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# A Strategy for Balanced Haplotype-Resolved De Novo Assembly of the Autotetraploid Genome of Medicago sativa


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## Background

- *Medicago sativa* (alfa-alfa, luzerne) is an **autotetraploid** forage crop with a large, **repeat-rich** genome.
  - Reference genome[1] : 4x8 CHRS; haplotype ~ 851 Mbp; N50 contig length = 459 kbp; L50 contig count = 1870
  - Haplotype-resolved assembly is difficult due to **high similarity between chromosomes**.
  - Assembly strategies usually adapted for diploids
- Hifiasm + Hi-C – enables de novo phased assembly also for polyploids

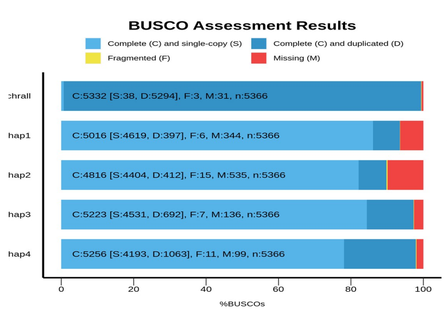
## Material & Methods

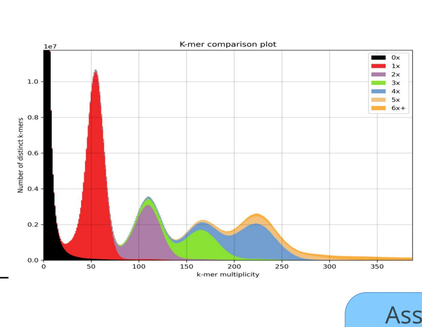
- Data: ~105× HiFi (PacBio) reads + 1 G Hi-C (OmniC) read pairs
- → Genomescope2[2]: ~3.2 Gbp, 86% repeats, 1.72% heterozygosity)
- **Contig assembly:** Hifiasm[3] v0.24.0 (with Hi-C integration, --*nhap* 4)
- l k-mer profile for completeness
- **Post-processing**: set of proteins aligned to contigs with minimap2[5] → contigs reassignment with Toulbar2[6] → Toulbar2 optimization[7] (modification of constraints)
- **Scaffolding**: Hi-C aligned with Juicer[8] (total Hi-C to all haplotypes) → kept within-haplotype aligned Hi-C → scaffolding with 3D-DNA[8] (only 1st round ; MAPQ=0) → manual curation in Juicebox[9]
- heck with DGenies[10] and against the reference genome[1]

## Results

### 1. Contig assembly

| Exp = 750 Mbp | Length (Mbp) |
|---|---|
| HAP1 | 784 |
| HAP2 | 733 |
| HAP3 | 866 |
| HAP4 | 899 |



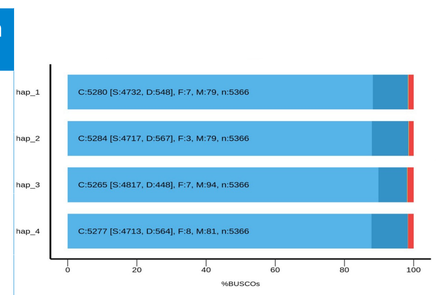



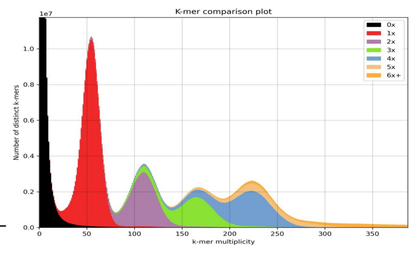

- Hifiasm produced :
  - ✓ Complete assembly – 4 haplotypes with no missing k-mers
  - ✗ Unbalanced haplotypes (varying haplotype lengths and BUSCO scores)

Assumption : Haplotypes in autopolyploids do not vary significantly between each other.

### plotype balancing post-processing

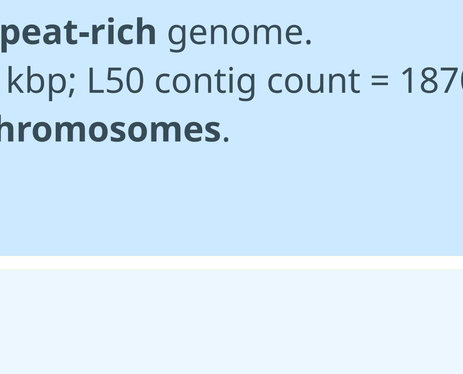
| Exp = 750 Mbp | Length (Mbp) |
|---|---|
| HAP1 | 798 |
| HAP2 | 785 |
| HAP3 | 787 |
| HAP4 | 800 |



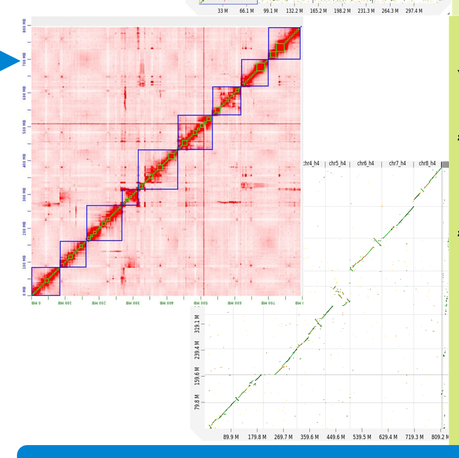

- Reassignment of contigs to haplotypes based on shared protein content :
  - ✓ More even haplotype lengths, better BUSCO balance
  - ✓ Some contigs 'lost', but no effect on the k-mer profile

### 2. Manual curation



- ✗ Chromosome arms not so clear (structure often broken), CHR4 almost completely missing in HAP4
- ✗ Dotplot with many small and big inversions

=> Extensive manual curation needed



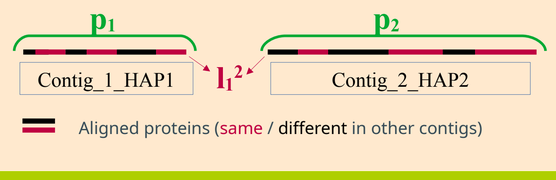
- ✓ All chromosomes present
- ✗ Chromosome arms even less visible, large missarangements
- ✗ Many large invertions, but less small ones

=> Manual curation still needed

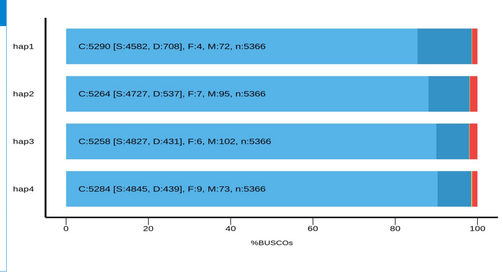
### Toulbar2 optimization

- **total cost = sum($I^2$) + sum(p)**

1. If two contigs are in the same haplotype => the cost is $I^2$ with I (number of shared proteins[2])
2. If a contig is moved from its haplotype of origin => the cost is p (number of proteins aligned in the contigs)



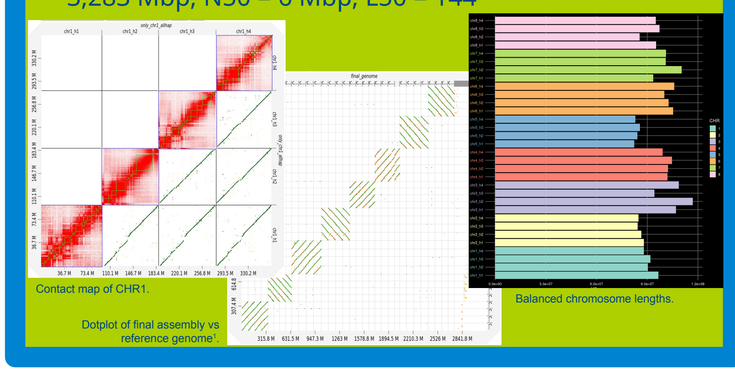

| Exp = 750 Mbp | Length (Mbp) |
|---|---|
| HAP1 | 770 |
| HAP2 | 780 |
| HAP3 | 760 |
| HAP4 | 766 |




- ➢ Toulbar2 is used to find the lowest cost possible.
- ➢ After modification of the two constraints :
- ➢ Toulbar2 reassigned between
- ➢ 21 and 43% contigs/per haplotype
- ➢ 8 and 17% haplotype length in bp
- ✓ Improved contig grouping
- ✓ Balanced haplotype lengths
- ✓ Retained all original contigs

### Final assembly

- ✓ Clear chromosome structure in all haplotypes
- ✓ Minimum inversions/misassemblies
- ✓ Improved collinearity with reference genome
- ➔ ~ 3,283 Mbp; N50 = 6 Mbp; L50 = 144




GITHUB REPO

PREPRINT[7]

## Conclusions

- ➢ Hifiasm + Hi-C produced unbalanced haplotype lengths and BUSCO scores, requiring extensive manual curation after scaffolding.
- ➢ Toulbar2 with added constraints enabled refined scaffolding requiring minimal curation.
- ➢ We obtained balanced haplotype lengths and collinearity despite the large, repetitive autotetraploid genome.

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