Computational Genomics

Michael Schatz

Oct 3, 2011
Frontiers in Genomics

Outline

Part 1: Schatz Lab Overview

Part 2: Sequence Alignment

Part 3: Genome Assembly

Part 4: Parallel & Cloud Computing

A Little About Me

Computational Biology

"Computer science is no more about computers than astronomy is about telescopes."
Edger Dijkstra

Computer Science = Science of Computation

- Compute solutions to problems, designing & building systems
- Computers are very, very dumb, but we can instruct them
  - Build complex systems out of simple components

Computational Biology = Thinking Computationally about Biology

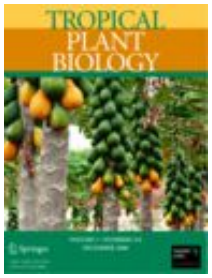
- Analysis: Make more powerful instruments, analyze results
- Design: experimental protocols, procedures, systems

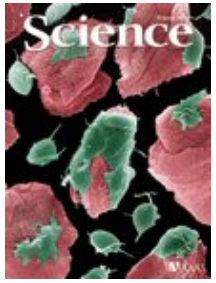
Computational Genomics

1. Alignment
2. Assembly
3. Expression
4. Comparative Genomics

Computational Thinking

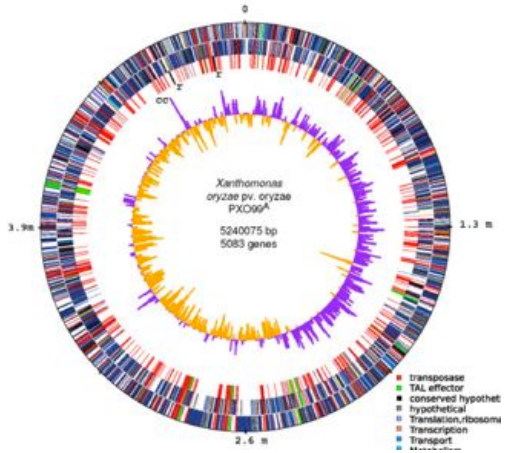
1. Algorithm
2. Data structure
3. Computational Analysis
4. Computational Modeling



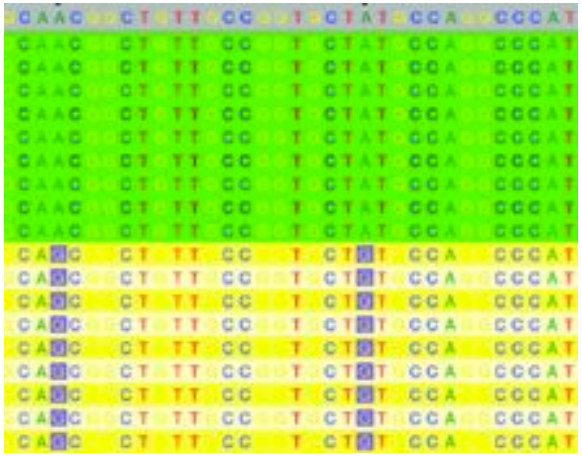


Genomics & Quantitative Biology

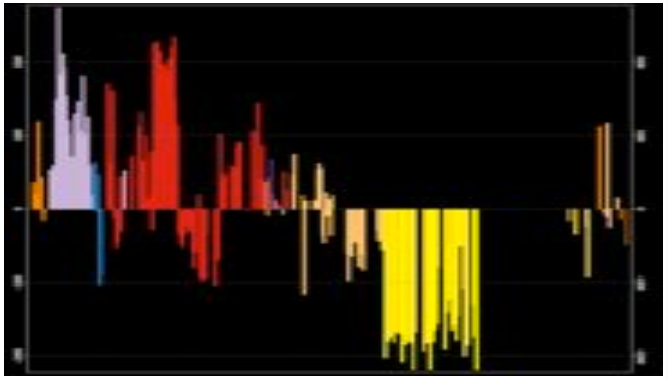
Genome Assembly



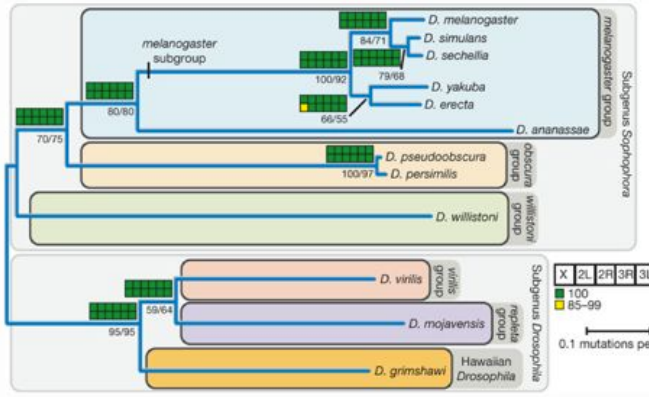
Mutations & Disease



Differential Analysis



Phylogeny & Evolution



Outline

Part 1: Schatz Lab Overview

Part 2: Sequence Alignment

- Exact Matching
- Suffix Arrays
- Bowtie and the BWT

Part 3: Genome Assembly

Part 4: Parallel & Cloud Computing

Searching for GATTACA

- Where is GATTACA in the human genome?
- Strategy 1: Brute Force

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	…
T	G	A	T	T	A	C	A	G	A	T	T	A	C	C	…
G	A	T	T	A	C	A									

No match at offset 1

Searching for GATTACA

- Where is GATTACA in the human genome?
- Strategy 1: Brute Force

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	…
T	G	A	T	T	A	C	A	G	A	T	T	A	C	C	…
	G	A	T	T	A	C	A								

Match at offset 2

Searching for GATTACA

- Where is GATTACA in the human genome?
- Strategy 1: Brute Force

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	…
T	G	A	T	T	A	C	A	G	A	T	T	A	C	C	…
		G	A	T	T	A	C	A	…						

No match at offset 3…

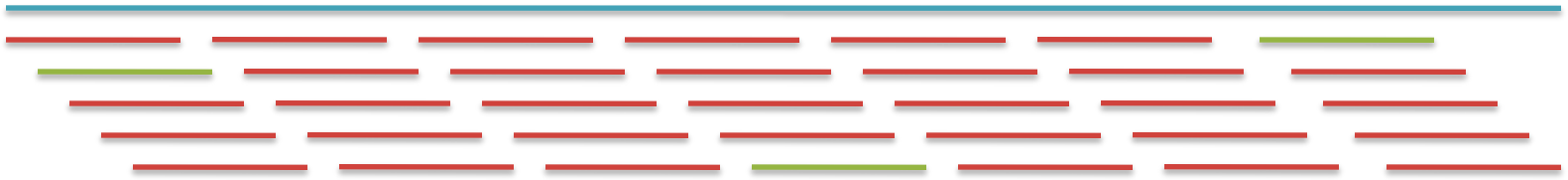
Searching for GATTACA

- Where is GATTACA in the human genome?
- Strategy 1: Brute Force

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	…
T	G	A	T	T	A	C	A	G	A	T	T	A	C	C	…
								G	A	T	T	A	C	A	

No match at offset 9 <- Checking each possible position takes time

Brute Force Analysis



- Brute Force:
  - At every possible offset in the genome:
    - Do all of the characters of the query match?

- Analysis
  - Simple, easy to understand
  - Genome length = n [3B]
  - Query length = m [7]
  - Comparisons: (n-m+1) * m [21B]

- Overall runtime: O(nm)

[How long would it take if we double the genome size, read length?]

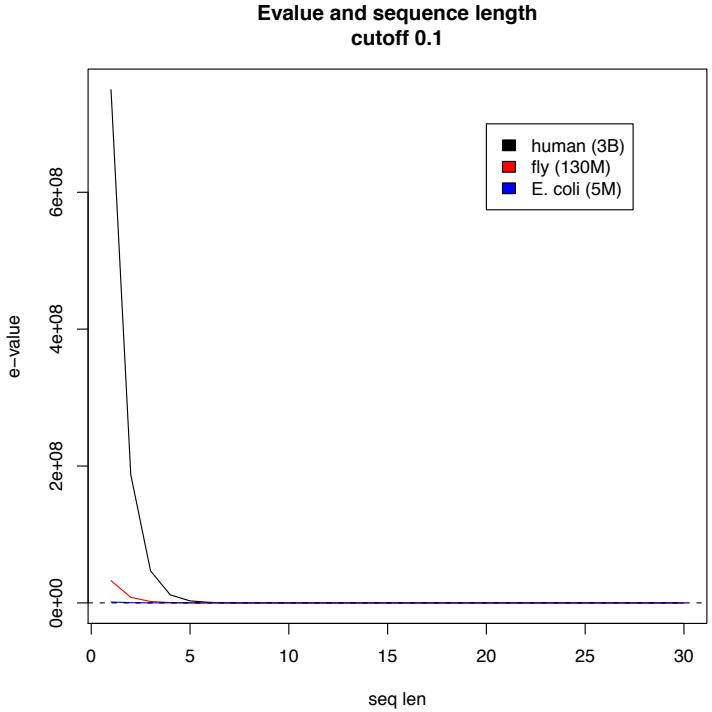
[How long would it take if we double both?]

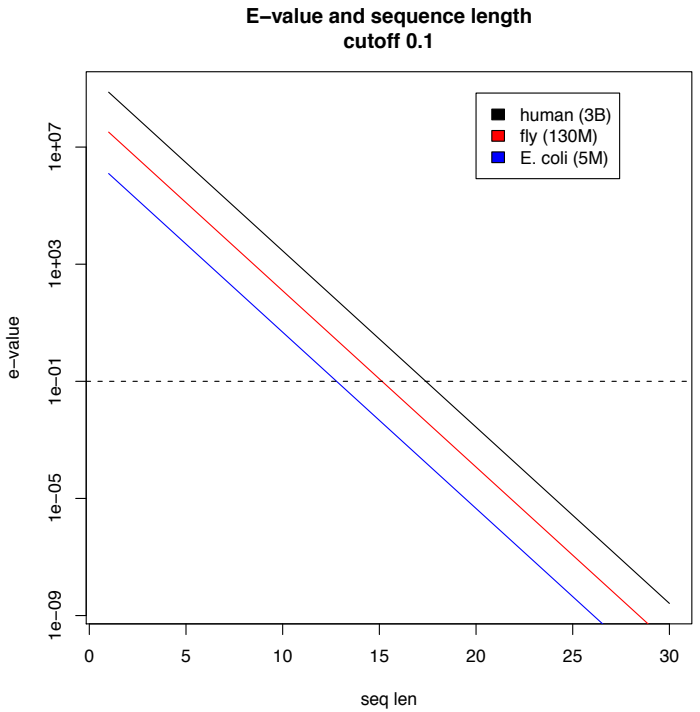
Expected Occurrences

The expected number of occurrences (e-value) of a given sequence in a genome depends on the length of the genome and inversely on the length of the sequence

- 1 in 4 bases are G, 1 in 16 positions are GA, 1 in 64 positions are GAT, …
- 1 in 16,384 should be GATTACA
- $E=n/(4^m)$ [183,105 expected occurrences]

[How long do the reads need to be for a significant match?]





Brute Force Reflections

Why check every position?

– GATTACA can't possibly start at position 15 [WHY?]

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	…
T	G	A	T	T	A	C	A	G	A	T	T	A	C	C	…
								G	A	T	T	A	C	A	

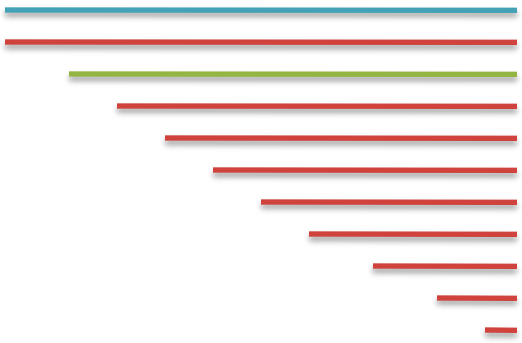
– Improve runtime to O(n + m) [3B + 7]
  - If we double both, it just takes twice as long
  - Knuth-Morris-Pratt, 1977
  - Boyer-Moyer, 1977, 1991

– For one-off scans, this is the best we can do (optimal performance)
  - We have to read every character of the genome, and every character of the query
  - For short queries, runtime is dominated by the length of the genome

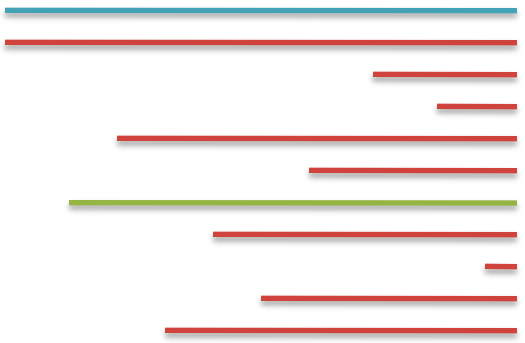
Suffix Arrays: Searching the Phone Book

- What if we need to check many queries?
  - We don't need to check every page of the phone book to find 'Schatz'
  - Sorting alphabetically lets us immediately skip 96% (25/26) of the book *without any loss in accuracy*

- Sorting the genome: Suffix Array (Manber & Myers, 1991)
  - Sort every suffix of the genome



Split into n suffixes



Sort suffixes alphabetically

[Challenge Question: How else could we split the genome?]

Searching the Index

- Strategy 2: Binary search
  - Compare to the middle, refine as higher or lower

- Searching for GATTACA
  - Lo = 1; Hi = 15;

Lo →

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
6	C…	15
7	CAGATTACC…	7
8	CC…	14
9	GATTACAGATTACC…	2
10	GATTACC…	9
11	TACAGATTACC…	5
12	TACC…	12
13	TGATTACAGATTACC…	1
14	TTACAGATTACC…	4
15	TTACC…	11

Hi →

Searching the Index

- Strategy 2: Binary search
  - Compare to the middle, refine as higher or lower

- Searching for GATTACA
  - Lo = 1; Hi = 15; Mid = (1+15)/2 = 8
  - Middle = Suffix[8] = CC

Lo →

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
6	C…	15
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Searching the Index

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- Searching for GATTACA
  - Lo = 1; Hi = 15; Mid = (1+15)/2 = 8
  - Middle = Suffix[8] = CC

    => Higher: Lo = Mid + 1

Lo →

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10	GATTACC…	9
11	TACAGATTACC…	5
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15	TTACC…	11

Hi →

Searching the Index

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- Searching for GATTACA
  - Lo = 1; Hi = 15; Mid = (1+15)/2 = 8
  - Middle = Suffix[8] = CC
    => Higher: Lo = Mid + 1

  - Lo = 9; Hi = 15;

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
6	C…	15
7	CAGATTACC…	7
8	CC…	14
9	GATTACAGATTACC…	2
10	GATTACC…	9
11	TACAGATTACC…	5
12	TACC…	12
13	TGATTACAGATTACC…	1
14	TTACAGATTACC…	4
15	TTACC…	11

Lo →

Hi →

Searching the Index

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  - Compare to the middle, refine as higher or lower

- Searching for GATTACA
  - Lo = 1; Hi = 15; Mid = (1+15)/2 = 8
  - Middle = Suffix[8] = CC

    => Higher: Lo = Mid + 1

  - Lo = 9; Hi = 15; Mid = (9+15)/2 = 12
  - Middle = Suffix[12] = TACC

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
6	C…	15
7	CAGATTACC…	7
8	CC…	14
9	GATTACAGATTACC…	2
10	GATTACC…	9
11	TACAGATTACC…	5
12	TACC…	12
13	TGATTACAGATTACC…	1
14	TTACAGATTACC…	4
15	TTACC…	11

Lo →

Hi →

Searching the Index

- Strategy 2: Binary search
  - Compare to the middle, refine as higher or lower

- Searching for GATTACA
  - Lo = 1; Hi = 15; Mid = (1+15)/2 = 8
  - Middle = Suffix[8] = CC
    => Higher: Lo = Mid + 1

  - Lo = 9; Hi = 15; Mid = (9+15)/2 = 12
  - Middle = Suffix[12] = TACC
    => Lower: Hi = Mid - 1

  - Lo = 9; Hi = 11;

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
6	C…	15
7	CAGATTACC…	7
8	CC…	14
9	GATTACAGATTACC…	2
10	GATTACC…	9
11	TACAGATTACC…	5
12	TACC…	12
13	TGATTACAGATTACC…	1
14	TTACAGATTACC…	4
15	TTACC…	11

Lo →

Hi →

Searching the Index

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- Searching for GATTACA
  - Lo = 1; Hi = 15; Mid = (1+15)/2 = 8
  - Middle = Suffix[8] = CC
    => Higher: Lo = Mid + 1

  - Lo = 9; Hi = 15; Mid = (9+15)/2 = 12
  - Middle = Suffix[12] = TACC
    => Lower: Hi = Mid - 1

  - Lo = 9; Hi = 11; Mid = (9+11)/2 = 10
  - Middle = Suffix[10] = GATTACC

	#	Sequence	Pos
	1	ACAGATTACC…	6
	2	ACC…	13
	3	AGATTACC…	8
	4	ATTACAGATTACC…	3
	5	ATTACC…	10
	6	C…	15
	7	CAGATTACC…	7
Lo →	8	CC…	14
	9	GATTACAGATTACC…	2
	10	GATTACC…	9
Hi →	11	TACAGATTACC…	5
	12	TACC…	12
	13	TGATTACAGATTACC…	1
	14	TTACAGATTACC…	4
	15	TTACC…	11

Searching the Index

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  - Compare to the middle, refine as higher or lower

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  - Lo = 1; Hi = 15; Mid = (1+15)/2 = 8
  - Middle = Suffix[8] = CC
    => Higher: Lo = Mid + 1

  - Lo = 9; Hi = 15; Mid = (9+15)/2 = 12
  - Middle = Suffix[12] = TACC
    => Lower: Hi = Mid - 1

  - Lo = 9; Hi = 11; Mid = (9+11)/2 = 10
  - Middle = Suffix[10] = GATTACC
    => Lower: Hi = Mid - 1

  - Lo = 9; Hi = 9;

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
6	C…	15
7	CAGATTACC…	7
8	CC…	14
9	GATTACAGATTACC…	2
10	GATTACC…	9
11	TACAGATTACC…	5
12	TACC…	12
13	TGATTACAGATTACC…	1
14	TTACAGATTACC…	4
15	TTACC…	11

Lo
Hi

Searching the Index

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  - Lo = 1; Hi = 15; Mid = (1+15)/2 = 8
  - Middle = Suffix[8] = CC
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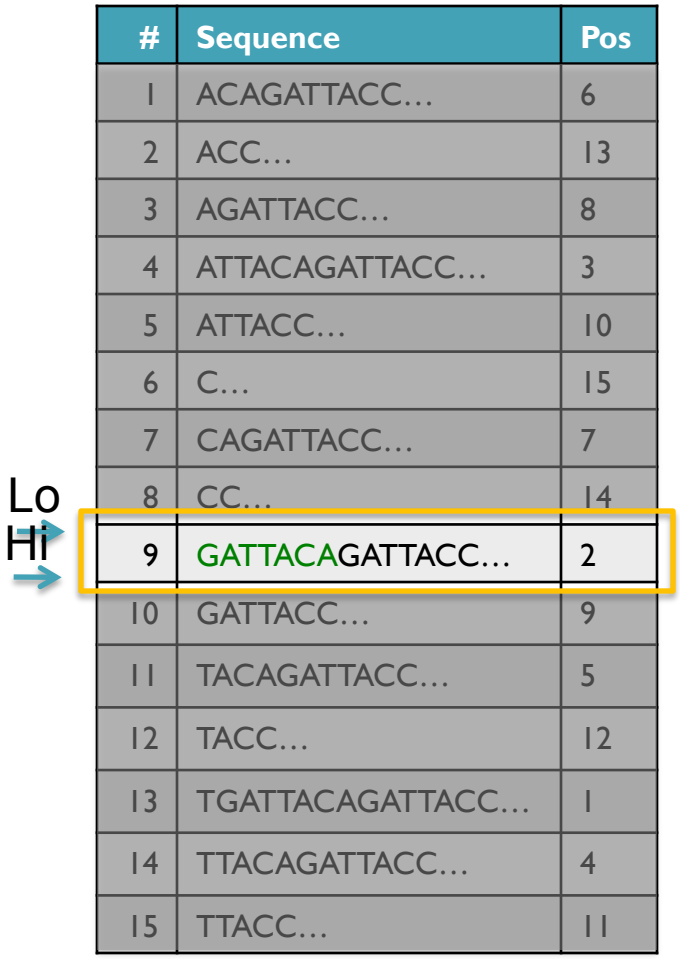
  - Lo = 9; Hi = 15; Mid = (9+15)/2 = 12
  - Middle = Suffix[12] = TACC
    => Lower: Hi = Mid - 1

  - Lo = 9; Hi = 11; Mid = (9+11)/2 = 10
  - Middle = Suffix[10] = GATTACC
    => Lower: Hi = Mid - 1

  - Lo = 9; Hi = 9; Mid = (9+9)/2 = 9
  - Middle = Suffix[9] = GATTACA…
    => Match at position 2!



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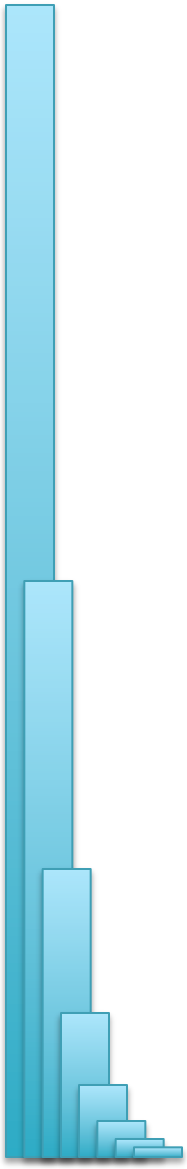
Binary Search Analysis



- Binary Search
  - Initialize search range to entire list
    - mid = (hi+lo)/2; middle = suffix[mid]
    - if query matches middle: done
    - else if query < middle: pick low range
    - else if query > middle: pick hi range
  - Repeat until done or empty range [WHEN?]

- Analysis
  - More complicated method
  - How many times do we repeat?
    - How many times can it cut the range in half?
    - Find smallest x such that: $n/(2^x) \leq 1$; $x = \lg_2(n)$ [32]

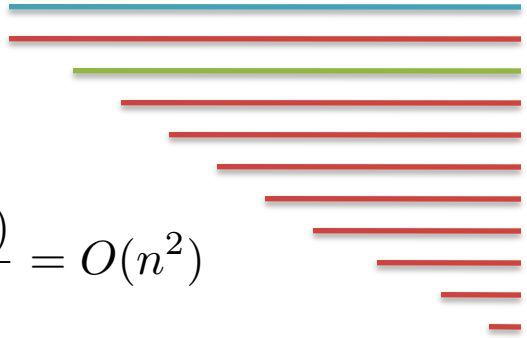
- Total Runtime: O(m lg n)
  - More complicated, but much faster!
  - Looking up a query loops 32 times instead of 3B

[How long does it take to search 6B or 24B nucleotides?]

Suffix Array Construction

- How can we store the suffix array?

  [How many characters are in all suffixes combined?]



$$S = 1 + 2 + 3 + \cdots + n = \sum_{i=1}^{n} i = \frac{n(n+1)}{2} = O(n^2)$$

  - Hopeless to explicitly store 4.5 billion billion characters
  - Instead use implicit representation
    - Keep 1 copy of the genome, and a list of sorted offsets
    - Storing 3 billion offsets fits on a server (12GB)

- Searching the array is very fast, but it takes time to construct
  - This time will be amortized over many, many searches
  - Run it once "overnight" and save it away for all future queries

Pos
6
13
8
3
10
15
7
14
2
9
5
12
1
4
11

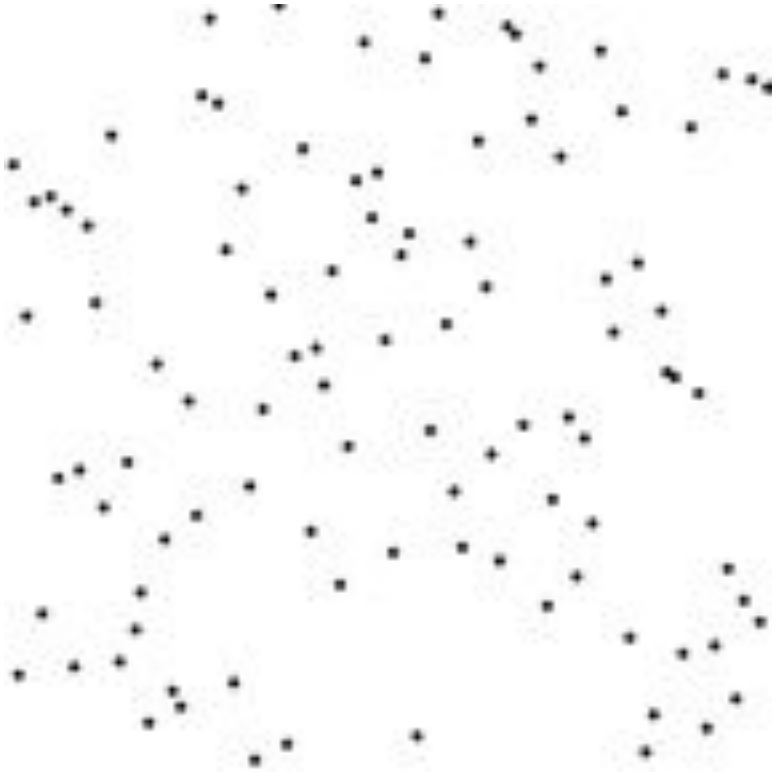
TGATTACAGATTACC

Sorting

Quickly sort these numbers into ascending order:
14, 29, 6, 31, 39, 64, 78, 50, 13, 63, 61, 19

[How do you do it?]

6, 13, 14, 29, 31, 39, 64, 78, 50, 63, 61, 19
6, 13, 14, 29, 31, 39, 64, 78, 50, 63, 61, 19
6, 13, 14, 19, 29, 31, 39, 64, 78, 50, 63, 61
6, 13, 14, 19, 29, 31, 39, 64, 78, 50, 63, 61
6, 13, 14, 19, 29, 31, 39, 64, 78, 50, 63, 61
6, 13, 14, 19, 29, 31, 39, 50, 64, 78, 63, 61
6, 13, 14, 19, 29, 31, 39, 50, 61, 64, 78, 63
6, 13, 14, 19, 29, 31, 39, 50, 61, 63, 64, 78
6, 13, 14, 19, 29, 31, 39, 50, 61, 63, 64, 78
6, 13, 14, 19, 29, 31, 39, 50, 61, 63, 64, 78
6, 13, 14, 19, 29, 31, 39, 50, 61, 63, 64, 78



http://en.wikipedia.org/wiki/Selection_sort

Selection Sort Analysis

- Selection Sort (Input: list of n numbers)

```
for pos = 1 to n
    // find the smallest element in [pos, n]
    smallest = pos
    for check = pos+1 to n
        if (list[check] < list[smallest]): smallest = check

    // move the smallest element to the front
    tmp = list[smallest]
    list[pos] = list[smallest]
    list[smallest] = tmp
```

- Complexity Analysis

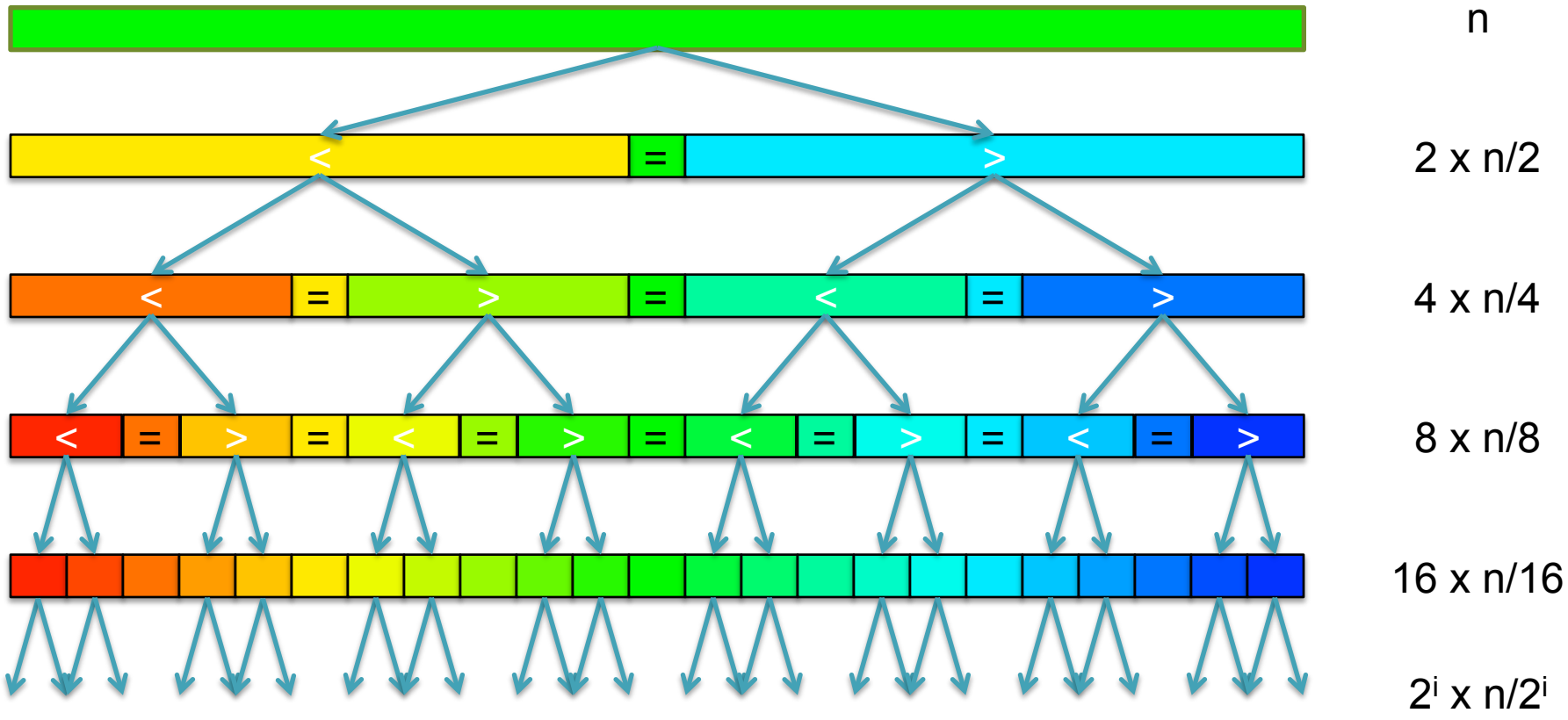
$$T = n + (n-1) + (n-2) + \cdots + 3 + 2 + 1 = \sum_{i=1}^{n} i = \frac{n(n+1)}{2} = O(n^2)$$

  - Outer loop: pos = 1 to n
  - Inner loop: check = pos to n
  - Running time: Outer * Inner = $O(n^2)$ [4.5 Billion Billion]

[Challenge Questions: Why is this slow? / Can we sort any faster?]

Divide and Conquer

- Selection sort is slow because it rescans the entire list for each element
  - How can we split up the unsorted list into independent ranges?
  - Hint 1: Binary search splits up the problem into 2 independent ranges (hi/lo)
  - Hint 2: Assume we know the median value of a list



[How many times can we split of n items a list in half?]

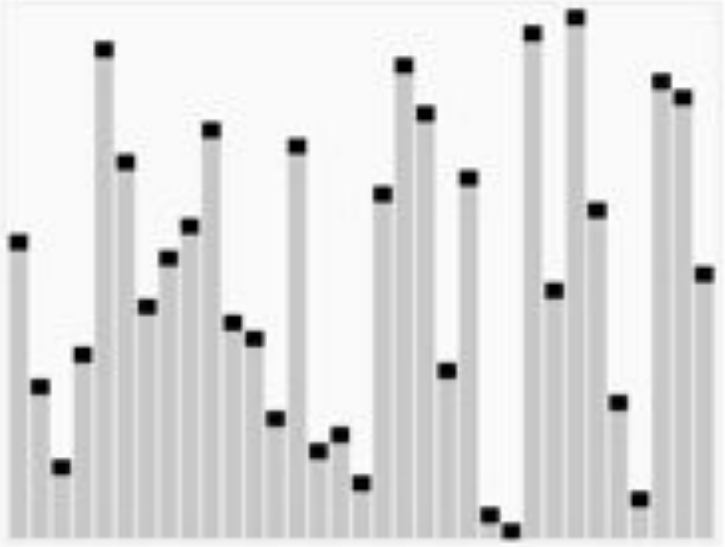
QuickSort Analysis

- QuickSort(Input: list of n numbers)

```
// see if we can quit
if (length(list)) <= 1): return list

// split list into lo & hi
pivot = median(list)
lo = {}; hi = {};
for (i = 1 to length(list))
      if (list[i] < pivot): append(lo, list[i])
      else:                 append(hi, list[i])

// recurse on sublists
return (append(QuickSort(lo), QuickSort(hi))
```



http://en.wikipedia.org/wiki/Quicksort

- Complexity Analysis (Assume we can find the median in O(n))

$$T(n) = \begin{cases} O(1) & \text{if } n \leq 1 \\ O(n) + 2T(n/2) & \text{else} \end{cases}$$

$$T(n) = n + 2(\frac{n}{2}) + 4(\frac{n}{4}) + \cdots + n(\frac{n}{n}) = \sum_{i=0}^{lg(n)} \frac{2^i n}{2^i} = \sum_{i=0}^{lg(n)} n = O(n \lg n)$$

[~94B]

QuickSort Analysis

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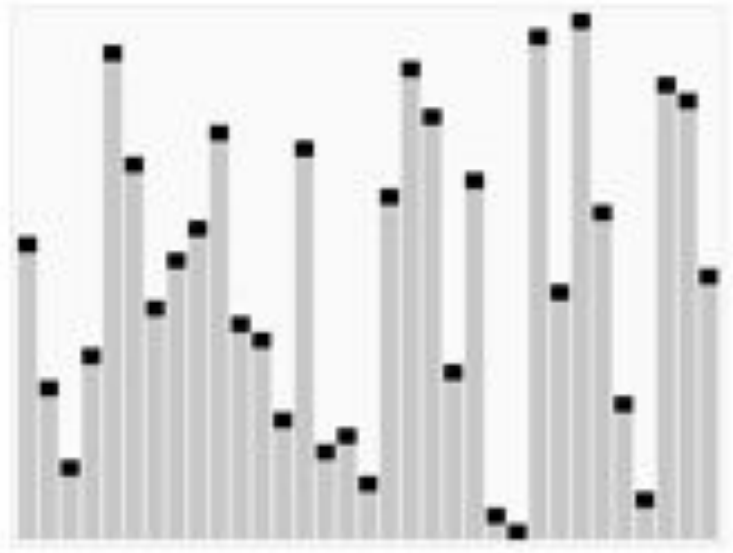


http://en.wikipedia.org/wiki/Quicksort

- Complexity Analysis (Assume we can find the median in O(n))

$$T(n) = \begin{cases} O(1) & \text{if } n \leq 1 \\ O(n) + 2T(n/2) & \text{else} \end{cases}$$

$$T(n) = n + 2(\frac{n}{2}) + 4(\frac{n}{4}) + \cdots + n(\frac{n}{n}) = \sum_{i=0}^{lg(n)} \frac{2^i n}{2^i} = \sum_{i=0}^{lg(n)} n = O(n \lg n)$$

[~94B]

In-exact alignment

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	…
T	G	A	T	T	A	C	A	G	A	T	T	A	C	C	…

- Where is GATTACA *approximately* in the human genome?
  - And how do we efficiently find them?

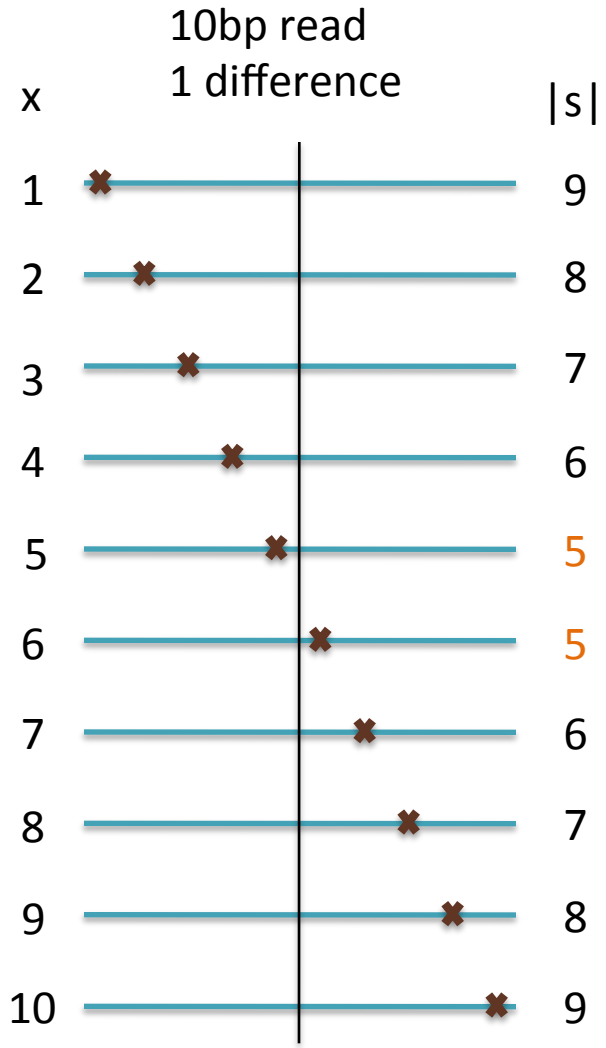
- It depends…
  - Define 'approximately'
    - Hamming Distance, Edit distance, or Sequence Similarity
    - Ungapped vs Gapped vs Affine Gaps, Global vs Local
  - Algorithm depends on the data characteristics & goals
    - Smith-Waterman: Exhaustive search for optimal alignments
    - BLAST: Hash-table based homology searches
    - Bowtie: BWT alignment for short read mapping

Seed-and-Extend Alignment

Theorem: An alignment of a sequence of length *m* with at most *k* differences ***must*** contain an exact match at least $s=m/(k+1)$ bp long
(*Baeza-Yates* and Perleberg, 1996)

- Proof: Pigeonhole principle
  - 1 pigeon can't fill 2 holes

- Seed-and-extend search
  - Use an index to rapidly find short exact alignments to seed longer in-exact alignments
    - BLAST, MUMmer, Bowtie, BWA, SOAP, …

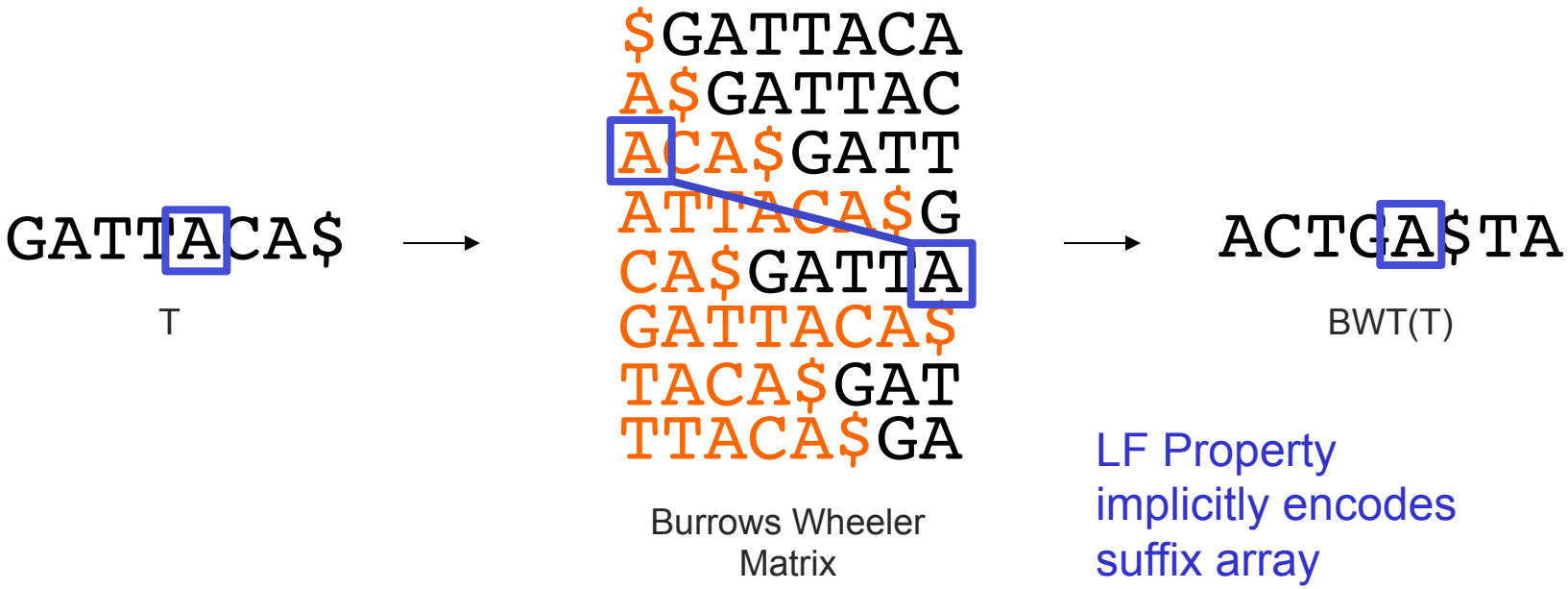


[How could you use seed-and-extend with a suffix array?]

Bowtie: Ultrafast and memory efficient alignment of short DNA sequences to the human genome

Slides Courtesy of Ben Langmead
(langmead@umiacs.umd.edu)

Burrows-Wheeler Transform



- Suffix Array is fast to search, but much larger than genome
  - BWT is a reversible permutation of the genome based on the suffix array
  - Core index for Bowtie (Langmead *et al.*, 2009) and most recent short read mapping applications

A block sorting lossless data compression algorithm.
Burrows M, Wheeler DJ (1994) *Digital Equipment Corporation, Palo Alto, CA* 1994, Technical Report 124

Bowtie algorithm

Reference

BWT(Reference)

Query:
A A T G A T A C G G C G A C C A C C G A G A T C T A

Bowtie algorithm

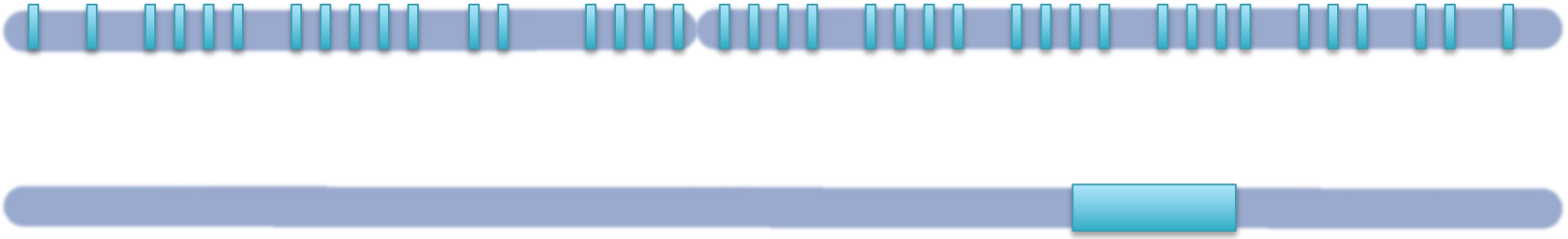
Reference

BWT(Reference)

Query:
A A T G A T A C G G C G A C C A C C G A G A T C T A

Bowtie algorithm

Reference

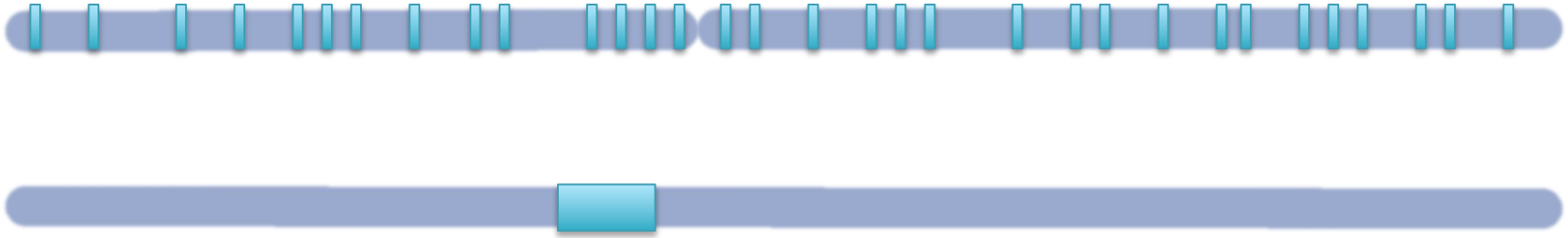


BWT(Reference)

Query:
AATGATACGGCGACCACCGAGATCTA

Bowtie algorithm

Reference

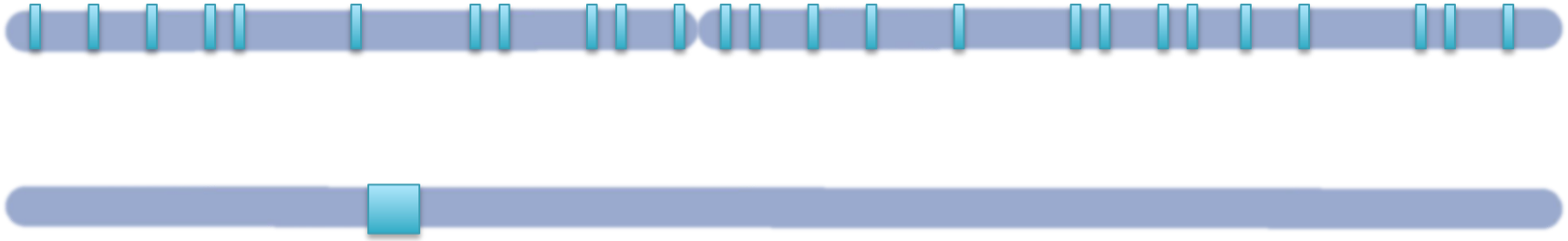


BWT(Reference)

Query:
AATGATACGGCGACCACCGAGATCTA

Bowtie algorithm

Reference



BWT(Reference)

Query:
AATGATACGGCGACCACCGAGATCTA

Bowtie algorithm

Reference

BWT(Reference)

Query:

A A T G A T A C G G C G A C C A C C G A G A T C T A

Bowtie algorithm

Reference

BWT(Reference)

Query:
AATGATACGGCGACCACCGAGATCTA

Bowtie algorithm

Reference

BWT(Reference)

Query:

A A T G T T A C G G C G A C C A C C G A G A T C T A

Bowtie algorithm

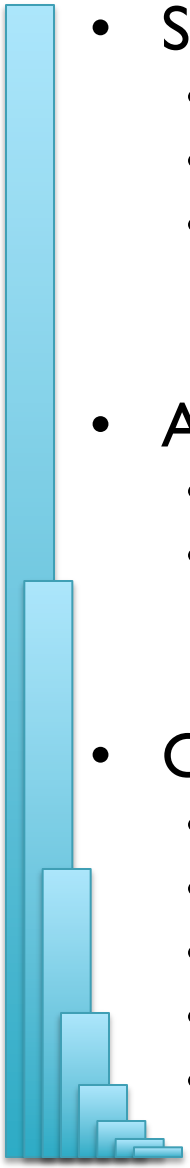
Reference

BWT(Reference)

Query:

A A T G T T A C G G C G A C C A C C G A G A T C T A

Part 1: Summary



- Short Read Mapping: Seed-and-extend search of the BWT
  - If we fail to reach the end, back-track and resume search
  - The beginning of the read is used as high confidence seed
  - 100s of times faster than competing approaches, works entirely in RAM
- Algorithms choreograph the dance of data inside the machine
  - Algorithms add provable precision to your method
  - A smarter algorithm can solve the same problem with much less work
- Computational Techniques
  - ***Binary search***: Fast lookup in any sorted list
  - ***Divide-and-conquer***: Split a hard problem into an easier problem
  - ***Recursion***: Solve a problem using a function of itself
  - ***Indexing***: Focus on just the important parts
  - ***Seed-and-extend***: Anchor the problem using a portion of it

Break


READING THE ENTIRE GENOME CODE...
Listen to this part: 'AGGCTAATC CGCATAACTG'
Wow!

Outline

Part 1: Schatz Lab Overview

Part 2: Sequence Alignment

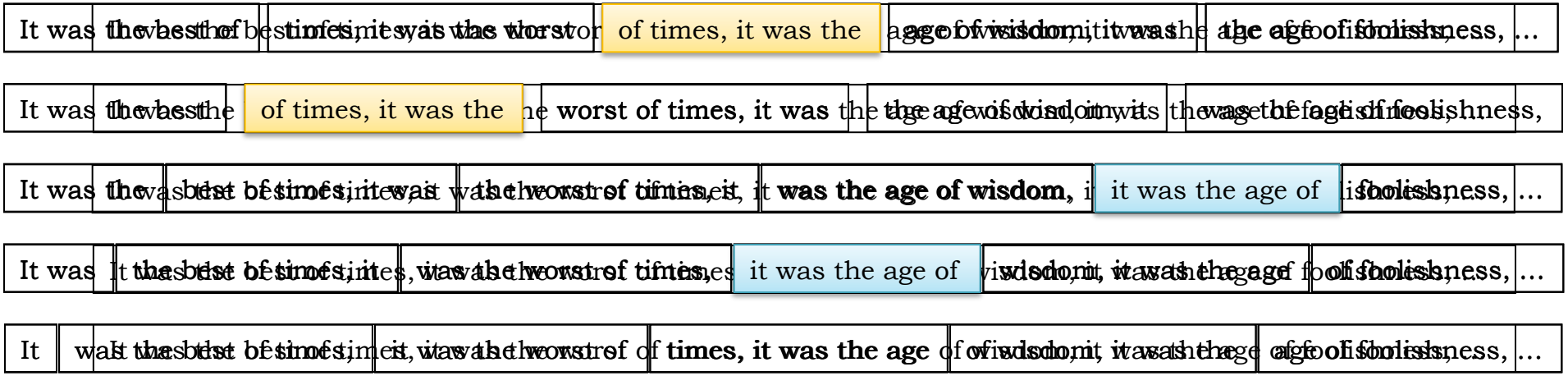
Part 3: Genome Assembly

- Assembly by analogy
- Coverage, read length, and repeats
- Contiging & Scaffolding
- Assembly Forensics

Part 4: Parallel & Cloud Computing

Shredded Book Reconstruction

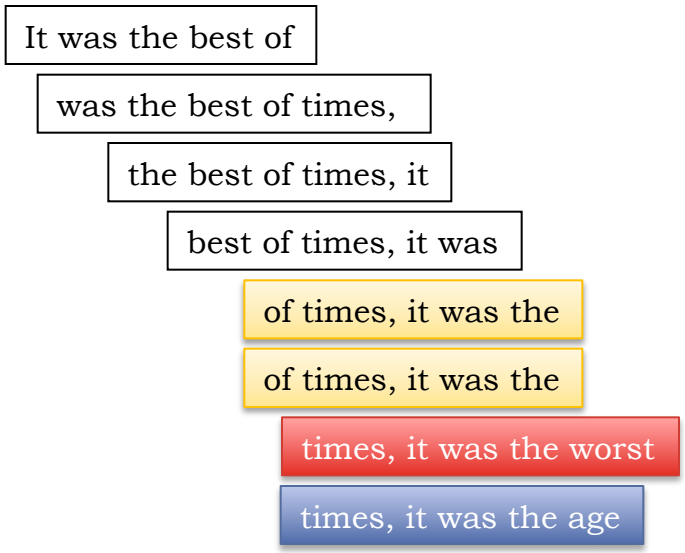
- Dickens accidentally shreds the first printing of A Tale of Two Cities
  - Text printed on 5 long spools



- How can he reconstruct the text?
  - 5 copies x 138, 656 words / 5 words per fragment = 138k fragments
  - The short fragments from every copy are mixed together
  - Some fragments are identical

Greedy Reconstruction

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



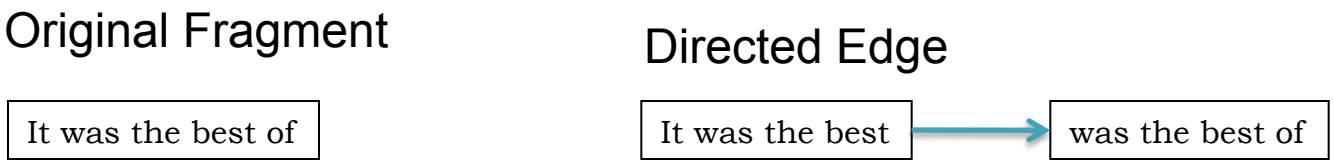
The repeated sequence make the correct reconstruction ambiguous

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

[Any ideas on how to proceed?]

de Bruijn Graph Construction

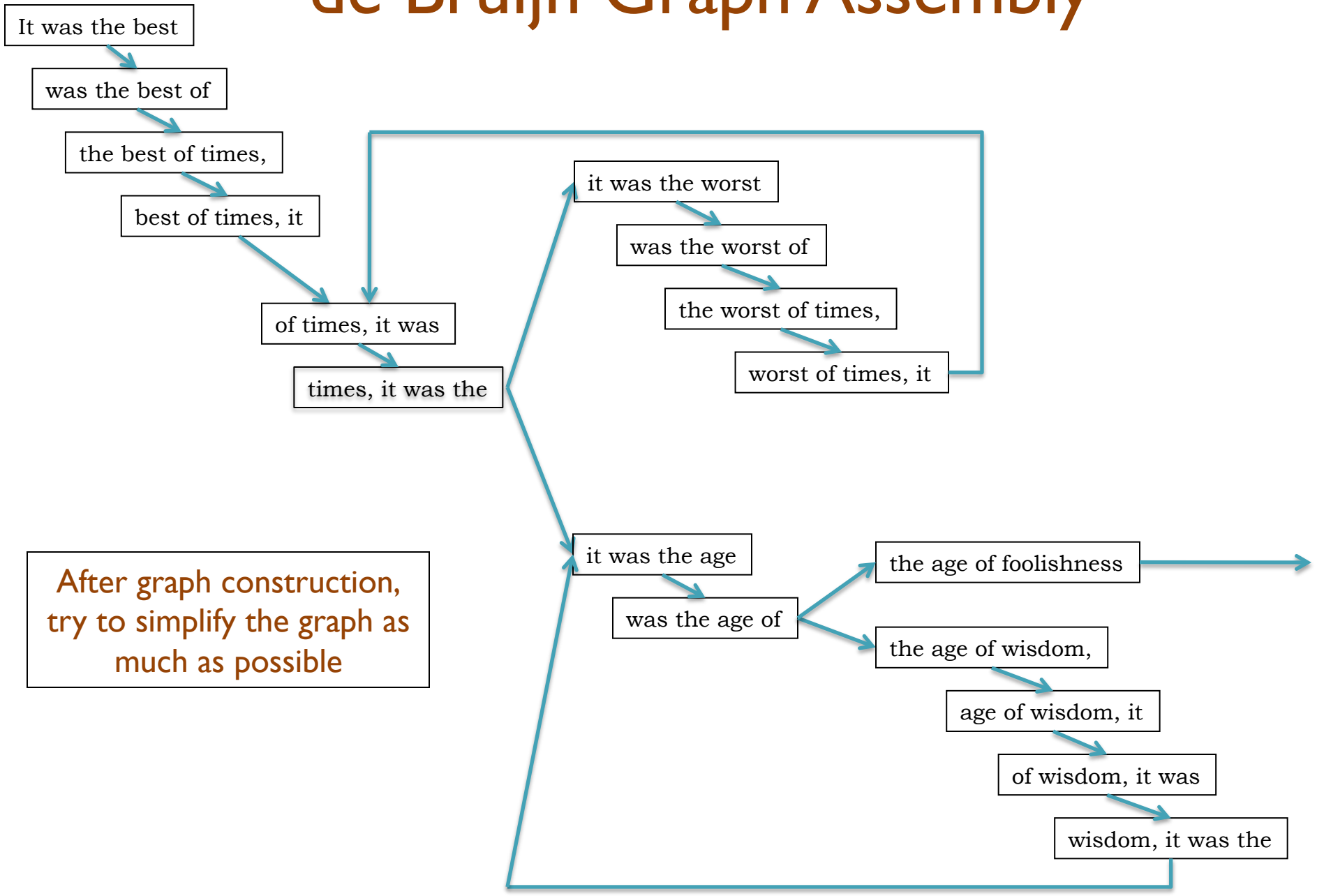
- $D_k = (V,E)$
  - V = All length-k subfragments (k < l)
  - E = Directed edges between consecutive subfragments
    - Nodes overlap by k-1 words



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

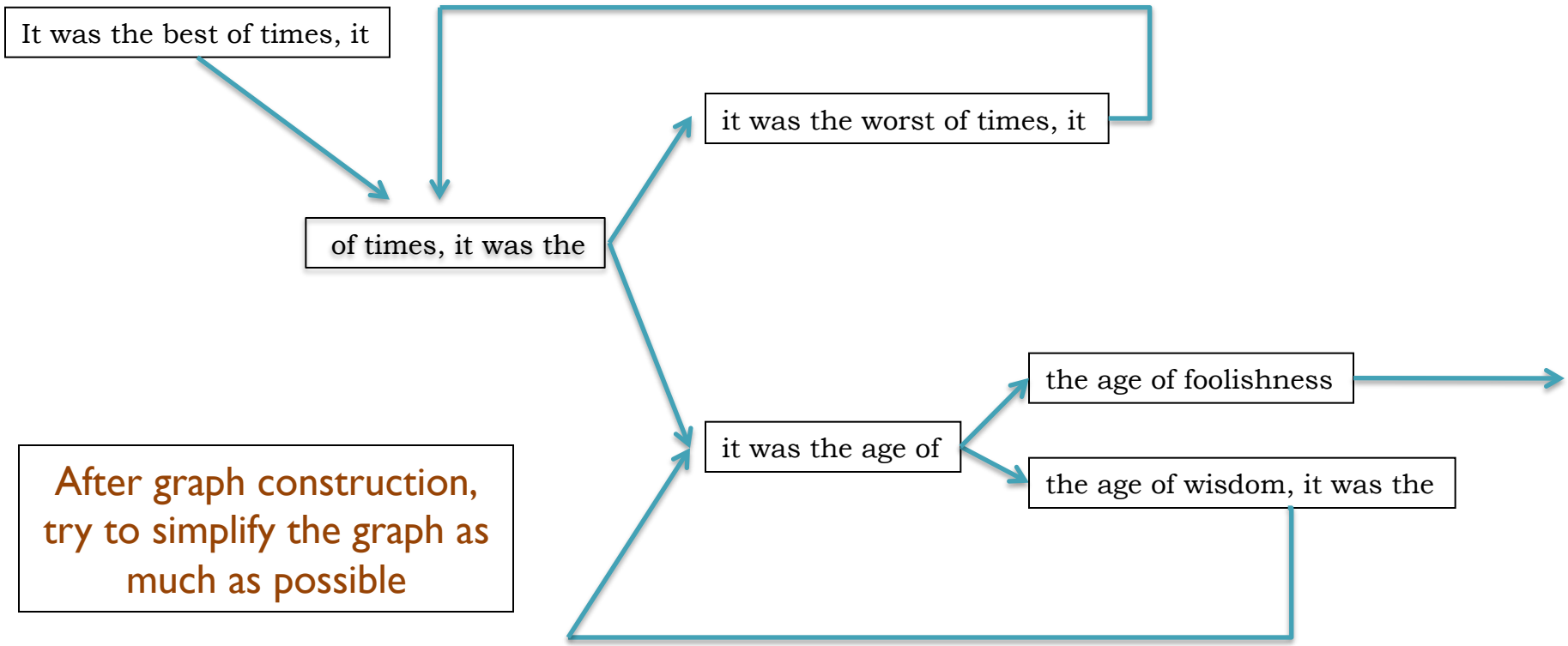
de Bruijn, 1946
Idury and Waterman, 1995
Pevzner, Tang, Waterman, 2001

de Bruijn Graph Assembly



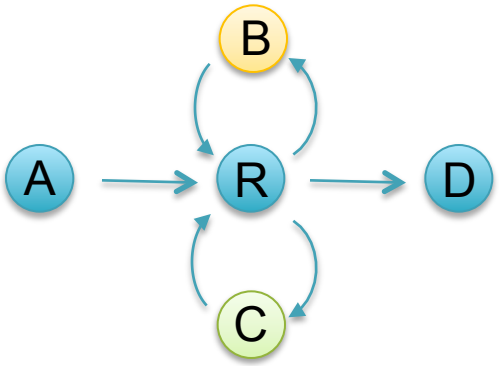
After graph construction, try to simplify the graph as much as possible

de Bruijn Graph Assembly



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

Counting Eulerian Tours



ARBRCRD
or
ARCRBRD

Generally an exponential number of compatible sequences

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

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

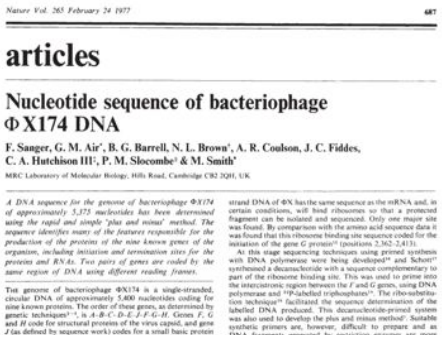
L = *n x n* matrix with r_u-a_{uu} along the diagonal and -a_{uv} in entry uv

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*.

Milestones in Genome Assembly



articles

Nucleotide sequence of bacteriophage ΦX174 DNA

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

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

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

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

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

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

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

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

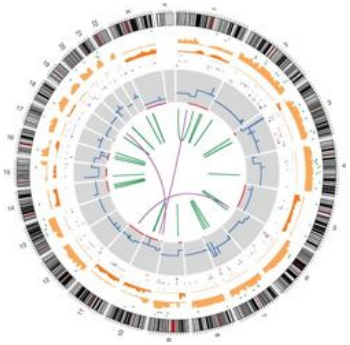
Like Dickens, we must computationally reconstruct a genome from short fragments

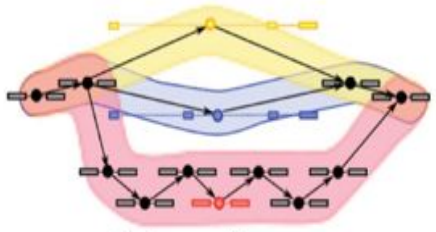
Current Applications

- Novel genomes

- Metagenomes

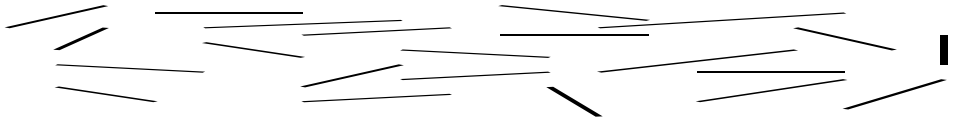
- Sequencing assays
  - Structural variations
  - Transcript assembly
  - …





Assembling a Genome

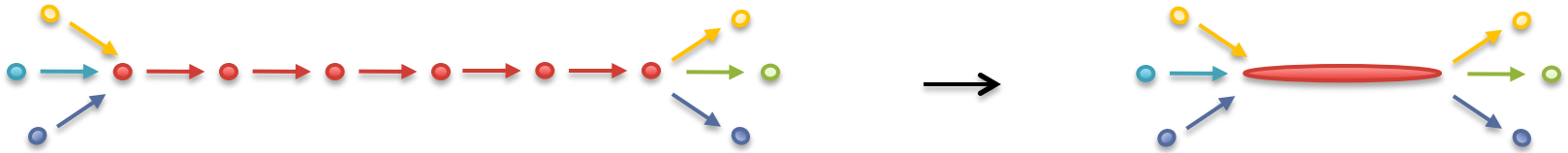
1. Shear & Sequence DNA



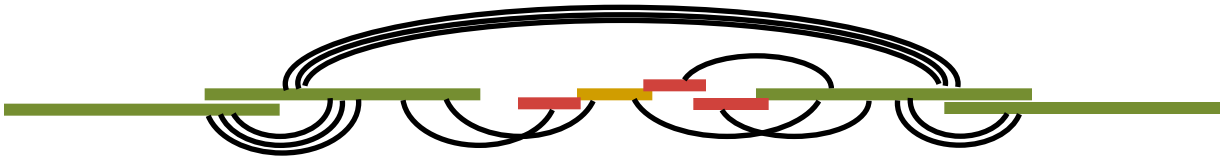
2. Construct assembly graph from overlapping reads

...AGCCTAGACCTACAGGATGCGCGACACGT
GGATGCGCGACACGTCGCATATCCGGT...

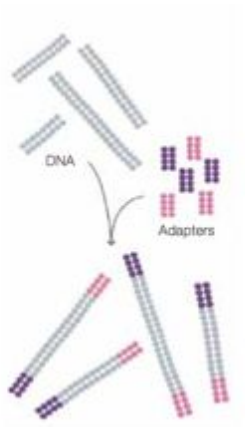
3. Simplify assembly graph



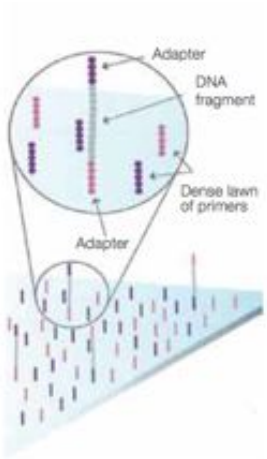
4. Detangle graph with long reads, mates, and other links



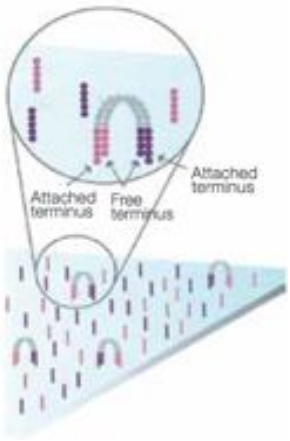
Illumina Sequencing by Synthesis



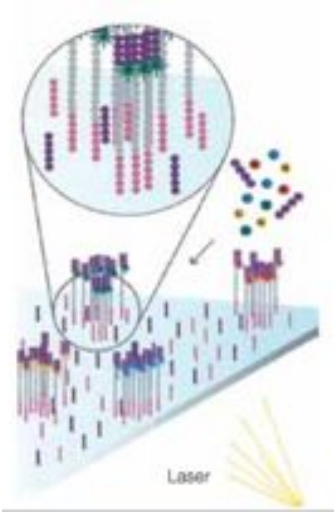
1. Prepare



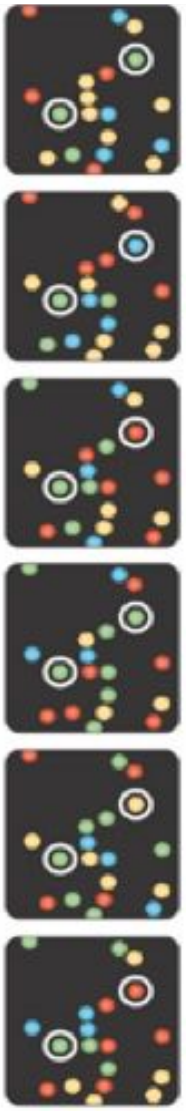
2. Attach



3. Amplify



4. Image



5. Basecall

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

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

Paired-end and Mate-pairs

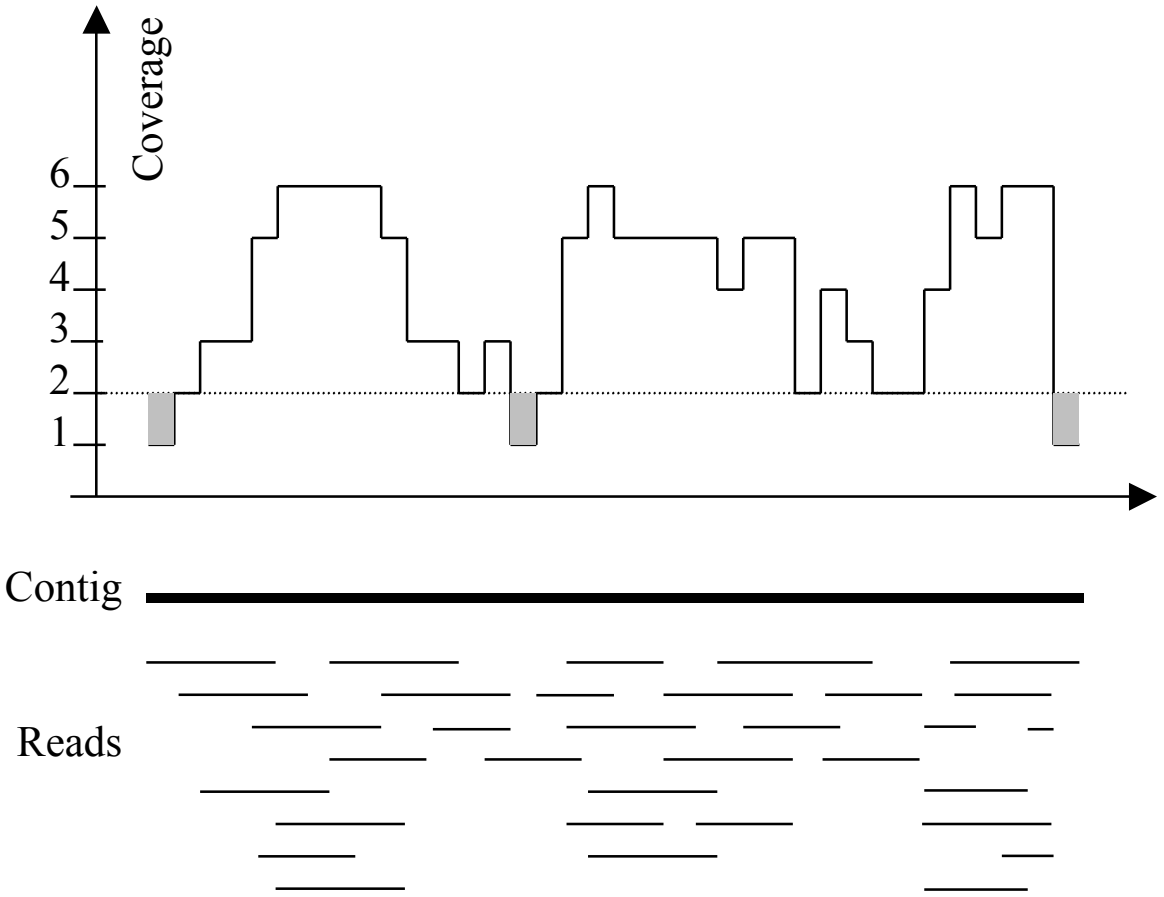
Paired-end sequencing

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

Mate-pair sequencing

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

Typical genome coverage

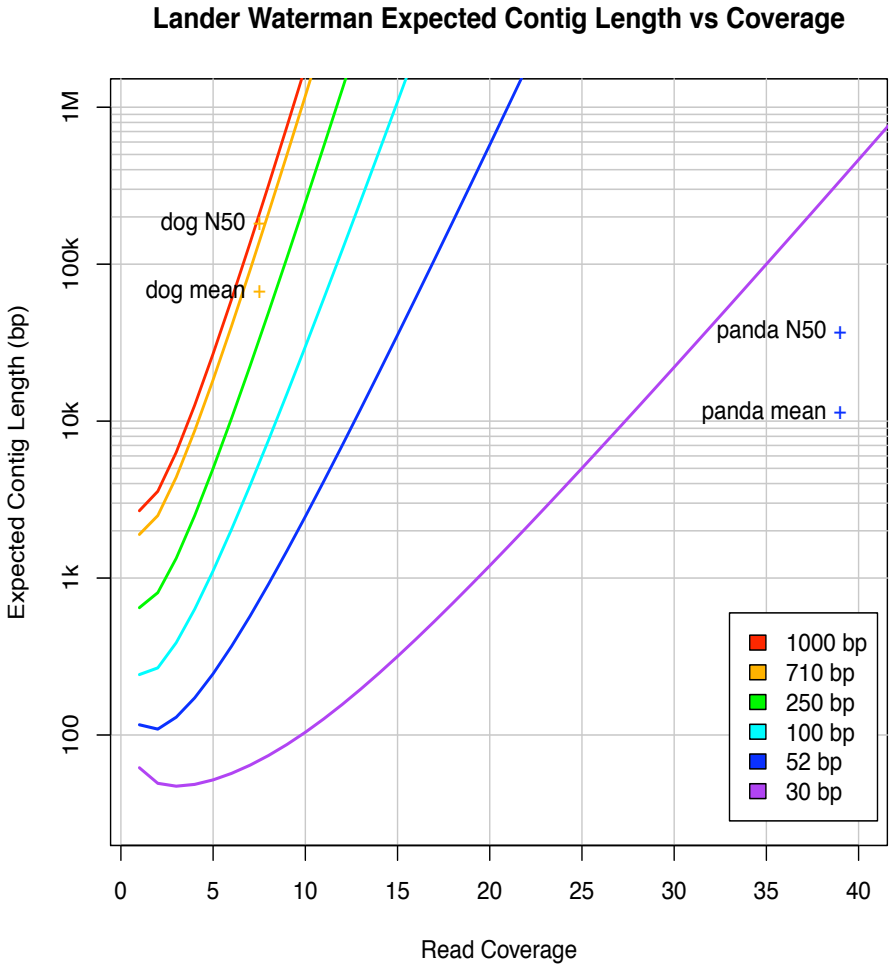


Imagine raindrops on a sidewalk

Coverage and Read Length

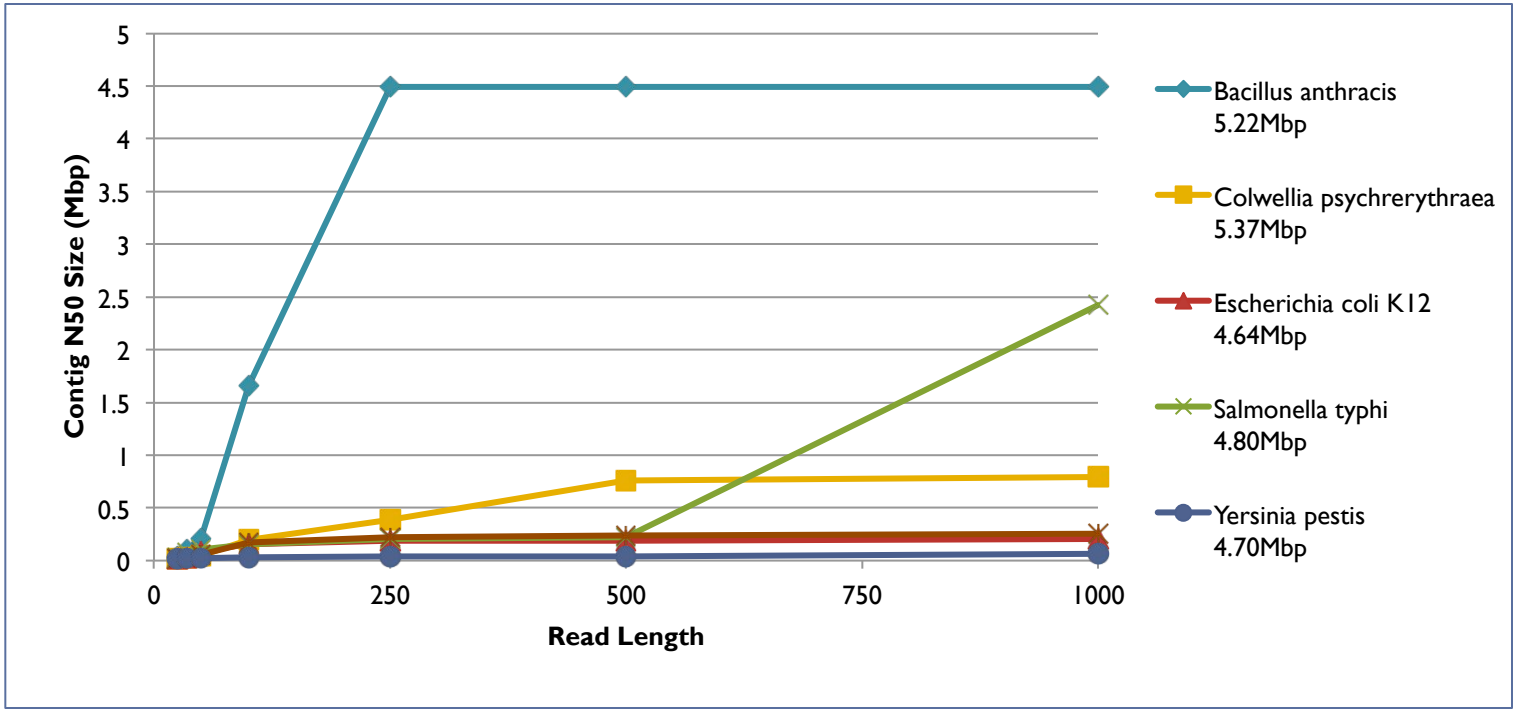
Idealized Lander-Waterman model

- Reads start at perfectly random positions
- Poisson distribution in coverage
  - Contigs end when there are no overlapping reads
- Contig length is a function of coverage and read length
  - Effective coverage reduced by *o/l*
  - Short reads require much higher coverage to reach same expected contig length



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

Repeats and Read Length



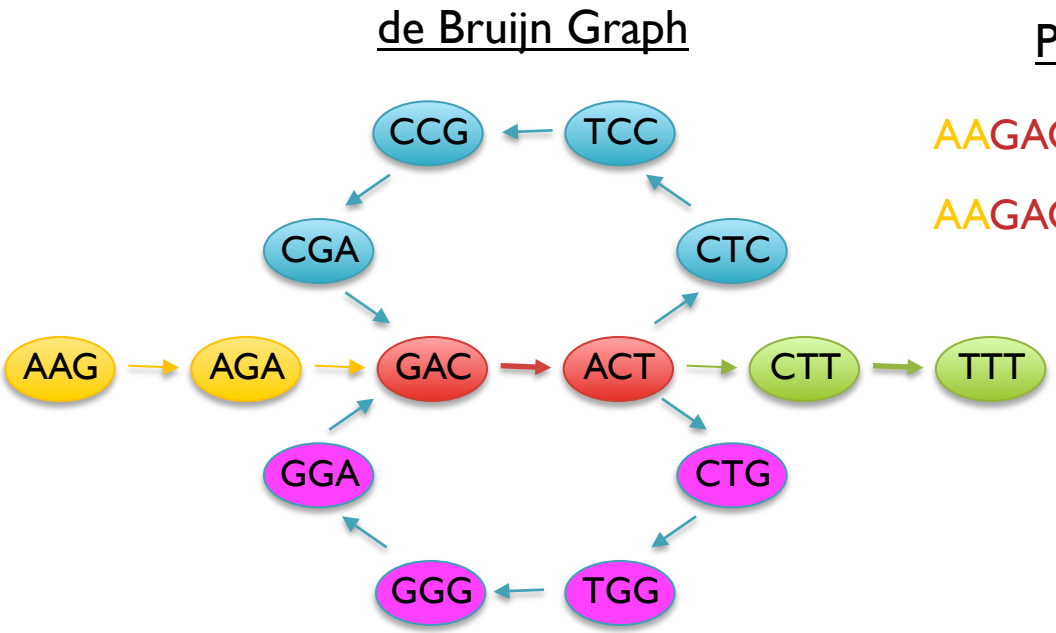
- Explore the relationship between read length and contig N50 size
  - Idealized assembly of read lengths: 25, 35, 50, 100, 250, 500, 1000
  - Contig/Read length relationship depends on specific repeat composition

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

Short Read Assembly

Reads

AAGA
ACTT
ACTC
ACTG
AGAG
CCGA
CGAC
CTCC
CTGG
CTTT
…



Potential Genomes

AAGACTCCGACTGGGACTTT

AAGACTGGGACTCCGACTTT

- Genome assembly as finding an Eulerian tour of the de Bruijn graph
  - Human genome: >3B nodes, >10B edges
- The new short read assemblers require tremendous computation
  - Velvet (Zerbino & Birney, 2008) serial: > 2TB of RAM
  - ABySS (Simpson *et al.*, 2009) MPI: 168 cores x ~96 hours
  - SOAPdenovo (Li *et al.*, 2010) pthreads: 40 cores x 40 hours, >140 GB RAM

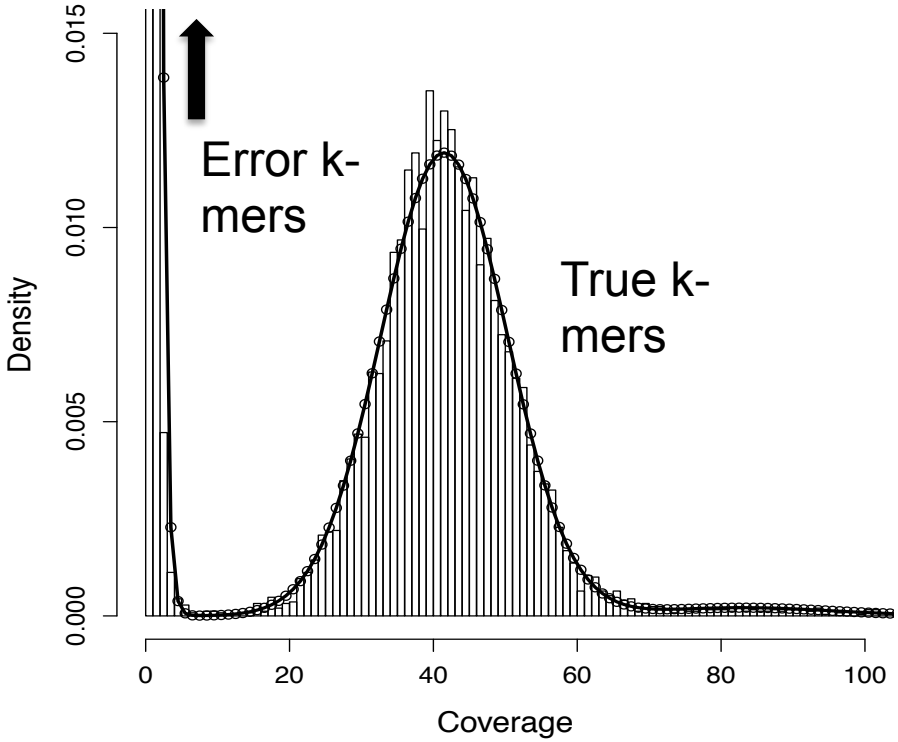
Error Correction with Quake

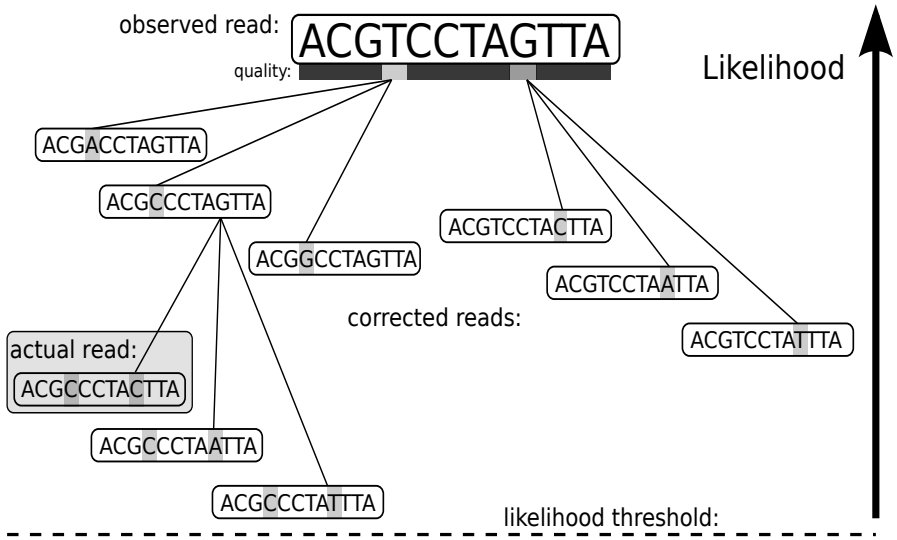
1. Count all "Q-mers" in reads

- Fit coverage distribution to mixture model of errors and regular coverage
- Automatically determines threshold for trusted k-mers

2. Correction Algorithm

- Considers editing erroneous kmers into trusted kmers in decreasing likelihood
- Includes quality values, nucleotide/nucleotide substitution rate

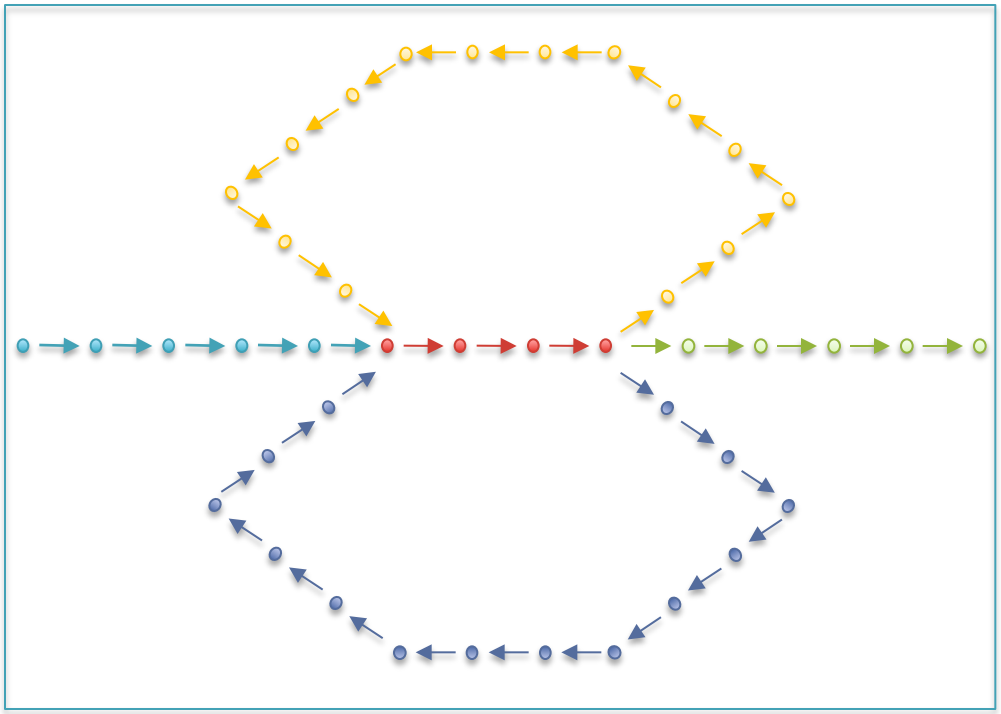


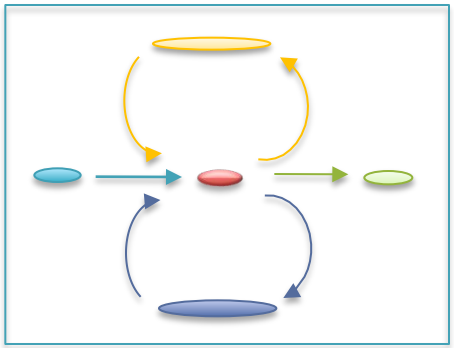


Quake: quality-aware detection and correction of sequencing reads.
Kelley, DR, Schatz, MC, Salzberg SL (2010) *Genome Biology*. 11:R116

Graph Compression

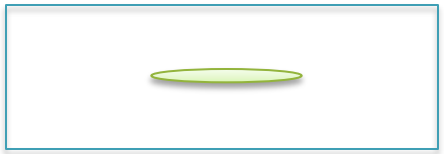
- After construction, many edges are unambiguous
  - Merge together compressible nodes
  - Error correction reduces number of nodes, number of false edges, and allows for longer word size



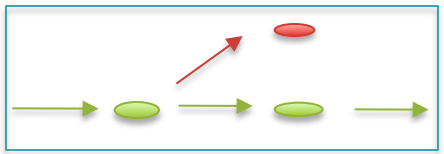


Node Types

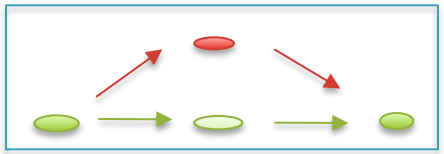
(Chaisson, 2009)



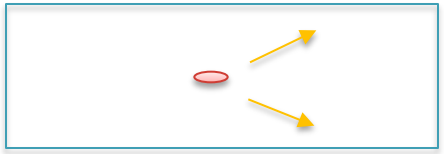
Isolated nodes (10%)



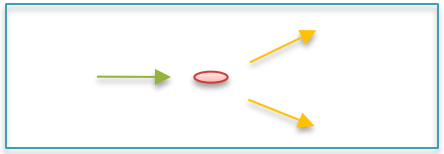
Tips (46%)



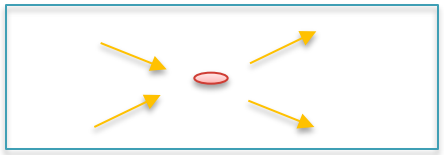
Bubbles/Non-branch (9%)



Dead Ends (.2%)



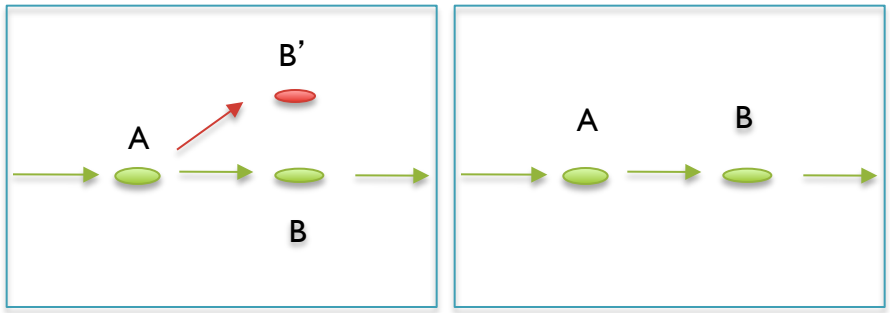
Half Branch (25%)



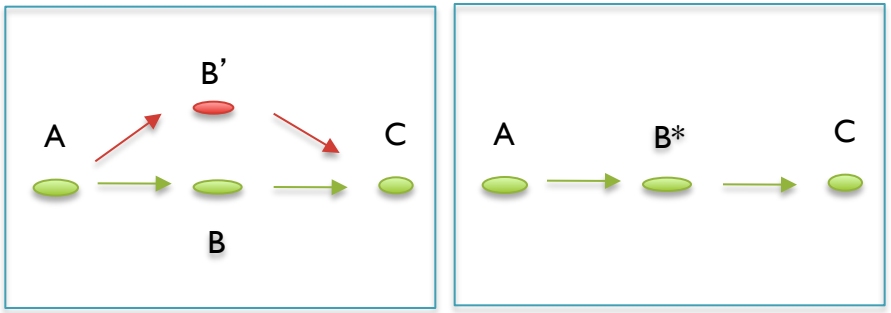
Full Branch (10%)

Graph Correction

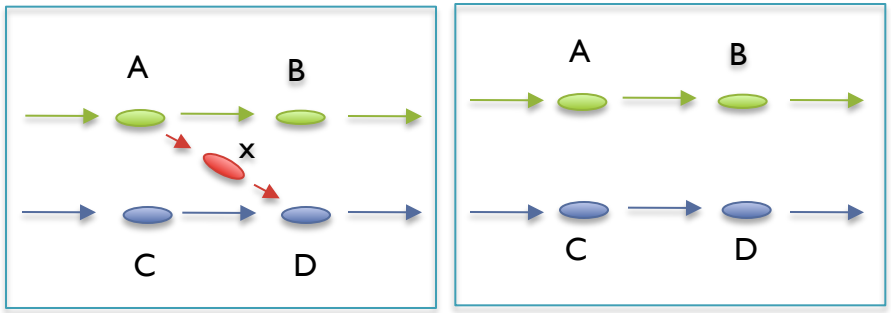
- Errors at end of read
  - Trim off ‘dead-end’ tips



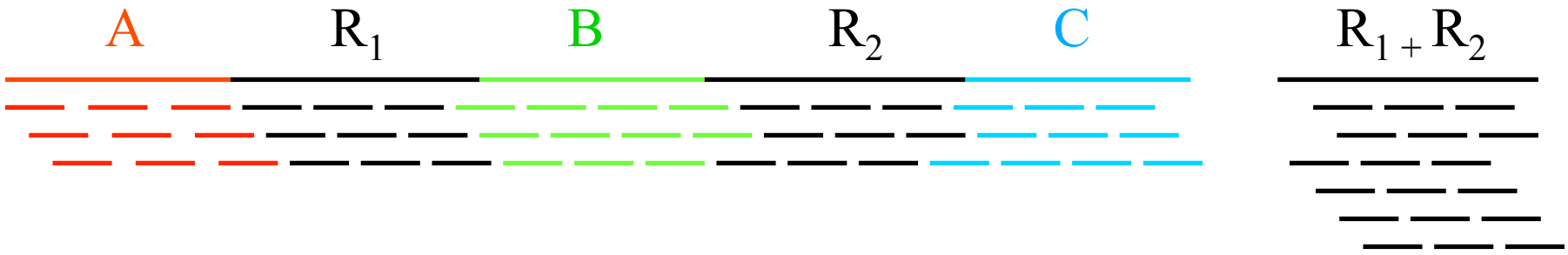
- Errors in middle of read
  - Pop Bubbles



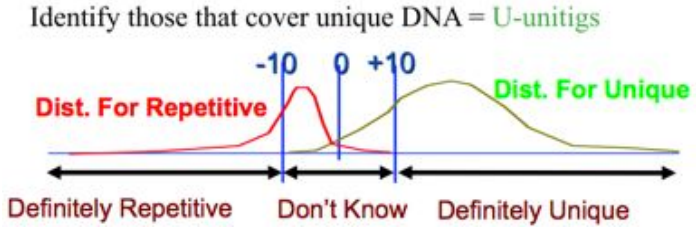
- Chimeric Edges
  - Clip short, low coverage nodes



Coverage Evaluation

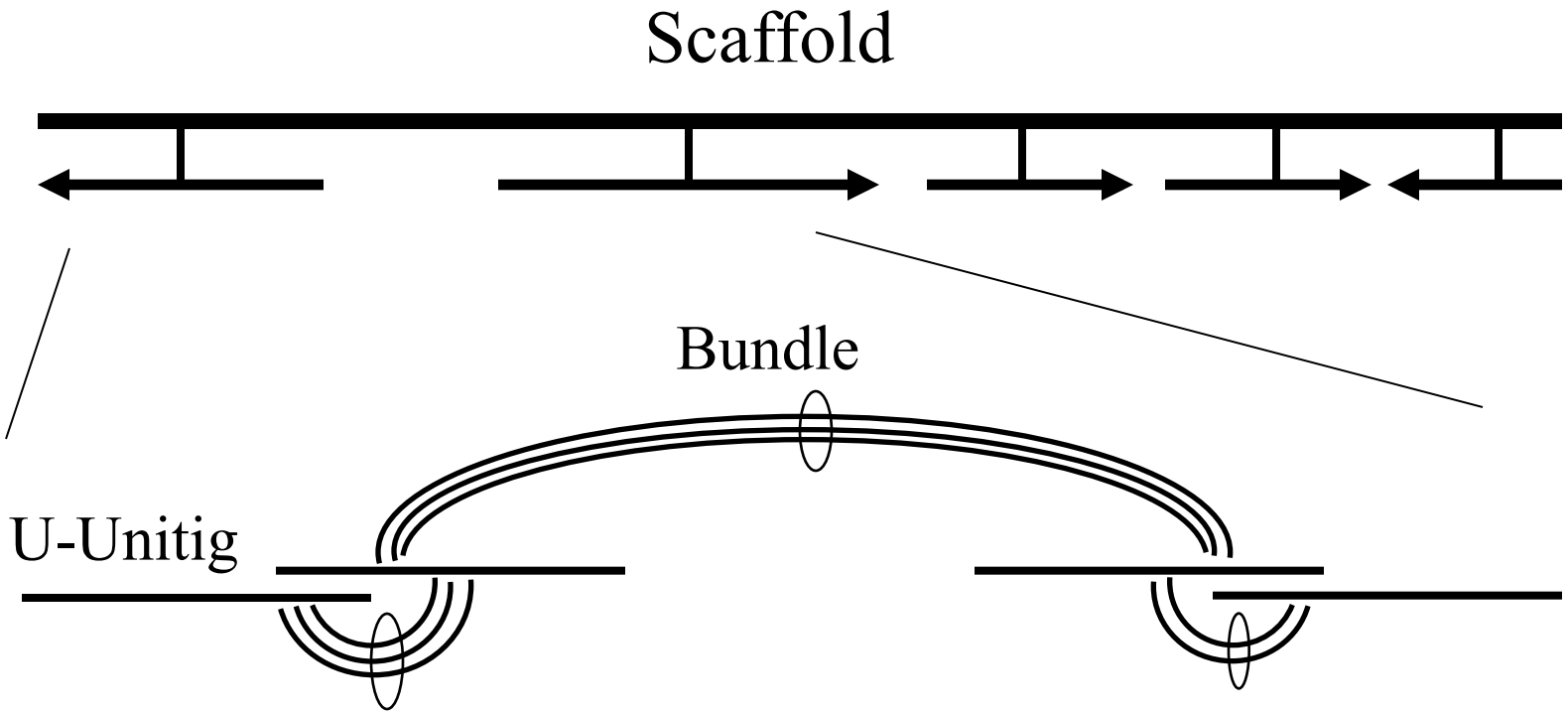


- If *n* reads are a uniform random sample of the genome of length *G*, we expect $k = n\Delta/G$ reads to start in a region of length Δ.
  - If we see many more reads than k (if the arrival rate is > A) , it is likely to be a collapsed repeat
  - Requires an accurate genome size estimate



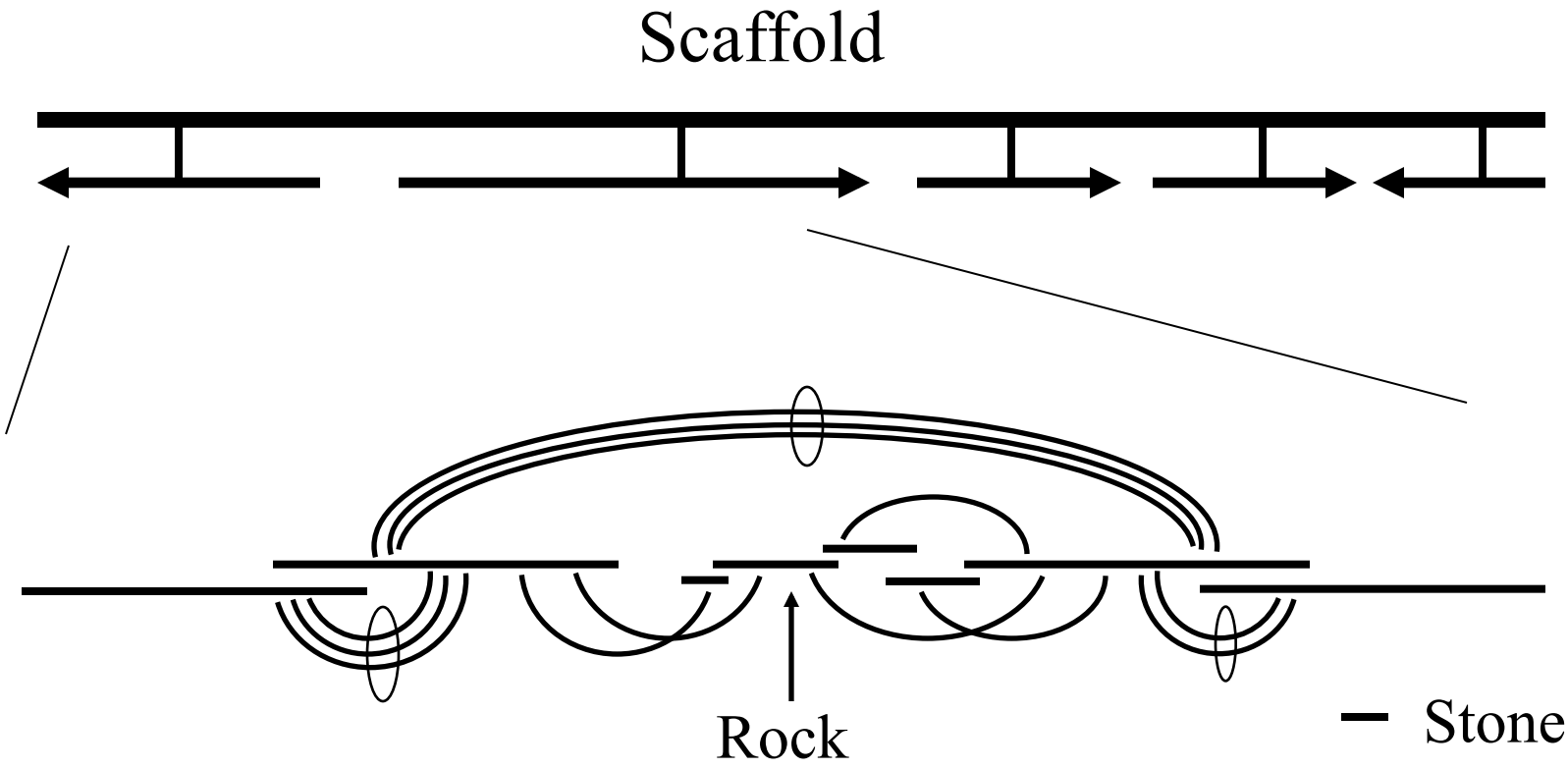
$$A(\Delta,k) = \ln\left(\frac{\Pr(1-copy)}{\Pr(2-copy)}\right) = \ln\left(\frac{\frac{(\Delta n/G)^k}{k!}e^{\frac{-\Delta n}{G}}}{\frac{(2\Delta n/G)^k}{k!}e^{\frac{-2\Delta n}{G}}}\right) = \frac{n\Delta}{G} - k\ln 2$$

Initial Scaffolding



Create an initial scaffold of basic contigs (“unitigs”) whose coverage indicates they are not repetitive (A-stat > 5).

Repeat Resolution



Then add in remaining repetitive contigs based on their mate relationships allowing repetitive sequences to be placed multiple times.

N50 size

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

Example:

1 Mbp genome
Contigs: 300k, 100k, 50k, 45k, 30k, 20k, 15k, 15k, 10k, ...

N50 size = 30 kbp
(300k+100k+50k+45k+30k = 525k >= 500kbp)

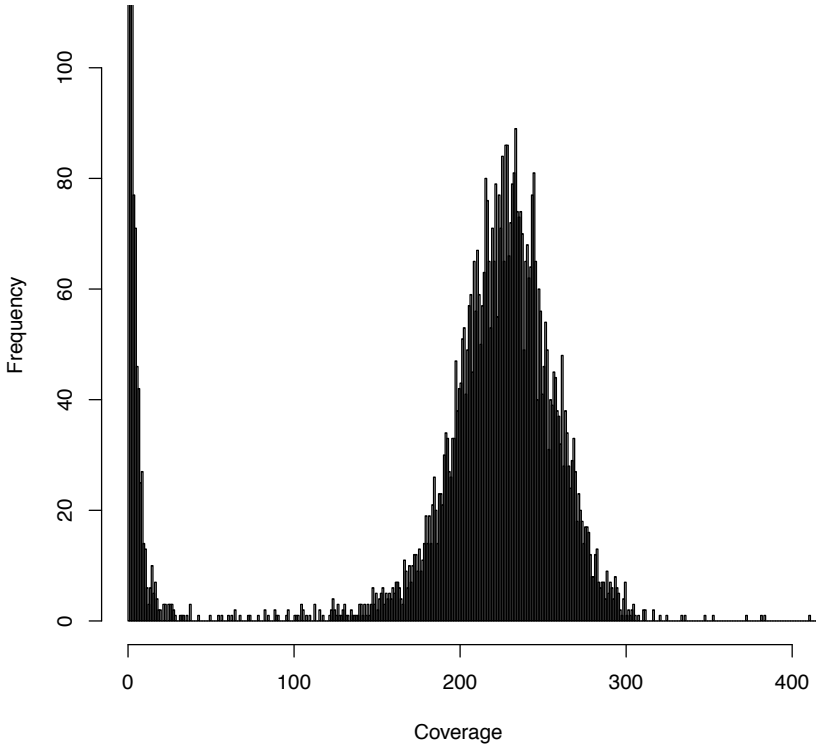
Note:

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

Illumina Sequencing & Assembly

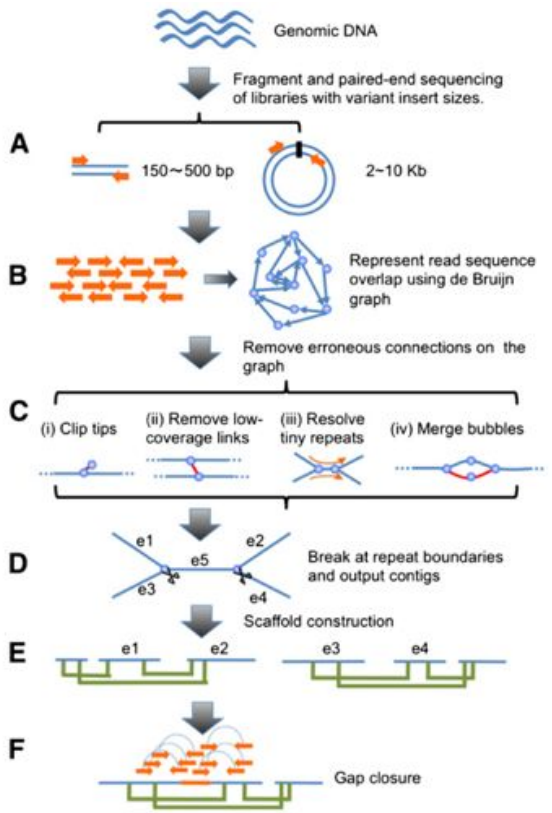
Quake Results

2x76bp @ 275bp
2x36bp @ 3400bp



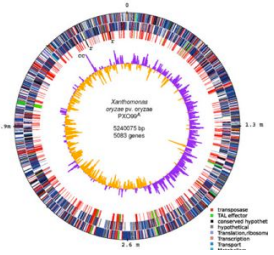
Validated	51,243,281	88.5%
Corrected	2,763,380	4.8%
Trim Only	3,273,428	5.6%
Removed	606,251	1.0%

SOAPdenovo Results



	# ≥ 100bp	N50 (bp)
Scaffolds	2,340	253,186
Contigs	2,782	56,374
Unitigs	4,151	20,772

Assembly realities

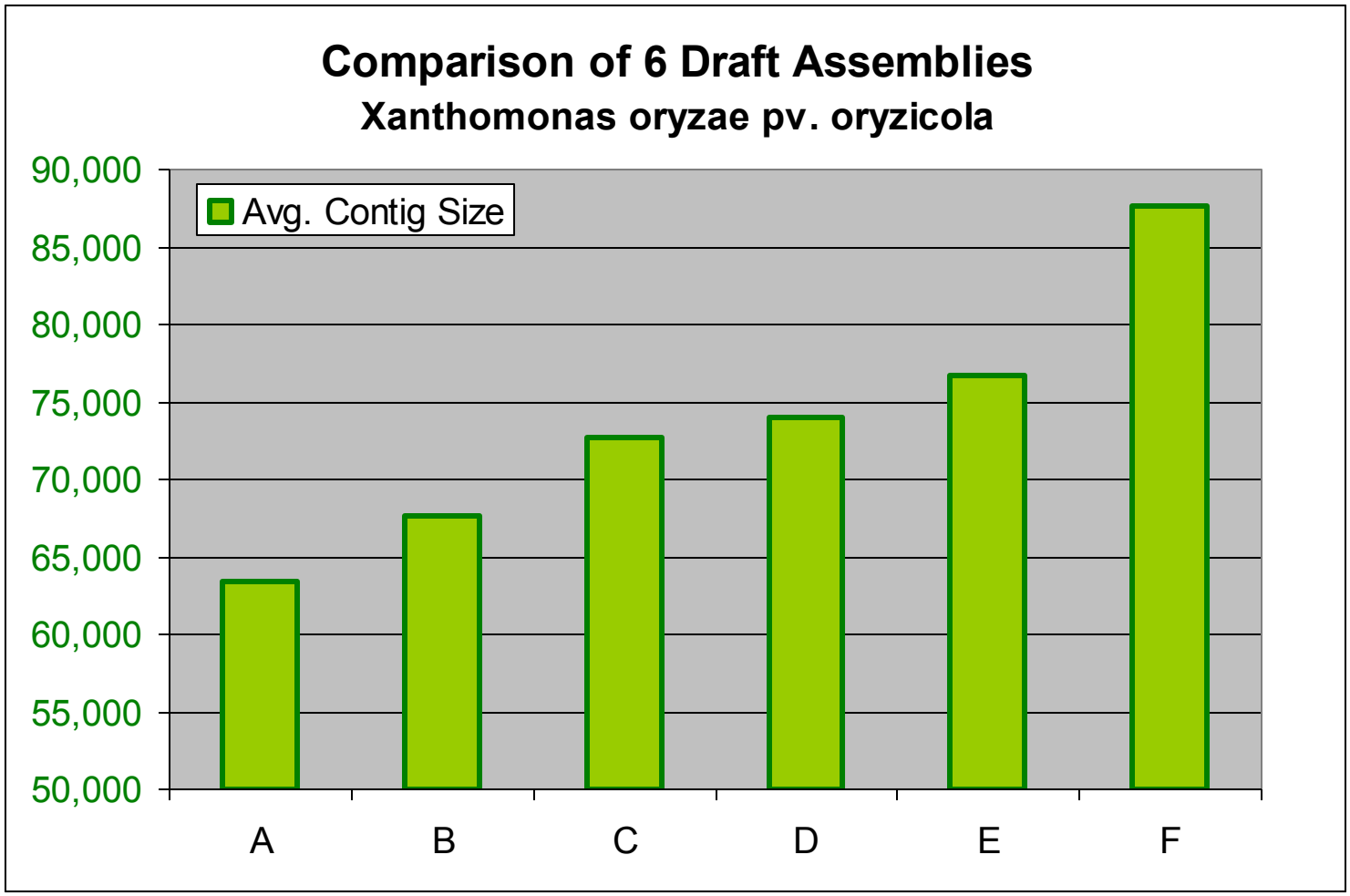


- Contigs are never as large as predicted
  - High coverage is a necessary but not sufficient condition
  - Error correction is required for good assembly
  - Sequencing is basically random, but sequence composition is not

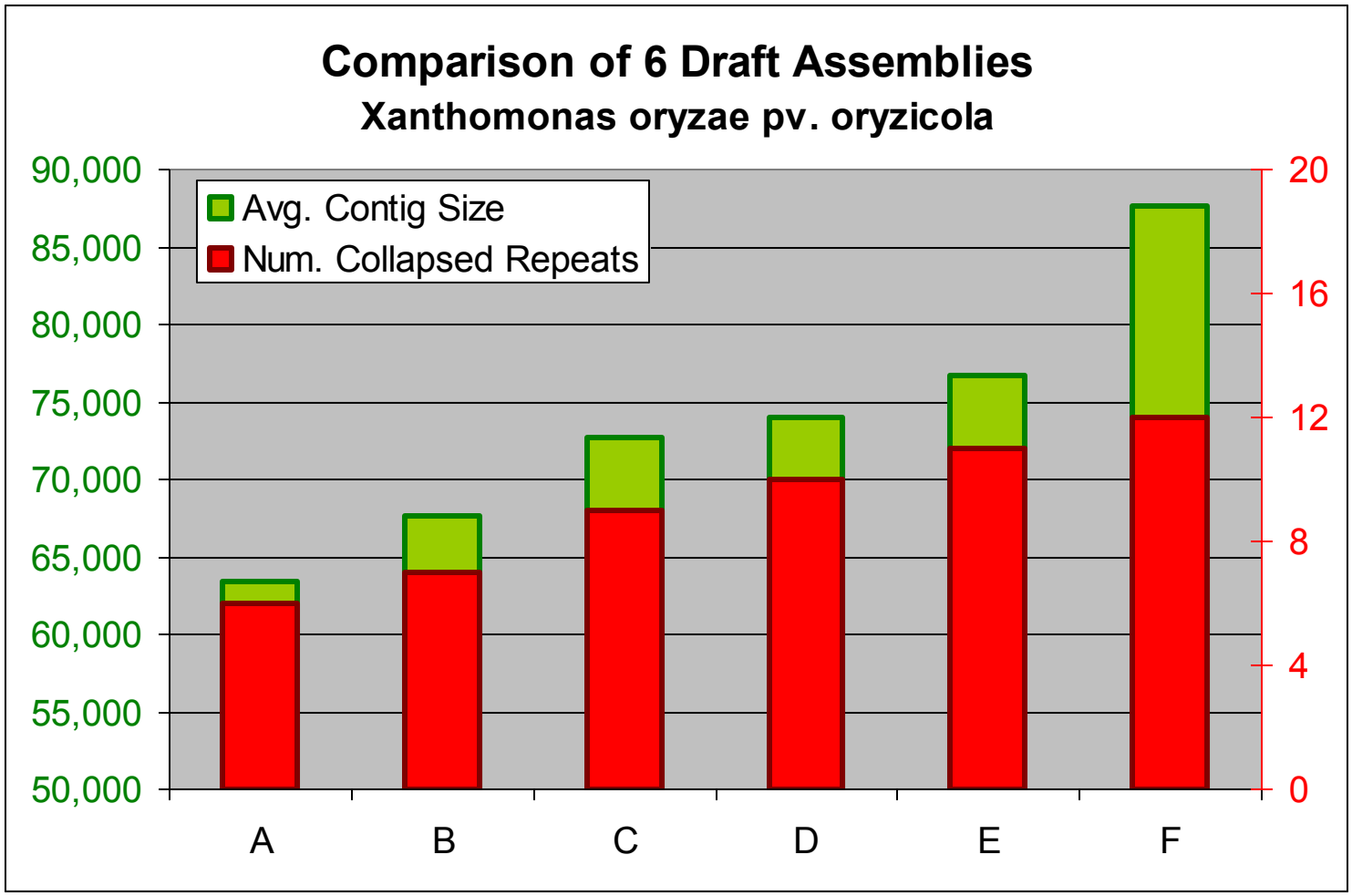
- Repeats control the quality of the assembly
  - Assemblers break contigs at ambiguous repeats
  - Highly repetitive genomes will be highly fragmented

- Assemblers make mistakes
  - Mis-assemblies confuse all downstream analysis
  - Tension between overlap error rate and repeat resolution

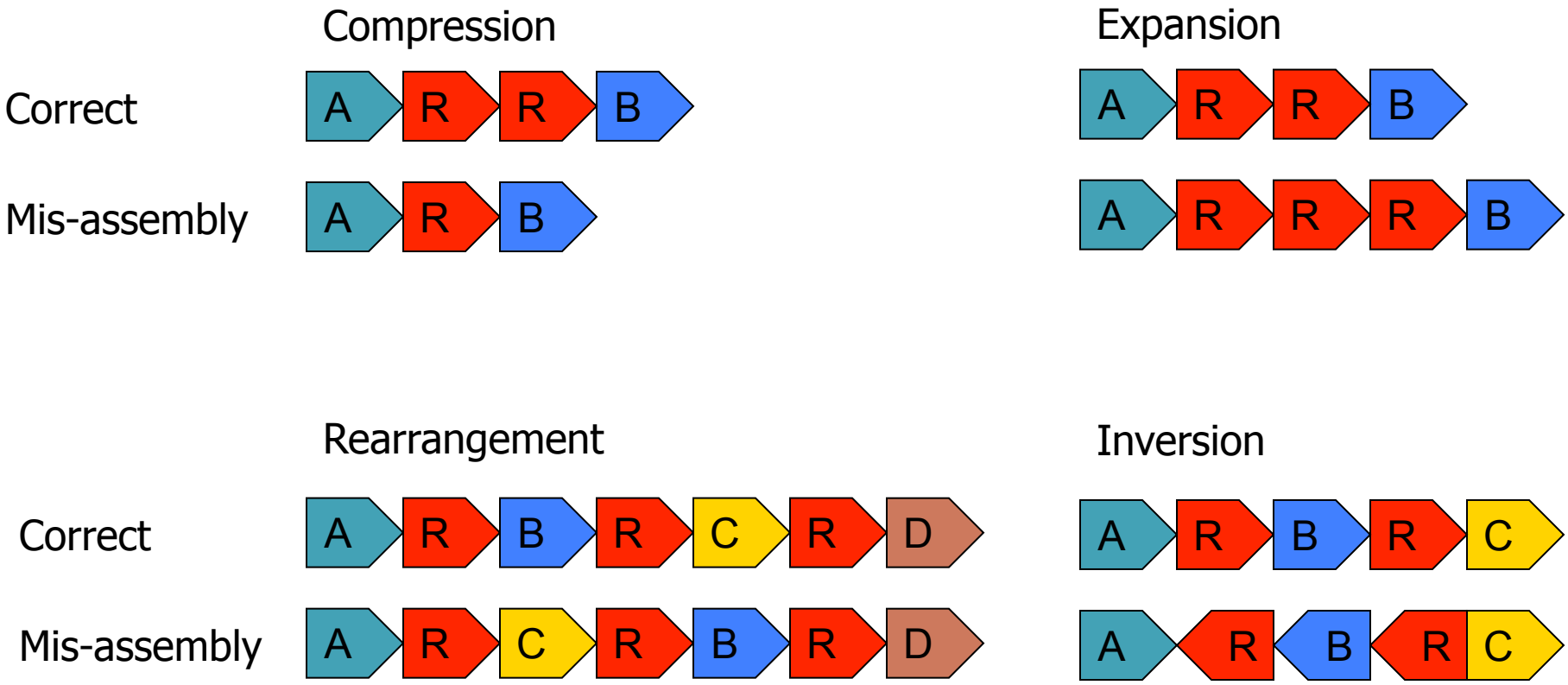
Assembly Evaluation



Assembly Evaluation



Mis-assembly Types



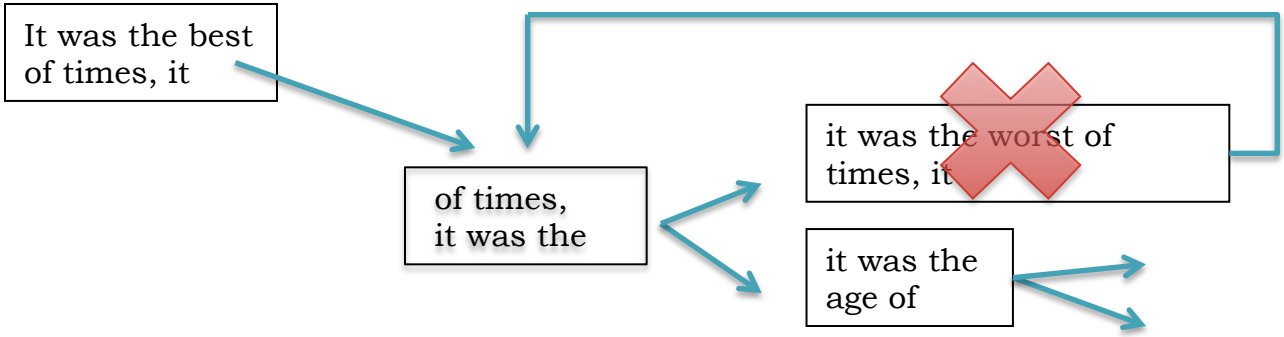
Basic mis-assemblies can be combined into more complicated patterns:
Insertions, Deletions, Giant Hairballs

Assembly Forensics

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

Assembly-validation pipeline

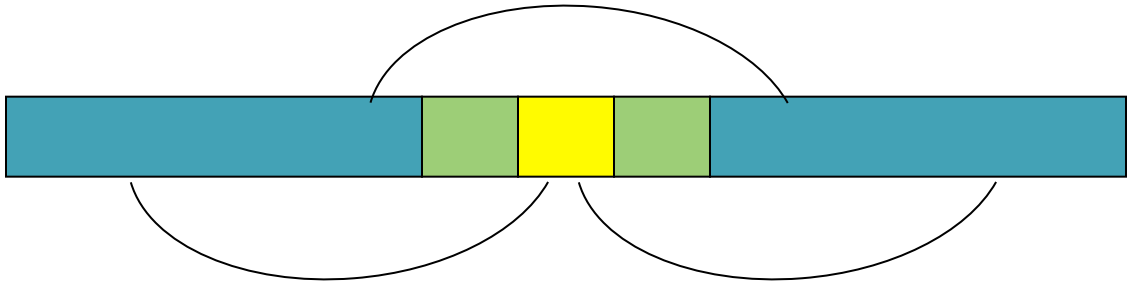
1. Evaluate Mate Pairs & Libraries
2. Evaluate Read Alignments
3. Evaluate Read Breakpoints
4. Analyze Depth of Coverage



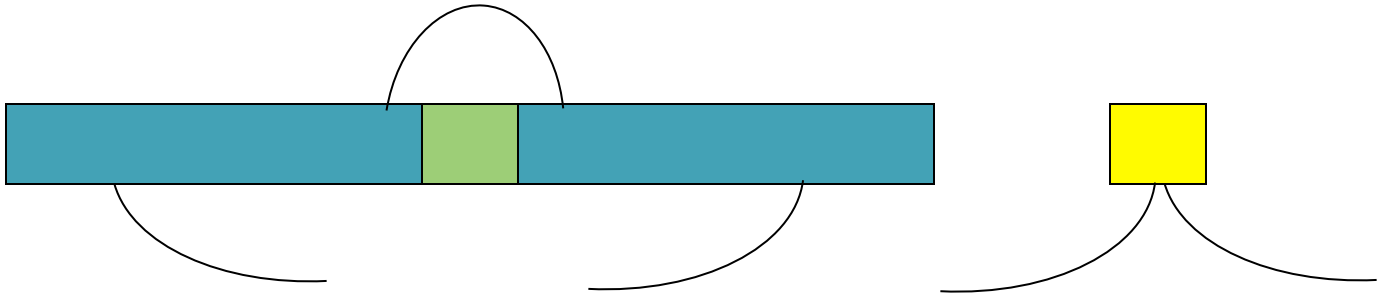
Genome Assembly forensics: finding the elusive mis-assembly.
Phillippy, AM, Schatz, MC, Pop, M. (2008) *Genome Biology* 9:R55.

Mate-Happiness: asmQC

- Excision: Skip reads between flanking repeats
  - Truth

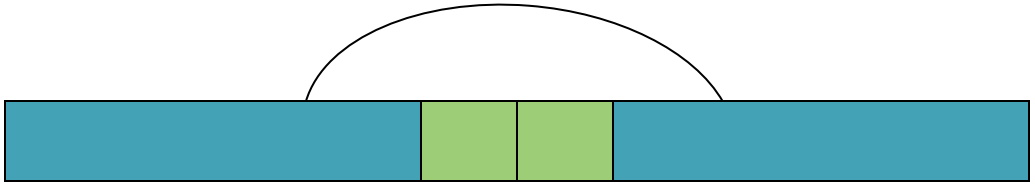


  - Misassembly: Compressed Mates, Missing Mates

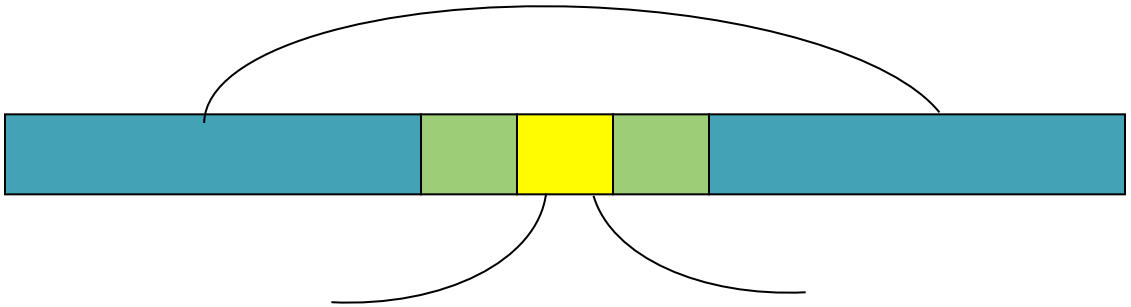


Mate-Happiness: asmQC

- Insertion: Additional reads between flanking repeats
  - Truth

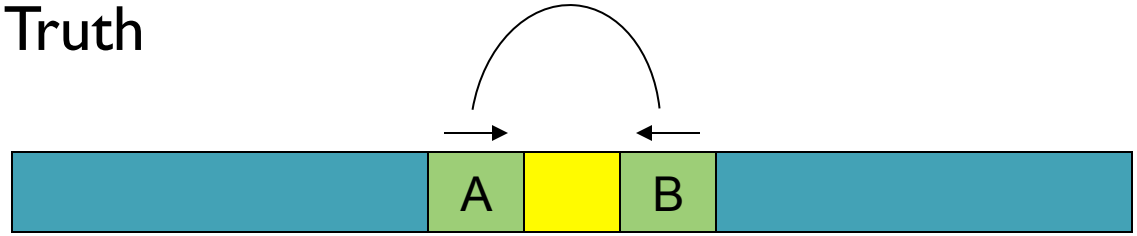


  - Misassembly: Expanded Mates, Missing Mates

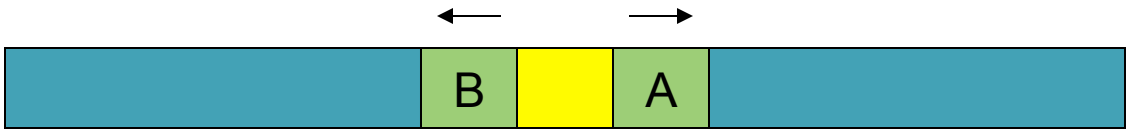


Mate-Happiness: asmQC

- Rearrangement: Reordering of reads
  - Truth

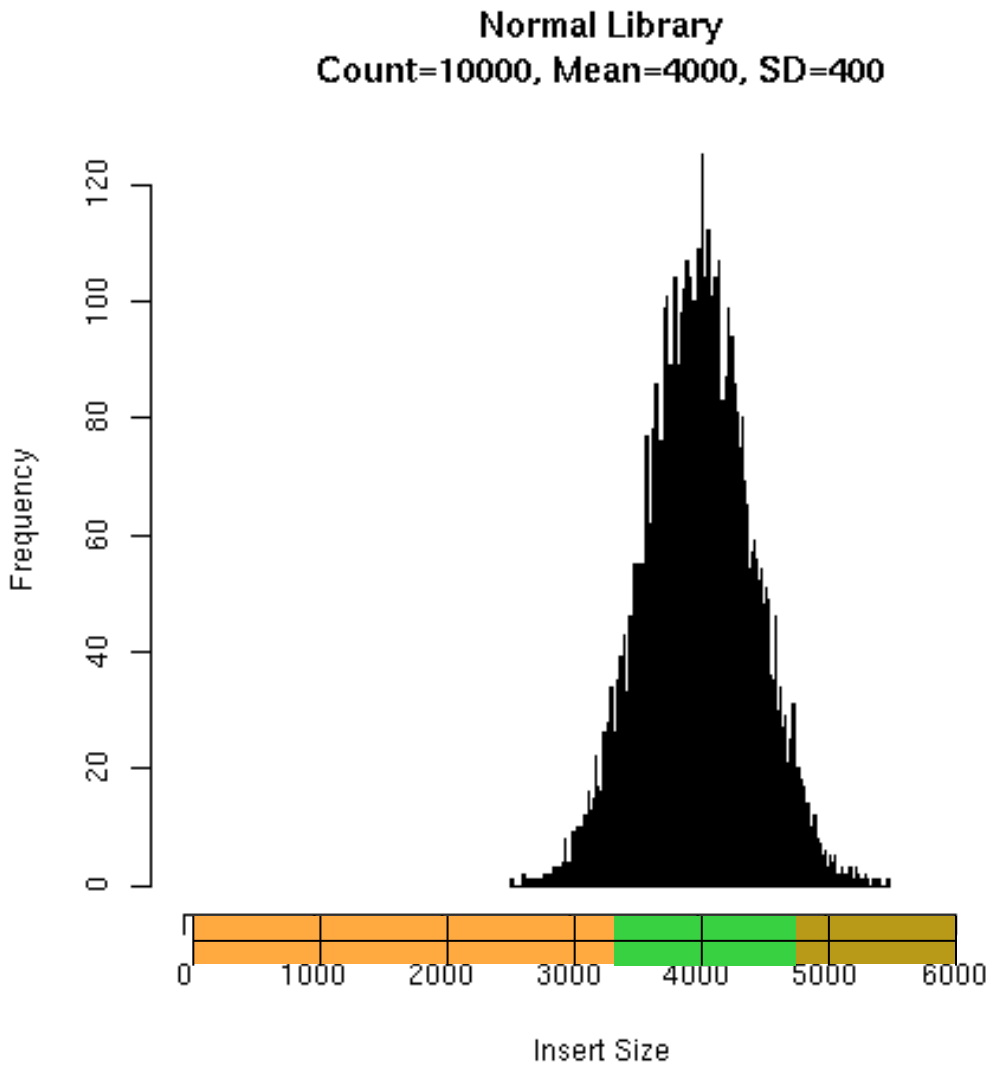


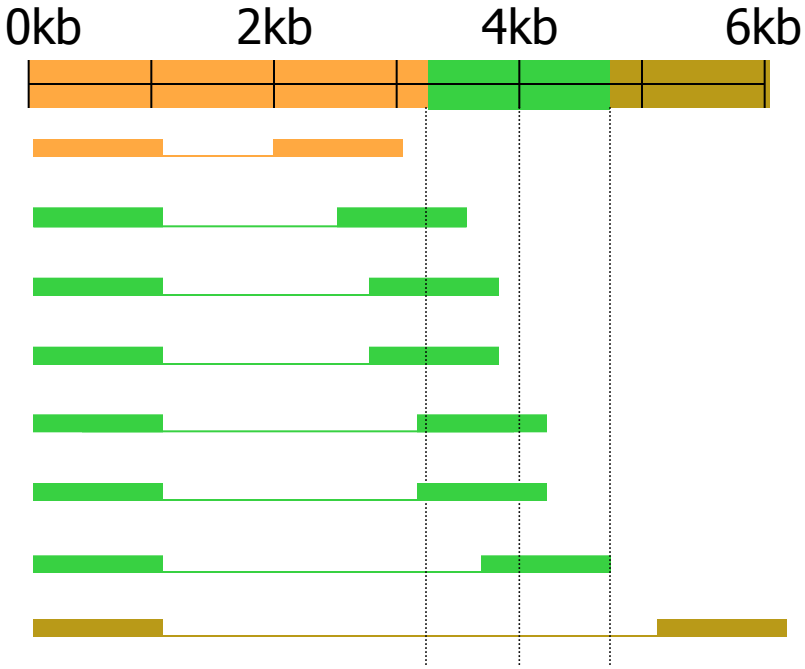
  - Misassembly: Misoriented Mates



Note: Unhappy mates may also occur for biological or technical reasons.

Sampling the Genome





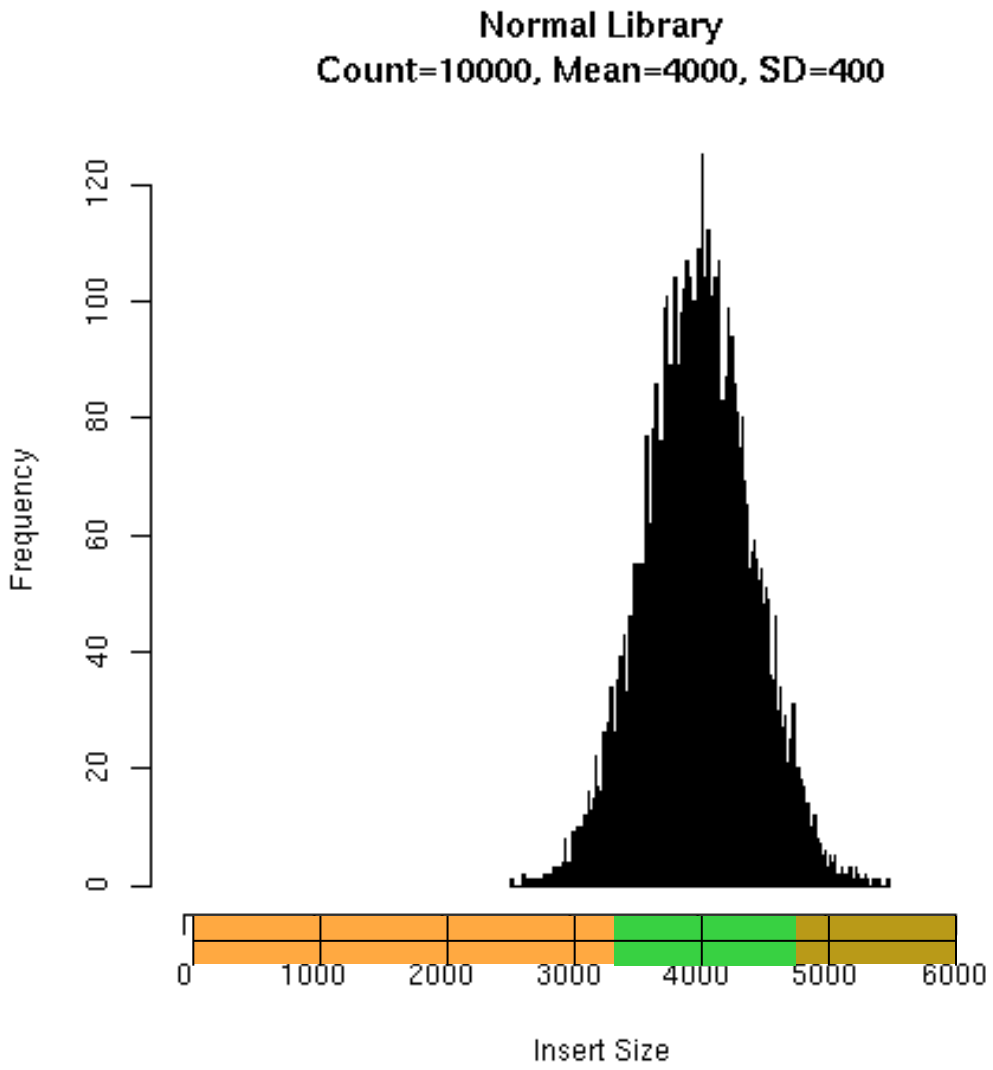
8 inserts: 3kb-6kb

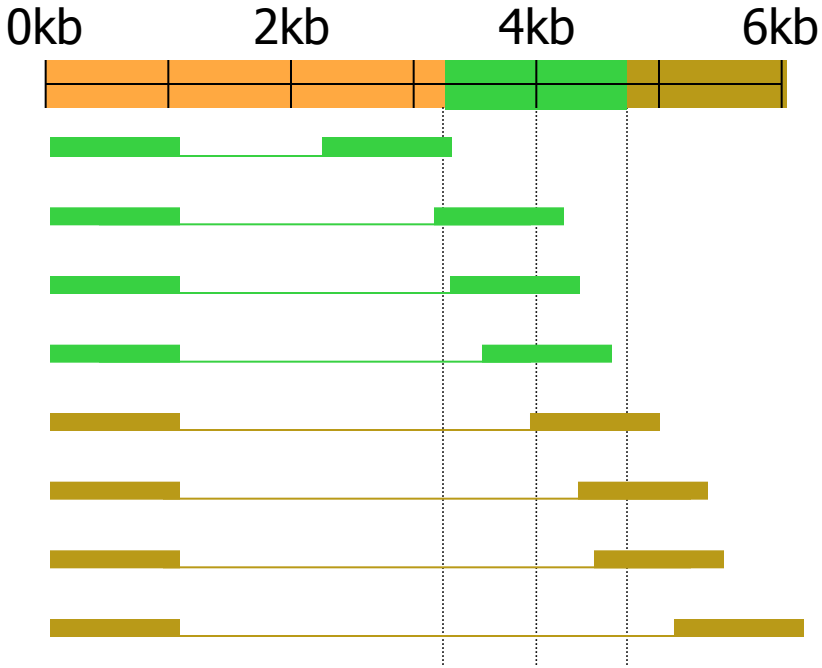
Local Mean: 4048

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

Near 0 indicates overall happiness

CE Statistic: Expansion





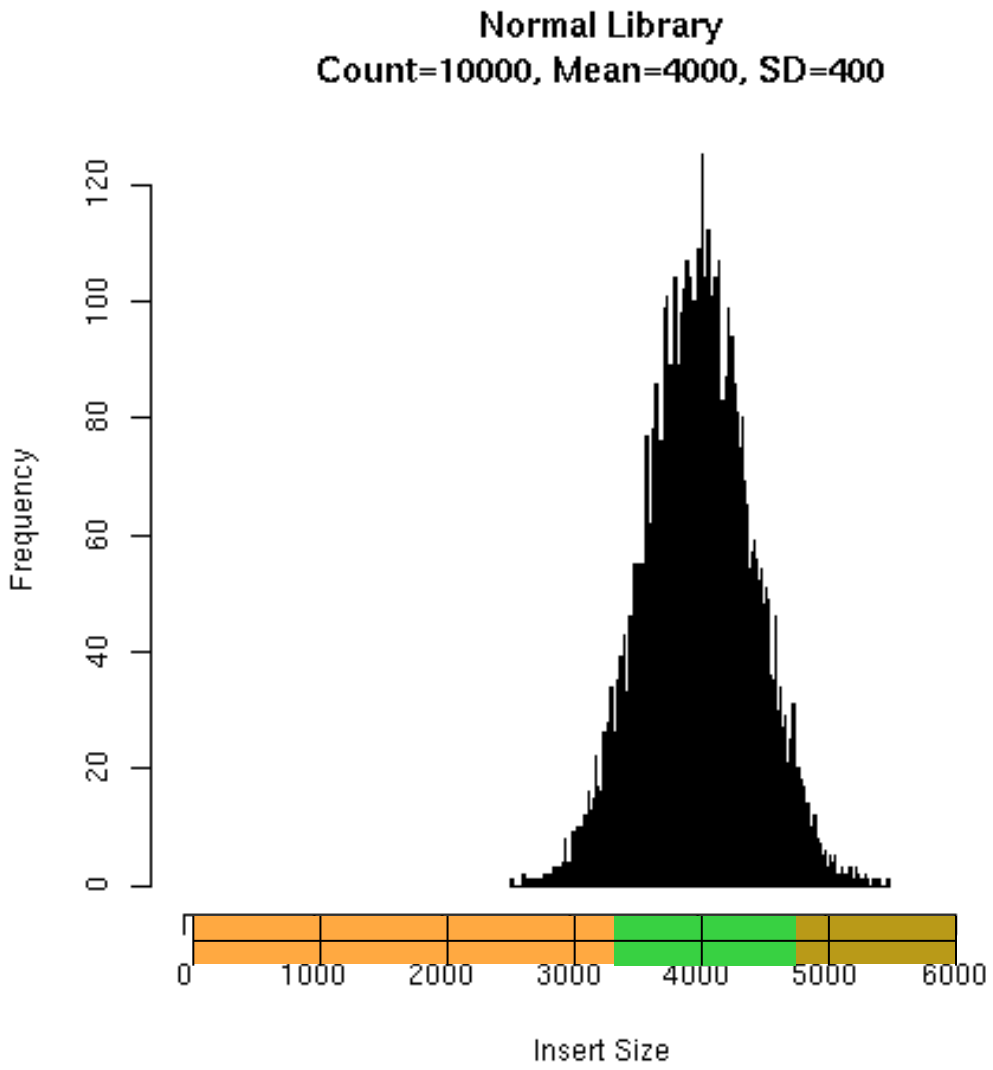
8 inserts: 3.2kb-6kb

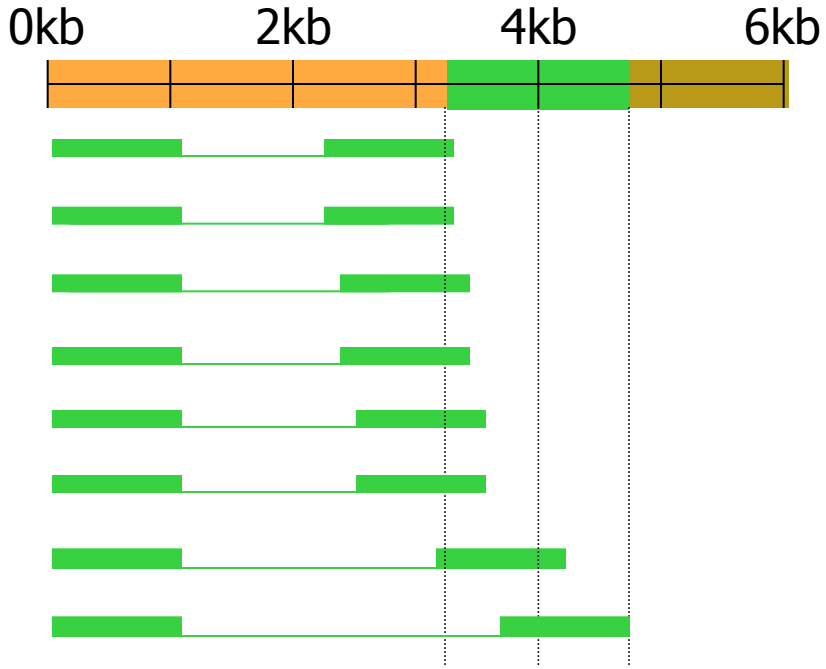
Local Mean: 4461

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

C/E Stat ≥ 3.0 indicates Expansion

CE Statistic: Compression





8 inserts: 3.2 kb-4.8kb

Local Mean: 3488

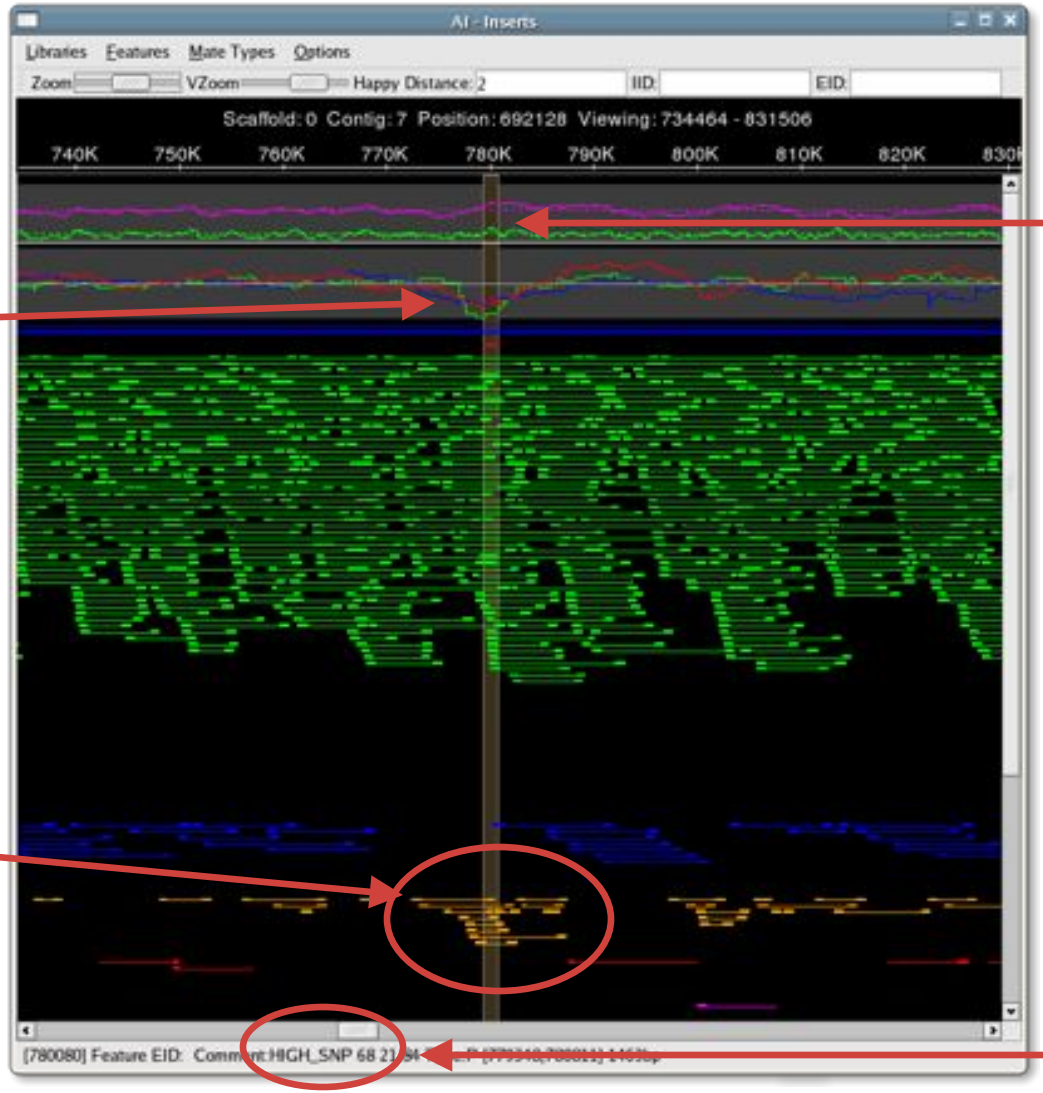
C/E Stat: $\frac{(3488-4000)}{(400 / \sqrt{8})} = -3.62$

C/E Stat ≤ -3.0 indicates Compression

Read Alignment

- Multiple reads with same conflicting base are unlikely
  - 1x QV 30: 1/1000 base calling error
  - 2x QV 30: 1/1,000,000 base calling error
  - 3x QV 30: 1/1,000,000,000 base calling error
- Regions of correlated SNPs are likely to be assembly errors or interesting biological events
  - Highly specific metric
- AMOS Tools: analyzeSNPs & clusterSNPs
  - Locate regions with high rate of correlated SNPs
  - Parameterized thresholds:
    - Multiple positions within 100bp sliding window
    - 2+ conflicting reads
    - Cumulative QV >= 40 (1/10000 base calling error)

Collapsed Repeat



-5.5 CE Dip

Read Coverage Spike

Compressed Mates Cluster

68 Correlated SNPs

Hawkeye: a visual analytics tool for genome assemblies.
Schatz, MC, Phillippy, AM, Shneiderman, B, Salzberg, SL. (2007) Genome Biology 8:R34.

Validation Accuracy

Table 1

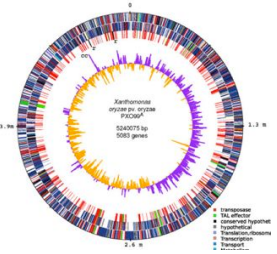
Accuracy of *amosvalidate* mis-assembly signatures and suspicious regions summarized for 16 bacterial genomes assembled with Phrap

				Mis-assembly signatures			Suspicious regions		
Species	Len	Ctgs	Errs	Num	Valid	Sens	Num	Valid	Sens
B. anthracis	5.2	87	2	1,336	21	100.0	127	2	100.0
B. suis	3.4	120	10	1,047	30	80.0	158	9	90.0
C. burnetii	2.0	55	22	1,375	70	100.0	124	19	100.0
C. caviae	1.4	270	12	625	16	83.3	50	8	66.7
C. jejuni	1.8	53	5	290	11	80.0	81	3	60.0
D. ethenogenes	1.8	632	12	686	22	91.7	88	9	100.0
F. succinogenes	4.0	455	21	1,670	27	95.2	266	14	66.7
L. monocytogenes	2.9	172	1	1,381	5	100.0	201	1	100.0
M. capricolum	1.0	17	3	83	0	0.0	16	0	0.0
N. sennetsu	0.9	16	0	91	0	NA	13	0	NA
P. intermedia	2.7	343	21	1,655	57	100.0	201	20	100.0
P. syringae	6.4	274	64	2,841	200	98.4	366	55	98.4
S. agalactiae	2.1	127	21	687	53	95.2	112	18	85.7
S. aureus	2.8	824	41	1,850	69	97.6	227	18	75.8
W. pipientis	1.3	2017	31	761	92	100.0	132	30	100.0
X. oryzae	5.0	50	151	2,569	379	100.0	100	69	100.0
Totals	46.8	5412	417	18,949	1,052	96.9	2,242	275	92.6

Species name, genome length (Len), number of assembled contigs (Ctgs), and alignment inferred mis-assemblies (Errs) are given in the first four columns. Number of mis-assembly signatures output by *amosvalidate* (Num) is given in column 5, along with the number of signatures coinciding with a known mis-assembly in column 6 (Valid), and percentage of known mis-assemblies identified by one or more signatures in column 7 (Sens). The same values are given in columns 8-10 for the suspicious regions output by *amosvalidate*. The suspicious regions represent at least two different, coinciding lines of evidence, whereas the signatures represent a single line of evidence. A signature or region is deemed 'validated' if its location interval overlaps a mis-assembled region identified by *dnadiff*. Thus, a single signature or region can identify multiple mis-assemblies, and vice versa, a single mis-assembly can be identified by multiple signatures or regions.

Phillippy *et al. Genome Biology* 2008 **9**:R55 doi:10.1186/gb-2008-9-3-r55

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

Break


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

Outline

Part 1: Schatz Lab Overview

Part 2: Sequence Alignment

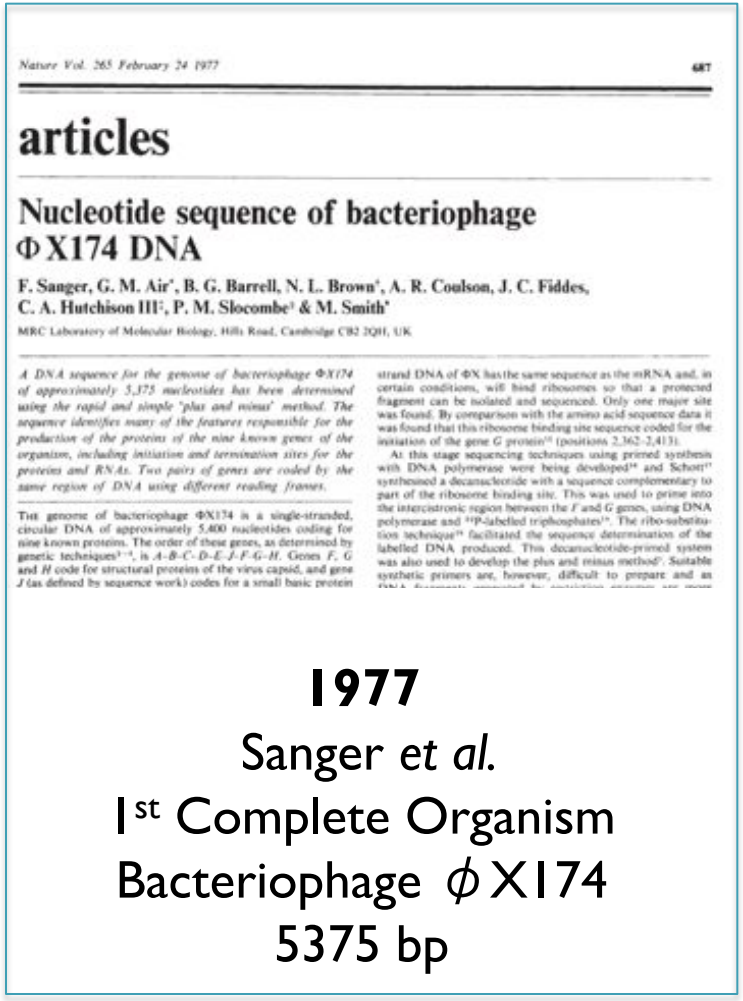
Part 3: Genome Assembly

Part 4: Parallel & Cloud Computing

- Milestones in DNA Sequencing
- Hadoop & Cloud Computing
- Sequence Analysis in the Clouds

Milestones in DNA Sequencing

1970 | 1980 | 1990 | 2000 | 2010



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[1-4], 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[10] (positions 2,362–2,413).

At this stage sequencing techniques using primed synthesis with DNA polymerase were being developed[11] and Schott[12] 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[13]. The ribo-substitution technique[14] facilitated the sequence determination of the labelled DNA produced. This decanucleotide-primed system was also used to develop the plus and minus method[5]. Suitable synthetic primers are, however, difficult to prepare and as

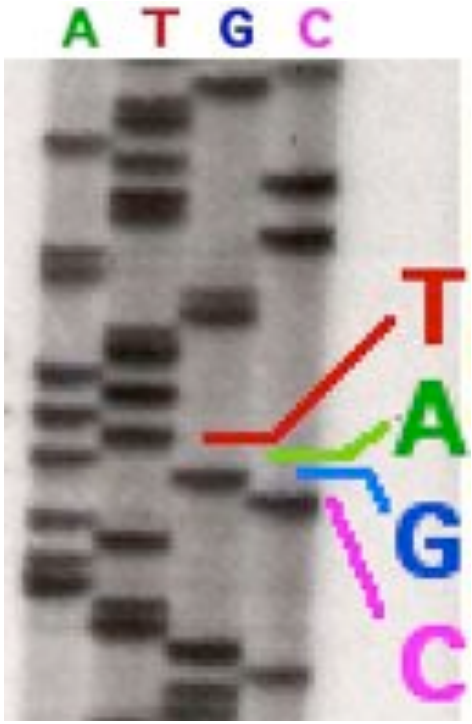
1977

Sanger *et al.*

1st Complete Organism

Bacteriophage ϕX174

5375 bp



Radioactive Chain Termination

5000bp / week / person

http://en.wikipedia.org/wiki/File:Sequencing.jpg

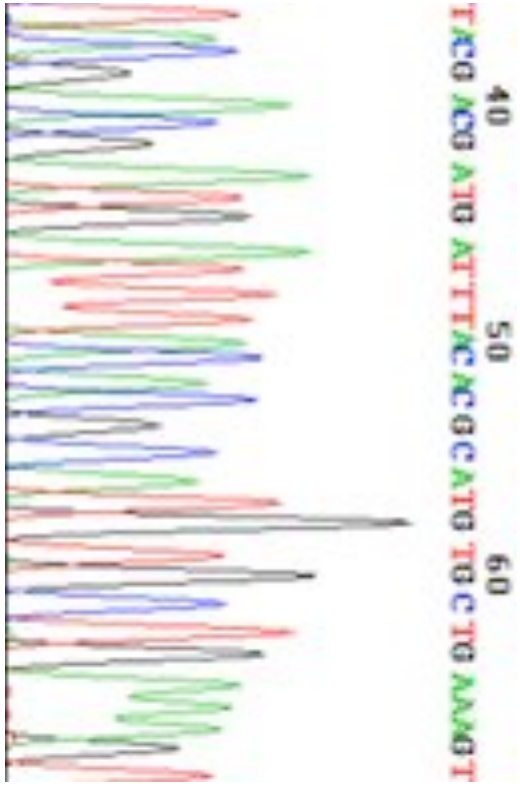
http://www.answers.com/topic/automated-sequencer

Milestones in DNA Sequencing

1970 | 1980 | 1990 | 2000 | 2010

1987
Applied Biosystems markets the ABI 370 as the first automated sequencing machine

http://commons.wikimedia.org/wiki/File:370A_automated_DNA_sequencer.jpg



Fluorescent Dye Termination
350bp / lane x 16 lanes =
5600bp / day / machine

http://www.answers.com/topic/automated-sequencer

Milestones in DNA Sequencing

1970 | 1980 | 1990 | 2000 | 2010

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

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

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

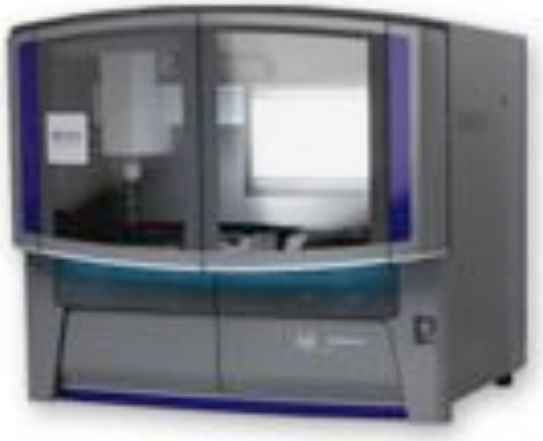
ABI 3700: 500 bp reads x 768 samples / day = 384,000 bp / day.
"The machine was so revolutionary that it could decode in a single day the same amount of genetic material that most DNA labs could produce in a year." J. Craig Venter

Milestones in DNA Sequencing

1970 1980 1990 2000 2010

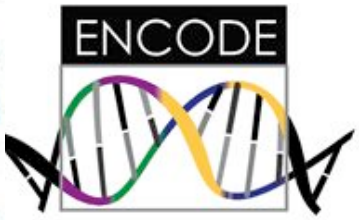
2004
454/Roche
Pyrosequencing
Current Specs (Titanium):
1M 400bp reads / run =
1Gbp / day

2007
Illumina
Sequencing by Synthesis
Current Specs (HiSeq 2000):
2.5B 100bp reads / run =
60Gbp / day

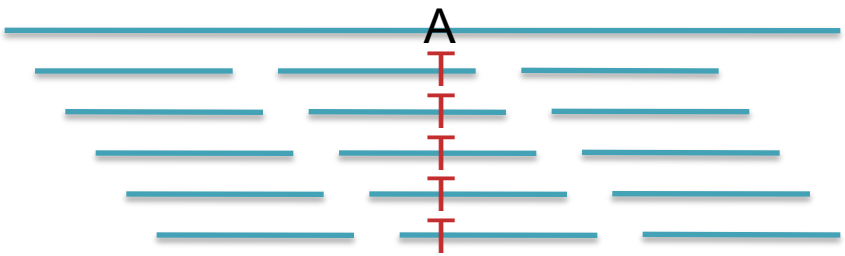


2008
ABI / Life Technologies
SOLiD Sequencing
Current Specs (5500xl):
5B 75bp reads / run =
30Gbp / day

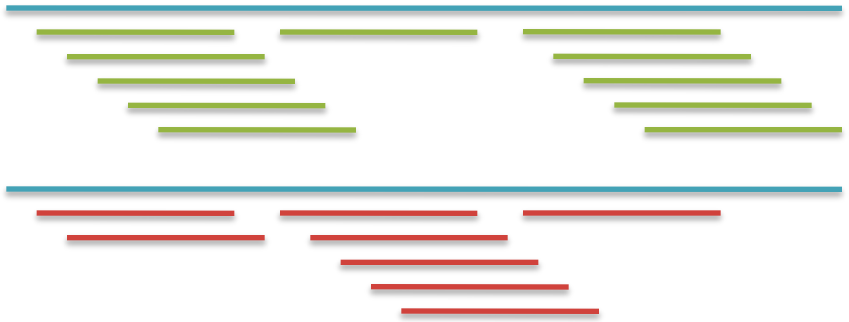
Second Generation Sequencing Applications



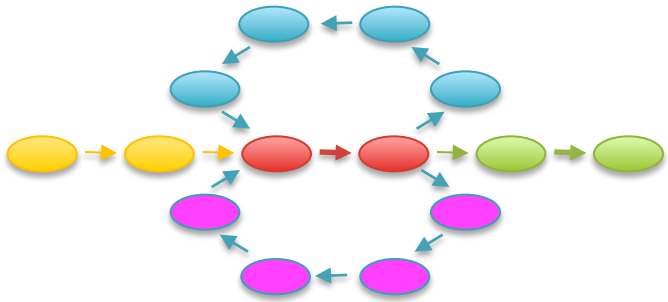
Alignment & Variations



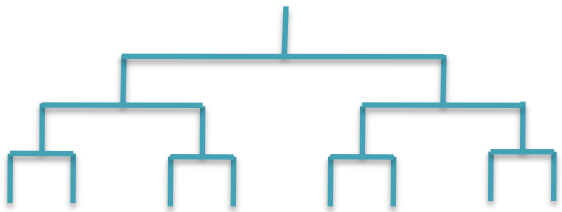
Differential Analysis



De novo Assembly



Phylogeny & Evolution

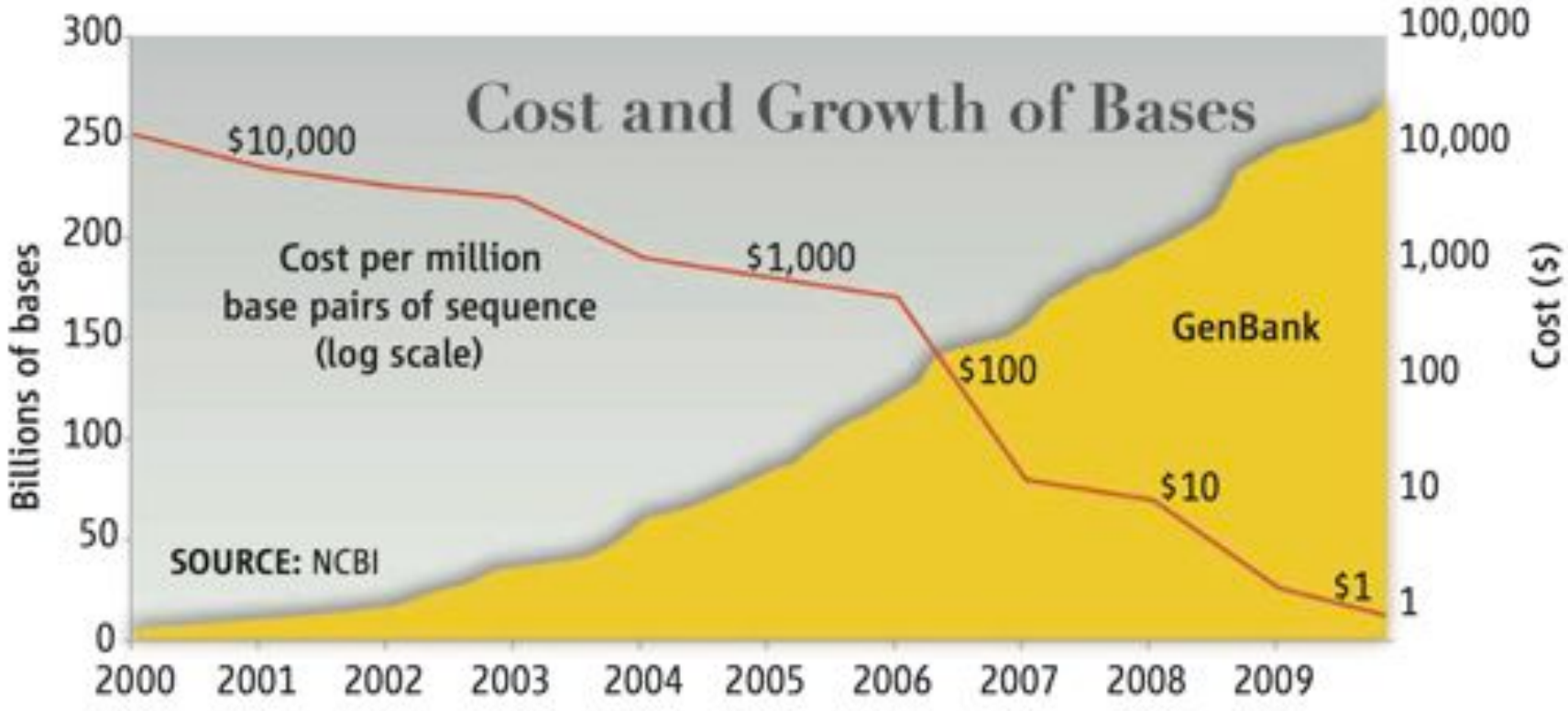


Sequencing Centers

Next Generation Genomics: World Map of High-throughput Sequencers
http://pathogenomics.bham.ac.uk/hts/

DNA Data Tsunami

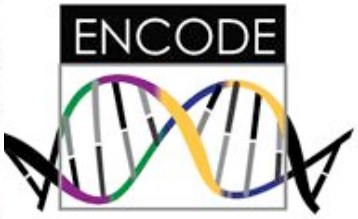
Current world-wide sequencing capacity exceeds 13Pbp/year and is growing at 5x per year!



"*Will Computers Crash Genomics?*"
Elizabeth Pennisi (2011) *Science*. 331(6018): 666-668.

Genomics and Parallel Computing





Current world-wide sequencing capacity exceeds 13Pbp/year and is growing at 5x per year!

Our best (only) hope is to use many computers:

- Parallel Computing aka Cloud Computing

- Now your programs will crash on 1000 computers instead of just 1 ☺

Amazon Web Services

http://aws.amazon.com

- All you need is a credit card, and you can immediately start using one of the largest datacenters in the world

- Elastic Compute Cloud (EC2)
  - On demand computing power
    - Support for Windows, Linux, & OpenSolaris
    - Starting at 2.0¢ / core / hour
- Simple Storage Service (S3)
  - Scalable data storage
    - 15¢ / GB monthly fee
- Plus many others

EC2 Architecture

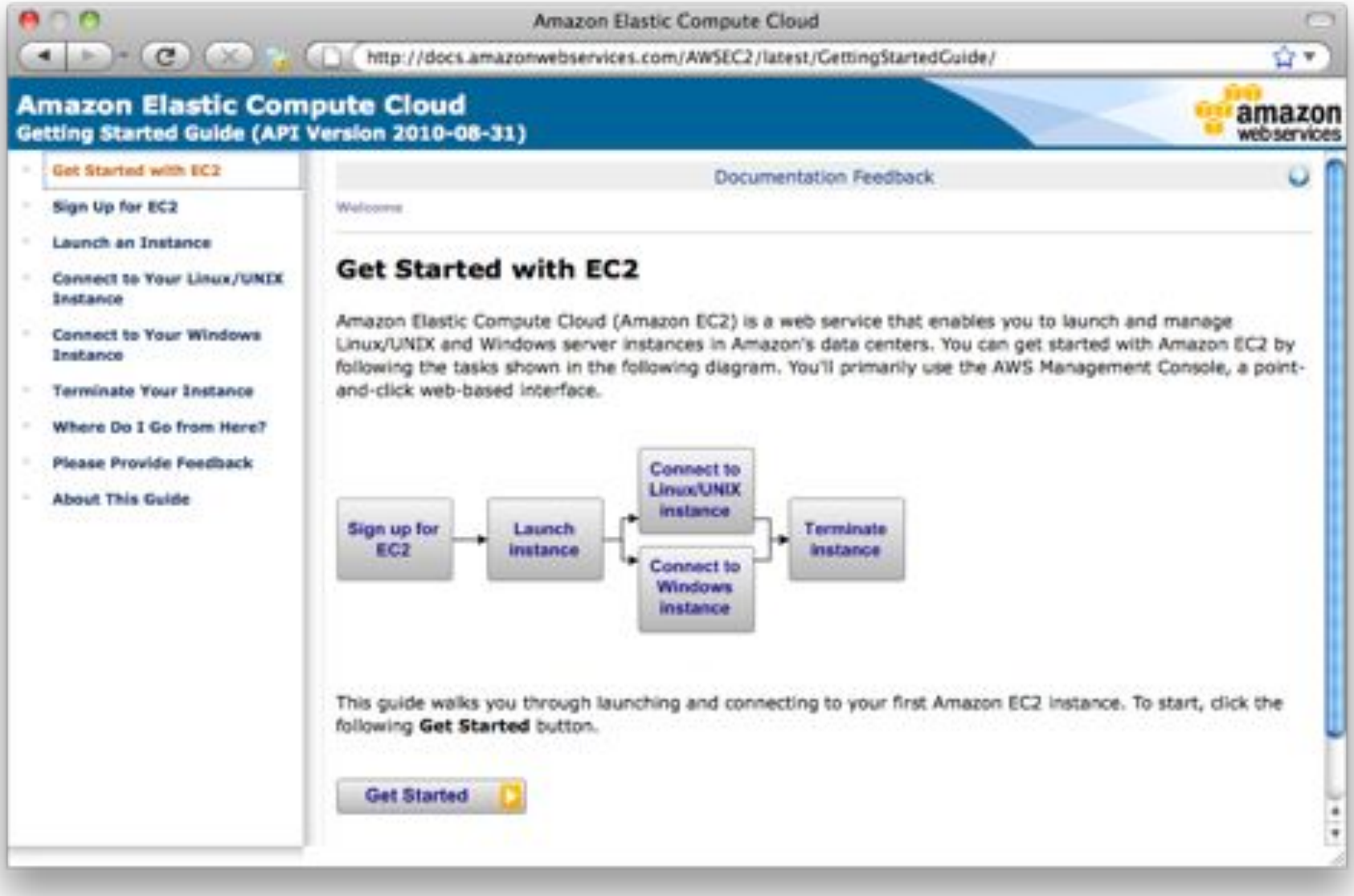
- Very large pool of machines
  - Effectively infinite resources
  - High-end servers with many cores and many GB RAM
- Machines run in a virtualized environment
  - Amazon can subdivide large nodes into smaller instances
  - You are 100% protected from other users on the machine
  - You get to pick the operating system, all installed software

Amazon Machine Images

- A few Amazon sponsored images
  - Suse Linux, Windows
- Many Community Images & Appliances
  - CloudBioLinux: Genomics Appliance
  - Crossbow: Hadoop, Bowtie, SOAPsnp
  - Galaxy: CloudMan
- Build you own
  - Completely customize your environment
  - You results could be totally reproducible

Getting Started

http://docs.amazonwebservices.com/AWSEC2/latest/GettingStartedGuide/



Hadoop MapReduce

http://hadoop.apache.org

- MapReduce is Google's framework for large data computations
  - Data and computations are spread over thousands of computers
    - Indexing the Internet, PageRank, Machine Learning, etc… (Dean and Ghemawat, 2004)
    - 946 PB processed in May 2010 (Jeff Dean at Stanford, 11.10.2010)
  - Hadoop is the leading open source implementation
    - Developed and used by Yahoo, Facebook, Twitter, Amazon, etc
    - GATK is an alternative implementation specifically for NGS

- Benefits
  - Scalable, Efficient, Reliable
  - Easy to Program
  - Runs on commodity computers

- Challenges
  - Redesigning / Retooling applications
    - Not Condor, Not MPI
    - Everything in MapReduce

Hadoop for NGS Analysis

CloudBurst

Highly Sensitive Short Read Mapping with MapReduce

100x speedup mapping on 96 cores @ Amazon

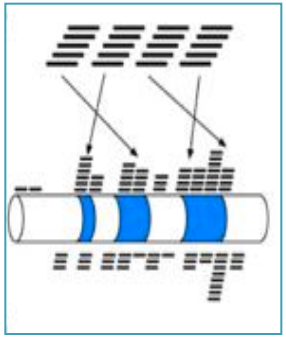
http://cloudburst-bio.sf.net

(Schatz, 2009)

Myrna

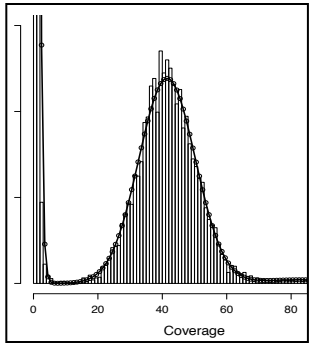
Cloud-scale differential gene expression for RNA-seq

Expression of 1.1 billion RNA-Seq reads in ~2 hours for ~$66



(Langmead, Hansen, Leek, 2010)

http://bowtie-bio.sf.net/myrna/



Quake

Quality-aware error correction of short reads

Correct 97.9% of errors with 99.9% accuracy

http://www.cbcb.umd.edu/software/quake/

(Kelley, Schatz, Salzberg, 2010)

Genome Indexing

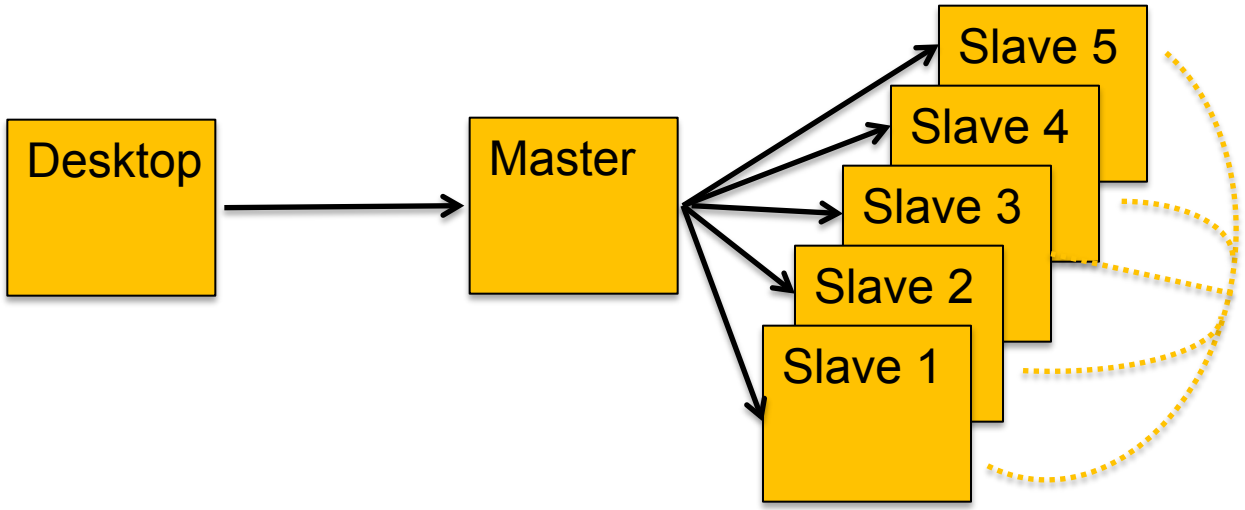
Rapid Parallel Construction of Genome Index

Construct the BWT of the human genome in 9 minutes

(Menon, Bhat, Schatz, 2011*)

http://code.google.com/p/genome-indexing/

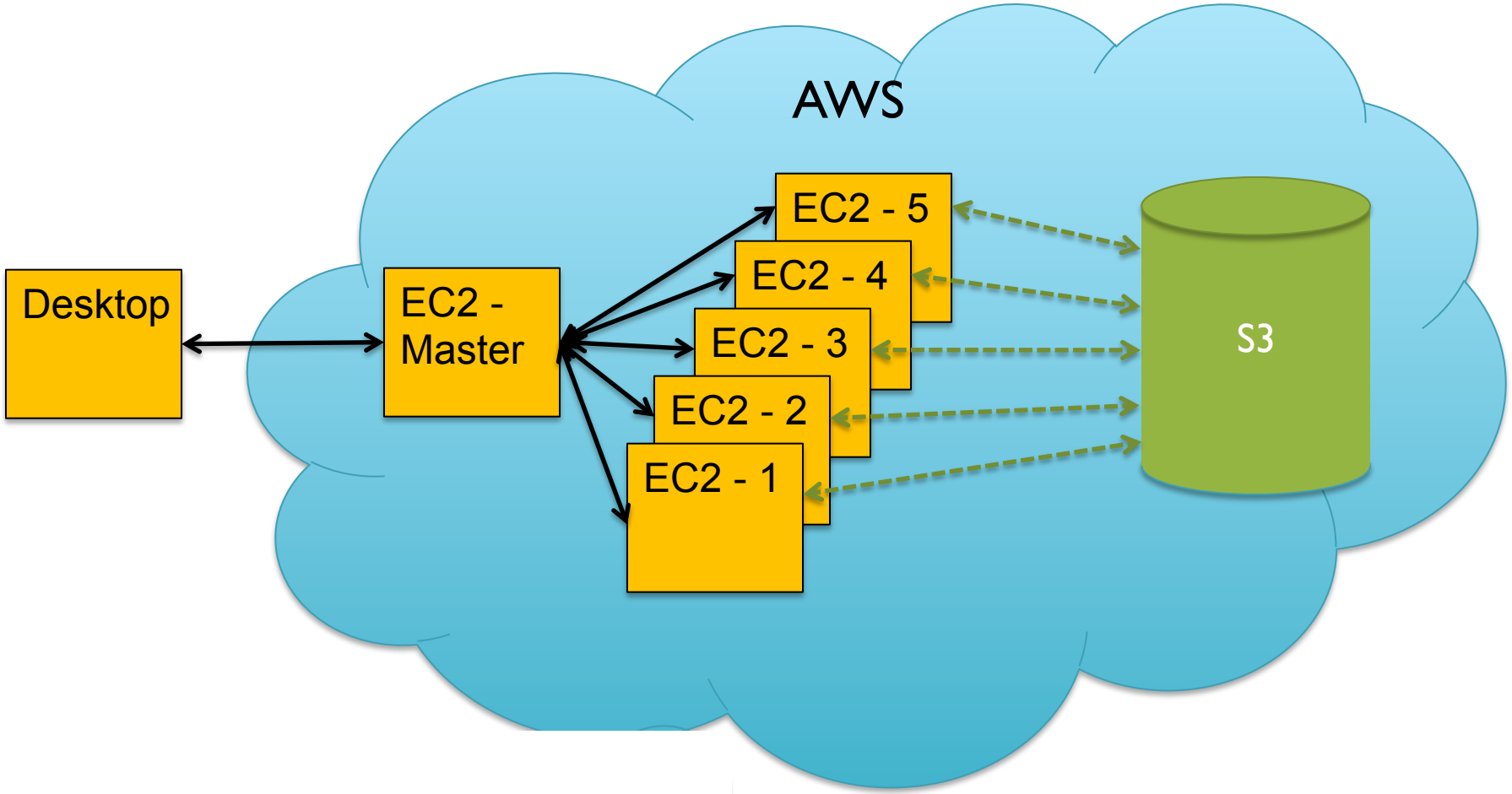
System Architecture



- Hadoop Distributed File System (HDFS)
  - Data files partitioned into large chunks (64MB), replicated on multiple nodes
  - Computation moves to the data, rack-aware scheduling

- Hadoop MapReduce system won the 2009 GreySort Challenge
  - Sorted 100 TB in 173 min (578 GB/min) using 3452 nodes and 4x3452 disks
  - Provides many disks in addition to many cores

Hadoop on AWS



If you don't have 1000s of machines, rent them from Amazon

- After machines spool up, ssh to master as if it was a local machine.
- Use S3 for persistent data storage, with very fast interconnect to EC2.

Parallel Algorithm Spectrum

Embarrassingly Parallel

Map-only

Each item is Independent

Loosely Coupled

MapReduce

Independent-Sync-Independent

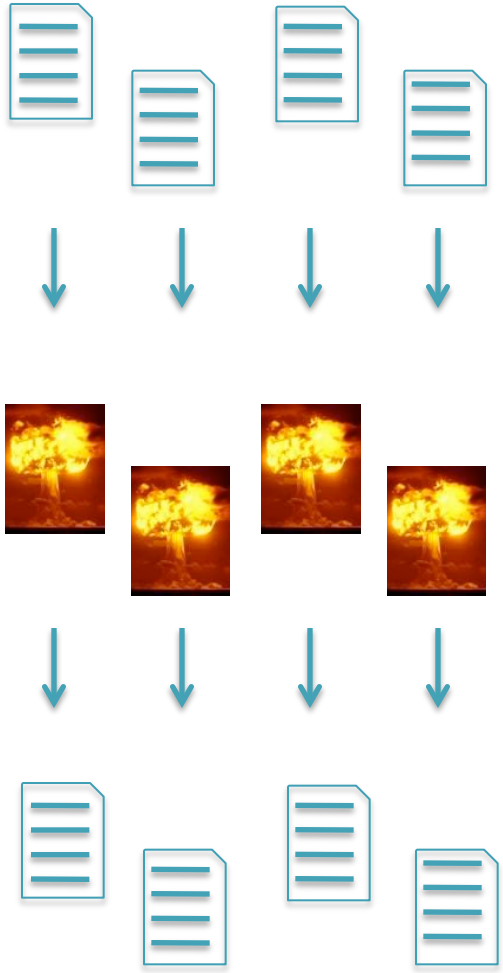
Tightly Coupled

Iterative MapReduce

Constant Sync

1. Embarrassingly Parallel

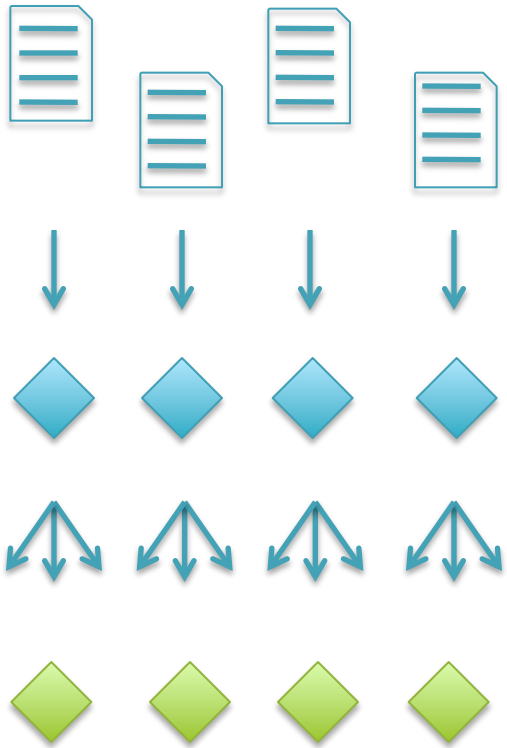
- Batch computing
  - Each item is independent
  - Split input into many chunks
  - Process each chunk separately on a different computer
- Challenges
  - Distributing work, load balancing, monitoring & restart
- Technologies
  - Condor, Sun Grid Engine
  - Amazon Simple Queue



Elementary School Dance

2. Loosely Coupled

- Divide and conquer
  - Independently process many items
  - Group partial results
  - Scan partial results into final answer
- Challenges
  - Batch computing challenges
  - + Shuffling of huge datasets
- Technologies
  - Hadoop, Elastic MapReduce, Dryad
  - Parallel Databases



Junior High Dance

Short Read Mapping

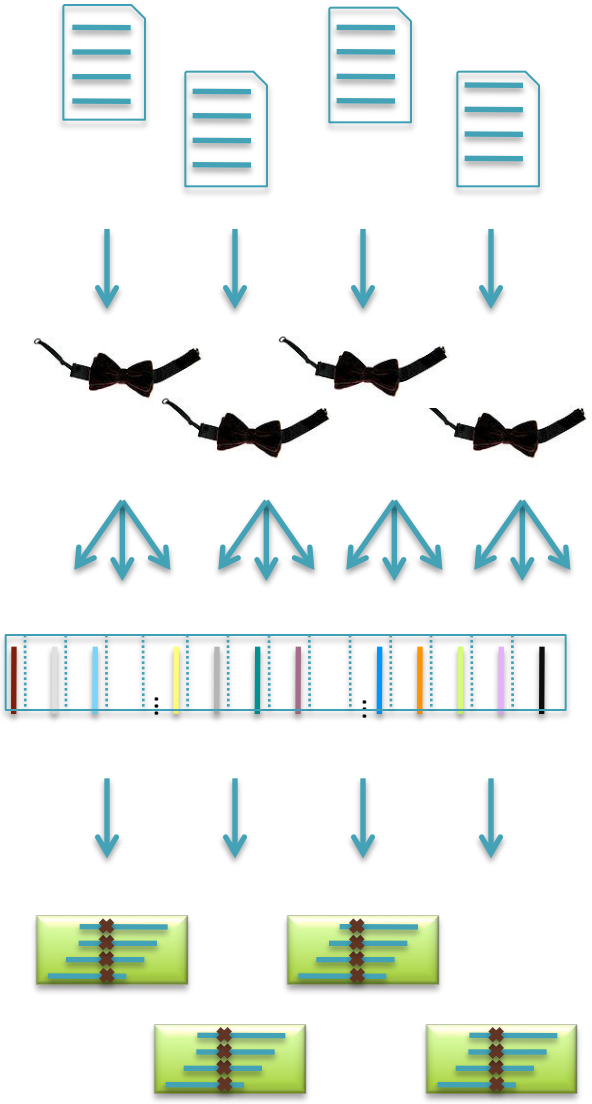
- Given a reference and many subject reads, report one or more "good" end-to-end alignments per alignable read
  - Find where the read most likely originated
  - Fundamental computation for many assays
    - Genotyping
    - Structural Variations
    - RNA-Seq
    - Chip-Seq
    - Methyl-Seq
    - Hi-C-Seq

- Desperate need for scalable solutions
  - Single human requires >1,000 CPU hours / genome

Crossbow

http://bowtie-bio.sourceforge.net/crossbow

- Align billions of reads and find SNPs
  - Reuse software components: Hadoop Streaming
- Map: Bowtie (Langmead *et al.*, 2009)
  - Find best alignment for each read
  - Emit (chromosome region, alignment)
- Shuffle: Hadoop
  - Group and sort alignments by region
- Reduce: SOAPsnp (Li *et al.*, 2009)
  - Scan alignments for divergent columns
  - Accounts for sequencing error, known SNPs



Performance in Amazon EC2

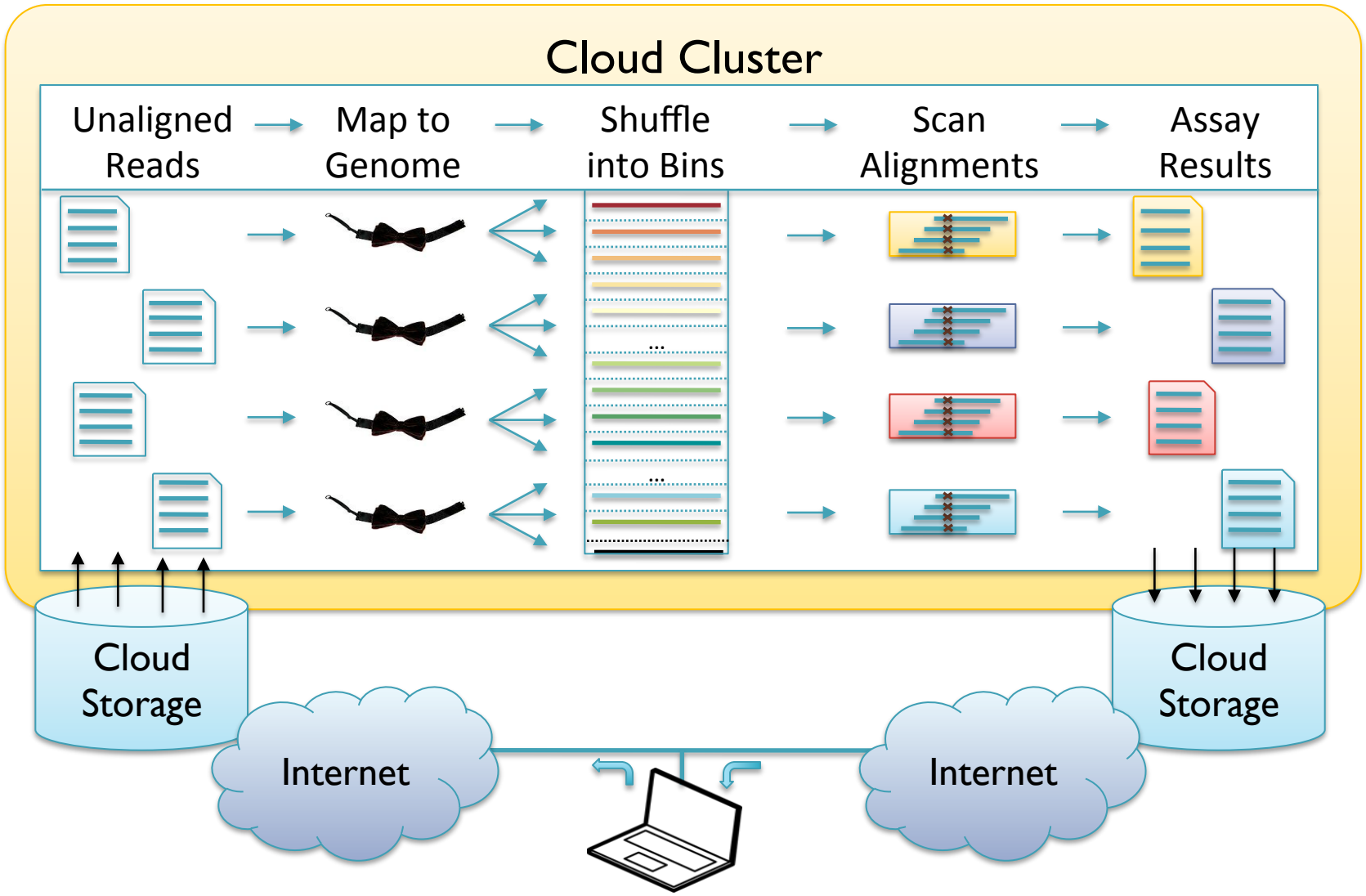
http://bowtie-bio.sourceforge.net/crossbow

	Asian Individual Genome		
Data Loading	3.3 B reads	106.5 GB	$10.65
Data Transfer	1h :15m	40 cores	$3.40
Setup	0h : 15m	320 cores	$13.94
Alignment	1h : 30m	320 cores	$41.82
Variant Calling	1h : 00m	320 cores	$27.88
End-to-end	4h : 00m		$97.69

Discovered 3.7M SNPs in one human genome for ~$100 in an afternoon.
Accuracy validated at >99%

Searching for SNPs with Cloud Computing.
Langmead B, Schatz MC, Lin J, Pop M, Salzberg SL (2009) *Genome Biology*. **10**:R134

Map-Shuffle-Scan for Genomics



Cloud Computing and the DNA Data Race.
Schatz, MC, Langmead B, Salzberg SL (2010) *Nature Biotechnology.* **28**:691-693

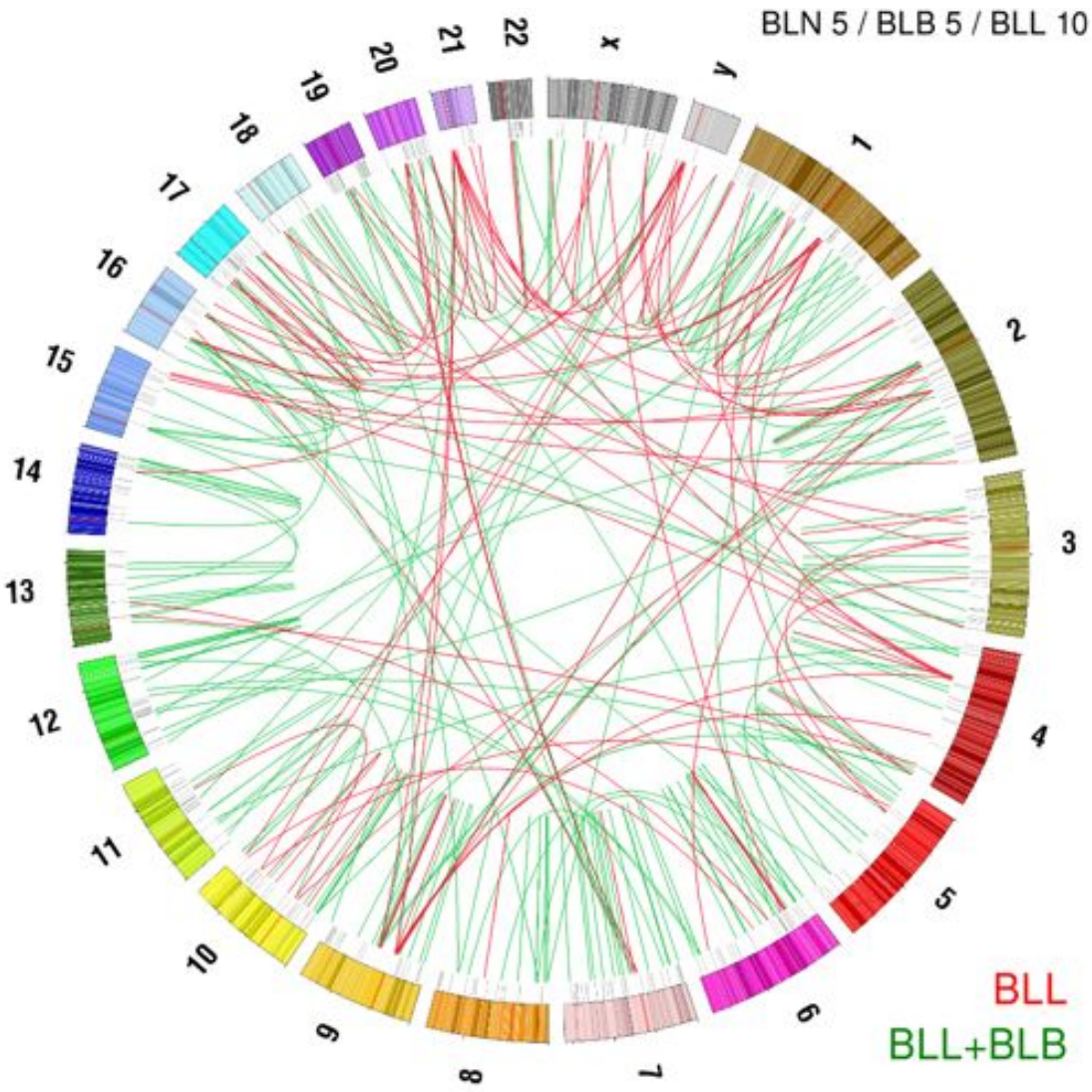
Jnomics Structural Variations

Circos plot of high confidence SVs specific to esophageal cancer sample

- Red: SVs specific to tumor
- Green: SVs in both diseased and tumor samples

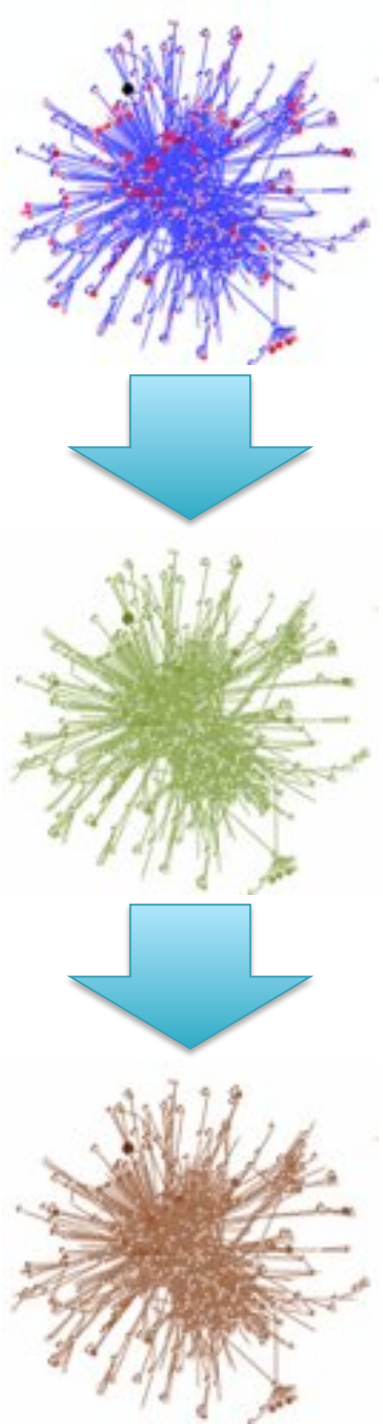
Detailed analysis of disrupted genes and fusion genes in progress

- Preliminary analysis shows many promising hits to known cancer genes



3. Tightly Coupled

- Computation that cannot be partitioned
  - Graph Analysis
  - Molecular Dynamics
  - Population simulations
- Challenges
  - Loosely coupled challenges
  - + Parallel algorithms design
- Technologies
  - MPI
  - MapReduce, Dryad, Pregel

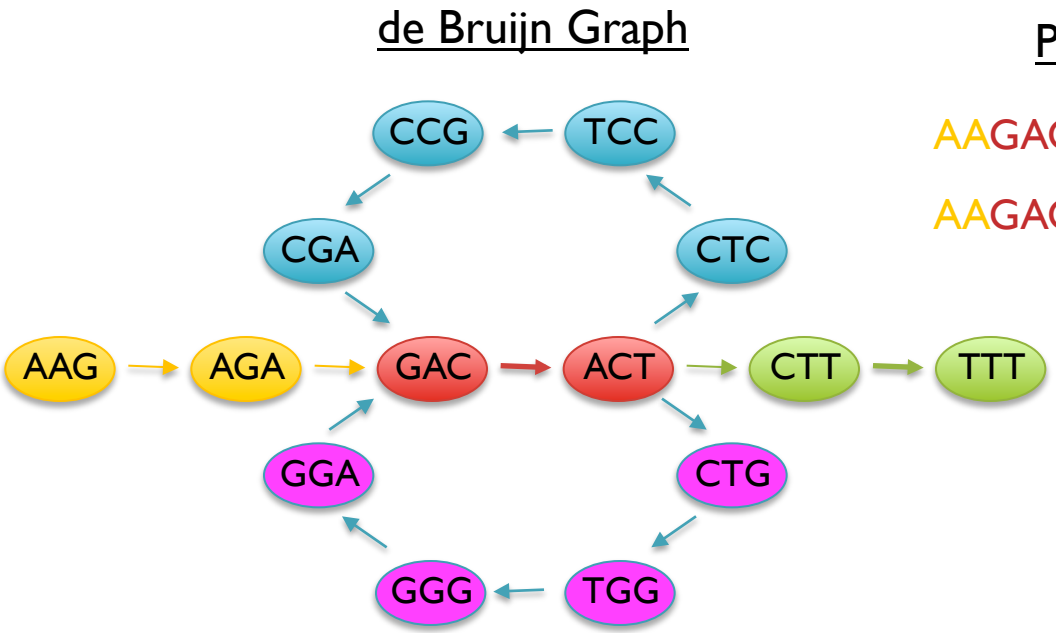


High School Dance

Short Read Assembly

Reads

AAGA
ACTT
ACTC
ACTG
AGAG
CCGA
CGAC
CTCC
CTGG
CTTT
…



Potential Genomes

AAGACTCCGACTGGGACTTT

AAGACTGGGACTCCGACTTT

- Genome assembly as finding an Eulerian tour of the de Bruijn graph
  - Human genome: >3B nodes, >10B edges
- The new short read assemblers require tremendous computation
  - Velvet (Zerbino & Birney, 2008) serial: > 2TB of RAM
  - ABySS (Simpson *et al.*, 2009) MPI: 168 cores x ~96 hours
  - SOAPdenovo (Li *et al.*, 2010) pthreads: 40 cores x 40 hours, >140 GB RAM

Warmup Exercise

Who here was born closest to Oct 3?

– You can only compare to 1 other person at a time

Find winner among 16 teams in just 4 rounds

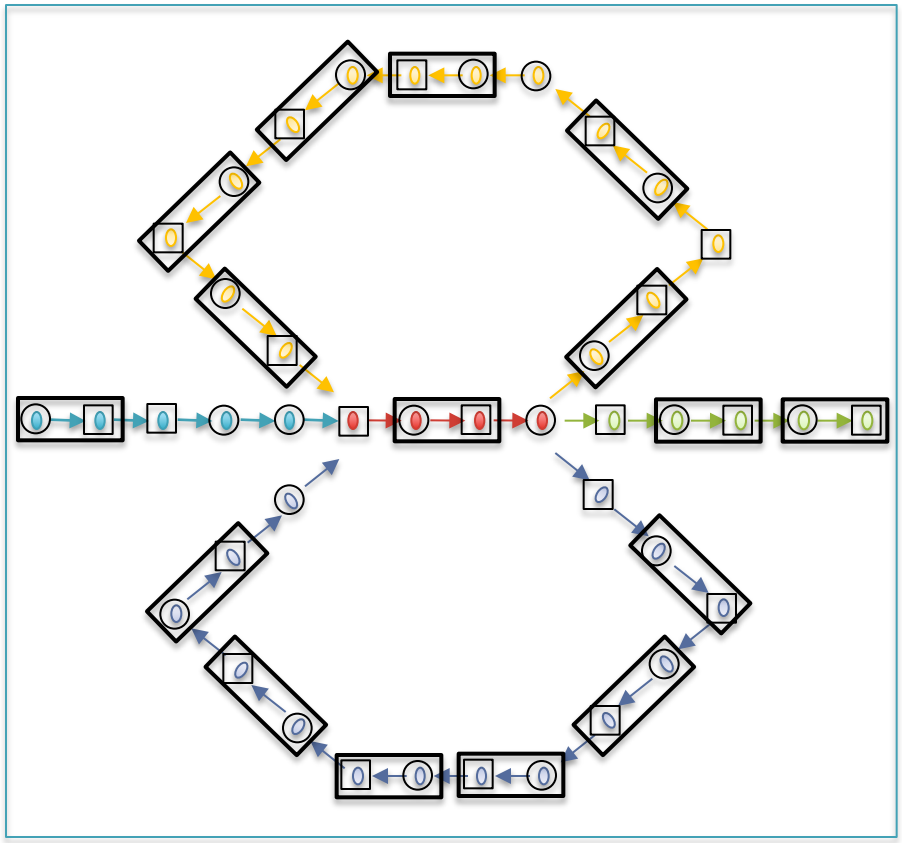
Fast Path Compression

Challenges

- Nodes stored on different computers
- Nodes can only access direct neighbors

Randomized List Ranking

- Randomly assign (H) / [T] to each compressible node
- Compress (H) → [T] links



Initial Graph: 42 nodes

Randomized Speed-ups in Parallel Computation.
Vishkin U. (1984) *ACM Symposium on Theory of Computation. 230-239.*

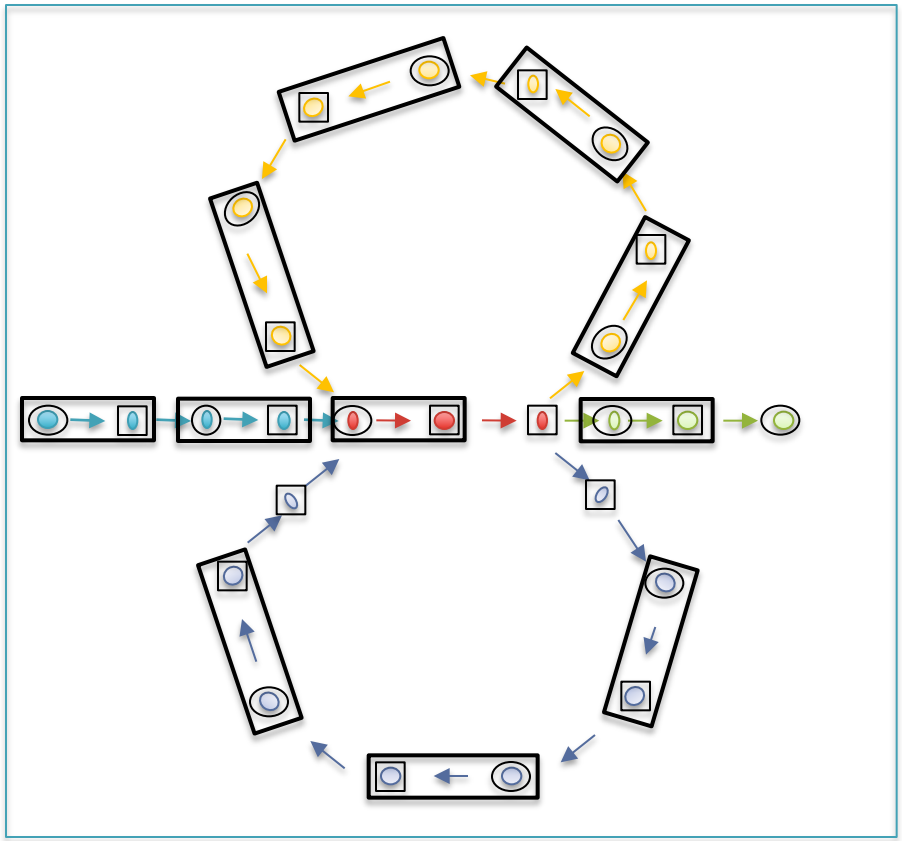
Fast Path Compression

Challenges

- Nodes stored on different computers
- Nodes can only access direct neighbors

Randomized List Ranking

- Randomly assign Ⓗ / [T] to each compressible node
- Compress Ⓗ → [T] links



Round 1: 26 nodes (38% savings)

Randomized Speed-ups in Parallel Computation.
Vishkin U. (1984) *ACM Symposium on Theory of Computation. 230-239.*

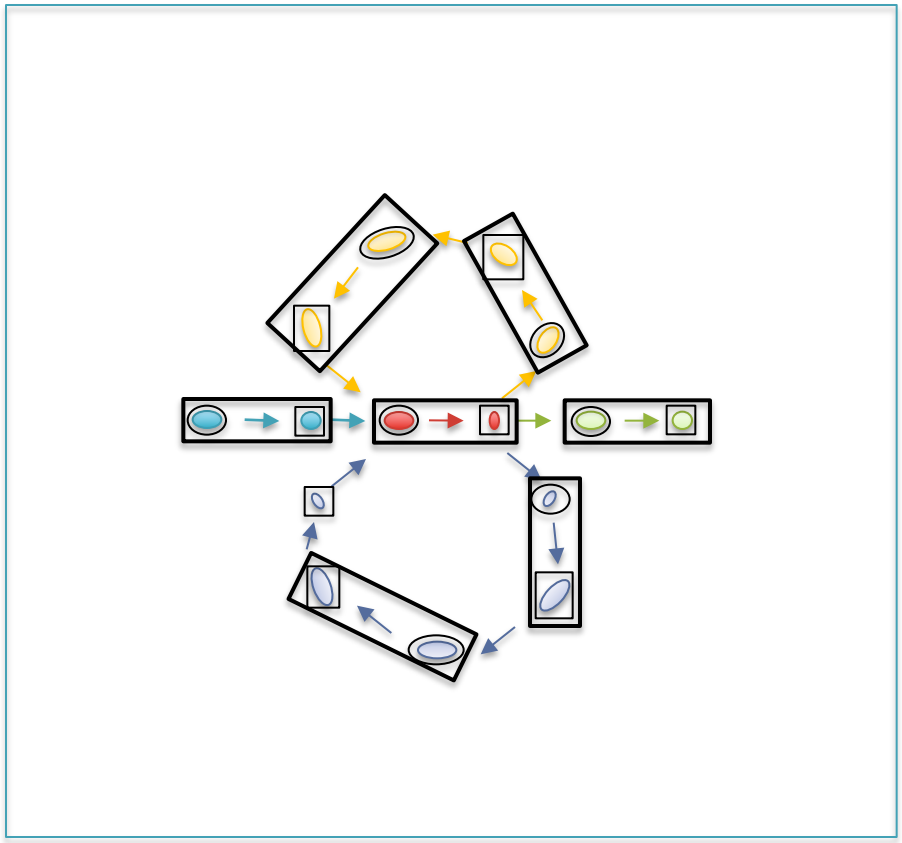
Fast Path Compression

Challenges

- Nodes stored on different computers
- Nodes can only access direct neighbors

Randomized List Ranking

- Randomly assign Ⓗ / [T] to each compressible node
- Compress Ⓗ → [T] links



Round 2: 15 nodes (64% savings)

Randomized Speed-ups in Parallel Computation.
Vishkin U. (1984) *ACM Symposium on Theory of Computation. 230-239.*

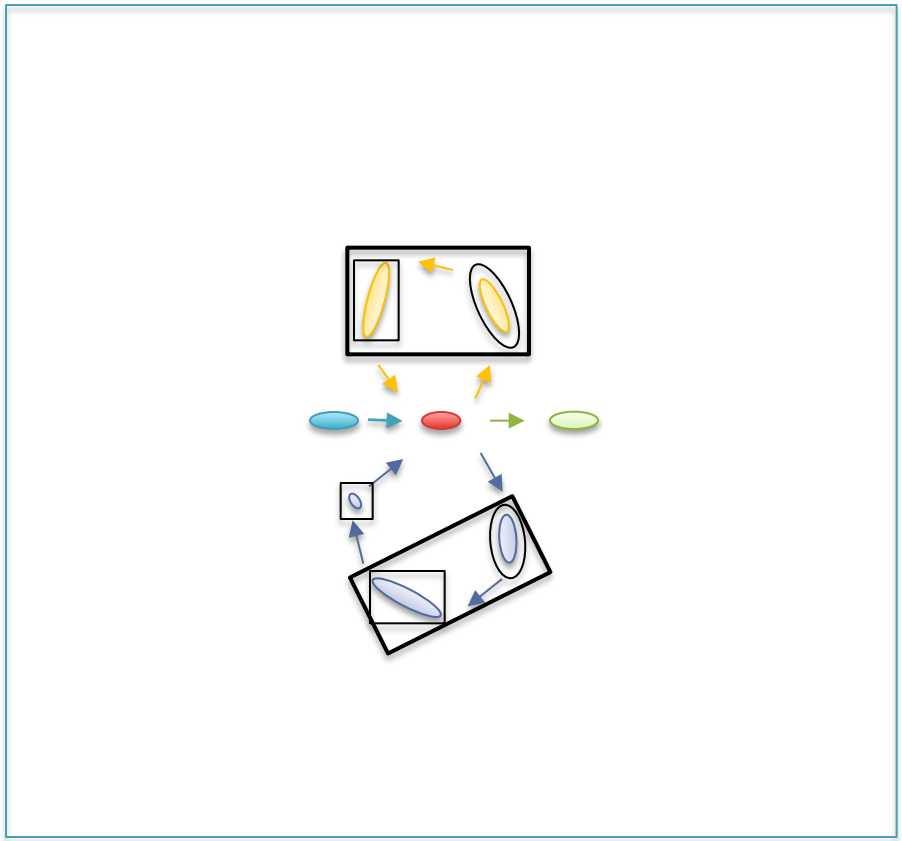
Fast Path Compression

Challenges

- Nodes stored on different computers
- Nodes can only access direct neighbors

Randomized List Ranking

- Randomly assign Ⓗ / [T] to each compressible node
- Compress Ⓗ → [T] links



Round 2: 8 nodes (81% savings)

Randomized Speed-ups in Parallel Computation.
Vishkin U. (1984) *ACM Symposium on Theory of Computation. 230-239.*

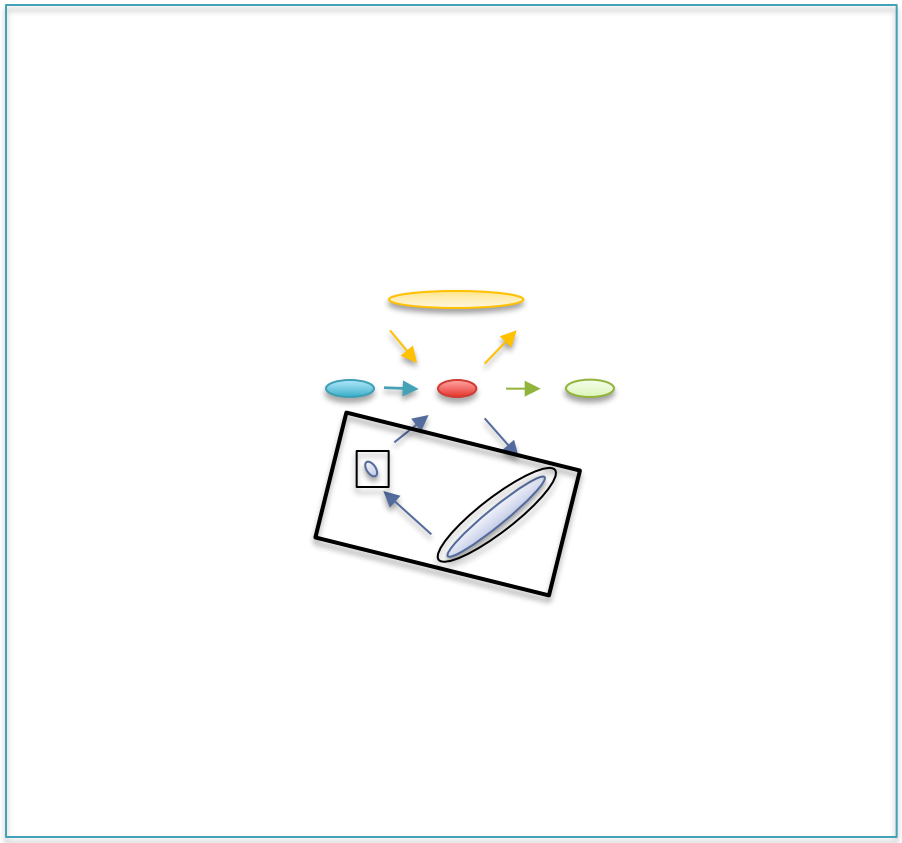
Fast Path Compression

Challenges

- Nodes stored on different computers
- Nodes can only access direct neighbors

Randomized List Ranking

- Randomly assign Ⓗ / [T] to each compressible node
- Compress Ⓗ → [T] links



Round 3: 6 nodes (86% savings)

Randomized Speed-ups in Parallel Computation.
Vishkin U. (1984) *ACM Symposium on Theory of Computation. 230-239.*

Fast Path Compression

Challenges

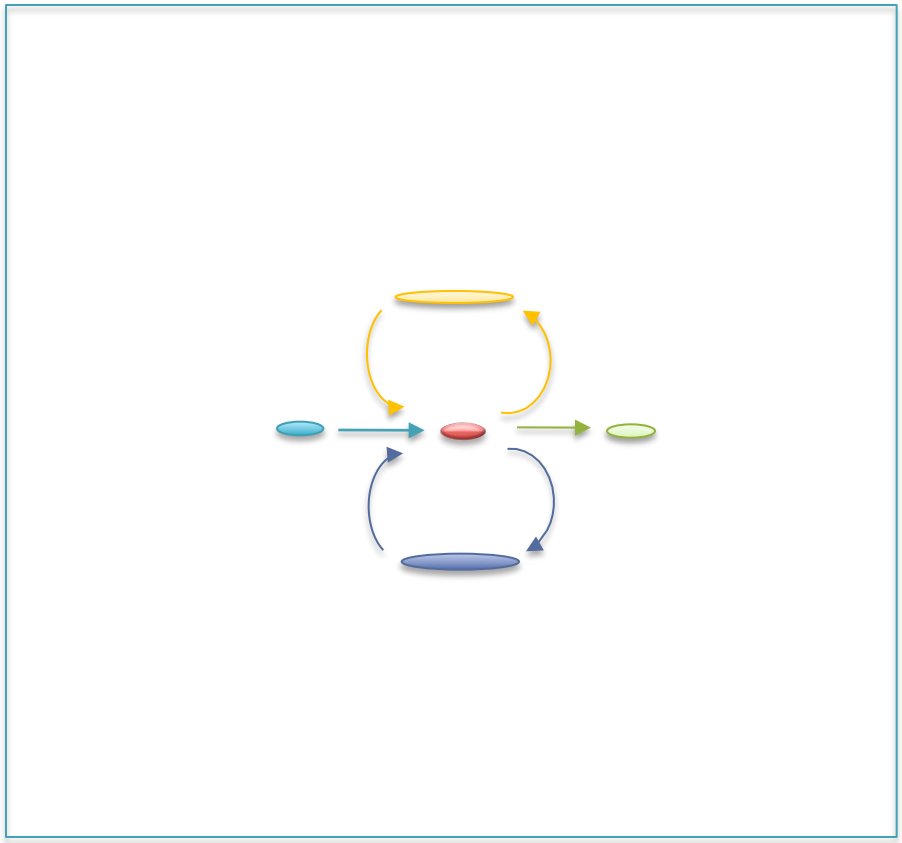
- Nodes stored on different computers
- Nodes can only access direct neighbors

Randomized List Ranking

- Randomly assign Ⓗ / [T] to each compressible node
- Compress Ⓗ → [T] links

Performance

- Compress all chains in log(S) rounds



Round 4: 5 nodes (88% savings)

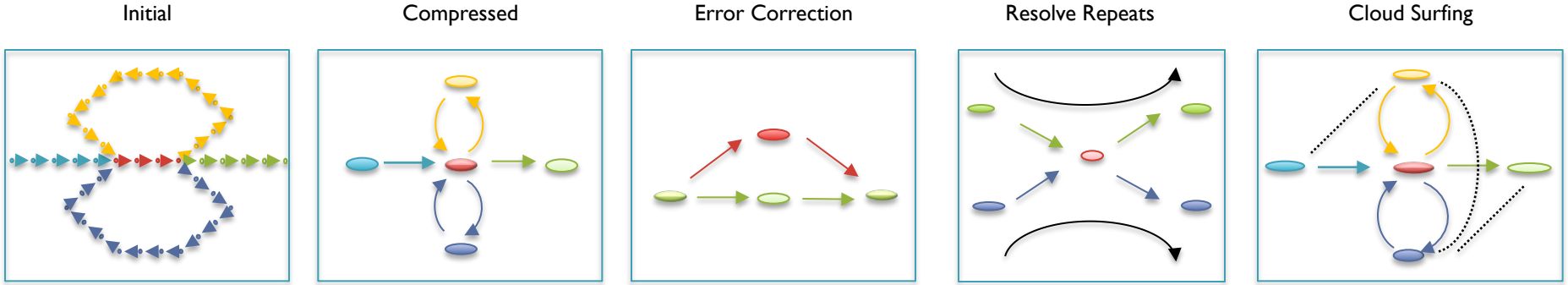
Randomized Speed-ups in Parallel Computation.
Vishkin U. (1984) *ACM Symposium on Theory of Computation. 230-239.*

Contrail

http://contrail-bio.sourceforge.net

De novo bacterial assembly

- *Genome: E. coli* K12 MG1655, 4.6Mbp
- *Input:* 20.8M 36bp reads, 200bp insert (~150x coverage)
- *Preprocessor*: Quake Error Correction



N	5.1 M	245,131	2,769	1,909	300
Max	27 bp	1,079 bp	70,725 bp	90,088 bp	149,006 bp
N50	27 bp	156 bp	15,023 bp	20,062 bp	54,807 bp

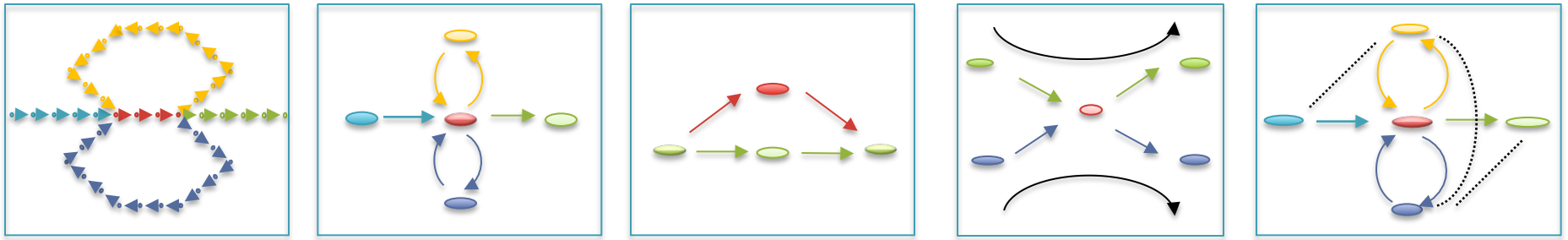
Assembly of Large Genomes with Cloud Computing.
Schatz MC, Sommer D, Kelley D, Pop M, *et al. In Preparation.*

Contrail

http://contrail-bio.sourceforge.net

De novo Assembly of the Human Genome

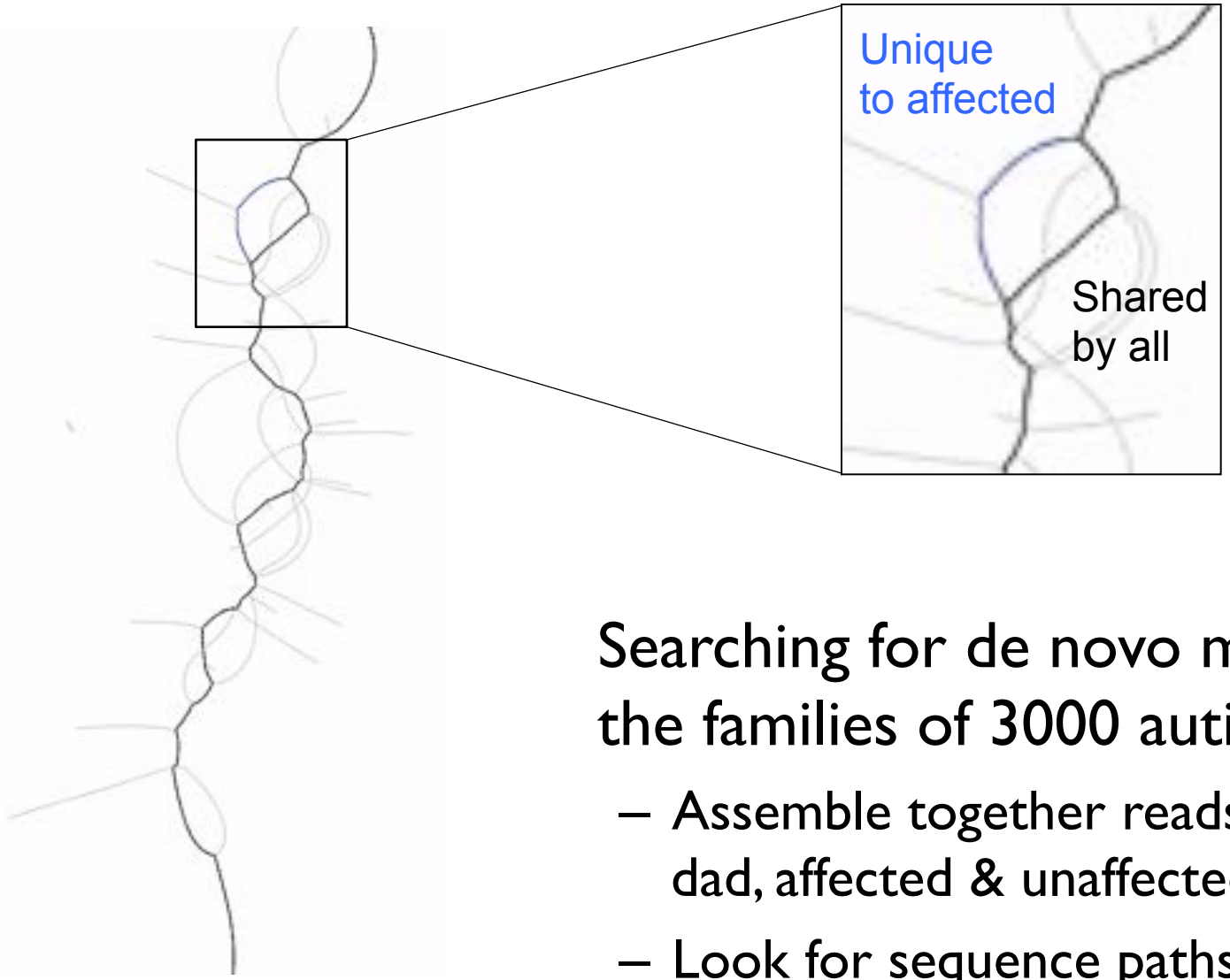
- *Genome:* African male NA18507 (SRA000271, Bentley *et al.*, 2008)
- *Input: 3.5*B 36bp reads, 210bp insert (~40x coverage)



N	>7 B	>1 B	4.2 M	4.1 M	3.3 M
Max	27 bp	303 bp	20,594 bp	20,594 bp	20,594 bp
N50	27 bp	< 100 bp	995 bp	1,050 bp	1,427 bp*

Assembly of Large Genomes with Cloud Computing.
Schatz MC, Sommer D, Kelley D, Pop M, *et al. In Preparation.*

De novo mutations and de Bruijn Graphs



COLEC12
C->A

Searching for de novo mutations in the families of 3000 autistic children.

- Assemble together reads from mom, dad, affected & unaffected children
- Look for sequence paths unique to affected child

Summary

- We are entering the digital age of biology
  - Next generation sequencing, microarrays, mass spectrometry, microscopy, ecology, etc
  - Parallel computing may be our only hope for keeping up with the pace of advance
- Modern biology requires (is) quantitative biology
  - Computational, mathematical, and statistical techniques applied to analyze, integrate, and interpret biological sensor data
- Don't let the data tsunami crash on you
  - Study, practice, collaborate with quantitative techniques

Watson School of Biological Sciences

Since opening in 1999, the WSBS has become a leading PhD program in the biological sciences, one whose fresh approach is quickly being emulated by other programs across the country.

- An innovative Ph.D. program designed for exceptional students
  - Approximately four years from matriculation to Ph.D. degree award
  - A first year with course work and laboratory rotations in separate phases
  - Emphasis on the principles of scientific reasoning and logic

- Learn more: http://www.cshl.edu/gradschool

Acknowledgements

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Sergey Koren

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Jimmy Lin
David Kelley
Dan Sommer
Cole Trapnell

Thank You!

http://schatzlab.cshl.edu
@mike_schatz