CHROMATIN CONFORMATION ANALYSIS AND ITS RELATIONSHIP WITH STRUCTURAL VARIANTS

JUMBO - Second Year Progress Report

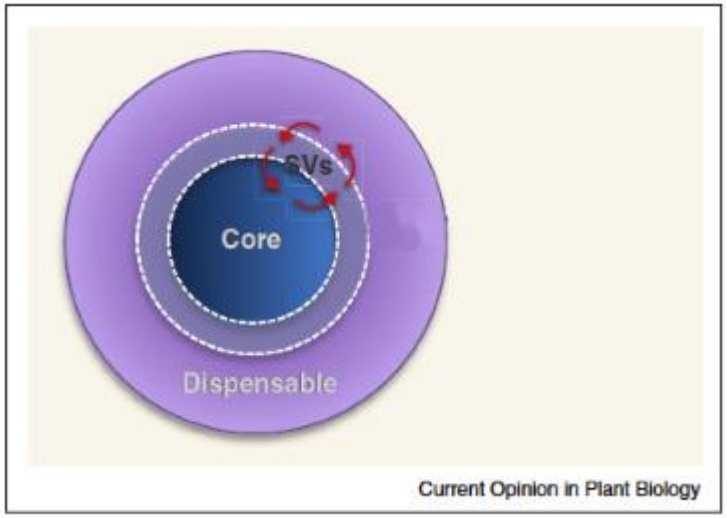
SISSA 13/10/2016

Supervisor: Prof. Michele Morgante

PhD Student: Aldo Tocci

UNIVERSITÀ DEGLI STUDI DI UDINE

The NOVABREED project



The plant Pan-Genome consists of:

- Core genomic features
- Dispensable Genome due to Structural Variants (SVs)

(Marroni, Pinosio and Morgante, 2014)

- Uncovering the composition, origin and structure of the SVs
- Understand the contribution of the SVs to the creation of new genetic variation in plant

Structural variants (SVs)

(Marroni, Pinosio and Morgante, 2014)

- **CNV**: Copy-Number Variants

- **PAV**: Presence-Absence Variants
  - » **deletions** and **insertions**

    (TEs movement and genomic rearrangements)

Aim

Assess the effect of the SVs on *Vitis vinifera* genome architecture

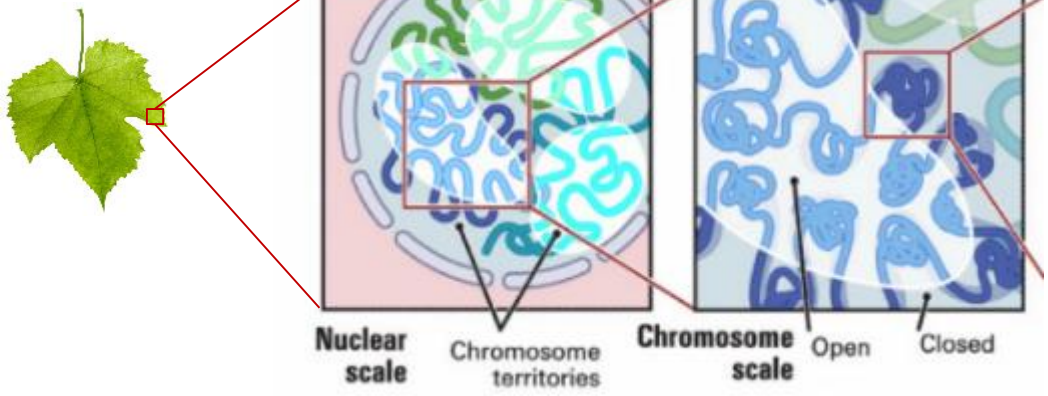
a. Investigate the role of genomic architecture in the interplay between structure, sequence and function

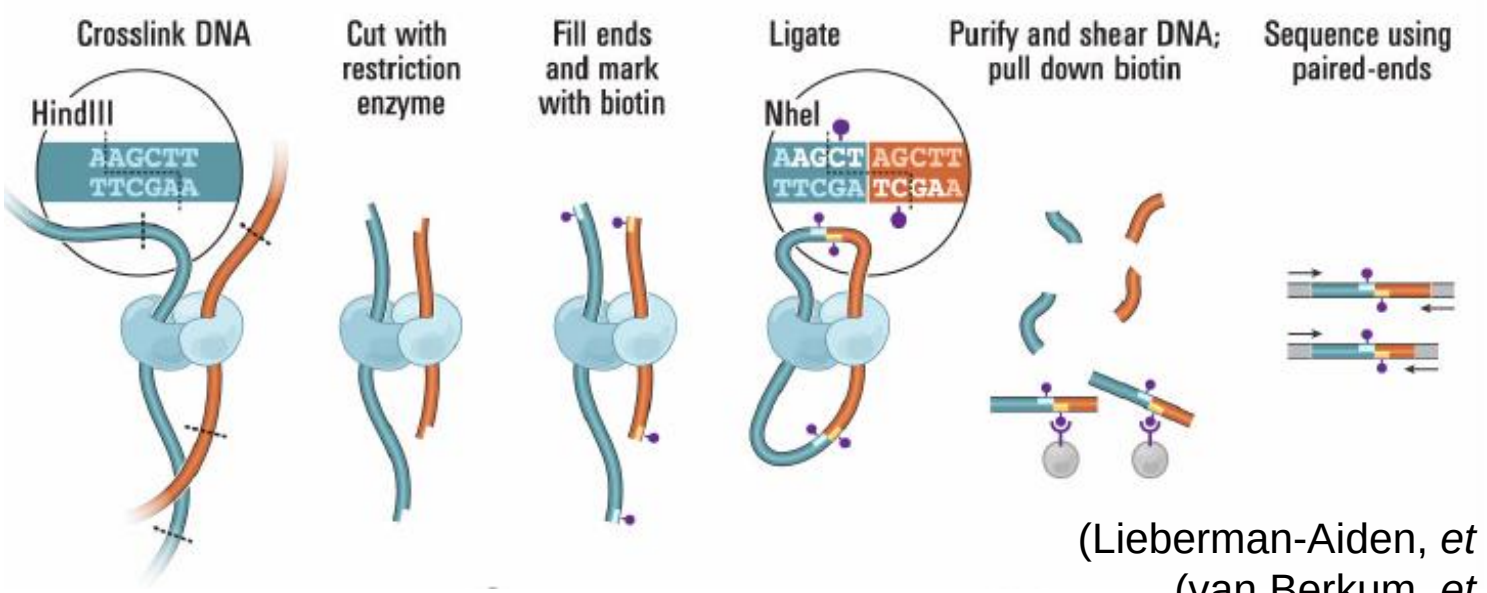
b. Investigate allele-specific chromatin structure:
   - its dependence on SVs in haplotypes
   - its effects on allele-specific regulation

c. Assess variation of genome architecture in different *Vitis vinifera* varieties

The standard Hi-C Method

V. vinifera
young leaf





(Lieberman-Aiden, *et al.* 2009)
(van Berkum, *et al.* 2010)

What has been done…

Variety: Pinot Noir

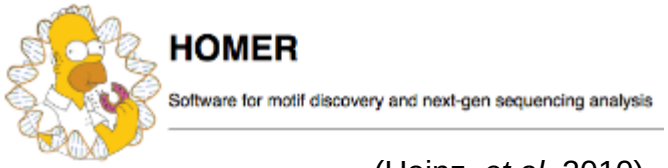
1. Contact maps reconstruction via Hi-C

2. Global Structural Domains (SDs) conformation analysis

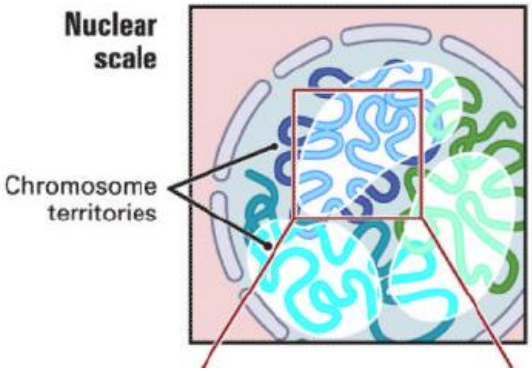
3. Hi-C data for scaffolding of an almost complete *Vitis vinifera* assembly

1. Contact Maps Reconstruction

Hi-C reads are aligned on current *Vitis vinifera* reference genome



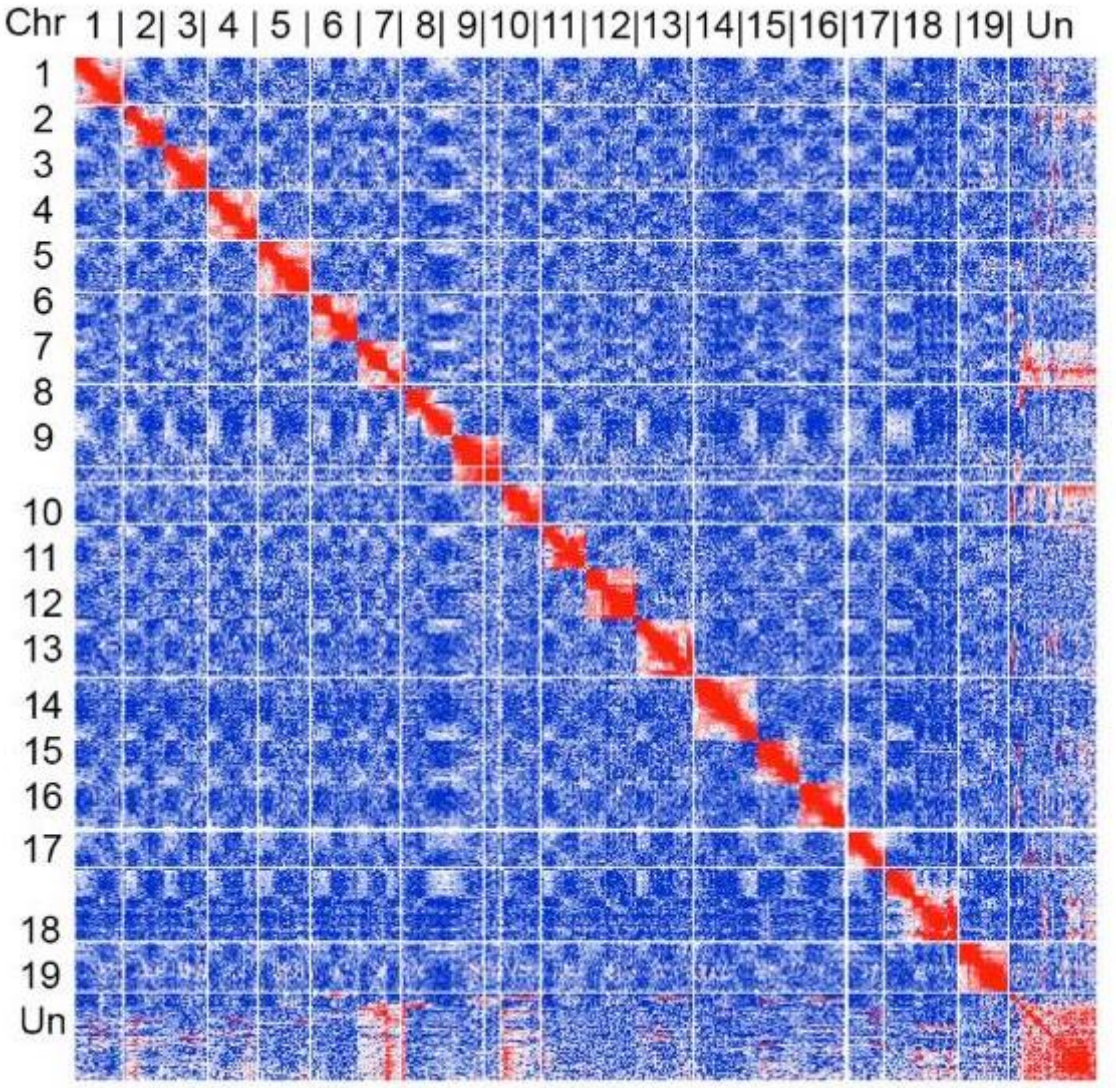
(Heinz, *et al.* 2010)



Resolution: 1 Mb

Red= high interaction frequency

Blue= low interaction frequency



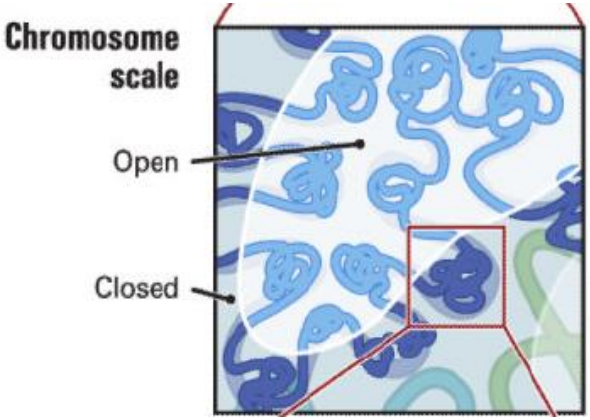
1. Contact Maps Reconstruction

There are Structural Domains (SDs) inside chromosomes

HOMER

Software for motif discovery and next-gen sequencing analysis

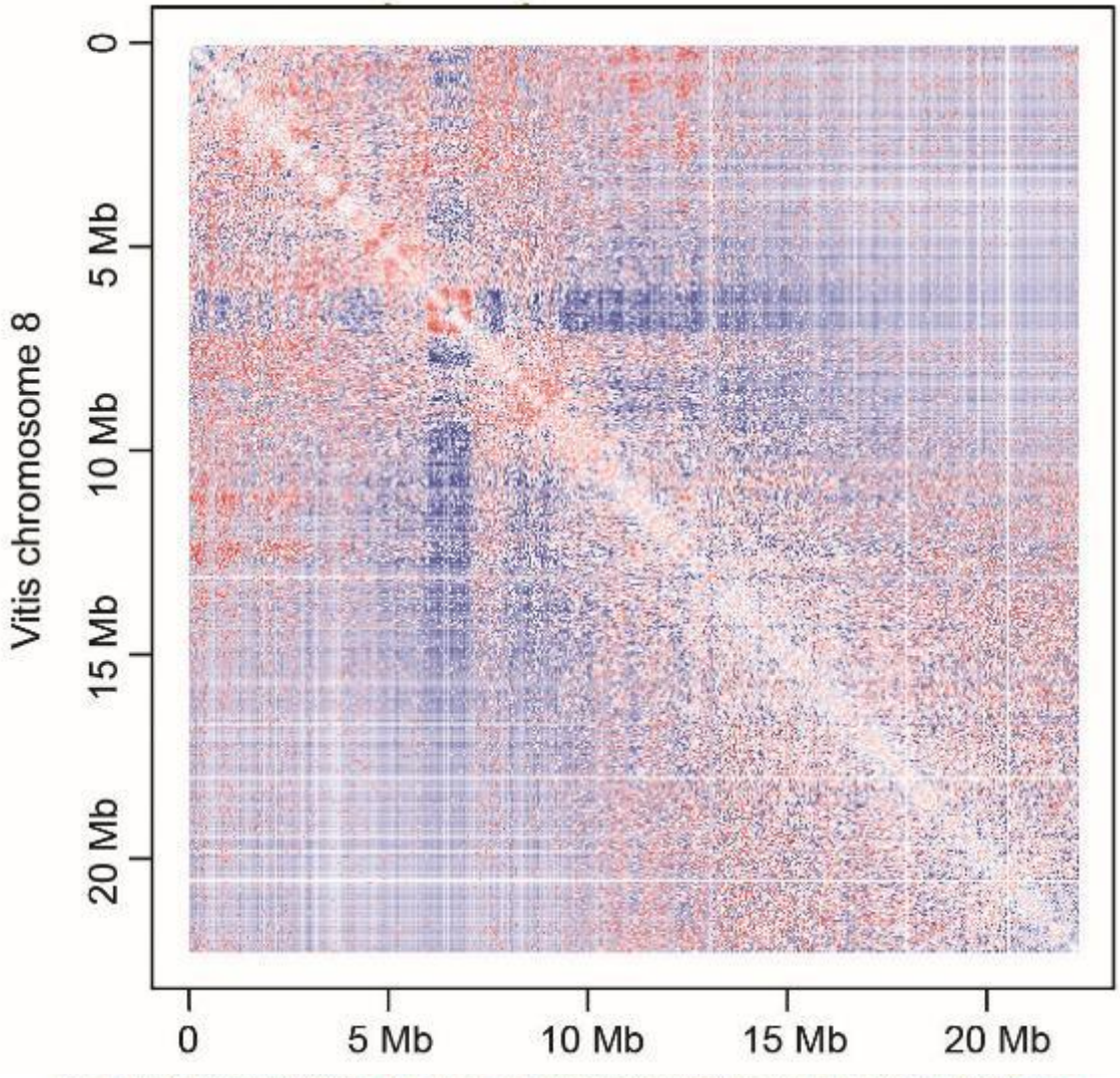
(Heinz, *et al.* 2010)



Resolution: 50 Kb

Red= high interaction frequency

Blue= low interaction frequency



2. Global Structural Domains (SDs) conformation analysis via PCA

Principal Component Analysis (PCA) simplifies the data into 2 sets of interactions: sparse and condensed, identifying two different SDs.

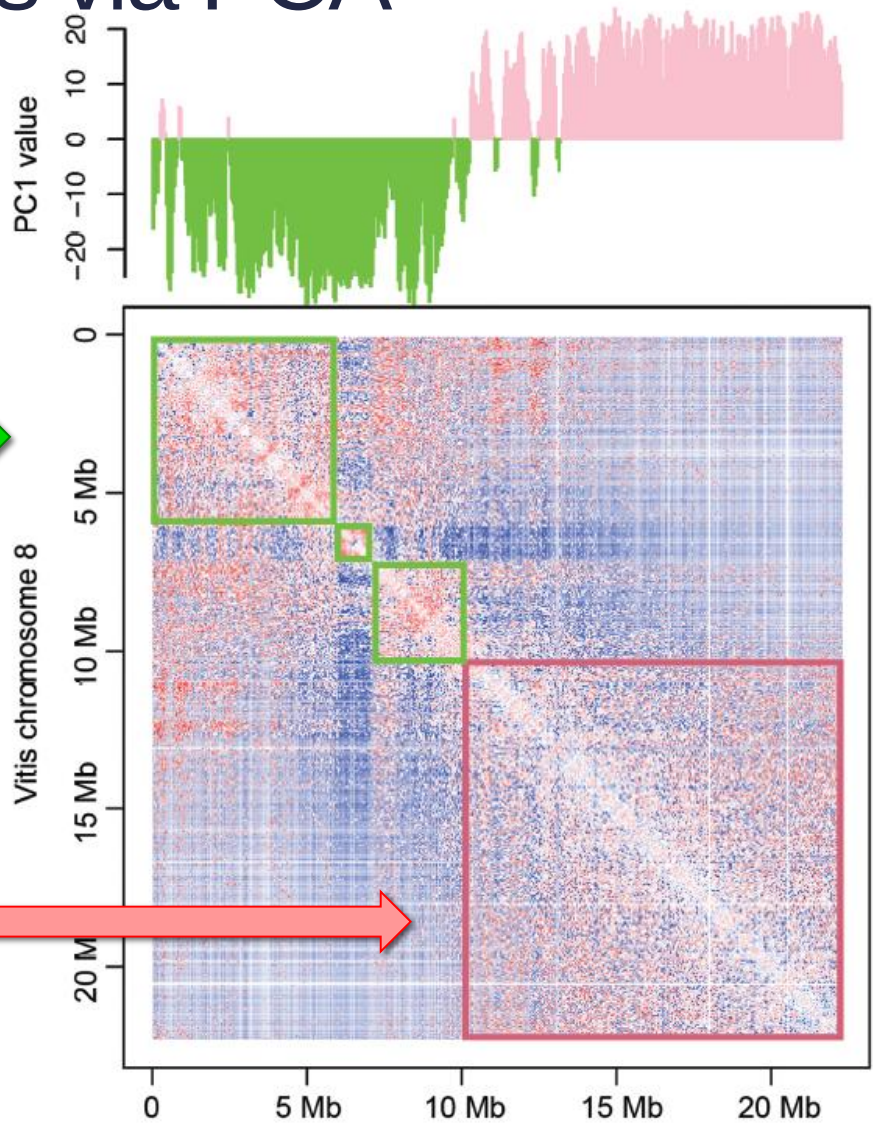
CSD

(Compacted Structural Domain): inactive epigenetic marks, low gene espression, presence of TEs and small RNAs.

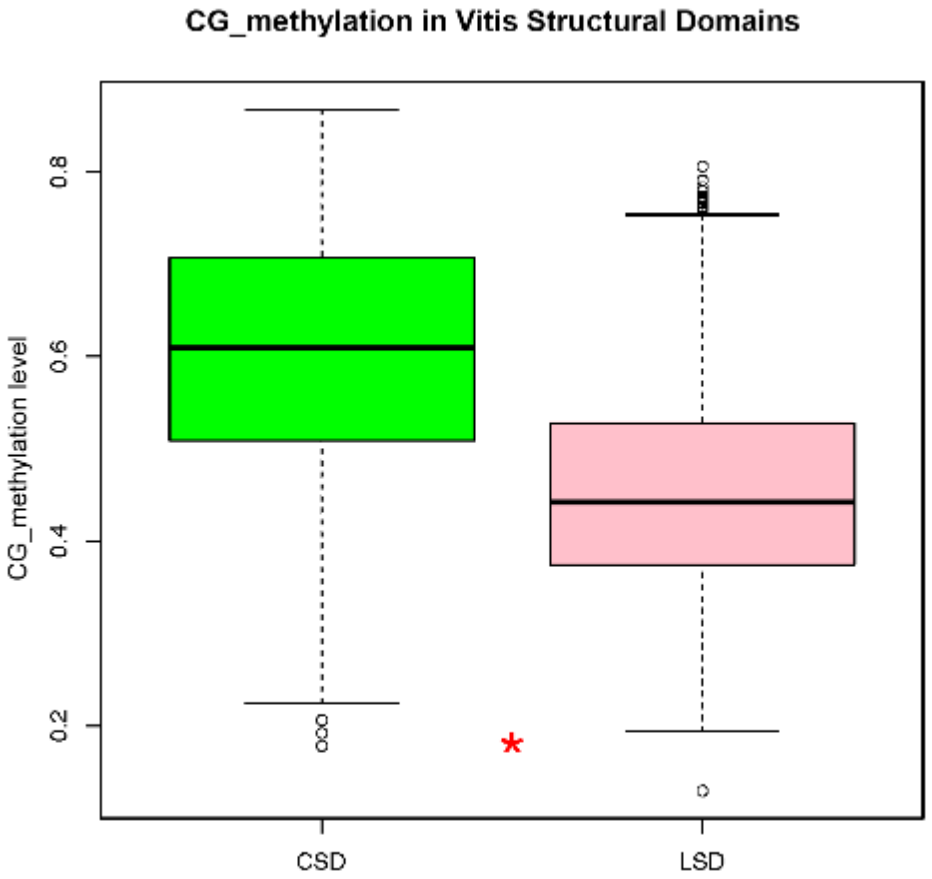
LSD

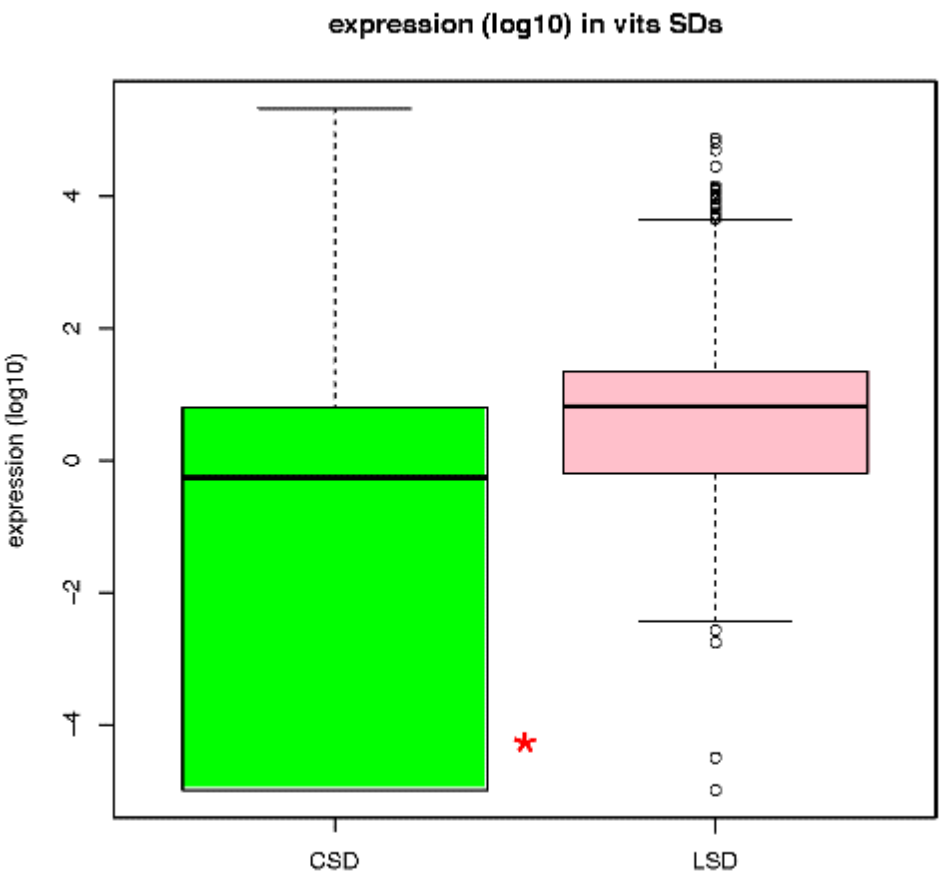
(Loose Structural Domain): active histone modifications, high transcription levels.

(Grob, Schmid and Grossniklaus, 2014)

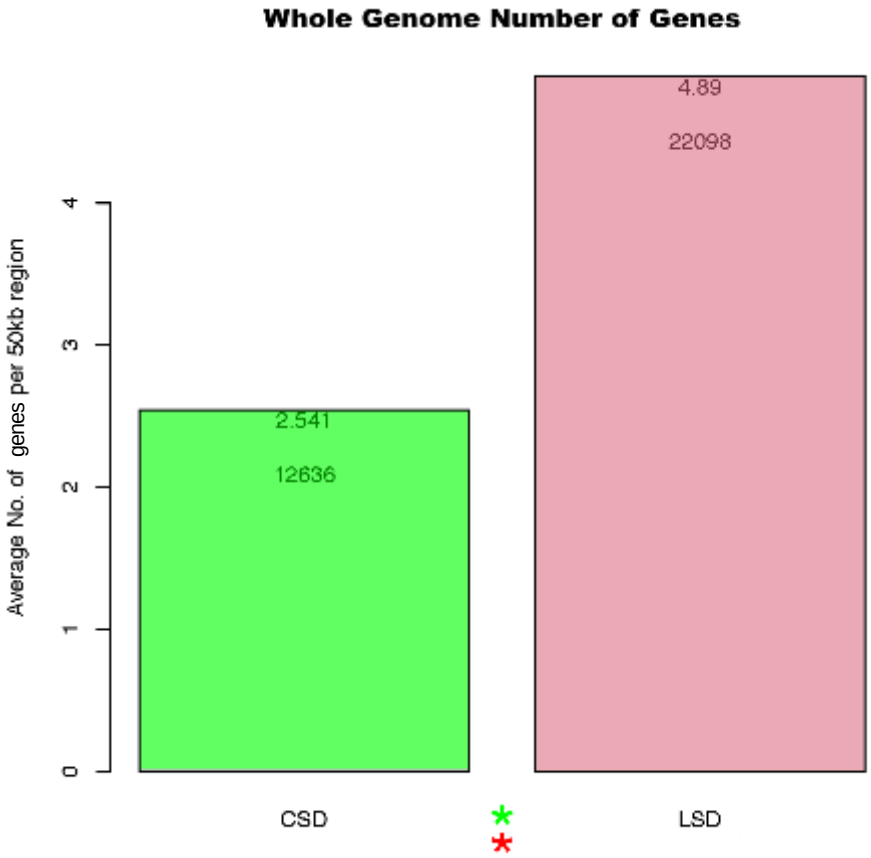


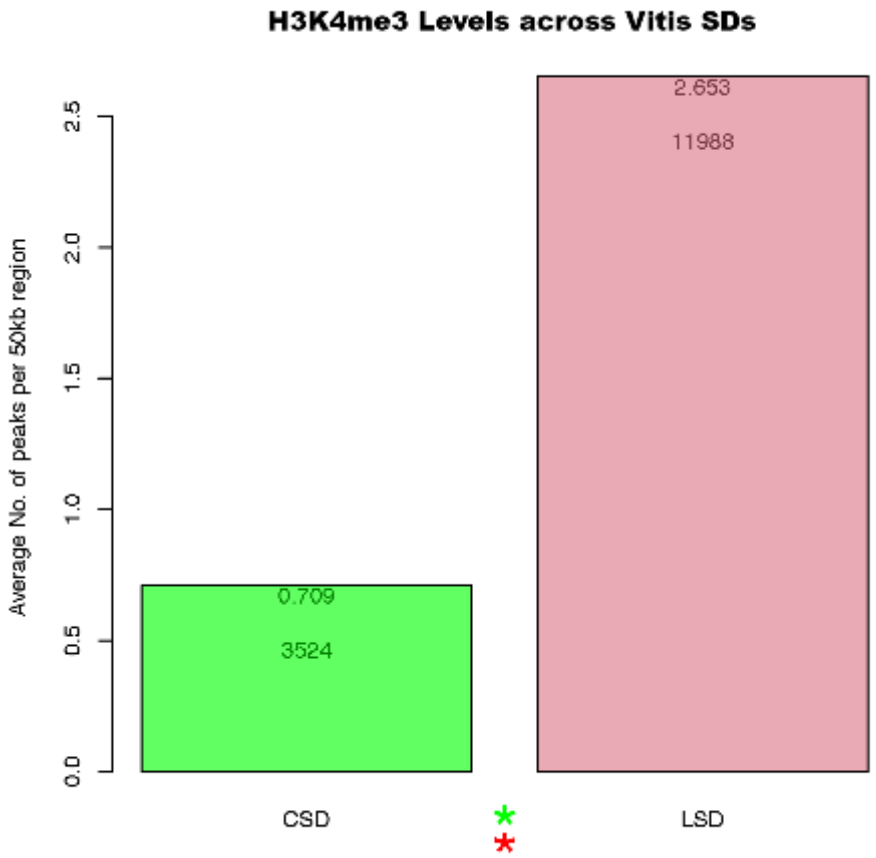
2.a Correlate SDs with the genomic features of Pinot noir





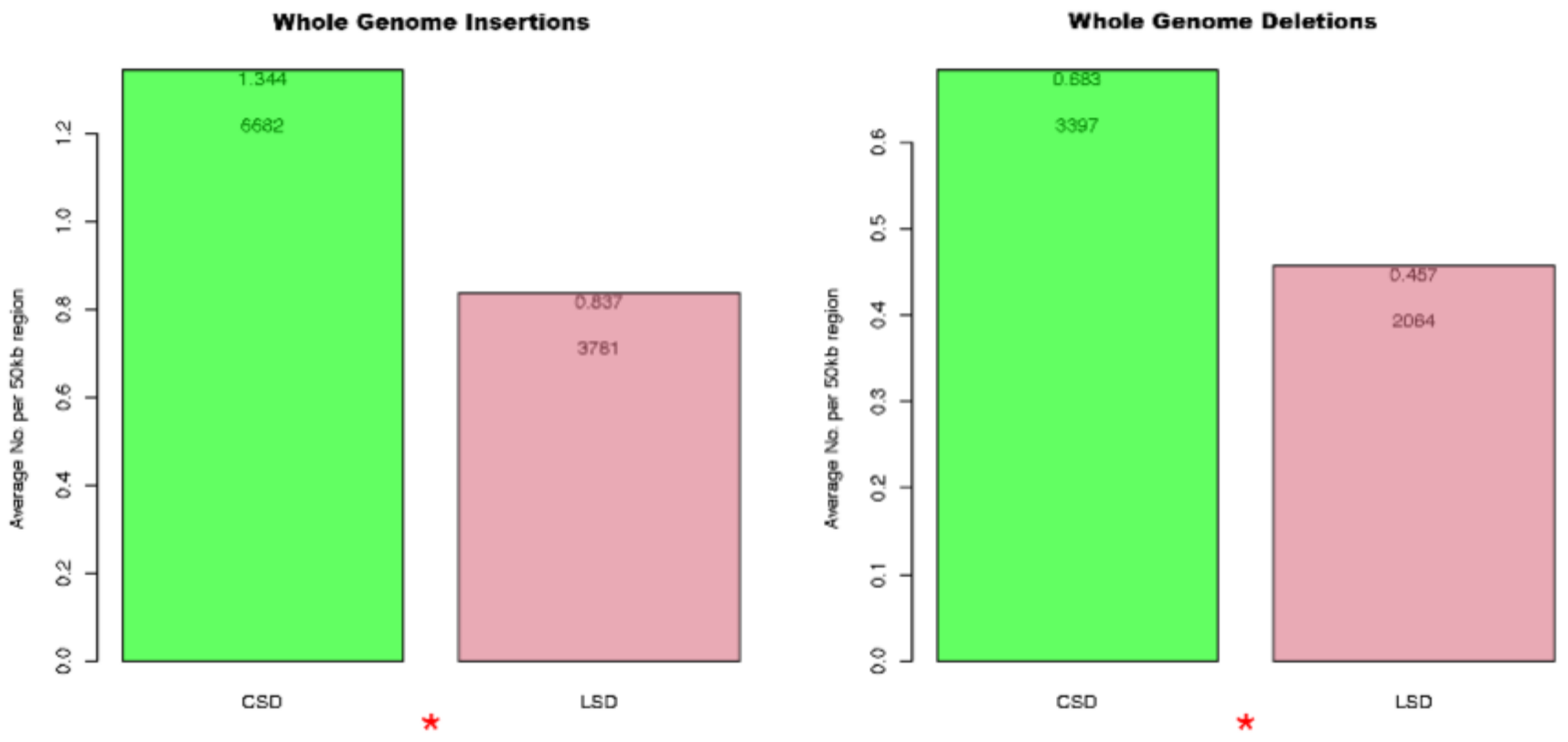
2.a Correlate SDs with the genomic features of Pinot noir





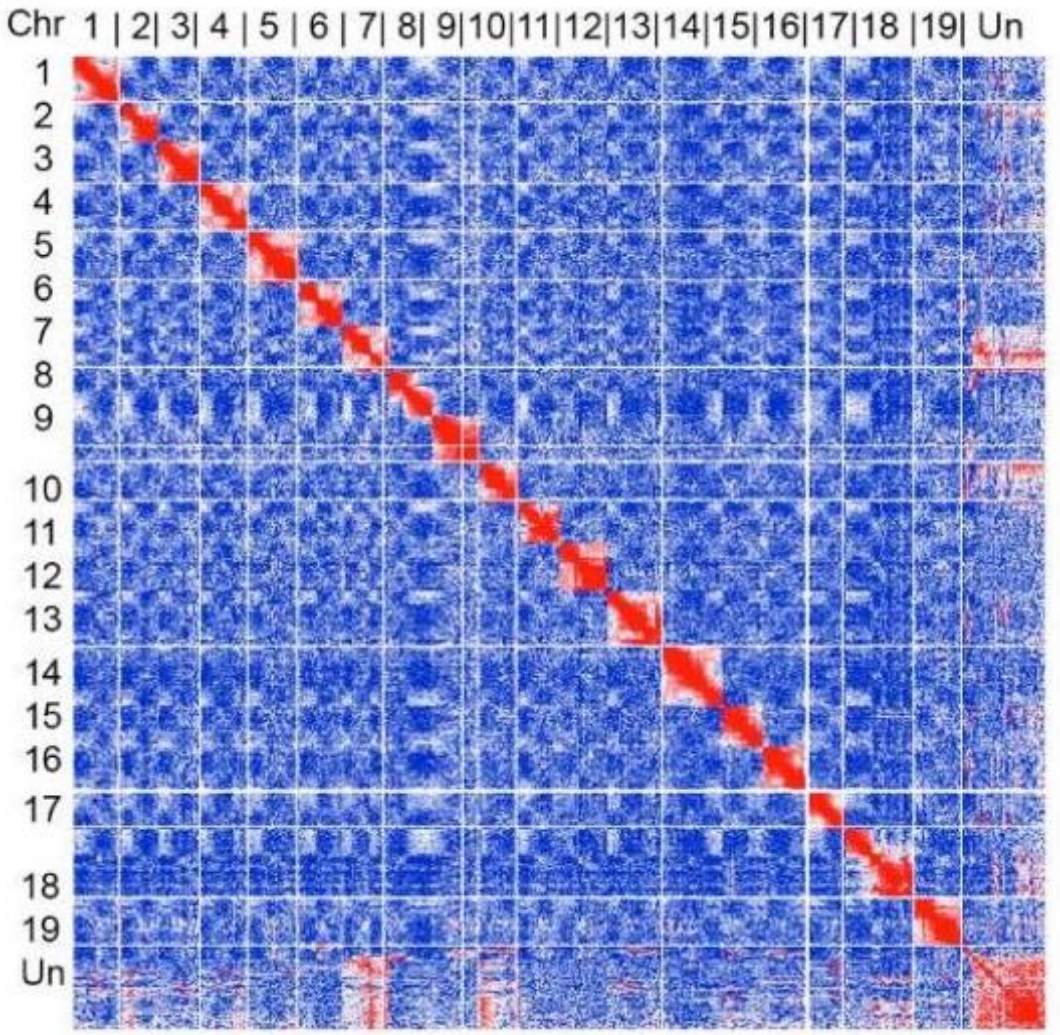
2.a Correlate SDs with the genomic features of Pinot noir

Vitis vinifera SVs distribution in SDs



3. Improve assembly using Hi-C interaction data

chrUn: set of scaffolds that could not be associated to any chromosome during the assembly



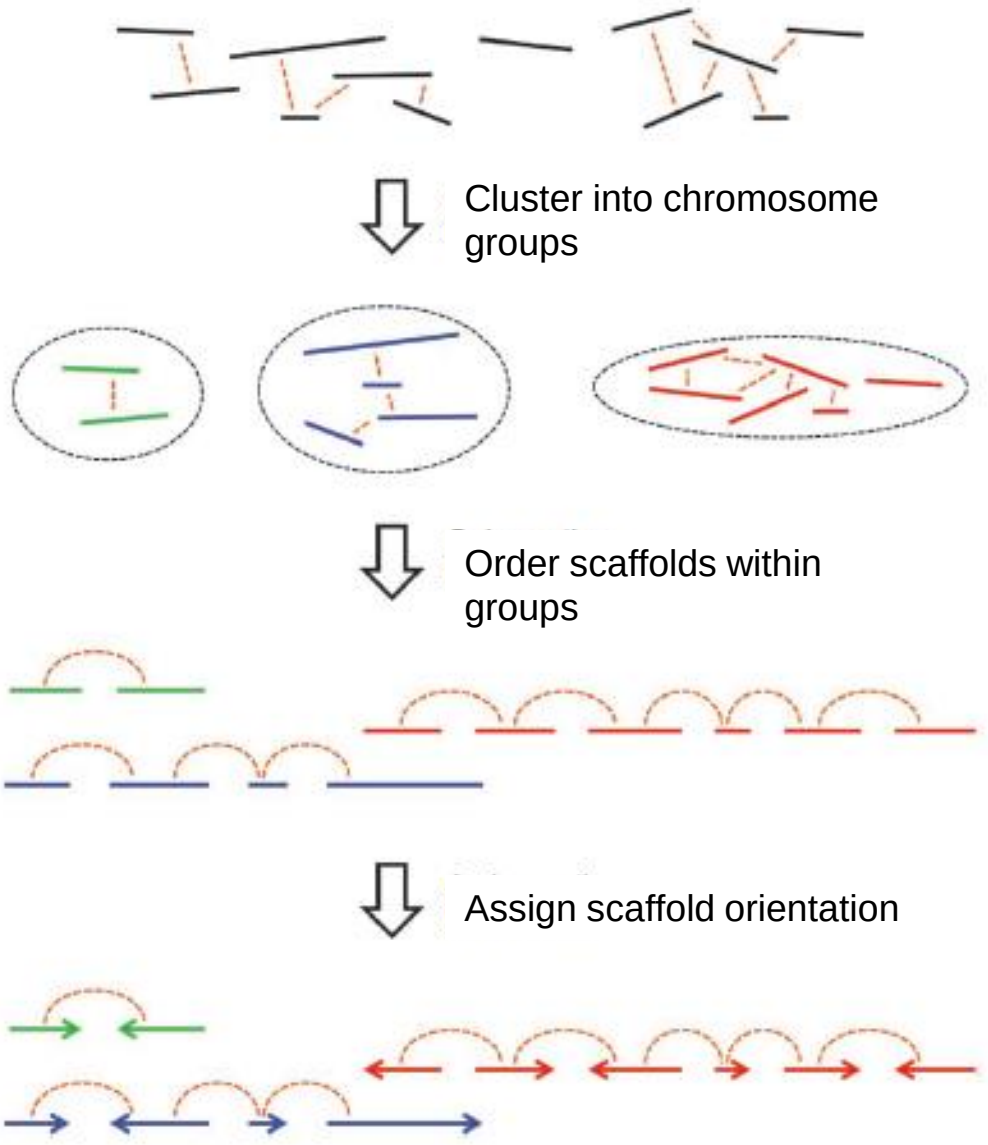
3. Improve assembly using Hi-C interaction data

LACHESIS

(Ligating Adjacent Chromatin Enables Scaffolding *In Situ*):
a computational method that exploits the genomic proximity signal in Hi-C data sets for ultra-long range scaffolding of *de novo* genome assemblies.

IMPORTANT: it doesn't require any reference genome, only Hi-C data!

(Burton, *et al.* 2013)



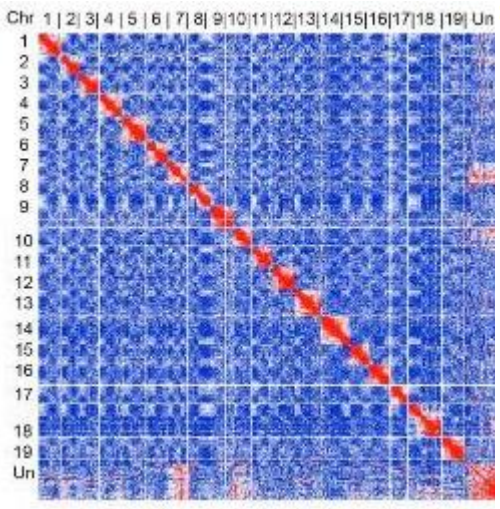
3. Improve assembly using Hi-C interaction data

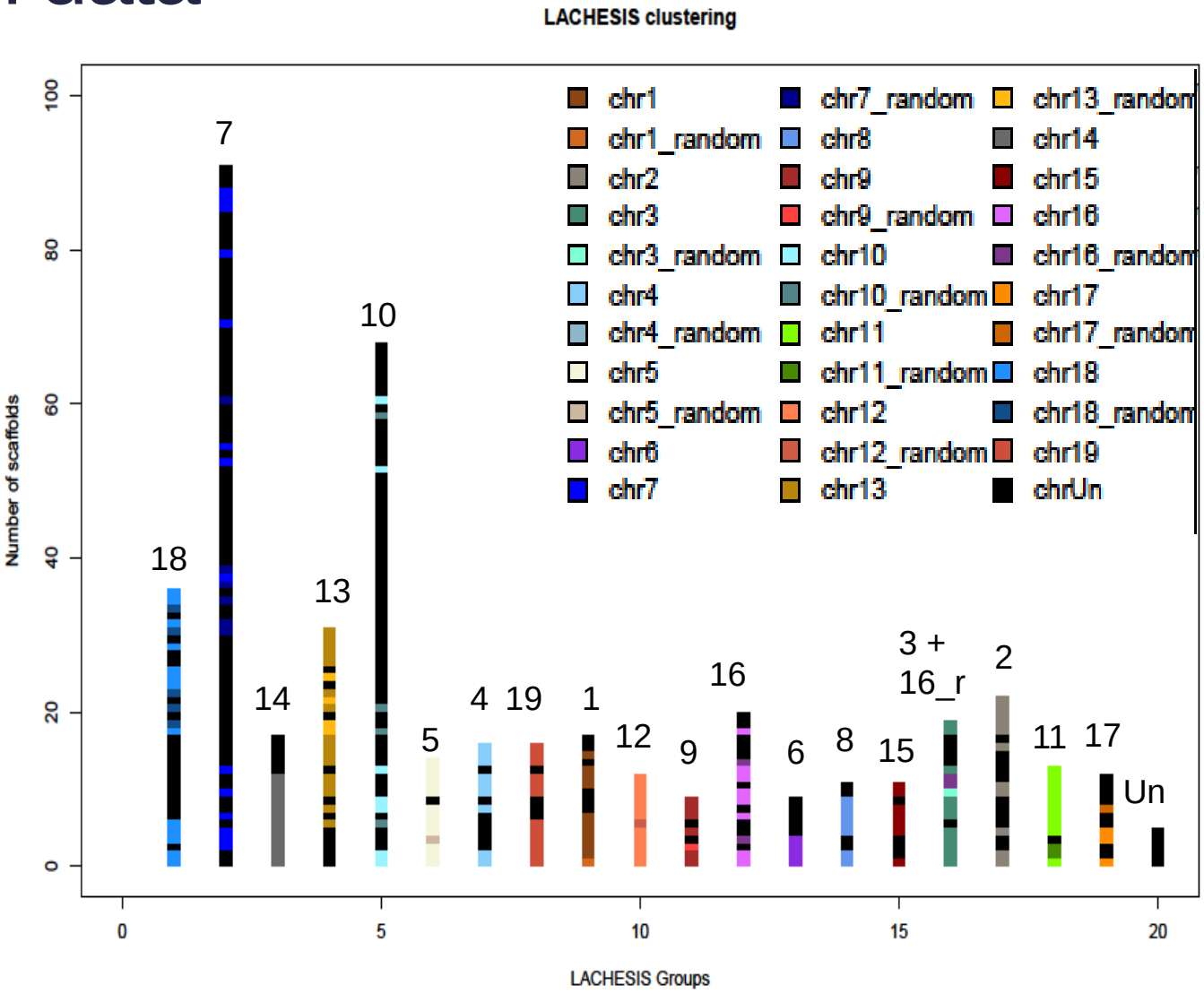
Clustering improvement:

Total scaffolds: 2059

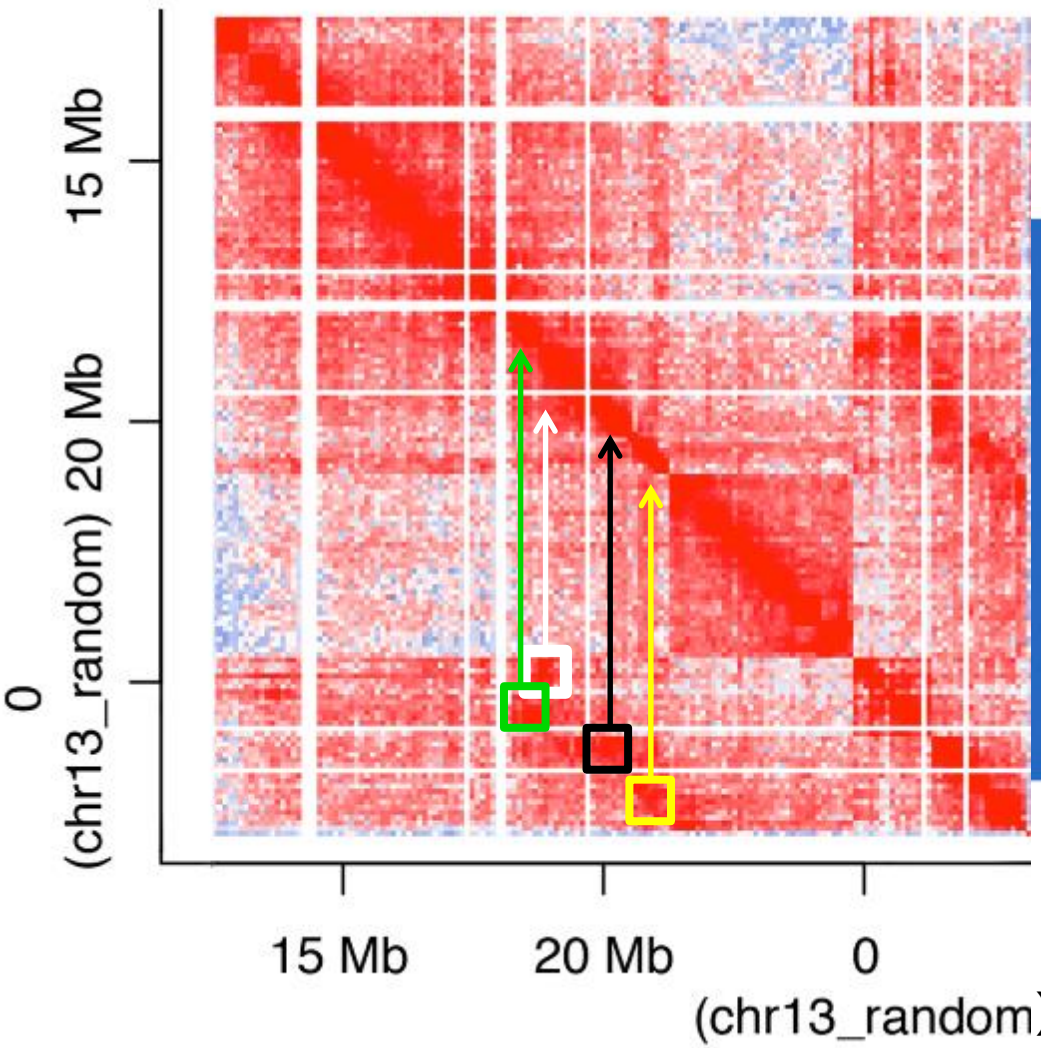
chrUn scaffolds: 1849/2059

Assigned chrUn scaffolds: 1834/1849
~ 39 Mb added





3. Improve assembly using Hi-C interaction data



Ordering improvement

scaffold_73	chr13	12897878	14847039
scaffold_175	chr13	14847539	15259454
scaffold_47	chr13	15259954	18426156
scaffold_221	chr13_random	1300885	1513631
scaffold_112	chr13_random	0	1300385
scaffold_724	chrUn	34657021	34677278
scaffold_84	chr13	18426656	20188594
scaffold_123	chr13_random	1514131	2566426
scaffold_139	chr13	20189094	20910598
scaffold_131	chrUn	2162969	3059925
scaffold_143	chr13_random	2566926	3268264
scaffold_467	chrUn	29778126	29820423
scaffold_158	chr13	20911098	21414819
scaffold_64	chr13	21415319	23748070
scaffold_156	chr13	23748570	24297419
scaffold_320	chr13	24297919	24396255

… what is going on…

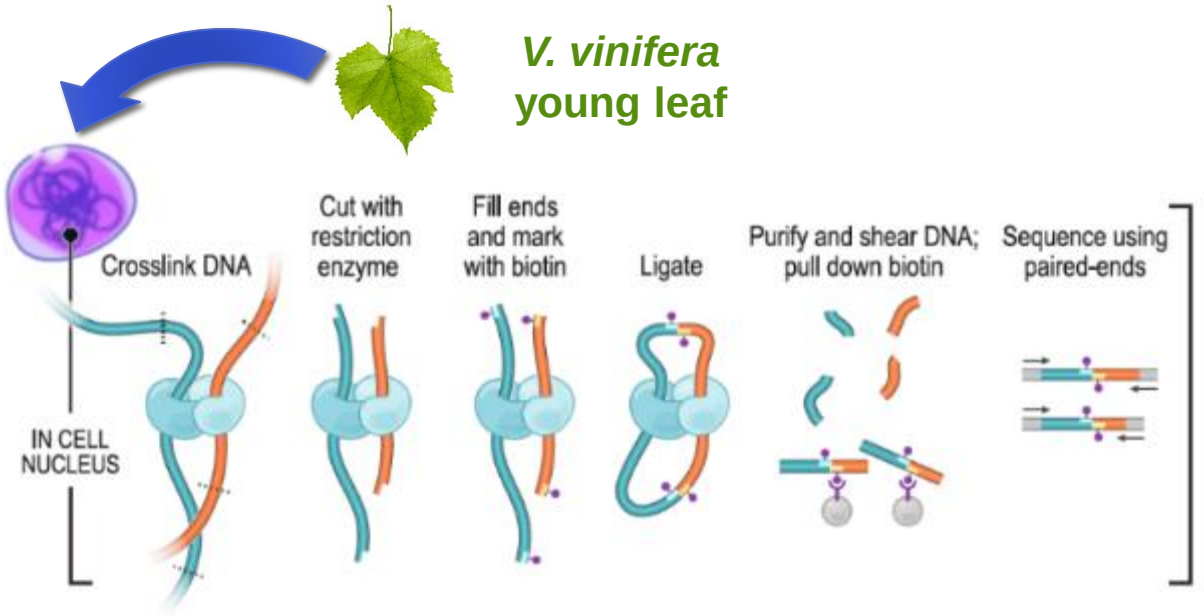
Variety: Rkatsiteli

1. Contact map reconstruction via *in situ* Hi-C

2. Lachesis for *de novo* assembly scaffolding, improving N50 and L50

3. Haplotype-specific Hi-C

1. *In Situ* Hi-C



What's different from classic Hi-C?

The DNA-DNA proximity ligation process happens **inside** the intact **nuclei** of permeabilized crosslinked cells.

Advantages:

- Reduced frequency of spurious contacts due to random ligation in diluted solution
- Faster protocol (requiring 3 days instead of 7)
- Enables higher resolution (up to ~1Kb)

(Rao, *et al.* 2014)

2. Improvement of *de novo* assembly

Rkatsiteli *de novo* assembly summary

Estimated genome size: 486,2 Mbp

Number of scaffolds: 10,089

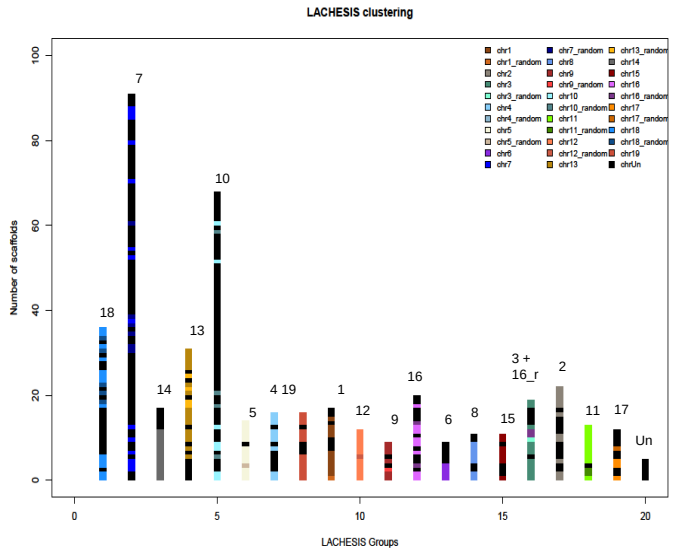
L50 scaffold length: 352,572 bp

N50 scaffold count: 612

(M. Vidotto, 2015)

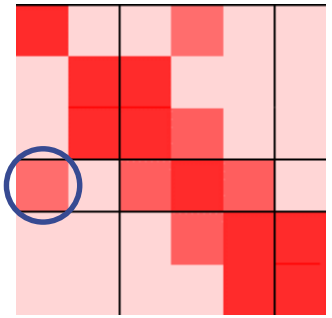
Rkatsiteli *in situ* Hi-C reads

LACHESIS

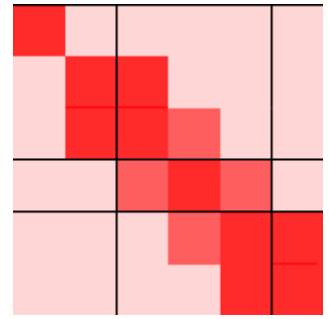


3. Obtain allele-specific versions of Hi-C maps

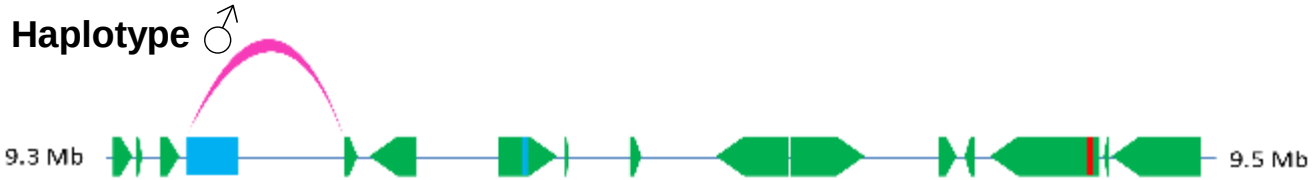
Rkatsiteli (heterozygous)

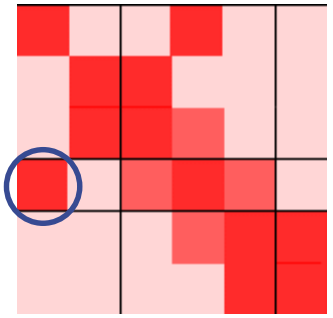


Haplotype ♀



Haplotype ♂





gene

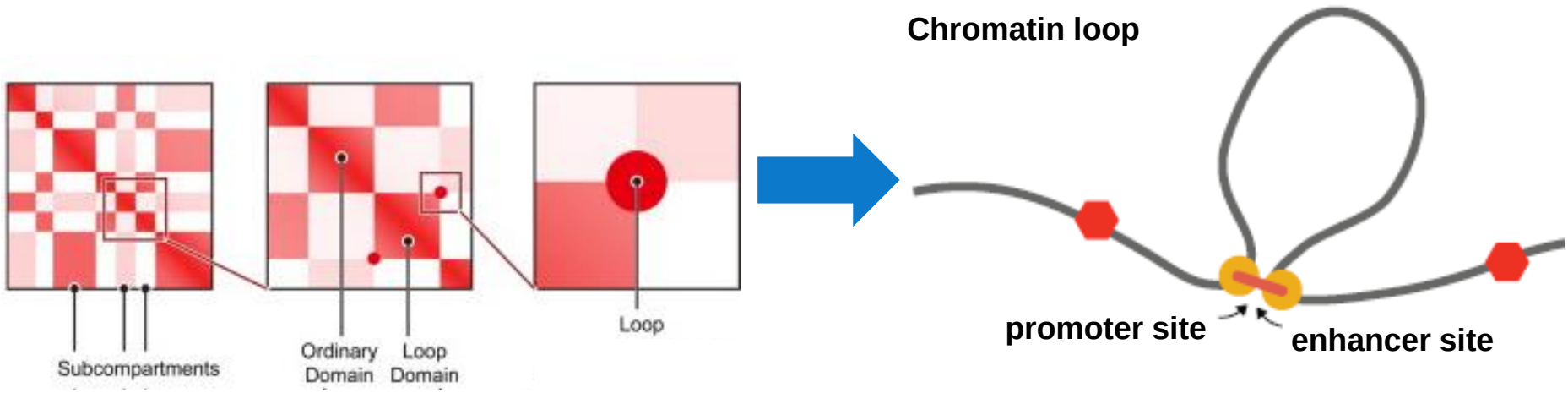
class I TE

class II TE

Future Perspectives

1. Finalize the ongoing works

2. Obtain high-resolution data from *in situ* Hi-C to identify promoter-enhancer interactions



Acknowledgments

Prof. Michele Morgante

Fabio Marroni
Rachel Schwope

Simone Scalabrin
Mirko Celii
Alice Fornasiero
Gabriele Magris
Ettore Zapparoli

Everyone from IGA (Istituto di Genomica Applicata) and Di4a (Dipartimento di Scienze AgroAlimentari, Ambientali e Animali - UNIUD)

THANKS
and Hi-C YOU
SOON!