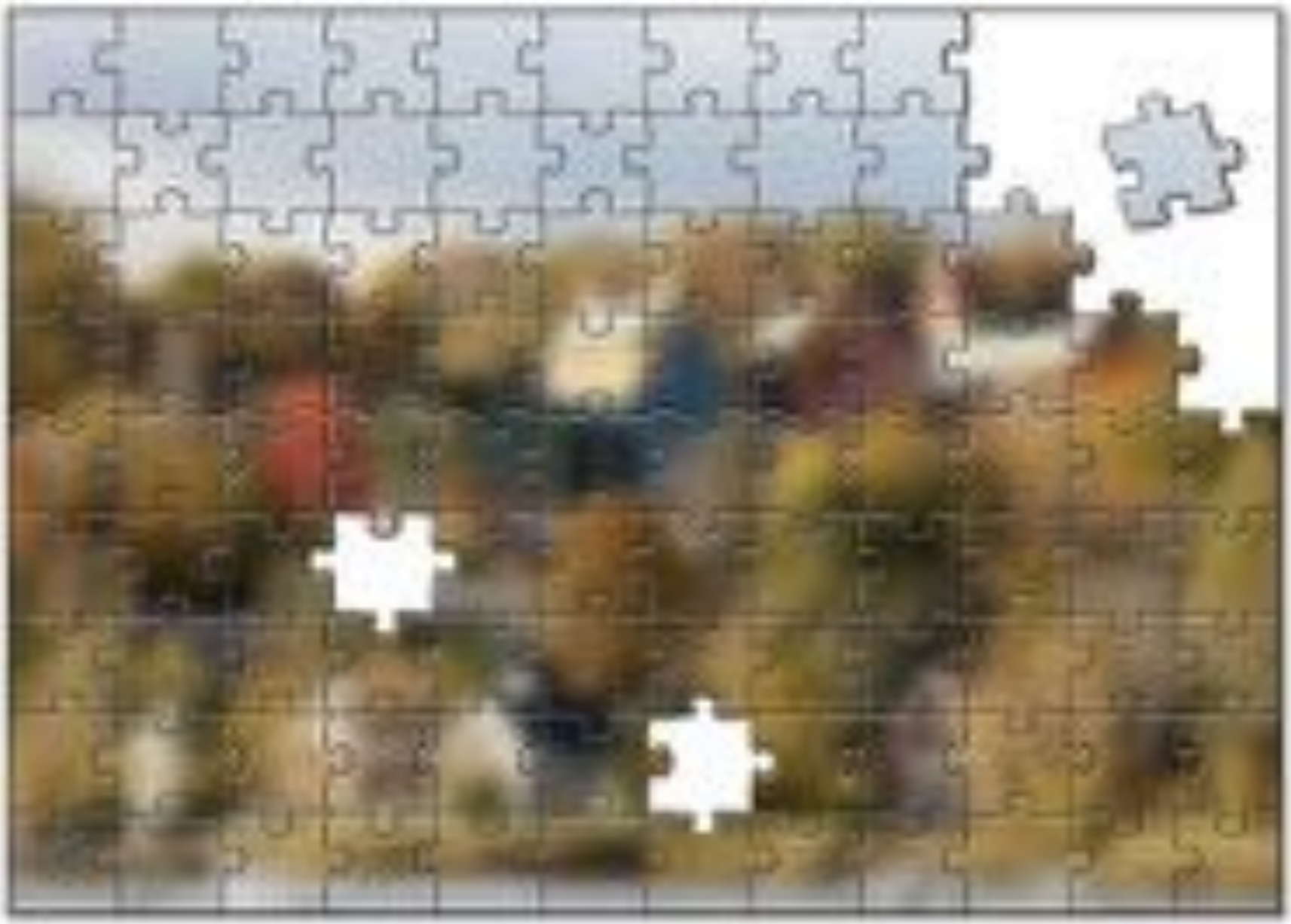




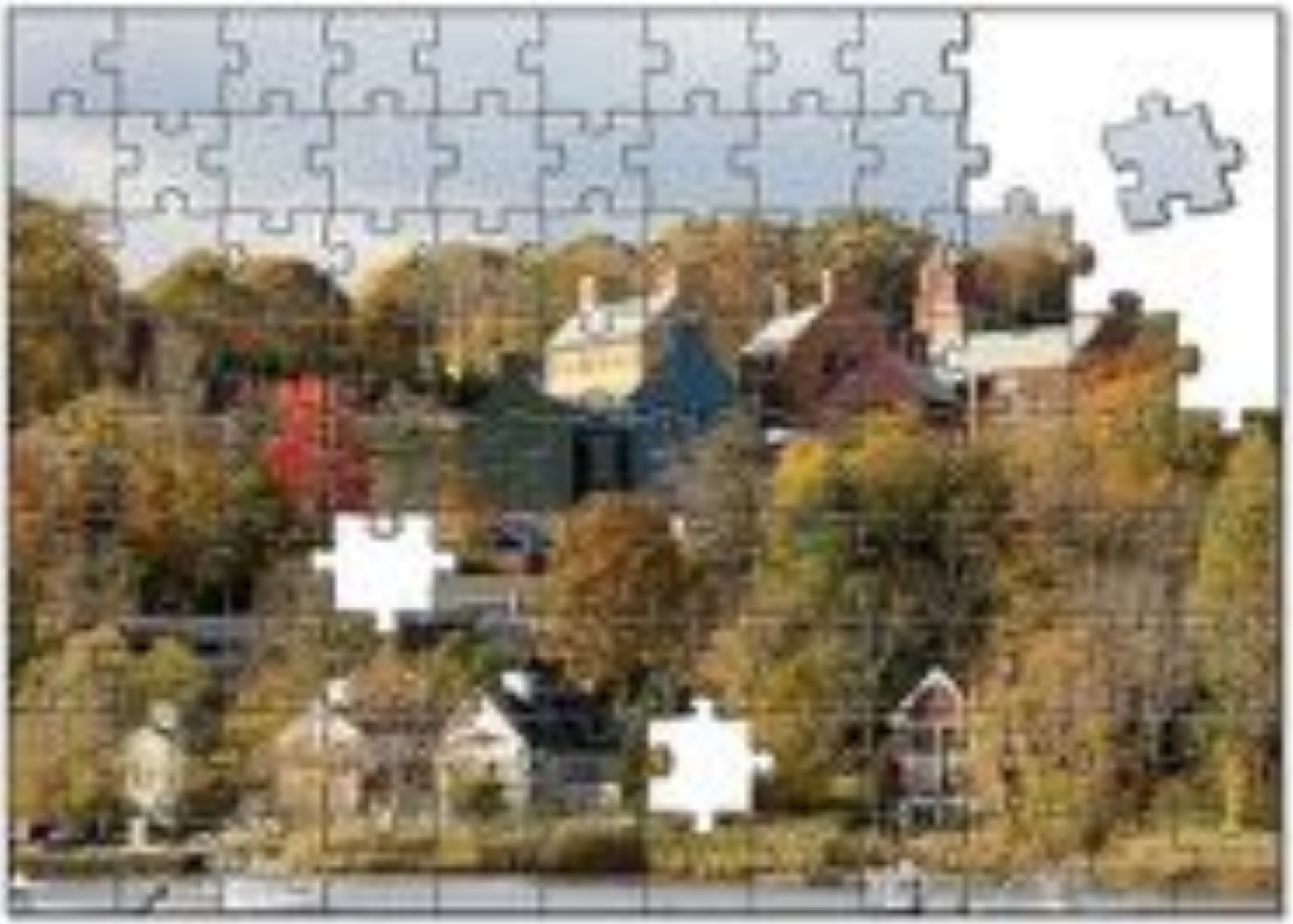


http://www.pacificbiosciences.com/assets/files/pacbio_technology_backgrounder.pdf

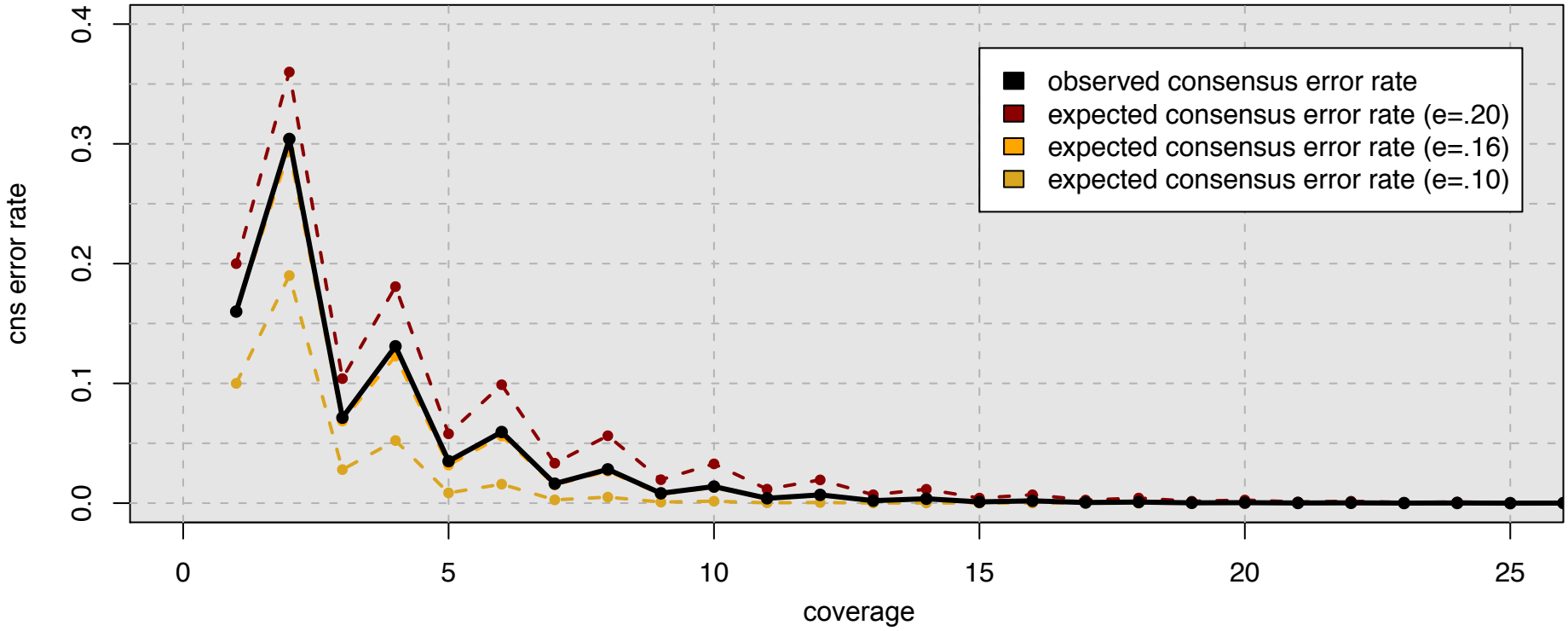
Single Molecule Sequences



“Corrective Lens” for Sequencing



Consensus Accuracy and Coverage



Coverage can overcome random errors

- Dashed: error model from binomial sampling
- Solid: observed accuracy

$$CNS\ Error = \sum_{i=\lceil c/2 \rceil}^{c} \binom{c}{i} (e)^{i} (1-e)^{n-i}$$

Koren, Schatz, *et al* (2012)
Nature Biotechnology. 30:693–700

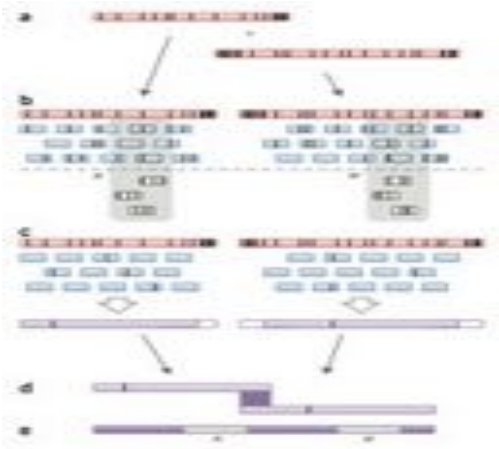
PacBio Assembly Algorithms

PBJelly

Gap Filling and Assembly Upgrade

English *et al* (2012)
PLOS One. 7(11): e47768

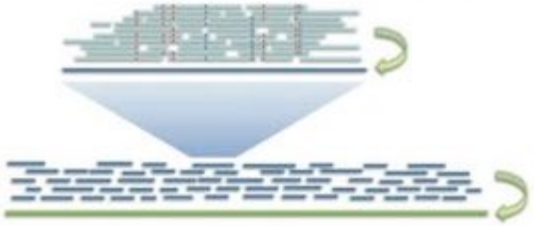
PacBioToCA & ECTools



Hybrid/PB-only Error Correction

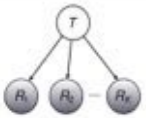
Koren, Schatz, *et al* (2012)
Nature Biotechnology. 30:693–700

HGAP/MHAP/Falcon & Quiver



$$\Pr(\mathbf{R} \mid T)$$

$$\Pr(\mathbf{R} \mid T) = \prod_k \Pr(R_k \mid T)$$



Quiver Performance Results
Comparison to Reference Genome
(M. ruber ; 3.1 MB ; SMRT® Cells)

	Initial Assembly	Quiver Consensus
QV	43.4	54.5
Accuracy	99.99540%	99.99964%
Differences	141	11

PB-only Correction & Polishing

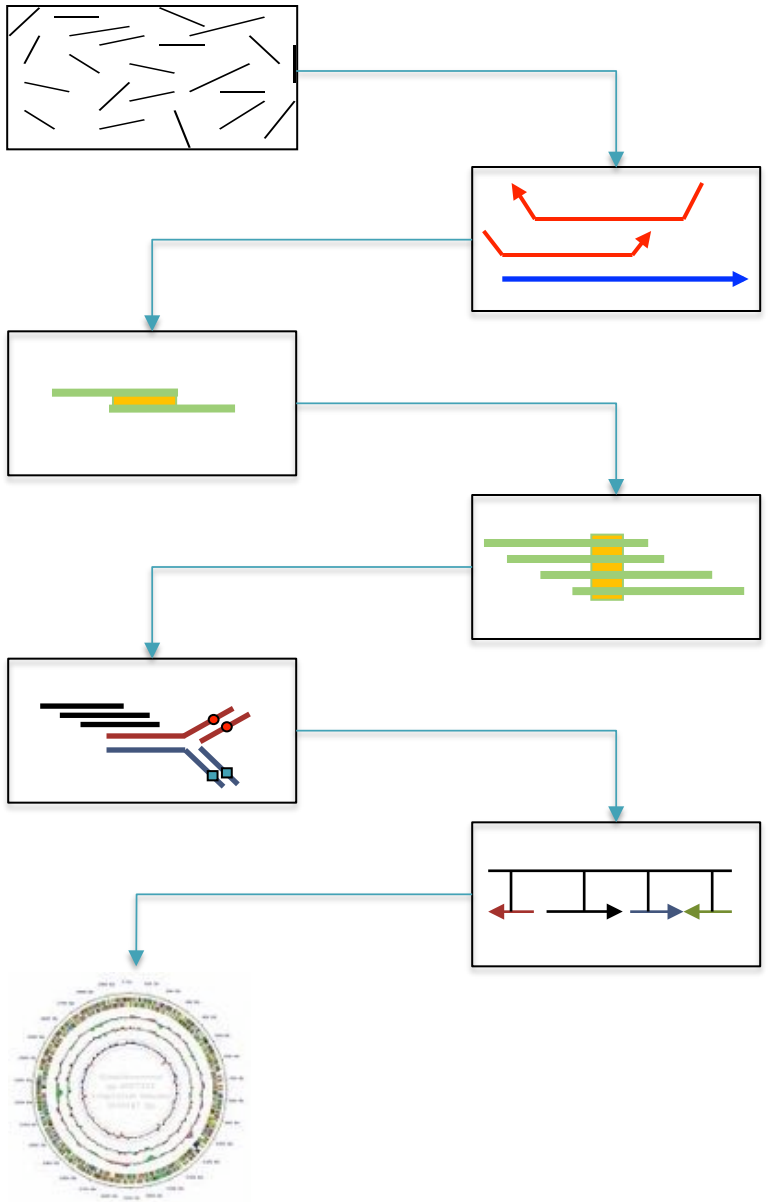
Chin *et al* (2013)
Nature Methods. 10:563–569

< 5x PacBio Coverage > 50x

Celera Assembler aka CANU

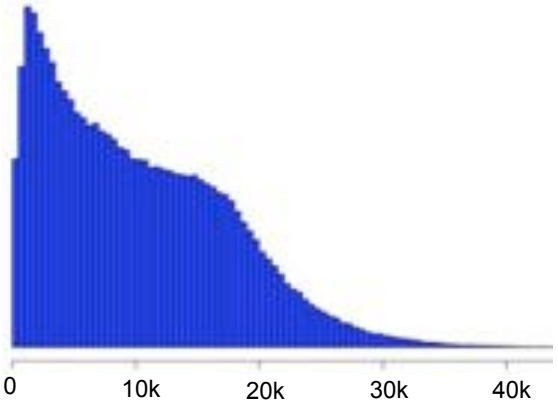
https://github.com/marbl/canu

1. Pre-overlap
   - Consistency checks
2. Trimming
   - Quality trimming & partial overlaps
3. Compute Overlaps
   - Find high quality overlaps
4. Error Correction
   - Evaluate difference in context of overlapping reads
5. Unitigging
   - Merge consistent reads
6. Scaffolding
   - Bundle mates, Order & Orient
7. Finalize Data
   - Build final consensus sequences

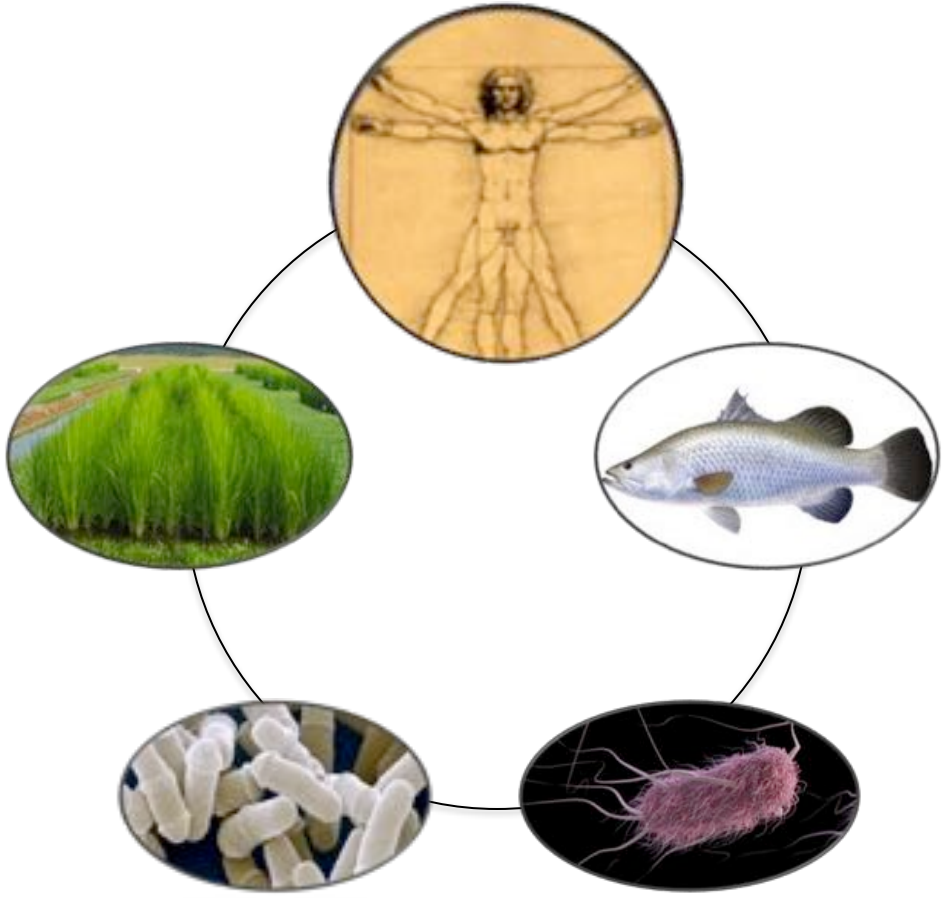


3rd Gen Long Read Sequencing

PacBio RS II

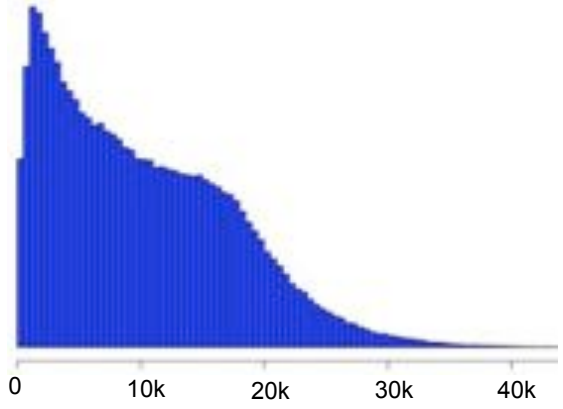


CSHL/PacBio

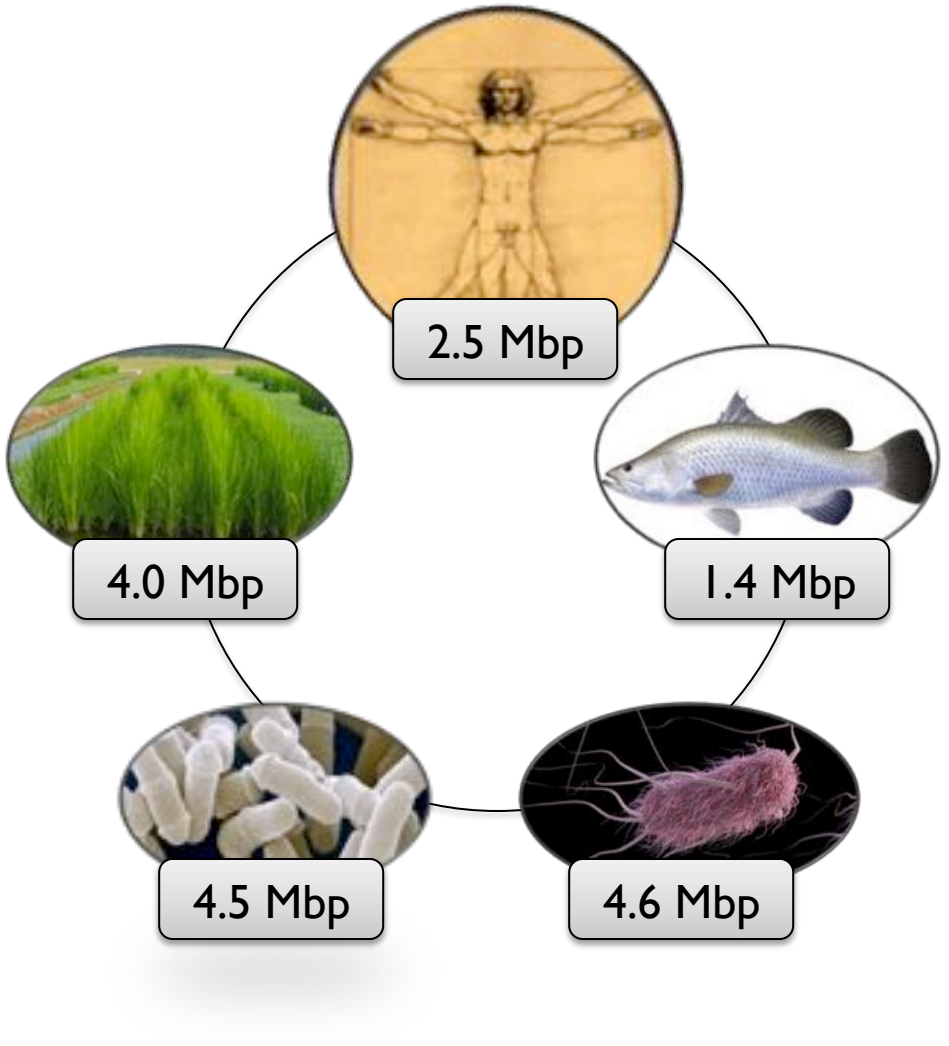


3rd Gen Long Read Sequencing

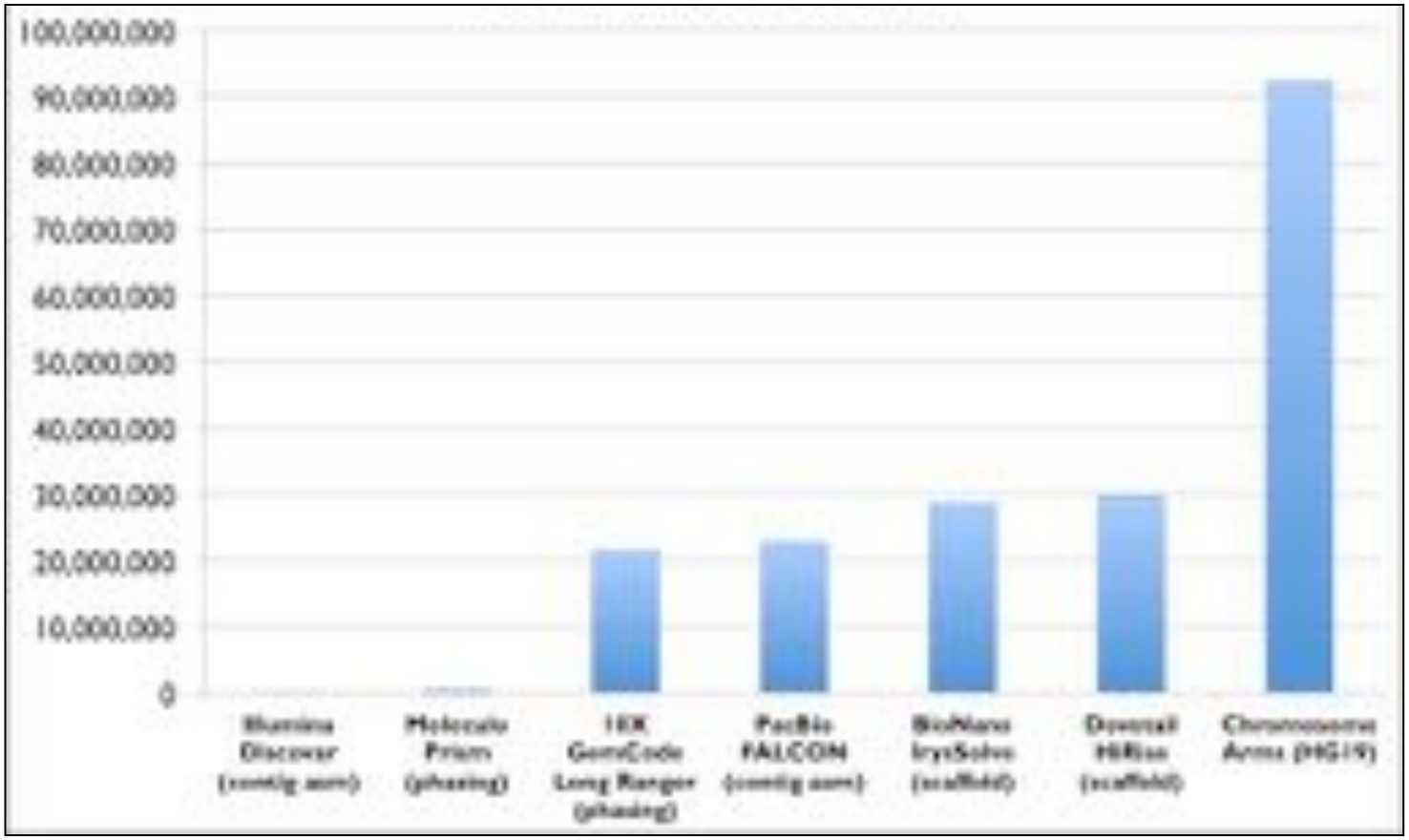
PacBio RS II



CSHL/PacBio



Human Analysis N50s*



Technology	Application	N50	Sample	Citation
Illumina Discovar	contig asm	178,000	NA12877	Putnam *et al.* (2015) arXiv:1502.05331
Moleculo Prism	phasing	563,801	NA12878	Kuleshov *et al.* (2014) Nature BioTech. doi:10.1038/nbt.2833
10X GemCode Long Ranger	phasing	21,600,000	GIAB	Zook *et al.* (2015) bioRxiv. doi: http://dx.doi.org/10.1101/026468
PacBio FALCON	contig asm	22,900,000	JCV-1	Jason Chin, PAG2016
BioNano IrysSolve	scaffold	28,800,000	NA12878	Pendleton *et al.* (2015) Nature Methods. doi:10.1038/nmeth.3454
Dovetail HiRise	scaffold	29,900,000	NA12878	Putnam *et al.* (2015) arXiv:1502.05331

*Cross analysis of different applications

PacBio Roadmap

PacBio RS II

$750k instrument cost
1895 lbs

~$75k / human @ 50x

SMRTcell

150k Zero Mode Waveguides
~10kb average read length
~1 GB / SMRTcell
~$500 / SMRTcell

PacBio Roadmap

PacBio Sequel

$350k instrument cost
841 lbs

~$15k / human @ 50x

SMRTcell v2

1M Zero Mode Waveguides
~15kb average read length
~10 GB / SMRTcell
~$1000 / SMRTcell

Oxford Nanopore

MinION

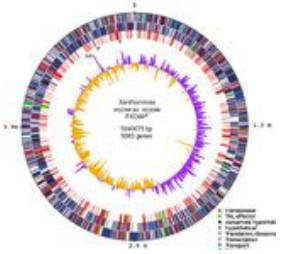
$2k / instrument
1 GB / day
~$300k / human @ 50x

PromethION

$75k / instrument
>>100GB / day
??? / human @ 50x

Oxford Nanopore sequencing, hybrid error correction, and de novo assembly of a eukaryotic genome
Goodwin, S, Gurtowski, J, Ethe-Sayers, S, Deshpande, P, Schatz MC, McCombie, WR (2015) Genome Research doi: 10.1101/gr.191395.115

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

Acknowledgements

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Rahul Amin
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James Gurtowski
Ke Jiang
Hayan Lee
Zak Lemmon
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Giuseppe Narzisi
Maria Nattestad
Aspyn Palatnick
Srividya Ramakrishnan
Fritz Sedlazeck
Rachel Sherman
Greg Vurture
Alejandro Wences

CSHL
Hannon Lab
Gingeras Lab
Jackson Lab
Hicks Lab
Iossifov Lab
Levy Lab
Lippman Lab
Lyon Lab
Martienssen Lab
McCombie Lab
Tuveson Lab
Ware Lab
Wigler Lab

OICR
Karen Ng
Timothy Beck
Yogi Sundaravadanam
John McPherson

NBACC
Adam Phillippy
Serge Koren

Oxford NANOPORE Technologies

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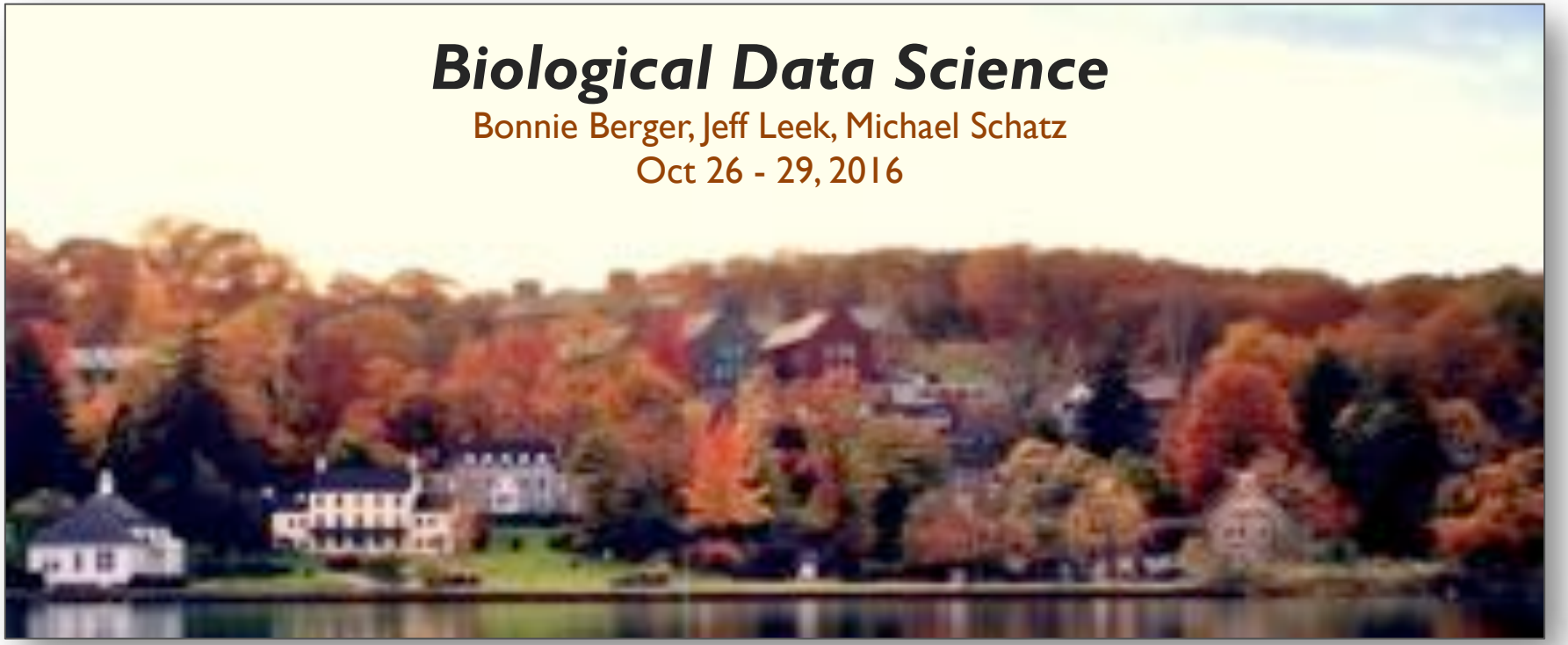
National Human Genome Research Institute

U.S. DEPARTMENT OF ENERGY

SFARI
SIMONS FOUNDATION AUTISM RESEARCH INITIATIVE

ALFRED P. SLOAN FOUNDATION



Thank you

http://www.cs.jhu.edu/~mschatz

@mike_schatz