


## CoolWine

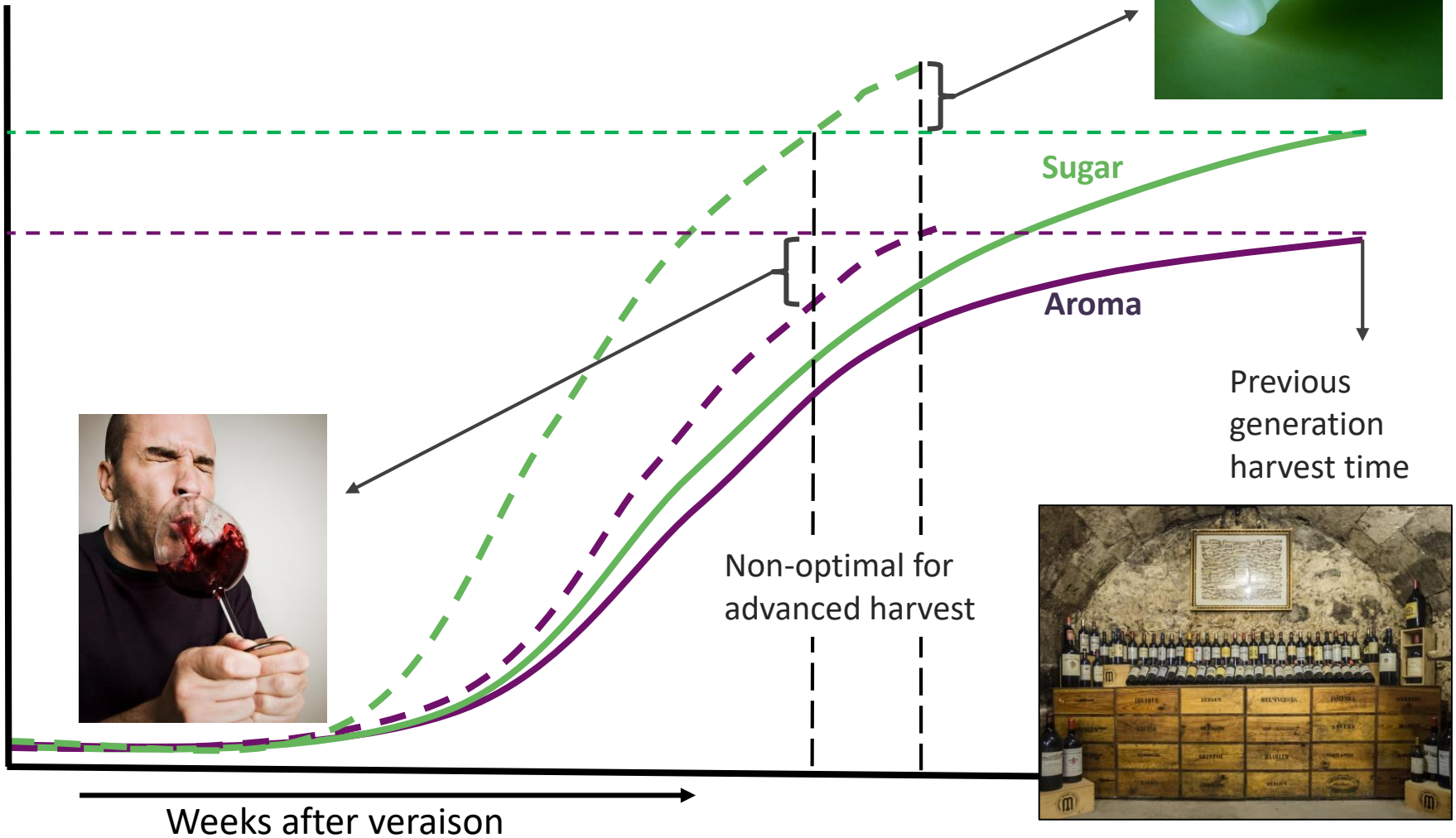
Model-guided evolution for balanced attenuation of wine ethanol content by developing non-GMO yeast strains and communities

Kiran R. Patil, EMBL / University of Cambridge  
On behalf of the CoolWine consortium



- 
- > €6 billion EU trade
  - 10% of agri. production for some EU countries
  - 20% of total agri. employment; >5 million jobs
  - European Landscape Convention: relevance of vineyards

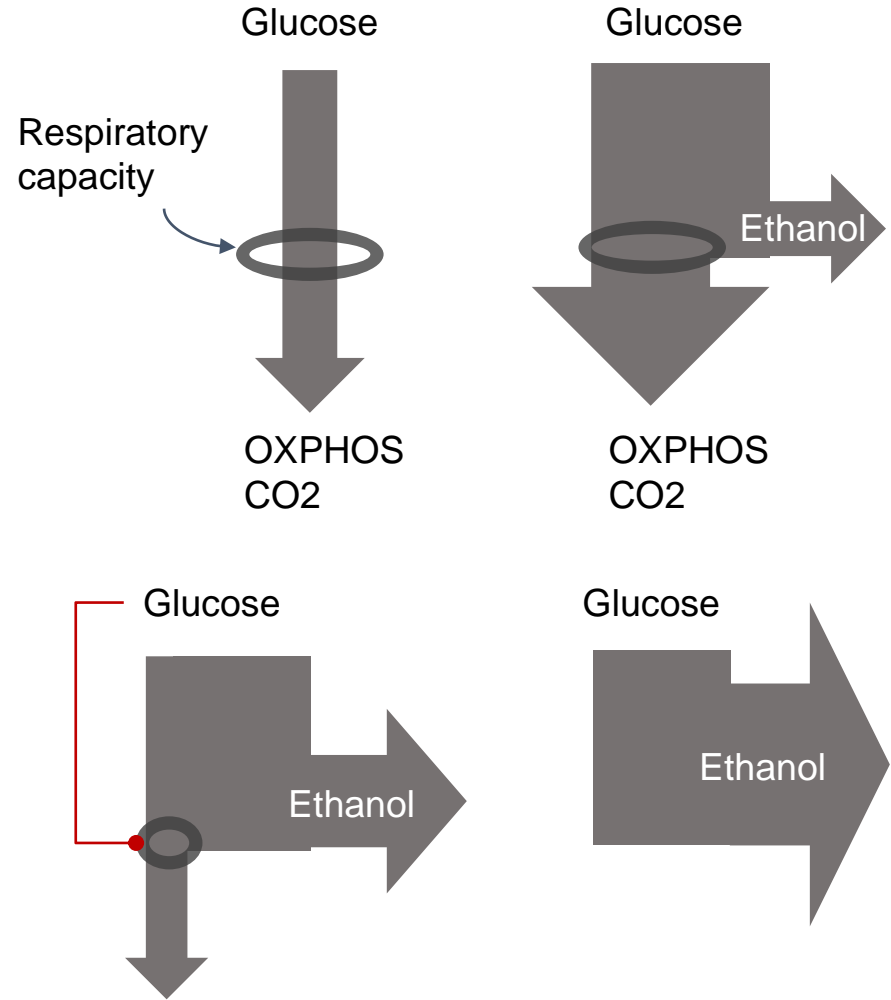
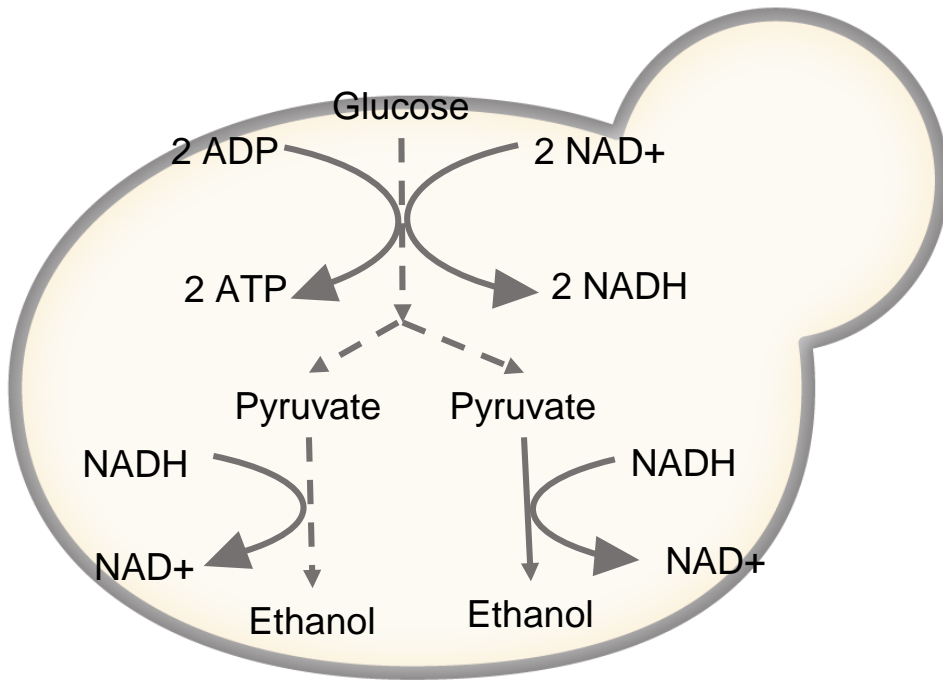
# Grape ripening is affected by climate change





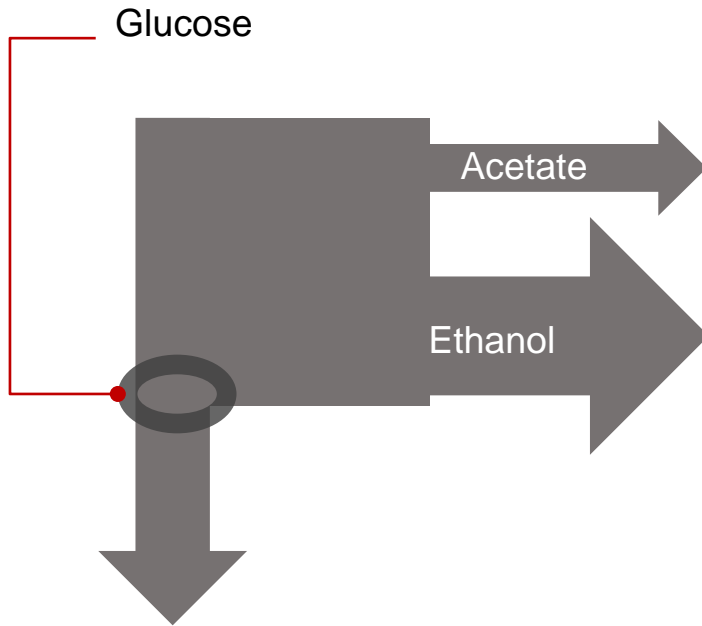


- Total increase of around 3% ABV since the eighties
- Impact on consumer acceptance and international trade
- Lower alcohol wine market growth ~30% per year
- Other producing countries (NZ) have started large research programs to address this issue
- Market not ready for GMO-based solutions

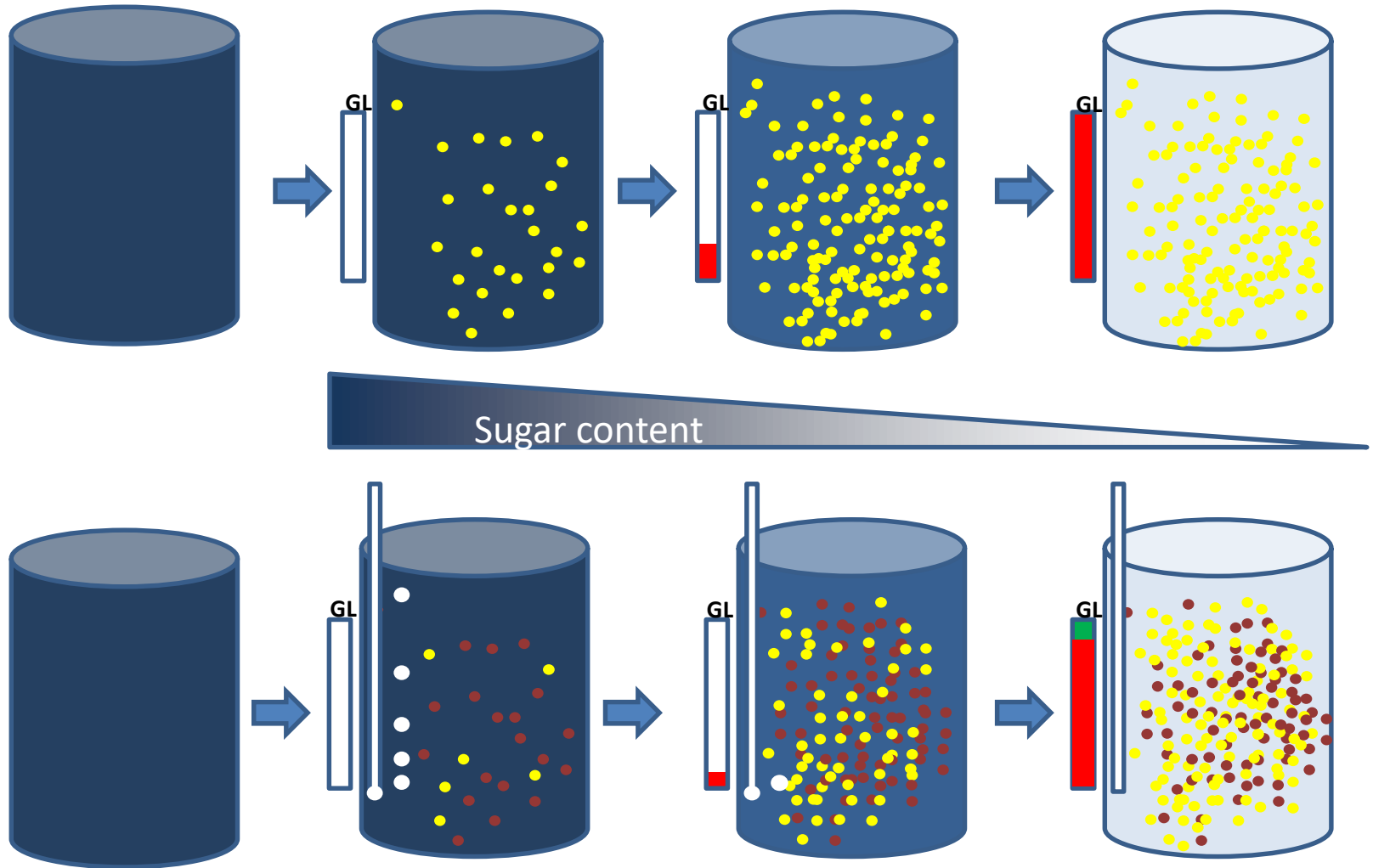




# ERA CoBioTech Aeration is (unfortunately) not a straightforward solution



# CoolWine approach: Co-inoculation with evolved Sacchs and non-Sacchs and/or aeration



## Key features

- Respiro-fermentation instead of standard anaerobic fermentation
- Targeting *S. cerevisiae* and non-*Saccharomyces* wine yeasts
- Massively parallel adaptive laboratory evolution (ALE) – **non-GMO solution**
- Computer modelling to design ALE conditions

## Problems to tackle

- Competitive fitness of Crabtree-negative yeasts
- Yeast strain/species compatibility
- Acetate production by *S. cerevisiae* (under aerobic conditions)





## Spain, Sweden, Norway and Germany

- Spanish Council for Scientific Research (CSIC) Ramon Gonzalez
- Rovira i Virgili University (URV) Gemma Beltrán / Albert Mas
- Norwegian University of Science and Technology (NTNU) Eivind Almaas
- European Molecular Biology Laboratory (EMBL) Kiran R. Patil
- University of Gothenburg (GU) Jonas Warringer
- Bodegas Roda S.A. (not funded) Esperanza Tomás



# CSIC

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS



UNIVERSITAT ROVIRA I VIRGILI



UNIVERSITY OF  
GOTHENBURG



# NTNU

# EMBL



BODEGAS  RODA

HARO • RIOJA ALTA • ESPAÑA





	TRACK 1	TRACK 2
WP1	<i>S. cerevisiae</i> mutant strain characterization. Plus data from WineSys	Non-Saccharomyces (NS) strain characterization in mono- and co-cultures
WP3	Improvement of <i>S. cerevisiae</i> metabolic models for aerobic fermentation	Metabolic modelling of NS and communities. Identification of target pathways
WP4	EvolveX guided high throughput ALE of <i>S. cerevisiae</i> industrial strains	EvolveX guided high and medium throughput ALE of NS wine yeast strains
WP5	Wine production assays with <i>S. cerevisiae</i> improved strains	Wine production assays with mixed starter cultures



# Project plan

**WP2. Omics.**  
**Main methods:** NGS, proteomics, metabolomics  
**Main partner:** EMBL, CSIC

**WP1.**  
**Yeast strains**

**Main methods:**  
Quantitative  
physiology,  
omics

**Main partners:**  
CSIC, URV, EMBL

**WP3.**  
**Modelling**

**Main methods:**  
EvolveX,  
SMETANA

**Main partners:**  
NTNU, EMBL

**WP4.**  
**Evolution**

**Main methods:**  
Massive scale  
exp. evolution,  
bioreactors

**Main partners:**  
EMBL, GU, CSIC

**WP5.**  
**Wine**

**Main methods:**  
Bioreactors,  
exp. cellar

**Main partners:**  
URV, CSIC,  
Agrovin, Roda

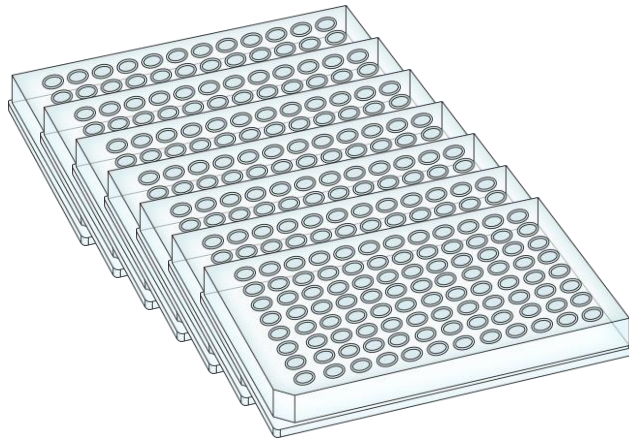
**WP6. Social impact**  
**Main methods:** Focus groups, in-depth interviews  
**Main partners:** URV, NTNU, CSIC

**WP7. Management.** CSIC



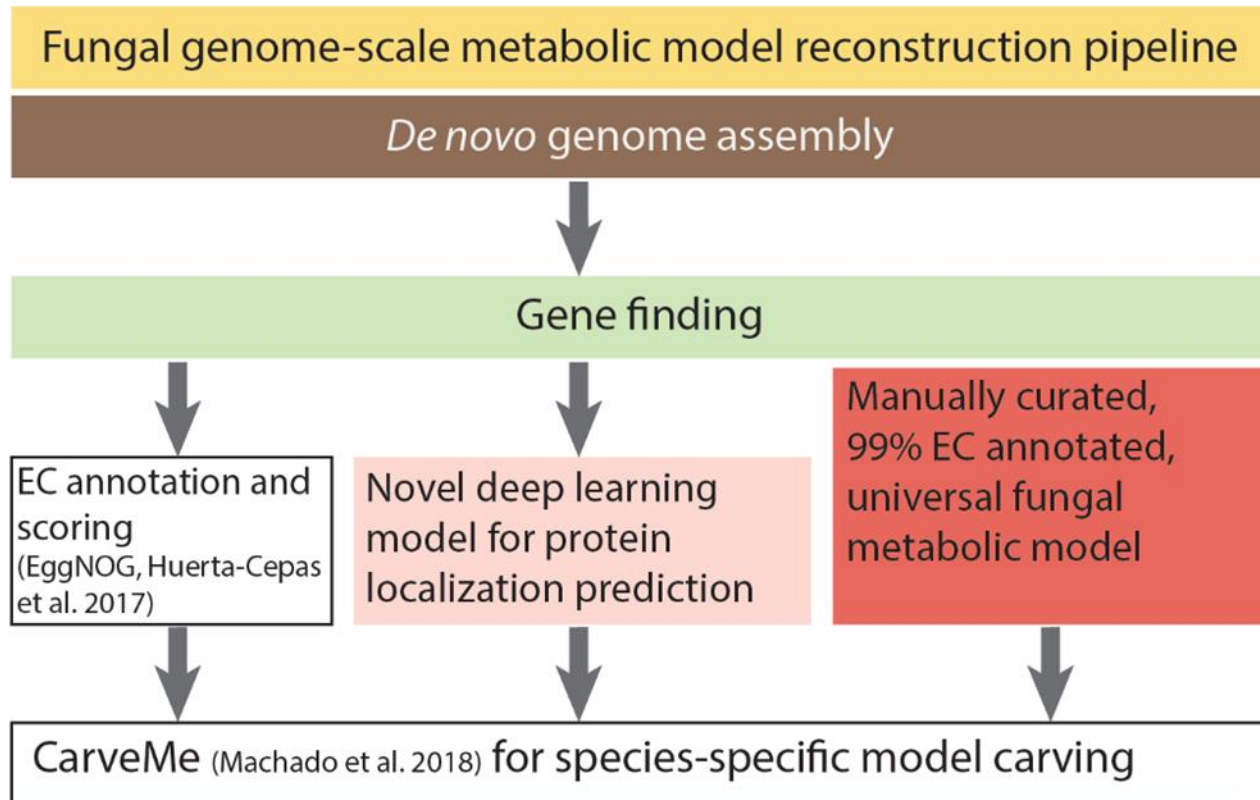


*Saccharomyces cerevisiae*  
(48 wine related strains)

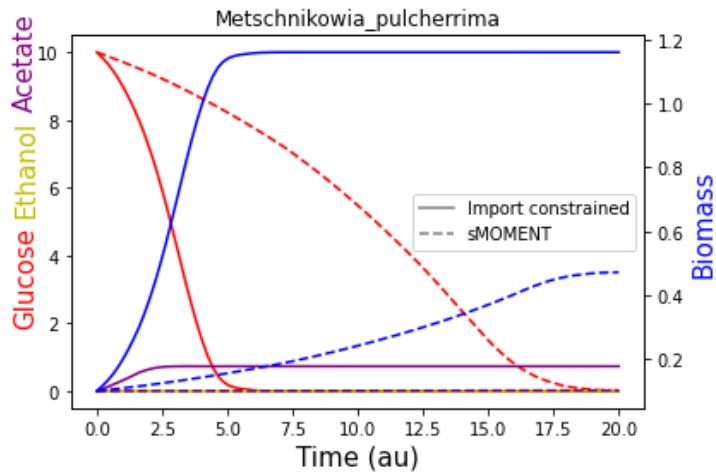


*Candida sake*  
*Candida boidinii*  
*Candida zemplinina*  
*Debaryomyces hansenii*  
*Hanseniaspora vinae*  
*Hanseniaspora guilliermondii*  
*Hanseniaspora osmophila*  
*Hanseniaspora uvarum*  
*Hanseniaspora vinae*  
*Hansenula polymorpha*  
*Issatchenkia terricola*  
*Kluyveromyces lactis*  
*Lachancea thermotolerans*  
*Metschnikowia pulcherrima*  
*Starmerella bombicola*  
*Torulaspora delbrueckii*  
*Zygosaccharomyces bailii*  
*Zygosaccharomyces rouxii*

Genome-scale metabolic model reconstruction automatically from genome

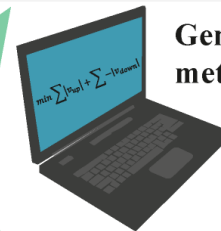






Up- and down-regulation target fluxes

Glycerol	Allantoin	Glucose...
1	0	0 ...
0	1	0 ...
1	0	0 ...
0	0	1 ...



Genome-scale metabolic model



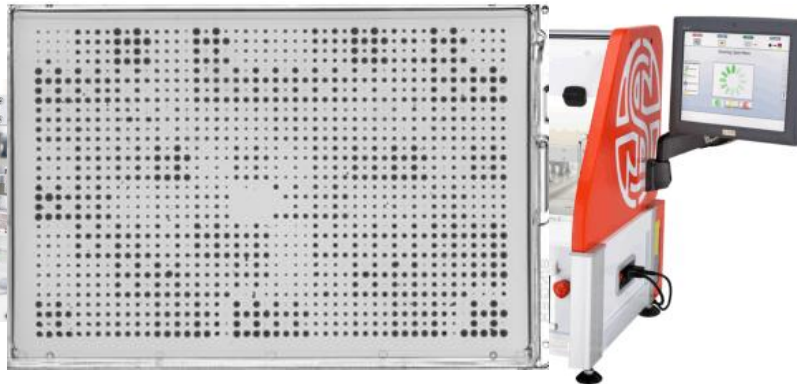
Mixed-integer linear problem (MILP) solver

Reproduction for optimization with Genetic Algorithm

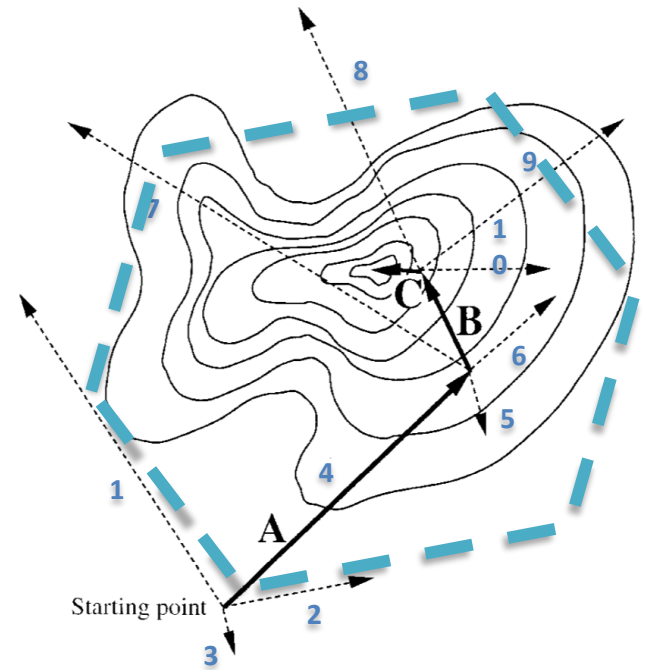
Scoring with **EvolveX**

Glycerol  
L-Phenylalanine  
2-Deoxyglucose  
...  
...

# Scan-o-matic: massively parallel experimental evolution

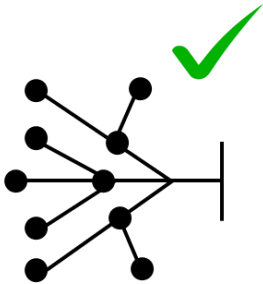


1536 format



**An evolutionary walk to the optimum fitness**

Elena SF, Lenski RE (2003) Nat Rev Genet 4: 457-469.



### Evolution

20 Cycles  
7 Environments  
(3: Sacch)  
(4: Non-Sacch)



### Scanning

**Starting-points**  
n:  $7 \cdot 48 \cdot 24 = 8064$   
**End-points**  
n:  $7 \cdot 1152 \cdot 4 = 32256$



### Analysis

**Feature extraction**  
90%  
**Quality control**  
90%



### Freeze down (in SGM)

n:  $7 \cdot 1152 = 8064$   
40%

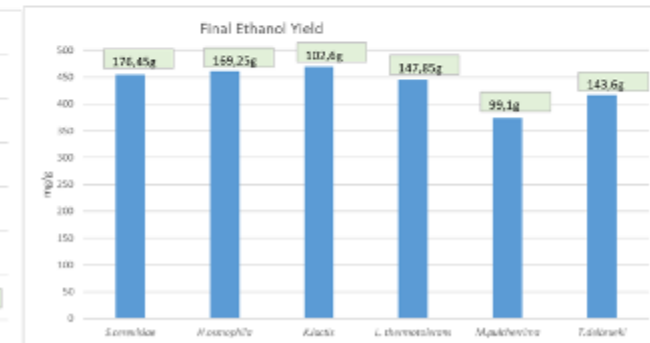
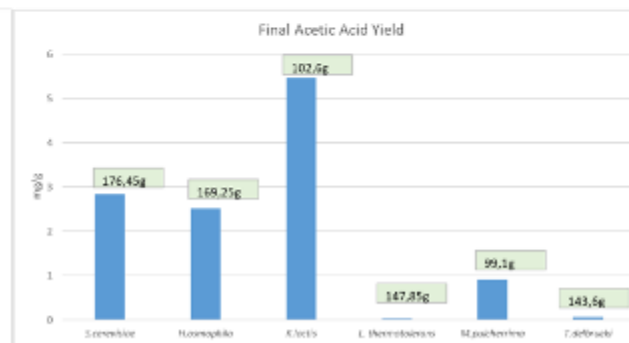
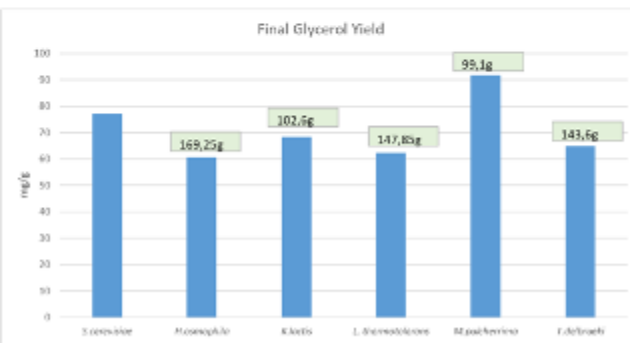
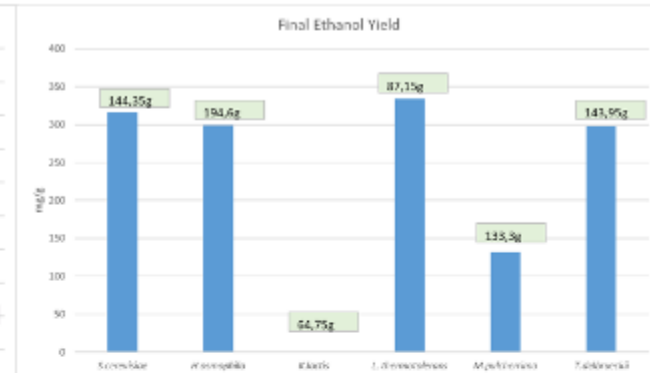
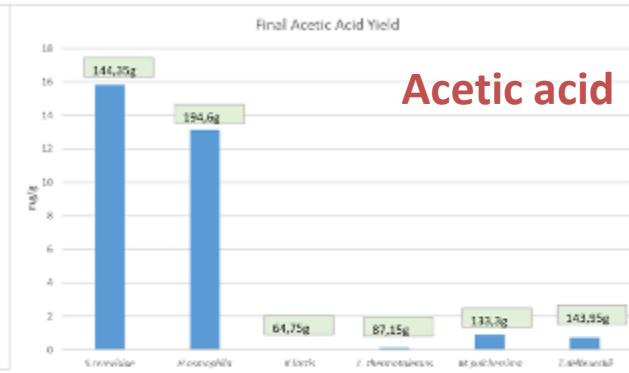
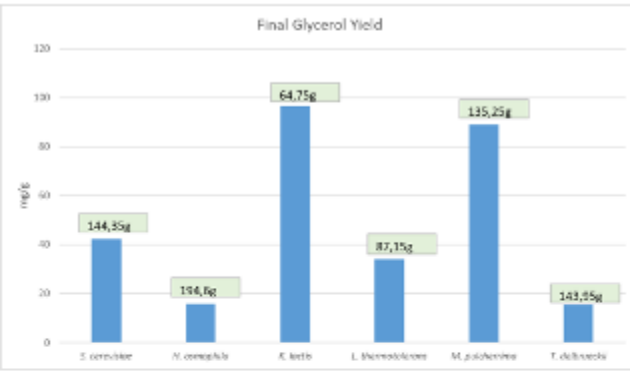


## Glycerol

AEROBIC  
CONTIDIONS

## Ethanol

Acetic acid

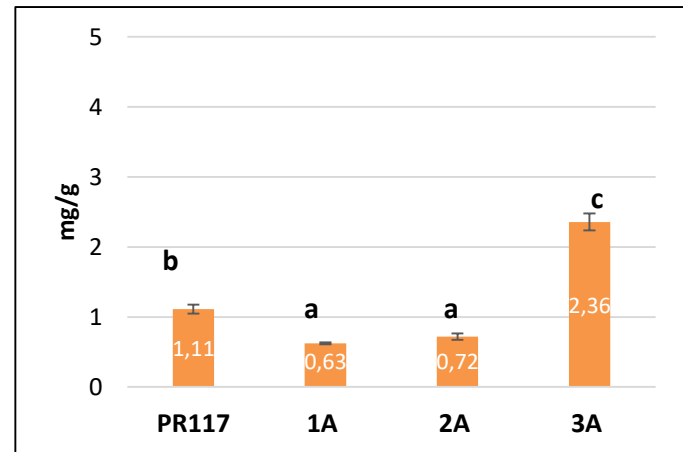
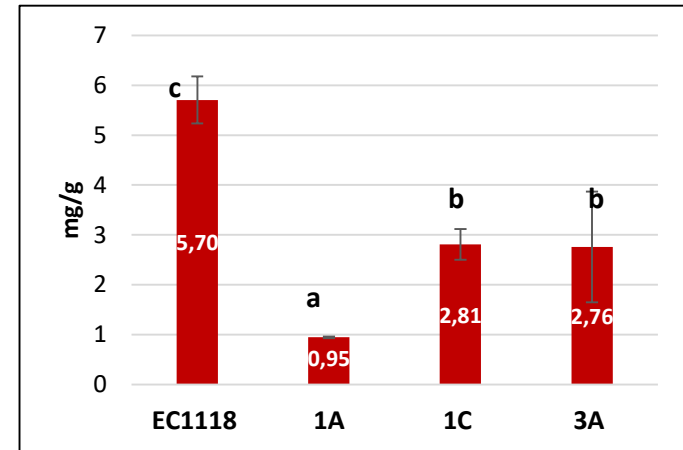
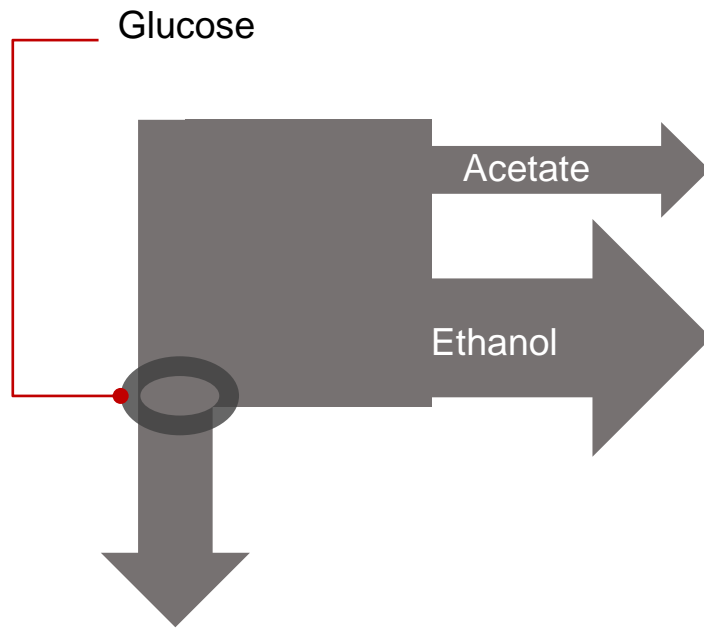


ANAEROBIC  
CONDITIONS

Non-sacchs

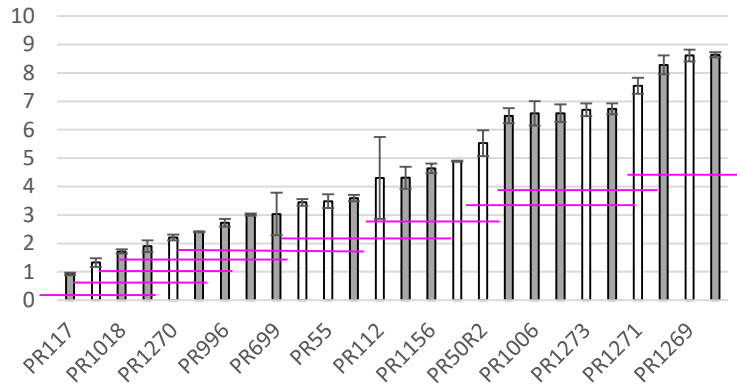
*S. cerevisiae*

# Experimental evolution to relieve glucose repression reduced acetic acid yield

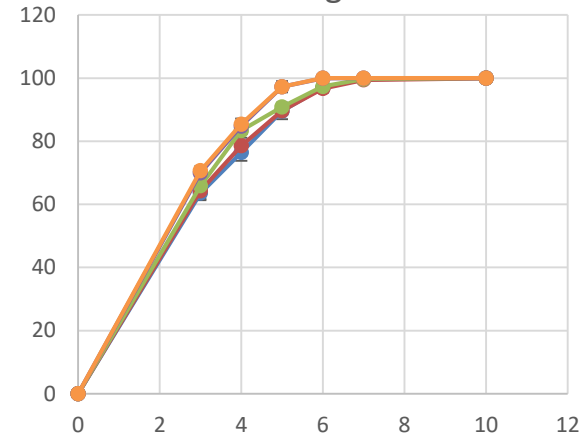


# *S. cerevisiae* strains with low acetic acid yield

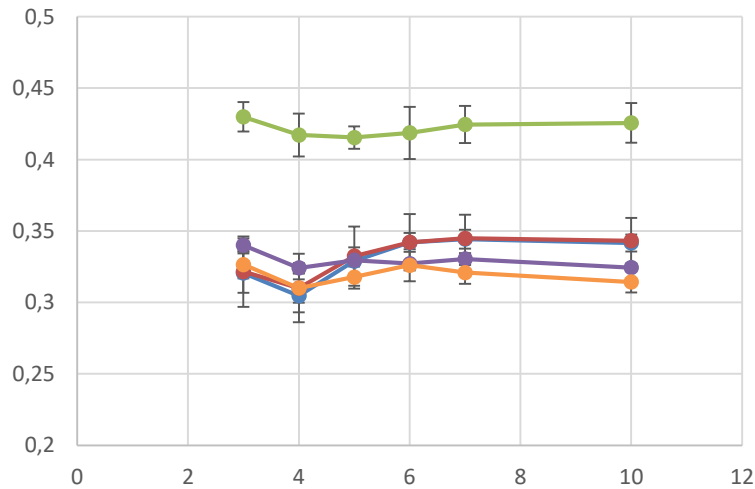
Acetic Acid Yield (mg/g)



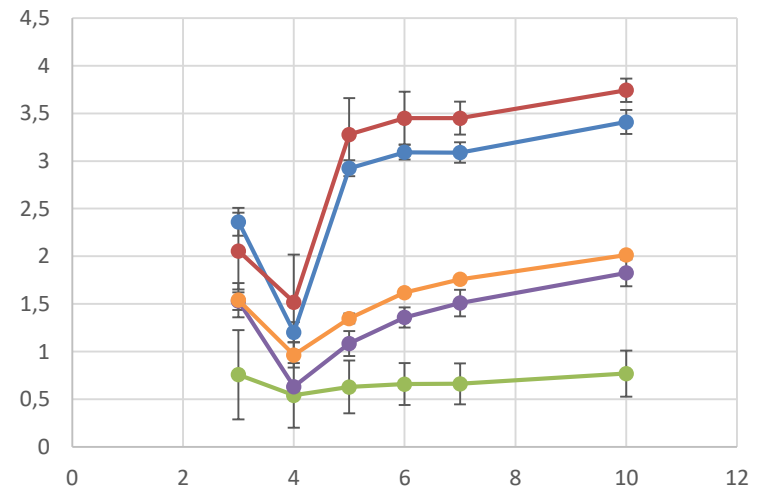
% Consumed sugars



Ethanol Yield (g/g)



Acetic acid Yield (mg/g)



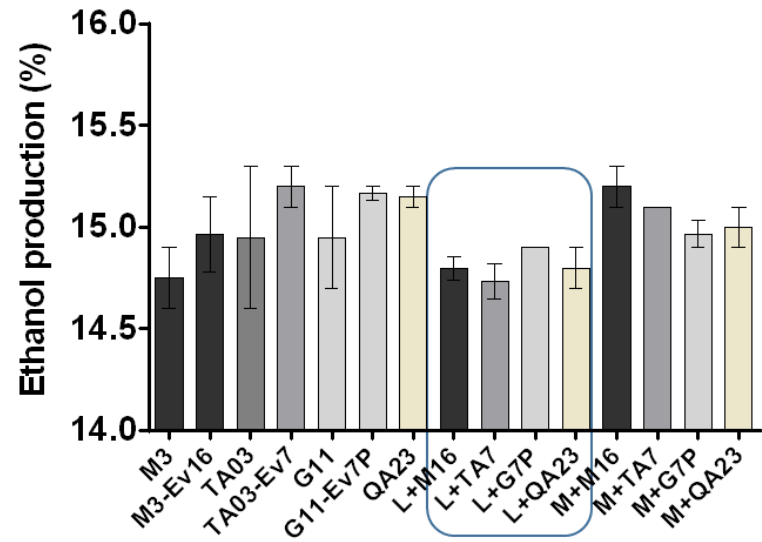
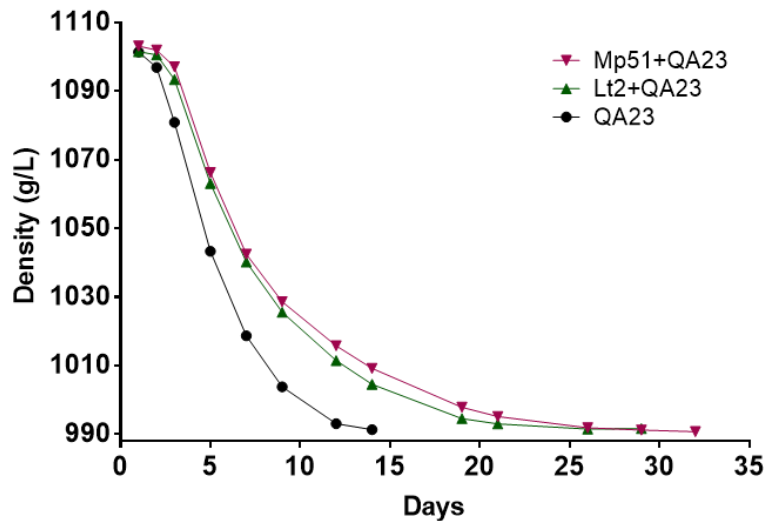




Sequential fermentations with selected Non-Sc and *S. cerevisiae* strains

<i>non-Saccharomyces</i>	<i>S. cerevisiae</i> (after 48h)
<i>L. thermotolerans</i> L. t 2	TA03 evolved 7 (butanol)
<i>M. pulcherrima</i> M.p 51	M3 evolved 16 (35% sugars)
	G11 evolved 7P (35% sugars)
	QA23

39 fermentations (5L)  
Natural must





## 7 FOCUS GROUPS

### -4 in Tarragona

- 4 wine consumers and non-consumers;
- 3 wine experts (biotechnologist, biochemist, oenologist);
- 2 wine sellers;
- 8 health professionals (doctor, nurses, psychologist, psychiatrist, social educator, policeman).

### -3 in Logroño

- 7 wine experts (biotechnologists, biochemists, oenologists, chemists);
- 4 wine producers;
- 6 health professionals (doctors, laboratory technicians; R&D manager; biochemist).

## 15 IN-DEPTH INTERVIEWS TO:

- 3 wine experts (biotechnologists, biochemists, environmental law expert);
- 2 wine sellers;
- 5 wine consumers and non-consumers;
- 3 wine producers;
- 1 yeast producer;
- 1 health professional (nurse involved in prevention program).

## OTHER SOURCES:

### Two participant observations:

- in a wine shop to observe criteria when choosing a wine;
- during a wine tasting that included a CoolWine wine.

**Questionnaire** completed by 16 participants to the wine tasting.

**Free-listing** completed by 34 participants to the focus groups.



- CoolWine yeast core collection
- Genome-scale metabolic models for wine yeasts beyond *S. cerevisiae*
- > 30 000 evolved populations obtained
- Evolved low acetate (aerobic) producing *S. cerevisiae* strains available
- Sequential inoculation wines made at pilot scale (TRL 6 / 7)
- Report on societal impact
- Three published papers, one submitted, 3-4 in the pipeline
- One patent application filed



- Project extended till June next year due to pandemic associated delays
- In-depth omics characterisation
- Another round of design-evolve-test cycle
- Another round of pilot scale fermentation





- Ramon Gonzalez (CSIC; Spain)
- Albert Mas/Gemma Beltran (URV; Spain)
- Maria I. Gracia (URV; Spain)
- Eivind Almaas (NTNU; Norway)
- Lars Ursin (NTNU; Norway)
- Jonas Warringer (Uni. Gothenburg; Sweden)
- Esperanza Tomás (Roda Cellar; Spain)
- Paula Jouhten (VTT; Finland)



UNIVERSITAT ROVIRA I VIRGILI



UNIVERSITY OF  
GOTHENBURG

ERA-CoBioTech and all National Funding Agencies

