Analysis of Structural Variants using 3rd generation Sequencing

Michael Schatz

January 12, 2016
Bioinformatics / PAG XXIV

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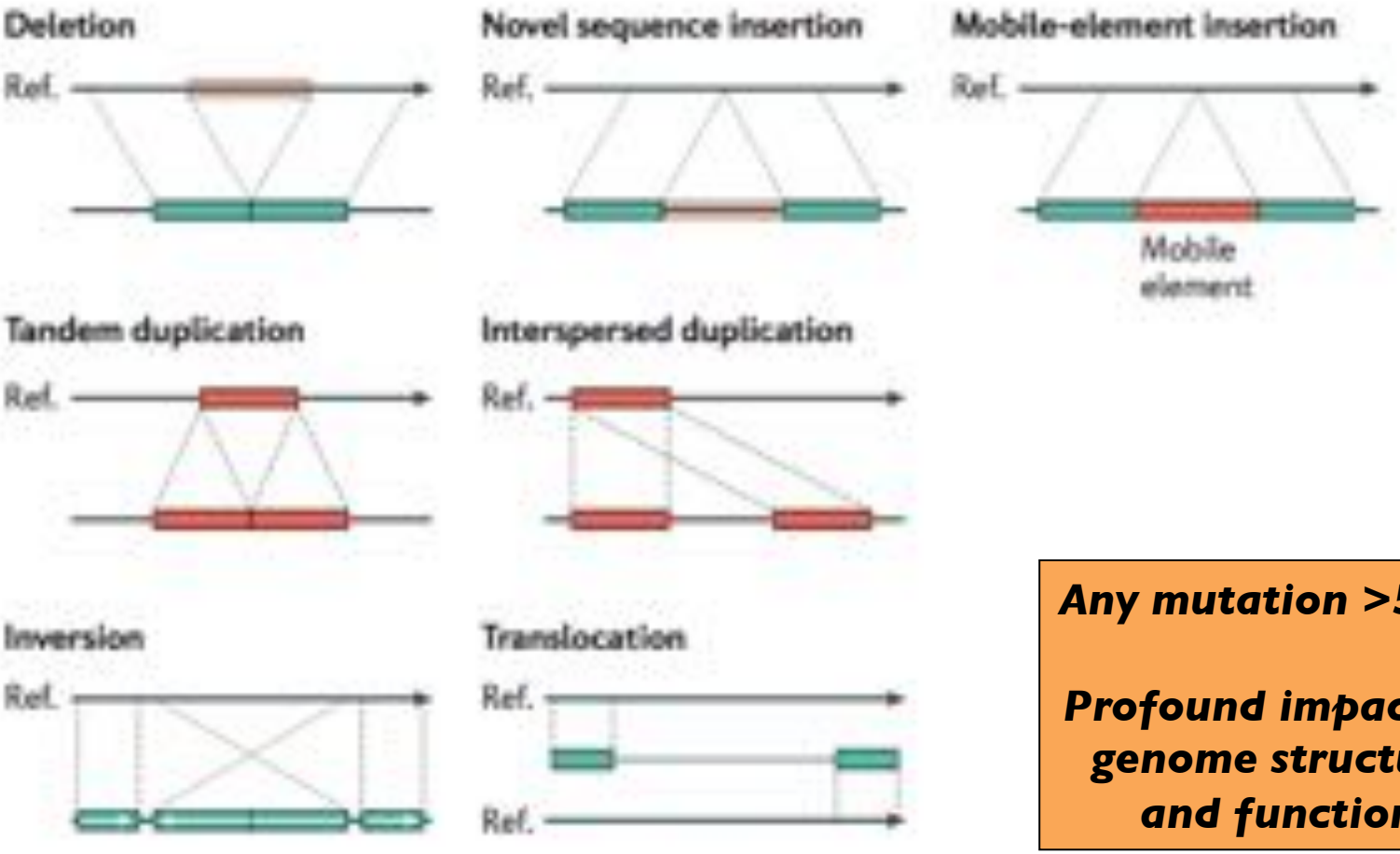
The Resurgence of Reference Quality Genomes

Michael Schatz & Daniel Rokhsar
Tuesday, January 12, 2016 @ 4pm – 6pm
Town & Country - Pacific Salon 1

4:00pm	***The Resurgence of Reference Quality Genomes*** Michael Schatz, CSHL + JHU
4:20pm	***High Quality, Highly Contiguous Genome Assemblies Now*** Richard Green, Dovetail Genomics
4:40pm	***Scalable Parallel Algorithms for de novo Assembly of Complex Genomes*** Aydin Buluc, Lawrence Berkeley National Laboratory
5:00pm	***Using PacBio Long Reads to Generate a High Quality Reference for the Allotetraploid Coffea arabica and its Maternal Diploid Ancestor Coffea eugeniodes*** Marcela Yepes, Cornell University
5:20pm	***MaSuRCA Mega-Reads Assembly Technique for Haplotype Resolved Genome Assembly of Hybrid PacBio and Illumina Data*** Aleksey Zimin, University of Maryland
5:40pm	***How to Compare and Cluster Every Known Genome in about an Hour*** Sergey Koren, NHGRI

Structural Variations



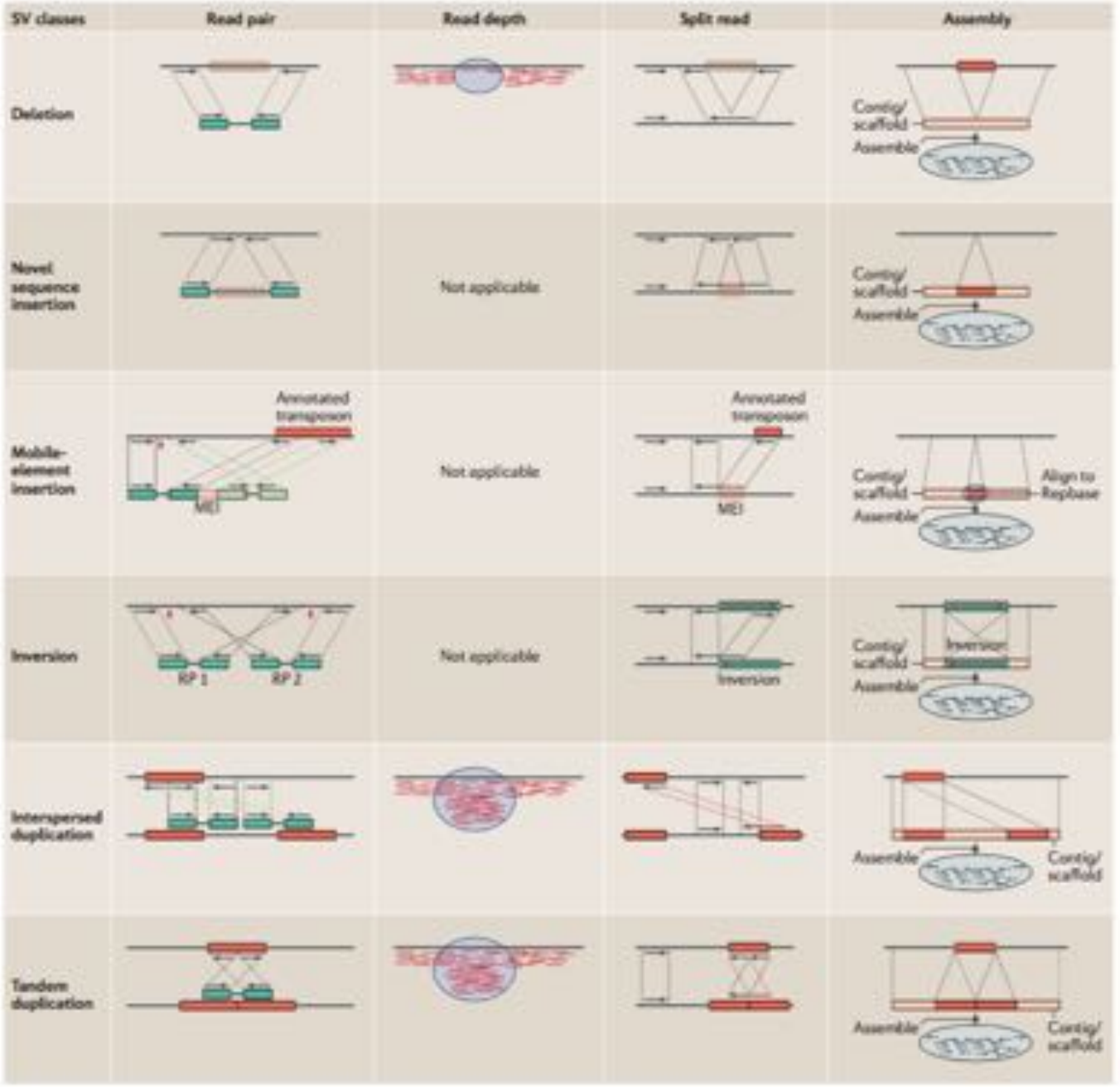
Any mutation >50bp

Profound impact on genome structure and function

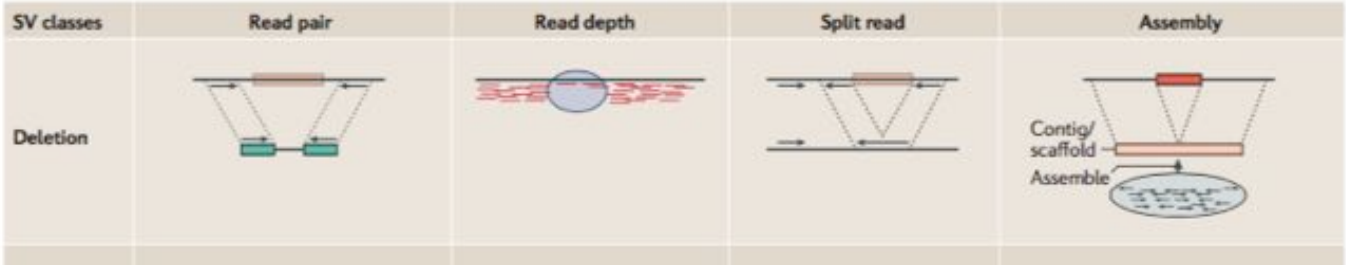
Genome structural variation discovery and genotyping

Alkan, C, Coe, BP, Eichler, EE (2011) *Nature Reviews Genetics*. May;12(5):363-76. doi: 10.1038/nrg2958.

Structural Variation Sequence Signatures



Structural Variation Sequence Signatures



PacBio Sequel

>10kbp Mean Read Lengths
~$15k / Mammalian-sized genome

Single Molecule Sequencing

- No amplification artifacts
- More uniform coverage
- Essentially no GC biases

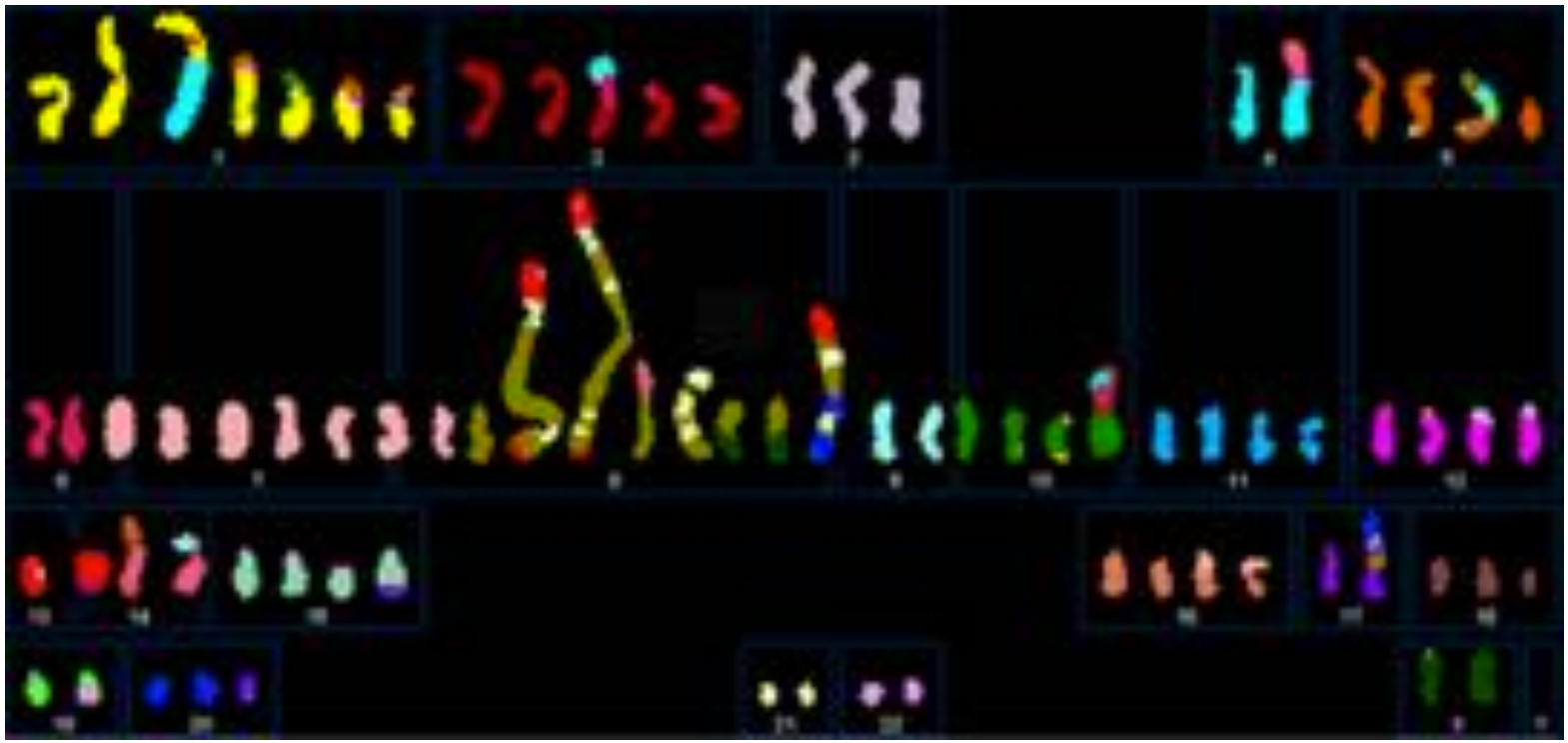
Long read lengths

- Improved mappability
- More likely to span breakpoints
- More robust split read analysis
- More robust assemblies

Basepair resolution for 50bp through 50Mbp events

SK-BR-3

Most commonly used Her2-amplified breast cancer cell line



(Davidson et al, 2000)

Highly-rearranged Mammalian genome

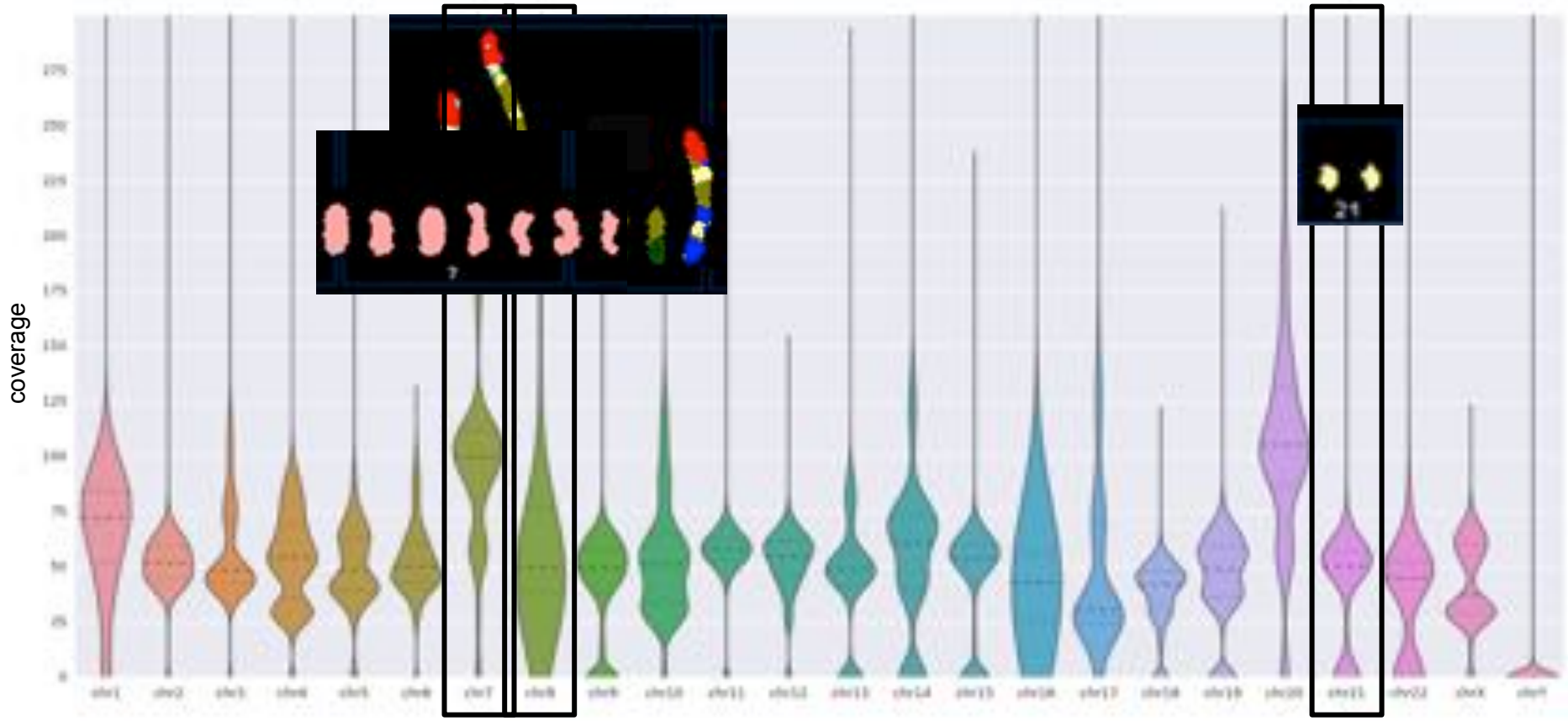
80 chromosomes instead of 46

Numerous chromosome fusions, rearrangements, other SVs

PacBio Long-Read Sequencing

mean read length: 9 kb
max read length: 71 kb

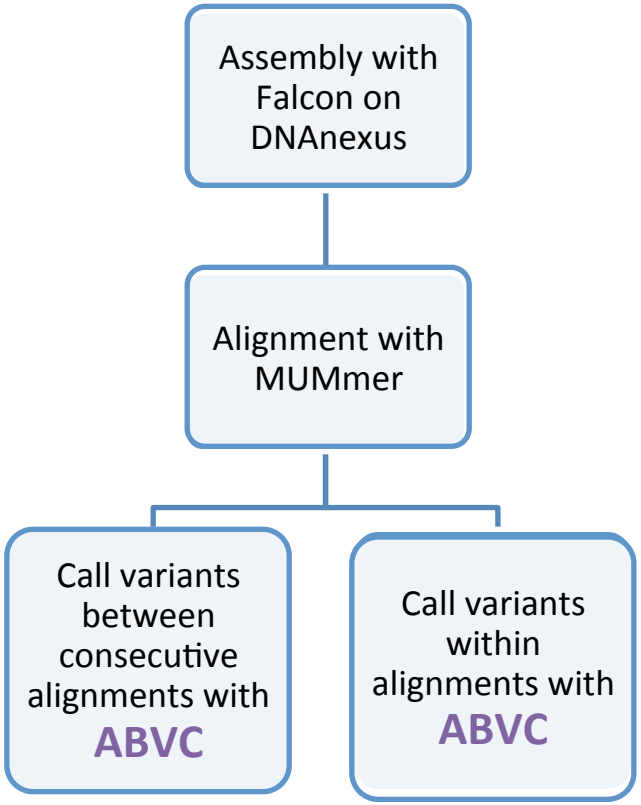
72X overall coverage



Genome-wide coverage averages around 54X
Coverage per chromosome varies greatly as expected from previous karyotyping results

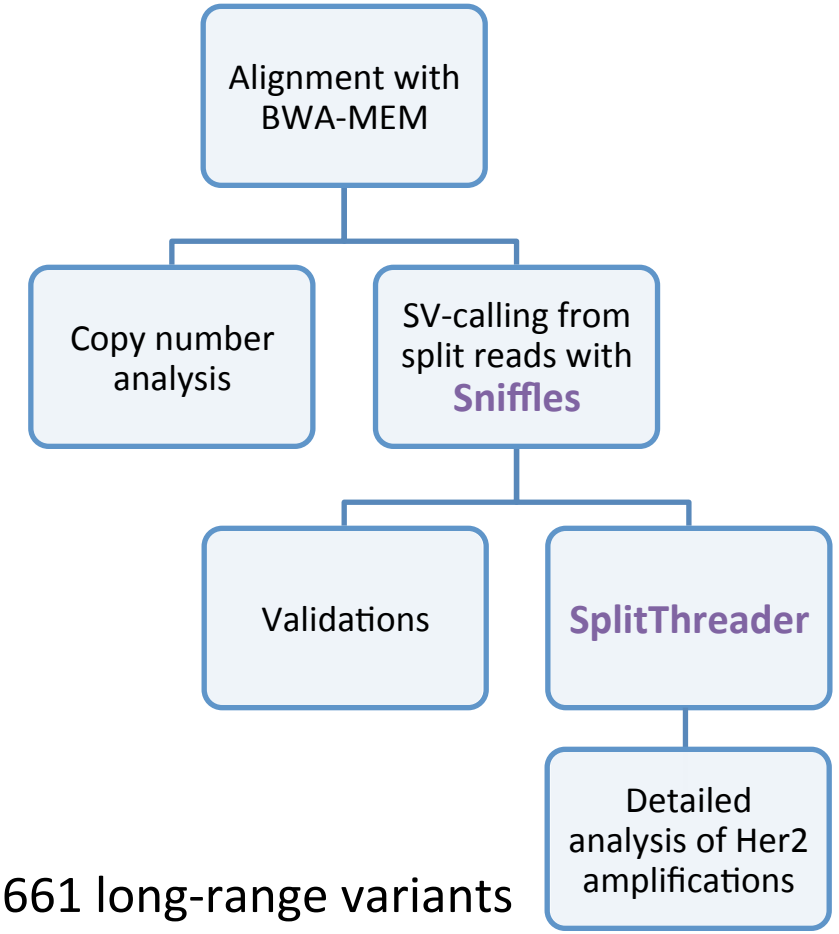
Genome structural analysis

Assembly-based



~ 11,000 local variants
50 bp < size < 10 kbp

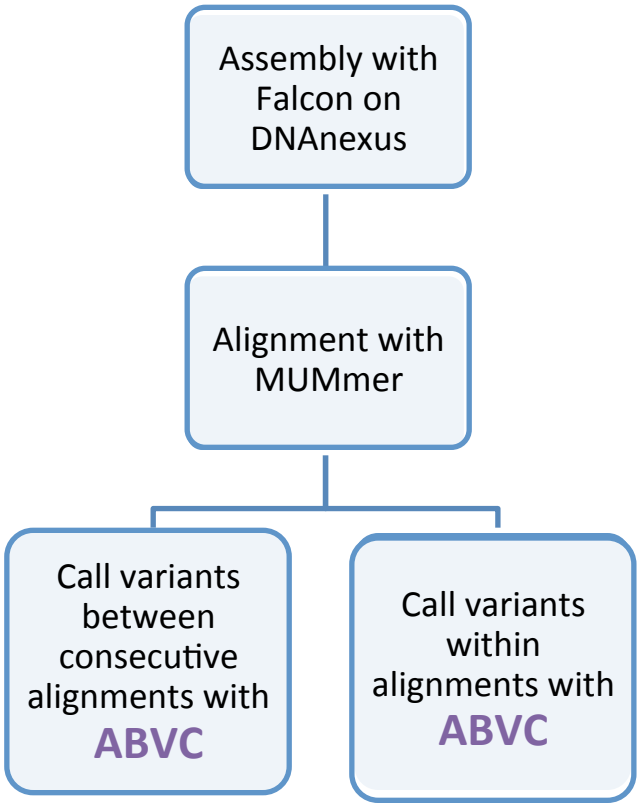
Alignment-based



661 long-range variants
(>10kb distance)

Genome structural analysis

Assembly-based



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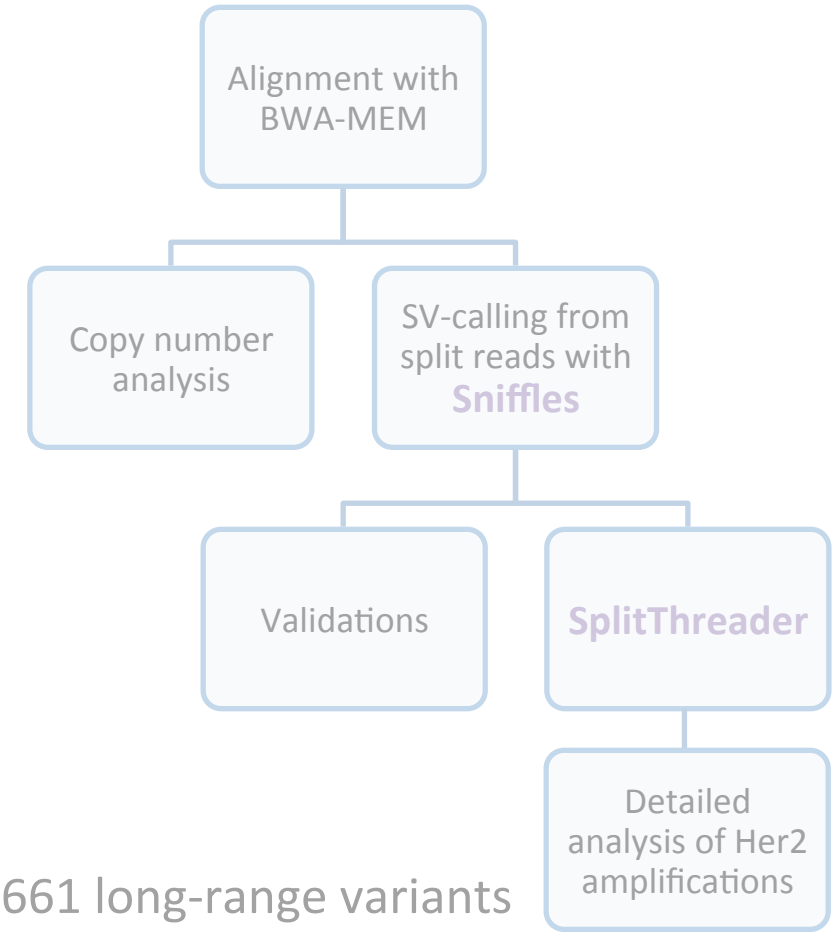
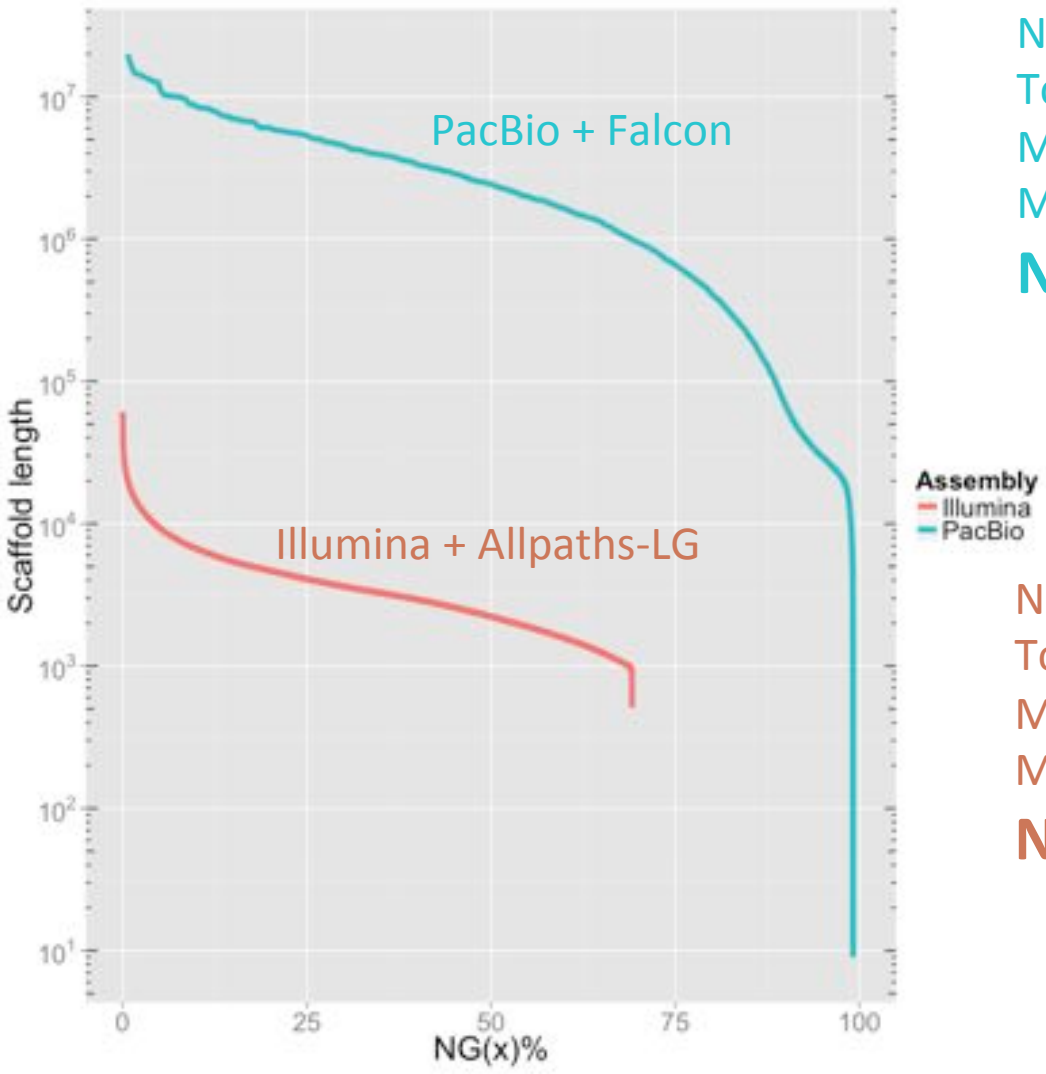


661 long-range variants
(>10kb distance)

Assembly using PacBio yields far better contiguity



Number of sequences: 13,532
Total sequence length: 2.97Gb
Mean: 266 kb
Max: 19.9 Mb

N50: 2.46 Mb

Relative to a genome size of 3 Gb

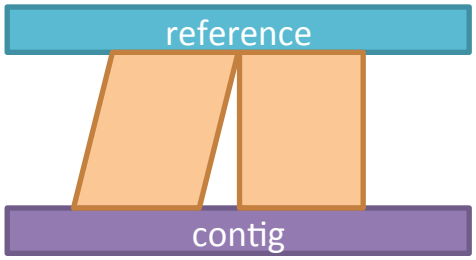
Number of sequences: 748,955
Total sequence length: 2.07 Gb
Mean: 2.8 kb
Max: 61 kb

N50: 3.3 kb

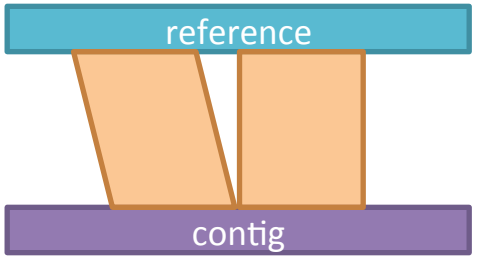
ABVC: Assembly-Based Variant-Caller

Defined point

Insertion

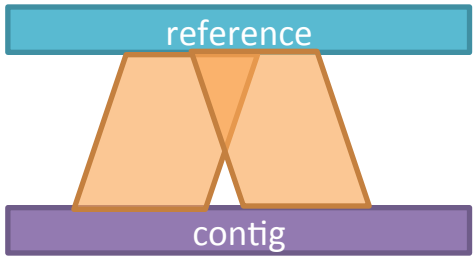


Deletion

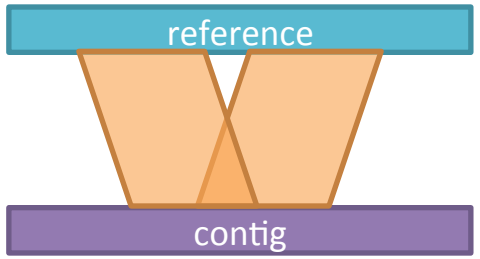


Overlapping alignments suggest tandem repeat

Tandem Expansions

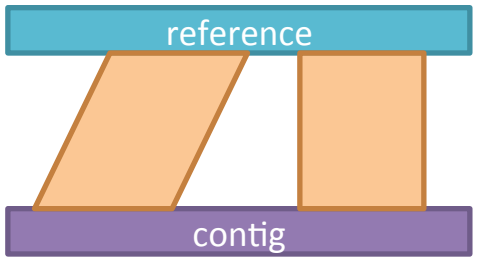


Tandem Contractions

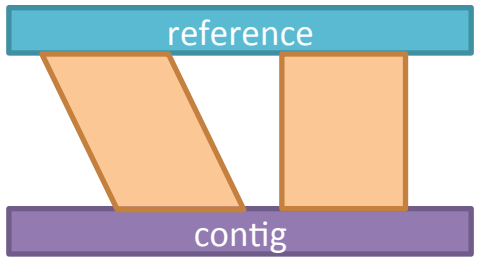


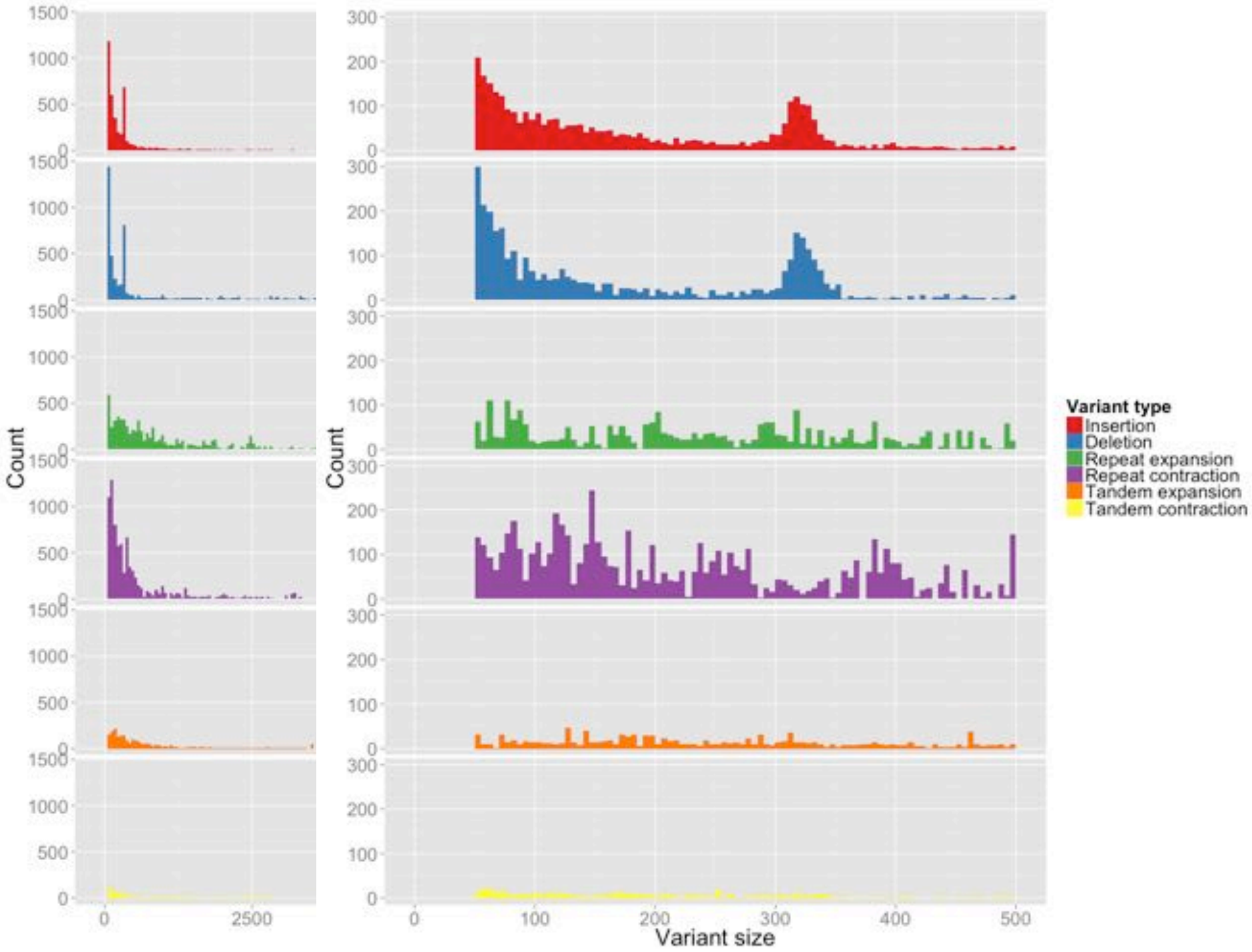
Gap where sequences do not align uniquely suggests a repeat

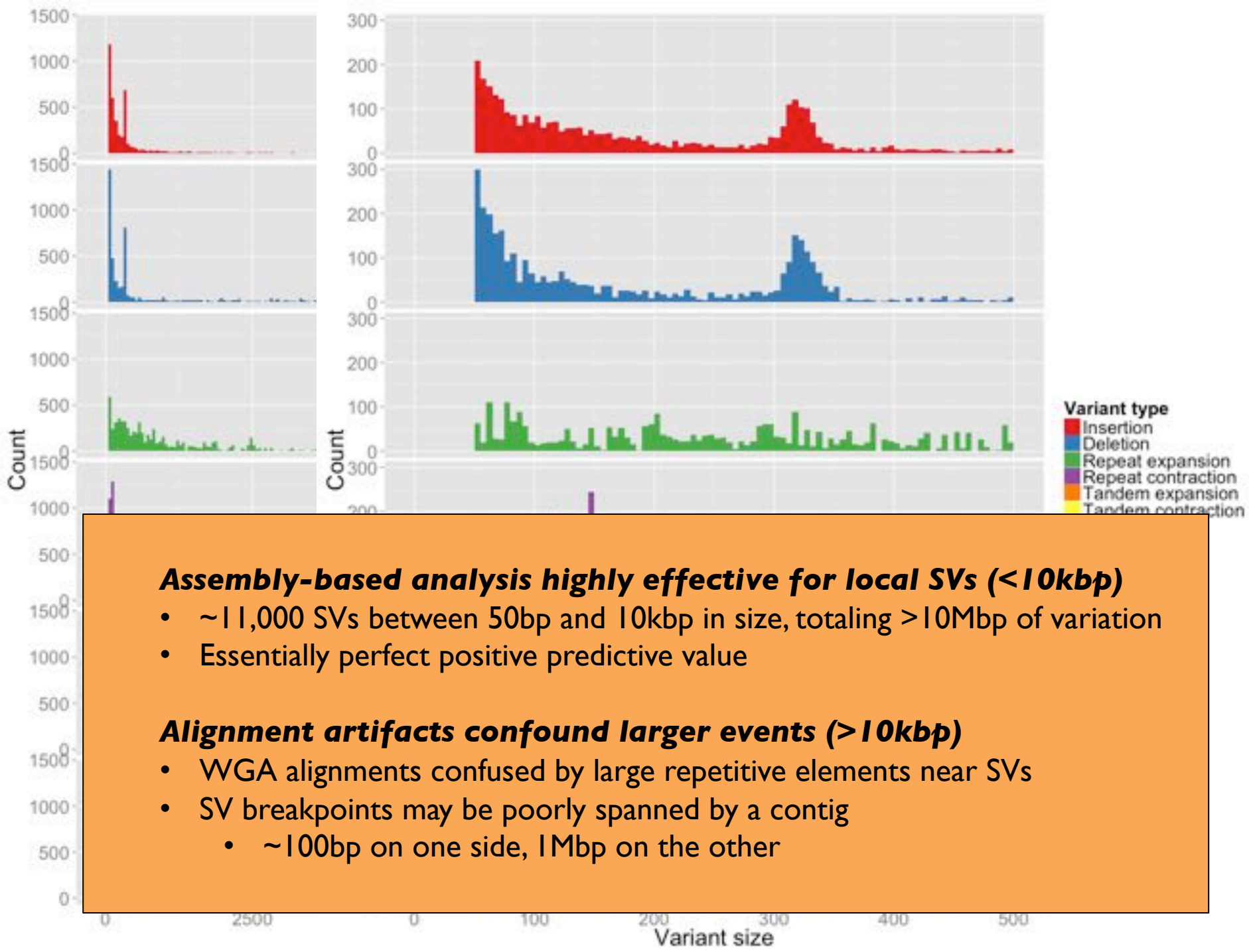
Repeat Expansions



Repeat Contractions




Count
Variant size
Variant type
Insertion
Deletion
Repeat expansion
Repeat contraction
Tandem expansion
Tandem contraction



Assembly-based analysis highly effective for local SVs (<10kbp)

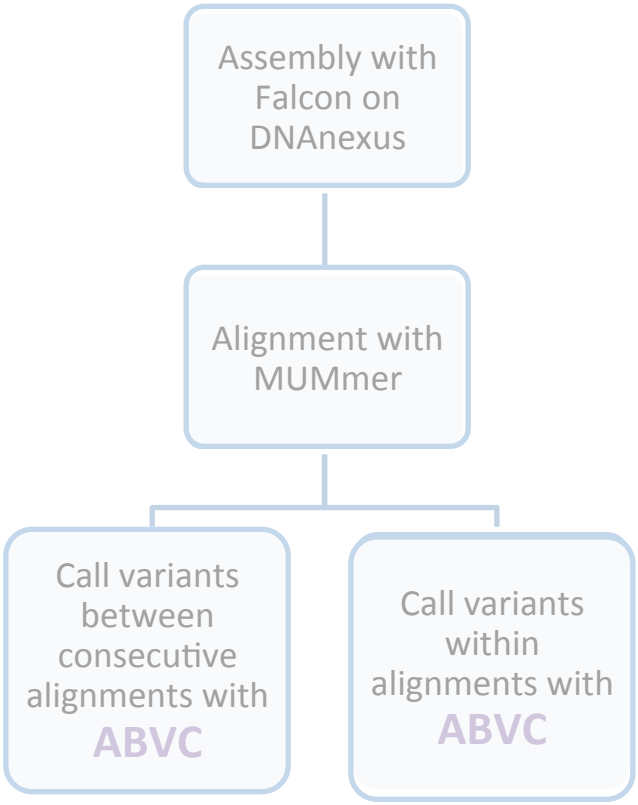
- ~11,000 SVs between 50bp and 10kbp in size, totaling >10Mbp of variation
- Essentially perfect positive predictive value

Alignment artifacts confound larger events (>10kbp)

- WGA alignments confused by large repetitive elements near SVs
- SV breakpoints may be poorly spanned by a contig
  - ~100bp on one side, 1Mbp on the other

Genome structural analysis

Assembly-based



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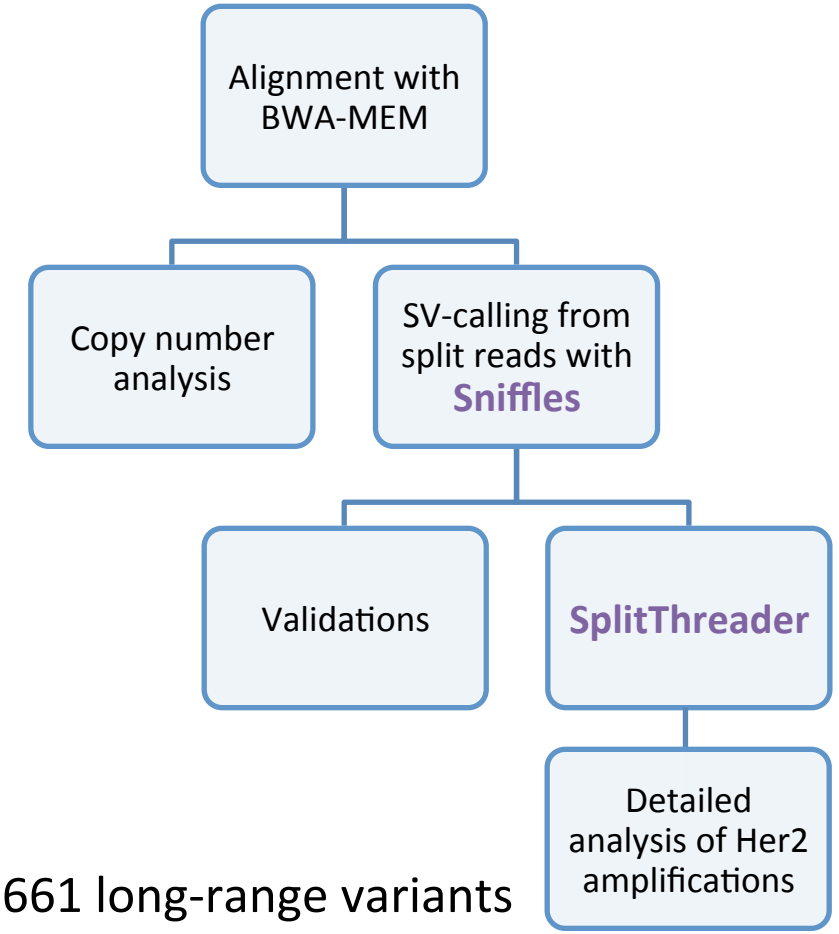
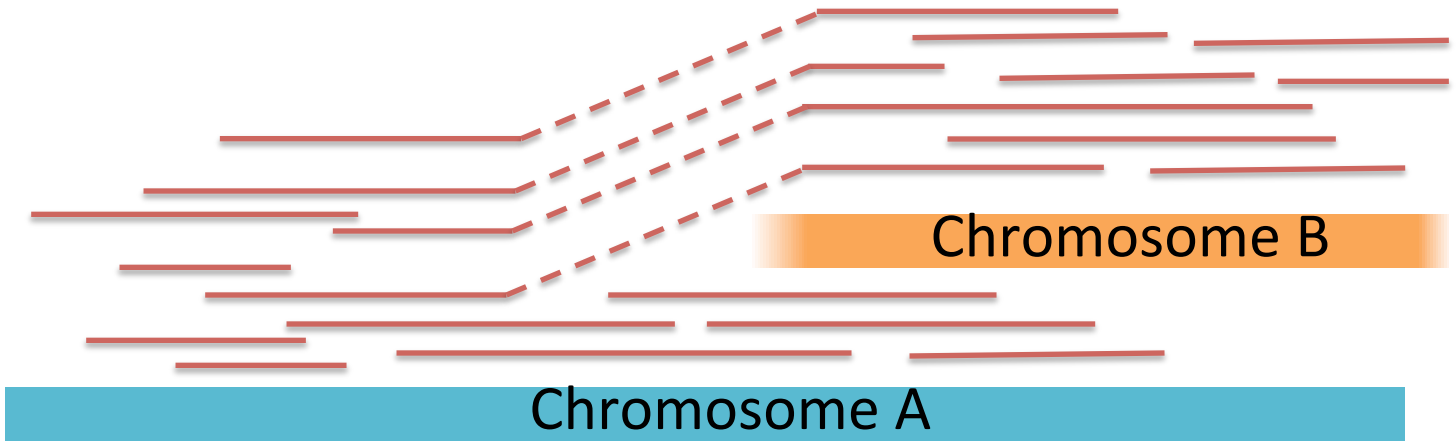
Alignment-based



661 long-range variants
(>10kb distance)

Long Read Structural Variation Analysis



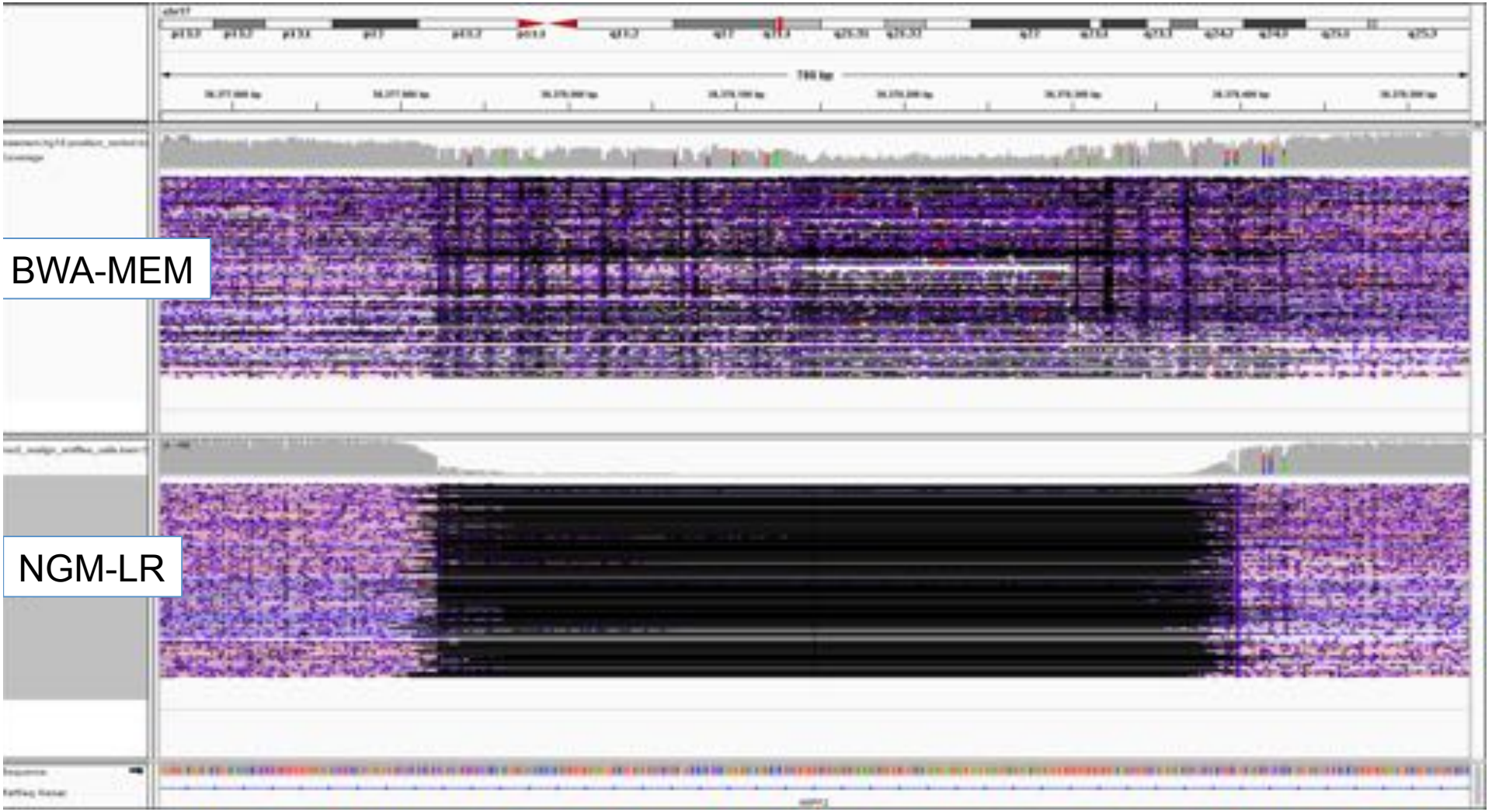
Split-read analysis greatly improved by long reads

- Improved chances of spanning event, including nested events
- However, many SVs lost due to poor alignments and poor PacBio support
  - LUMPY fails on reads that span more than 1 breakpoint, poor localization

New methods in development: NGM-LR + Sniffles

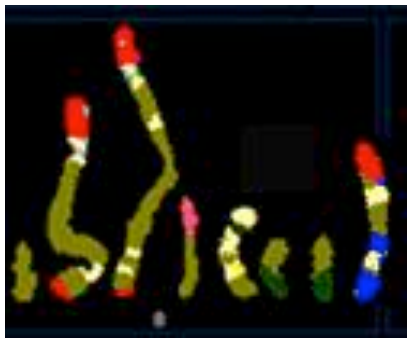
- ***NGM-LR***: Improve mapping of noisy long reads
- ***Sniffles***: Integrates SV evidence from split alignments, alignment fidelity (CIGAR string and MD tag)

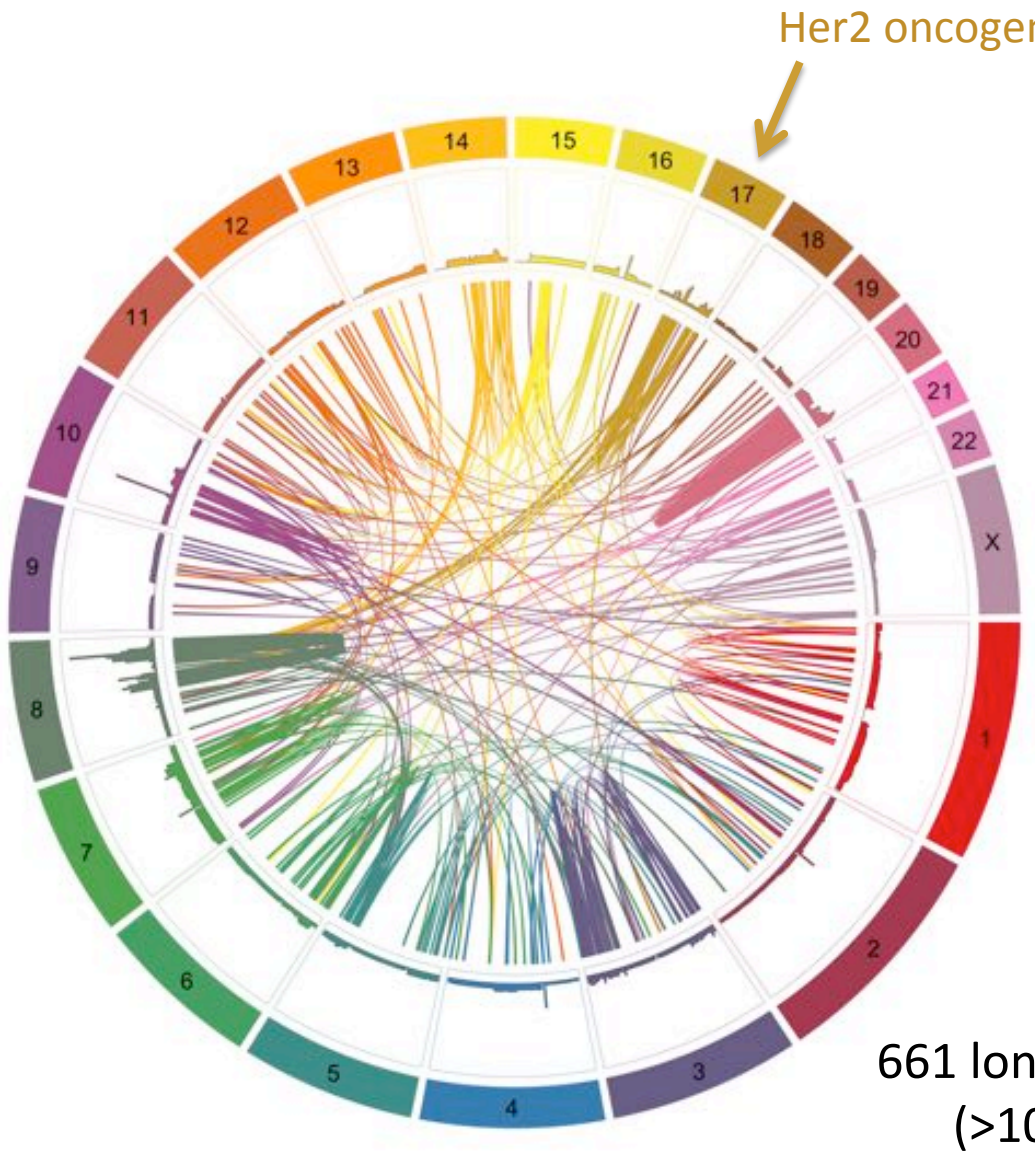
Mapping a ~500bp deletion



Similar issues for insertions, inversions; or Nanopore sequencing
Improved seeding, improved gap scoring: convex instead of affine

Long-range structural variants found by Sniffles





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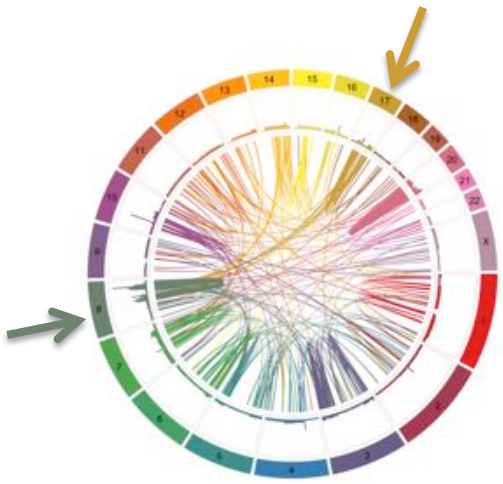
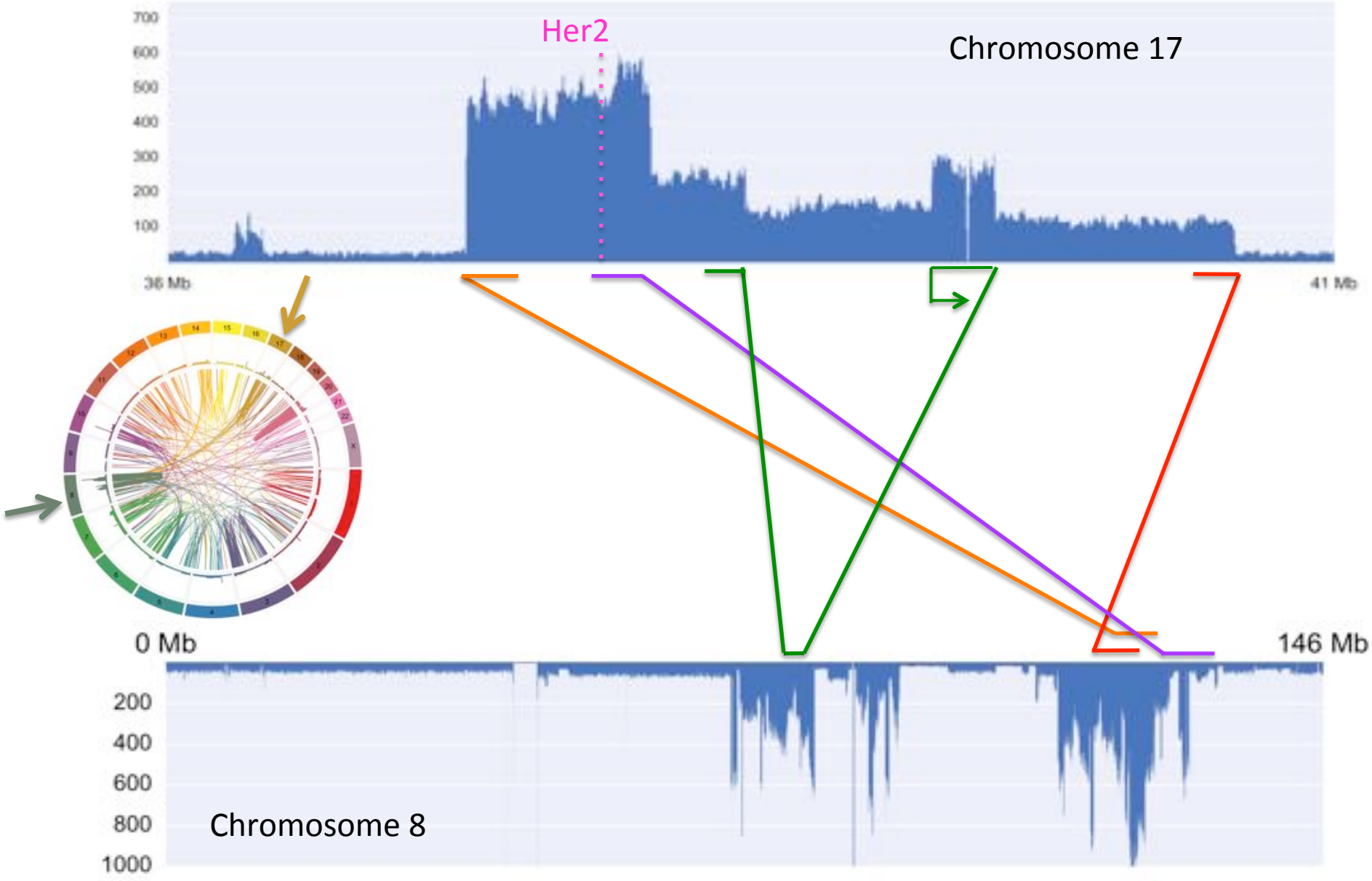
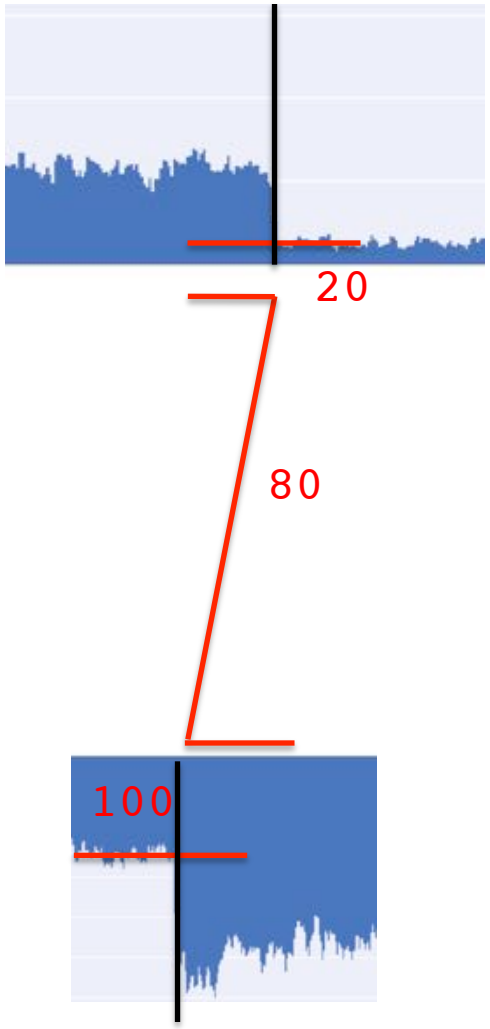


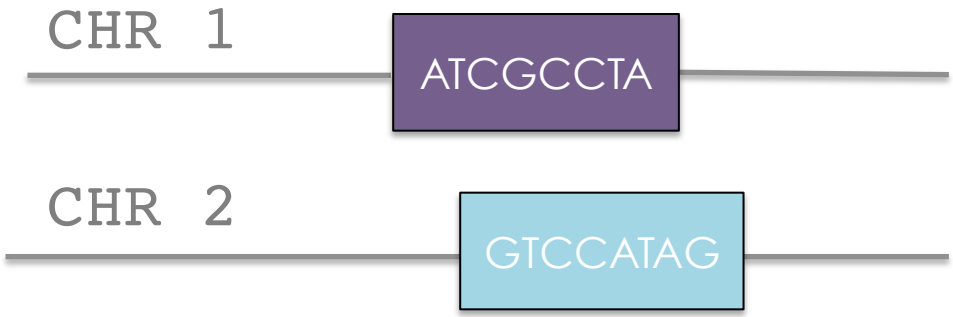
Long-range structural variants found by Sniffles

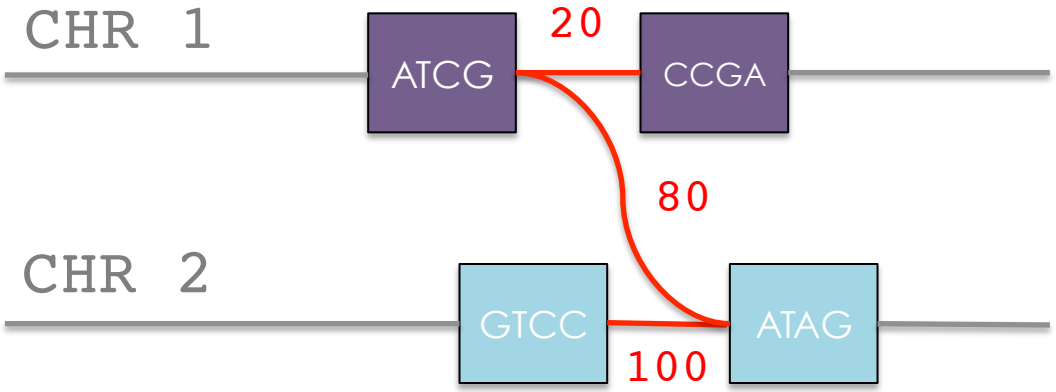


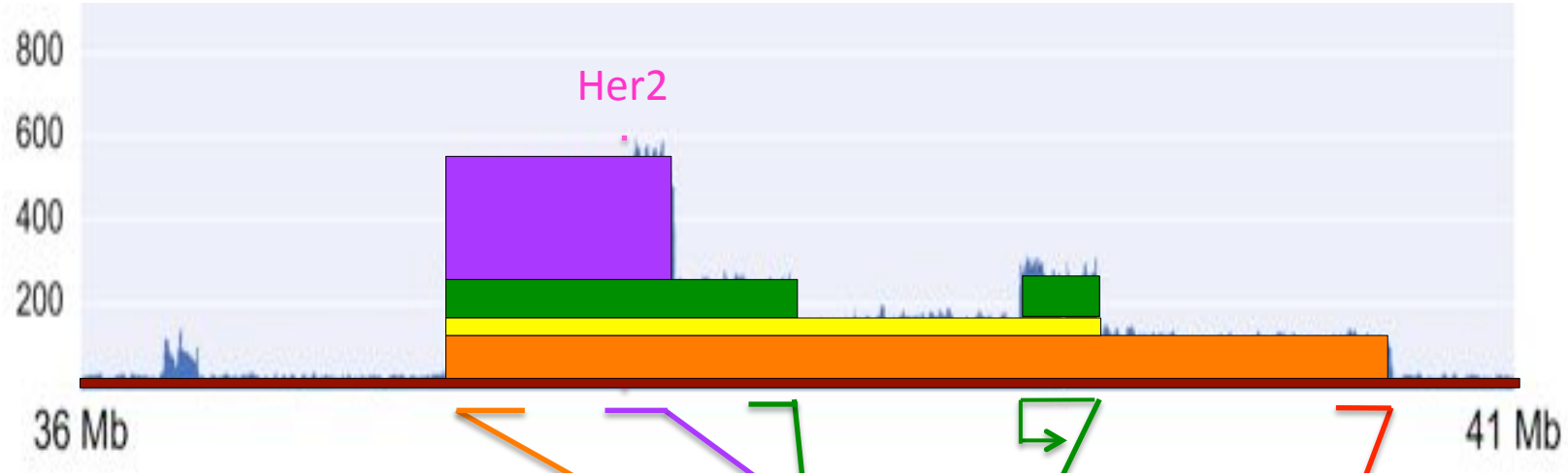
SplitThreader

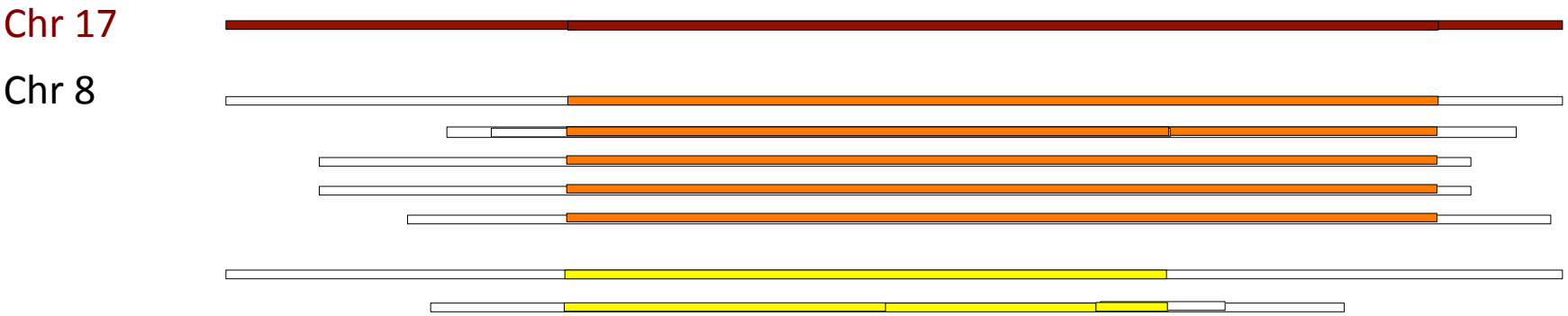
Threading SV breakpoints to
Infer the history of rearrangements in complex genomes



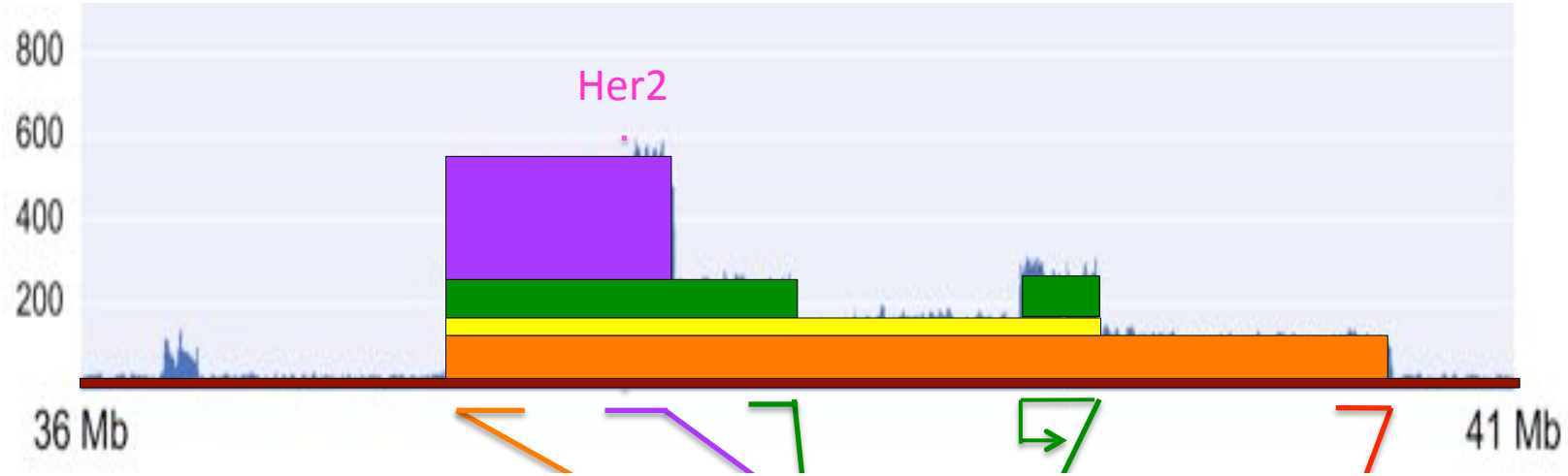






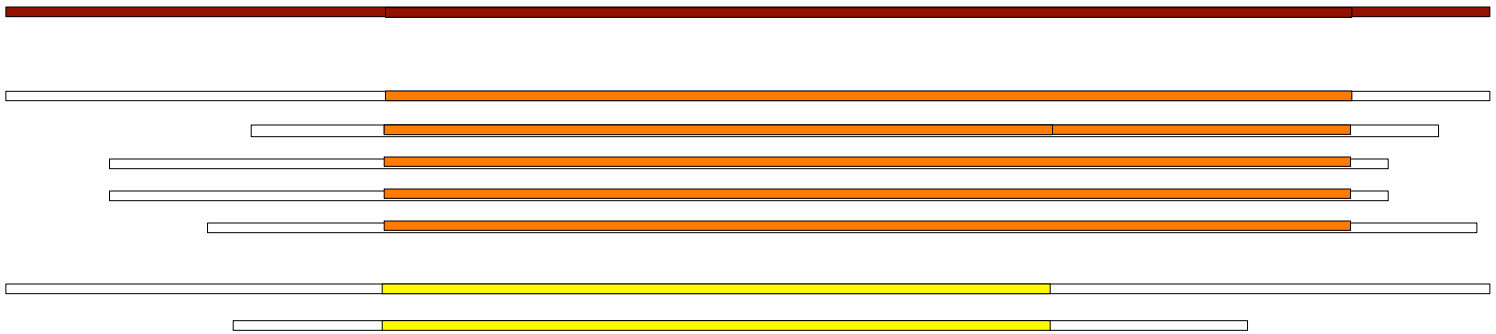


1. Healthy chromosome 17 & 8
2. Translocation into chromosome 8
3. Translocation within chromosome 8
4. Complex variant and inverted duplication within chromosome 8
5. Translocation within chromosome 8



Chr 17

Chr 8



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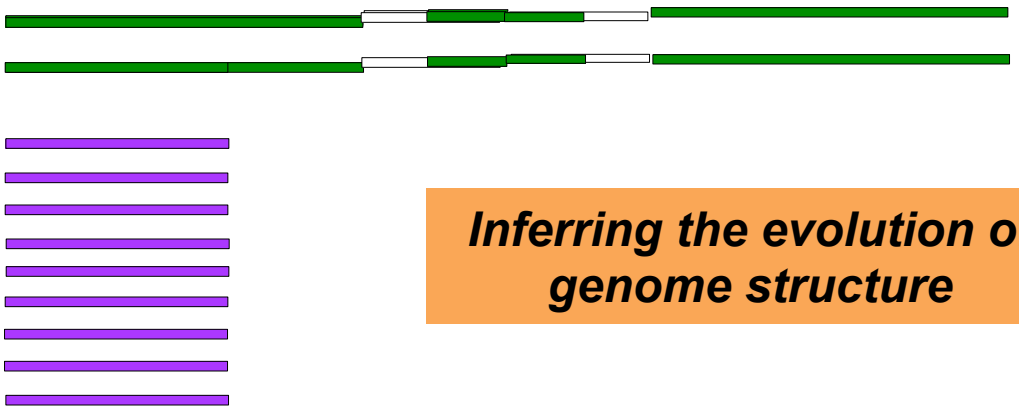

Inferring the evolution of genome structure

Summary & Acknowledgements

GitHub

ABVC + SplitThreader by Maria Nattestad

- Assembly-based variant analysis is efficient and accurate
  - 10s of thousands variants present in mammalian-sized genomes
- SplitThreader infers the evolution to genome structure
  - Additional context as genes are moved next to new promoters and other regulatory elements

NGM-LR + Sniffles by Fritz Sedazeck

- Correct long read mapping is essential for SV analysis
  - Design the mapping strategy for the error model of the data
- Integrate all available information for robust SV calling
  - Currently extending to other long-range mapping technologies: Oxford Nanopore, BioNano, 10X Genomics

Special thanks to Dick McCombie (CSHL), John McPherson (OICR), PacBio

Funding by NSF, NIH, DOE, Sloan Foundation

Thank you!

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