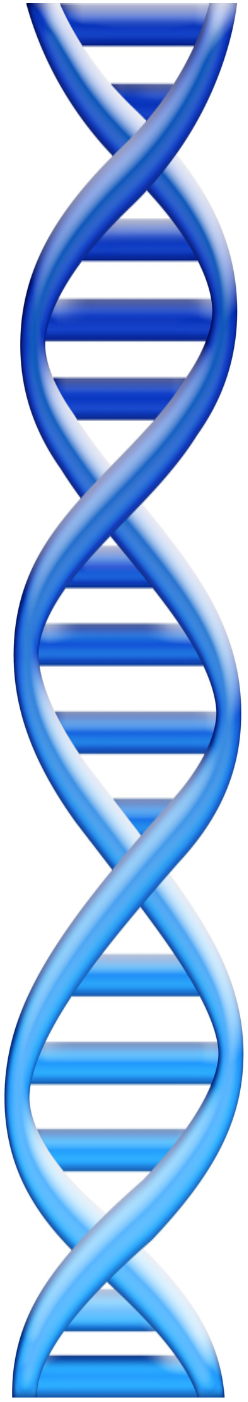
Biology in the Clouds

Michael Schatz

July 30, 2012
Science Cloud Summer School



Outline

1. Milestones in genomics
   1. Quick primer on molecular biology
   2. The evolution of sequencing

2. Hadoop Applications for Genomics
   1. Mapping & Jnomics
   2. Assembly & Contrail

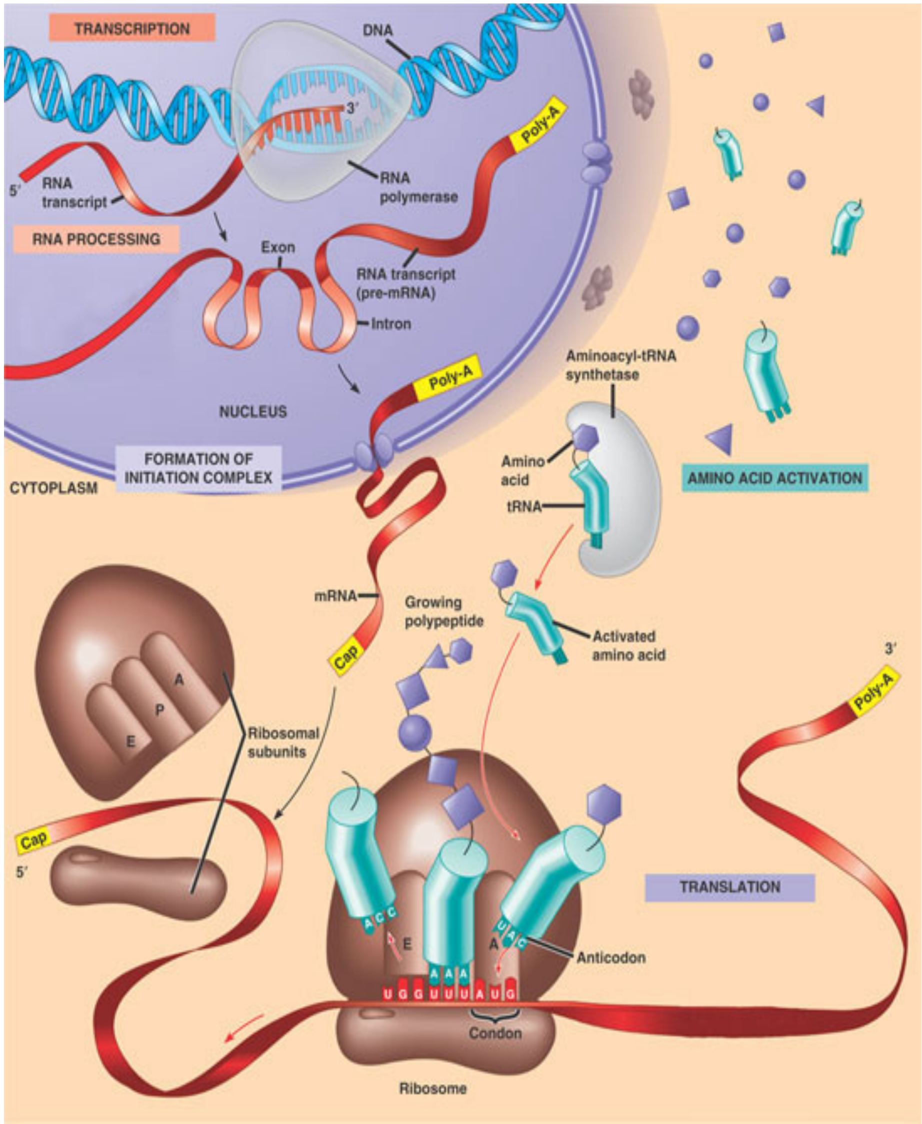
Milestones in Genomics

In 1953 James Watson and Francis Crick determined the double helix structure of DNA as a long series of 4 different nucleotides. In 1958 Francis Crick established the **Central Dogma** of Biology:

1. Genetic information is transmitted from generation to generation by the sequence of nucleotides in your ***DNA***.

2. Active regions called genes, are transcribed into ***messenger RNAs*** that are sent to cellular machines called ribosomes for processing

3. RNA messages are translated by the ribosomes into ***proteins*** that do work in the cell



http://compbio.pbworks.com/w/page/16252897/Introduction%20and%20Basic%20Molecular%20Biology

Milestones in Genomics

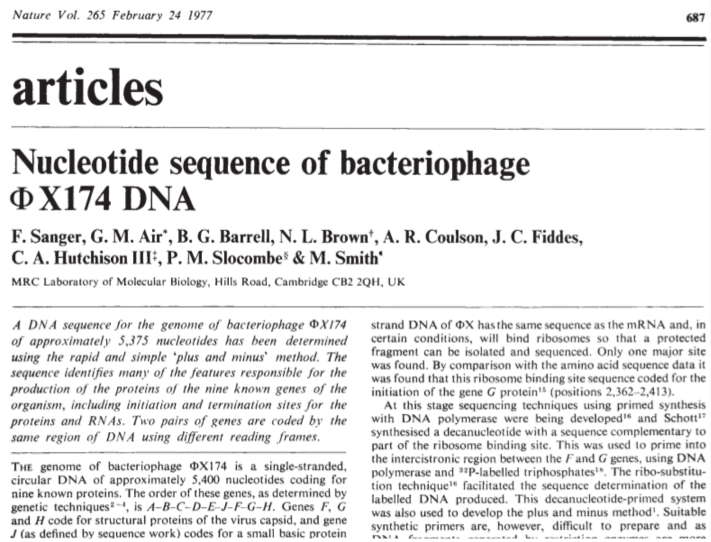
Your genome and environment define who you are:

- Human with 5 fingers & 5 toes
- Hair, eye & skin color
- Susceptibility to diseases, responses to drugs
- Personality and social disorders
- …

There is tremendous interest to sequence genomes:

- What is your genome sequence?
- How does your genome compare to my genome?
- Where are the genes and how active are they?
- How does gene activity change under development?
- Where do proteins bind and regulate genes?
- How has the disease mutated your genome?
- What virus and microbes are living inside you?
- What drugs should we give you?
- …

Milestones in Genomics:
Zeroth Generation Sequencing



Nature Vol. 265 February 24 1977 687

articles

Nucleotide sequence of bacteriophage ΦX174 DNA

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

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

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

THE genome of bacteriophage ΦX174 is a single-stranded, circular DNA of approximately 5,400 nucleotides coding for nine known proteins. The order of these genes, as determined by genetic techniques[2–4], is *A–B–C–D–E–J–F–G–H*. Genes *F*, *G* and *H* code for structural proteins of the virus capsid, and gene *J* (as defined by sequence work) codes for a small basic protein strand DNA of ΦX has the same sequence as the mRNA and, in certain conditions, will bind ribosomes so that a protected fragment can be isolated and sequenced. Only one major site was found. By comparison with the amino acid sequence data it was found that this ribosome binding site sequence coded for the initiation of the gene *G* protein[15] (positions 2,362–2,413).

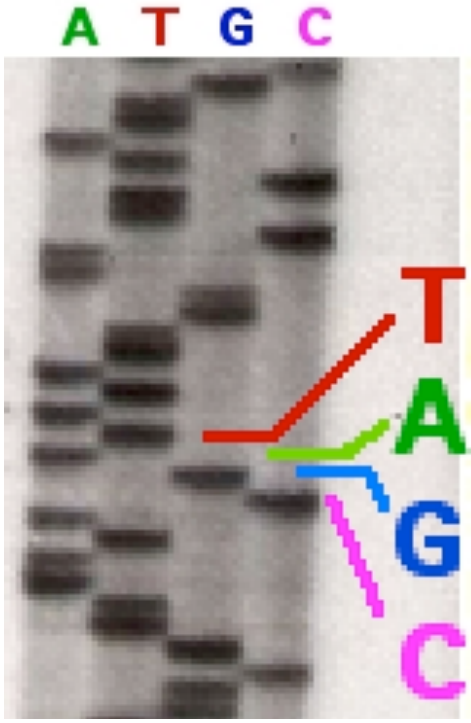
At this stage sequencing techniques using primed synthesis with DNA polymerase were being developed[16] and Schott[17] synthesised a decanucleotide with a sequence complementary to part of the ribosome binding site. This was used to prime into the intercistronic region between the *F* and *G* genes,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

1977

1st Complete Organism

Bacteriophage ϕX174

5375 bp

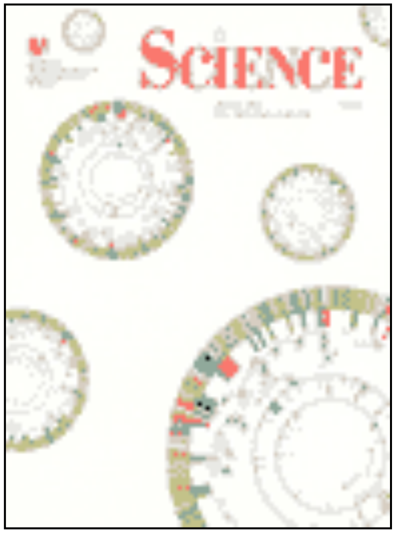


Radioactive Chain Termination
5000bp / week / person

http://en.wikipedia.org/wiki/File:Sequencing.jpg
http://www.answers.com/topic/automated-sequencer

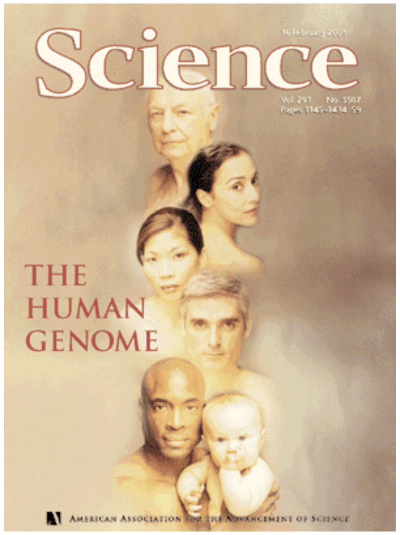
Nucleotide sequence of bacteriophage ϕX174 DNA
Sanger, F. et al. (1977) *Nature. 265: 687 - 695*

Milestones in Genomics: First Generation Sequencing



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.* / IHGSC
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

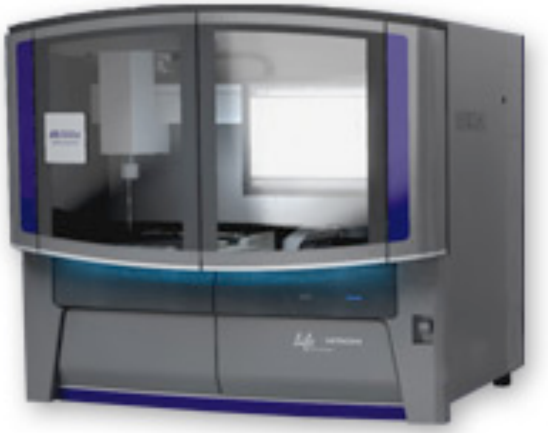
Milestones in Genomics: Second Generation Sequencing

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

Milestones in Genomics: Third Generation Sequencing

2010
Ion Torrent
Postlight Sequencing

Current Specs (Ion 318):
11M 300bp reads / run =
>1Gbp / day

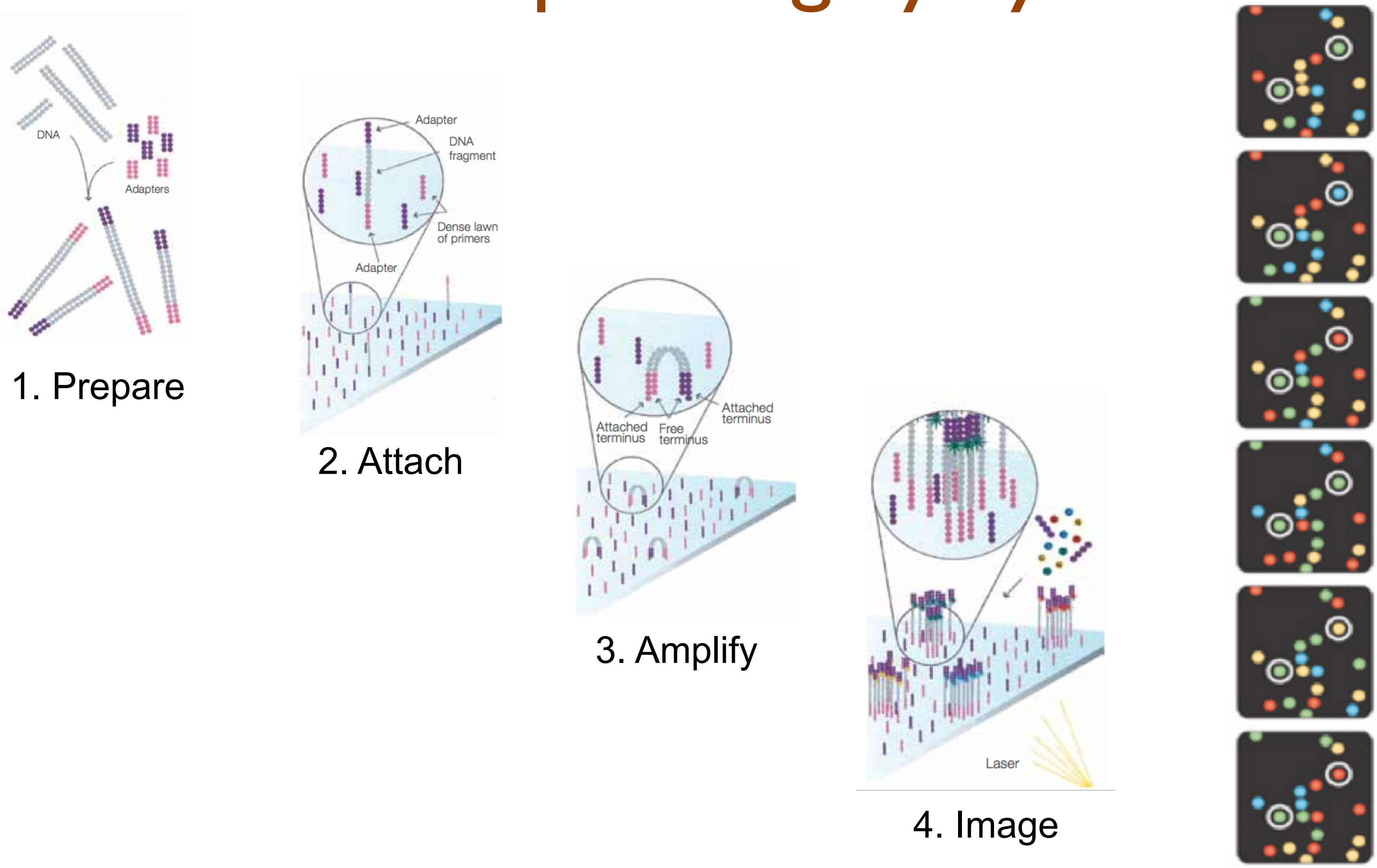
2011
Pacific Biosciences
SMRT Sequencing

Current Specs (RS):
50k 10kbp reads / run =
>500Mbp / day

2012
Oxford Nanopore
Nanopore sensing

Many GB / day?
Very Long Reads?

Illumina Sequencing by Synthesis



1. Prepare

2. Attach

3. Amplify

4. Image

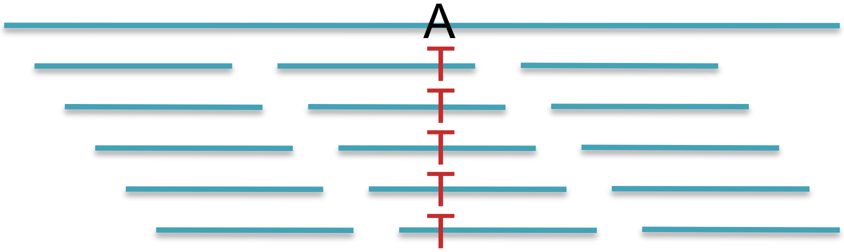
5. Basecall

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

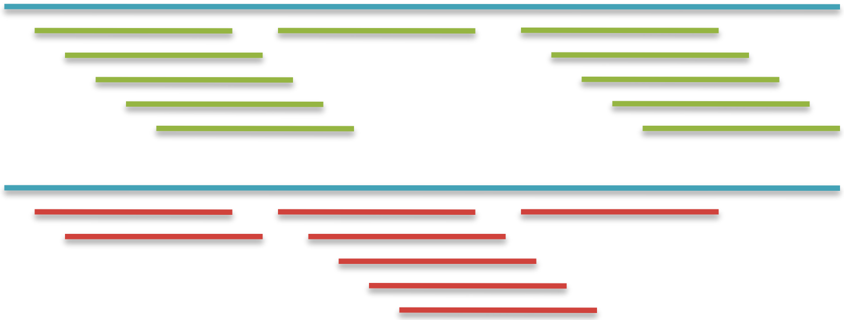
http://www.youtube.com/watch?v=l99aKKHcxC4

Milestones in Genomics

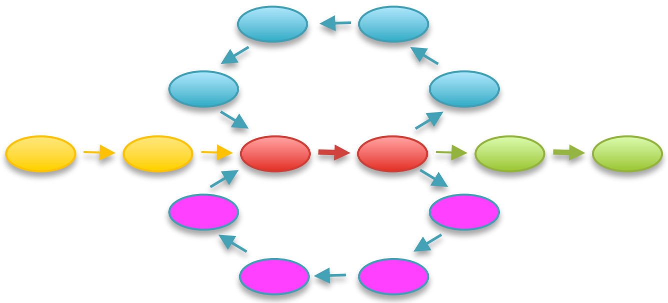
Alignment & Variations



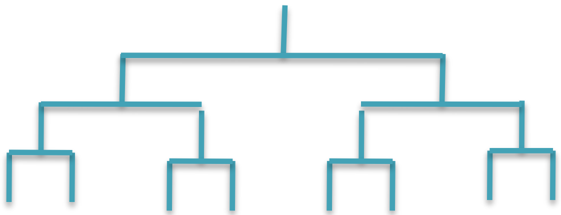
Differential Analysis



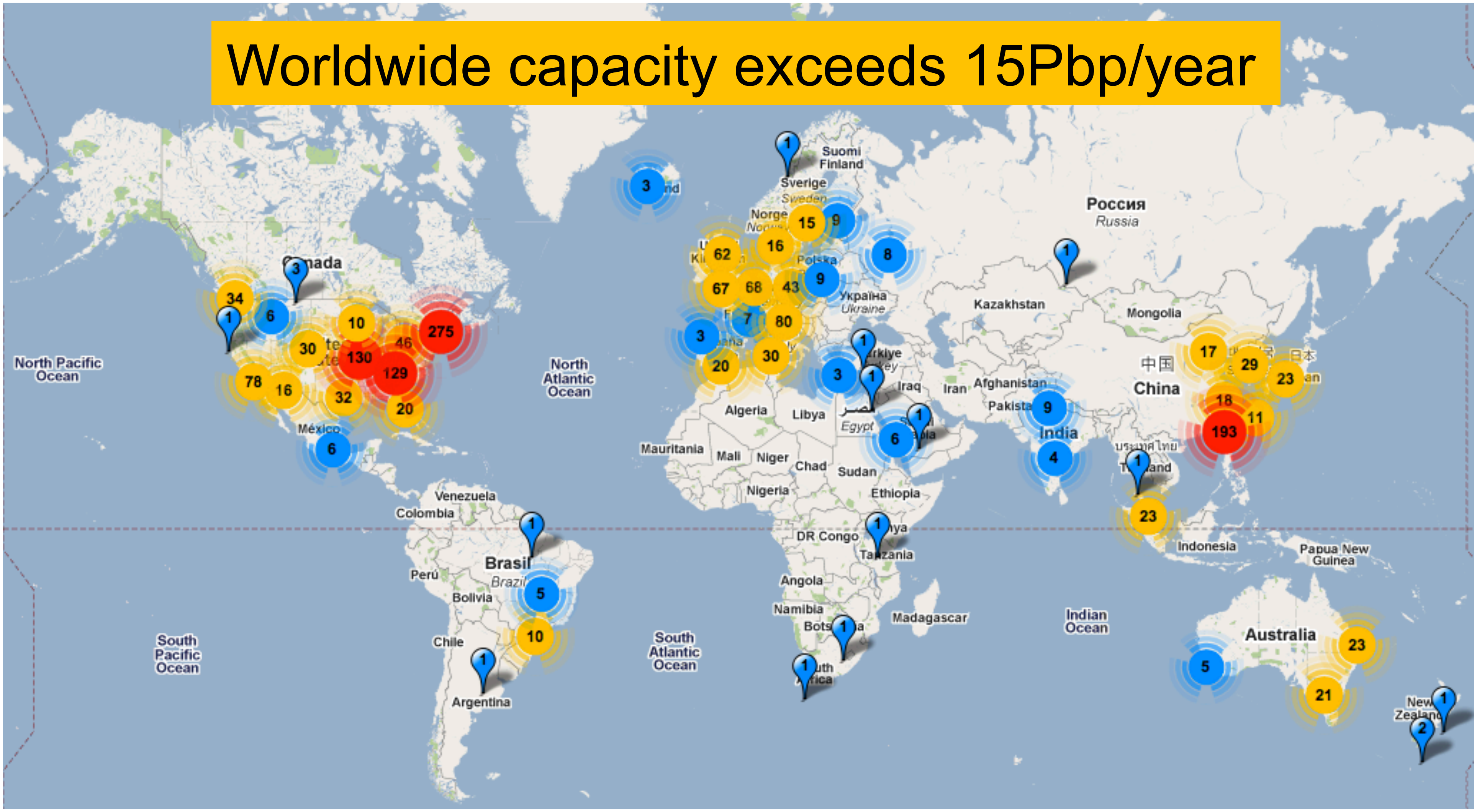
De novo Assembly



Phylogeny & Modeling



Sequencing Centers



Worldwide capacity exceeds 15Pbp/year

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

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)
    - 946PB 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

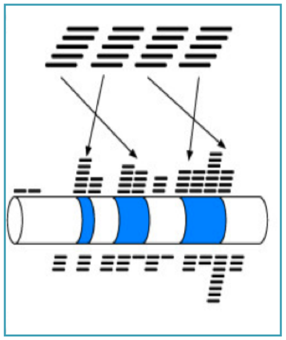
http://cloudburst-bio.sf.net

(Schatz, 2009)

Myrna

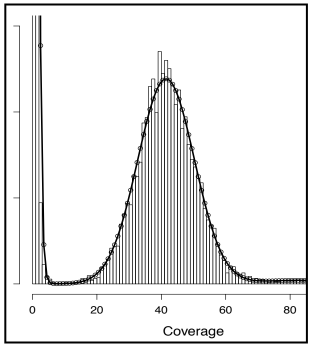
Cloud-scale differential gene expression for RNA-seq

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

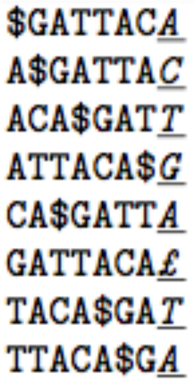
http://www.cbcb.umd.edu/software/quake/

(Kelley, Schatz,
Salzberg, 2010)

Genome Indexing

Rapid Parallel Construction of Genome Index

Construct the BWT of the human genome in 9 minutes

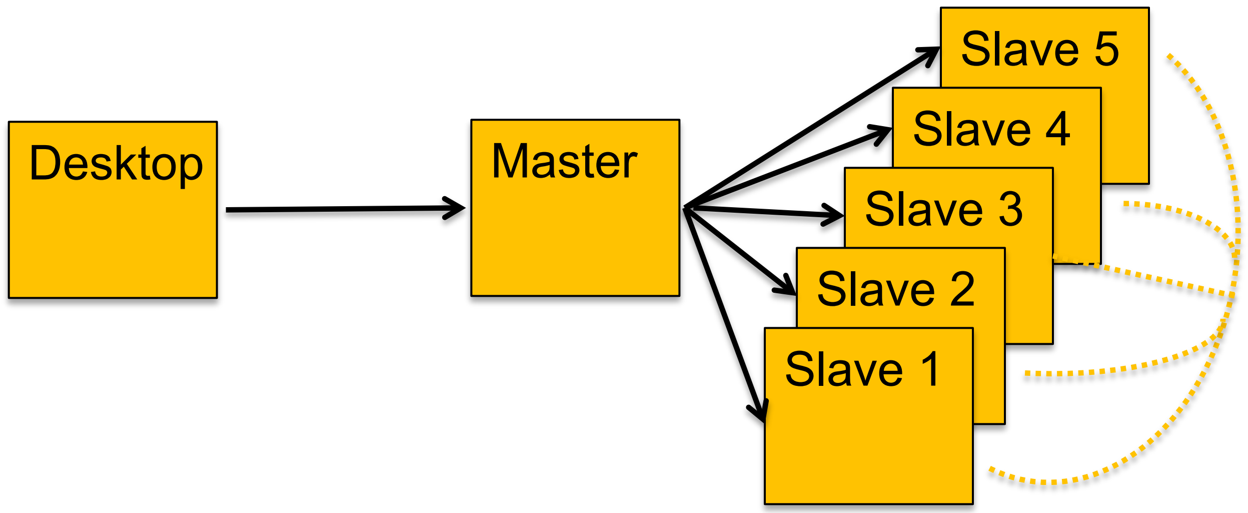


```
$GATTACA
A$GATTAC
ACA$GATT
ATTACA$G
CA$GATTA
GATTACA£
TACA$GAT
TTACA$GA
```

(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

Parallel Algorithm Spectrum

Embarrassingly Parallel

Map-only

Each item is Independent

Loosely Coupled

MapReduce

Independent-Sync-Independent

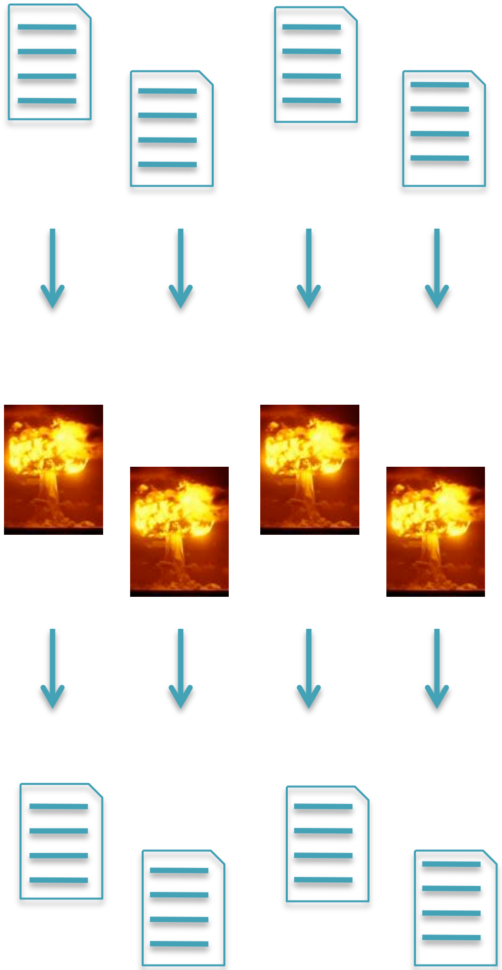
Tightly Coupled

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

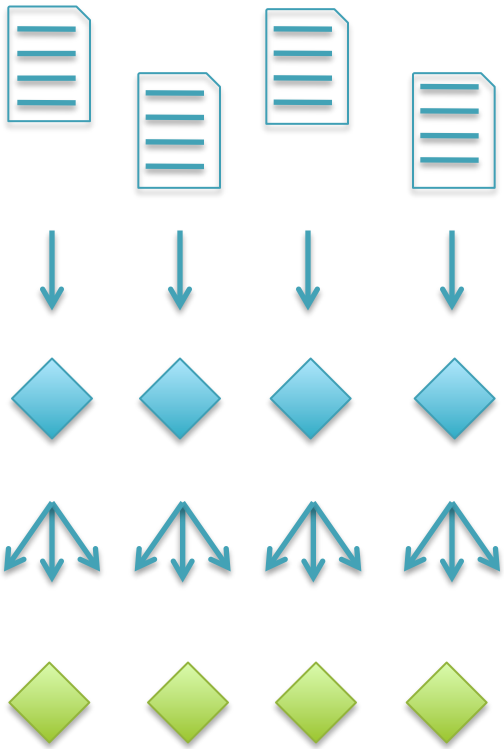


- Challenges
  - Distributing work, load balancing, monitoring & restart
- Technologies
  - Condor, Sun Grid Engine
  - Amazon Simple Queue

Elementary School Dance

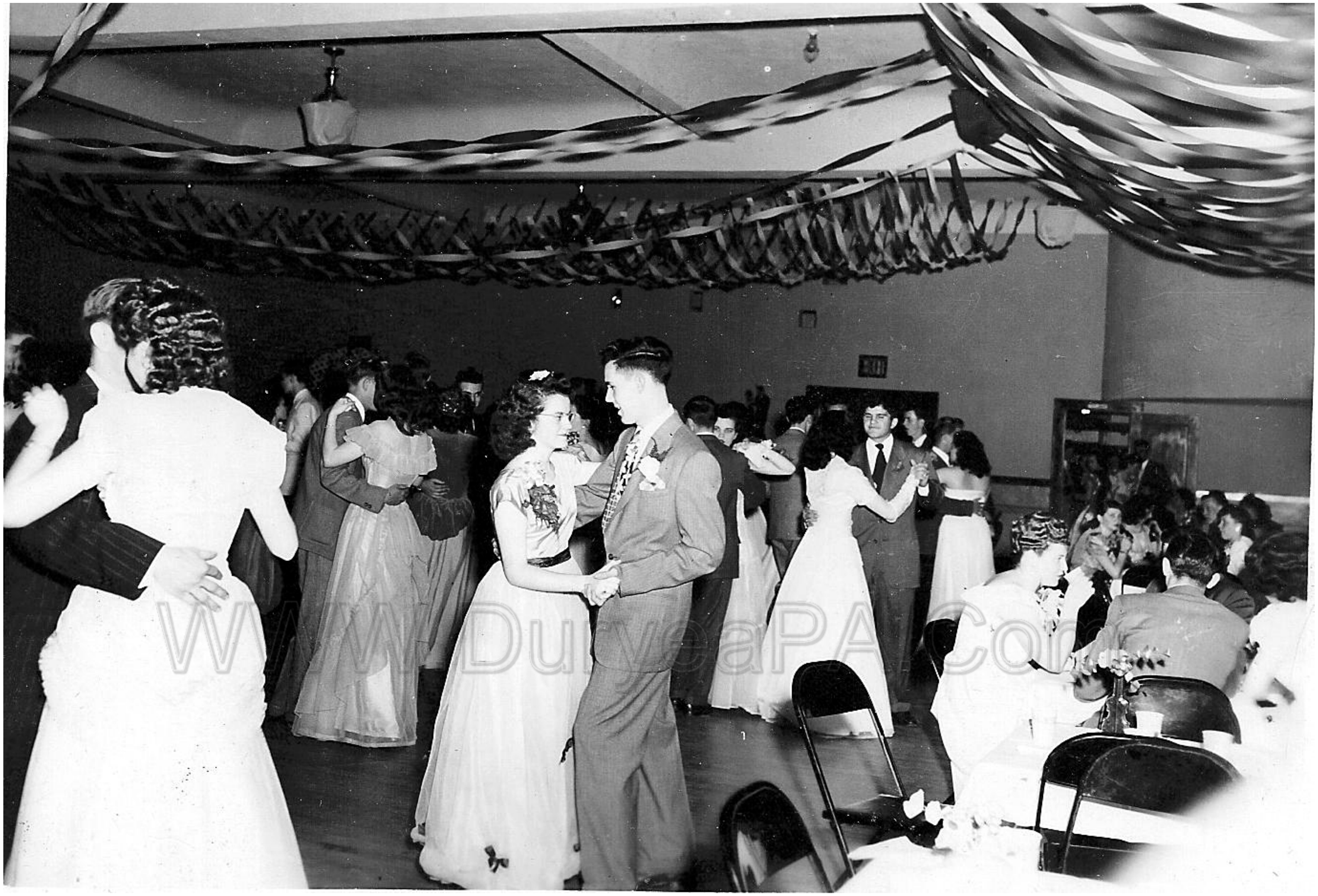
2. Loosely Coupled

- Divide and conquer
  - Independently process many items
  - Group partial results
  - Scan partial results into final answer

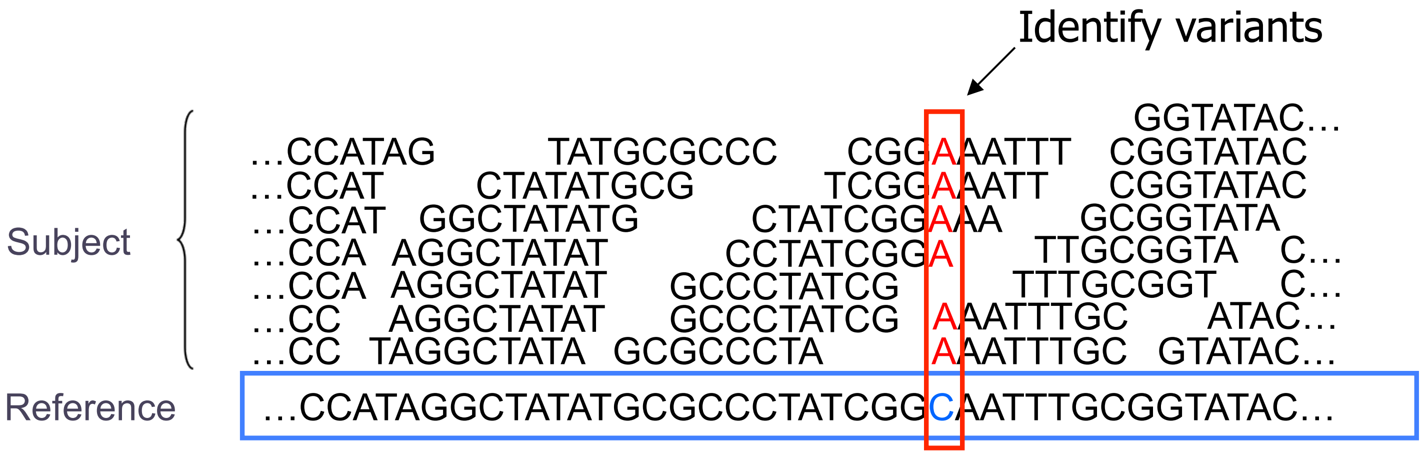


- Challenges
  - Batch computing challenges
  - + Shuffling of huge datasets
- Technologies
  - Hadoop, Elastic MapReduce, Dryad
  - Parallel Databases

Junior High Dance



Short Read Mapping



Identify variants

Subject

```
                                  GGTATAC…
…CCATAG    TATGCGCCC   CGGAAATTT CGGTATAC
…CCAT   CTATATGCG     TCGGAAATT  CGGTATAC
…CCAT GGCTATATG    CTATCGGAAA   GCGGTATA
…CCA AGGCTATAT    CCTATCGGA   TTGCGGTA  C…
…CCA AGGCTATAT  GCCCTATCG    TTTGCGGT   C…
…CC  AGGCTATAT  GCCCTATCG AAATTTGC   ATAC…
…CC TAGGCTATA GCGCCCTA    AAATTTGC GTATAC…
```

Reference

```
…CCATAGGCTATATGCGCCCTATCGGCAATTTGCGGTATAC…
```

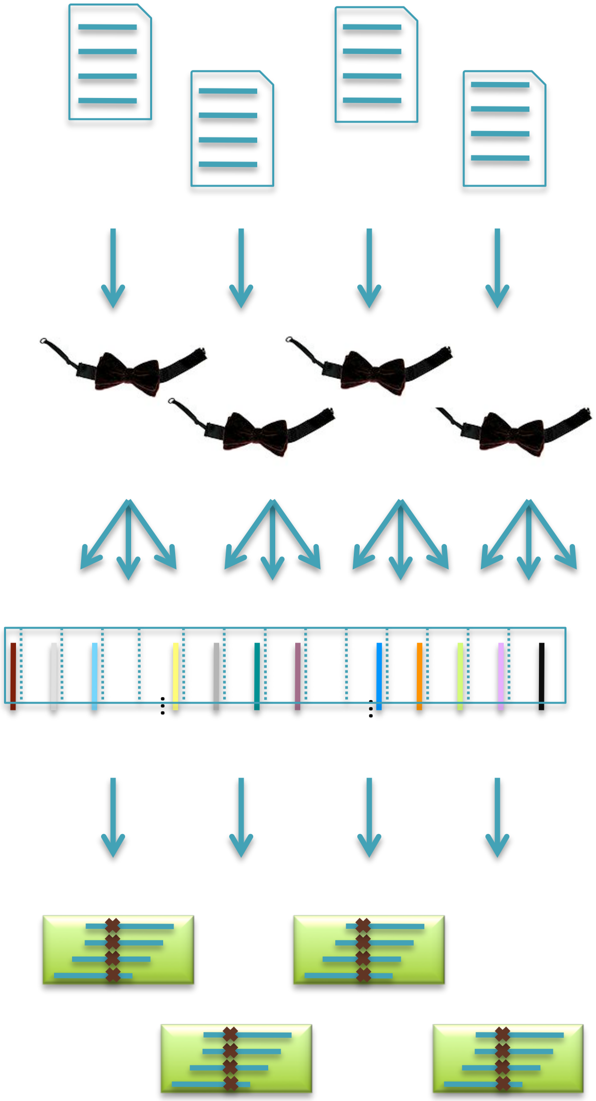
- Given a reference and many subject reads, report one or more "good" end-to-end alignments per alignable read
  - Find where the read most likely originated
  - Fundamental computation for many assays
    - Genotyping RNA-Seq Methyl-Seq
    - Structural Variations Chip-Seq Hi-C-Seq

- 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

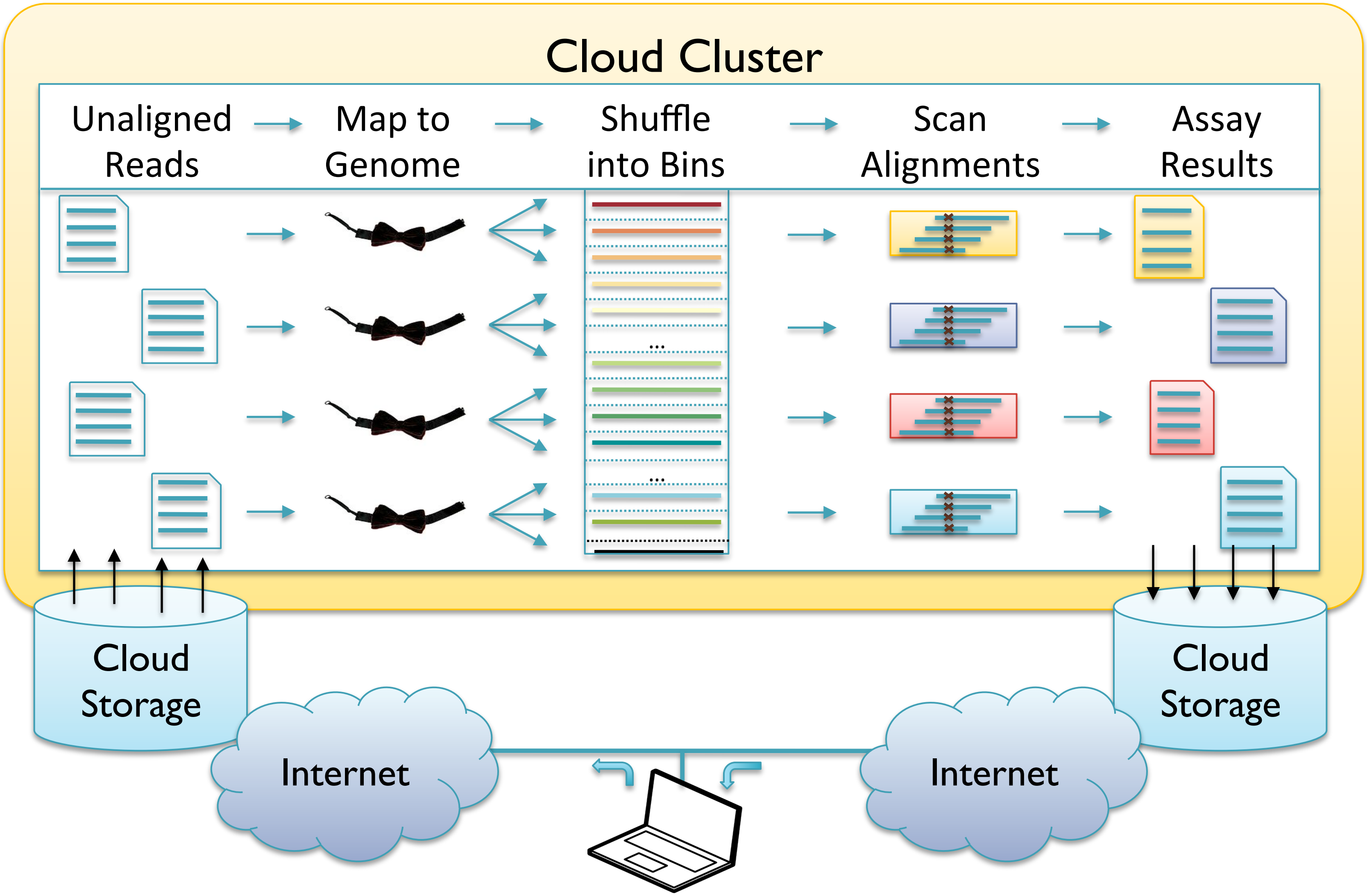
http://bowtie-bio.sourceforge.net/crossbow

	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

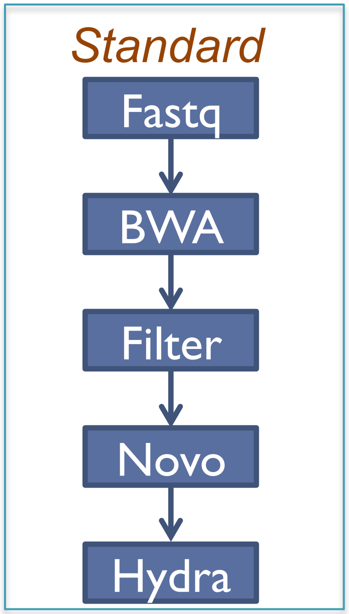
Map-Shuffle-Scan for Genomics

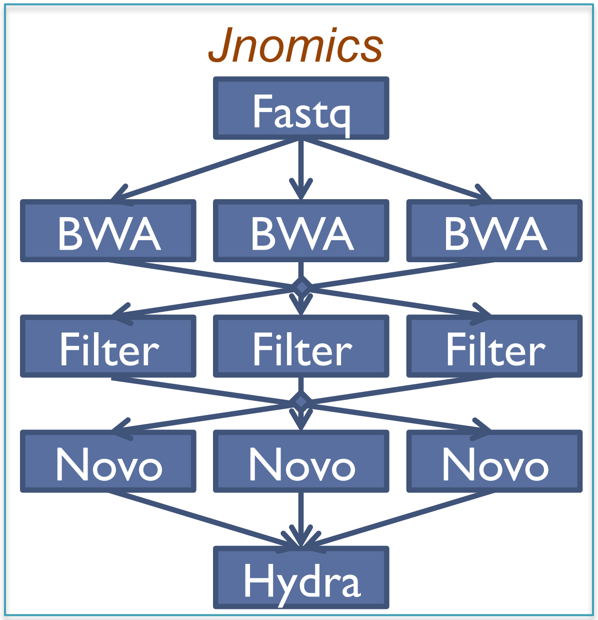


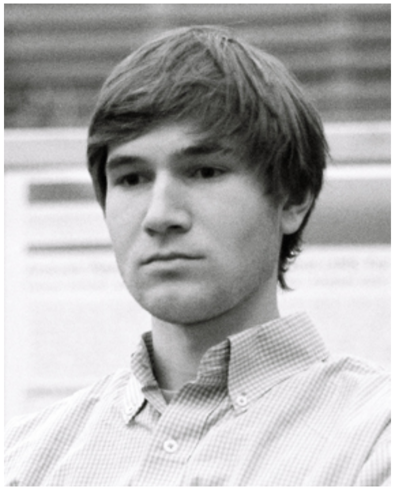
Cloud Computing and the DNA Data Race.
Schatz, MC, Langmead B, Salzberg SL (2010) *Nature Biotechnology.* **28**:691-693

Jnomics: Cloud-scale genomics

James Gurtowski, Matt Titmus, Michael Schatz







- Rapid parallel execution of NGS analysis pipelines
  - FASTX, BWA, Bowtie, Novoalign, SAMTools, Hydra
  - Sorting, merging, filtering, selection, of BAM, SAM, BED, fastq
  - Population analysis: Clustering, GWAS, Trait Inference
- Used for rapidly analyzing human diseases and plants

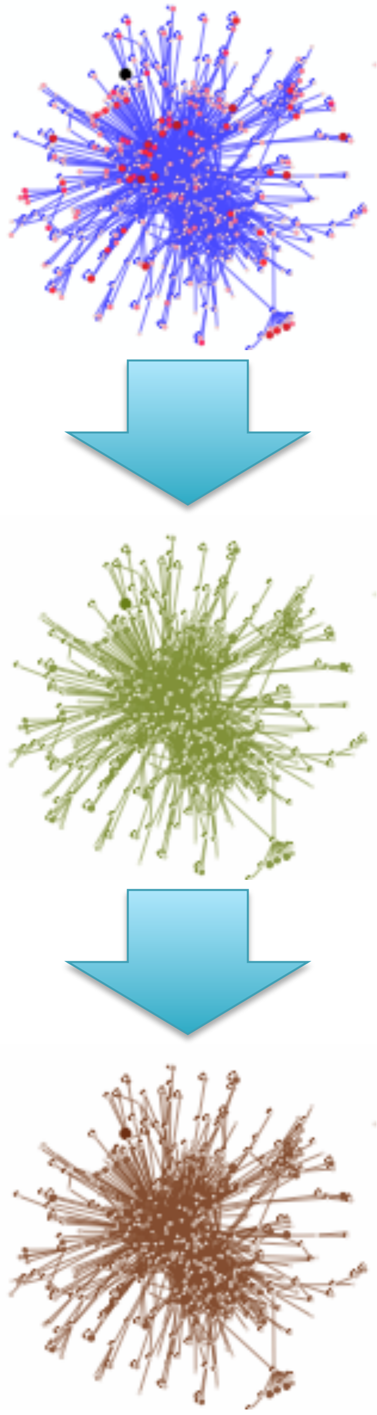
Answering the demands of digital genomics
Titmus, M.A.., Gurtowski, J, Schatz, M.C.. (2012) *Under Review*

3. Tightly Coupled

- Computation that cannot be partitioned
  - Graph Analysis
  - Molecular Dynamics
  - Population simulations

- Challenges
  - Loosely coupled challenges
  - + Parallel algorithms design

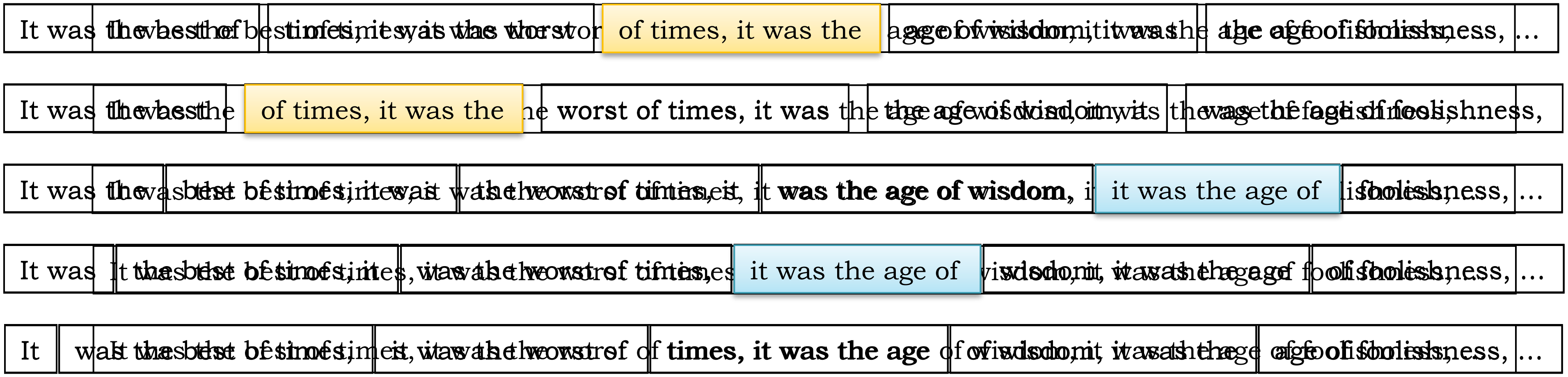
- Technologies
  - MPI
  - MapReduce, Dryad, Pregel



High School Dance

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,

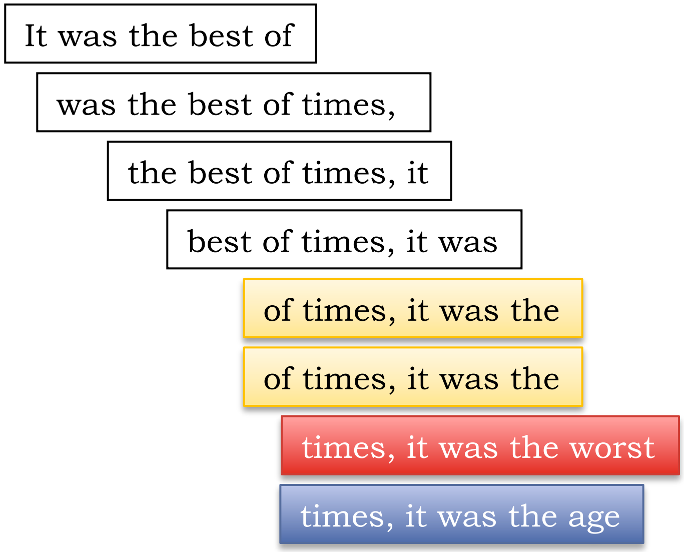
was the age of foolishness,

was the best of times,

was the worst of times,

wisdom, it was the age

worst of times, it was



It was the best of

was the best of times,

the best of times, it

best of times, it was

of times, it was the

of times, it was the

times, it was the worst

times, it was the age

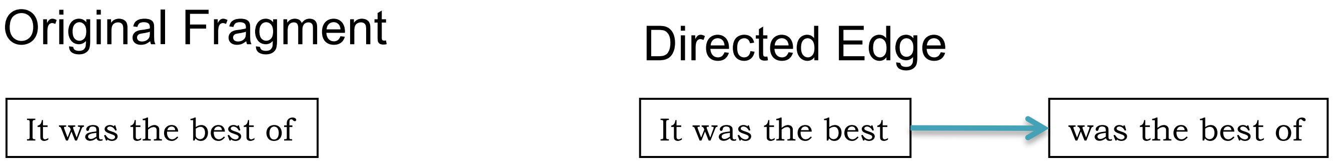
The repeated sequence make the correct reconstruction ambiguous

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

Model sequence reconstruction as a graph problem.

de Bruijn Graph Construction

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



Original Fragment

It was the best of

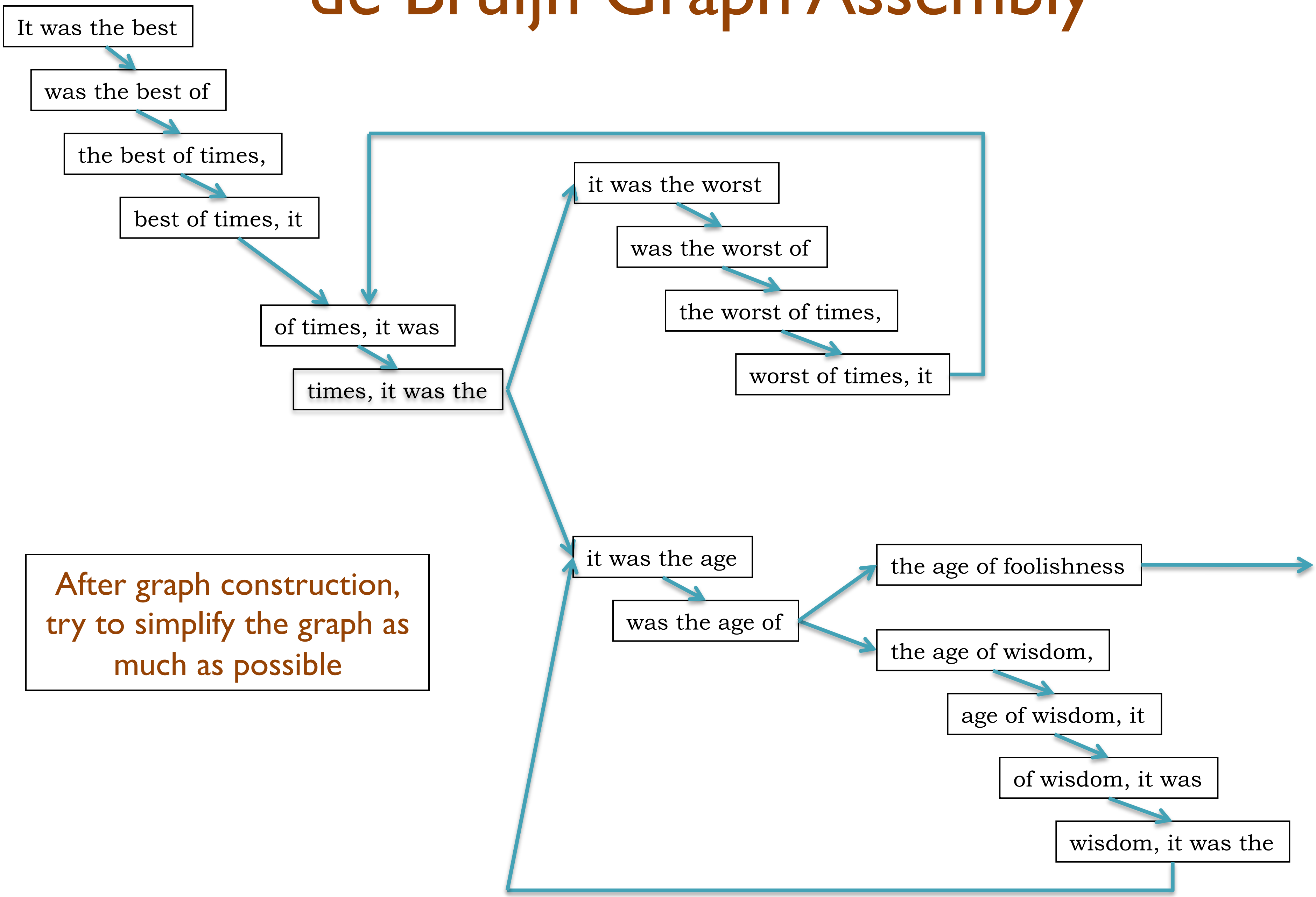
Directed Edge

It was the best → was the best of

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

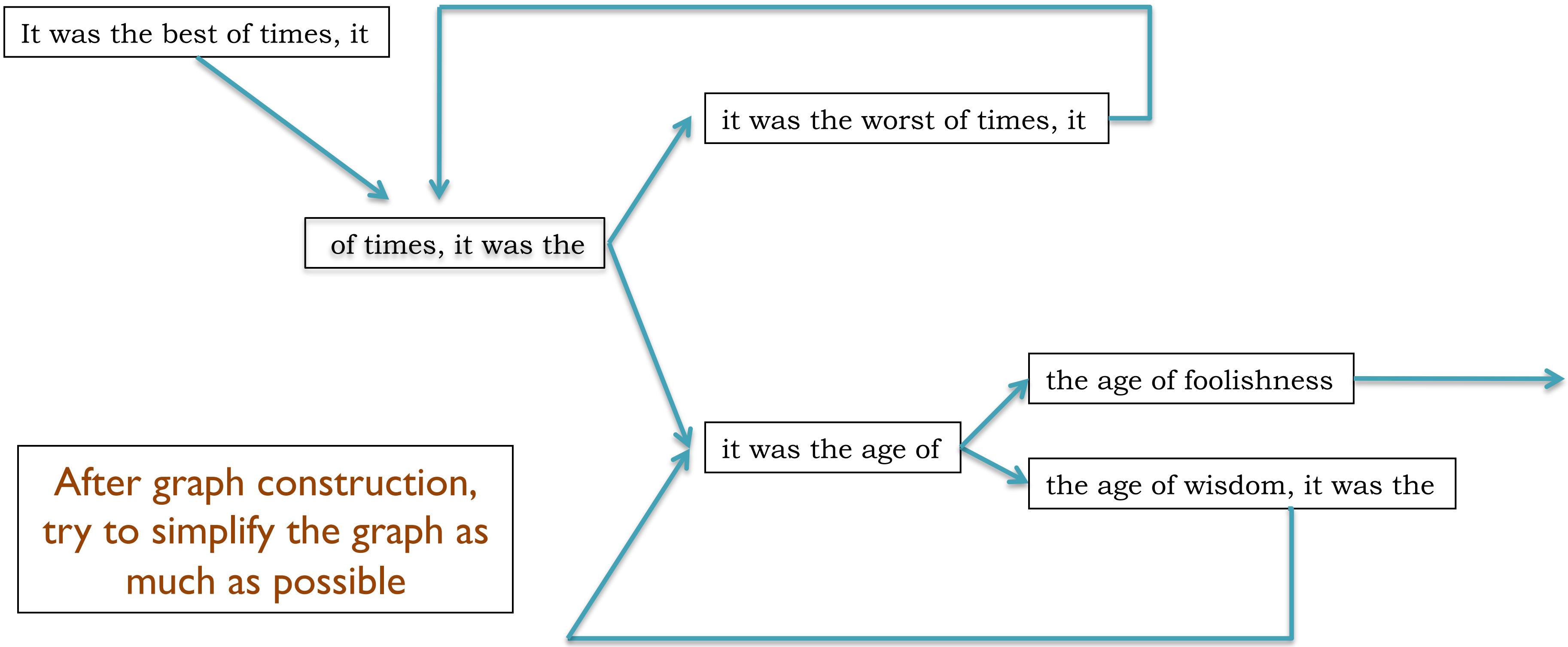
de Bruijn, 1946
Idury and Waterman, 1995
Pevzner, Tang, Waterman, 2001

de Bruijn Graph Assembly



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

de Bruijn Graph Assembly



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

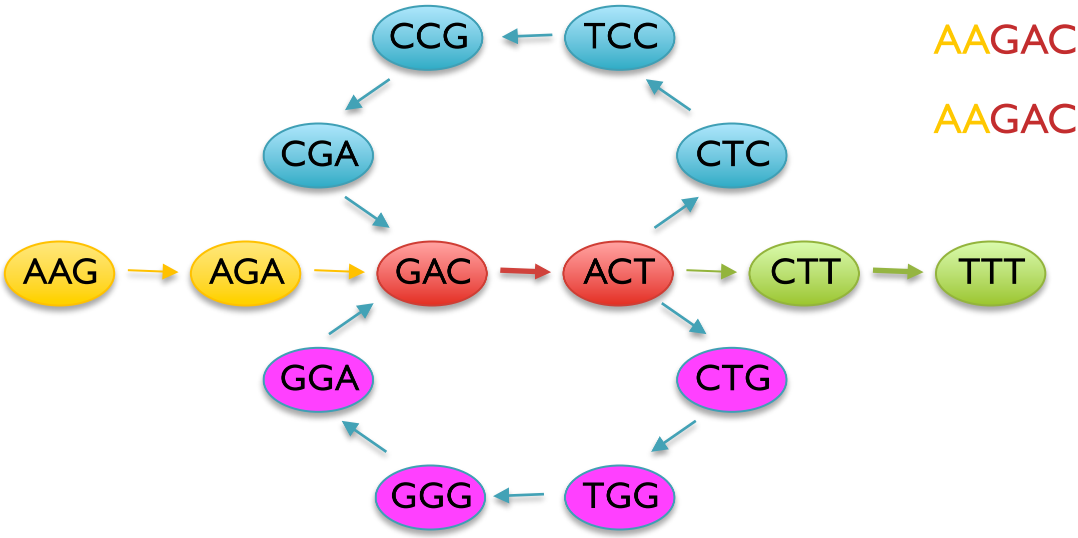
After graph construction, try to simplify the graph as much as possible

Genome Assembly

Reads

AAGA
ACTT
ACTC
ACTG
AGAG
CCGA
CGAC
CTCC
CTGG
CTTT
…

de Bruijn Graph



Potential Genomes

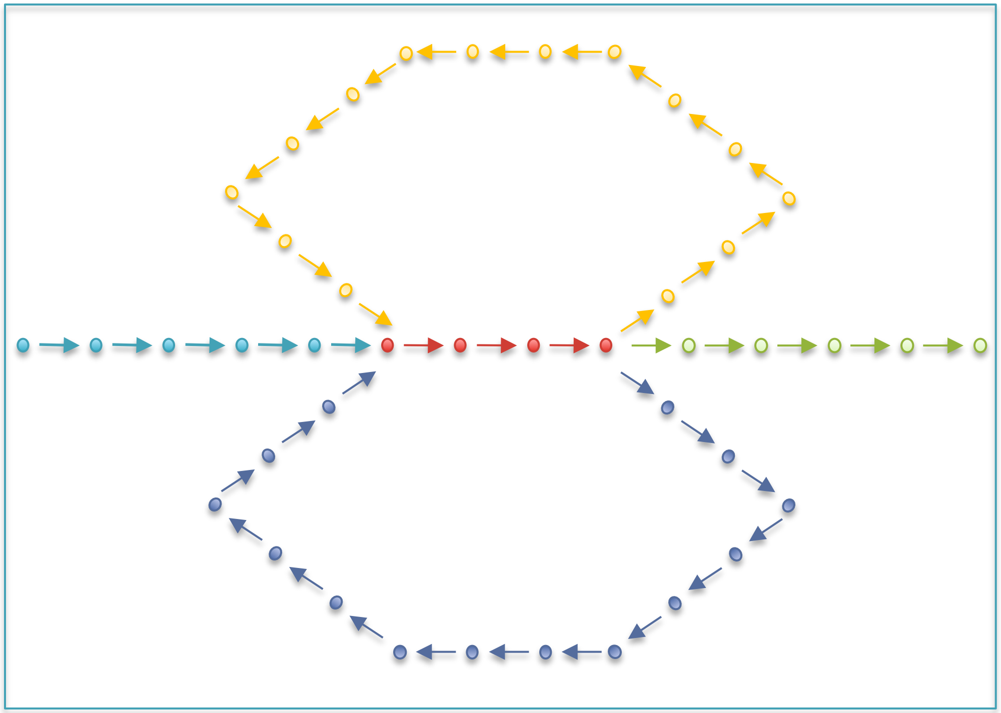
AAGACTCCGACTGGGACTTT

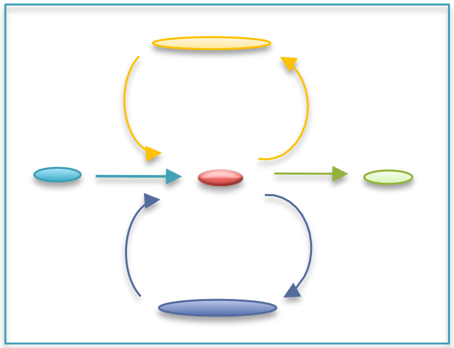
AAGACTGGGACTCCGACTTT

- 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

Graph Compression

- After construction, many edges are unambiguous
  - Merge together compressible nodes
  - Graph physically distributed over hundreds of computers

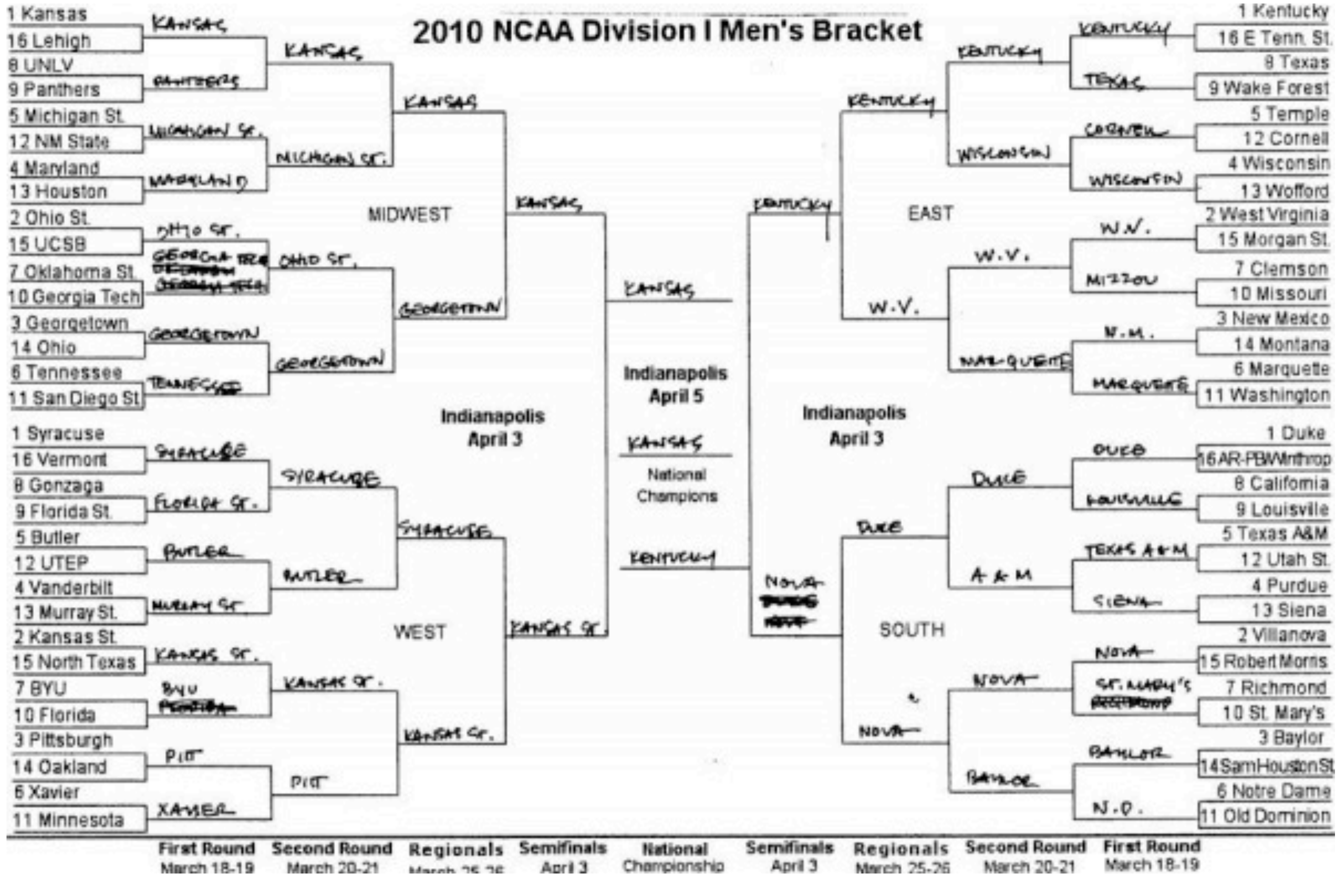




Design Patterns for Efficient Graph Algorithms in MapReduce.
Lin, J., Schatz, M.C. (2010) Workshop on Mining and Learning with Graphs Workshop (KDD-2010)

Warmup Exercise

- Who here was born closest to July 30?
  - You can only compare to 1 other person at a time



Find winner among 64 teams in just 6 rounds

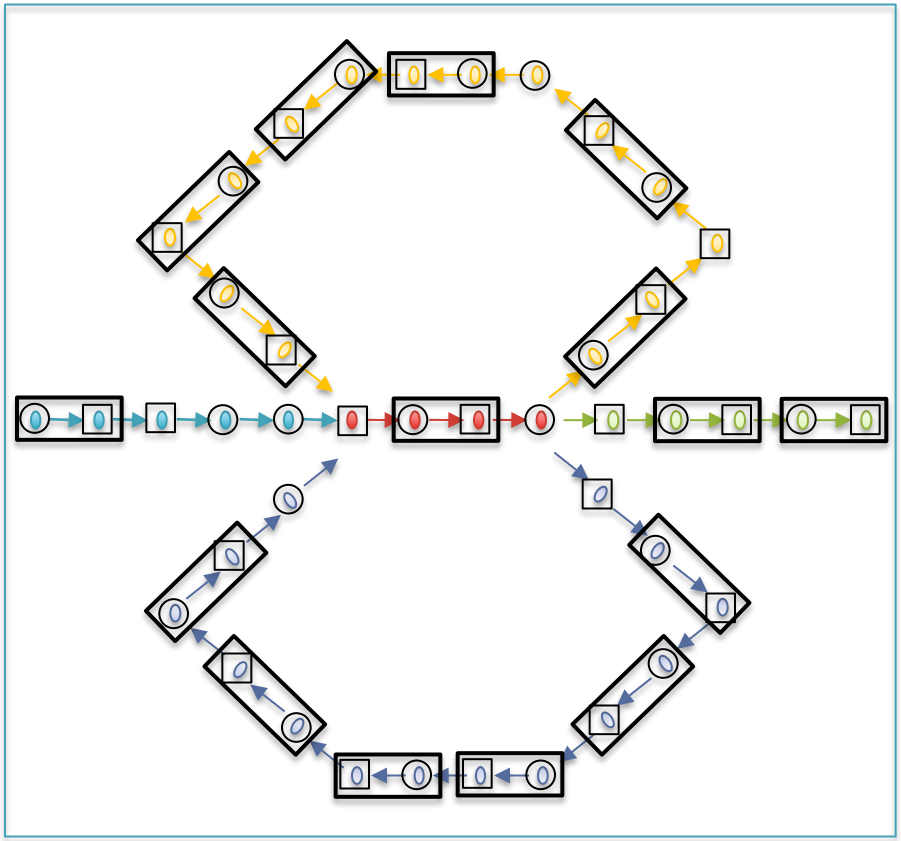
Fast Path Compression

Challenges

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

Randomized List Ranking

- Randomly assign Ⓗ / $\boxed{\text{T}}$ to each compressible node
- Compress Ⓗ → $\boxed{\text{T}}$ links



Initial Graph: 42 nodes

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

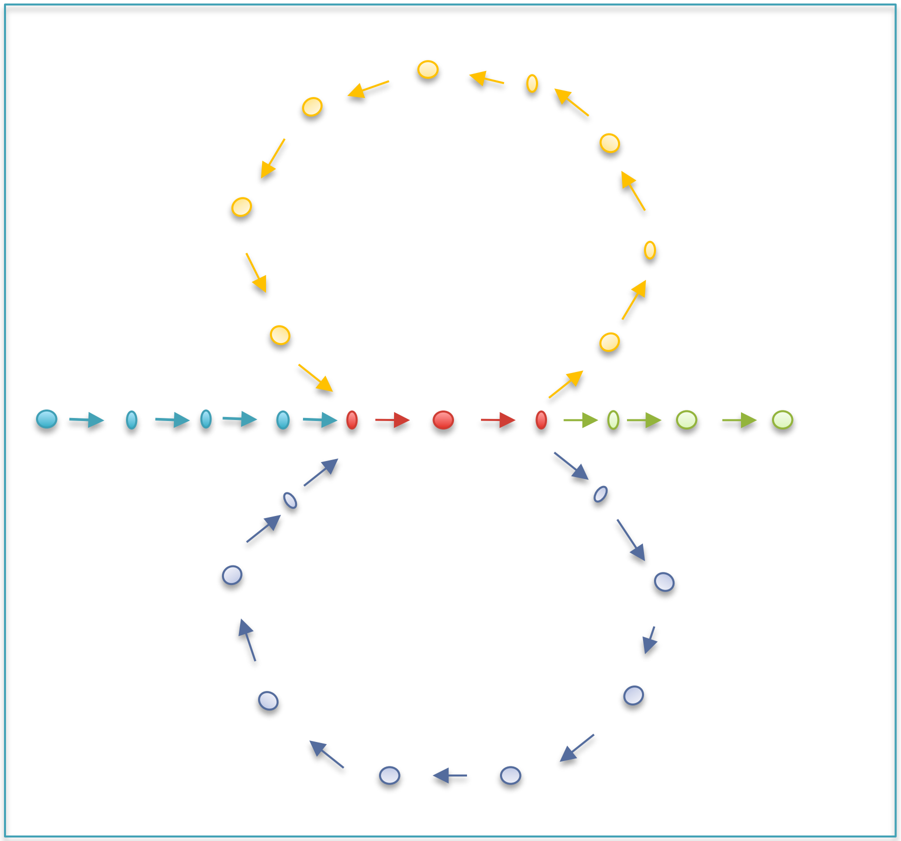
Fast Path Compression

Challenges

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

Randomized List Ranking

- Randomly assign Ⓗ/ $\boxed{\mathrm{T}}$ to each compressible node
- Compress Ⓗ→$\boxed{\mathrm{T}}$ links



Round 1: 26 nodes (38% savings)

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

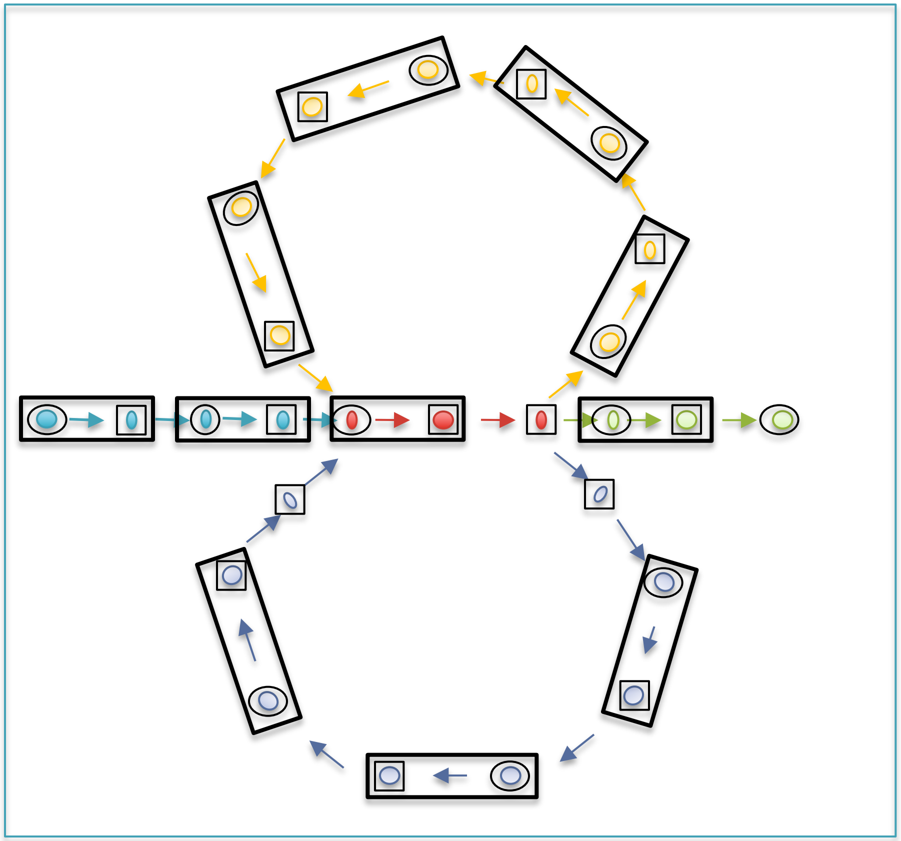
Fast Path Compression

Challenges

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

Randomized List Ranking

- Randomly assign $\textcircled{H}$/ $\boxed{T}$ to each compressible node
- Compress $\textcircled{H} \rightarrow \boxed{T}$ links



Round 1: 26 nodes (38% savings)

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

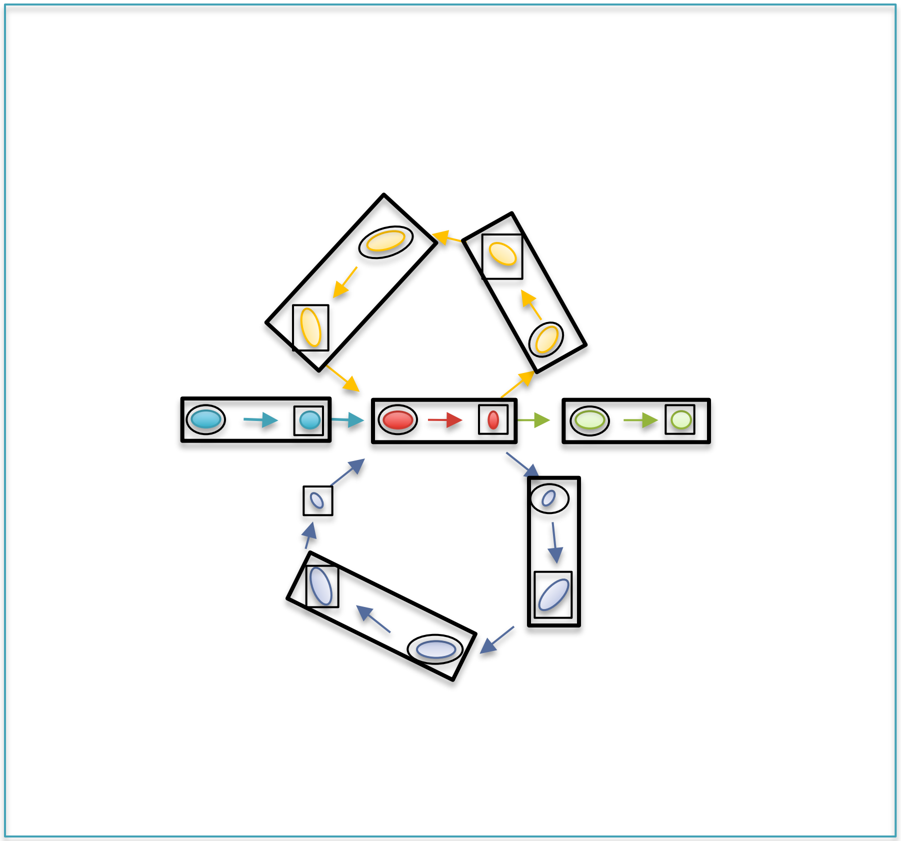
Fast Path Compression

Challenges

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

Randomized List Ranking

- Randomly assign Ⓗ/ $\boxed{\text{T}}$ to each compressible node
- Compress Ⓗ➔$\boxed{\text{T}}$ links



Round 2: 15 nodes (64% savings)

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

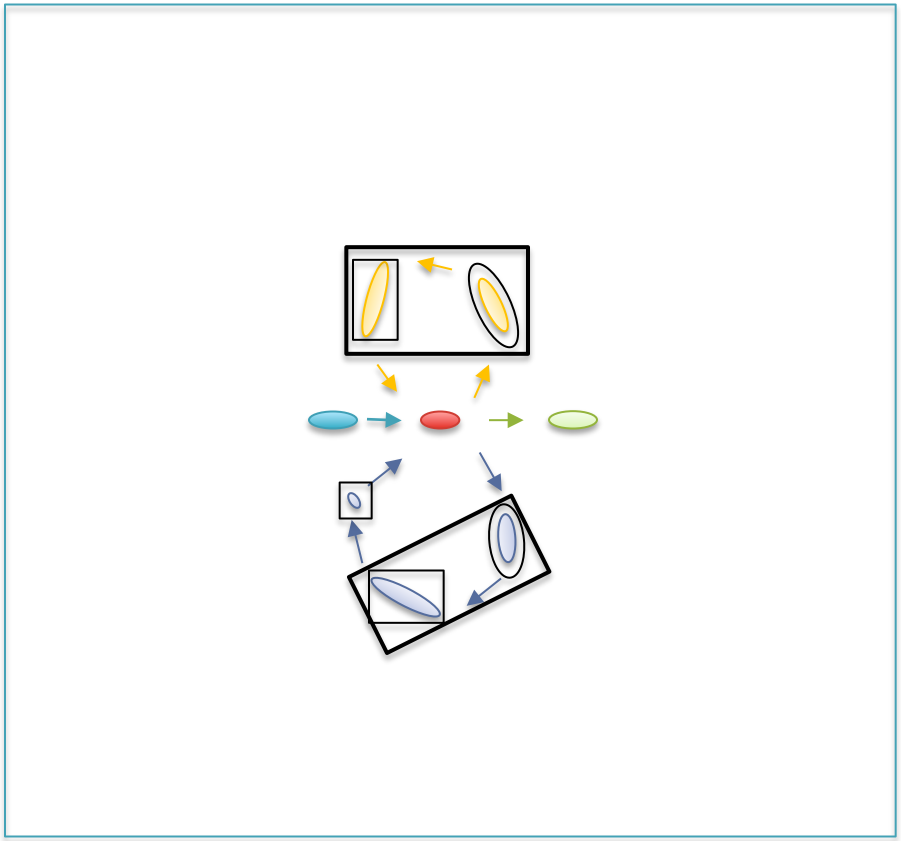
Fast Path Compression

Challenges

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

Randomized List Ranking

- Randomly assign Ⓗ/ $\boxed{T}$ to each compressible node
- Compress Ⓗ→$\boxed{T}$ links



Round 2: 8 nodes (81% savings)

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

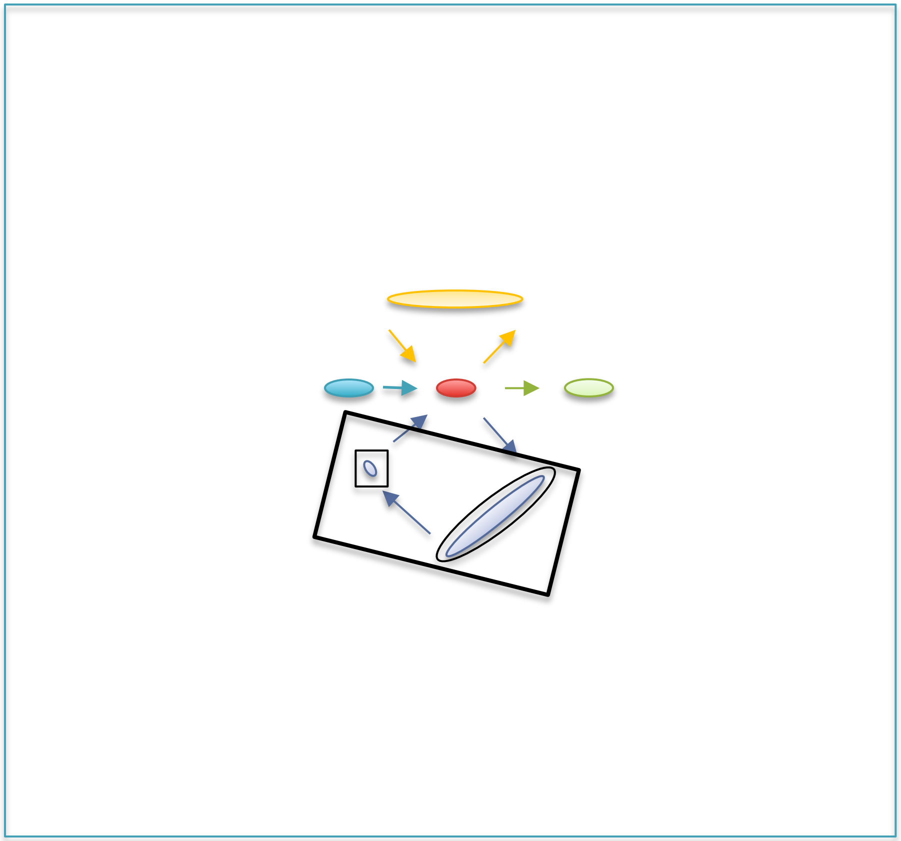
Fast Path Compression

Challenges

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

Randomized List Ranking

- Randomly assign $\textcircled{H}$/ $\boxed{T}$ to each compressible node
- Compress $\textcircled{H}\rightarrow\boxed{T}$ 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

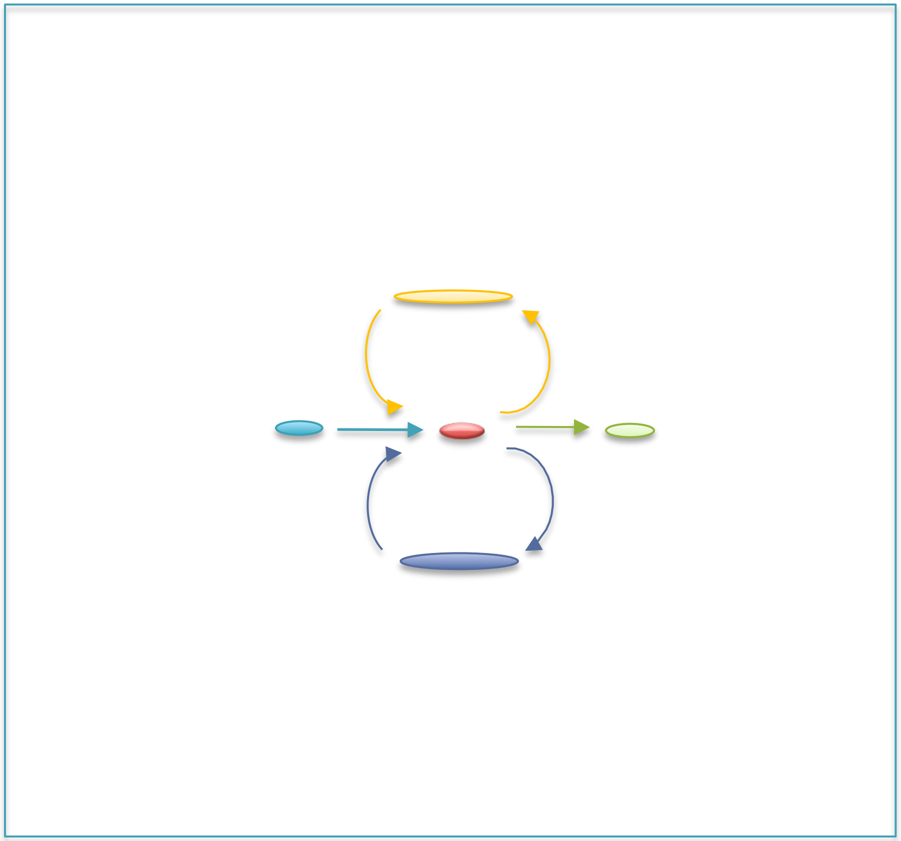
- Nodes stored on different computers
- Nodes can only access direct neighbors

Randomized List Ranking

- Randomly assign Ⓗ/ $\boxed{\text{T}}$ to each compressible node
- Compress Ⓗ→$\boxed{\text{T}}$ links

Performance

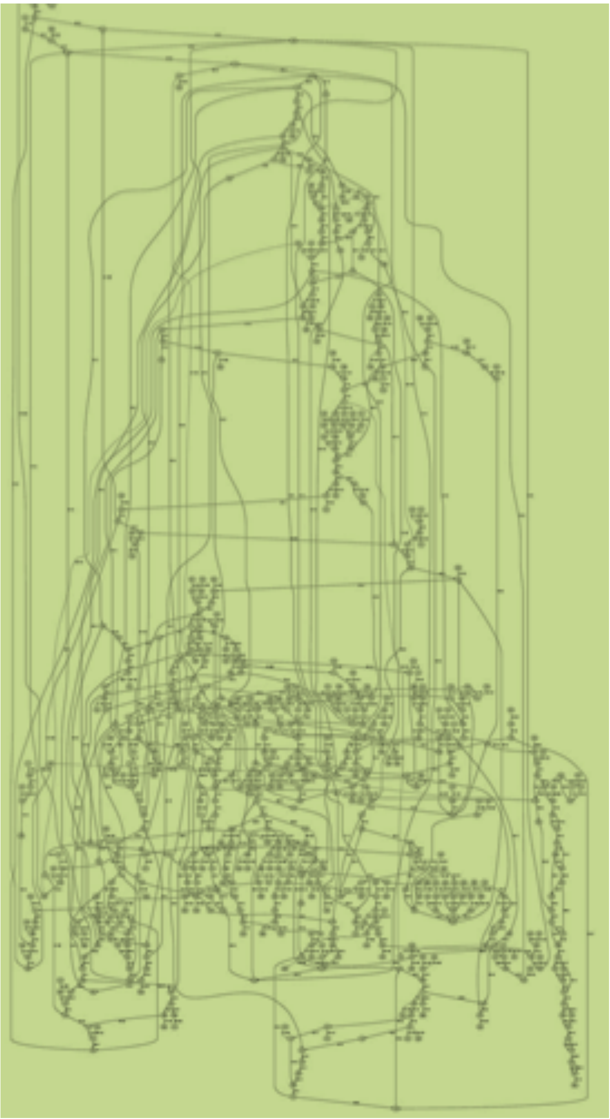
- Compress all chains in log(S) rounds



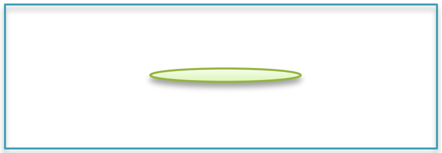
Round 4: 5 nodes (88% savings)

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

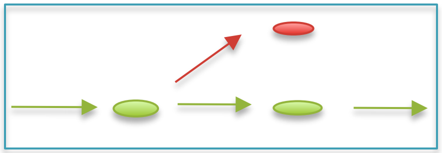
Node Types



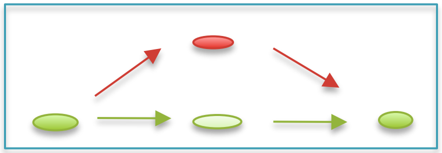
(Chaisson, 2009)



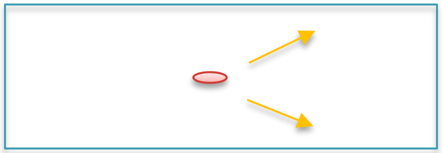
Isolated nodes (10%)



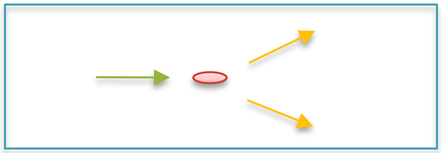
Tips (46%)



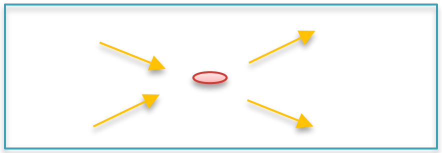
Bubbles/Non-branch (9%)



Dead Ends (.2%)



Half Branch (25%)



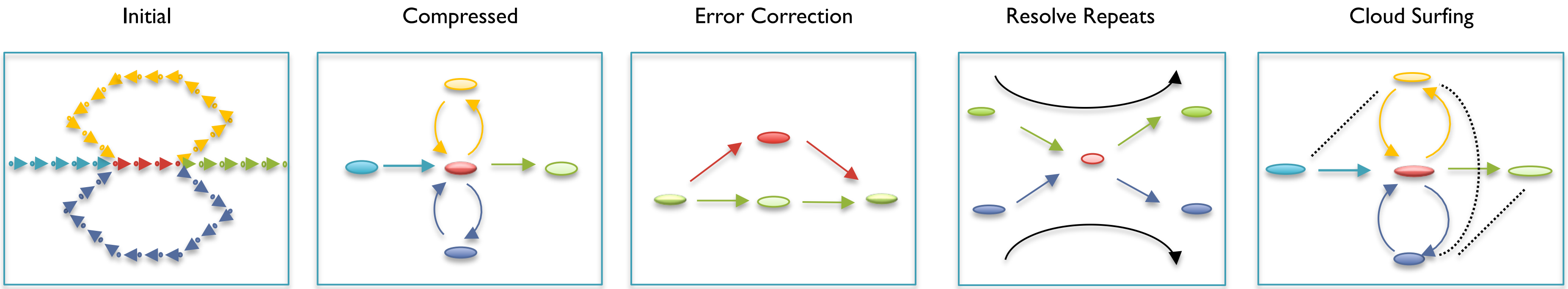
Full Branch (10%)

Contrail

http://contrail-bio.sourceforge.net

De novo Assembly of the Human Genome

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



Initial Compressed Error Correction Resolve Repeats Cloud Surfing

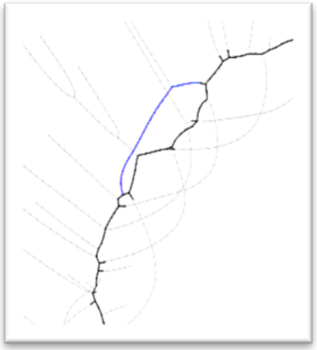
N	>7 B	>1 B	4.2 M	4.1 M	3.3 M
Max	27 bp	303 bp	20,594 bp	20,594 bp	20,594 bp
N50	27 bp	< 100 bp	995 bp	1,050 bp	1,427 bp*

Assembly of Large Genomes with Cloud Computing.
Schatz MC *et al. In Preparation.*

Scalpel: Haplotype Microassembly

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

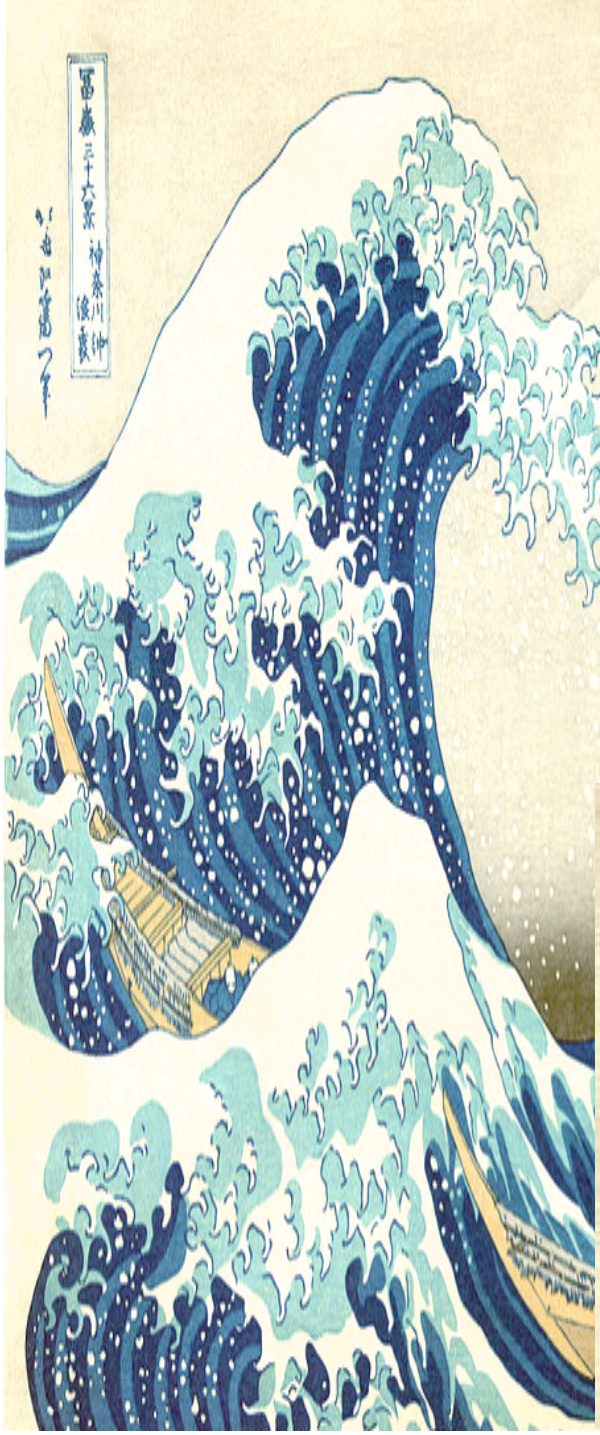
- Use assembly techniques to identify complex variations from short reads
  - Improved power to find indels
  - Trace candidate haplotypes sequences as paths through assembly graphs



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

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

6bp heterozygous indel at chr13:25280526 ATP12A



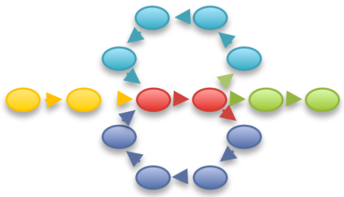
Summary

- We are in 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

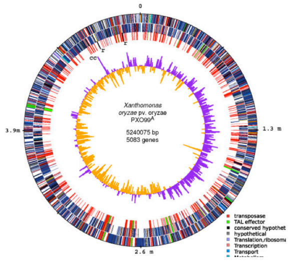
Acknowledgements



Mitch Bekritsky
Giuseppe Narzisi

Ivan Iossifov
Wigler Lab

SFARI
SIMONS FOUNDATION
AUTISM RESEARCH INITIATIVE

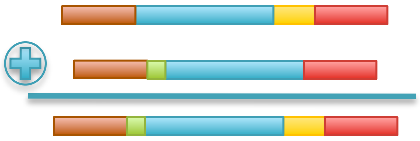


Hayan Lee
James Gurtowski

Ware Lab
McCombie Lab

Adam Phillippy (NBACC)
Sergey Koren (NBACC)

KBASE
predictive biology
DOE Systems Biology Knowledgebase



Paul Baranay
Eric Biggers
Robert Aboukhalil

Scott Emrich (ND)
Steven Salzberg (JHU)
Mihai Pop (UMD)
Ben Langmead (JHU)

National Human
Genome Research
Institute

Thank You!

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