VISUALIZING ASSEMBLY FOR NGS

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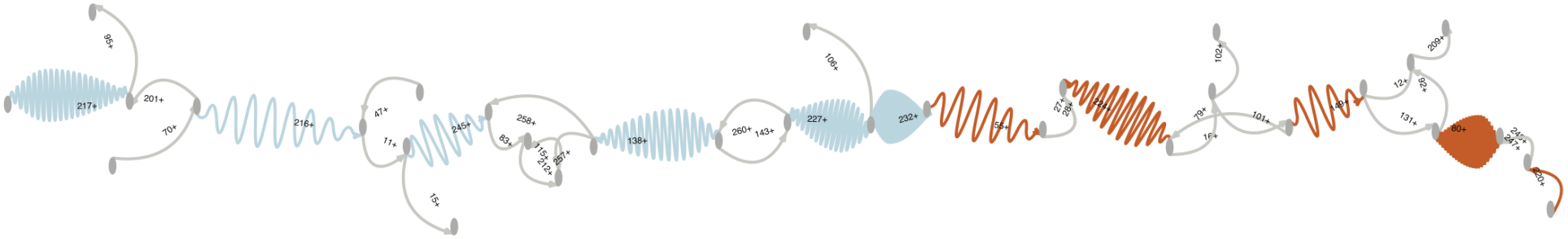


Genome Sequencing

Sample preparation

↓

Physical sequencing

↓

Assembly

Sequencing Technologies

First Generation	Second Generation	Third Generation
 ABI 3730	 Roche/454	 Pacific Biosciences SMRT
	 Life Technologies SOLiD	 Oxford Nanopore MinION
	 Illumina HiSeq	
	. . .	. . .

Sequencing Technologies

Second Generation

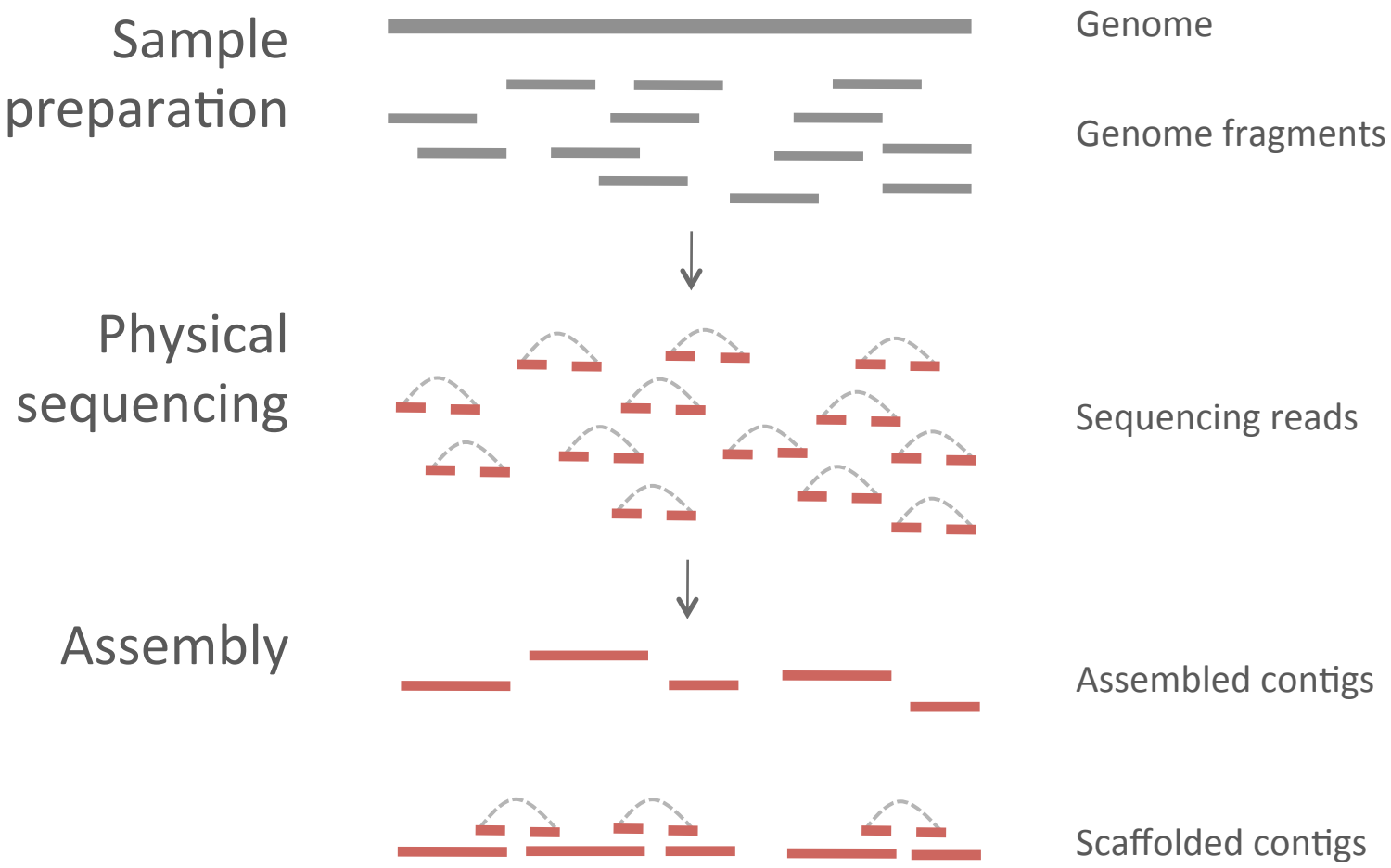
Roche/454

Life Technologies SOLiD

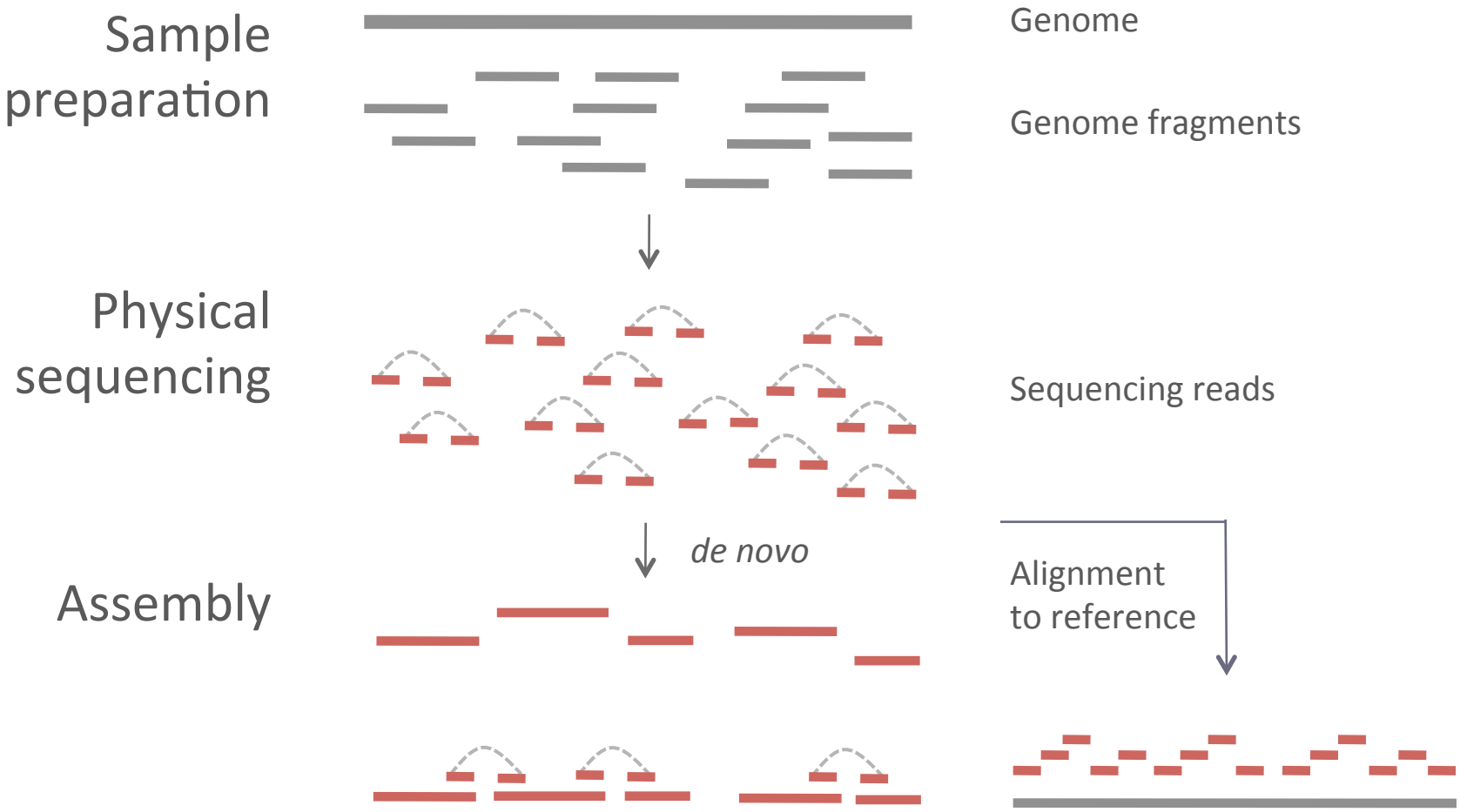
Illumina HiSeq

Produce sequencing reads that are tens to hundreds of bases long

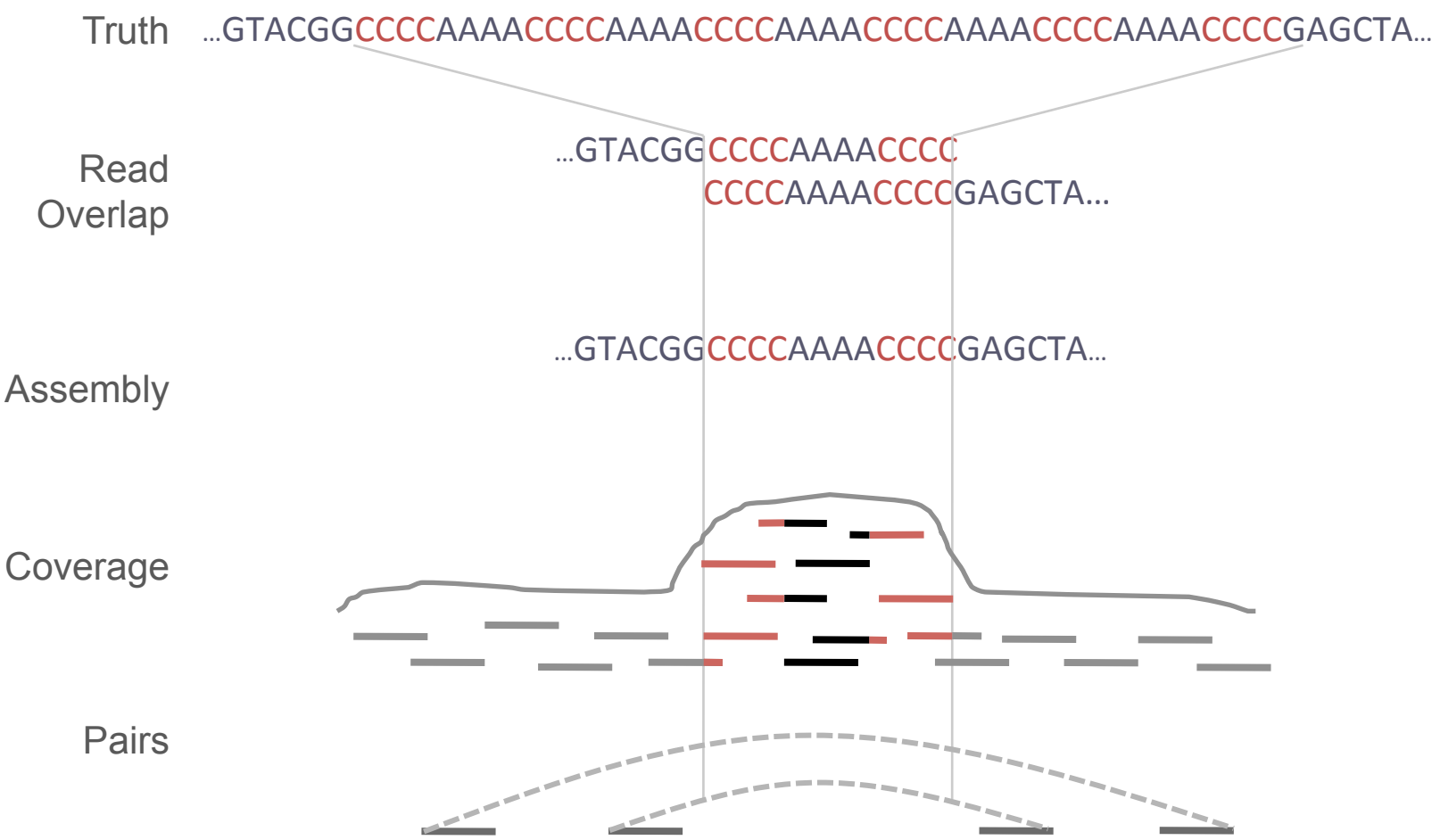
Genome Sequencing



Genome Sequencing



Genome Assembly Challenges



Adapted from Schatz *et al.*
Briefings in Bioinformatics, 2011

Assembly Visualization: Applications

- Finishing
  - involves closing gaps, correcting misassemblies and improving the error probabilities of consensus bases
  - often done manually; can be labour intensive and costly
- Algorithm development and iteration
  - often valuable to inspect potential assembly errors
- Investigation of structural variation
  - Detailed analysis of biologically relevant events in resequencing data

Task 1 | Examining Nucleotide-Level Discrepancies

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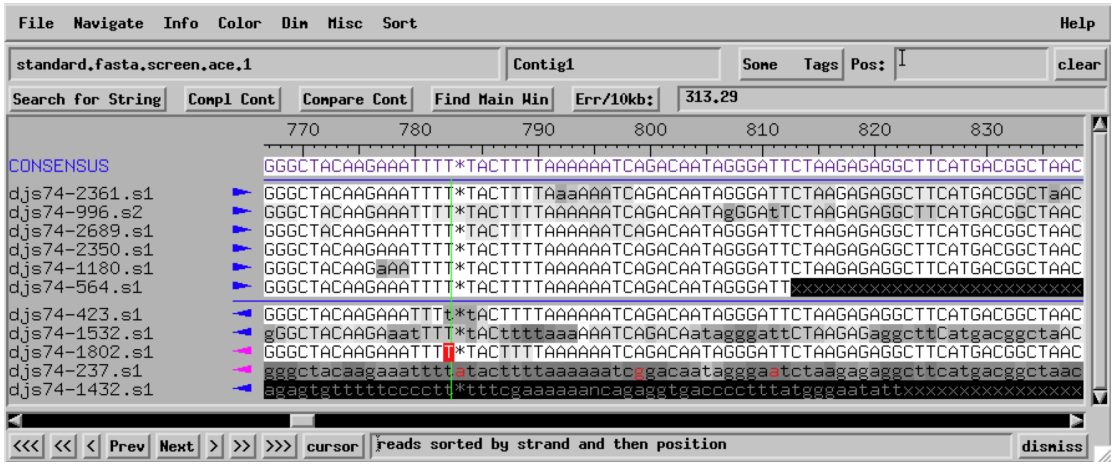
Aligned Reads Window



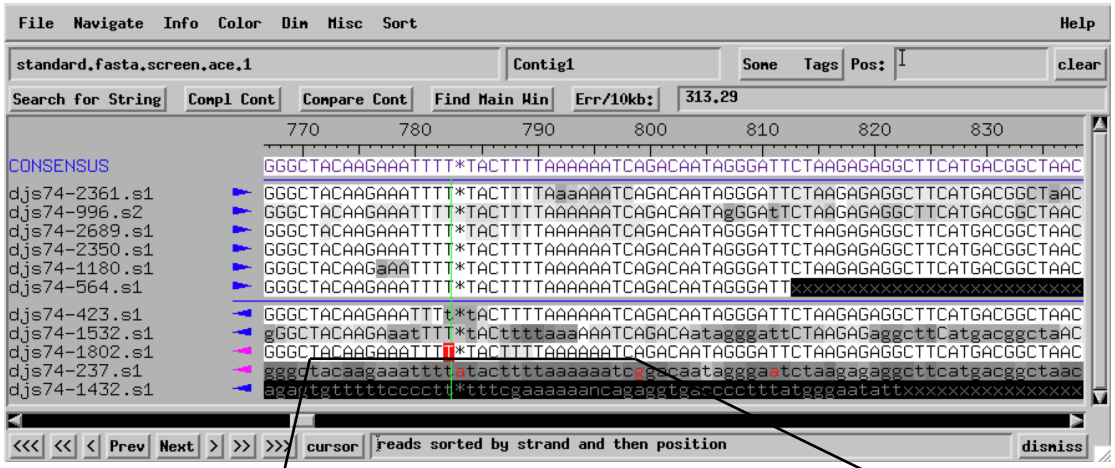
- Initially designed for Sanger sequencing (8-10x coverage of 500- to 1000-base reads)
- Introduction of quality values (log transformed error probabilities) was a significant contribution in providing an objective criterion to guide finishing

Consed

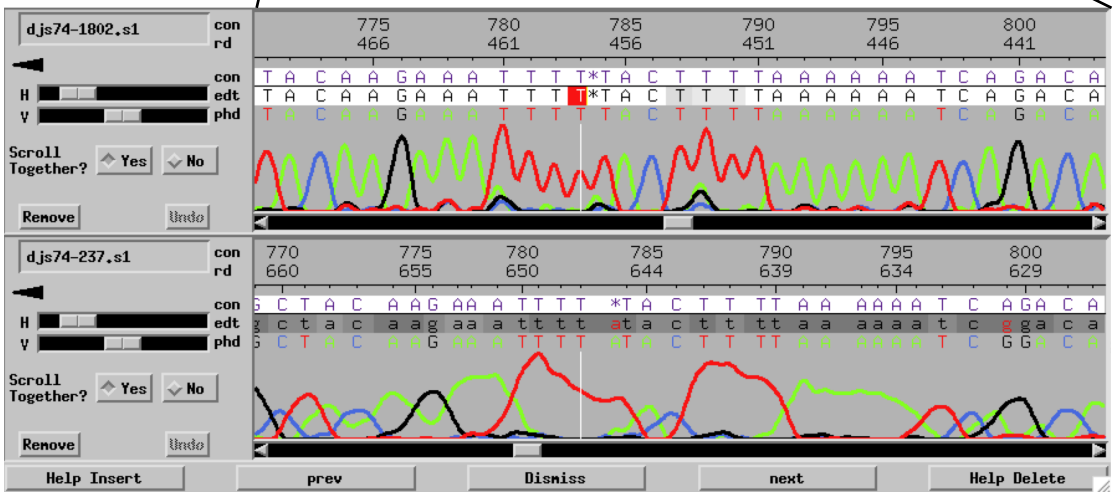
David Gordon and Phil Green

Task 1 | Examining Nucleotide-Level Discrepancies

Aligned Reads Window



Trace Window



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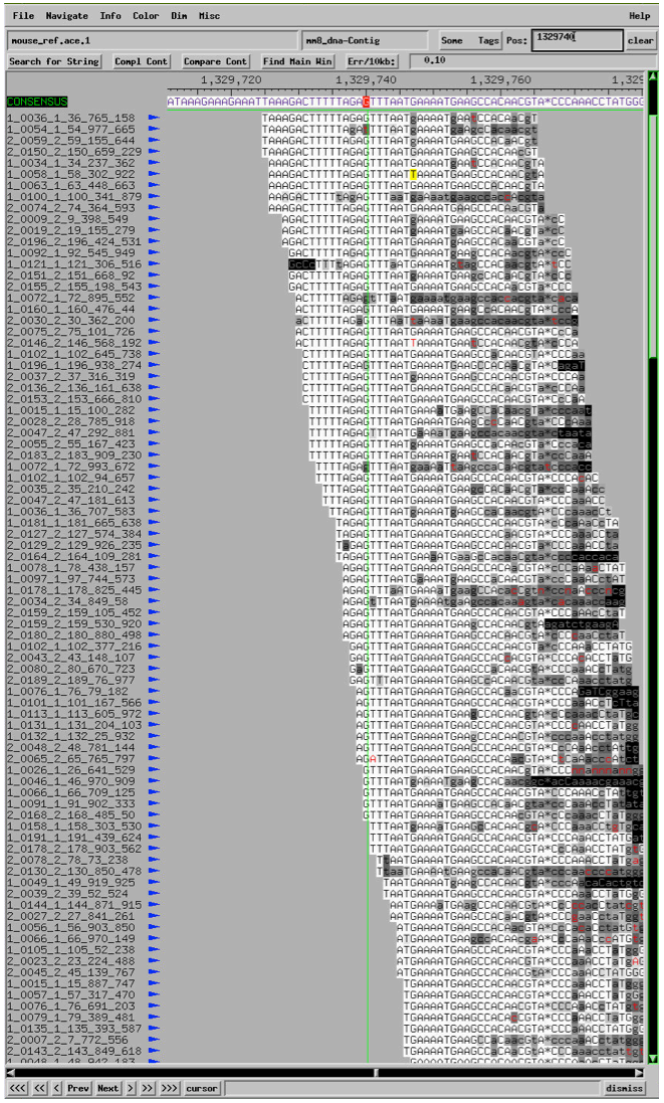
Task 1 | Examining Nucleotide-Level Discrepancies

Aligned Reads Window



30-100x coverage of 50- to 100-base reads from second generation technologies pose a challenge

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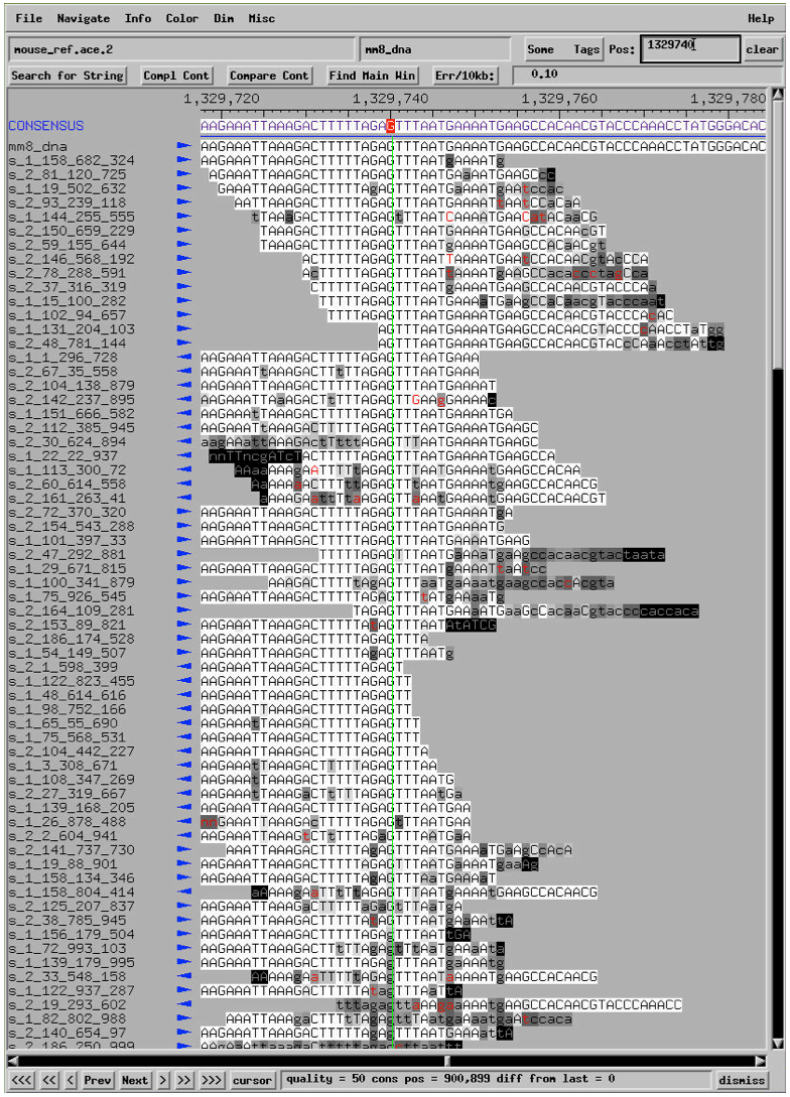
Task 1 | Examining Nucleotide-Level Discrepancies

Aligned Reads Window



Sorting by quality value is a useful guide

No longer need to inspect raw data underlying an individual read

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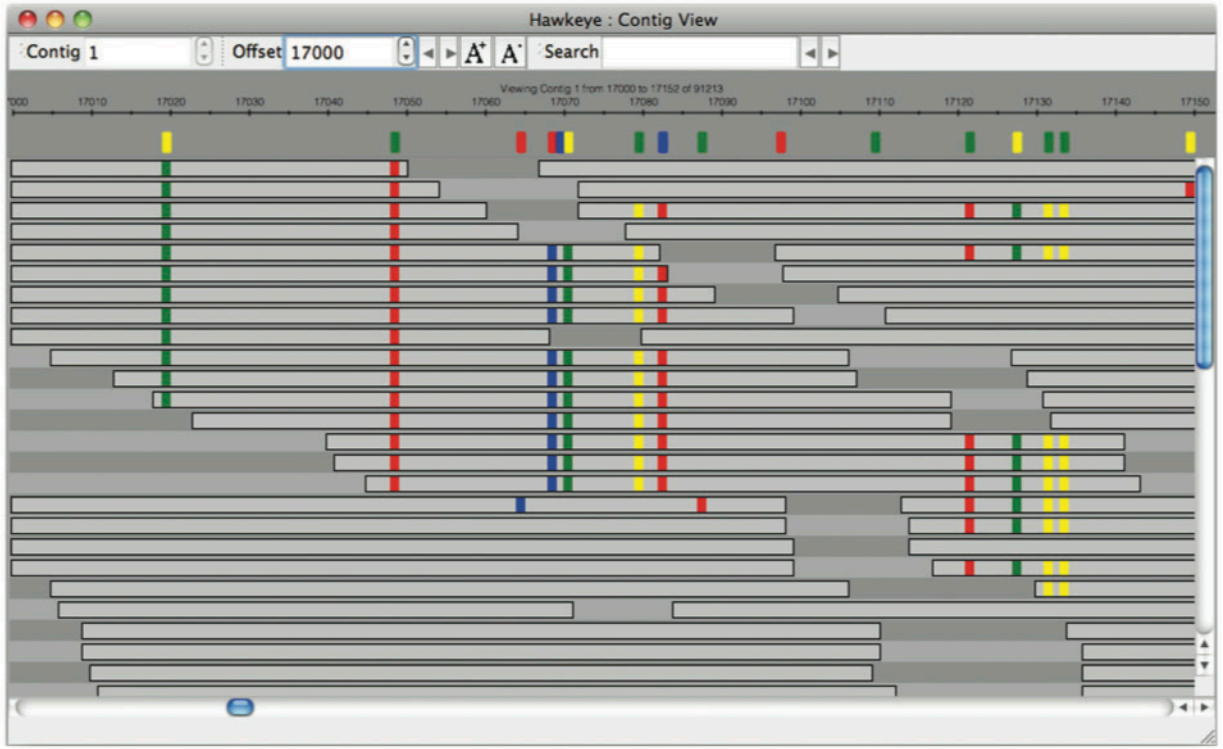
Task 1 | Examining Nucleotide-Level Discrepancies

Contig View

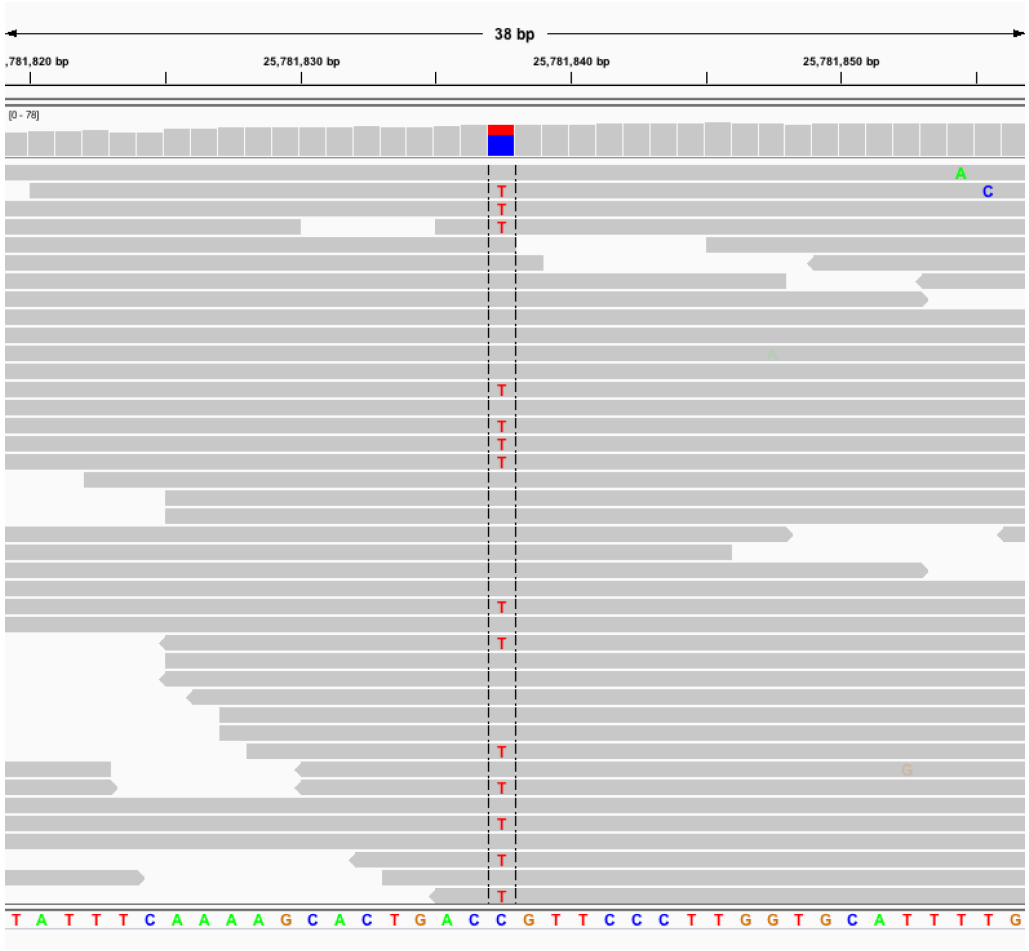


- High quality discrepancies can be indicative of misassemblies or nucleotide variants

Hawkeye

Schatz *et al.*, 2007; 2011

Task 1 | Examining Nucleotide-Level Discrepancies



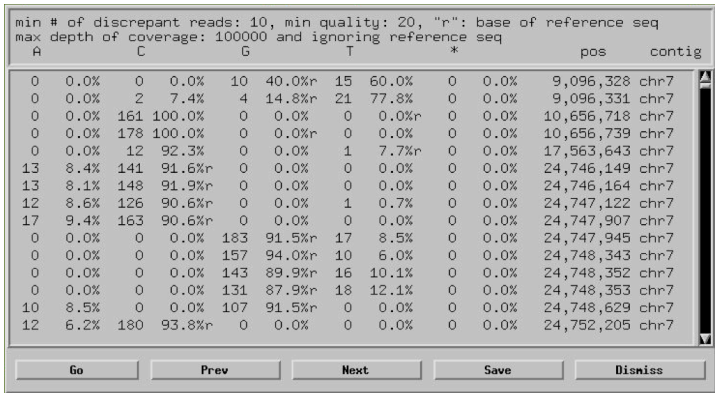
Integrative genomics viewer (IGV)

Robinson *et al.*, 2011

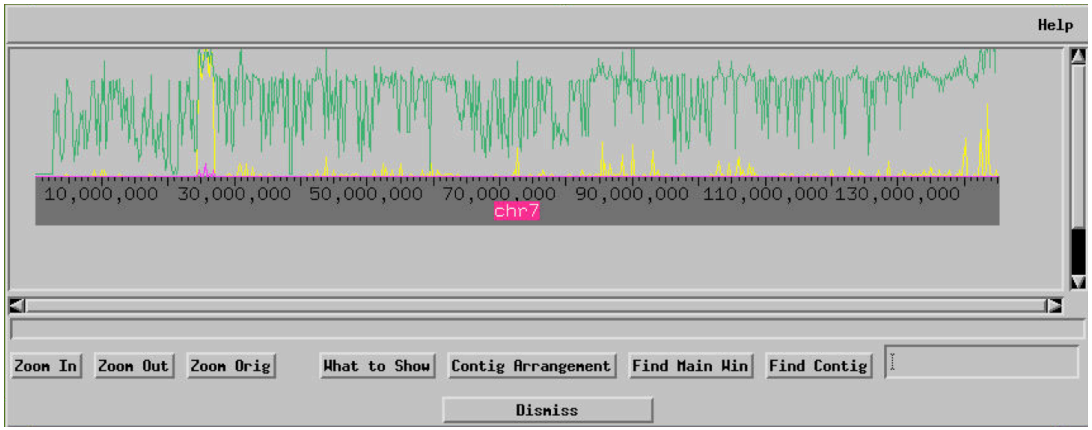
Task 1 | Examining Nucleotide-Level Discrepancies

Navigating to discrepant positions

Highly Discrepant Positions Table



Assembly View



Coverage (green) Indels (magenta) Non-indel discrepancies (yellow)

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Task 2 | Inspecting Deviant Read Pairs

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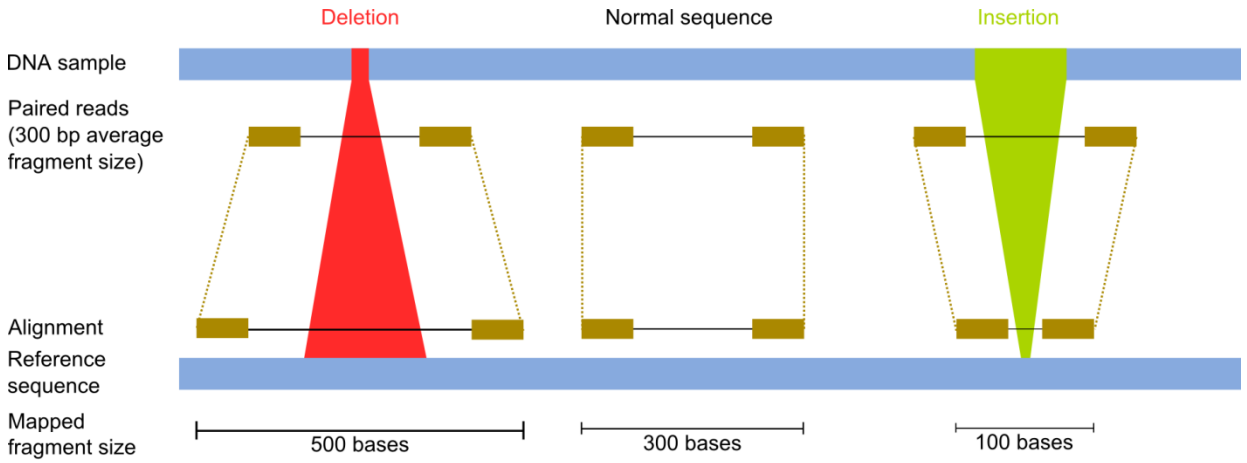


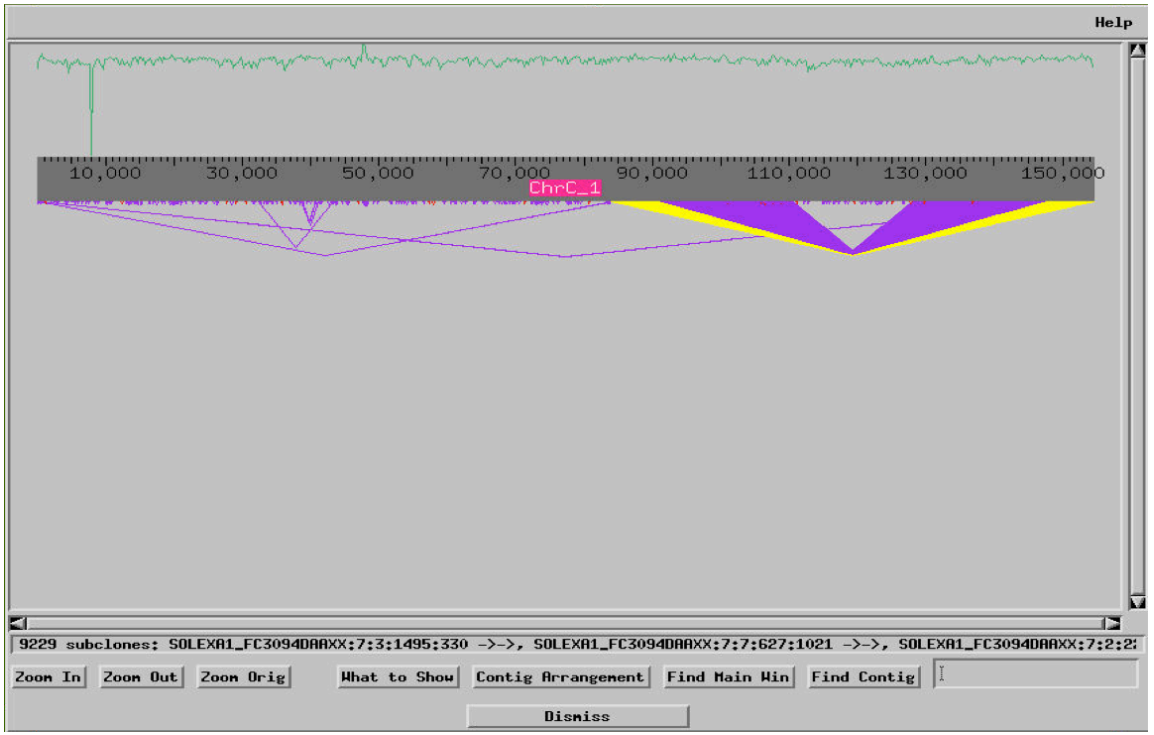
From Manske and Kwiatkowski,
Genome Research, 2009

Task 2 | Inspecting Deviant Read Pairs

Indicate deviations as overlaid arcs

Assembly View

Filtered inconsistent pairs shown



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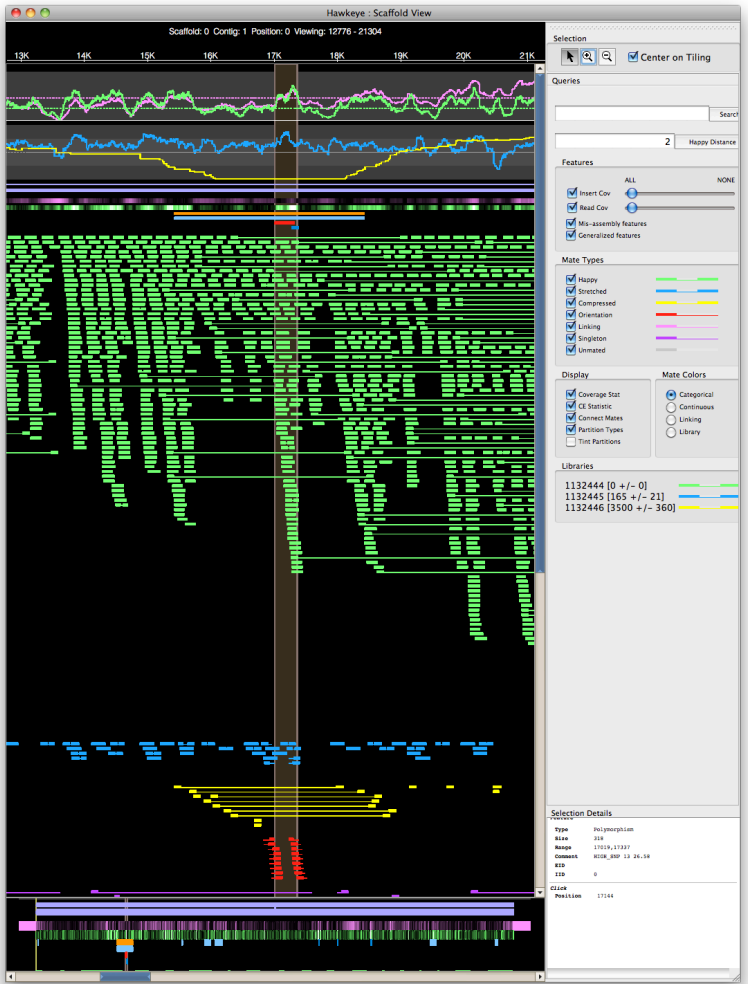
and Phil Green

Task 2 | Inspecting Deviant Read Pairs

Indicate deviations with colour and clustering

Scaffold View



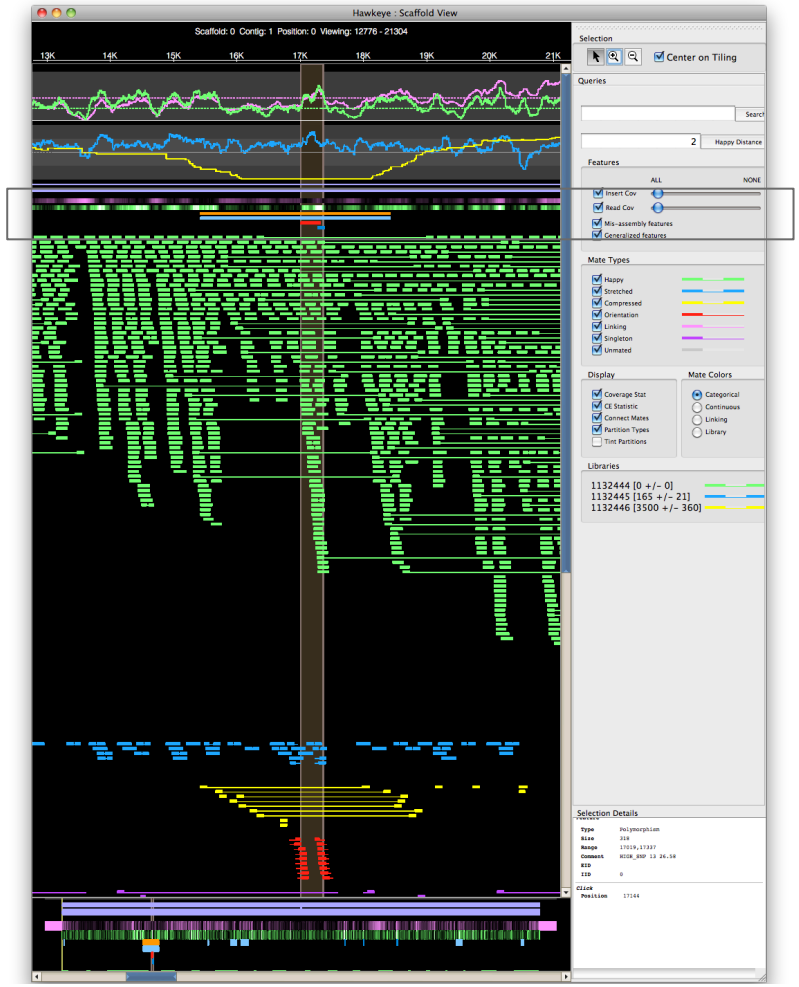
Hawkeye

Schatz *et al.*, 2011

Task 2 | Inspecting Deviant Read Pairs

Indicate deviations with colour and clustering

Scaffold View



Evidence for a misassembly:

Orange bar: suspicious region (by AMOSvalidate)

Light blue bar: compression

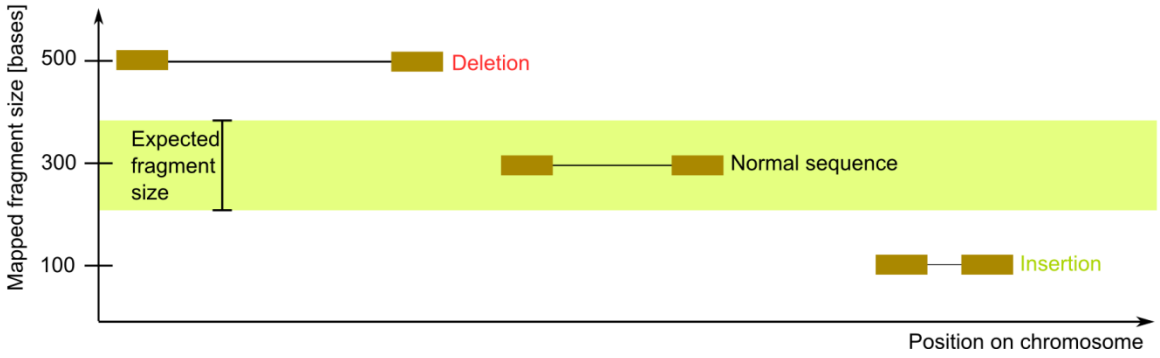
Red: high density of heterogeneous SNPs

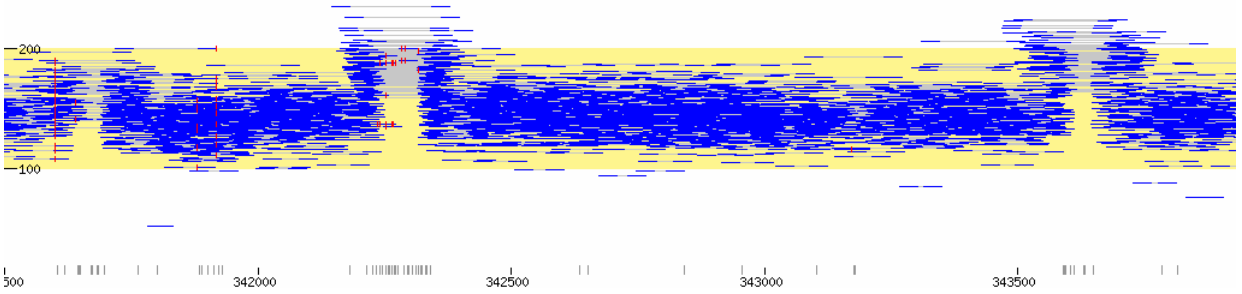
Hawkeye

Schatz *et al.*, 2011

Task 2 | Inspecting Deviant Read Pairs

Use the y-axis to indicate insert size





LookSeq

Manske and Kwiatkowski, 2009

Task 3 | Investigating Contig Connectivity

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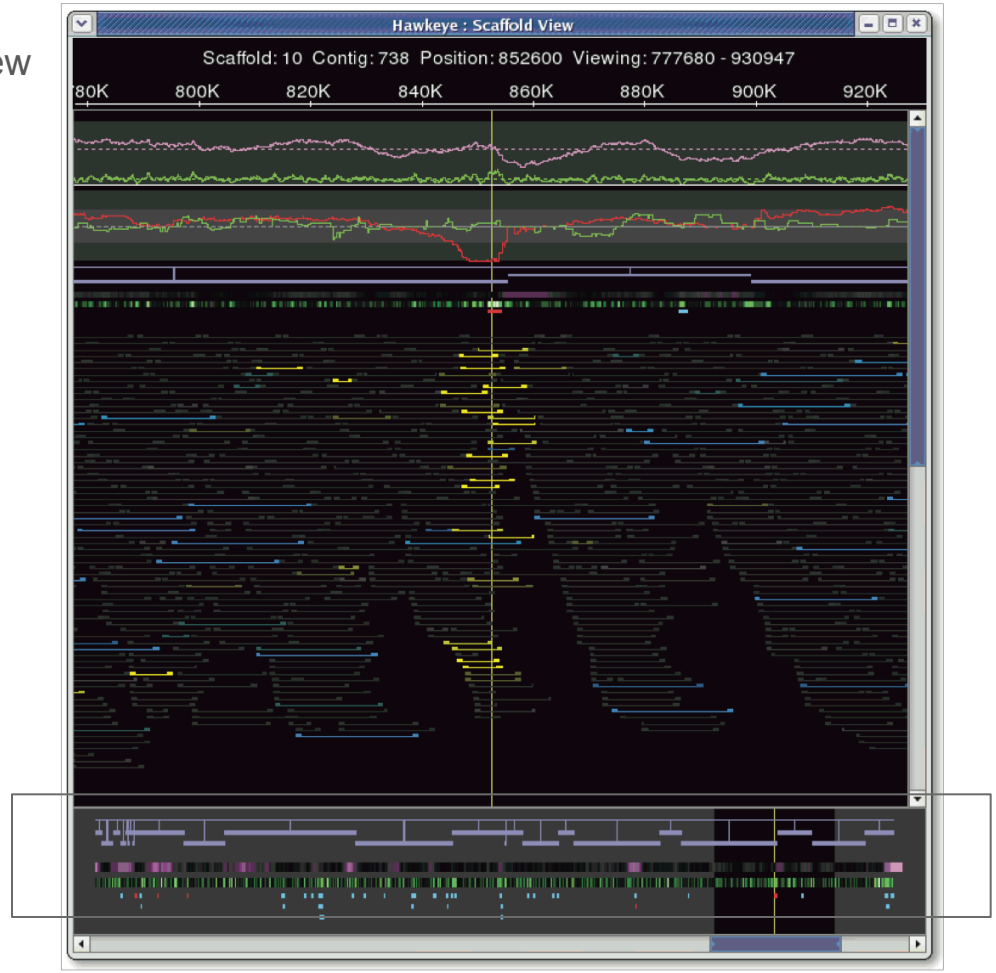
Scaffold View



Contig order in the scaffold displayed in overview panel

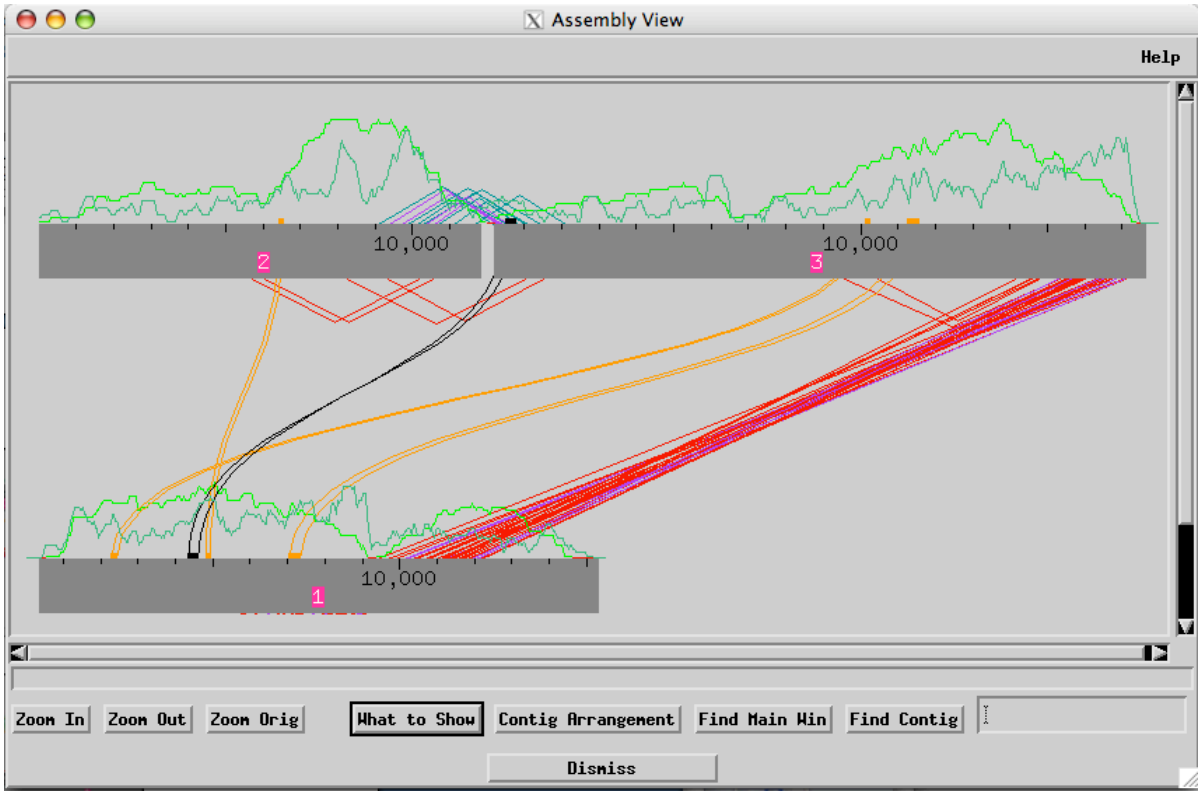
Hawkeye

Schatz *et al.*, 2007

Task 3 | Investigating Contig Connectivity

Inconsistent read pairs (red) indicate a misassembly

Assembly View



Read coverage: line plots

Read pairs: angled lines

Sequence similarity: curved lines

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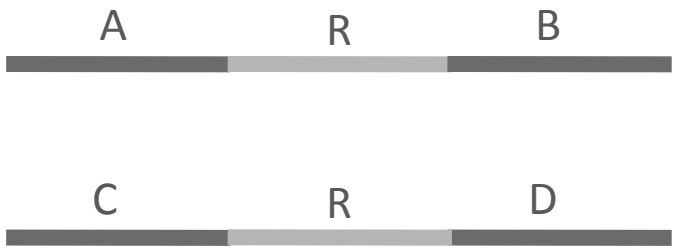
and Phil Green

Task 3 | Investigating Contig Connectivity

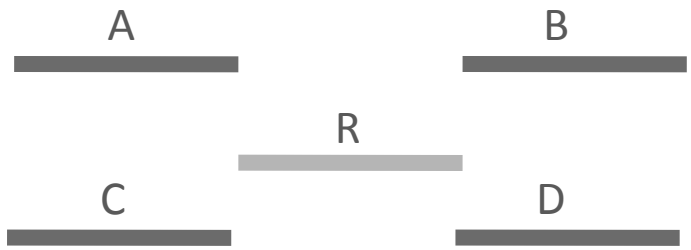
Source DNA



Repeat R

Task 3 | Investigating Contig Connectivity

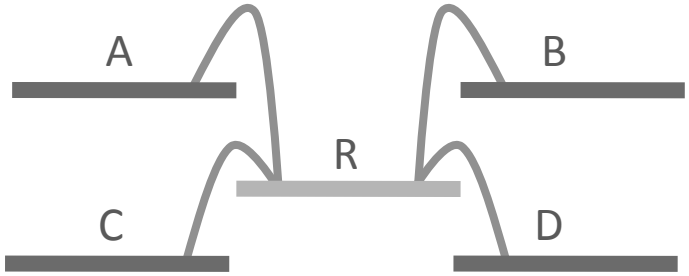
Assembled contigs



Collapsed repeat R

Task 3 | Investigating Contig Connectivity

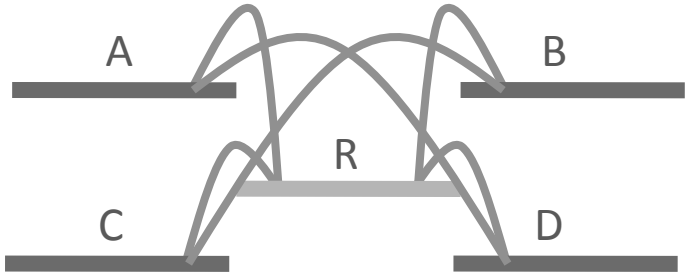
Assembled contigs



Collapsed repeat R
Arcs = Read Pairs

Task 3 | Investigating Contig Connectivity

Assembled contigs



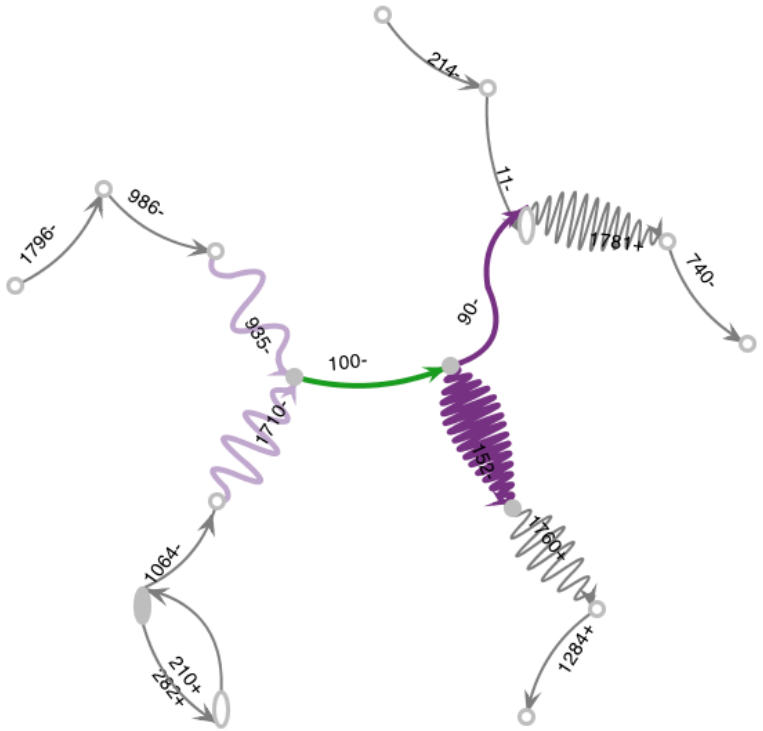
Collapsed repeat R
Arcs = Read Pairs

Arcs + linear ordering : gets complicated fast

Task 3 | Investigating Contig Connectivity

ABySS-Explorer

Nielsen *et al.*, InfoVis 2009

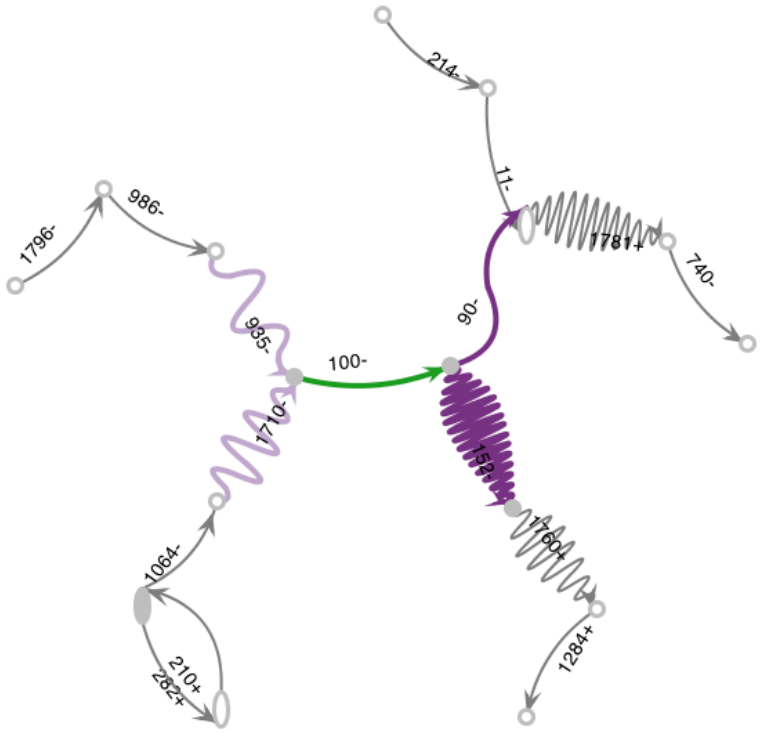


Edge = contig

Vertex = overlap

Squiggle = contig length (one oscillation per 1000 bps)

Task 3 | Investigating Contig Connectivity



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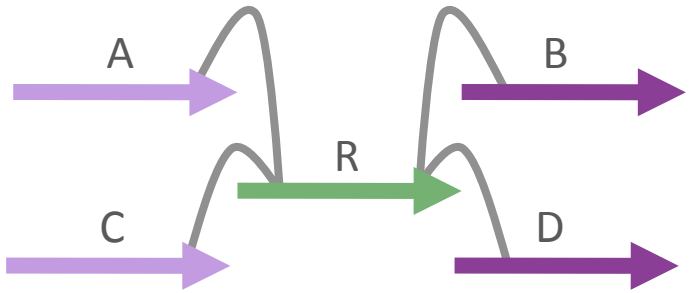
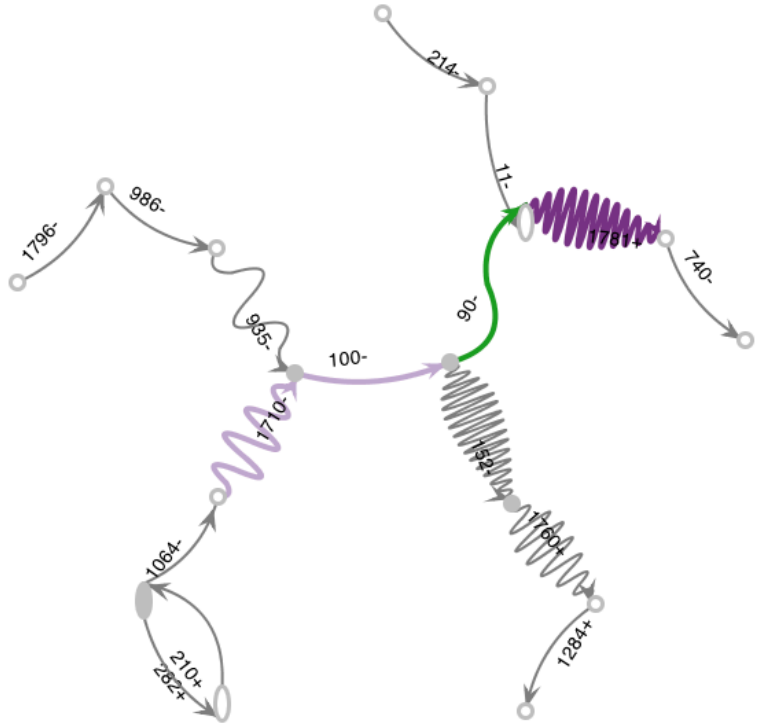


Green = selected contig

Light Purple = selected contig has upstream paired reads in this contig

Dark Purple = selected contig has downstream paired reads in this contig

Task 3 | Investigating Contig Connectivity



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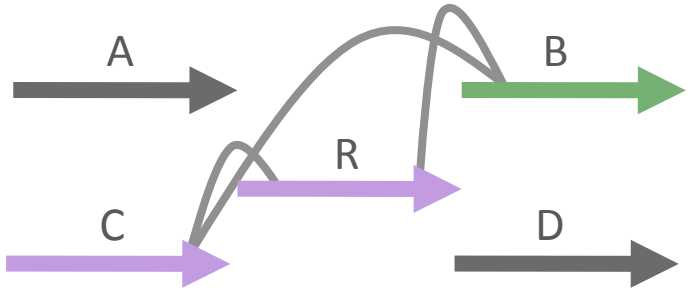
Nielsen *et al.*, InfoVis 2009



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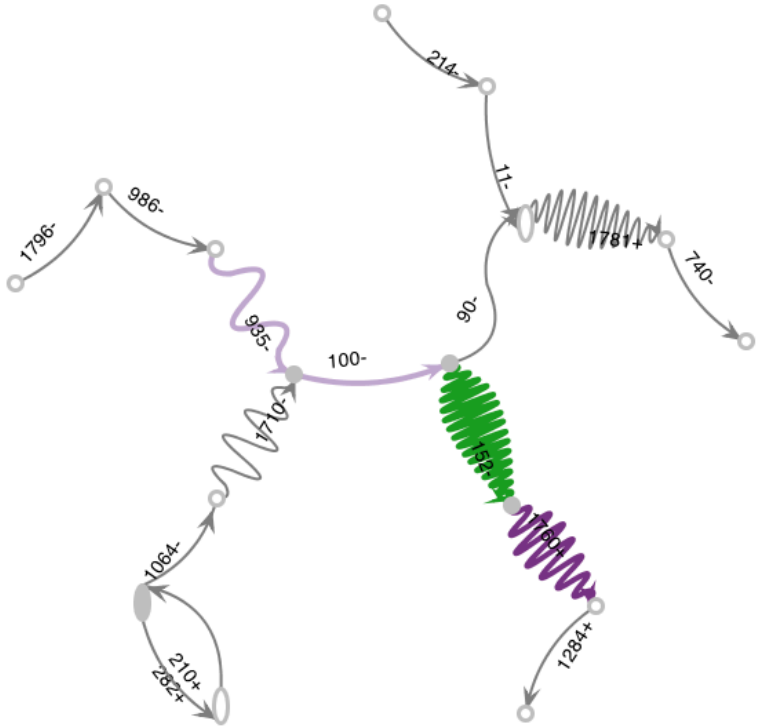

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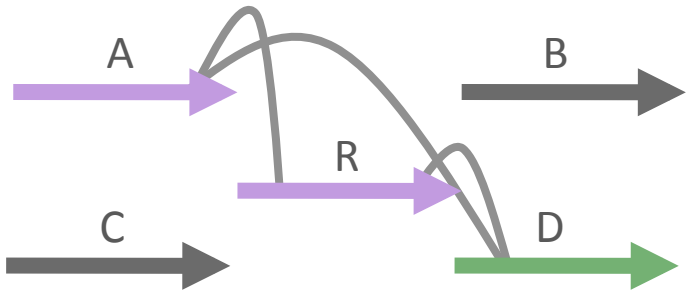

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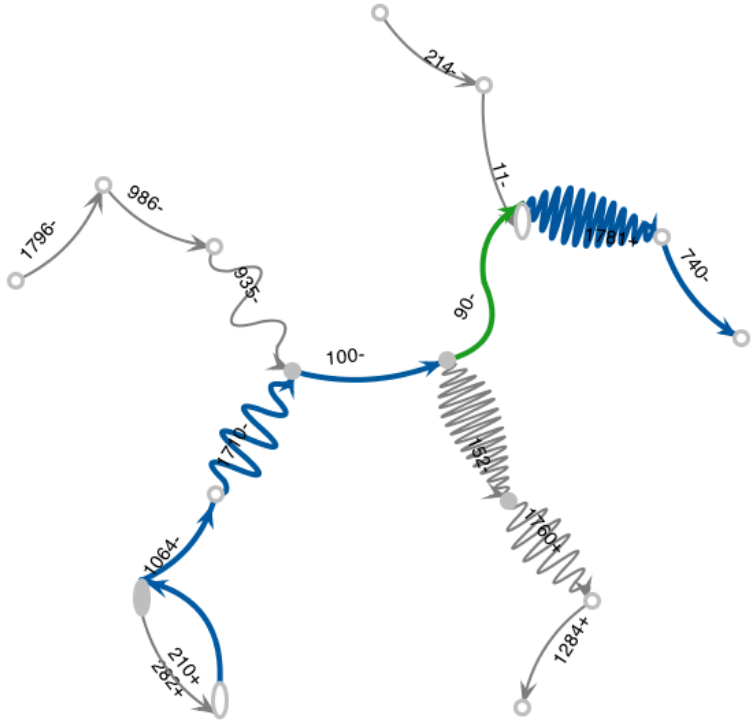

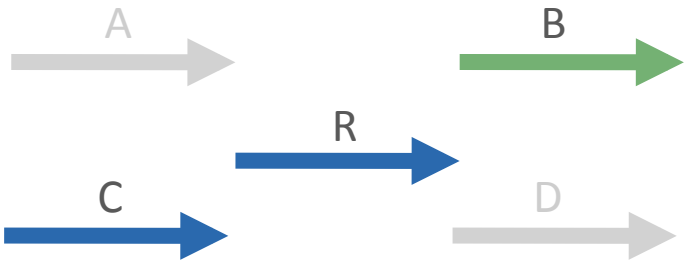

Edge = contig

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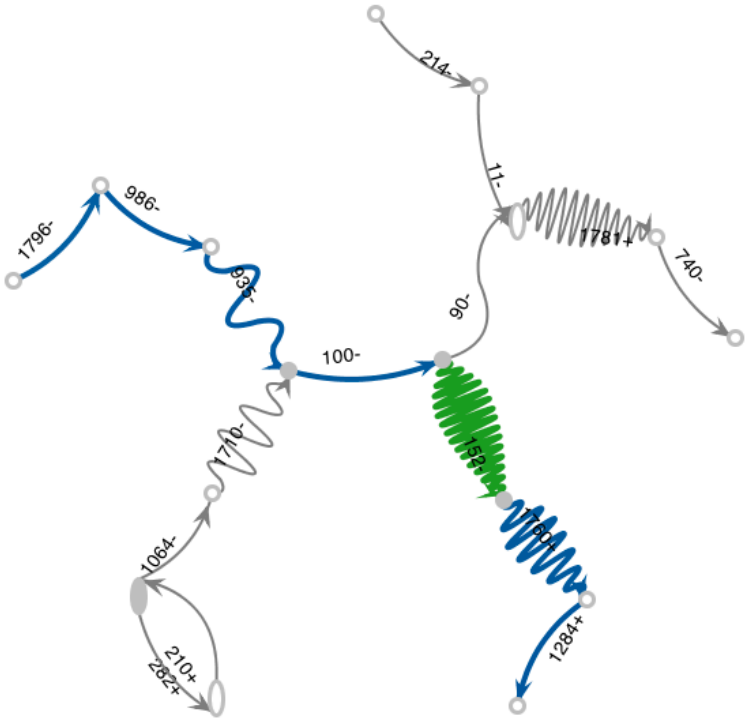
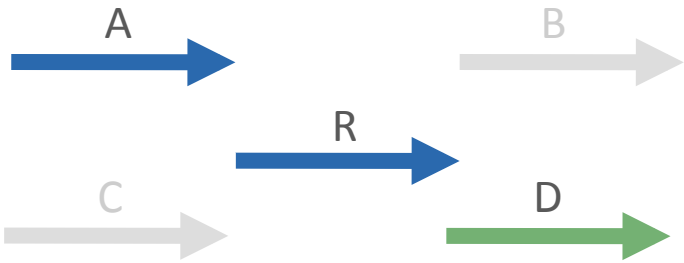
Blue = predicted scaffold

Task 3 | Investigating Contig Connectivity

ABySS-Explorer

Nielsen *et al.*, InfoVis 2009





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Task 4 | Assembly Evaluation

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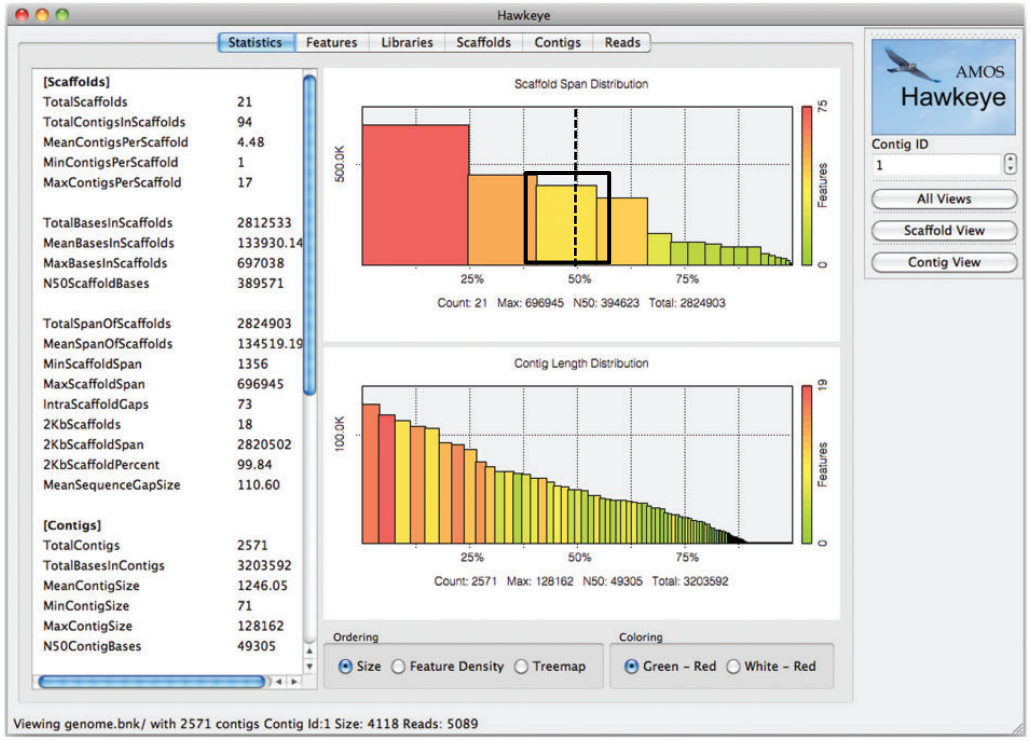
Contig size is a common metric of assembly quality

Scaffold N50

Half of the genome has been assembled into scaffolds larger than the N50 value

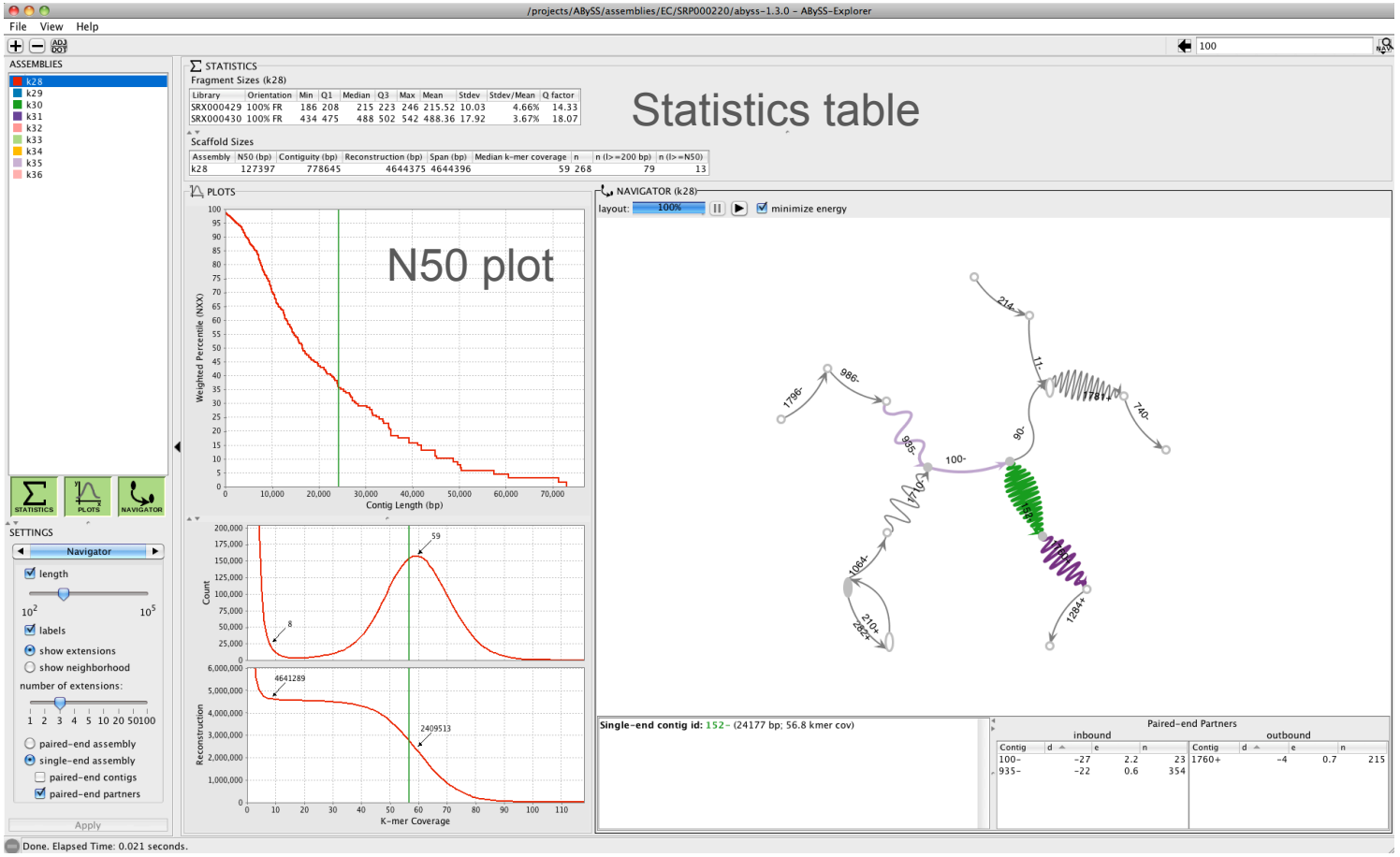


Bar height = contig size
Bar width = relative fraction of the genome size

Hawkeye

Schatz *et al.*, 2007; 2011

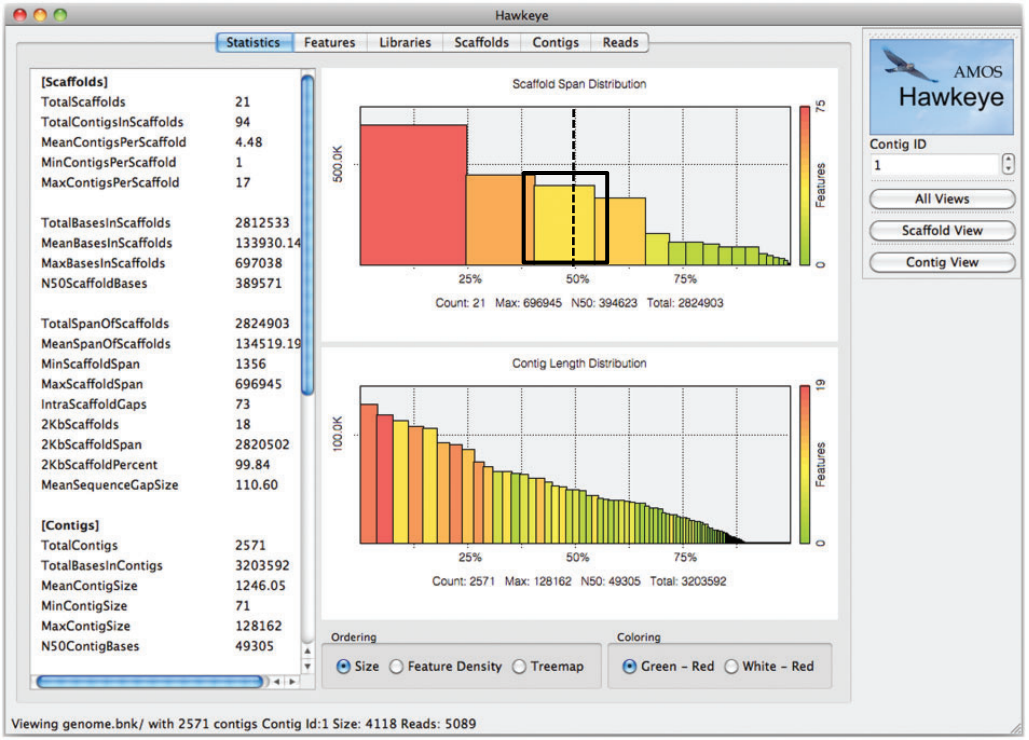
Task 4 | Assembly Evaluation



Task 4 | Assembly Evaluation

Contig quality also an important consideration

Bar colour indicates number of misassembly features discovered by AMOSvalidate



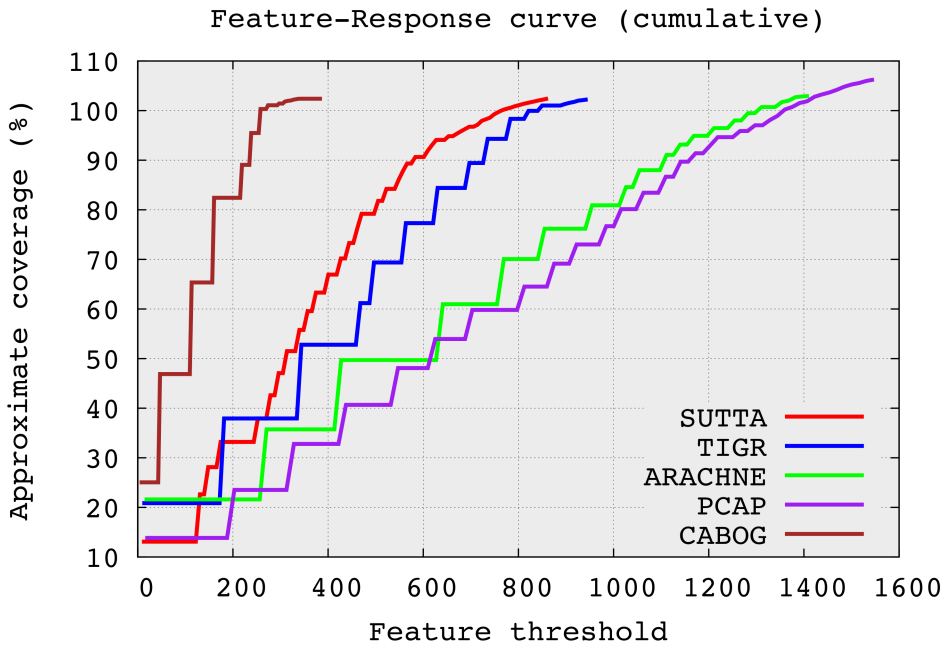
Bar height = contig size
Bar width = relative fraction of the genome size

Hawkeye

Schatz *et al.*, 2007; 2011

Task 4 | Assembly Evaluation

FRCurve - simultaneously measure connectivity and quality



- Each contig has a number of misassembly features (detected by AMOSvalidate)
- Sort contigs by size (largest first) and tally genome coverage for contigs with < threshold missassembly feature counts

From Schatz *et al*.
Briefings in Bioinformatics, 2011

Task 5 | Making Sense of Complex Structures

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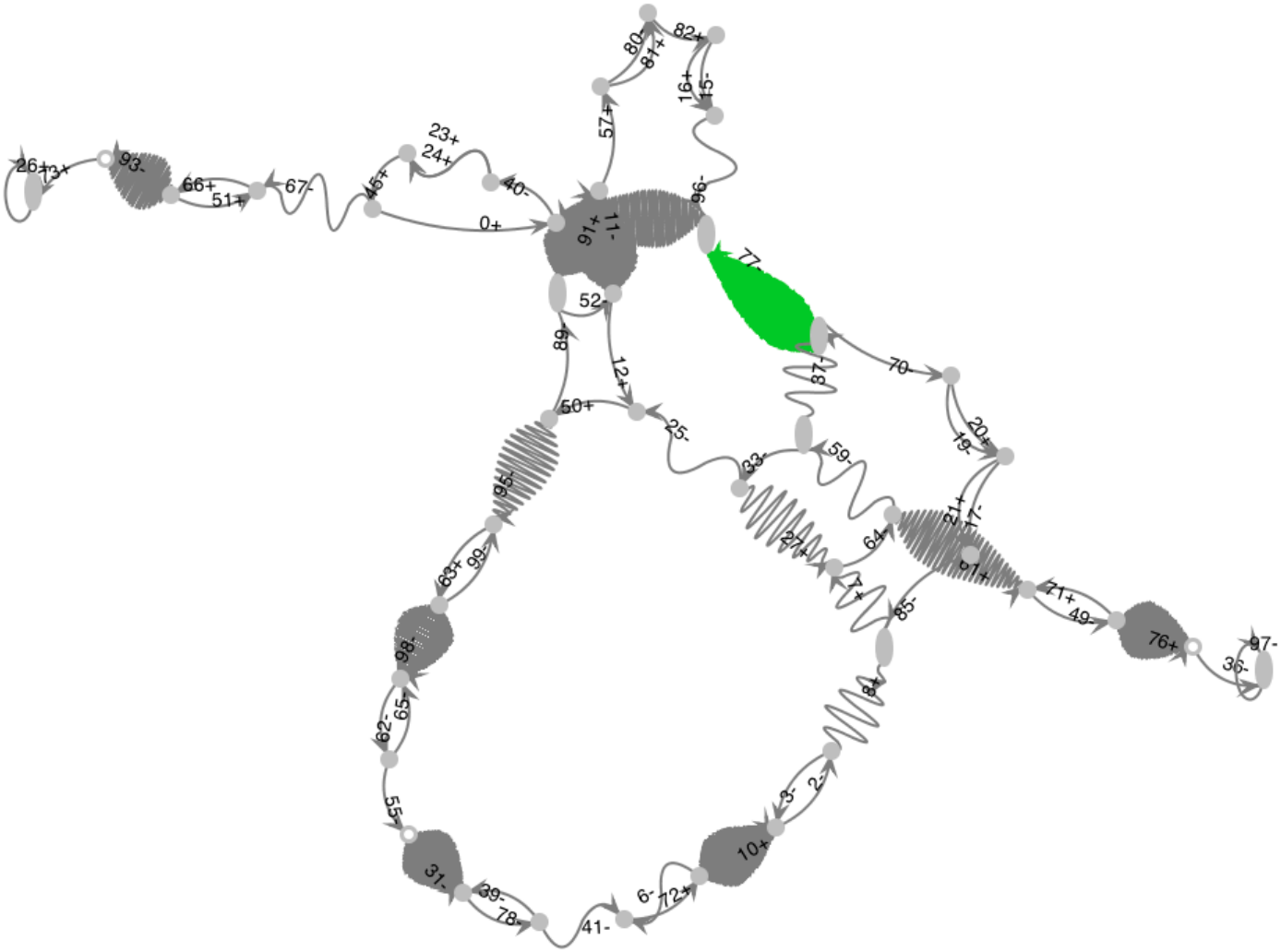
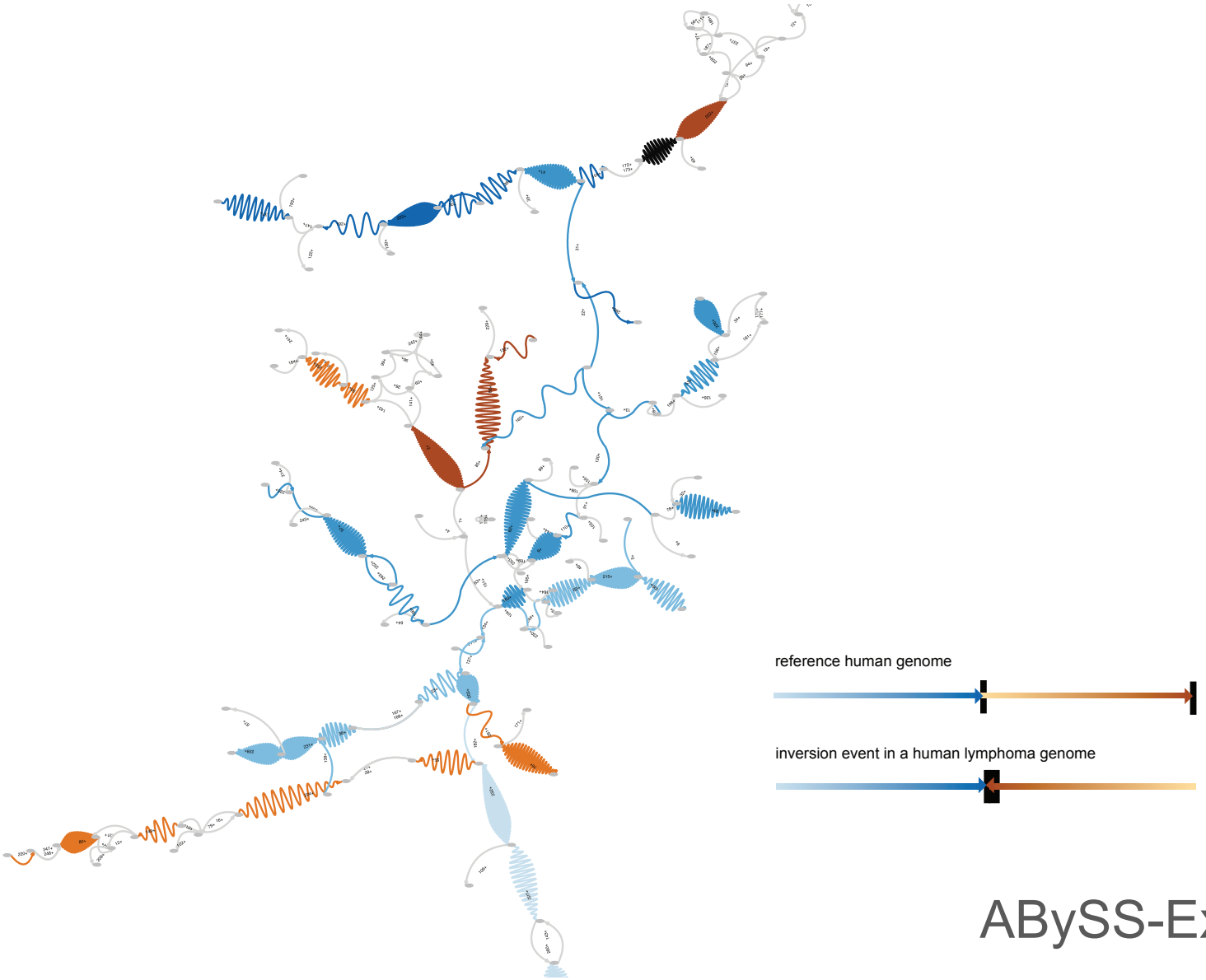


1 Mbp *Mycoplasma capricolum* genome

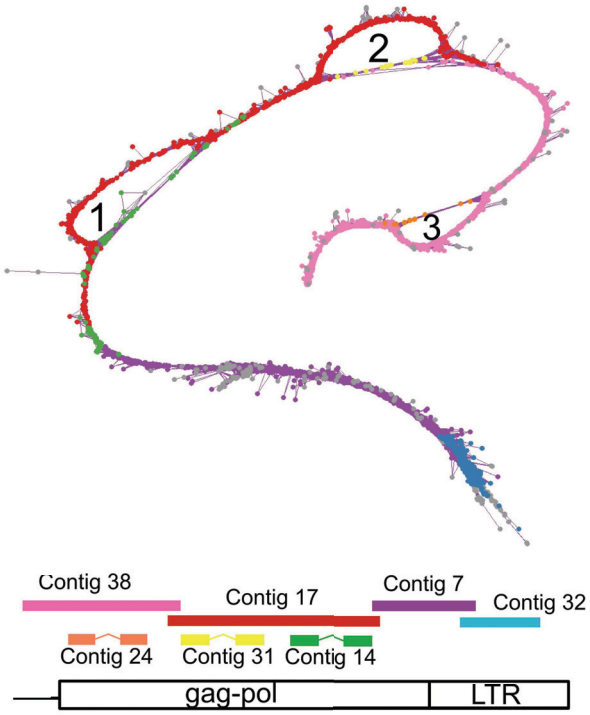
ABySS-Explorer

Task 5 | Making Sense of Complex Structures



ABySS-Explorer

Task 5 | Making Sense of Complex Structures



Vertex = sequencing read

Edge = overlap

- Use read clustering (not assembly *per se*) based on sequence similarity to identify structures of repeat families
- 5320 reads from the *Pisum sativum* (pea) genome representing the Ty1/copia LTR-retrotransposon Angela (CAP3 assembled contigs shown below)
- Enabled identification of the most common form of the Angela element and three less frequent deletions

From Novák *et al*.
BMC Bioinformatics, 2010

Summary | Where visualization is used

1. Nucleotide-Level Discrepancies
2. Deviant Read Pairs
3. Contig Connectivity
4. Assembly Evaluation
5. Complex Structures

Summary | Challenges

1. Must address multiple levels of resolution
2. Large data sets pose computational and performance challenges
3. Rapidly changing field directly affected by innovations in sequencing technology

Acknowledgements

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Sujai Kumar

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Michael Schatz

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