Investigating Non-Infectious Bud Failure Using Hi-Fi Sequencing for Genome Assemblies and Methylation Profiling

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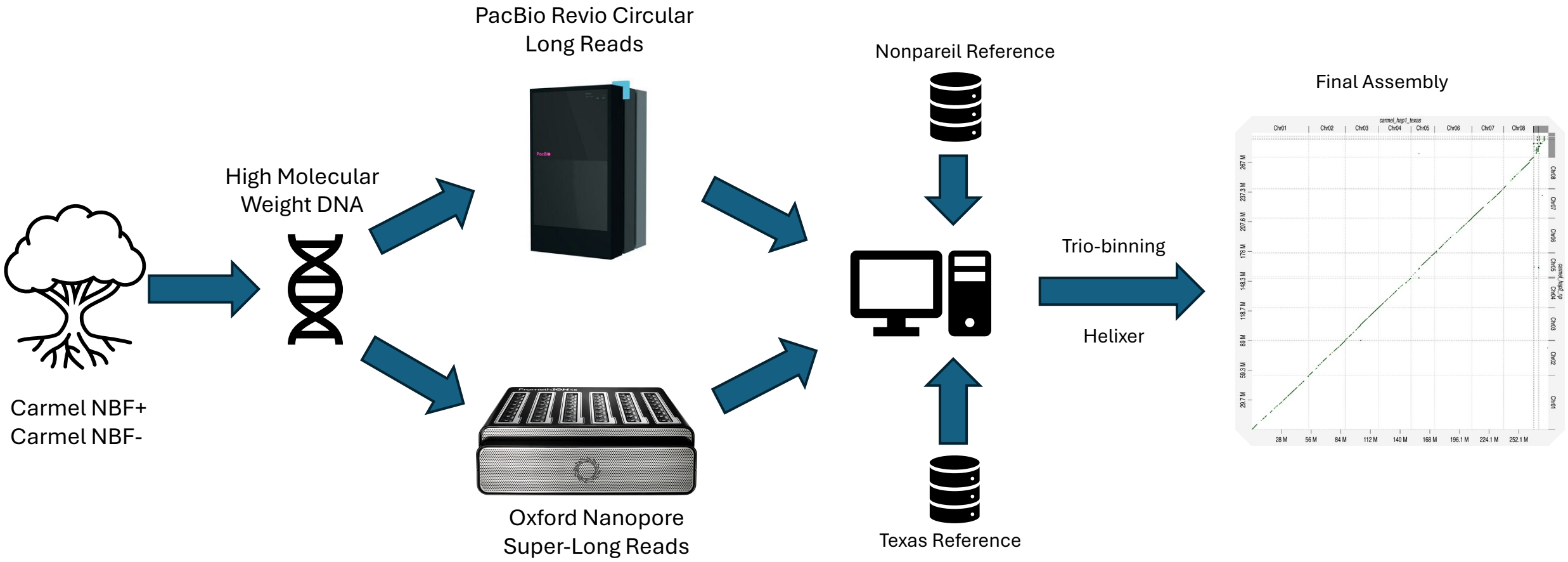
Overview

- Project Proposal
  - Goals
  - Experimental Design
- Results
  - New Carmel Genome Assembly and Annotation
  - Improvements to DNA Methylation and RNA Sequencing
  - Long Read DNA Methylation
- Next Steps

Proposal: Project Goals

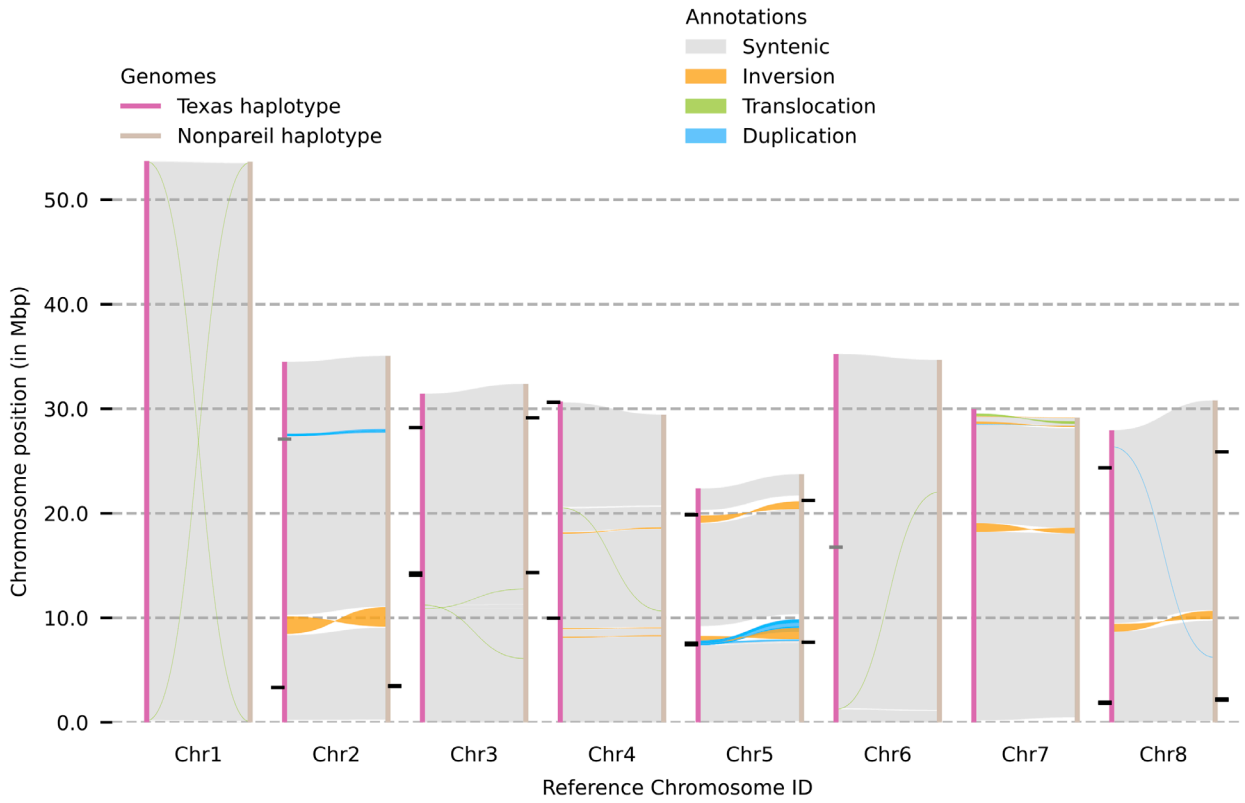
- Develop a new reference assembly for almond to improve our ability to detect bud failure
- Identify parental haplotypes to narrow down genetic origin of bud failure
- Extract new methylation data from the assembly using cutting-edge technologies
- Reexamine previous data sets using the new assembly to gain new insights on bud failure

Proposal: Experimental Design

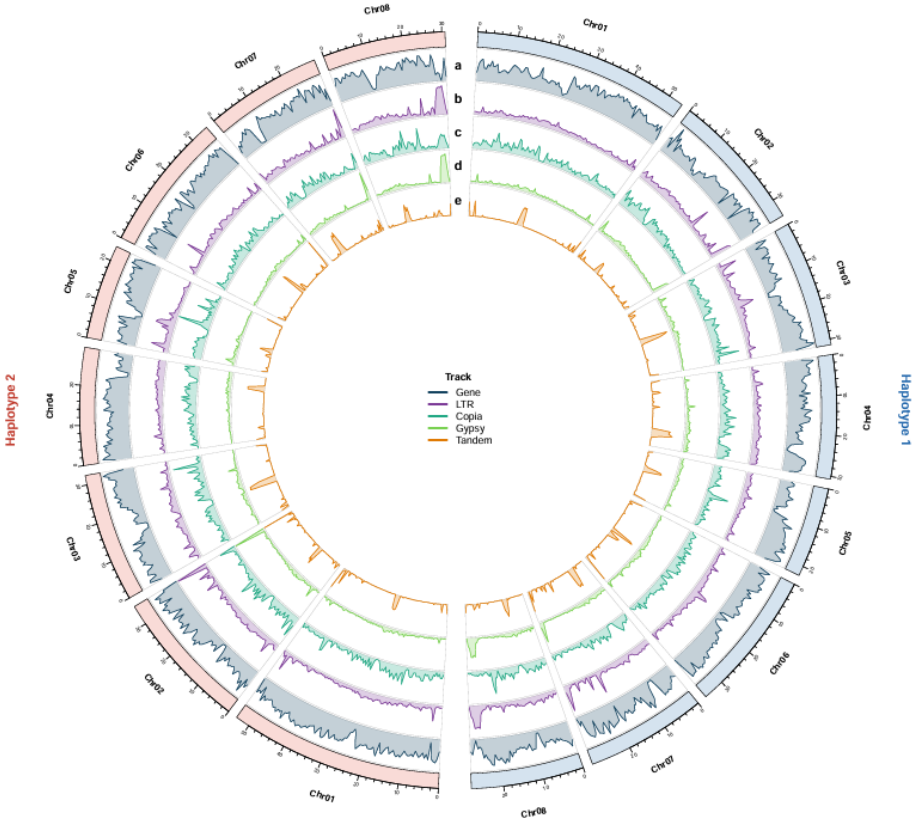


Results: Assembly

- Near telomere to telomere assembly for almond var. Carmel
- Haplotype phasing between two parents, Texas and Nonpareil
  - High BUSCO Completeness for both haplotypes
  - High syntony between the two haplotypes

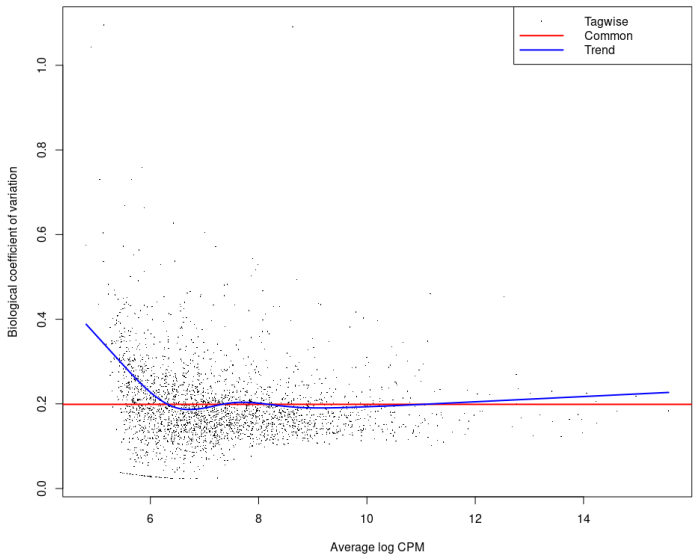


Results: Assembly

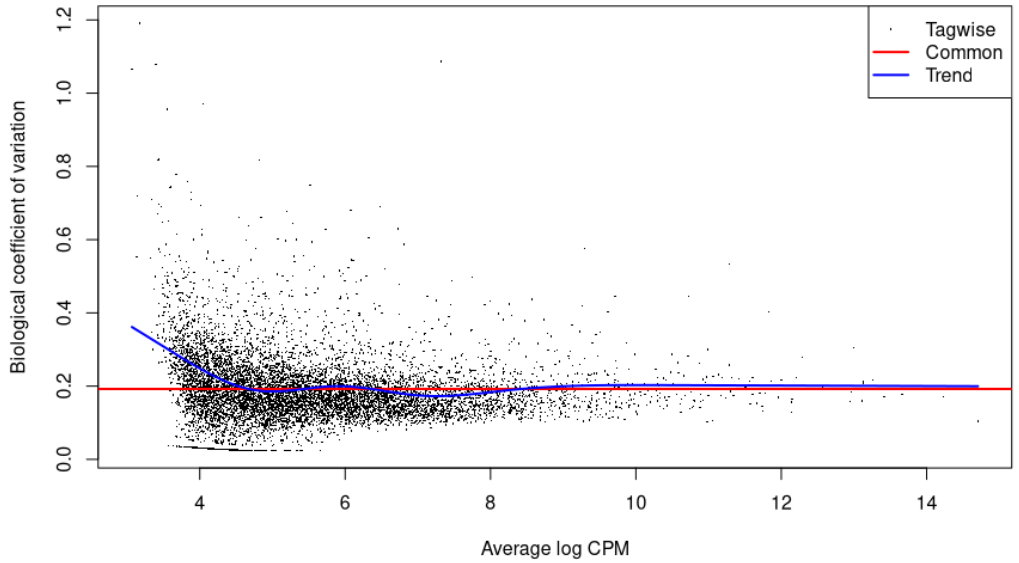


- Improved annotation with haplotype phasing
  - Hap1 (Texas):**38376 genes in 8 chr**
  - Hap2(Nonpareil): **43,429 genes in 8 chr**
  - **Old NP annotation contain only 22,000 genes**
- Identification of 5` UTR and transposable elements
- Allows for haplotype aware alignment of genomic data
  - Allele Specific Expression
  - Allele Specific methylation

Results: Improved RNA Seq and MethylSeq

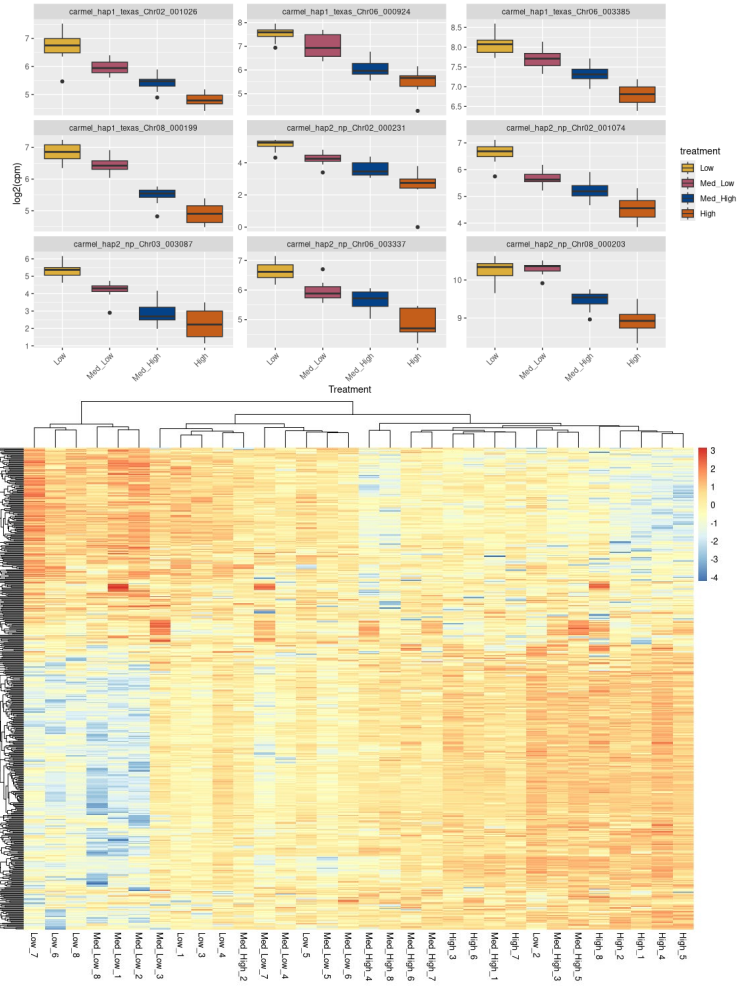


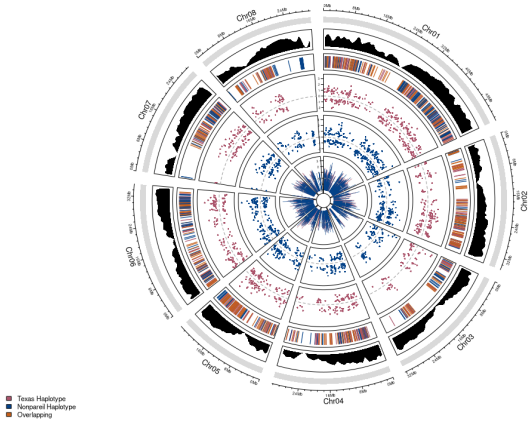
RNA Seq with Old Assembly

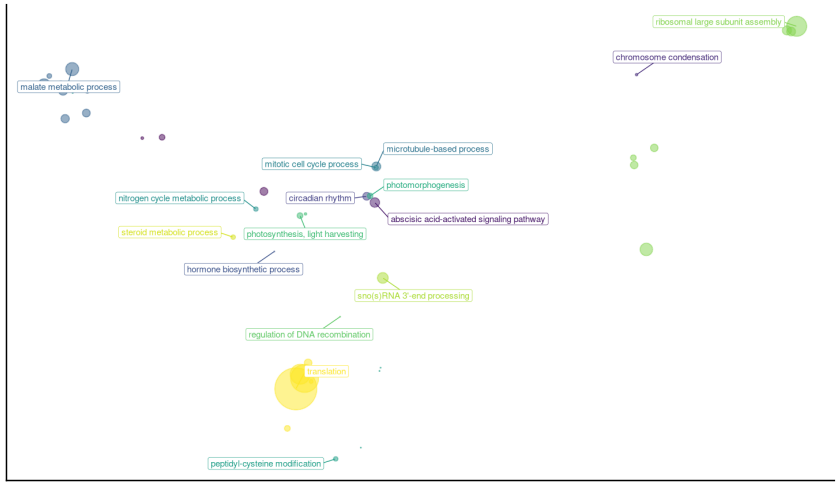


RNA Seq with New Assembly

Results: Improved RNA Seq and MethylSeq

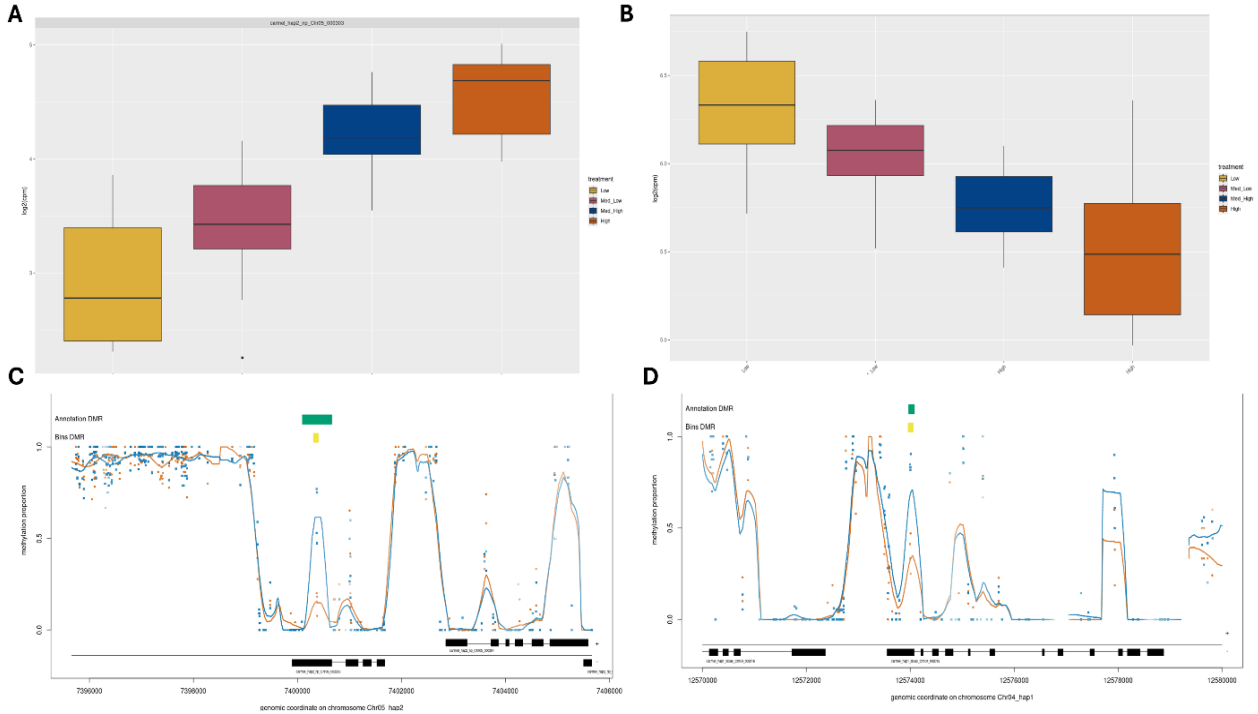




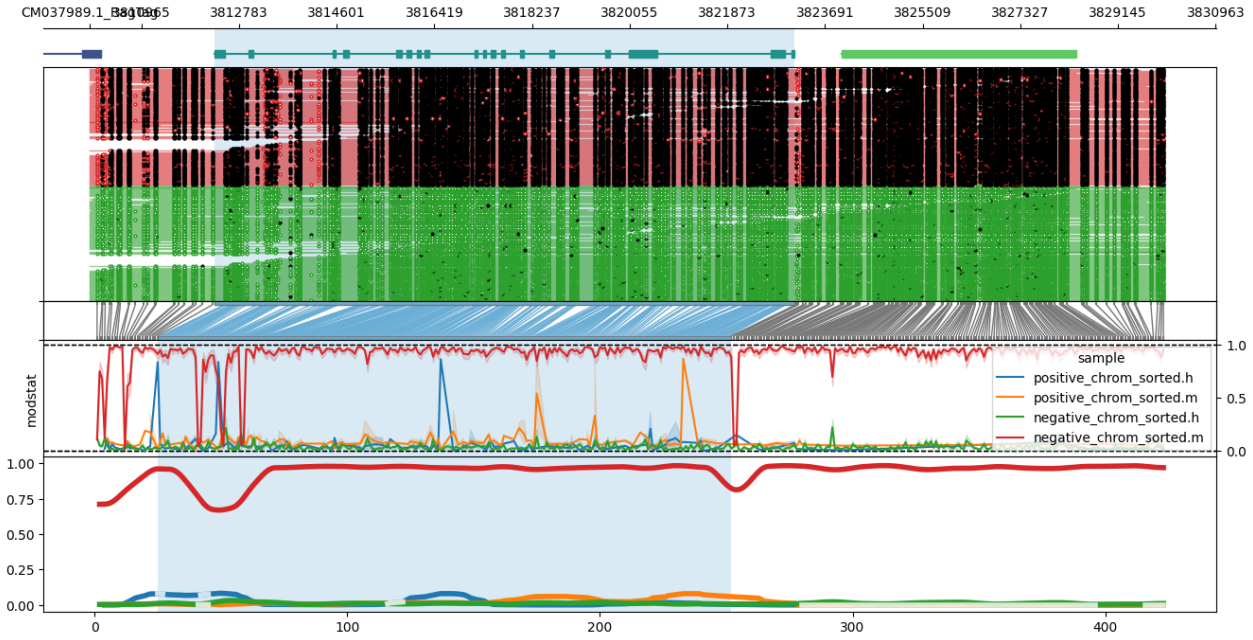


Results: Improved RNA Seq and MethylSeq

- Improved annotation and assembly has improved results of previous IAB funded projects:
  - 2093 Total differentially expressed genes between the two haplotypes
  - 20 Differentially methylated regions
  - 2 DE genes overlap with 2 DMRs



Results: DMRs From Long Reads



- Using newer sequences, we can extract methylation data directly from DNA
- Other types of Methylation (hydroxy-methylation, 6-Adenine methylation)
- No additional lab steps which can cause error
- High coverage but fewer samples…

Conclusions and Next Steps

- A new assembly has expanded our ability to analyze bud failure from a molecular genetics perspective
- New resources for breeders
- New targets for genetic assays for NBF
- Alternative hypotheses to methylation as a cause of NBF