Graphs and Genomes

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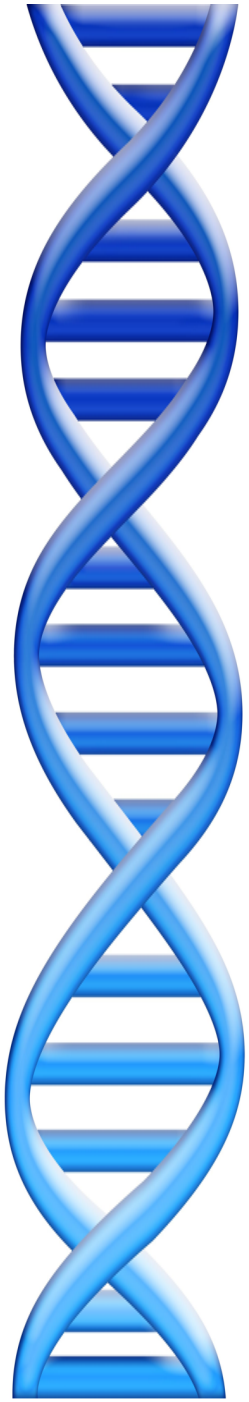
July 27, 2012

CSHL Undergraduate Research Program



Outline

1. Graph Searching
2. Assembly by analogy
3. Genome Assembly

Biological Networks

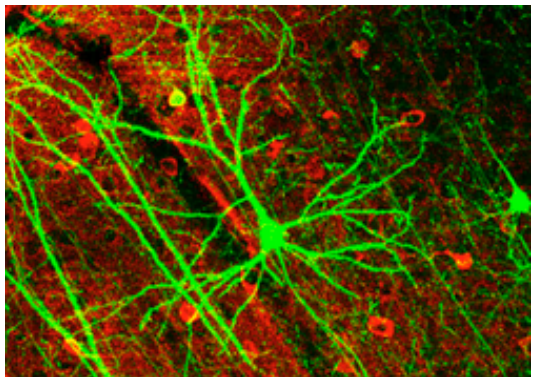
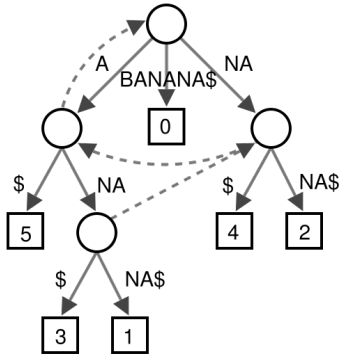
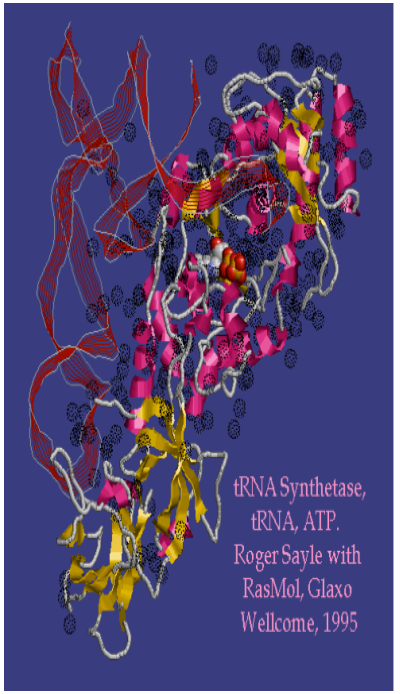
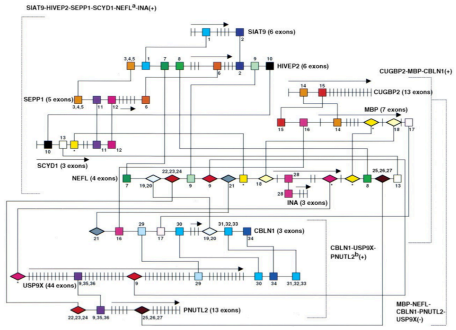


Figure 5 Putative regulatory elements shared between groups of correlated and anticorrelated genes

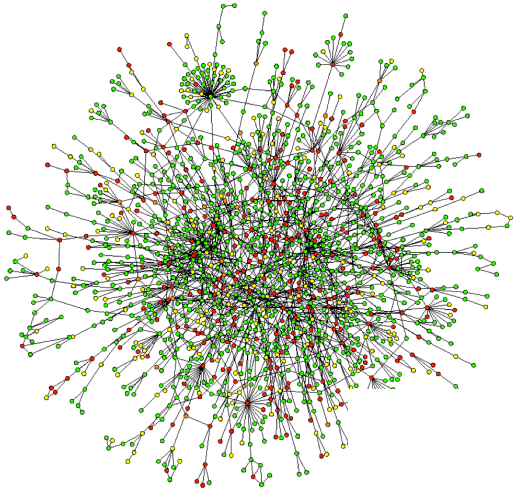


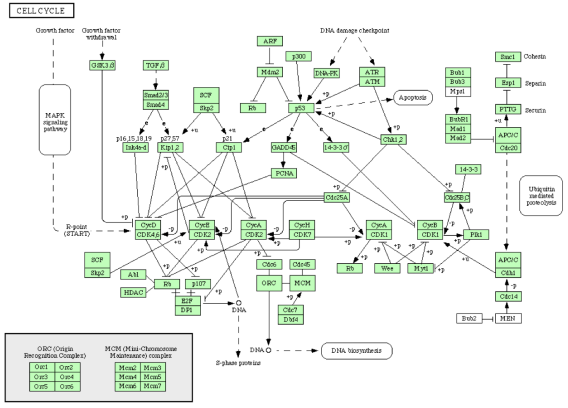


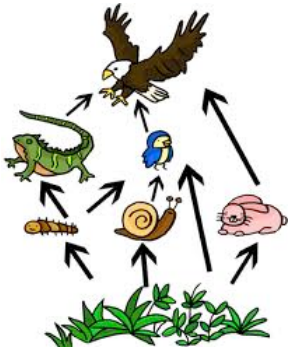


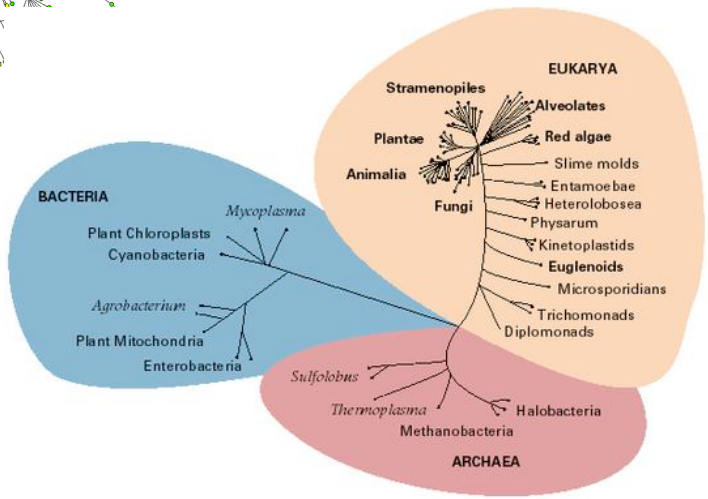
Vanessa M. Brown et al. Genome Res. 2002; 12: 868-884

Cold Spring Harbor Laboratory Press

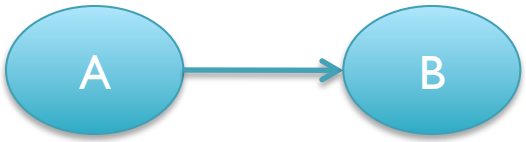






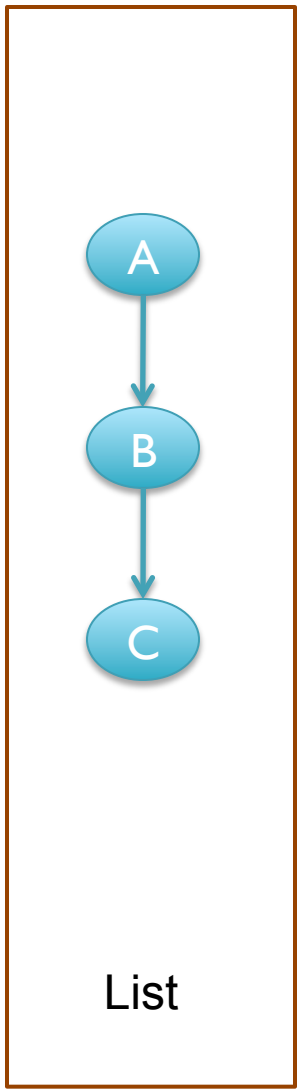


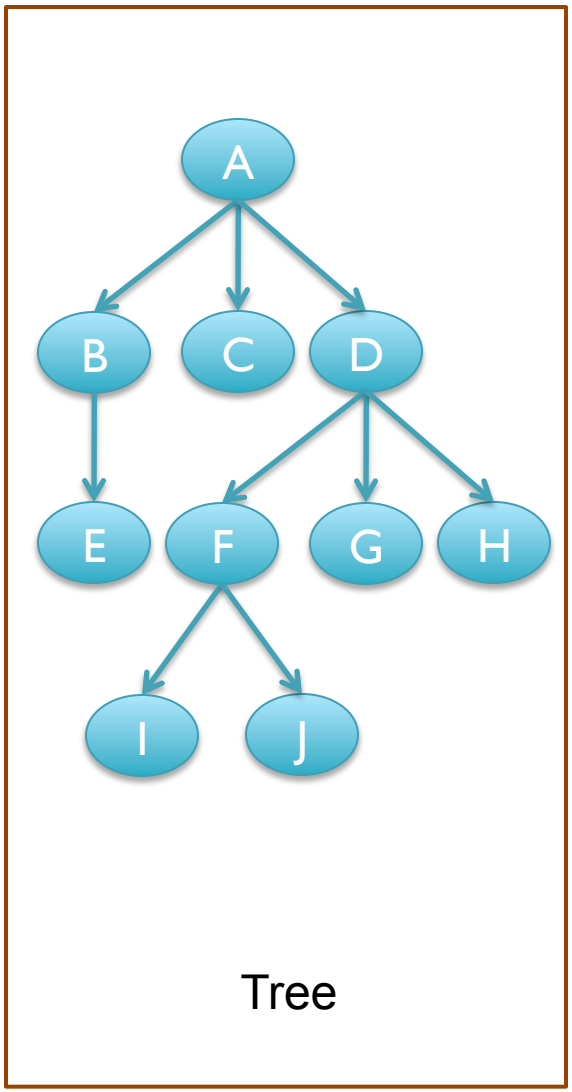
Graphs

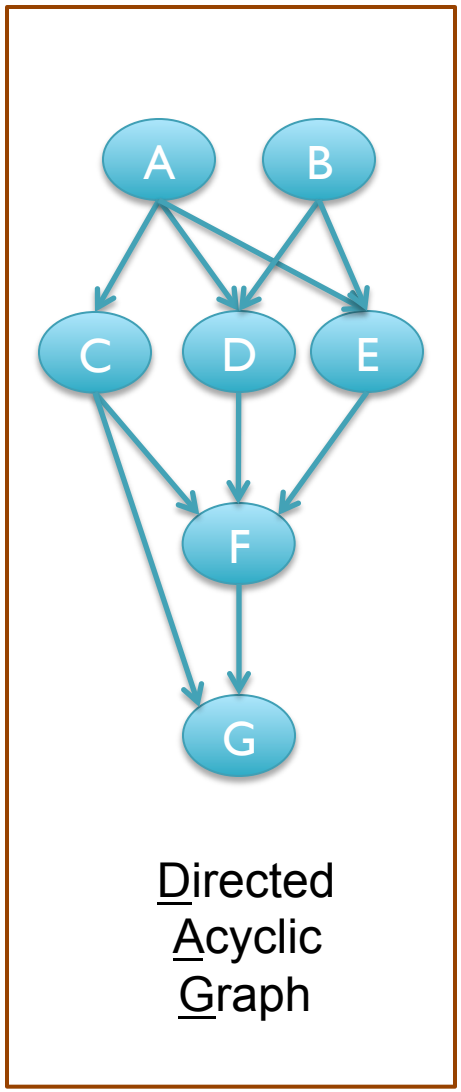


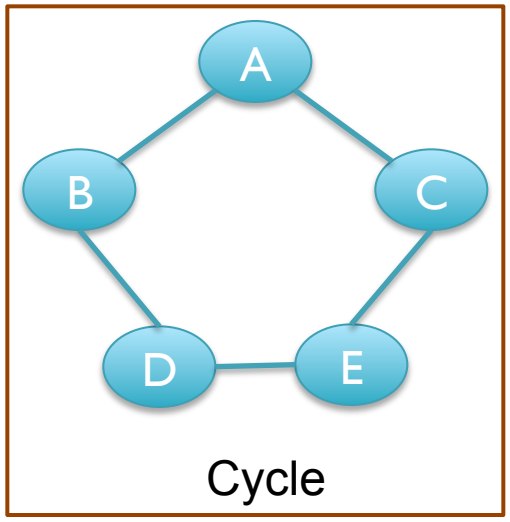
- Nodes
  - People, Proteins, Genes, Neurons, Sequences, Numbers, …
- Edges
  - A is connected to B
  - A is related to B
  - A regulates B
  - A precedes B
  - A interacts with B
  - A activates B
  - …

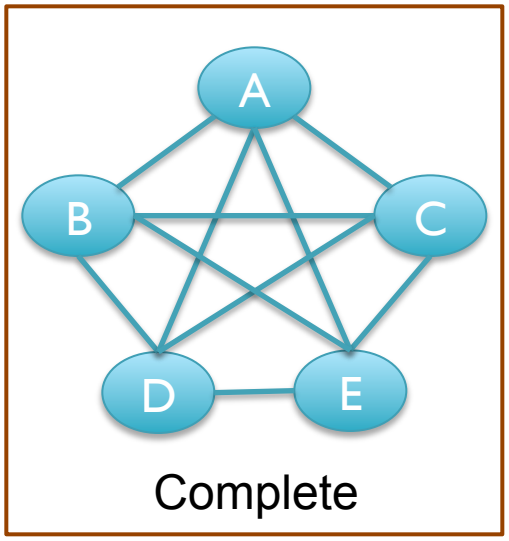
Graph Types


A
B
C
List


A
B
C
D
E
F
G
H
I
J
Tree


A
B
C
D
E
F
G
Directed
Acyclic
Graph


A
B
C
D
E
Cycle

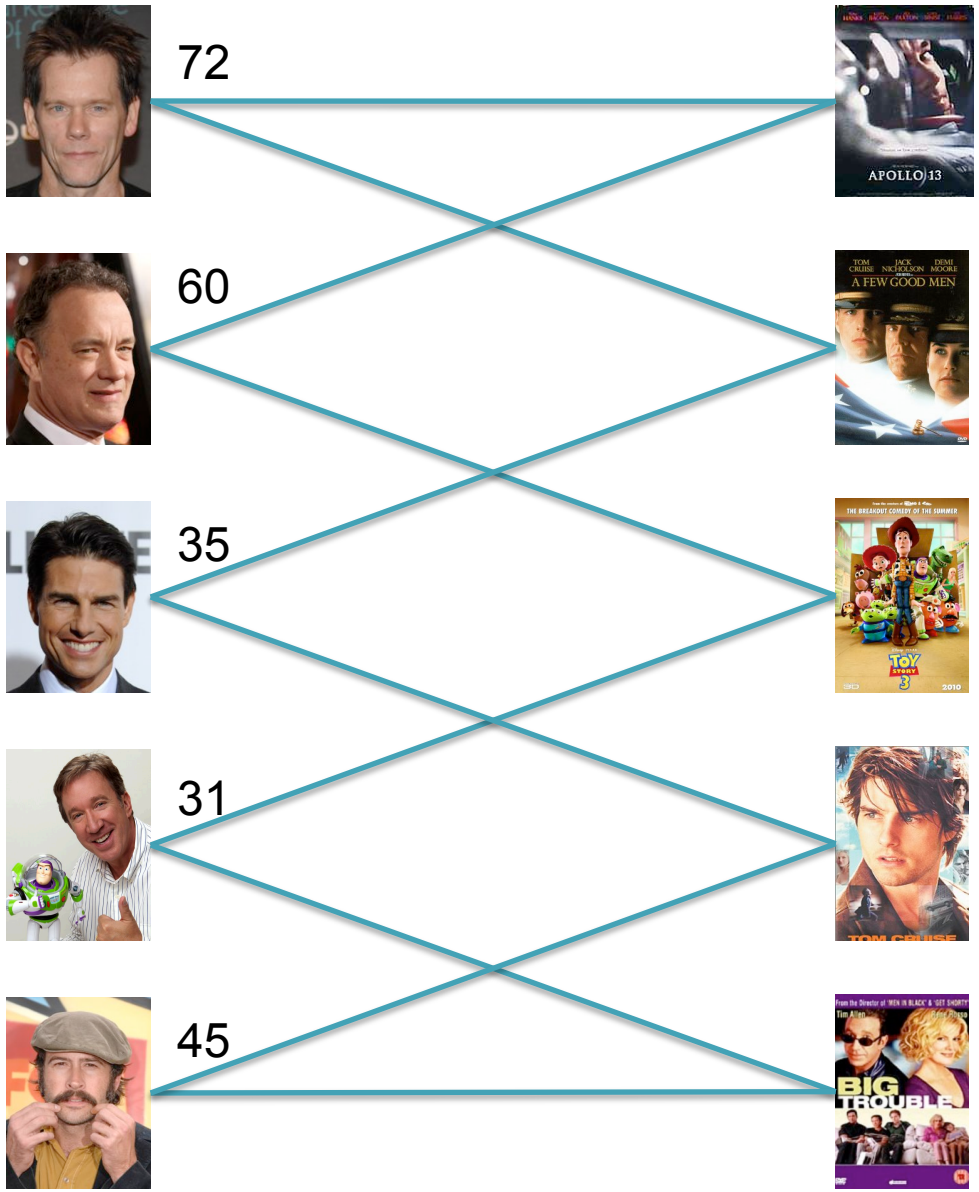

A
B
C
D
E
Complete

Kevin Bacon and Bipartite Graphs

Find the ***shortest*** path from Kevin Bacon to Jason Lee

Breadth First Search: 4 hops

Bacon Distance: 2



BFS and TSP

- BFS computes the shortest path between a pair of nodes in $O(|E|) = O(|N|^2)$

- What if we wanted to compute the shortest path visiting every node once?
  - Traveling Salesman Problem

ABDCA: 4+2+5+3 = 14

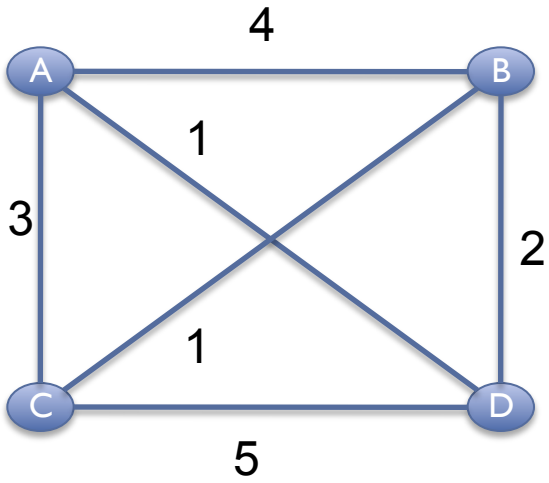
ACDBA: 3+5+2+4 = 14*

ABCDA: 4+1+5+1 = 11

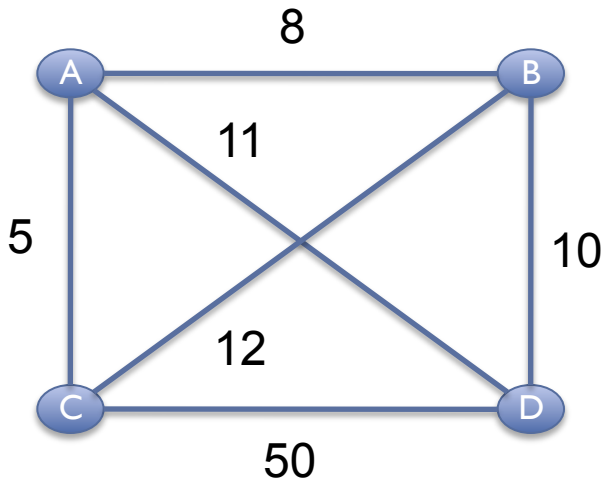
ADCBA: 1+5+1+4 = 11*

ACBDA: 3+1+2+1 = 7

ADBCA: 1+2+1+3= 7 *



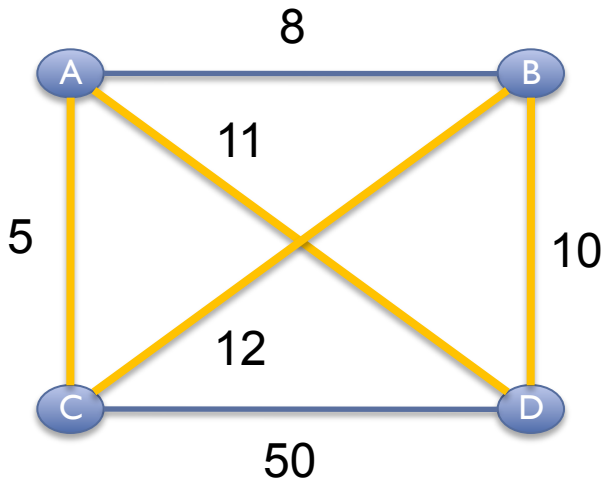
Greedy Search



Greedy Search

Greedy Search

```
cur=graph.randNode()
while (!done)
    next=cur.getNextClosest()
```



Greedy: ABDCA = 5+8+10+50= 73

Optimal: ACBDA = 5+11+10+12 = 38

Greedy finds the global optimum only when

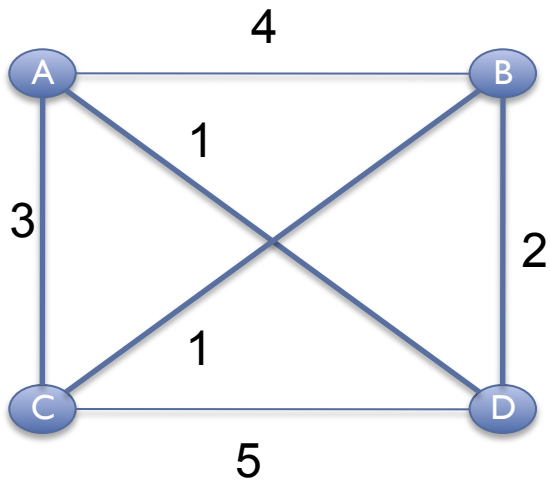
1. Greedy Choice: Local is correct without reconsideration
2. Optimal Substructure: Problem can be split into subproblems

Optimal Greedy: Making change with the fewest number of coins

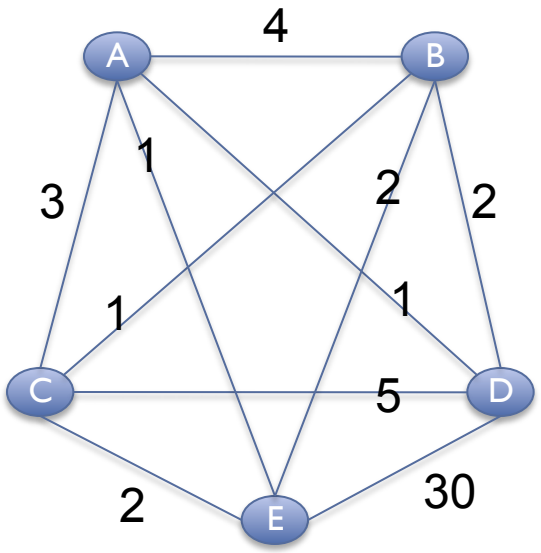
TSP Complexity

- No fast solution
  - Knowing optimal tour through n cities doesn't seem to help much for n+1 cities

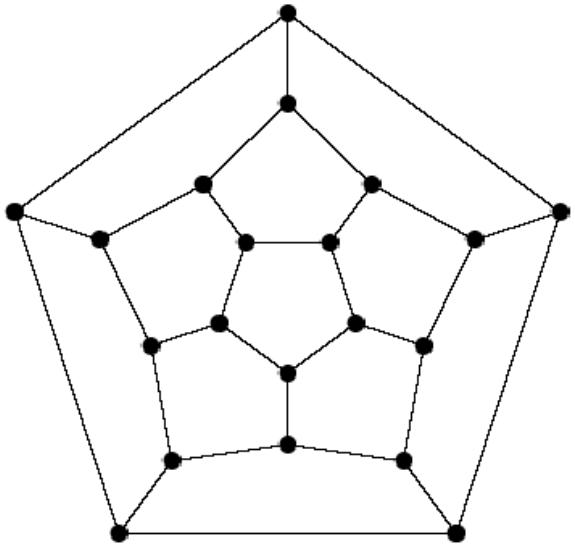
[How many possible tours for n cities?]



- Extensive searching is the only provably correct algorithm
  - Brute Force: O(n!)
    - ~20 cities max
    - $20! = 2.4 \times 10^{18}$
  - Branch-and-Bound can sometimes help

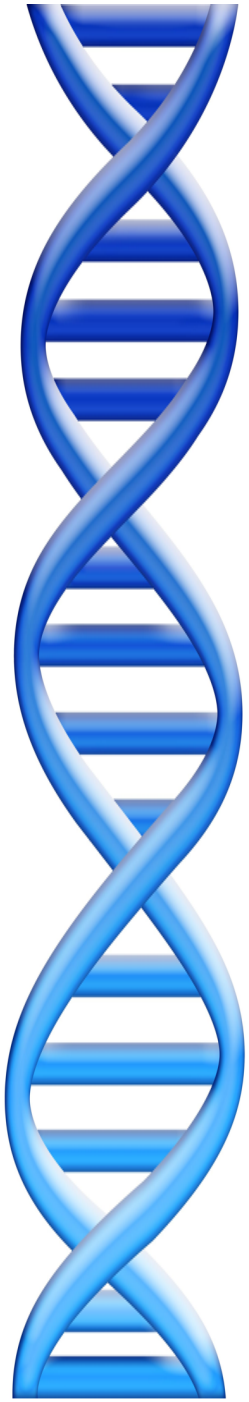


TSP and NP-complete



- TSP is one of many extremely hard problems of the class NP-complete
  - Extensive searching is the only way to find an exact solution
  - Often have to settle for approx. solution

- WARNING: Many biological problems are in this class
  - Find a tour the visits every node once (Genome Assembly)
  - Find the smallest set of vertices covering the edges (Essential Genes)
  - Find the largest clique in the graph (Protein Complexes)
  - Find the highest mutual information encoding scheme (Neurobiology)
  - Find the best set of moves in tetris
  - …
  - http://en.wikipedia.org/wiki/List_of_NP-complete_problems

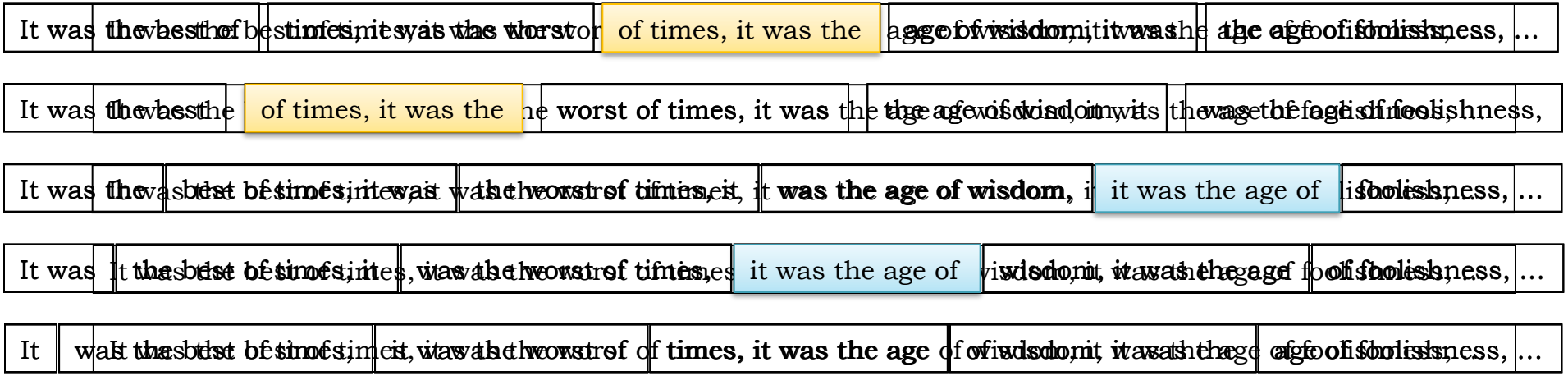


Outline

1. Graph Searching
2. Assembly by analogy
3. Genome Assembly

Shredded Book Reconstruction

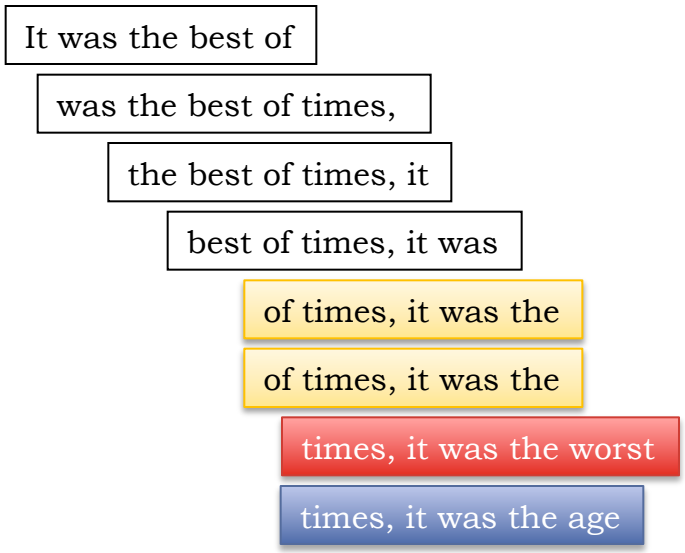
- Dickens accidentally shreds the first printing of A Tale of Two Cities
  - Text printed on 5 long spools



- 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



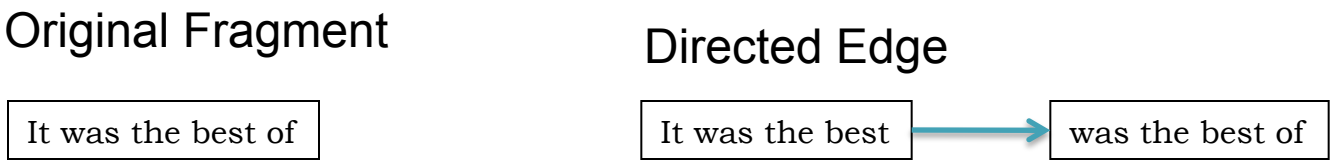
The repeated sequence make the correct reconstruction ambiguous

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

Model sequence reconstruction as a graph problem.

de Bruijn Graph Construction

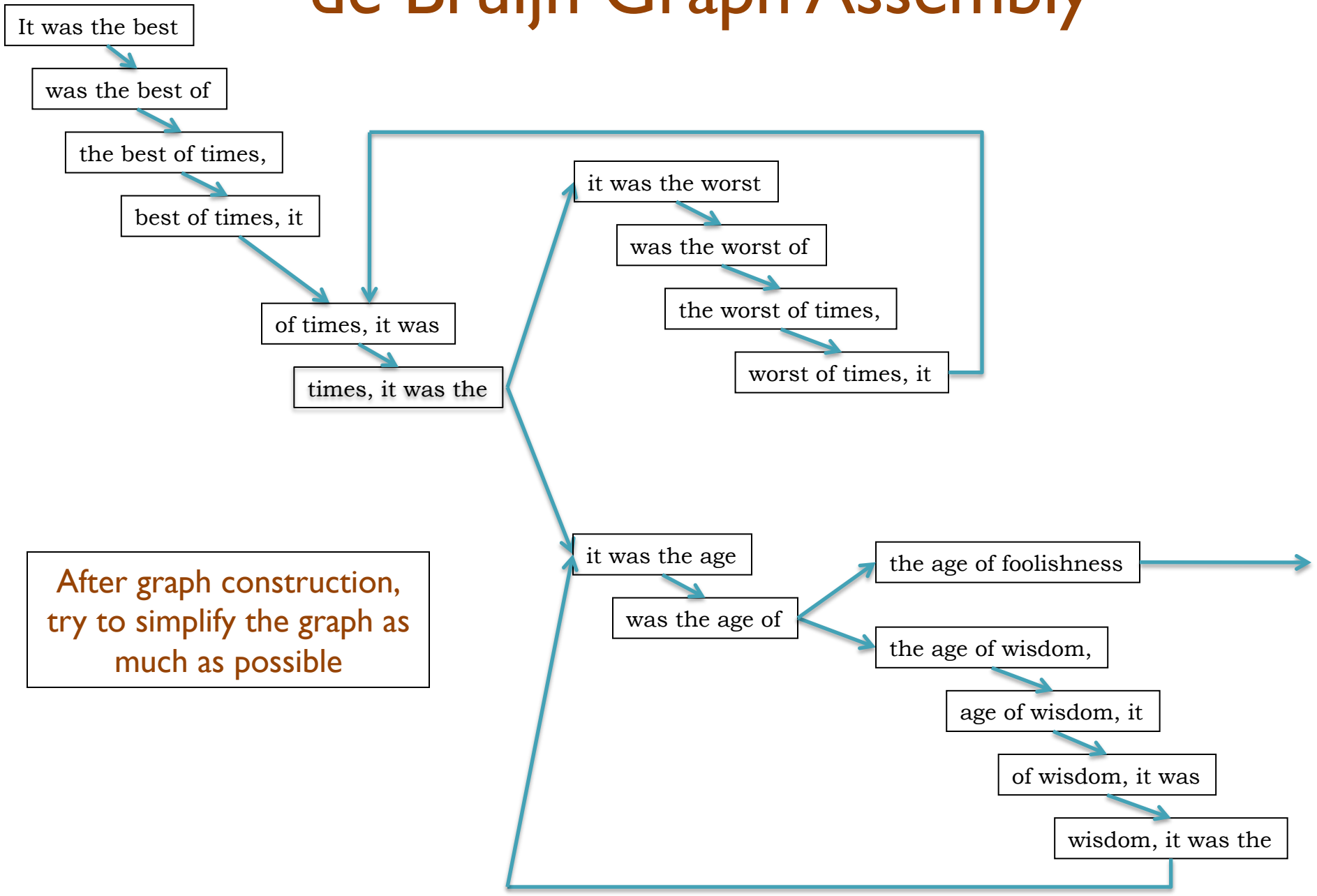
- $D_k = (V,E)$
  - V = All length-k subfragments (k < l)
  - E = Directed edges between consecutive subfragments
    - Nodes overlap by k-1 words



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

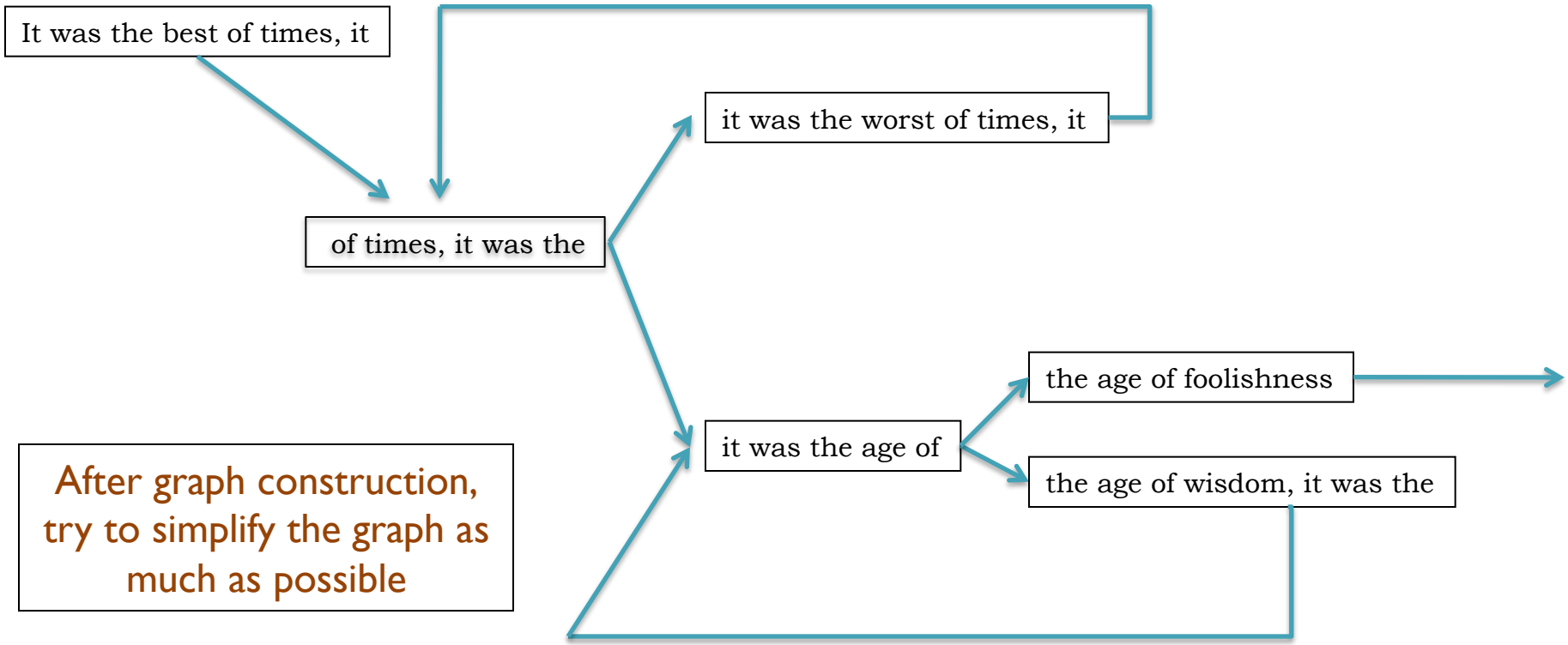
de Bruijn, 1946
Idury and Waterman, 1995
Pevzner, Tang, Waterman, 2001

de Bruijn Graph Assembly

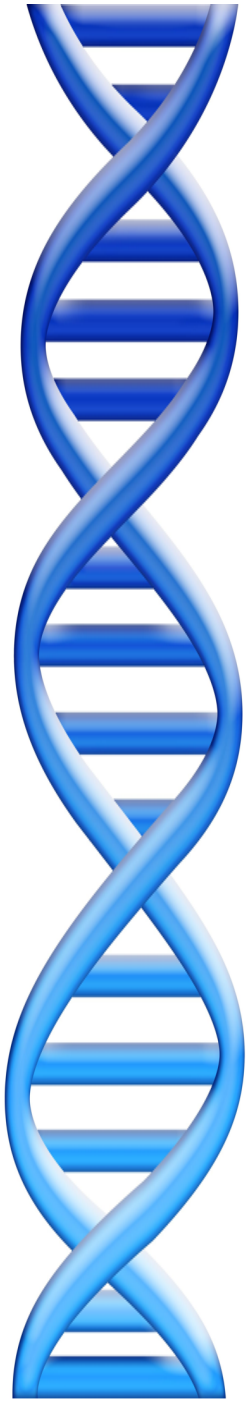


After graph construction, try to simplify the graph as much as possible

de Bruijn Graph Assembly



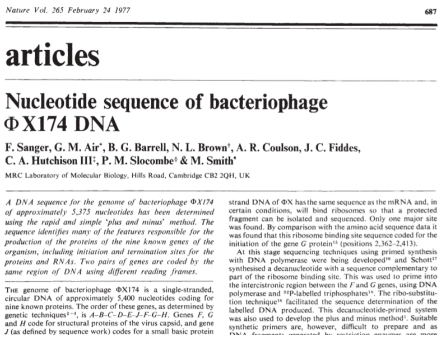
After graph construction, try to simplify the graph as much as possible



Outline

1. Genome Assembly by Analogy
2. Graph Searching
3. Genome Assembly

Milestones in Genome Assembly



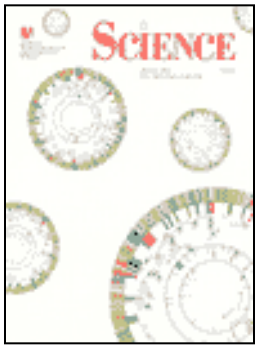
articles

Nucleotide sequence of bacteriophage ΦX174 DNA

F. Sanger, G. M. Air*, B. G. Barrell, N. L. Brown†, A. R. Coulson, J. C. Fiddes, C. A. Hutchison III‡, P. M. Slocombe‡ & M. Smith*

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

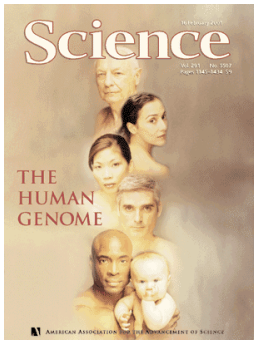
1977. Sanger *et al.*
1st Complete Organism
5375 bp



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

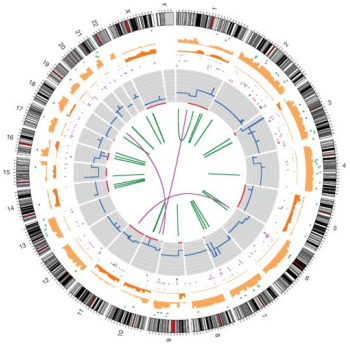
Like Dickens, we must computationally reconstruct a genome from short fragments

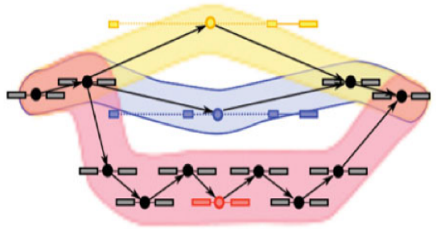
Current Applications

- Novel genomes

- Metagenomes

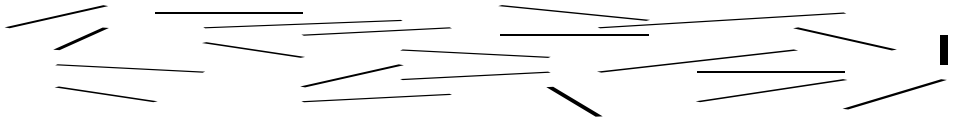
- Sequencing assays
  - Structural variations
  - Transcript assembly
  - …





Assembling a Genome

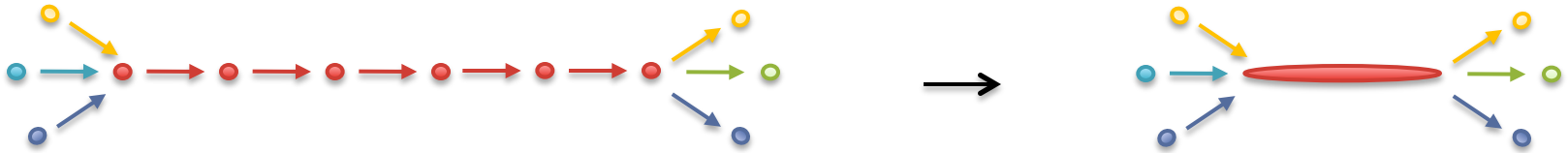
1. Shear & Sequence DNA



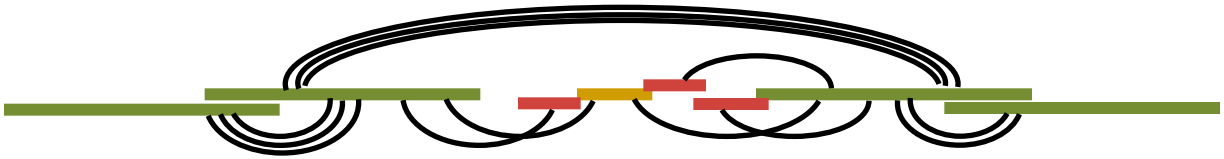
2. Construct assembly graph from overlapping reads

...AGCCTAGACCTACAGGATGCGCGACACGT
GGATGCGCGACACGTCGCATATCCGGT...

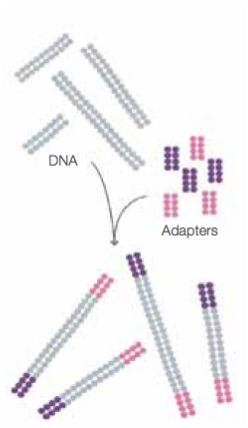
3. Simplify assembly graph



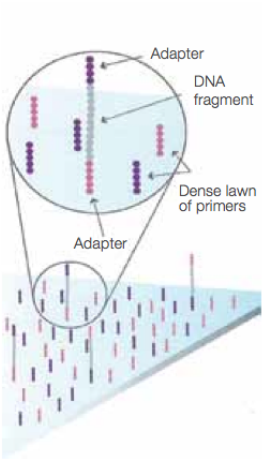
4. Detangle graph with long reads, mates, and other links



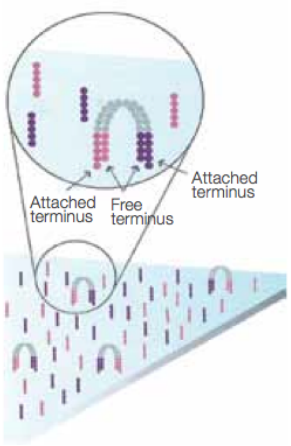
Illumina Sequencing by Synthesis



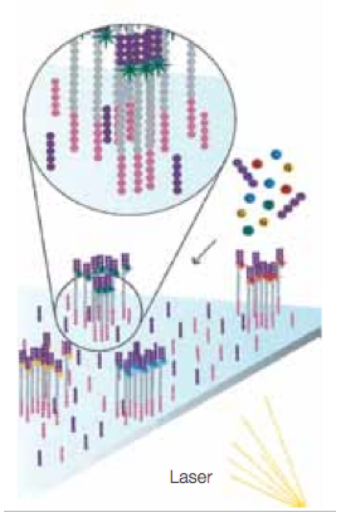
1. Prepare



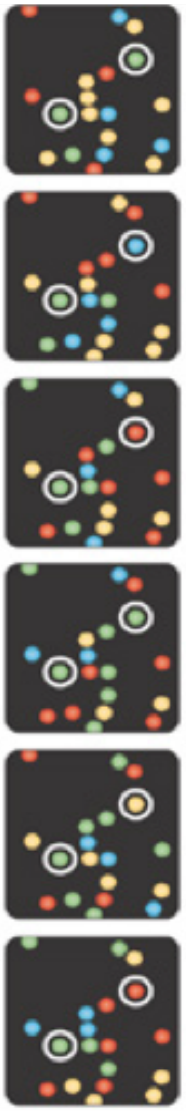
2. Attach



3. Amplify



4. Image



5. Basecall

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

http://www.illumina.com/documents/products/techspotlights/techspotlight_sequencing.pdf

Paired-end and Mate-pairs

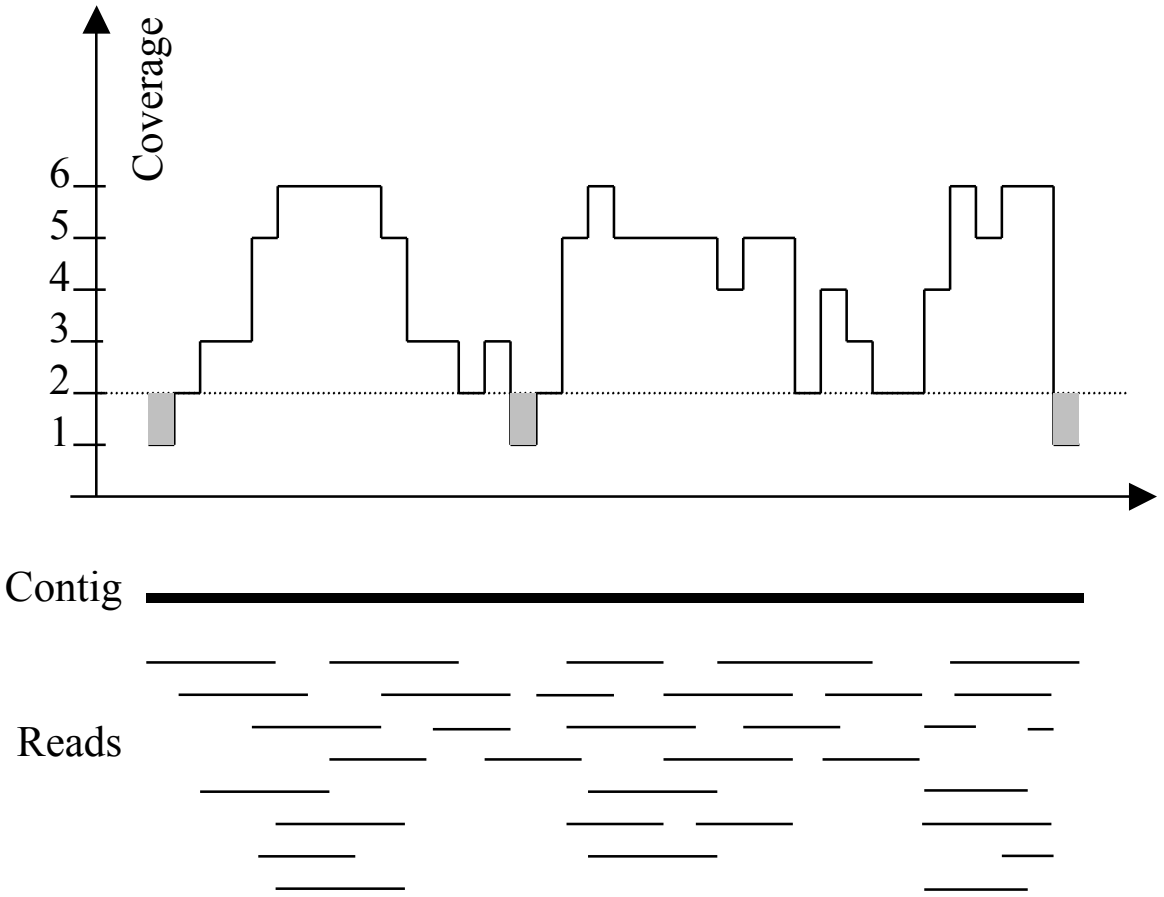
Paired-end sequencing

- 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

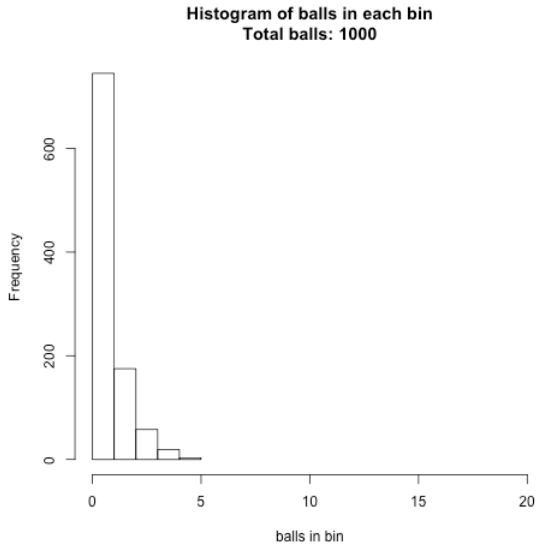
- Circularize long molecules (1-10kbp), shear into fragments, & sequence
- Mate failures create short paired-end reads

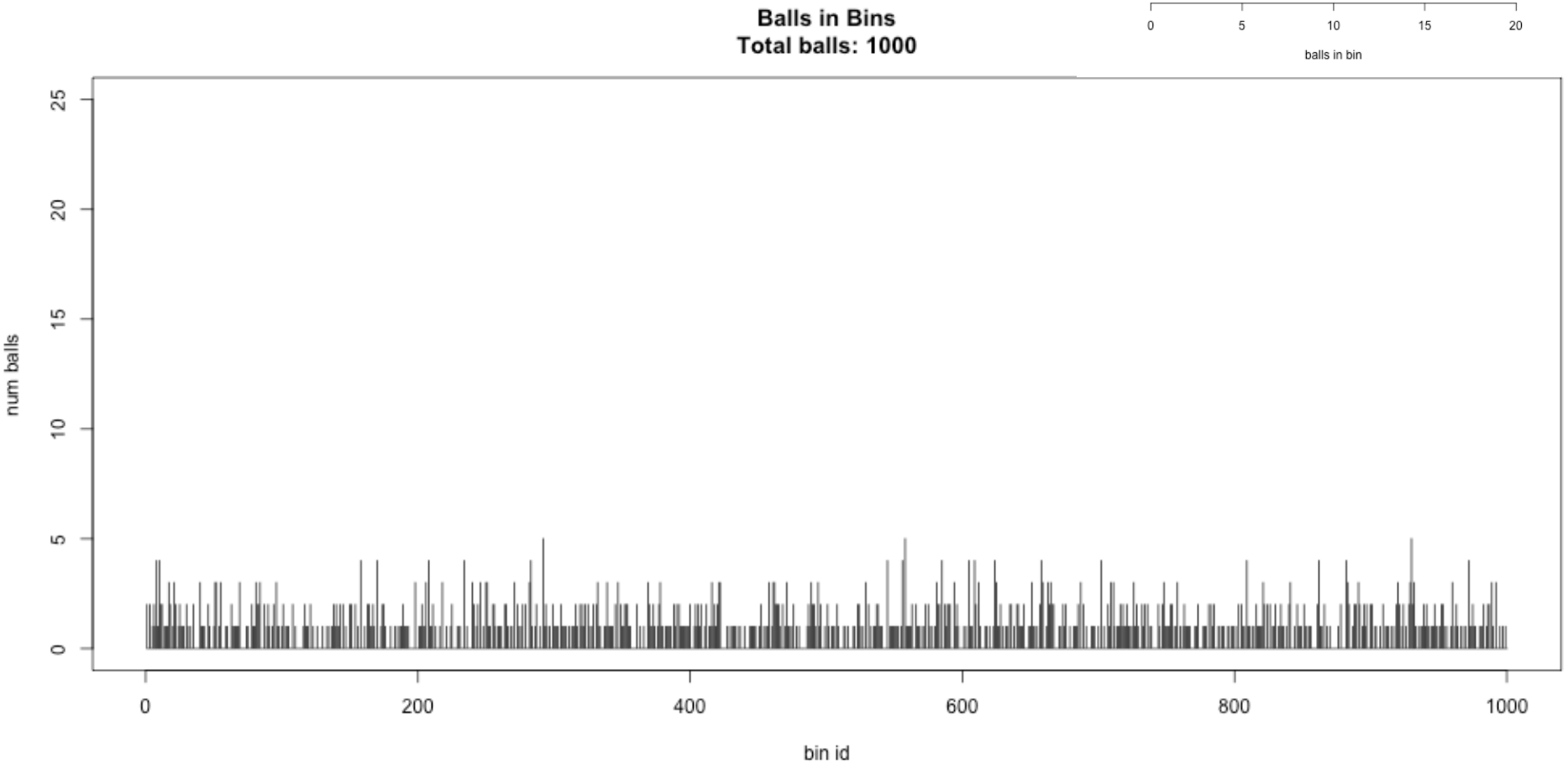
Typical contig coverage



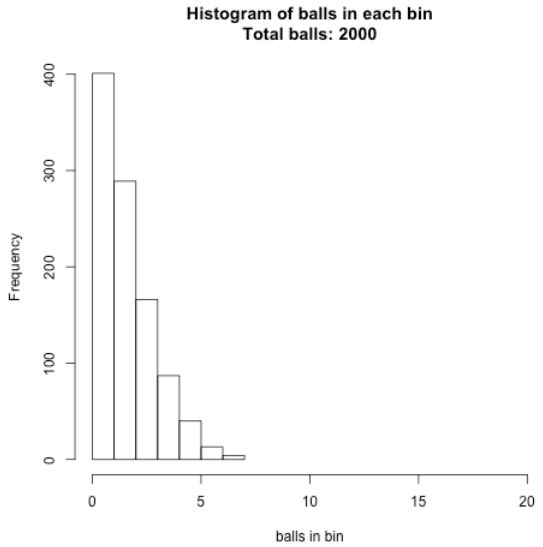
Imagine raindrops on a sidewalk

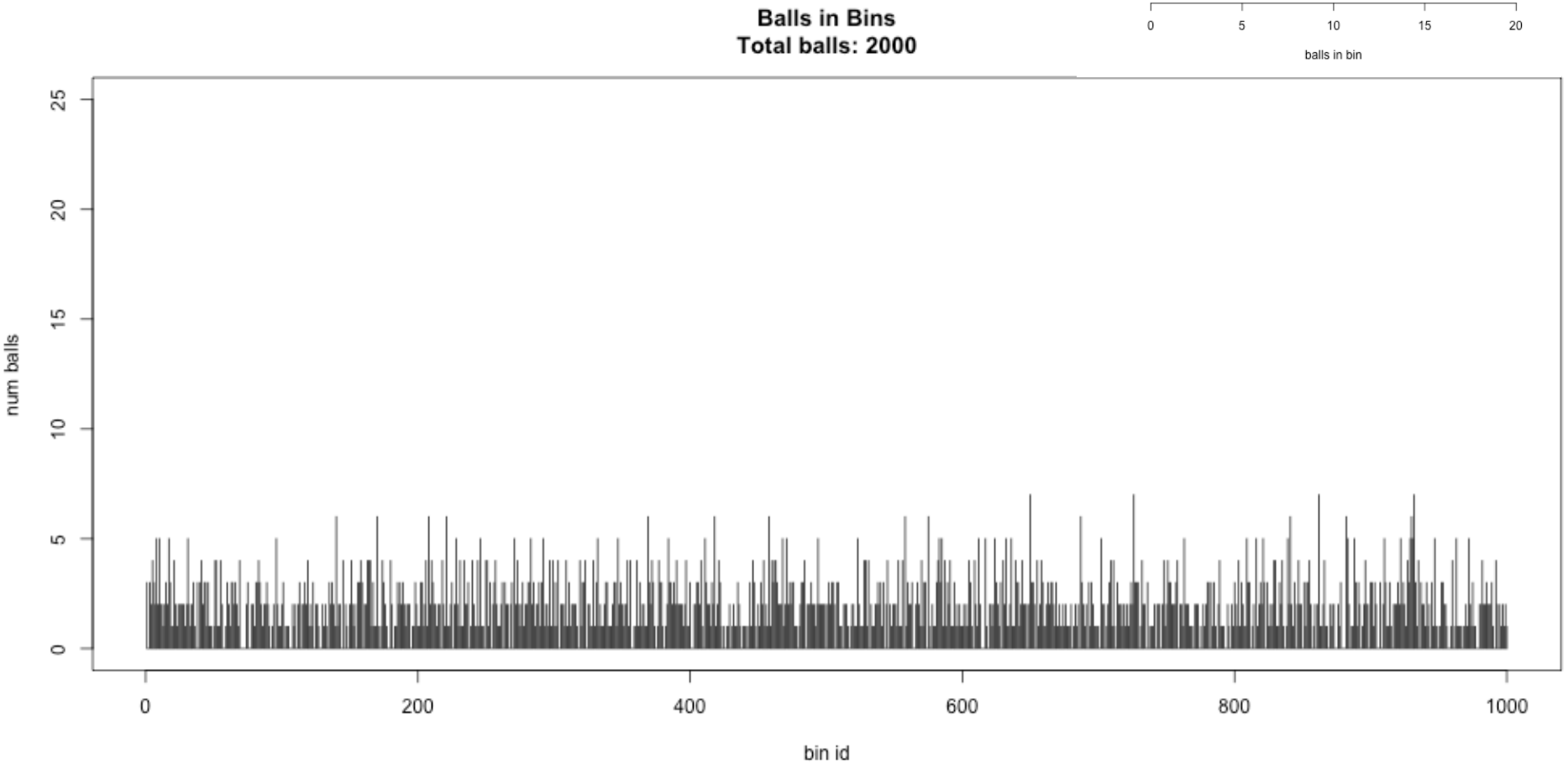
Balls in Bins 1x



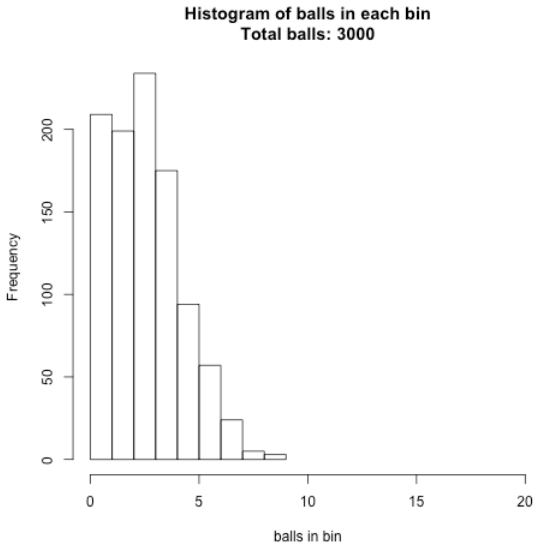


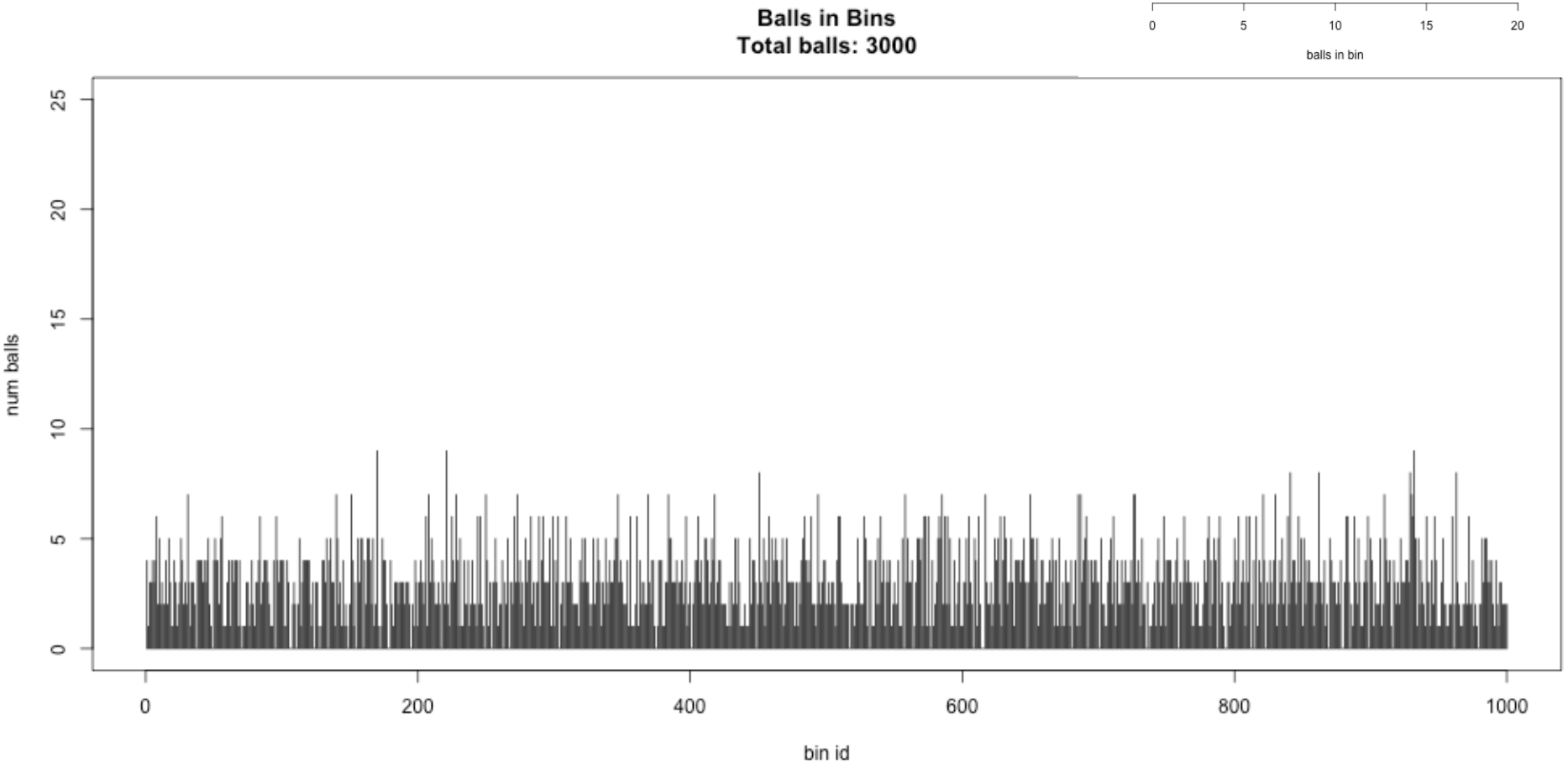
Balls in Bins 2x



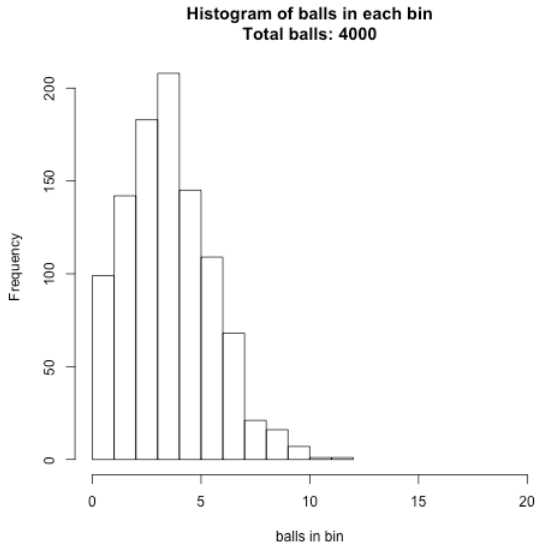


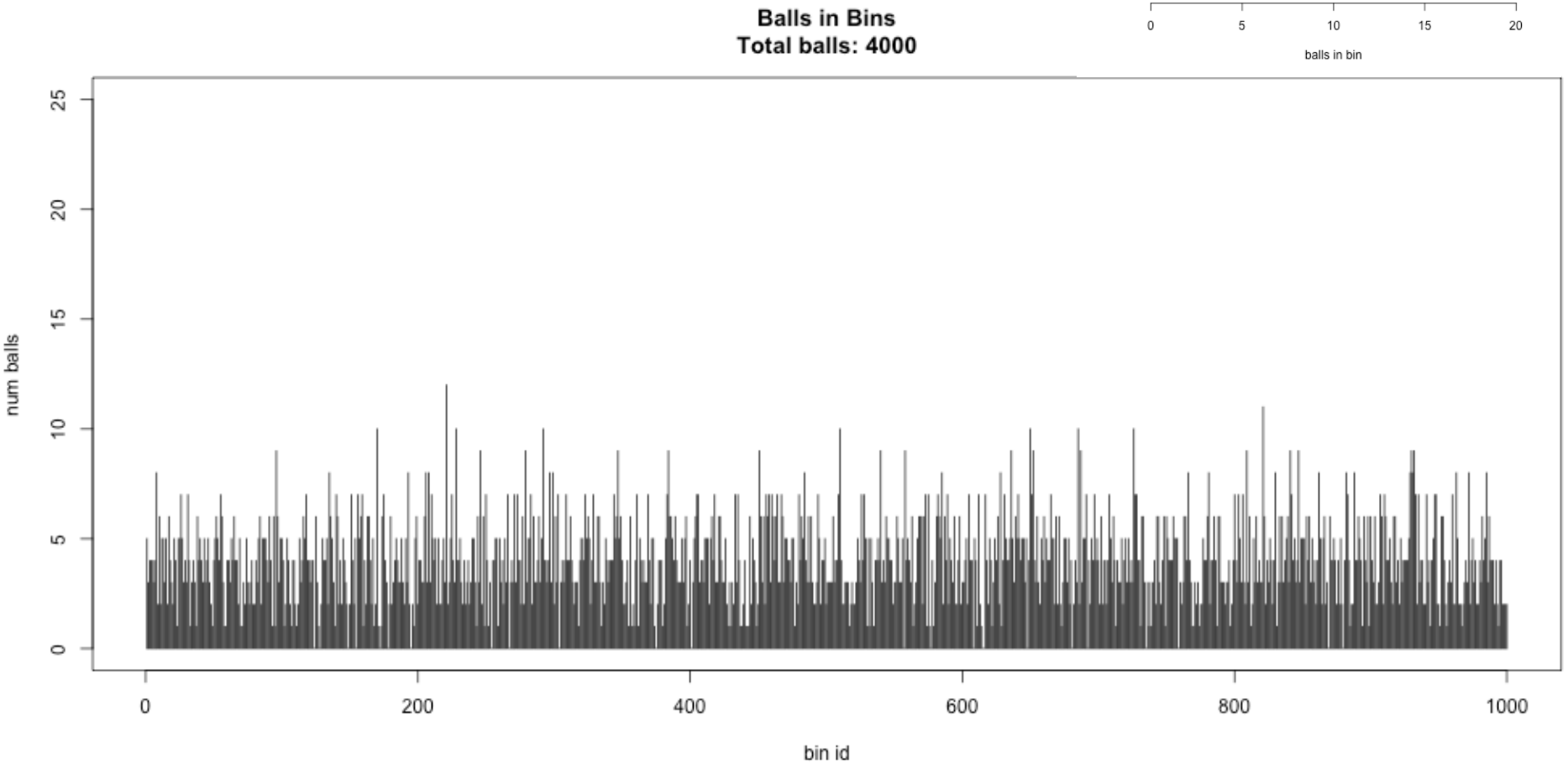
Balls in Bins 3x



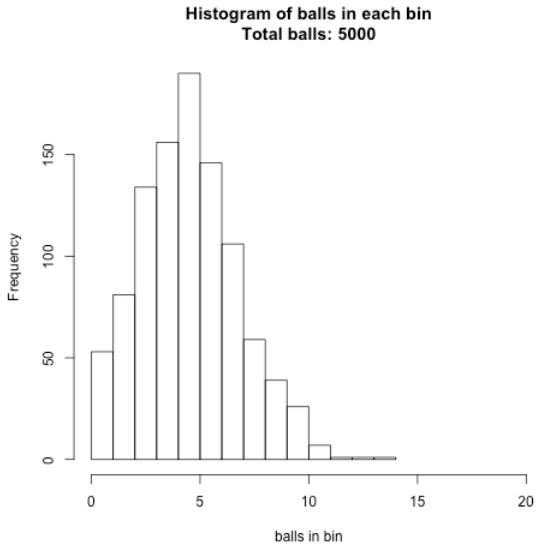


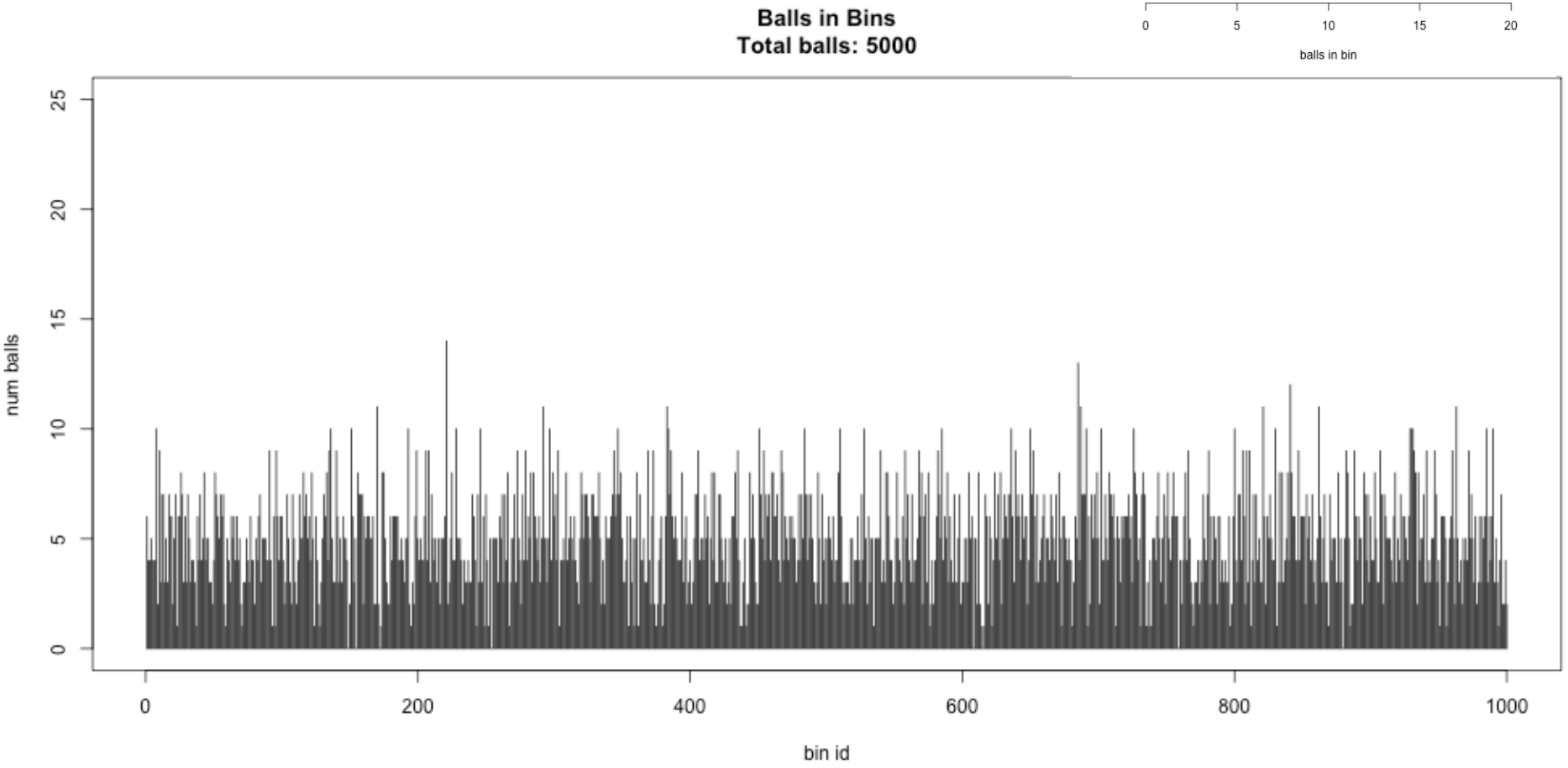
Balls in Bins 4x



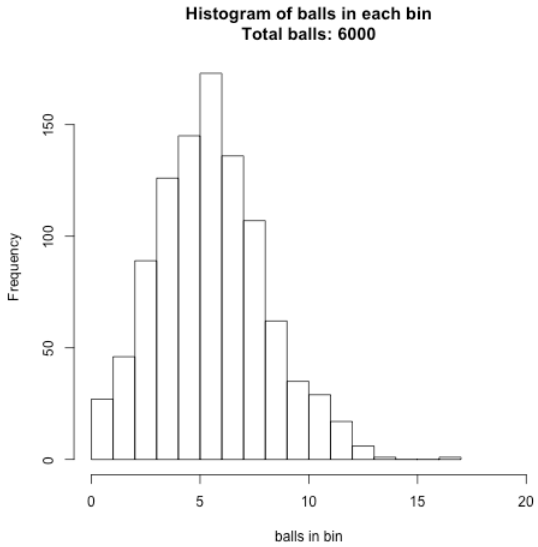


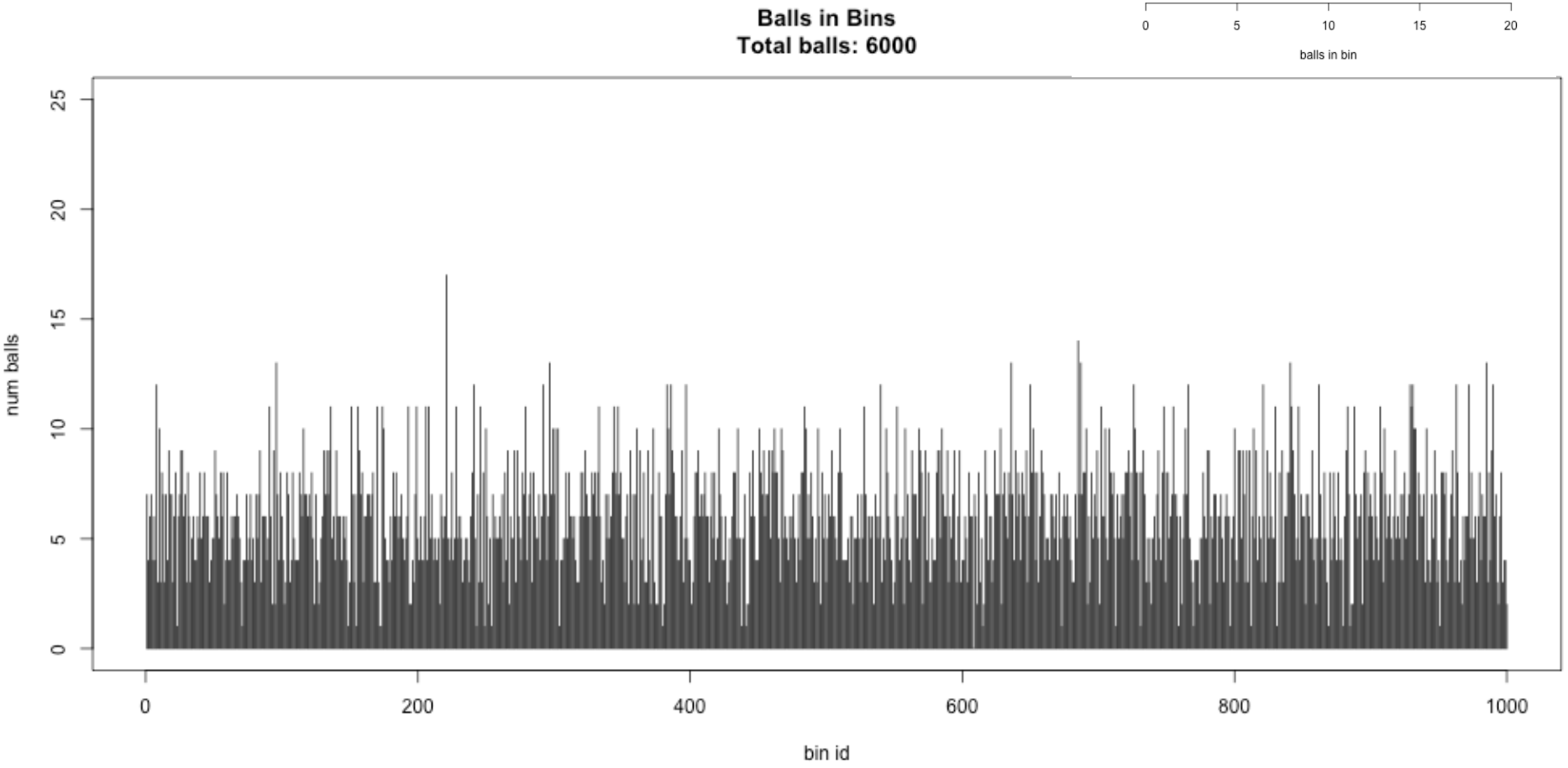
Balls in Bins 5x



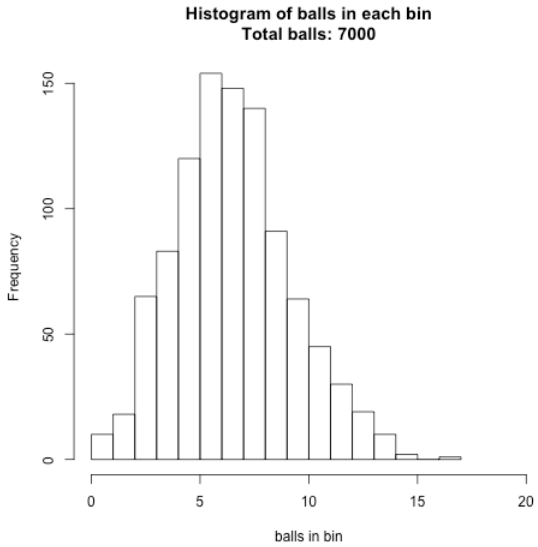


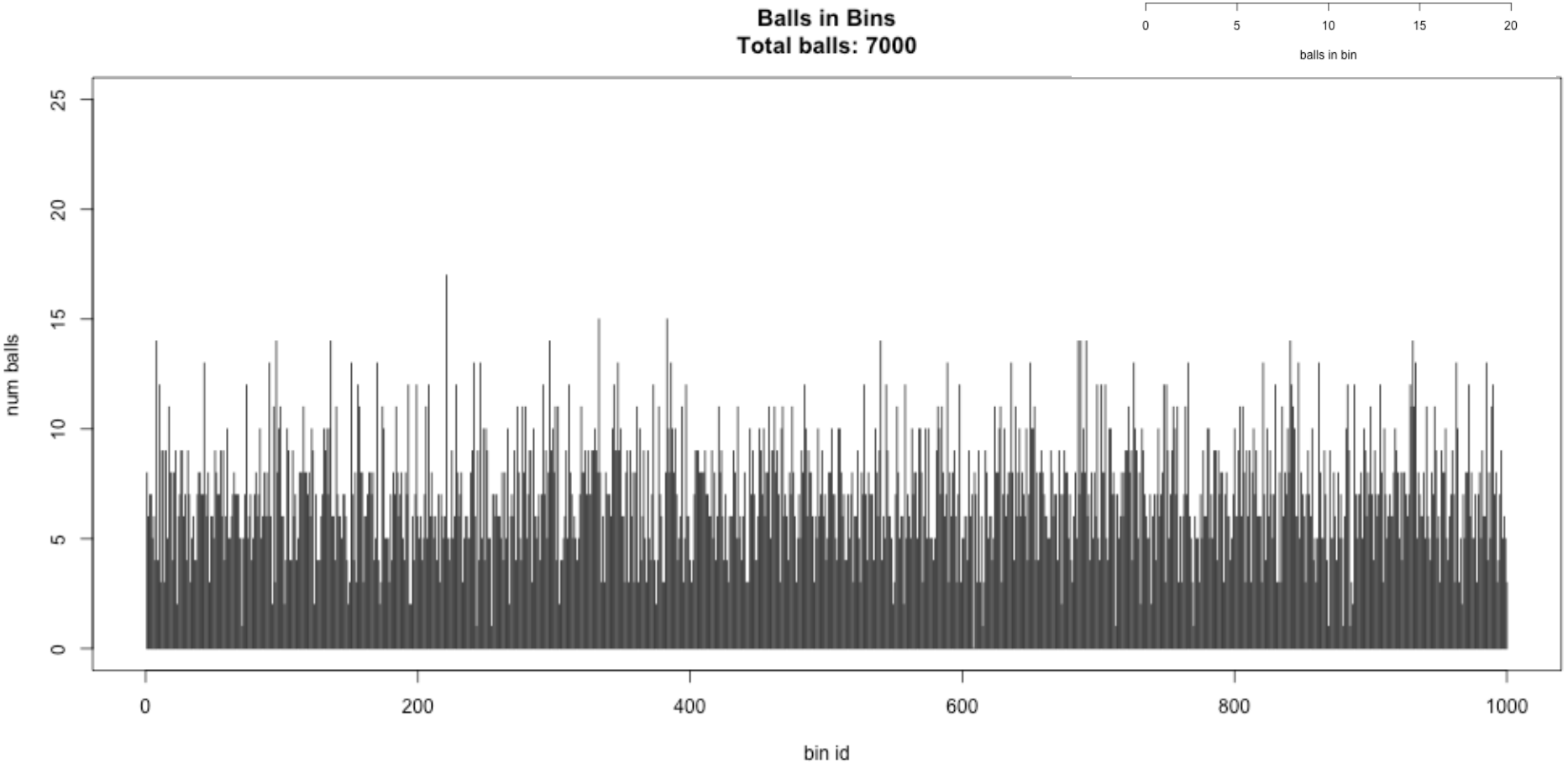
Balls in Bins 6x



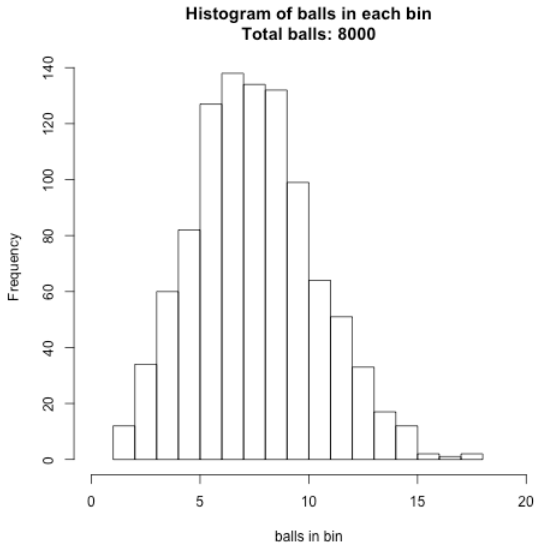


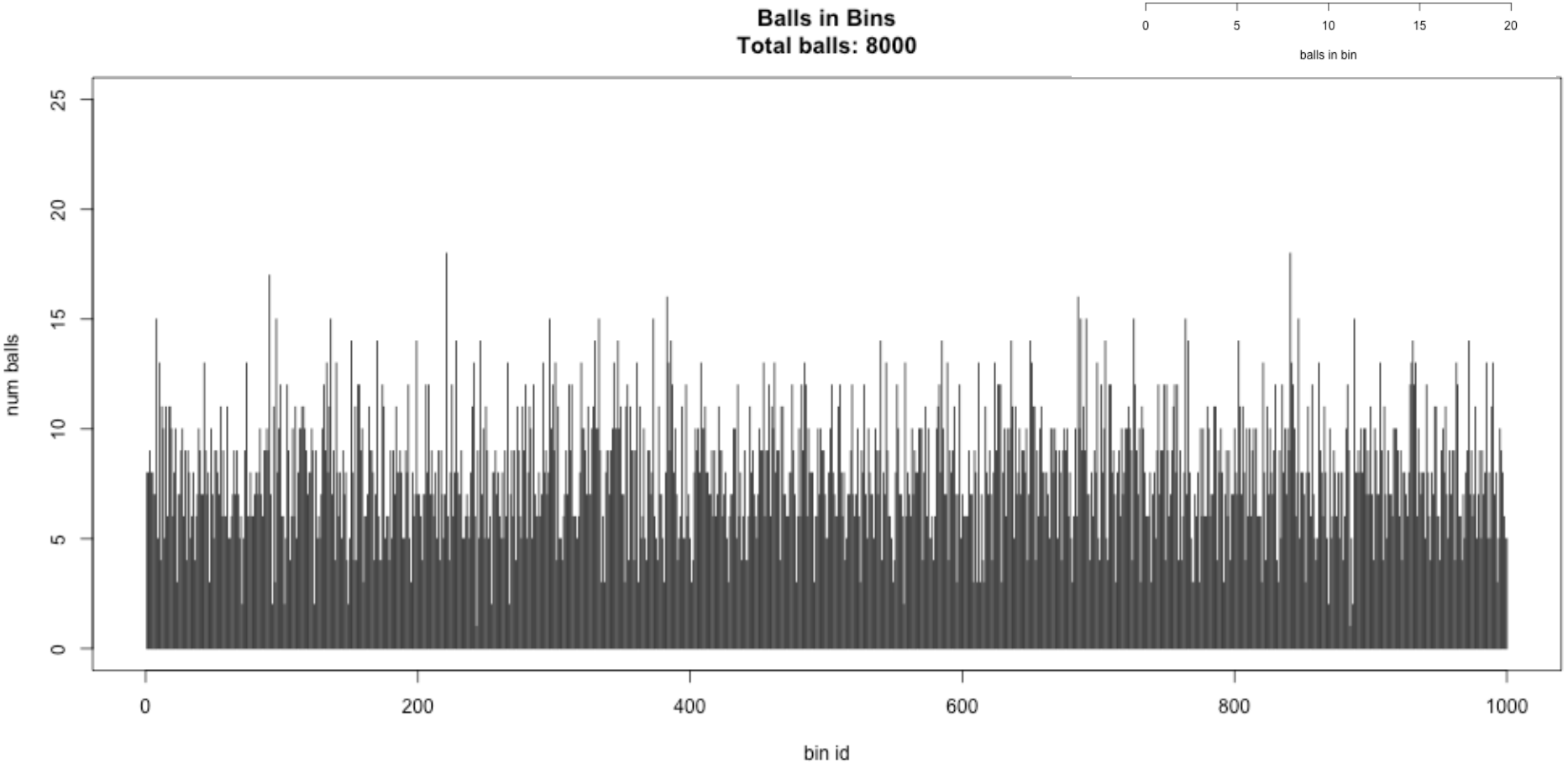
Balls in Bins 7x



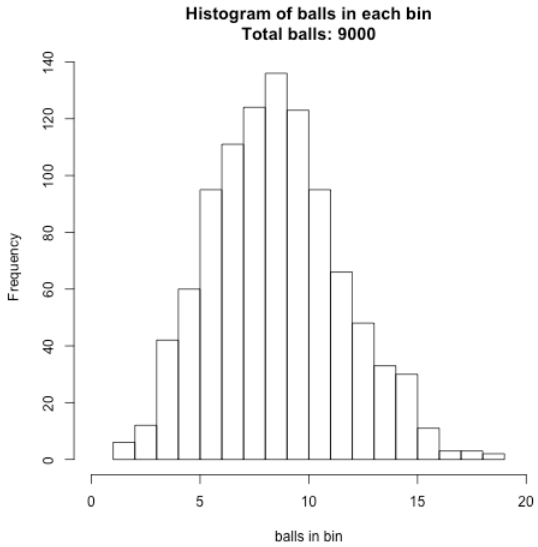


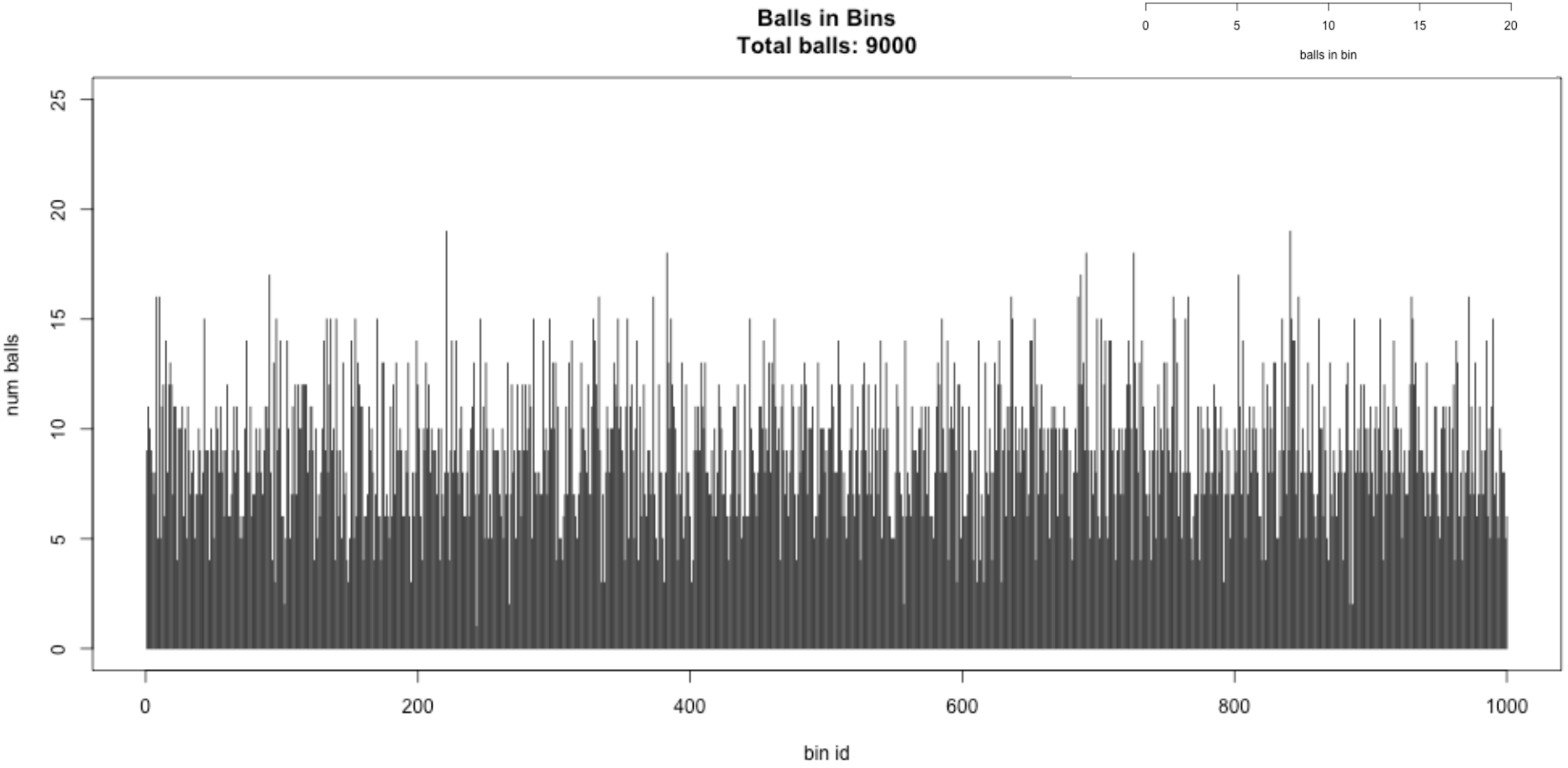
Balls in Bins 8x



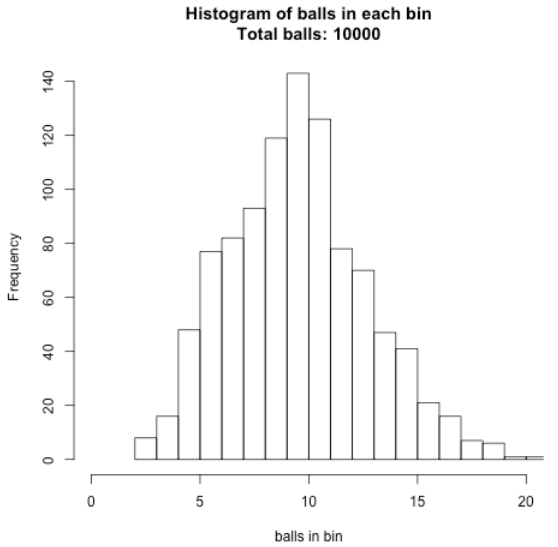


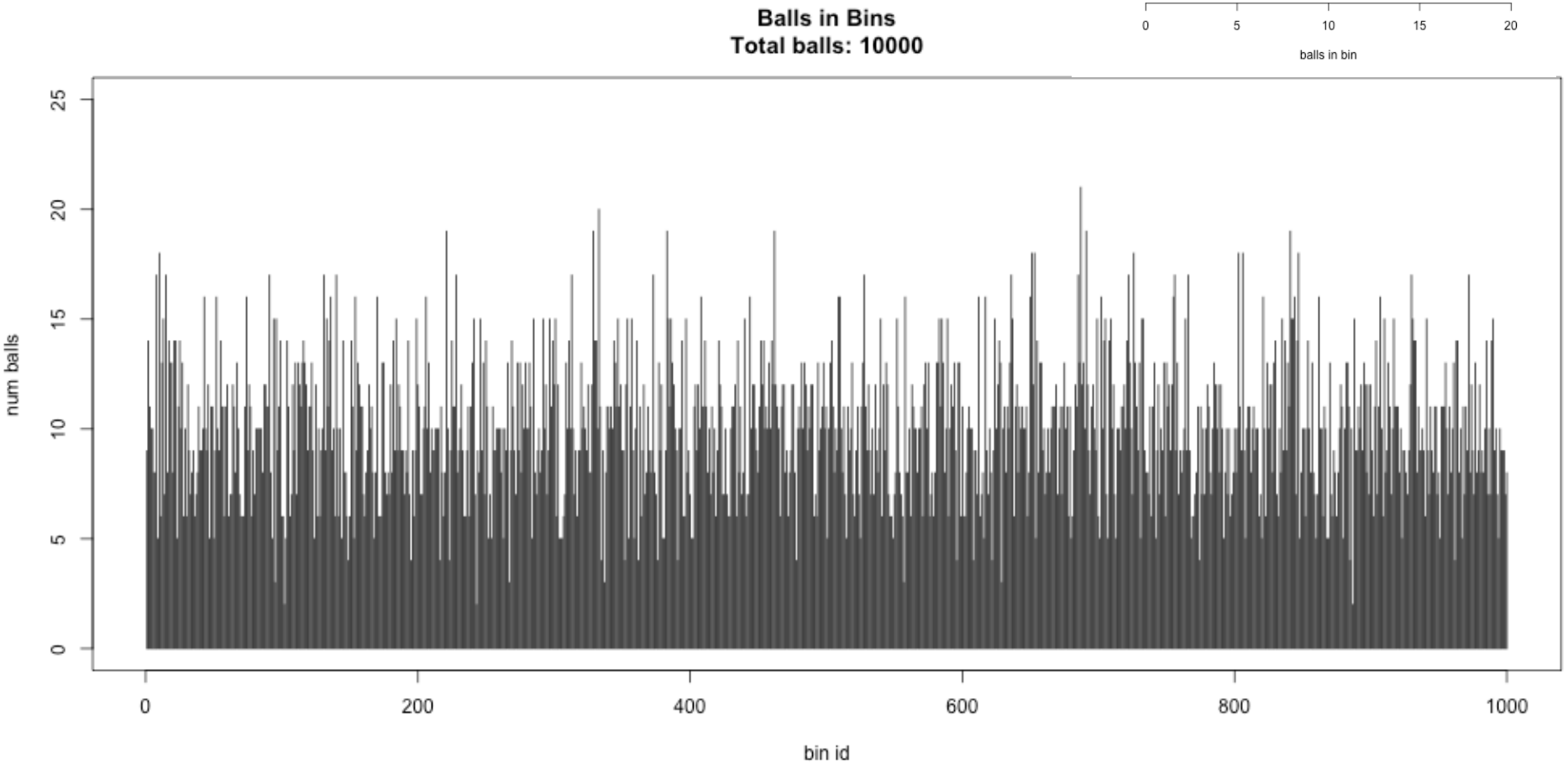
Balls in Bins 9x



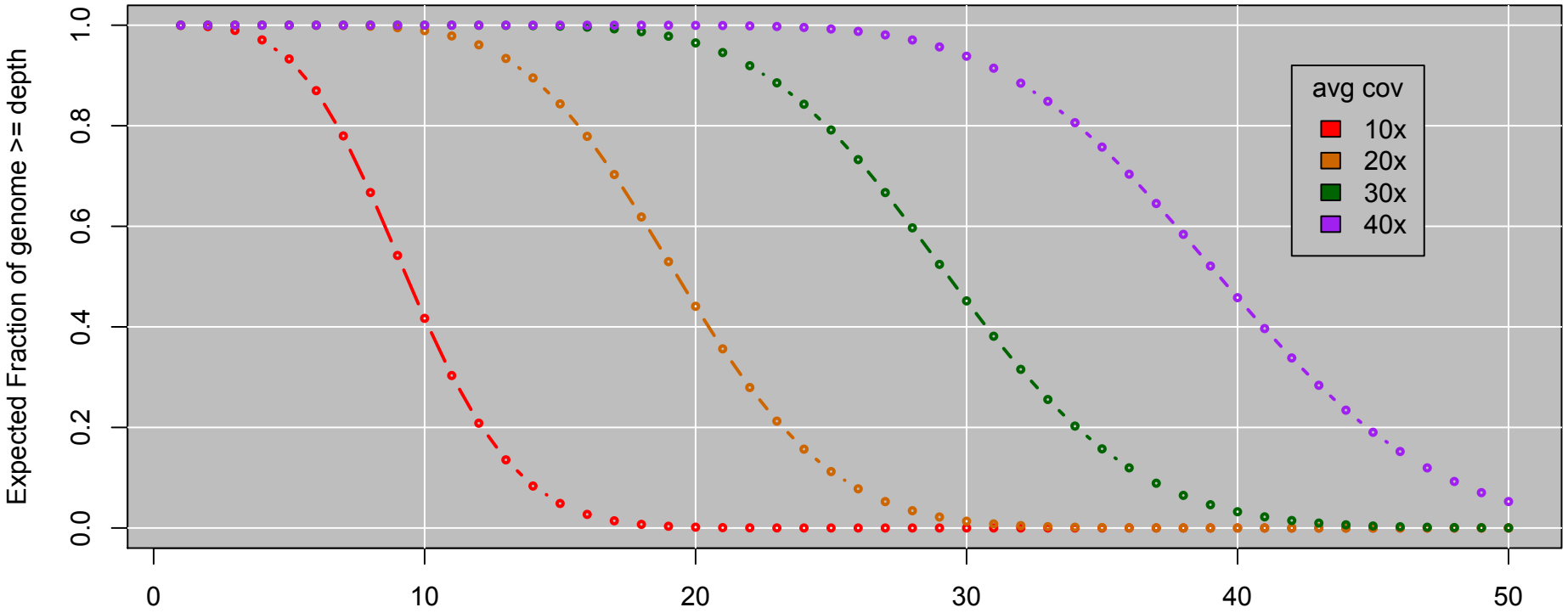


Balls in Bins 10x





Genome Coverage Distribution



Expect Poisson distribution on depth

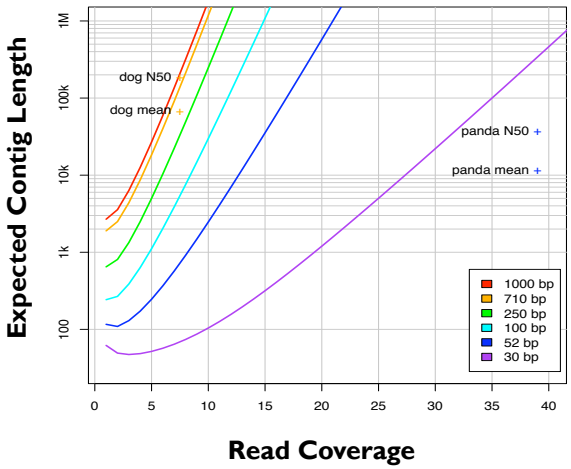
Standard Deviation = sqrt(cov)

This is the mathematically model => reality may be much worse

Double your coverage for diploid genomes

Ingredients for a good assembly

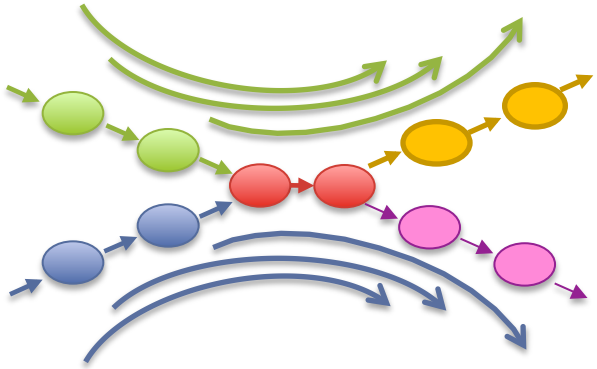
Coverage



High coverage is required

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

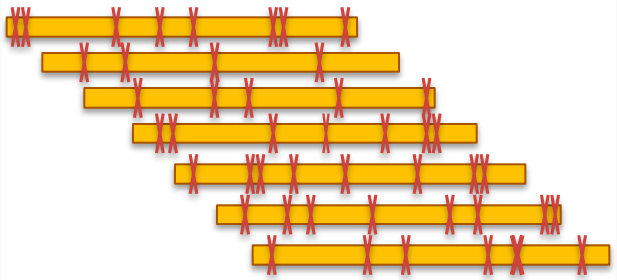
Read Length



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



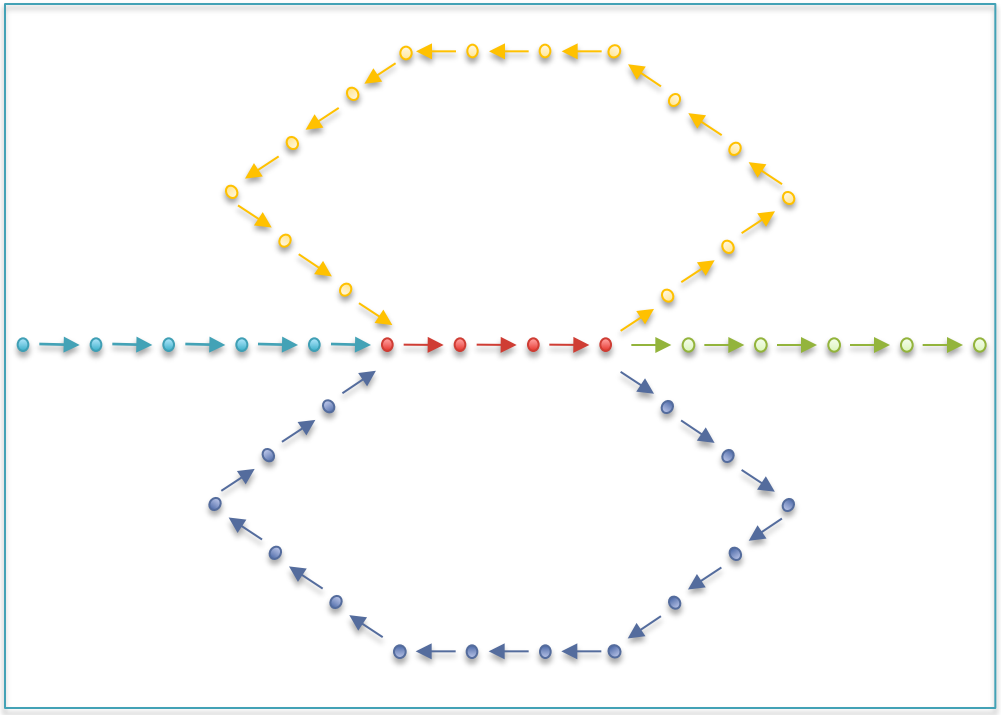
Errors obscure overlaps

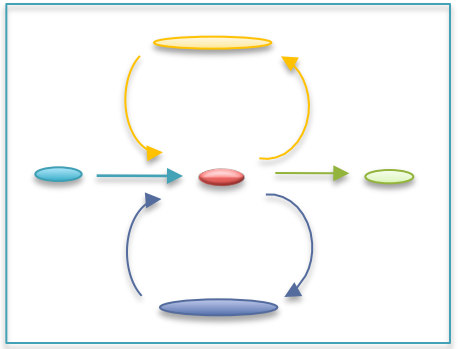
- Reads are assembled by finding kmers shared in pair of reads
- High error rate requires very short seeds, increasing complexity and forming assembly hairballs

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

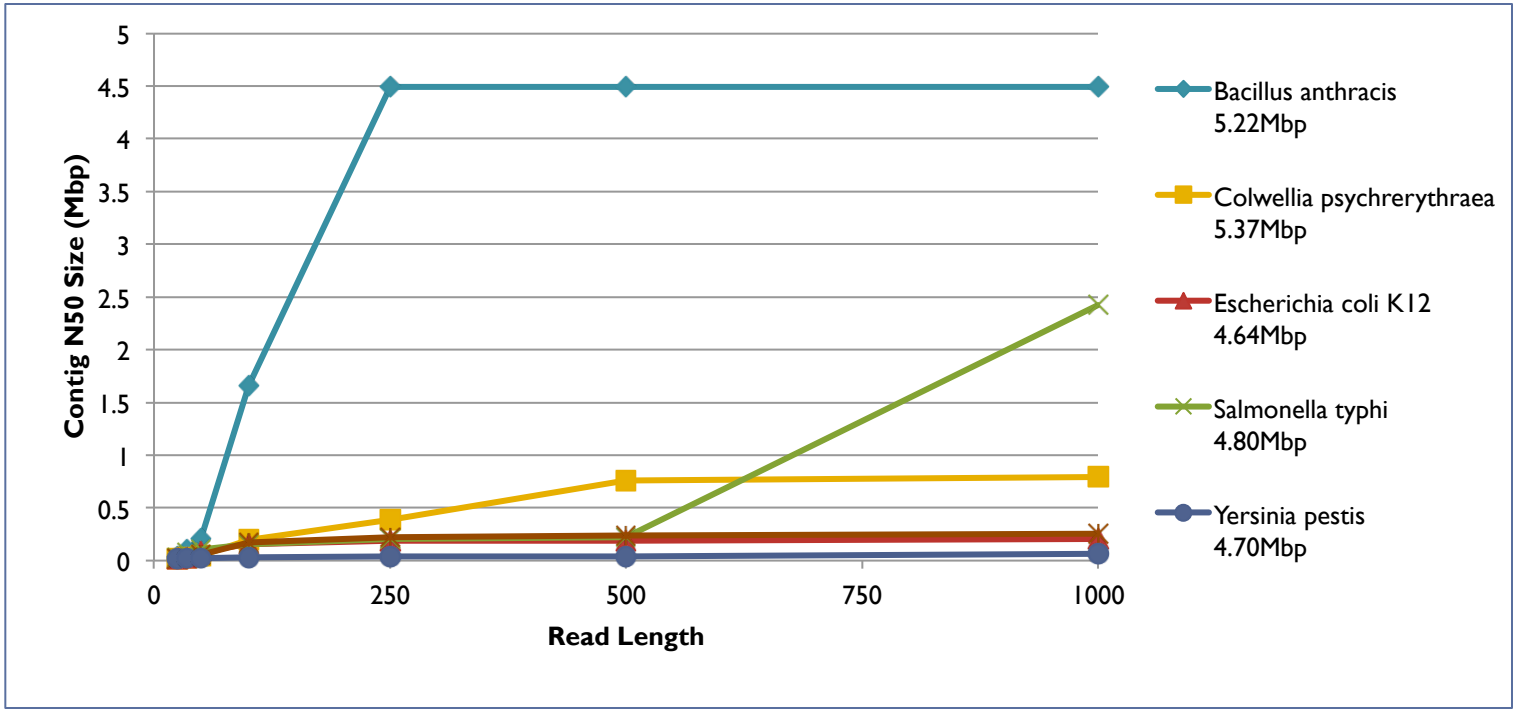
Initial Contigs

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





Repeats and Read Length



- Explore the relationship between read length and contig N50 size
  - Idealized assembly of read lengths: 25, 35, 50, 100, 250, 500, 1000
  - Contig/Read length relationship depends on specific repeat composition

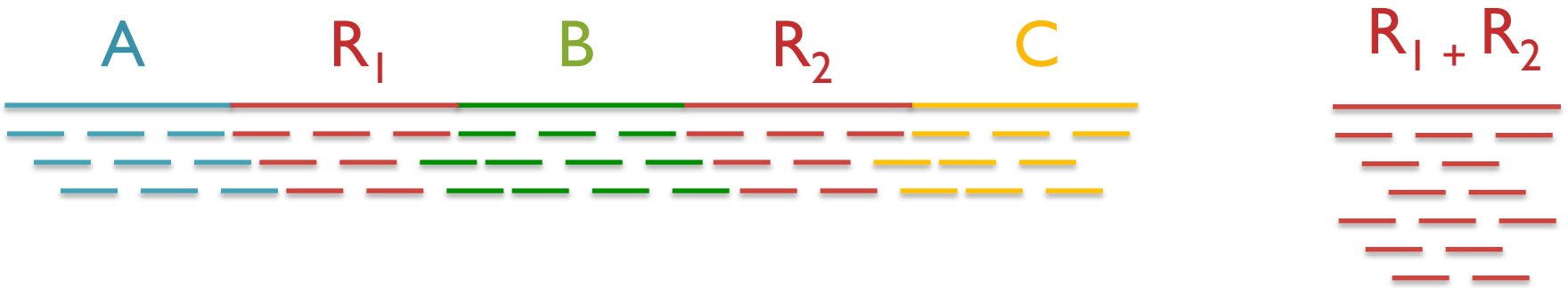
Assembly Complexity of Prokaryotic Genomes using Short Reads.
Kingsford C, Schatz MC, Pop M (2010) *BMC Bioinformatics*. 11:21.

Repetitive regions

- Over 50% of the human genome is repetitive

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%

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

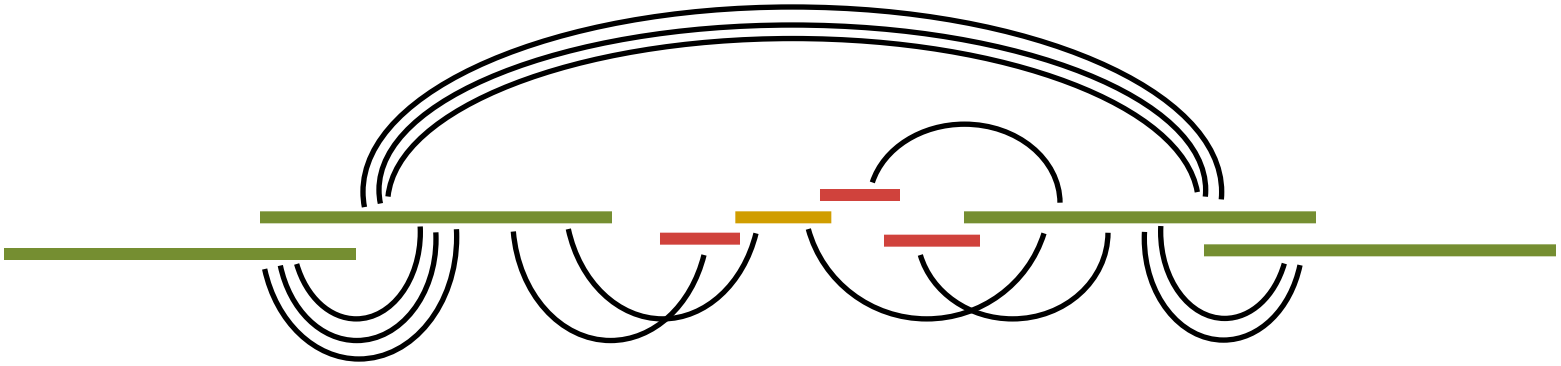
$$\Pr(X-copy)=\binom{n}{k}\left(\frac{X\Delta}{G}\right)^{k}\left(\frac{G-X\Delta}{G}\right)^{n-k}$$

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

Scaffolding

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

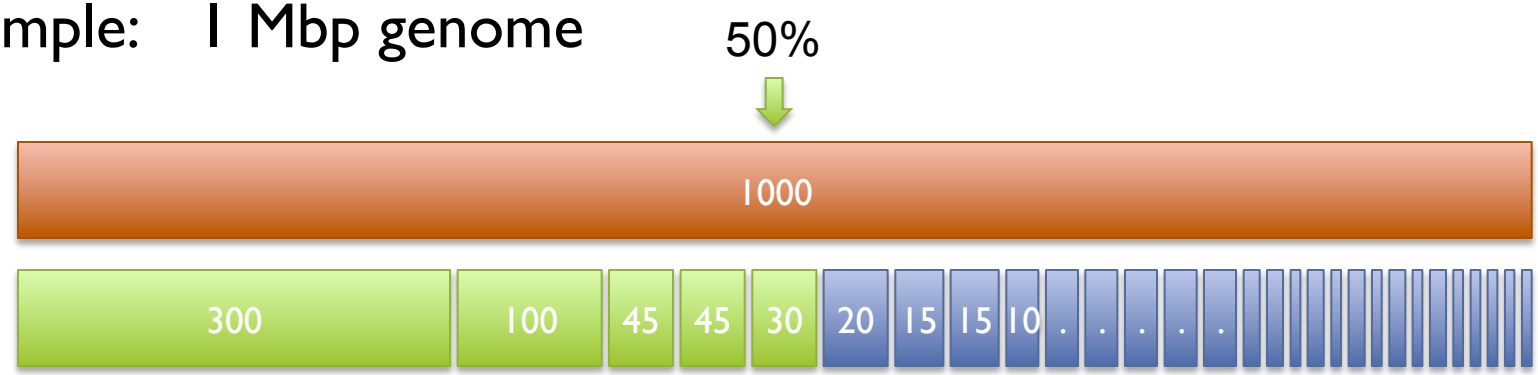
- Iteratively resolve longest, 'most unique' contigs
  - Both overlap graph and de Bruijn assemblers initially collapse repeats into single copies
  - Uniqueness measured by a statistical test on coverage



N50 size

Def: 50% of the genome is in contigs larger than N50

Example: 1 Mbp genome



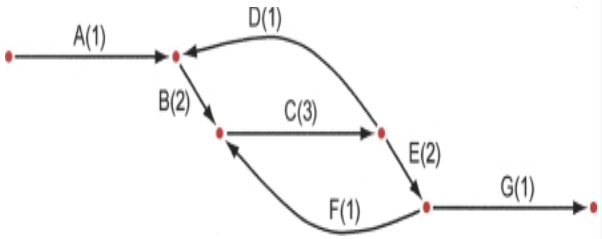
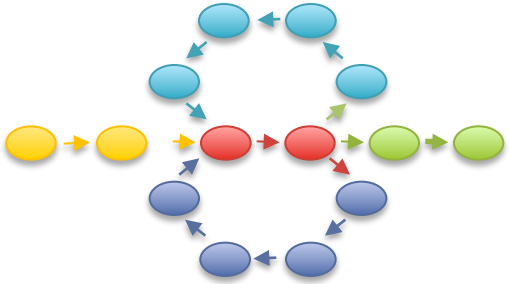
N50 size = 30 kbp

(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

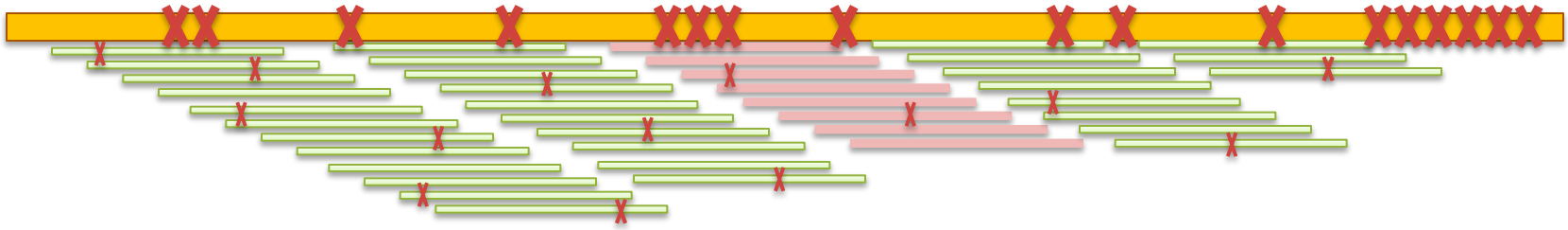
ALLPATHS-LG	SOAPdenovo	Celera Assembler
		
Broad's assembler (Gnerre et al. 2011)	BGI's assembler (Li et al. 2010)	JCVI's assembler (Miller et al. 2008)
De bruijn graph Short + PacBio (patching)	De bruijn graph Short reads	Overlap graph Medium + Long reads
Easy to run if you have compatible libraries	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

PacBio Error Correction & Assembly

http://wgs-assembler.sf.net

1. Correction Pipeline
   1. Map short reads (SR) to long reads (LR)
   2. Trim LRs at coverage gaps
   3. Compute consensus for each LR

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, Walenz, BP, Martin, J, Howard, J, Ganapathy, G, Wang, Z, Rasko, DA, McCombie, WR, Jarvis, ED, Phillippy, AM. (2012) *Nature Biotechnology.* doi:10.1038/nbt.2280

Assembly of Heterozygous Genomes

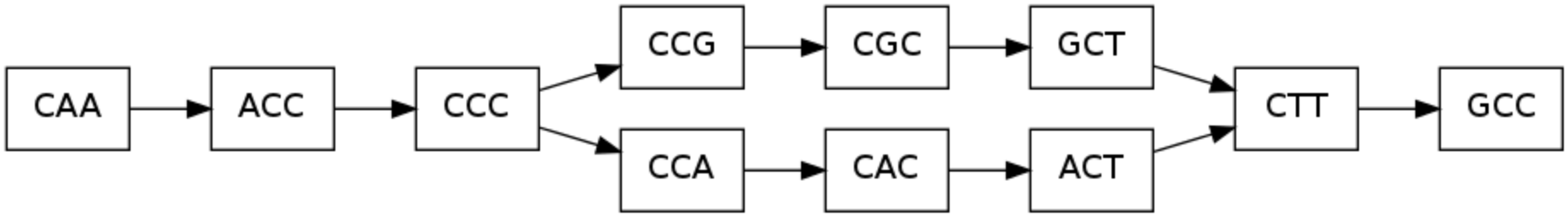
E. Biggers, M. Schatz

Genome assemblers developed to assembly genomes with low rates of heterozygosity

- 0-.1% (similar to human)

Assembly becomes more complicated with higher rates

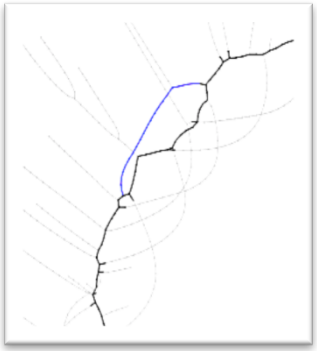
Preprocess the reads to "smooth" the heterozygosity, assemble, and then restore variants



Scalpel: Haplotype Microassembly

G. Narzisi, D. Levy, I. Iossifov, J. Kendall, M. Wigler, M. Schatz

- Use assembly techniques to identify complex variations from short reads
  - Improved power to find indels
  - Trace candidate haplotypes sequences as paths through assembly graphs

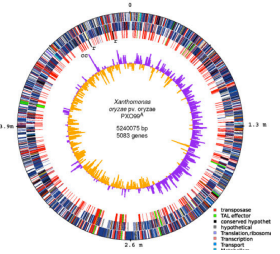


```
Ref:     ...TCAGAACAGCTGGATGAGATCTTAGCCAACTACCAGGAGATTGTCTTTGCCCGGA...

Father:  ...TCAGAACAGCTGGATGAGATCTTAGCCAACTACCAGGAGATTGTCTTTGCCCGGA...
Mother:  ...TCAGAACAGCTGGATGAGATCTTAGCCAACTACCAGGAGATTGTCTTTGCCCGGA...
Sib:     ...TCAGAACAGCTGGATGAGATCTTAGCCAACTACCAGGAGATTGTCTTTGCCCGGA...
Aut(1):  ...TCAGAACAGCTGGATGAGATCTTAGCCAACTACCAGGAGATTGTCTTTGCCCGGA...
Aut(2):  ...TCAGAACAGCTGGATGAGATCTTACC------CCGGAGATTGTCTTTGCCCGGA...
```

6bp heterozygous indel at chr13:25280526 ATP12A

Assembly Summary



Graphs are ubiquitous in the world

- Pairwise searching is easy, finding features is hard

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

Genomics Challenges

The foundations of genomics will continue to be *observation*, *experimentation*, and *interpretation*

- Technology will continue to push the frontier
- Measurements will be made *digitally* over large populations, at extremely high resolution, and for diverse applications

Rise in Quantitative and Computational Demands

1. *Experimental design*: selection, collection & metadata
2. *Observation*: measurement, storage, transfer, computation
3. *Integration*: multiple samples, assays, analyses
4. *Discovery*: visualizing, interpreting, modeling

Ultimately limited by the human capacity to execute extremely complex experiments and interpret results

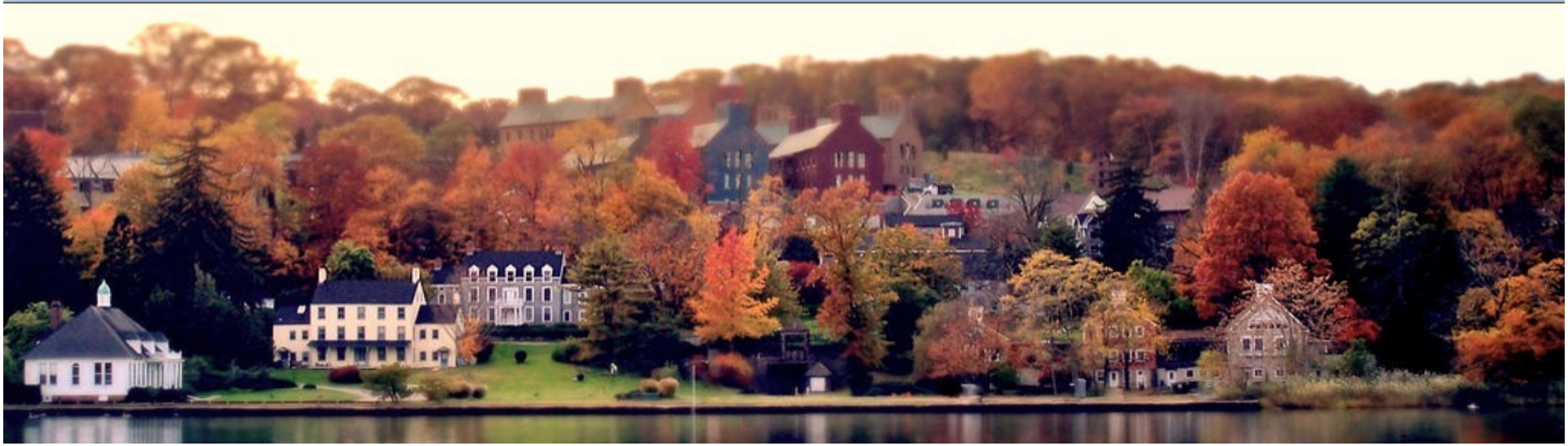
Acknowledgements

Schatzlab
Eric Biggers
Hayan Lee
Mitch Bekritsky
James Gurtowski
Rushil Gupta
Giuseppe Narzisi
Rob Aboukhalil

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

NBACC
Adam Phillippy
Sergey Koren

UMD
Steven Salzberg
Mihai Pop
Ben Langmead
Cole Trapnell



Thank You






http://schatzlab.cshl.edu/teaching/
@mike_schatz