Assembly and validation of a draft genome of *Brassica napus* using skim genotyping by sequencing

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Overview

- Genome assembly challenges
- Validating genome assemblies
- Genotyping by sequencing
- Placing missing contigs
- Clustering based validation

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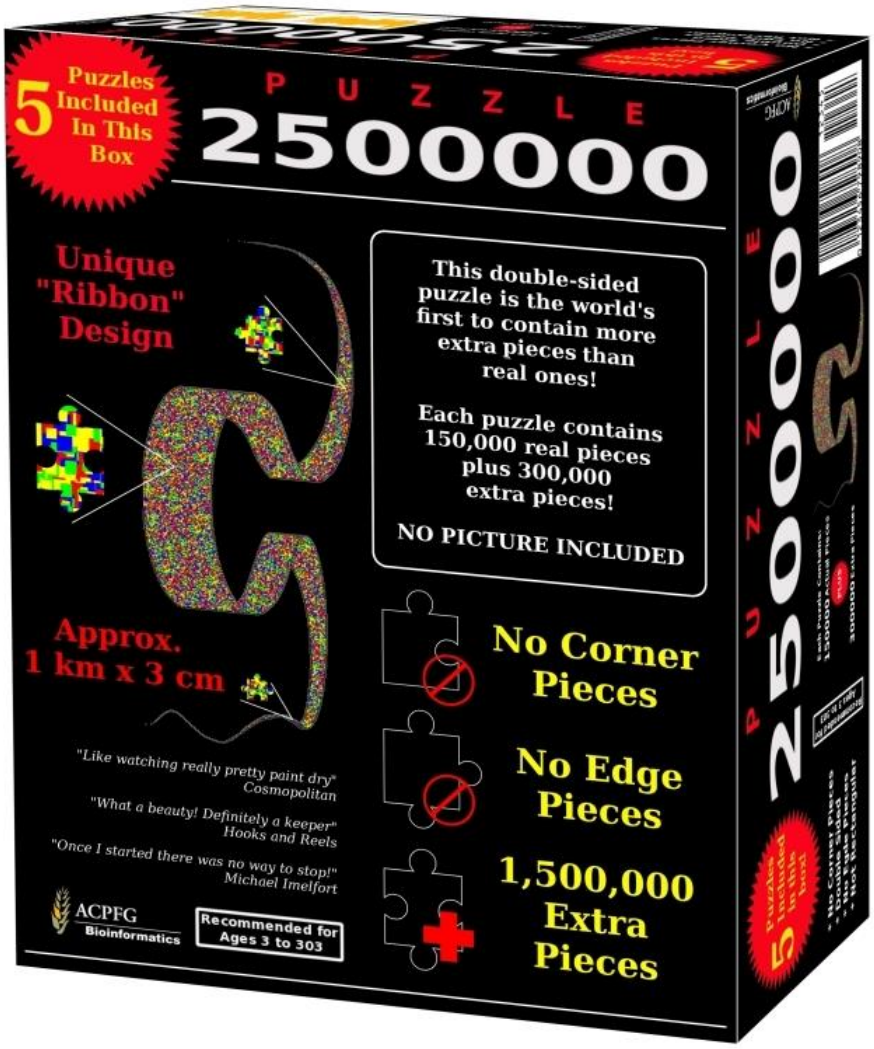
The challenge of genome sequencing

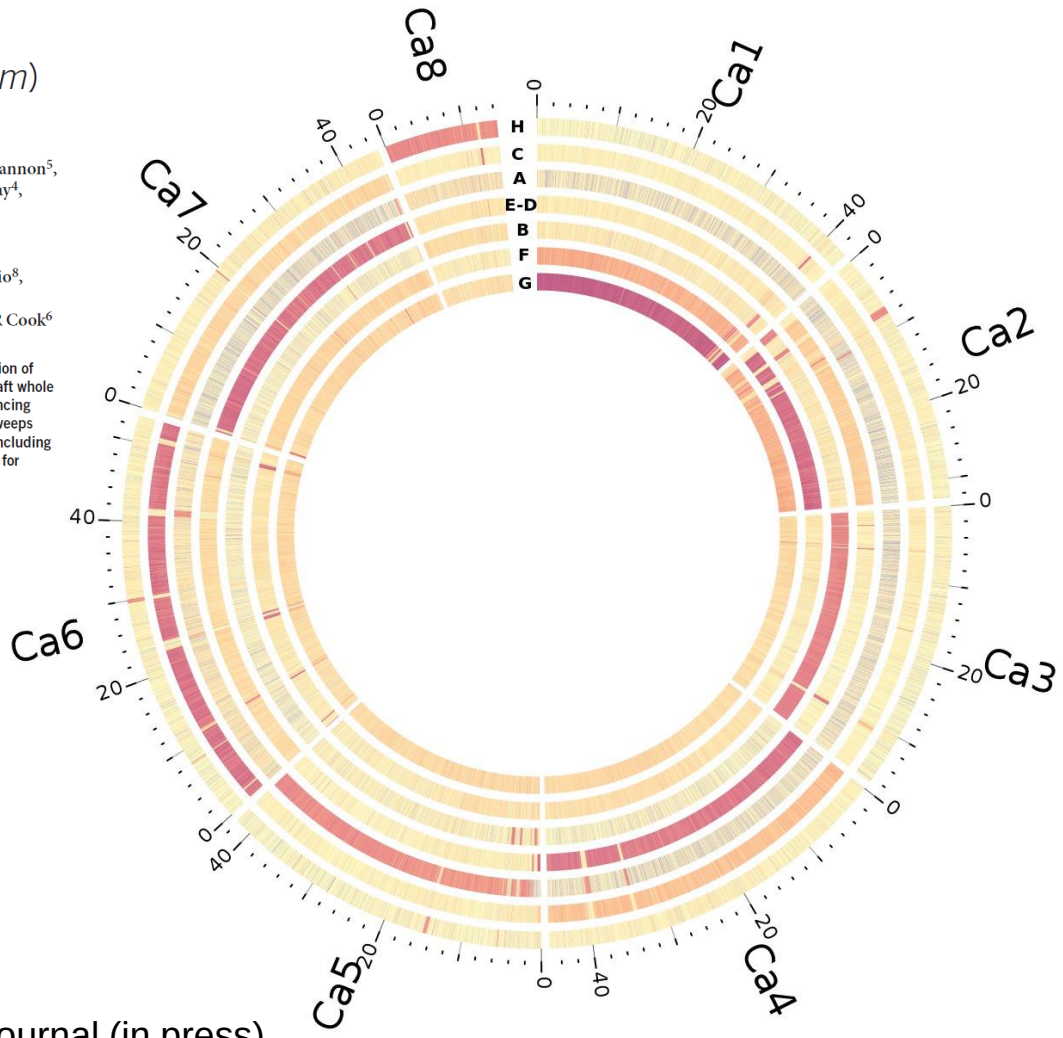


The challenge of genome sequencing

Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement

Rajeev K Varshney[1,2], Chi Song[3], Rachit K Saxena[1], Sarwar Azam[1], Sheng Yu[3], Andrew G Sharpe[4], Steven Cannon[5], Jongmin Baek[6], Benjamin D Rosen[6], Bunyamin Tar'an[7], Teresa Millan[8], Xudong Zhang[3], Larissa D Ramsay[4], Aiko Iwata[9], Ying Wang[3], William Nelson[10], Andrew D Farmer[11], Pooran M Gaur[1], Carol Soderlund[10], R Varma Penmetsa[6], Chunyan Xu[3], Arvind K Bharti[11], Weiming He[3], Peter Winter[12], Shancen Zhao[3], James K Hane[13], Noelia Carrasquilla-Garcia[6], Janet A Condie[4], Hari D Upadhyaya[1], Ming-Cheng Luo[6], Mahendar Thudi[1], C L L Gowda[1], Narendra P Singh[14], Judith Lichtenzveig[15], Krishna K Gali[4], Josefa Rubio[8], N Nadarajan[16], Jaroslav Dolezel[17], Kailash C Bansal[18], Xun Xu[3], David Edwards[19], Gengyun Zhang[3], Guenter Kahl[20], Juan Gil[8], Karam B Singh[13,21], Swapan K Datta[22], Scott A Jackson[9], Jun Wang[3,23] & Douglas R Cook[6]

Chickpea (*Cicer arietinum*) is the second most widely grown legume crop after soybean, accounting for a substantial proportion of human dietary nitrogen intake and playing a crucial role in food security in developing countries. We report the ~738-Mb draft whole genome shotgun sequence of CDC Frontier, a *kabuli* chickpea variety, which contains an estimated 28,269 genes. Resequencing and analysis of 90 cultivated and wild genotypes from ten countries identifies targets of both breeding-associated genetic sweeps and breeding-associated balancing selection. Candidate genes for disease resistance and agronomic traits are highlighted, including traits that distinguish the two main market classes of cultivated chickpea—*desi* and *kabuli*. These data comprise a resource for chickpea improvement through molecular breeding and provide insights into both genome diversity and domestication.



Ruperao et al. The Plant Biotechnology Journal (in press)

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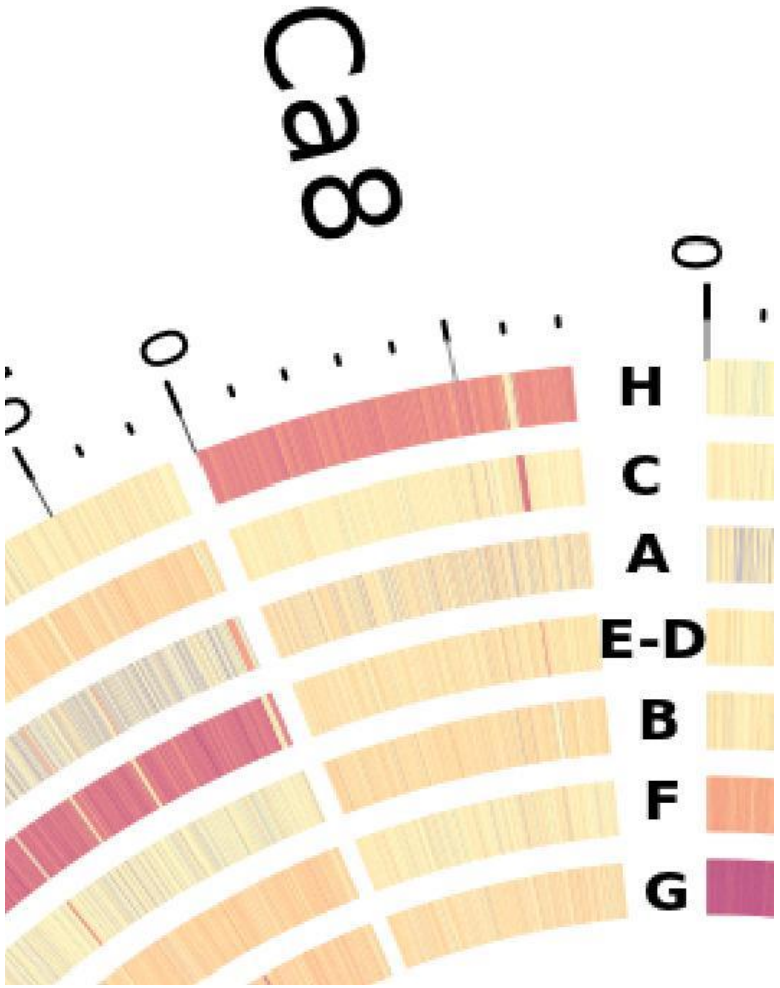
Kabuli chickpea reference



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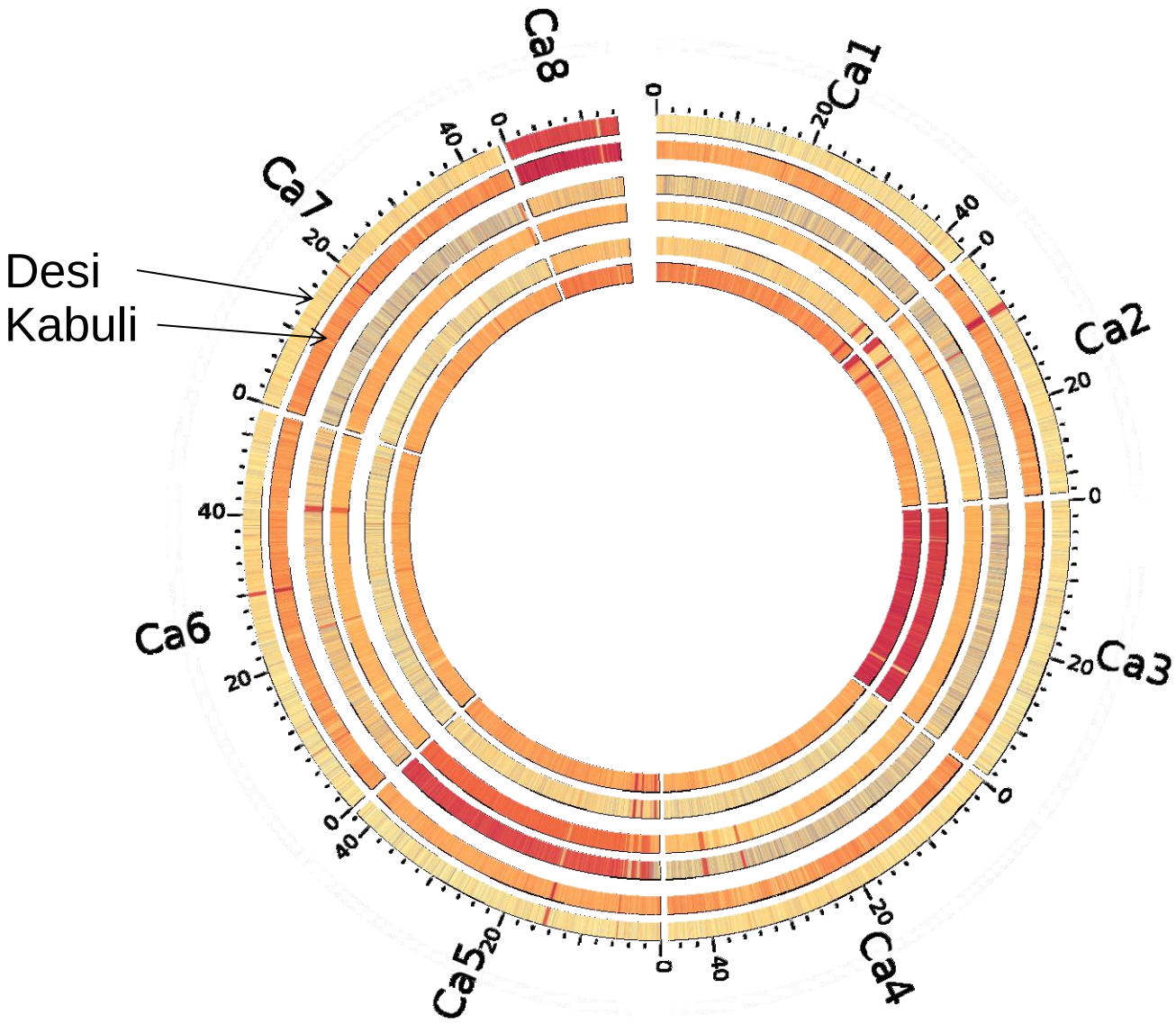
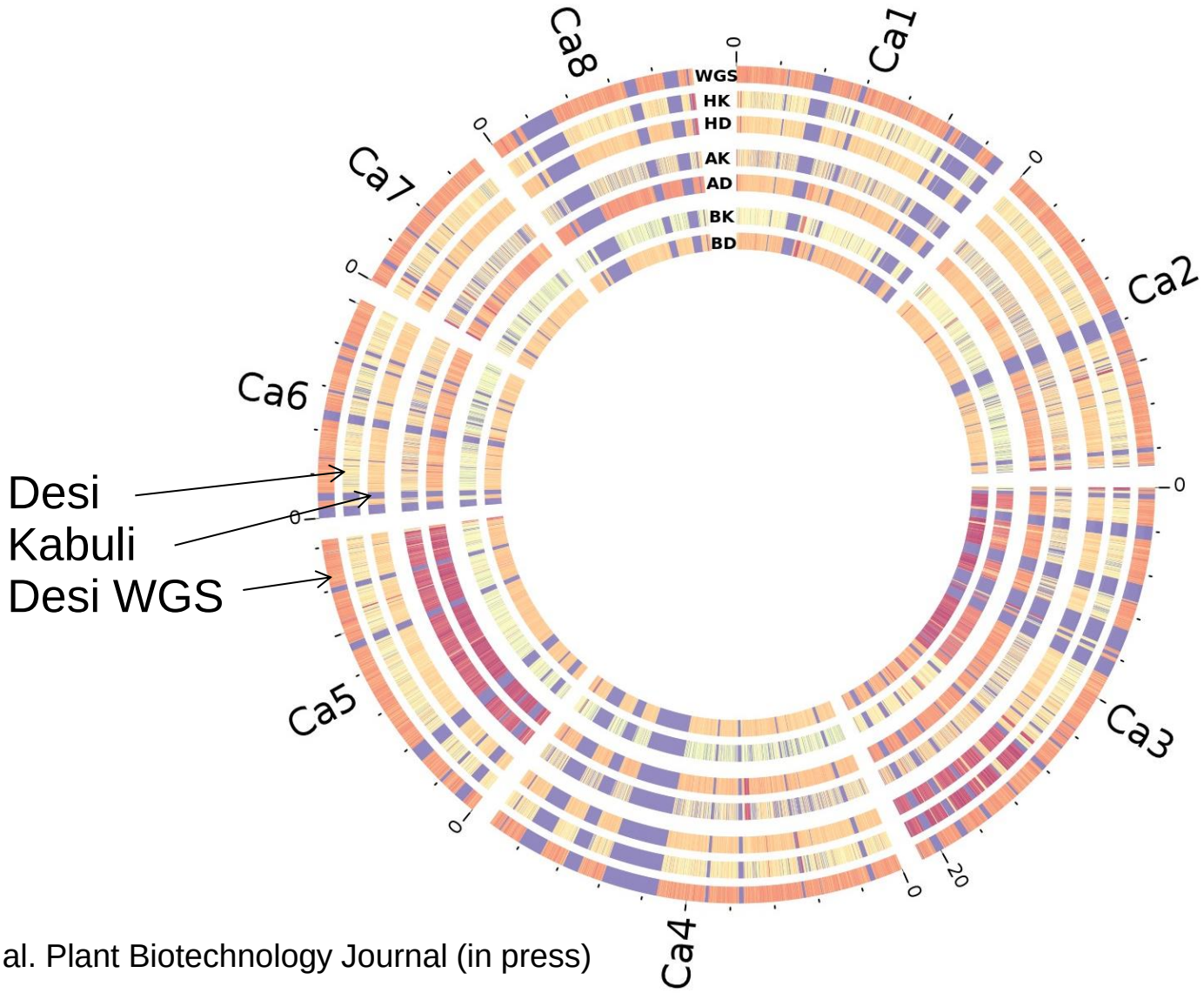
Kabuli chickpea reference



Desi chickpea reference



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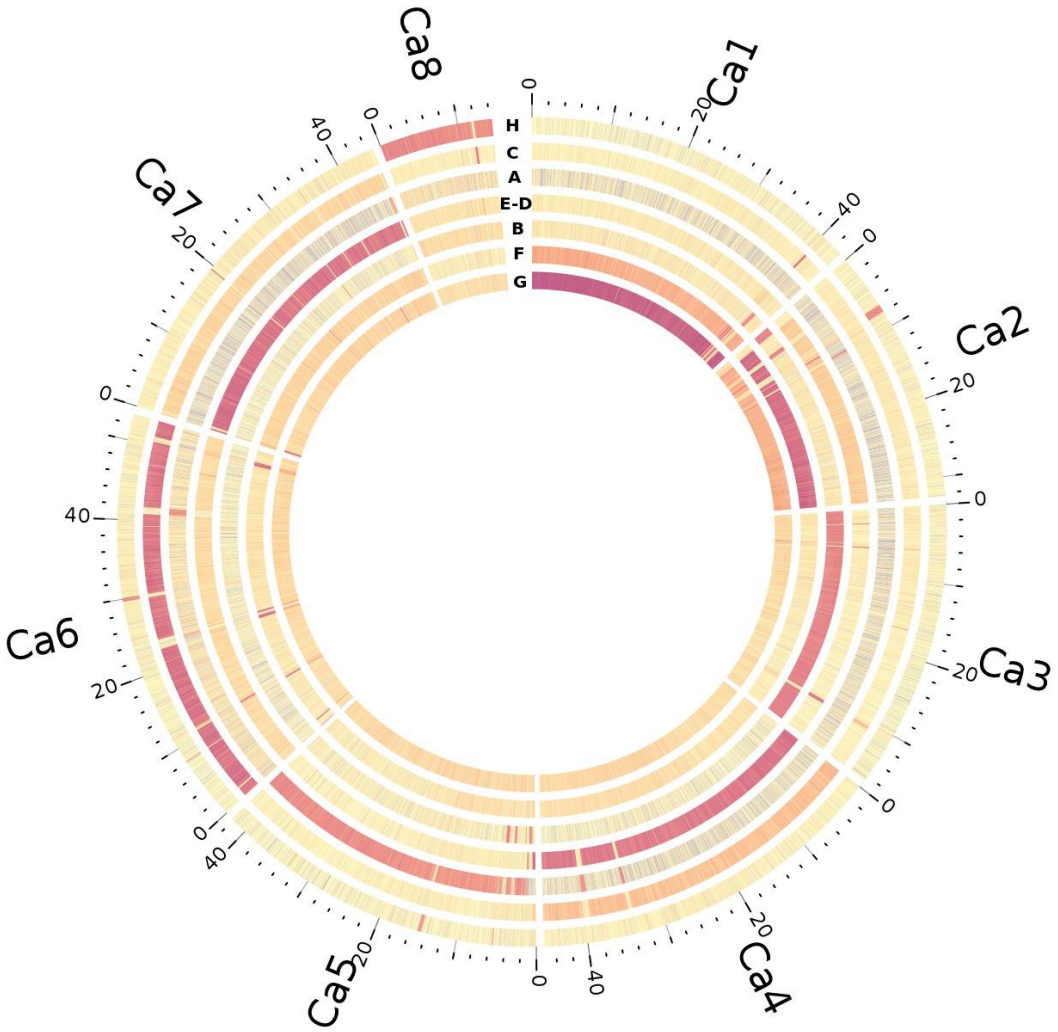
How do we validate and fix a reference genome



Skim GBS

- Determine SNPs by sequencing parents and running SGSautoSNP

- Low coverage skim sequence segregating population
- Map reads to the reference genome
- Call genotype where reads cover previously defined SNP
- Impute and clean to define haplotype blocks

Genotype calling

Call genotype of previously predicted SNPs

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Haplotype blocks

T	A	G	G	T	C	C	A	G	G	A	T	A	A	T
N	T	C	C	A	G	G	C	T	C	G	C	G	G	C
TN1	A	G	G	T	C	C	A	G	G	A	T	A	A	T
TN2	A	G	G	T	C	C	A	G	G	A	T	A	A	T
TN3	T	C	C	A	G	G	C	G	G	A	T	A	A	T
TN4	A	G	G	T	C	C	A	G	G	A	T	A	A	T
TN5	T	C	C	A	G	G	C	T	C	G	C	G	G	C
TN6	A	G	G	T	C	C	A	G	G	A	T	A	A	T
TN7	T	C	C	A	G	G	C	T	C	G	C	G	G	C

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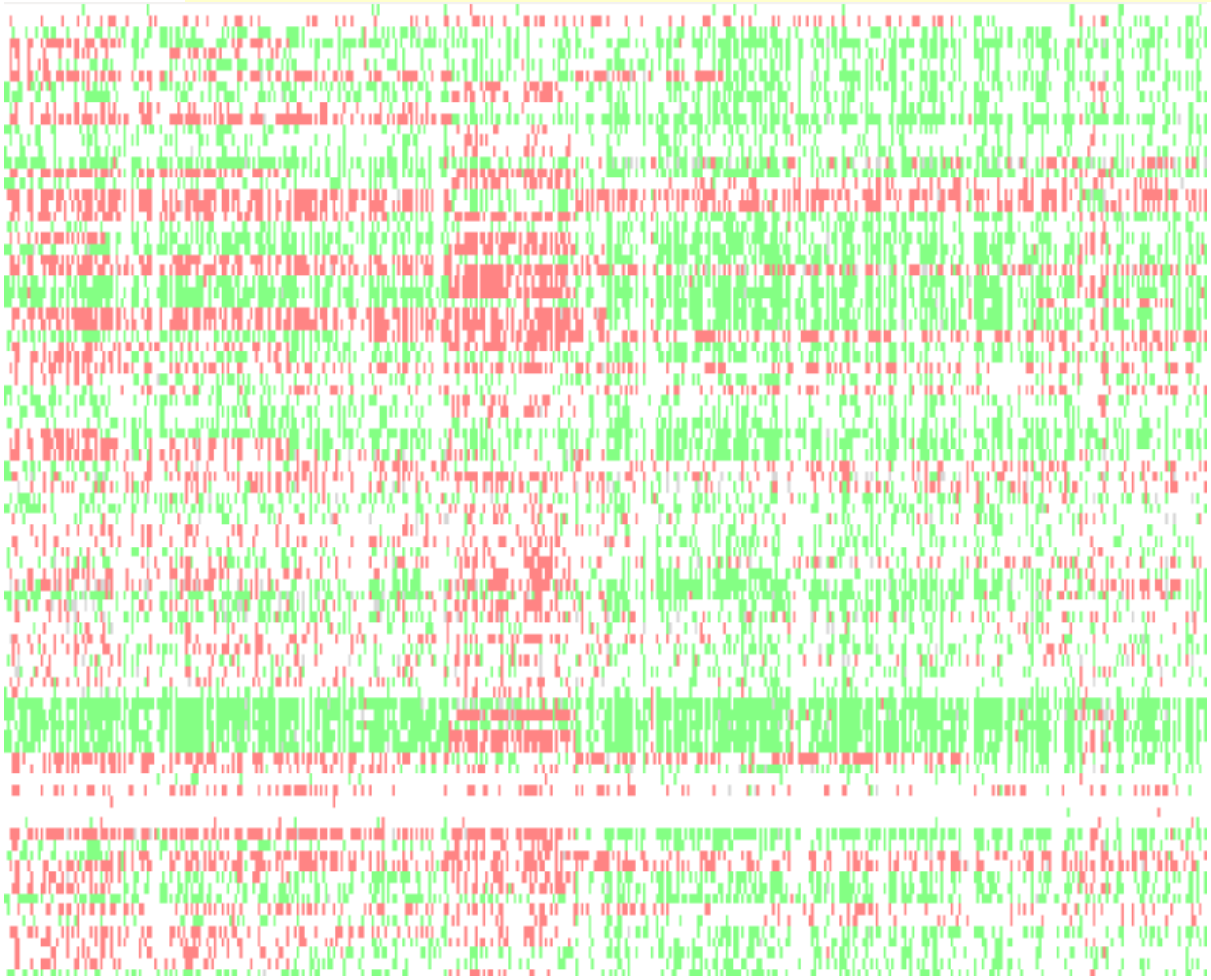
Pre imputation



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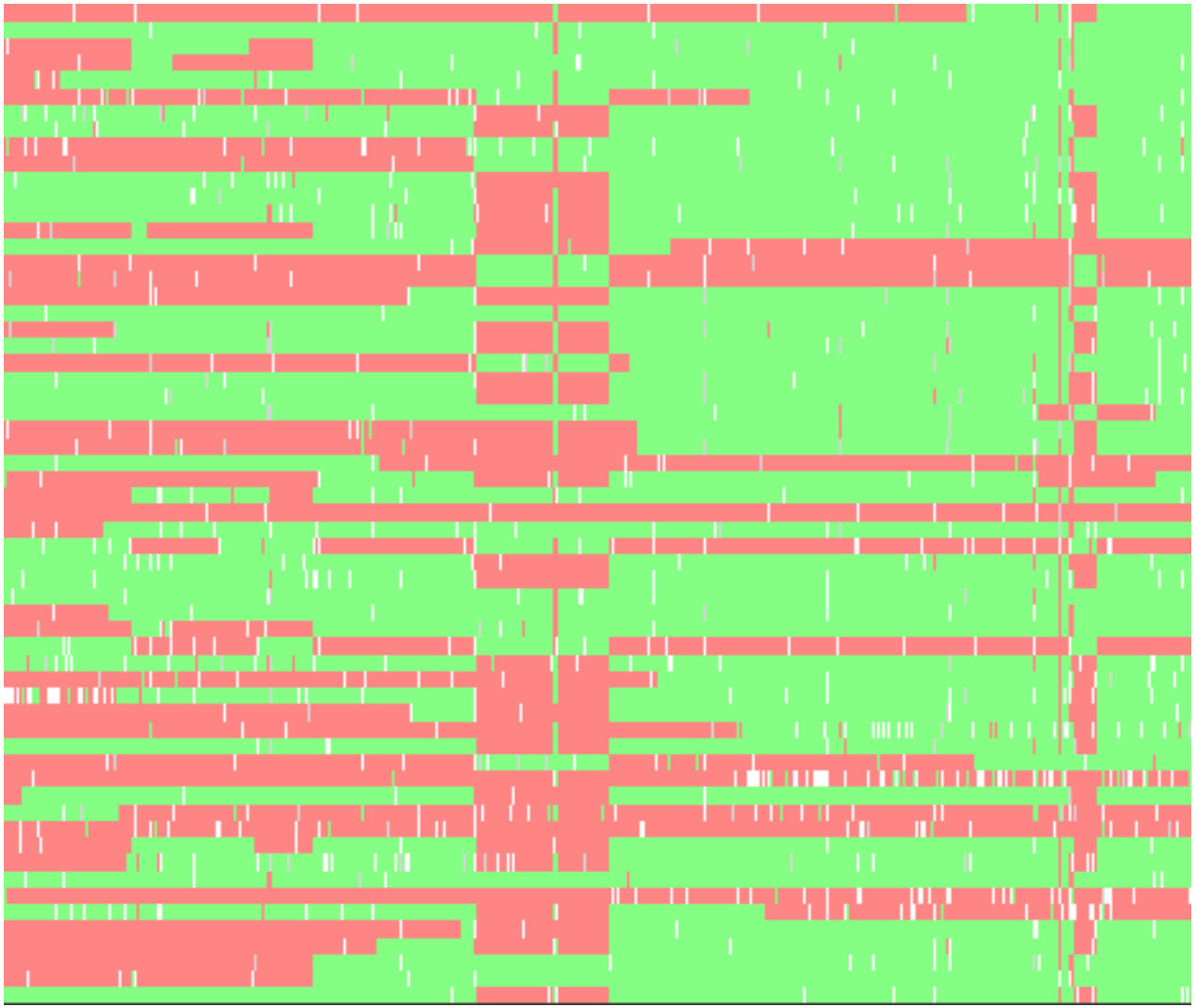
After imputation and cleaning



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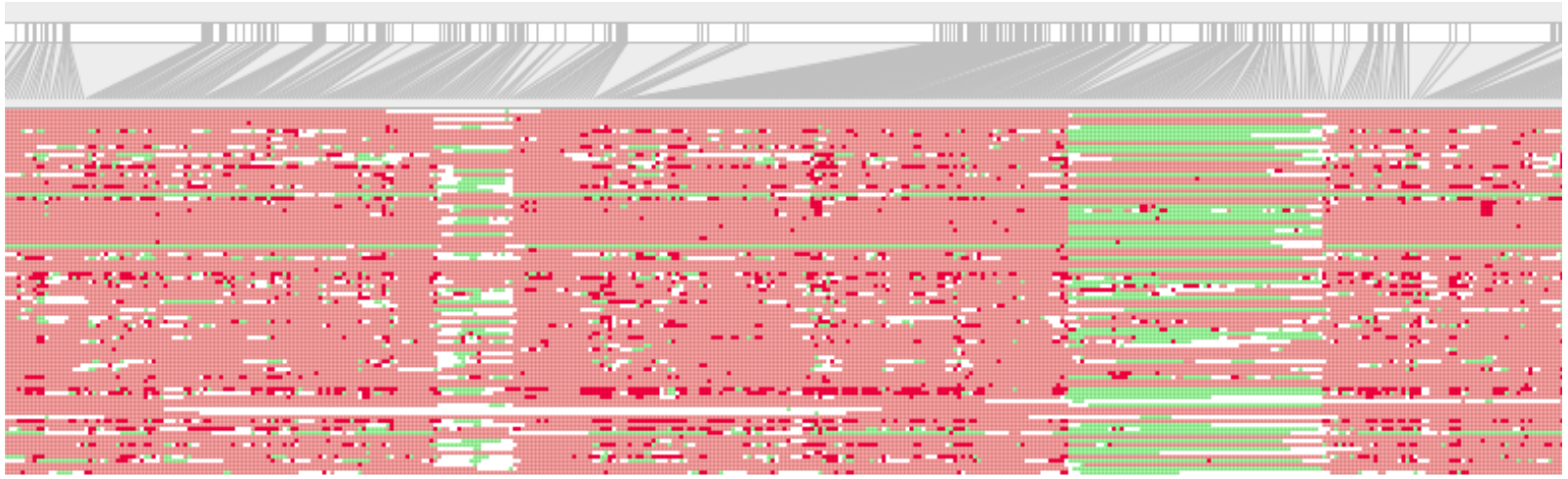
Genotyping by sequencing

- 92 double haploid Tapidor x Ningyou individuals
- Called SNPs for parents, assigned parental genotypes to all alleles in the population
- Used these alleles to
  - create genetic maps
  - place unplaced contigs
  - identify misplaced and chimeric contigs

GBS applications

- Check for misplaced contigs based on recombination events shared between individuals



- Red:Tapidor, green: Ningyou, dark red: heterozygous allele, white: missing

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Placing unplaced contigs: contigPlacer

- Compare unplaced contigs with all placed contigs
- Use metaSNPs
- Penalized Hamming distance to compare alleles between two SNPs
- Places unplaced contig next to the best possible placed contig
- Possible to reverse contig if latter half of contig fits better to best partner than first half

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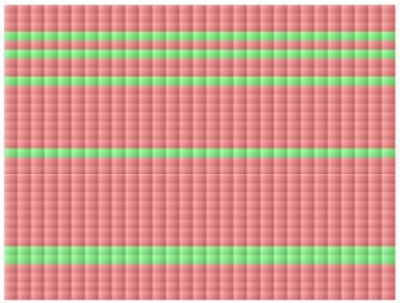
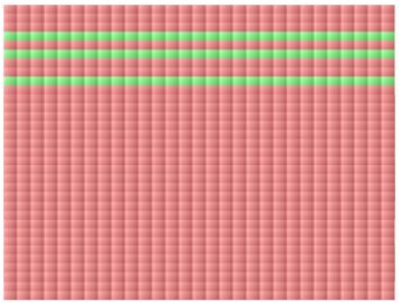
contigPlacer 1

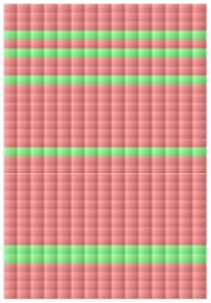
Placed contig 1



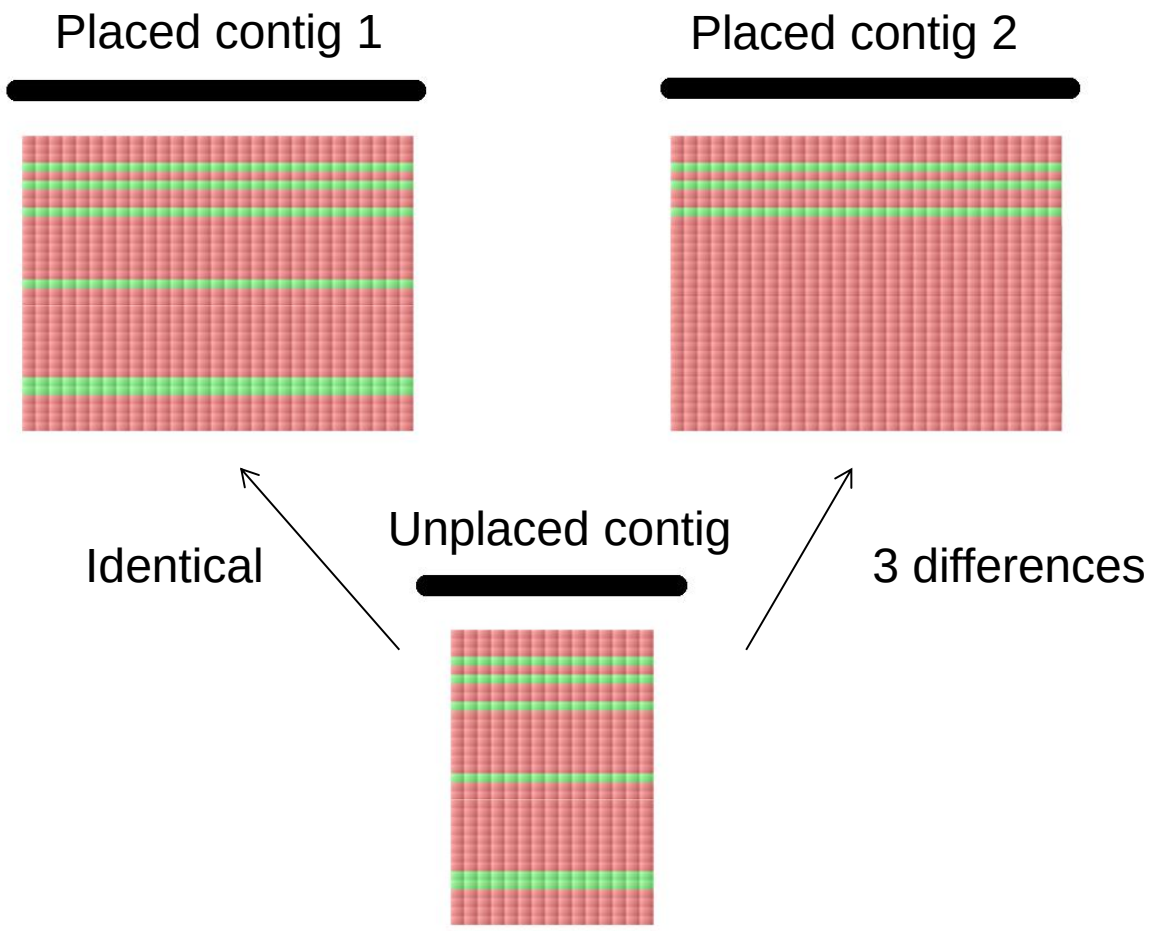
Placed contig 2



Unplaced contig



contigPlacer 2

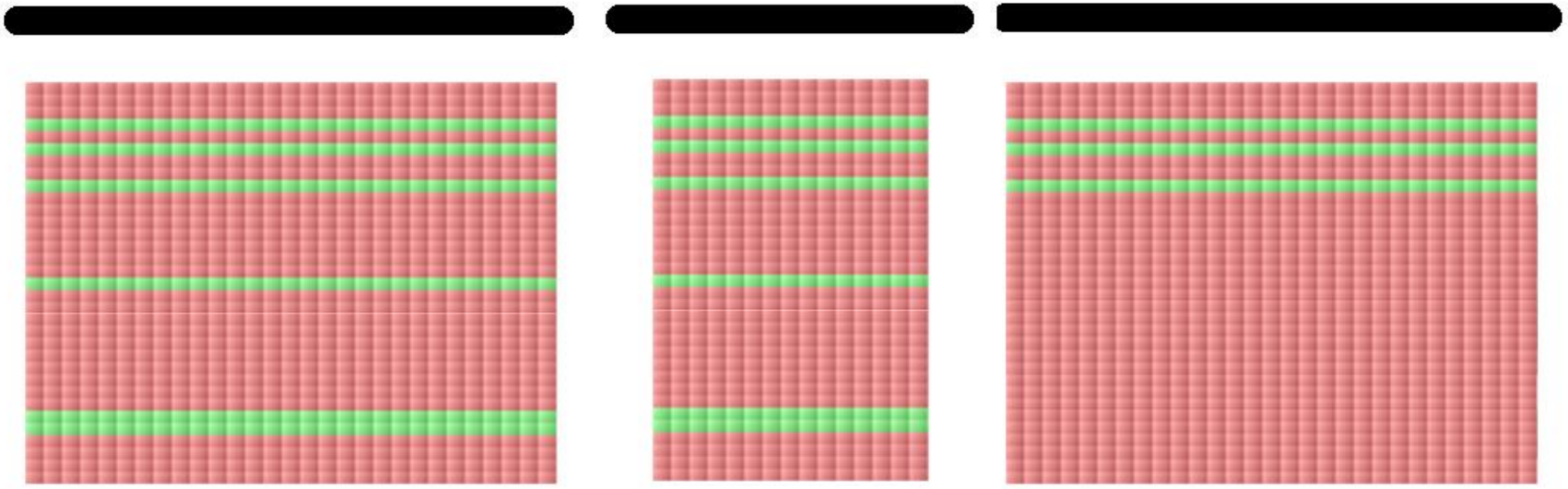


contigPlacer 3

Placed contig 1

Now placed contig

Placed contig 2



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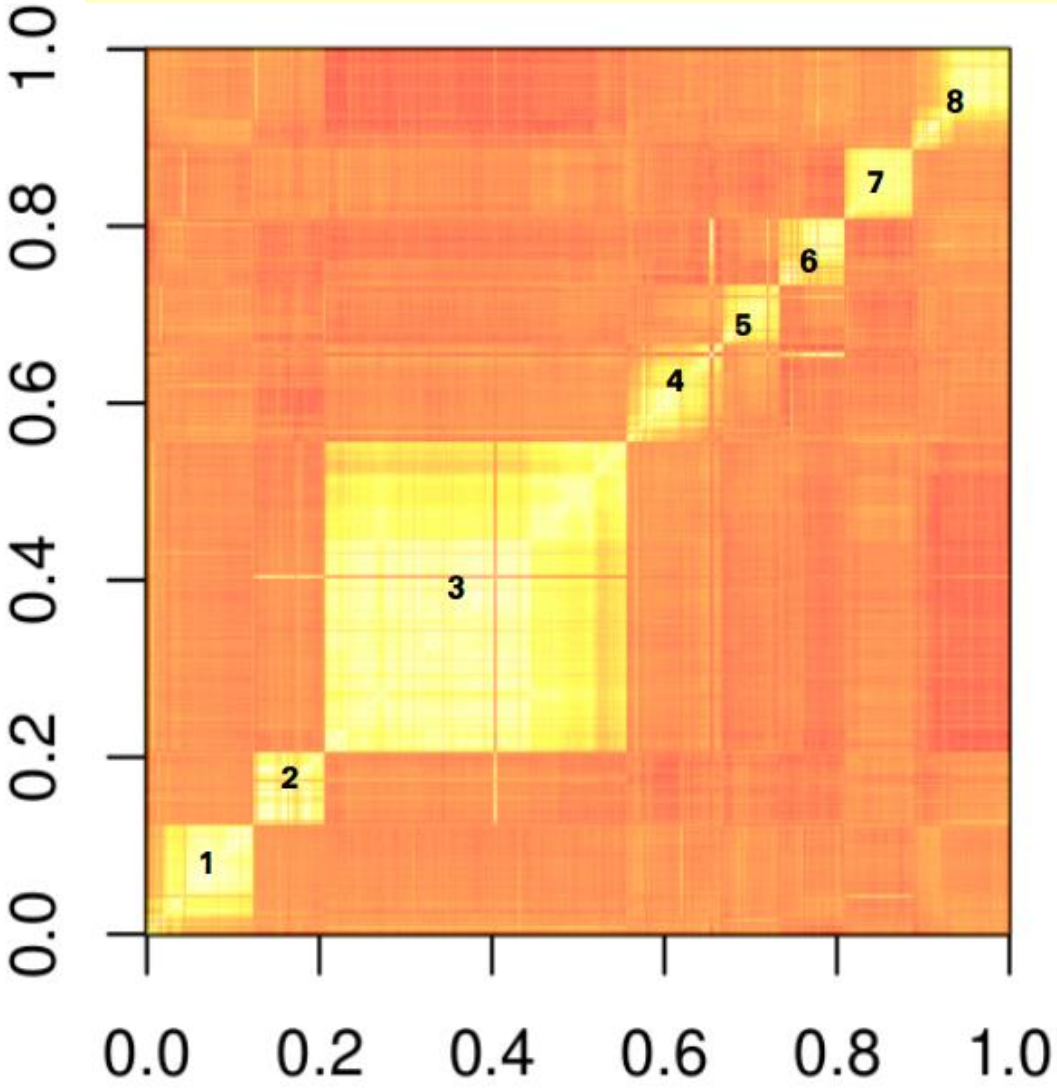
Clustering



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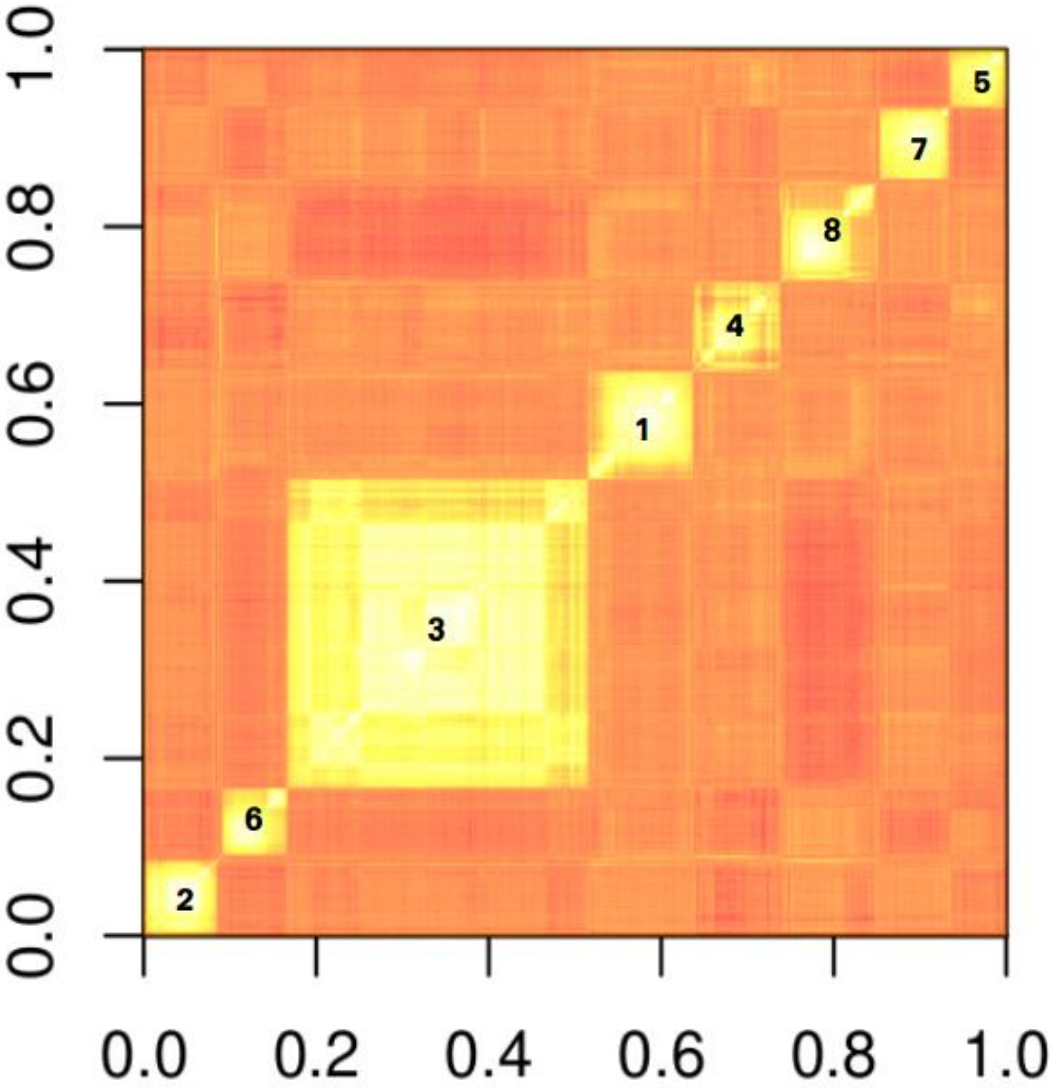

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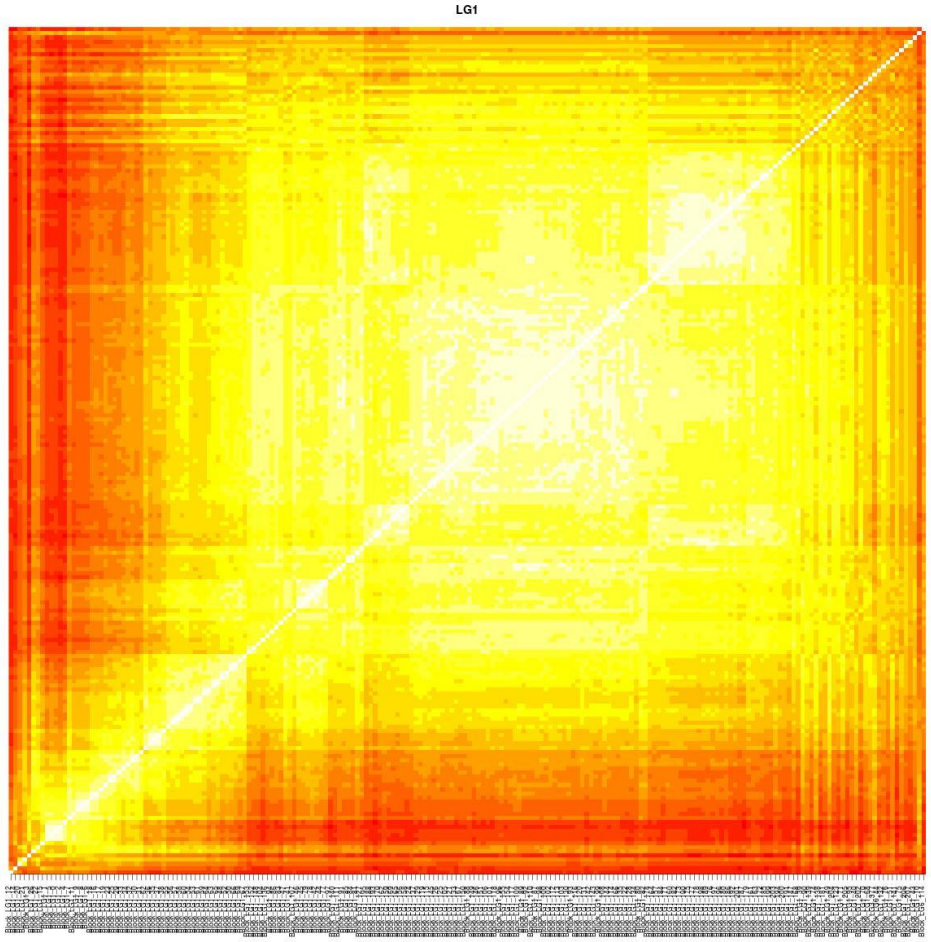

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Placing contigs in Darmor

- Total size: 850 Mbp
- Was 645 Mbp placed in pseudomolecules
- Now 800 Mbp placed in pseudomolecules,
- 50 Mbp unplaced (contigs with no SNPs or chimeric)
- Genes on pseudomolecules from 63,904 to 75,955
- Only 3,528 genes remain unplaced

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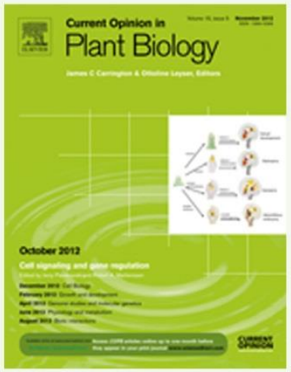

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