PACIFIC BIOSCIENCES®

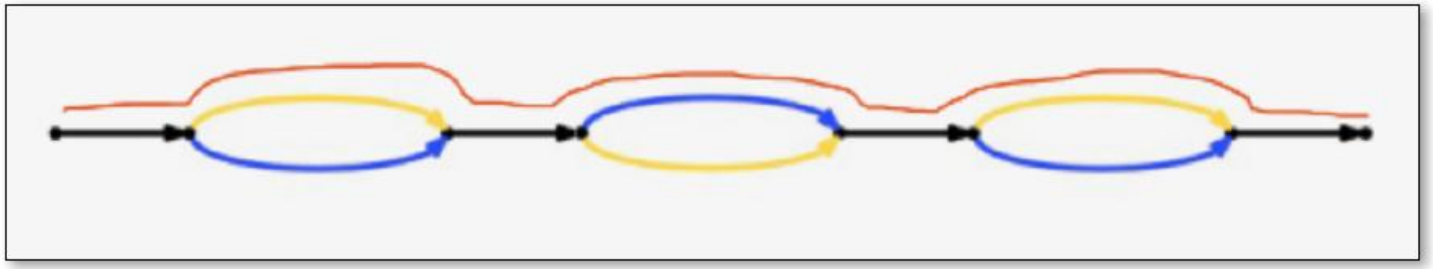
# FALCON-Phase: a diploid assembler for PacBio and Hi-C data

Collaboration between PacBio and Phase Genomics, 2018

# THE NEED FOR HAPLOTYPE-RESOLVED ASSEMBLIES

## Persistent Paradigm in Genome Assembly

- Choose an inbred individual to make assembly simpler and more attainable
- When encountering heterozygosity in genome, “collapse” haplotypes into a single mosaic consensus representation of the genome



Longest path mixing haplotypes (blue and yellow) to generate a “pseudohaplotype”

Image from Weisenfeld et al. 2017

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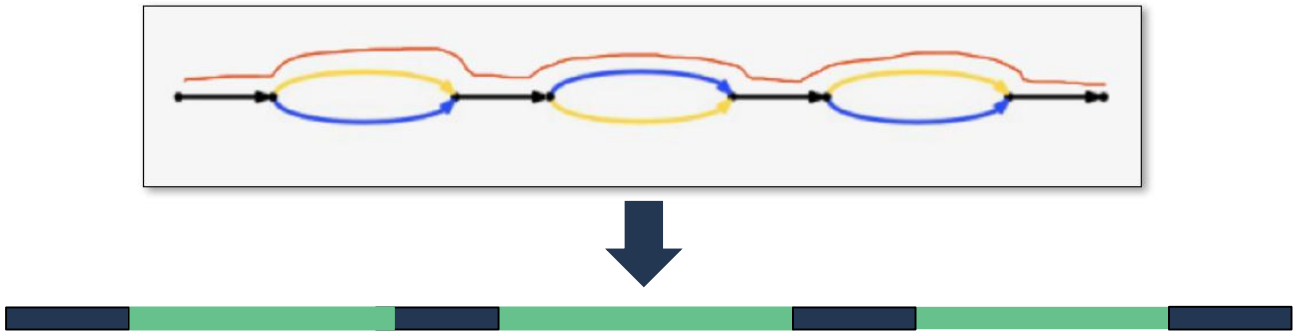
## Issues with Paradigm

- Choosing an inbred individual misrepresents the genetic diversity present within the organism and/or species
- Collapsed haplotype assemblies result in “Franken-haplotypes” that do not accurately represent the genome of interest



Resulting linear genome of haplotype collapsing approach to assembly

Image from Rhie, Koren, Phillippy PAG 2018 and 10X Genomics

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## Advantages to Phased Diploid Assembly

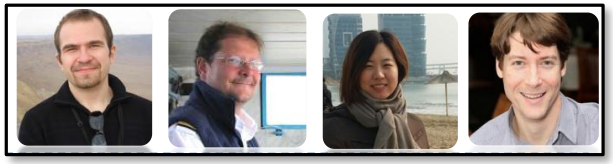
- SNP and SV characterization can be done in context with haplotype
- Gene models and annotations will be more accurate
- Targeted use of the genome will be less error-prone

Phased diploid assembly

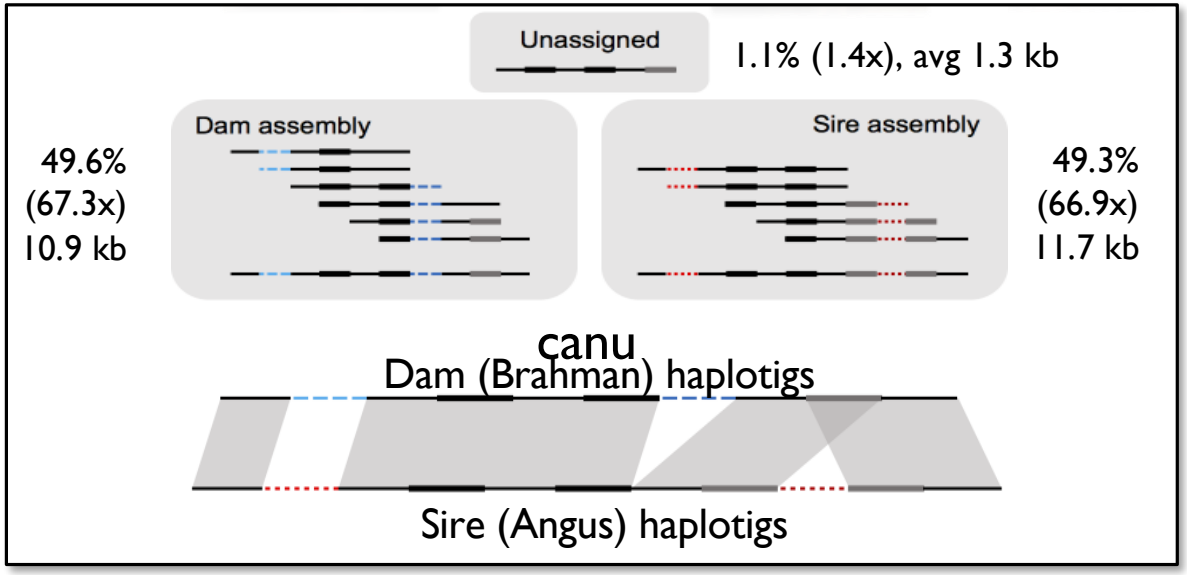
# CURRENT APPROACHES TO LONG READ DIPLOID ASSEMBLY

## 1. Trio Binning with TrioCanu

- PacBio data for F1
- ILMN data for parents
- Identify parent-specific markers to bin PacBio reads
- Perform two haploid Canu assemblies



Example on F1 cattle hybrid




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

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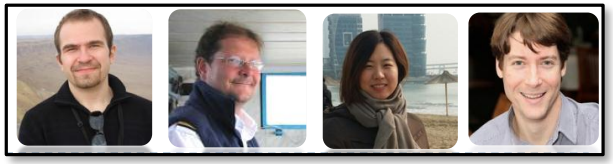
**Requires a second data type & both parents of individual of interest**

**Additional BFX for identifying parent-specific k-mers**

**Additional compute required to run two full assemblies**



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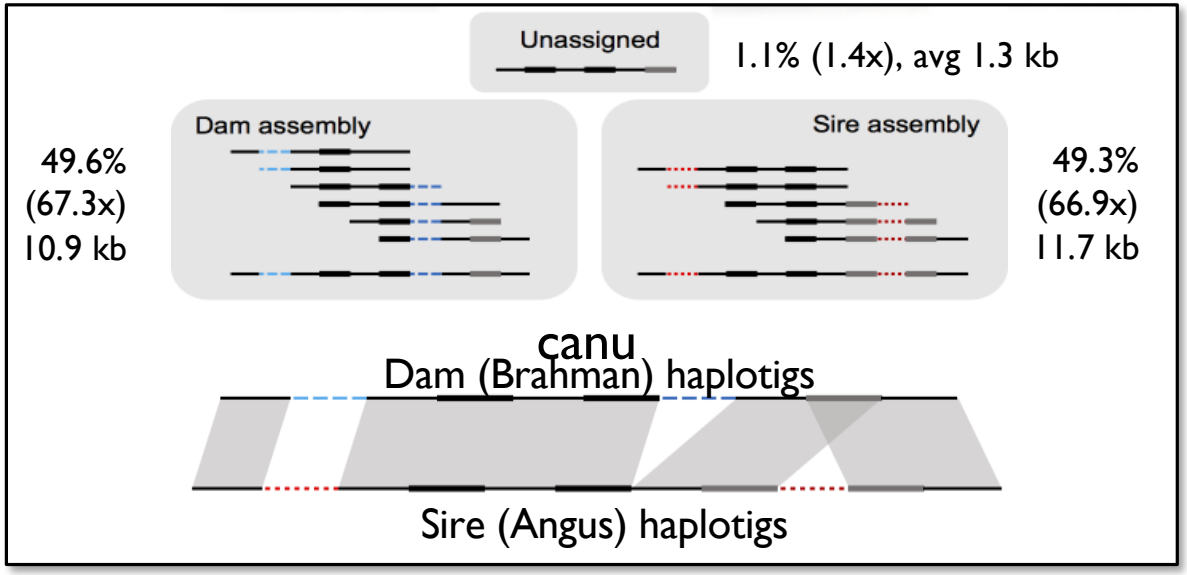


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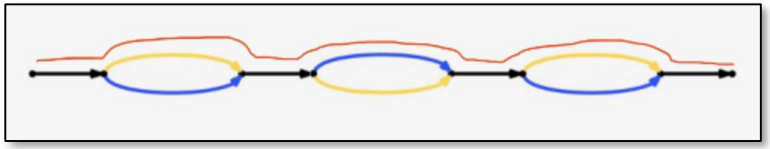
# CURRENT APPROACHES TO LONG READ DIPLOID ASSEMBLY

## 2. Haplotype assembly with FALCON-Unzip

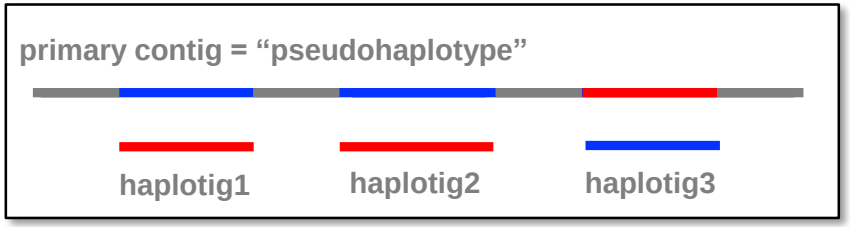
- PacBio data for diploid individual
- Perform FALCON and FALCON-Unzip assemblies
- Outputs primary contigs (longest path through bubbles and collapsed regions) and haplotigs (divergent alleles from bubble regions)

Phase reads in Assembly Graph



Weisenfeld et al. 2017

Emit Psuedohaplotype and Haplotigs

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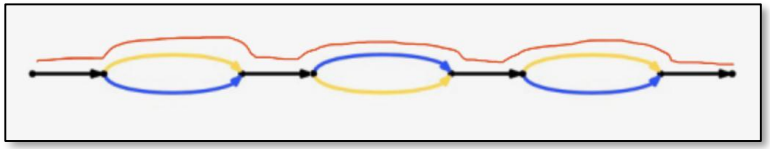
**Length of phase block is a function of heterozygosity (biology) and read length (technology)**

**Phase-switches can occur along primary contig**

Phase reads in Assembly Graph



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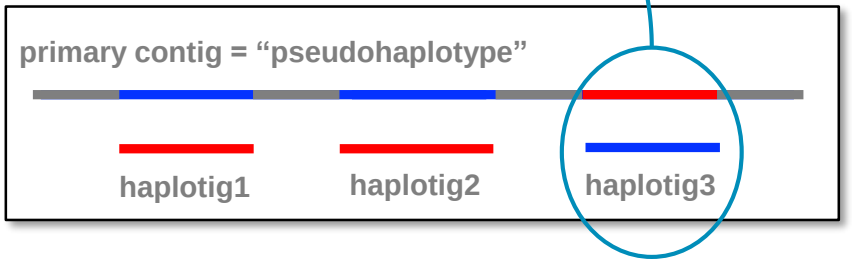


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# *NEW!* FALCON-PHASE FOR PHASED DIPLOID GENOME ASSEMBLIES

**Features:**

- Addresses haplotype/phase switches in FALCON-Unzip assemblies improving base accuracy, gene predictions, scaffolding errors, and eliminating “Franken-haplotypes”
- Increases the length of phase blocks in FALCON-Unzip assemblies
- Utilizes long-range HiC data for phasing & can be run iteratively on contigs and scaffolds
- Easy-to-use, open source software available on Github
- Collaboration with Phase Genomics

PHASE GENOMICS

Kronenberg, Z. et al. (2018). FALCON-Phase: Integrating PacBio and Hi-C data for phased diploid genomes. bioRxiv. Available from: https://doi.org/10.1101/327064

# *NEW!* FALCON-PHASE INTEGRATES HI-C WITH FALCON-UNZIP

## FALCON-Phase Process

***Input*** a FALCON-Unzip assembly & HiC data

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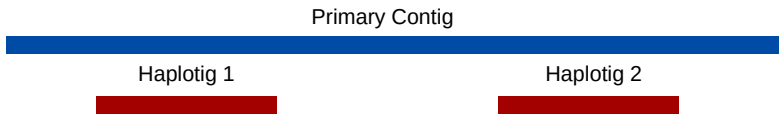


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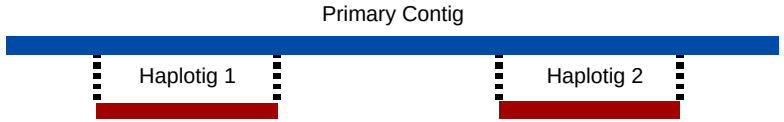

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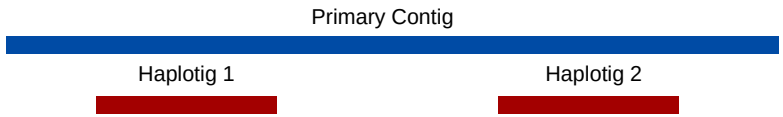


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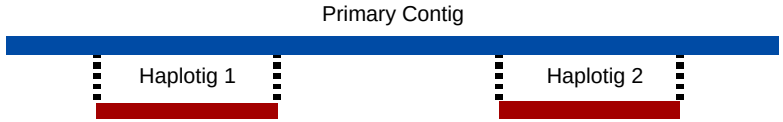


2. Mince primary contigs at edges of haplotig placements to separate homologous haplotigs and collapsed haplotype

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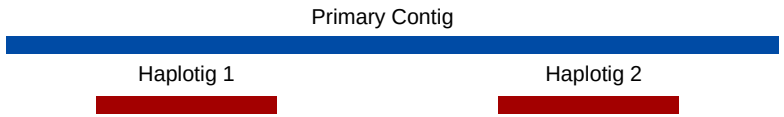


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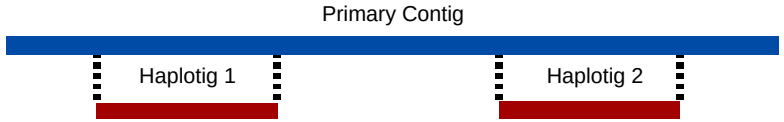


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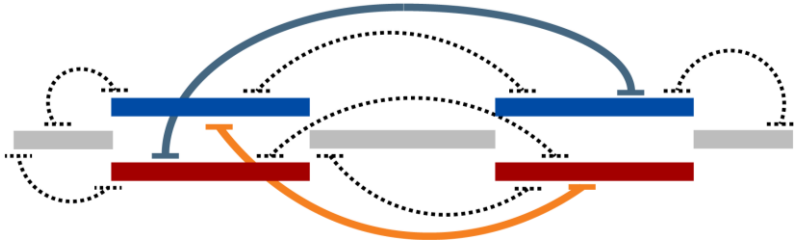
3. Map paired Hi-C reads to minced contigs to identify haplotype switches



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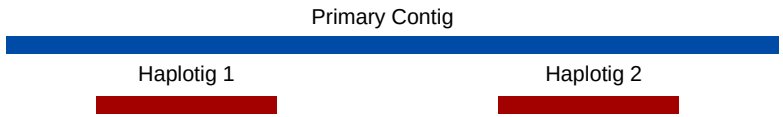


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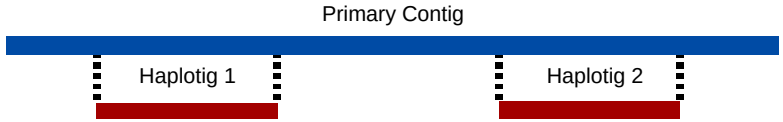


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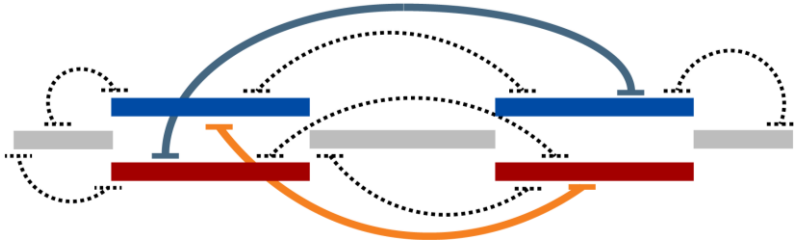
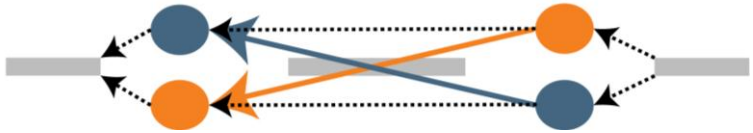


4. Combine same-phase haplotigs and collapsed haplotype contigs into gap-less phased contigs



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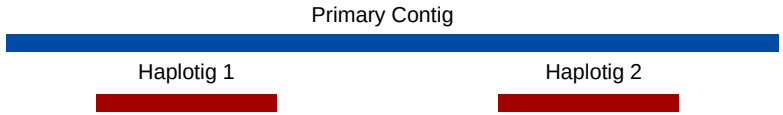


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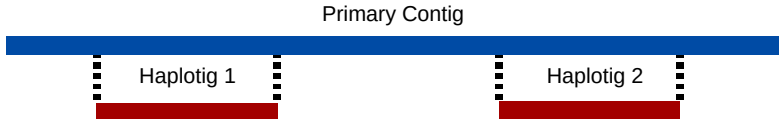


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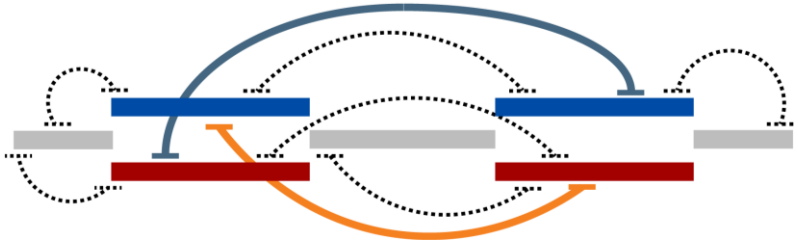

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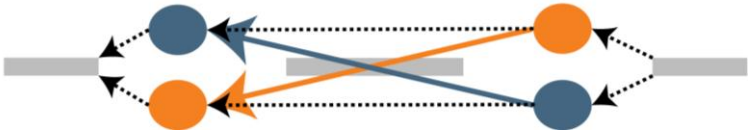
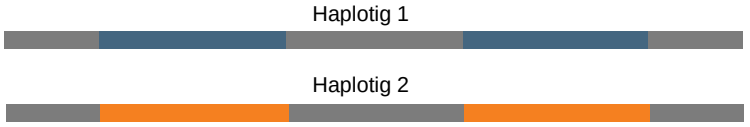


***Output*** a phased diploid assembly



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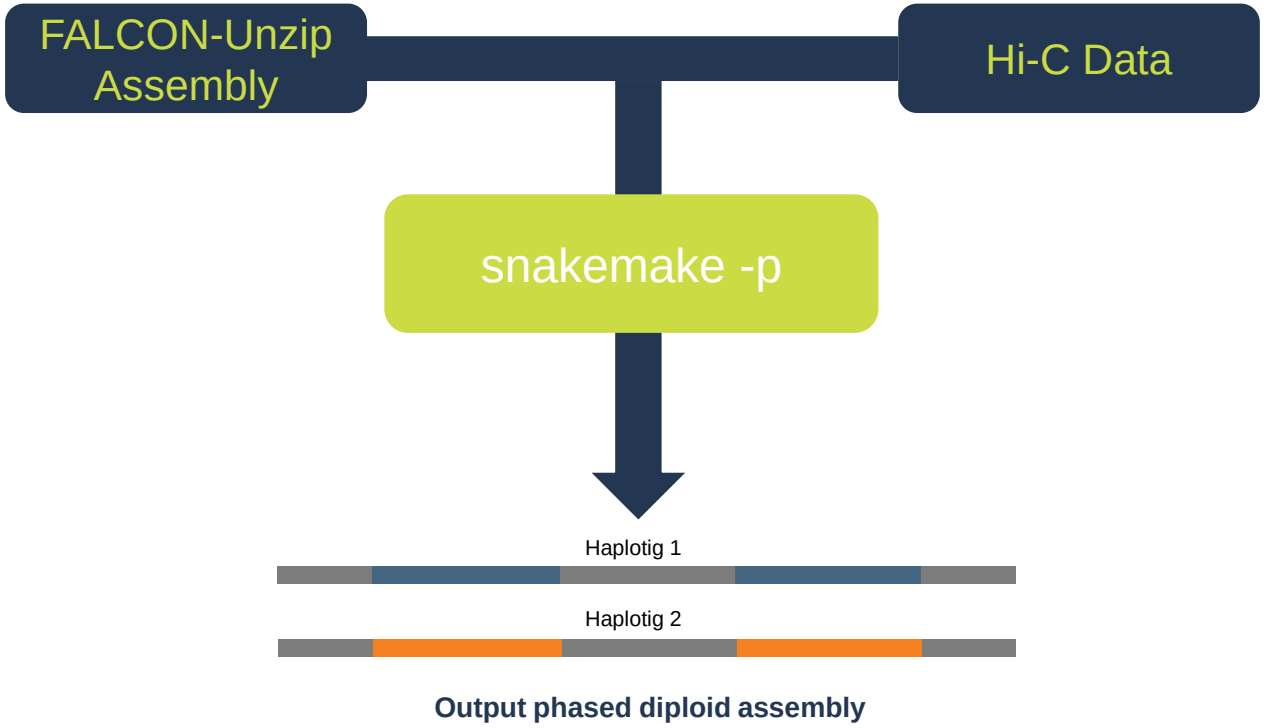
## FALCON-Phase Workflow




**Output phased diploid assembly**

While it is a command line tool, it can be run on a laptop with no compute cluster and uses standard bioinformatics dependencies, making it as easy-to-use as it is powerful for phased genome assembly.

Kronenberg, Z. et al. (2018). FALCON-Phase: Integrating PacBio and Hi-C data for phased diploid genomes. bioRxiv. Available from: https://doi.org/10.1101/327064

# FALCON-PHASE FOR PHASED DIPLOID GENOME ASSEMBLIES

## Conclusions:

- Only method to combine the megabase size contigs of PacBio assemblies and long range HiC information to give phased diploid assemblies
- Easy-to-use single command tool that can be run iteratively on contigs and scaffolds
- Does ***not*** require compute cluster resources
- Can be run retroactively on FALCON-Unzip assemblies with HiC data from any source

For more information on FALCON-Phase please see the the GitHub page and the preprint on bioRxiv.

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