Graphs and Genomes

Michael Schatz

Bioinformatics Lecture 3
Quantitative Biology 2014

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

```
ABC...XY-
DEF....UV
```

α

Left

```
ABC....XY
DEF...UV-
```

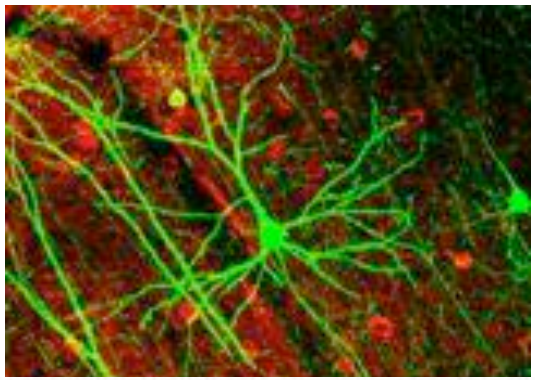
β

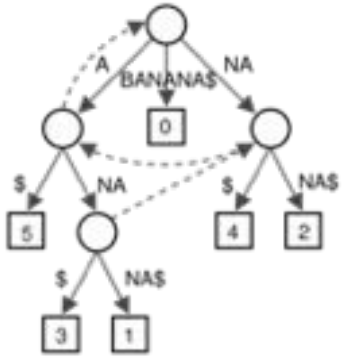
Diagonal

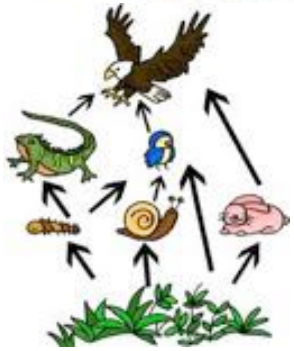
```
ABC...XY
DEF...UV
```

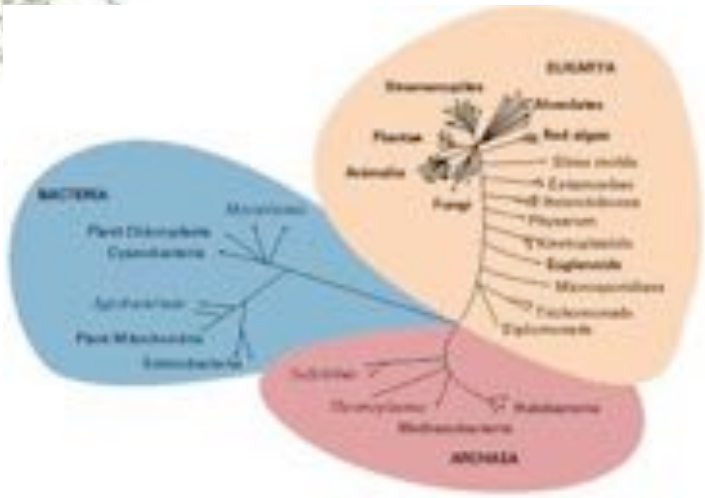
γ

Biological Networks

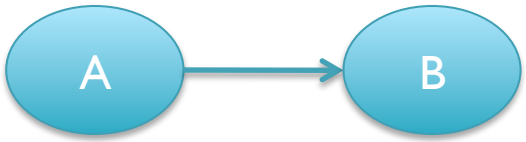






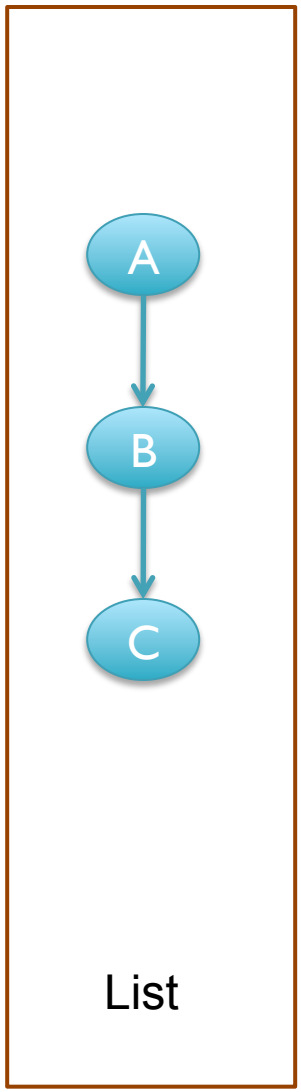


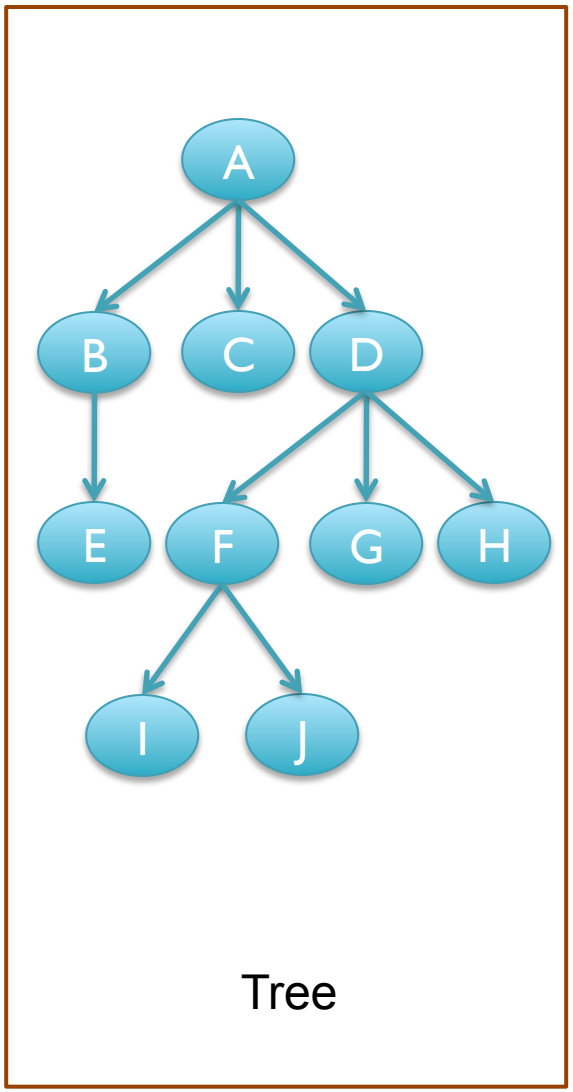
Graphs

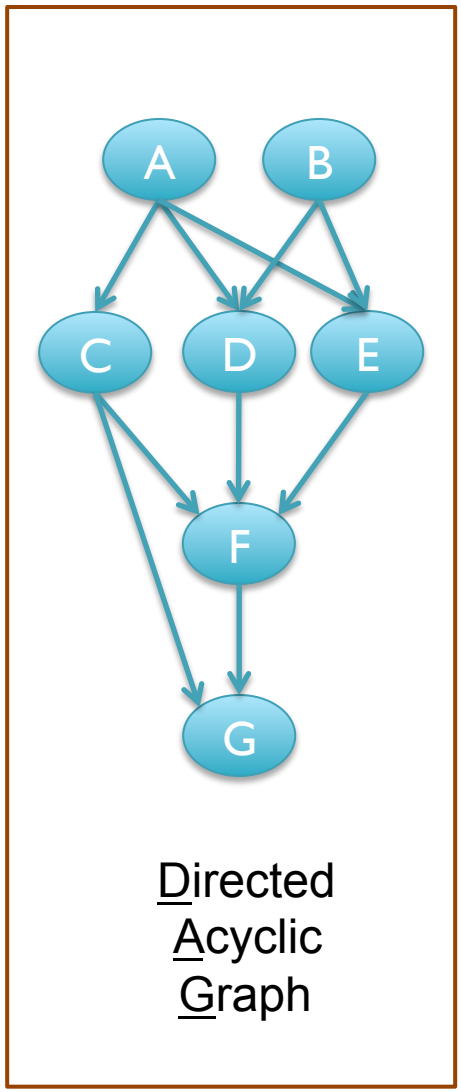


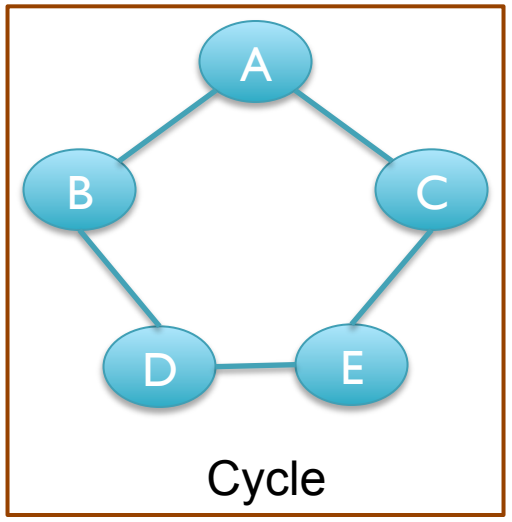
- Nodes
  - People, Proteins, Genes, Neurons, Sequences, Numbers, …
- Edges
  - A is connected to B
  - A is related to B
  - A regulates B
  - A precedes B
  - A interacts with B
  - A activates B
  - …

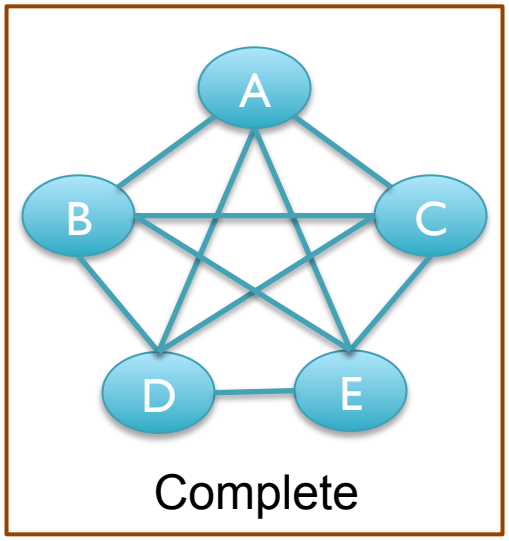
Graph Types


A
B
C
List

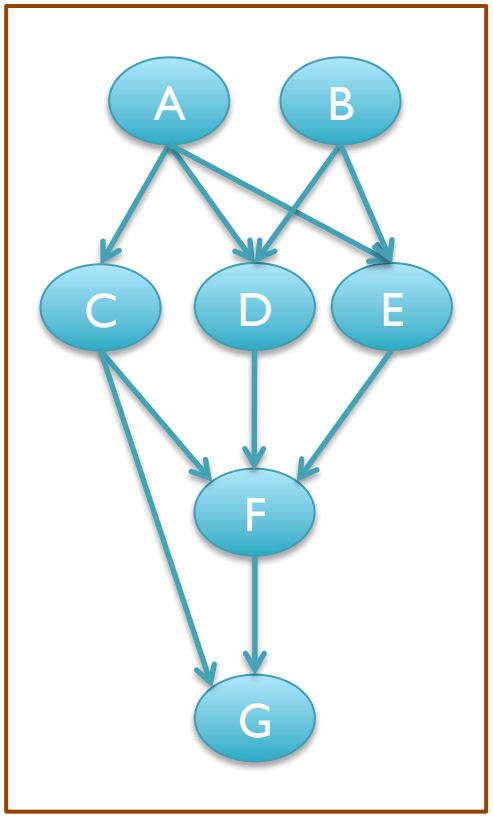

A
B
C
D
E
F
G
H
I
J
Tree


A
B
C
D
E
F
G
Directed
Acyclic
Graph


A
B
C
D
E
Cycle


A
B
C
D
E
Complete

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

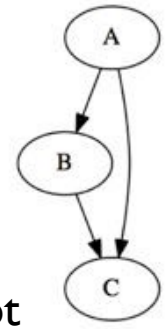
Easy, good if you (mostly) need to iterate through the edges
8 bytes / edge

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

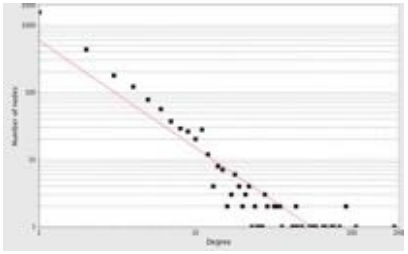
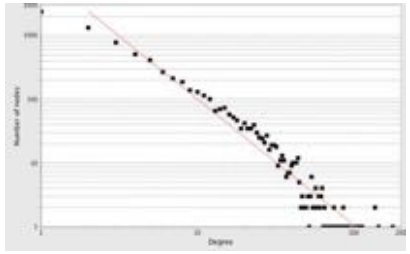
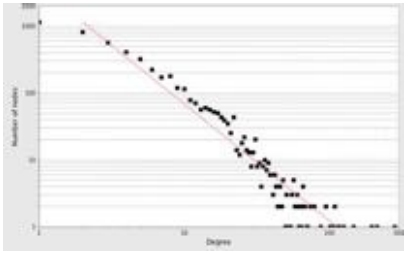
Tools

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
```



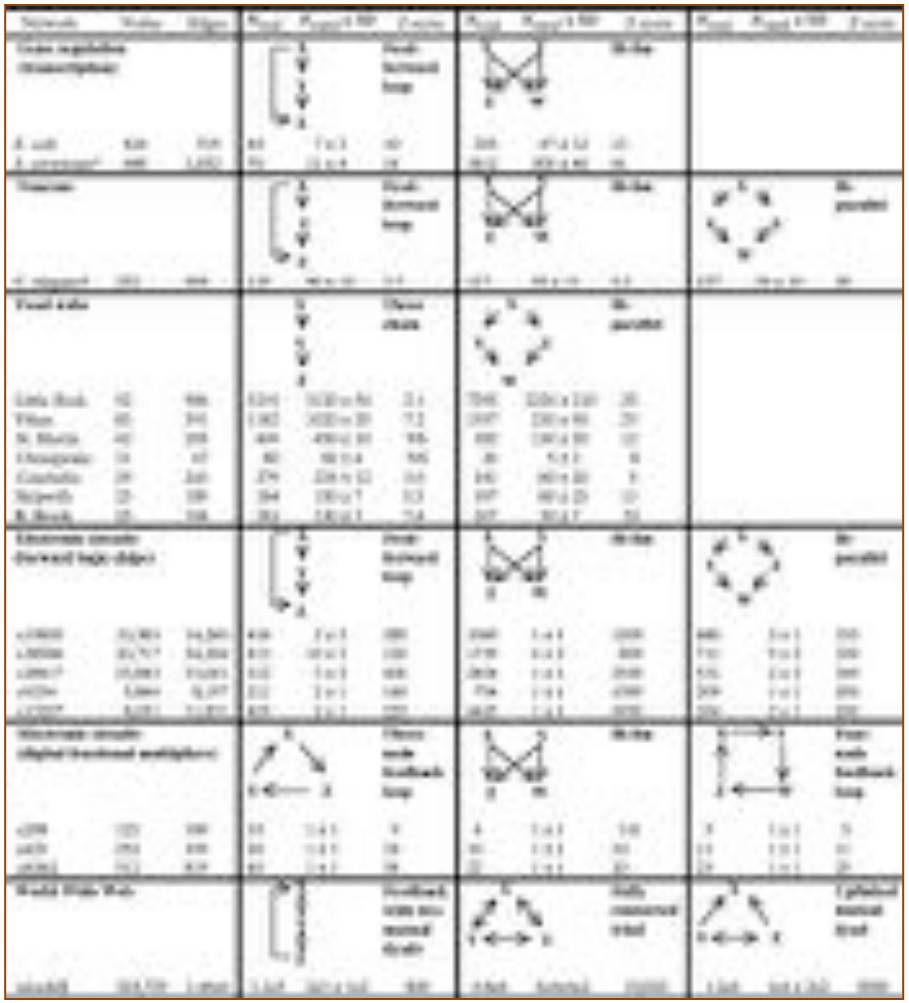
Network Characteristics

	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

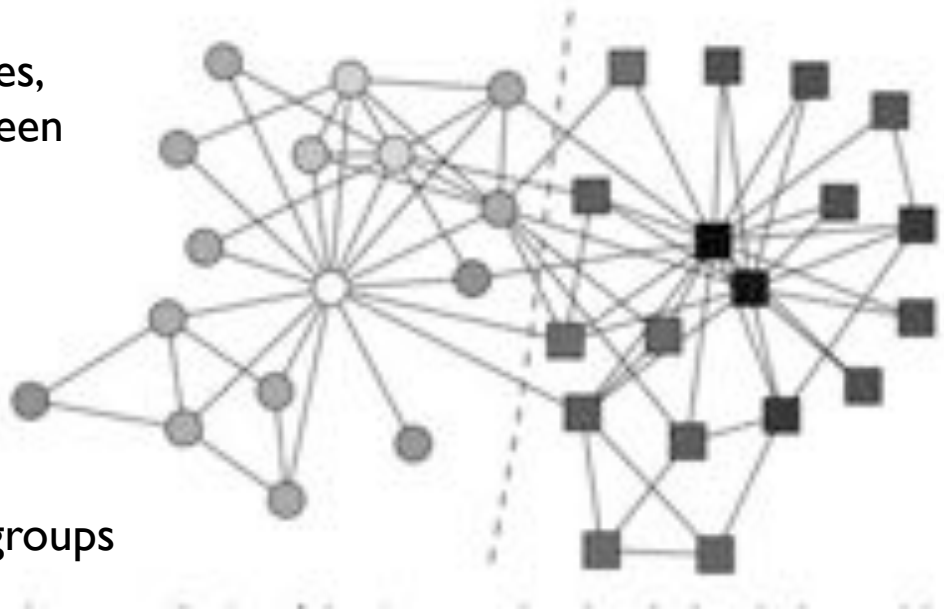
- Network Motif
  - Simple graph of connections
  - Exhaustively enumerate all possible 1, 2, 3, … k node motifs
- Statistical Significance
  - Compare frequency of a particular network motif in a real network as compared to a randomized network
- Certain motifs are "characteristic features" of the network



Network 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

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

Normalization factor

Adjacency matrix

Random Prob. (product of degrees)

Indicates same group

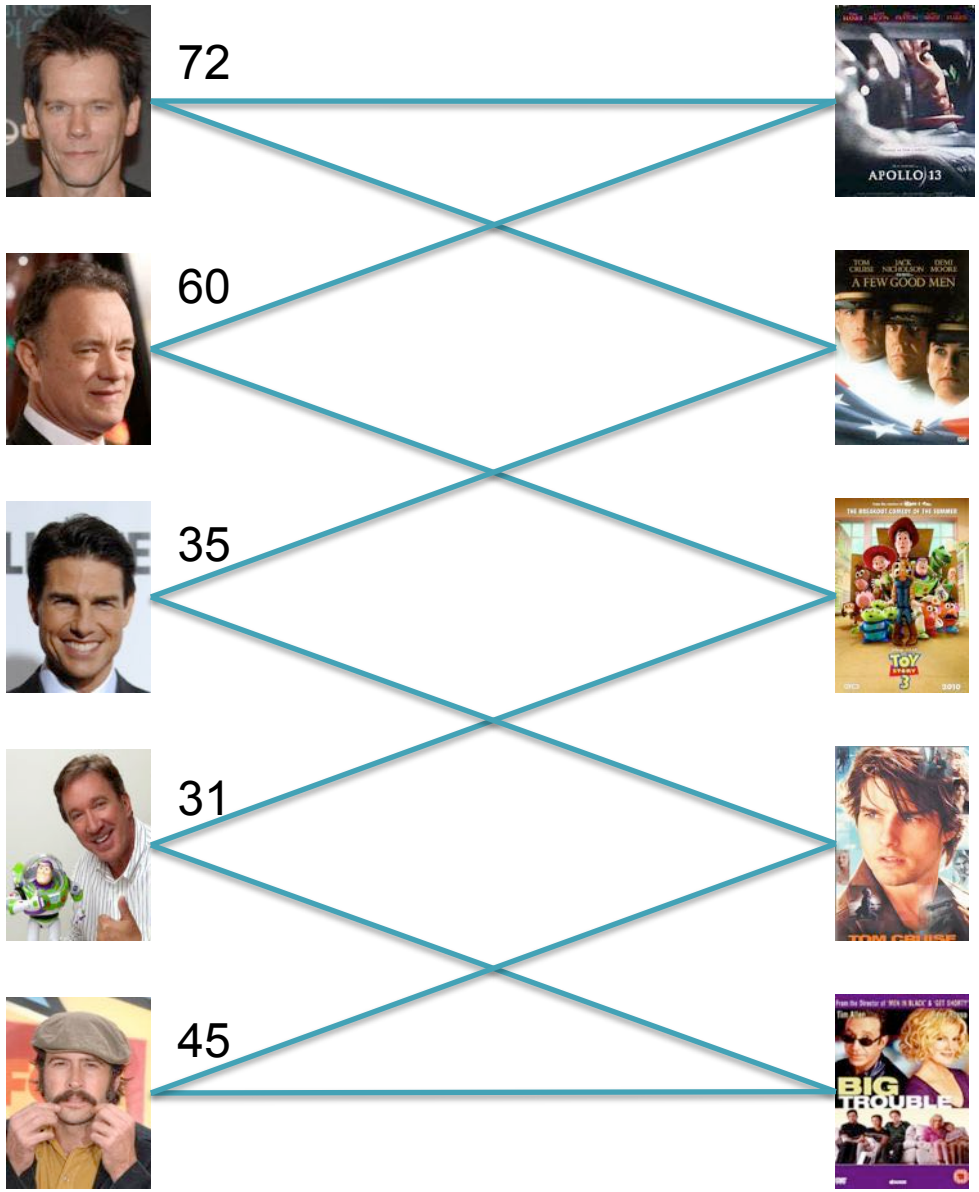
Modularity and community structure in networks.
Newman ME (2006) *PNAS. 103(23) 8577-8582*

Kevin Bacon and Bipartite Graphs

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

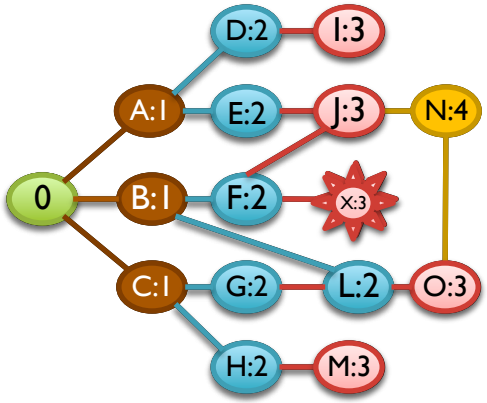
Breadth First Search: 4 hops

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)
```

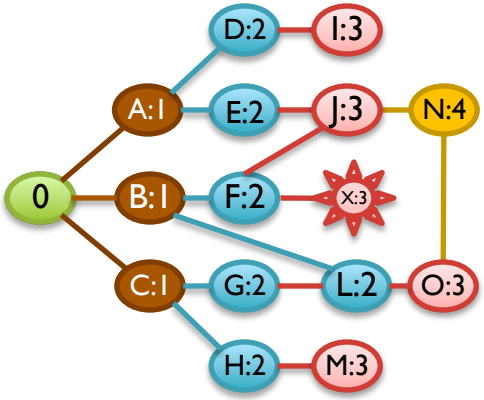
0

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

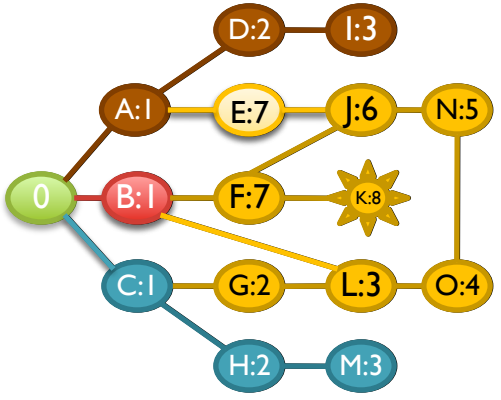
A,B,C

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



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

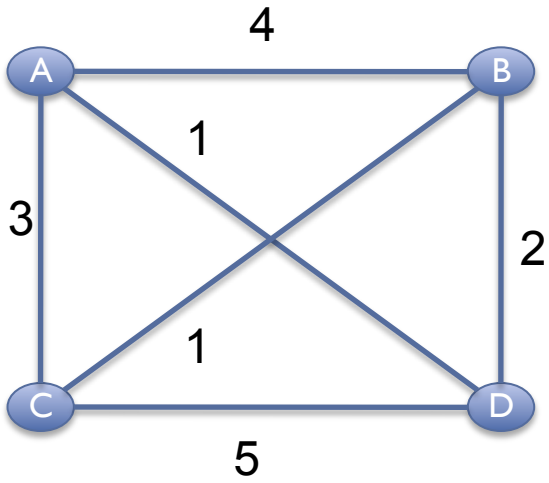
ACDBA: 3+5+2+4 = 14*

ABCDA: 4+1+5+1 = 11

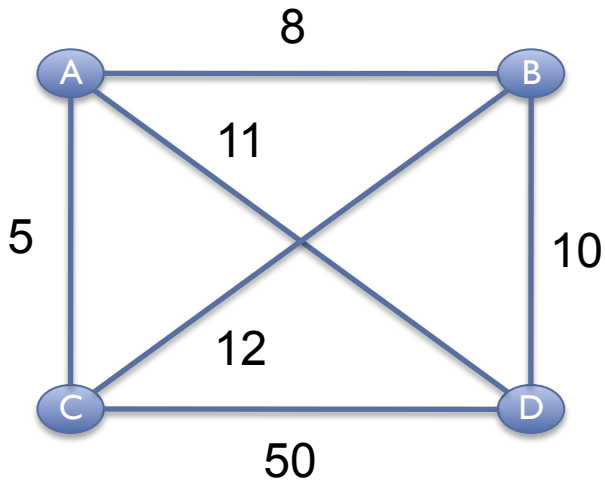
ADCBA: 1+5+1+4 = 11*

ACBDA: 3+1+2+1 = 7

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



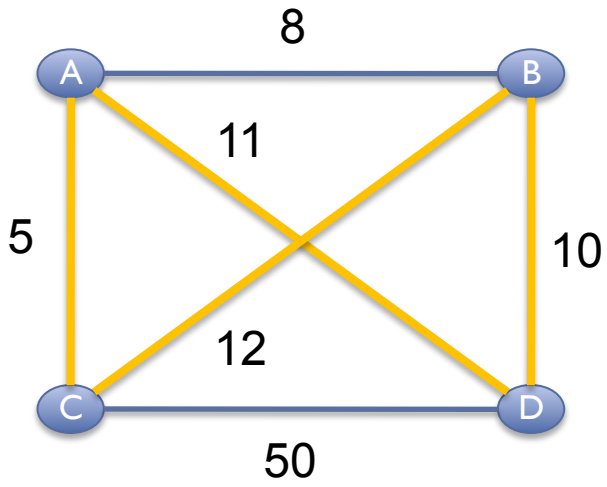
Greedy Search



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

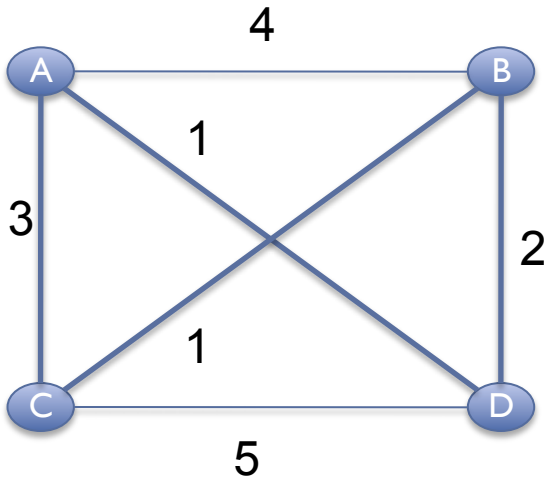
1. Greedy Choice: Local is correct without reconsideration
2. Optimal Substructure: Problem can be split into subproblems

Optimal Greedy: Making change with the fewest number of coins

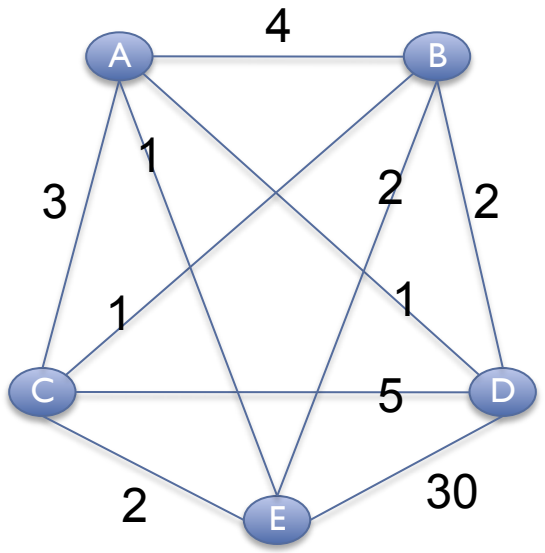
TSP Complexity

- 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?]

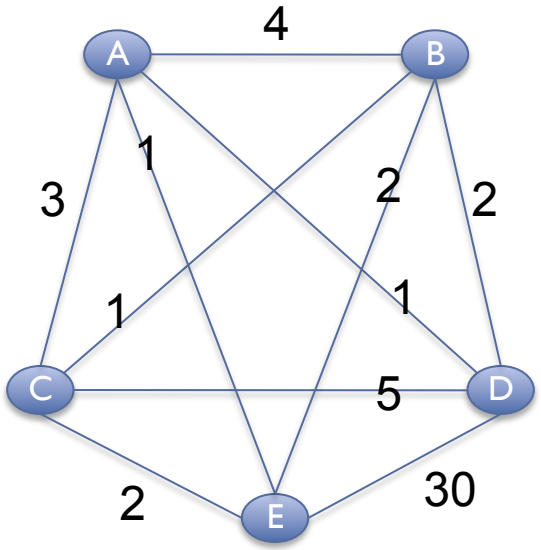


- Extensive searching is the only provably correct algorithm
  - Brute Force: O(n!)
    - ~20 cities max
    - 20! = 2.4 x 10^{18}



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
  - …

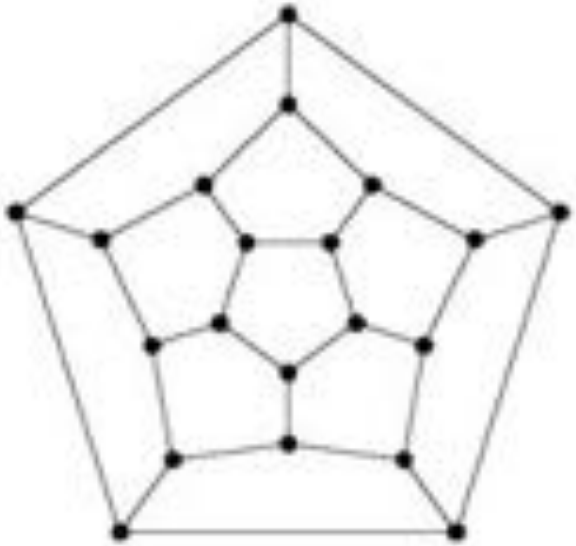


- Performance Heuristic
  - Always gives the optimal answer
  - Doesn't always help performance, but often does
  - Current TSP record holder:
    - 85,900 cities
    - 85900! = 10^{386526}

[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

Break

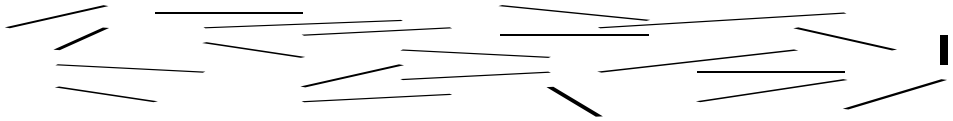

PHASE TWO: INTERPRETATION
I THINK I FOUND A CORNER PIECE.
3 BILLION PIECES
GENOME

What is your genome?

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

Sequencing a Genome

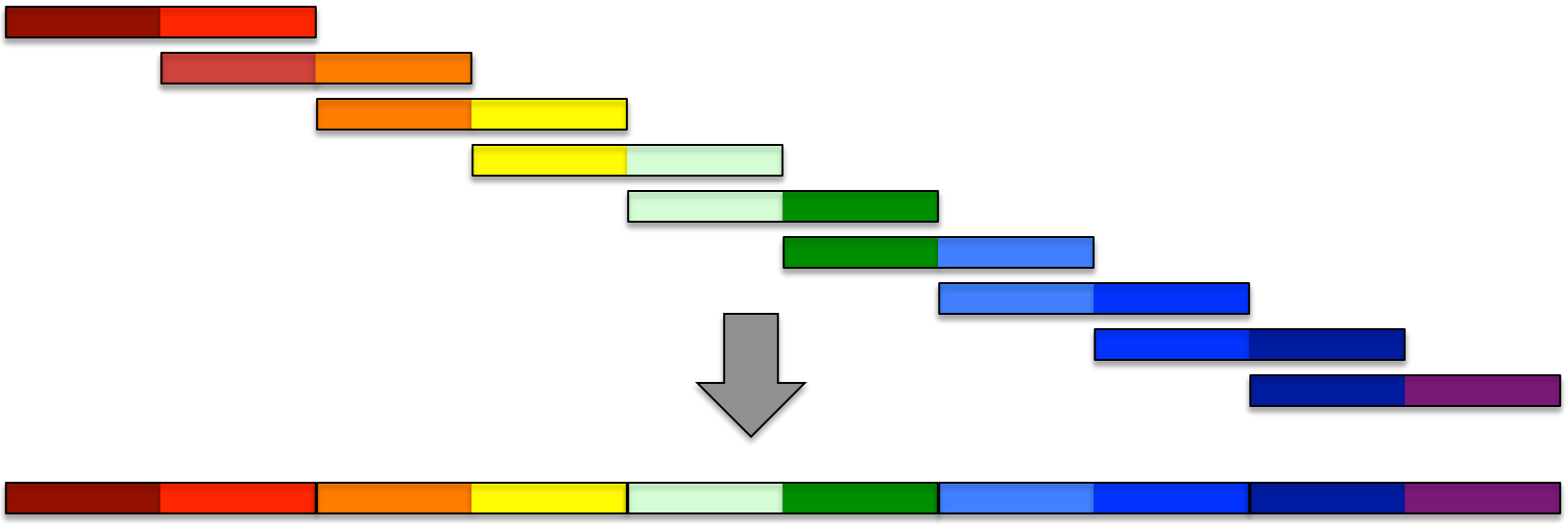
1. Shear & Sequence DNA



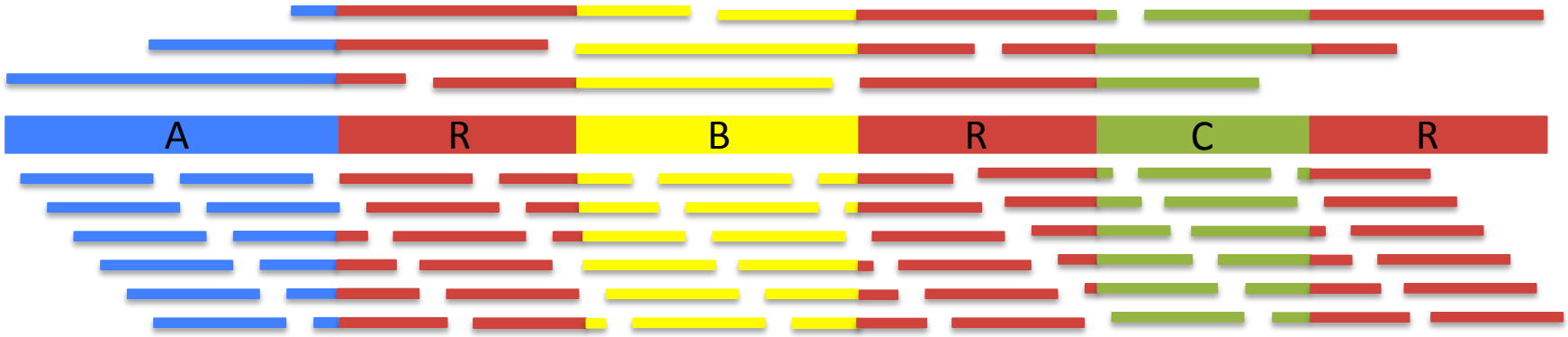
2. Construct assembly graph from overlapping reads

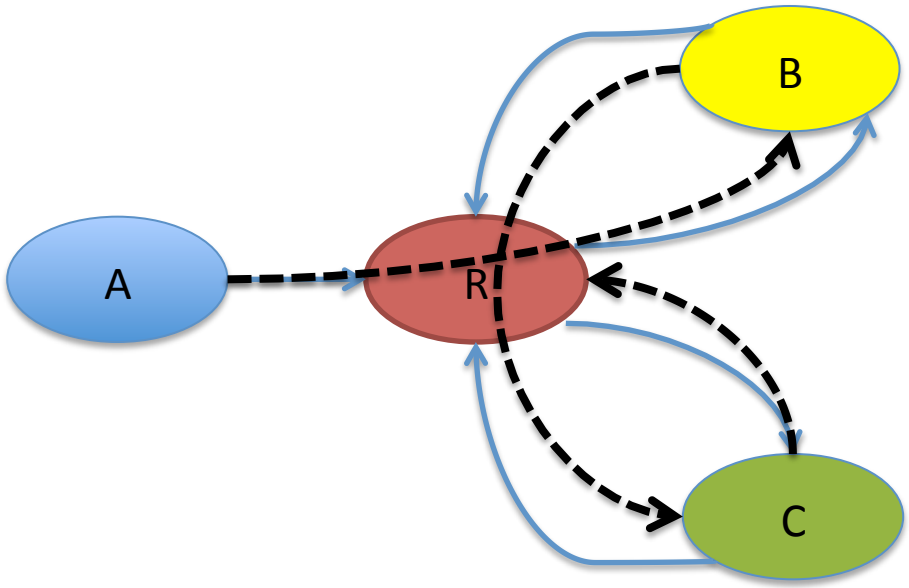
...AGCCTAGGGATGCGCGACACGT
GGATGCGCGACACGTCGCATATCCGGTTTGGTCAACCTCGGACGGAC
CAACCTCGGACGGACCTCAGCGAA...

3. Simplify assembly graph

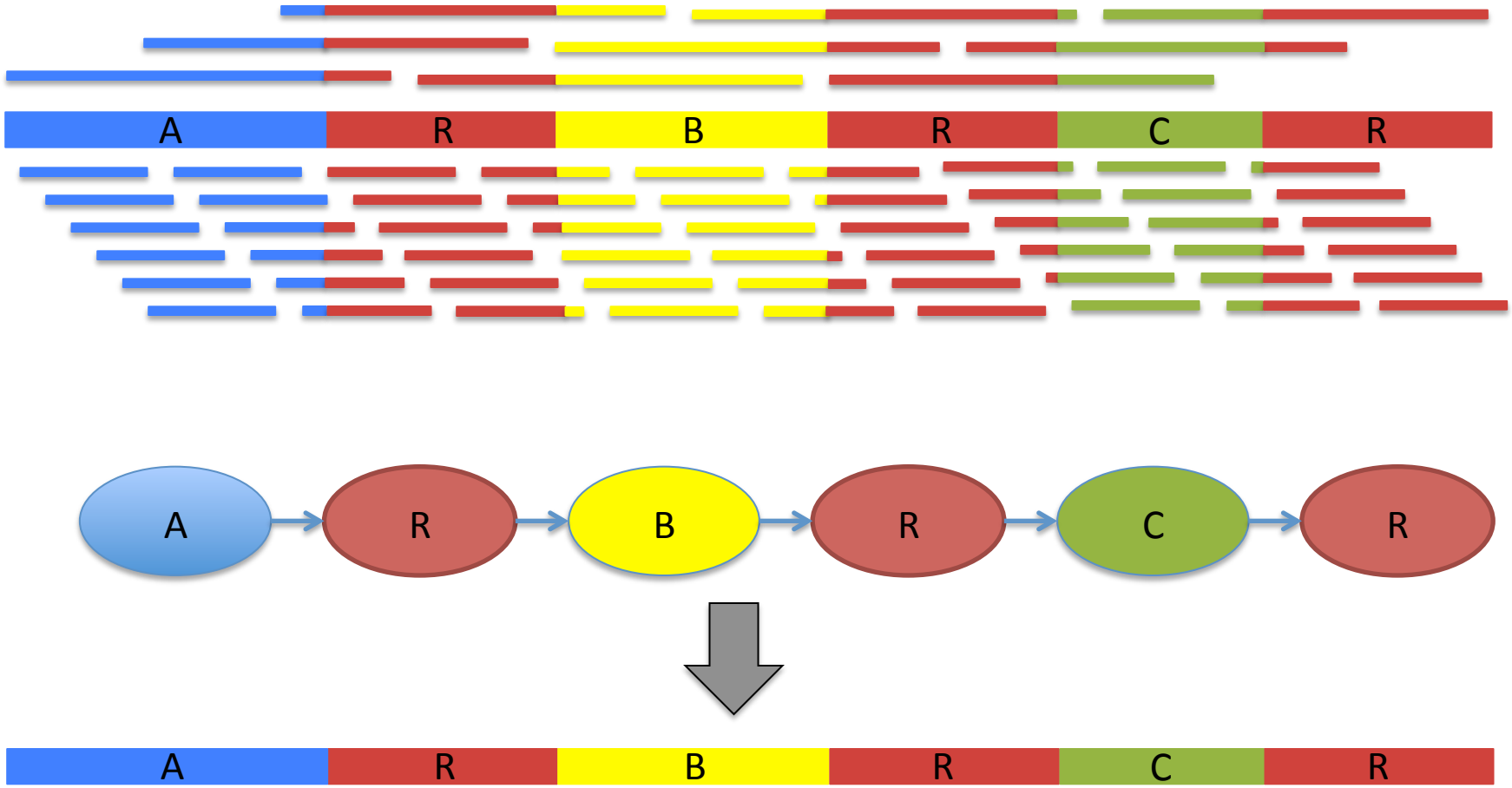


Assembly Complexity





Assembly Complexity



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

Milestones in Genome Assembly


articles

Nucleotide sequence of bacteriophage ΦX174 DNA

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

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

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

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

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

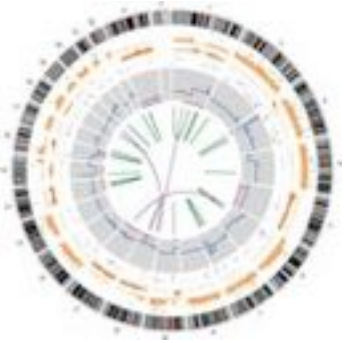
2010. Li *et al.*
1st Large SGS Assembly.
SOAPdenovo 2.2 Gbp

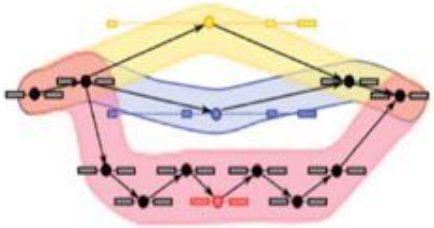
Assembly Applications

- Novel genomes

- Metagenomes

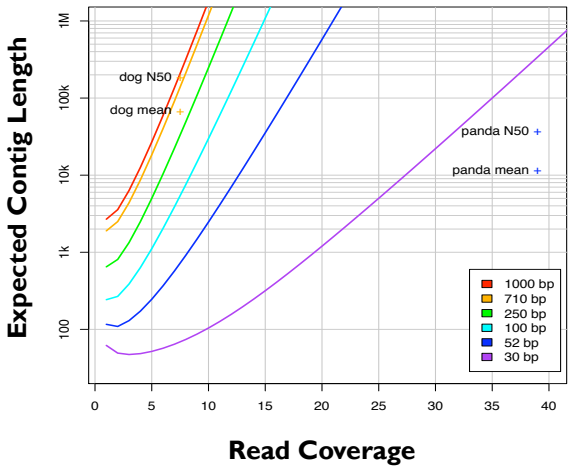
- Sequencing assays
  - Structural variations
  - Transcript assembly
  - …





Ingredients for a good assembly

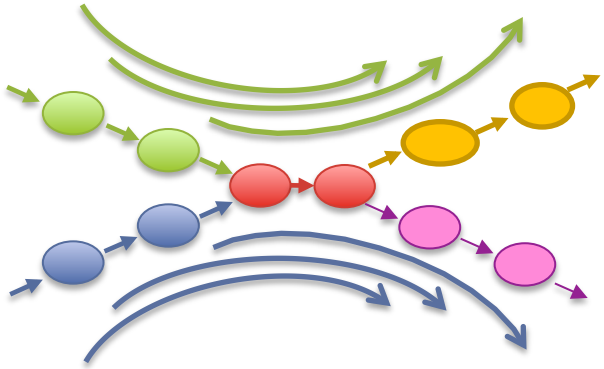
Coverage



High coverage is required

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

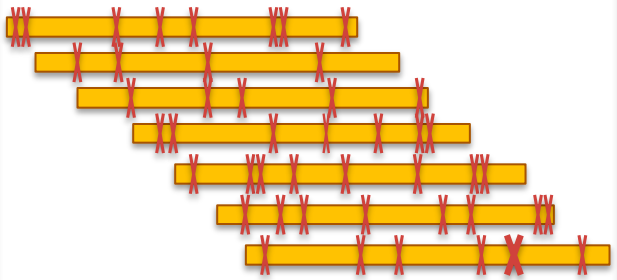
Read Length



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

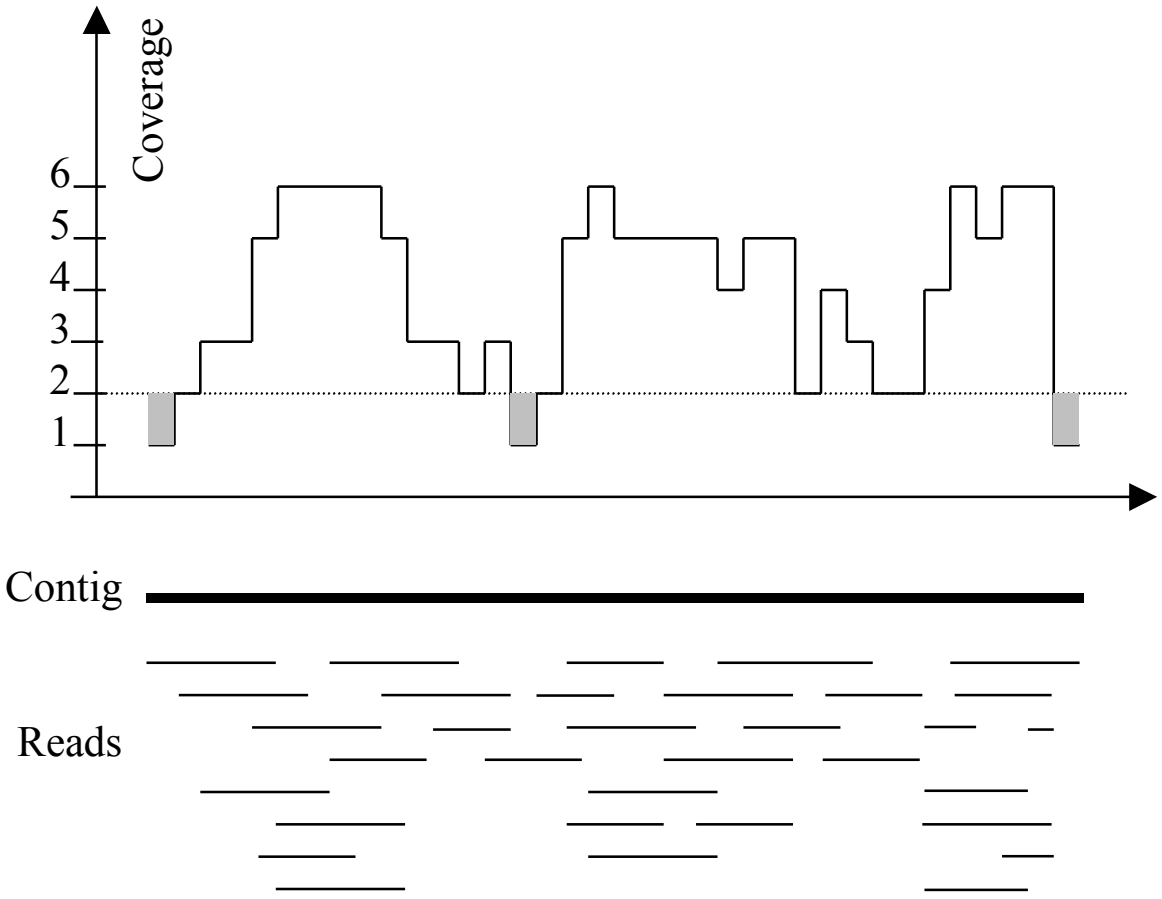


Errors obscure overlaps

- 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

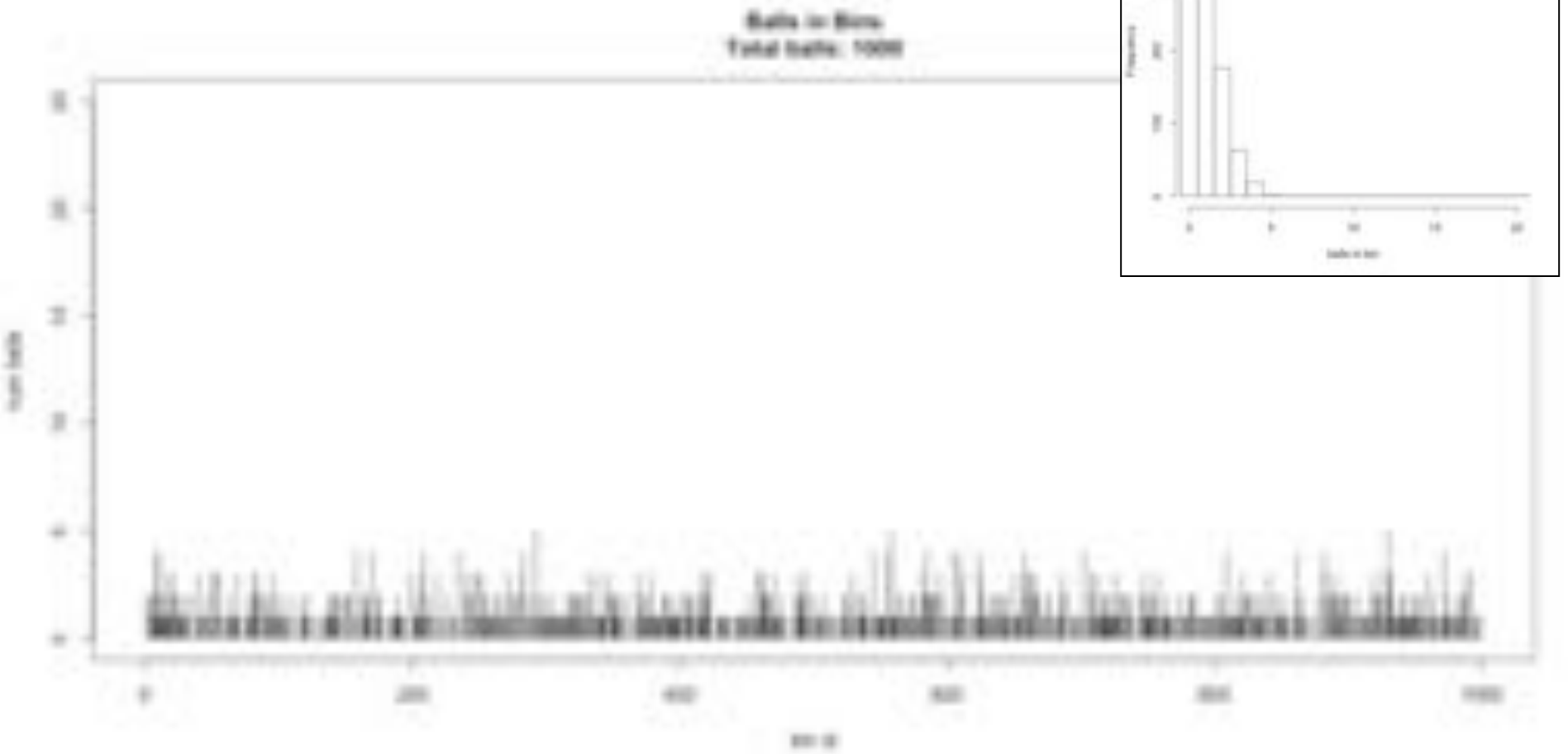
Typical sequencing coverage



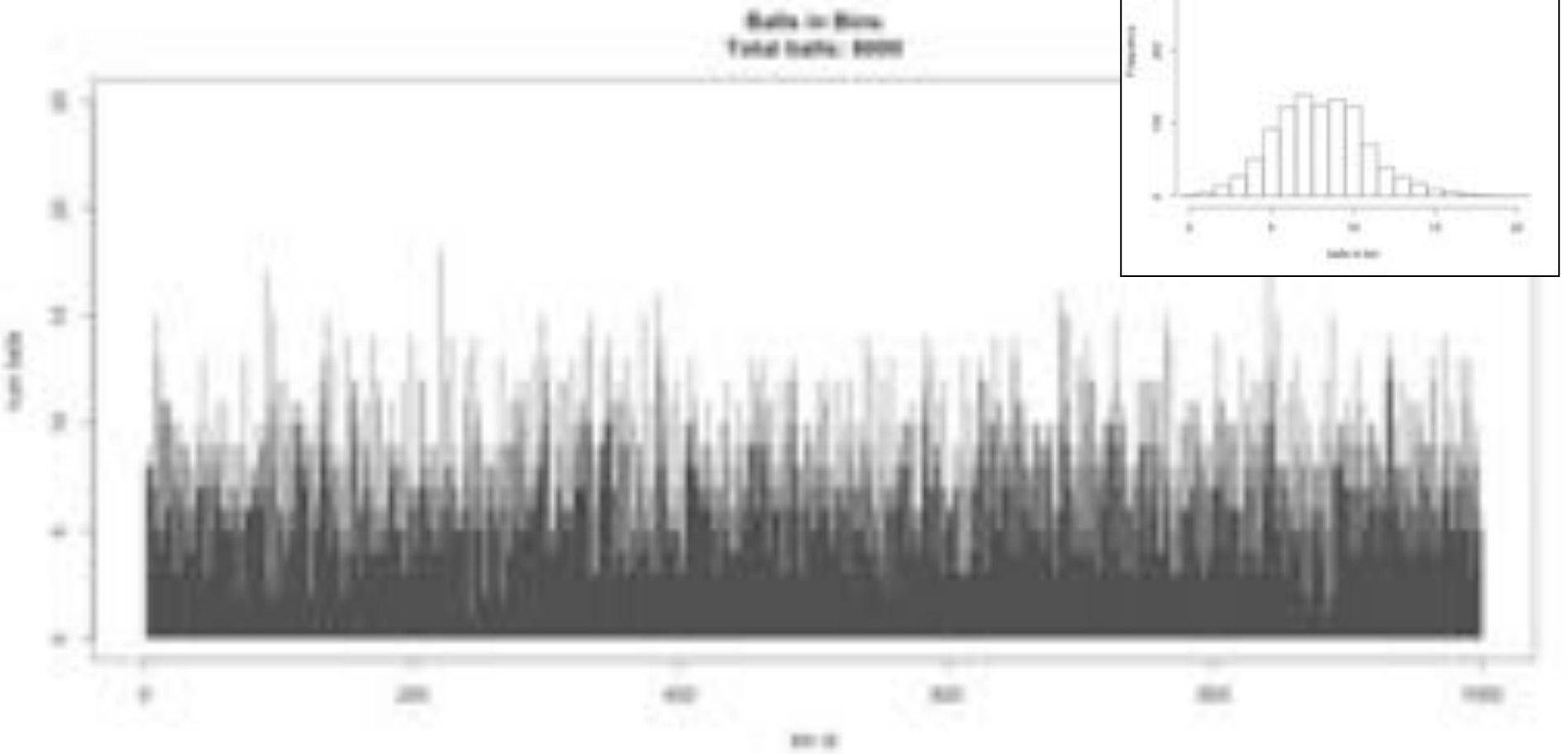
Imagine raindrops on a sidewalk

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

1x sequencing



8x sequencing



Poisson Distribution

The probability of a given number of events occurring in a fixed interval of time and/or space if these events occur with a known average rate and independently of the time since the last event.

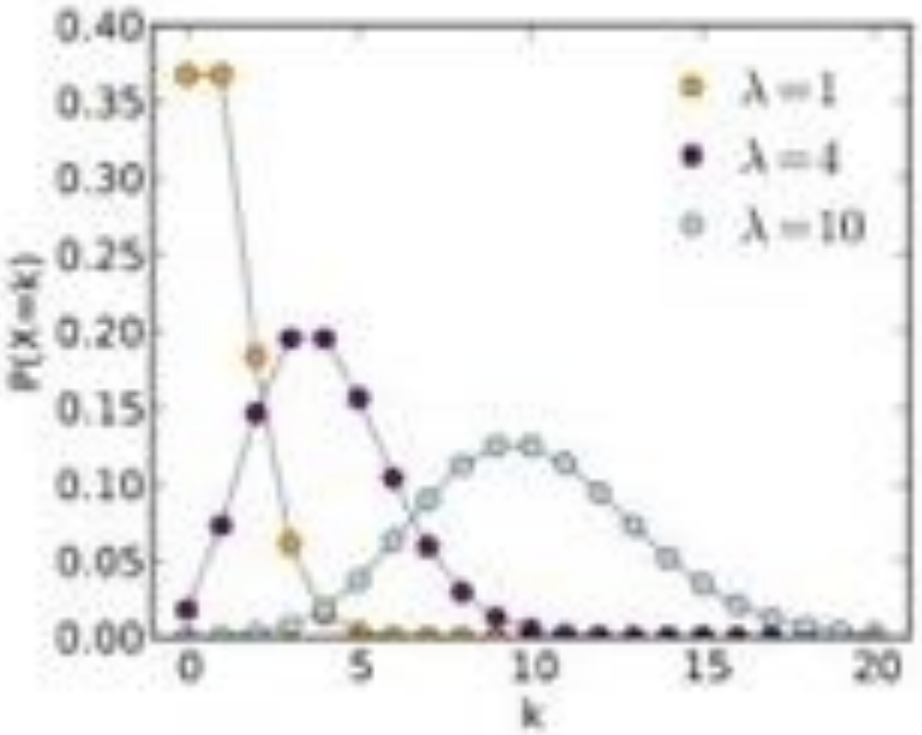
$$P(k) = \frac{\lambda^k}{k!} e^{-\lambda}$$

Formulation comes from the limit of the binomial equation

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

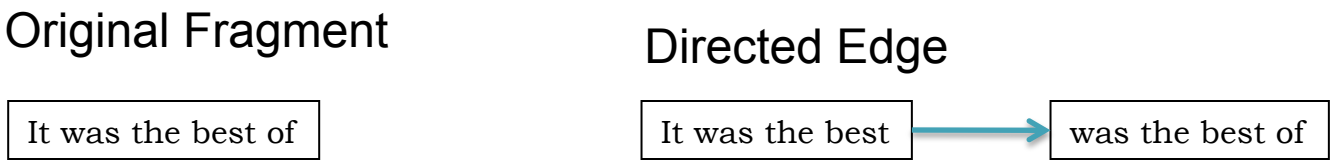
Key property:

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



de Bruijn Graph Construction

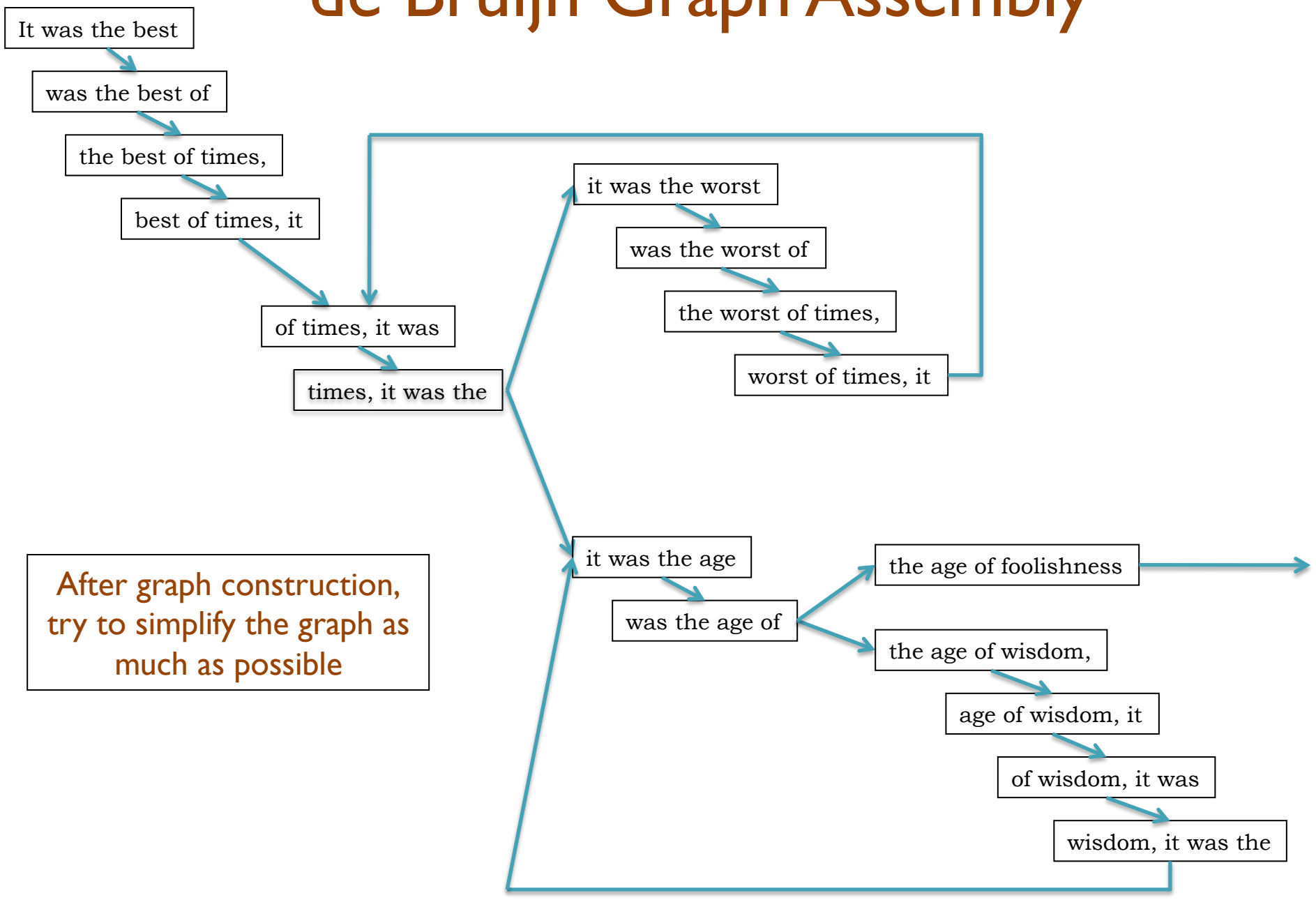
- $D_k = (V,E)$
  - V = All length-k subfragments (k < l)
  - E = Directed edges between consecutive subfragments
    - Nodes overlap by k-1 words



- 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



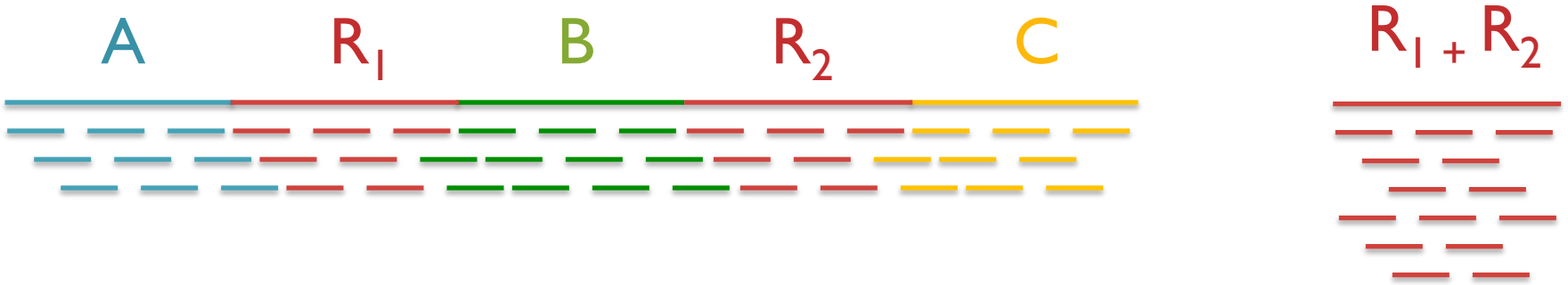
After graph construction, try to simplify the graph as much as possible

Repetitive regions

Repeat Type	Definition / Example	Prevalence
Low-complexity DNA / Microsatellites	$(b_1 b_2 \ldots b_k)^N$ where $1 \leq k \leq 6$ CACACACACACACACACACA	2%
SINEs (Short Interspersed Nuclear Elements)	*Alu* sequence (~280 bp) Mariner elements (~80 bp)	13%
LINEs (Long Interspersed Nuclear Elements)	~500 – 5,000 bp	21%
LTR (long terminal repeat) retrotransposons	Ty1-copia, Ty3-gypsy, Pao-BEL (~100 – 5,000 bp)	8%
Other DNA transposons		3%
Gene families & segmental duplications		4%

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

Repeats and Coverage Statistics



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

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

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

The fragment assembly string graph
Myers, EW (2005) Bioinformatics. 21(suppl 2): ii79-85.

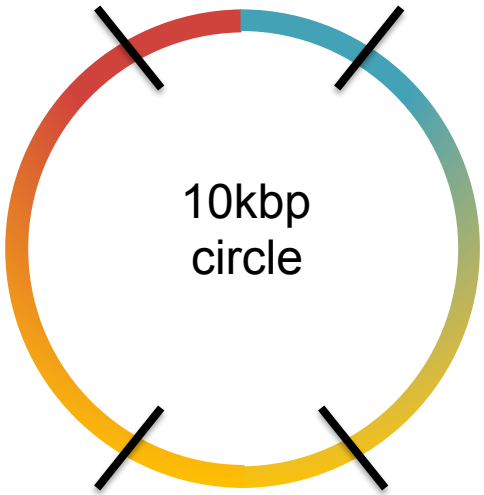
Paired-end and Mate-pairs

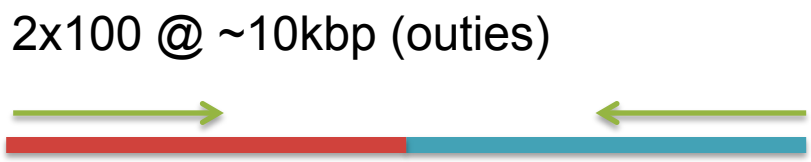
Paired-end sequencing

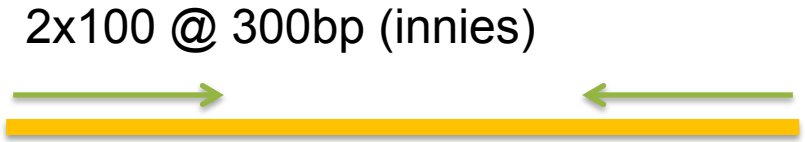
- Read one end of the molecule, flip, and read the other end
- Generate pair of reads separated by up to 500bp with inward orientation

Mate-pair sequencing

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

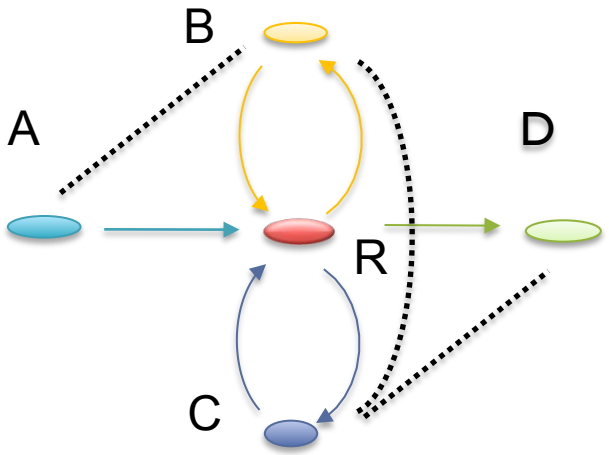




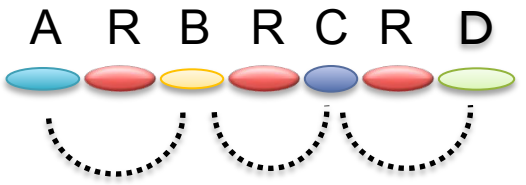


Scaffolding

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



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

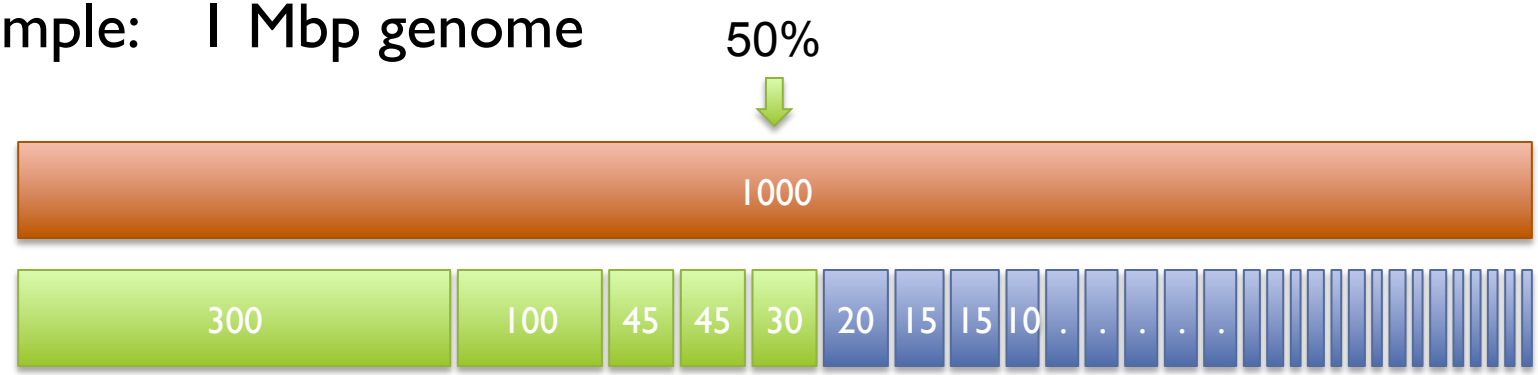


- Final scaffold may have internal gaps called sequencing gaps
  - We know the order, orientation, and spacing, but just not the bases. Fill with Ns instead

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:

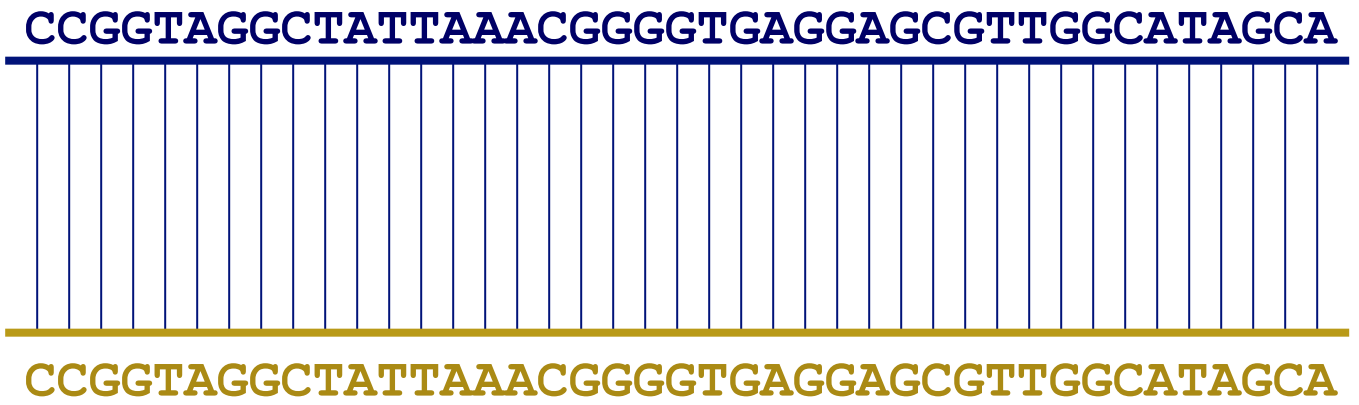
N50 values are only meaningful to compare when base genome size is the same in all cases

Whole Genome Alignment with MUMmer

Slides Courtesy of Adam M. Phillippy
University of Maryland

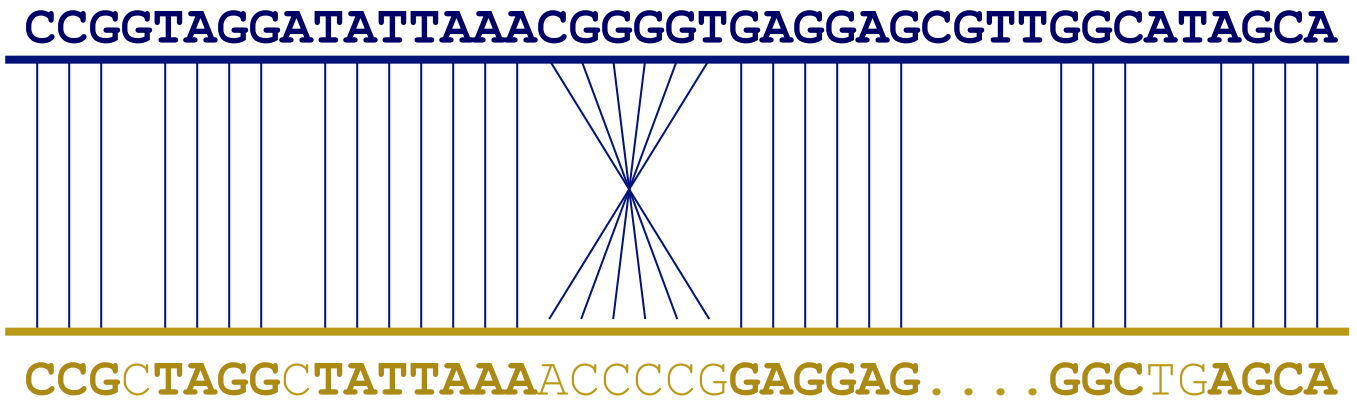
Goal of WGA

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



Not so fast...

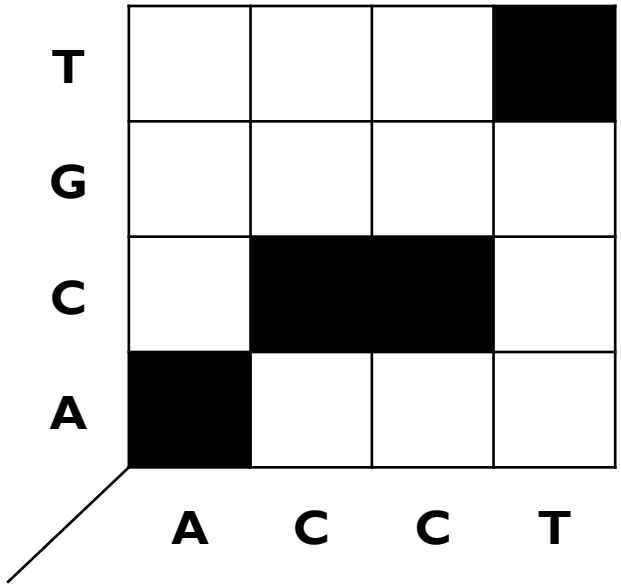
- Genome *A* may have insertions, deletions, translocations, inversions, duplications or SNPs with respect to *B* (sometimes all of the above)



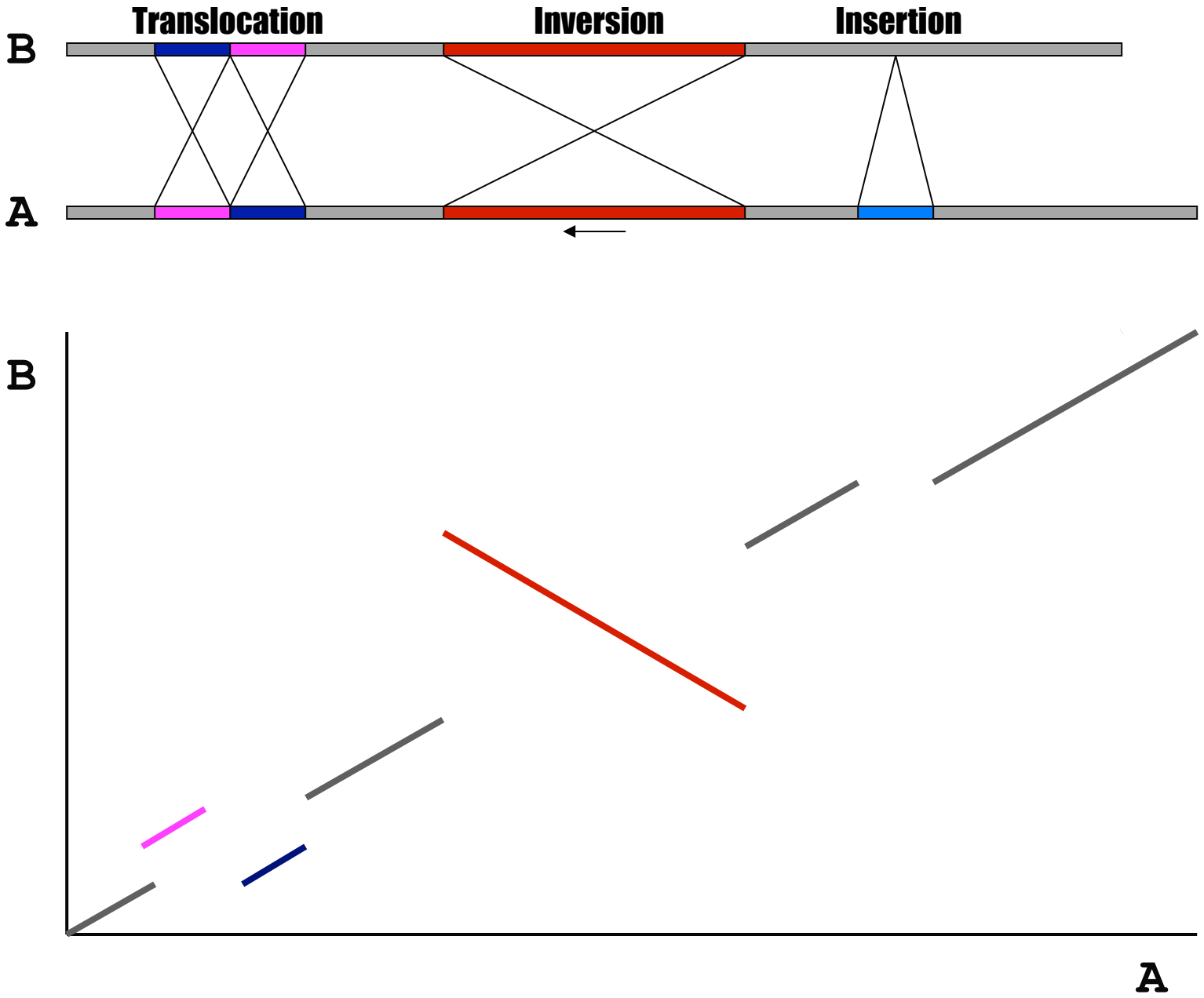
WGA visualization

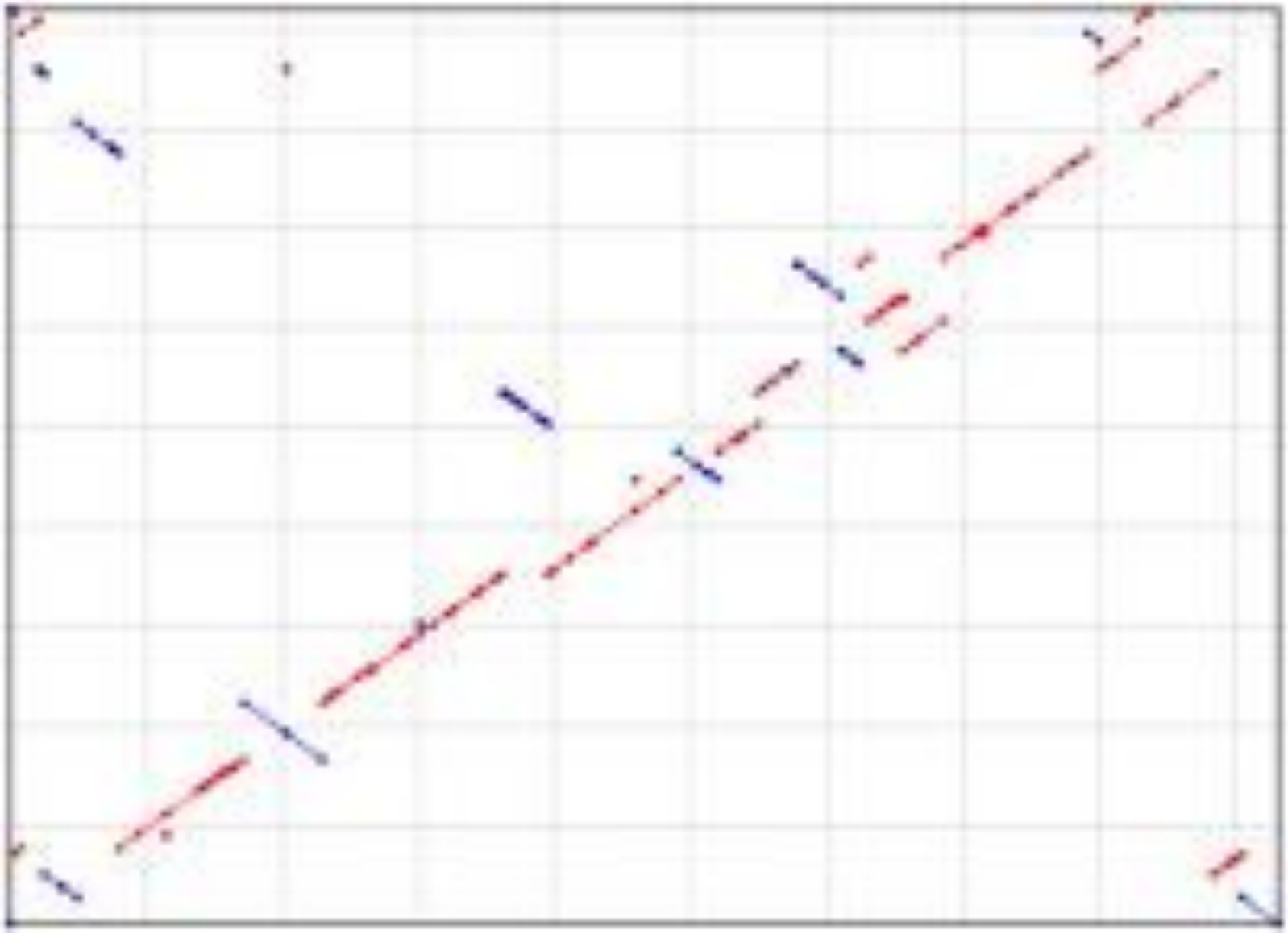
- How can we visualize *whole* genome alignments?

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



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

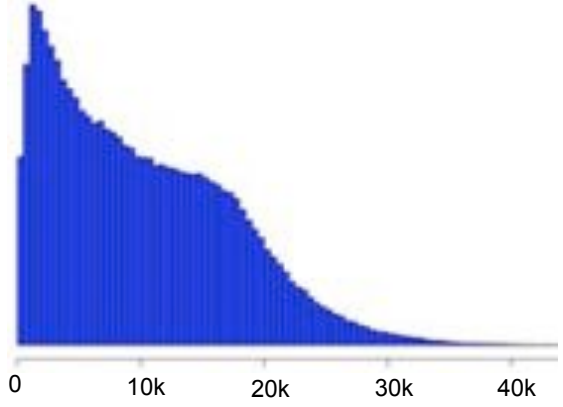

Translocation
Inversion
Insertion
B
A
B
A



Alignment of 2 strains of *Y. pestis*
http://mummer.sourceforge.net/manual/

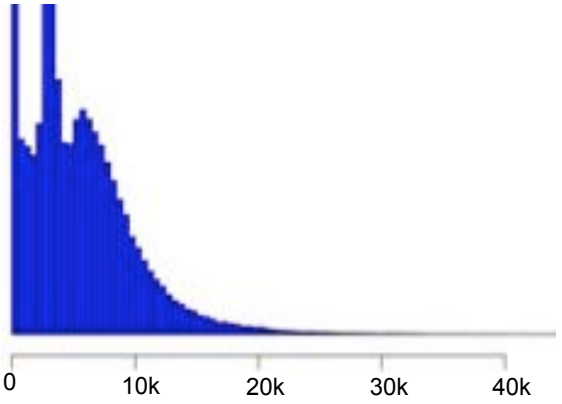
3rd Gen Long Read Sequencing

PacBio RS II



CSHL/PacBio

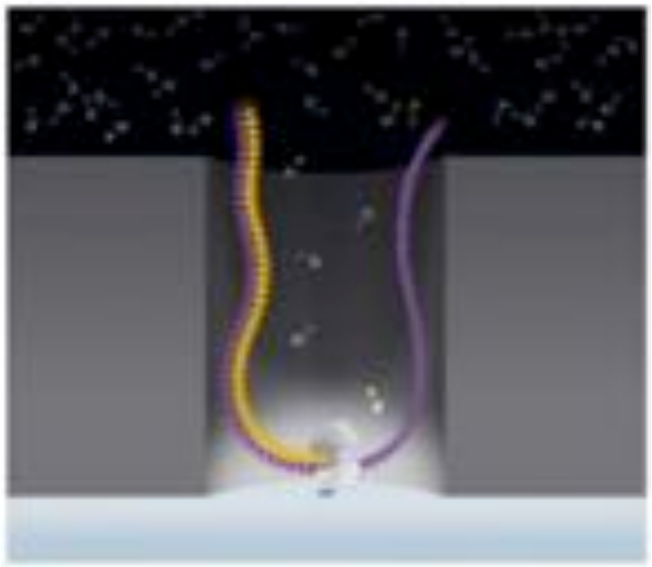
Oxford Nanopore

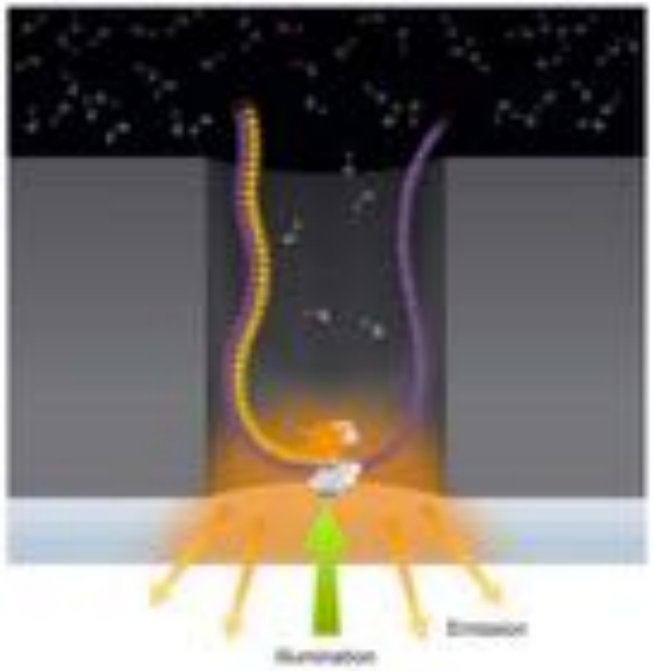


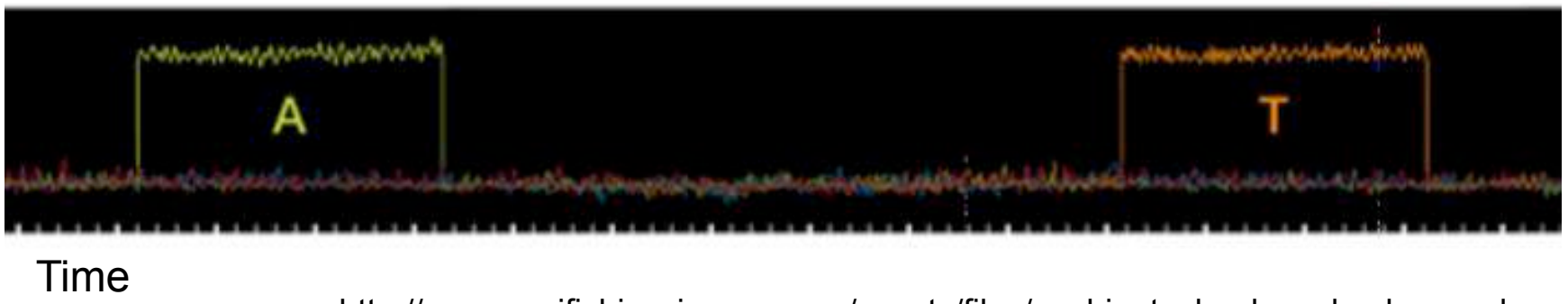
CSHL/ONT

PacBio SMRT Sequencing

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

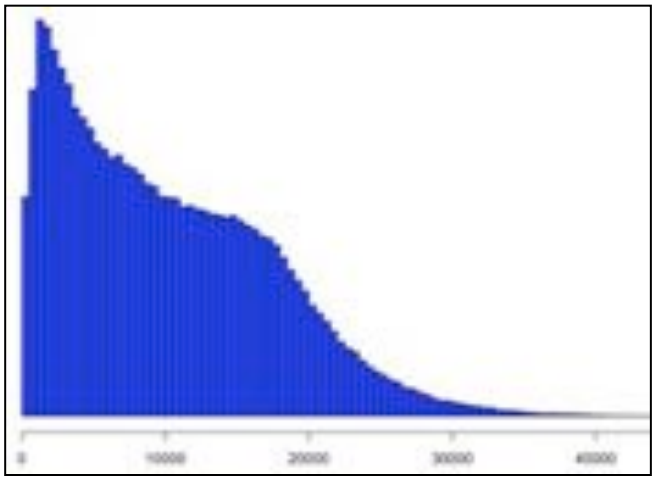






http://www.pacificbiosciences.com/assets/files/pacbio_technology_backgrounder.pdf

SMRT Sequencing Data



Match	83.7%
Insertions	11.5%
Deletions	3.4%
Mismatch	1.4%

```
TTGTAAGCAGTTGAAAACTATGTGTGGATTTAGAATAAAGAACATGAAAG
|||||||||||||||||||||||||| ||||||| |||||||||||| |||
TTGTAAGCAGTTGAAAACTATGTGT-GATTTAG-ATAAAGAACATGGAAG

ATTATAAA-CAGTTGATCCATT-AGAAGA-AAACGCAAAAGGCGGCTAGG
| |||||| |||||||||||||| |||| | |||||| |||||| |||||||
A-TATAAATCAGTTGATCCATTAAGAA-AGAAACGC-AAAGGC-GCTAGG

CAACCTTGAATGTAATCGCACTTGAAGAACAAGATTTTATTCCGCGCCCG
| |||||| |||| ||  ||||||||||||||||||||||||||||||||
C-ACCTTG-ATGT-AT--CACTTGAAGAACAAGATTTTATTCCGCGCCCG

TAACGAATCAAGATTCTGAAAACACAT-ATAACAACCTCCAAAA-CACAA
| ||||||| |||||||||||||| || ||    |||||||||| |||||
T-ACGAATC-AGATTCTGAAAACA-ATGAT----ACCTCCAAAAGCACAA

-AGGAGGGGAAAGGGGGGAATATCT-ATAAAAGATTACAAATTAGA-TGA
 ||||||   ||     |||||||| || |||||||||||||| || |||
GAGGAGG---AA-----GAATATCTGAT-AAAGATTACAAATT-GAGTGA

ACT-AATTCACAATA-AATAACACTTTTA-ACAGAATTGAT-GGAA-GTT
|||  ||||||||| | |||||||||||| ||| ||||||| |||| |||
ACTAAATTCACAA-ATAATAACACTTTTAGACAAAATTGATGGGAAGGTT

TCGGAGAGATCCAAAACAATGGGC-ATCGCCTTTGA-GTTAC-AATCAAA
|| ||||||||| ||||||| ||| |||| |||||| ||||| |||||||
TC-GAGAGATCC-AAACAAT-GGCGATCG-CTTTGACGTTACAAATCAAA

ATCCAGTGGAAAATATAATTTATGCAATCCAGGAACTTATTCACAATTAG
||||||| ||||||||  |||||| ||||| ||||||||||||||||||
ATCCAGT-GAAAATATA--TTATGC-ATCCA-GAACTTATTCACAATTAG
```

Sample of 100k reads aligned with BLASR requiring >100bp alignment

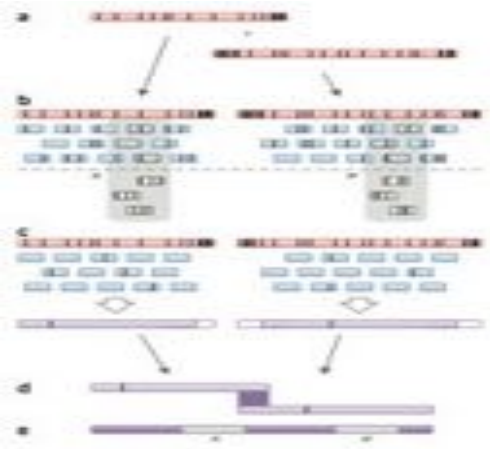
PacBio Assembly Algorithms

PBJelly

Gap Filling and Assembly Upgrade

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

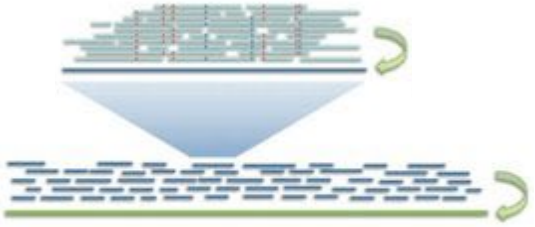
PacBioToCA & ECTools



Hybrid/PB-only Error Correction

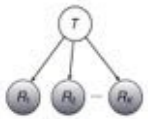
Koren, Schatz, *et al* (2012)
Nature Biotechnology. 30:693–700

HGAP & Quiver



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

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



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

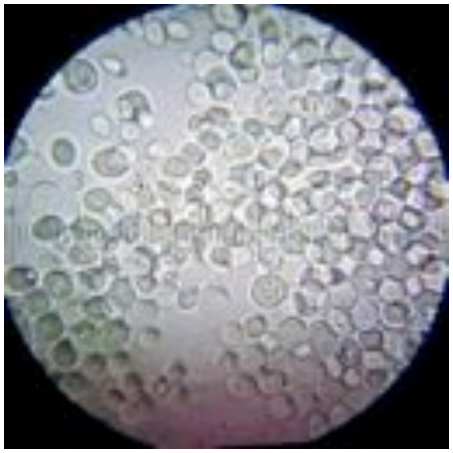
	Initial Assembly	Quiver Consensus
QV	43.4	54.5
Accuracy	99.99540%	99.99964%
Differences	141	11

PB-only Correction & Polishing

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

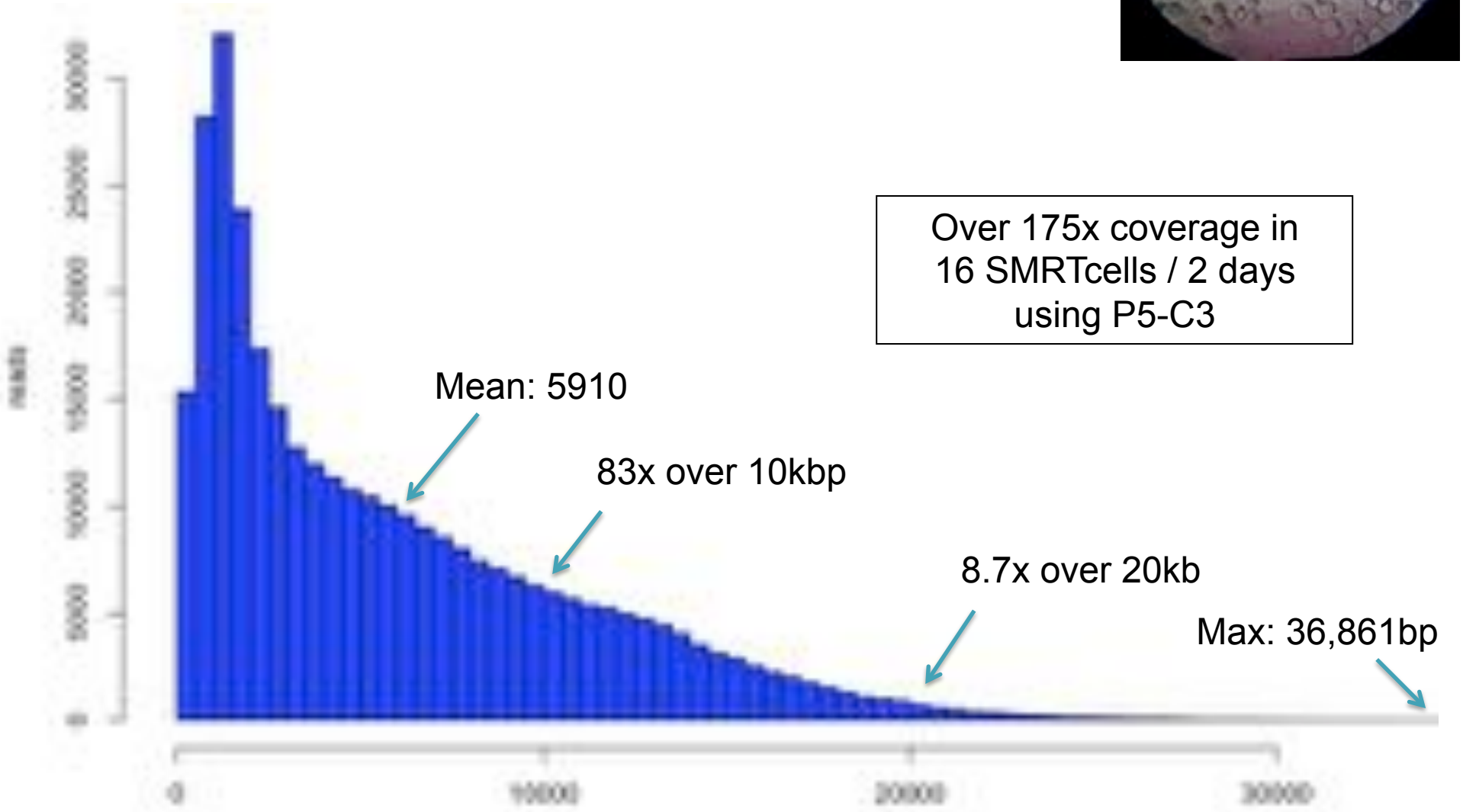
< 5x PacBio Coverage > 50x

S. cerevisiae W303

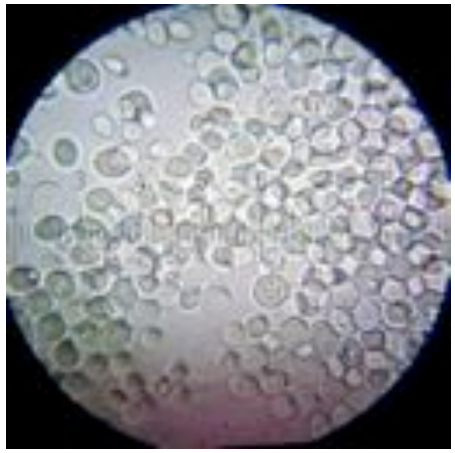


PacBio RS II sequencing at CSHL in the McCombie Lab

- Size selection using an 7 Kb elution window on a BluePippin™ device from Sage Science



S. cerevisiae W303

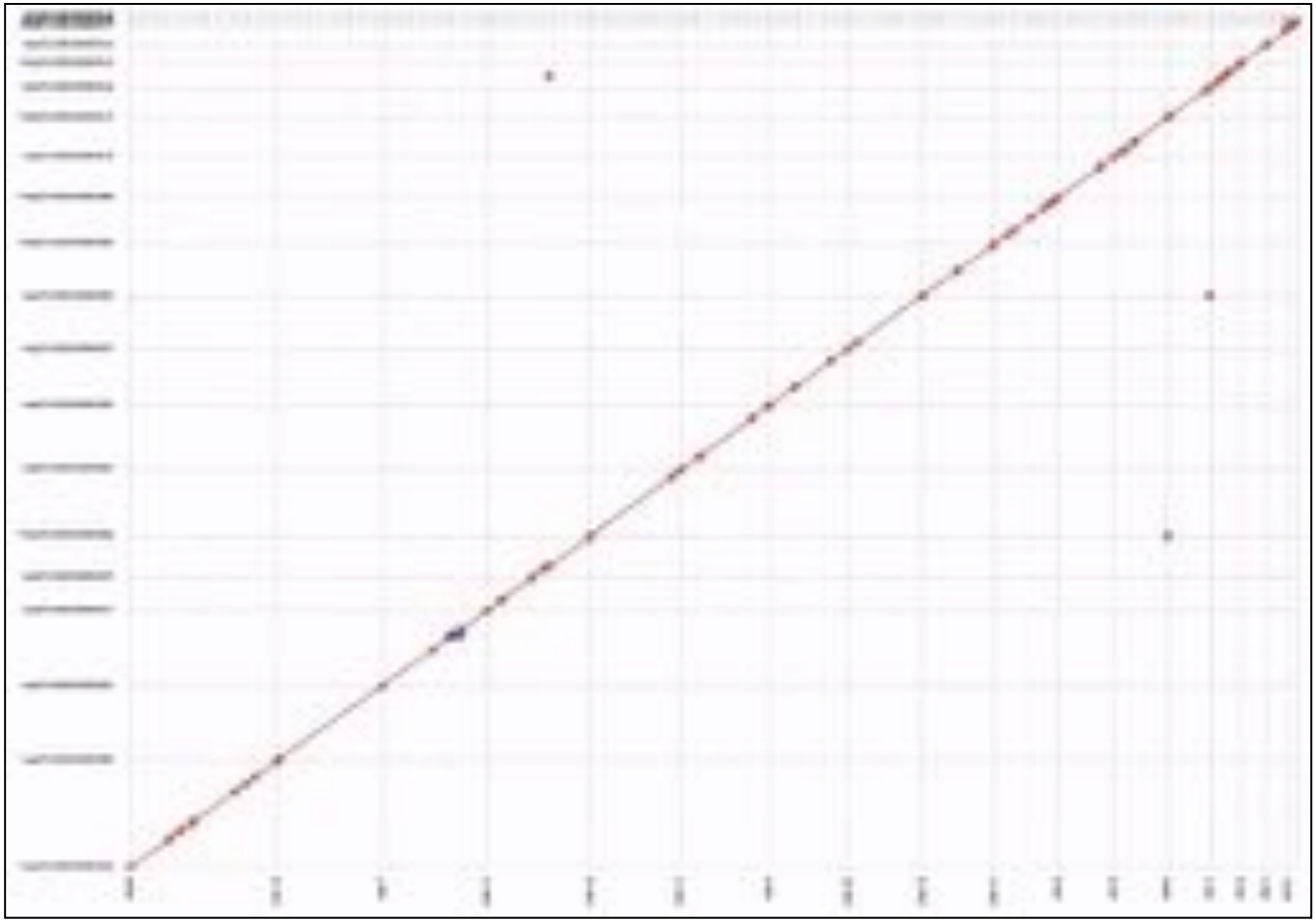


S288C Reference sequence

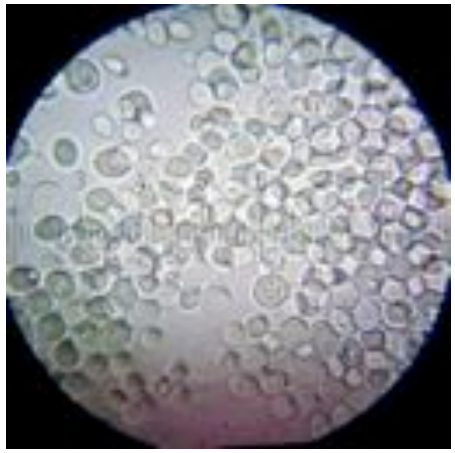
- 12.1Mbp; 16 chromo + mitochondria; N50: 924kbp

PacBio assembly using HGAP + Celera Assembler

- 12.4Mbp; 21 non-redundant contigs; N50: 811kbp; >99.8% id



S. cerevisiae W303



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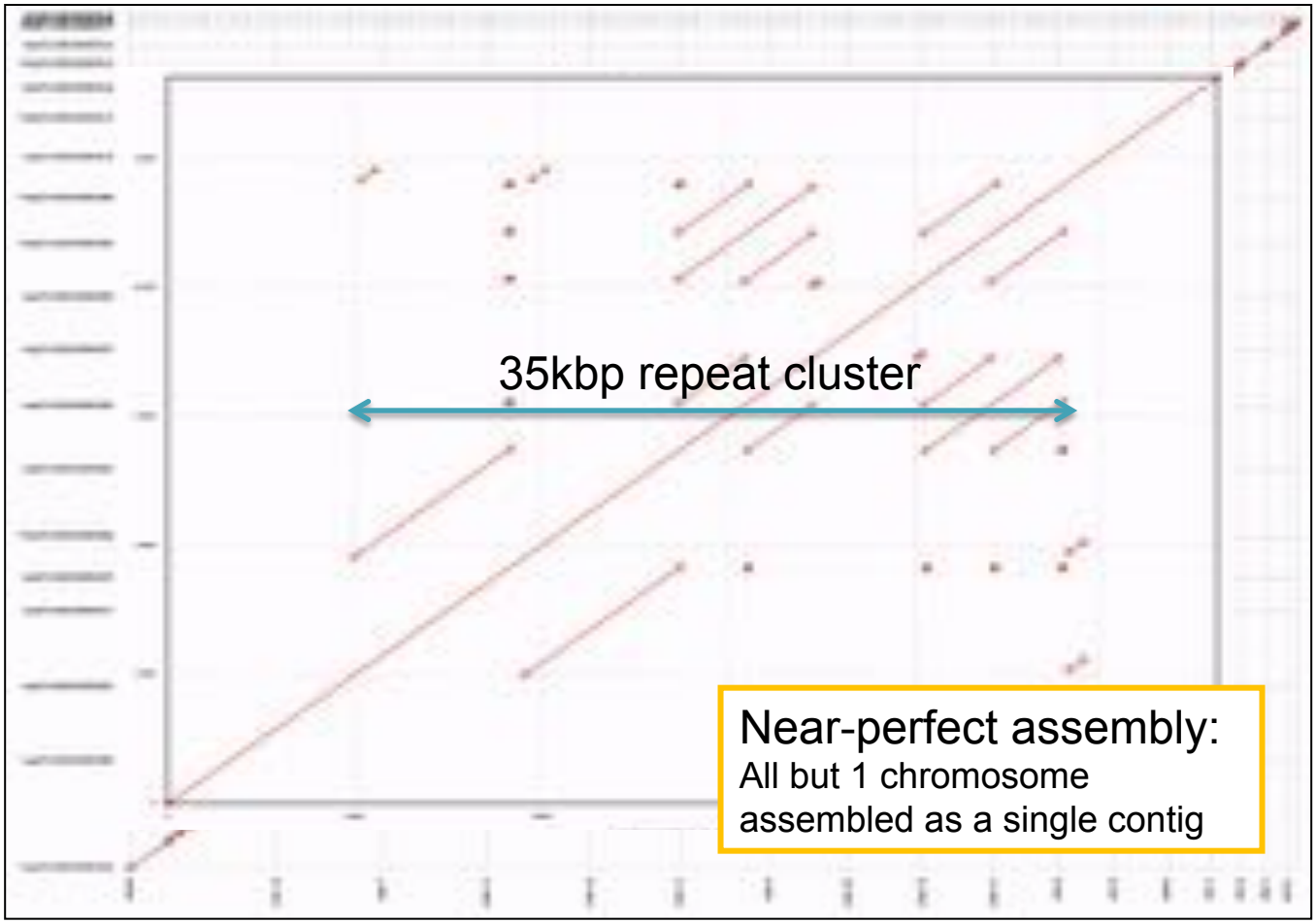
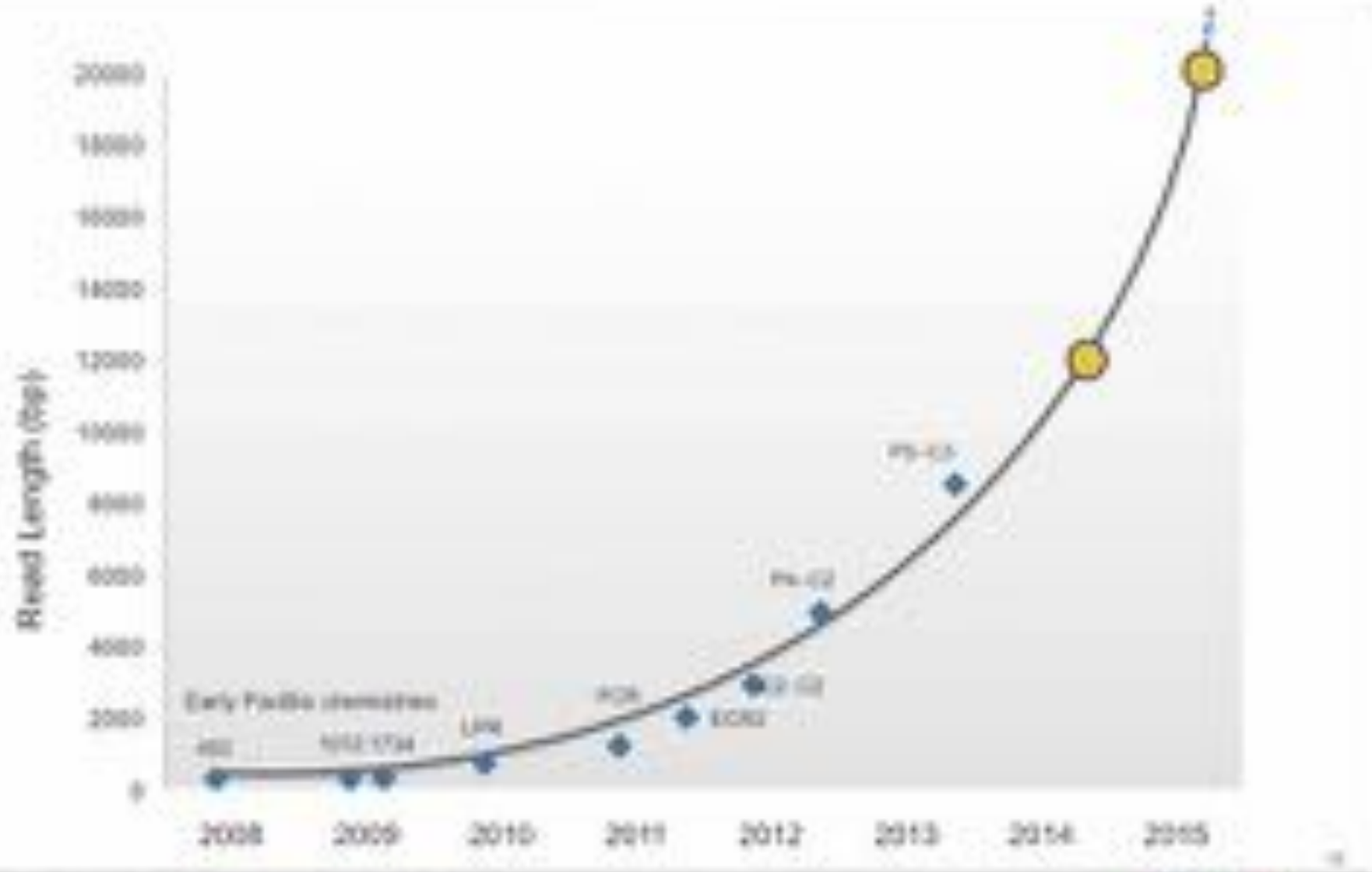
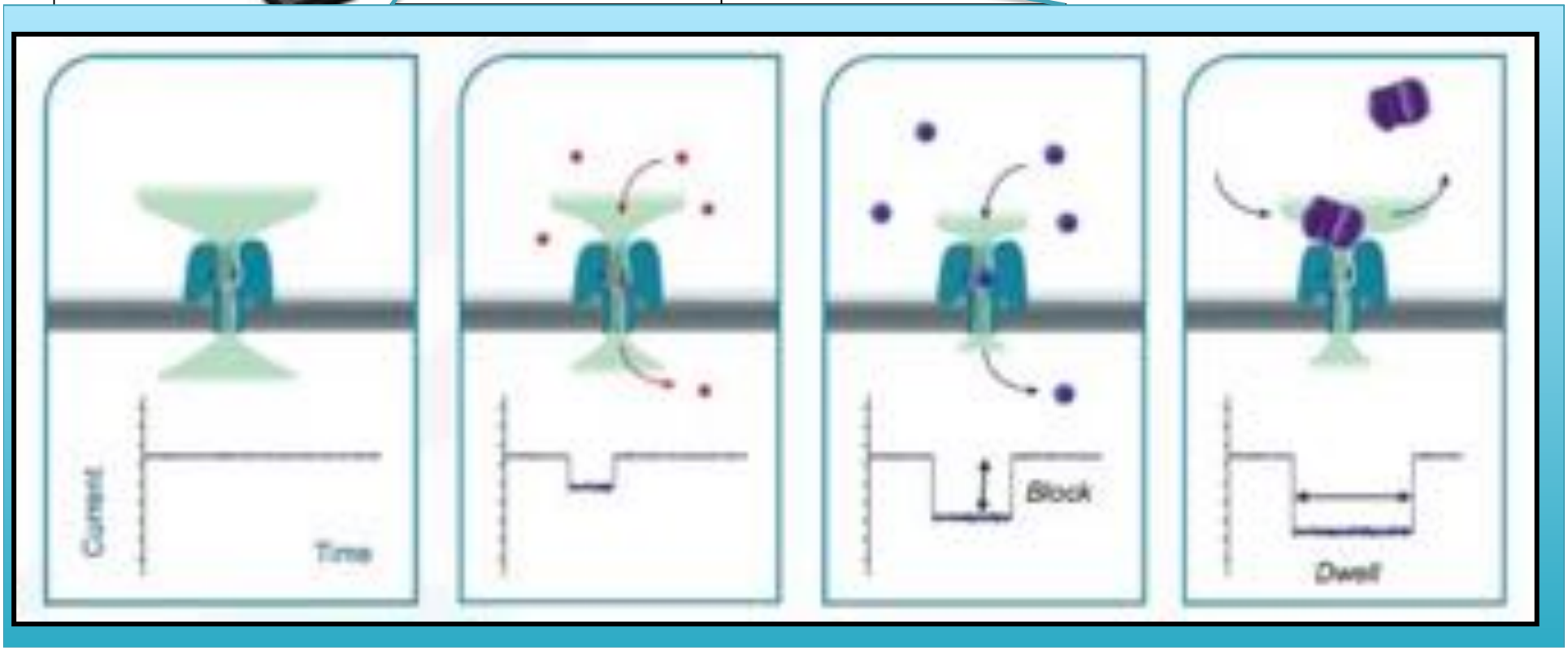


PacBio® Advances in Read Length

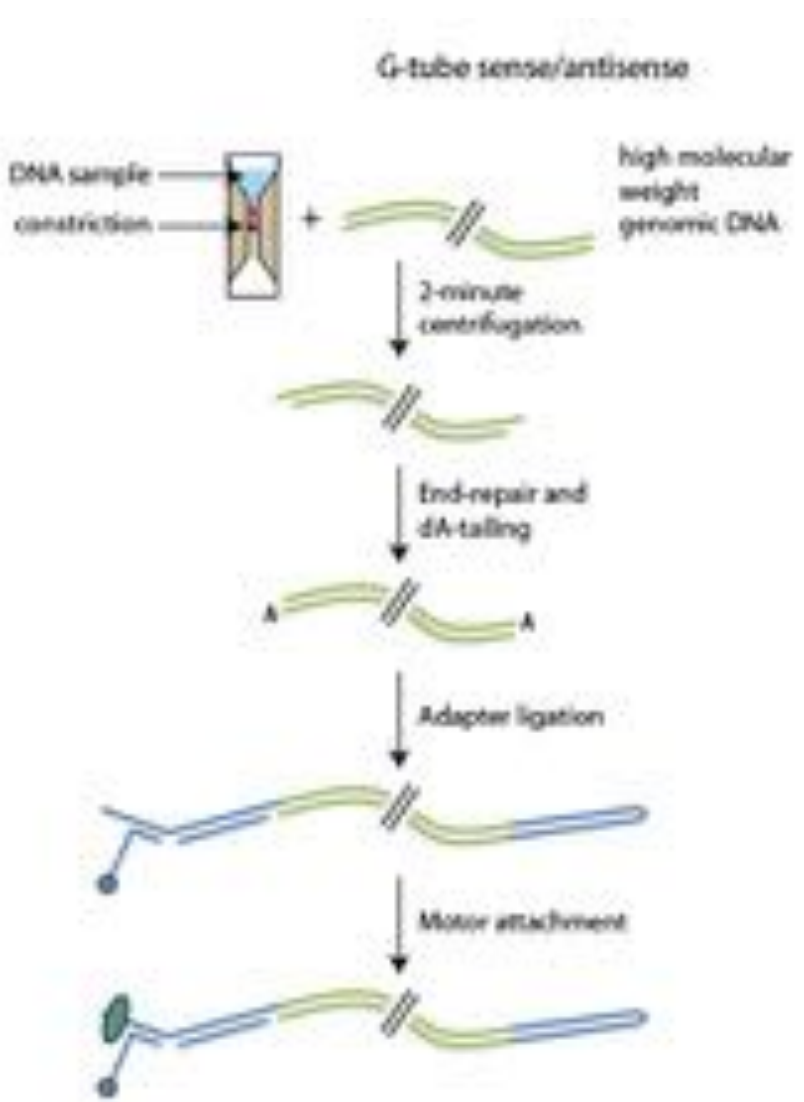


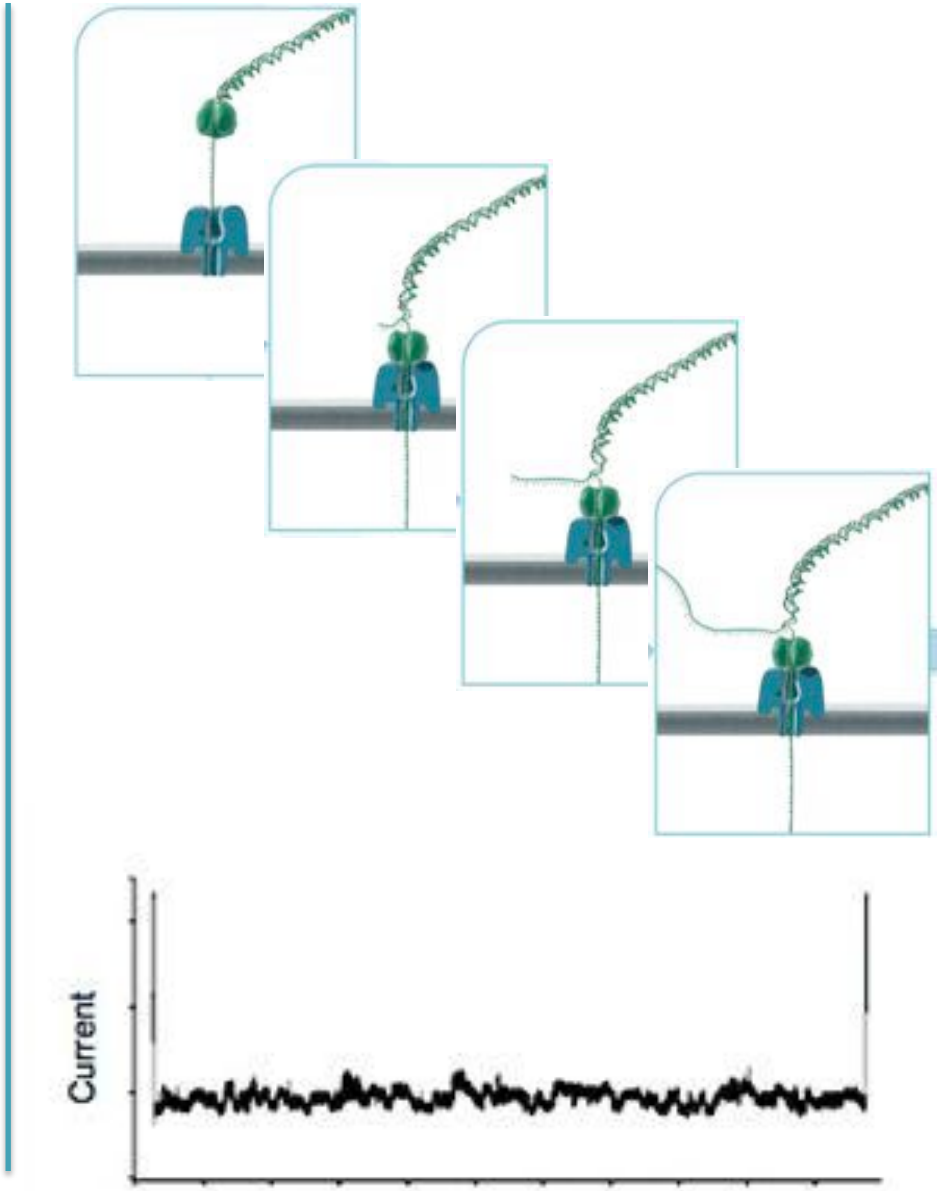
Oxford Nanopore MinION

- Thumb drive sized sequencer powered over USB
- Capacity for 512 reads at once
- Senses DNA by measuring changes to ion flow

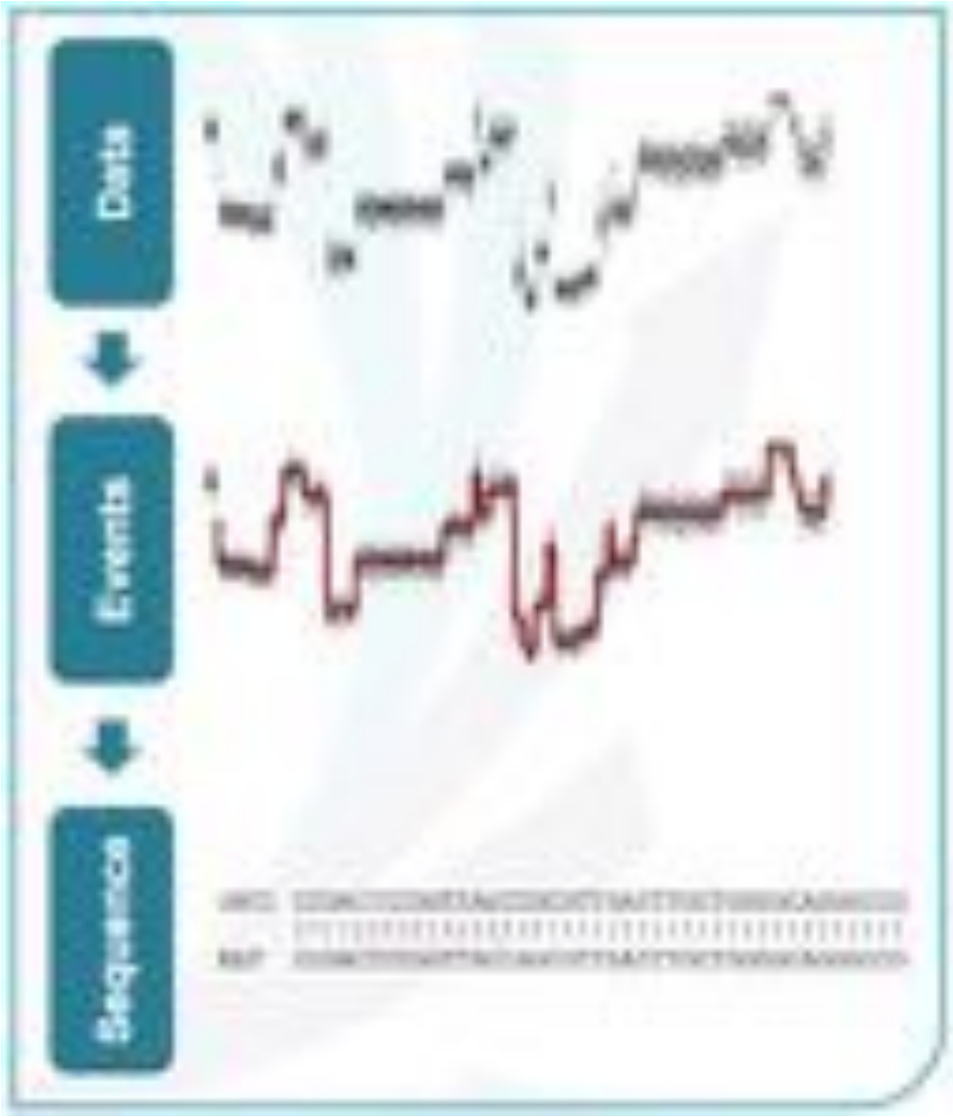


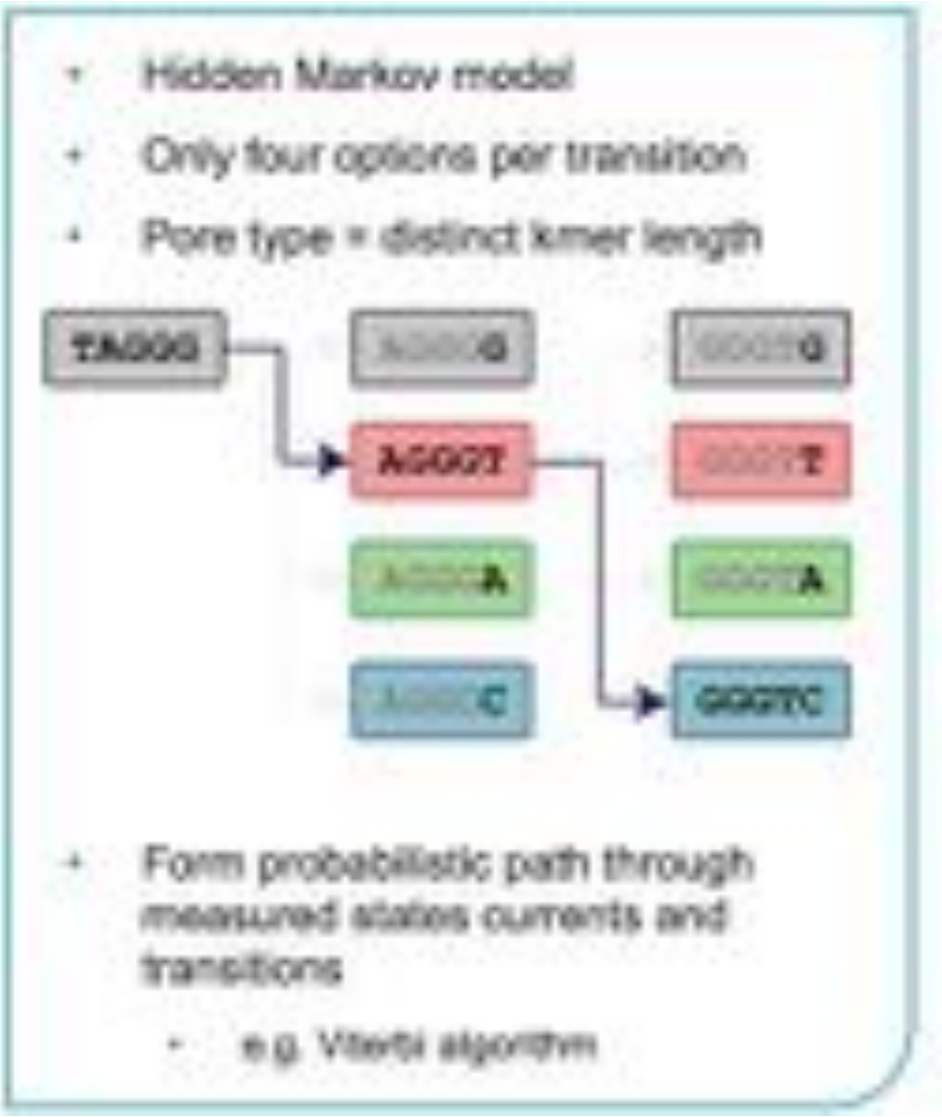
Nanopore Sequencing





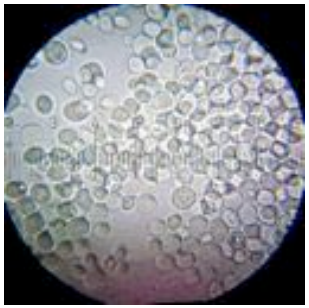
Nanopore Basecalling





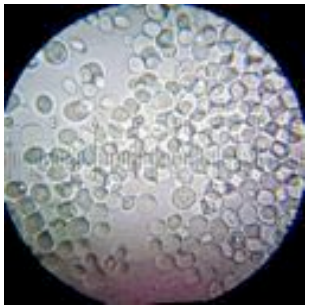
Basecalling currently performed at Amazon with frequent updates to algorithm

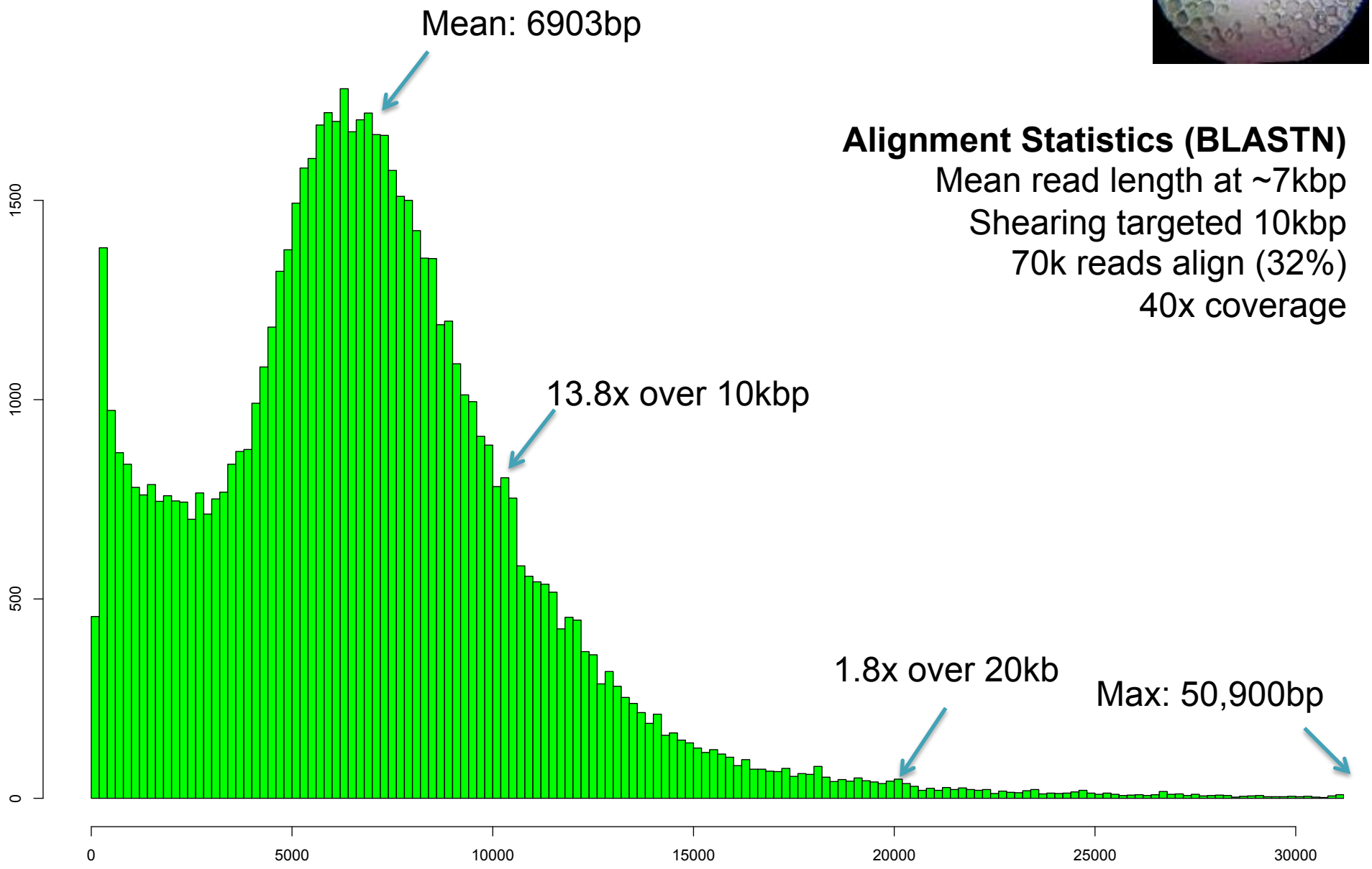
Nanopore Readlengths



Oxford Nanopore Sequencing at CSHL
30 runs, 267k reads, 122x total coverage
Between 11 and 73k reads per run!
Mean flow cell: 50 Mbp in 2 days
Max flow cell: 446Mbp in 2 days

Nanopore Alignments





Alignment Statistics (BLASTN)

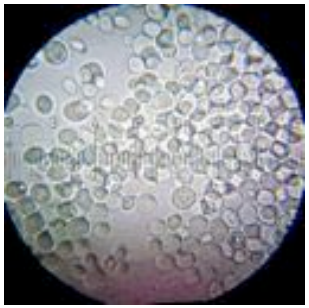
Mean read length at ~7kbp

Shearing targeted 10kbp

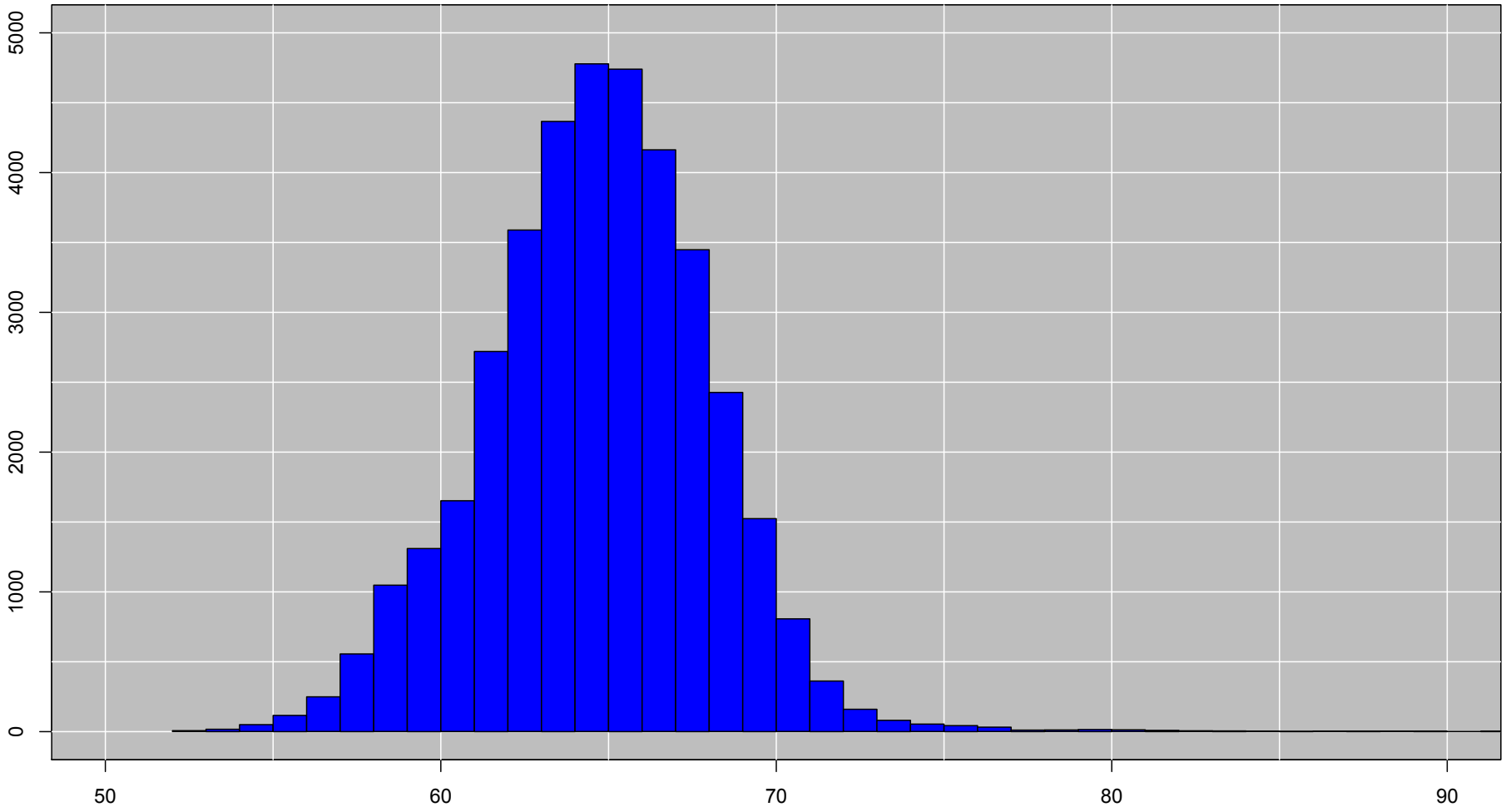
70k reads align (32%)

40x coverage

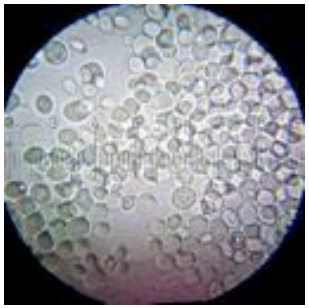
Nanopore Accuracy



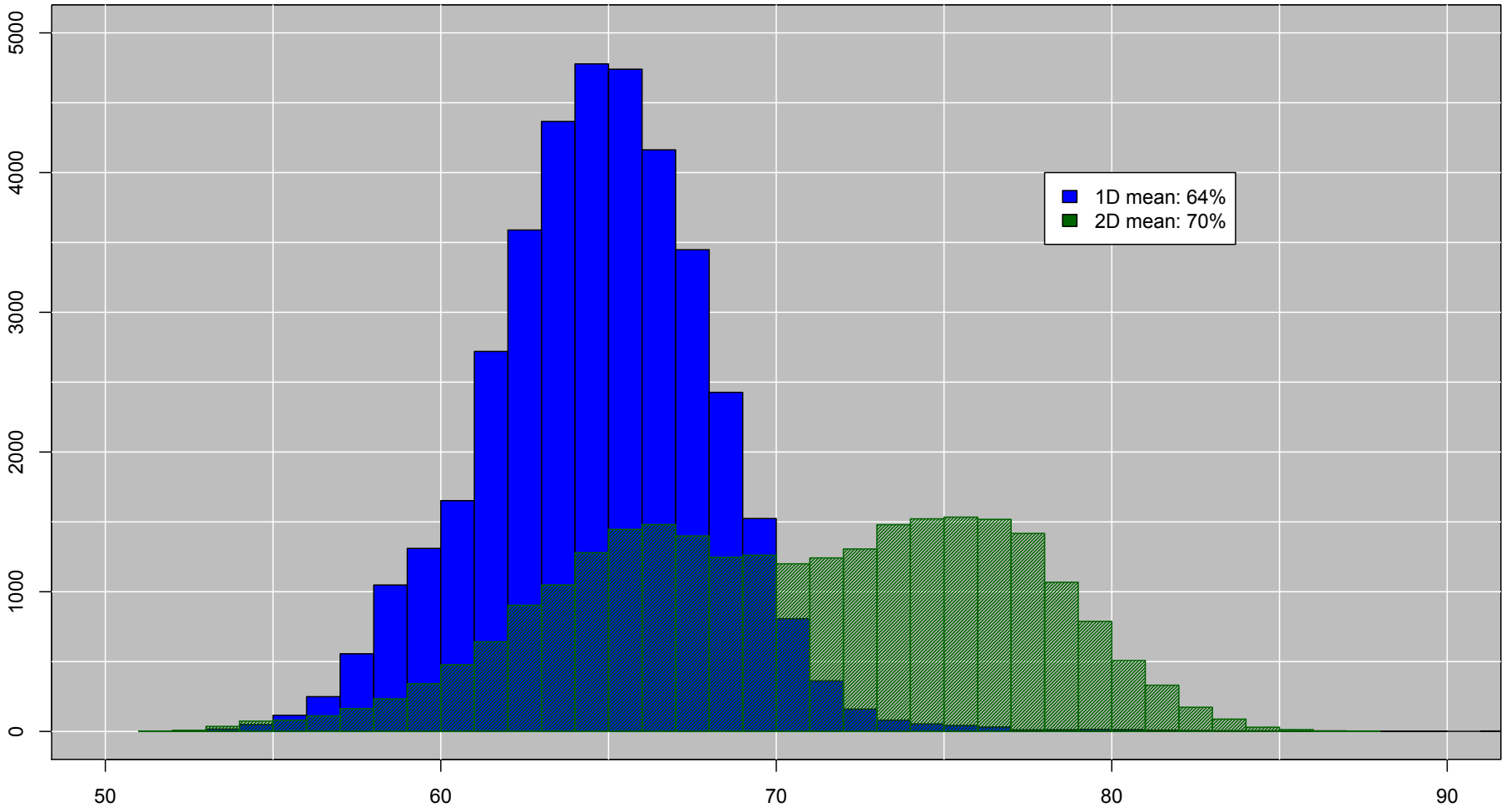
Alignment Quality (BLASTN)
Of reads that align, average ~64% identity



Nanopore Accuracy



Alignment Quality (BLASTN)
Of reads that align, average ~64% identity
"2D base-calling" improves to ~70% identity



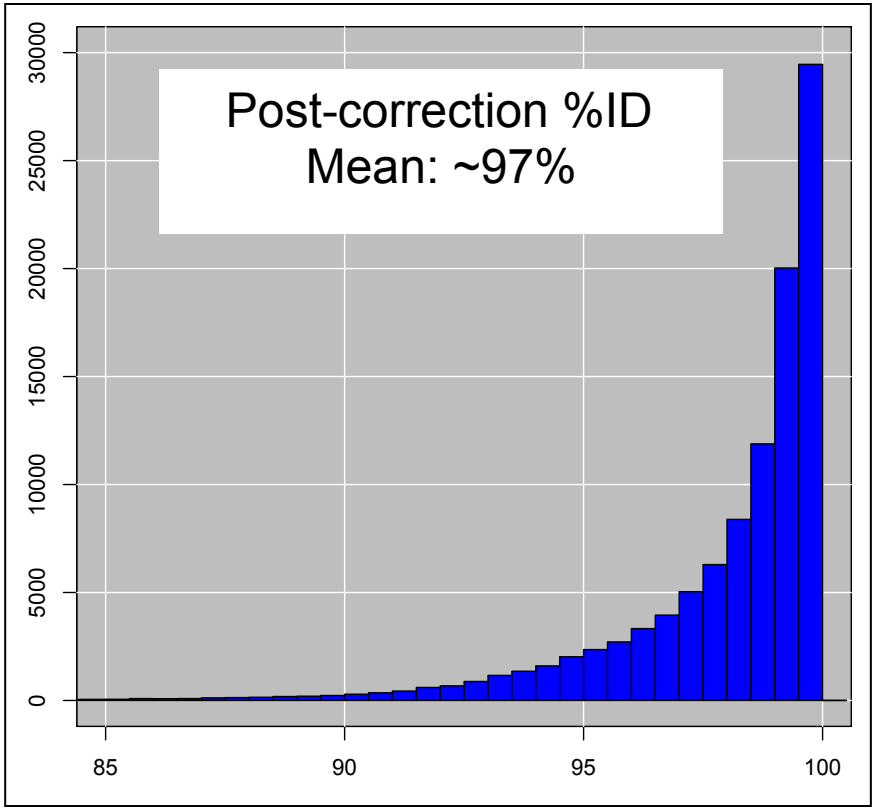
NanoCorr: Nanopore-Illumina Hybrid Error Correction

https://github.com/jgurtowski/nanocorr

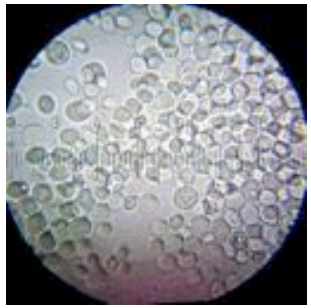
1. BLAST Miseq reads to all raw Oxford Nanopore reads

2. Select non-repetitive alignments
   - First pass scans to remove "contained" alignments
   - Second pass uses Dynamic Programming (LIS) to select set of high-identity alignments with minimal overlaps

3. Compute consensus of each Oxford Nanopore read
   - Currently using Pacbio's pbdagcon



Long Read Assembly



S288C Reference sequence

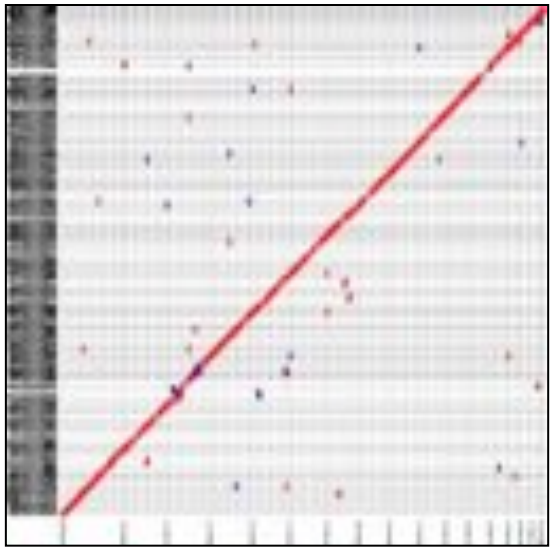
- 12.1Mbp; 16 chromo + mitochondria
- Chromosome N50: 924kbp

Illumina MiSeq

30x, 300bp PE (Flashed)

Celera Assembler

- 6953 non-redundant contigs
- N50: 59kb >99.9% id

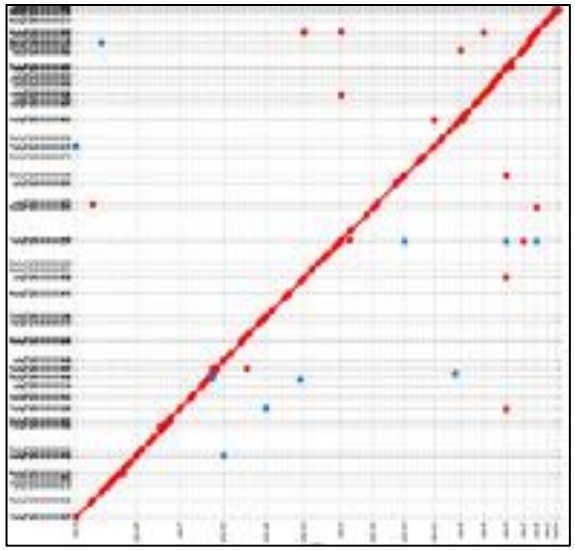


Oxford Nanopore

30x corrected reads > 6kb

NanoCorr + Celera Assembler

- 234 non-redundant contigs
- N50: 362kbp >99.78% id

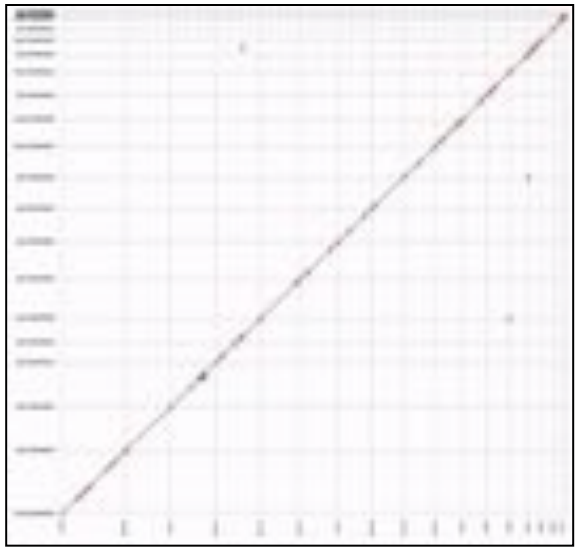


Pacific Biosciences

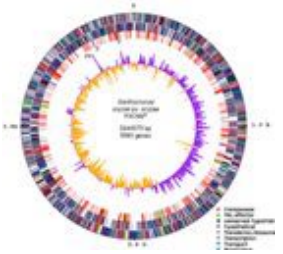
25x corrected reads > 10kb

HGAP + Celera Assembler

- 21 non-redundant contigs
- N50: 811kb >99.8% id



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