

Intra-strain phenotypic and genomic variability of the commercial *Saccharomyces cerevisiae* strains Zymaflore VL1 recovered from vineyard environments

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M.A.S. Santos², M. Casal¹, D. Schuller¹

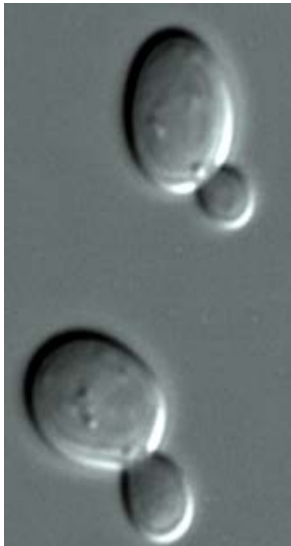
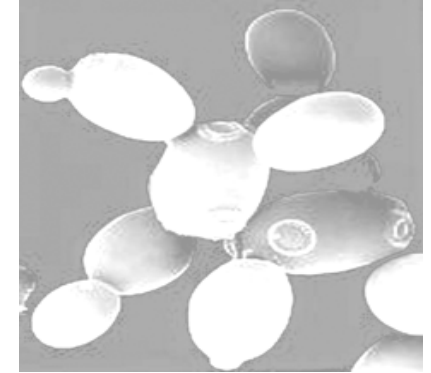
1 - Centre of Molecular and Environmental Biology (CBMA), Braga, Portugal

2 - RNA Biology Laboratory, CESAM, Aveiro, Portugal

3 - UMR Sciences pour l'Oenologie, Microbiologie, INRA, Montpellier, France



- ❖ World's premier commercial microorganism for biotechnological applications
- ❖ Genome shaped through the action of multiple independent rounds of wild yeast domestication



- ❖ Excellent model to study how the divergent selective pressures can shape the genomic content and phenotypic characteristics
- ❖ Knowledge about ecological and geographical distribution of yeasts, as well as about population structure, but a deeper exploration of natural populations and how they adapt to the environment is required

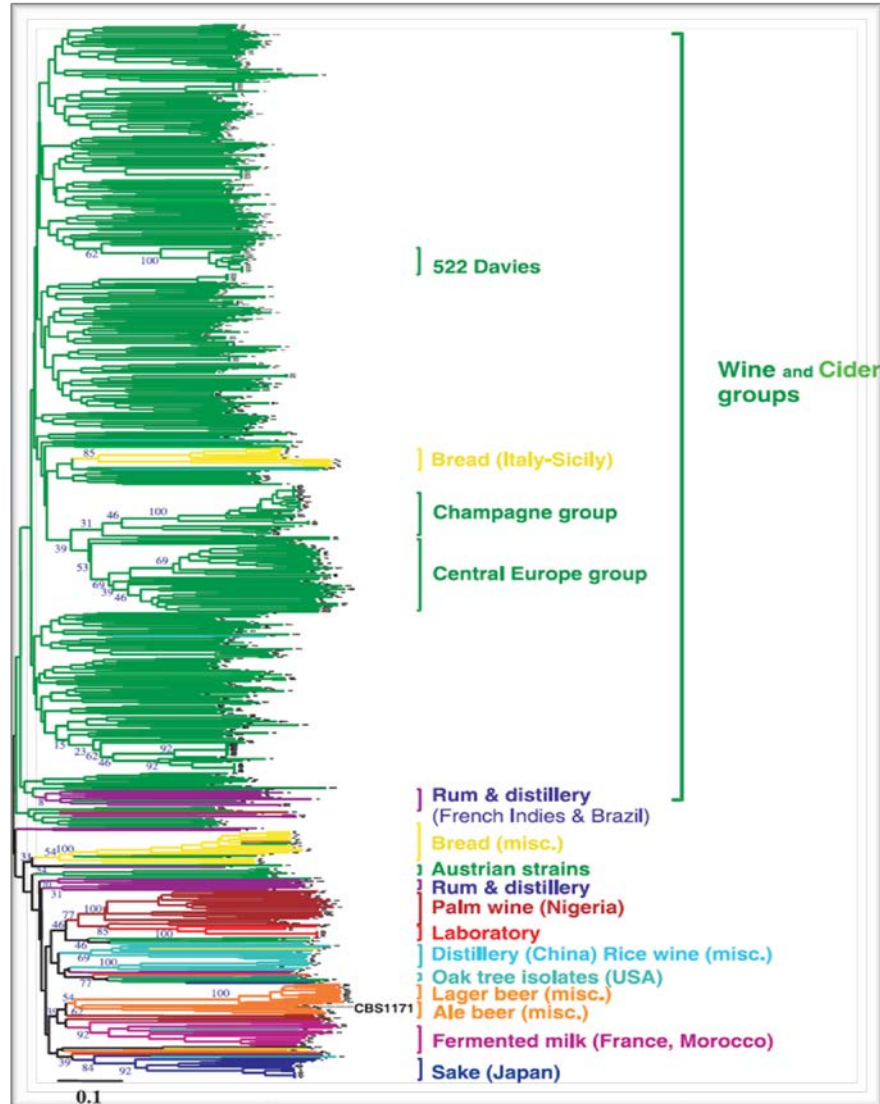
INTRODUCTION

The population structure of *Saccharomyces cerevisiae*

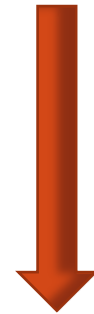
Consensus tree of *S. cerevisiae* populations based on F_{ST} genetic distances

- 651 strains
- 56 origins

12
polymorphic
microsatellites



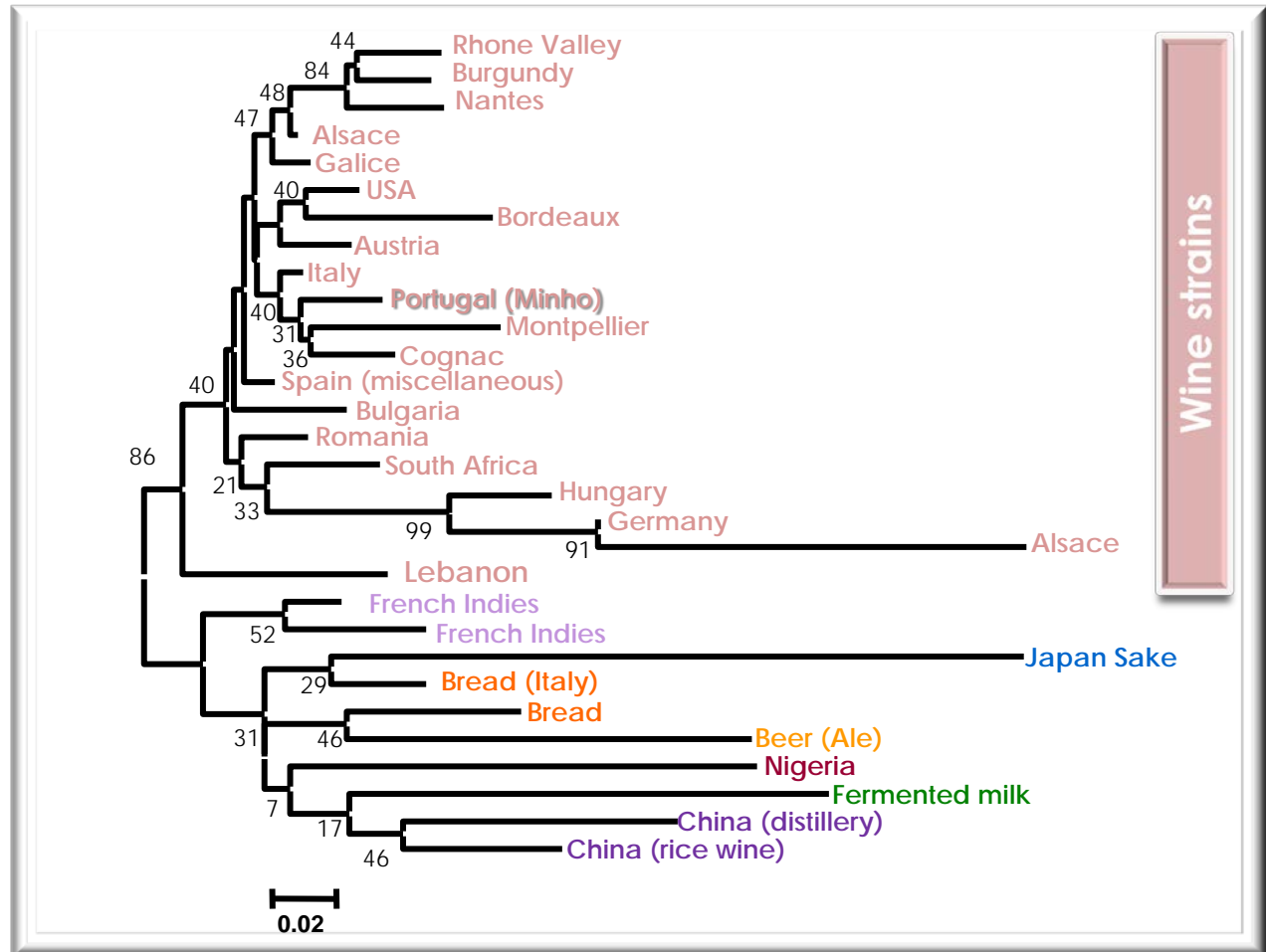
28% of genetic diversity associated with geographical differences



local domestications

Legras et. al., Mol. Ecol. 2007

Consensus tree of *S. cerevisiae* populations based on F_{ST} genetic distances

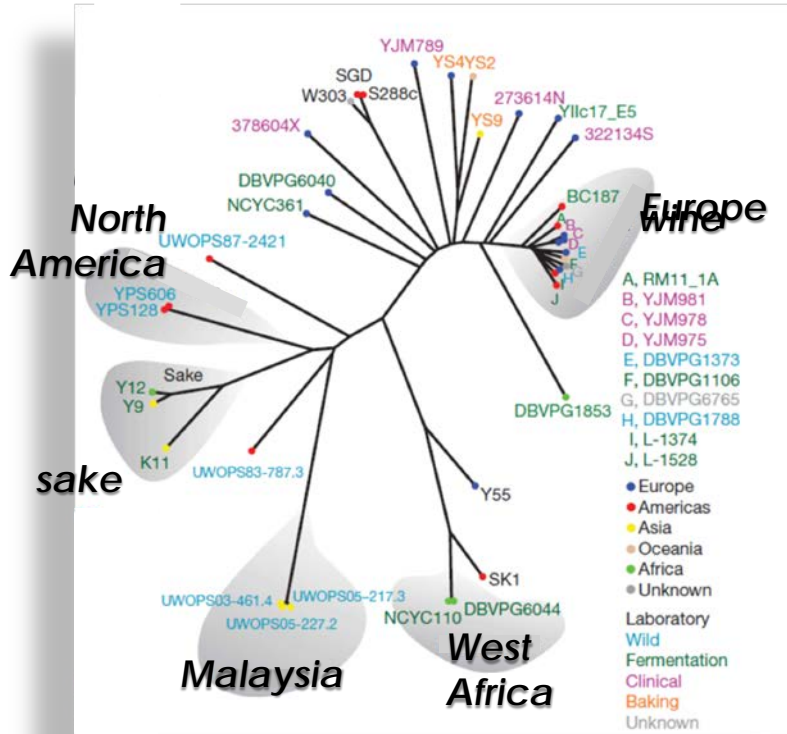


Legras et. al., Mol. Ecol. 2007

INTRODUCTION

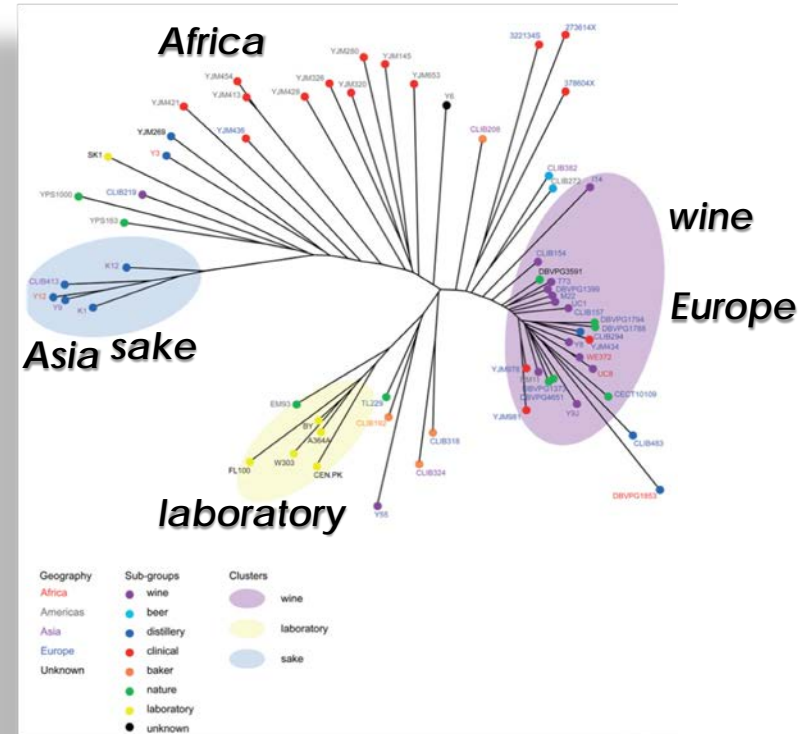
The population structure of *Saccharomyces cerevisiae*

Liti et al., Nature, 2009



235,127 SNPs
 14,051 nucleotide insertions or deletions

Schacherer et al., Nature, 2009



1.89 x 10⁶ SNP (30,097 SNPs per strain)
 3,985 deletions (200 bp length)

- low coverage whole genome sequencing
- high density arrays

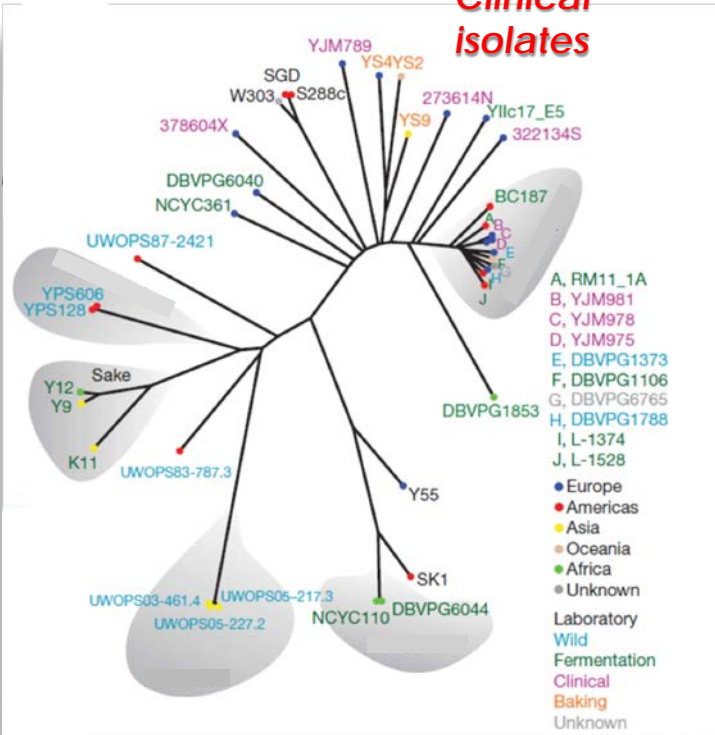
- few well-defined geographically isolated lineages
- many different mosaics of these lineages (wine, laboratory and sake strains)

INTRODUCTION

The population structure of *Saccharomyces cerevisiae*

Liti et al., Nature, 2009

Clinical isolates

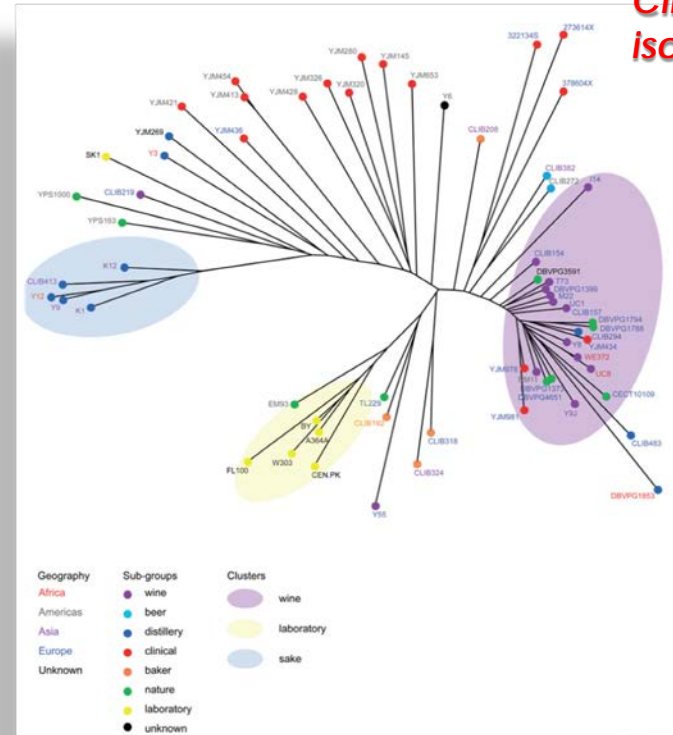


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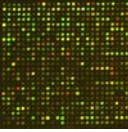
Intraspecific genome diversity of *Saccharomyces cerevisiae*

16 yeast strains representative of distinct genotypic clusters

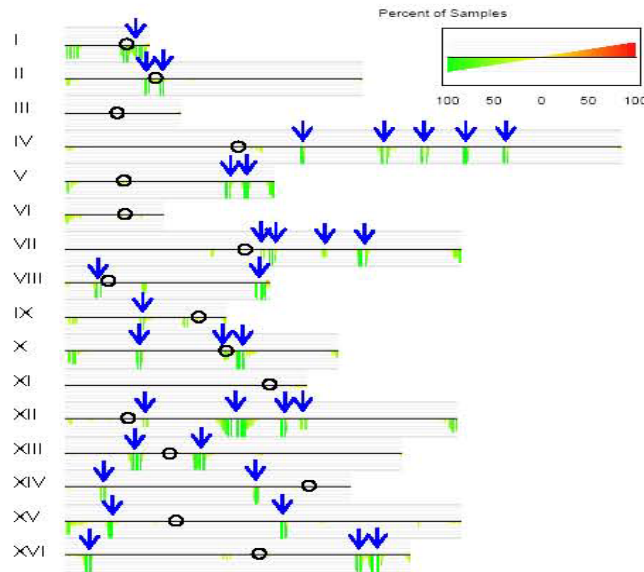
clusters genotypic of distinct representative strains 16 yeast

Carreto et al. 2008, BMC Genomics

Nacional Facility for DNA Microarrays

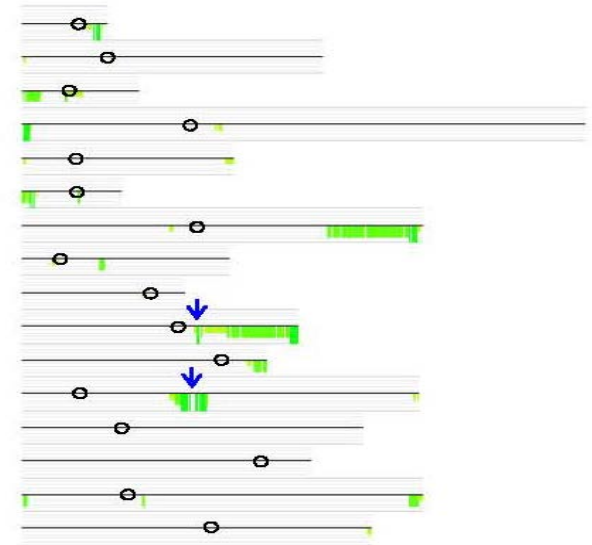


S. cerevisiae winemaking strains



➤ *Ty* element insertion

S. cerevisiae clinical strains



➤ Sub-telomeric instability

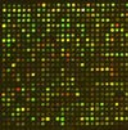
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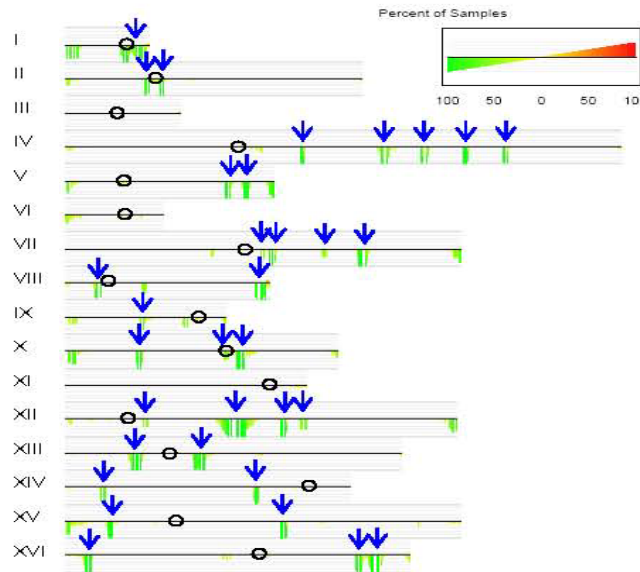
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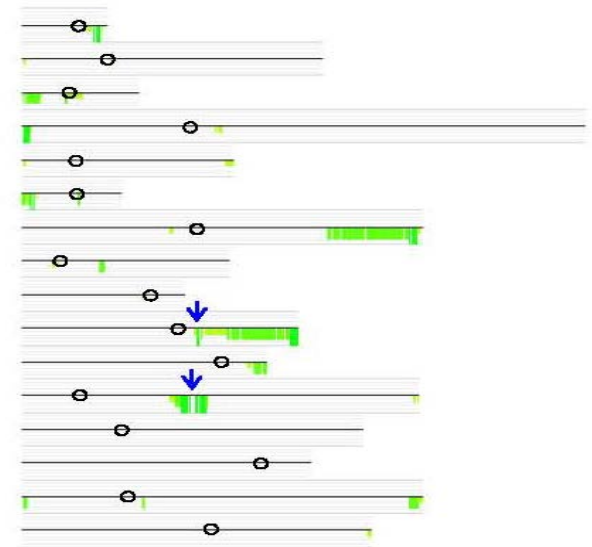
Nacional Facility
for DNA Microarrays



S. cerevisiae winemaking strains



S. cerevisiae clinical strains



clusters
genotypic
of distinct
representative
strains
16 yeast

- Transmembrane transport
- Sugar and alcohol metabolism
- Drug resistance

Gene families
with copy
number changes

INTRODUCTION

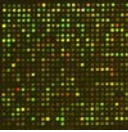
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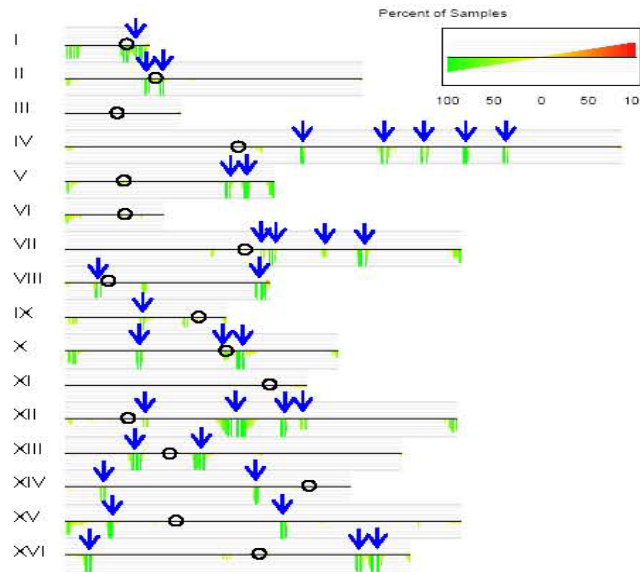
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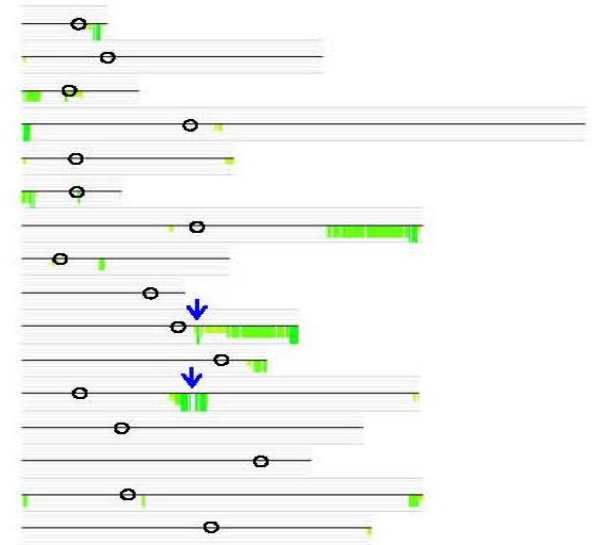
Nacional Facility for DNA Microarrays



S. cerevisiae winemaking strains



S. cerevisiae clinical strains



Intra-specific natural genome diversity



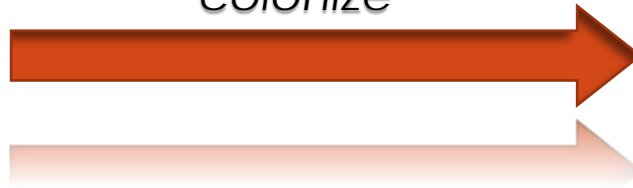
How natural selection shapes yeast genome

- ✓ Extensive use of commercial *S. cerevisiae* wine strains



S. cerevisiae
commercial
strains

colonize



vineyards

- ✓ Extensive use of commercial *S. cerevisiae* wine strains

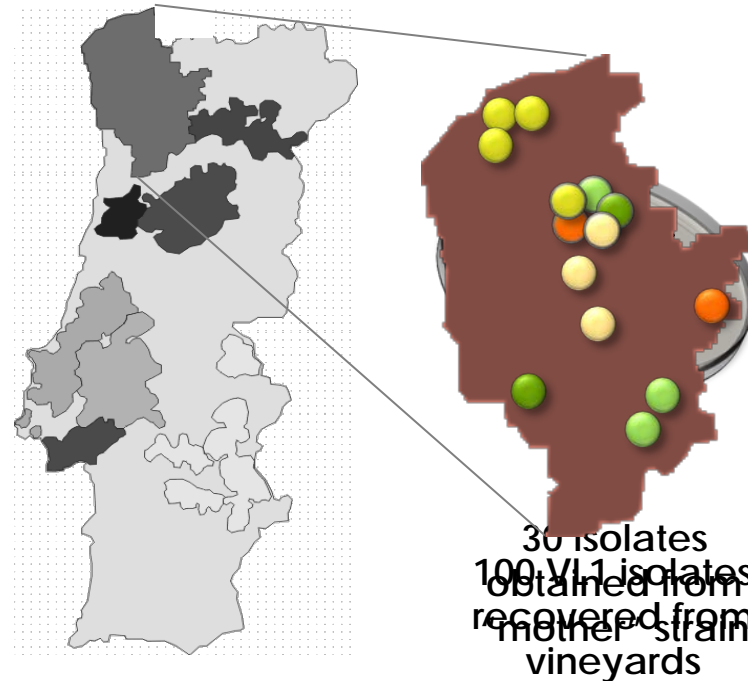


- ✓ Such strains are disseminated from the winery and can be recovered from locations in close proximity (10-200m)

Valero et al., 2005



Zymaflore VL1

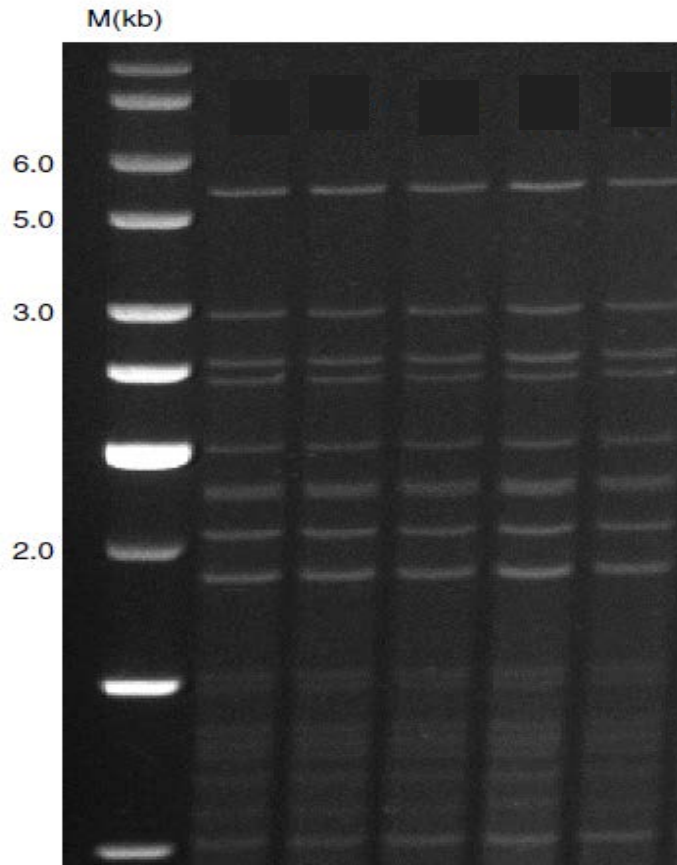


Portuguese
demarcated
wine regions

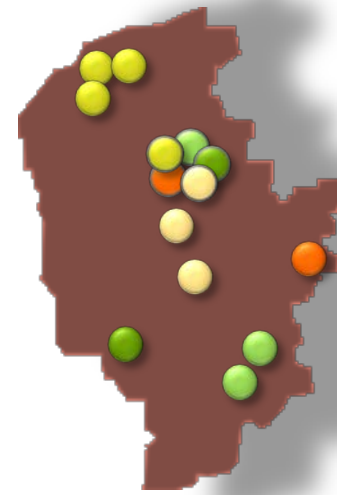
- ✓ Re-isolation of 100 isolates of the **commercial strain VL1** from vineyards close to the winery where this strain has been used during many years

Schuller and Casal, 2007

Mitochondrial DNA restriction patterns



Schuller *et al.* 2007

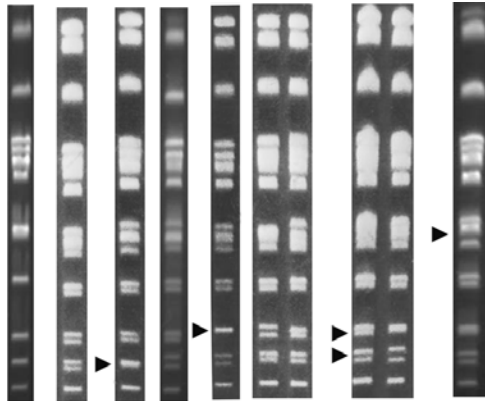


100 isolates of Zymaflore VL1

INTRODUCTION

S. cerevisiae commercial winemaking strains

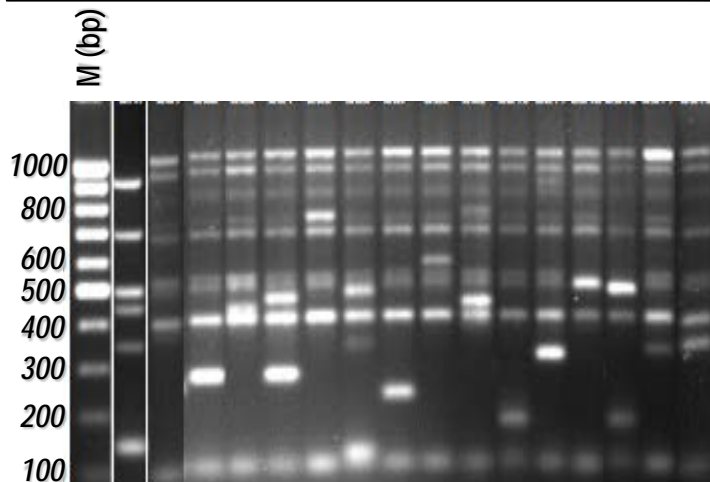
Karyotype patterns



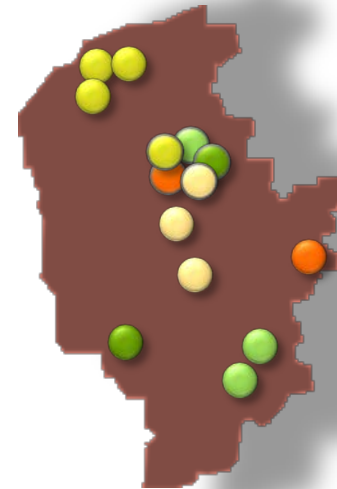
Microsatellite patterns

Loci	Alleles (bp) of distinct microsatellite patterns						
	M1	M2	M4	M5	M6	M7	M8
ScAAT1	204/219	219	204	204/219	204/219	204/219	204/219
ScAAT2	372/381	372	384	381	372	372/381	372/381
ScAAT3	265	265	265	265	265	265	265
ScAAT4	329	329	329	329	329	329	329
ScAAT5	219/222	222	219	219/222	219/222	222	219/222
ScAAT6	256/259	256	256	256/259	256/259	256/259	259

Interdelta sequence patterns



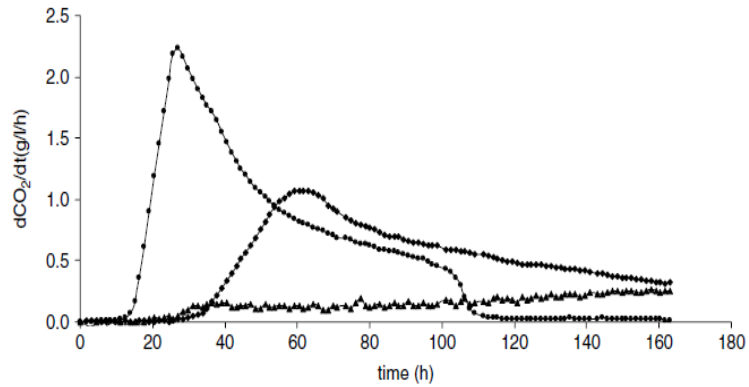
Schuller et al. 2007



100 isolates of Zymaflore VL1

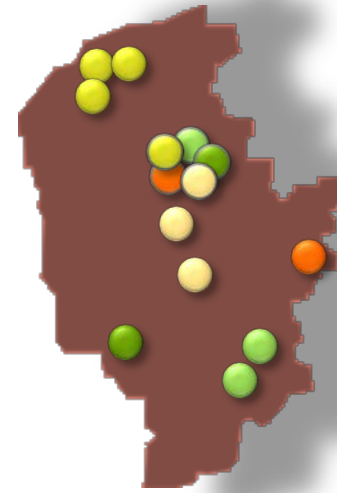
INTRODUCTION

S. cerevisiae commercial winemaking strains



Fermentation profiles

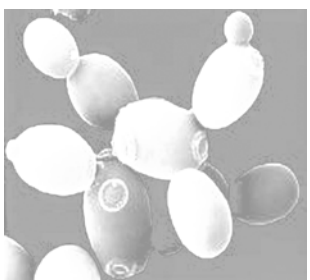
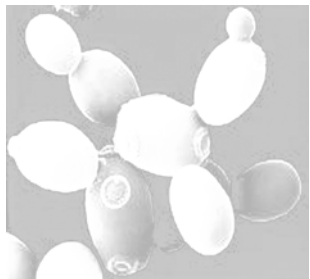
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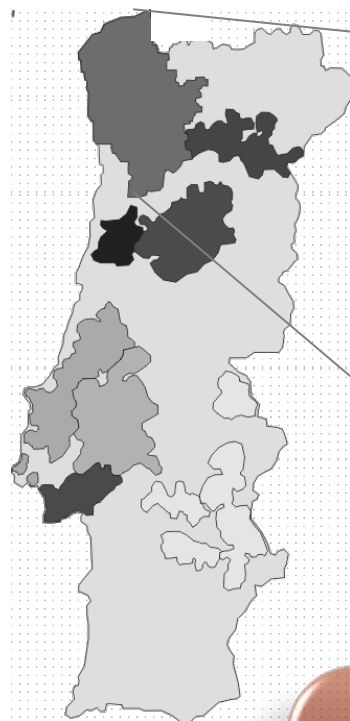


100 isolates of Zymaflore VL1

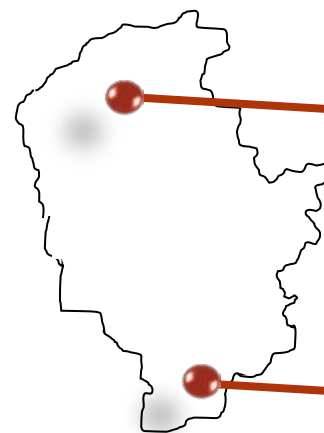
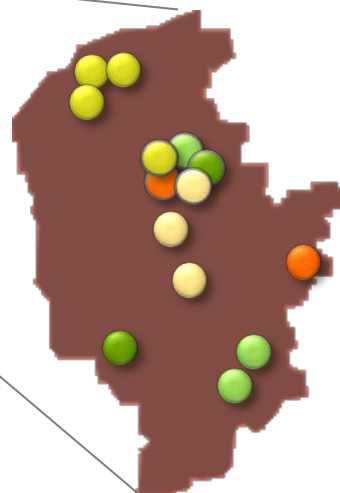
Objectives

- ✓ Evaluation of genome variations among isogenic isolates of the commercial strain *Saccharomyces cerevisiae* Zymaflore VL1 that were re-isolated from vineyards surrounding the wineries where this industrial strain was applied
 - Comparative Genome Hybridization on array (aCGH)
 - Further SNP analysis
- ✓ Phenotypic characterization
- ✓ Conclude about adaptive mechanisms that occur during the strain's permanence in vineyard environments





Portuguese demarcated wine regions



VL1 099 and VL1 108




.1 020

VL1 isolates recovered from vineyards

Reference

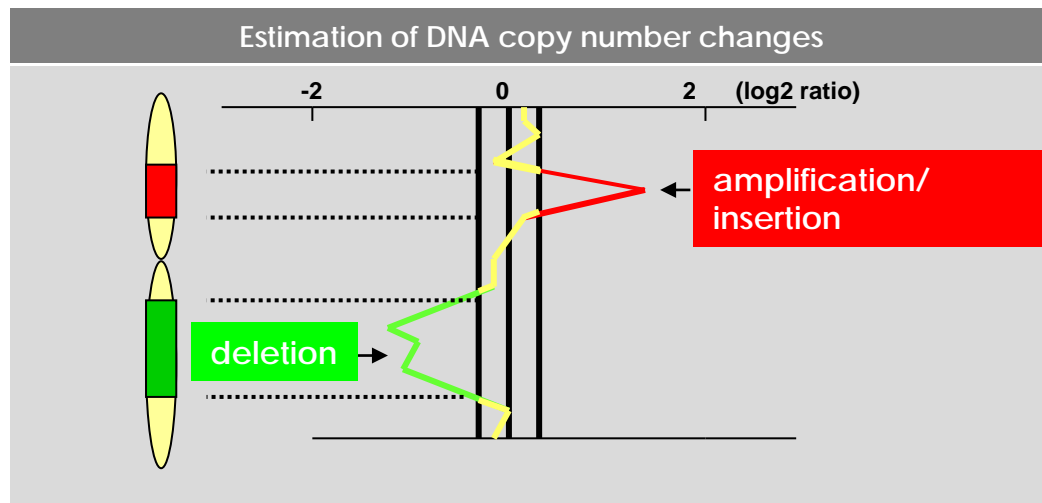
1. Commercial VL1 "mother" strain
2. VM06 (Isolate obtained through clonal expansion of the "mother" strain)

Reference DNA
"VL1 Mother strain" 

Test DNA
VM 06
VL 018
VL 020
VL 099
VL 108 

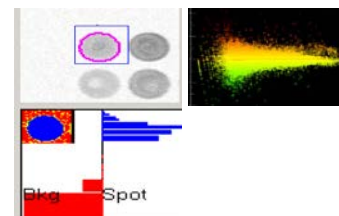


Dye swap hybridizations



QuantArray software

Image analysis - data extraction

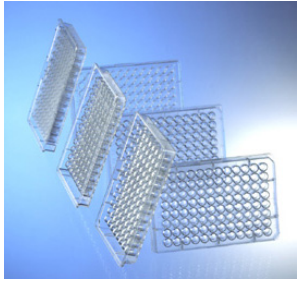


BrB software

Normalization of data

MeV software

Graphical displays of log ratios and visual representation of data
Significance Analysis for Microarrays



- Wine must + compound
- 30 °C
- 200 rpm
- O.D. after 22h of growth
- quadruplicate

Phenotypes tested:

- ✓ Temperatures (18, 30 and 40 °C)
- ✓ Tolerance to stress
 - pH values (2 and 8)
 - Osmotic/saline stress (KCl 0.75M and NaCl 1.5M)
 - Growth in finished wines supplemented with glucose (0.5% and 1%)
- ✓ Growth in the presence of
 - Potassium bisulfite (150 and 300 mg/L)
 - Copper sulphate (5mM)
 - Sodium dodecyl sulphate (0.01% w/v)
 - Cycloheximide (0.05 $\mu\text{g}/\text{mL}$ and 0.1 $\mu\text{g}/\text{mL}$)
 - Iprodion (0.05 $\mu\text{g}/\text{mL}$ and 0.1 $\mu\text{g}/\text{mL}$)
 - Procymidon (0.05 mg/mL and 0.1 mg/mL)

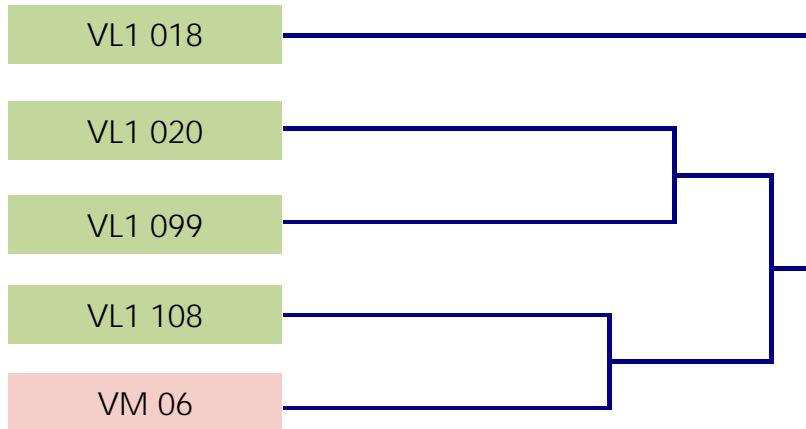
RESULTS

Clustering of aCGH profiles

aCGH profiles of 5 strains (4 + 1) in comparison with "mother" strain

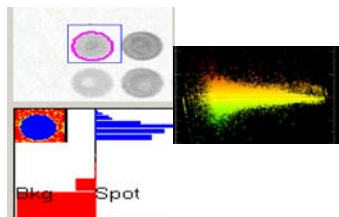


Clustering



No clear separation between VL1 isolates obtained from nature (●) and the isolate derived from the "mother" strain (●)

Hierarchical clustering
Pearson correlation
Average linkage



RESULTS

Gene Copy number alterations – SAM analysis

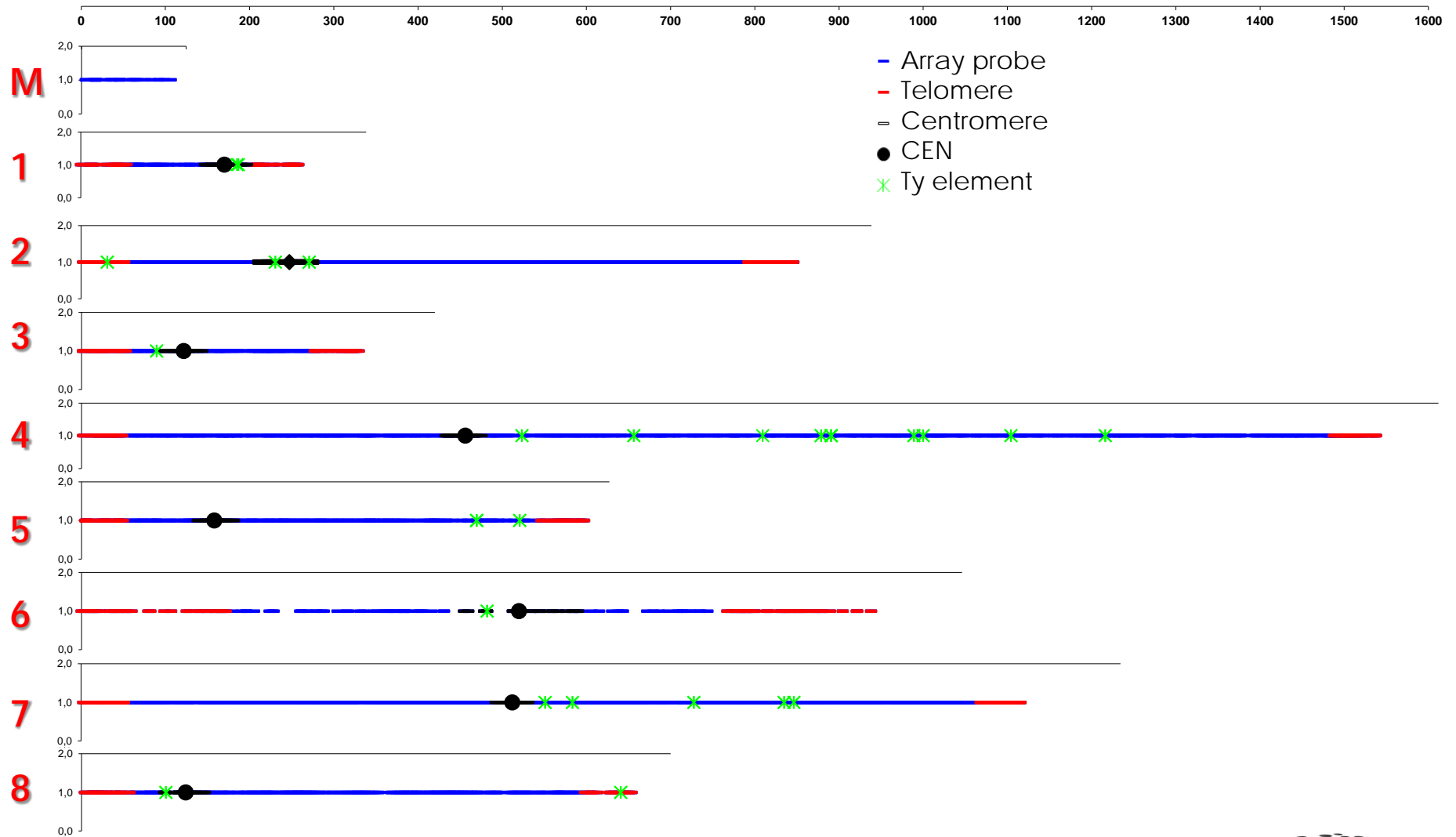
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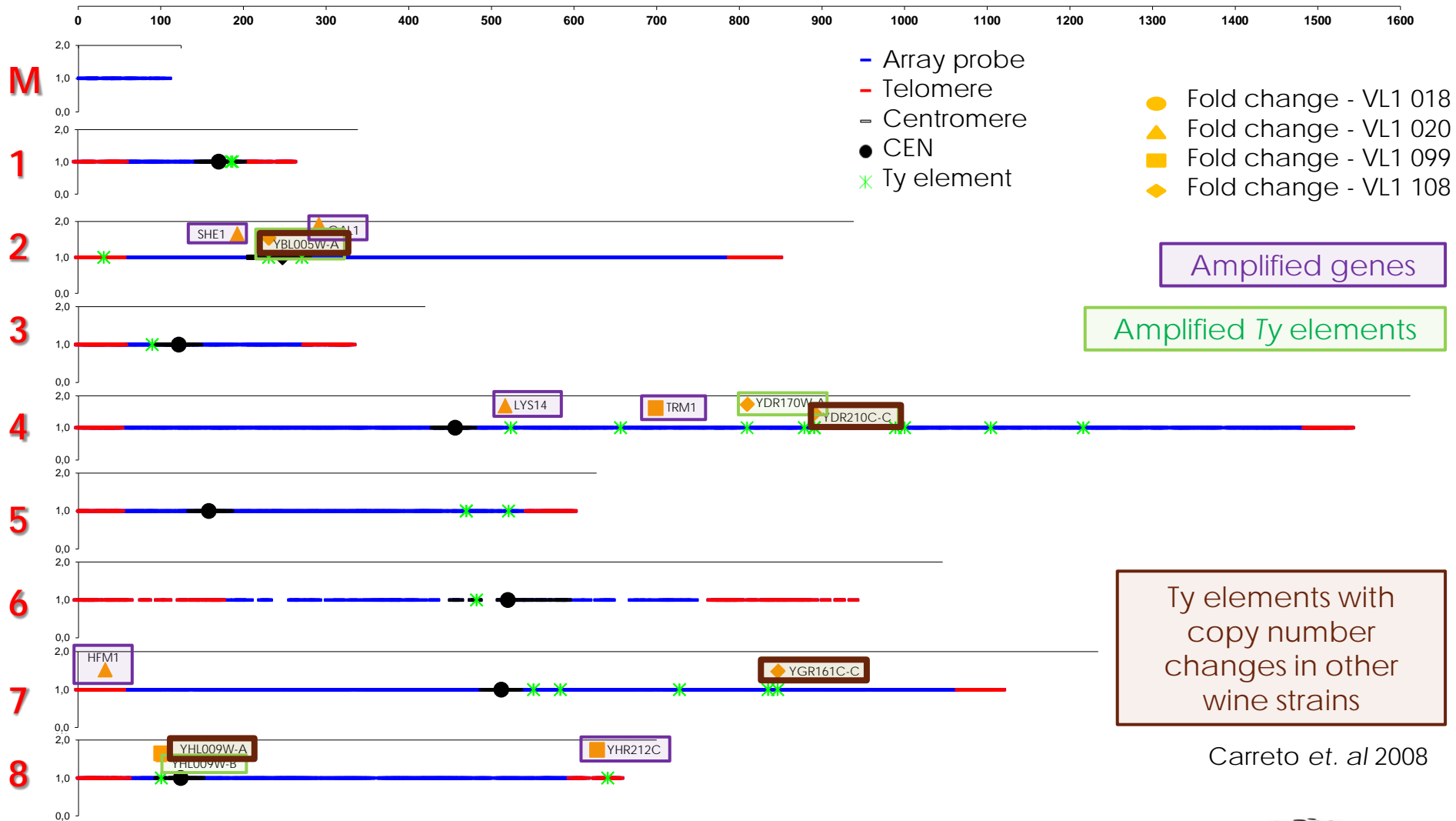
RESULTS

Gene Copy number alterations – SAM analysis



RESULTS

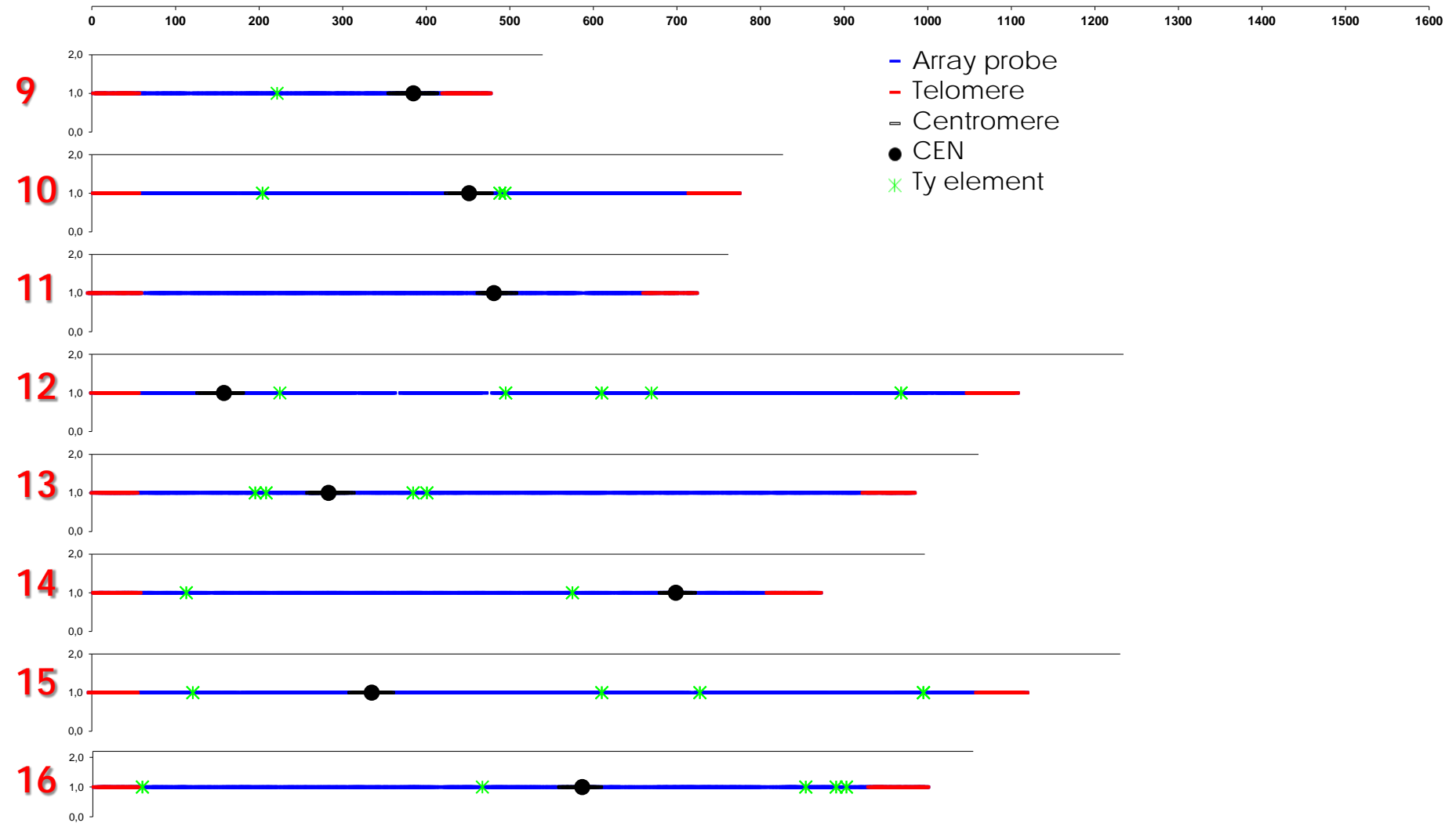
Gene Copy number alterations – SAM analysis



Carreto et. al 2008

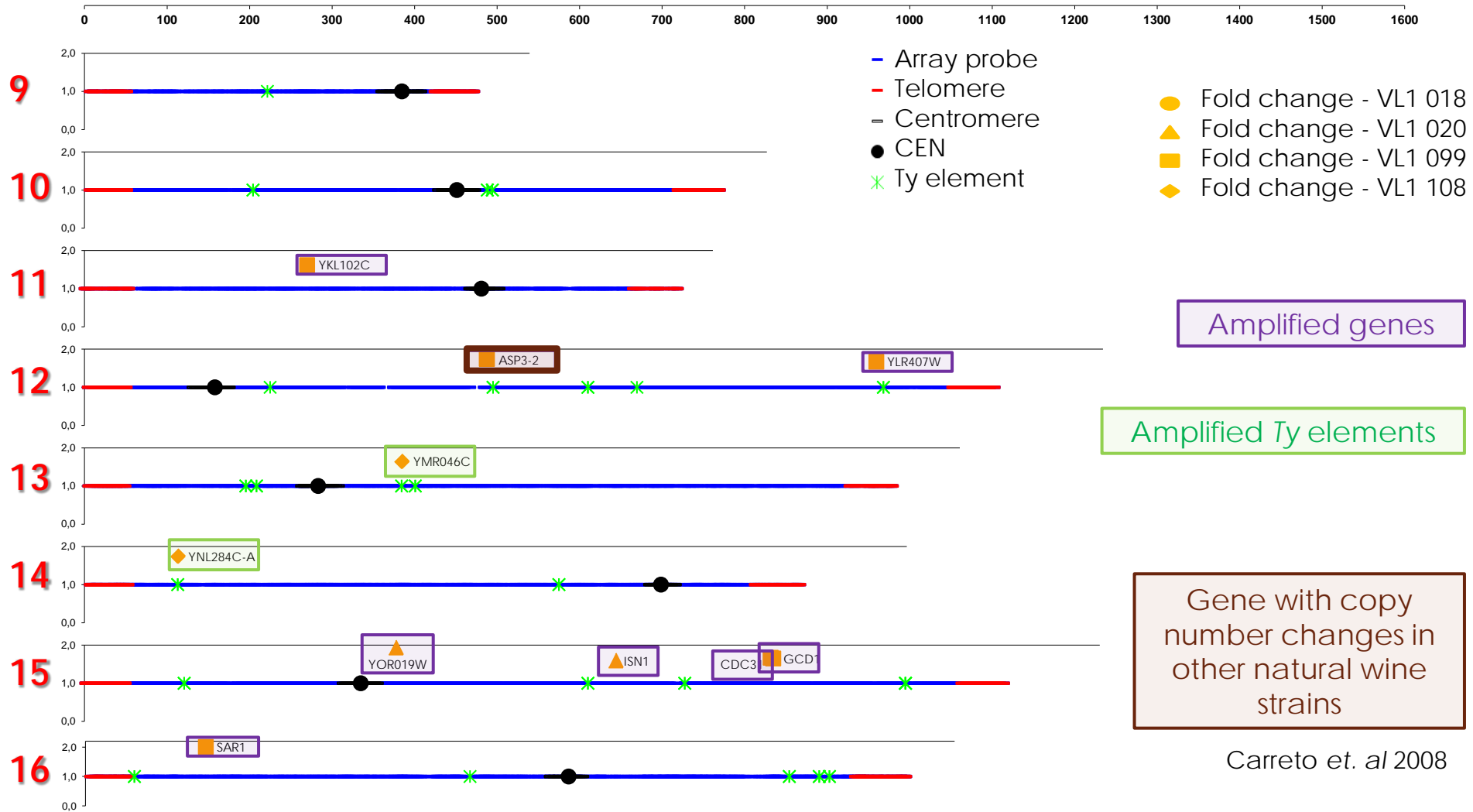
RESULTS

Gene Copy number alterations – SAM analysis



RESULTS

Gene Copy number alterations – SAM analysis



Carreto *et. al* 2008

RESULTS

Gene Copy number alterations – amplified genes

Amplified genes (14):

copy number fold changes

Systematic Name	Gene	VL1 018	VL1 020	VL1 099	VL1 108
YBL031W	<i>SHE1</i>		1.7		
YOR019W			1.9		
YGL251C	<i>HFM1/MER3</i>		1.5		
YOR155C	<i>ISN1</i>		1.6		
YDR034C	<i>LYS14</i>		1.7		
YBR020W	<i>GAL1</i>		1.9		
YDR120C	<i>TRM1</i>			1.7	
YLR407W				1.7	
YOR260W	<i>GCD1/TRA3</i>			1.7	
YKL102C				1.6	
YOR257W	<i>CDC31/DSK1</i>			1.7	
YHR212C				1.7	
YLR157C	<i>ASP3-2</i>			1.7	
YPL218W	<i>SAR1</i>			2.0	

RESULTS

Gene Copy number alterations – amplified genes

Amplified genes (14):

Systematic Name	Gene
YBL031W	<i>SHE1</i>
YOR019W	
YGL251C	<i>HFM1/MER3</i>
YOR155C	<i>ISN1</i>
YDR034C	<i>LYS14</i>
YBR020W	<i>GAL1</i>
YDR120C	<i>TRM1</i>
YLR407W	
YOR260W	<i>GCD1/TRA3</i>
YKL102C	
YOR257W	<i>CDC31/DSK1</i>
YHR212C	
YLR157C	<i>ASP3-2</i>
YPL218W	<i>SAR1</i>



mitosis



meiosis



lysine biosynthesis



galactose catabolism



asparagine catabolism

RESULTS

Gene Copy number alterations – Ty elements amplification

Amplified Ty elements (8):

copy number fold changes

Systematic Name	Chromosome	VL1 018	VL1 020	VL1 099	VL1 108
YMR046C	13	1.6			1.7
YHL009W-A	8			1.6	
YYHL009W-B	8			1.6	
YGR161C-C	7				1.5
YBL005W-A	2				1.5
YDR210C-C	4				1.5
YDR170W-A	4				1.7
YNL284C-A	14				1.7

RESULTS

Gene Copy number alterations – Ty elements amplification

Amplified Ty elements (9):

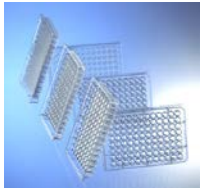
Systematic Name	Chromosome
YMR046C	13
YHL009W-A	8
YYHL009W-B	8
YGR161C-C	7
YBL005W-A	2
YDR210C-C	4
YDR170W-A	4
YNL284C-A	14

Induced in *S. cerevisiae* by various stresses:

- DNA damage
- UV-light exposure
- ionizing radiation
- adenine starvation
- **nitrogen starvation**

RESULTS

Phenotypic characterization



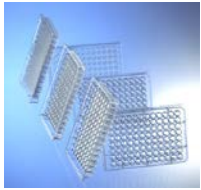
- Wine must + compound
- 30 °C
- 200 rpm
- 22h of growth
- quadruplicate

- 0 – Abs_{640nm} 0.1
- 1 – Abs_{640nm} 0.2-0.4
- 2 – Abs_{640nm} 0.5-1.2
- 3 – Abs_{640nm} ≥1.3

Strain	Phenotypic tests																			
	30°C	18°C	40°C	pH 2	pH 8	KCl 0.75M	CuSO ₄ 5mM	SDS 0.01%	NaCl 1.5M	Ethanol 6%	Ethanol 10%	Ethanol 14%	Iprodion (0.05mg/mL)	Iprodion (0.1mg/mL)	Procymidon (0.05mg/mL)	Procymidon (0.1mg/mL)	KHSO ₃ (150 mg/L)	KHSO ₃ (300 mg/L)	Wine + glucose 0.5%	Wine + glucose 1%
VL1 018	3	1	3	0	2	2	0	0	1	3	2	1	3	3	3	3	3	1	1	1
VL1 020	3	1	3	0	2	3	0	0	1	3	2	1	3	3	3	3	3	1	1	1
VL1 099	3	1	3	0	2	2	0	0	1	3	2	1	3	3	3	3	3	2	0	0
VL1 108	3	1	3	0	2	2	0	0	0	3	2	1	3	3	3	3	3	2	0	0
VM 06	3	1	3	0	2	2	0	0	1	3	2	1	3	3	3	3	3	2	1	1
"mother" strain	3	0	3	0	2	2	1	1	1	3	2	1	3	3	3	3	3	2	0	1

RESULTS

Phenotypic characterization



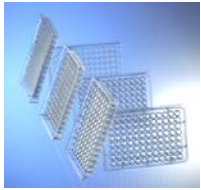
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VL1 018	3	1	3	0	2	2	0	0	1	3	2	1	3	3	3	3	3	1	1	1
VL1 020	3	1	3	0	2	3	0	0	1	3	2	1	3	3	3	3	3	1	1	1
VL1 099	3	1	3	0	2	2	0	0	1	3	2	1	3	3	3	3	3	2	0	0
VL1 108	3	1	3	0	2	2	0	0	0	3	2	1	3	3	3	3	3	2	0	0
VM06	3	1	3	0	2	2	0	0	1	3	2	1	3	3	3	3	3	2	1	1
"mother" strain	3	0	3	0	2	2	1	1	1	3	2	1	3	3	3	3	3	2	0	1

RESULTS

Phenotypic characterization



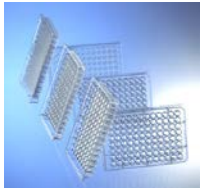
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- 3 – Abs_{640nm} ≥1.3

Strain	Phenotypic tests																			
	30°C	18°C	40°C	pH 2	pH 8	KCl 0.75M	CuSO ₄ 5mM	SDS 0.01%	NaCl 1.5M	Etanol 6%	Etanol 10%	Etanol 14%	Iprodion (0.05mg/mL)	Iprodion (0.1mg/mL)	Procymidon (0.05mg/mL)	Procymidon (0.1mg/mL)	KHSO ₃ (150 mg/l)	KHSO ₃ (300 mg/l)	Wine + glucose 0.5%	Wine + glucose 1%
VL1 018	3	1	3	0	2	2	0	0	1	3	2	1	3	3	3	3	3	1	1	1
VL1 020	3	1	3	0	2	3	0	0	1	3	2	1	3	3	3	3	3	1	1	1
VL1 099	3	1	3	0	2	2	0	0	1	3	2	1	3	3	3	3	3	2	0	0
VL1 108	3	1	3	0	2	2	0	0	0	3	2	1	3	3	3	3	3	2	0	0
VM06	3	1	3	0	2	2	0	0	1	3	2	1	3	3	3	3	3	2	1	1
"mother" strain	3	0	3	0	2	2	1	1	1	3	2	1	3	3	3	3	3	2	0	1

RESULTS

Phenotypic characterization



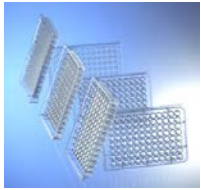
- Wine must + compound
- 30 °C
- 200 rpm
- 22h of growth
- quadruplicate

- 0 – Abs_{640nm} 0.1
- 1 – Abs_{640nm} 0.2-0.4
- 2 – Abs_{640nm} 0.5-1.2
- 3 – Abs_{640nm} ≥1.3

Strain	Phenotypic tests																			
	30°C	18°C	40°C	pH 2	pH 8	KCl 0.75M	CuSO ₄ 5mM	SDS 0.01%	NaCl 1.5M	Etanol 6%	Etanol 10%	Etanol 14%	Iprodion (0.05mg/mL)	Iprodion (0.1mg/mL)	Procymidon (0.05mg/mL)	Procymidon (0.1mg/mL)	KHSO ₃ (150 mg/l)	KHSO ₃ (300 mg/l)	Wine + glucose 0.5%	Wine + glucose 1%
VL1 018	3	1	3	0	2	2	0	0	1	3	2	1	3	3	3	3	3	1	1	1
VL1 020	3	1	3	0	2	3	0	0	1	3	2	1	3	3	3	3	3	1	1	1
VL1 099	3	1	3	0	2	2	0	0	1	3	2	1	3	3	3	3	3	2	0	0
VL1 108	3	1	3	0	2	2	0	0	0	3	2	1	3	3	3	3	3	2	0	0
VM06	3	1	3	0	2	2	0	0	1	3	2	1	3	3	3	3	3	2	1	1
"mother" strain	3	0	3	0	2	2	1	1	1	3	2	1	3	3	3	3	3	2	0	1

RESULTS

Phenotypic characterization

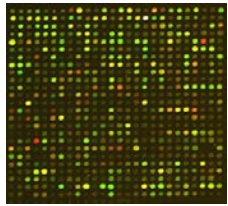


- Wine must + compound
- 30 °C
- 200 rpm
- 22h of growth
- quadruplicate

- 0 – Abs_{640nm} 0.1
- 1 – Abs_{640nm} 0.2-0.4
- 2 – Abs_{640nm} 0.5-1.2
- 3 – Abs_{640nm} ≥1.3

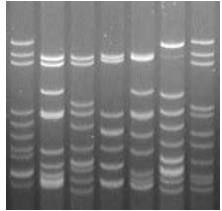
Strain	Phenotypic tests																			
	30°C	18°C	40°C	pH 2	pH 8	KCl 0.75M	CuSO ₄ 5mM	SDS 0.01%	NaCl 1.5M	Etanol 6%	Etanol 10%	Etanol 14%	Iprodion (0.05mg/mL)	Iprodion (0.1mg/mL)	Procymidon (0.05mg/mL)	Procymidon (0.1mg/mL)	KHSO ₃ (150 mg/l)	KHSO ₃ (300 mg/L)	Wine + glucose 0.5%	Wine + glucose 1%
VL1 018	3	1	3	0	2	2	0	0	1	3	2	1	3	3	3	3	3	1	1	1
VL1 020	3	1	3	0	2	3	0	0	1	3	2	1	3	3	3	3	3	1	1	1
VL1 099	3	1	3	0	2	2	0	0	1	3	2	1	3	3	3	3	3	2	0	0
VL1 108	3	1	3	0	2	2	0	0	0	3	2	1	3	3	3	3	3	2	0	0
VM06	3	1	3	0	2	2	0	0	1	3	2	1	3	3	3	3	3	2	1	1
"mother" strain	3	0	3	0	2	2	1	1	1	3	2	1	3	3	3	3	3	2	0	1

SUMMARY AND CONCLUSIONS

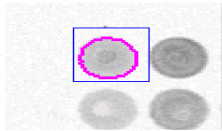
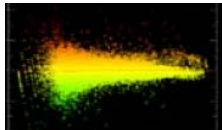


❖ *Isogenic isolates of the commercial wine yeast strain Zymaflore VL1 recovered from nature showed genetic differences in comparison with the “mother” strain:*

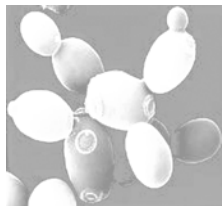
- *Gene amplifications*
- *Ty element amplifications*
- *Apparent stochastic distribution*



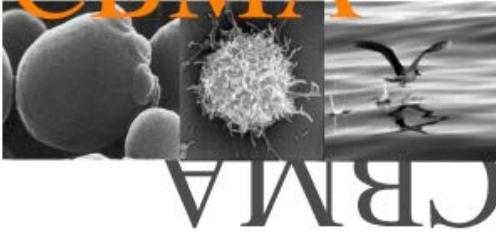
❖ *Generation of intra-strain phenotypic variability*



The transition from nutrient-rich musts to nutritionally scarce natural environments is correlated with microevolutionary changes that may reflect adaptive responses

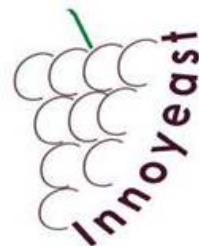


changes that may reflect adaptive responses
natural environments is correlated with microevolutionary



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- Dorit Schuller
- Inês Mendes
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- Eugénia Vieira



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