

Whole Genome Assembly and Alignment

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CSHL Advanced Sequencing





Outline

- I. Assembly theory
 1. Assembly by analogy
 2. De Bruijn and Overlap graph
 3. Coverage, read length, errors, and repeats
2. Genome assemblers
 1. ALLPATHS-LG
 2. SOAPdenovo
 3. Celera Assembler
3. Whole Genome Alignment with MUMmer
4. Assembly Tutorial

Shredded Book Reconstruction

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

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

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

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

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

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

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

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

Greedy Reconstruction

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 the assembly problem as a graph problem

de Bruijn Graph Construction

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

Original Fragment

It was the best of

Directed Edge

It was the best → was the best of

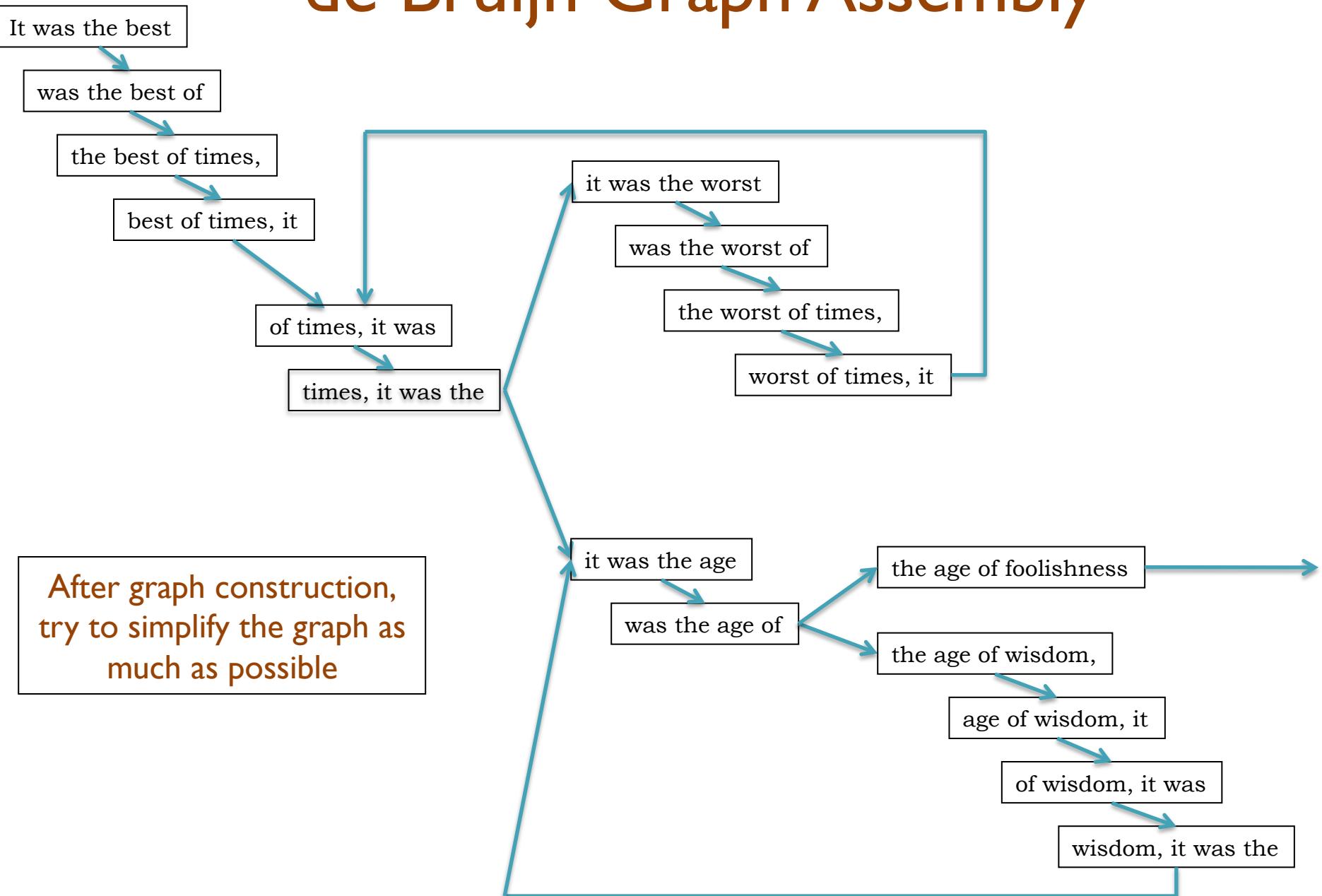
- Locally constructed graph reveals the global sequence structure
 - Overlaps between sequences implicitly computed

de Bruijn, 1946

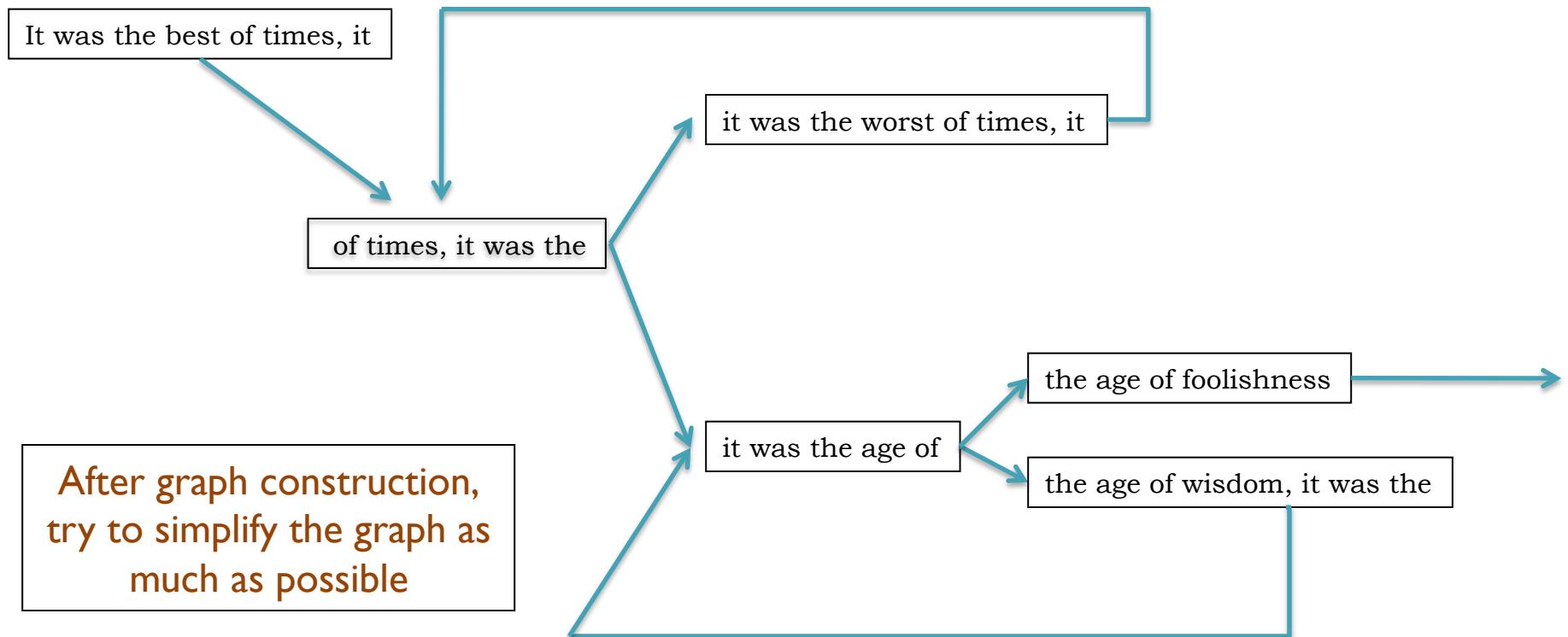
Idury and Waterman, 1995

Pevzner, Tang, Waterman, 2001

de Bruijn Graph Assembly



de Bruijn Graph Assembly



The full tale

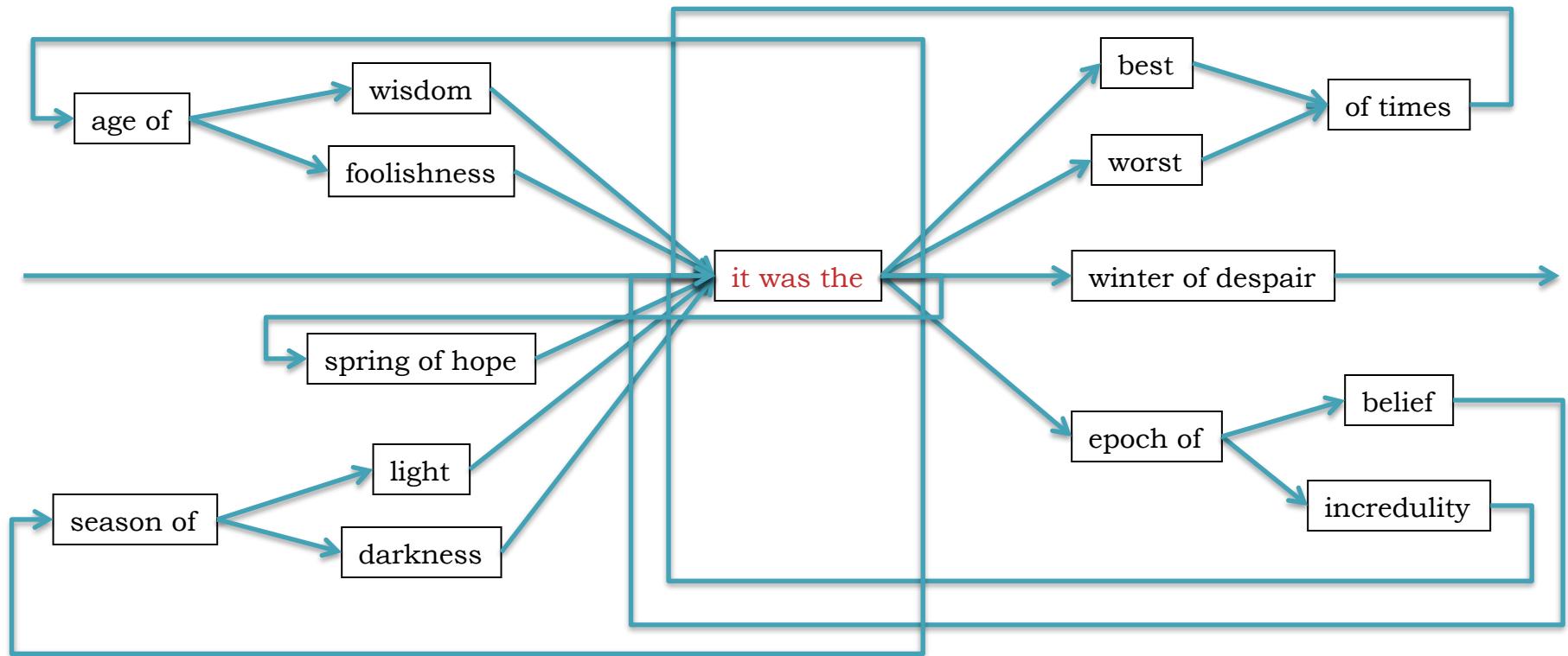
... it was the best of times it was the worst of times ...

... it was the age of wisdom it was the age of foolishness ...

... it was the epoch of belief it was the epoch of incredulity ...

... it was the season of light it was the season of darkness ...

... it was the spring of hope it was the winter of despair ...



N50 size

Def: 50% of the genome is in contigs as large as the N50 value

Example: 1 Mbp genome



N50 size = 30 kbp

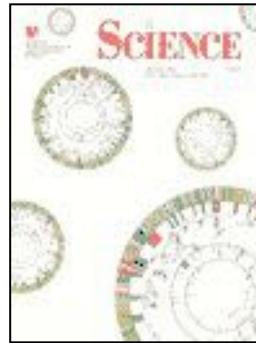
$$(300k+100k+45k+45k+30k = 520k \geq 500\text{ kbp})$$

Note:

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

Milestones in Genome Assembly

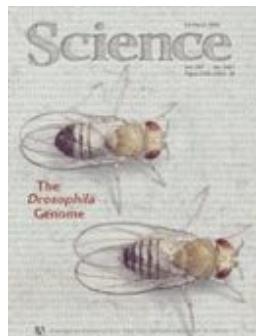
1977. Sanger et al.
1st Complete Organism
5375 bp



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



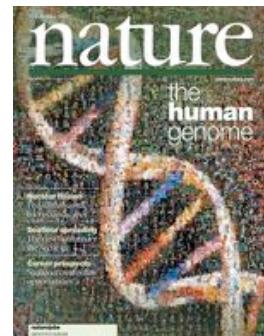
1998. *C.elegans* SC
1st Multicellular Organism
BAC-by-BAC Phrap. 97Mbp



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



The image shows the front cover of the journal 'nature'. The title 'nature' is written in a large, lowercase, serif font at the top. Below the title is a black and white photograph of a giant panda's face and upper body, looking slightly to the right. The background of the cover consists of several bamboo stalks. At the bottom of the cover, there is a dark rectangular area containing text.

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

Assembly Applications

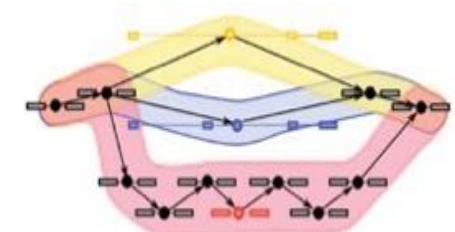
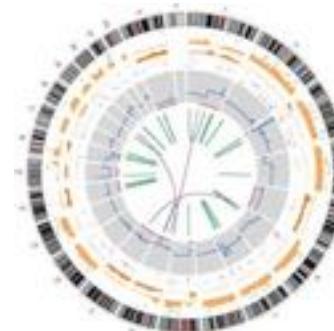
- Novel genomes



- Metagenomes



- Sequencing assays
 - Structural variations
 - Transcript assembly
 - ...



Why are genomes hard to assemble?

1. **Biological:**

- (Very) High ploidy, heterozygosity, repeat content



2. **Sequencing:**

- (Very) large genomes, imperfect sequencing

3. **Computational:**

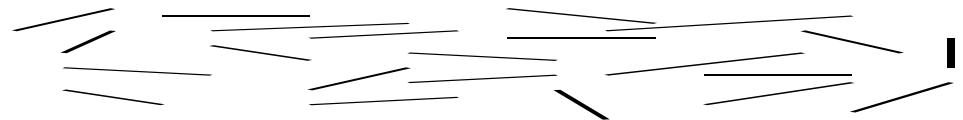
- (Very) Large genomes, complex structure

4. **Accuracy:**

- (Very) Hard to assess correctness

Assembling a Genome

1. Shear & Sequence DNA



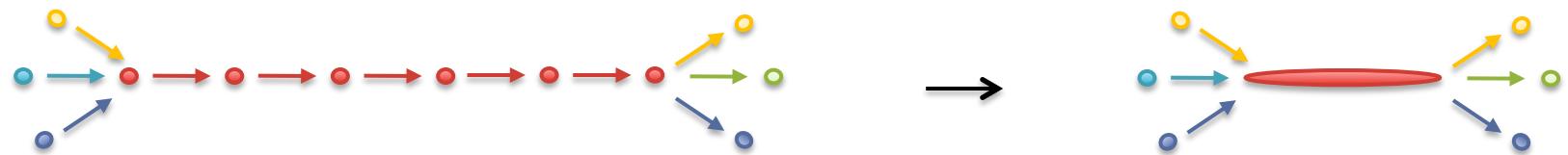
2. Construct assembly graph from overlapping reads

...AGCCTAGGGATGCGCGACACGT

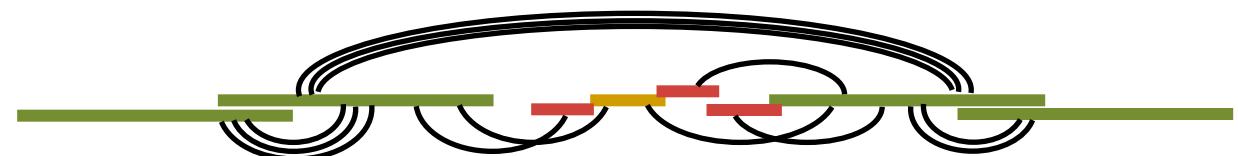
GGATGCGCGACACGT CGCATATCCGGTTGGT CAACCTCGGACGGAC

CAACCTCGGACGGAC CTCAGCGAA...

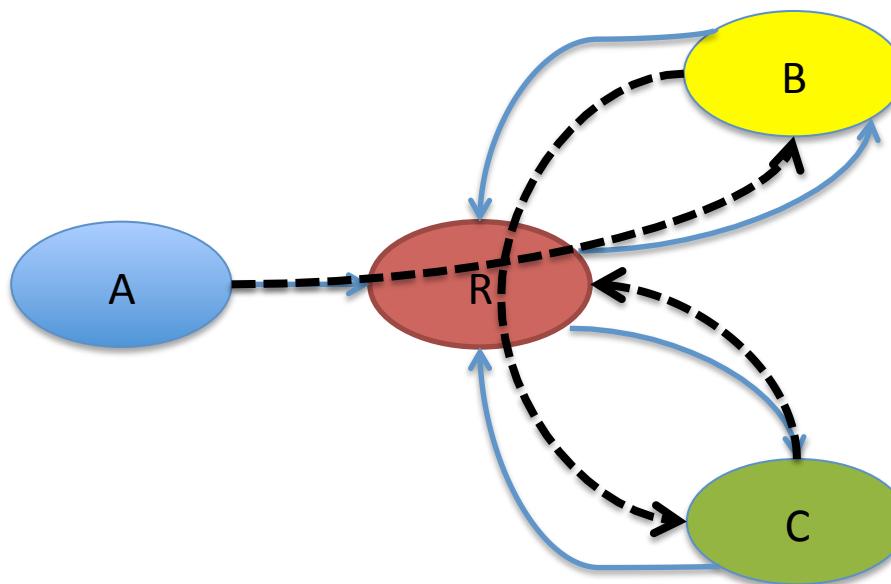
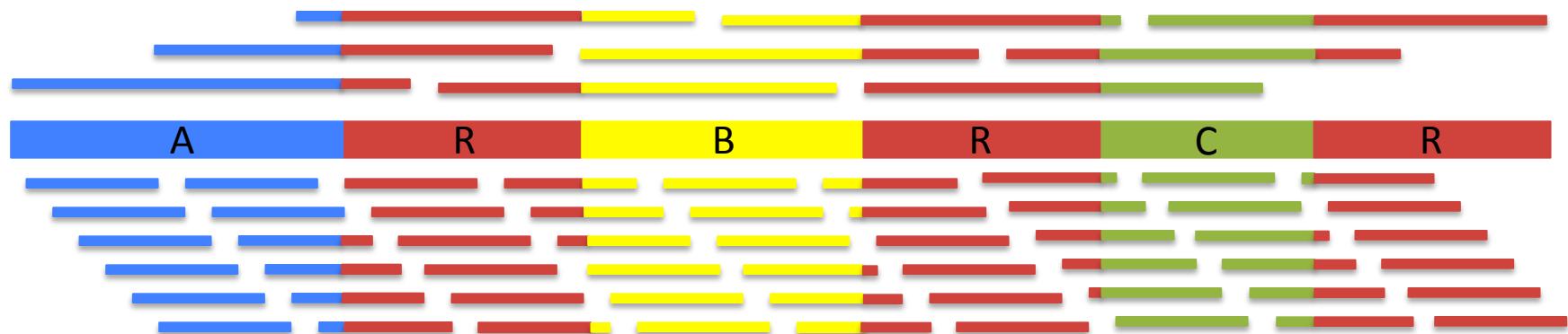
3. Simplify assembly graph



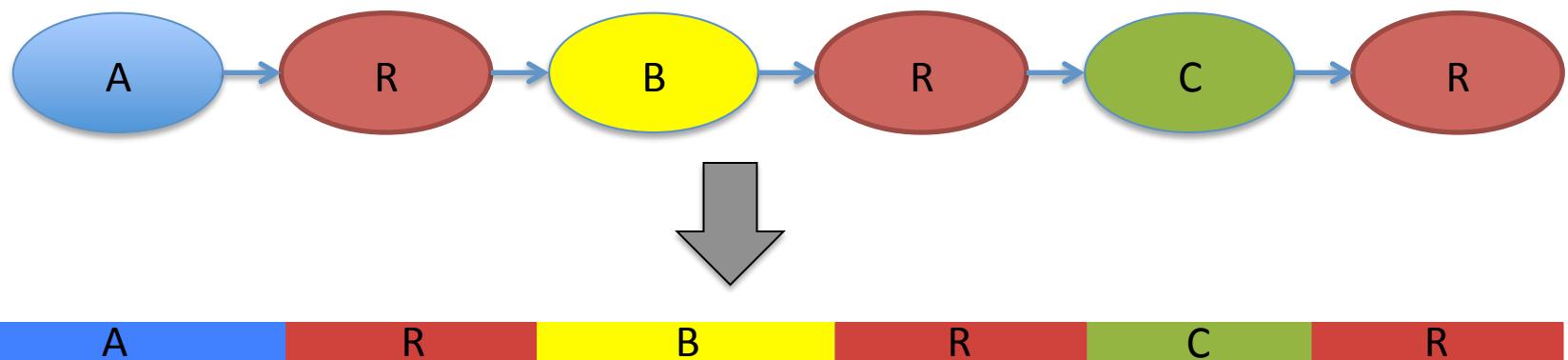
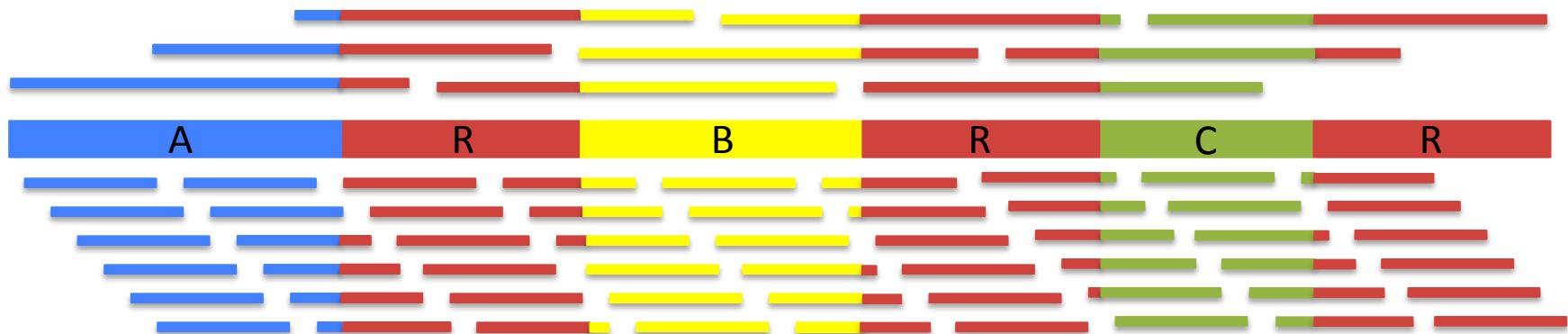
4. Detangle graph with long reads, mates, and other links



Assembly Complexity

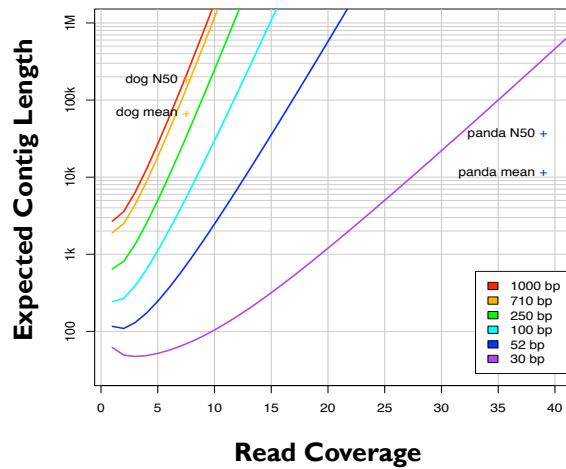


Assembly Complexity



Ingredients for a good assembly

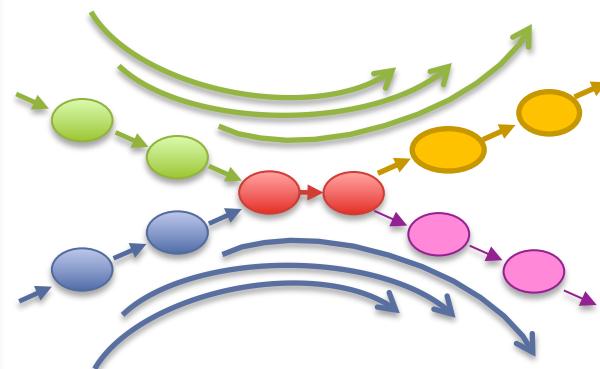
Coverage



High coverage is required

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

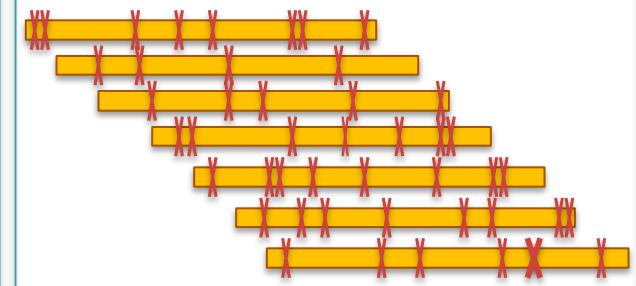
Read Length



Reads & mates must be longer than the repeats

- Short reads will have **false overlaps** forming hairball assembly graphs
- With long enough reads, assemble entire chromosomes into contigs

Quality

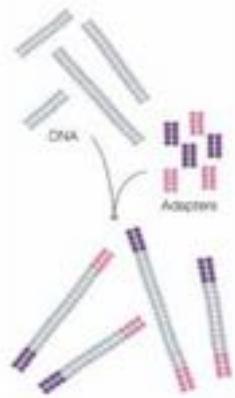


Errors obscure overlaps

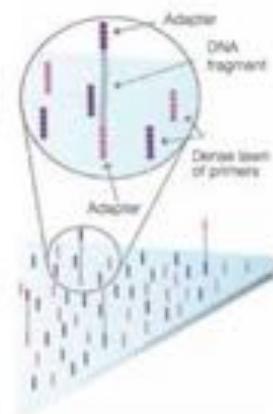
- Reads are assembled by finding kmers shared in pair of reads
- High error rate requires very short seeds, increasing complexity and forming assembly hairballs

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

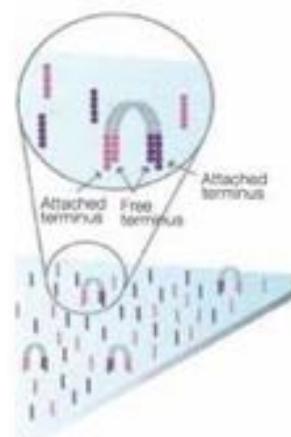
Illumina Sequencing by Synthesis



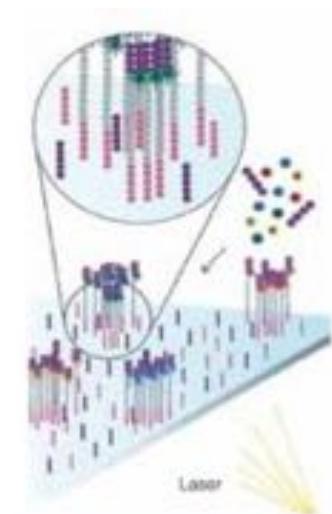
1. Prepare



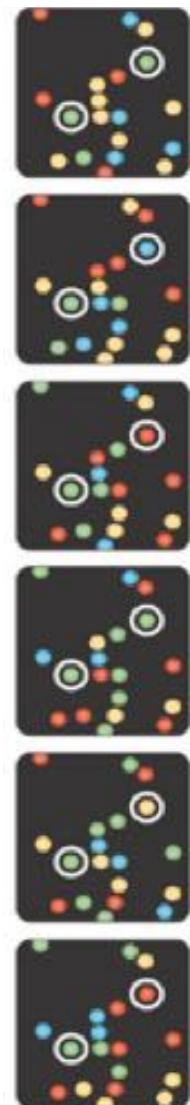
2. Attach



3. Amplify



4. Image



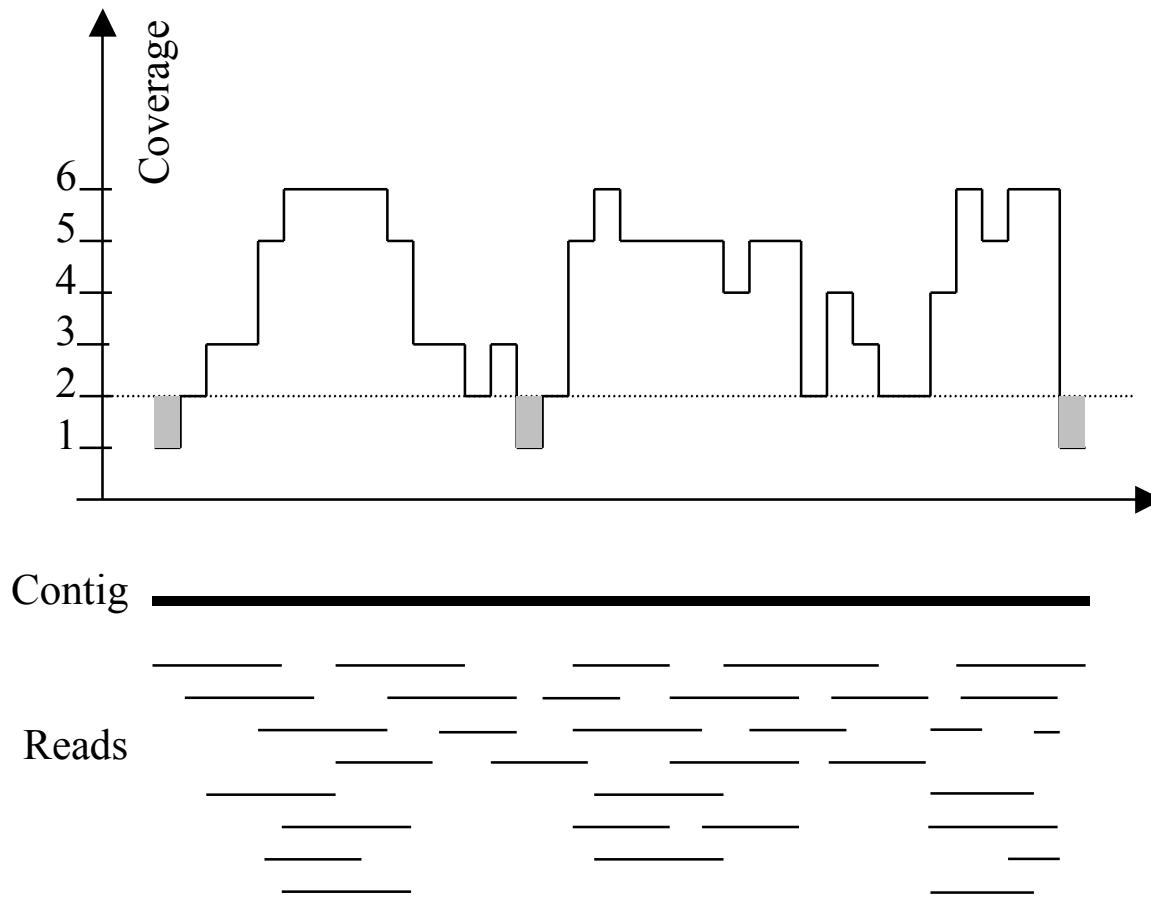
5. Basecall

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

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

Coverage

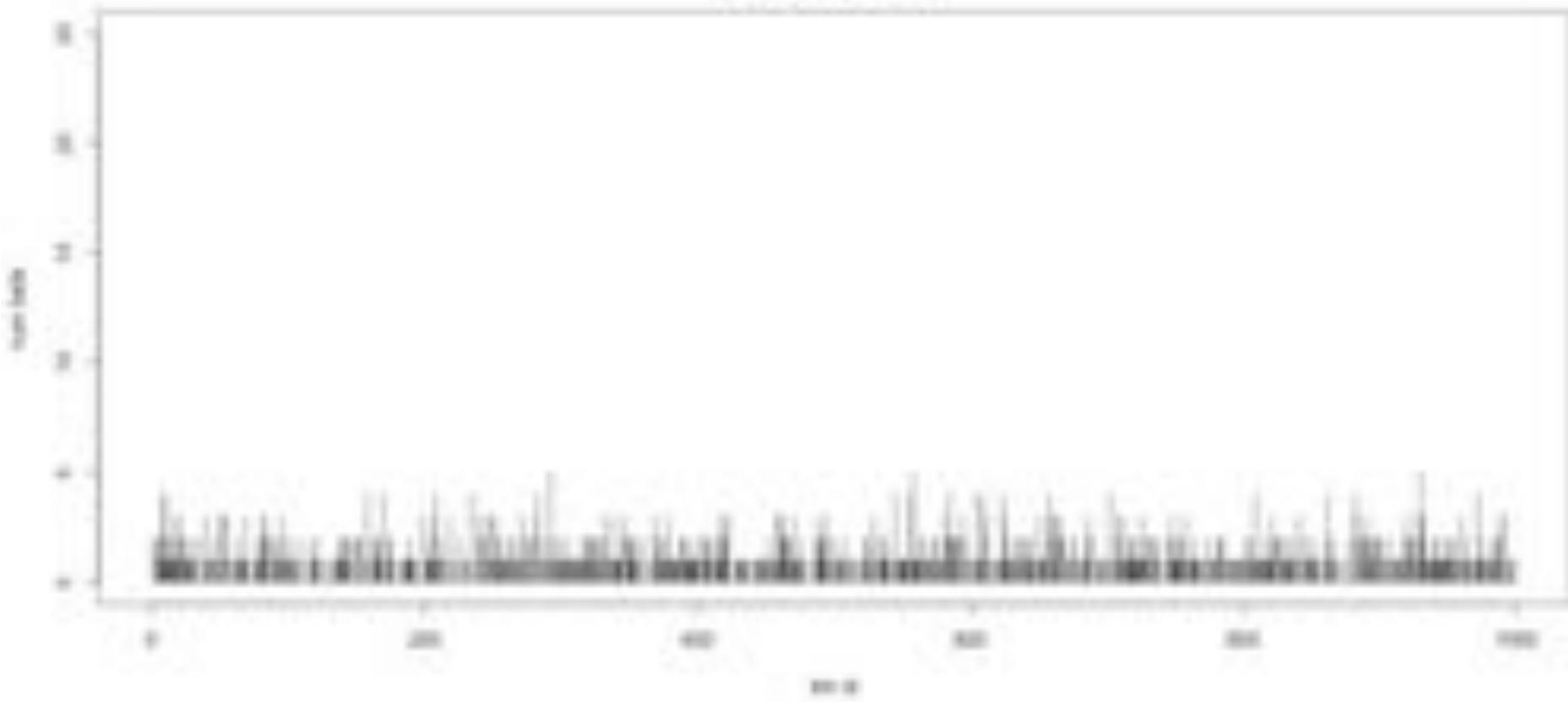
Typical contig coverage



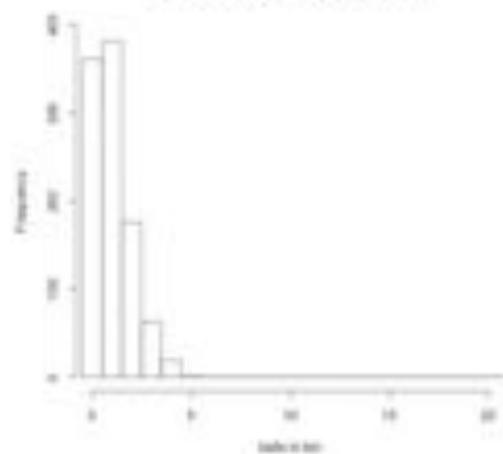
Imagine raindrops on a sidewalk

Balls in Bins IX

Balls in Bins
Total balls: 1000

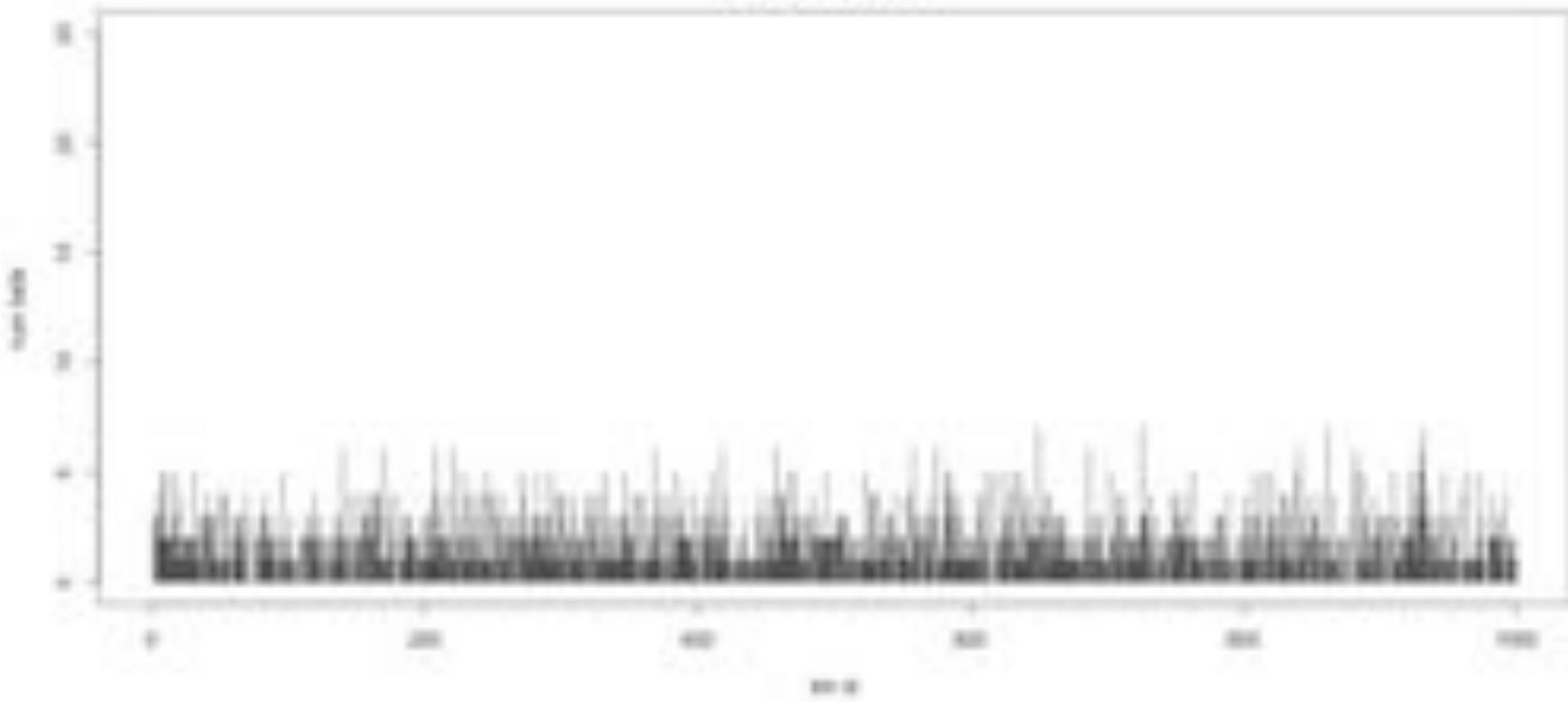


Histogram of balls in each bin
Total balls: 1000. Empty bins: 361

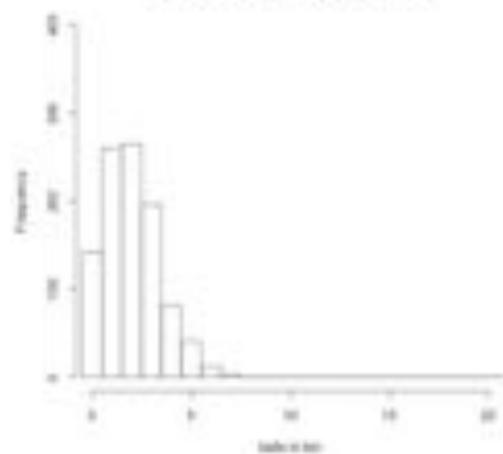


Balls in Bins 2x

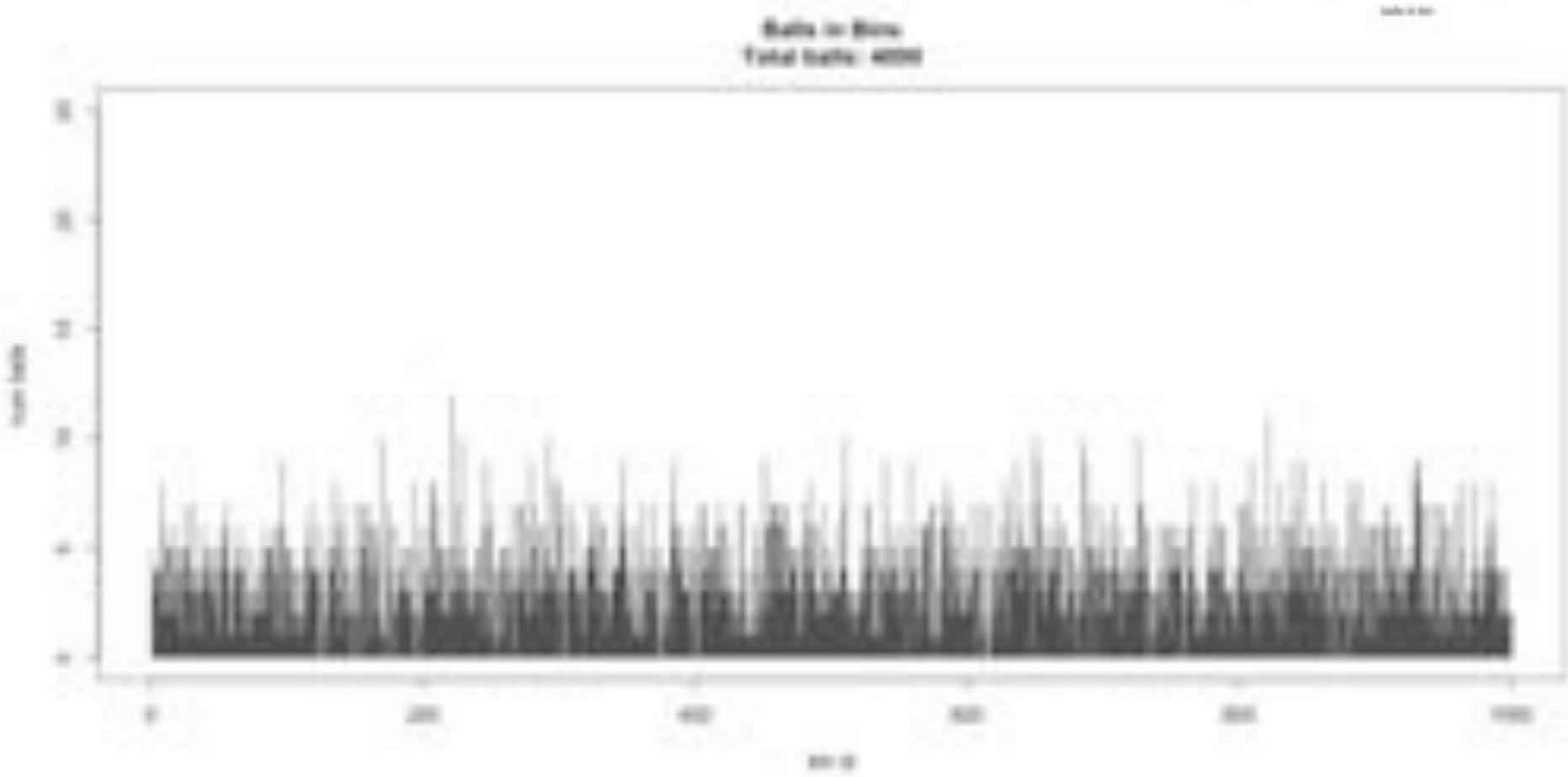
Balls in Bins
Total balls: 2000



Histogram of balls in each bin
Total balls: 2000. Empty bins: 142

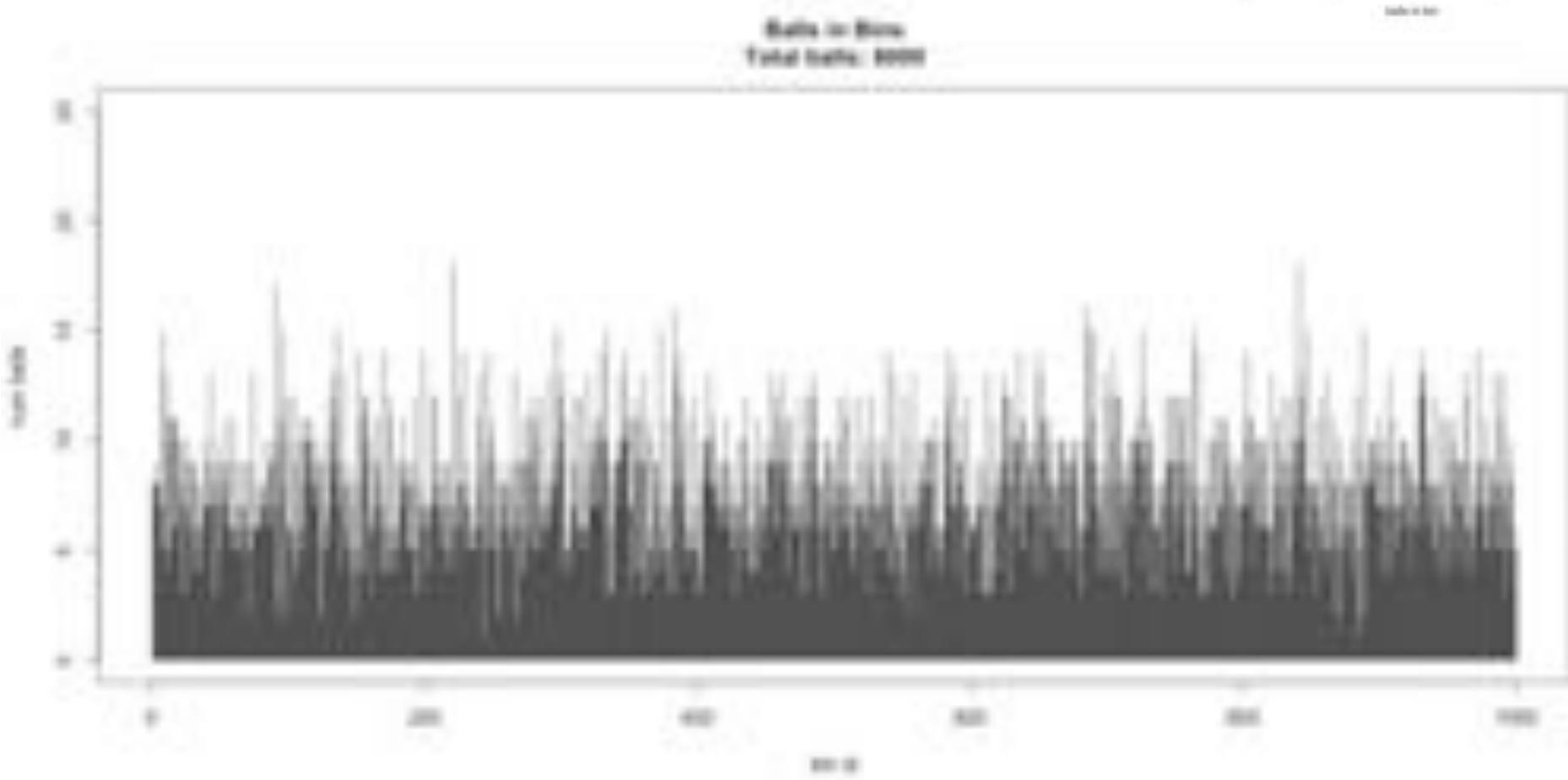


Balls in Bins 4x

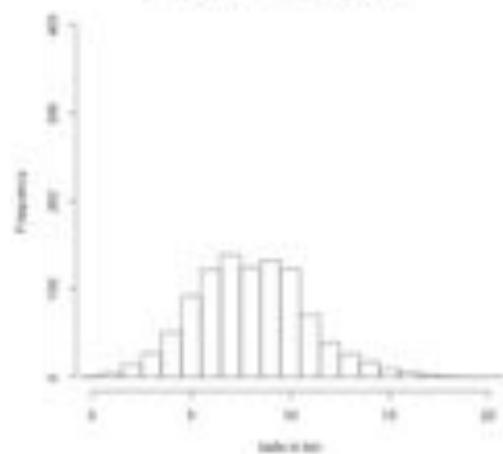


Histogram of balls in each bin
Total balls: 4000 Empty bins: 17

Balls in Bins 8x



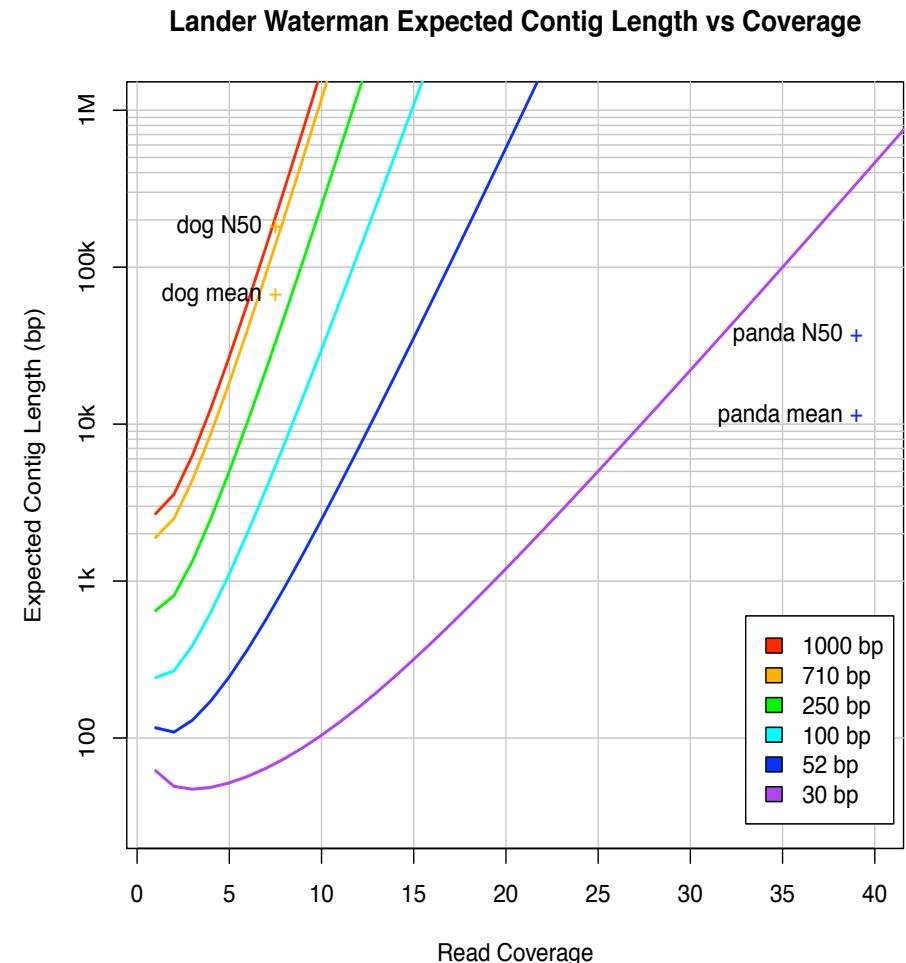
Histogram of balls in each bin
Total balls: 8000 Empty bins: 0



Coverage and Read Length

Idealized Lander-Waterman model

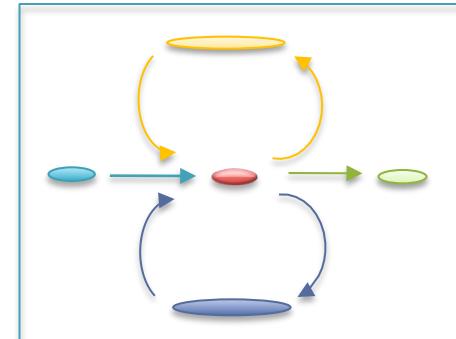
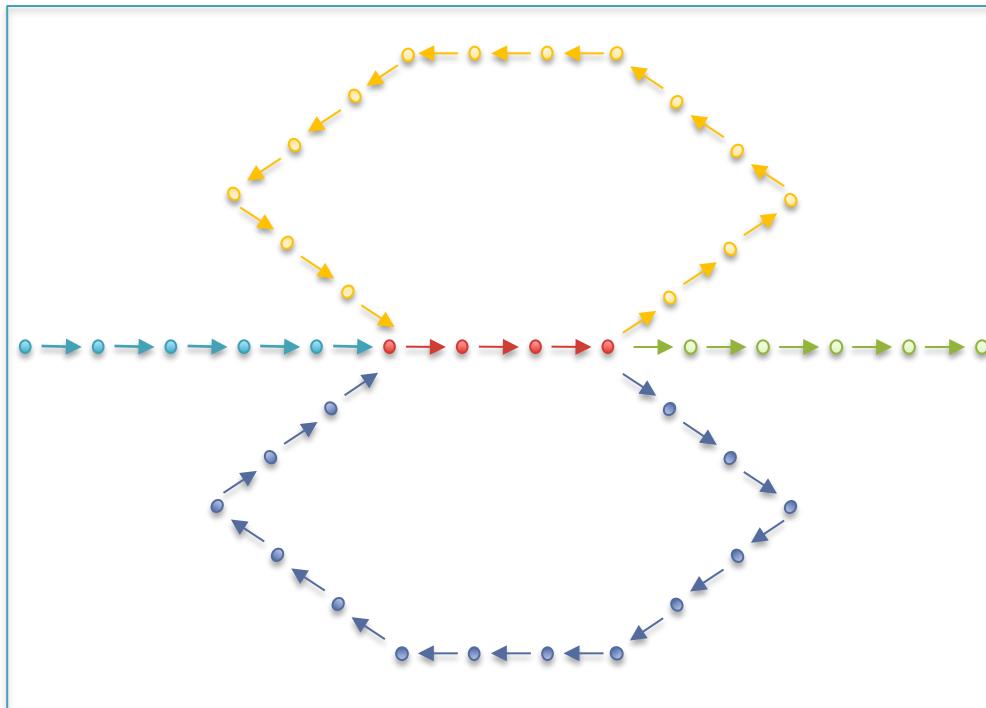
- Reads start at perfectly random positions
- Contig length is a function of coverage and read length
 - Short reads require much higher coverage to reach same expected contig length
- Need even high coverage for higher ploidy, sequencing errors, sequencing biases
 - Recommend 100x coverage



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

Unitigging / Unipathing

- After simplification and correction, compress graph down to its non-branching initial contigs
 - Aka “unitigs”, “unipaths”
 - Unitigs end because of (1) lack of coverage, (2) errors, and (3) repeats



Errors in the graph



(Chaisson, 2009)

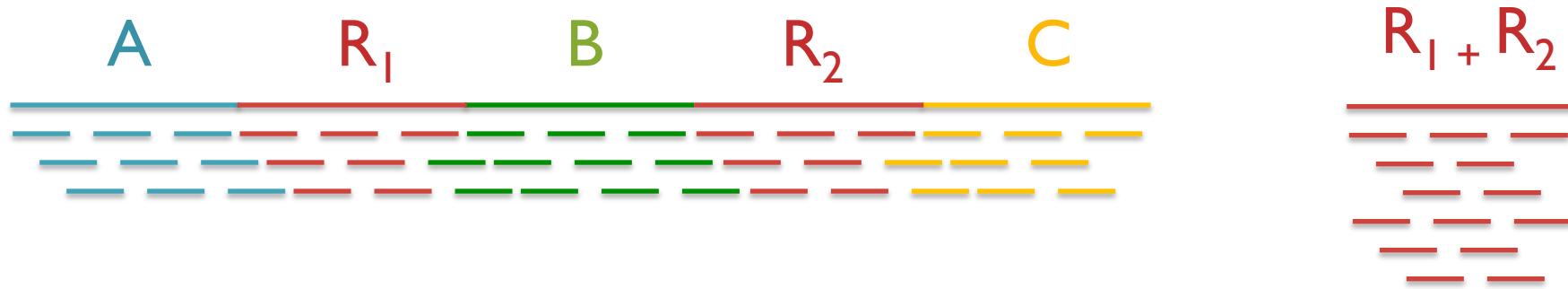
Clip Tips	Pop Bubbles
<p>was the worst of times,</p> <p>was the worst of tymes,</p> <p>the worst of times, it</p>	<p>was the worst of times,</p> <p>was the worst of tymes,</p> <p>times, it was the age</p> <p>tymes, it was the age</p>
<p>the worst of tymes,</p> <p>was the worst of</p> <p>the worst of times,</p> <p>worst of times, it</p>	<p>tymes,</p> <p>was the worst of</p> <p>it was the age</p> <p>times,</p>

Repetitive regions

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

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

Repeats and Coverage Statistics



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

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

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

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

Paired-end and Mate-pairs

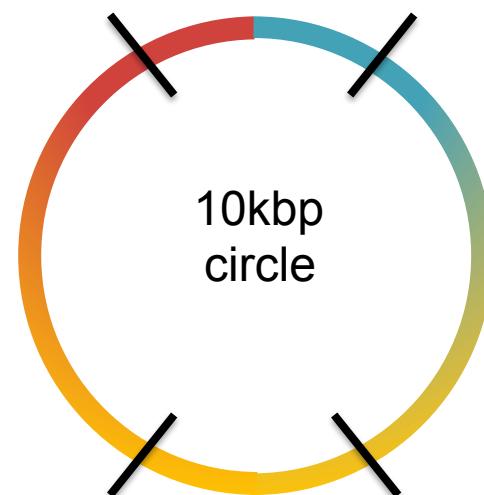
Paired-end sequencing

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



Mate-pair sequencing

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



2x100 @ ~10kbp (outies)

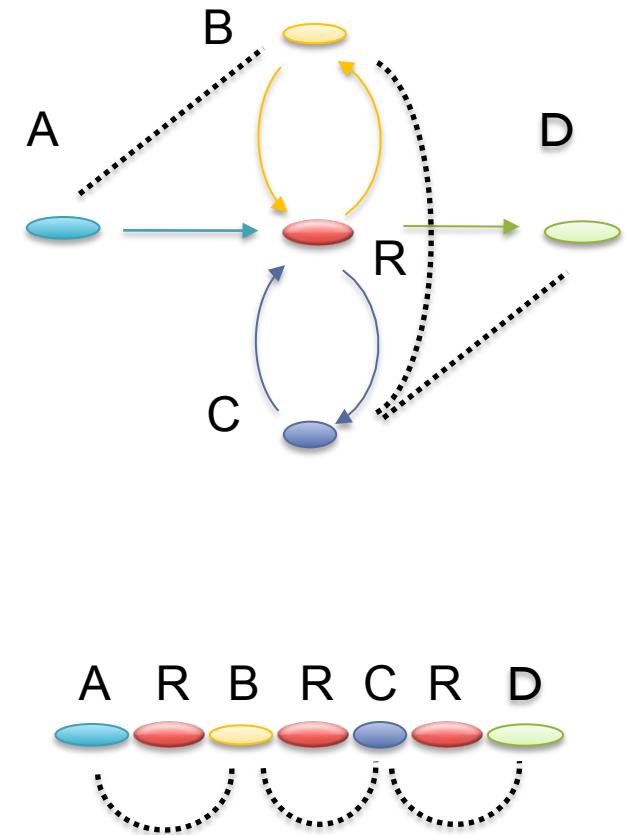


2x100 @ 300bp (innies)



Scaffolding

- Initial contigs (*aka* unipaths, unitigs) terminate at
 - Coverage gaps: especially extreme GC
 - Conflicts: errors, repeat boundaries
- Use mate-pairs to resolve correct order through assembly graph
 - Place sequence to satisfy the mate constraints
 - Mates through repeat nodes are tangled
- Final scaffold may have internal gaps called sequencing gaps
 - We know the order, orientation, and spacing, but just not the bases. Fill with Ns instead



Annotation

After assembly:

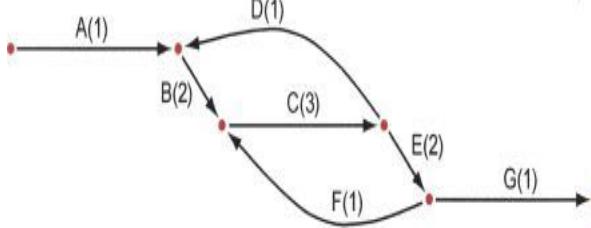
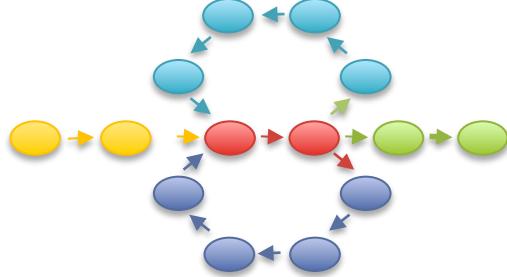
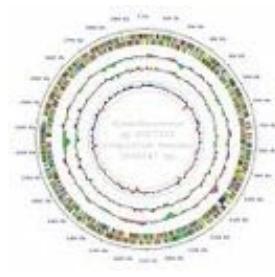
- Validation
- WGA
- BLAST
- CEGMA
- Gene Finding
- Repeat mask
- RNA-seq
- *-seq
- ...
- Publish! ☺

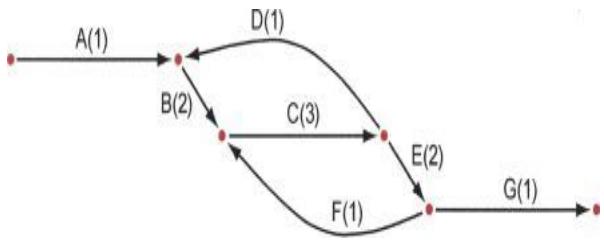


Break



Assembly Algorithms

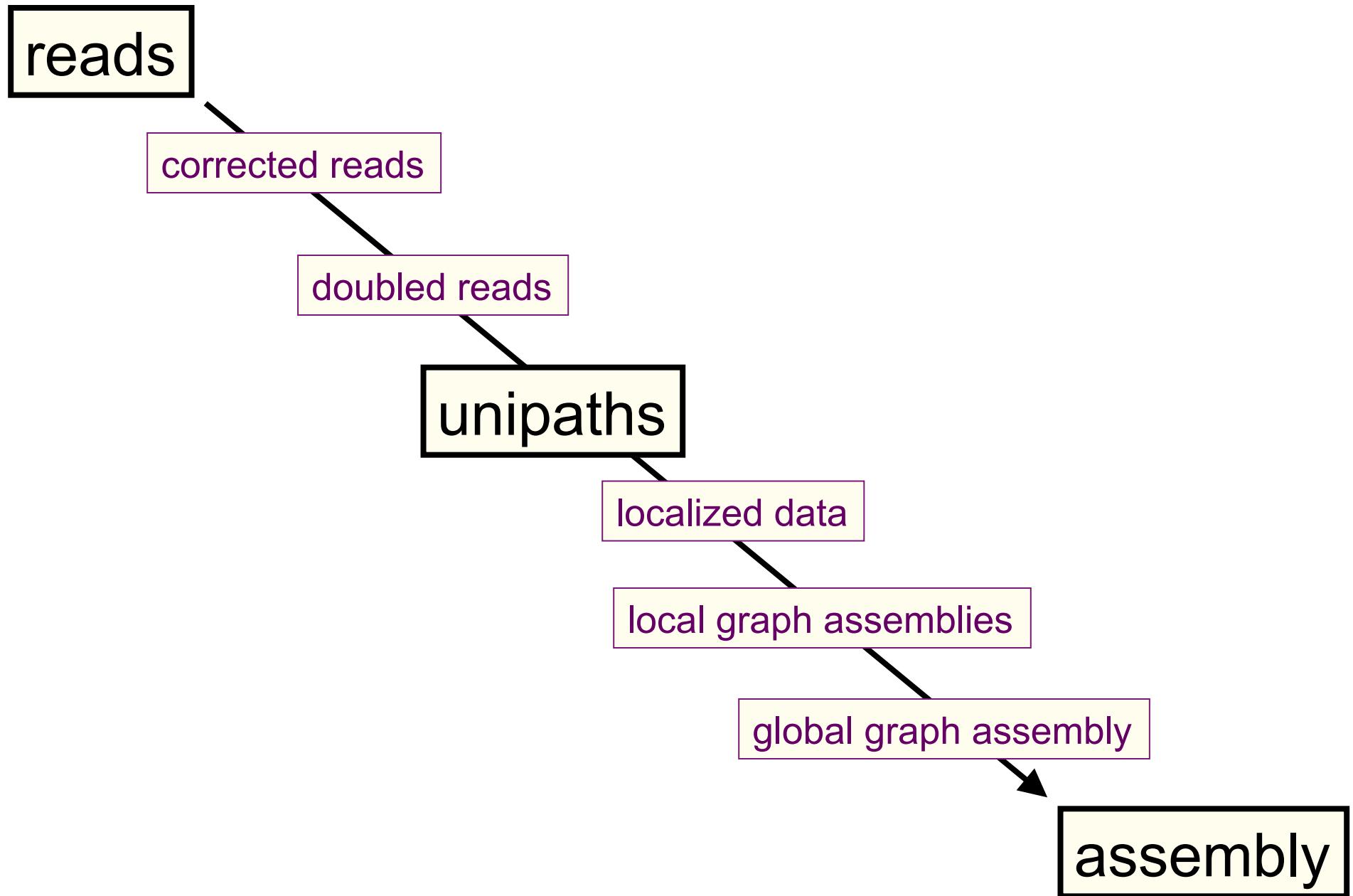
ALLPATHS-LG	SOAPdenovo	Celera Assembler
		
Broad's assembler (Gnerre et al. 2011)	BGI's assembler (Li et al. 2010)	JCVI's assembler (Miller et al. 2008)
De bruijn graph Short + PacBio (patching)	De bruijn graph Short reads	Overlap graph Medium + Long reads
Easy to run if you have compatible libraries	Most flexible, but requires a lot of tuning	Supports Illumina/454/PacBio Hybrid assemblies
http://www.broadinstitute.org/ software/allpaths-lg/blog/	http://soap.genomics.org.cn/ soapdenovo.html	http://wgs-assembler.sf.net



Genome assembly with ALLPATHS-LG

Iain MacCallum

How ALLPATHS-LG works



ALLPATHS-LG sequencing model

Libraries (insert types)	Fragment size (bp)	Read length (bases)	Sequence coverage (x)	Required
Fragment	180*	≥ 100	45	yes
Short jump	3,000	≥ 100 preferable	45	yes
Long jump	6,000	≥ 100 preferable	5	no**
Fosmid jump	40,000	≥ 26	1	no**

*See next slide.

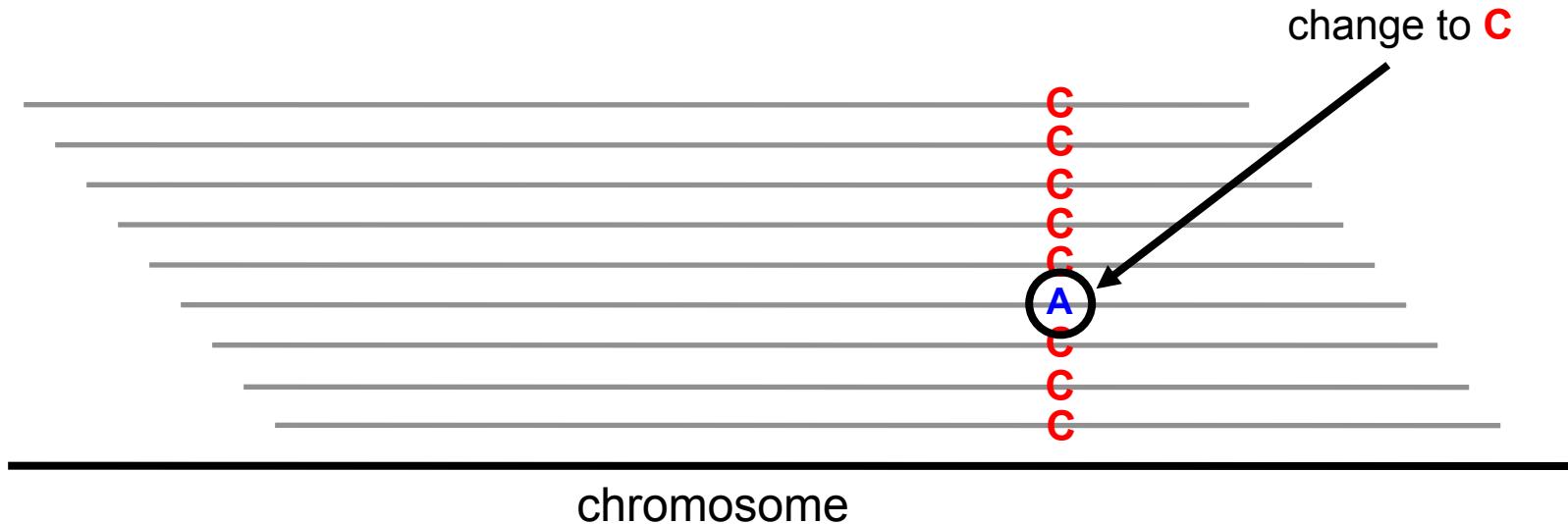
**For best results. Normally not used for small genomes.
However essential to assemble long repeats or duplications.

Cutting coverage in half still works, with some reduction in quality of results.

All: protocols are either available, or in progress.

Error correction

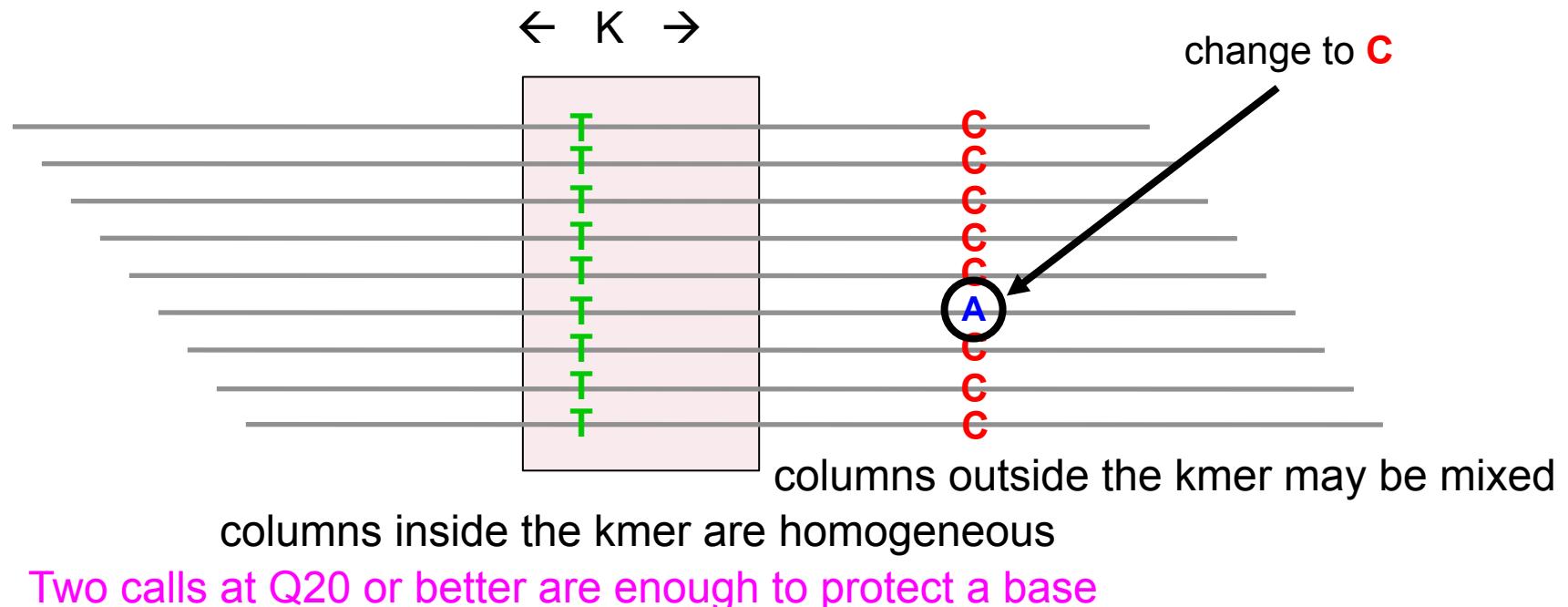
Given a crystal ball, we could stack reads on the chromosomes they came from (with homologous chromosomes separate), then let each column ‘vote’:



But we don't have a crystal ball....

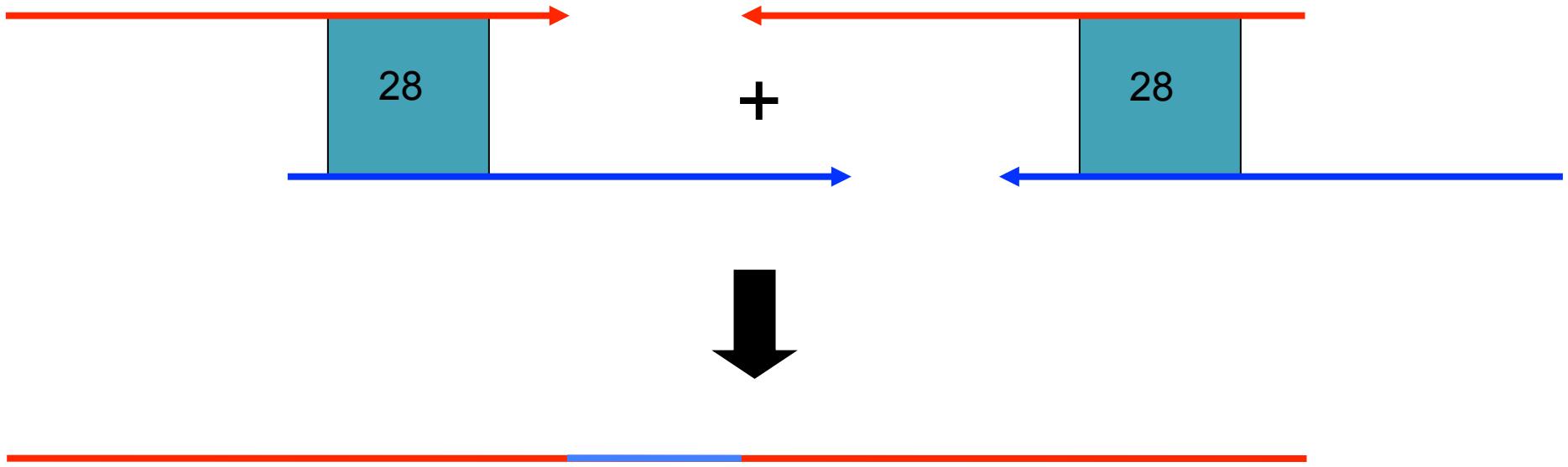
Error correction

ALLPATHS-LG. For every K-mer, examine the stack of all reads containing the K-mer. Individual reads may be edited if they differ from the overwhelming consensus of the stack. If a given base on a read receives conflicting votes (arising from membership of the read in multiple stacks), it is not changed. (K=24)



Read doubling

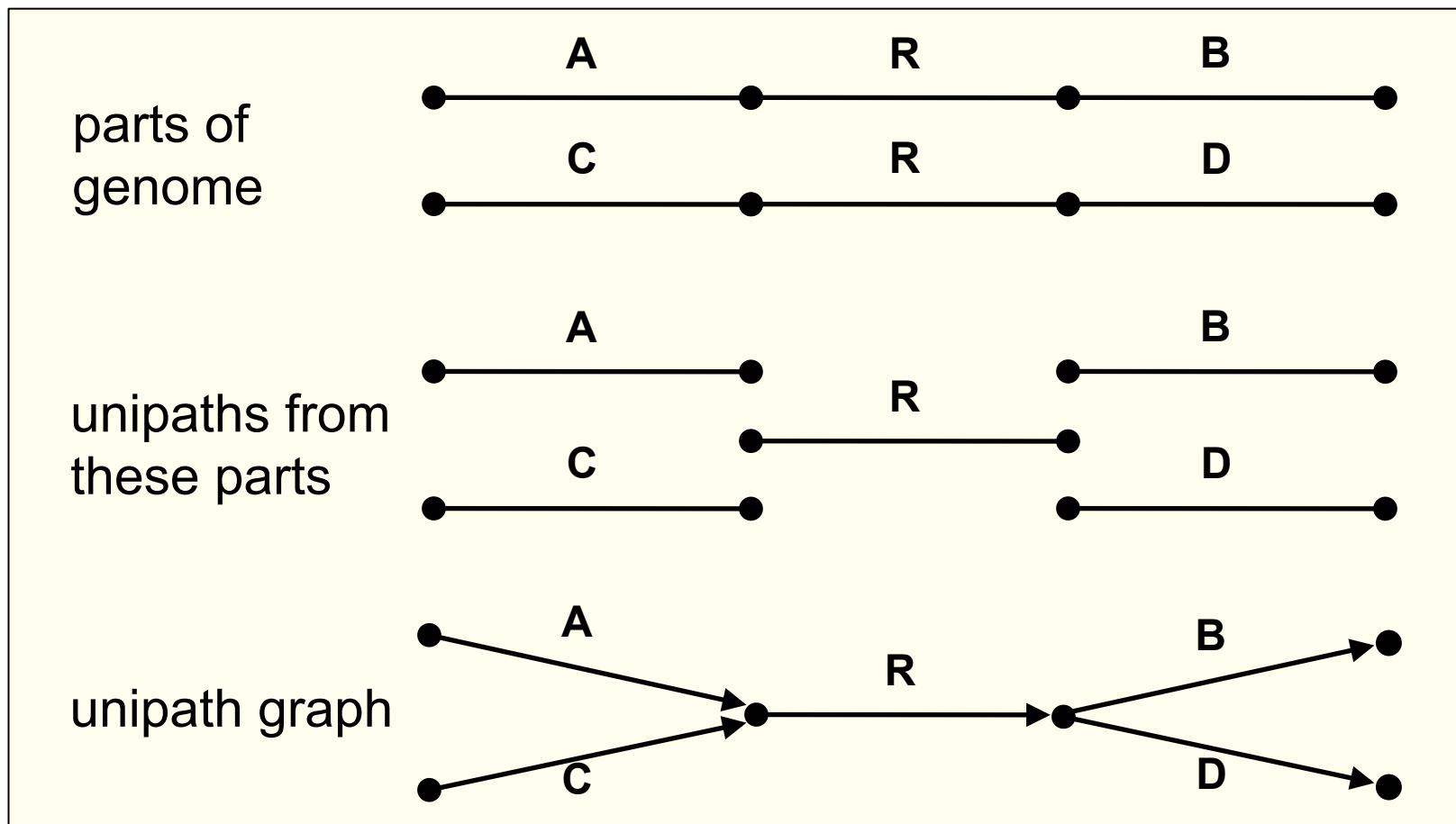
To close a read pair (red), we require the existence of another read pair (blue), overlapping perfectly like this:



More than one closure allowed (but rare).

Unipaths

Unipath: unbranched part of genome – squeeze together perfect repeats of size $\geq K$



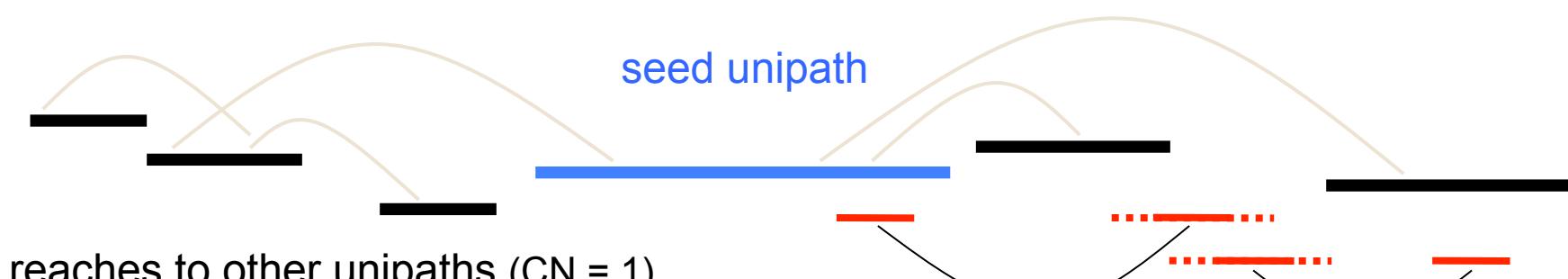
Adjacent unipaths overlap by $K-1$ bases

Localization

- I. Find ‘seed’ unipaths, evenly spaced across genome**
(ideally long, of copy number CN = 1)



- II. Form neighborhood around each seed**



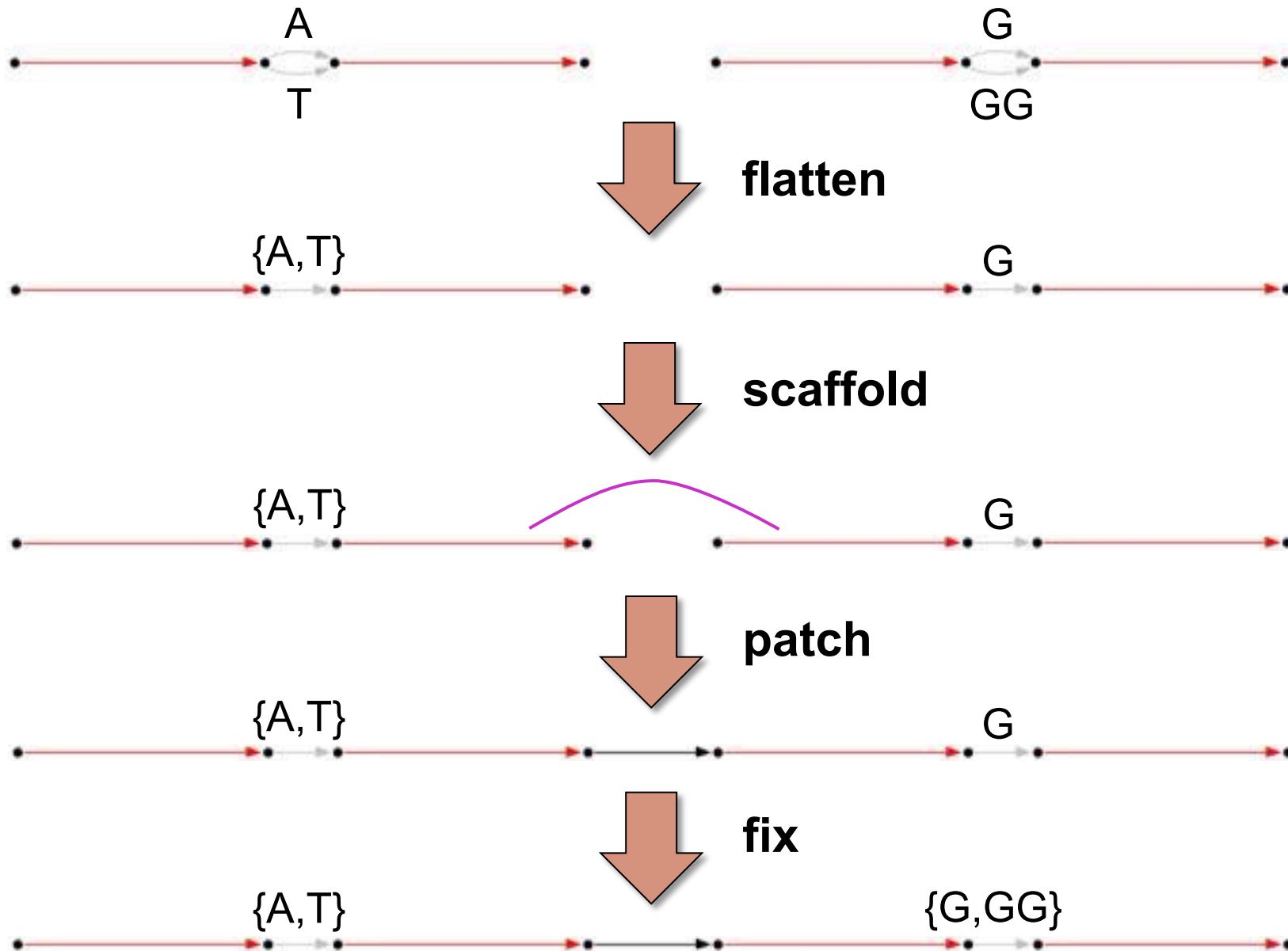
reaches to other unipaths (CN = 1)
directly and indirectly

read pairs reach into repeats

and are extended by other
unipaths

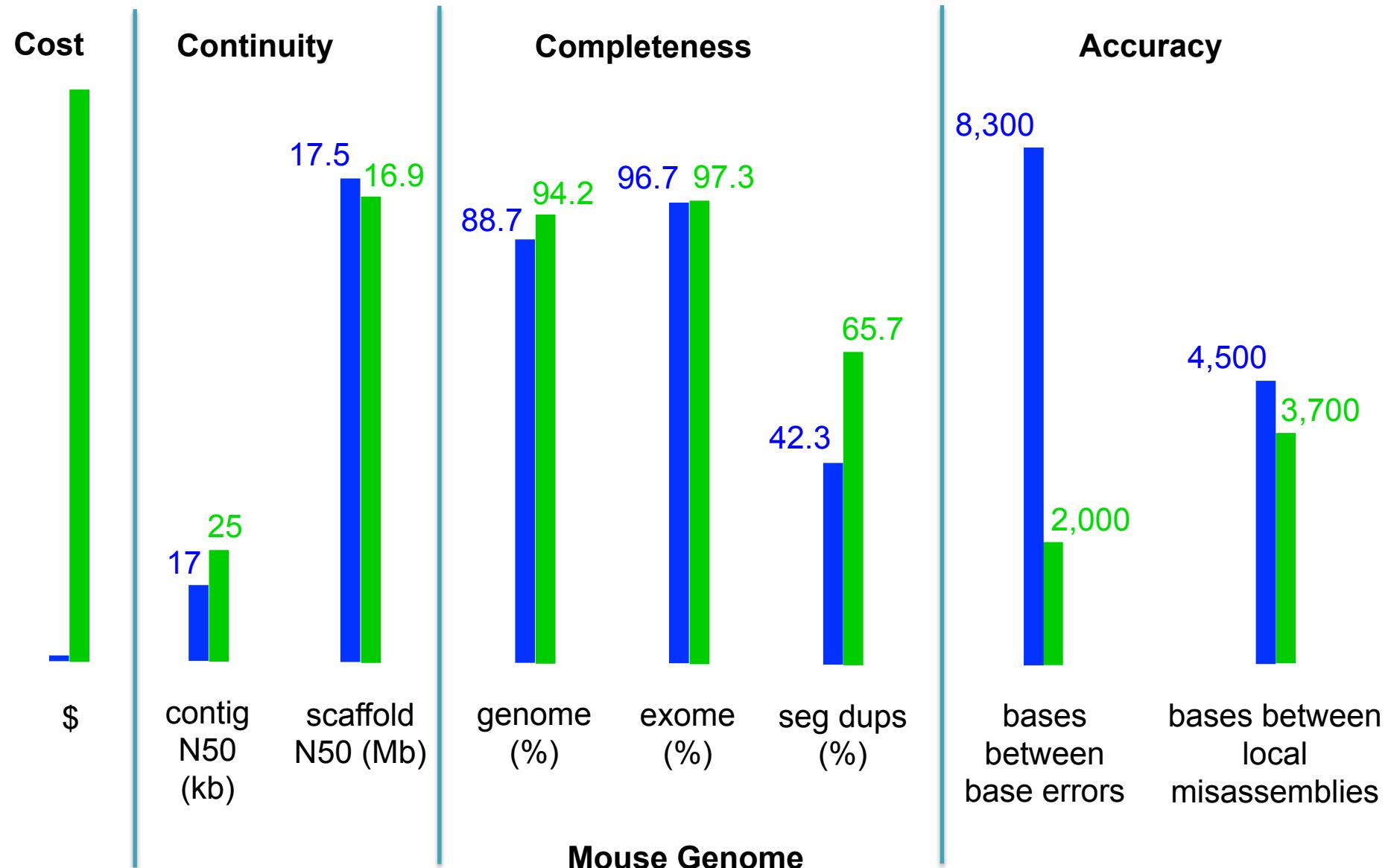
.....

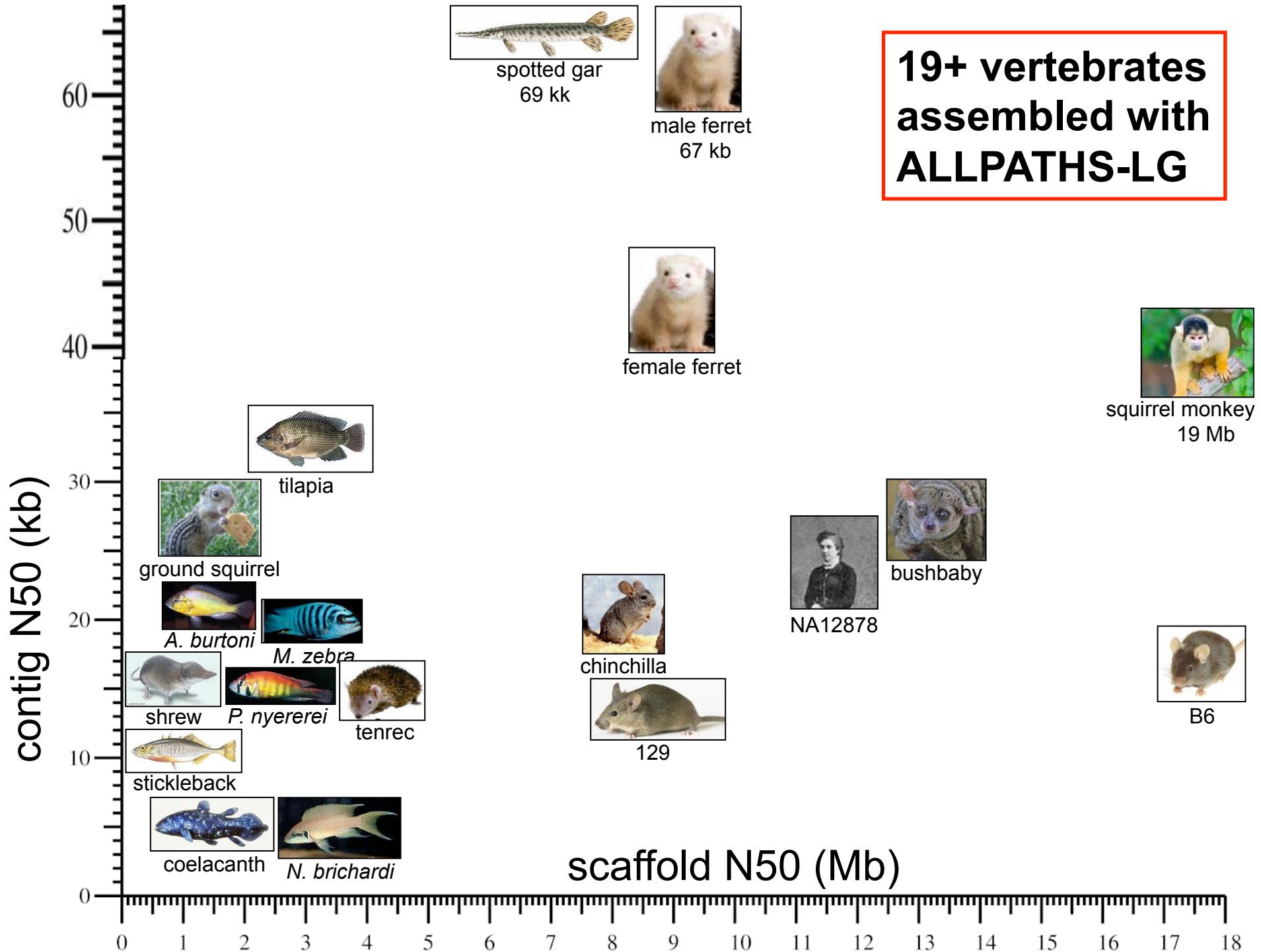
Create assembly from global assembly graph

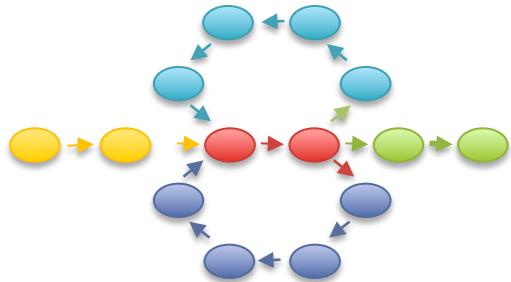




Large genome recipe: ALLPATHS-LG vs capillary







Genome assembly with SOAPdenovo

Short Read Assembly

Reads

AAGA
ACTT
ACTC
ACTG
AGAG
CCGA
CGAC
CTCC
CTGG
CTTT
...

de Bruijn Graph

Potential Genomes

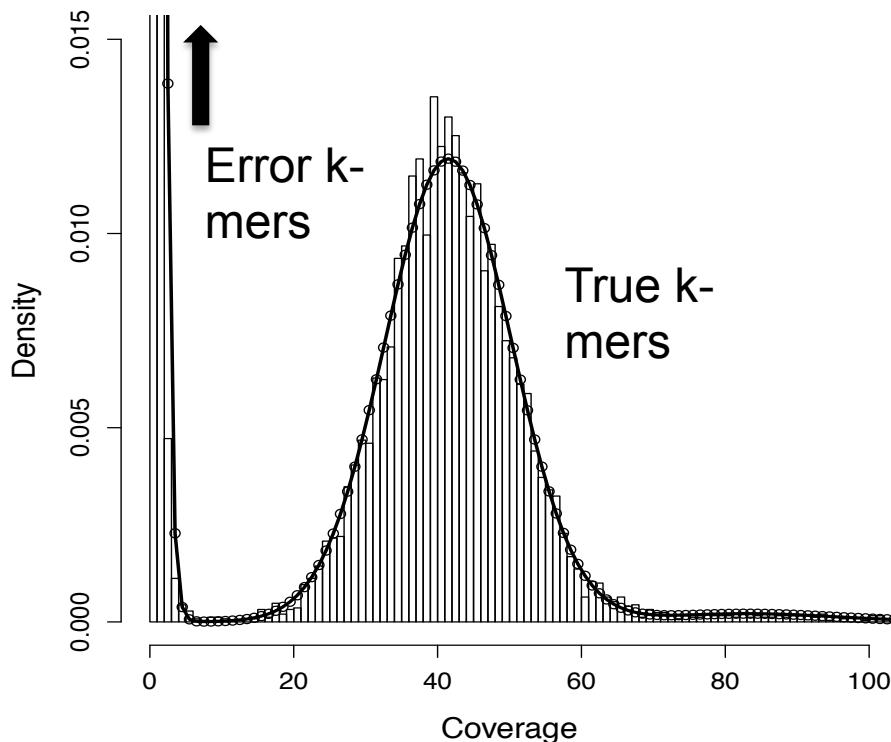
AAGACTCCGACTGGGACTTT
AAGACTGGGACTCCGACTTT

- 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

Error Correction with Quake

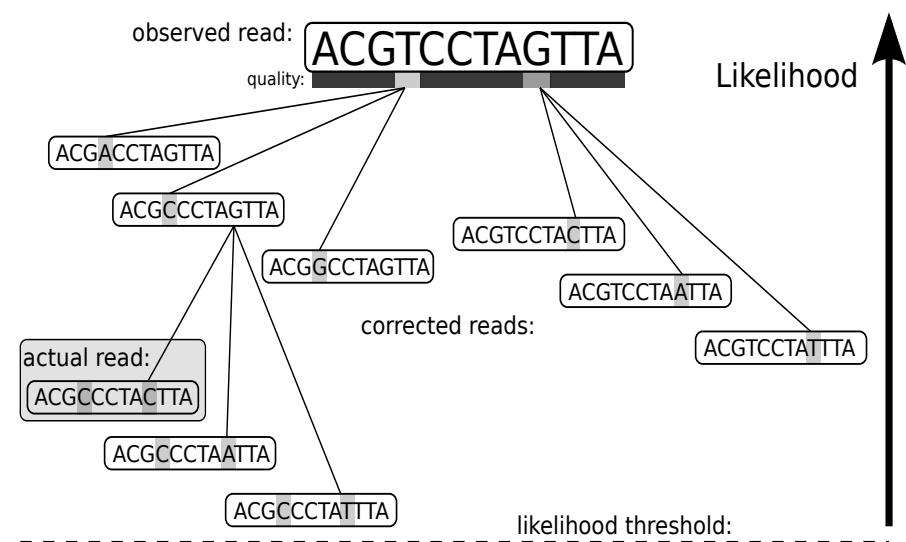
I. Count all “Q-mers” in reads

- 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

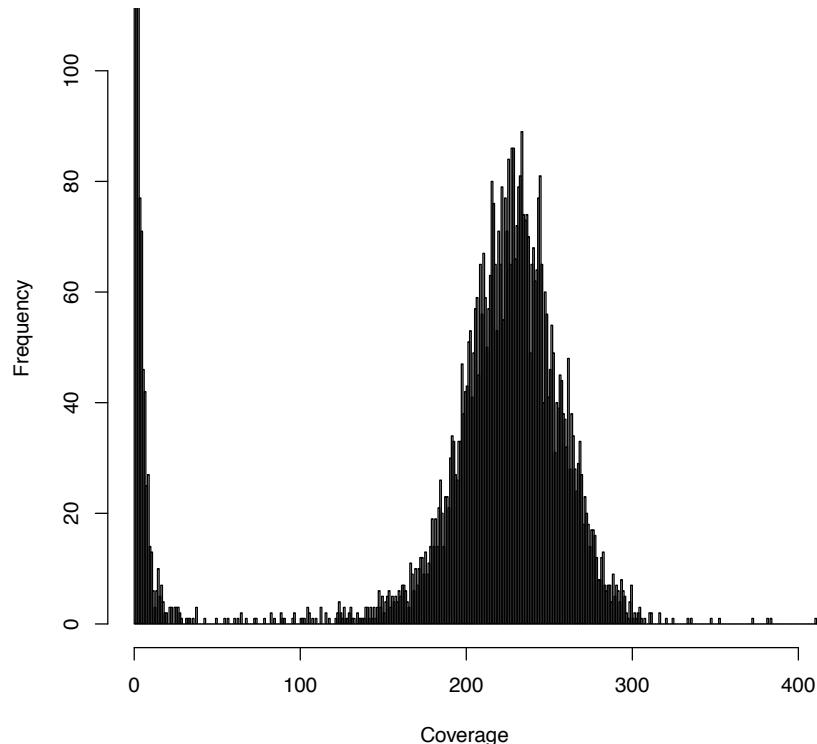


Quake: quality-aware detection and correction of sequencing reads.
Kelley, DR, Schatz, MC, Salzberg SL (2010) *Genome Biology*. 11:R116

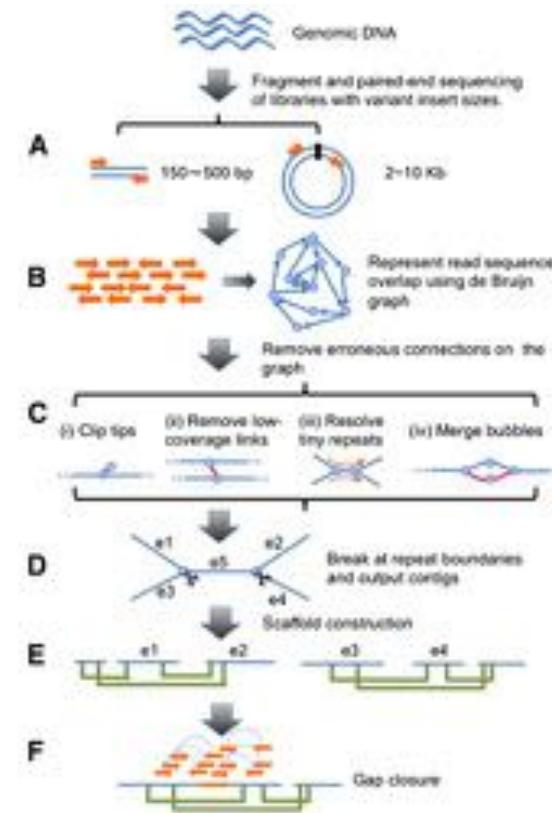
Illumina Sequencing & Assembly

Quake Results

2x76bp @ 275bp
2x36bp @ 3400bp

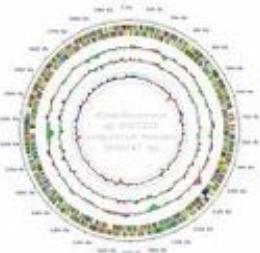


SOAPdenovo Results



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

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

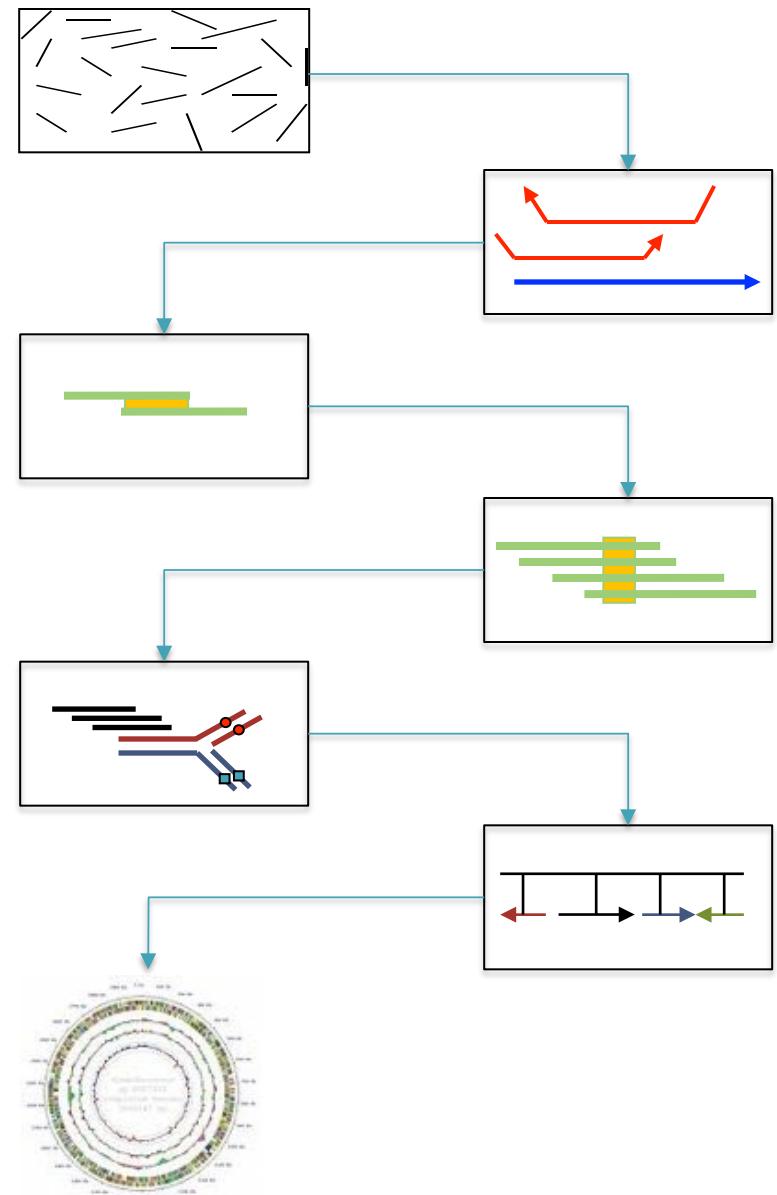


Genome assembly with the Celera Assembler

Celera Assembler

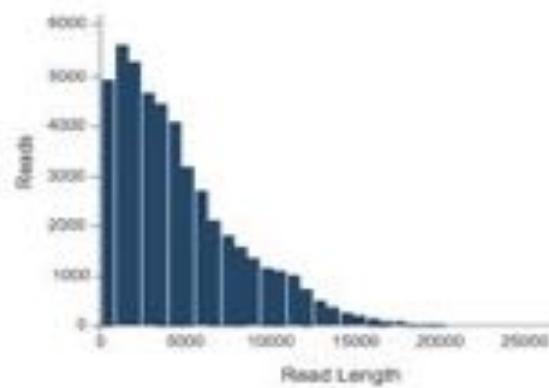
<http://wgs-assembler.sf.net>

1. Pre-overlap
 - Consistency checks
2. Trimming
 - Quality trimming & partial overlaps
3. Compute Overlaps
 - Find high quality overlaps
4. Error Correction
 - Evaluate difference in context of overlapping reads
5. Unitigging
 - Merge consistent reads
6. Scaffolding
 - Bundle mates, Order & Orient
7. Finalize Data
 - Build final consensus sequences

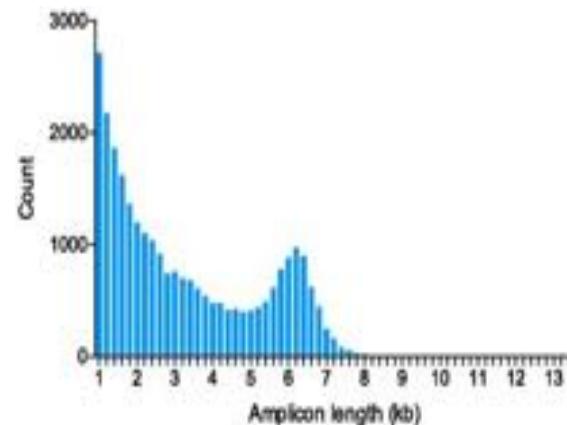


Single Molecule Sequencing Technology

PacBio RS II



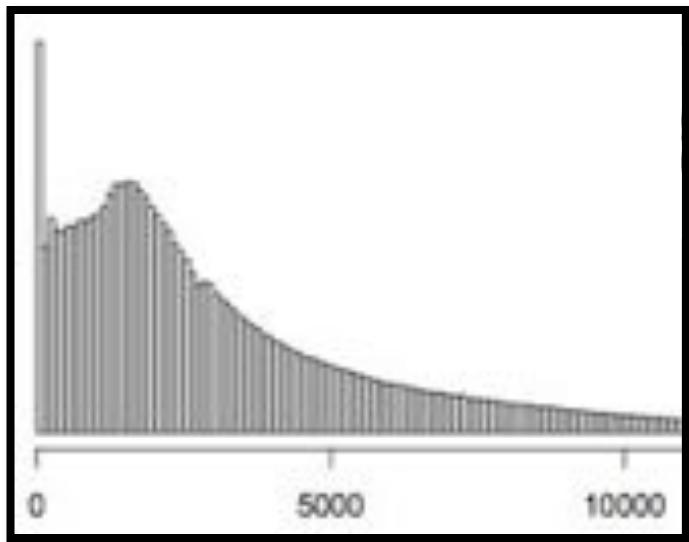
Moleculo



Oxford Nanopore



SMRT Sequencing Data



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

TTGTAAGCAGTTGAAACTATGTGT GGATTAGATAAAGAACATGAAAG
TTGTAAGCAGTTGAAACTATGTGT - GATTAG-ATAAAGAACATGGAAAG

ATTATAAAA-CAGTTGATCCATT-AGAAGA-AAACGCAAAAGGC GGCTAGG
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
A-TATAAAATCAGTTGATCCATTAGAA-AGAAACGC-AAAGGC-GCTAGG

CACACCTTGAATGT AATCGCACTTGAAAGAACAGATT TATTCCGGGCCCG
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
C-ACCTTG-ATGT-AT--CACTTGAAAGAACAGATT TATTCCGGGCCCG

TAACGAATCAAGATTCTGAAACACAT-AT ACAACCTCCAAAA-CACAA
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
T-ACGAATC-AGATTCTGAAACACATGAT---ACCTCCAAAA GCACAA

-AGGAGGGGAAAGGGGGGAATATCT-ATAAAAGATTACAAATT AGA-TGA
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
GAGGAGG-AA---GAATATCTGAT-AAAGATTACAAATT-GAGTGA

ACT-AATTACACAAATA-AATAACACTTTA-ACAGAATTGAT-GGAA-GTT
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
ACTAAATTACACAA-ATAATAACACTTTAGACAAATTGATGGAAAGGTT

TCGGAGAGATCCAACAAATGGGC-ATCGCCTTGA-GTTAC-AATCAA
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
TC-GAGAGATCC-AAACAAT-GGC GATCG-CTTGACGTTACAAATCAA

ATCCAGTGGAAAATATAATTTATGCAATCCAGGAACCTATTACAATTAG
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
ATCCAGT-GAAAATATA--TTATGC-ATCCA-GAACCTATTACAATTAG

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

PacBio Error Correction: HGAP



- With 50-100x of Pacbio coverage, virtually all of the errors can be eliminated
 - Works well for Microbial genomes: single contig per chromosome routinely achieved
 - Difficult to scale up for use with eukaryotic genomes

Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data
Chin, CS et al. (2013) *Nature Methods*. 10: 563-569

Hybrid Sequencing



Illumina
Sequencing by Synthesis

High throughput (60Gbp/day)
High accuracy (~99%)
Short reads (~100bp)



Pacific Biosciences
SMRT Sequencing

Lower throughput (1 Gbp/day)
Lower accuracy (~85%)
Long reads (5 kbp+)

Hybrid Error Correction: PacBioToCA

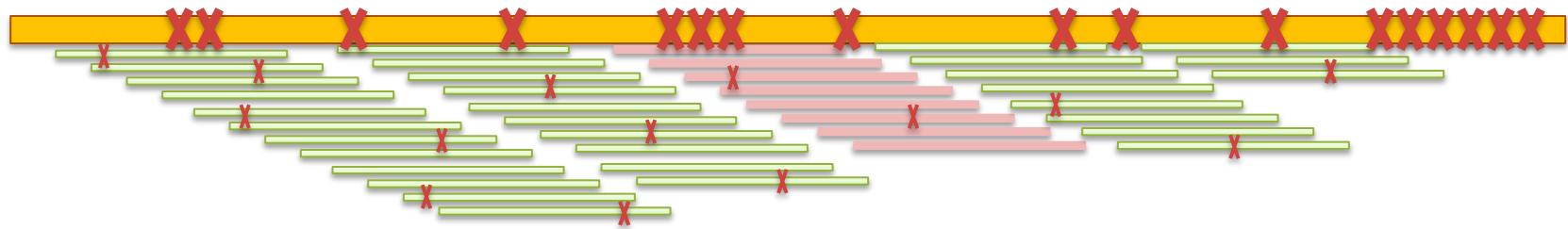
<http://wgs-assembler.sf.net>

I. Correction Pipeline

1. Map short reads to long reads
2. Trim long reads at coverage gaps
3. Compute consensus for each long read



2. Error corrected reads can be easily assembled, aligned

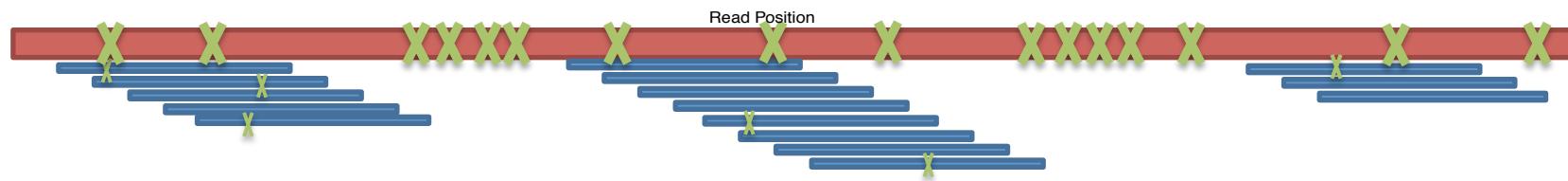


Hybrid error correction and de novo assembly of single-molecule sequencing reads.
Koren, S, Schatz, MC, et al. (2012) *Nature Biotechnology*. doi:10.1038/nbt.2280

Enhanced PacBio Error Correction

PacBioToCA fails in complex regions

1. Simple Repeats – Kmer Frequency Too High to Seed Overlaps
2. Error Dense Regions – Difficult to compute overlaps with many errors
3. Extreme GC – Lacks Illumina Coverage

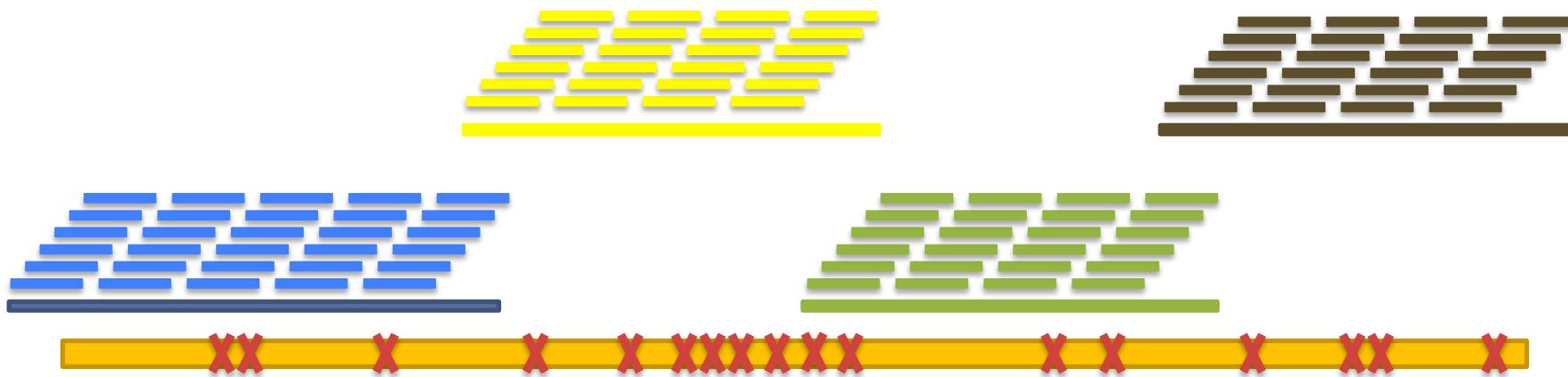


Assembly complexity of long read sequencing

Lee, H*, Gurtowski, J*, Yoo, S, Marcus, S, McCombie, WR, Schatz MC et al. (2013) *In preparation*

Error Correction with pre-assembled Illumina reads

<https://github.com/jgurtowski/pbtools>



Short Reads -> Assemble Unitigs -> Align & Select -> Error Correct

Unitigs:

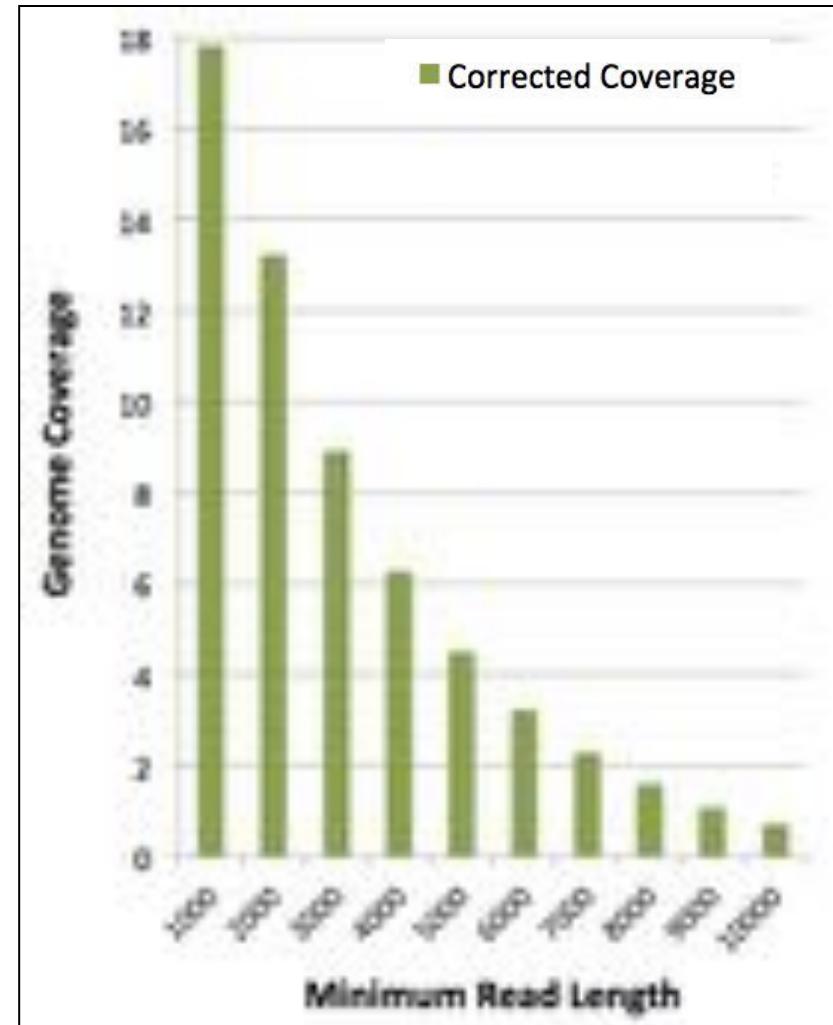
High quality contigs formed from unambiguous, unique overlaps of reads
Each read is placed into a single unitig

Can Help us overcome:

- 1. Simple Repeats – Kmer Frequency Too High to Seed Overlaps**
- 2. Error Dense Regions – Difficult to compute overlaps with many errors**

Preliminary Rice Assemblies

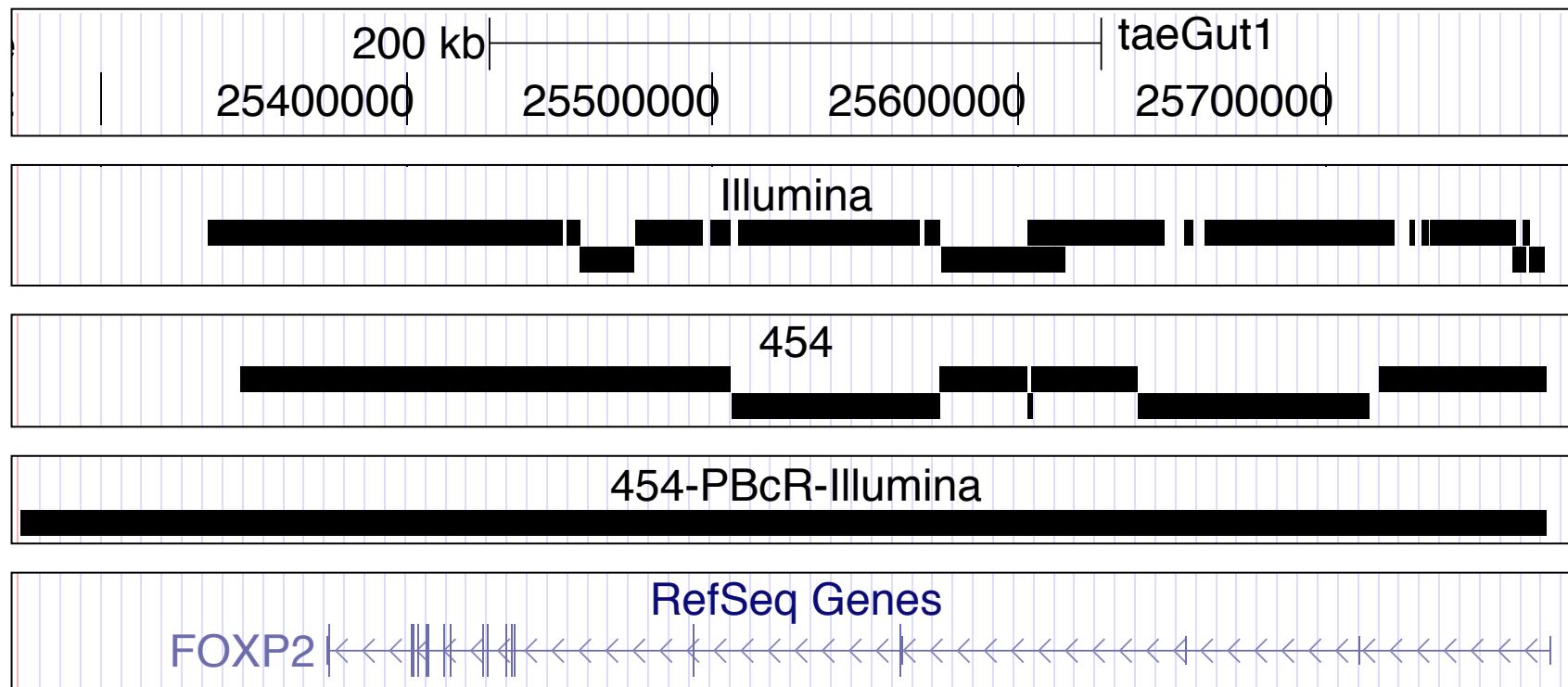
Assembly	Contig NG50
HiSeq Fragments 50x 2x100bp @ 180	3,925
MiSeq Fragments 23x 459bp 8x 2x251bp @ 450	6,332
“ALLPATHS-recipe” 50x 2x100bp @ 180 36x 2x50bp @ 2100 51x 2x50bp @ 4800	18,248
PBeCR Reads 19x @ 3500 ** MiSeq for correction	50,995
Enhanced PBeCR 19x @ 3500 ** MiSeq for correction	155,695



In collaboration with McCombie & Ware labs @ CSHL

Improved Gene Reconstruction

FOXP2 assembled in a single contig in the PacBio parrot assembly

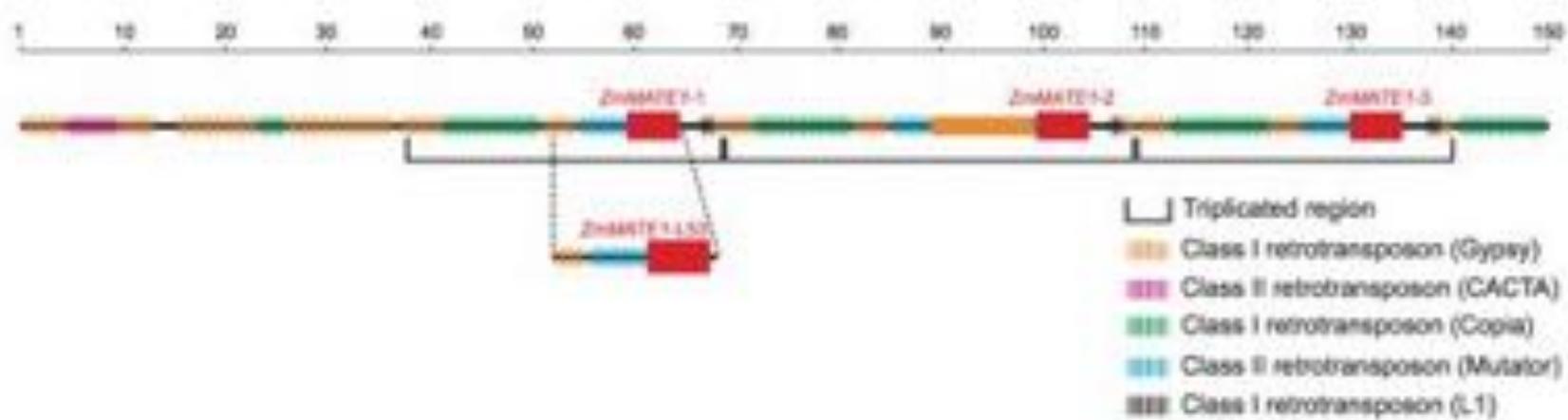


Hybrid error correction and de novo assembly of single-molecule sequencing reads.
Koren, S, Schatz, MC, et al. (2012) *Nature Biotechnology*. doi:10.1038/nbt.2280

Long Read CNV Analysis

Aluminum tolerance in maize is important for drought resistance and protecting against nutrient deficiencies

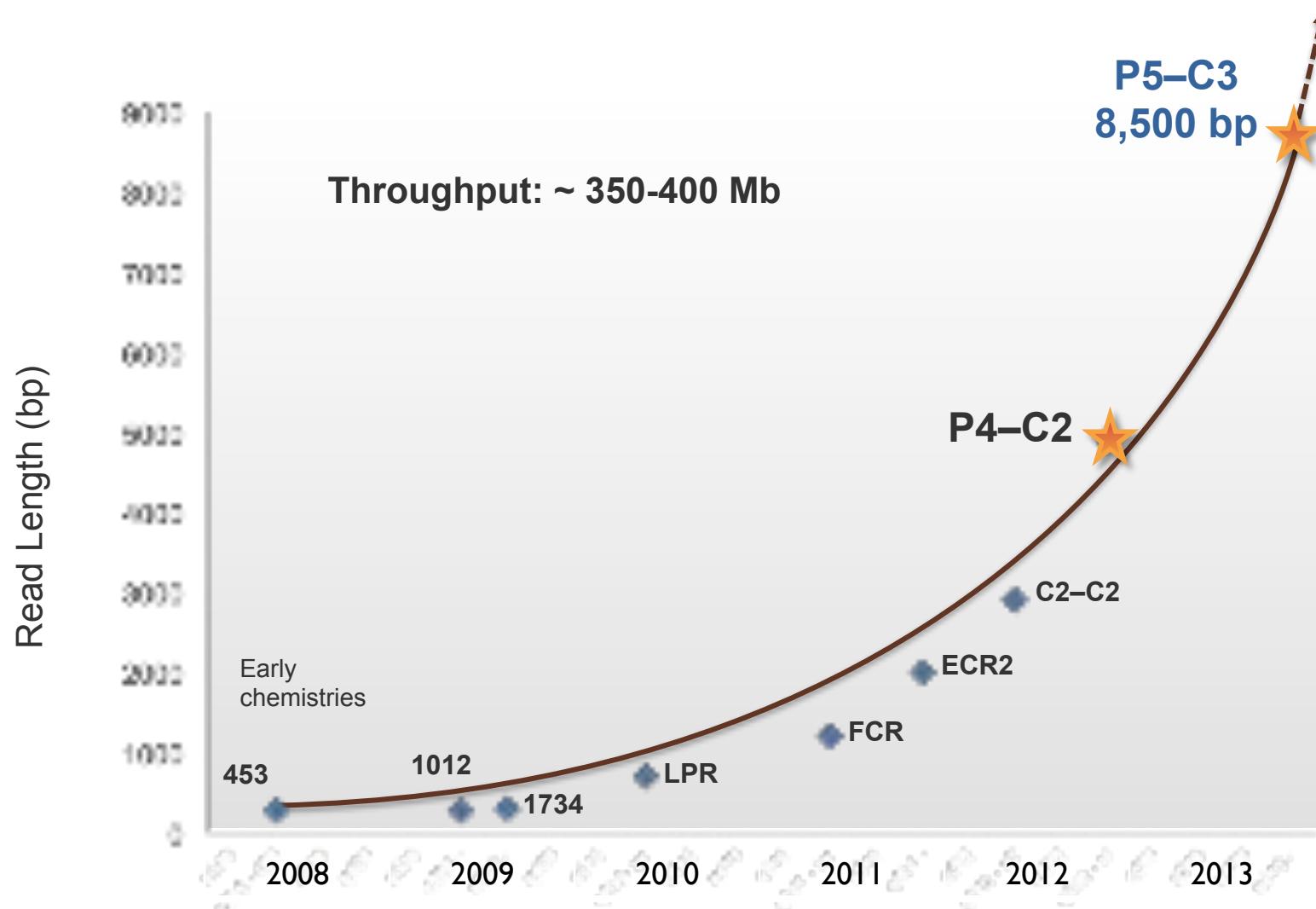
- Segregating population localized a QTL on a BAC, but unable to genotype with Illumina sequencing because of high repeat content and GC skew
- Long read PacBio sequencing corrected by CCS reads revealed a triplication of the ZnMATE1 membrane transporter



A rare gene copy-number variant that contributes to maize aluminum tolerance and adaptation to acid soils

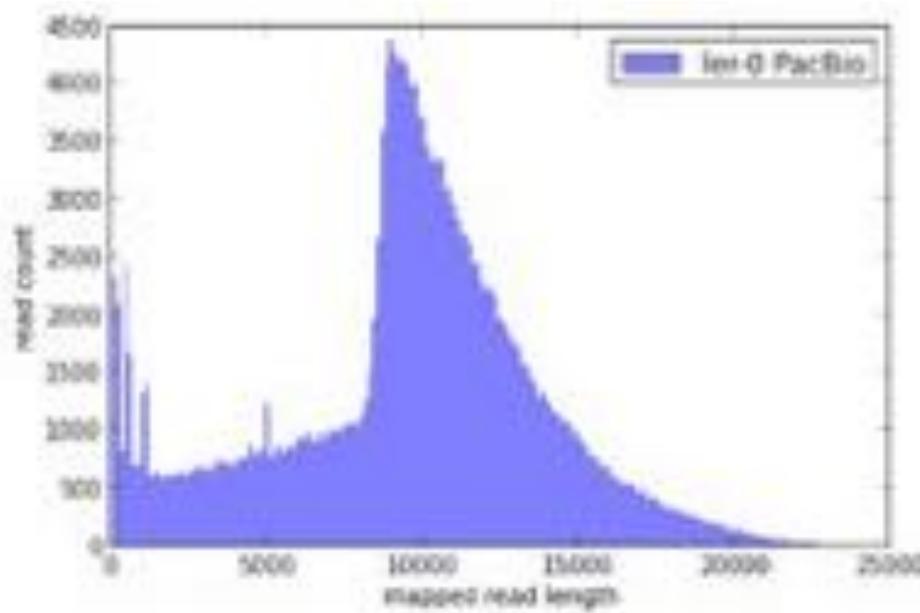
Maron, LG et al. (2013) PNAS doi: 10.1073/pnas.1220766110

P5-C3 Chemistry Read Lengths



De novo assembly of Arabidopsis

<http://blog.pacificbiosciences.com/2013/08/new-data-release-arabidopsis-assembly.html>



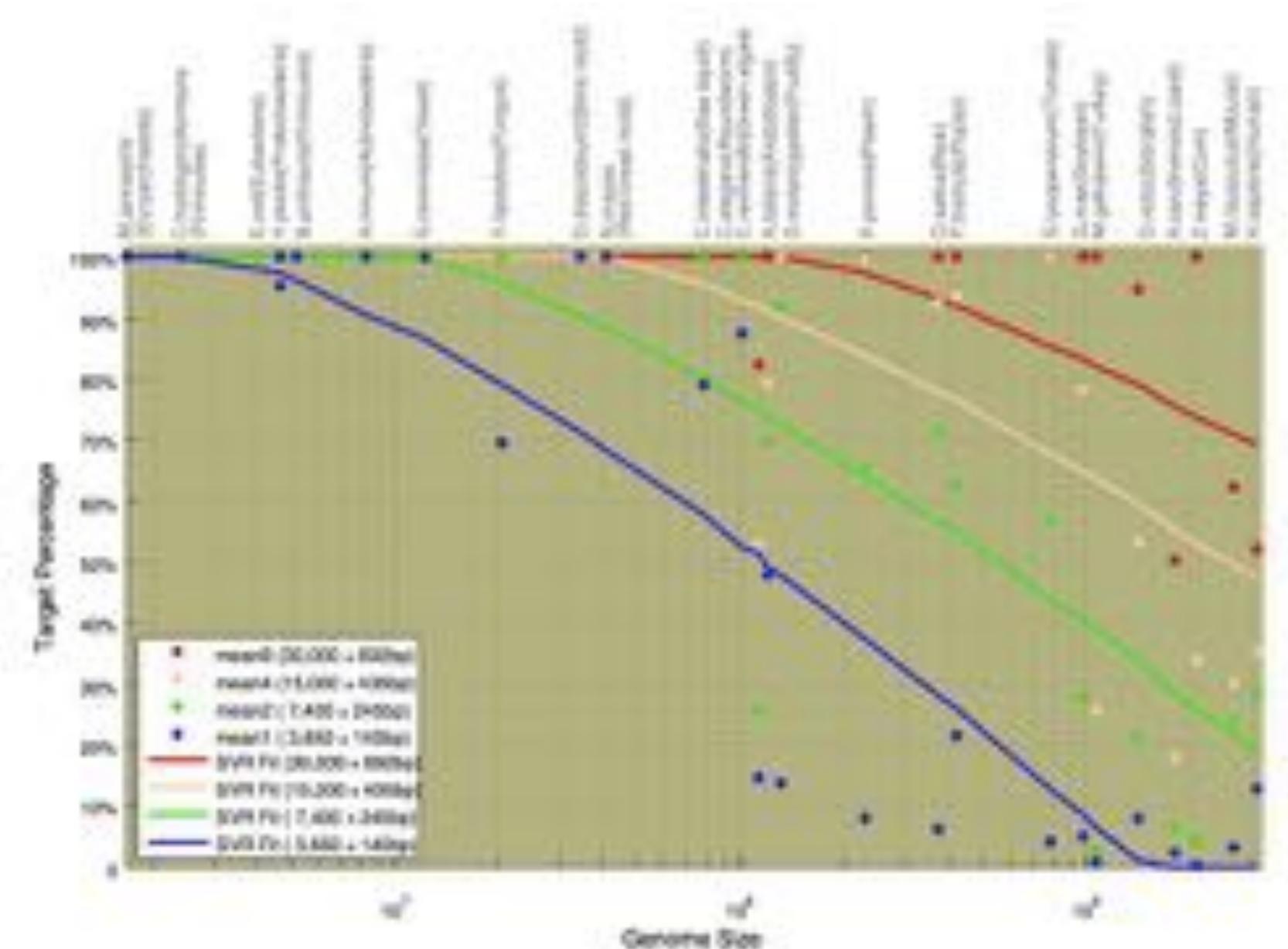
A. thaliana Ler-0 sequenced at PacBio

- Sequenced using the latest P4 enzyme and C2 chemistry
- Size selection using an 8 Kb to 50 Kb elution window on a BluePippin™ device from Sage Science
- Total coverage >100x

Genome size: 124.6 Mb
GC content: 33.92%
Raw data: 11 Gb
Assembly coverage: 15x over 9kbp

Sum of Contig Lengths: 149.5Mb
Number of Contigs: 1788
Max Contig Length: 12.4 Mb
N50 Contig Length: 8.4 Mb

Assembly Complexity of Long Reads



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
 - Reads -> unitigs -> mates -> scaffolds
-> optical / physical / genetic maps
-> chromosomes
 - Recommendations:
 - ALLPATH-LG for Illumina-only
 - HGAP for PacBio-only, CA for Hybrid assembly
 - See Assemblathon papers for a more extensive analysis





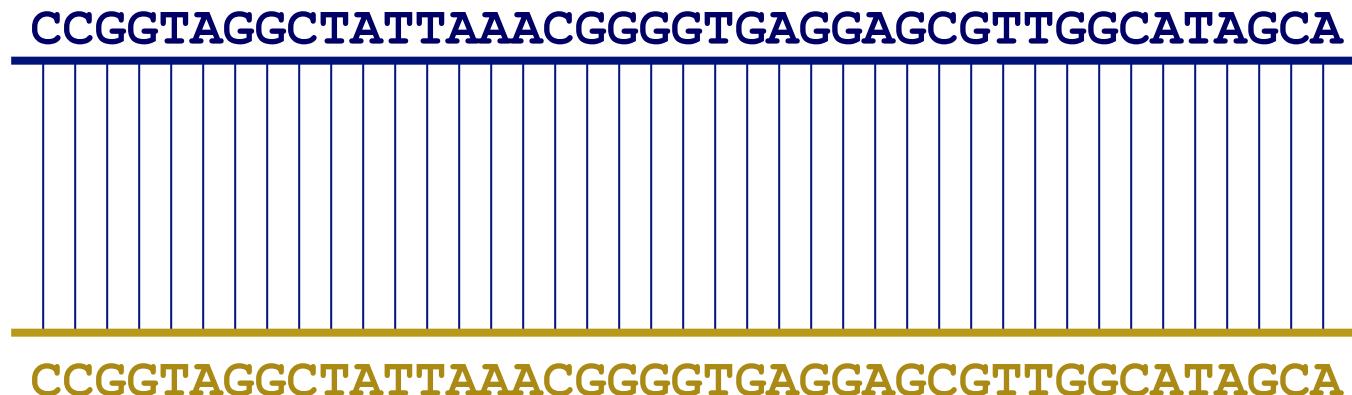
Whole Genome Alignment with MUMmer

Slides Courtesy of Adam M. Phillippy

amp@umics.umd.edu

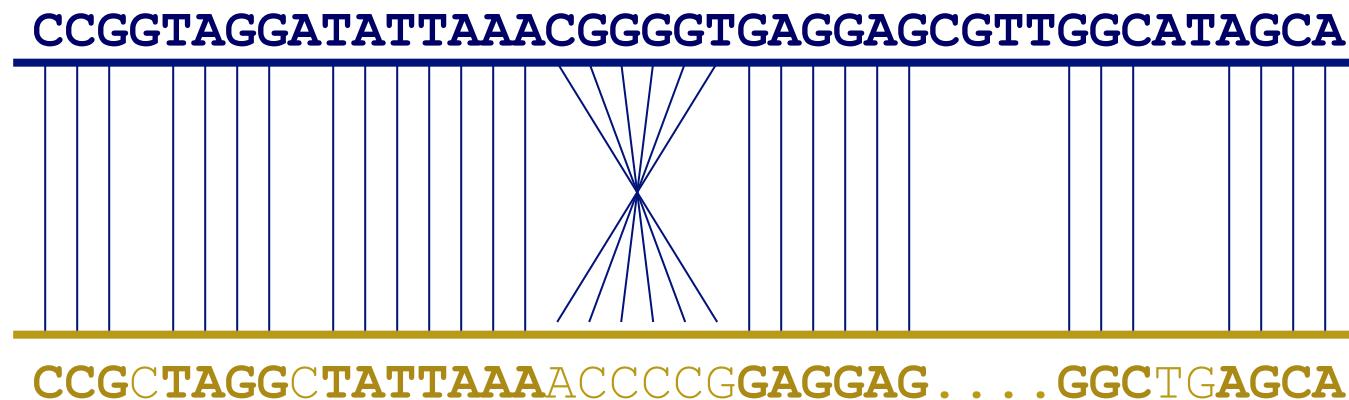
Goal of WGA

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



Not so fast...

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



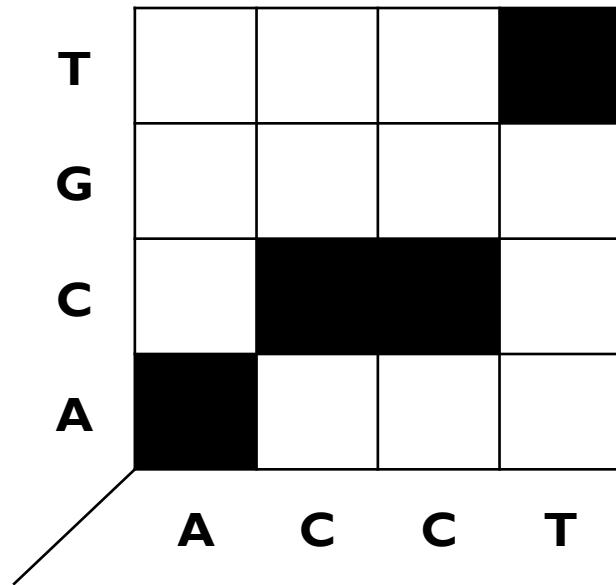
WGA visualization

- How can we visualize *whole genome* alignments?

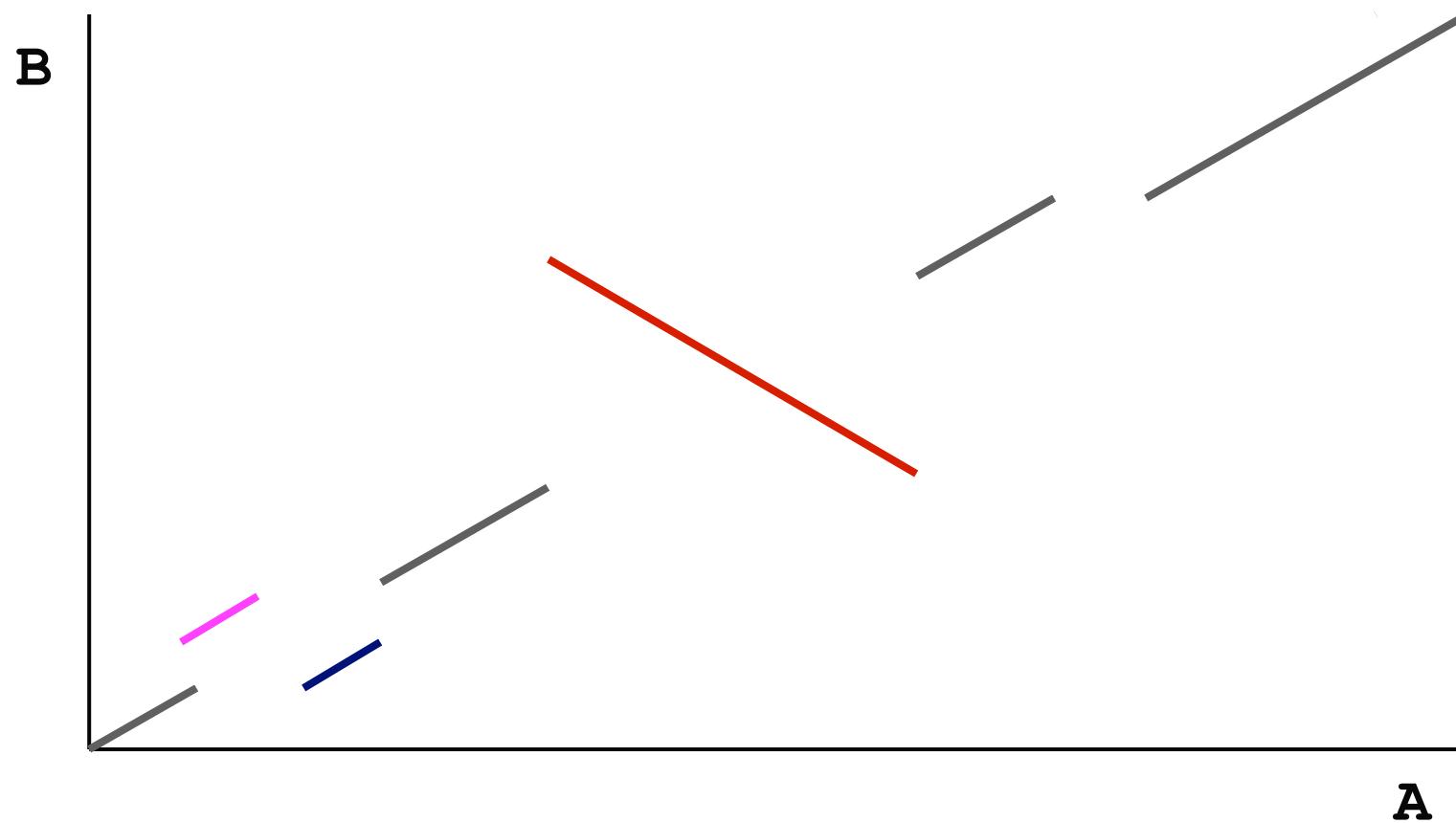
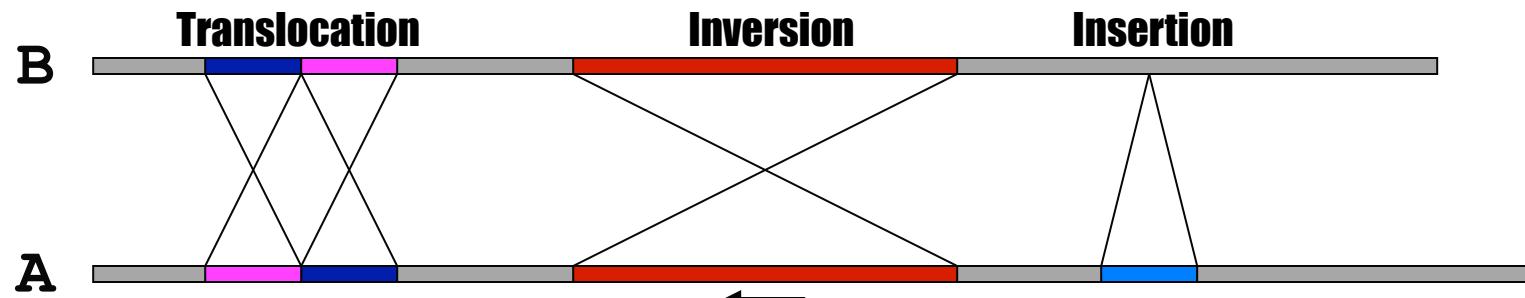
- With an alignment dot plot

- $N \times M$ matrix

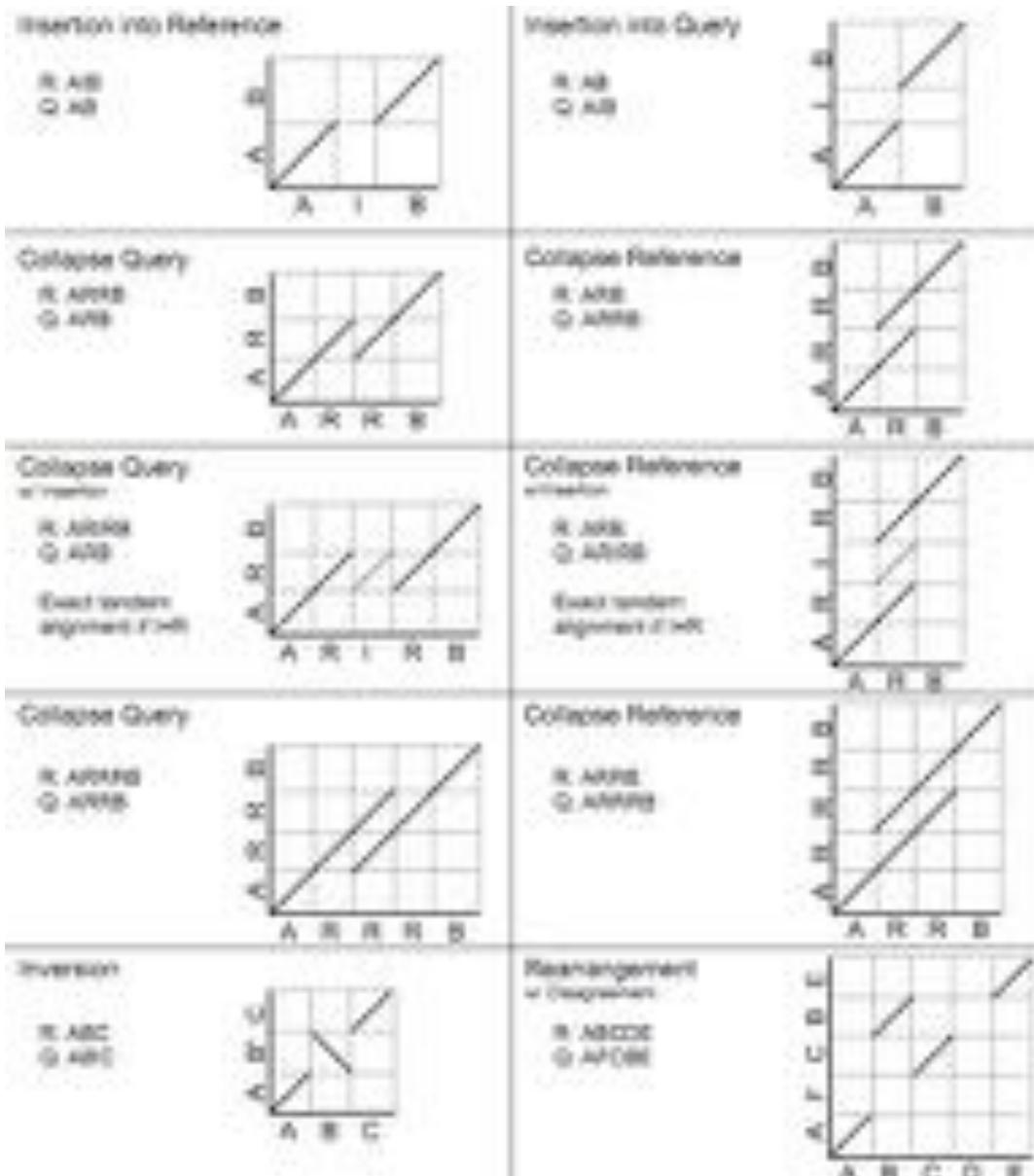
- Let i = position in genome A
 - Let j = position in genome B
 - Fill cell (i,j) if A_i shows similarity to B_j



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



SV Types



- Different structural variation types / misassemblies will be apparent by their pattern of breakpoints
- Most breakpoints will be at or near repeats
- Things quickly get complicated in real genomes

[http://mummer.sf.net/manual/
AlignmentTypes.pdf](http://mummer.sf.net/manual/AlignmentTypes.pdf)

Seed-and-extend with MUMmer

How can quickly align two genomes?

I. Find maximal-unique-matches (MUMs)

- ◆ Match: exact match of a minimum length
- ◆ Maximal: cannot be extended in either direction without a mismatch
- ◆ Unique
 - ◆ occurs only once in both sequences (MUM)
 - ◆ occurs only once in a single sequence (MAM)
 - ◆ occurs one or more times in either sequence (MEM)

2. Cluster MUMs

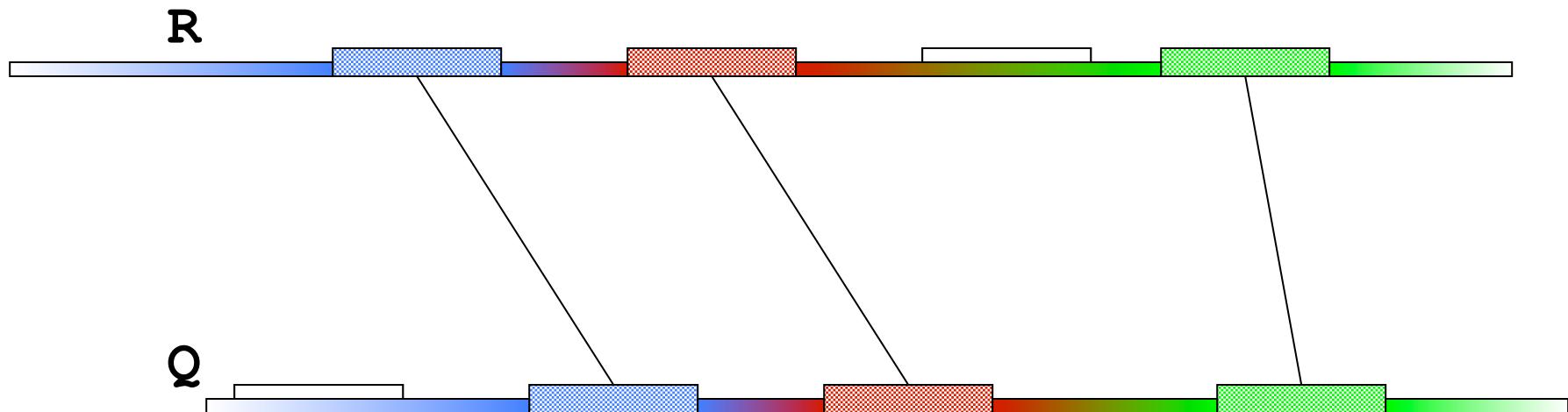
- ◆ using size, gap and distance parameters

3. Extend clusters

- ◆ using modified Smith-Waterman algorithm

Seed and Extend visualization

FIND all MUMs
CLUSTER consistent MUMs
EXTEND alignments



WGA example with nucmer

- *Yersina pestis* CO92 vs. *Yersina pestis* KIM
 - High nucleotide similarity, 99.86%
 - Two strains of the same species
 - Extensive genome shuffling
 - Global alignment will not work
 - Highly repetitive
 - Many local alignments

WGA Alignment

nucmer -maxmatch CO92.fasta KIM.fasta

-maxmatch Find maximal exact matches (MEMs)

delta-filter -m out.delta > out.filter.m

-m Many-to-many mapping

show-coords -r out.delta.m > out.coords

-r Sort alignments by reference position

dnadiff out.delta.m

Construct catalog of sequence variations

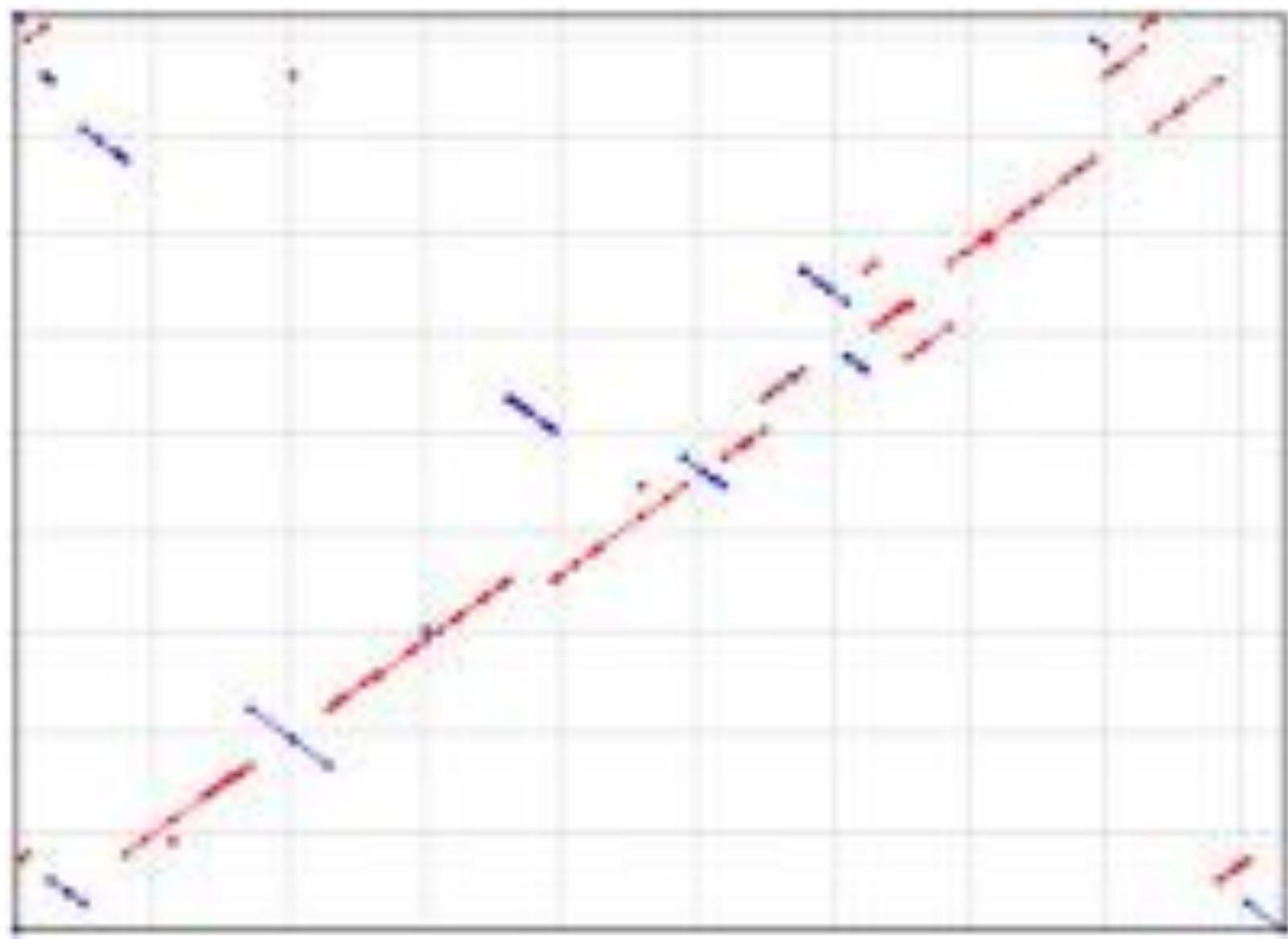
mummerplot --large --layout out.delta.m

--large Large plot

--layout Nice layout for multi-fasta files

--x11 Default, draw using x11 (--postscript, --png)

*requires gnuplot



References

- Documentation
 - <http://mummer.sourceforge.net>
 - » publication listing
 - <http://mummer.sourceforge.net/manual>
 - » documentation
 - <http://mummer.sourceforge.net/examples>
 - » walkthroughs
- Email
 - mummer-help@lists.sourceforge.net