Graphs and Genomes

Michael Schatz

Bioinformatics Lecture 3

Quantitative Biology 2012

Dynamic Programming Matrix

Compute the optimal alignment of ABC…XY..N and DEF…UV…M

	0	A	B	C	…	X	Y	…	N
0									
D									
E									
F									
…									
U									
V									
…									
M									

Dynamic Programming Matrix

Compute the optimal alignment of ABC…XY..N and DEF…UV…M

	0	**A**	**B**	**C**	**…**	**X**	**Y**	**…**	**N**
0	0	1	2	3		X	X+1		N
D	1								
E	2								
F	3								
…									
U	U								
V	U+1								
…									
M	M								

Top row and first column are easy: it takes L-edits to transform and empty string into a length L string

Dynamic Programming Matrix

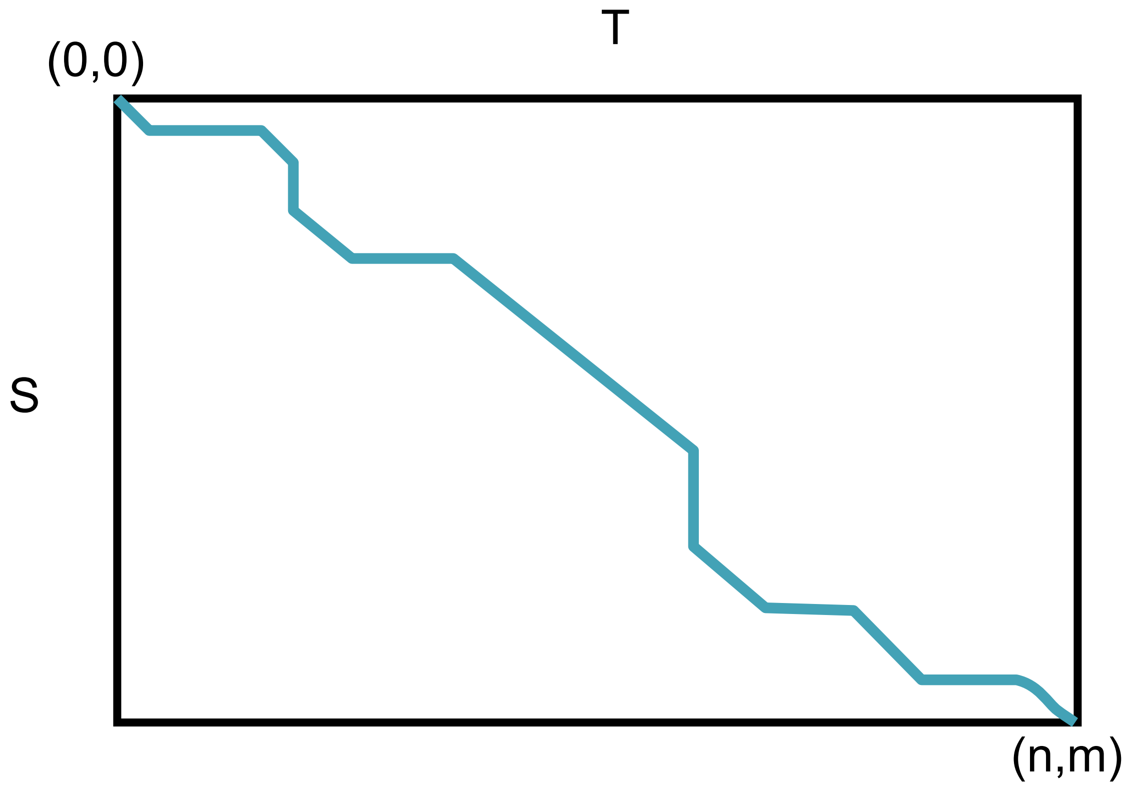
Compute the optimal alignment of “ABC…XY..N” and “DEF…UV…M”

	0	**A**	**B**	**C**	**…**	**X**	**Y**	**…**	**N**
0	0	1	2	3		X	X+1		N
D	1								
E	2								
F	3								
…									
U	U					γ	α		
V	U+1					β	Ω		
…									
M	M								

$$\Omega = \min \begin{cases} \text{“Up” + 1} & \alpha+1 \\ \text{“Left+ + 1} & \beta+1 \\ \text{“Diagonal” +0/1} & \gamma+1 \end{cases}$$

Up	Left	Diagonal
ABC...XY**-**	ABC....X**Y**	ABC...X**Y**
DEF....U**V**	DEF...UV**-**	DEF...U**V**
α	β	γ

Global Alignment Schematic

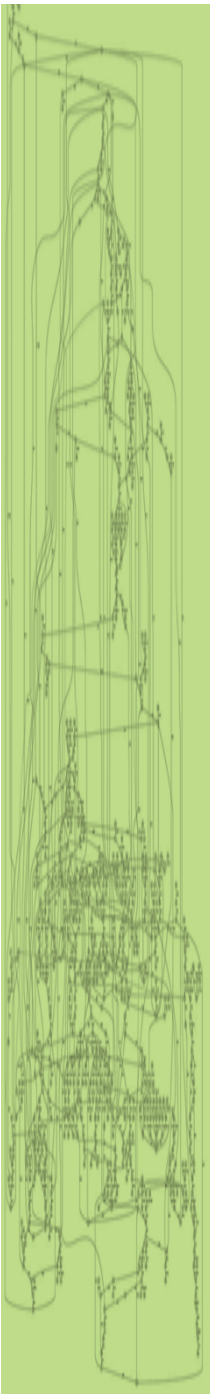


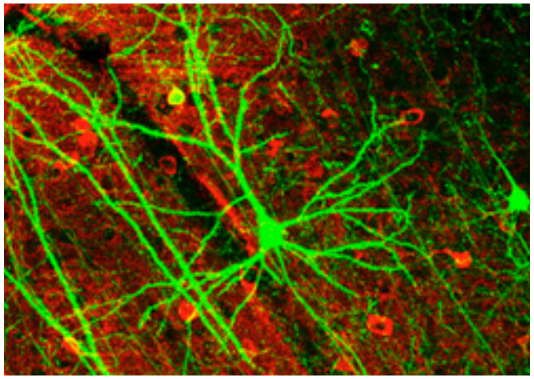
Evaluate all NxM cells in O(NxM) time.
Value in cell D[n,m] is the edit distance.
D[AGCACACA,ACACACTA] = 2

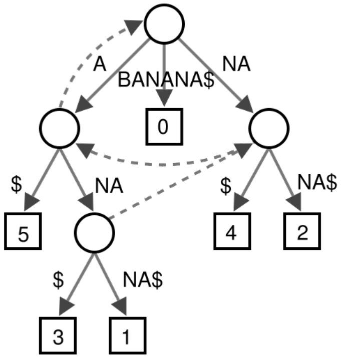
```
AGCACAC-A
|*|||||*|
A-CACACTA
```

Nathan Edwards

Biological Networks







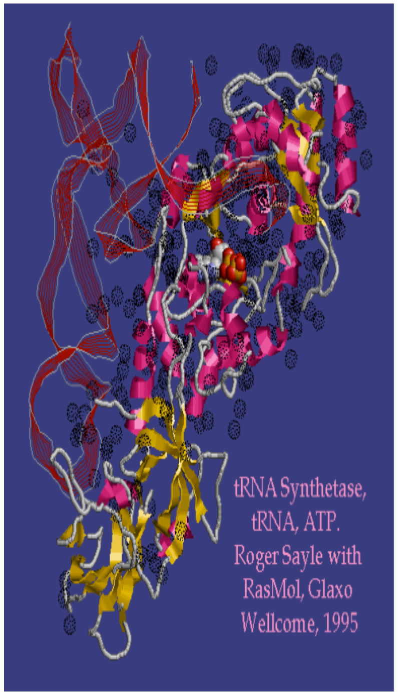
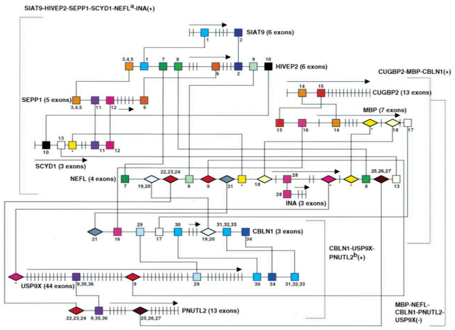
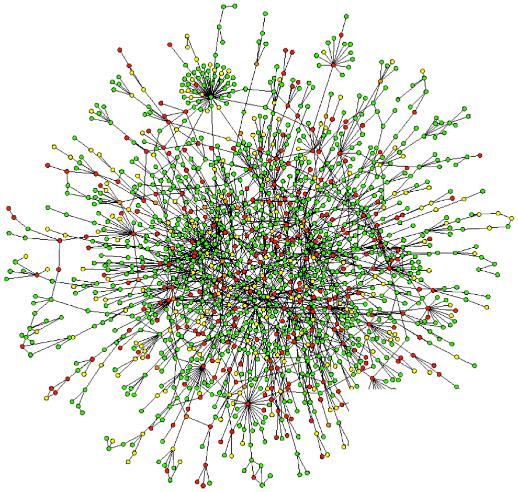

tRNA Synthetase, tRNA, ATP. Roger Sayle with RasMol, Glaxo Wellcome, 1995

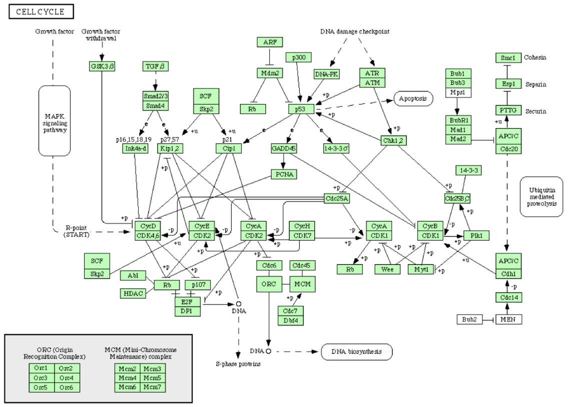
Figure 5 Putative regulatory elements shared between groups of correlated and anticorrelated genes

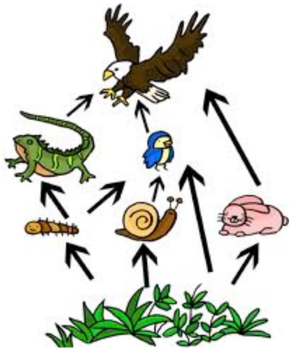


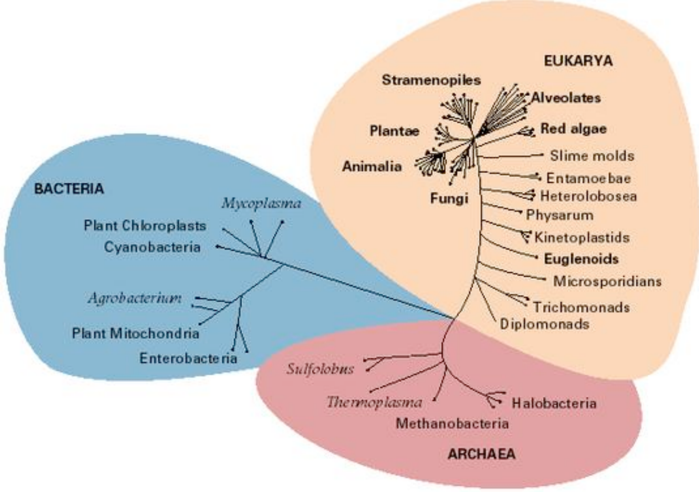
Vanessa M. Brown et al. Genome Res. 2002; 12: 868-884

Cold Spring Harbor Laboratory Press

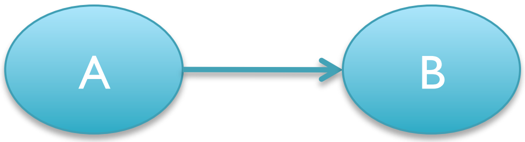








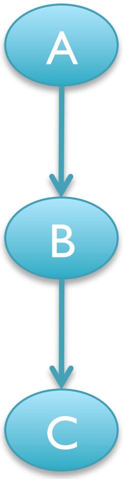
Graphs



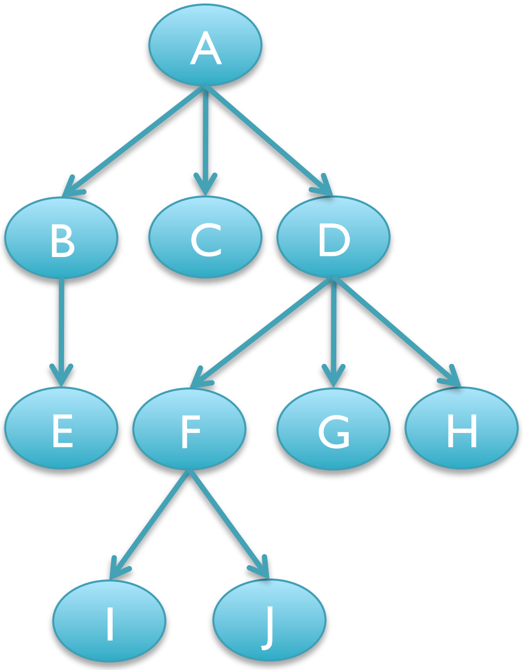
- Nodes
  - People, Proteins, Genes, Neurons, Sequences, Numbers, …

- Edges
  - A is connected to B
  - A is related to B
  - A regulates B
  - A precedes B
  - A interacts with B
  - A activates B
  - …

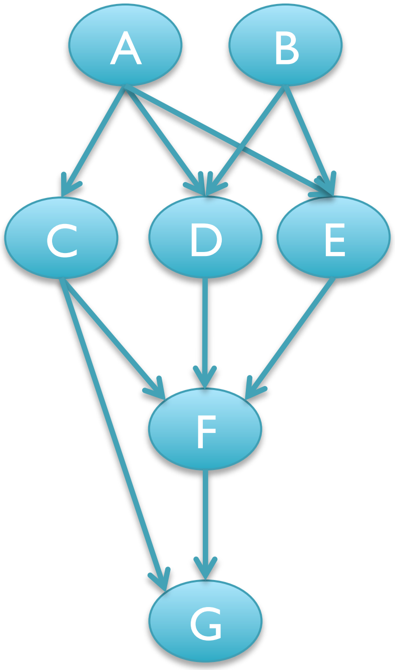
Graph Types



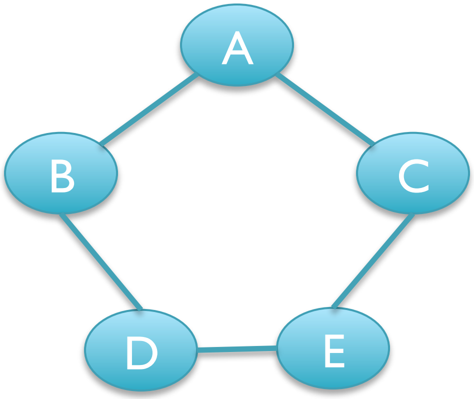
List



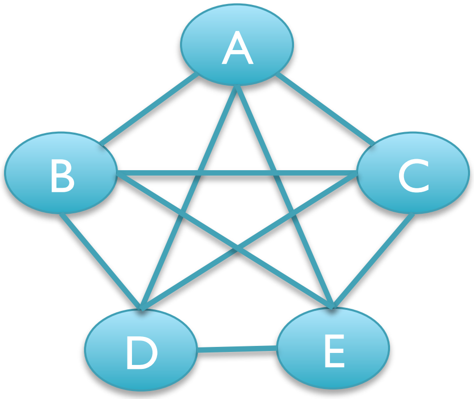
Tree



Directed
Acyclic
Graph

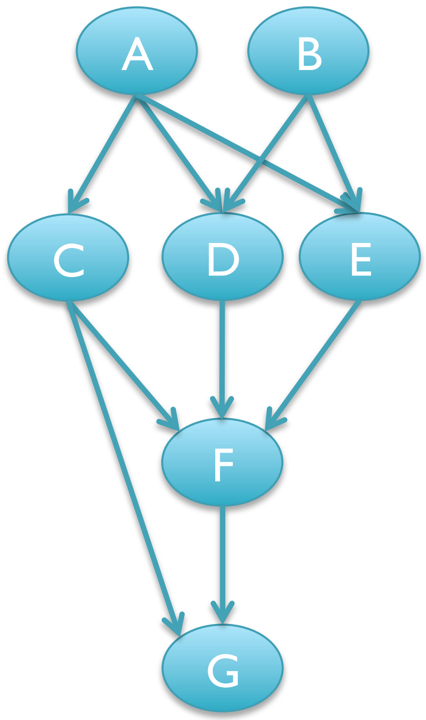


Cycle



Complete

Representing Graphs



Adjacency Matrix

Good for dense graphs

Fast, Fixed storage: N^2 bits

	A	B	C	D	E	F	G
A			1	1	1		
B				1	1		
C						1	1
D						1	
E						1	
F							1
G							

Adjacency List

Good for sparse graphs

Compact storage: 4 bytes/edge

A: C, D, E
B: D, E
C: F, G
D: F
E: F
G:

Edge List

Easy, good if you (mostly) need to iterate through the edges

8 bytes / edge

A,C
A,D
A,E
B,C
B,D
B,E
C,F
C,G
D,F
E,F
F,G

Tools

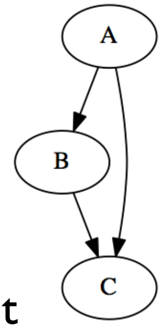
Matlab: http://www.mathworks.com/

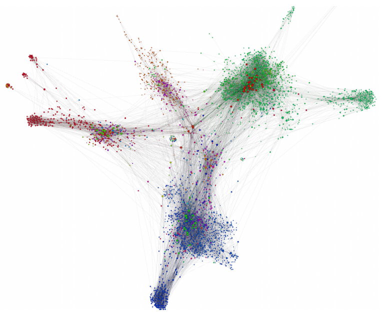
Graphviz: http://www.graphviz.org/

Gephi: https://gephi.org/

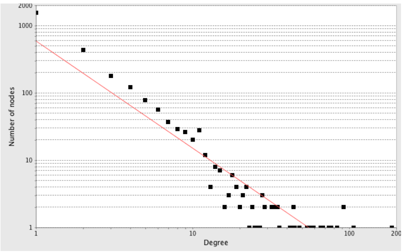
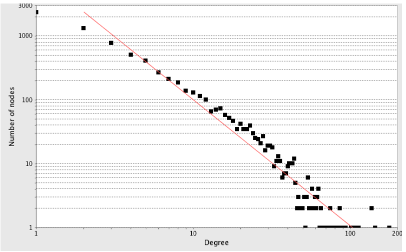
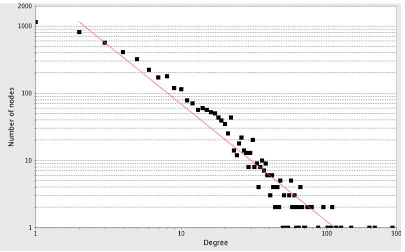
Cytoscape: http://www.cytoscape.org/

```
digraph G {
      A->B
      B->C
      A->C
}
dot –Tpdf -og.pdf g.dot
```





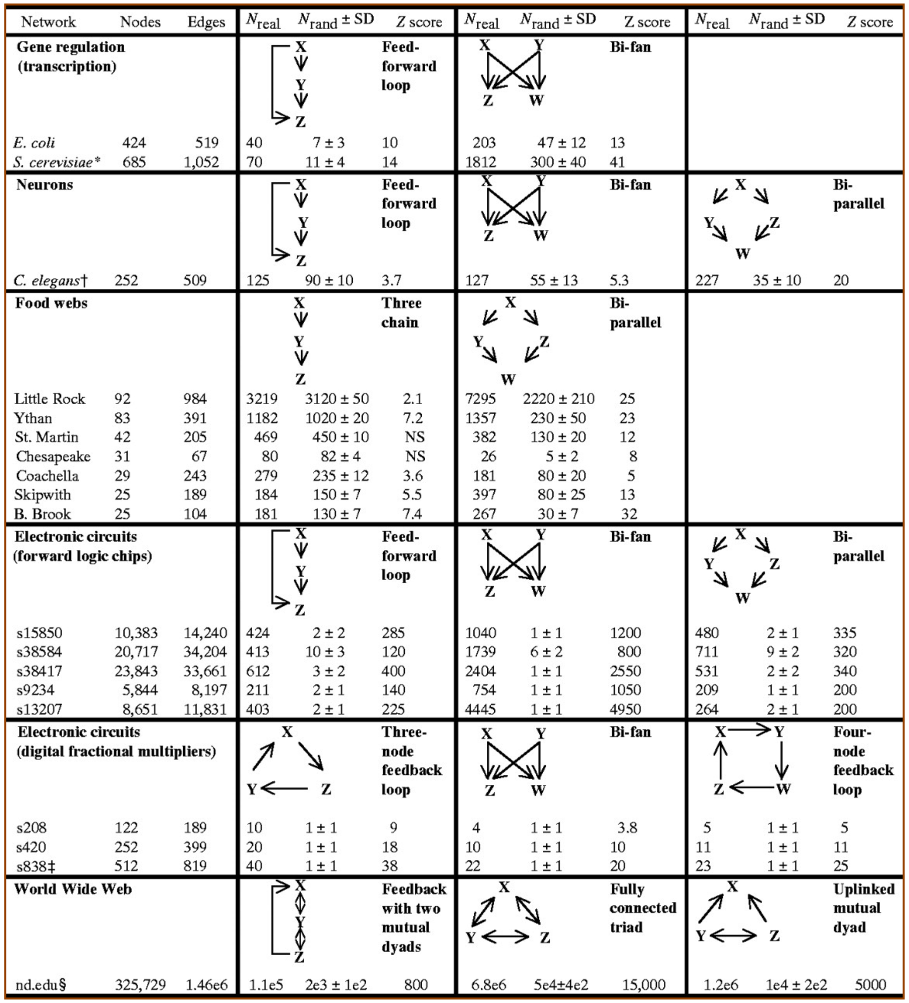
Network Characteristics

	C. elegans	*D. melanogaster*	*S. cerevisiae*
# Nodes	2646	7464	4965
# Edges	4037	22831	17536
Avg. / Max Degree	3.0 / 187	6.1 / 178	7.0 / 283
# Components	109	66	32
Largest Component	2386	7335	4906
Diameter	14	12	11
Avg. Shortest Path	4.8	4.4	4.1
Data Sources	2H	2x2H, TAP-MS	8x2H, 2xTAP, SUS
Degree Distributions			

Small World: Avg. Shortest Path between nodes is small
Scale Free: Power law distribution of degree – preferential attachment

Network Motifs

- Network Motif
  - Simple graph of connections
  - Exhaustively enumerate all possible 1, 2, 3, … k node motifs
- Statistical Significance
  - Compare frequency of a particular network motif in a real network as compared to a randomized network
- Certain motifs are “characteristic features” of the network

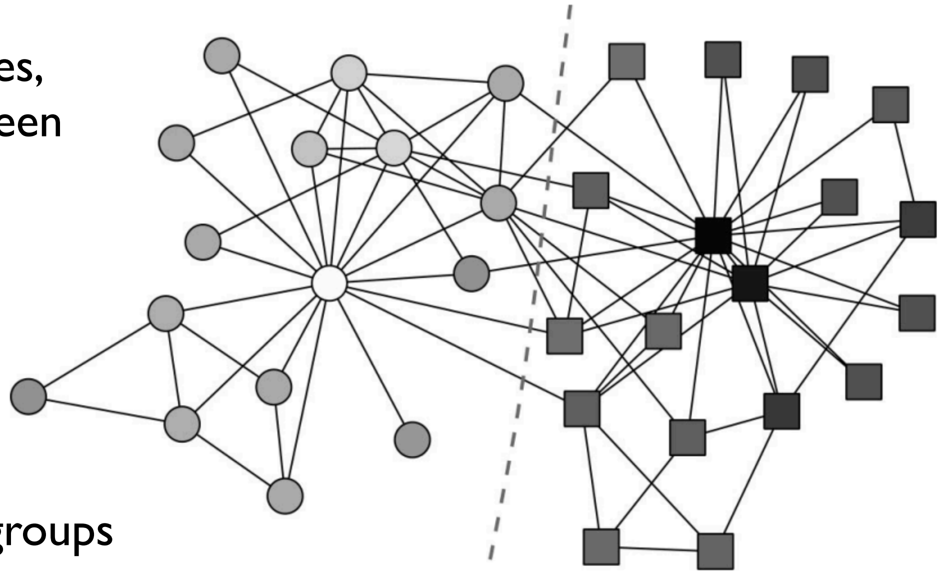


Network	Nodes	Edges	N_{real}	N_{rand} ± SD	Z score	N_{real}	N_{rand} ± SD	Z score	N_{real}	N_{rand} ± SD	Z score
Gene regulation (transcription)					**Feed-forward loop**			**Bi-fan**			
E. coli	424	519	40	7 ± 3	10	203	47 ± 12	13			
*S. cerevisiae**	685	1,052	70	11 ± 4	14	1812	300 ± 40	41			
Neurons					**Feed-forward loop**			**Bi-fan**			**Bi-parallel**
C. elegans†	252	509	125	90 ± 10	3.7	127	55 ± 13	5.3	227	35 ± 10	20
Food webs					**Three chain**			**Bi-parallel**			
Little Rock	92	984	3219	3120 ± 50	2.1	7295	2220 ± 210	25			
Ythan	83	391	1182	1020 ± 20	7.2	1357	230 ± 50	23			
St. Martin	42	205	469	450 ± 10	NS	382	130 ± 20	12			
Chesapeake	31	67	80	82 ± 4	NS	26	5 ± 2	8			
Coachella	29	243	279	235 ± 12	3.6	181	80 ± 20	5			
Skipwith	25	189	184	150 ± 7	5.5	397	80 ± 25	13			
B. Brook	25	104	181	130 ± 7	7.4	267	30 ± 7	32			
Electronic circuits (forward logic chips)					**Feed-forward loop**			**Bi-fan**			**Bi-parallel**
s15850	10,383	14,240	424	2 ± 2	285	1040	1 ± 1	1200	480	2 ± 1	335
s38584	20,717	34,204	413	10 ± 3	120	1739	6 ± 2	800	711	9 ± 2	320
s38417	23,843	33,661	612	3 ± 2	400	2404	1 ± 1	2550	531	2 ± 2	340
s9234	5,844	8,197	211	2 ± 1	140	754	1 ± 1	1050	209	1 ± 1	200
s13207	8,651	11,831	403	2 ± 1	225	4445	1 ± 1	4950	264	2 ± 1	200
Electronic circuits (digital fractional multipliers)					**Three-node feedback loop**			**Bi-fan**			**Four-node feedback loop**
s208	122	189	10	1 ± 1	9	4	1 ± 1	3.8	5	1 ± 1	5
s420	252	399	20	1 ± 1	18	10	1 ± 1	10	11	1 ± 1	11
s838‡	512	819	40	1 ± 1	38	22	1 ± 1	20	23	1 ± 1	25
World Wide Web					**Feedback with two mutual dyads**			**Fully connected triad**			**Uplinked mutual dyad**
nd.edu§	325,729	1.46e6	1.1e5	2e3 ± 1e2	800	6.8e6	5e4±4e2	15,000	1.2e6	1e4 ± 2e2	5000

Network Motifs: Simple Building Blocks of Complex Networks
Milo et al (2002) *Science. 298:824-827*

Modularity

- Community structure
  - Densely connected groups of vertices, with only sparser connections between groups
  - Reveals the structure of large-scale network data sets



- Modularity
  - The number of edges falling within groups minus the expected number in an equivalent network with edges placed at random
  - Larger positive values => Stronger community structure
  - Optimal assignment determined by computing the eigenvector of the modularity matrix

$$Q = \boxed{\frac{1}{4m}} \sum_{ij} \boxed{\Big(A_{ij}} - \boxed{\frac{k_i k_j}{2m} \Big)} \boxed{(s_i s_j + 1)}$$

Normalization factor

Adjacency matrix

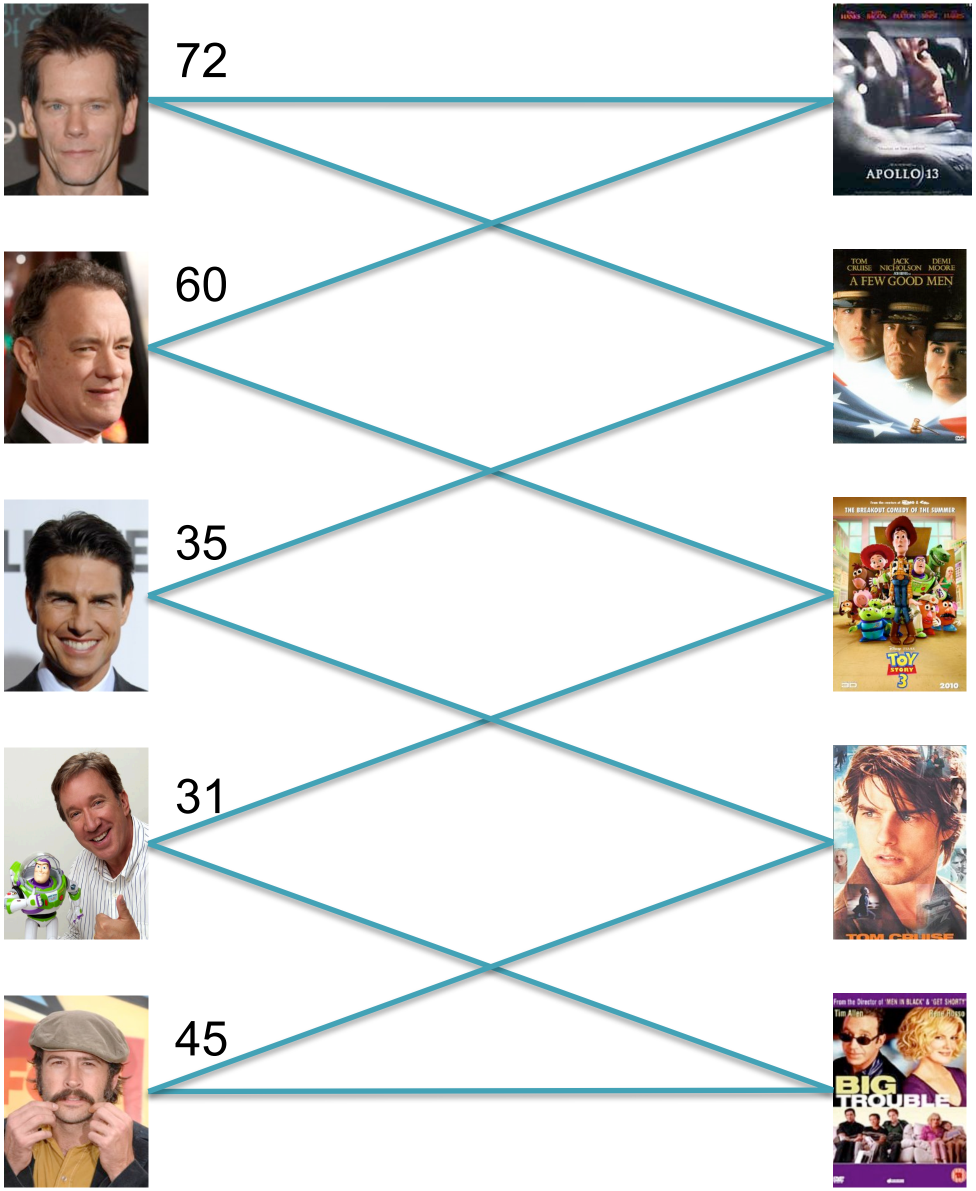
Random Prob. (product of degrees)

Indicates same group

Modularity and community structure in networks. Newman ME (2006) *PNAS. 103(23) 8577-8582*

Kevin Bacon and Bipartite Graphs

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



Breadth First Search:
4 hops

Bacon Distance:
2

BFS

```
BFS(start, stop)
// initialize all nodes dist = -1
start.dist = 0
list.addEnd(start)
while (!list.empty())
   cur = list.begin()
   if (cur == stop)
      print cur.dist;
   else
      foreach child in cur.children
         if (child.dist == -1)
            child.dist = cur.dist+1
            list.addEnd(child)
```

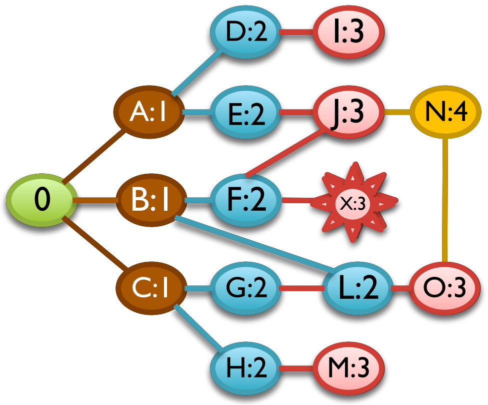
0

A,B,C
B,C,D,E
C,D,E,F,L

D,E,F,L,G,H
E,F,L,G,H,I
F,L,G,H,I,J
L,G,H,I,J,X
G,H,I,J,X,O
H,I,J,X,O

I,J,X,O,M
J,X,O,M
X,O,M,N
O,M,N
M,N

N



[How many nodes will it visit?]

[What's the running time?]

[What happens for disconnected components?]

BFS

```
BFS(start, stop)
// initialize all nodes dist = -1
start.dist = 0
list.addEnd(start)
while (!list.empty())
   cur = list.begin()
   if (cur == stop)
      print cur.dist;
   else
      foreach child in cur.children
         if (child.dist == -1)
            child.dist = cur.dist+1
            list.addEnd(child)
```

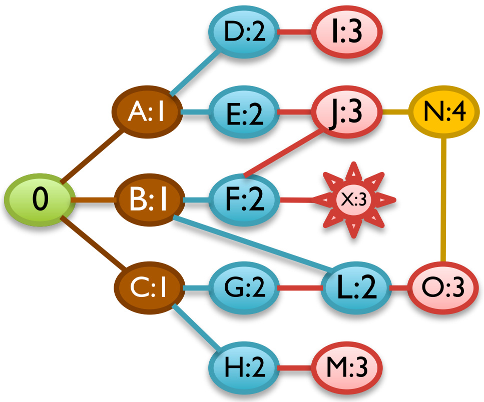
0

A,B,C
B,C,D,E
C,D,E,F,L

D,E,F,L,G,H
E,F,L,G,H,I
F,L,G,H,I,J
L,G,H,I,J,X
G,H,I,J,X,O
H,I,J,X,O

I,J,X,O,M
J,X,O,M
X,O,M,N
O,M,N
M,N

N



DFS

```
DFS(start, stop)
// initialize all nodes dist = -1
start.dist = 0
list.addEnd(start)
while (!list.empty())
   cur = list.end()
   if (cur == stop)
      print cur.dist;
   else
      foreach child in cur.children
         if (child.dist == -1)
            child.dist = cur.dist+1
            list.addEnd(child)
```

0

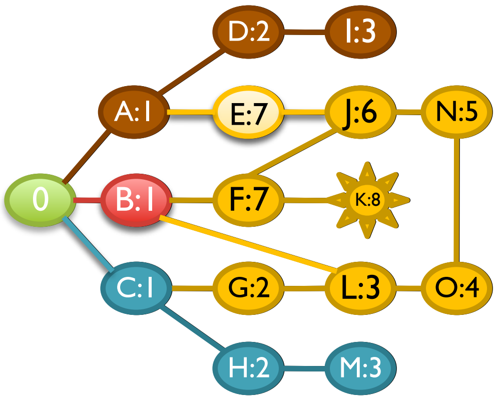
A,B,C

A,B,G,H
A,B,G,M

A,B,G
A,B,L
A,B,O
A,B,N
A,B,J
A,B,E,F
A,B,E,K
A,B,E

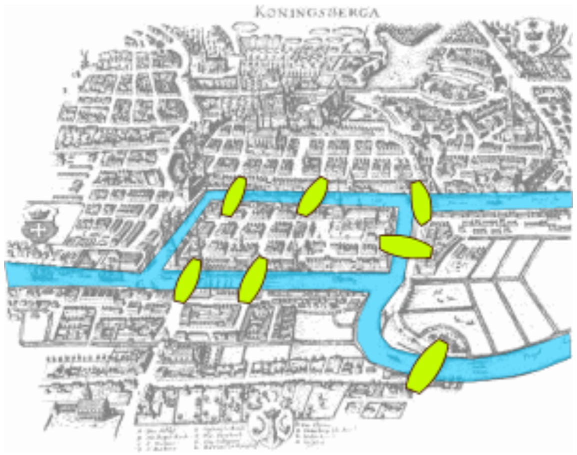
A,B

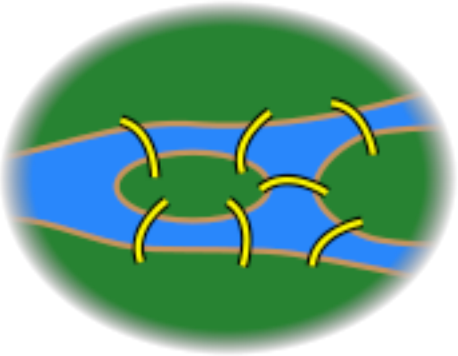
A
D
I

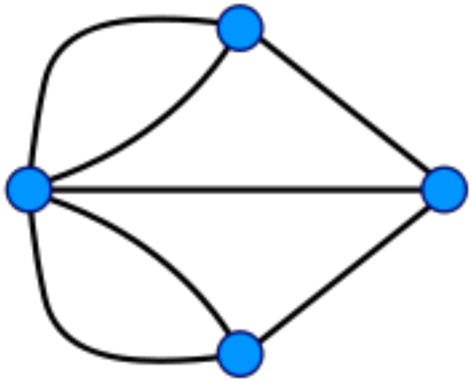


Eulerian Cycle Problem

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







[Can you find the cycle?]

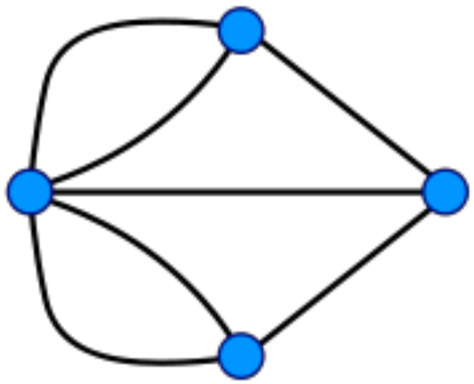
bioalgorithms.info

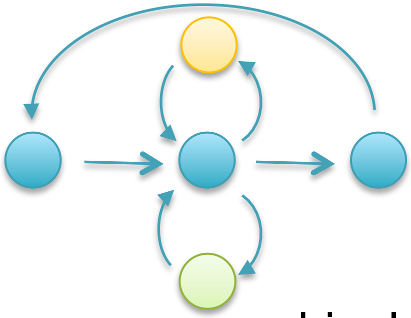
Euler Theorem

- A graph is ***balanced*** if for every vertex the number of incoming edges equals to the number of outgoing edges:

$$in(v)=out(v)$$

- **Theorem**: *A connected graph is Eulerian if and only if each of its vertices is balanced.*

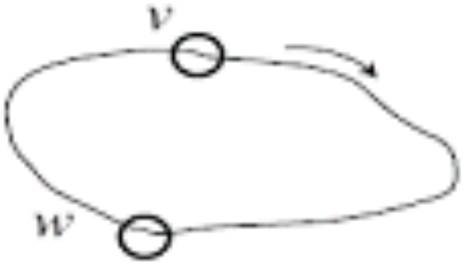




bioalgorithms.info

Algorithm for Constructing an Eulerian Cycle

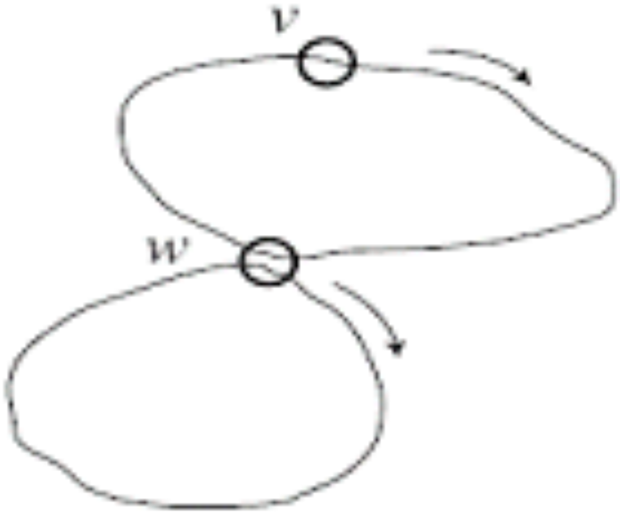
a. Start with an arbitrary vertex *v* and form an arbitrary cycle with unused edges until a dead end is reached. Since the graph is Eulerian this dead end is necessarily the starting point, i.e., vertex *v*.



(a)

Algorithm for Constructing an Eulerian Cycle (cont’d)

b. If cycle from (a) above is not an Eulerian cycle, it must contain a vertex *w*, which has untraversed edges. Perform step (a) again, using vertex *w* as the starting point. Once again, we will end up in the starting vertex *w*.

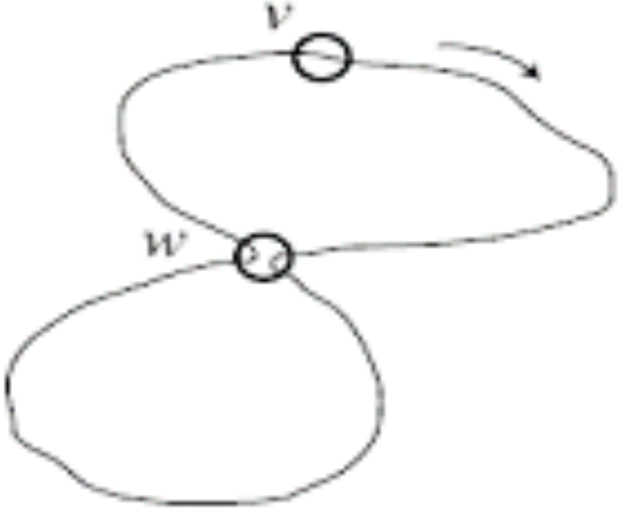


(b)

bioalgorithms.info

Algorithm for Constructing an Eulerian Cycle (cont' d)

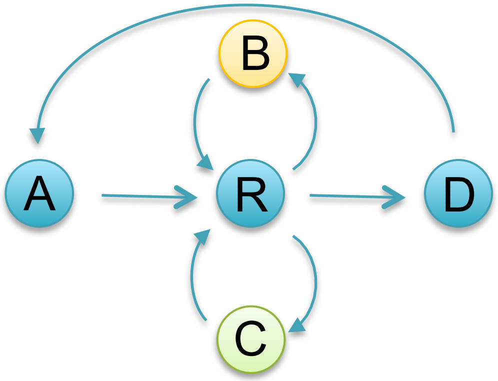
c. Combine the cycles from (a) and (b) into a single cycle and iterate step (b).



(c)

bioalgorithms.info

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*.

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

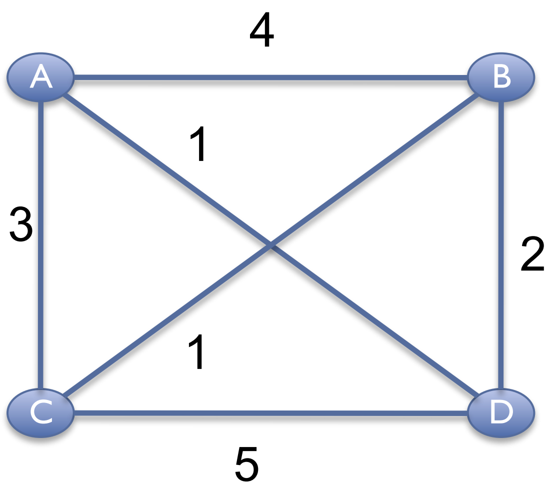
ACDBA: 3+5+2+4 = 14*

ABCDA: 4+1+5+1 = 11

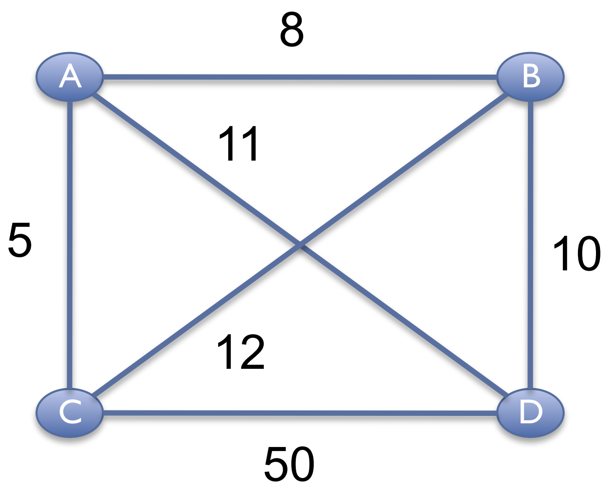
ADCBA: 1+5+1+4 = 11*

ACBDA: 3+1+2+1 = 7

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



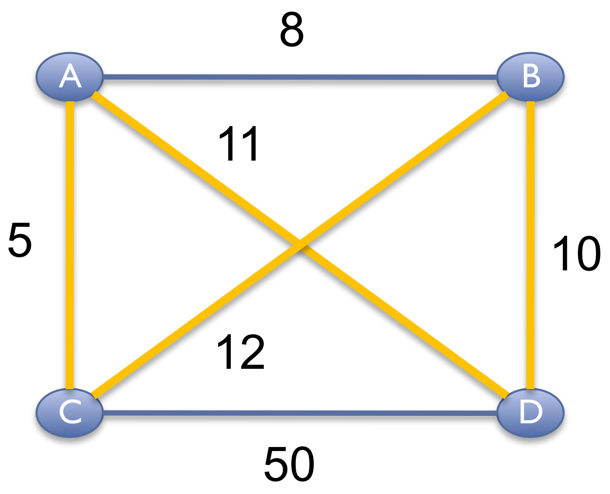
Greedy Search



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

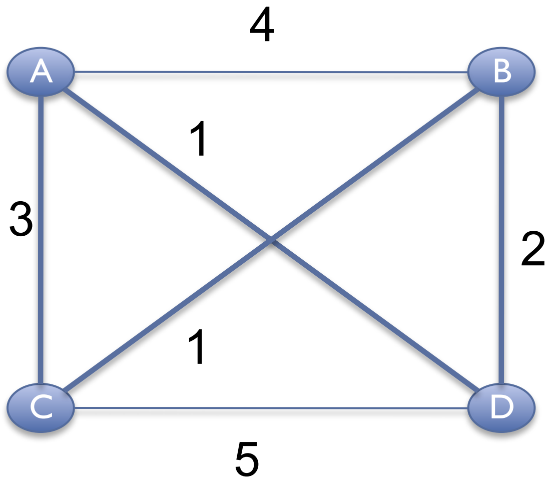
1. Greedy Choice: Local is correct without reconsideration
2. Optimal Substructure: Problem can be split into subproblems

Optimal Greedy: Making change with the fewest number of coins

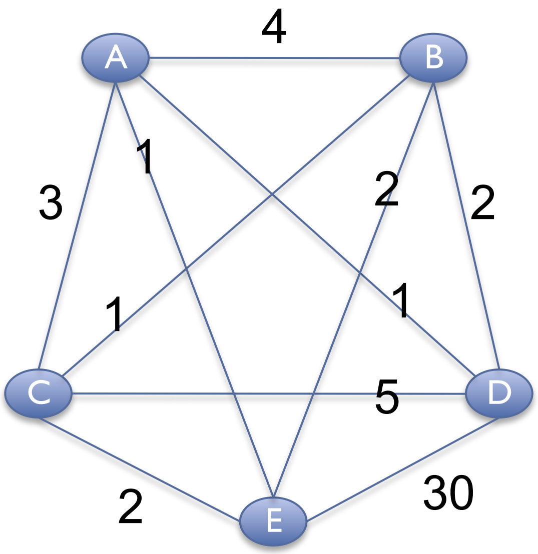
TSP Complexity

- 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?]

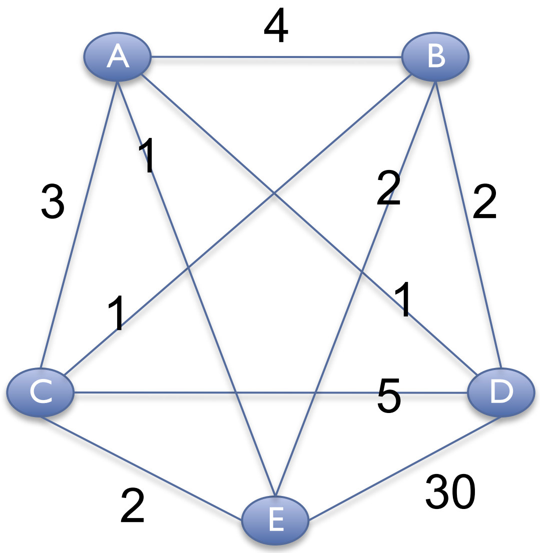


- Extensive searching is the only provably correct algorithm
  - Brute Force: O(n!)
    - ~20 cities max
    - 20! = 2.4 x 10^{18}



Branch-and-Bound

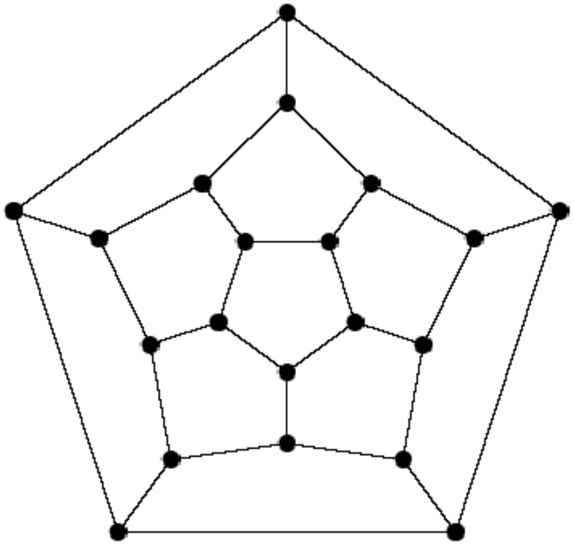
- Abort on suboptimal solutions as soon as possible
  - ADBECA = 1+2+2+2+3 = 10
  - ABDE = 4+2+30 > 10
  - ADE = 1+30 > 10
  - AED = 1+30 > 10
  - …



- Performance Heuristic
  - Always gives the optimal answer
  - Doesn't always help performance, but often does
  - Current TSP record holder:
    - 85,900 cities
    - 85900! = 10^{386526}

[When not?]

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

Shortest Common Superstring

Given: $S = \{s_1, \ldots, s_n\}$

Problem: Find minimal length superstring of S

s_1 CACCC

s_2 CCGGGTGC

s_3 CCACC

$s_{1,}s_2,s_3$ = CACCCGGGTGCCACC 15

s_1,s_3,s_2 = CACCCACCGGGTGC14

s_2,s_1,s_3 = CCGGGTGCACCCACC 15

s_2,s_3,s_1 = CCGGGTGCCACCC 13

s_3,s_1,s_2 = CCACCCGGGTGC 12

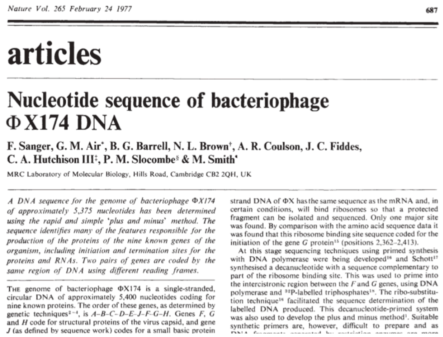
s_3,s_2,s_1 = CCACCGGGTGCACCC 15

NP-Complete by reduction from Vertex-Cover and later Directed-Hamiltonian-Path

Break

PHASE TWO : INTERPRETATION
I THINK I FOUND A CORNER PIECE.
3 BILLION PIECES
GENOME
SHEINMAN The Star-Ledger

Milestones in Genome Assembly



Nature Vol. 265 February 24 1977 687

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

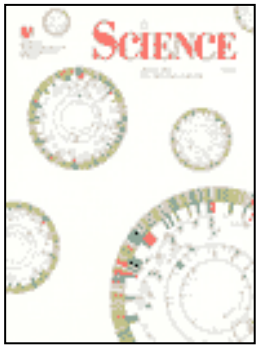
A DNA sequence for the genome of bacteriophage ΦX174 of approximately 5,375 nucleotides has been determined using the rapid and simple 'plus and minus' method. The sequence identifies many of the features responsible for the production of the proteins of the nine known genes of the organism, including initiation and termination sites for the proteins and RNAs. Two pairs of genes are coded by the same region of DNA using different reading frames.

THE genome of bacteriophage ΦX174 is a single-stranded, circular DNA of approximately 5,400 nucleotides coding for nine known proteins. The order of these genes, as determined by genetic techniques[2-4], is *A–B–C–D–E–J–F–G–H*. Genes *F*, *G* and *H* code for structural proteins of the virus capsid, and gene *J* (as defined by sequence work) codes for a small basic protein

strand DNA of ΦX has the same sequence as the mRNA and, in certain conditions, will bind ribosomes so that a protected fragment can be isolated and sequenced. Only one major site was found. By comparison with the amino acid sequence data it was found that this ribosome binding site sequence coded for the initiation of the gene *G* protein[15] (positions 2,362–2,413).

At this stage sequencing techniques using primed synthesis with DNA polymerase were being developed[16] and Schott[17] synthesised a decanucleotide with a sequence complementary to part of the ribosome binding site. This was used to prime into the intercistronic region between the *F* and *G* genes, using DNA polymerase and ^{32}P-labelled triphosphates[18]. The ribo-substitution technique[16] facilitated the sequence determination of the labelled DNA produced. This decanucleotide-primed system was also used to develop the plus and minus method[1]. Suitable synthetic primers are, however, difficult to prepare and as

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

nature
the human genome
Nuclear fission
Five-dimensional energy landscapes
Seafloor spreading
The view from under the Arctic ice
Career prospects
Sequence creates new opportunities
naturejobs
genomics special

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

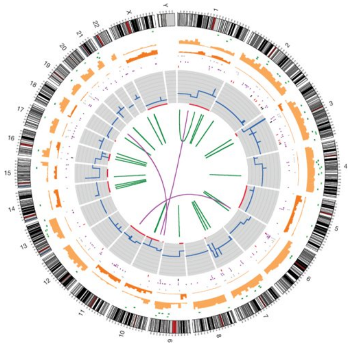
Like Dickens, we must computationally reconstruct a genome from short fragments

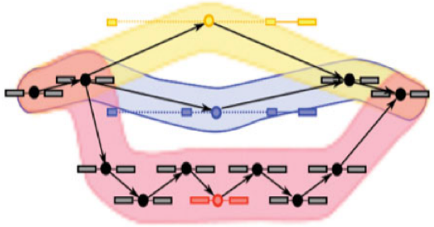
Assembly Applications

- Novel genomes

- Metagenomes

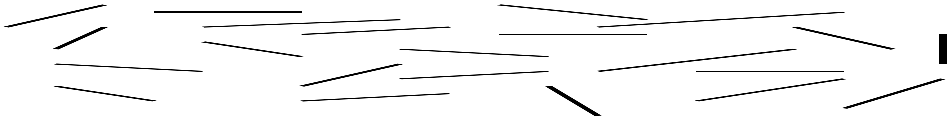
- Sequencing assays
  - Structural variations
  - Transcript assembly
  - …





Assembling a Genome

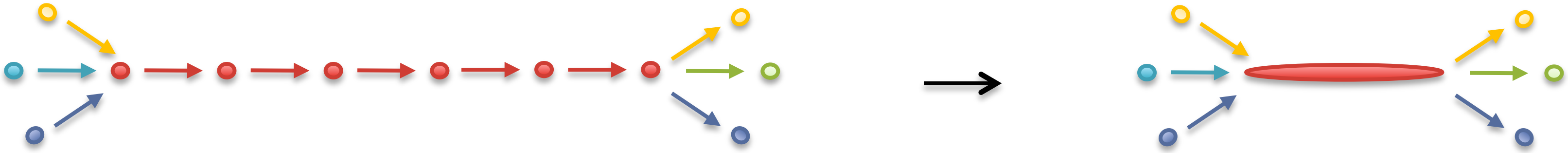
1. Shear & Sequence DNA



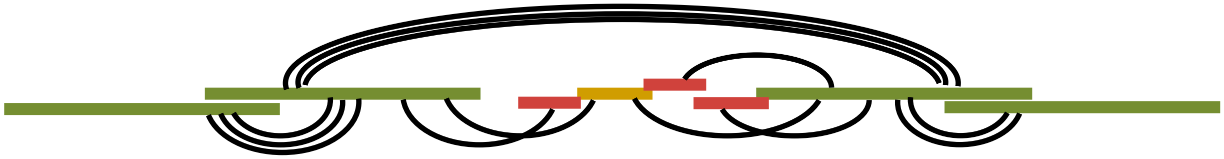
2. Construct assembly graph from overlapping reads

...AGCCTAGACCTACAGGATGCGCGACACGT
GGATGCGCGACACGTCGCATATCCGGT...

3. Simplify assembly graph



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

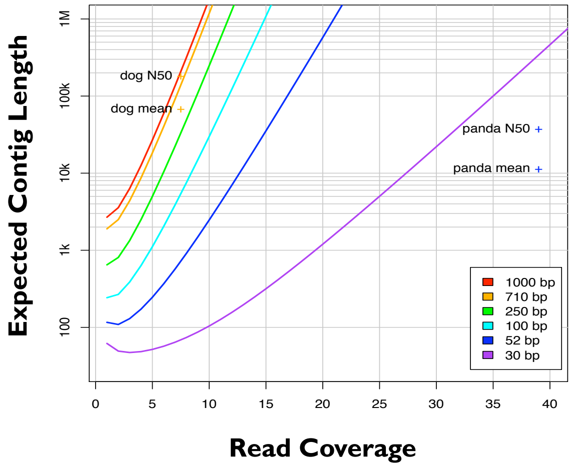


Why are genomes hard to assemble?

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

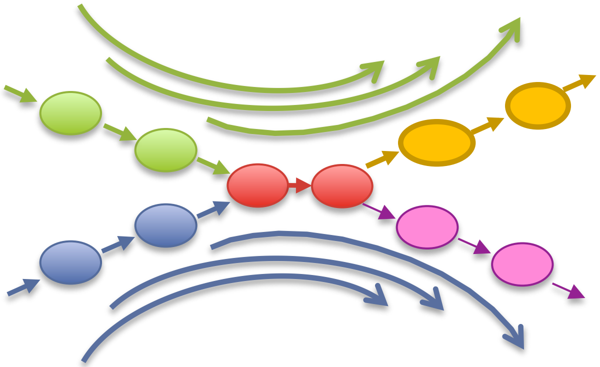
Coverage



High coverage is required

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

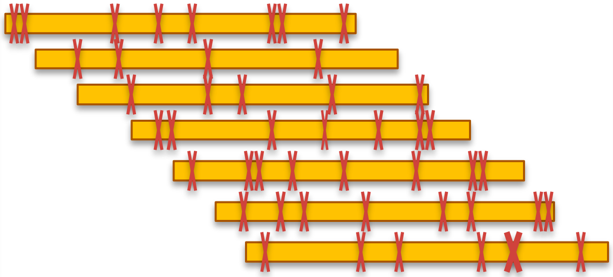
Read Length



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

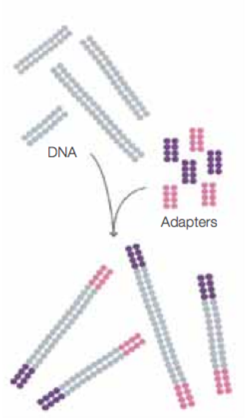


Errors obscure overlaps

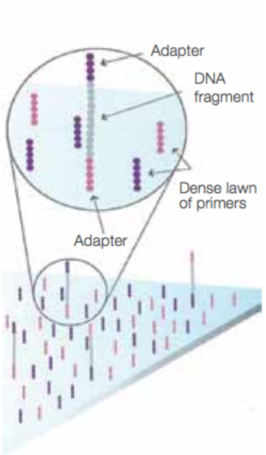
- Reads are assembled by finding kmers shared in pair of reads
- High error rate requires very short seeds, increasing complexity and forming assembly hairballs

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

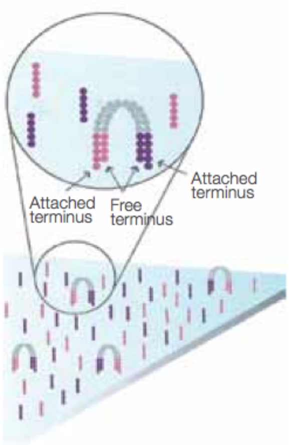
Illumina Sequencing by Synthesis



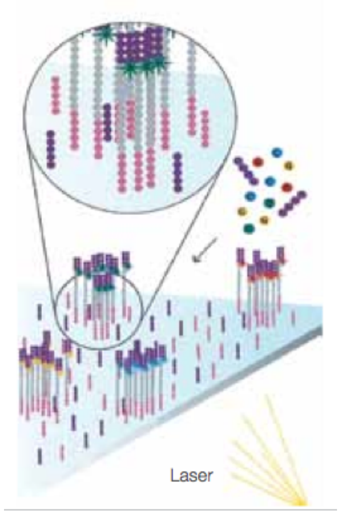
1. Prepare



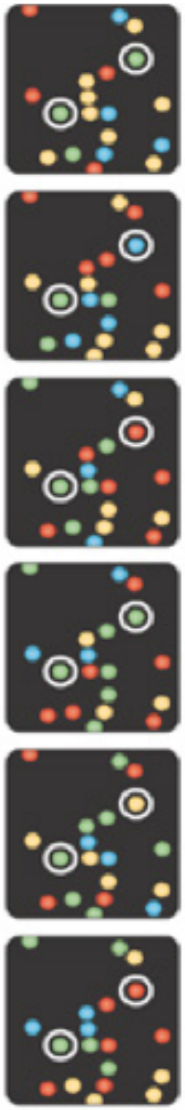
2. Attach



3. Amplify



4. Image



5. Basecall

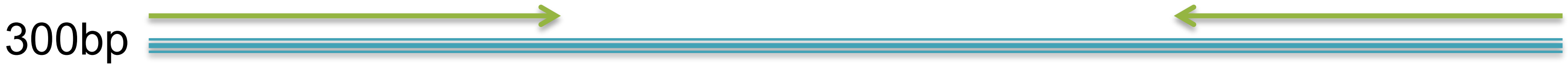
Metzker (2010) Nature Reviews Genetics 11:31-46

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

Paired-end and Mate-pairs

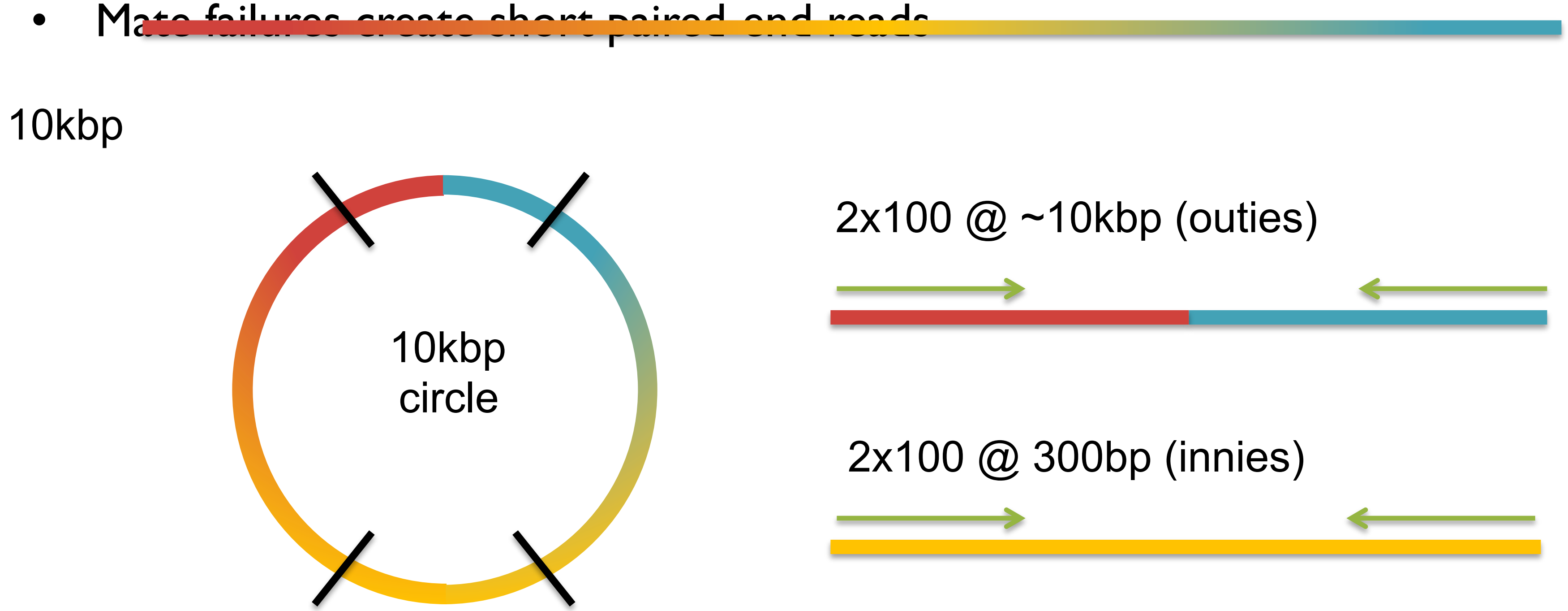
Paired-end sequencing

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

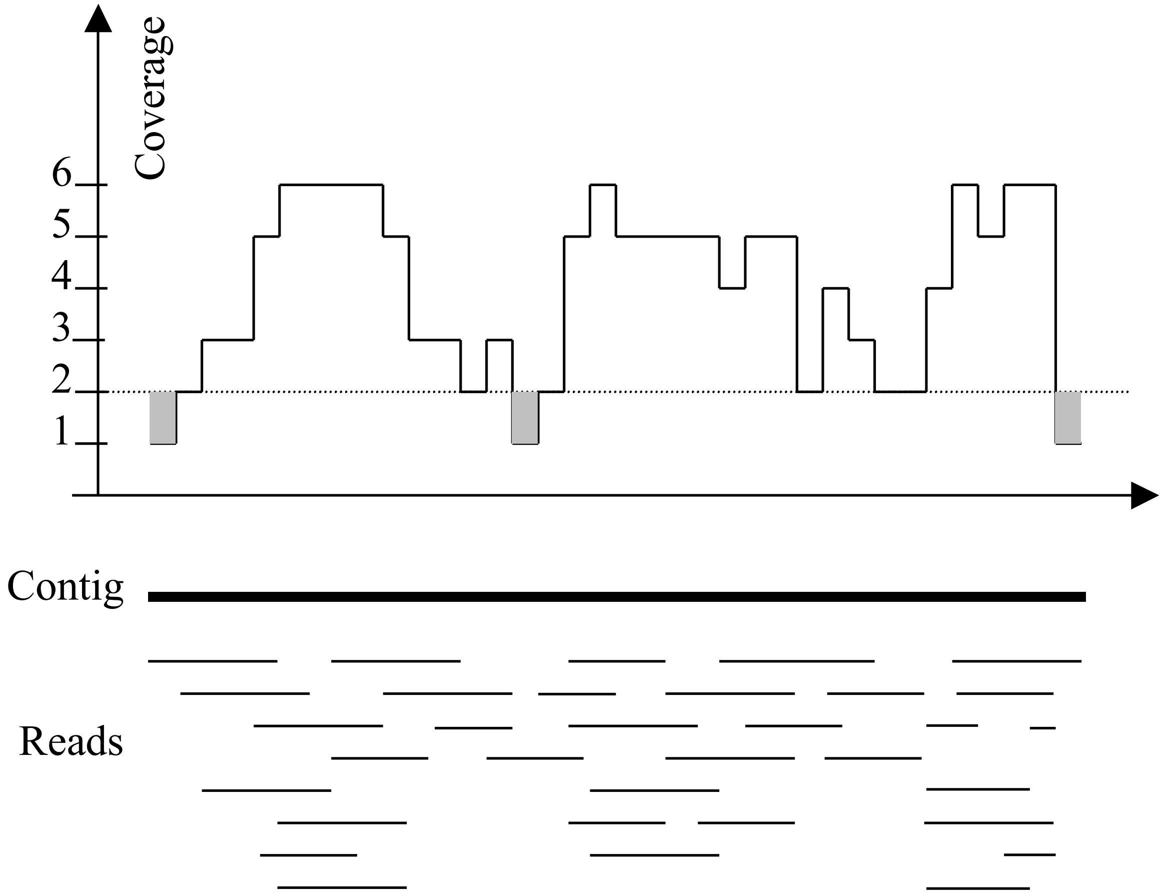


Mate-pair sequencing

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

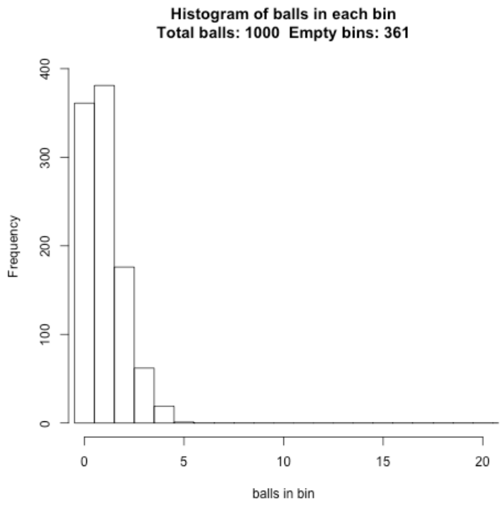


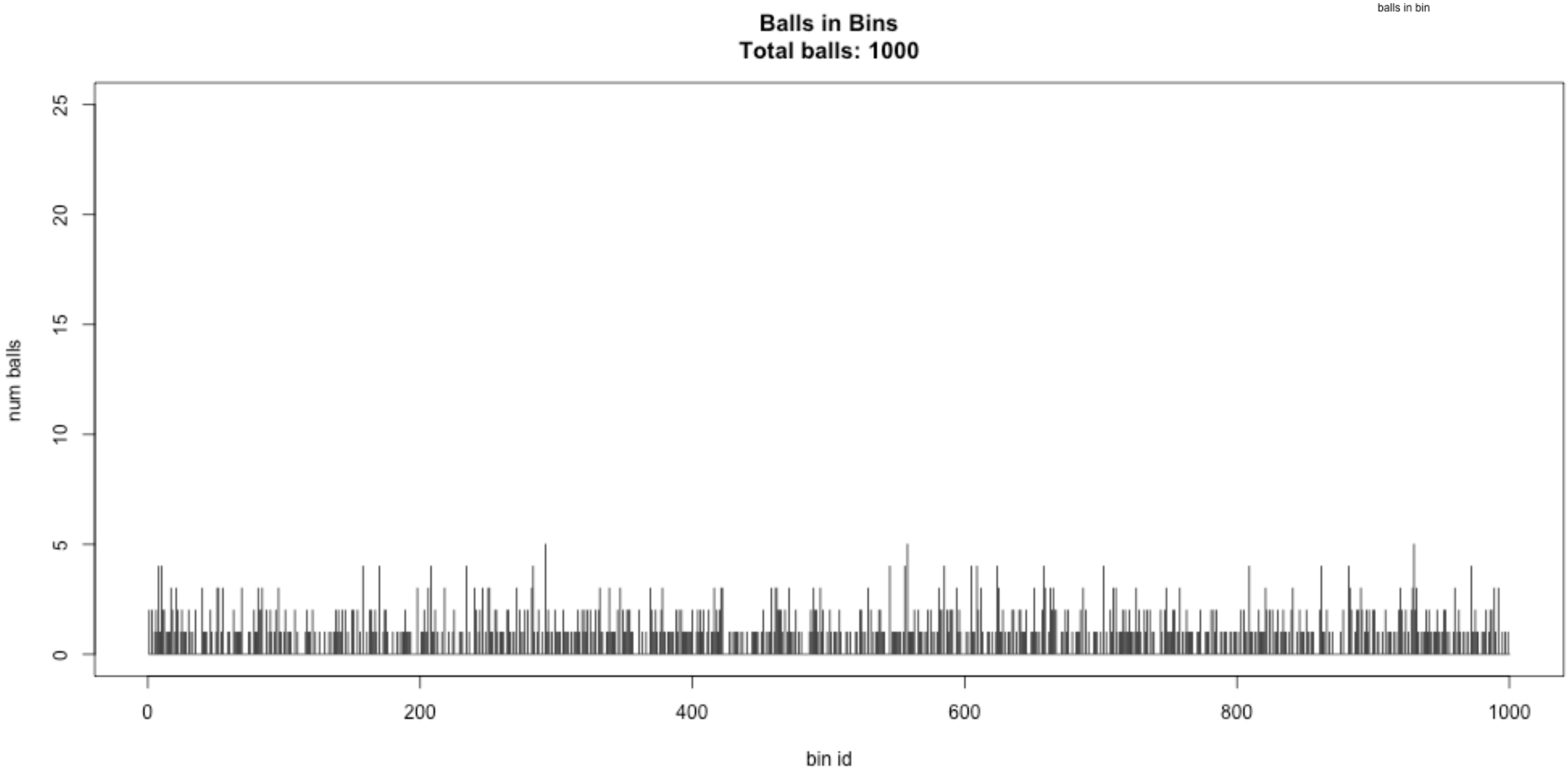
Typical contig coverage



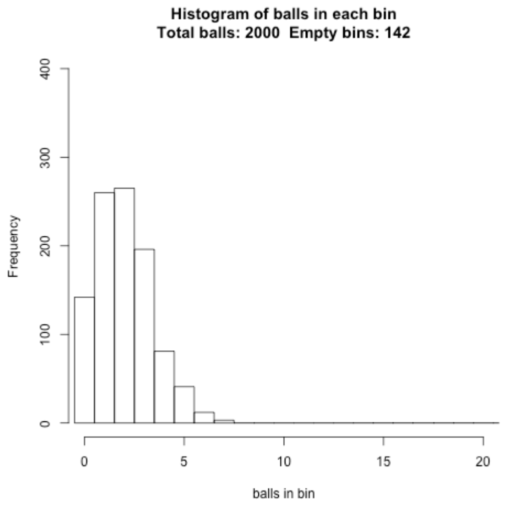
Imagine raindrops on a sidewalk

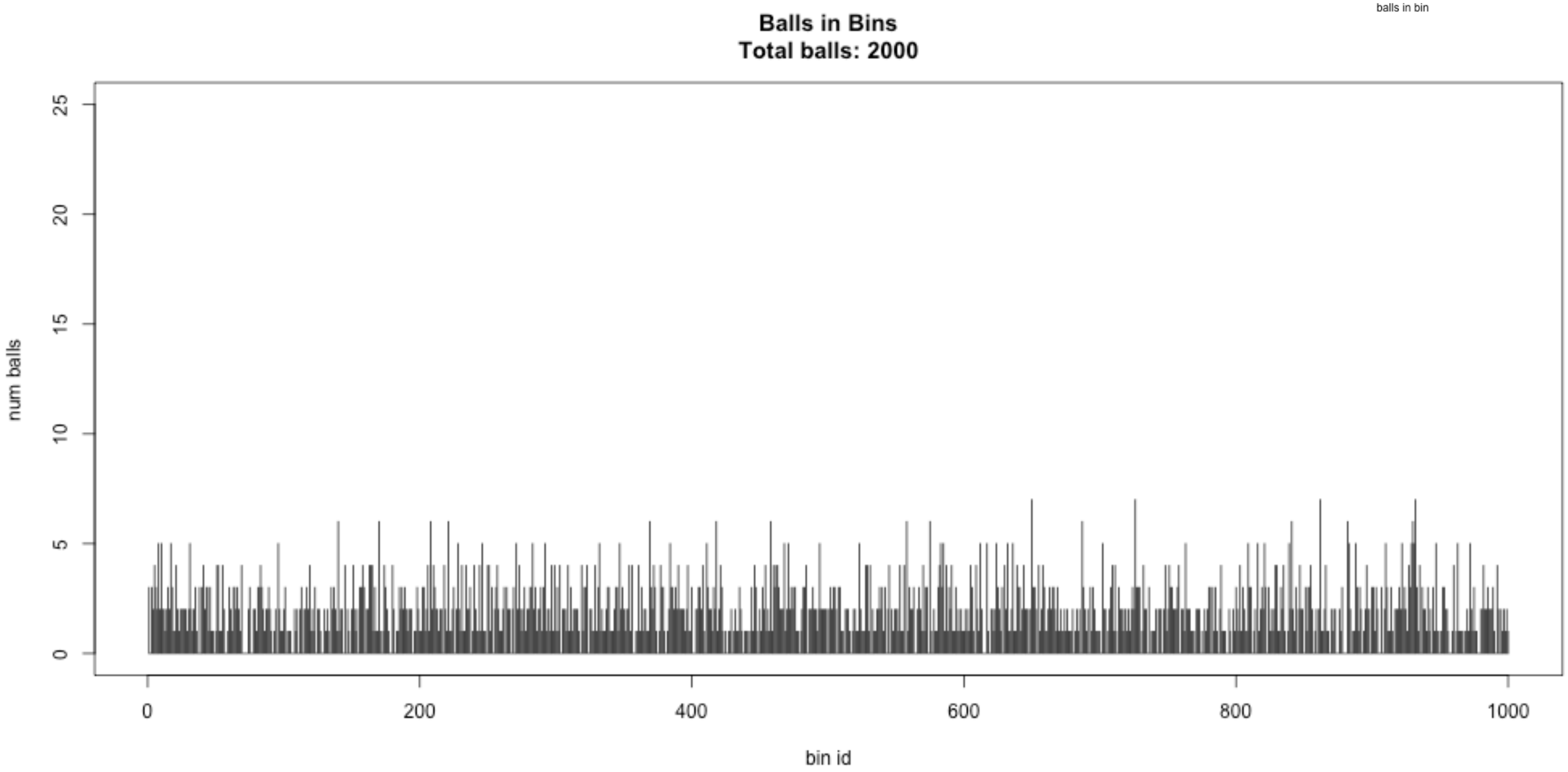
Balls in Bins 1x



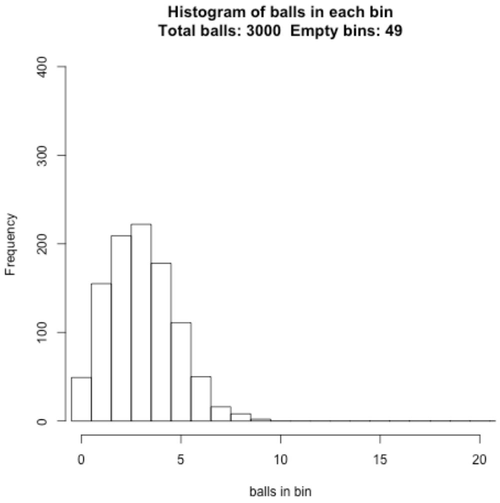


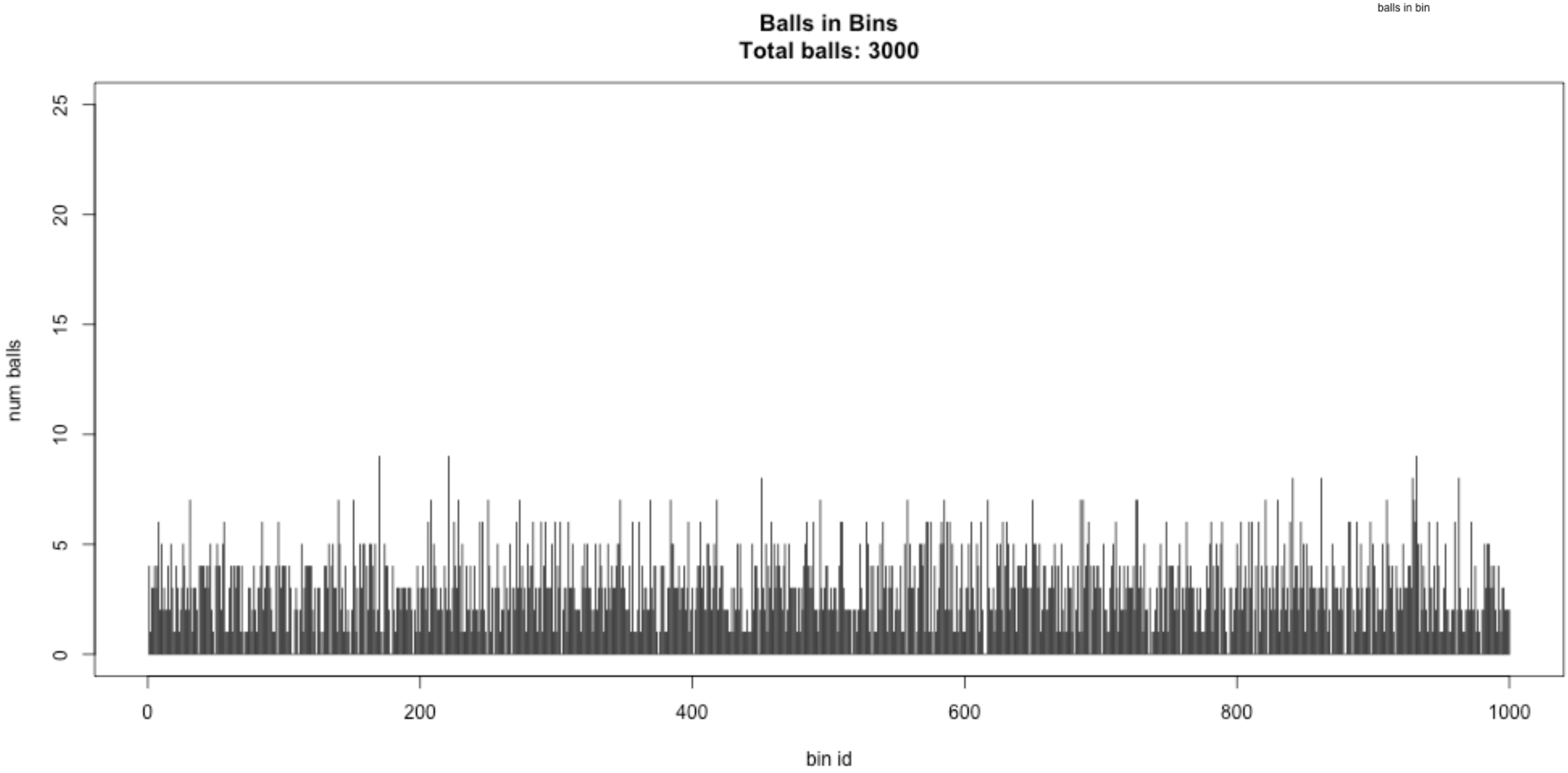
Balls in Bins 2x



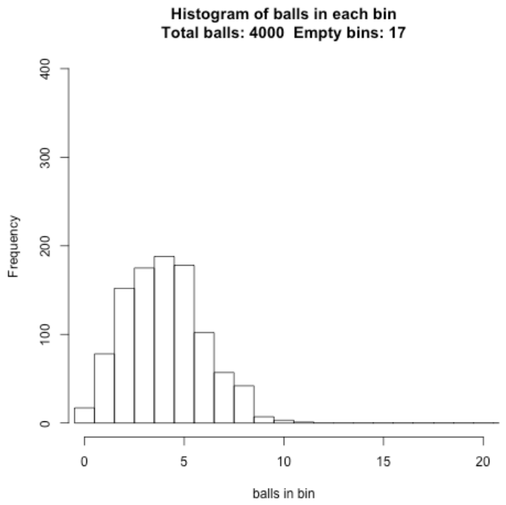


Balls in Bins 3x

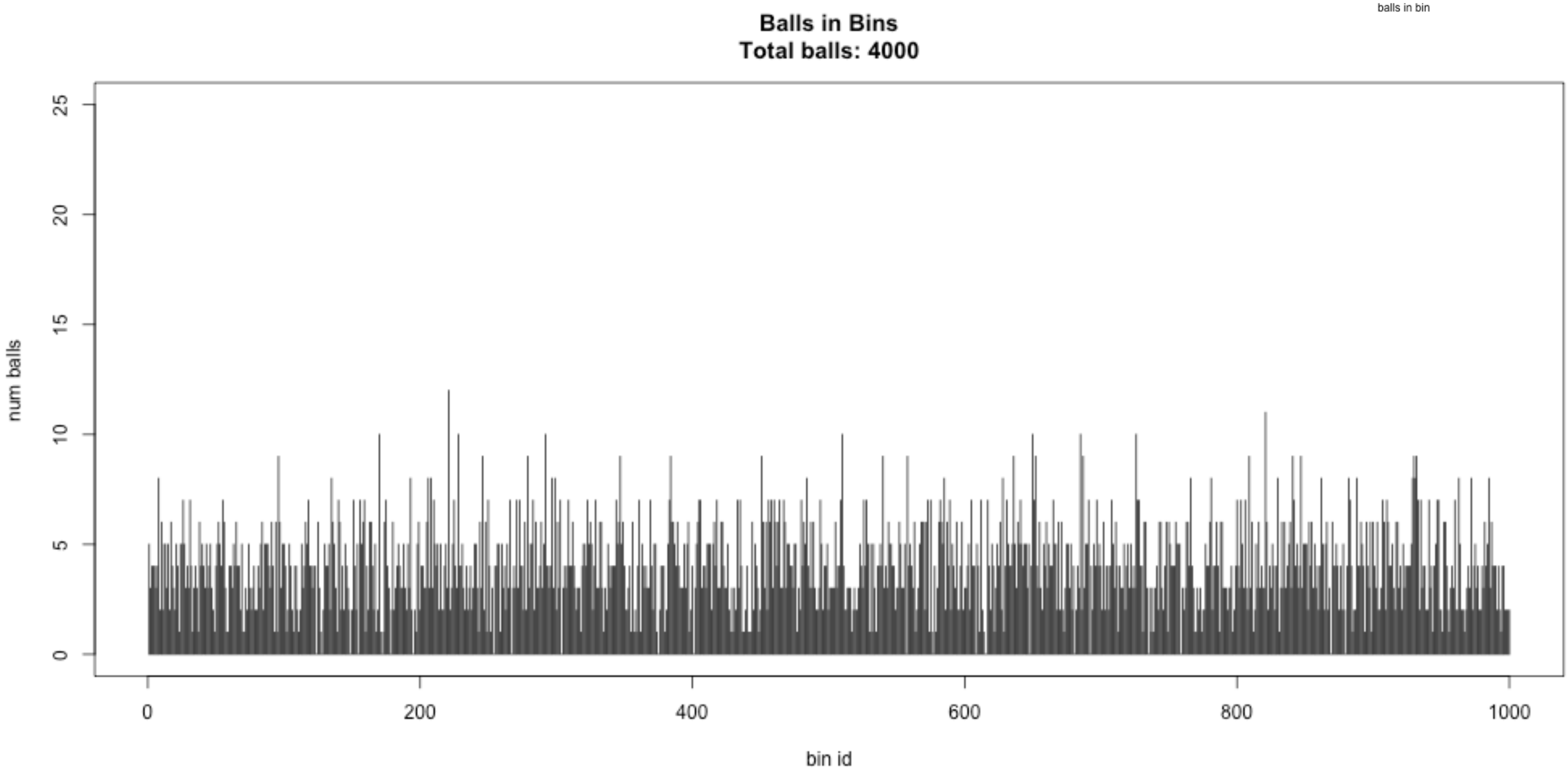




Balls in Bins 4x

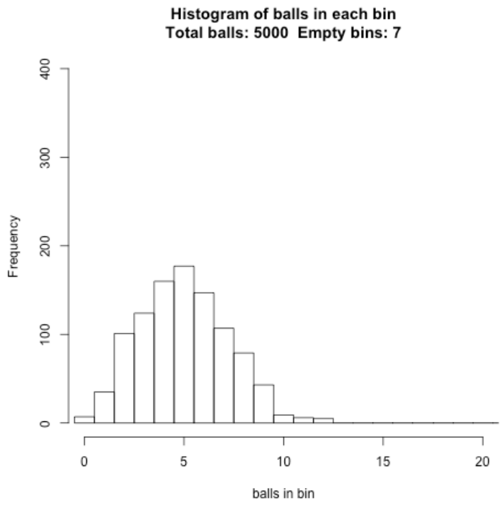


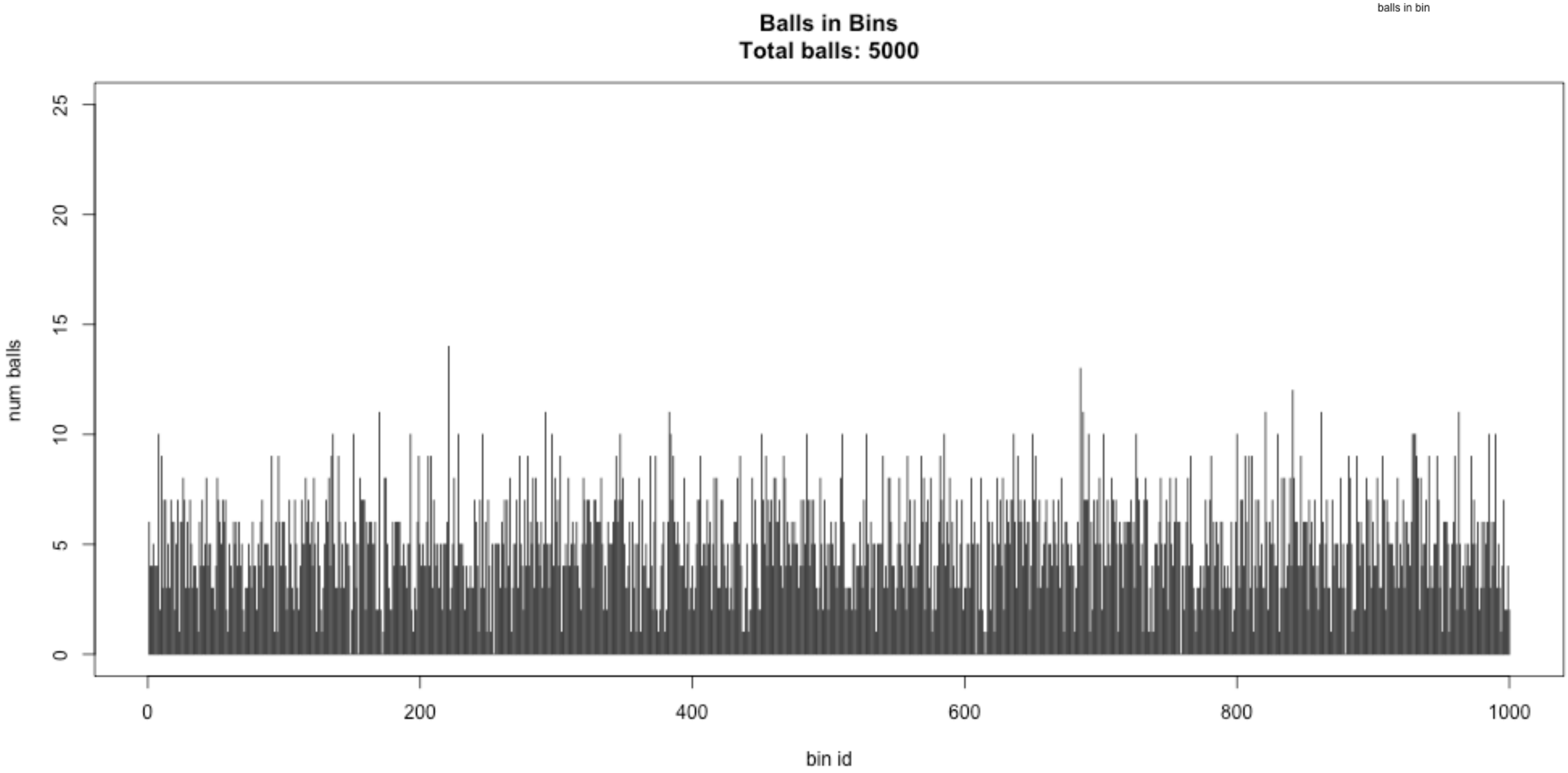
Histogram of balls in each bin
Total balls: 4000 Empty bins: 17



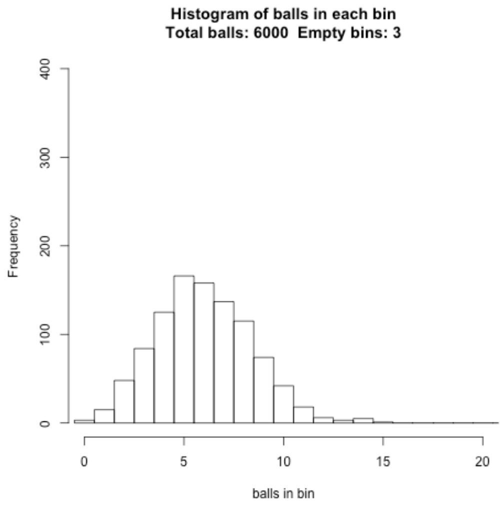
Balls in Bins
Total balls: 4000

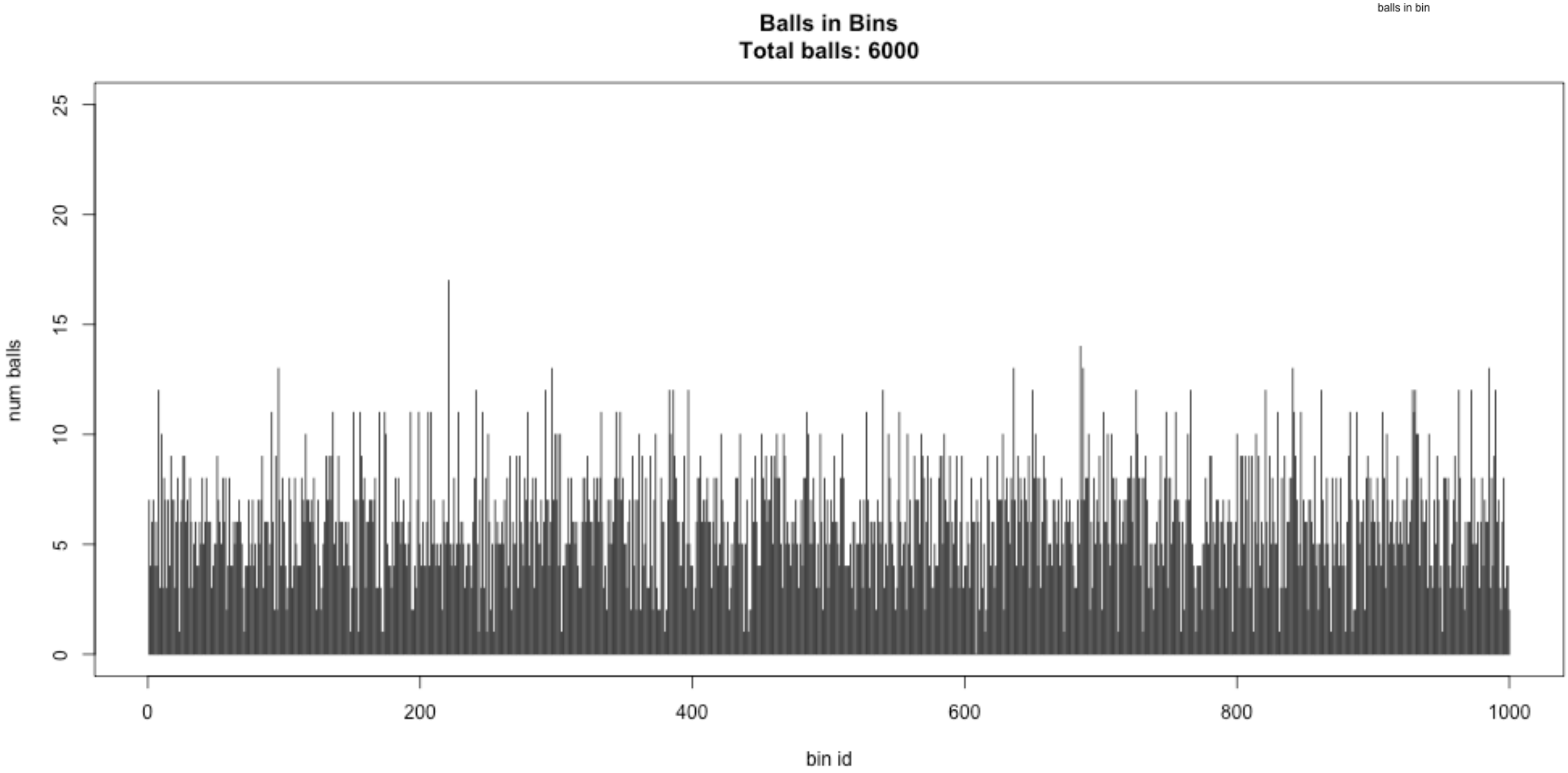
Balls in Bins 5x



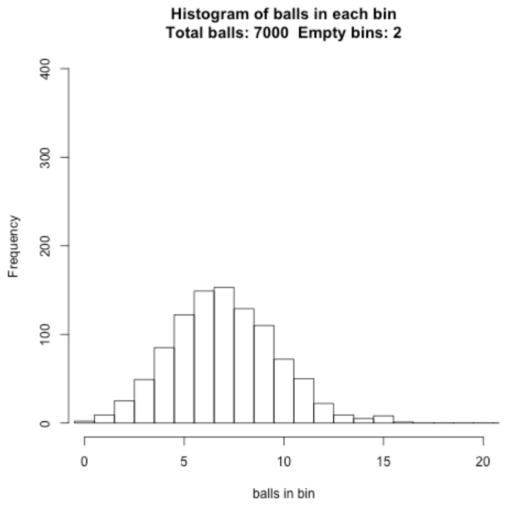


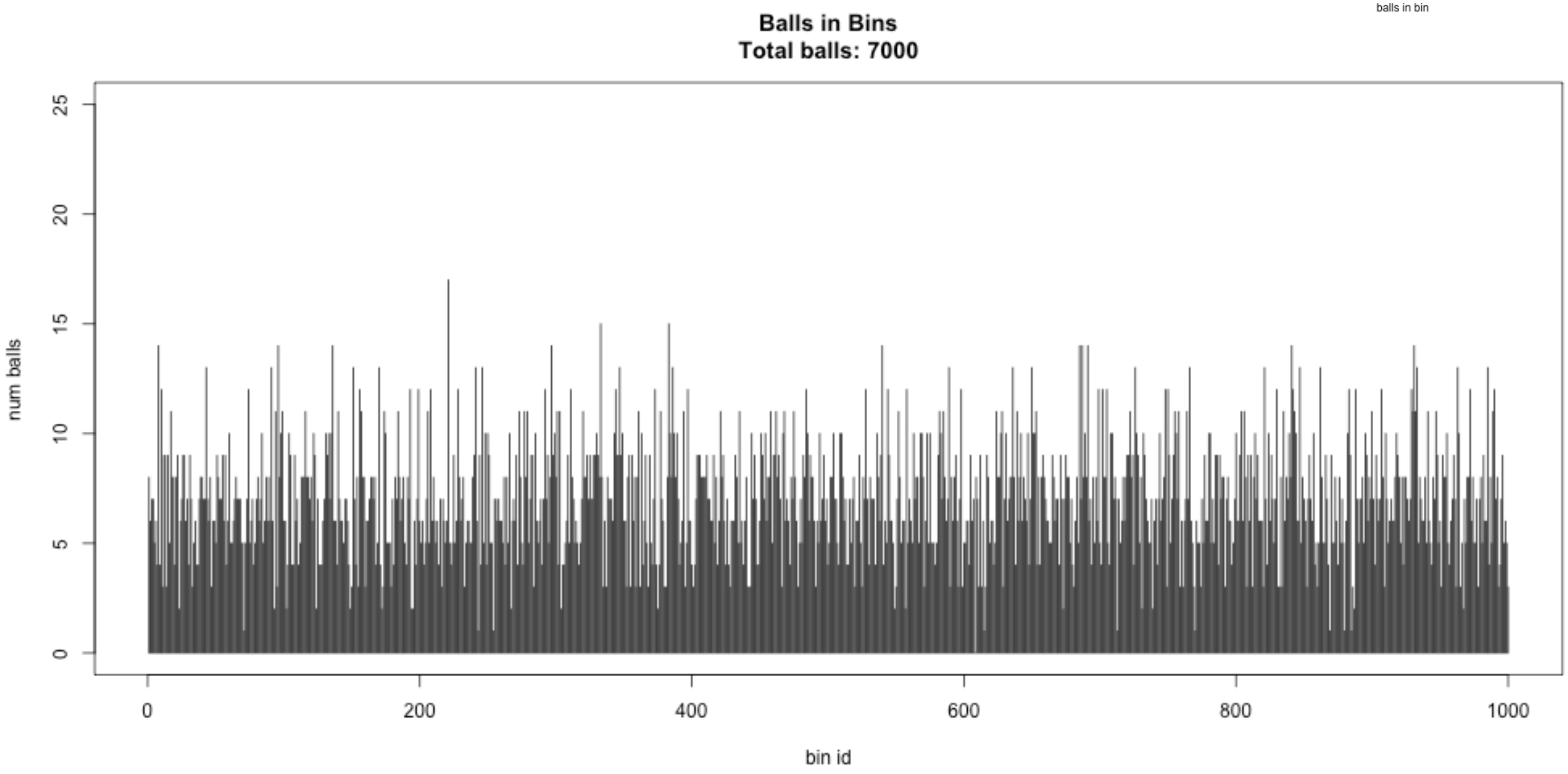
Balls in Bins 6x



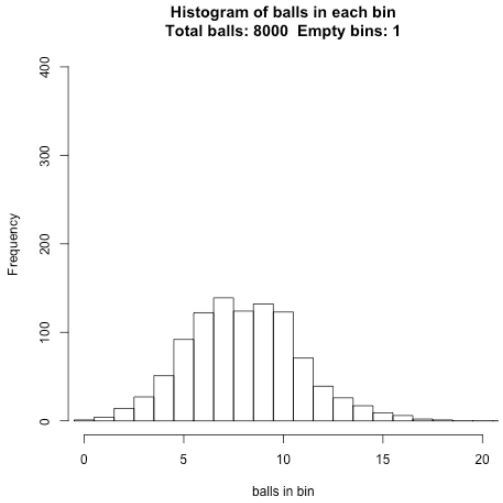


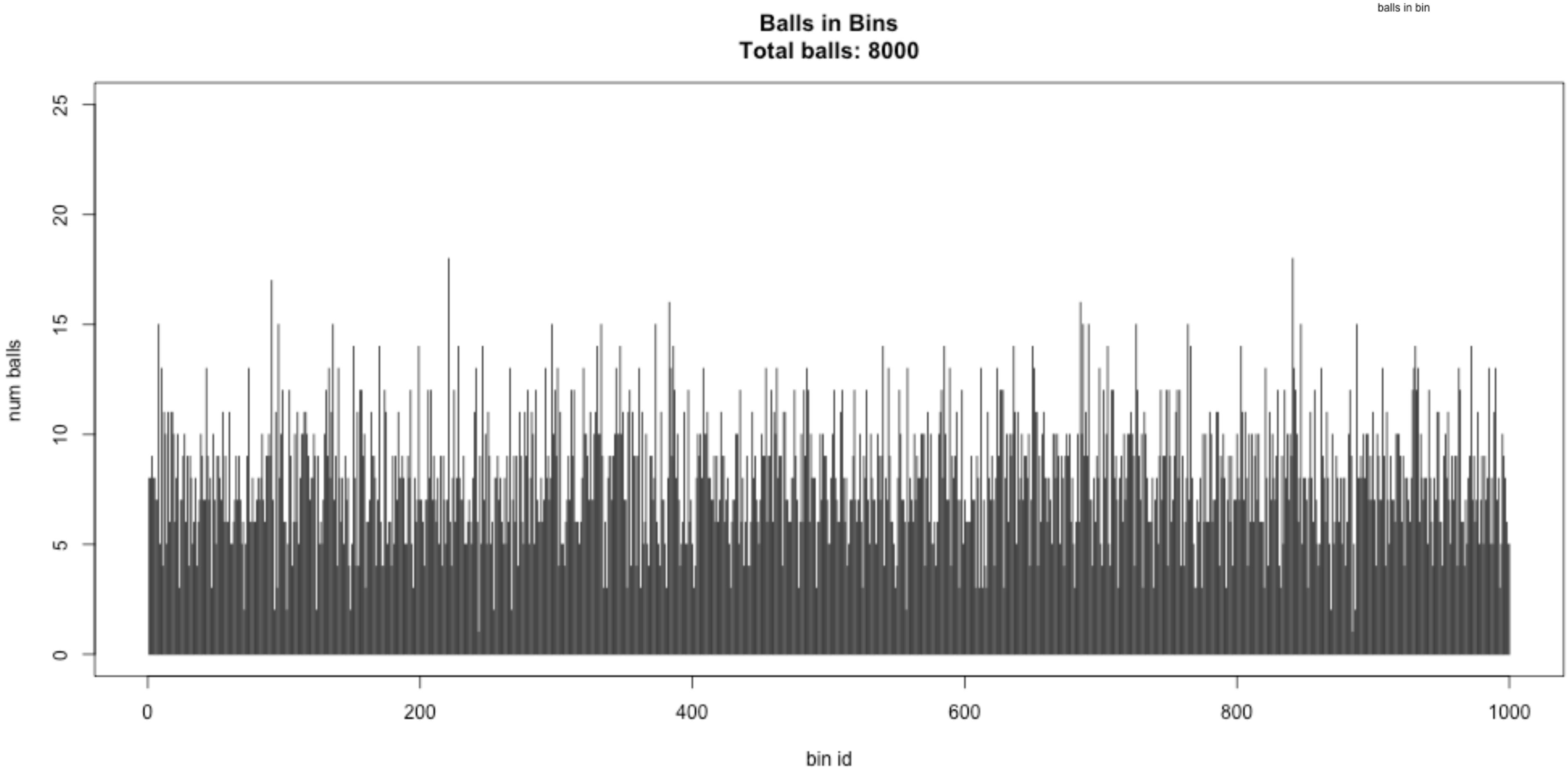
Balls in Bins 7x





Balls in Bins 8x





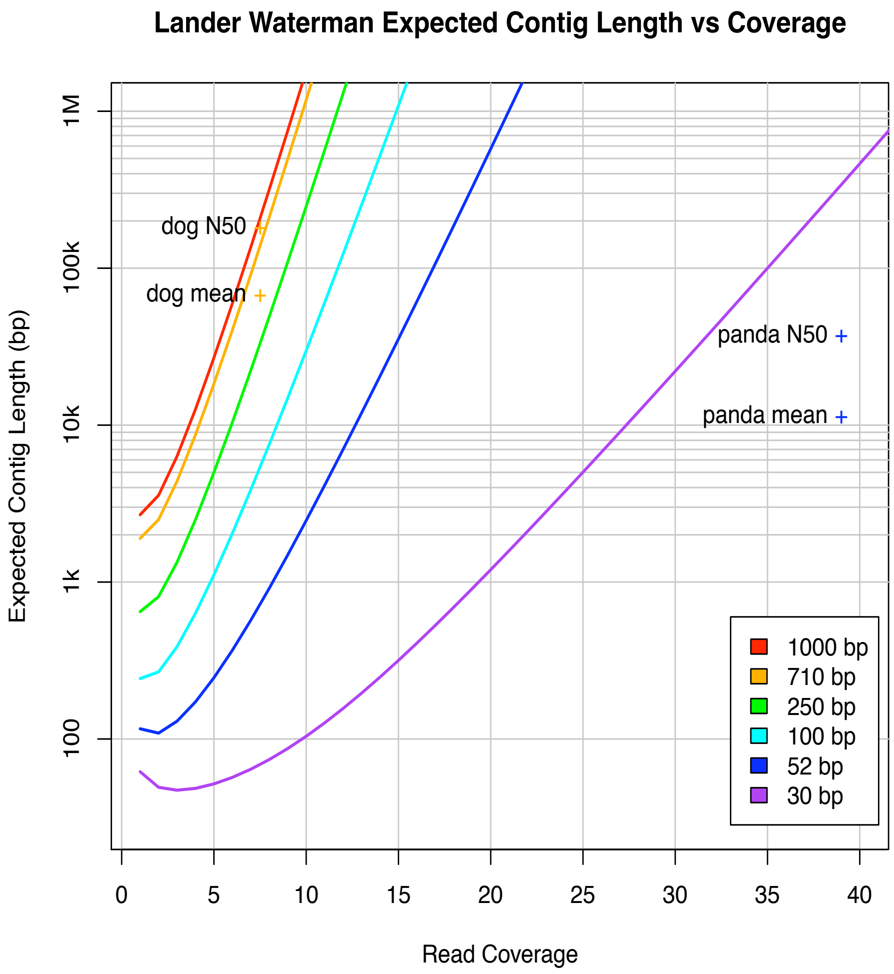
Coverage and Read Length

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

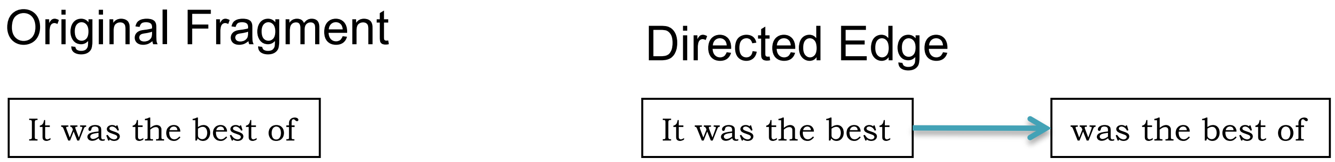


Lander Waterman Expected Contig Length vs Coverage

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

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



Original Fragment

It was the best of

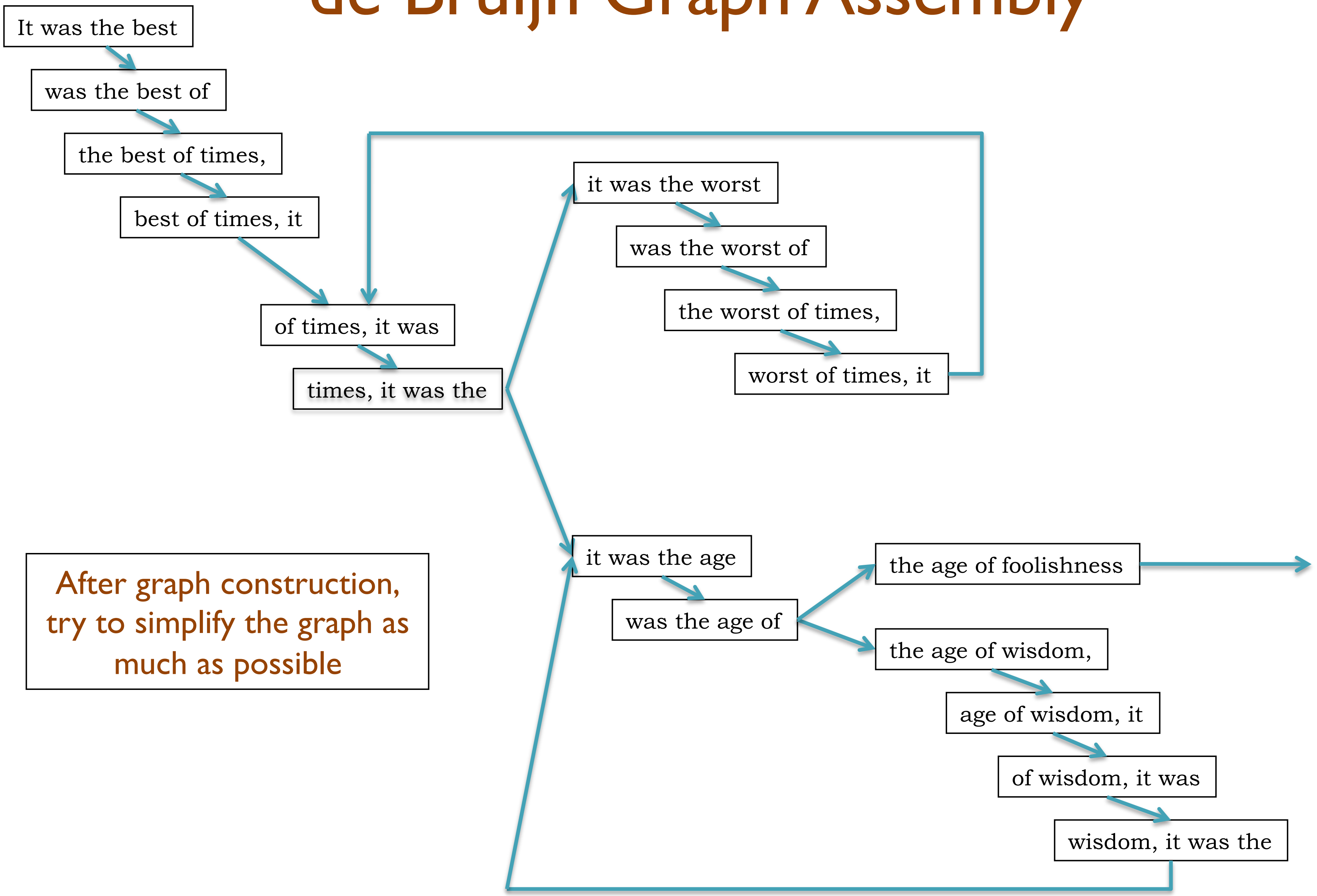
Directed Edge

It was the best → was the best of

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

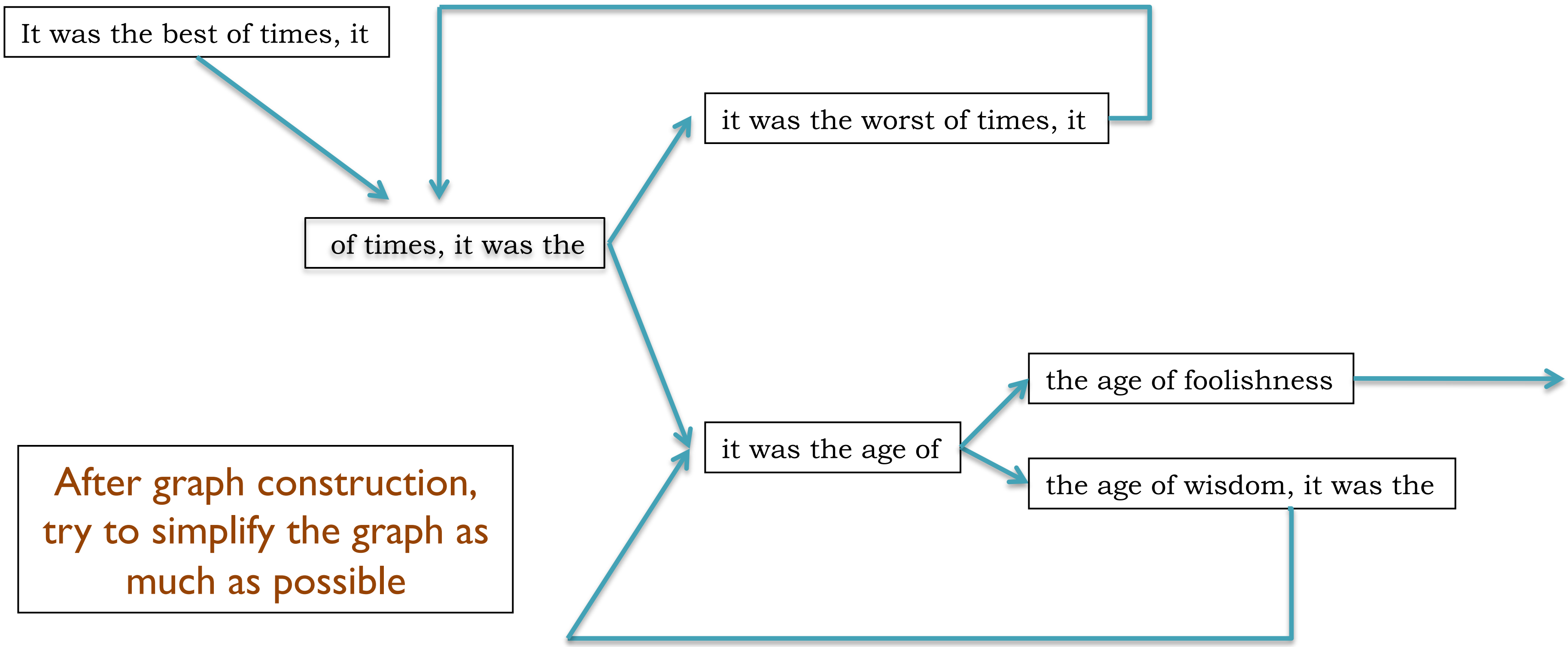
de Bruijn, 1946
Idury and Waterman, 1995
Pevzner, Tang, Waterman, 2001

de Bruijn Graph Assembly



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

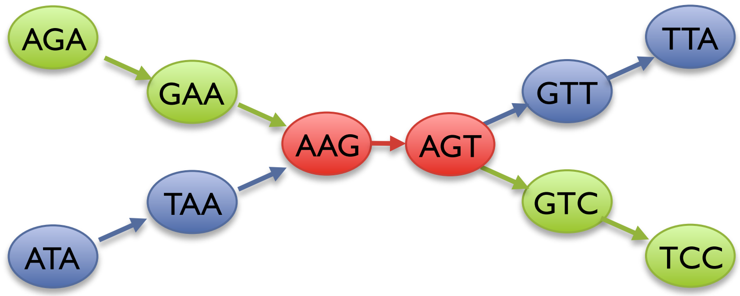
de Bruijn Graph Assembly


It was the best of times, it
of times, it was the
it was the worst of times, it
it was the age of
the age of foolishness
the age of wisdom, it was the

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

Two Paradigms for Assembly

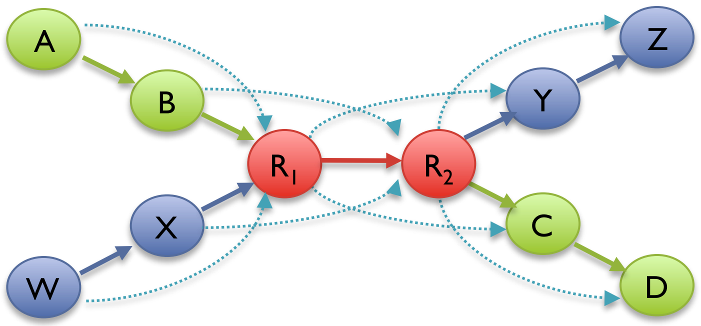
de Bruijn Graph



Short read assemblers

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

Overlap Graph

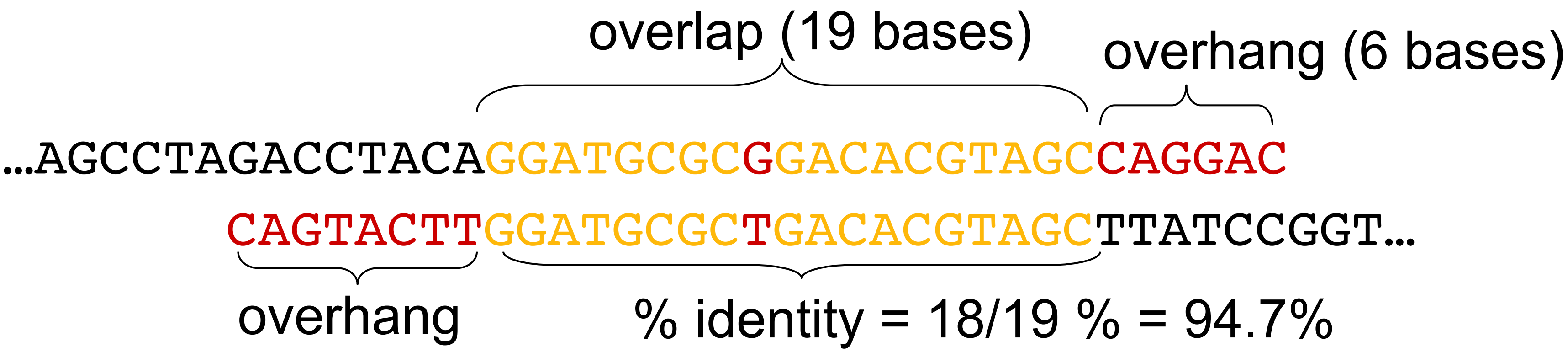


Long read assemblers

- 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.

Overlap between two sequences



overlap (19 bases) overhang (6 bases)

```
...AGCCTAGACCTACAGGATGCGCGGACACGTAGCCAGGAC
         CAGTACTTGGATGCGCTGACACGTAGCTTATCCGGT...
```

overhang % identity = 18/19 % = 94.7%

overlap - region of similarity between regions
overhang - un-aligned ends of the sequences

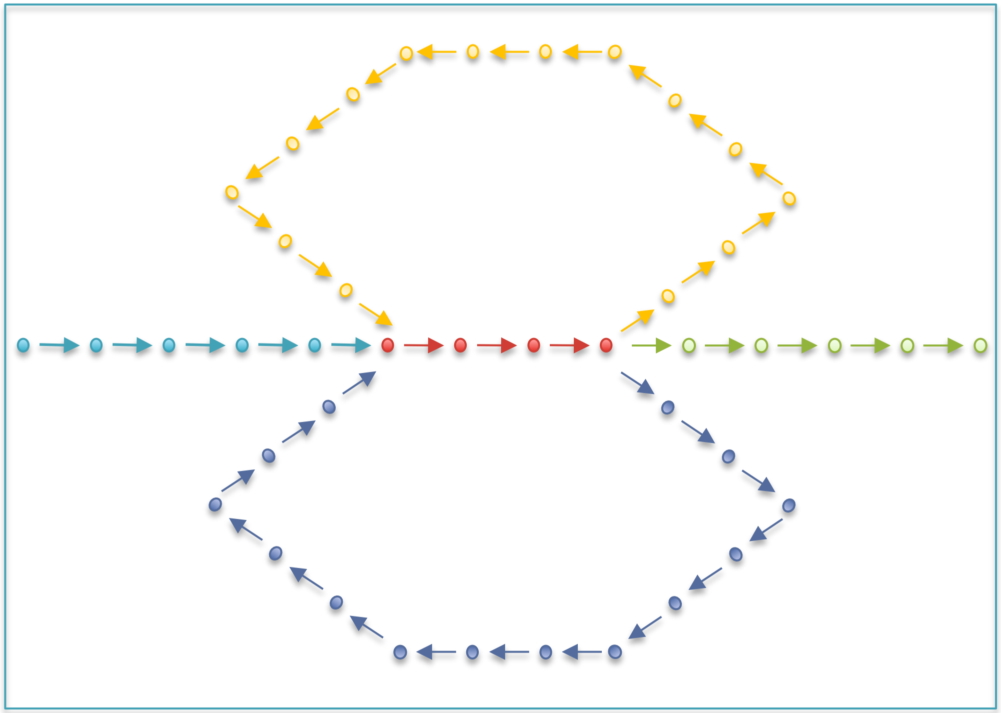
The assembler screens merges based on:

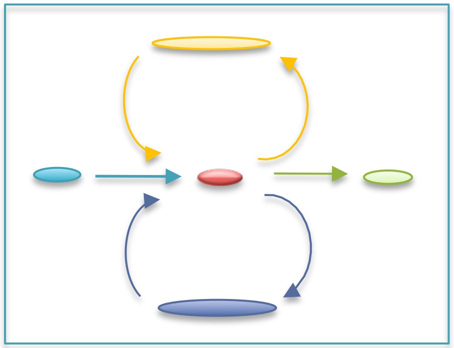
- length of overlap
- % identity in overlap region
- maximum overhang size.

[How do we compute the overlap?]

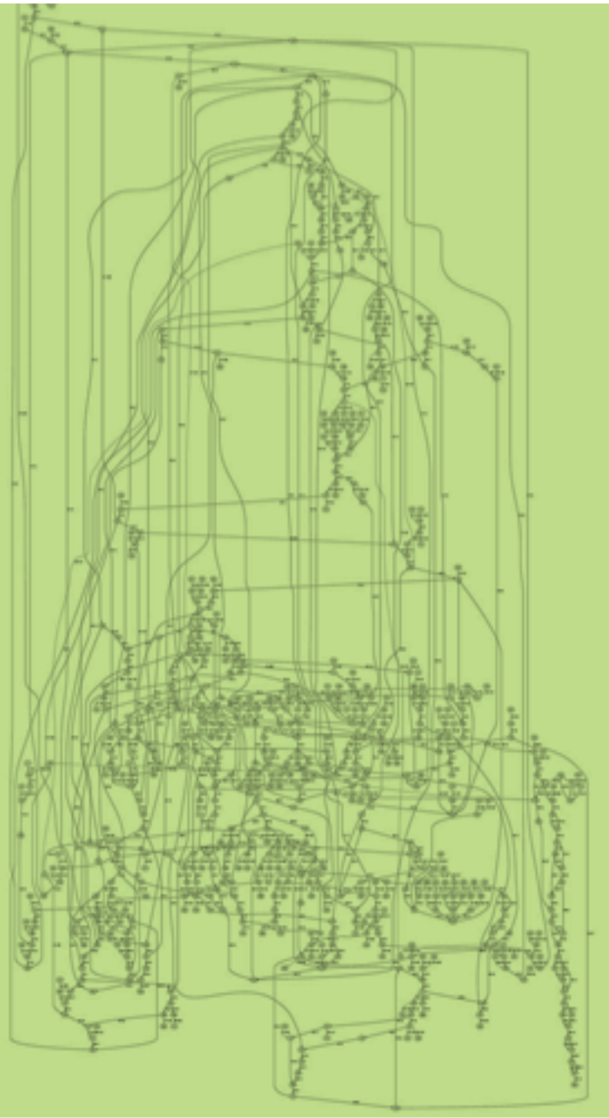
Unitigging / Unipathing

- 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

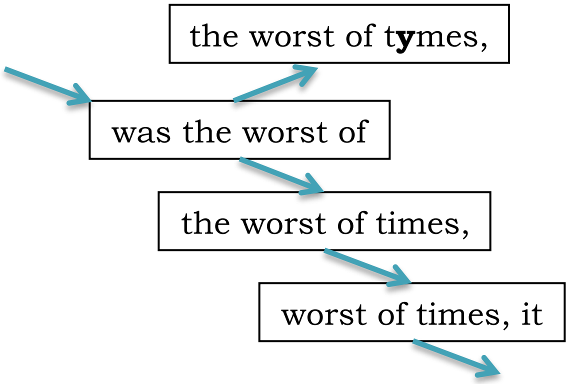
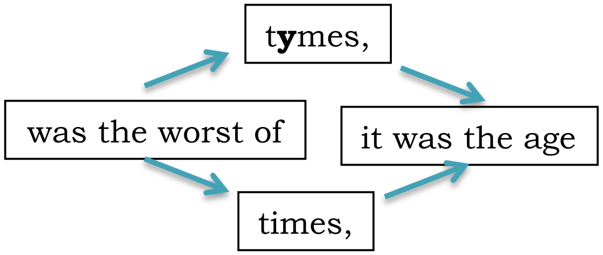




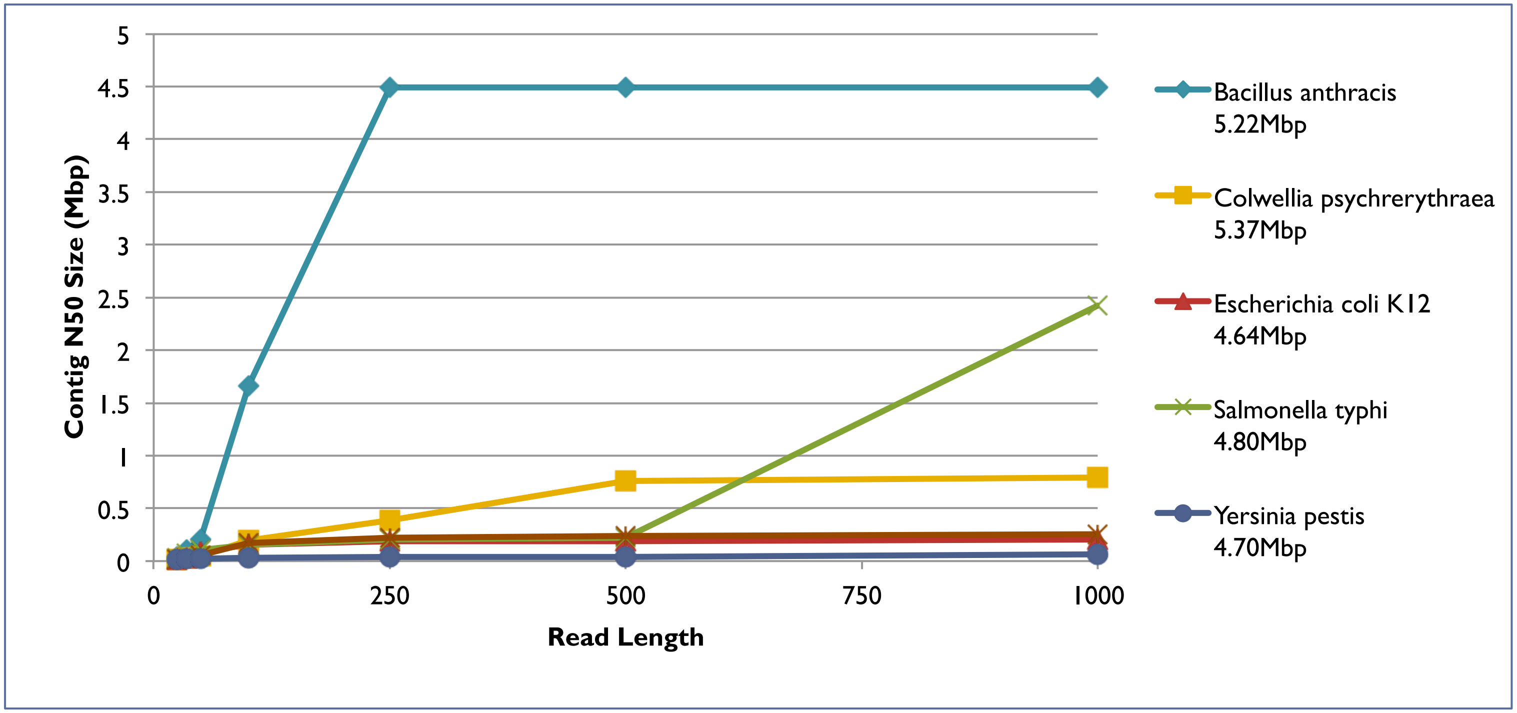
Errors in the graph



(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



- 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

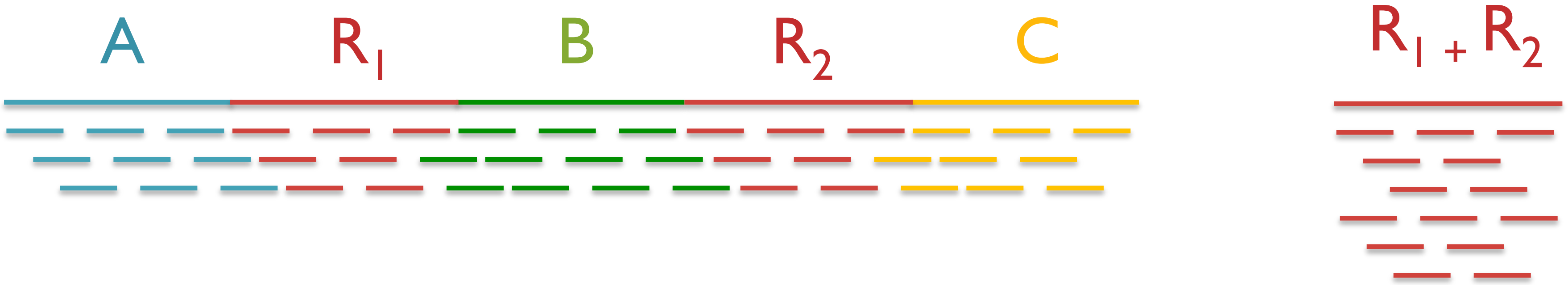
Assembly Complexity of Prokaryotic Genomes using Short Reads.
Kingsford C, Schatz MC, Pop M (2010) *BMC Bioinformatics*. 11:21.

Repetitive regions

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

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

Repeats and Coverage Statistics

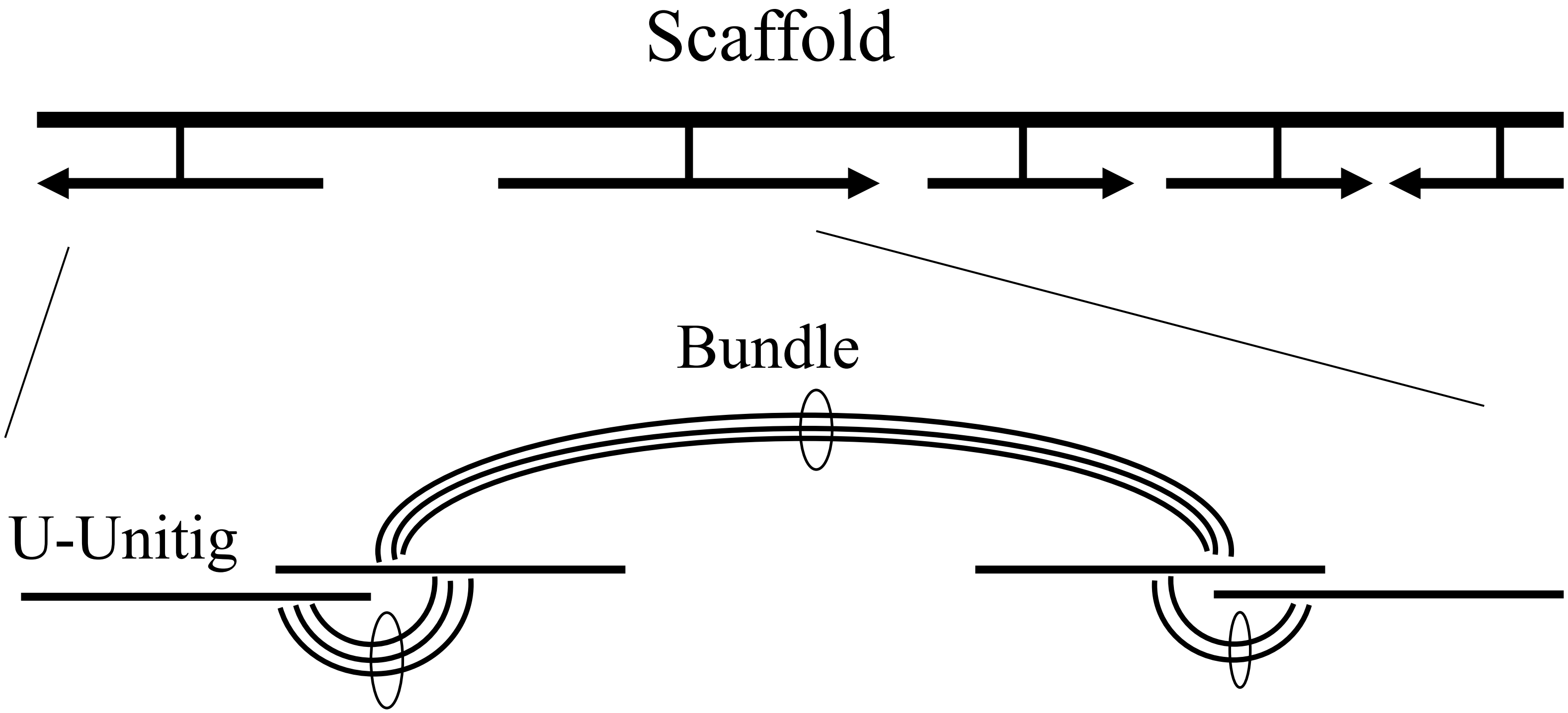


- If *n* reads are a uniform random sample of the genome of length *G*, we expect *k*=*n Δ* /*G* reads to start in a region of length *Δ* .
  - If we see many more reads than k (if the arrival rate is > A) , it is likely to be a collapsed repeat
  - Requires an accurate genome size estimate

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

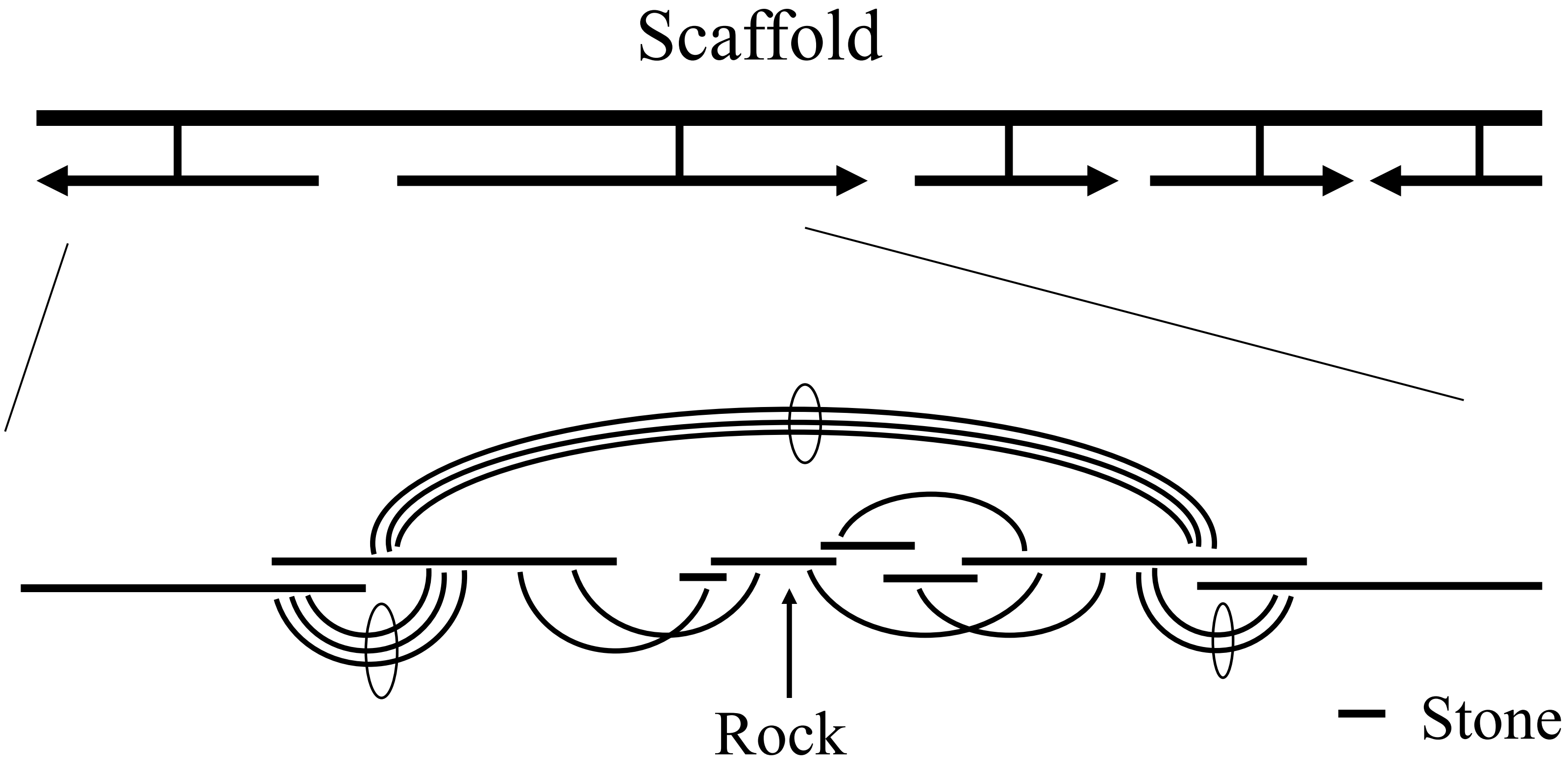
$$A(\Delta,k)=\ln\left(\frac{\Pr(1-copy)}{\Pr(2-copy)}\right)=\ln\left(\frac{\dfrac{(\Delta n/G)^{k}}{k!}e^{\frac{-\Delta n}{G}}}{\dfrac{(2\Delta n/G)^{k}}{k!}e^{\frac{-2\Delta n}{G}}}\right)=\frac{n\Delta}{G}-k\ln 2$$

Initial Scaffolding



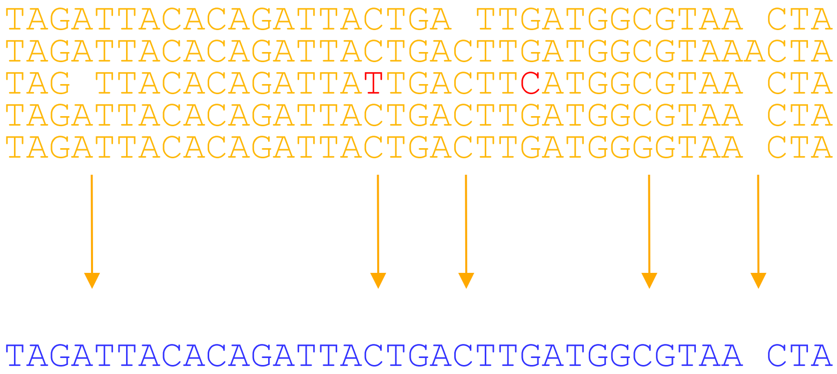
Create a initial scaffold of unique unitigs (U-Unitigs) whose A-stat > 5. Also recruit borderline unitigs whose A-stat is > 2 and have consistent mates with the U-Unitigs.

Repeat Resolution



Place rocks (A-stat > 0 with multiple consistent mates), and stones (single mate and overlap path with placed objects) into the gaps. Pebbles, unitigs lackings mates, are no longer incorporated regardless of overlap qualities.

Derive Consensus Sequence



```
TAGATTACACAGATTACTGA TTGATGGCGTAA CTA
TAGATTACACAGATTACTGACTTGATGGCGTAAACTA
TAG TTACACAGATTATTGACTTCATGGCGTAA CTA
TAGATTACACAGATTACTGACTTGATGGCGTAA CTA
TAGATTACACAGATTACTGACTTGATGGGGTAA CTA

TAGATTACACAGATTACTGACTTGATGGCGTAA CTA
```

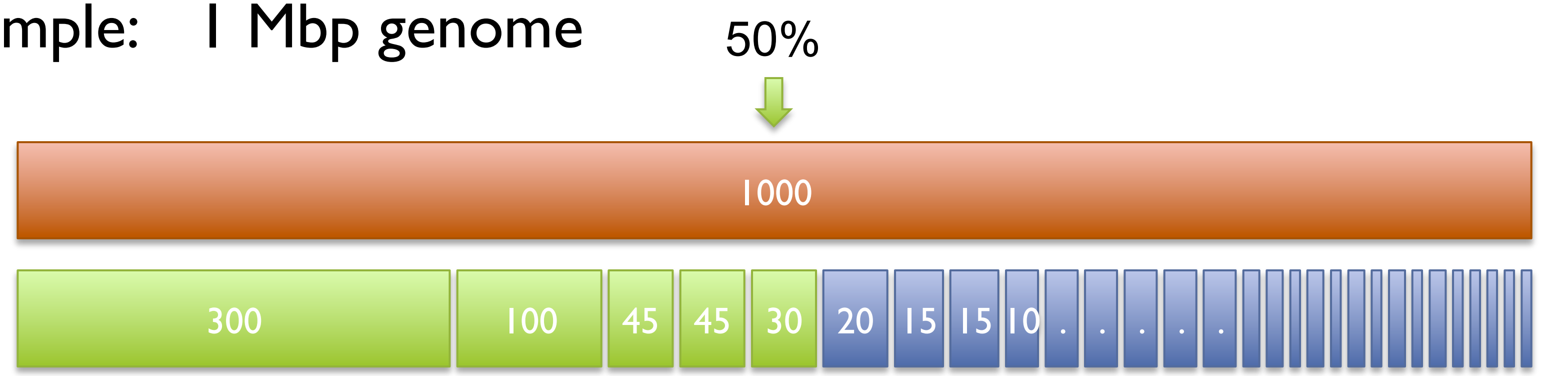
Derive multiple alignment from pairwise read alignments

Derive each consensus base by weighted voting

N50 size

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

Example: 1 Mbp genome



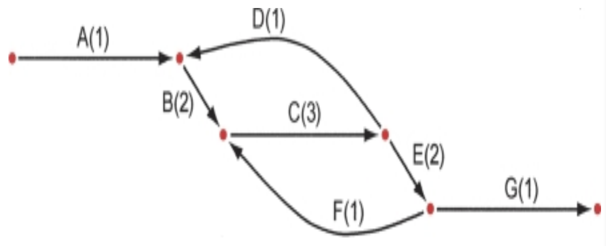
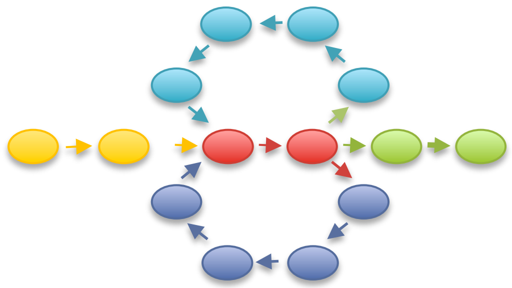
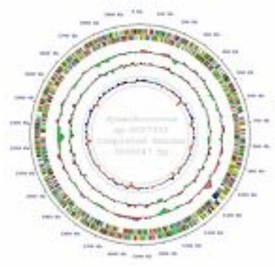
N50 size = 30 kbp

(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

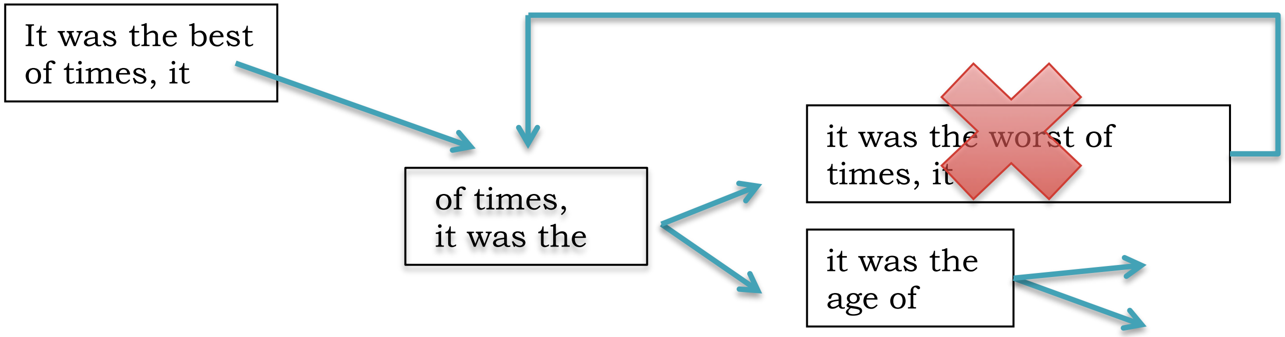
ALLPATHS-LG	SOAPdenovo	Celera Assembler
		
Broad's assembler (Gnerre et al. 2011)	BGI's assembler (Li et al. 2010)	JCVI's assembler (Miller et al. 2008)
De bruijn graph Short + PacBio (patching)	De bruijn graph Short reads	Overlap graph Medium + Long reads
Easy to run if you have compatible libraries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

Assembly Validation

Automatically scan an assembly to locate misassembly signatures for further analysis and correction

Assembly-validation pipeline

1. Evaluate Mate Pairs & Libraries
2. Evaluate Read Alignments
3. Evaluate Read Breakpoints
4. Analyze Depth of Coverage



It was the best of times, it

of times, it was the

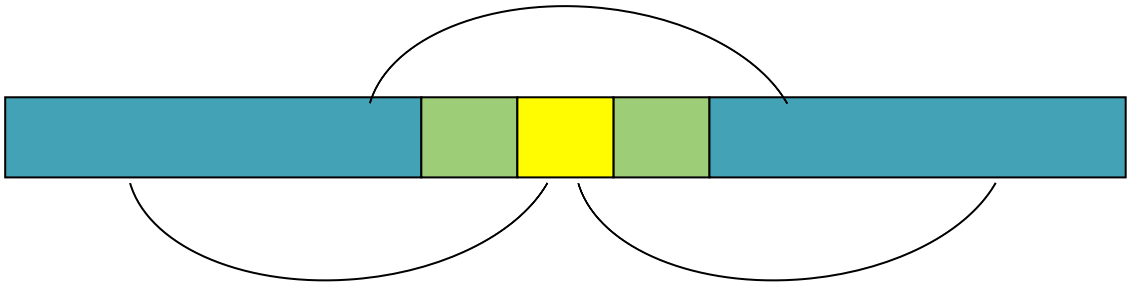
it was the worst of times, it

it was the age of

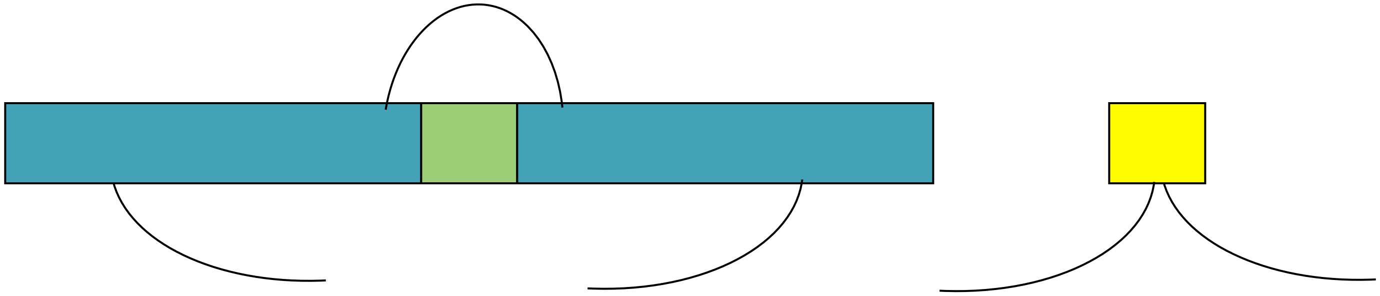
Genome Assembly forensics: finding the elusive mis-assembly.
Phillippy, AM, Schatz, MC, Pop, M. (2008) *Genome Biology* 9:R55.

Mate-Happiness: asmQC

- Excision: Skip reads between flanking repeats
  - Truth



  - Misassembly: Compressed Mates, Missing Mates

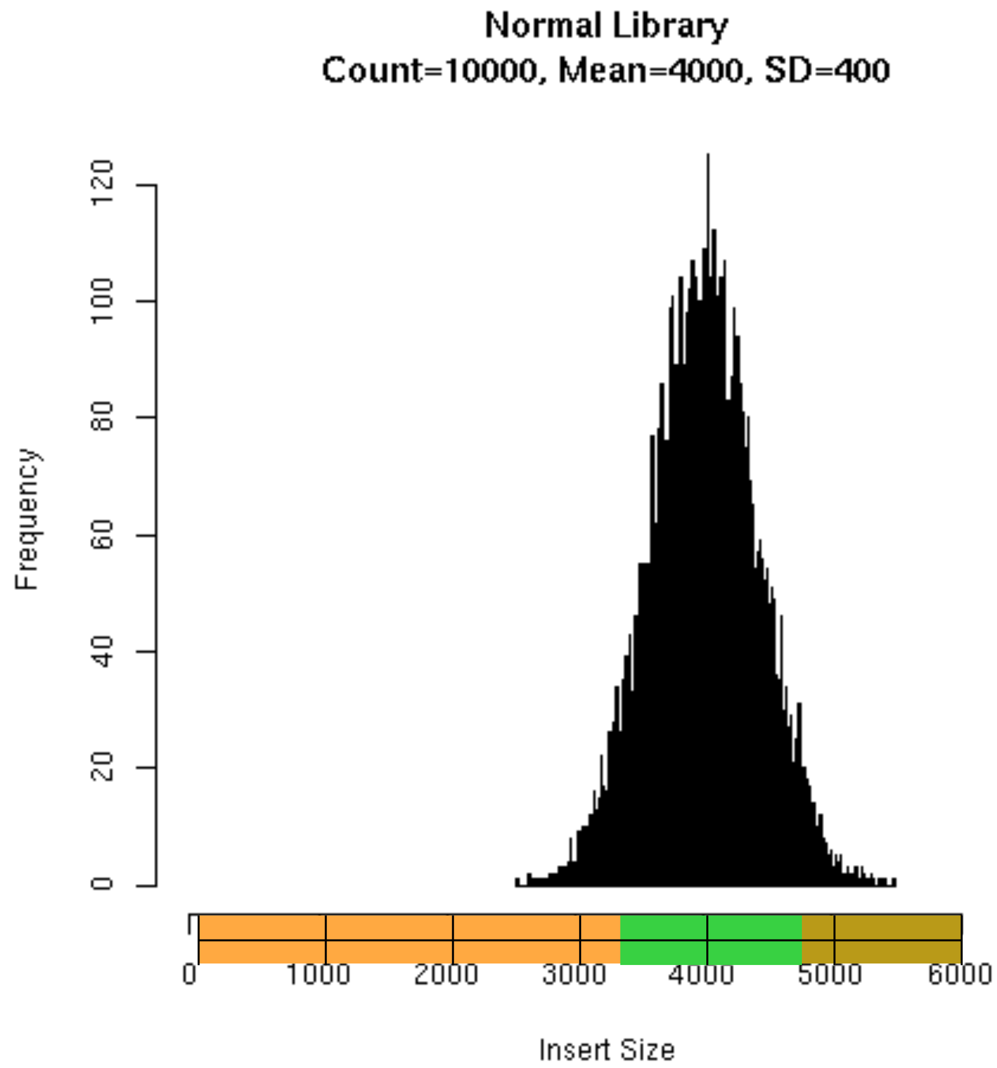


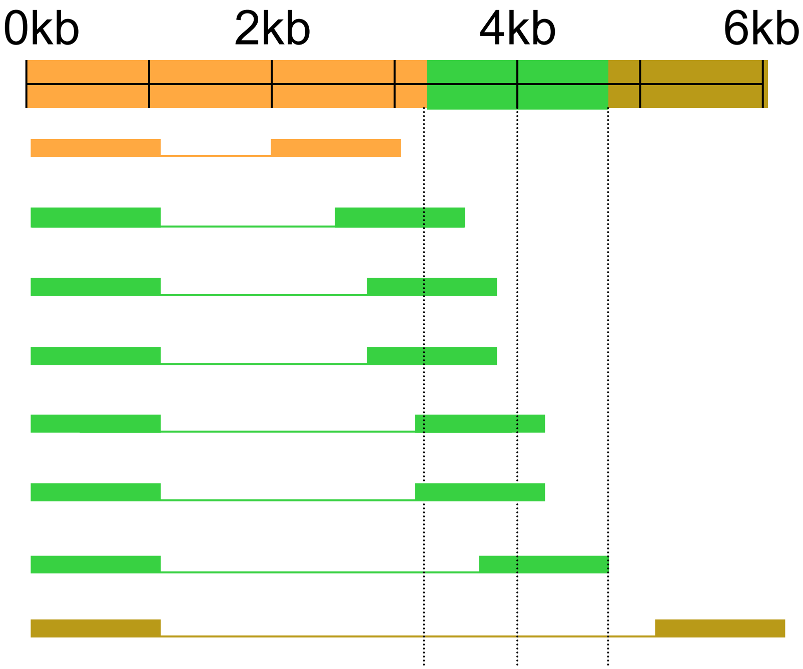
C/E Statistic

- The presence of individual compressed or expanded mates is rare but expected.
- Do the inserts spanning a given position differ from the rest of the library?
  - Flag large differences as potential misassemblies
  - Even if each individual mate is "happy"
- Compute the statistic at all positions
  - (Local Mean – Global Mean) / Scaling Factor
- Introduced by Jim Yorke's group at UMD

Forensics

Sampling the Genome





8 inserts: 3kb-6kb

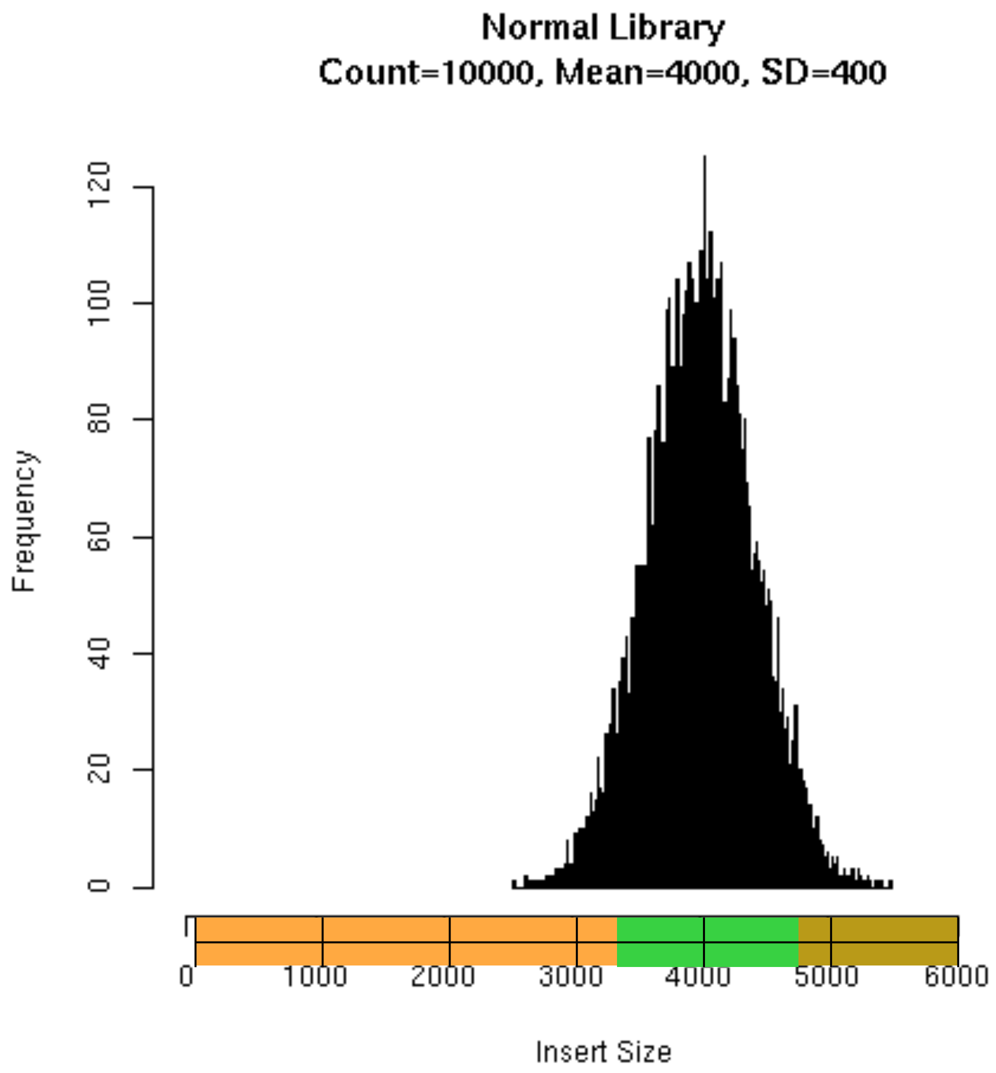
Local Mean: 4048

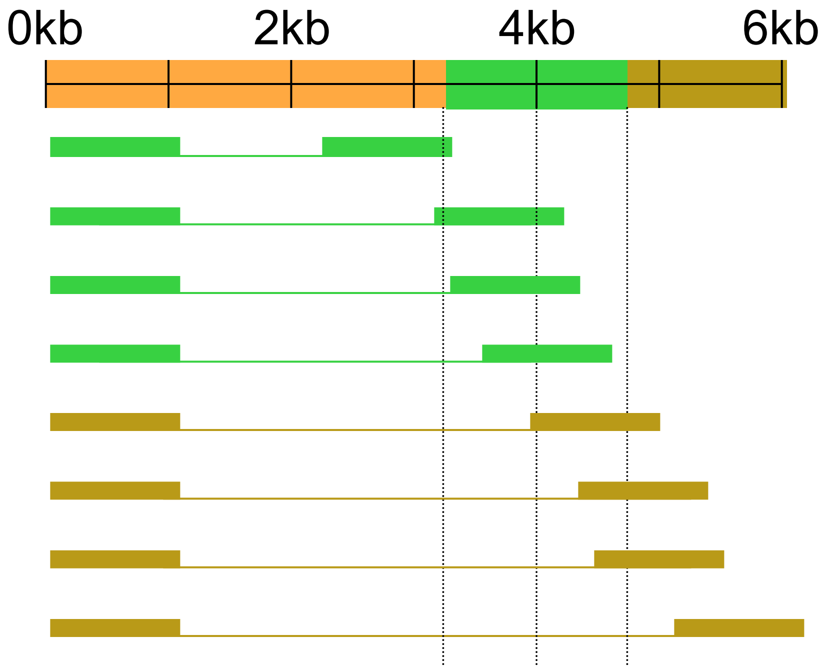
C/E Stat: $\frac{(4048-4000)}{(400 / \sqrt{8})} = +0.33$

Near 0 indicates overall happiness

Forensics

C/E-Statistic: Expansion





8 inserts: 3.2kb-6kb

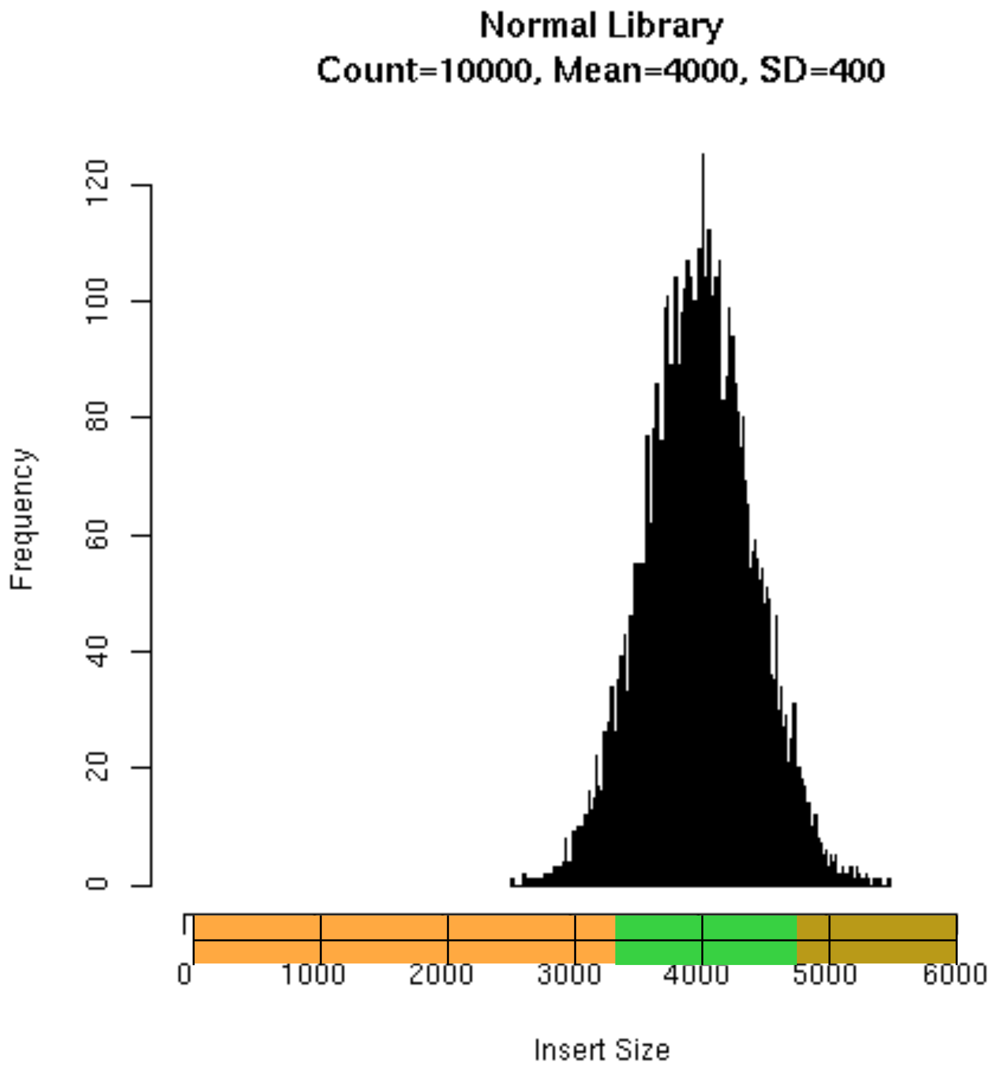
Local Mean: 4461

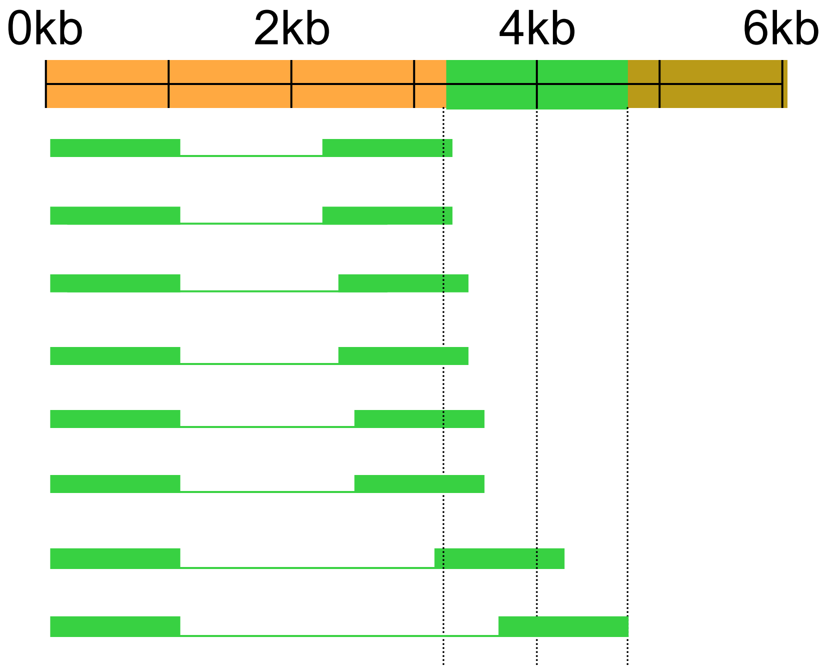
C/E Stat: $\dfrac{(4461-4000)}{(400 / \sqrt{8})}$ = +3.26

C/E Stat ≥ 3.0 indicates Expansion

Forensics

C/E-Statistic: Compression





8 inserts: 3.2 kb-4.8kb

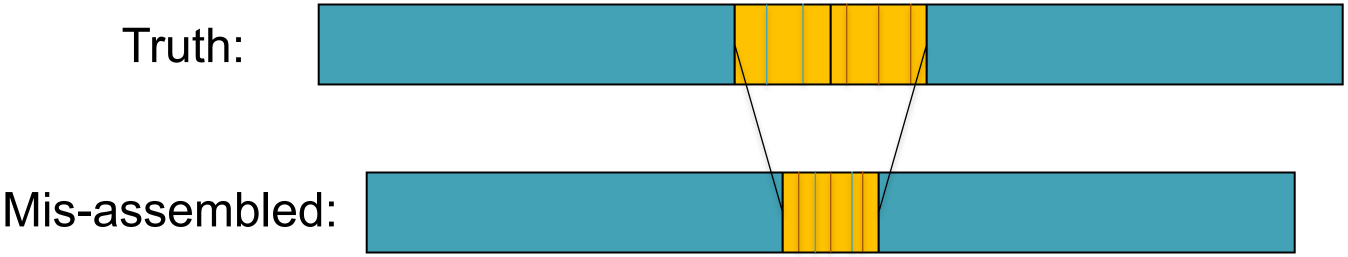
Local Mean: 3488

C/E Stat: $\frac{(3488-4000)}{(400 / \sqrt{8})}$ = -3.62

C/E Stat ≤ -3.0 indicates Compression

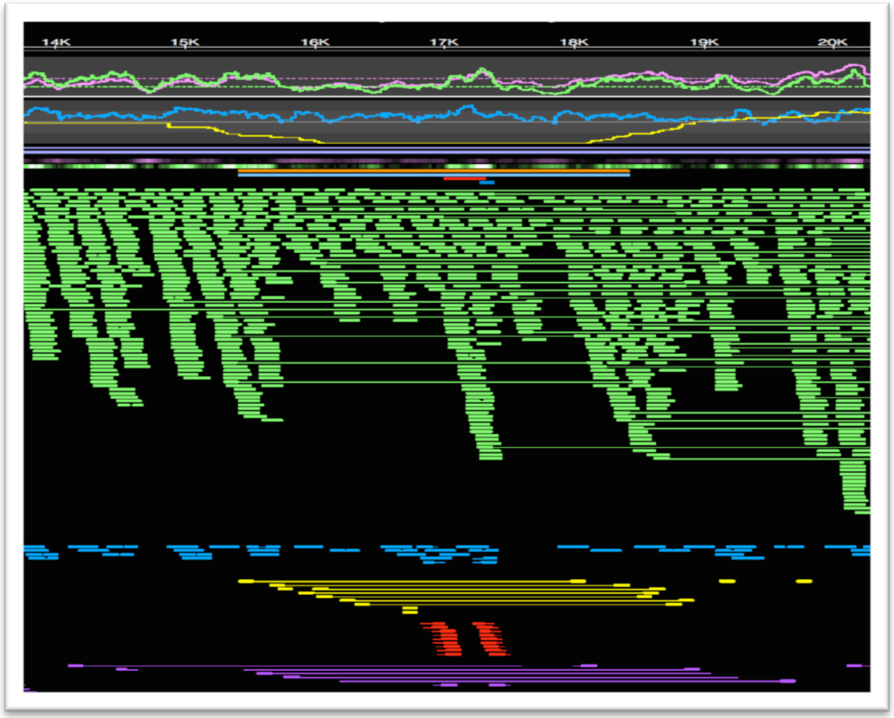
Forensics

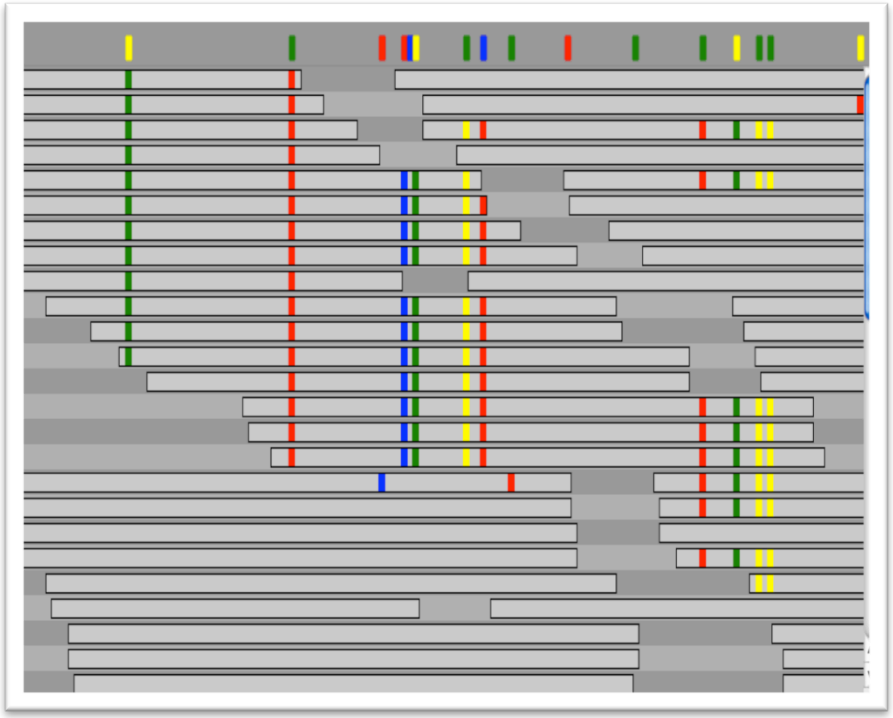
Assembly Forensics



Truth:

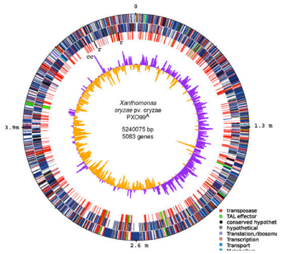
Mis-assembled:





Hawkeye & AMOS:Visualizing and assessing the quality of genome assemblies
Schatz, M.C. *et al.* (2011) *Briefings in Bioinformatics.* In Press.

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

Thank You

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

@mike_schatz