Principles and problems of de novo genome assembly

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Material adapted from slides provided by Lex Nederbragt

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What is this thing called 'genome assembly'?

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What is a genome assembly?

A hierarchical data structure

that maps the sequence data

to a putative reconstruction of the target

Miller et al 2010, Genomics 95 (6): 315-327

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Hierarchical structure

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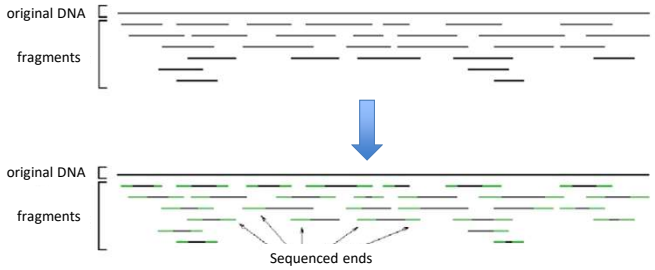
Sequence data

reads
contigs
scaffolds

Reads



http://www.cbcb.umd.edu/research/assembly_primer.shtml

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Reads!

reads
contigs
scaffolds

http://www.sciencephoto.com/media/210915/enlarge

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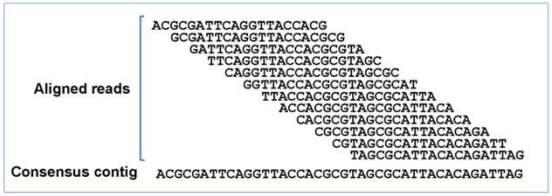
Contigs

reads
contigs
scaffolds

Building contigs



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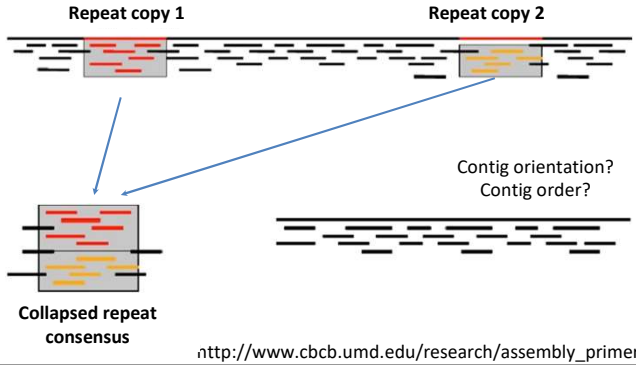
Contigs

reads
contigs
scaffolds

Building contigs



http://www.cbcb.umd.edu/research/assembly_primer.shtml

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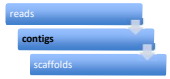
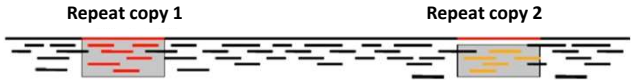
Contigs

Repeats: major problem





http://www.cbcb.umd.edu/research/assembly_primer.shtml

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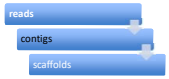
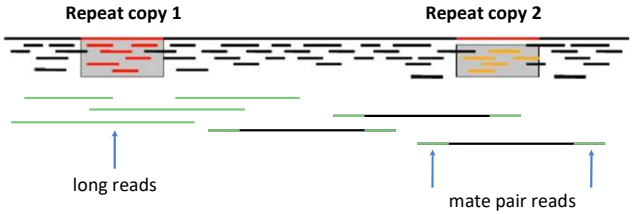
Long reads

Other read type





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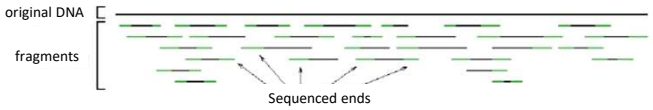
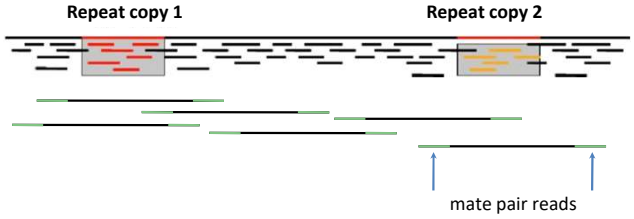
Mate pairs

Paired end reads → 100-500 bp insert



Mate pairs → 2-20 kb insert



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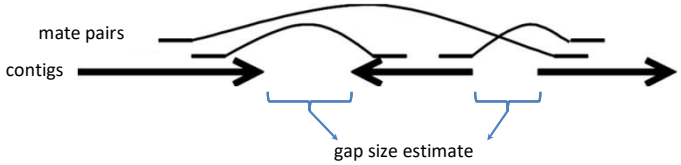
Scaffolds

Ordered, oriented contigs



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Hierarchical structure

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Assembly

How to do this?

Algorithms

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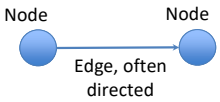
Algorithms

All are graph-based



Graph-theory!

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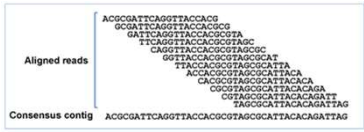
Algorithms

All are graph-based



Graph-theory!

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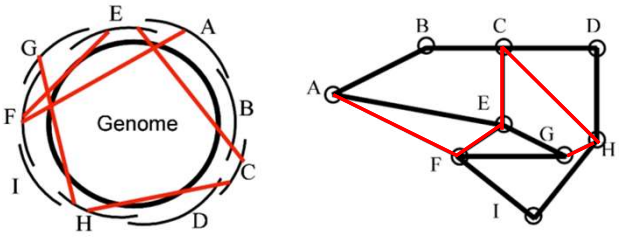
Algorithms

Hamiltonian path

– a path that contains all the nodes



https://www.cbcb.umd.edu/research/assembly_primer

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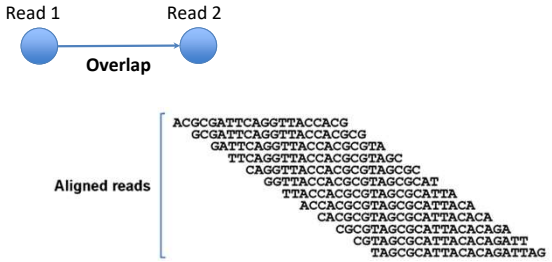
Algorithms

Overlap calculation (alignment)

– computationally intensive



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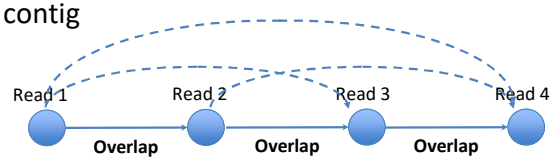
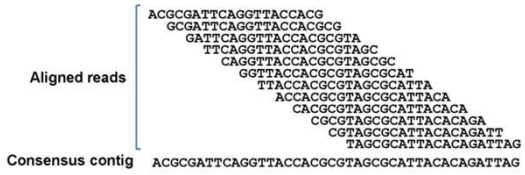
Algorithms

Path through the graph

→ contig





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Algorithms

Many flavors

Abandoned

- Greedy extension

Two most used

- Overlap Layout Consensus
- de Bruijn graph

http://www.waialuasodaworks.com/images/flavors2009.jpg

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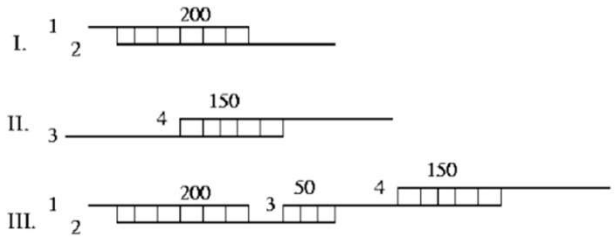
Greedy extension

Oldest



https://www.cbcb.umd.edu/research/assembly_primer

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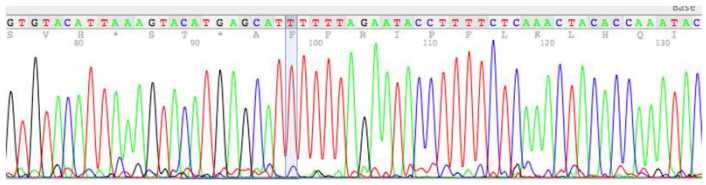
Overlap-Layout-Consensus

Developed for Sanger-type reads (longer reads)



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Overlap-Layout-Consensus

Steps

- Overlap computation
- Layout: graph simplification
- Consensus: sequence

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Overlap-Layout-Consensus

Overlap phase: find "similar enough" reads

Comparing all against all: expensive

Trick for finding "similar enough" reads:

- Split reads into k-mers

K-mer: substring of length *k* from a longer string

- Make list over which read has which k-mers
- If two reads share k-mers, test for similarity

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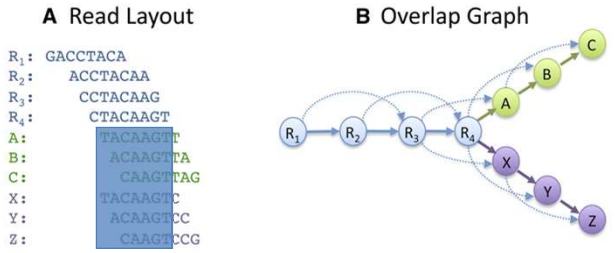
Overlap-Layout-Consensus



Schatz M C et al. Genome Res. 2010;20:1165-1173

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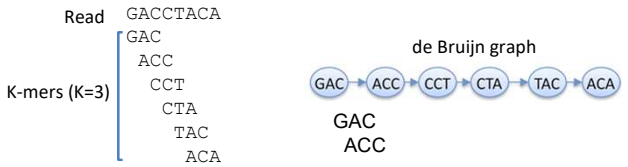
de Bruijn graphs

Developed outside of DNA-related work

– Best solution for short(er) reads



K-1 bases overlap

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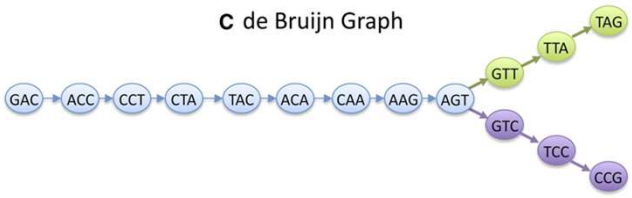
Graphs



Schatz M C et al. Genome Res. 2010;20:1165-1173

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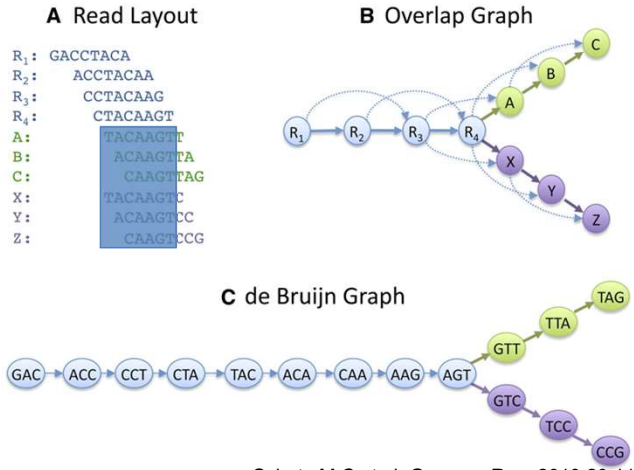
Graphs



Schatz M C et al. Genome Res. 2010;20:1165-1173

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Graphs

Simplify the graph

Add scaffolding information

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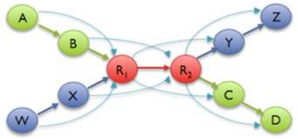
de Bruijn Graphs

Overlap Graph



Long read assemblers

- Repeats depends on read length
- Read coherency, placements kept
- Tangled by high coverage

de Bruijn Graph

Short read assemblers

- Repeats depends on word length
- Read coherency, placements lost
- Robust to high coverage

Mike Schatz

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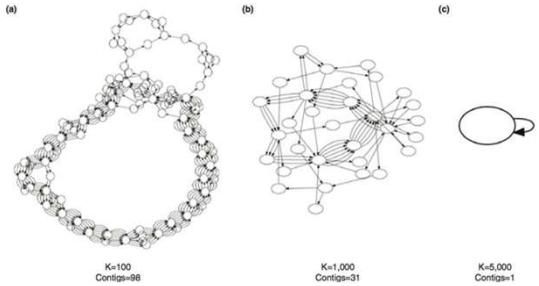
Read length matters

5.2 Mb circular genome, infinite error-free reads



Roberts et al (2013) doi:10.1186/gb-2013-14-6-405

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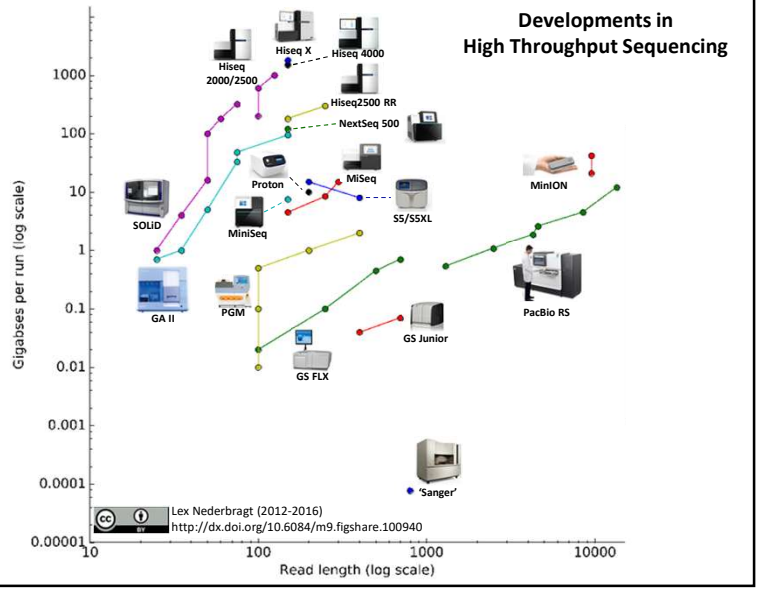

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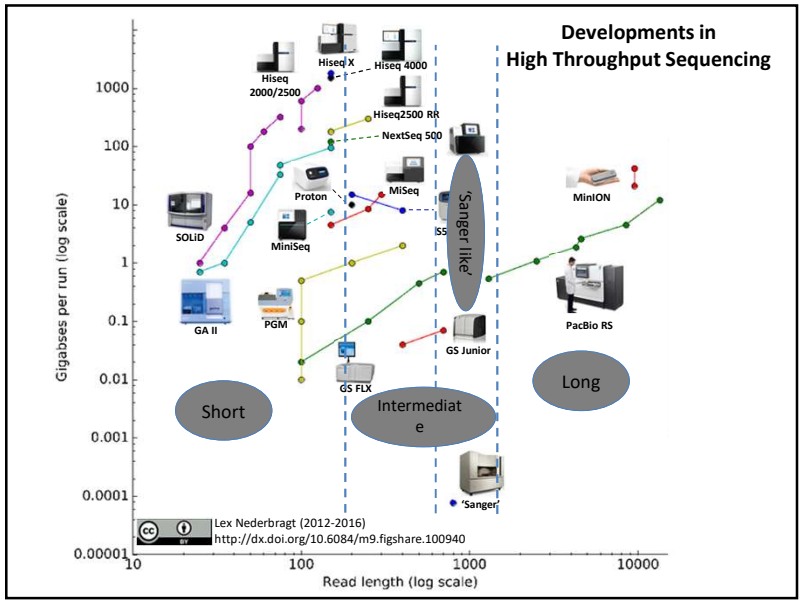

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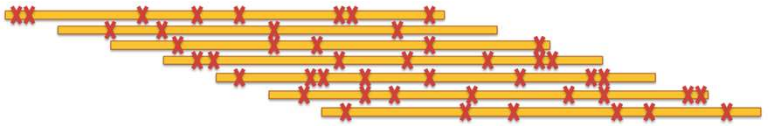
Quality matters

Too many errors → hard to find overlaps



http://schatzlab.cshl.edu/presentations/2012-01-17.PAG.SMRTassembly.pdf

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Why is genome assembly such a difficult problem?

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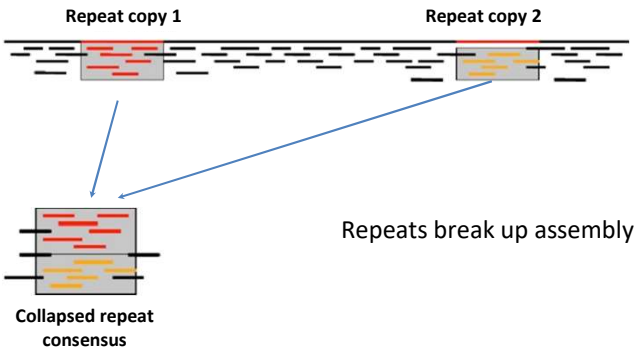
1) Repeats



Repeats break up assembly

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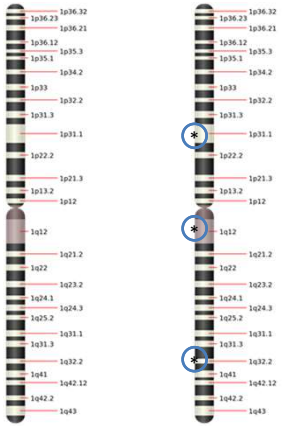
2) Diploidy



Differences between sister chromosomes

↓

'heterozygosity'

http://commons.wikimedia.org/wiki/File:Chromosome_1.svg

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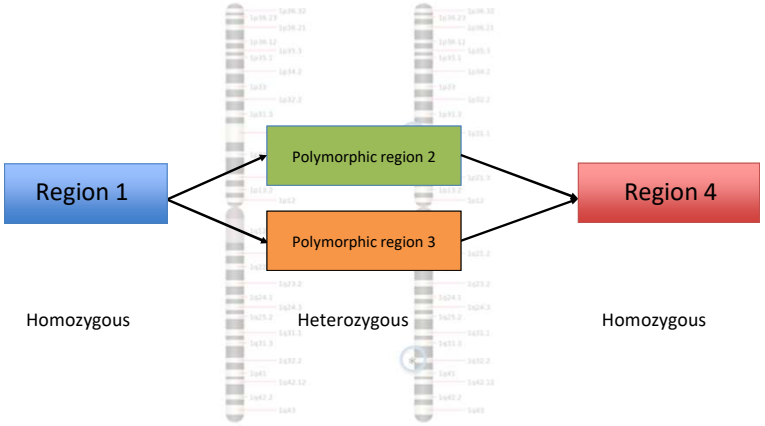
2) Diploidy



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2) Diploidy

http://www.astraean.com/borderwars/wp-content/uploads/2012/04/heterozygoats.jpg
and many other sites

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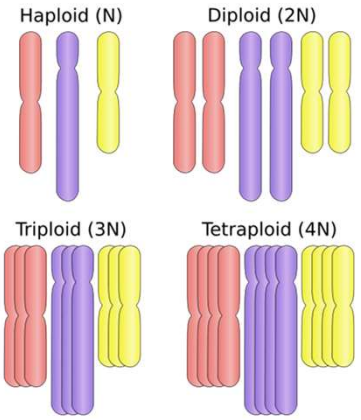
3) Polyploidy



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

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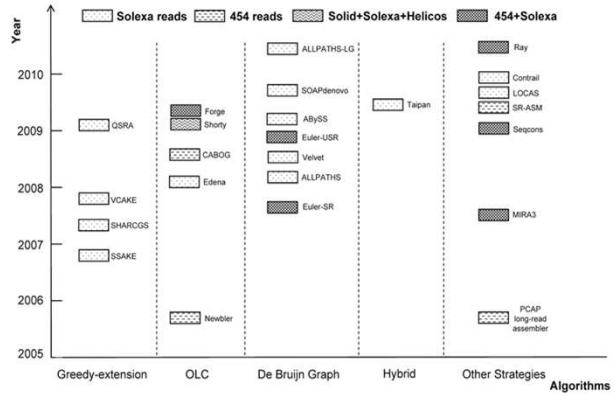
4) Many programs to choose from



Zhang et al. PLoSOne 2011

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Assembly with noisy single molecule sequencing data

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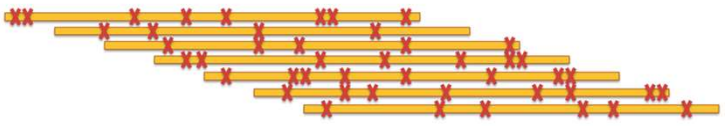
Usage of long reads



- Problem: higher error rates
- Overlaps more difficult/expensive to find
- OLC more commonly used than for 2nd generation data

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Long read assembly strategies

- Alt 0: Scaffolding short read asms
- Alt 1: Correct reads, then assemble
- Alt 2: Assemble reads, then correct

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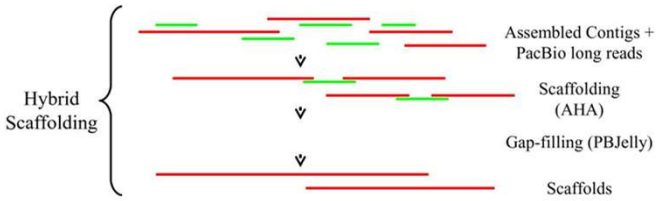
Scaffolding and gap closing (hybrid)



Powers *et.al.*, BMC genomics 2013

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Correct, assemble

- Do pairwise comparison, find shorter reads that support the longer
- Align supporting reads, correct longer reads
- Overlap-Layout-Consensus on corrected reads
- Polish assembly

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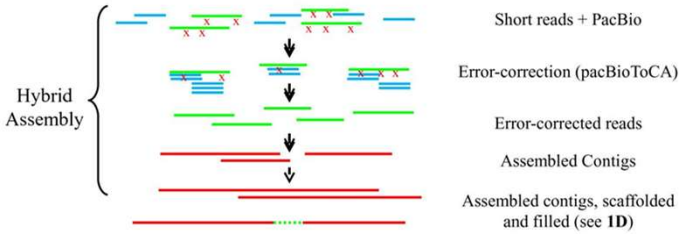
Mapping and error correcting (hybrid)



Powers *et.al.*, BMC genomics 2013

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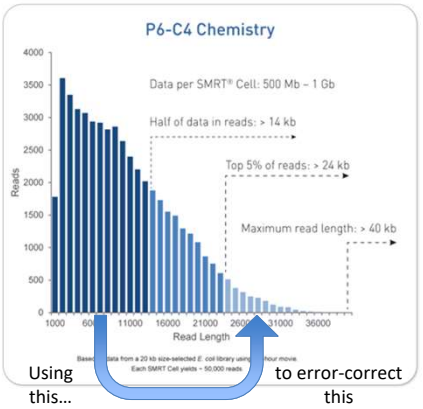
Hierarchical approach (self-correcting)



https://genome.duke.edu/cores-and-services/sequencing-and-genomic-technologies/pacbio

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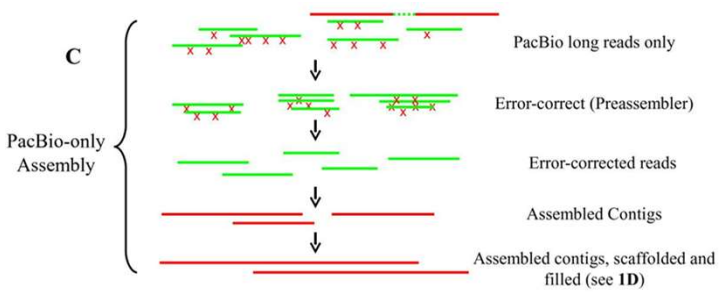
Short read error correction



Powers *et.al.*, BMC genomics 2013

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Assemble, correct

- Compare reads, find overlaps
- Assemble reads, knowing things will be wrong
- Align reads to assembly
- Correct assembly

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Questions?

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