Genome Sequencing & Assembly

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May 2, 2013

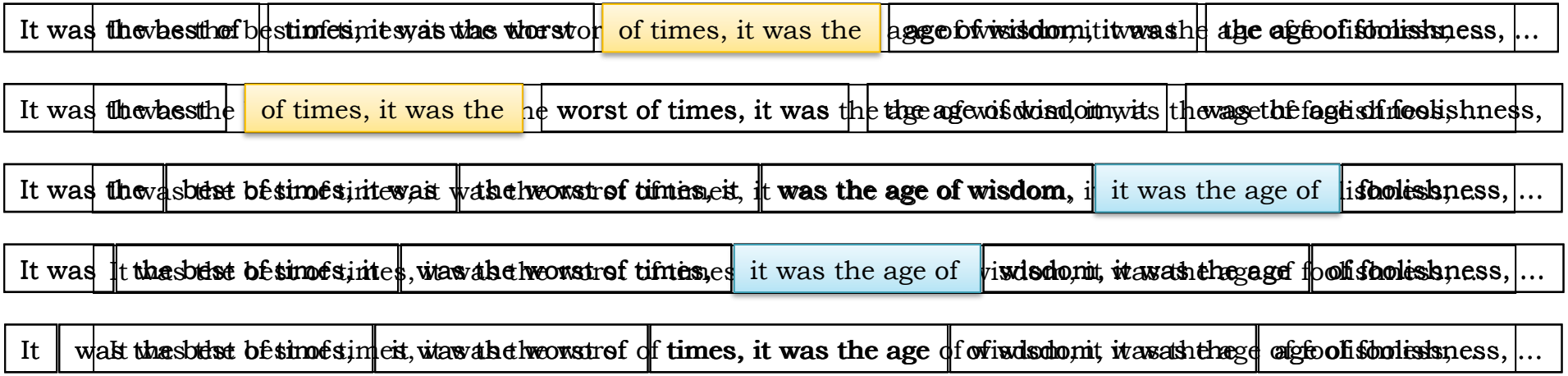
Human Microbiome Consortium

Outline

1. Assembly theory
    1. Assembly by analogy
    2. De Bruijn and Overlap graph
    3. Coverage, read length, errors, and repeats
2. Genome assemblers
    1. Assemblathon 1 & 2
    2. Hybrid assembly with the Celera Assembler
3. Resources

Shredded Book Reconstruction

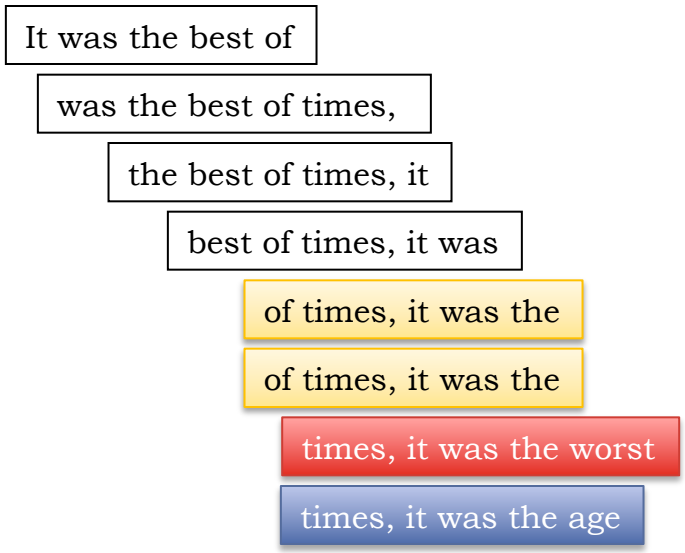
- Dickens accidentally shreds the first printing of A Tale of Two Cities
  - Text printed on 5 long spools



- 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



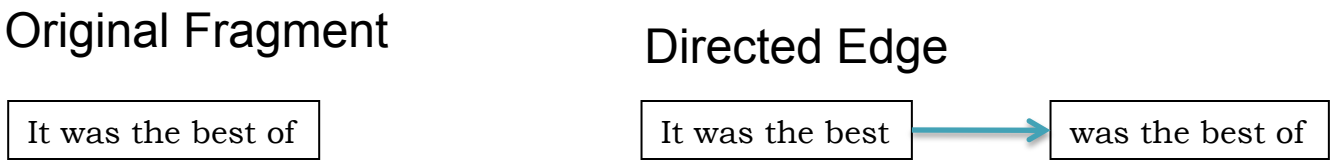
The repeated sequence make the correct reconstruction ambiguous

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

Model the assembly problem as a graph problem

de Bruijn Graph Construction

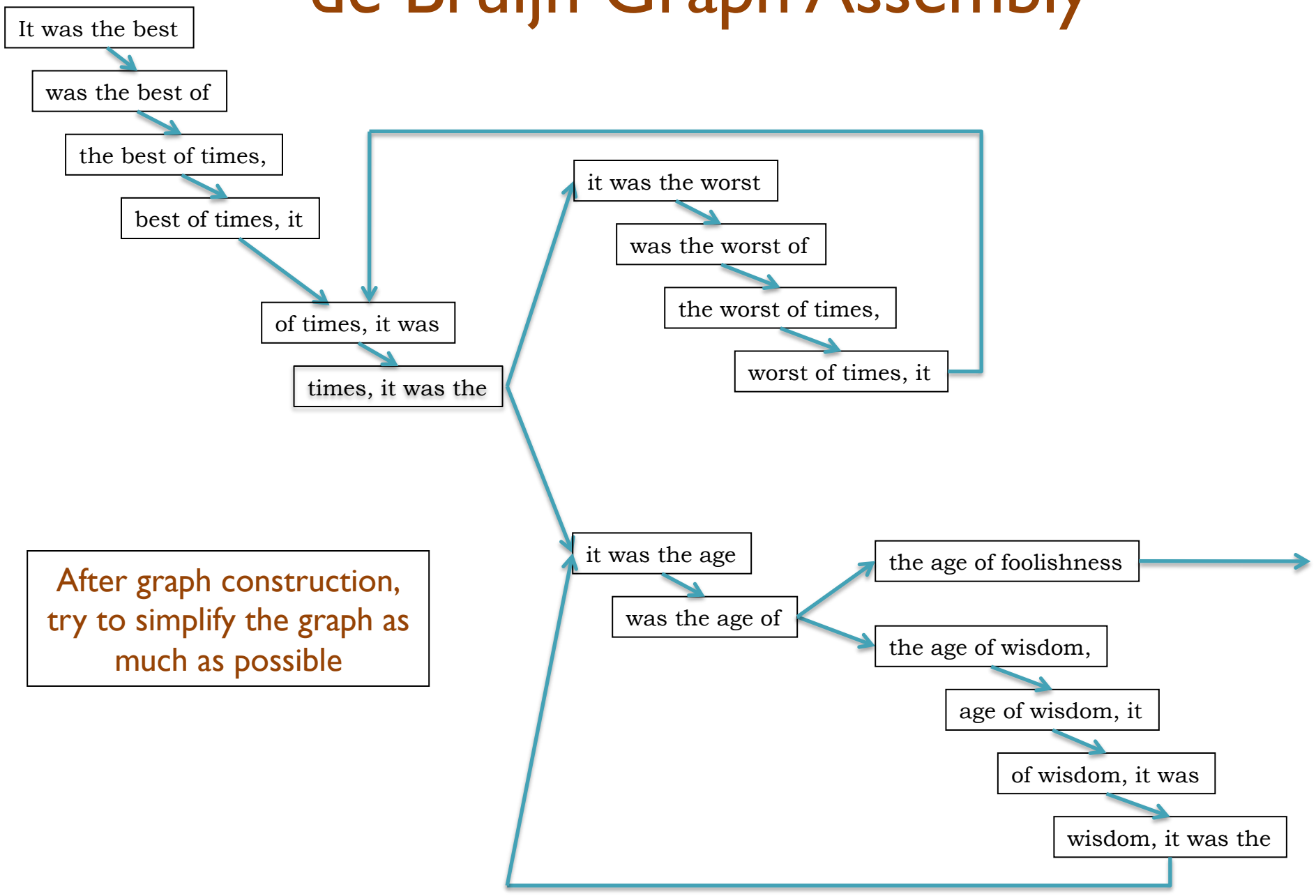
- $D_k = (V,E)$
  - V = All length-k subfragments (k < l)
  - E = Directed edges between consecutive subfragments
    - Nodes overlap by k-1 words



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

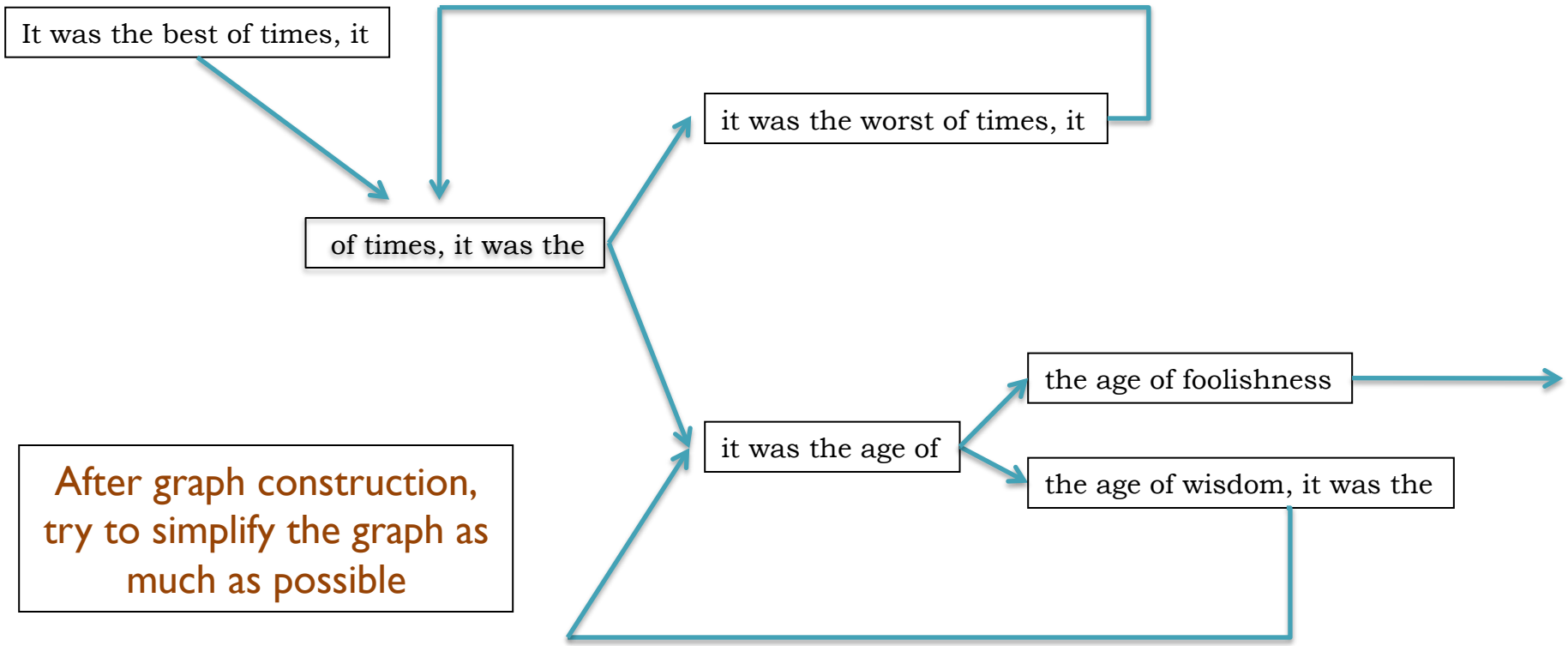
de Bruijn, 1946
Idury and Waterman, 1995
Pevzner, Tang, Waterman, 2001

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

The full tale

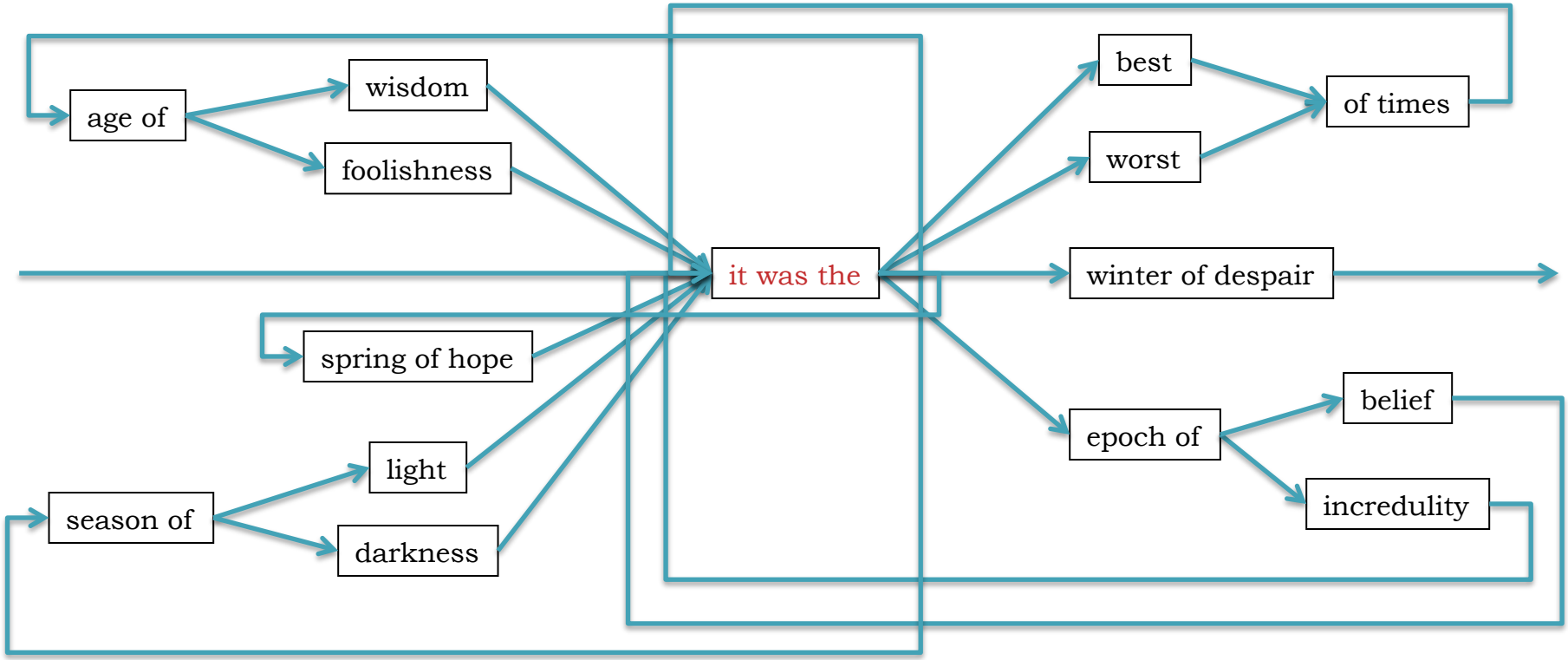
… it was the best of times it was the worst of times …

… 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 …

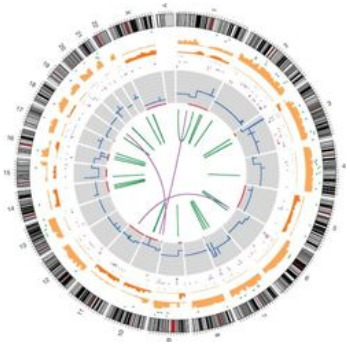


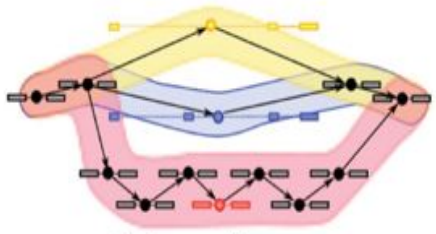
Assembly Applications

- Novel genomes

- Metagenomes

- Sequencing assays
  - Structural variations
  - Transcript assembly

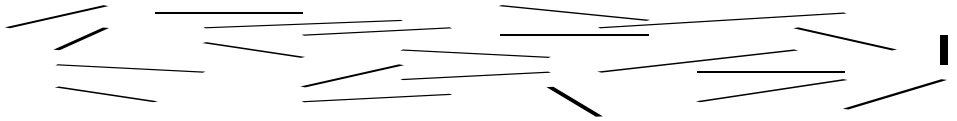




Like Dickens, have to reconstruct from short fragments

Assembling a Genome

1. Shear & Sequence DNA



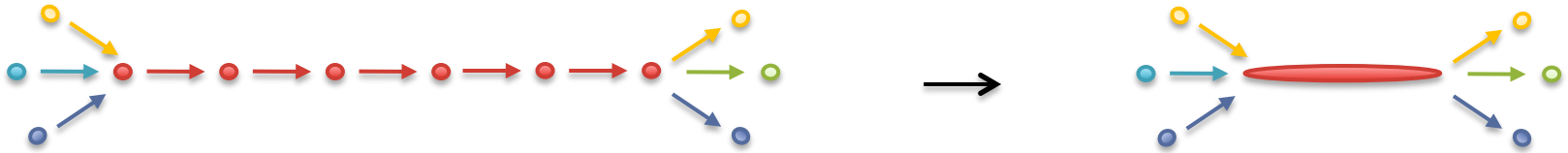
2. Construct assembly graph from overlapping reads

...AGCCTAGGGATGCGCGACACGT

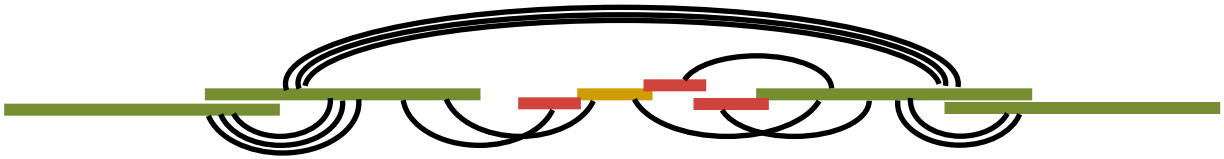
GGATGCGCGACACGTCGCATATCCGGTTTGGTCAACCTCGGACGGAC

CAACCTCGGACGGACCTCAGCGAA...

3. Simplify assembly graph

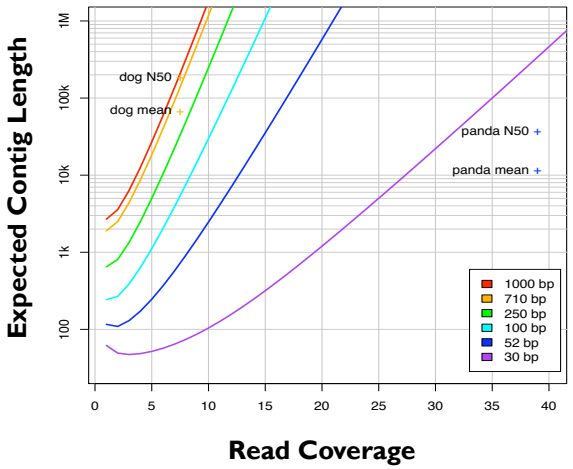


4. Detangle graph with long reads, mates, and other links



Ingredients for a good assembly

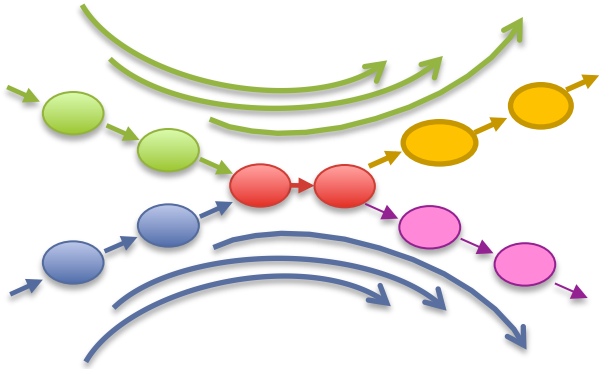
Coverage



High coverage is required

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

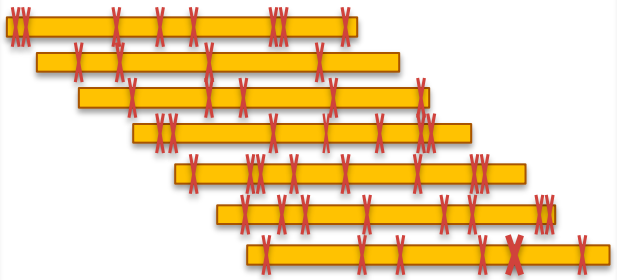
Read Length



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

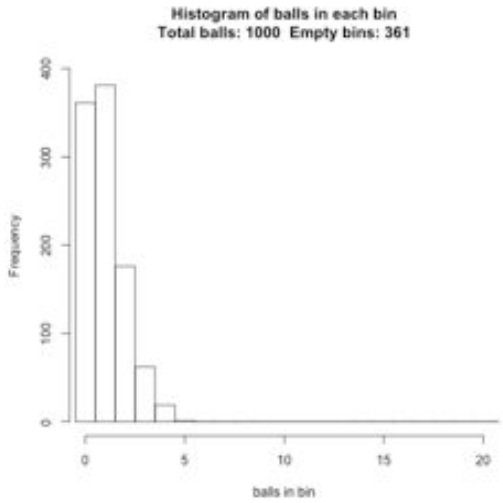


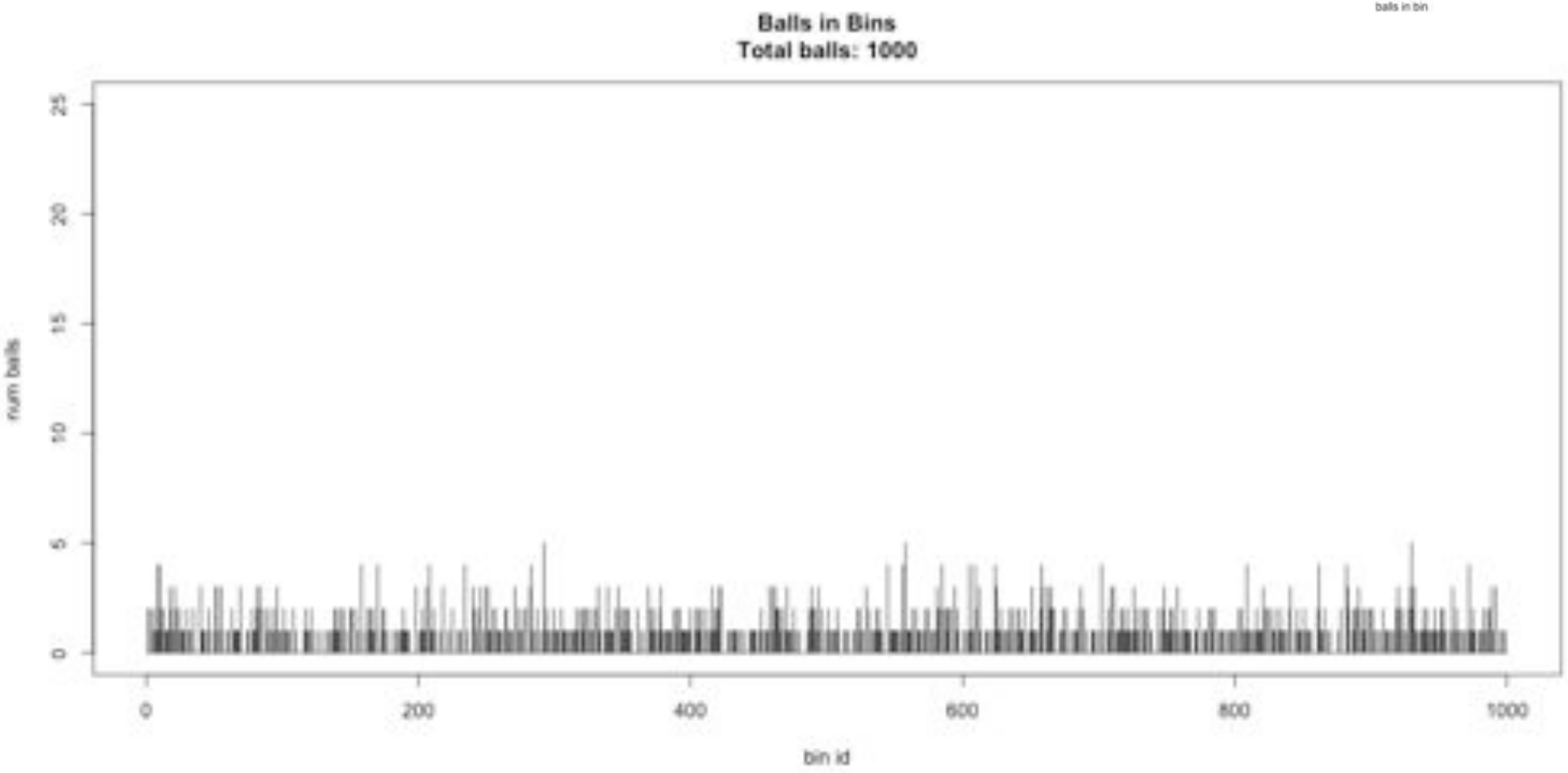
Errors obscure overlaps

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

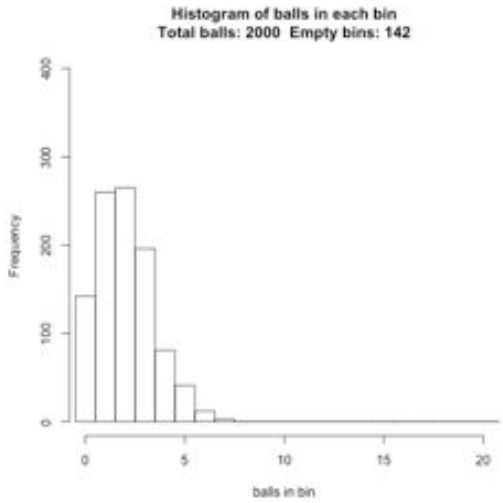
Current challenges in *de novo* plant genome sequencing and assembly
Schatz MC, Witkowski, McCombie, WR (2012) *Genome Biology*. 12:243

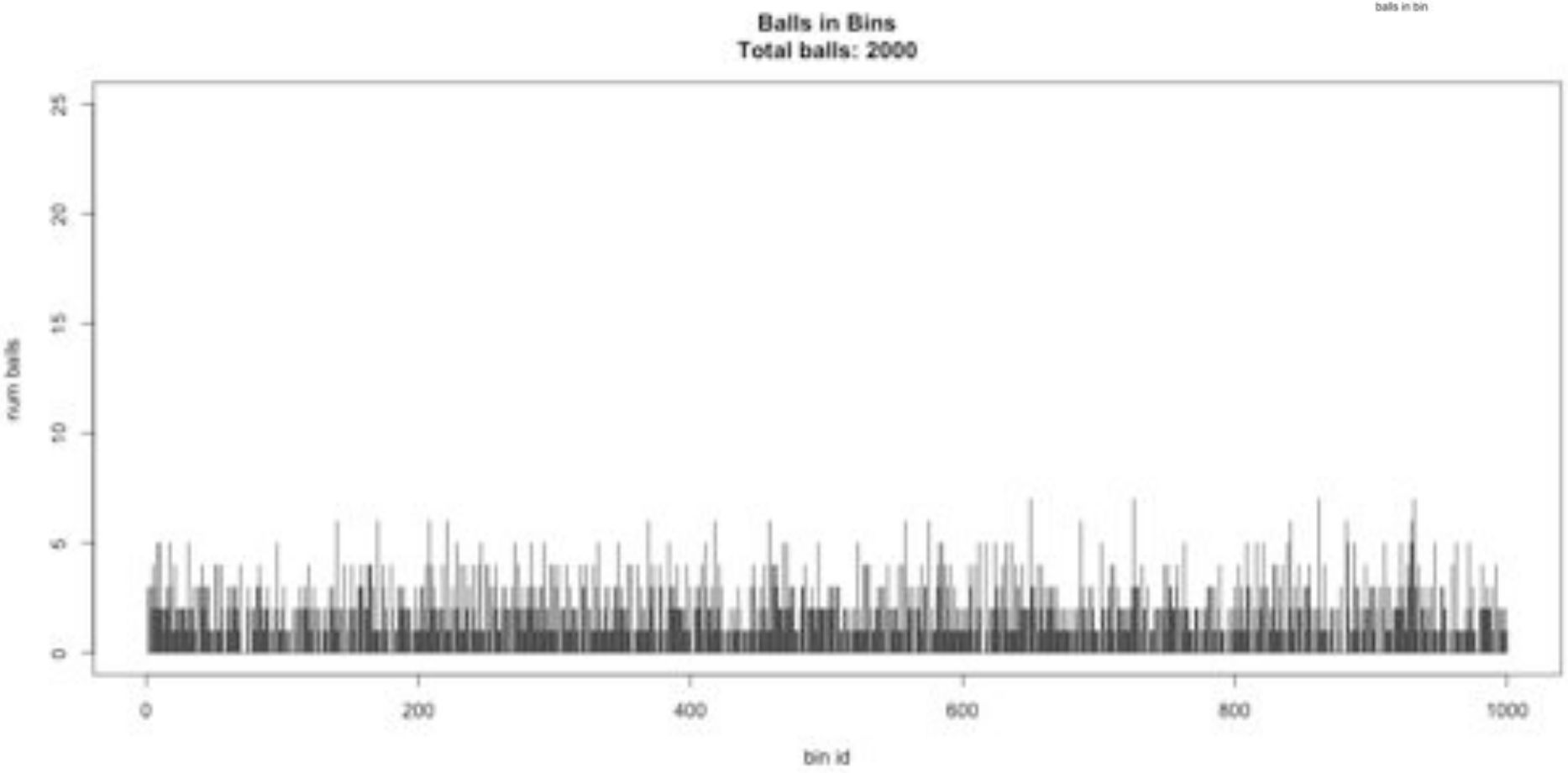
Balls in Bins 1x



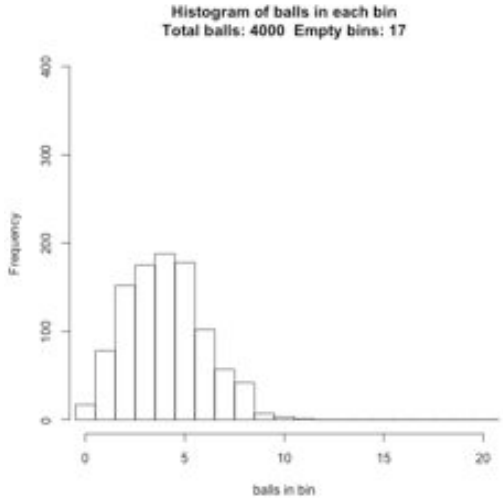


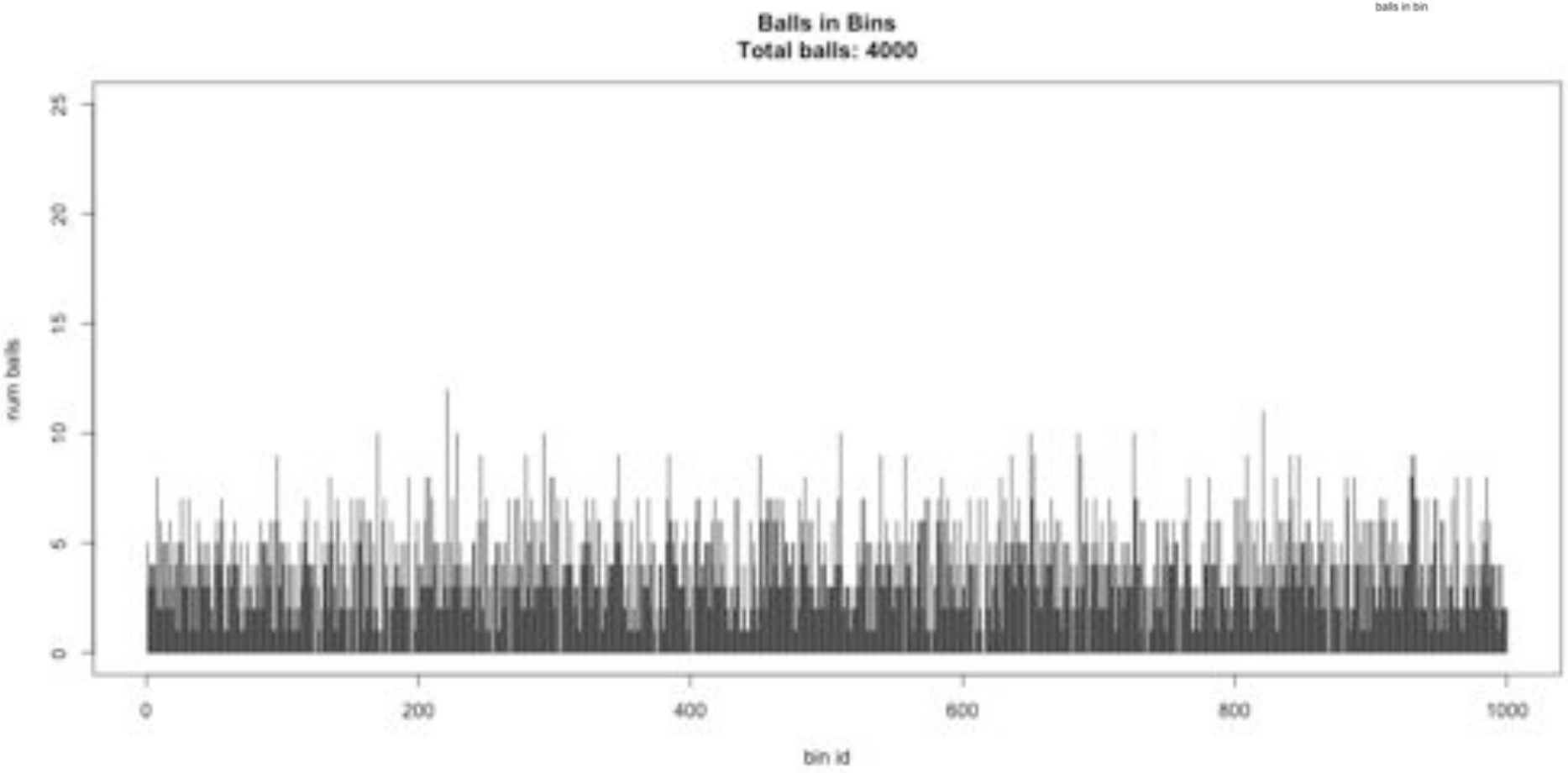
Balls in Bins 2x



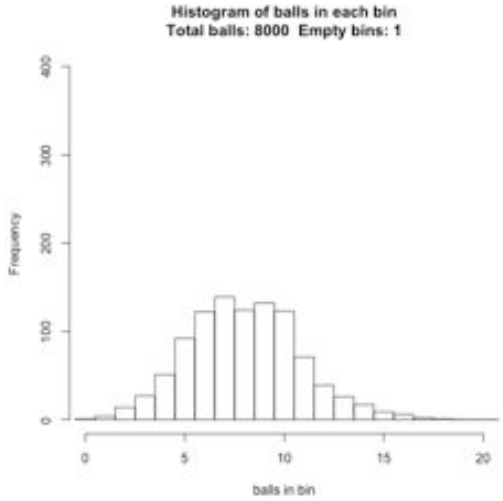


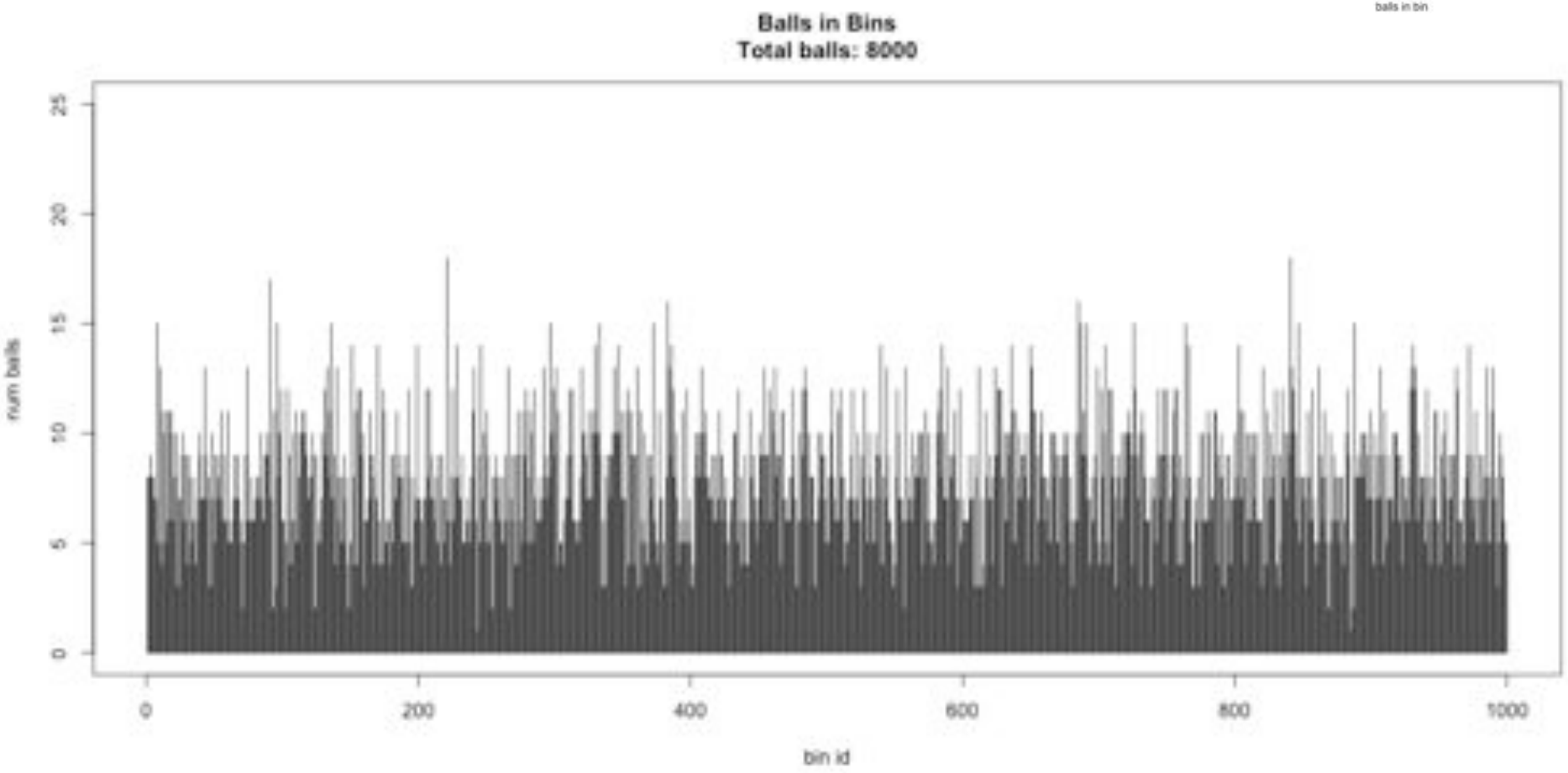
Balls in Bins 4x





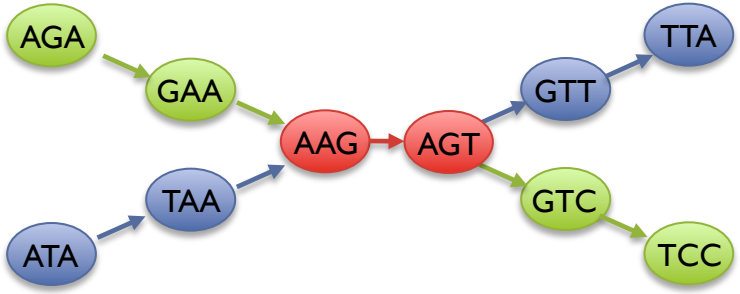
Balls in Bins 8x





Two Paradigms for Assembly

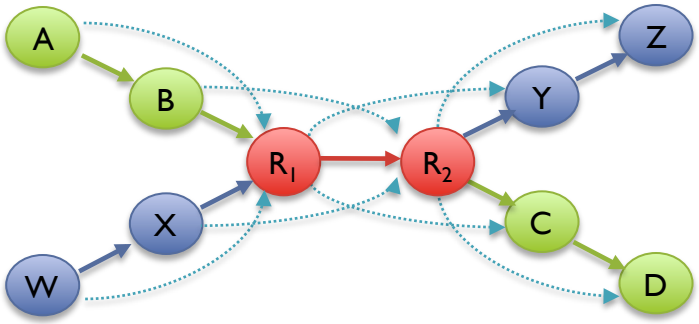
de Bruijn Graph



Short read assemblers

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

Overlap Graph



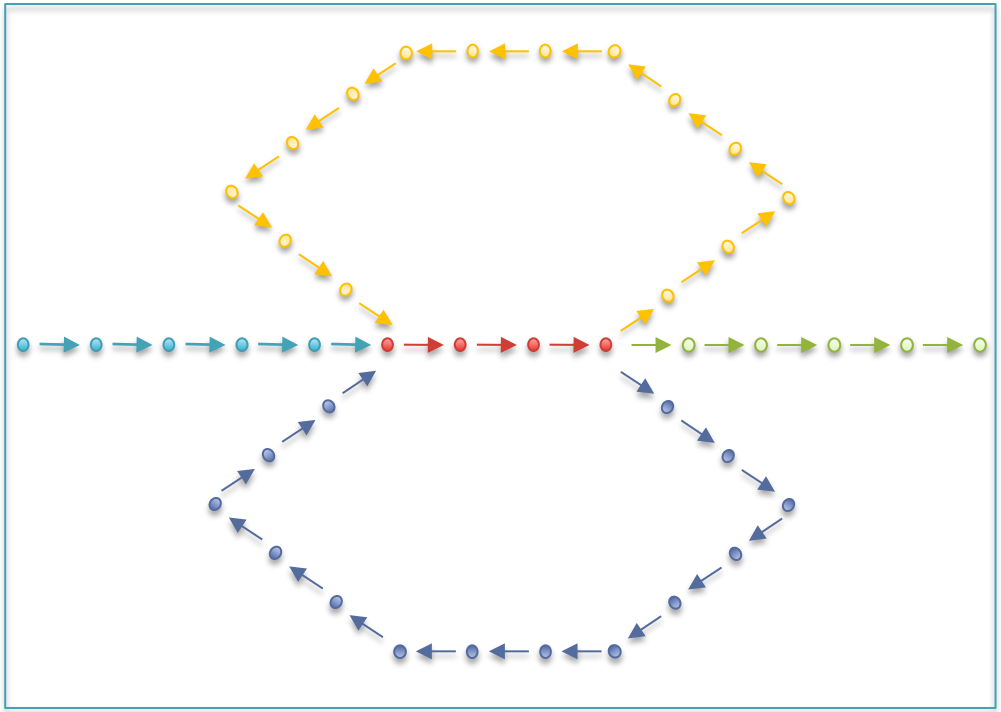
Long read assemblers

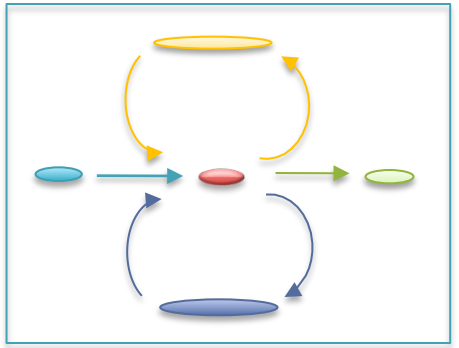
- Repeats depends on read length
- Read coherency, placements kept
- Tangled by high coverage

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

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, and (3) repeats



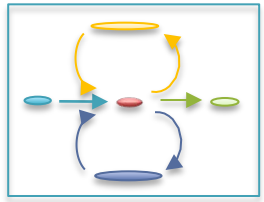


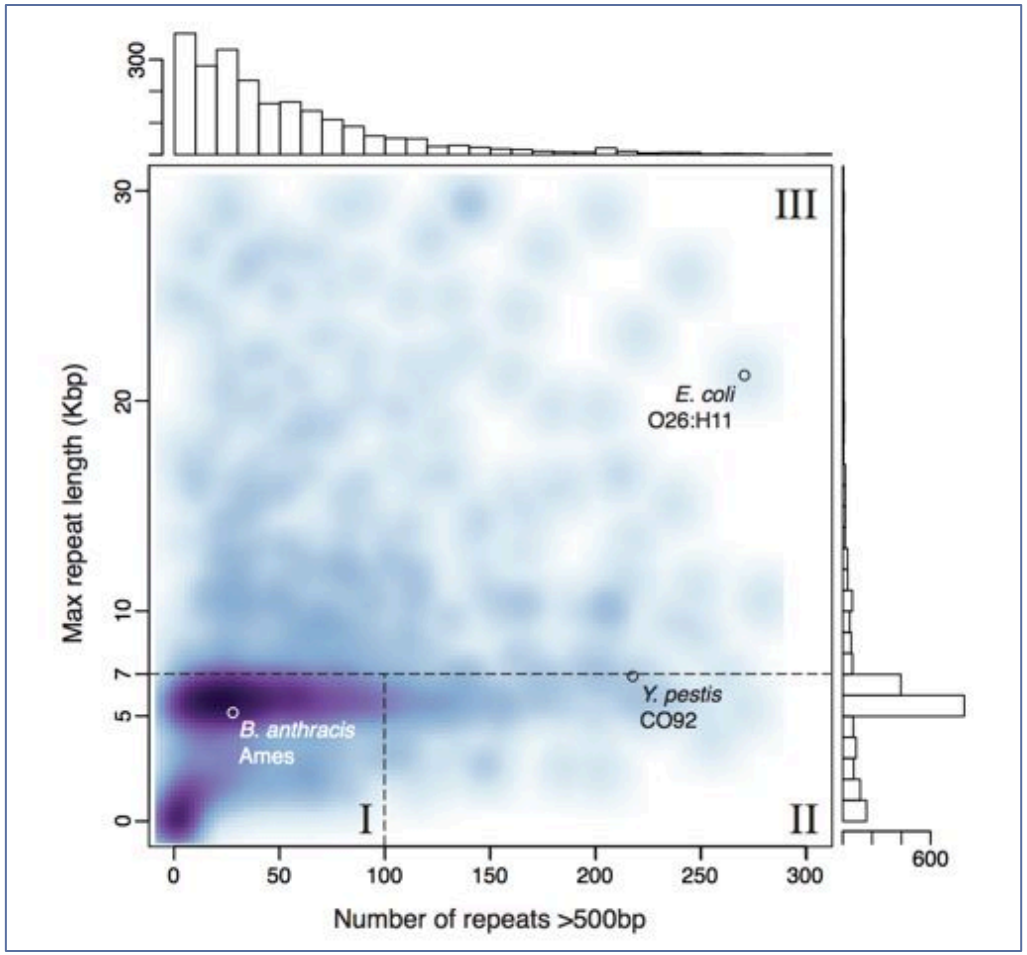
Errors in the graph

(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
the worst of t**y**mes, was the worst of the worst of times, worst of times, it	t**y**mes, was the worst of it was the age times,

Repeats and Read Length

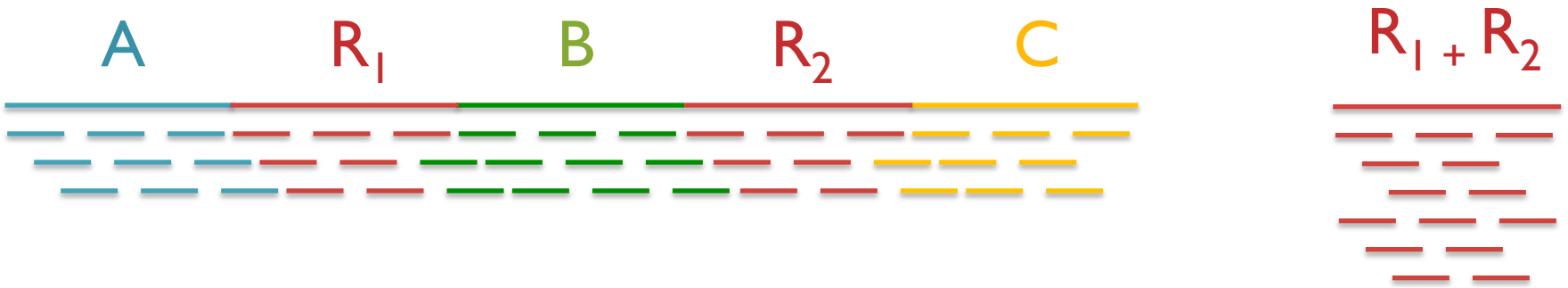




- All microbes have repeats
  - Analyzed all 2,267 available microbial genomes
  - Most are < 7kbp in length and occur in < 100 copies
  - Most repeats are rRNA operons or IS elements
- With enough coverage, contig sizes will be determined by the repeats
  - 5-50kbp contig N50 sizes are common

Reducing assembly complexity of microbial genomes with single-molecule sequencing
Koren S. *et al.* (2013) *Under Review.* http://arxiv.org/abs/1304.3752

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
  - Requires an accurate genome size estimate

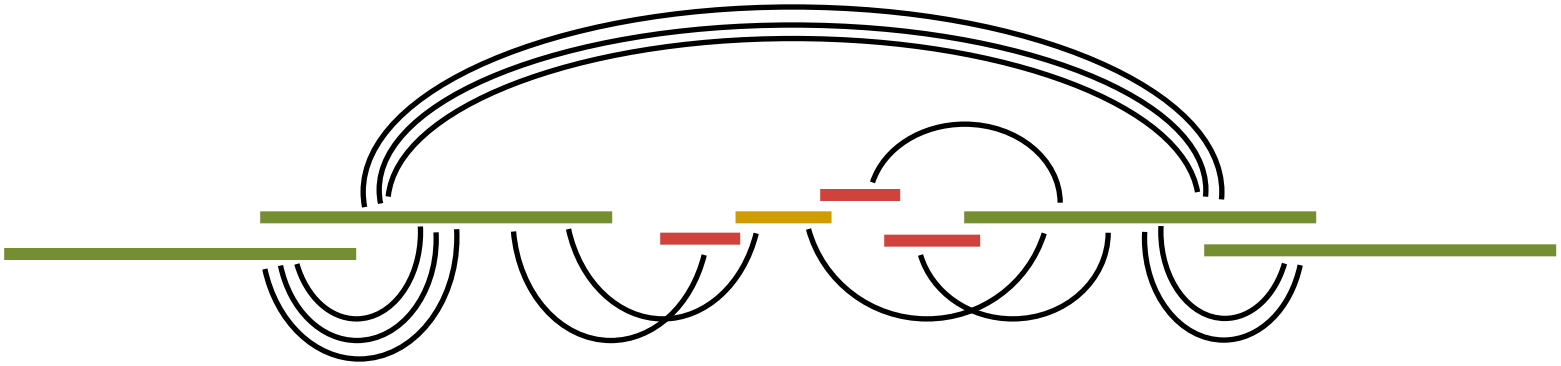
$$\Pr(X-copy)=\binom{n}{k}\left(\frac{X\Delta}{G}\right)^{k}\left(\frac{G-X\Delta}{G}\right)^{n-k}$$

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

Scaffolding

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

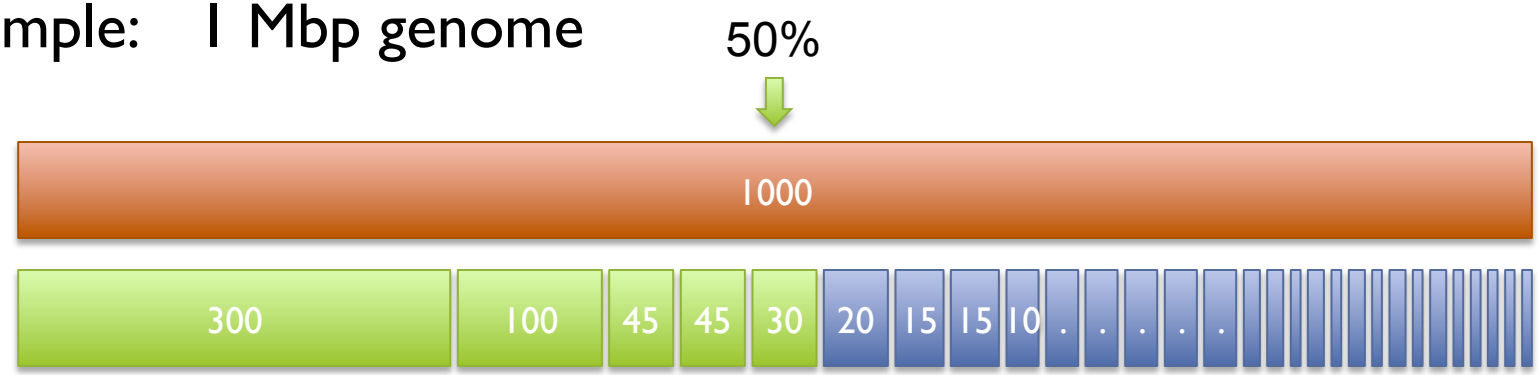
- Iteratively resolve longest, 'most unique' contigs
  - Both overlap graph and de Bruijn assemblers initially collapse repeats into single copies
  - Uniqueness measured by a statistical test on coverage



N50 size

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

Example: 1 Mbp genome



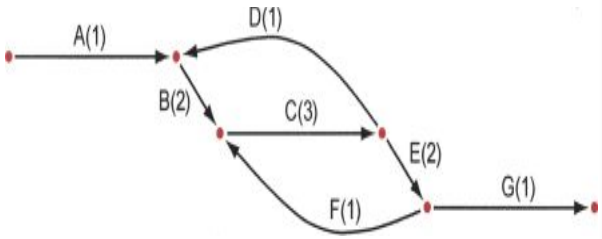
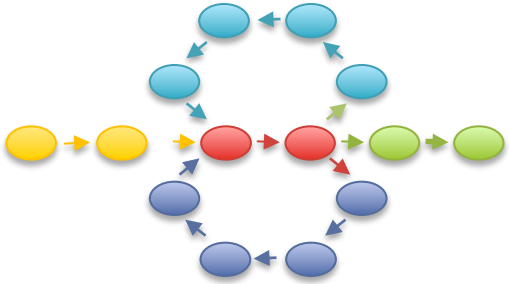
N50 size = 30 kbp

(300k+100k+45k+45k+30k = 520k >= 500kbp)

Note:

N50 values are only meaningful to compare when base genome size is the same in all cases

Assembly Algorithms

ALLPATHS-LG	SOAPdenovo	Celera Assembler
		
Broad's assembler (Gnerre et al. 2011)	BGI's assembler (Li et al. 2010)	JCVI's assembler (Miller et al. 2008)
De bruijn graph Short + PacBio (patching)	De bruijn graph Short reads	Overlap graph Medium + Long reads
Easy to run if you have compatible libraries	Most flexible, but requires a lot of tuning	Supports Illumina/454/PacBio Hybrid assemblies
http://www.broadinstitute.org/software/allpaths-lg/blog/	http://soap.genomics.org.cn/soapdenovo.html	http://wgs-assembler.sf.net

THE ASSEMBLATHON

- Attempt to answer the question:
  "What makes a good assembly?"

- Organizers provided simulated sequence data
  - Simulated 100 base pair Illumina reads from simulated diploid organism
  - 41 submissions from 17 groups

Assemblathon 1: A competitive assessment of de novo short read assembly methods.
Earl, DA, et al. (2011) *Genome Research.* doi: 10.1101/gr.126599.111

Final Rankings

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								
WTSI-P	64							☆	
EBI	64						★		
CRACS	64					☆			

- ALLPATHS and SOAPdenovo came out neck-and-neck followed closely behind by SGA, Celera Assembler, ABySS
  - My recommendation for "typical" short read assembly is to use ALLPATHS
  - See Assemblathon 2 paper for more discussion

Assemblathon 2: evaluating de novo methods of genome assembly in three vertebrate species
Bradman, KR. (2013) *Under Review.* http://arxiv.org/abs/1301.5406

Hybrid Sequencing

Illumina

Sequencing by Synthesis

High throughput (60Gbp/day)
High accuracy (~99%)
Short reads (~100bp)

Pacific Biosciences

SMRT Sequencing

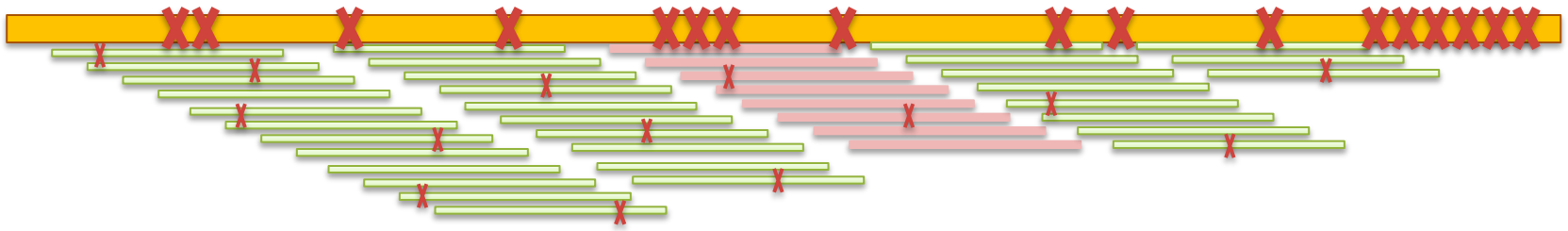
Lower throughput (600Mbp/day)
Lower accuracy (~85%)
Long reads (2-25kbp)

PacBio Error Correction

http://wgs-assembler.sf.net

1. Correction Pipeline
    1. Map short reads to long reads
    2. Trim long reads at coverage gaps
    3. Compute consensus for each long read

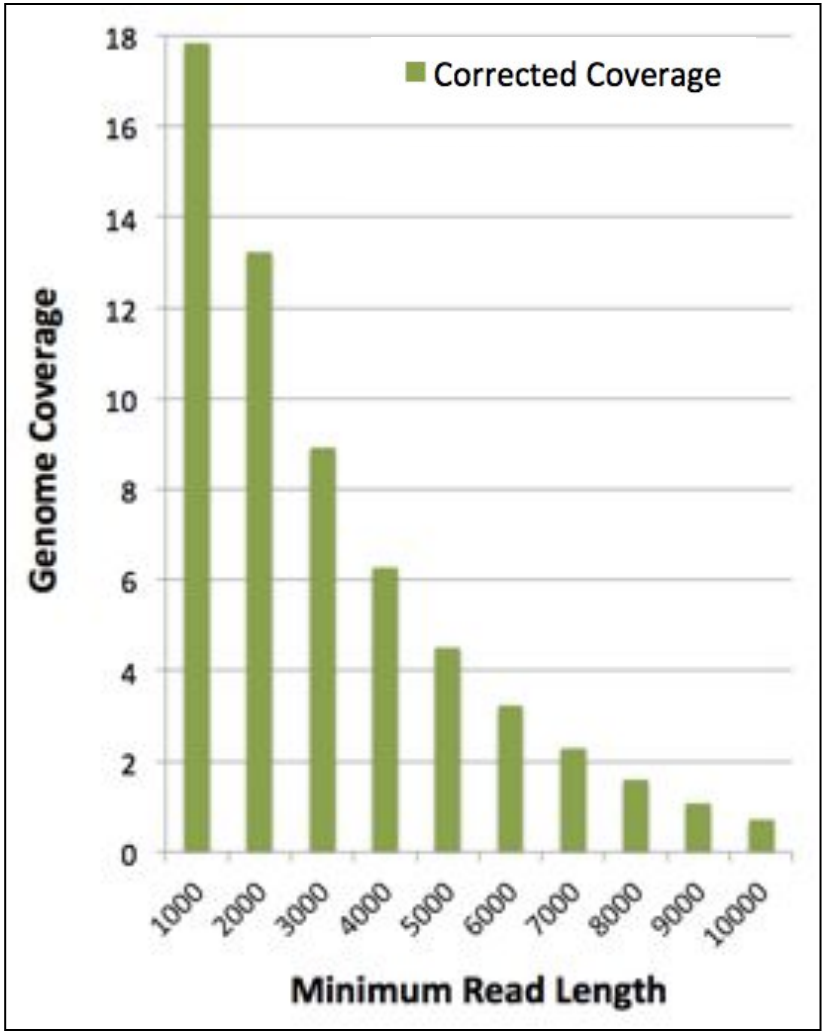
2. Error corrected reads can be easily assembled, aligned



Hybrid error correction and de novo assembly of single-molecule sequencing reads.
Koren, S, Schatz, MC, et al. (2012) *Nature Biotechnology*. doi:10.1038/nbt.2280

Preliminary Rice Assemblies

Assembly	Contig NG50
HiSeq Fragments 50x 2x100bp @ 180	3,925
MiSeq Fragments 23x 459bp 8x 2x251bp @ 450	6,332
"ALLPATHS-recipe" 50x 2x100bp @ 180 36x 2x50bp @ 2100 51x 2x50bp @ 4800	18,248
PBeCR Reads 7x @ 3500 ** MiSeq for correction	50,995
PBeCR + Illumina Shred 7x @ 3500 ** MiSeq for correction 5x @ 3000bp shred	59,695

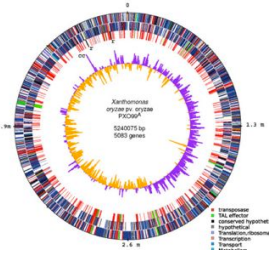


In collaboration with McCombie & Ware labs @ CSHL

Other Resources

Resource	URL	Description
Google	http://www.google.com	Internet Search
Google Scholar	http://scholar.google.com/	Literature Searches
SeqAnswers	http://seqanswers.com/	Bioinformatics Forum
Wikipedia	http://www.wikipedia.org/	Overview on anything
Clovr	http://clovr.org/	Automated Sequence Analysis
Circos	http://circos.ca/	Circular Genome Plots
Galaxy	http://usegalaxy.org	Sequence Analysis in the clouds
GraphViz	http://www.graphviz.org/	Graph Visualization
IGV	http://www.broadinstitute.org/igv/	Read Mapping Viz
R	http://www.r-project.org/	Stats & Visualizations
Schatz Lab	http://schatzlab.cshl.edu/teaching/	Exercises and Lectures

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

Schatz Lab
Giuseppe Narzisi
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Hayan Lee
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Mitch Bekritsky
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Rushil Gupta
Avijit Gupta
Shishir Horane
Deepak Nettem
Varrun Ramani
Kelly Moffat
Eric Biggers
Aspyn Palatnick

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

IT Department

NBACC
Adam Phillippy
Sergey Koren

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Thank You

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