Phased diploid genomes using short, long, and linked reads

Michael Schatz

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PAG G10k Workshop

@mike_schatz / #PAGXXVI

Selected Tools

1. Pre-assembly QC
2. SV Detection & Phasing
3. Post-assembly

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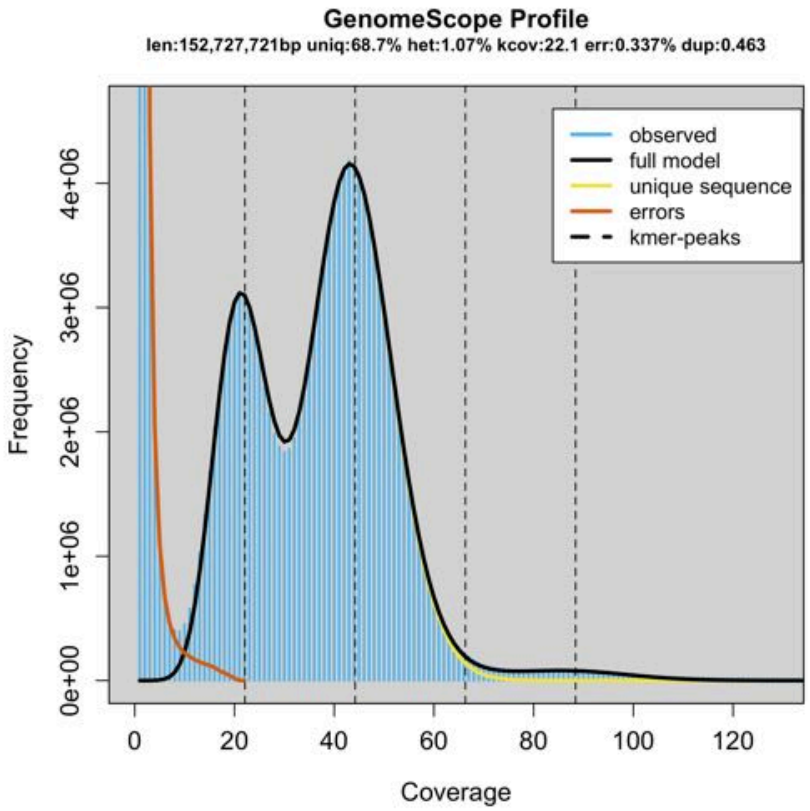
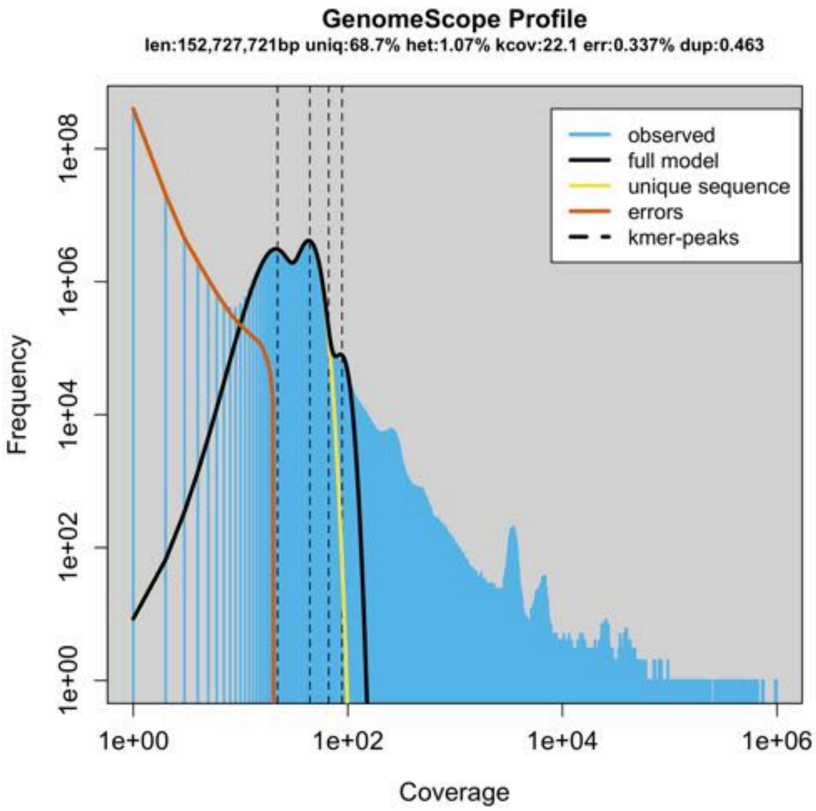
GenomeScope: Fast reference-free genome profiling

http://genomescope.org





Infer the properties of unassembled genomes from raw sequencing data:

- *Genome Size, Repeat Content, Rate of Heterozygosity*
- *Coverage, Read Error Rate, Rate of PCR Duplications*
- *Analysis of polypoid genomes in development*

Vurture *et al.* (2017) *Bioinformatics. doi: https://doi.org/10.1093/bioinformatics/btx153*

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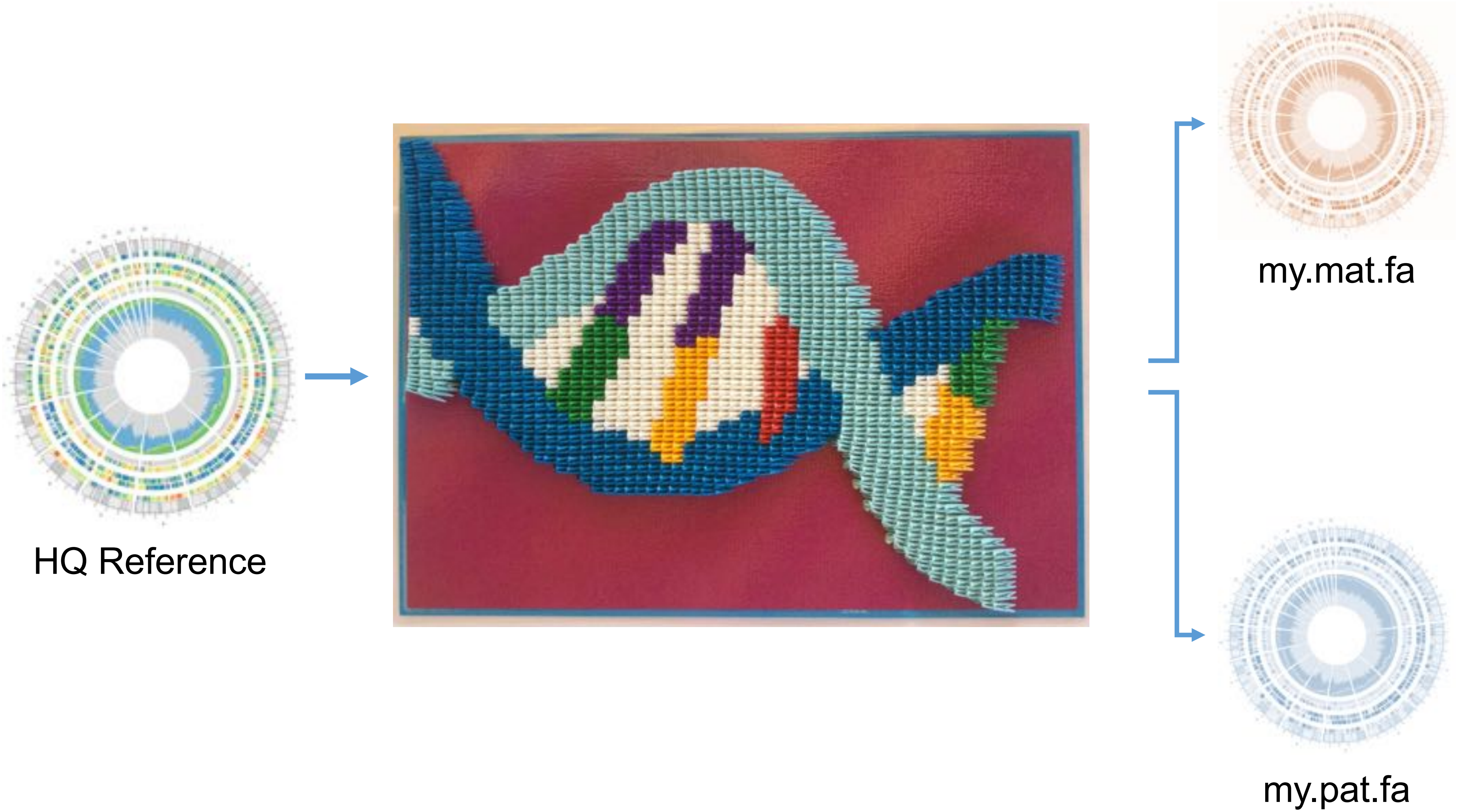
CrossStitch

https://github.com/schatzlab/crossstitch



HQ Reference

my.mat.fa

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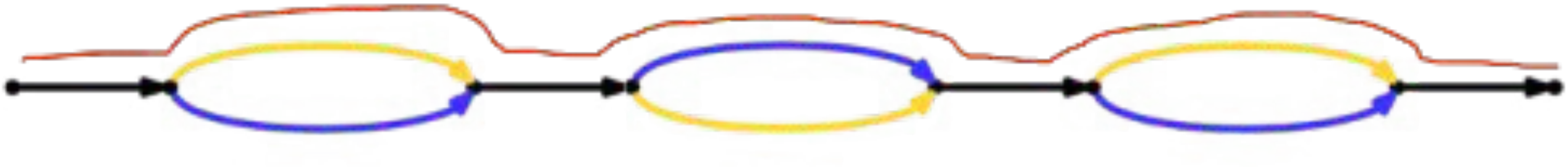
In collaboration with Sedlazeck, Gingeras, Guido, Ring, & Gerstein labs

CrossStitch for de novo assembly



Rather than a reference genome, start from a "pseudohap" draft assembly

- Native output for FALCON and SuperNova (and regular Canu?)
- Will need to be careful to correctly recognize and traverse the bubbles

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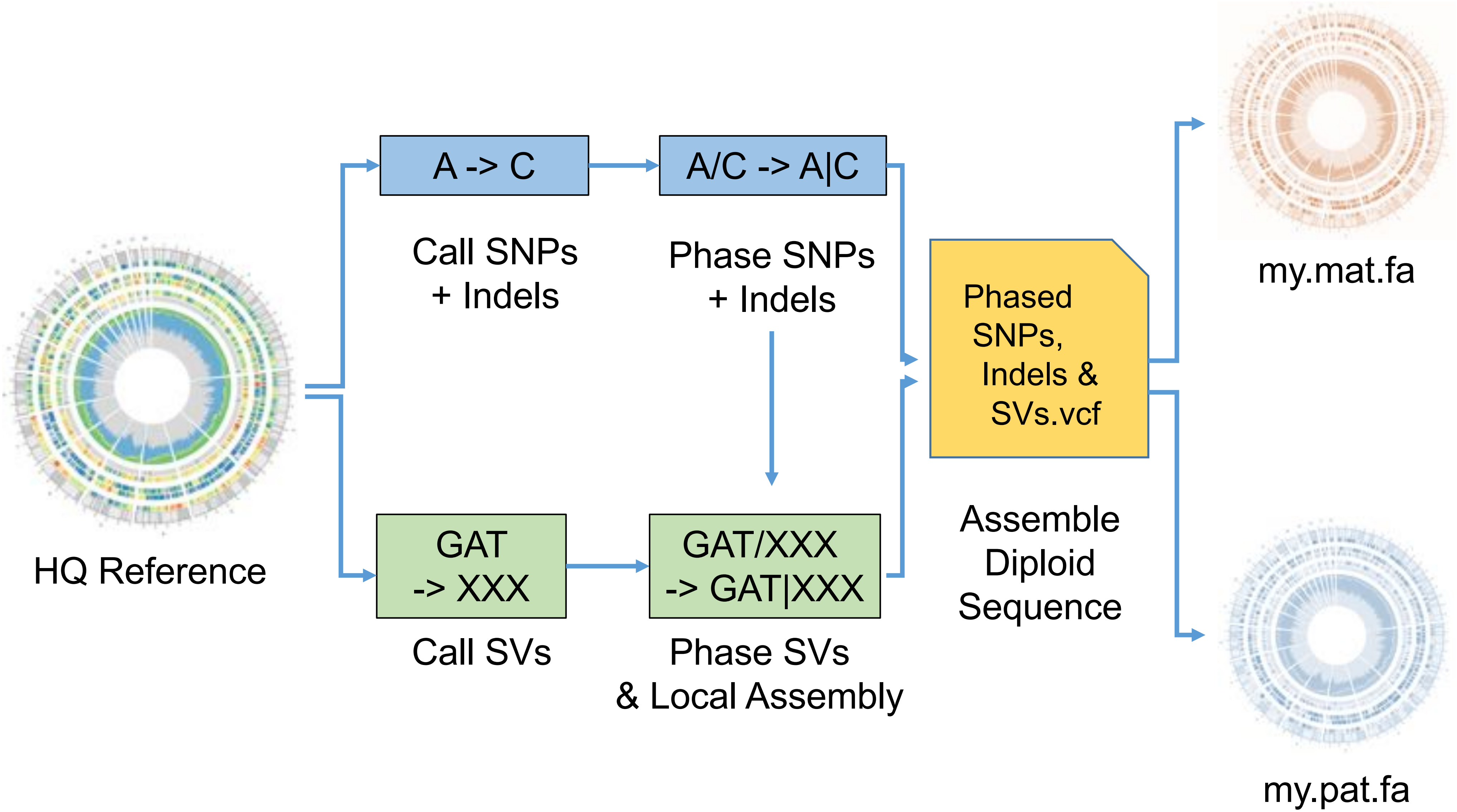

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A -> C

Call SNPs
+ Indels

A/C -> A|C

Phase SNPs
+ Indels

GAT
-> XXX

Call SVs

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Phase SVs
& Local Assembly

Phased
SNPs,
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SVs.vcf

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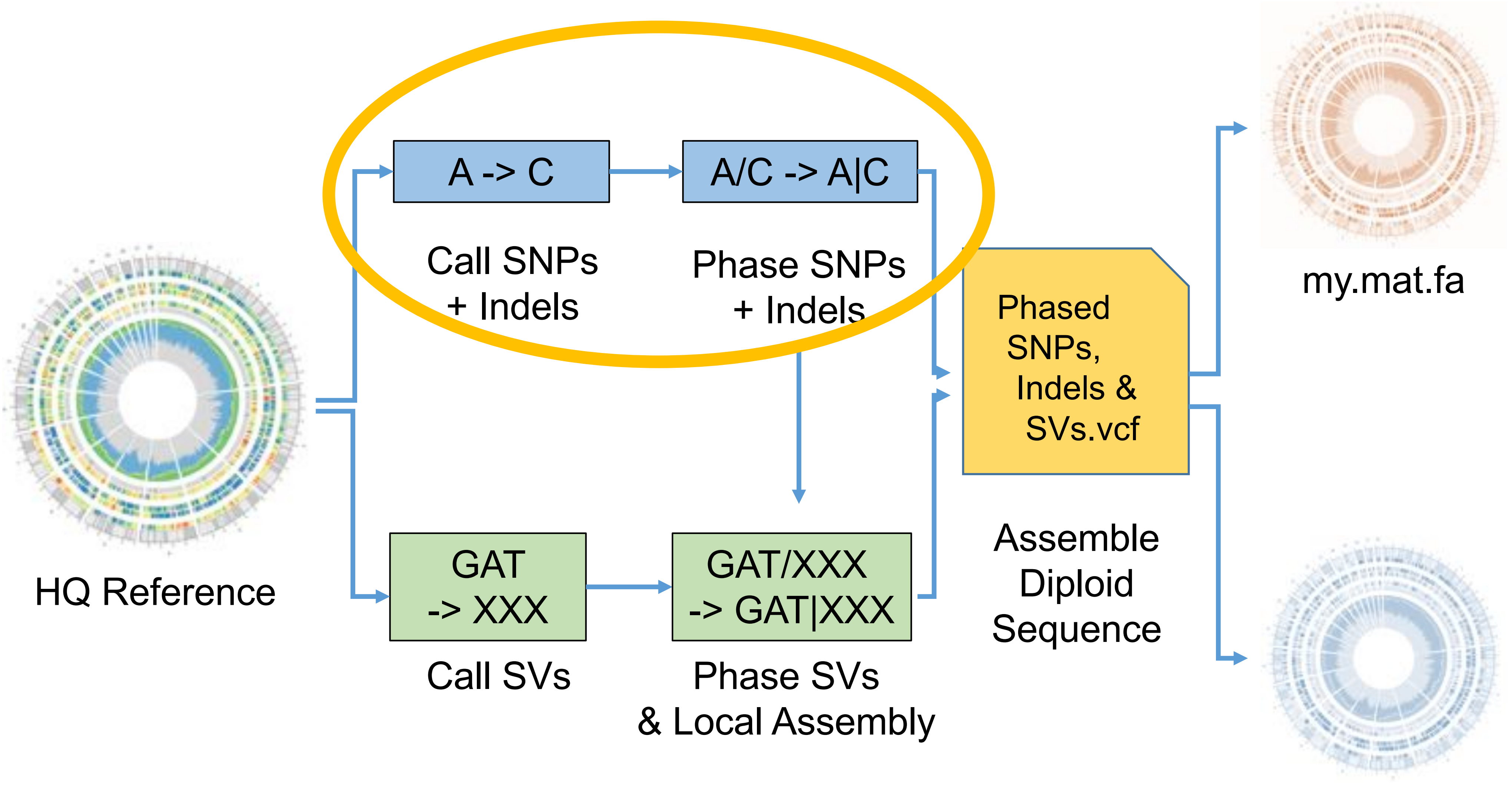

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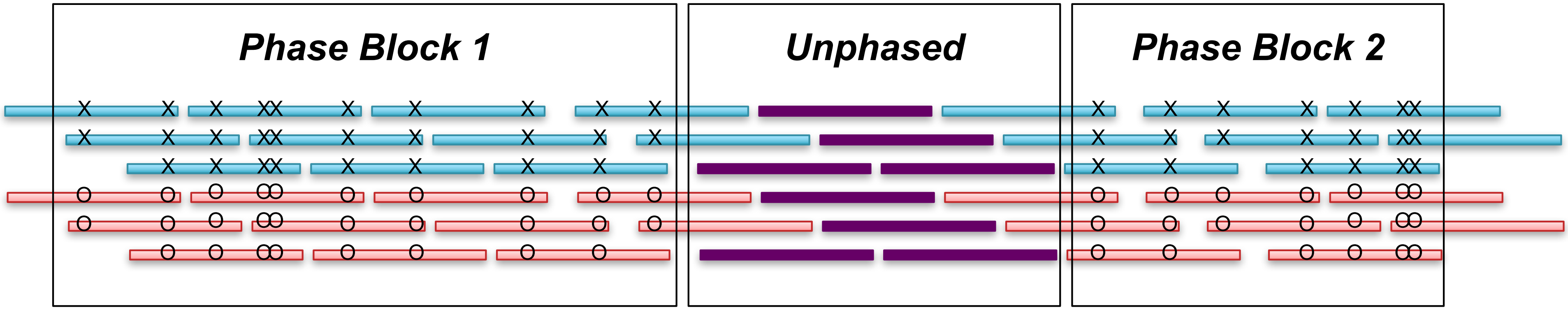
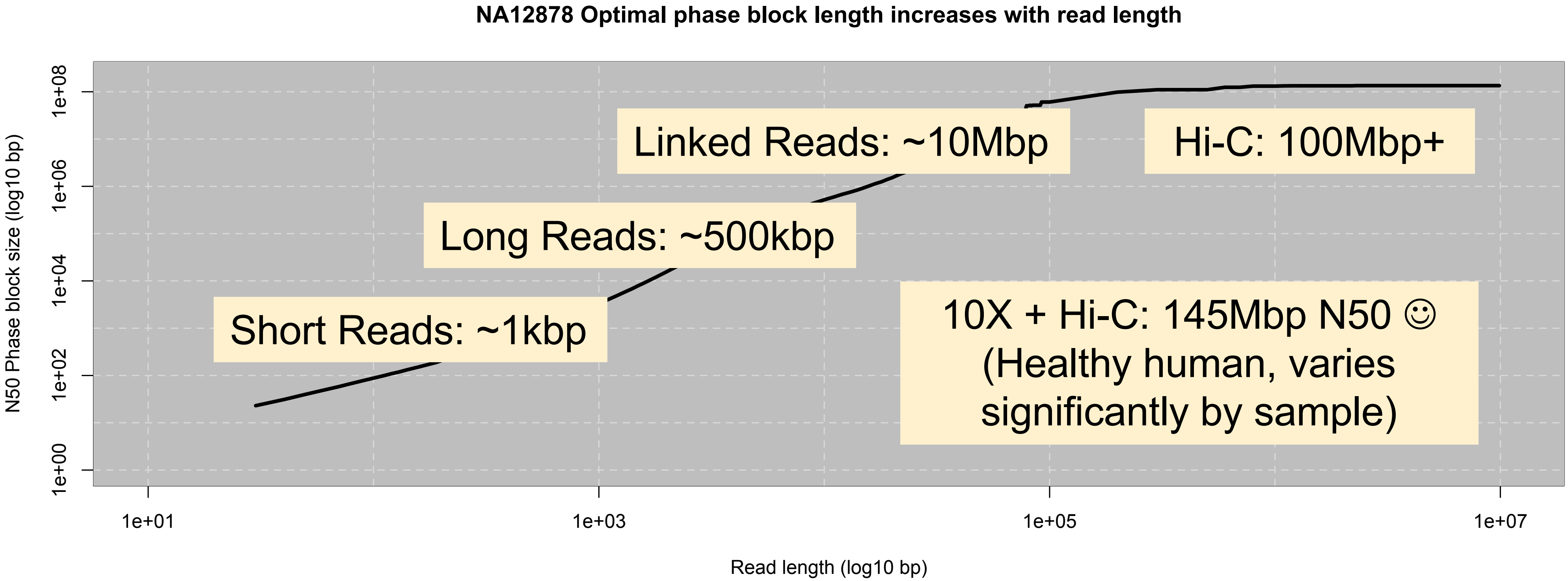
Phasing Results




NA12878 Optimal phase block length increases with read length

Short Reads: ~1kbp

Long Reads: ~500kbp

Linked Reads: ~10Mbp

Hi-C: 100Mbp+

10X + Hi-C: 145Mbp N50 ☺
(Healthy human, varies significantly by sample)

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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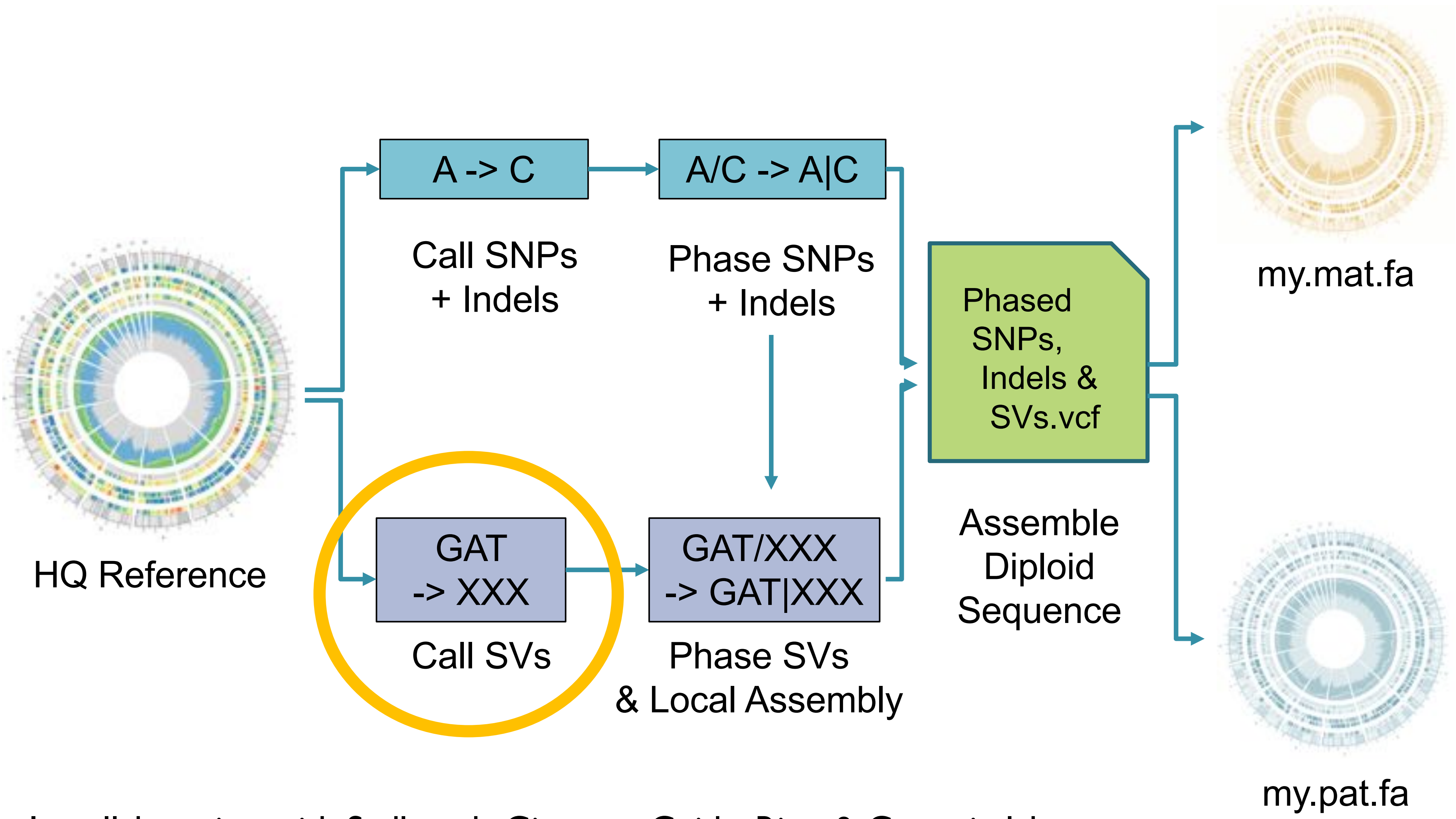

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SVs using Short, Long and Linked Reads

	Sniffles	Falcon	LongRanger	SuperNova	SURVIVOR2	MegaHit
Sniffles	17,139					
Falcon	7,857	12,241				
LongRanger	2,823	1,946	3,785			
SuperNova	3,394	2,837	1,486	18,862		
SURVIVOR2	3,291	2,163	2,274	1,646	6,631	
MegaHit	1,858	1,529	569	1,378	687	3,855

PacBio

10X Genomics

Illumina

Main Diagonal

- Calls per tool

Outer triplets

- Concordance by Technology

Inner triplets

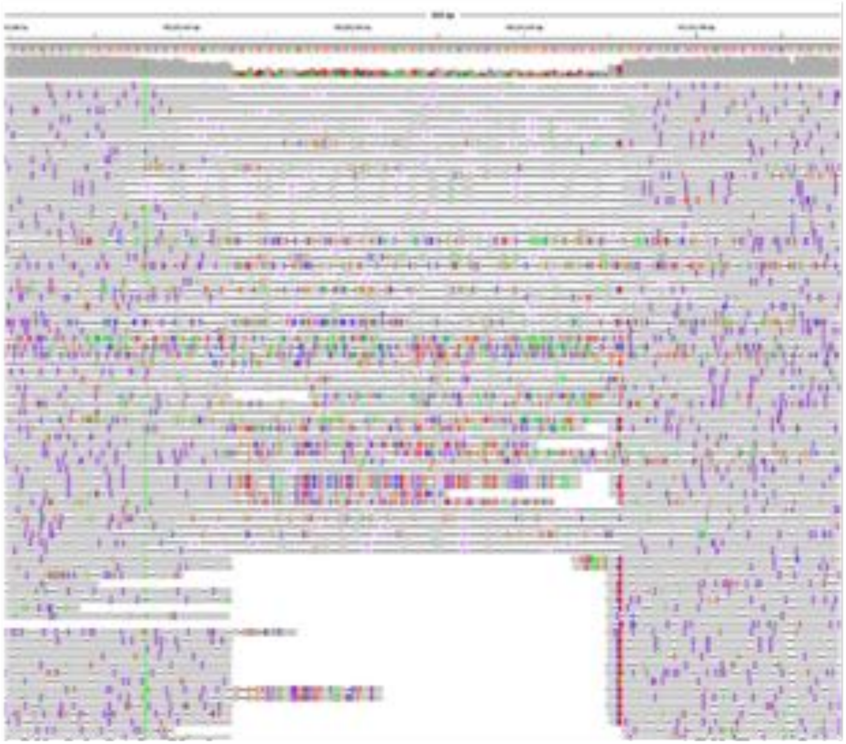
- Concordance by Assembly
- Concordance by Mappers

Overall:

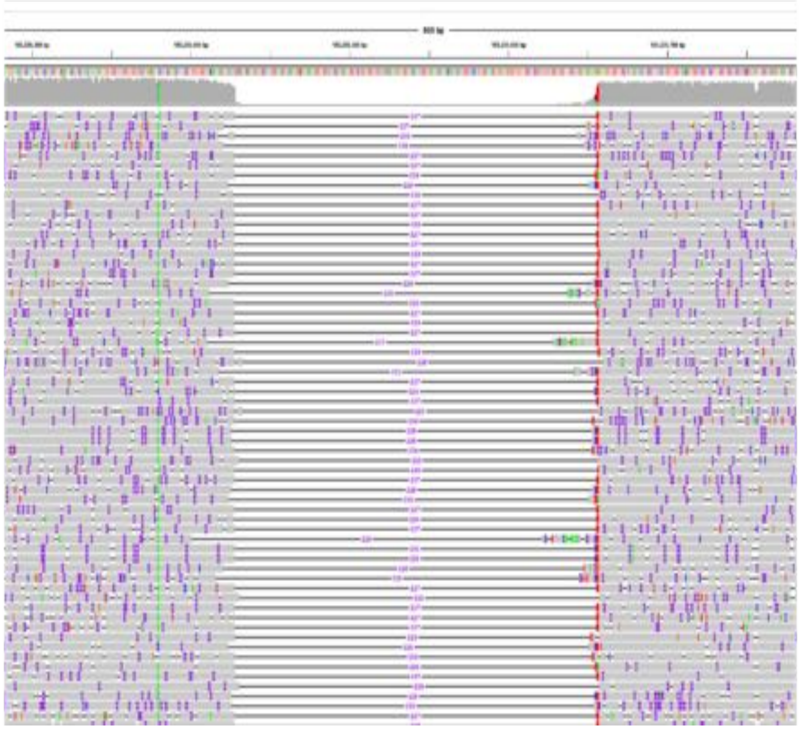
- Lonnnnnnng reads give the most variants with the best concordance ☺

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*

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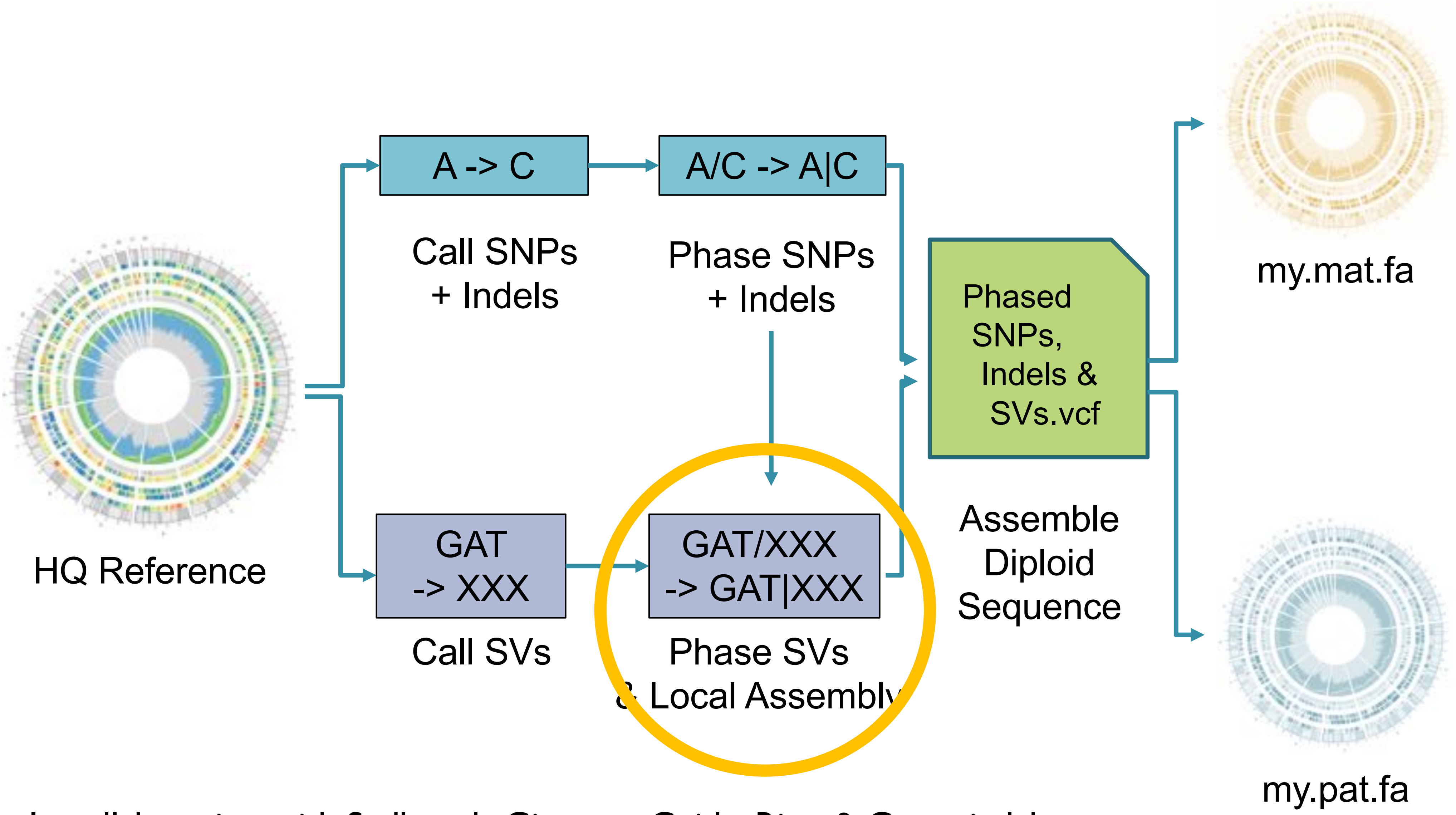

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Phase SVs & Local Assembly

Phased SNPs, Indels & SVs.vcf

Assemble Diploid Sequence

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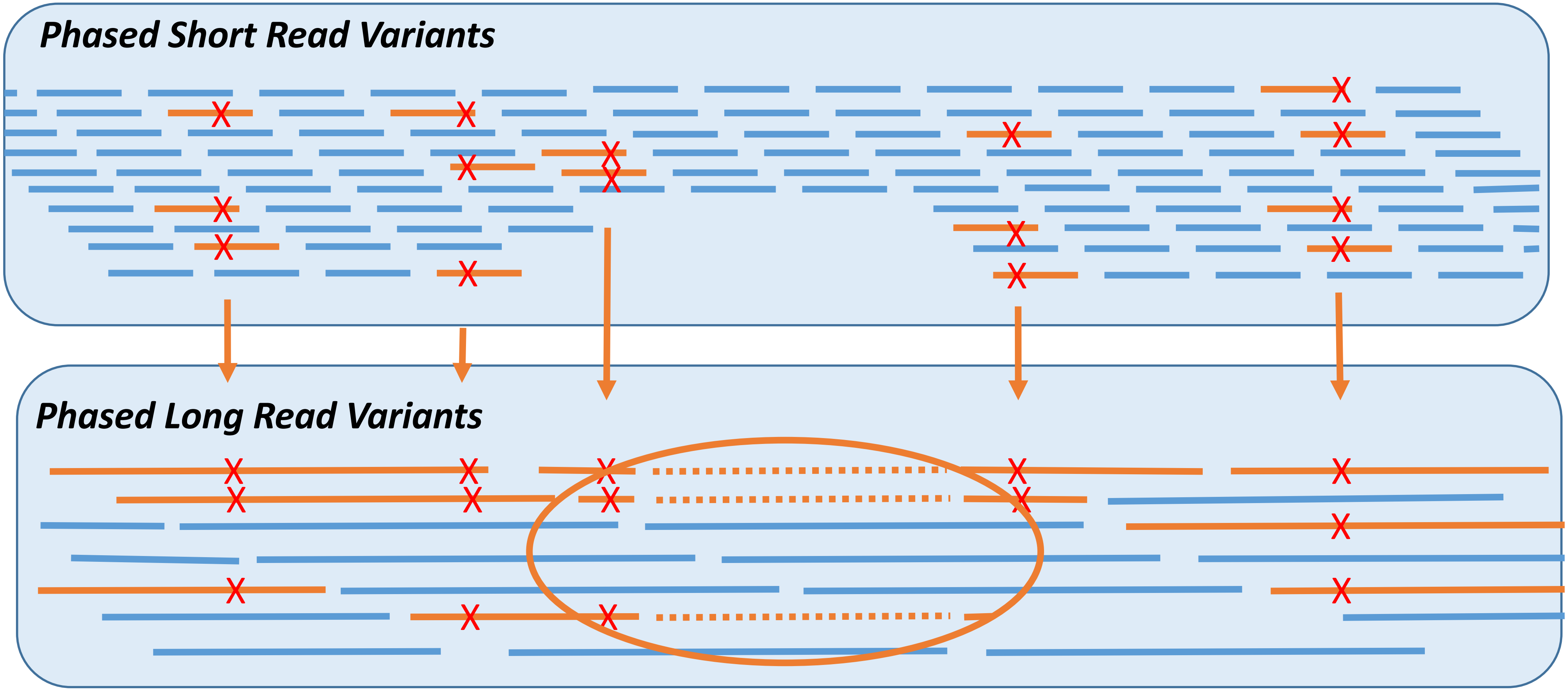
Local Assembly and SV Phasing

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

CrossStitch

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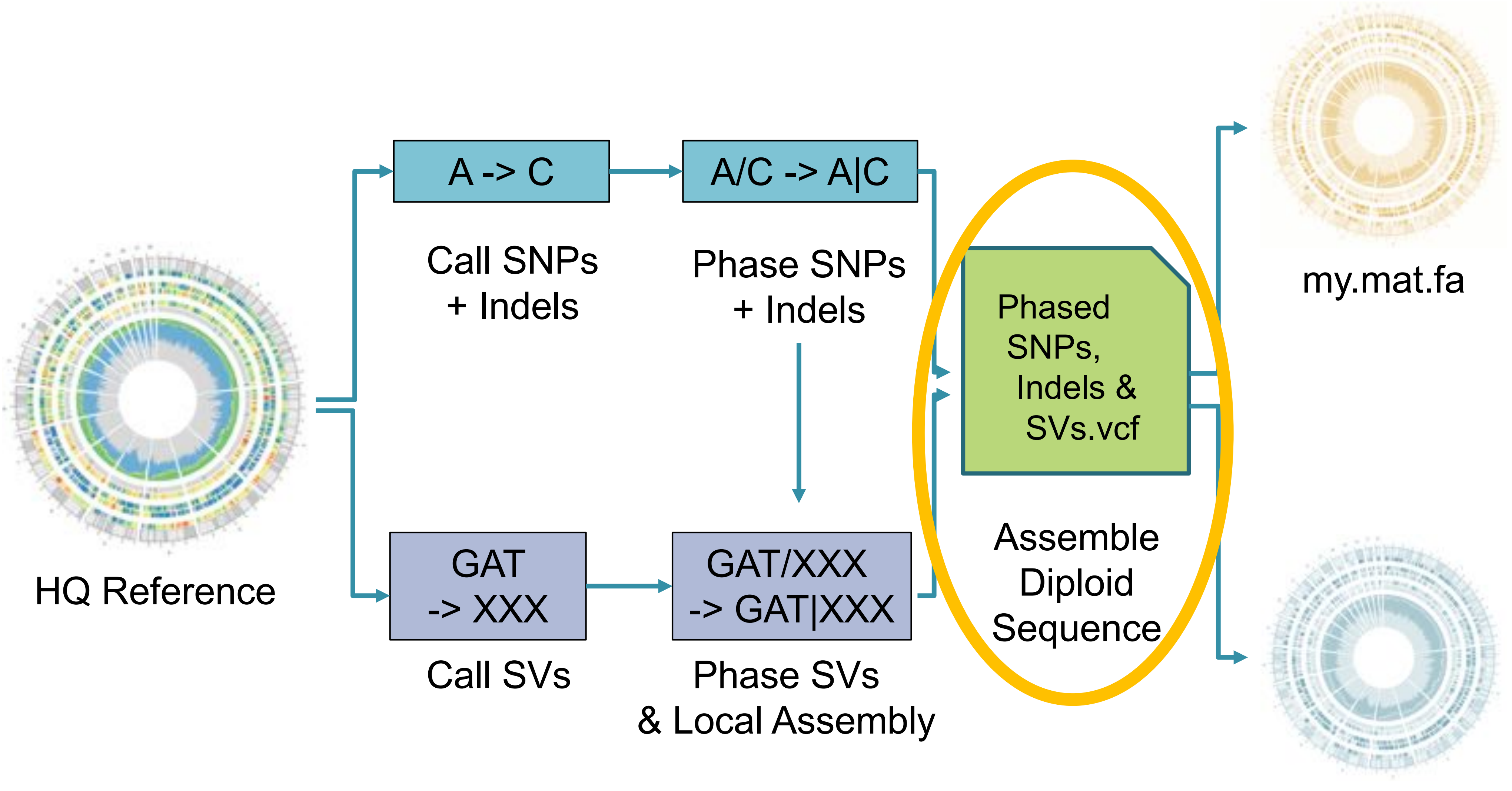

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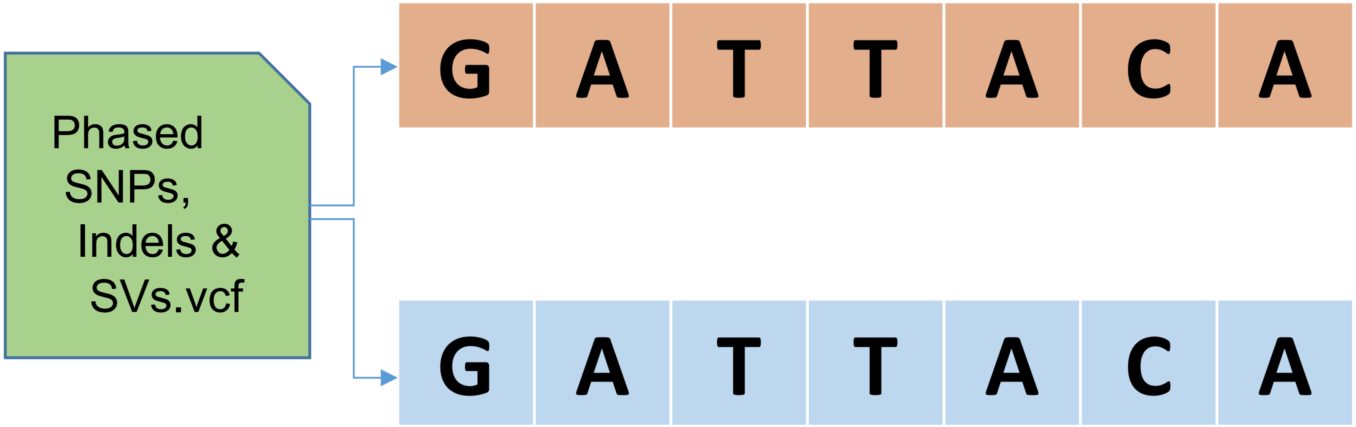
Assembling a “Perfect” Personalized Diploid Genome

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



Phased SNPs, Indels & SVs.vcf

G	A	T	T	A	C	A

G	A	T	T	A	C	A

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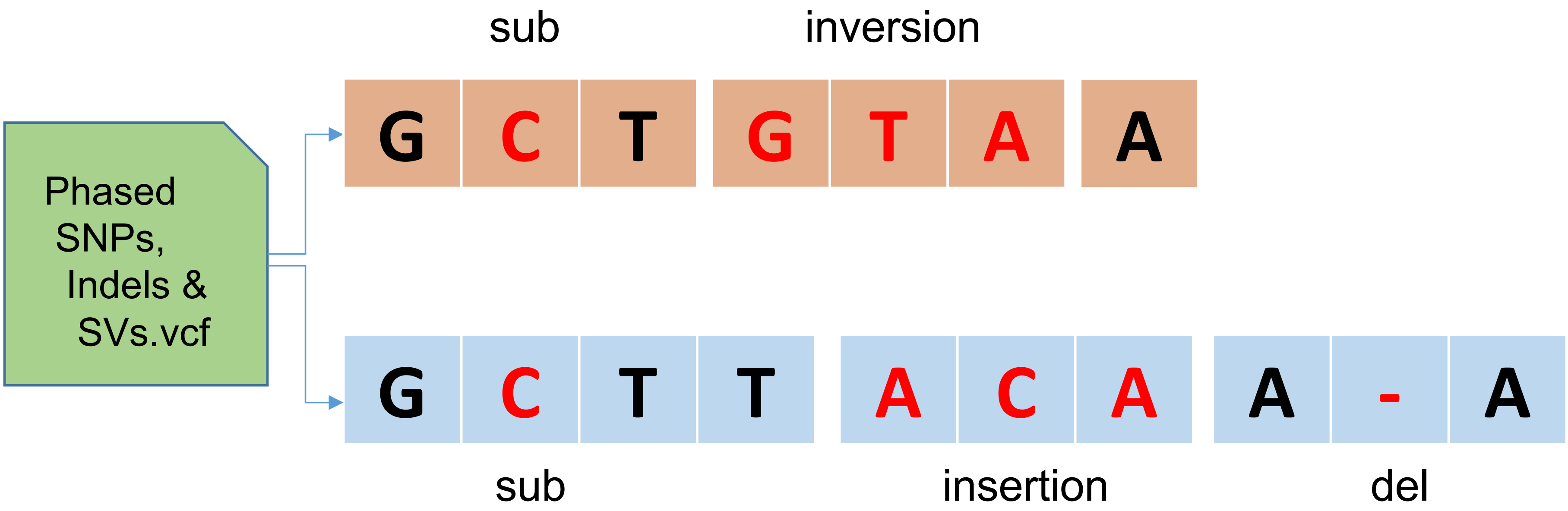

Phased SNPs, Indels & SVs.vcf

sub inversion

G C T G T A A

G C T T A C A A - A

sub insertion del

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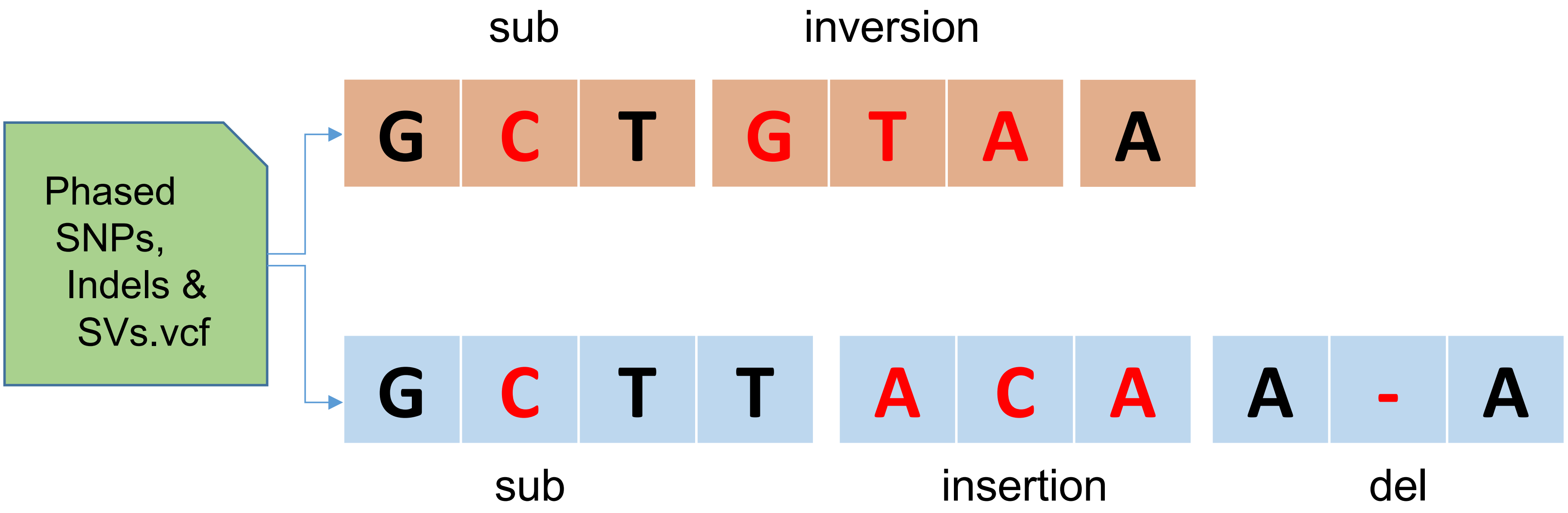

Stitching based on AlleleSeq pipeline enhanced for SVs (Rozowsky et al, 2011)

- 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

ENCODE

MaizeCODE

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)

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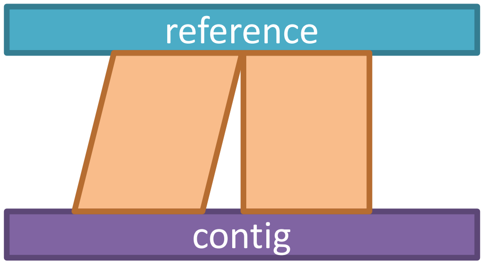
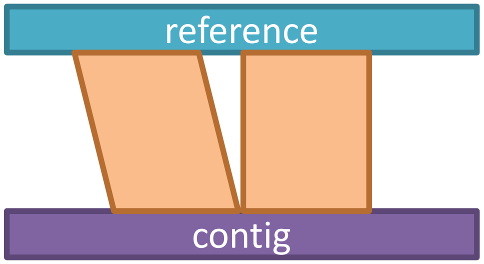
Assemblytics: Assembly-Based Variant-Caller

Defined point

Insertion

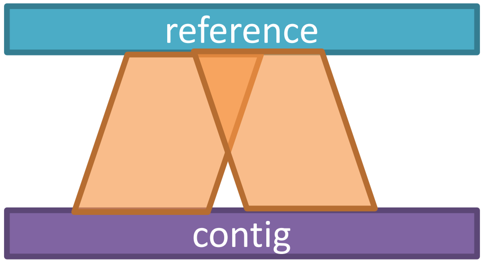


Deletion

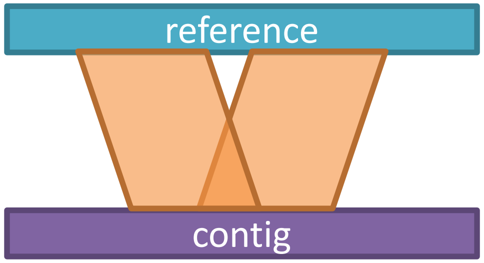


Overlapping alignments suggest tandem repeat

Tandem Expansions

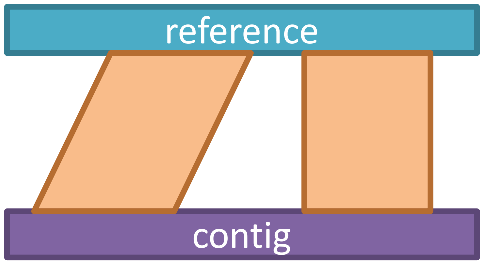


Tandem Contractions

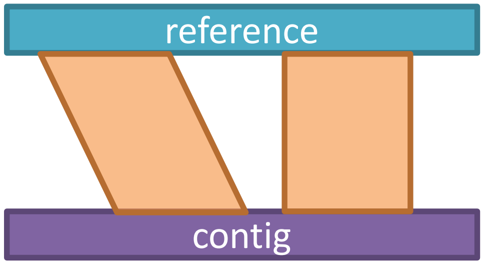


Gap where sequences do not align uniquely suggests a repeat

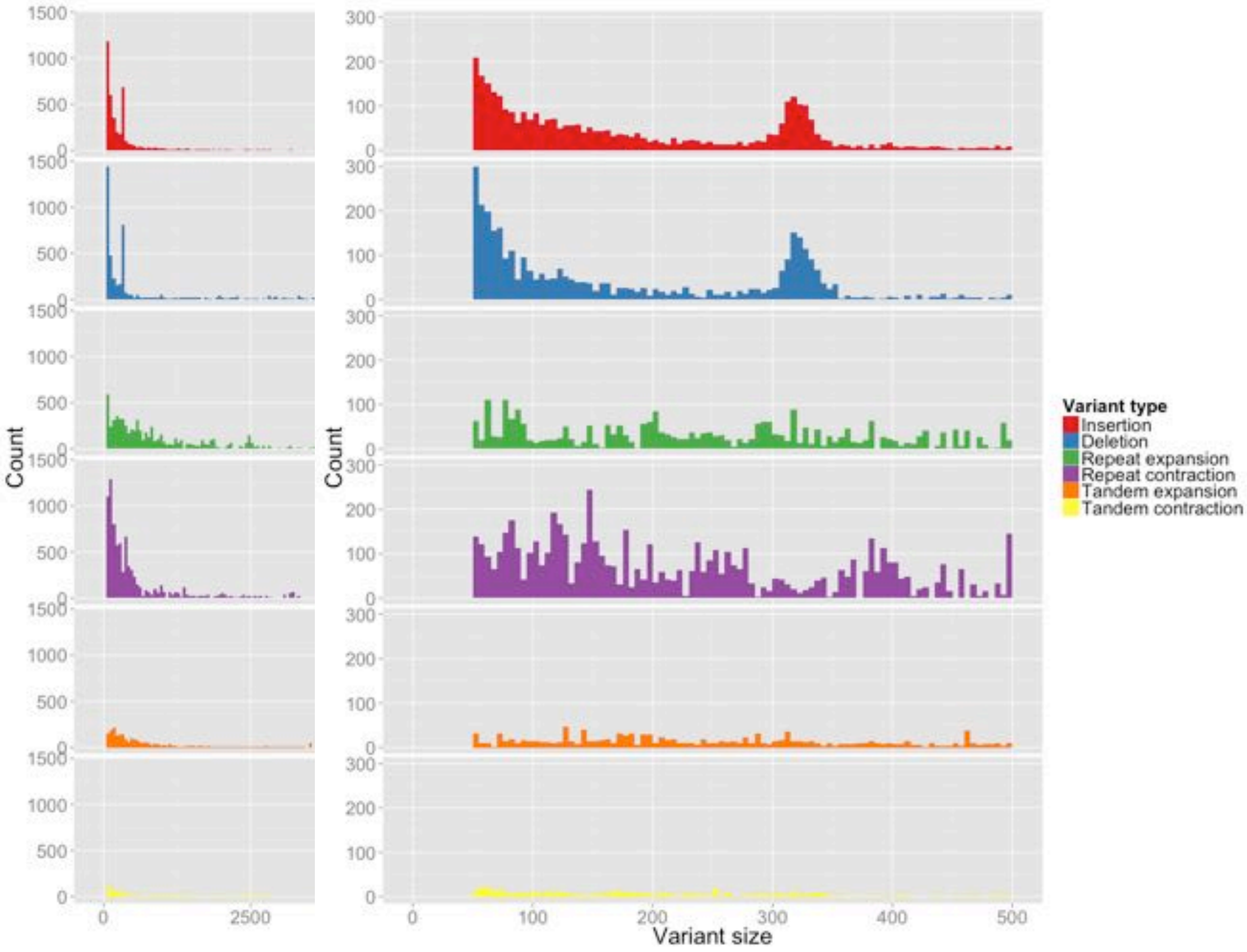
Repeat Expansions

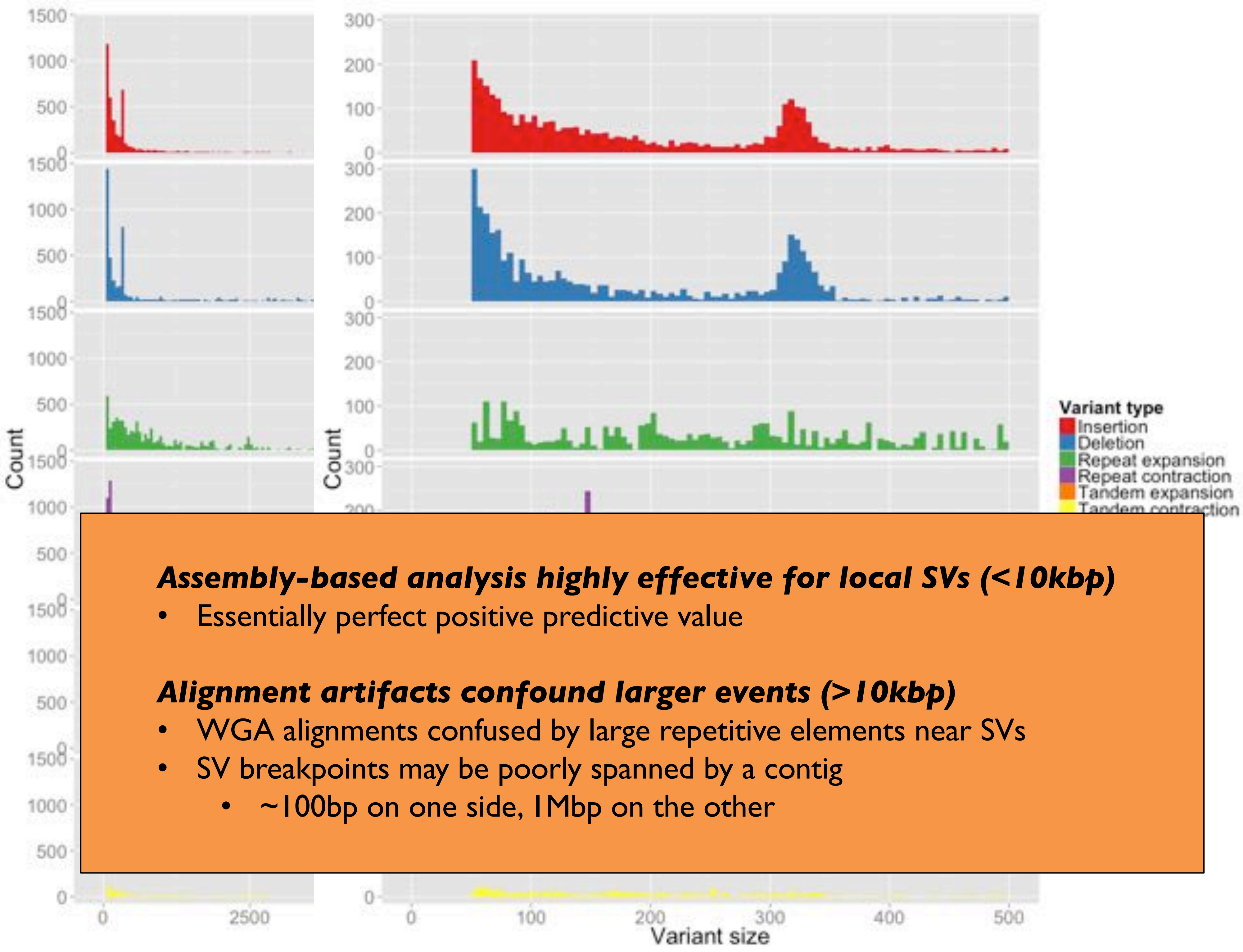


Repeat Contractions



Assemblytics: a web analytics tool for the detection of variants from an assembly
Nattestad, M, Schatz, MC (2016) Bioinformatics doi: 10.1093/bioinformatics/btw369


Count
1500
1000
500
0
0
2500
Count
300
200
100
0
0
100
200
300
400
500
Variant size
Variant type
🟥 Insertion
🟦 Deletion
🟩 Repeat expansion
🟪 Repeat contraction
🟧 Tandem expansion
🟨 Tandem contraction



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

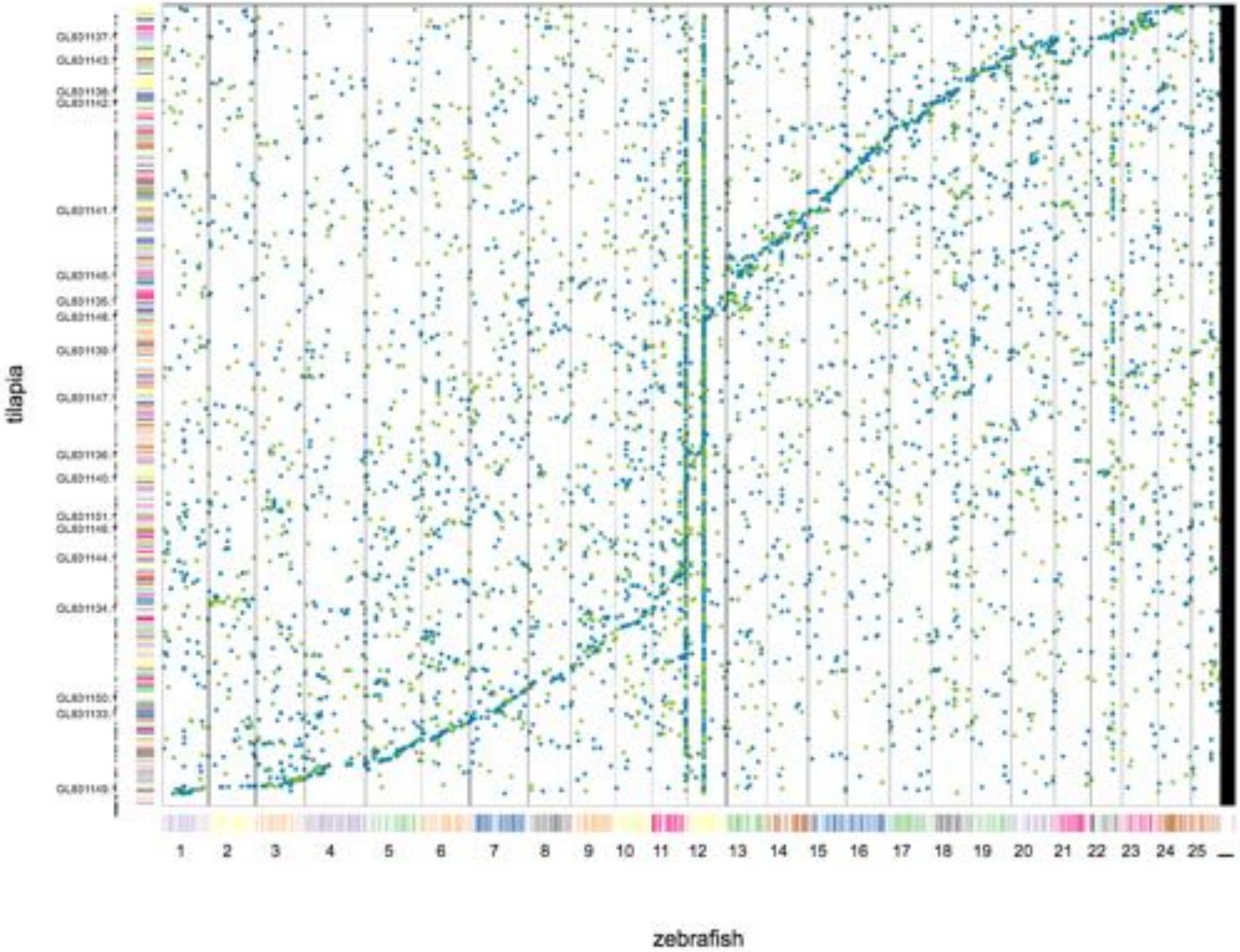
- Essentially perfect positive predictive value

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

Dot: Interactive Dot plots for Comparative Genomics

https://github.com/dnanexus/dot



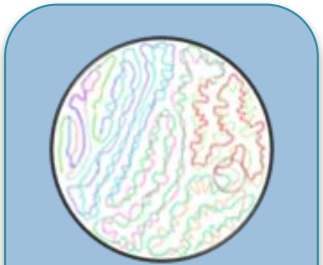
In pursuit of perfect genome sequencing

- ***Strive for Perfection: 100% Correct and 100% Complete***
  - The key for perfect genomes is lonnnnnnnnng reads ☺
  - Expect new insights on the causes of diseases, forces of evolution

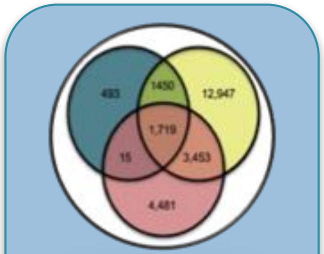
- ***Multiple sequencing technologies & approaches needed***
  - *PacBio*: Best Resolution of SVs
  - *10X/HIC*: Best Phasing
  - *De novo*: Best Resolution of small SVs
  - *Mapping*: Best resolution of large SVs

- ***We have just begun to explore the universe of variants present***
  - Tens of thousands of SVs per person, many megabases of variation
  - Also need to push these ideas into single cell and population scale analysis

CrossStitch

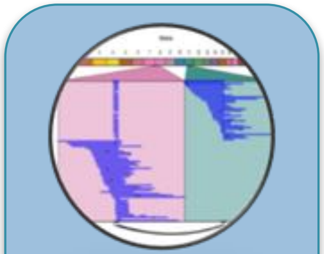


FALCON

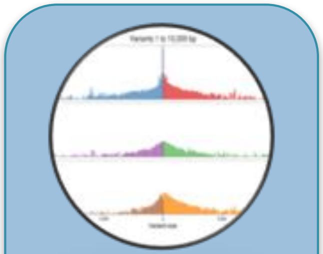


SURVIVOR

NGM+Sniffles



Ribbon



Assemblytics

http://schatz-lab.org

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Schatz Lab
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Your Name Here

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Fritz Sedlazeck

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DNAnexus
Maria Nattestad

CSHL
Gingeras Lab
Jackson Lab
Lippman Lab
Lyon Lab
Martienssen Lab
McCombie Lab
Tuveson Lab
Ware Lab
Wigler Lab

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Skiena Lab
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Gerstein Lab

JHU
Battle Lab
Langmead Lab
Leek Lab
Salzberg Lab
Taylor Lab
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PacBio
Greg Concepcion

NSF

National Human
Genome Research
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AUTISM RESEARCH INITIATIVE

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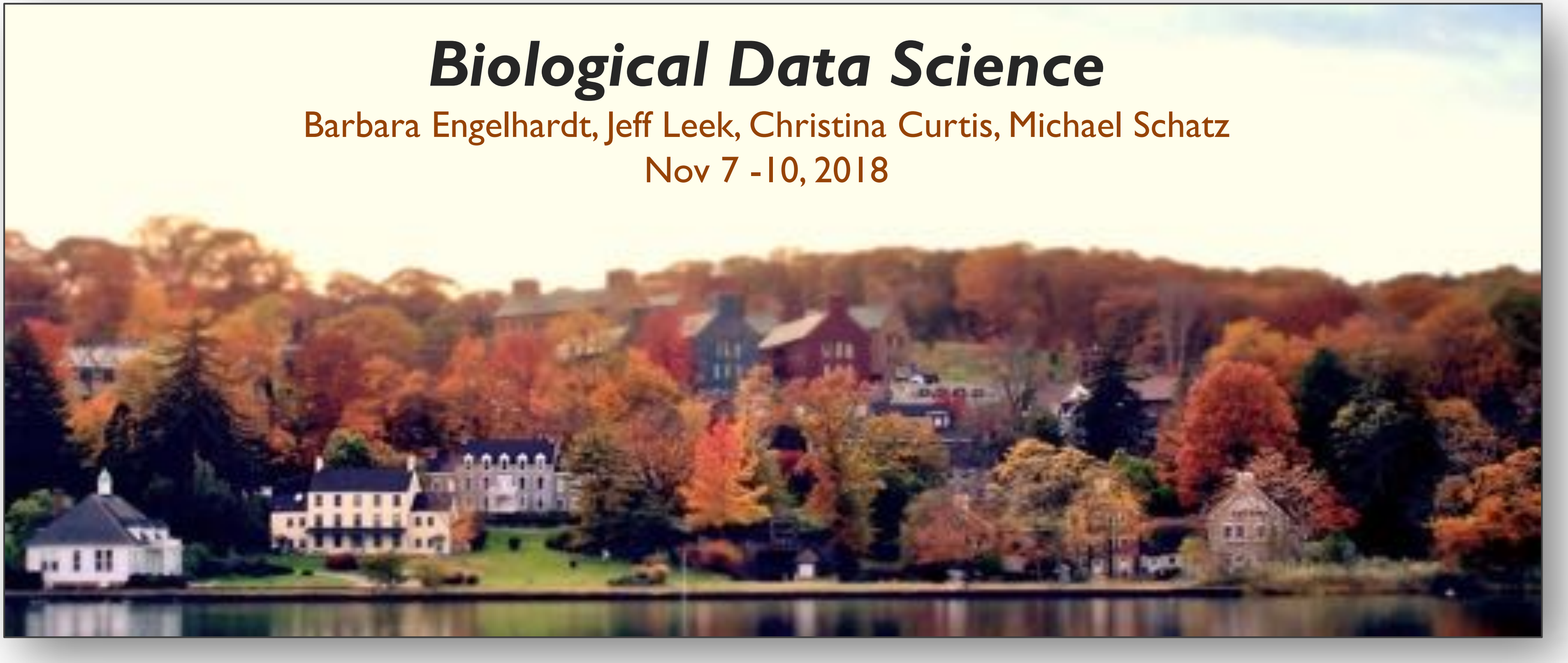

Biological Data Science

Barbara Engelhardt, Jeff Leek, Christina Curtis, Michael Schatz

Nov 7 -10, 2018

Thank you!

@mike_schatz