Lectures 18, 19: Sequence Assembly

Spring 2017
April 13, 18, 2017

Outline

- Introduction
- Sequence Assembly Problem
- Different Solutions:
  - Overlap-Layout-Consensus Assembly Algorithms
  - De Bruijn Graph Based Assembly Algorithms
- Resolving Repeats
- Introduction to Single-Cell Sequencing

Whole Genome Shotgun Sequencing

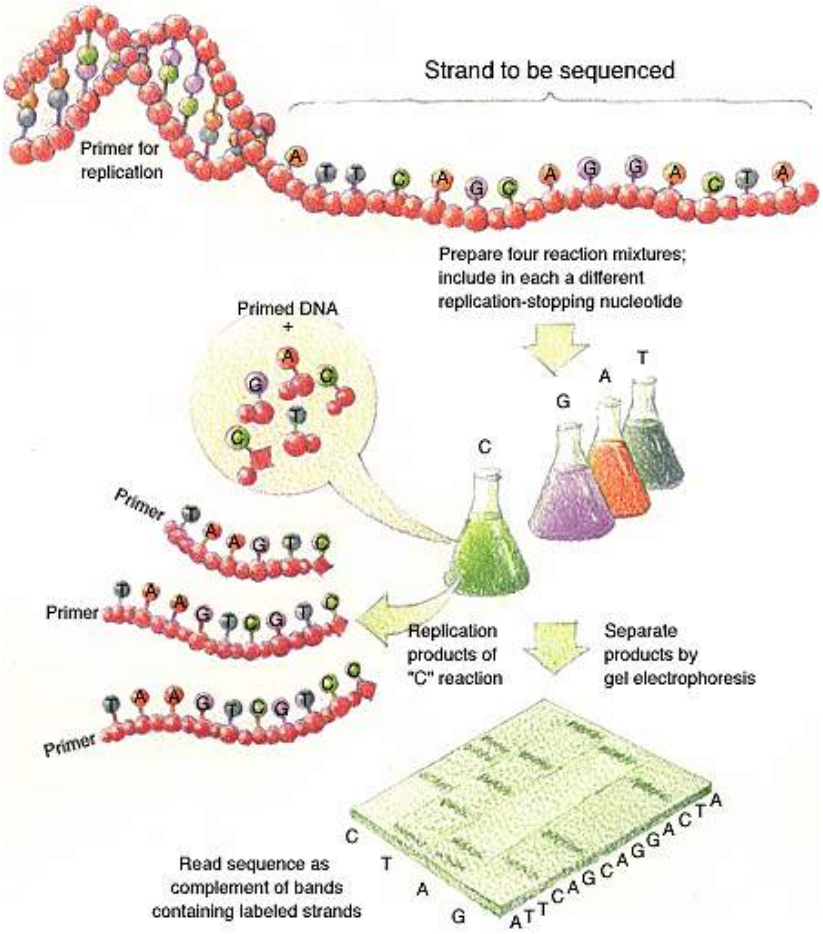
- Frederick Sanger (and others) shared a Nobel Prize in Chemistry in 1980 for developing a method to sequence short regions of DNA.

- There is no current technology to simply read the whole genome sequence from one end to the other.

- The human genome is 3 billion nucleotides long. Sequencing it requires breaking it into little pieces, sequencing the pieces separately, and fitting them back together, like a jigsaw puzzle.

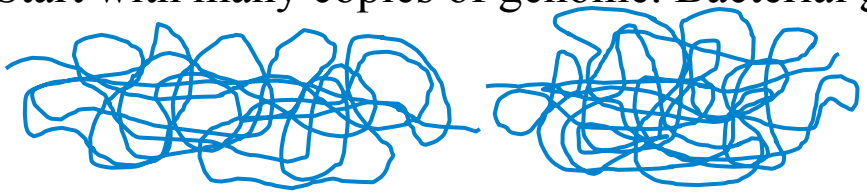
DNA Sequencing

- Shear DNA into millions of small fragments
- Read 500 – 700 nucleotides at a time from the small fragments (Sanger method)

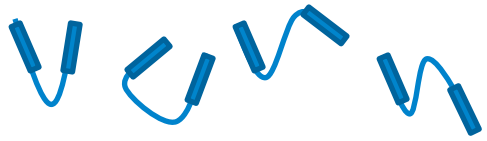


Whole Genome Shotgun Sequencing

Start with many copies of genome. Bacterial genome length: ~5 million.



Fragment them and sequence reads at both ends. Read length: 35 to 1000 bp.



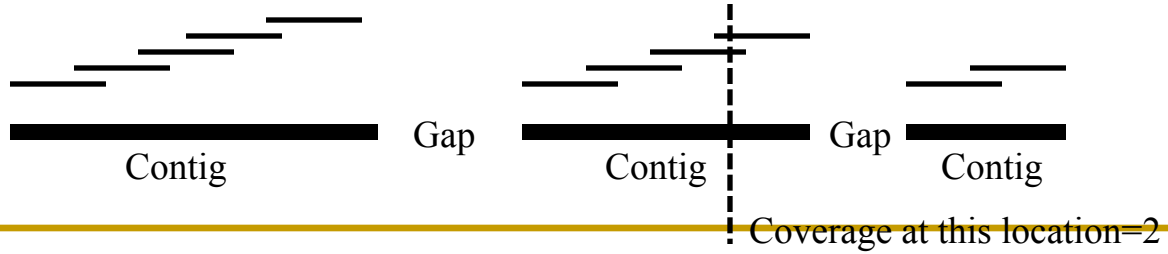
Find overlapping reads.

ACGTAGAATCGACCATG...

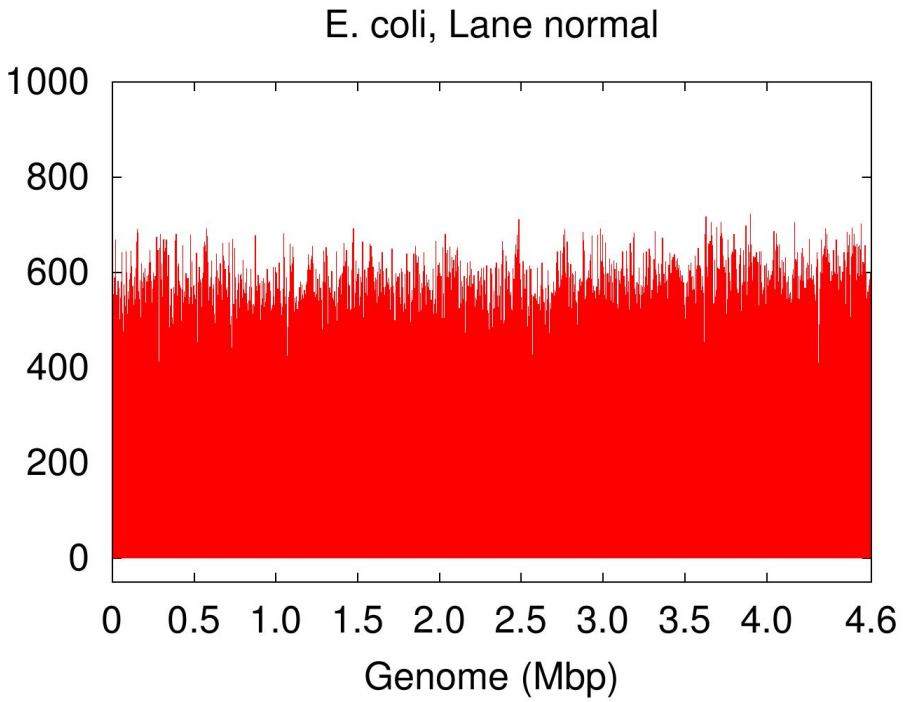
...AACATAGTTGACGTAGAATC

Merge overlapping reads into contigs.

...AACATAGTTGACGTAGAATCGACCATG...



Sequencing Coverage



Number of reads: ~28 million, read length: 100 bp, genome size: 4.6 Mbp, coverage: ~600x

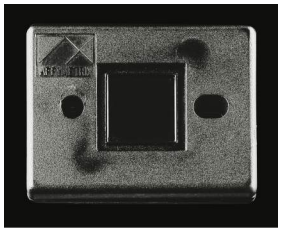
H. Chitsaz, et al., *Nature Biotech (2011)*

Sequencing by Hybridization (SBH): History

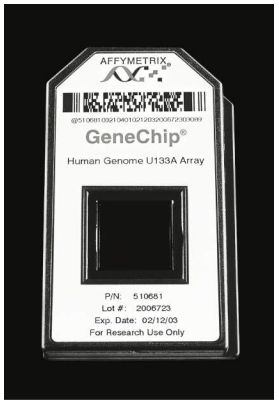
- 1988: SBH suggested as an an alternative sequencing method. Nobody believed it will ever work
- 1991: Light directed polymer synthesis developed by Steve Fodor and colleagues.
- 1994: Affymetrix develops first 64-kb DNA microarray

First microarray prototype (1989)

First commercial DNA microarray prototype w/16,000 features (1994)



500,000 features per chip (2002)



How SBH Works

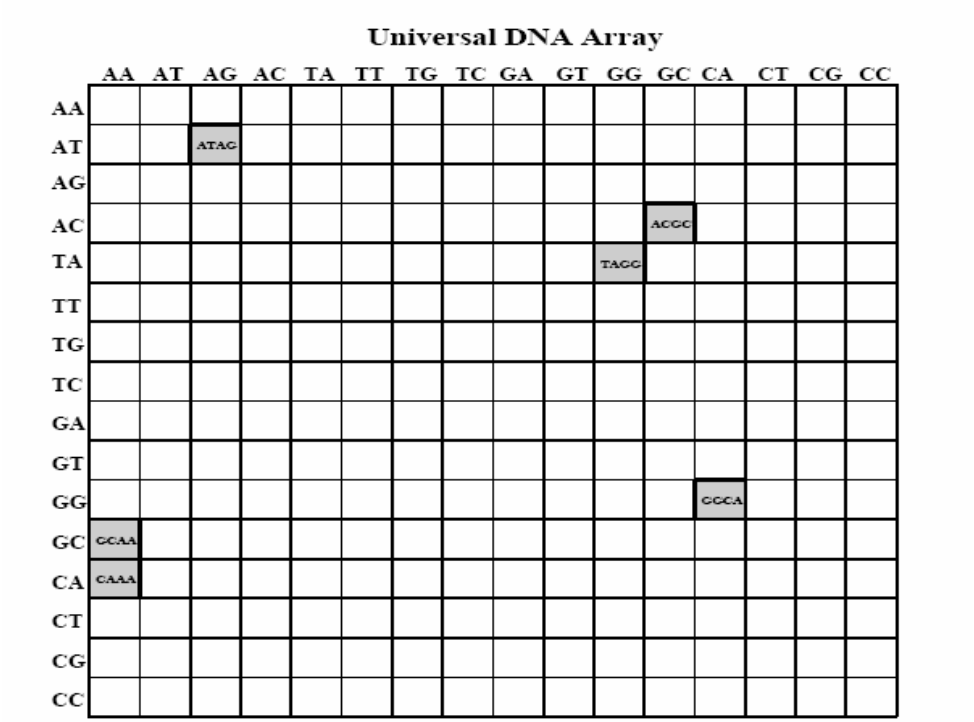
- Attach all possible DNA probes of length *l* to a flat surface, each probe at a distinct and known location. This set of probes is called the DNA array.
- Apply a solution containing fluorescently labeled DNA fragment to the array.
- The DNA fragment hybridizes with those probes that are complementary to substrings of length *l* of the fragment.

How SBH Works (cont'd)

- Using a spectroscopic detector, determine which probes hybridize to the DNA fragment to obtain the *l*–mer composition of the target DNA fragment.

- Apply the combinatorial algorithm (below) to reconstruct the sequence of the target DNA fragment from the *l* – mer composition.

Hybridization on DNA Array



DNA target TATCCGTTT (complement of ATAGGCAAA)

hybridizes to the array of all 4-mers:

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

l-mer composition

- ***Spectrum (s, l)*** - *unordered* multiset of all possible *(n – l + 1)* *l*-mers in a string *s* of length *n*
- The order of individual elements in *Spectrum (s, l)* does not matter
- For *s* = TATGGTGC all of the following are equivalent representations of *Spectrum (s, 3):*

  {TAT, ATG, TGG, GGT, GTG, TGC}

  {ATG, GGT, GTG, TAT, TGC, TGG}

  {TGG, TGC, TAT, GTG, GGT, ATG}

Different sequences – the same spectrum

- Different sequences may have the same spectrum:

  Spectrum(GTATCT,2)=

  Spectrum(GTCTAT,2)=

  {AT, CT, GT, TA, TC}

The SBH Problem

- Goal: Reconstruct a string from its *l*-mer composition
- Input: A set *S*, representing all *l*-mers from an (unknown) string *s*
- Output: String *s* such that *Spectrum (s,l) = S*

Some Applications of Sequencing

- 1000 Human Genomes Project

  An international effort to map variability in the genome

  The 1000 Genomes Project Consortium, *Nature (Oct 2010) 467: 1061–1073*

- Prostate Cancer Genomics

  M.F. Berger et al., *Nature (Feb 2011) 470: 214-220*

- Genome 10K Project

  - A continuation of Human (2001), Mouse (2002), Rat (2004), Chicken (2004), Dog (2005), Chimpanzee (2005), Macaque (2007), Cat (2007), Horse (2007), Elephant (2009), Turkey (2011), etc. genomes.
  - An international effort to sequence, *de novo* assemble, and annotate 10,000 vertebrate genomes; 300+ species to be started in 2011.

  Genome 10K Community of Scientists, *J Heredity (Sep 2009) 100 (6): 659-674*

De Novo Genome Assembly

Problem: given a collection of reads, i.e. short subsequences of the genomic sequence in the alphabet "A, C, G, T", completely reconstruct the genome from which the reads are derived.

Challenges:

- Repeats in the genome

…ACCCAGTT*GACTGGGAT*CCTTTTTAAA*GACTGGGAT*TTTAACGCG…

CAGTT*GACTG*

*ACTGGGAT*CC

*GACTGGGAT*T

} Sample reads

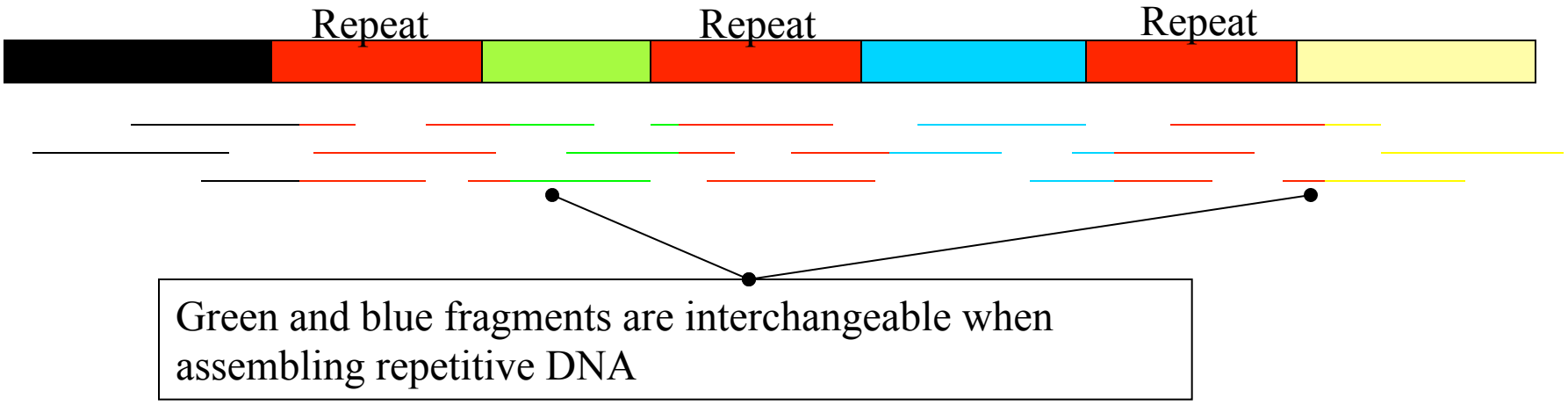
- Sequencing errors: substitutions, insertions, deletions, and others.

TTTTTATA*GA* (substitution), CCTT—TAAACG (deletion and insertion)

- Size of the data, e.g. 1.5 billion reads in 110GB FASTA file.

Challenges in Fragment Assembly

- Repeats: A **major** problem for fragment assembly
- > 50% of human genome are repeats:
  - over 1 million *Alu* repeats (about 300 bp)
  - about 200,000 LINE repeats (1000 bp and longer)



Repeat Types

- **Low-Complexity DNA** (e.g. ATATATATACATA…)
- **Microsatellite repeats** $(a_1 \ldots a_k)^N$ where k ~ 3-6
  (e.g. CAGCAGTAGCAGCACCAG)
- **Transposons/retrotransposons**
  - **SINE** Short Interspersed Nuclear Elements
    (e.g., *Alu*: ~300 bp long, 10^6 copies)
  - **LINE** Long Interspersed Nuclear Elements
    ~500 - 5,000 bp long, 200,000 copies
  - **LTR retroposons** Long Terminal Repeats (~700 bp) at each end
- **Gene Families** genes duplicate & then diverge
- **Segmental duplications** ~very long, very similar copies

Triazzle: A Fun Example

The puzzle looks simple

BUT there are repeats!!!

The repeats make it very difficult.

Try it

De Novo Genome Assembly
Current solutions

- Overlap-layout-consensus (*Celera*, *Newbler*)
  - Suitable for low coverage, long reads
  - Highly parallelizable

- De Bruijn graph construction (*ALLPATHS-LG*, *ABySS*, *Velvet*, *SOAPdenovo*, *EULER-SR, SPAdes,* and *HyDA*)
  - Suitable for high coverage, short reads
  - Fast but memory-intensive
  - Sensitive to sequencing errors
  - Mathematically elegant repeat classification

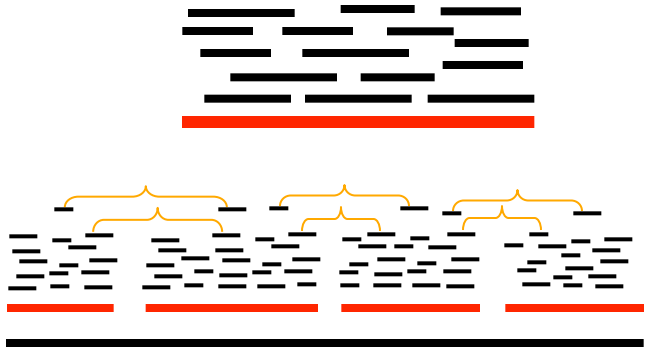
Overlap-Layout-Consensus Assembly

Overlap-Layout-Consensus

Assemblers: SGA, ARACHNE, PHRAP, CAP, TIGR, CELERA

Overlap: find potentially overlapping reads

Layout: merge reads into contigs and contigs into supercontigs



Consensus: derive the DNA sequence and correct read errors

..ACGATTACAATAGGTT..

Overlap

- Find the best match between the suffix of one read and the prefix of another
- Due to sequencing errors, need to use dynamic programming to find the optimal *overlap alignment*
- Apply a filtration method to filter out pairs of fragments that do not share a significantly long common substring

Overlapping Reads

- Sort all *k*-mers in reads ($k \sim 24$)
- Find pairs of reads sharing a *k*-mer
- Extend to full alignment – throw away if not >95% similar

Overlapping Reads and Repeats

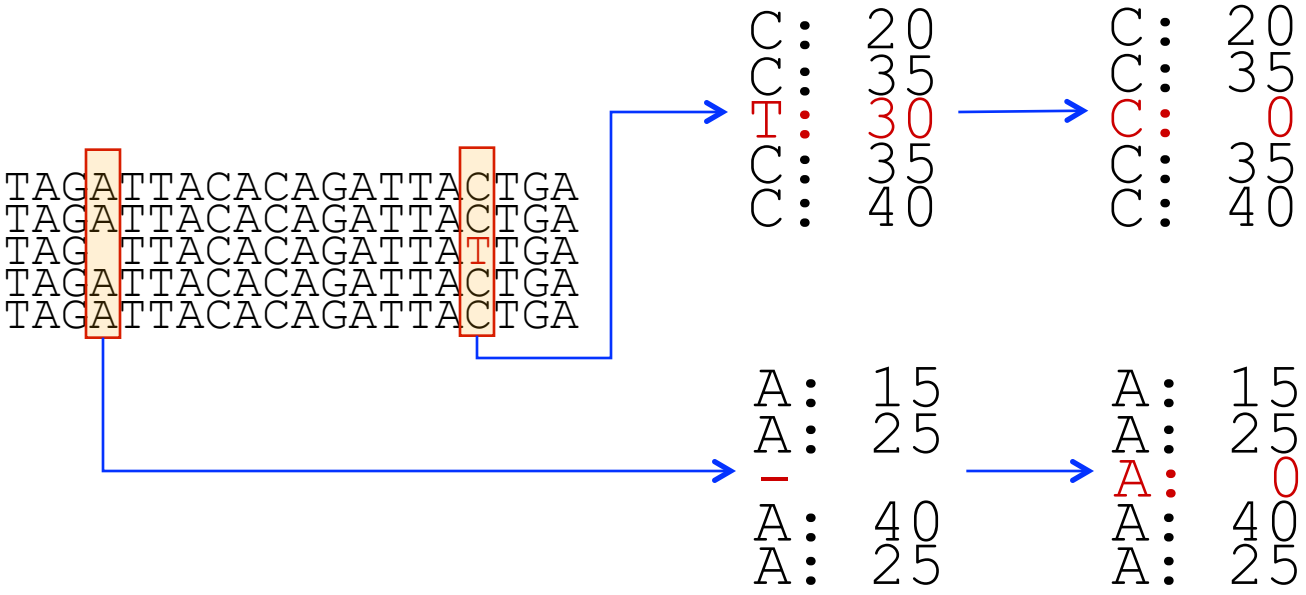
- A *k*-mer that appears N times, initiates N^2 comparisons
- For an *Alu* that appears 10^6 times → 10^{12} comparisons – too much
- **Solution:**
  Discard all *k*-mers that appear more than
  $t \times$ Coverage, ($t \sim 10$)

Finding Overlapping Reads

Create local multiple alignments from the overlapping reads

Finding Overlapping Reads (cont'd)

- Correct errors using multiple alignment

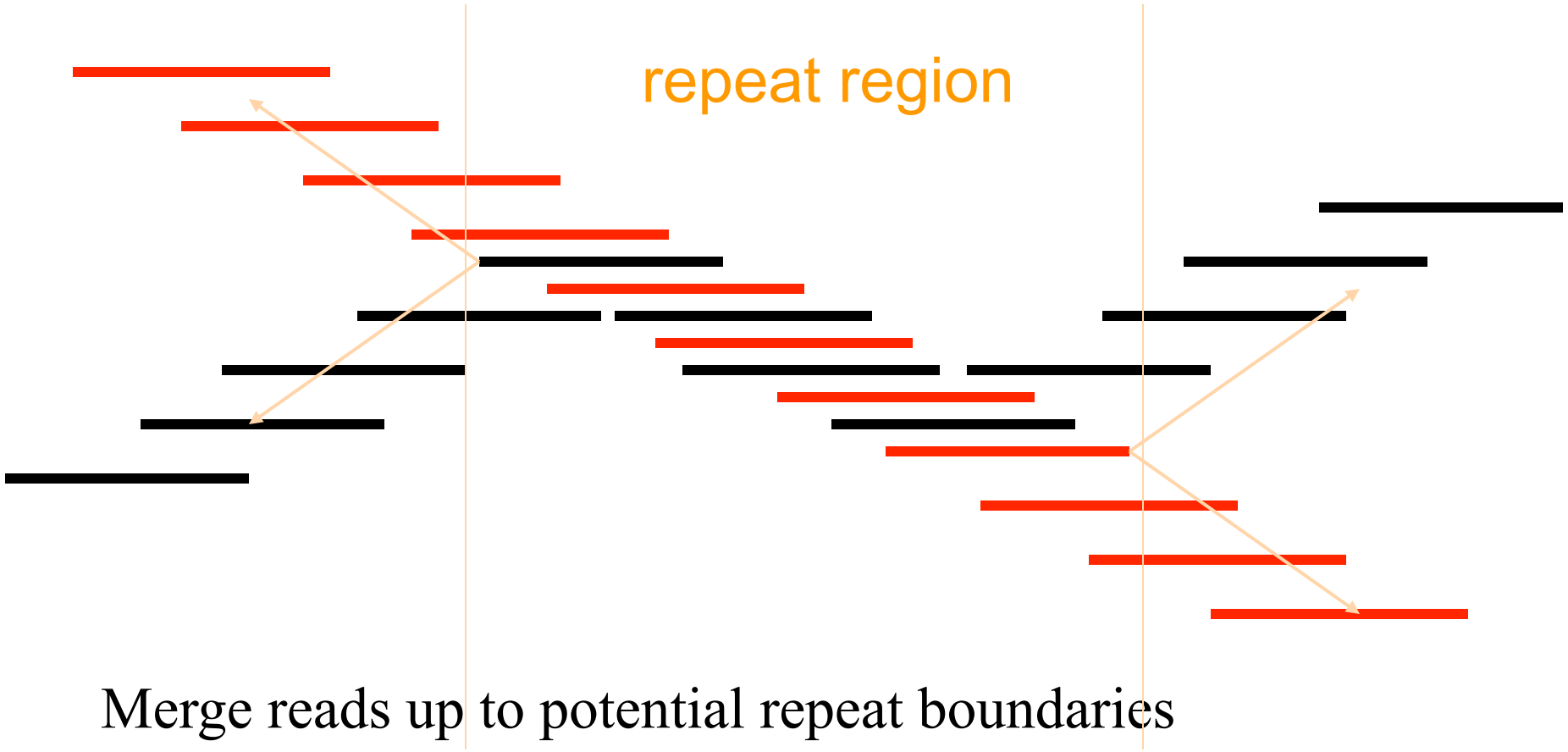


- Score alignments
- Accept alignments with good scores

Layout

- Repeats are a major challenge.
- Do two aligned fragments really overlap, or are they from two copies of a repeat?
- Solution: repeat masking – hide the repeats!!!
- Masking results in high rate of misassembly (up to 20%).
- Misassembly means alot more work at the finishing step.

Merge Reads into Contigs



Merge reads up to potential repeat boundaries

Repeats, Errors, and Contig Lengths

- Repeats shorter than read length are OK.
- Repeats with more base pair differences than sequencing error rate are OK.
- To make a smaller portion of the genome **appear** repetitive, try to:
  - Increase read length.
  - Decrease sequencing error rate.

De Bruijn Graph Based Assembly

De Bruijn Graph Example

Shred reads into k-mers (k = 3)

Read 1

G G A C T A A A

G G A

G A C

A C T

C T A

T A A

A A A

Read 2

G A C C A A A T

G A C

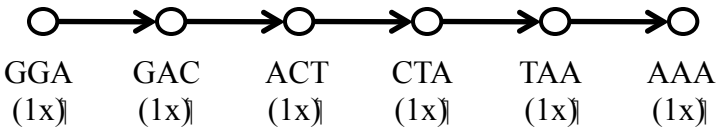
A C C

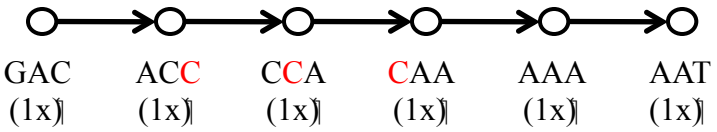
C C A

C A A

A A A

A A T





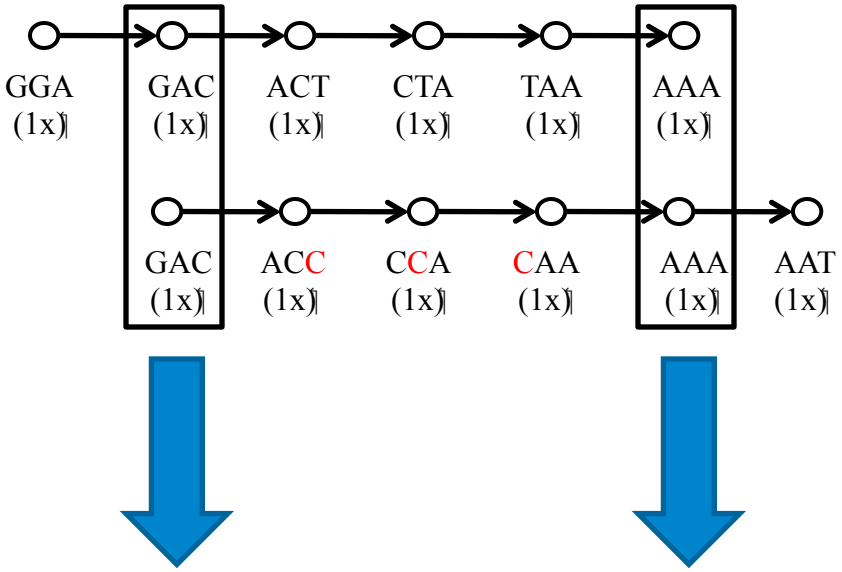
P. Pevzner, *J Biomol Struct Dyn (1989) 7:63–73*
R. Idury, M. Waterman, *J Comput Biol (1995) 2:291–306*

De Bruijn Graph Example

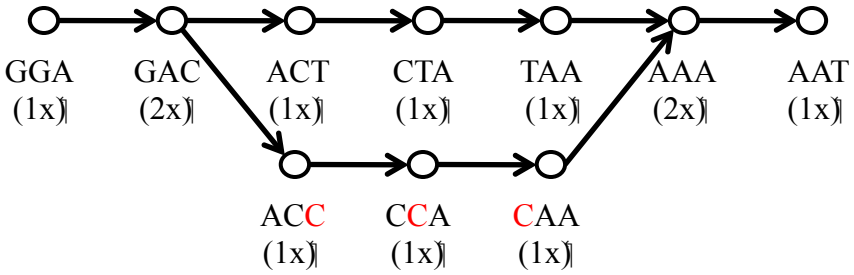
Merge vertices labeled by identical k-mers

Read 1:

Read 2:

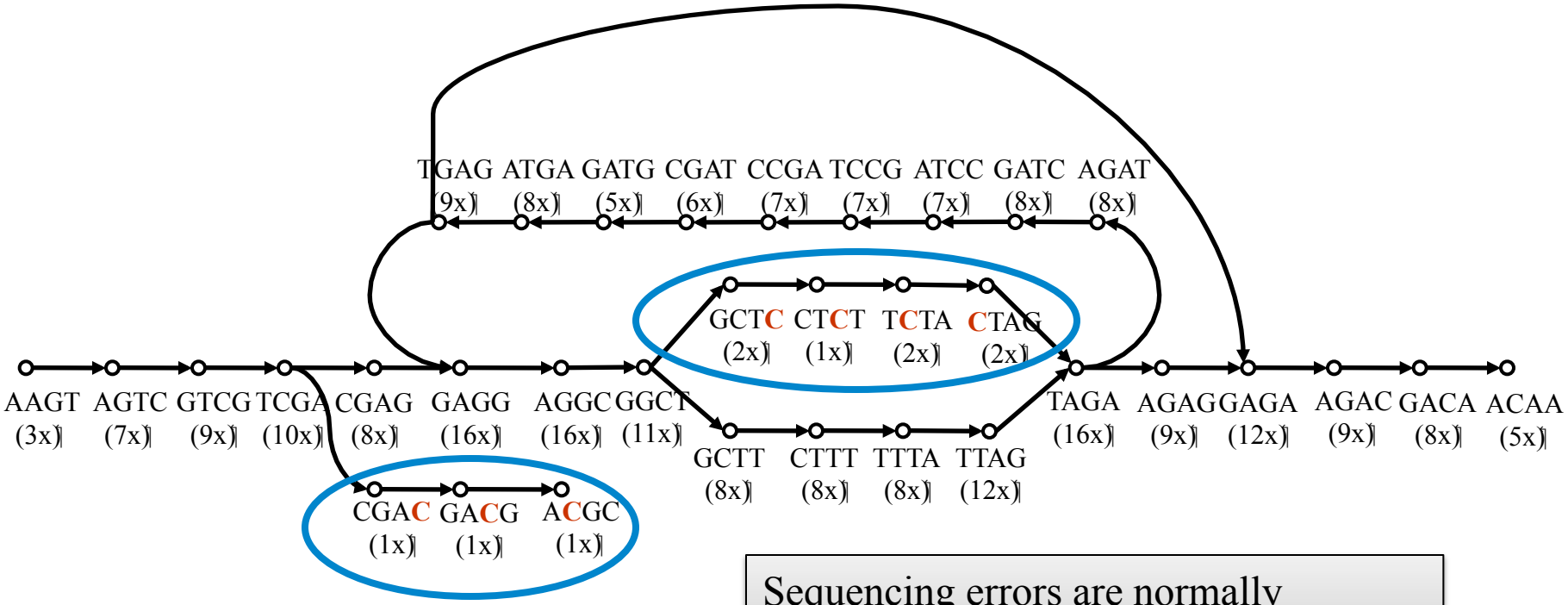


Resulting Graph:



Another Example

Constructing the graph (k = 4)

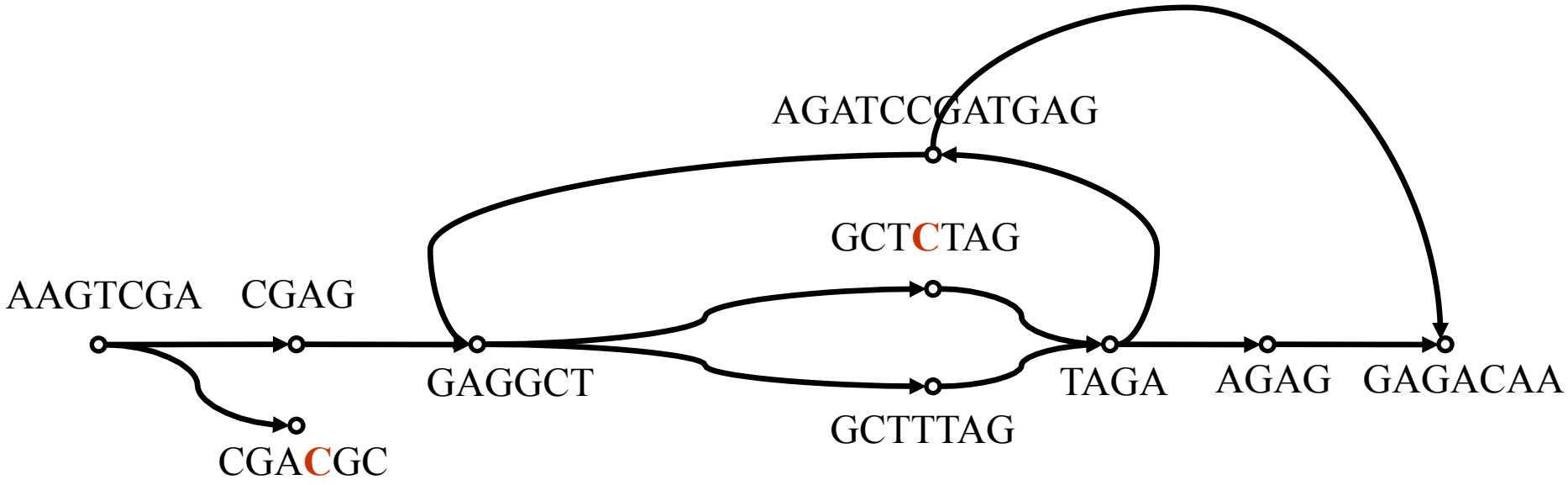


Sequencing errors are normally detected by a coverage cutoff threshold

A branching vertex is caused by either a repeat in the original sequence or a sequencing error

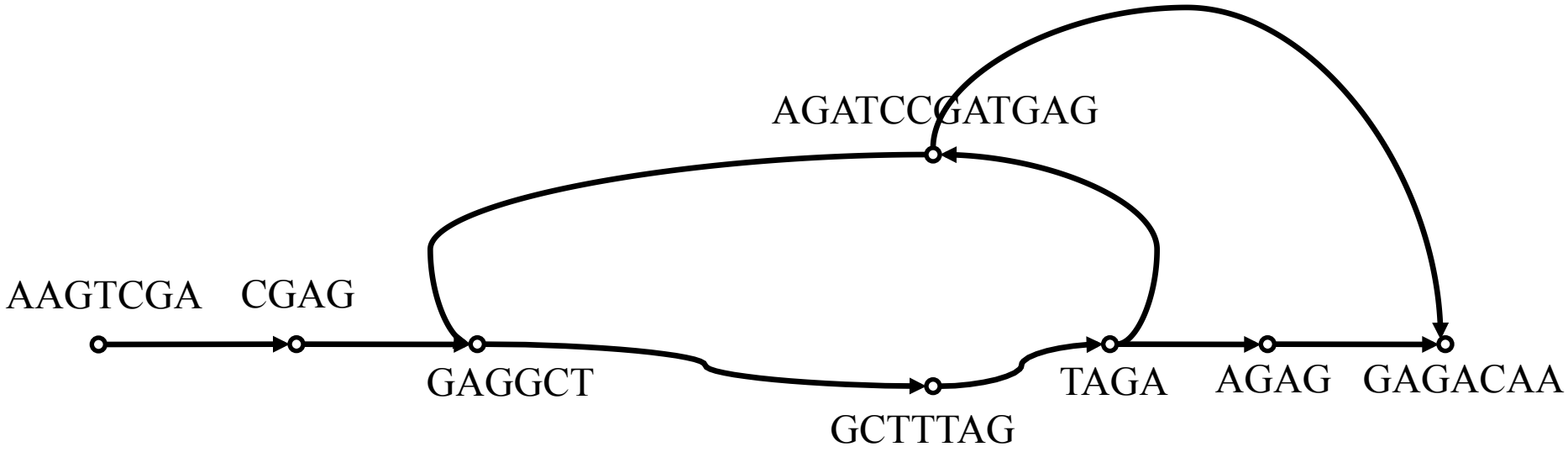
Example

After condensation

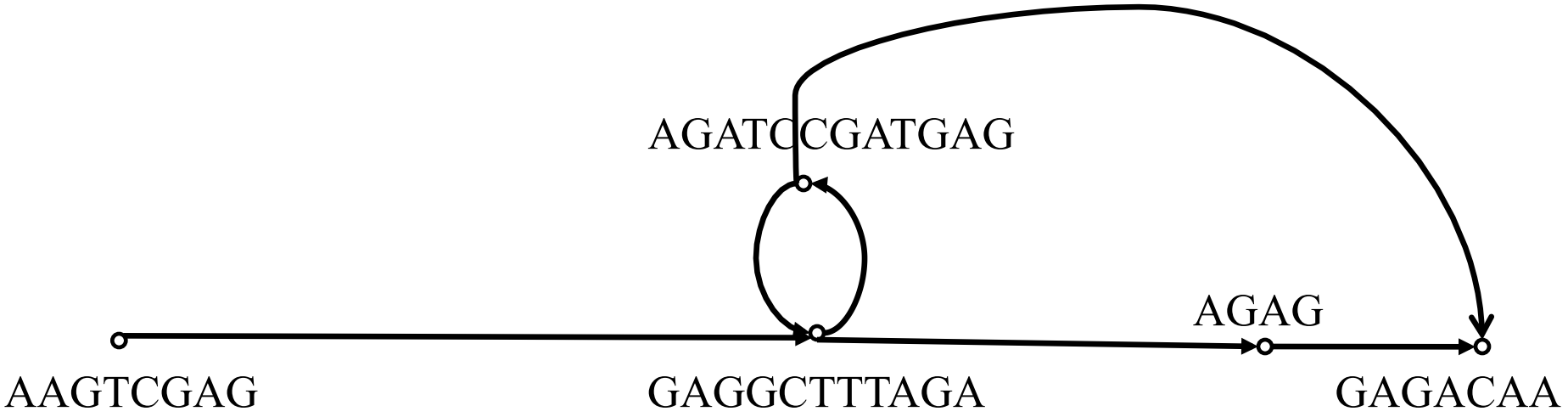


Example

After error removal



Example
After recondensation



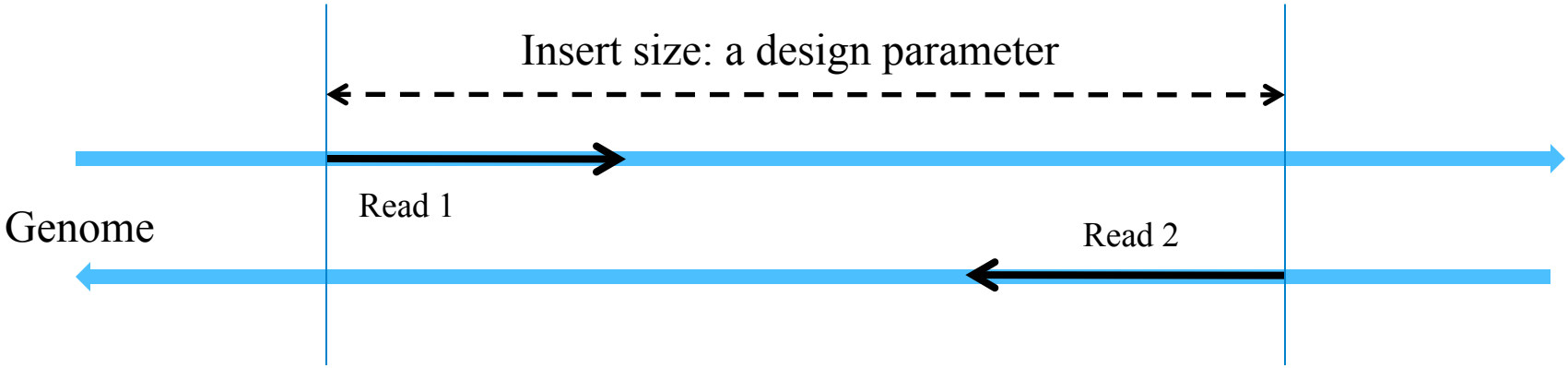
Any non-branching path in this graph corresponds to a contig in the original sequence.

Taking the risk of following arbitrary branching paths may create chimeric species

Source: Serafim Batzoglou

Resolving Repeats

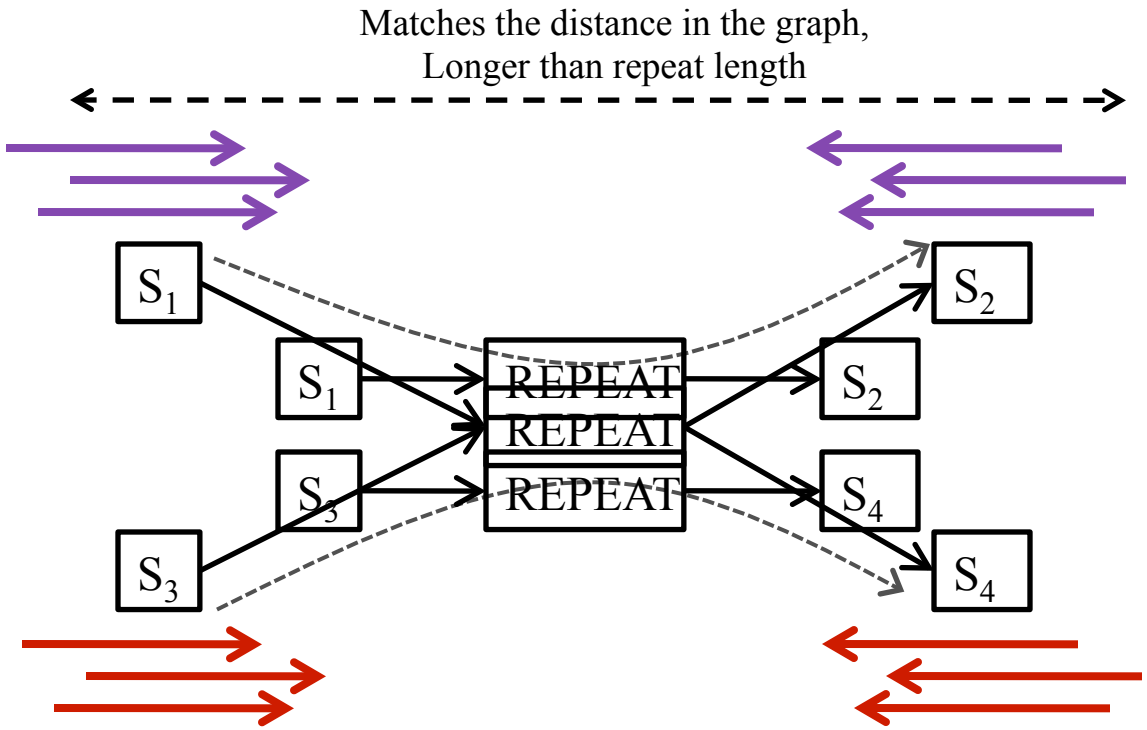
Using paired reads



Resolving Repeats

Equivalent transformations

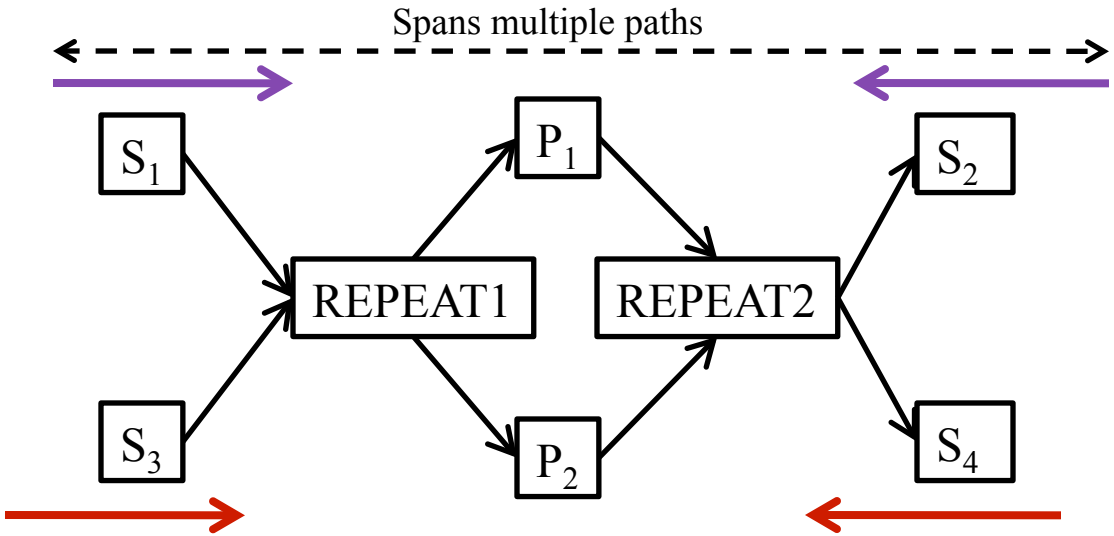
Genome: … S_1 REPEAT S_2 ……. S_3 REPEAT S_4 …



P. Pevzner and H. Tang, *Bioinformatics (2001) Suppl1:S225-33*

Resolving Repeats

Failure



Mate pair transformation (*Velvet, ABySS, EULER-SR*)

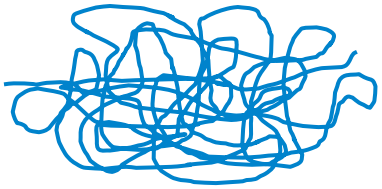
- Find a unique path between mates in the graph.
- **When multiple paths match the distance between mate-pairs, mate pair transformation fails.**

To resolve a repeat, insert size must be larger than the repeat length and smaller than the length of potential conjugate paths (same length paths passing through the repeat).

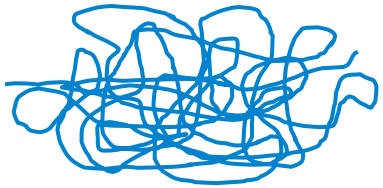
Single Cell Sequencing

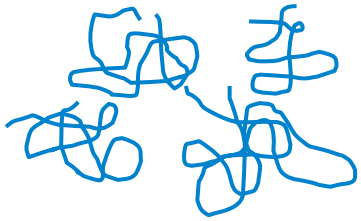
Whole genome amplification

Start with a single copy of genome.



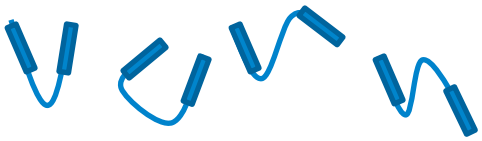
Amplify (copy) the genome using multiple displacement amplification (MDA) technique invented by Roger Lasken at J. Craig Venter Institute.





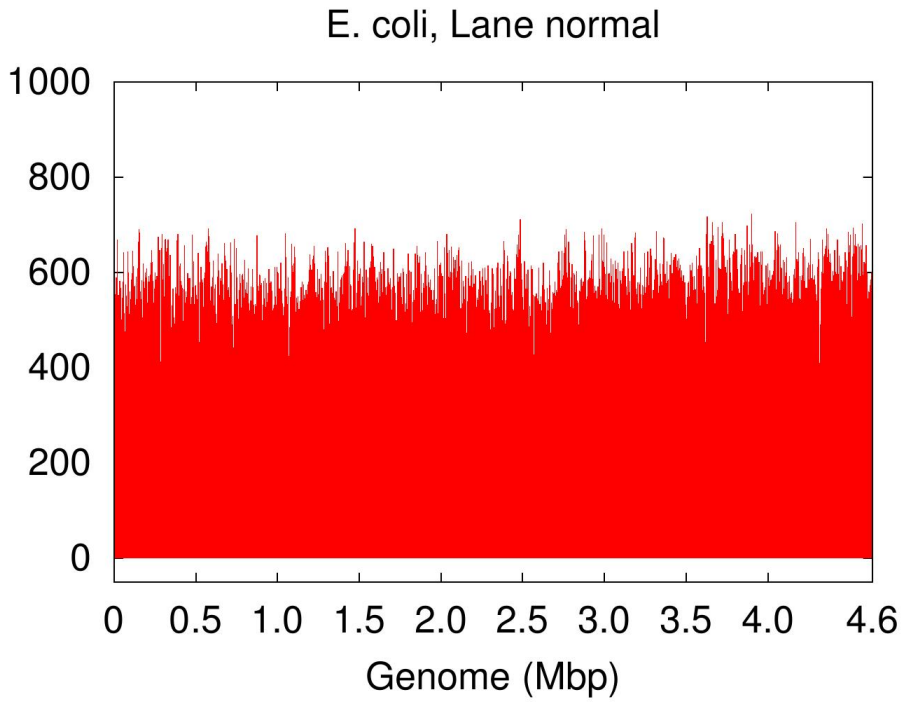
F.B. Dean ,et al., *PNAS (2002) 99(8): 5261-6*

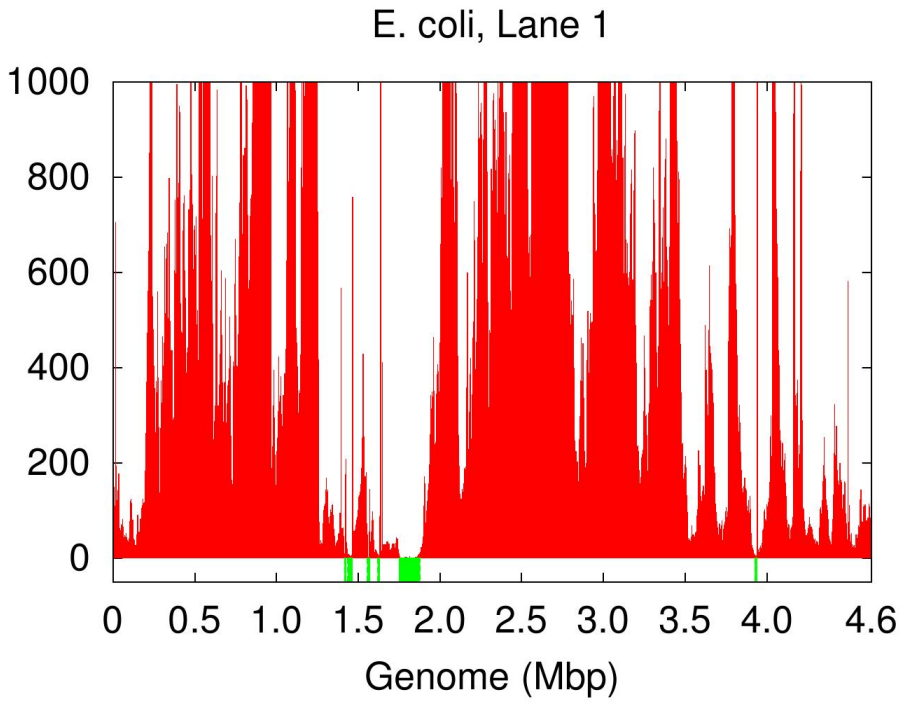
Fragment them and sequence reads at both ends.



Sequencing Coverage

Normal multicell vs. single cell



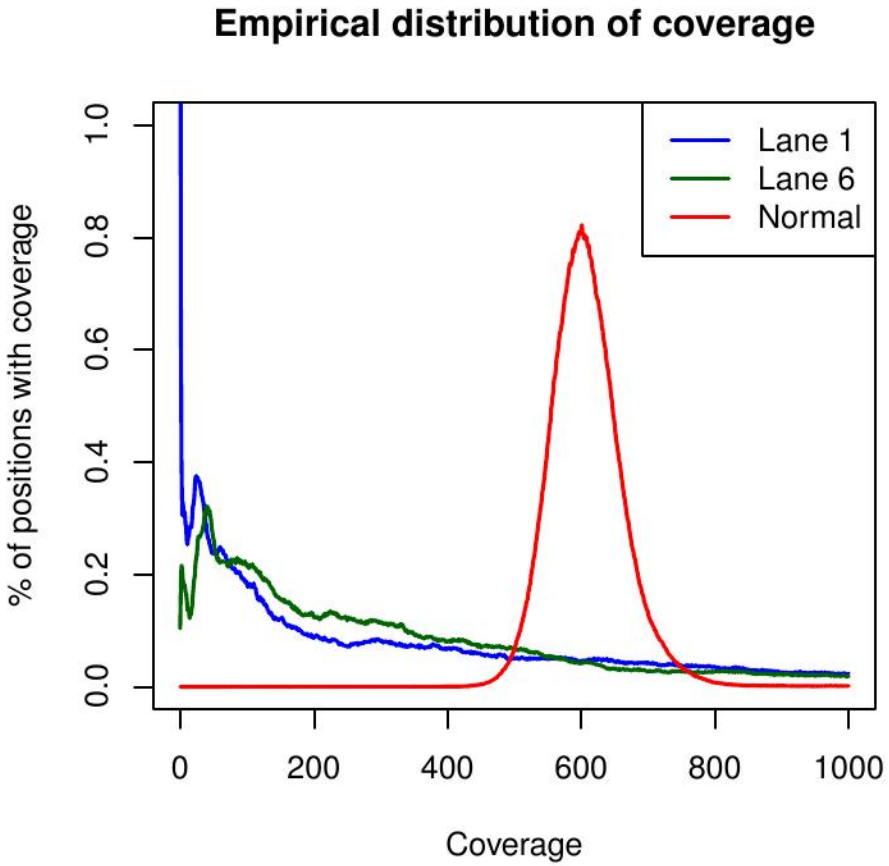


Green regions are blackout

Number of reads: ~28 million, read length: 100 bp, genome size: 4.6 Mbp, coverage: ~600x

H. Chitsaz, et al., *Nature Biotech (2011)*

Distribution of Coverage



A cutoff threshold will eliminate about 25% of valid data in the single cell case, whereas it eliminates noise in the normal multicell case.

H. Chitsaz, et al., *Nature Biotech (2011)*