

Genome-wide characterization of gene-families in Resequencing projects – *Challenges and Perspectives*

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E P G V

Etude du Polymorphisme des Génomes Végétaux

US1279 - BAP

Introduction

- *Genomics Era* – Low Cost - Resequencing large genotypes
- More NGS tools for data mining – Mapping – SNP detection – downstream analysis.
- Paucity of robust tools in the context of gene families and distantly placed species from the reference genome.
- Gene family size variation is an important mechanism that shapes the natural variation for adaptation in various species.
- The pattern of gene family size variation in plant species are still not well understood.

Vitis and Muscadine species

GrapeReSeq

Large scale re-sequencing in the *Vitis* genus for identification of resistance genes, SNP discovery and high throughput genotyping

Research project of Transnational Plant Alliance for Novel Technologies toward implementing the Knowledge Based Bio-Economy (PLANT - KBBE2008) in Europe (France, Germany and Spain)

Muscarea

Comparative genomics of *Vitis vinifera* and *Muscadinia rotundifolia* for the study of disease resistance in the Vitaceae

ANR funded project ANR-08-GENM-007, with the support of CNIV (Comité National des Interprofessions des Vins d'appellation d'origine)

<i>Vitis vinifera</i>	<i>Vitis sylvestris</i> (wild)	<i>Vitis</i> (other) species	<i>Muscadinia</i> species
Araklinos	Dirmstein-male	<i>V.aestivalis.aestivalis-1</i>	<i>M.carlos</i>
Carbernet-franc	Lambrusque-campmarcel	<i>V.aestivalis.aestivalis-2</i>	<i>M.Dulcet</i>
Carignan-noir	Lambrusque-PSL2	<i>V.aestivalis.aestivalis-3</i>	<i>M.fry</i>
Castellana-blanca	Teulere-pied-sauvage	<i>V.amurensis.colmar</i>	<i>M.mchx</i>
Chardhchi-blanc		<i>V.amurensis.rupprecht</i>	<i>Regale</i>
Chouchillon		<i>V.berlandieri.planchon</i>	<i>M.trayshed</i>
Colorino		<i>V.berlandieri-Resseguier</i>	<i>M.yugax-carlos</i>
Espadeiro-tinto		<i>V.berlandieri-10594</i>	
Hebén		<i>V.cinerea.cinerea-1</i>	
Jaén		<i>V.cinerea.cinerea-2</i>	
Lambrusque-E		<i>V.cinerea.cinerea-3</i>	
Listàn-Prieto		<i>V.labrusca.concord</i>	
Malvasia-di-Sardegna		<i>V.labrusca.fredonia</i>	
Maska		<i>V.linccumii</i>	
Médouar			
Orlovi-nogti			
PN40024 -TruSeq			
Riesling			
Savagnin			
Tsolikoouri			
20	4	14	7
Total		45	

Mapping Assembly Tool



CLC GENOMICS WORKBENCH

A comprehensive and user-friendly analysis package for analyzing, comparing, and visualizing next generation sequencing data.



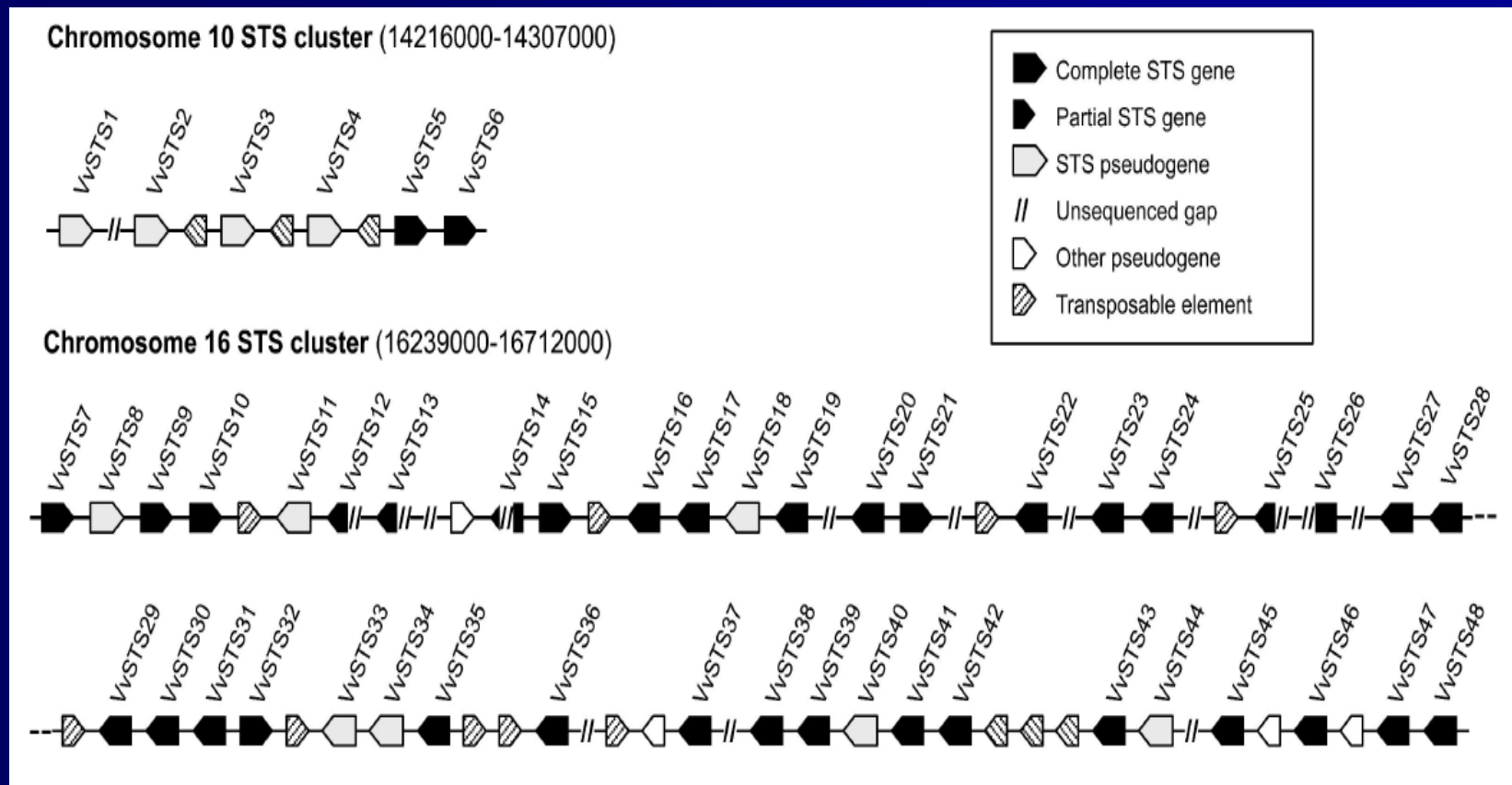
CLC ASSEMBLY CELL

Fast and accurate command-line tool for read mapping or *de novo* assembly of large and complex NGS datasets.

Stilbene Synthase (STS)

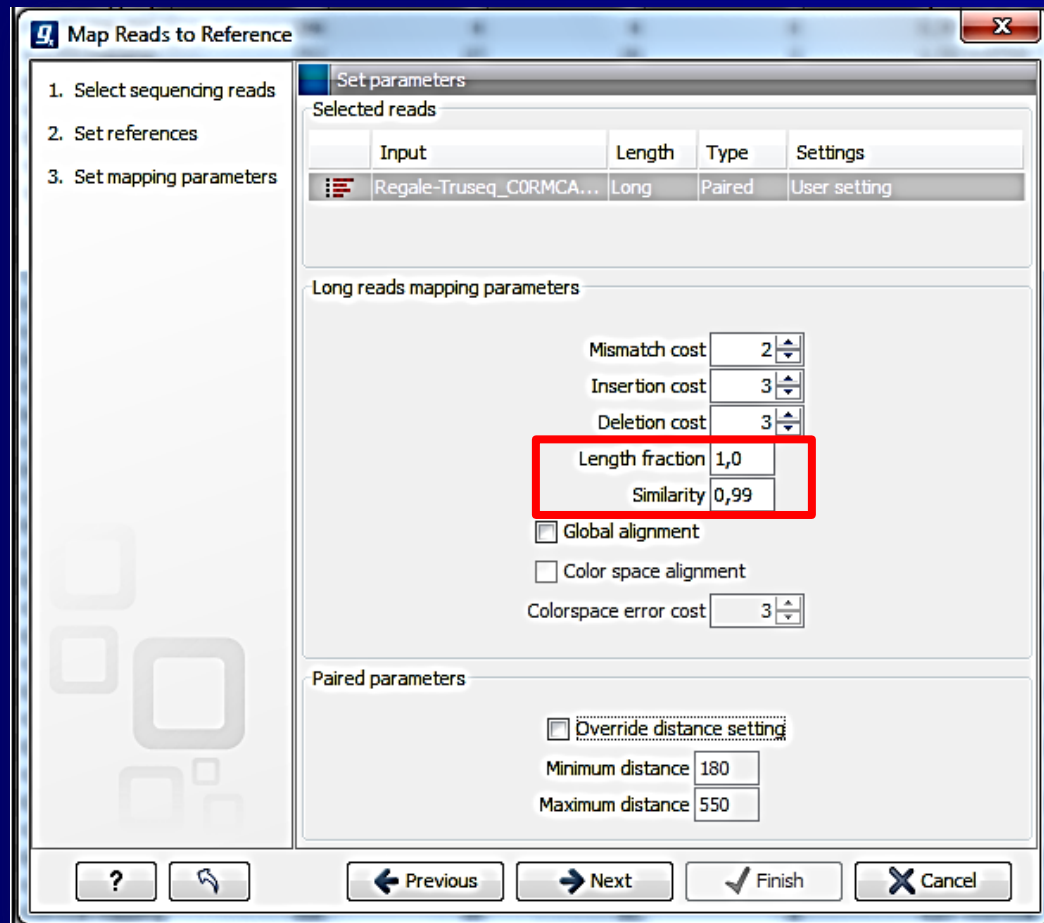
- ❑ Stilbenes belong to a small family of phenylpropanoids
- ❑ Produced in numerous plant spp. against several biotic and abiotic stresses, including to UV and chemical treatments.
- ❑ Stilbenes such as resveratrol represent an imperative medicinal property –
 - Anticancer
 - Antinflammation
 - Diabetes
 - Anti-Ageing (*French Paradox*)
- ❑ Mysterically grapevine possess numerous STS genes (~ 45 genes) unlike in other plant species, makes the molecular evolutionist and plant breeders ever more curious.

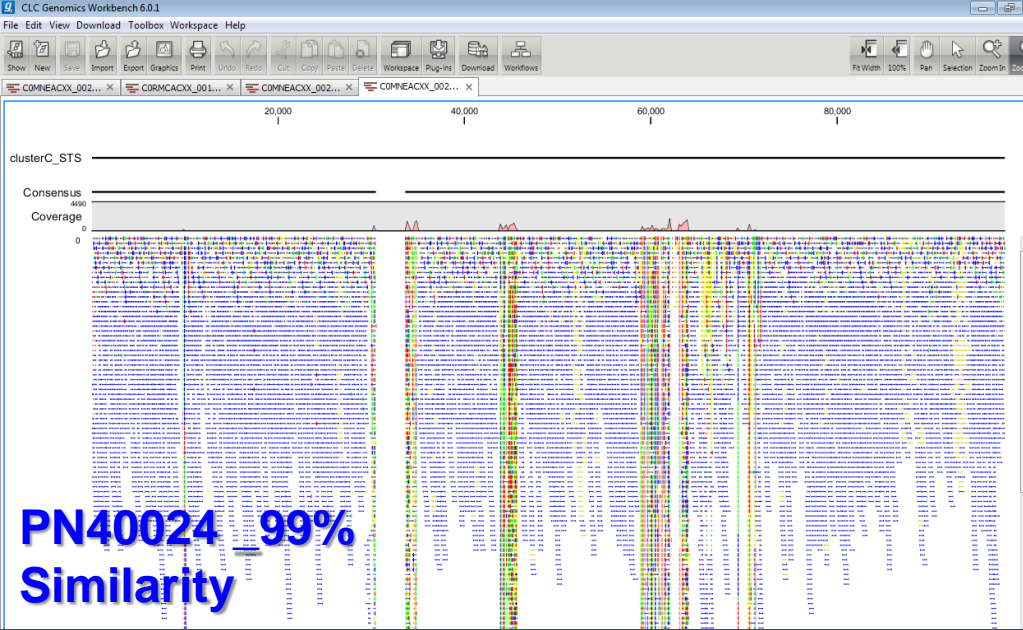
The STS clusters were annotated in the reference grape genome PN40024



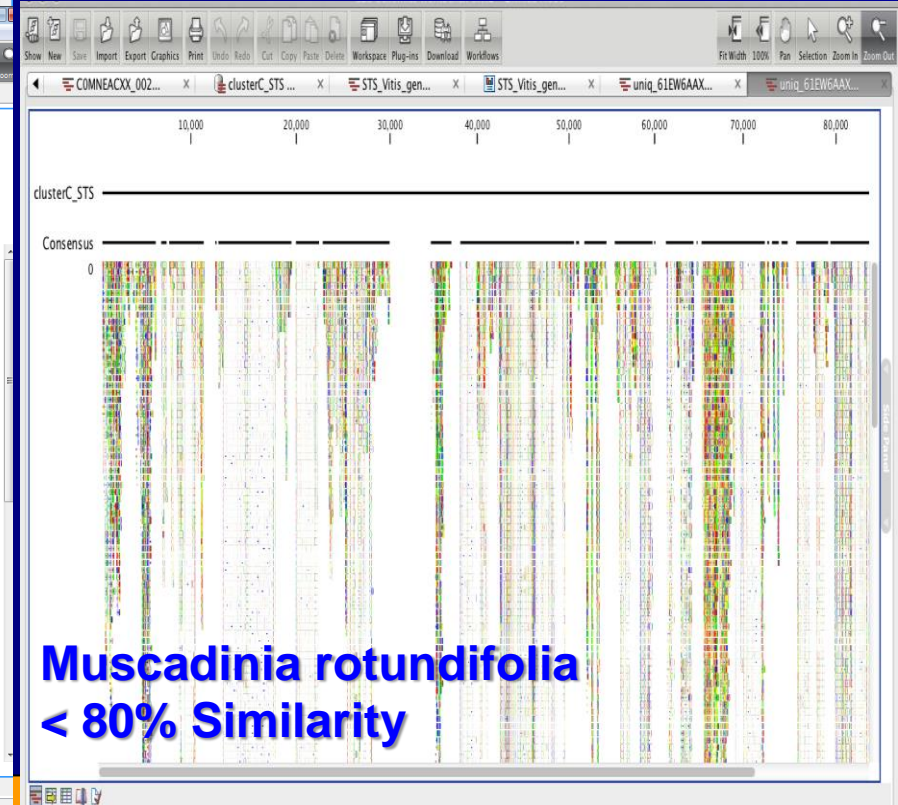
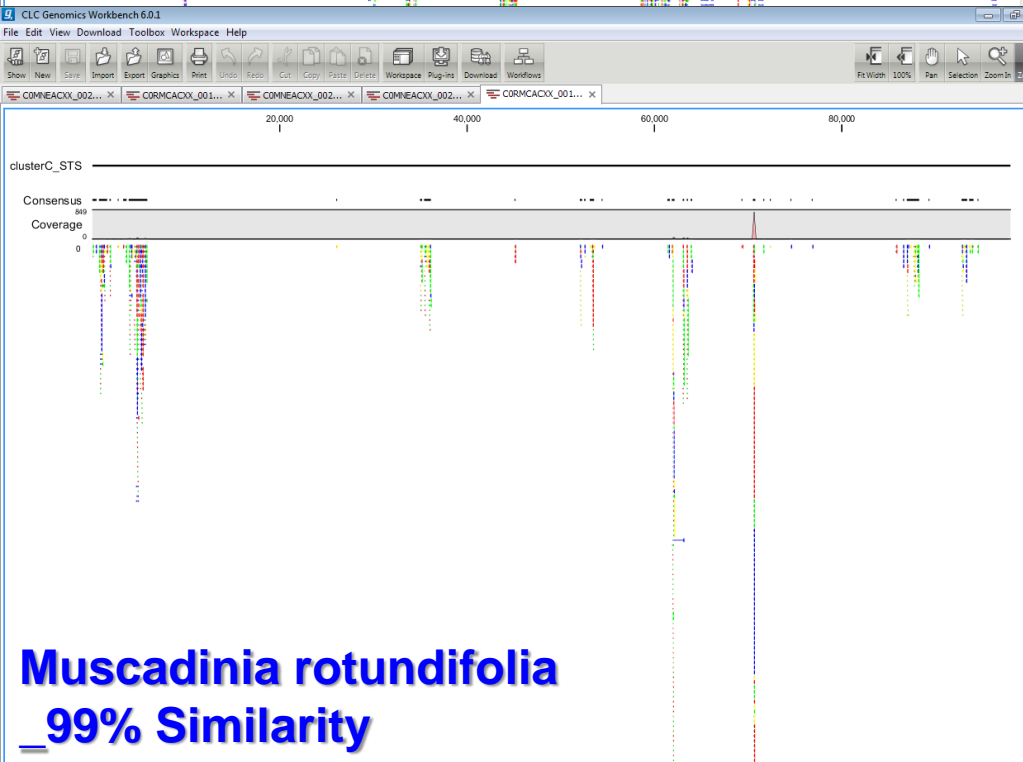
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STS – Cluster C



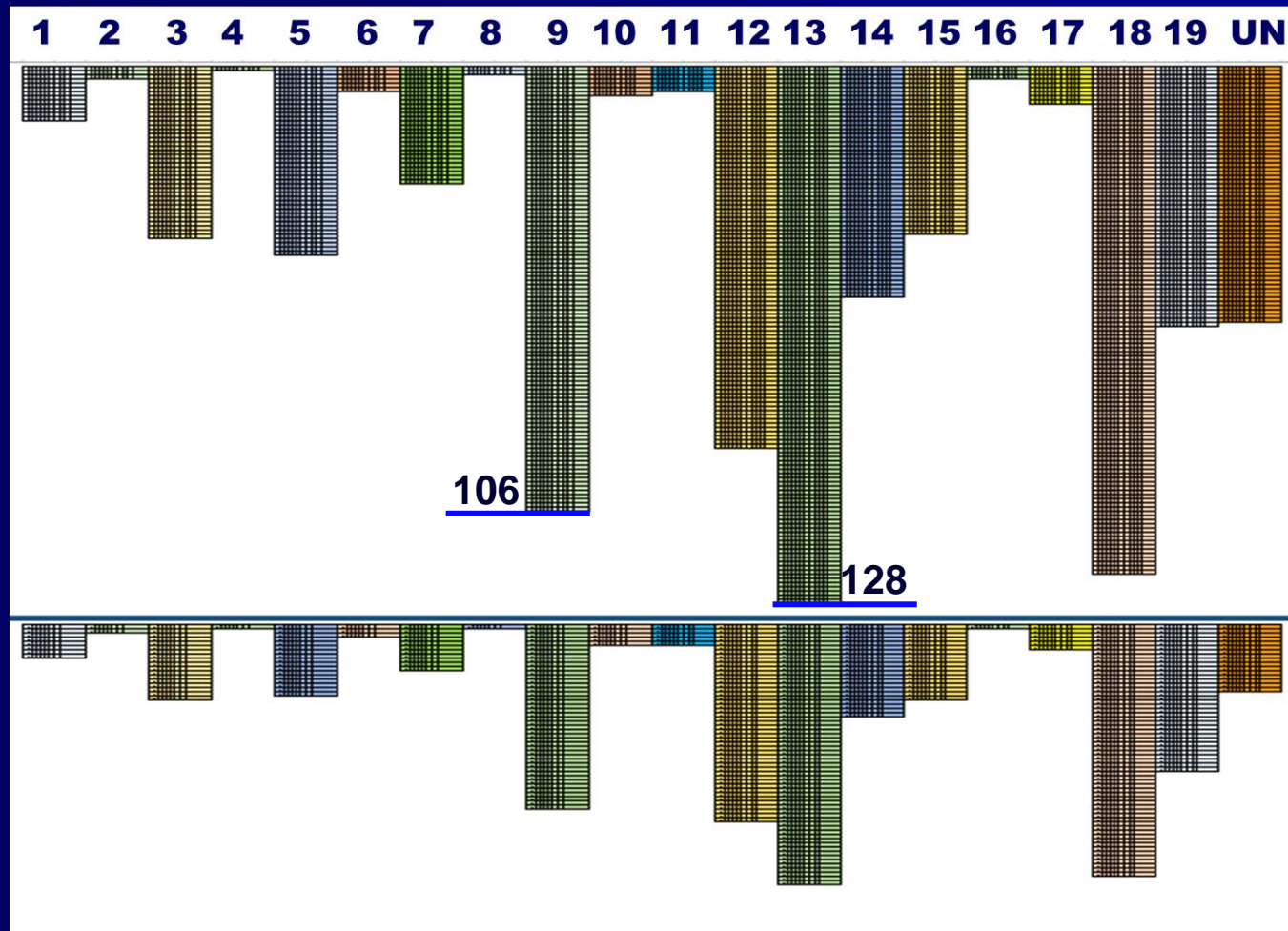
Annotation of RGA genes in PN40024



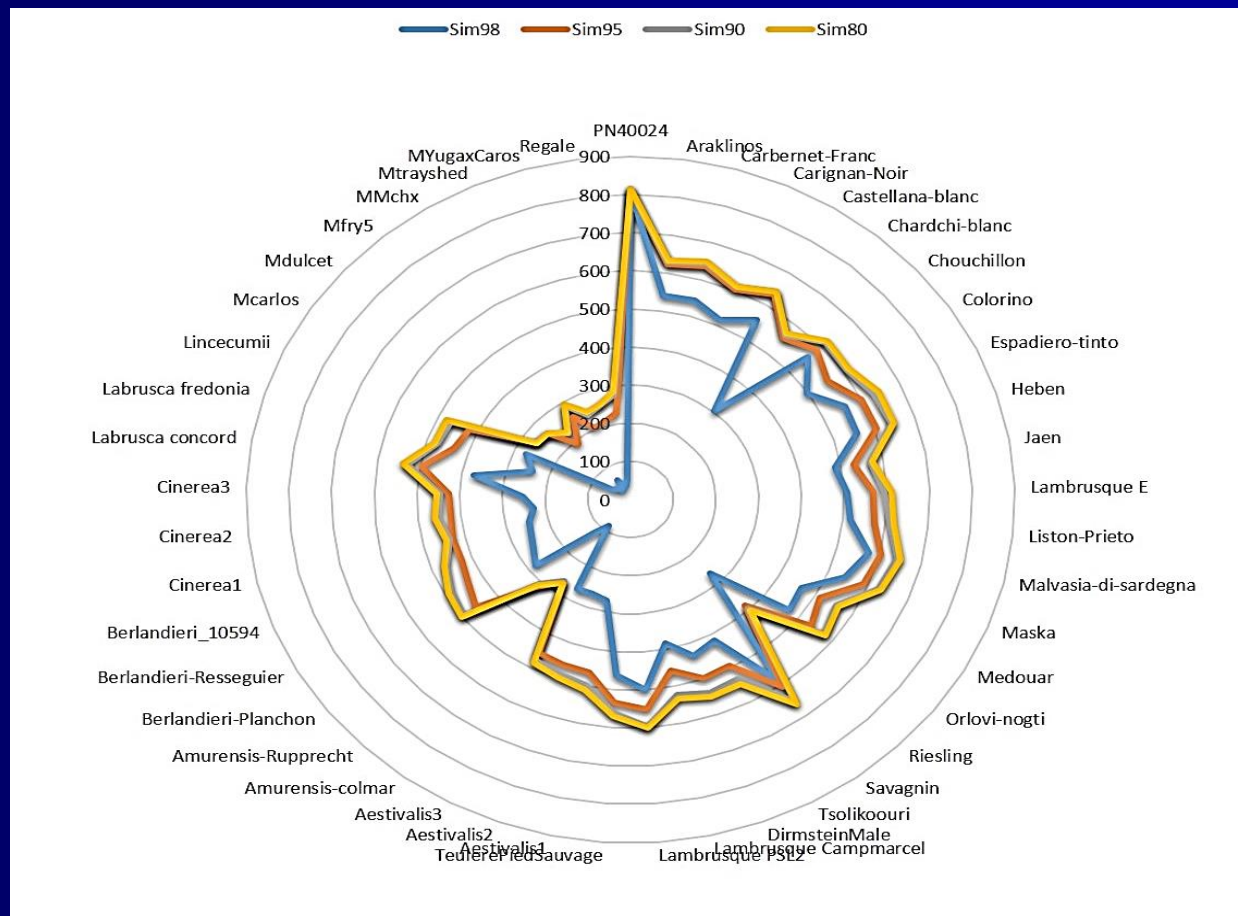
URGV - Plant Genomics Research
Unité de Recherche en Genomique Végétale

- The 12x *Vitis vinifera* (PN40024) genome has been explored exhaustively using a similarity search approach based on a reference bank made with previously characterized NBS-LRR proteins.
- A total of **828 NBS-LRR genes** were identified and characterized in PN40024.
- These 828 NBS genes are chosen to study their variations, presence-absence among the genotypes.

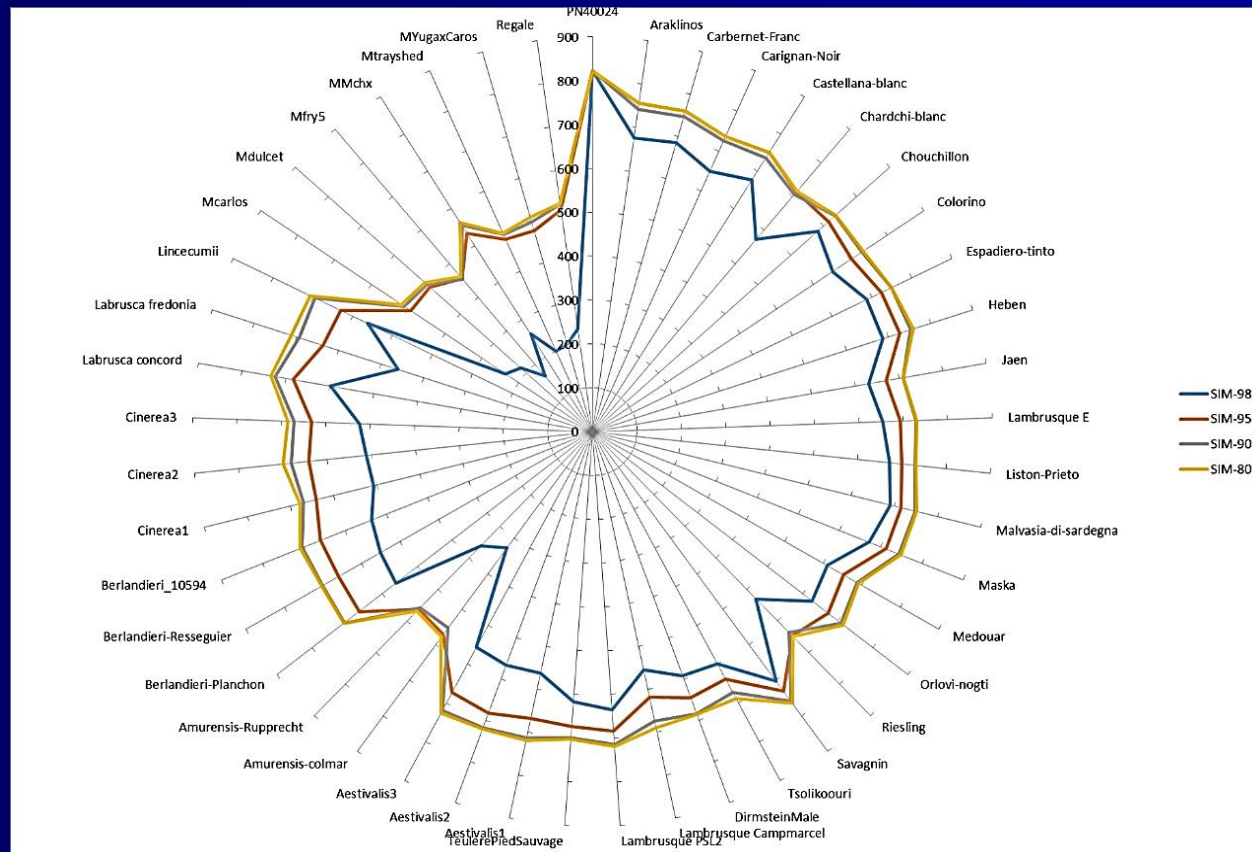
Global view of RGA's scattered across the 19 chromosomes of PN40024



Reference genes covered by length over 90%



Reference genes covered by length over 65%



Limitations and Challenges

- Only Reference genes can be compared
- Species specific haplotypes / gene sequences are ignored
- Tedious validation
- Heterozygosity - creating false segmental duplications in assemblies.

Perspectives

- **Local Assembly based strategy**
- **Longer-reads**
- **Motif-based mapping strategy**

In-search of ideas and solutions



GenScale
Scalable Optimized and Parallel Algorithms for Genomics

INSTITUT NATIONAL
DE RECHERCHE
EN INFORMATIQUE
ET EN AUTOMATIQUE



INRIA

centre de recherche **RENNES - BRETAGNE ATLANTIQUE**

- Local softwares and algorithms didnt meet our requirements
 - Paired-end reads
 - Large data sets
 - Longer-reads??

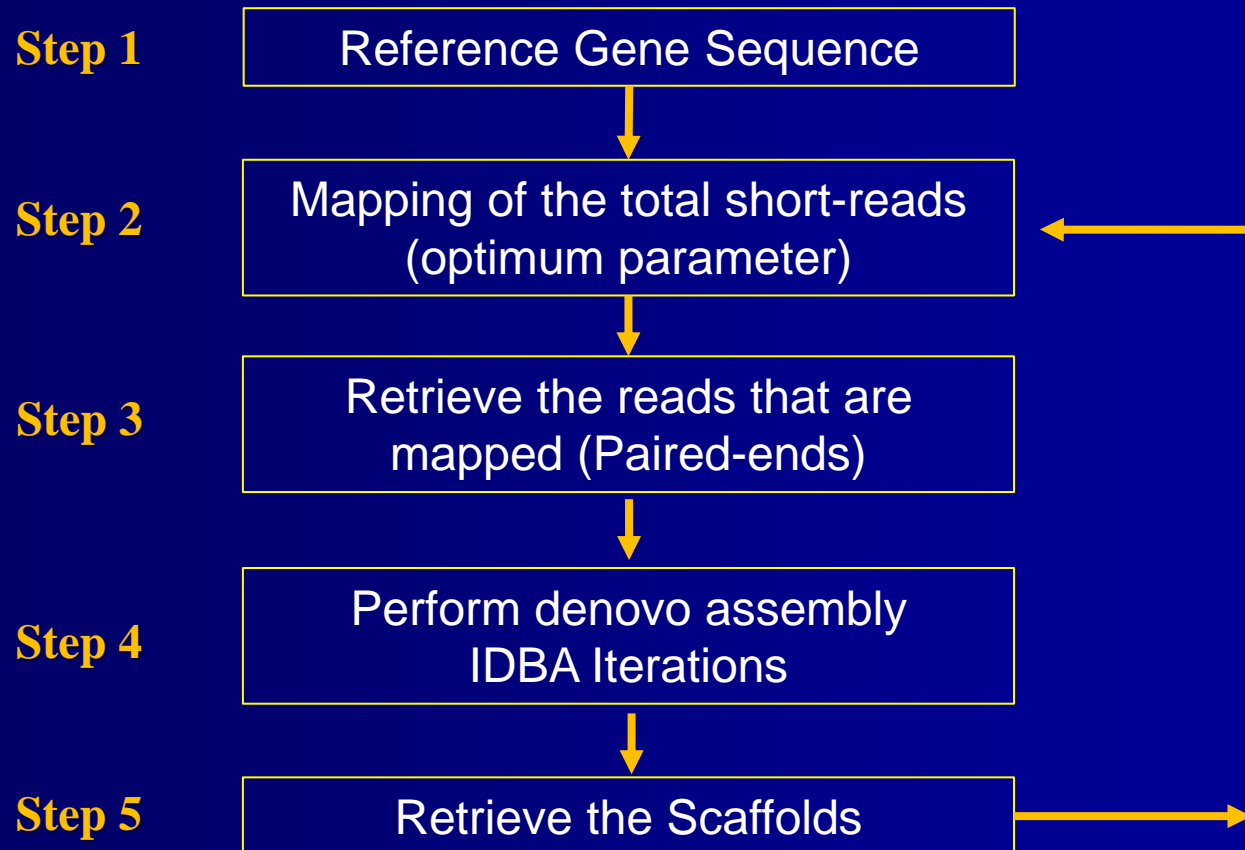


hku-idba

A Practical Iterative de Bruijn Graph De Novo Assembler

Customized CLC-IDBA Iteration approach

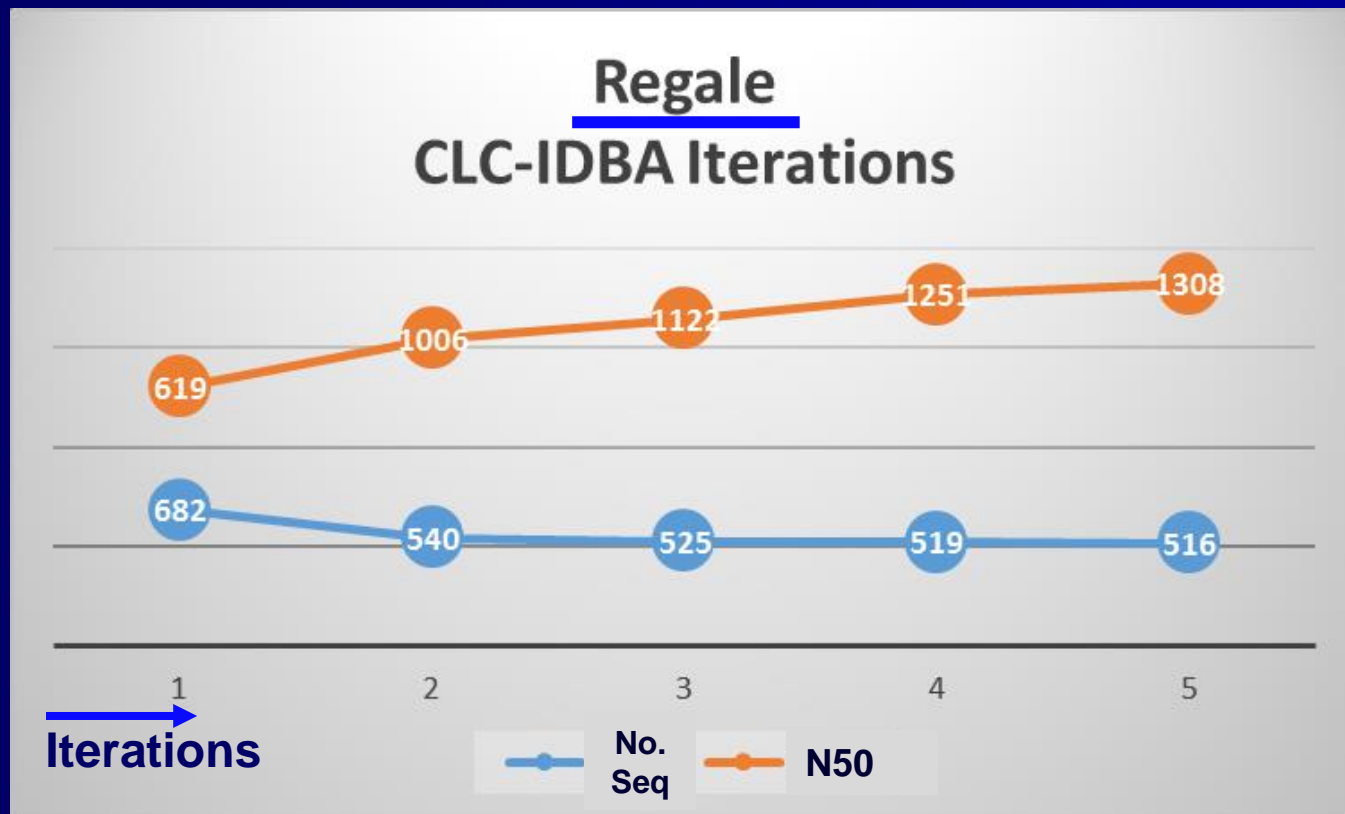
- Based on Christelle Aluome's experiences (at EPGV) in Genespace (Pea)



In Progress...

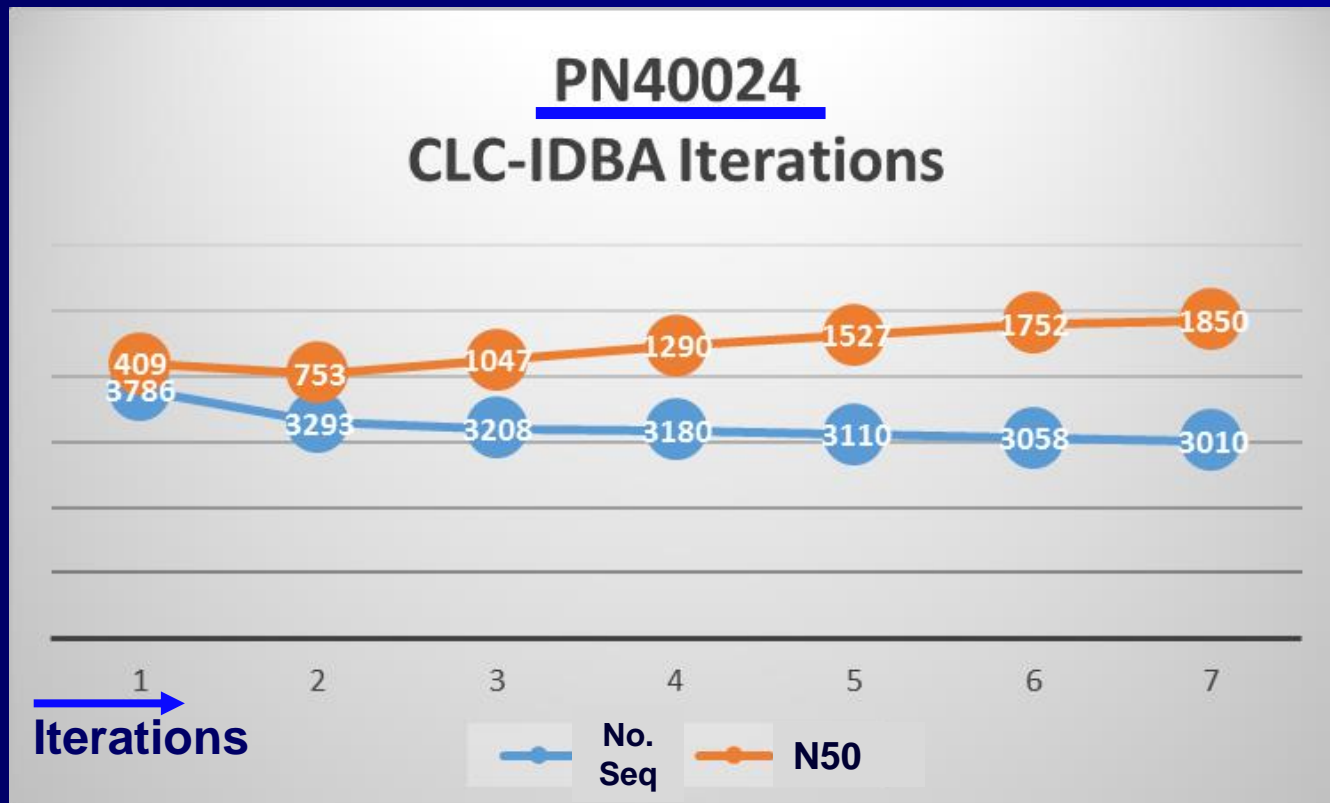
(hopeful signs..)

828 RGAs



In Progress...

(hopeful signs..)



Conclusion

- **Rigorous increase in sequencing scales - Paucity of tools to analyse resequenced data in regards to gene families**
- **Need more improvement in species-specific haplotypes and gene discoveries**
- **Efficient Local assembly tools – need of the hour!**

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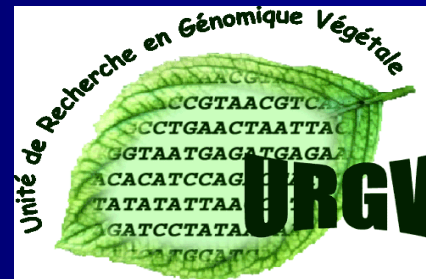
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Philippe Huguenev

Didier Merdinoglu

EPGV

Etude du Polymorphisme
des Génomes Végétaux



Santé de la Vigne
et Qualité du Vin
(SVQV)

Thank You !