FALCON-Phase: Integrating PacBio and Hi-C data for phased diploid genomes

SB Kingan, Staff Scientist, Bioinformatics, PacBio

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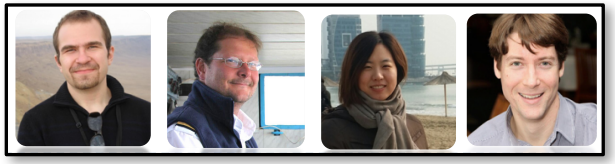
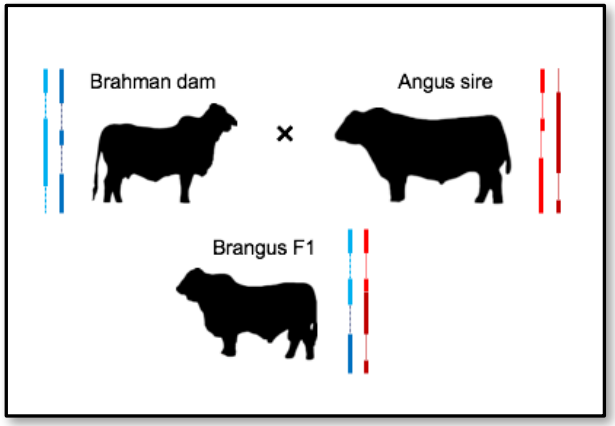
CURRENT APPROACHES TO LONG READ DIPLOID ASSEMBLY

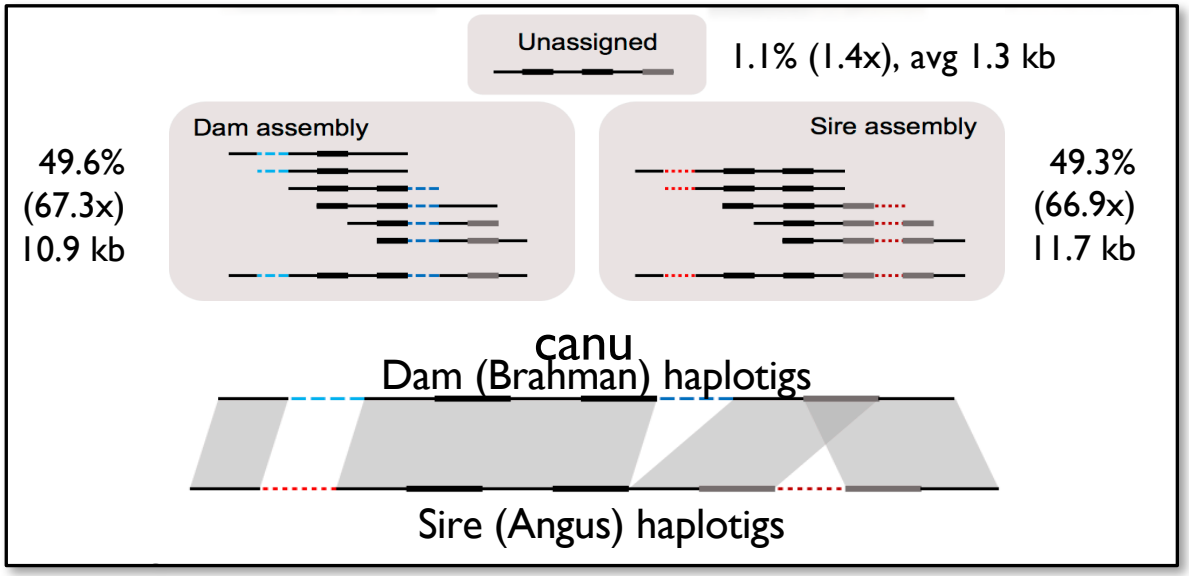
1. Separate Reads with Trio Binning (TrioCanu)

- PacBio data for F1
- ILMN data for parent-specific k-mers
- Bin PacBio reads with k-mers
- Perform two haploid Canu assemblies



EXAMPLE ON F1 BULL



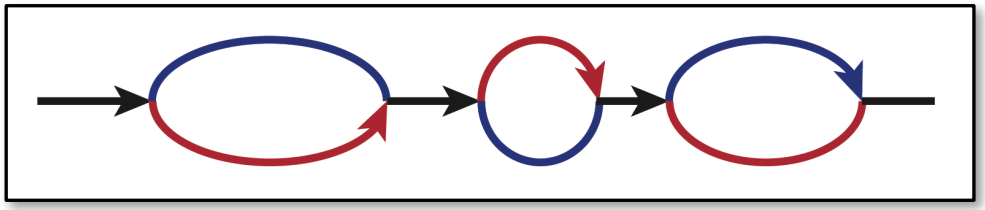


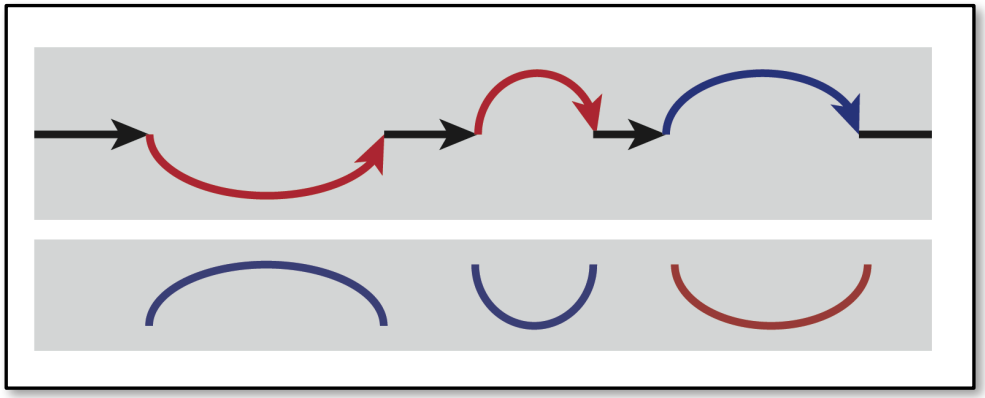
Koren, S. et al. (2018). Complete assembly of parental haplotypes with trio binning. bioRxiv. Available from: https://doi.org/10.1101/271486

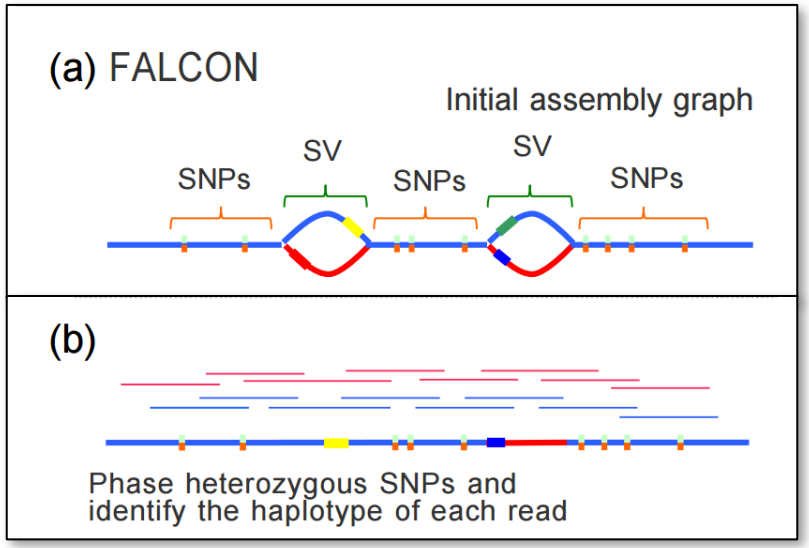
CURRENT APPROACHES TO LONG READ DIPLOID ASSEMBLY

2. Separate Haplotypes During Assembly with FALCON-Unzip

- PacBio data for diploid individual (no trio)
- Phase PacBio reads using SNPs identified in initial assembly graph
- Output phased and collapsed regions in high contiguity contigs



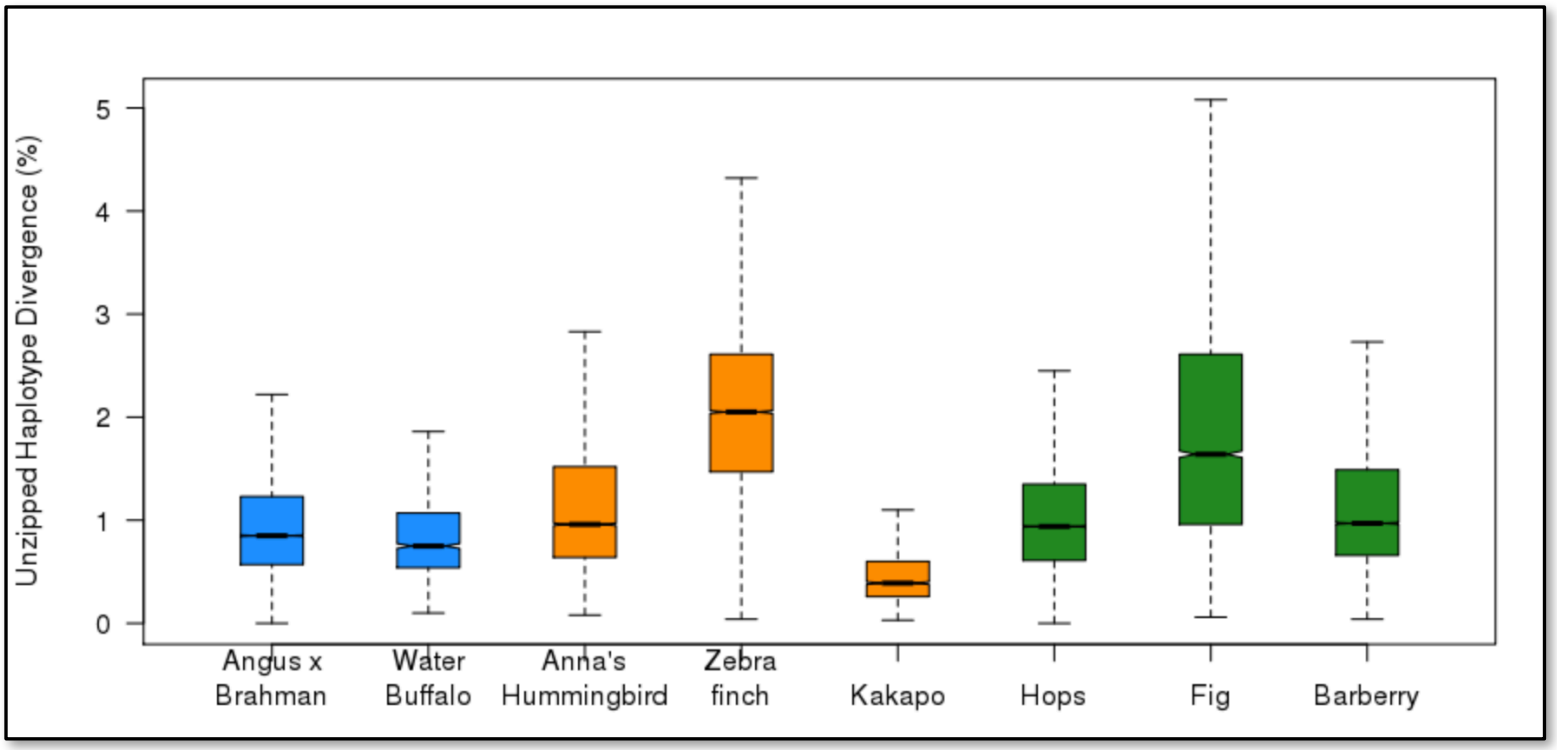




Chin, C.S. et al. (2016). Phased diploid genome assembly with single-molecule real-time sequencing. *Nature Methods*. 13(12), 1050.

THE “SWEET SPOT” FOR FALCON-UNZIP

Up to 5% divergence can be unzipped



Data Credits: Tim Smith, John Williams, Lloyd Low, Erich Jarvis, John Henning, David Hendrix, Carlos Machado, Iago Hale

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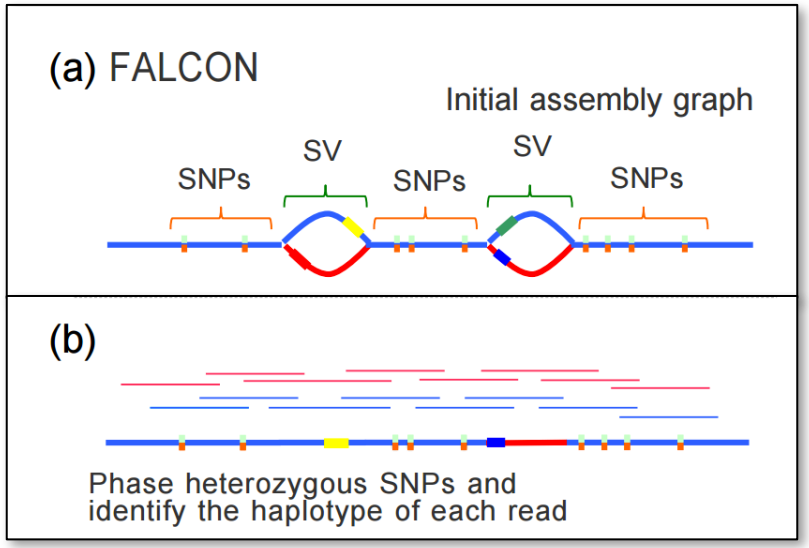
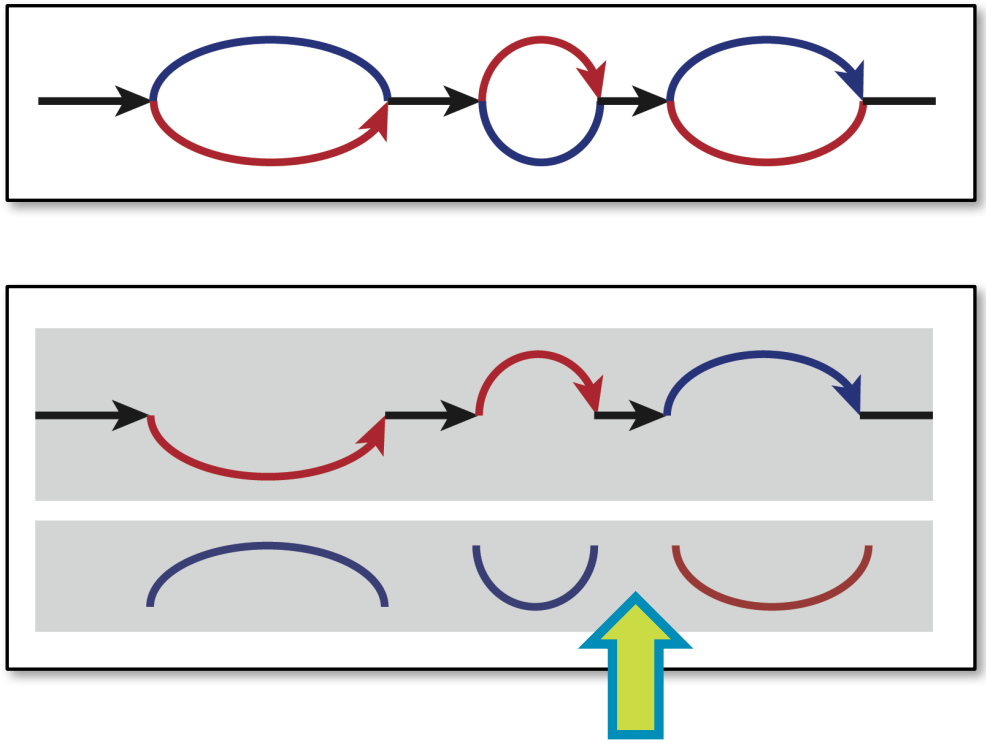




“Phase/Haplotype Switch”

Chin, C.S. et al. (2016). Phased diploid genome assembly with single-molecule real-time sequencing. *Nature Methods*. 13(12), 1050.

FALCON-PHASE: MOTIVATIONS AND GOALS

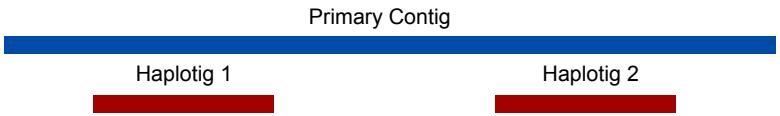
- FALCON-Unzip phase blocks are small
  - Phasing is function of heterozygosity, read depth, read length
  - Phase switches between haplotype blocks are nearly random
- Haplotype/phase switches are problematic
  - “Franken-haplotypes” impact base accuracy, gene prediction
  - Scaffolding errors
- Hi-C contains long-range haplotype information
- FALCON-Phase Tool
  - Open-source snakemake pipeline
  - Co-development project between PacBio and Phase Genomics
  - ***Can be applied at contig and scaffold scale***

Zev Kronenberg

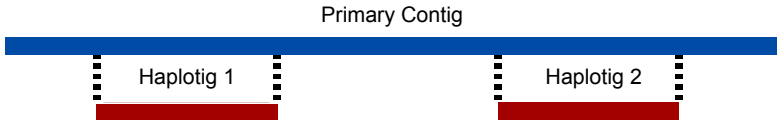
Logo Credit: Kaylee Mueller

FALCON-PHASE WORKFLOW

Input: FALCON-Unzip assembly & HiC data

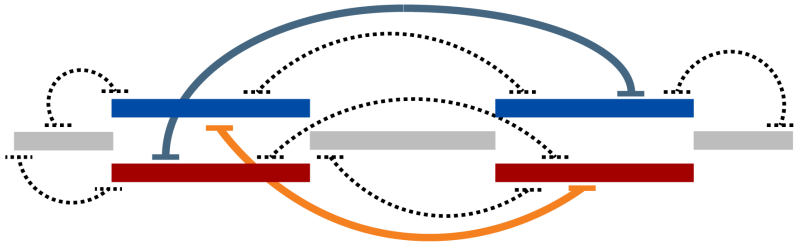


1. Identify **haplotig placement** on primary contigs

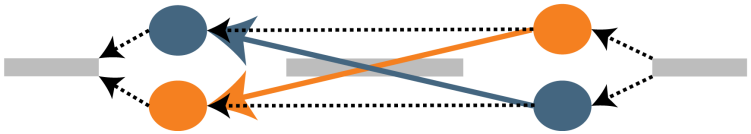


2. **Mince** primary contigs: separate **haplotig pairs** and **collapsed haplotypes**

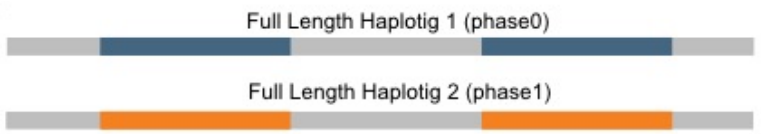
3. **Map** paired HiC reads to minced contigs and generate normalized **contact matrix**



4. **Phase** haplotigs along each primary contig



Output: phased full length pseudohaplotypes



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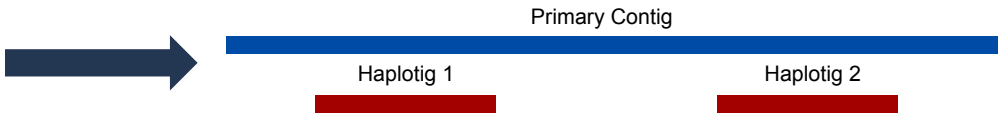
Input: FALCON-Unzip assembly & HiC data



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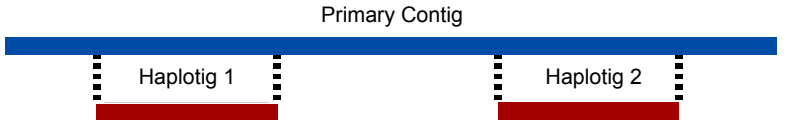

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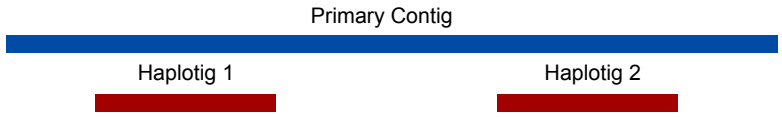

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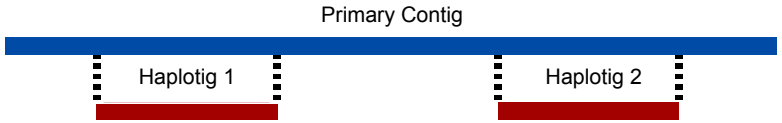

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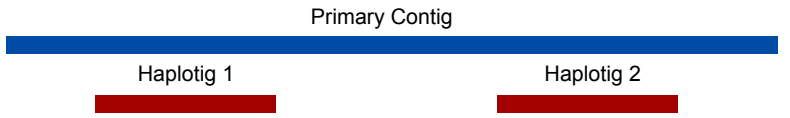

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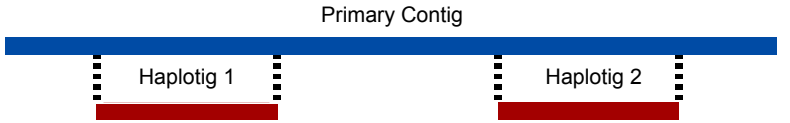

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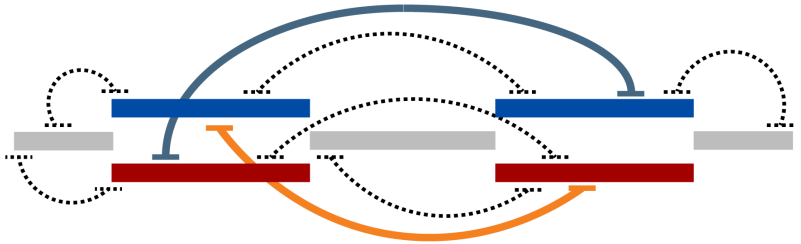

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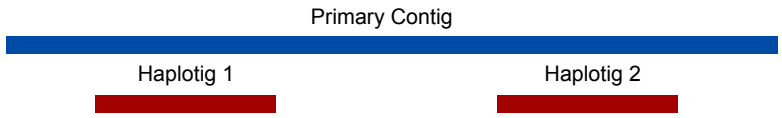

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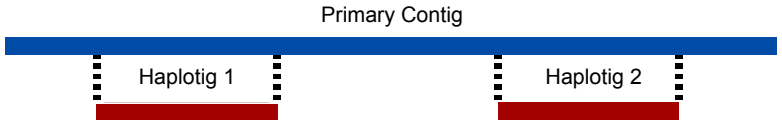

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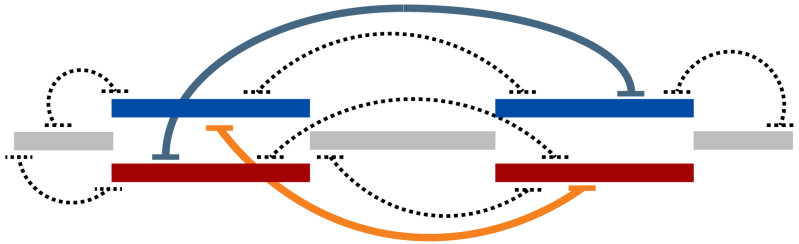

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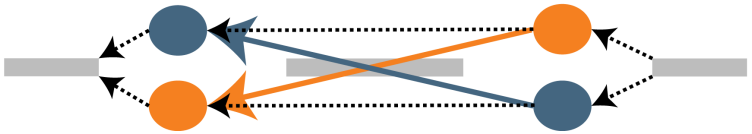

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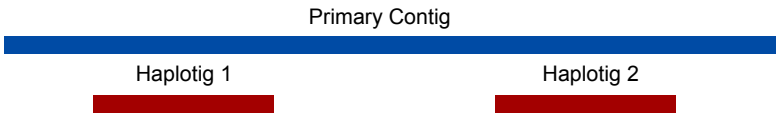

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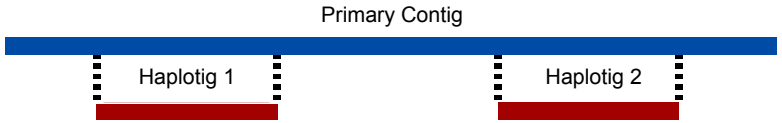

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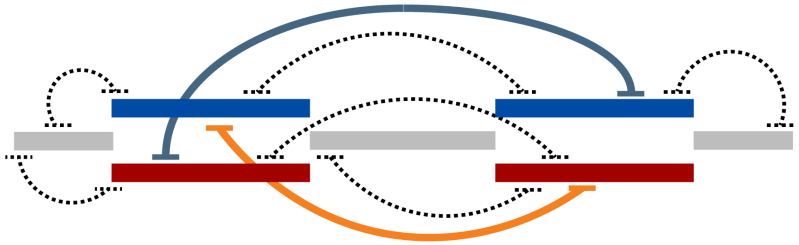

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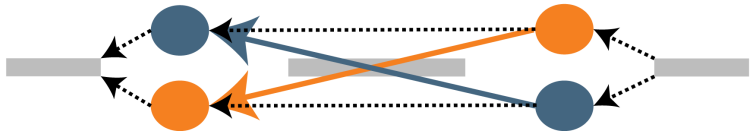

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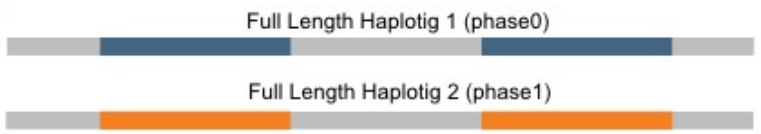
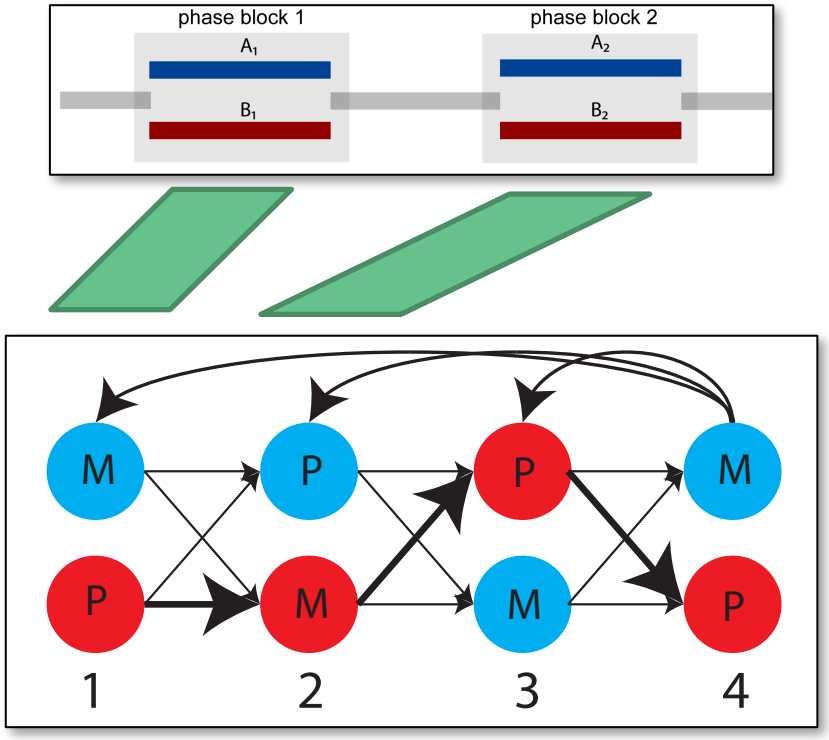


PHASING ALGORITHM: INPUTS AND OUTPUTS

- FALCON-Unzip Input
  - Order and pairing of phase blocks along primary contig

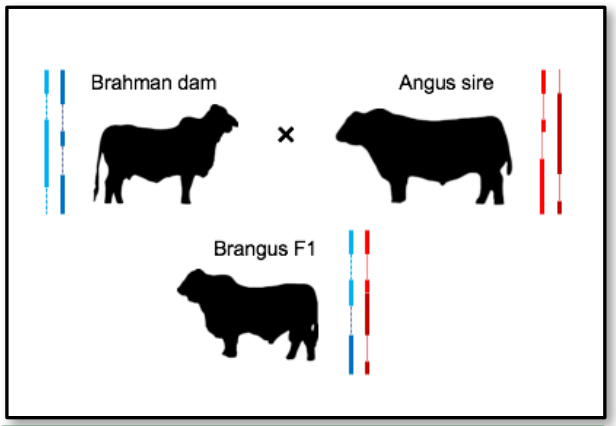


- Hi-C Input
  - Normalized contact matrix between each phase block

	A_1	B_1	A_2	B_2
A_1	18	.	.	.
B_1	1	15	.	.
A_2	2	9	15	.
B_2	7	0	3	12

- Output
  - Majority phase assignment configuration for haplotigs along primary contig

VALIDATION DATASET: ANGUS-BRAHMAN F1 BULL



Data: Tim Smith (USDA), John Williams and Stefan Hiendleder (U Adelaide)
Canu Asms: Adam Phillippy, Sergey Koren, Arang Rhie (NHGRI)

FALCON-Unzip: 90% Unzipped

CONTIGS	NUMBER	LENGTH	N50
PRIMARY	1427	2.71 Gb	31.4 Mb
HAPLOTIGS	5879	2.45 Gb	2.48 Mb

Phase Genomics Hi-C

- 200 million read pairs

TrioCanu Assemblies

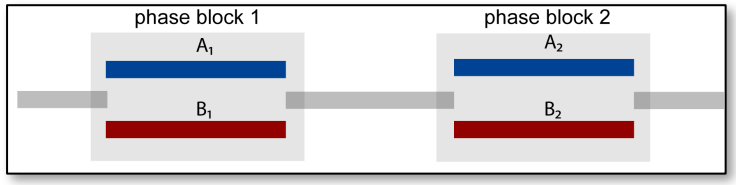
CONTIGS	NUMBER	LENGTH	N50
ANGUS DAM	1747	2.57 Gb	26.7 Mb
BRAHMAN SIRE	1040	2.68 Gb	23.3 Mb

Parental SNP Calls

- 20-25x coverage ILM PE
- read mapping with bwa mem
- SNV calls with freebayes

Koren et al. 2018

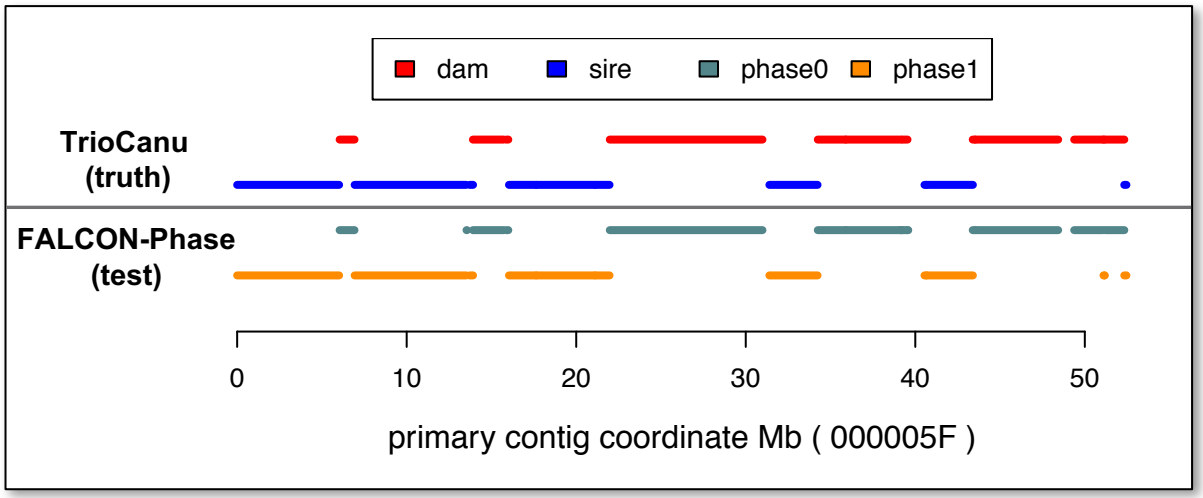
PHASE ASSIGNMENT ACCURACY: PARENTAL ASSIGNMENT



- Minimap to Canu Asms
- Highest PID for longest alignment
- Required concordance between pairs

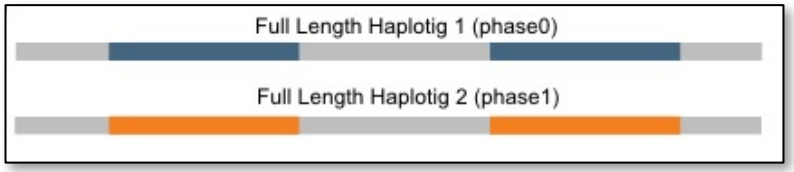
Contig Assignment	Count	Length (%)	Mean Length
Dam	2,305	2.32 Gb (42 %)	1.01 Mb
Sire	2,305	2.32 Gb (42 %)	1.01 Mb
No Parent	1,704	116 Mb (2.1 %)	68.1 kb
Collapsed	3,934	374 Mb (6.8 %)	88.2 kb

RESULTS FOR PRIMARY CONTIG 000005F



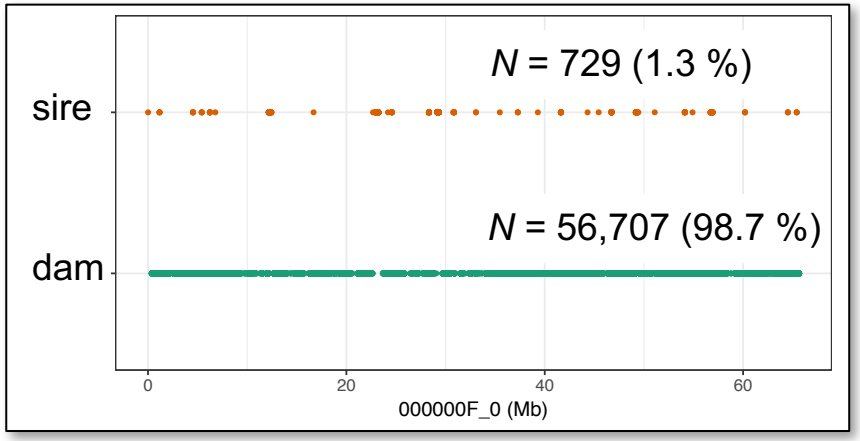
overall accuracy: 96.72%

PHASE ASSIGNMENT ACCURACY: PARENTAL SNV CALLS

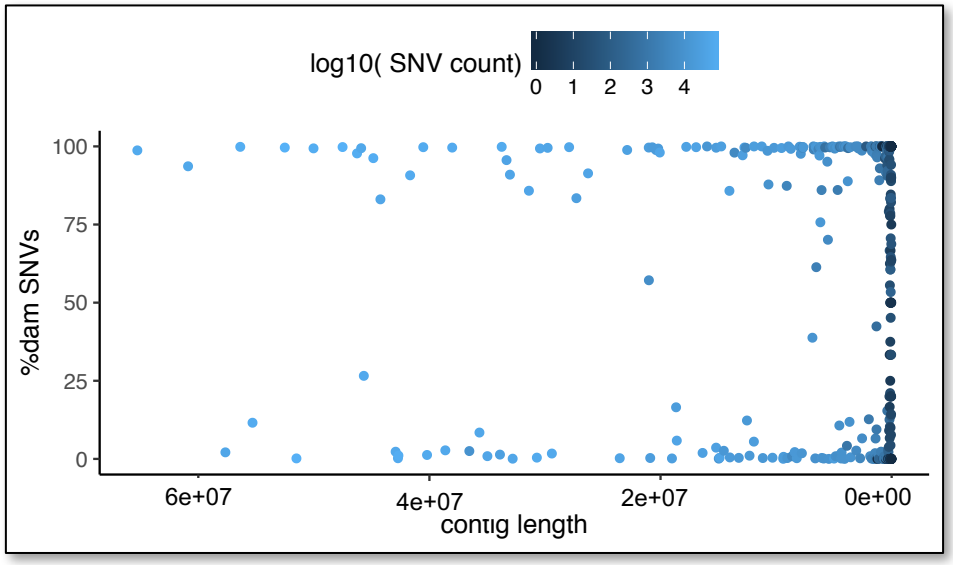


- bwa mem alignment of parental PE ILM data to phase0 haplotigs
- Variant calling with Freebayes
- SNV filtered for homozygous sites that differ between parents

PRIMARY CONTIG 000000F_0 (DAM)



ACCURACY BY PRIMARY CONTIG LENGTH

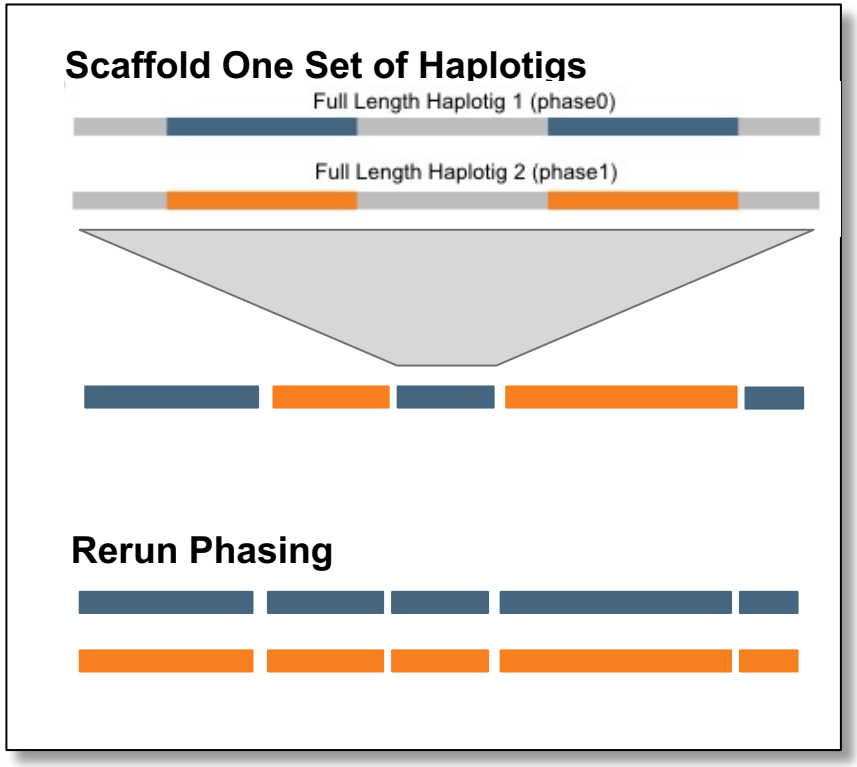


OVERALL PERFORMANCE

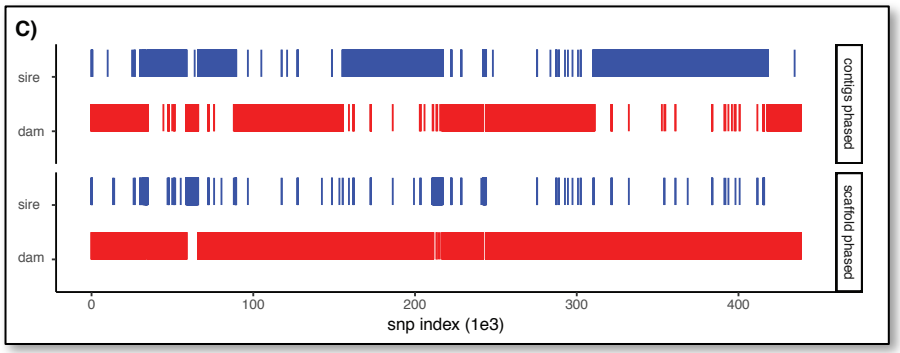
	SNV Count	Correct
dam	2,031,334	97.4 %
sire	1,464,748	95.8 %
total	3,496,082	**96.7 %**

PHASING CHROMOSOME-SCALE SCAFFOLDS

- Scaffold one set of full-length haplotigs with Proximo (Phase Genomics)
- Scaffolds are chromosome-scale
- We know:
  - order of contigs along scaffold
  - pairing of phase 0 and phase 1
- FALCON-Phase Scaffolds



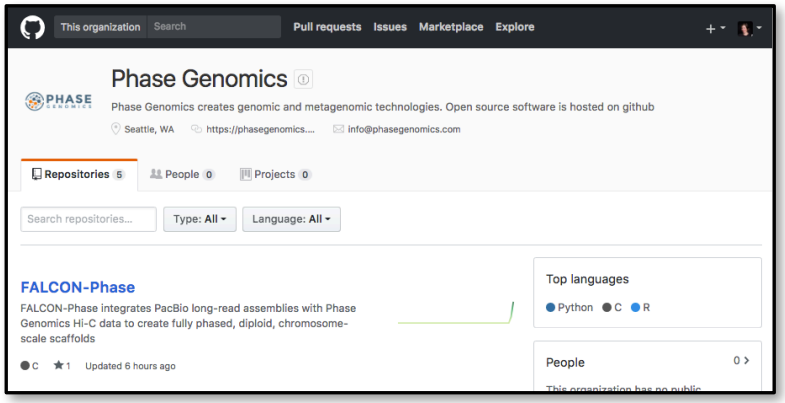
PARENTAL SNVS AFTER SCAFFOLD PHASING

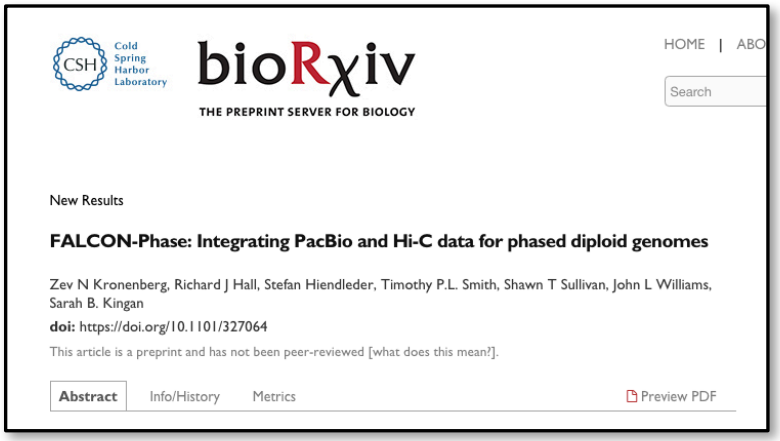


Output: Chromosome-scale, phased, diploid assembly!

SUMMARY

- FALCON-Phase is highly accurate
  - > 96% accuracy when tested against parental assemblies or SNVs
- FALCON-Phase implemented in snakemake pipeline
  - Run locally or on cluster, Open source
- PacBio plus HiC is all you need to produce, phased, chromosome-scale diploid assembly
- More Info:





ACKNOWLEDGEMENTS

- Coauthors
  - Zev Kronenberg (Phase)
  - Richard Hall (PacBio)
  - Stefan Hiendleder (U Adelaide)
  - Timothy Smith (USDA)
  - Shawn Sullivan (Phase)
  - John Williams (U Adelaide)

PACBIO®

Greg Concepcion
Jonas Korlach
Billy Rowell
Ivan Sovic
Liz Tseng
Michelle Vierra

PHASE GENOMICS

Ivan Liachko
Kaylee Mueller
Max Press
Andrew Wiser

Jason Chin
Mark Chaisson
Luke Harmon
Ryan Layer