Reference-quality diploid genomes without *de novo* assembly

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PAG Bioinformatics Workshop

@mike_schatz / #PAGXXVI

Why NOT *de novo*?

De novo is necessary for the first genome of a species, but is it really necessary for genomes 2 through N?

1. ***De novo is slow:*** Large inputs and intermediate files. Algorithms are complex to compare all the reads to each other. Mammalian genomes need thousands of core hours and terabytes of space

2. ***De novo is demanding:*** Make the libraries just right, sequence the reads just right, set the parameters just right, launch and relaunch to optimize

3. ***De novo is unpredictable:*** Add a little more (or a little less) data, and your contig N50 drops in half. Errors creep in ranging from SNPs to SVs. Heuristics break when the data or genome structure are unexpected.

4. ***De novo is just the beginning.*** Annotation from scratch is really hard, and to use it (variant analysis/selection analysis/regulatory analysis) you will probably need to align to a reference anyways.

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2. ***D…*** …eads ju… …ize

3. ***D…*** …nd y… …SVs. H… …d.

4. ***D…*** …ard, an… …you will probably need to align to a reference anyways.

Wouldn't it be great if we could assemble high quality genome sequences and capture all the genetic diversity in a species without this complexity?

Reference-Guided Assembly

1. ***High quality reference***
   - Contig N50 over 1Mbp
   - Scaffold N50 over 10Mbp
   - High Quality Gene Annotation (See VGP definition)
   - Your sample is sufficiently similar (~99% identity)

2. ***Sample specific data***
   - *SNPs and Indels*: Illumina-based (Illumina PE or 10X)
   - *Structural Variants*: Long Reads (PacBio or ONT)
   - *Phasing Data*: 10X and/or HiC; trios when available

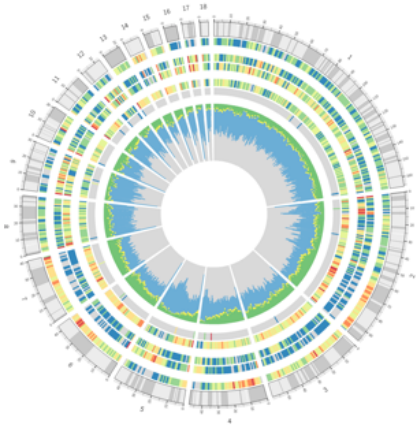
Data requirements similar to de novo, but less demanding, more accurate, and more predictable

Comparative Genome Assembly ("AMOScmp")

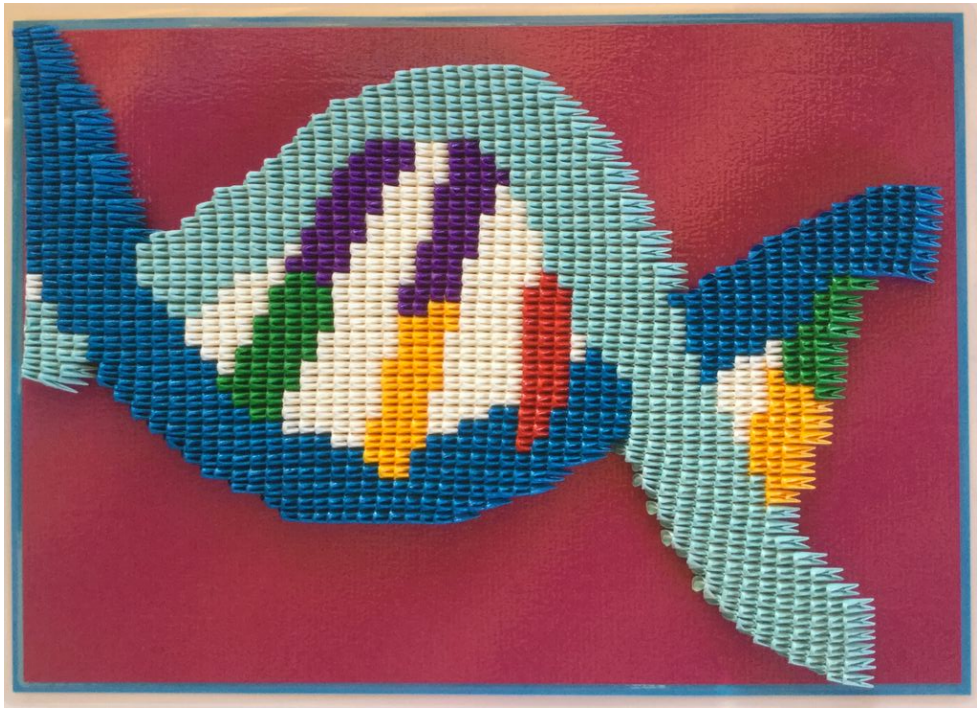
Pop *et al* (2004) *Briefings in Bioinformatics. Sep;5(3):237-48.*

CrossStitch

https://github.com/schatzlab/crossstitch



HQ Reference



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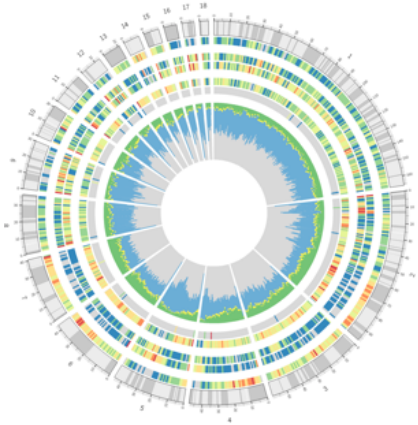

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Call SNPs
+ Indels

A/C -> A|C

Phase SNPs
+ Indels

GAT
-> XXX

Call SVs

GAT/XXX
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Phase SVs
& Local Assembly

Phased
SNPs,
Indels &
SVs.vcf

Assemble
Diploid
Sequence

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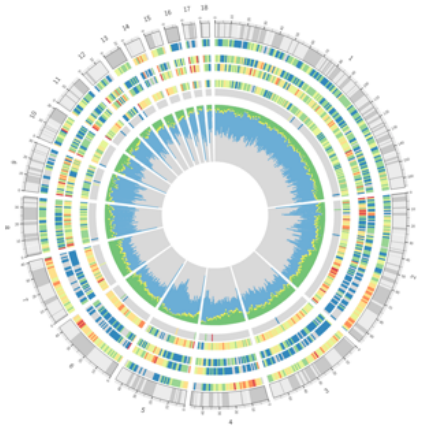

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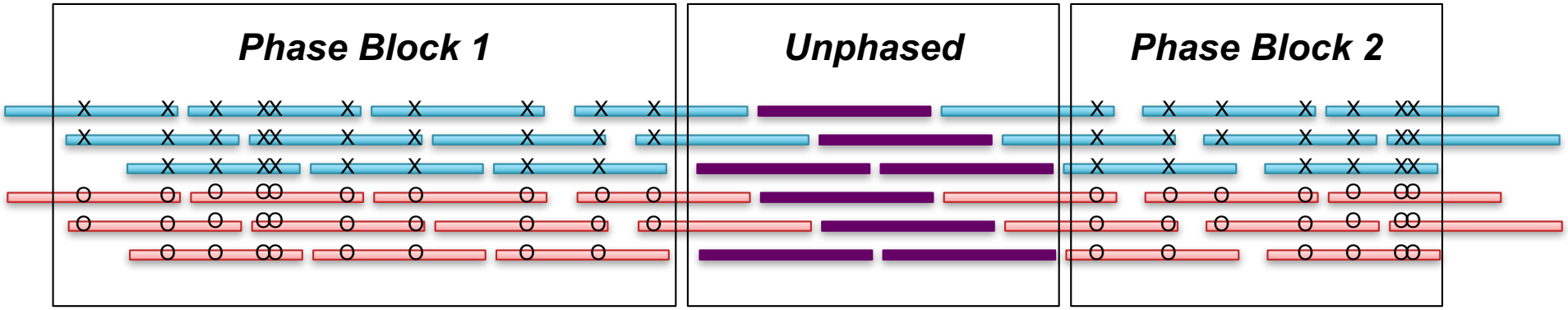
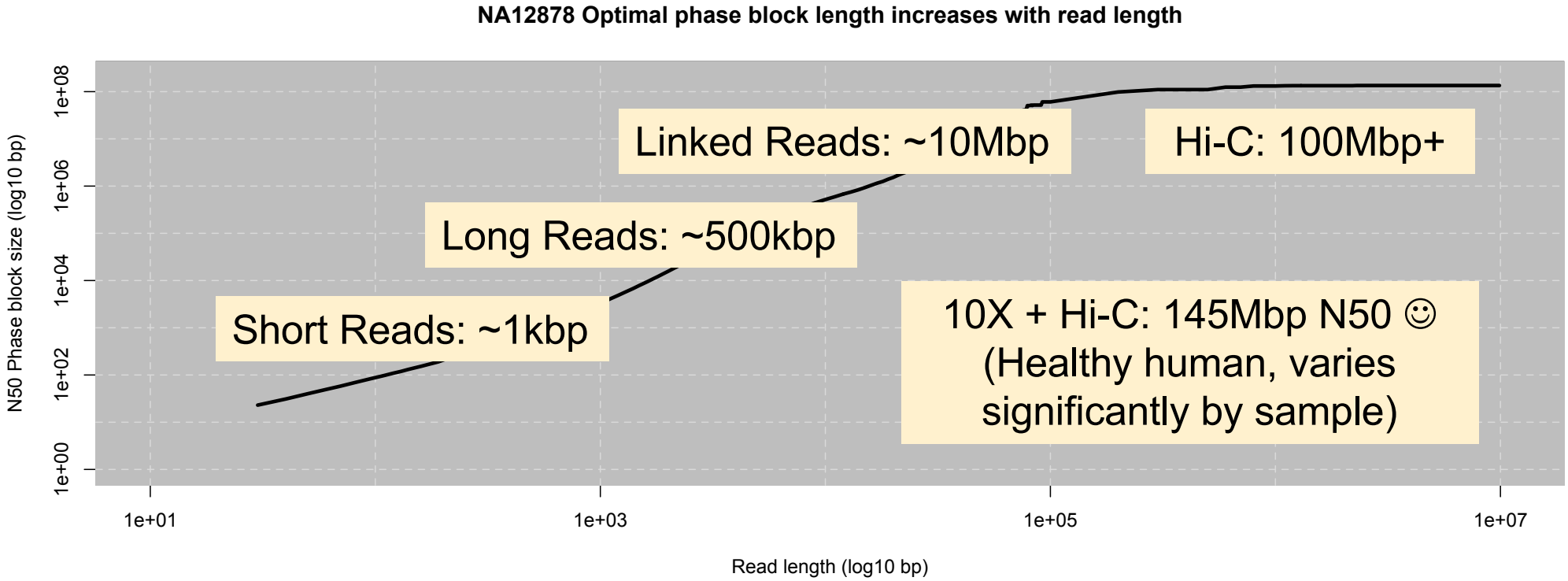
Phasing Results





HapCUT2: robust and accurate haplotype assembly for diverse sequencing technologies
Edge, P, Bafna, V, Bansal, V (2016) *Genome Research*. doi: 10.1101/gr.213462.116

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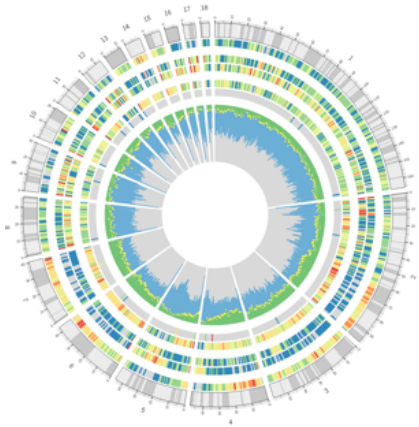

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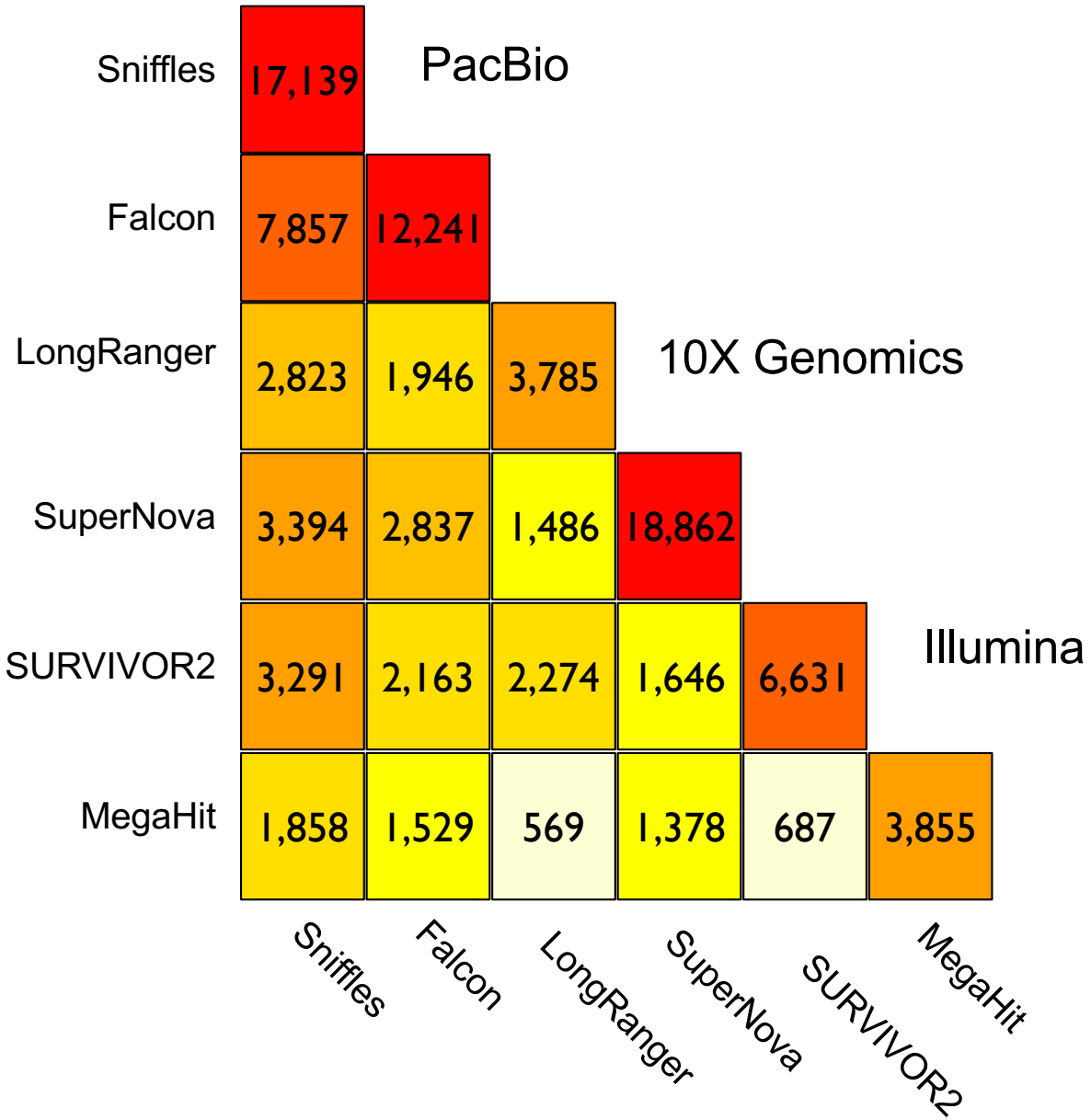
SVs using Short, Long and Linked Reads



Main Diagonal

- Calls per tool

Outer triplets

- Concordance by Technology

Inner triplets

- Concordance by Assembly
- Concordance by Mappers

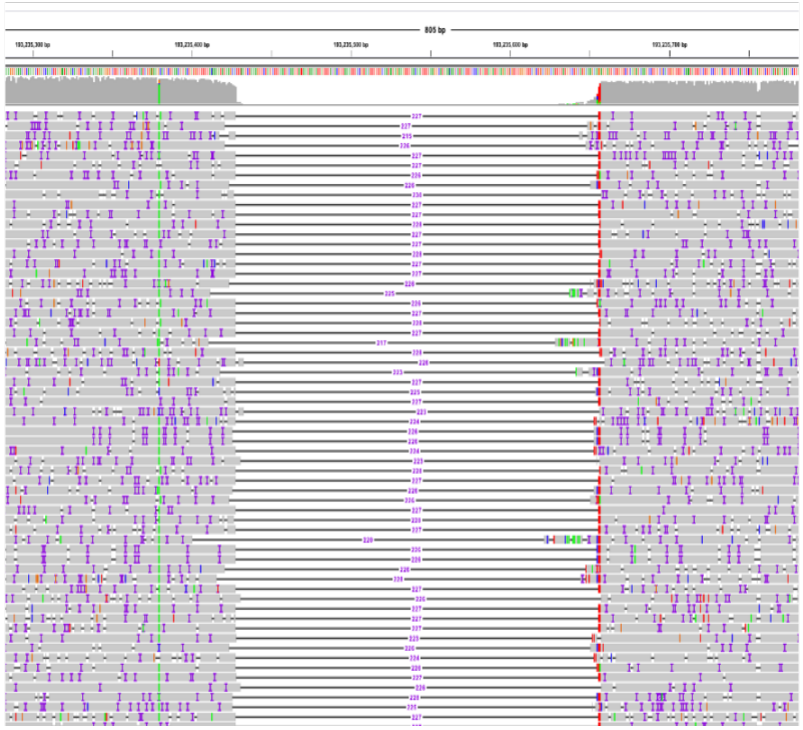
Overall:

- Lonnnnnnng reads give the most variants with the best concordance ☺

NGMLR + Sniffles

BWA-MEM:

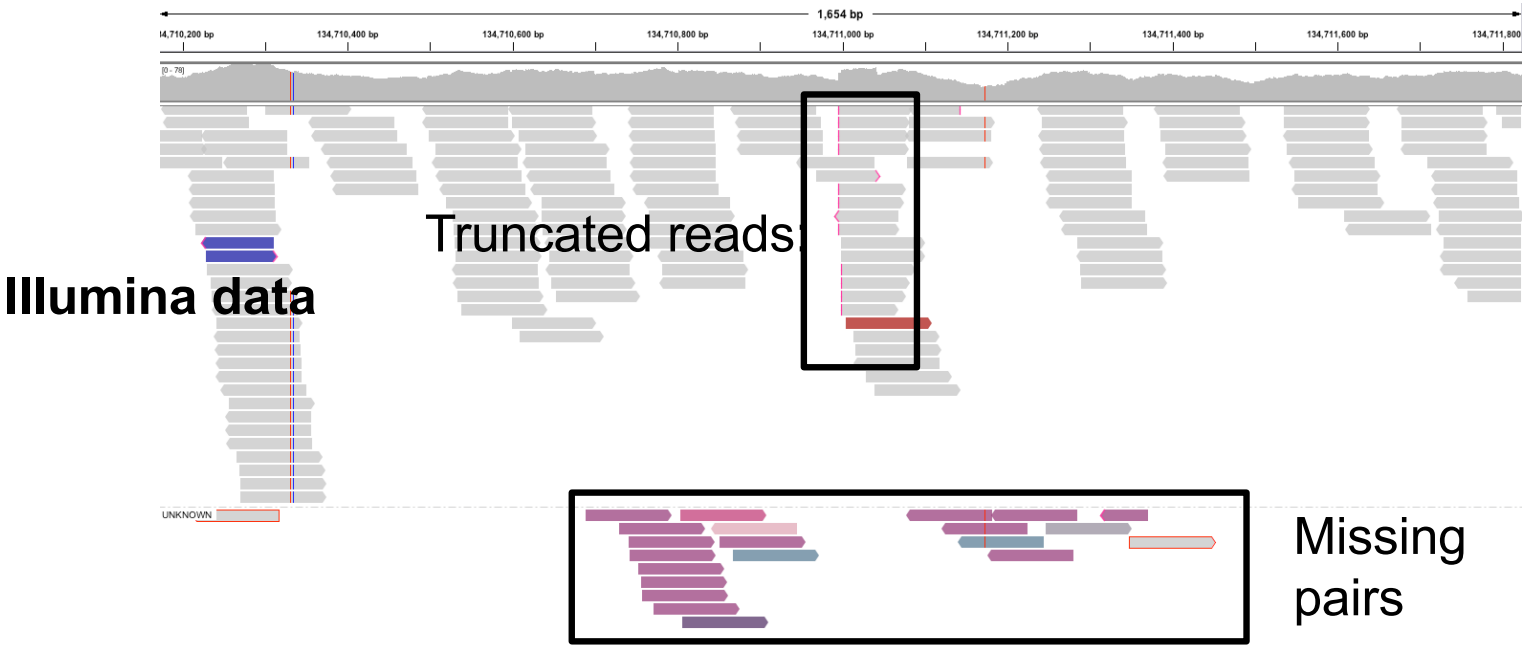
NGMLR:



NGMLR: Convex gap penalty to balance frequent small sequencing errors with larger SVs
Sniffles: Scan within and between split reads to accurately find SVs (Ins, Del, Dup, Inv, Trans)
Mendelian concordance >95%, experimental validation also very high

Accurate detection of complex structural variations using single molecule sequencing
Sedlazeck, Rescheneder et al (2017) *bioRxiv https://doi.org/10.1101/169557*

No more false positives!



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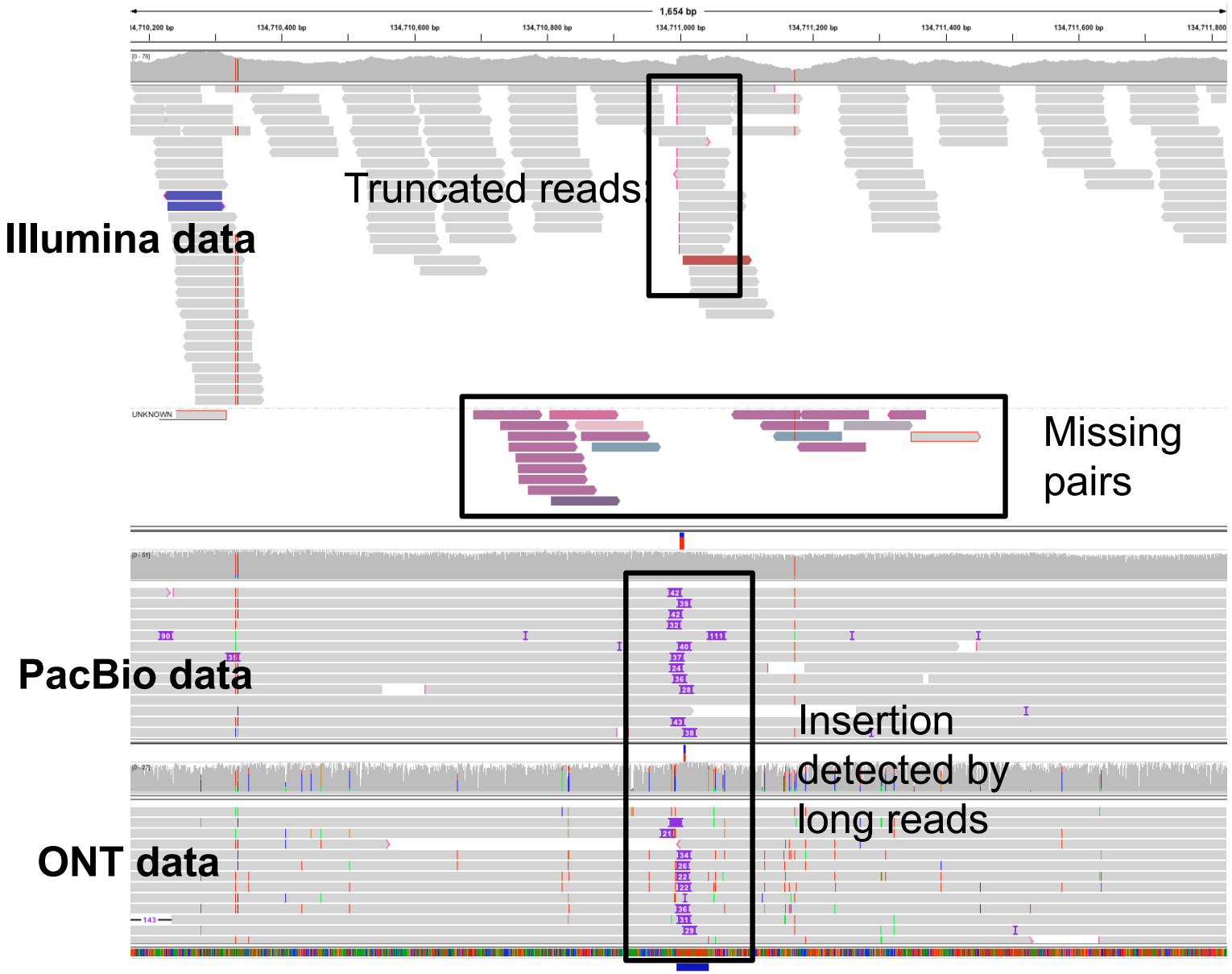

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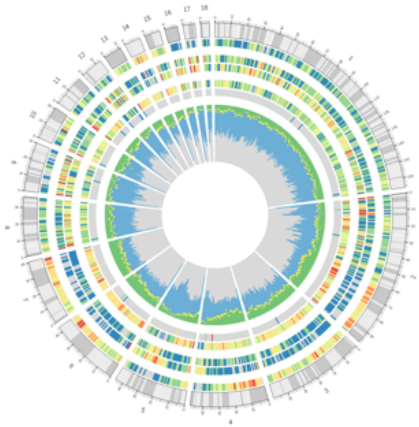

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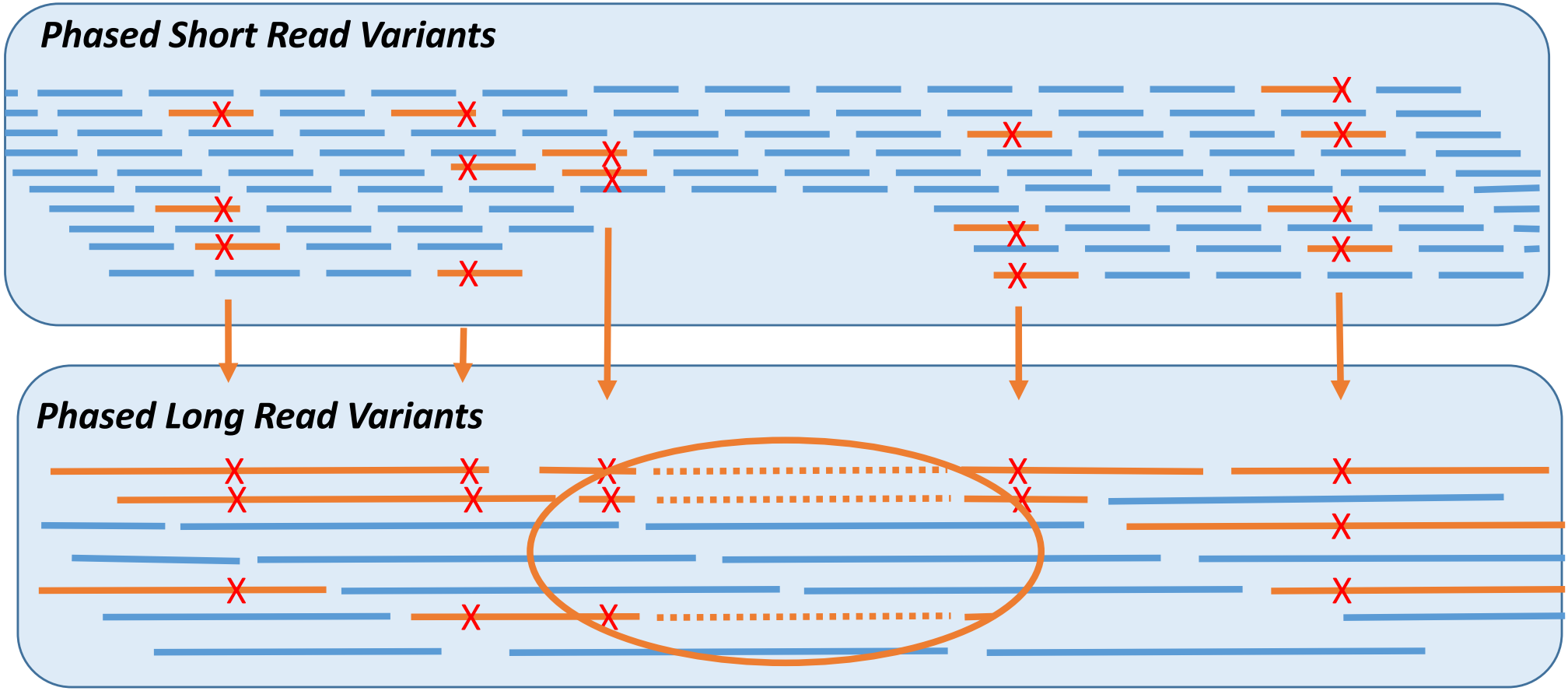
Local Assembly and SV Phasing

Transfer the phasing of the short read variants to the long reads
The phased long reads allow the SVs to be phased



Phase SVs: Make sure SVs are associated with the correct haplotype
Local Assembly: Refine sequence of insertions, resolve complex nested variants

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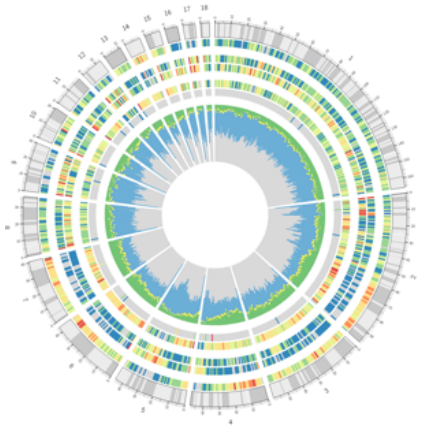

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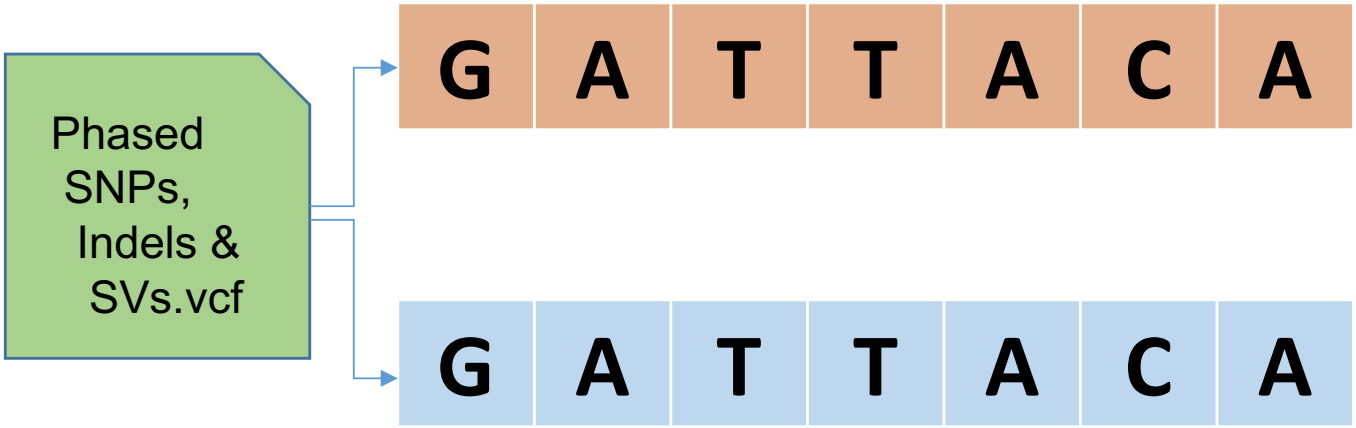
Assembling a "Perfect" Personalized Diploid Genome

Carefully "stitch" the phased variants into the reference genome at the right position to create a pair of phased chromosome fasta files



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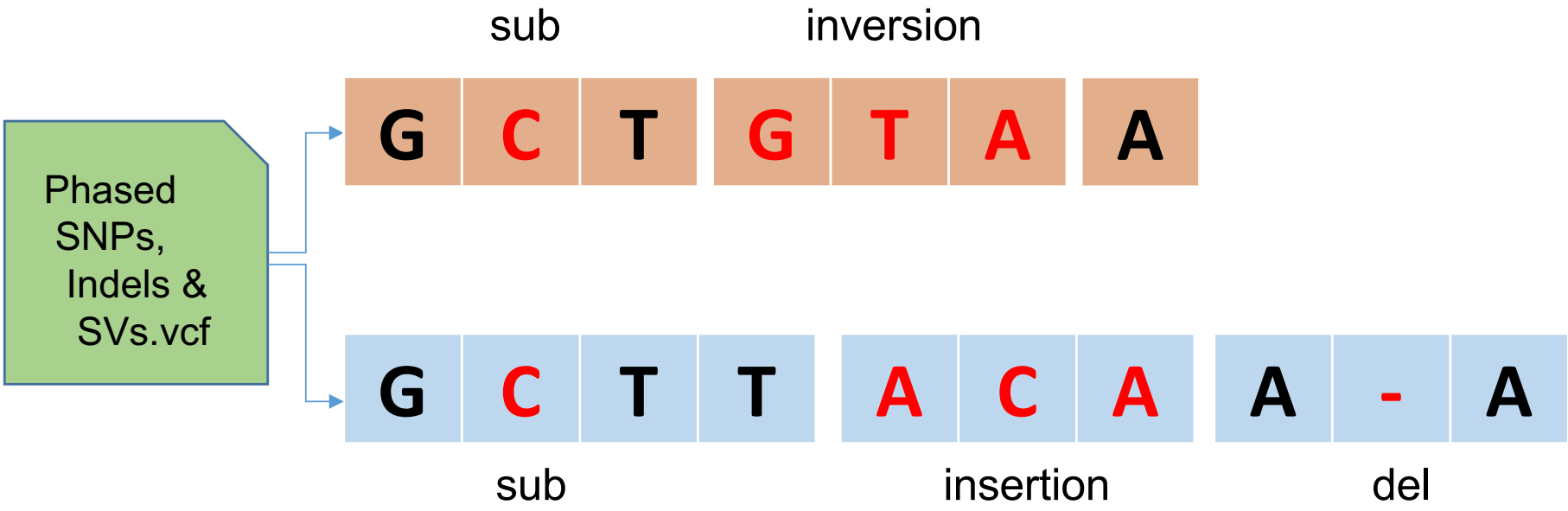

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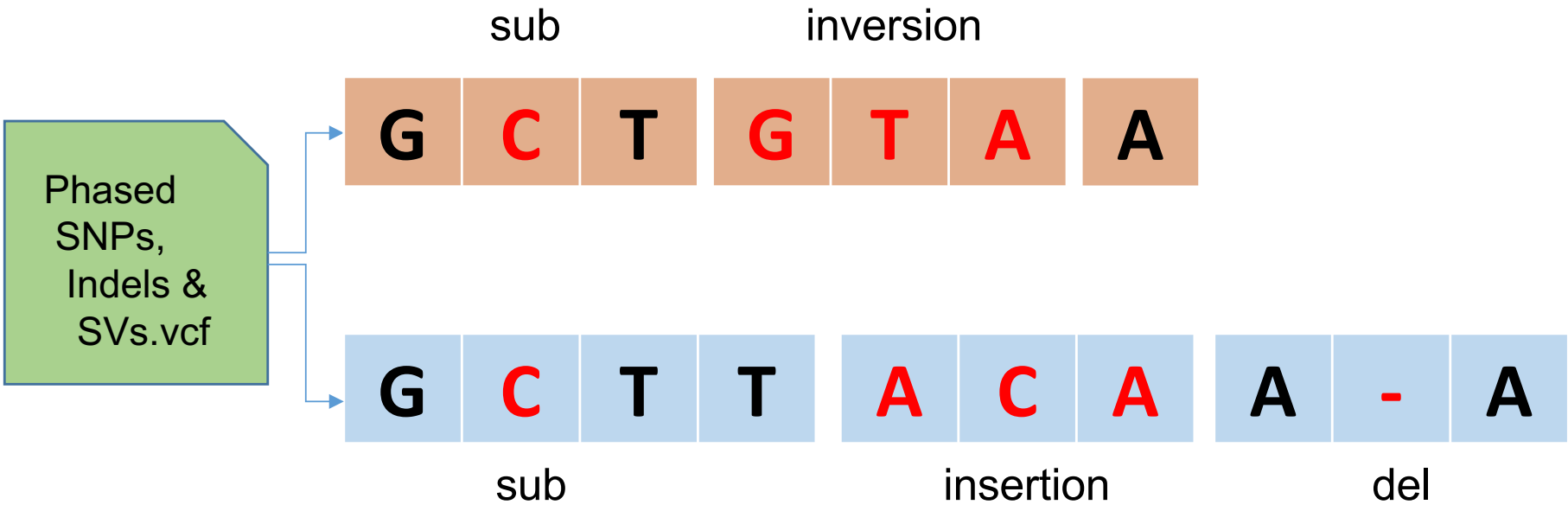


Stitching based on AlleleSeq pipeline enhanced for SVs (Rozowsky et al, 2011)

- Maintains a mapping from reference to personal genome coordinates to make lift over of annotation straightforward to compute

Using 10X + HiC + PacBio, assemble essentially perfect diploid human genomes with haplotypes spanning entire chromosomes

- Phased diploid genome can be aligned or aligned against just like a de novo genome assembly

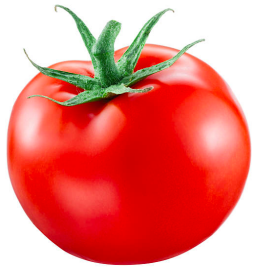
Applications

Expression & Regulation

Foundation for mapping functional data

- Discover novel genes and gene fusions
- Analyze differential expression in CNVs
- Discover new regulatory regions
- Analyze allele-specific expression

Population Genetics



Framework for GWAS of Structural Variations

- Identified SVs in >900 accessions using short reads
- Assembling the top 50 lines using long & linked reads
- Perform GWAS of breeding traits

Polyploidy

Studying heterozygosity in sugarcane

- Have a high quality PacBio-based assembly of POJ2878 using FALCON (140kbp N50)
- Developing new methods for phasing (9-14 copies of each chromosome)

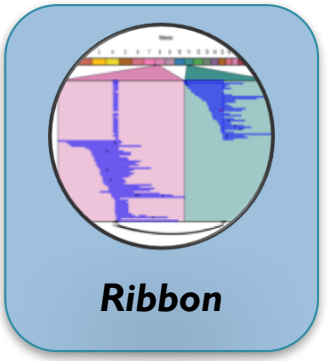
Reference-quality Genomes without *de novo* assembly

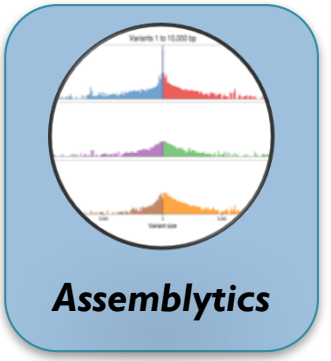
De novo assembly is essential for exploring new species

- Reference-free or mapping to a distant reference is difficult to impossible

But once the first genome of a species has been assembled, shouldn't the second genome be a little easier?

- Use the right combination of data to capture and phase all types of variants
- Overnight analysis to create a high quality personalized genome that are more accurate, more predictable, and easier to use than a de novo assembly
- The personalized diploid genome will be a platform for functional and evolutionary analysis in many species





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Your Name Here

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Fritz Sedlazeck

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CSHL
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Lyon Lab
Martienssen Lab
McCombie Lab
Tuveson Lab
Ware Lab
Wigler Lab

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Sunset Room, 9am-5pm, tomorrow

Thank you!

@mike_schatz