

# Generating wine yeasts for the production of low alcohol wines

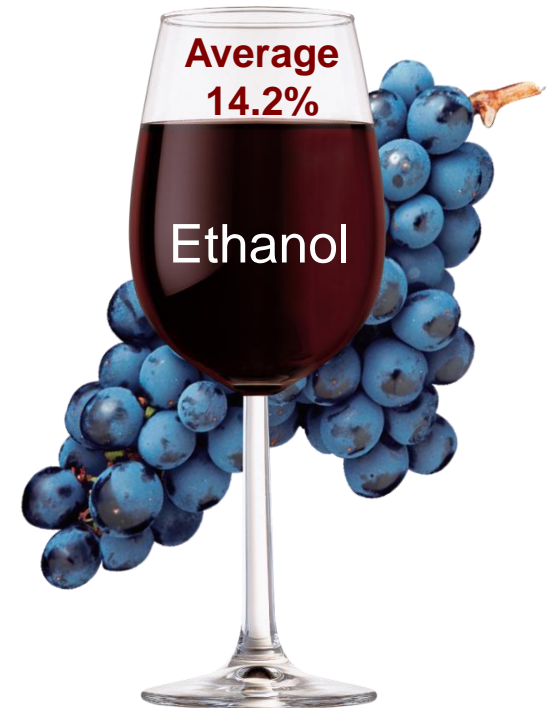
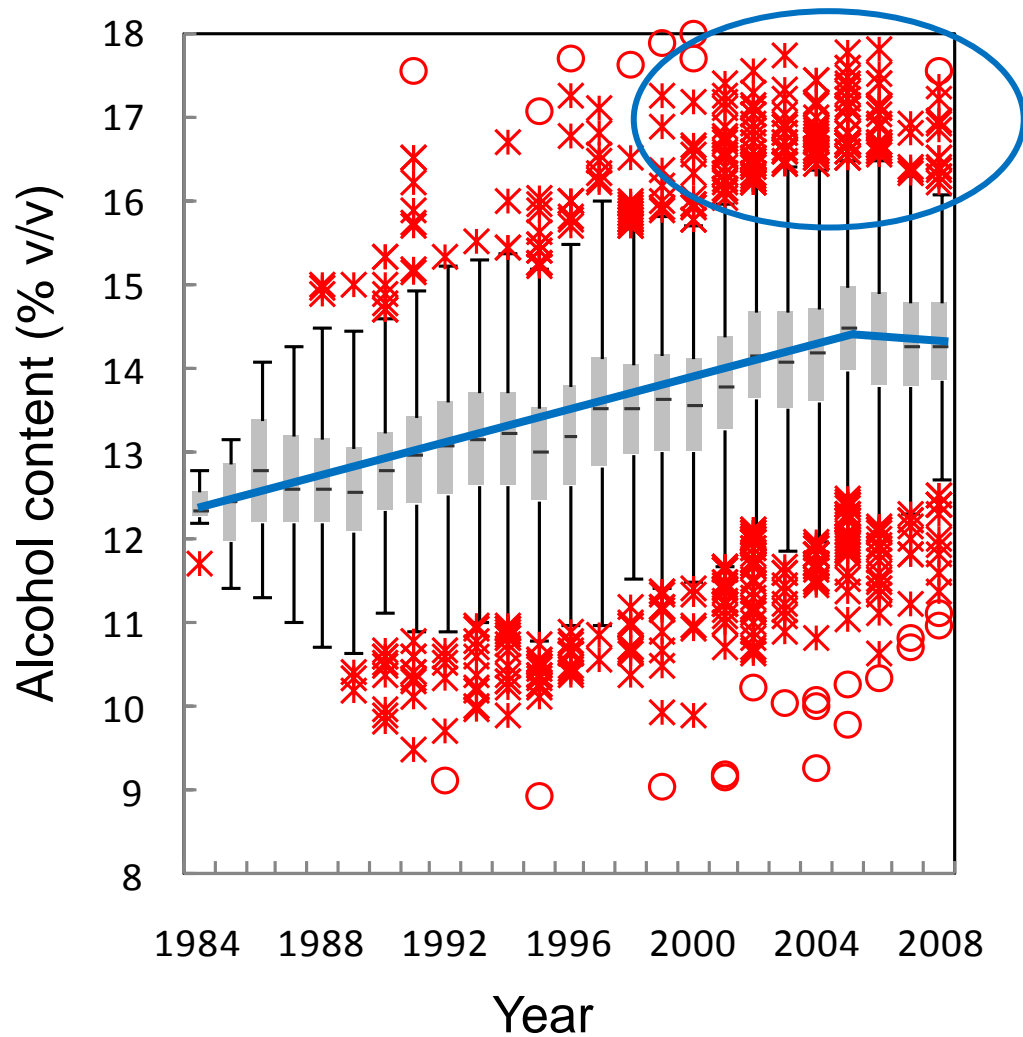
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Schmidt, Anthony Borneman, Paul  
Henschcke, Jens Krömer, Alamgir Khan, Paul  
Chambers & The Australian Wine Yeast  
Systems Biology Consortium



# Alcohol in Australian Wine



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# Why reduce alcohol?



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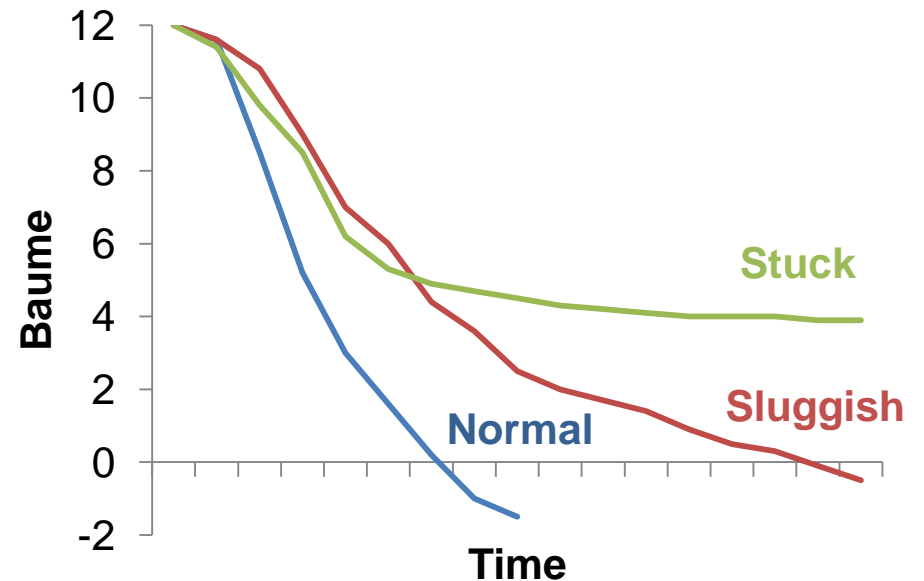


**15% 14% 13% 12%**  
**Ethanol content**



**Wine & society**

**Alcohol abuse**



# How to reduce alcohol?



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**Viticultural  
practices**



**Winemaking  
practices**



**Fermentation  
practices**

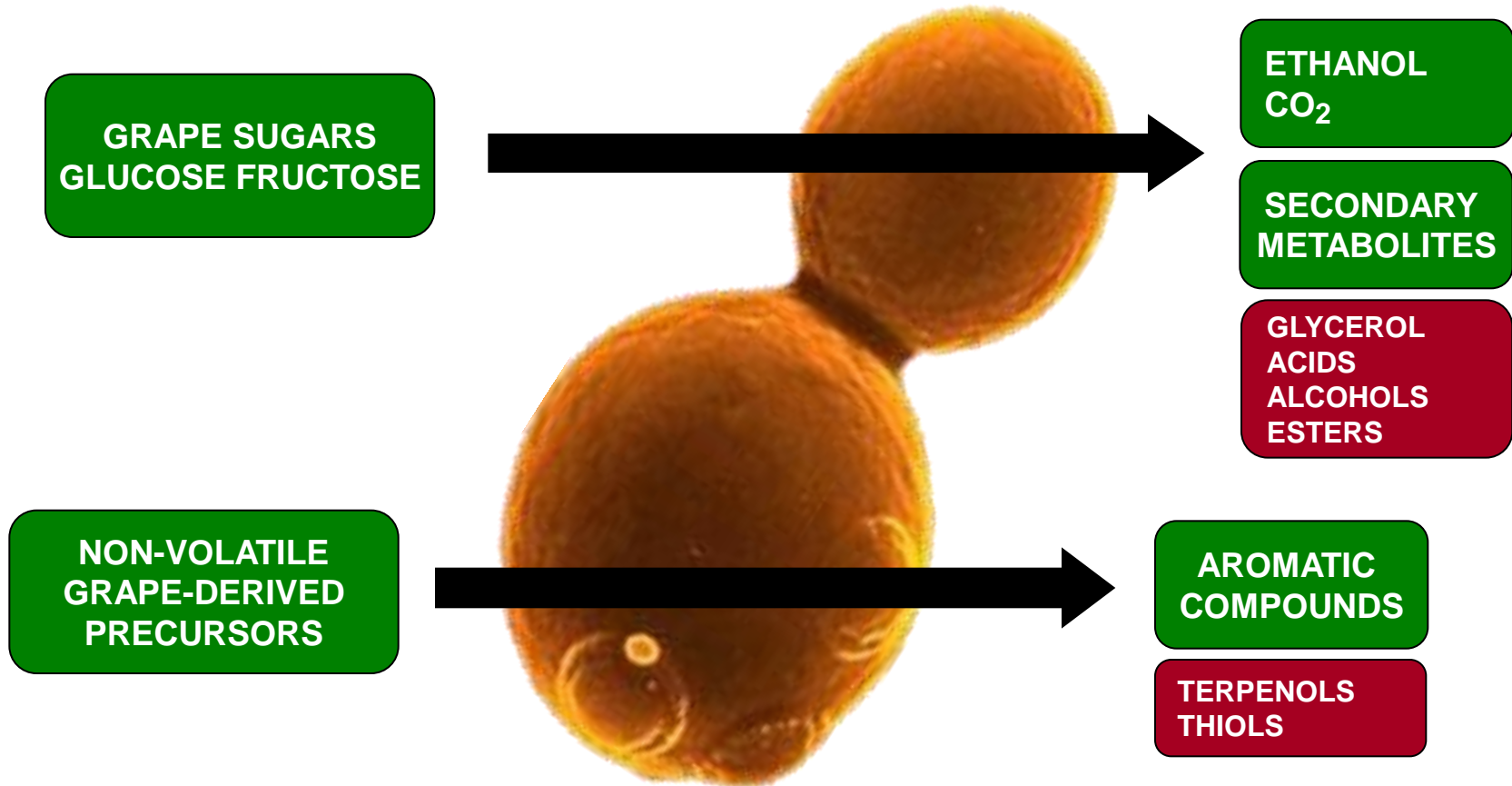


**Post-fermentation  
technologies**

# What is the role of wine yeast?



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# Aim



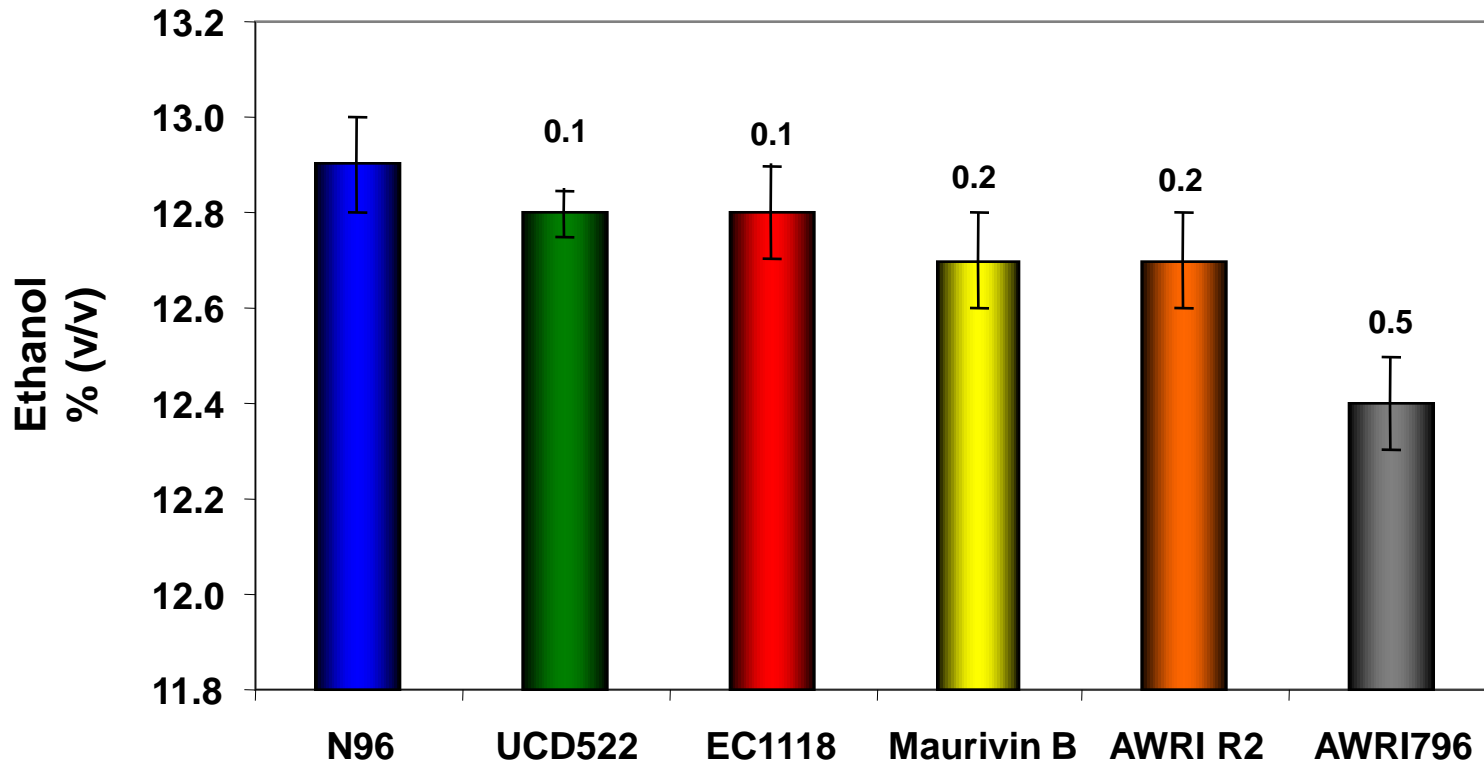
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Produce an industrial wine yeast strain that generates less ethanol than existing strains

# Existing variation between wine yeasts



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# Generating yeasts that make less ethanol



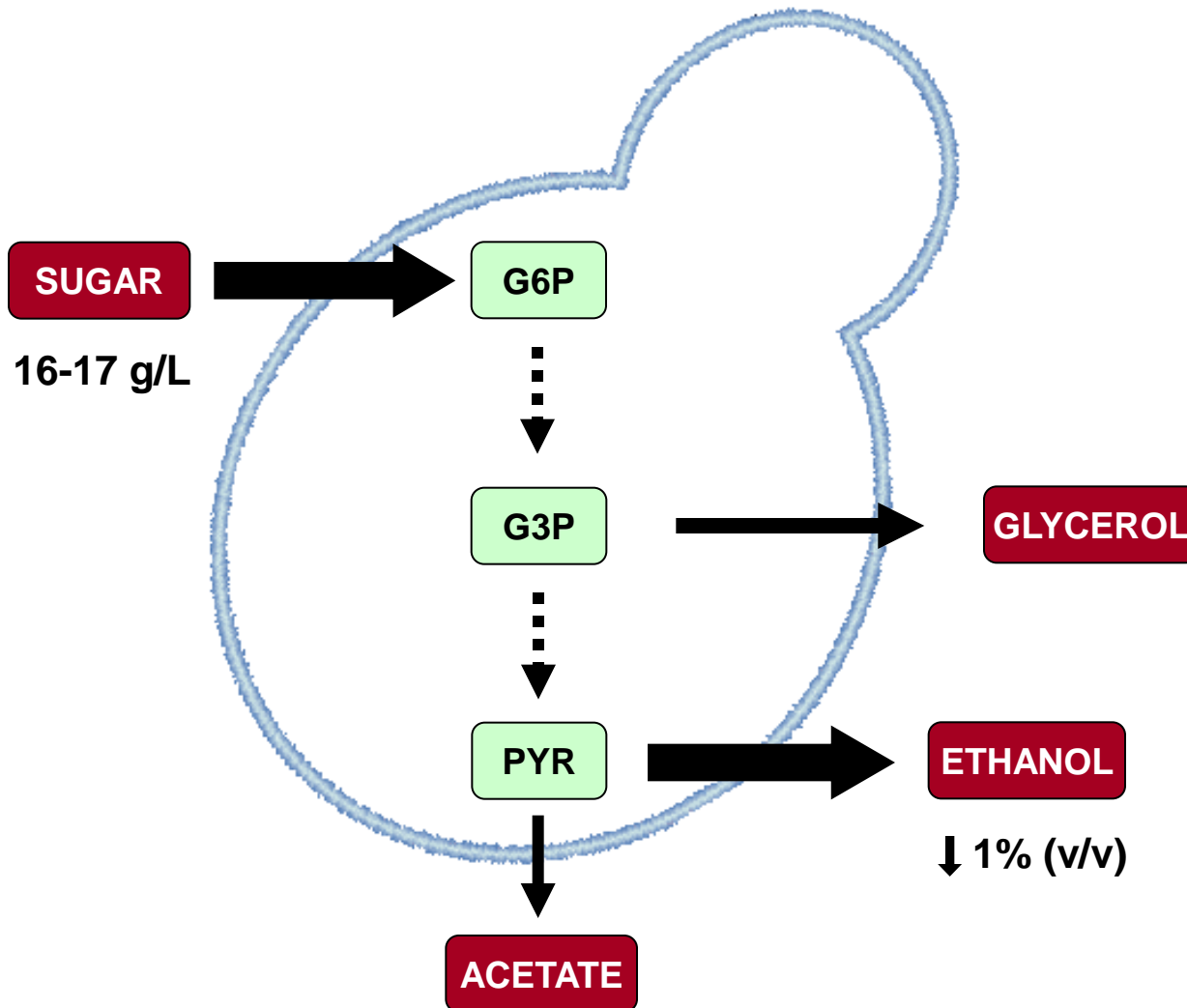
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1. GMO                      Genetic Engineering
2. Non-GMO                Adaptive Evolution

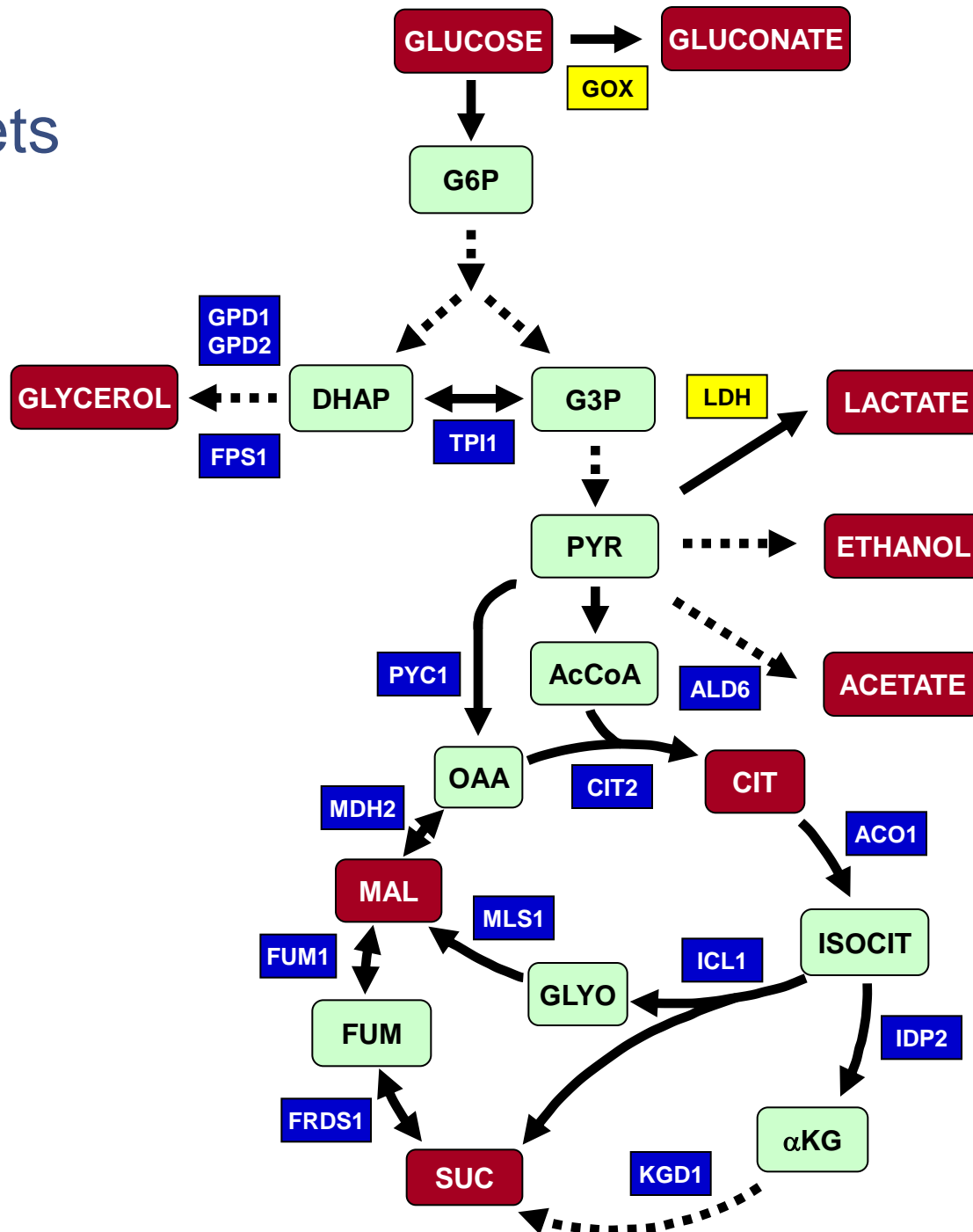
# Where does variation arise?



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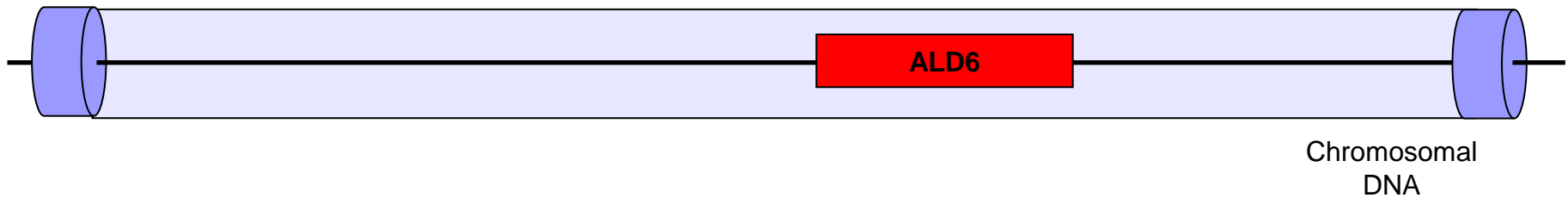
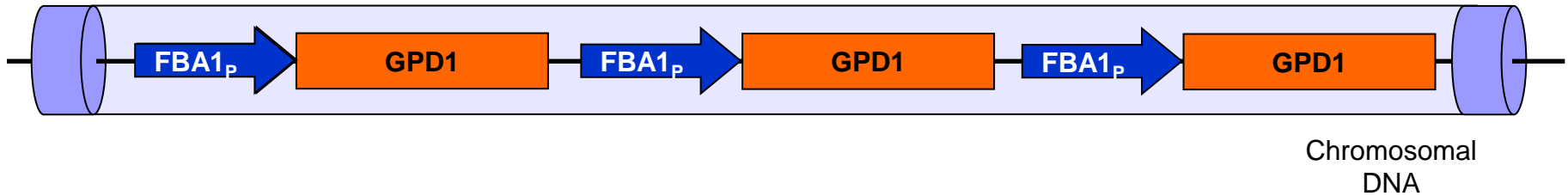
# GM targets



# GM strategy



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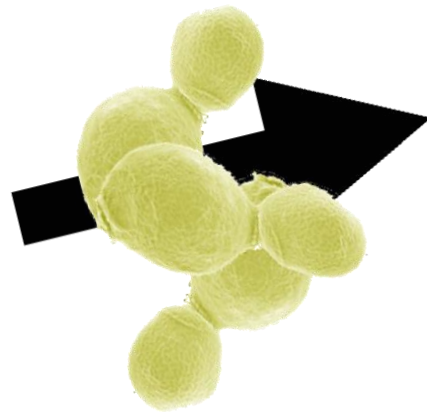
**AWRI2531**  
**FBA1p-GPD1 (two copies)**  
 **$\Delta$ ALD6**

**AWRI2532**  
**FBA1p-GPD1 (three copies)**  
 **$\Delta$ ALD6**

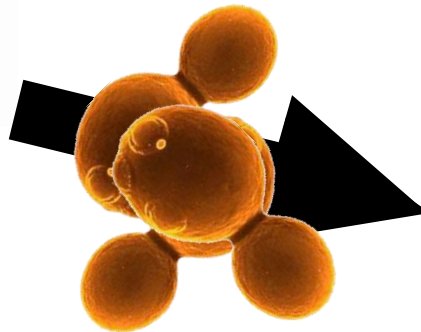
# Best GM low-alcohol yeasts



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**Wine yeast**



**GM yeast**



**AWRI1631**  
**Ethanol**  
**15.7 %(v/v)**

Glycerol  
9 g/L  
Acetic acid  
0.2 g/L



**AWRI2531**  
**Ethanol**  
**13.3 %(v/v)**

Glycerol  
30 g/L  
Acetic acid  
0.2 g/L

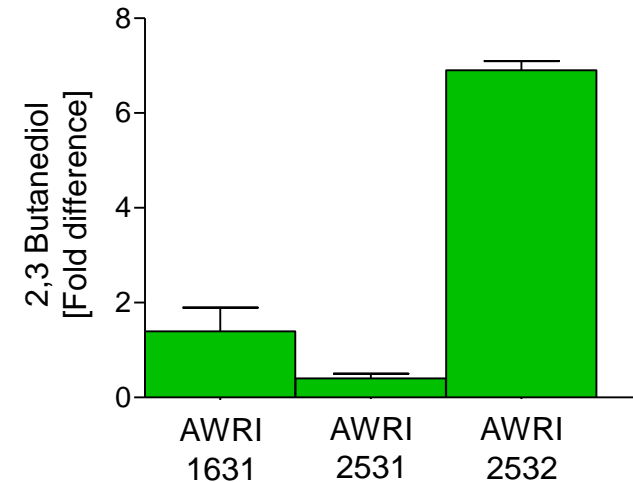
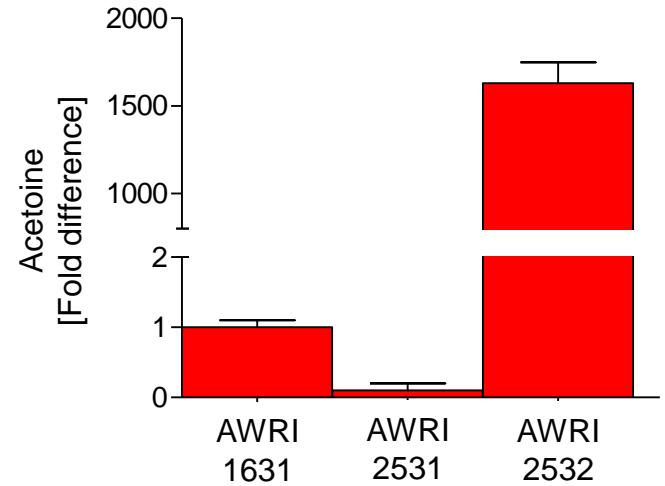
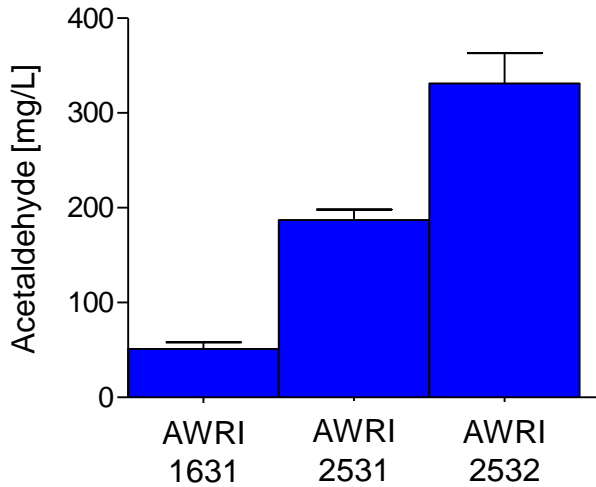
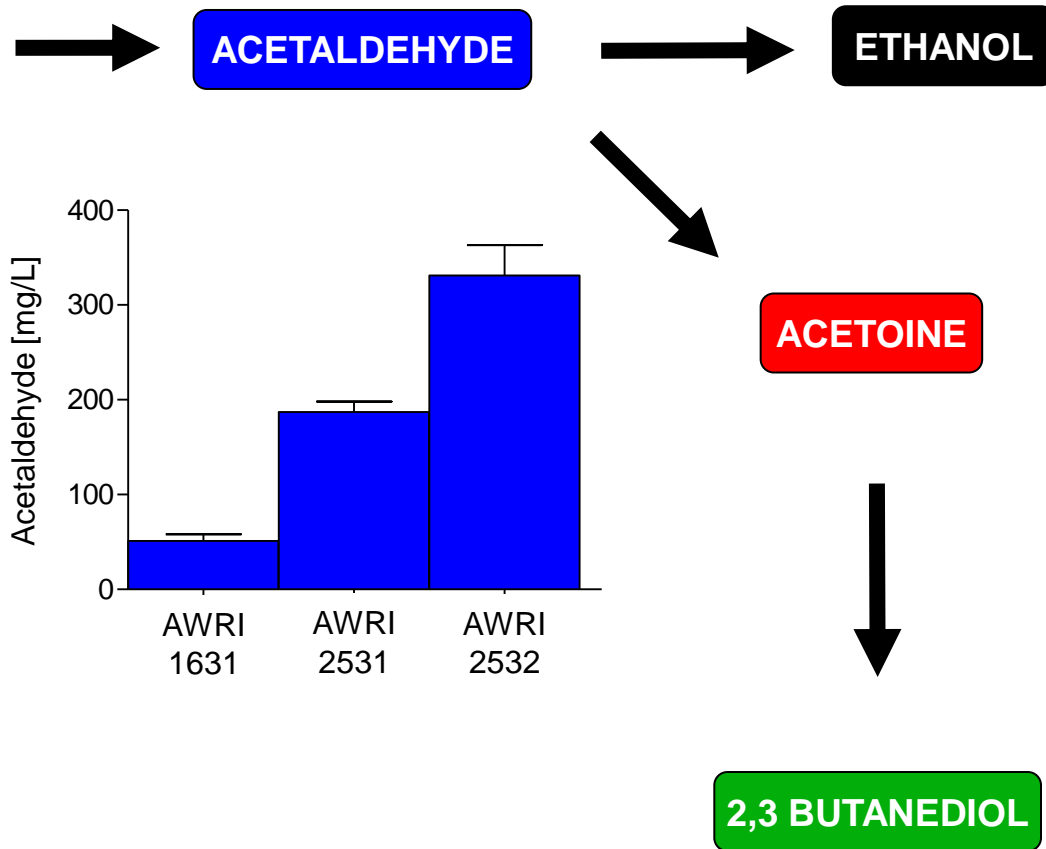
**AWRI2532**  
**Ethanol**  
**12.2 %(v/v)**

Glycerol  
40 g/L  
Acetic acid  
0.5 g/L

# GM yeast metabolism



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# Summary



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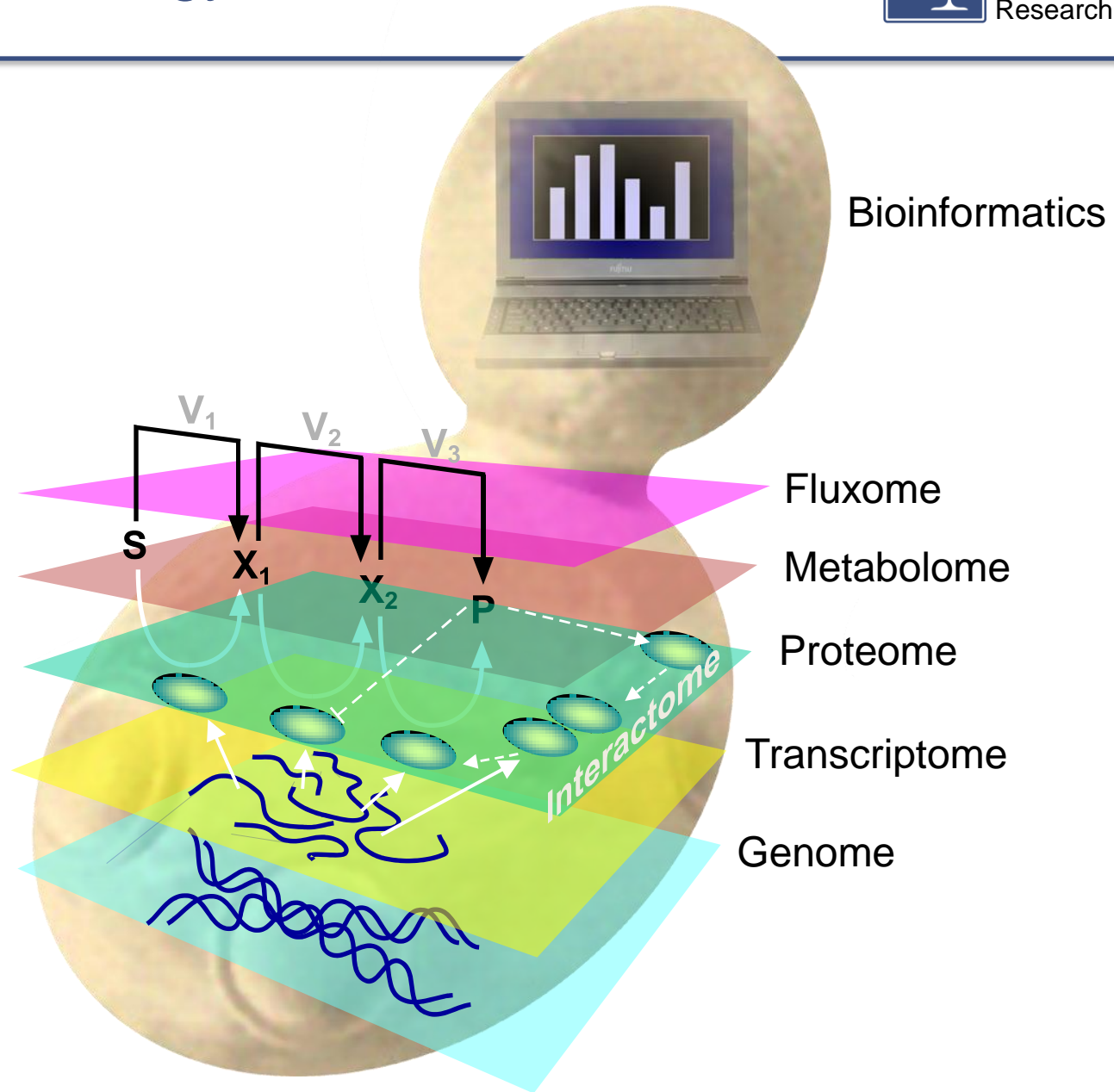
- AWRI2532 produces 3.5% v/v less ethanol, might have quality-sensory issues
- AWRI2531 produces 2.6% v/v less ethanol and shows less quality-sensory issues

# Reductionist vs System approaches



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# Experimental overview



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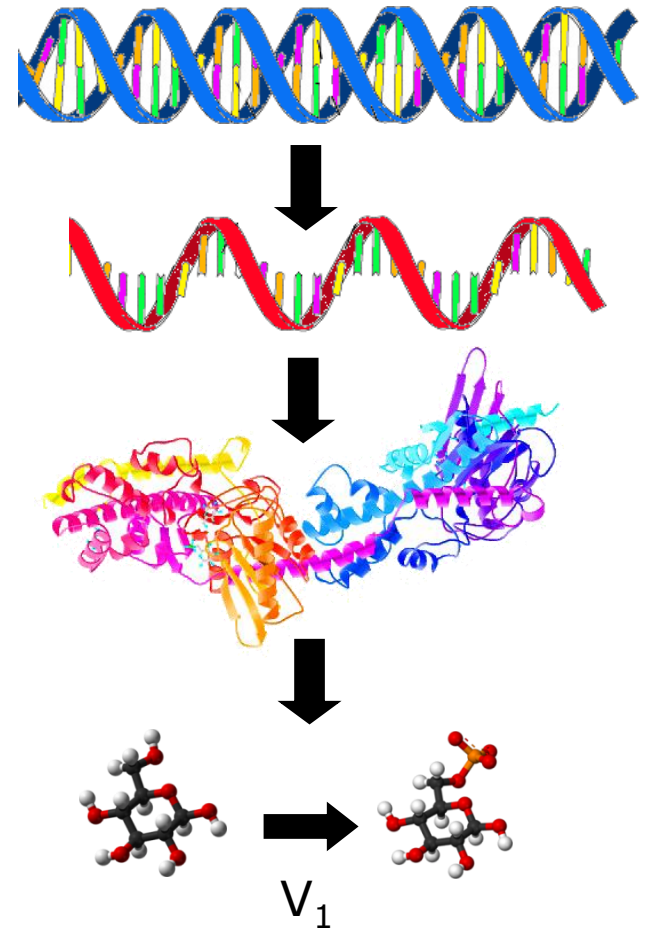
- Chemically defined grape juice
- Conditions resembling white wine fermentation
- Bioreactors: on-line control and auto sampling
- Parental strain AWRI1631 and low ethanol AWRI2531

# Sample analyses



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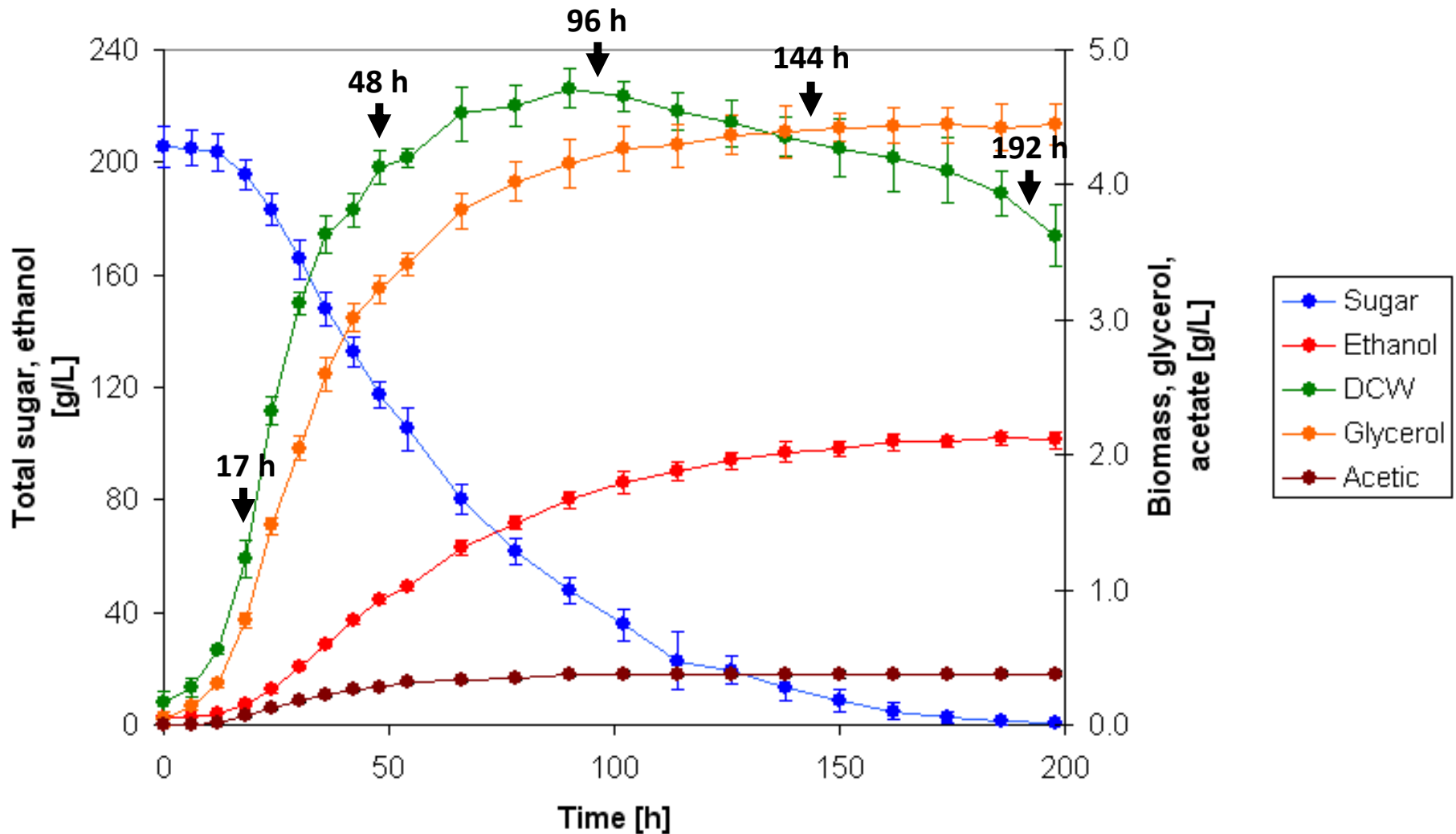
- Genome (complete seq)
- Transcriptome (RNAseq)
- Proteome (iTRAQ and 2Dgels)
- Metabolome (GC-MS, LC-MS)
- Fluxome ( $^{13}\text{C}$  label cultures)



# Model fermentation



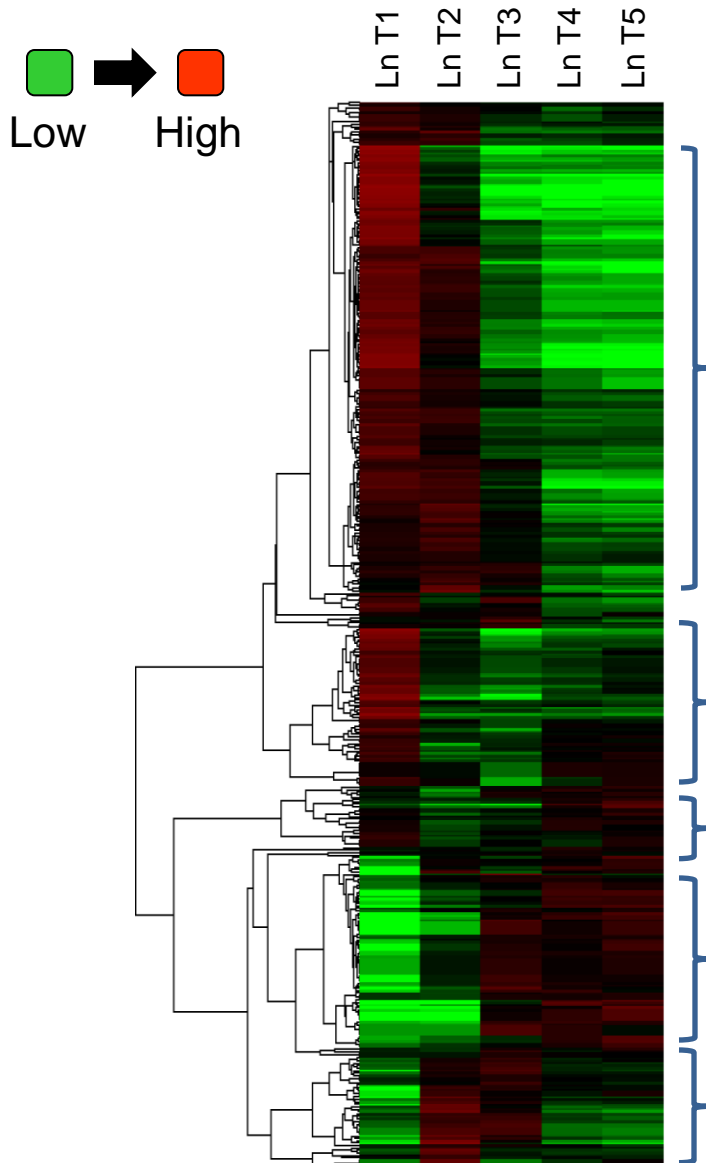
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# Transcriptional profiling



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## Gene ontology - Function

### *Oxidoreductase activity*

Including amino acid biosynthesis, mitochondrial enzymes and ergosterol biosynthesis

### *Ligase and transferase activity*

Including purine biosynthesis, amino acid biosynthesis

### *Transferase activity*

Including amino acid biosynthesis, sulphur transfer

### *Oxidoreductase and membrane transporter*

Thioredoxin and cytochrome c related, ATP synthase subunits

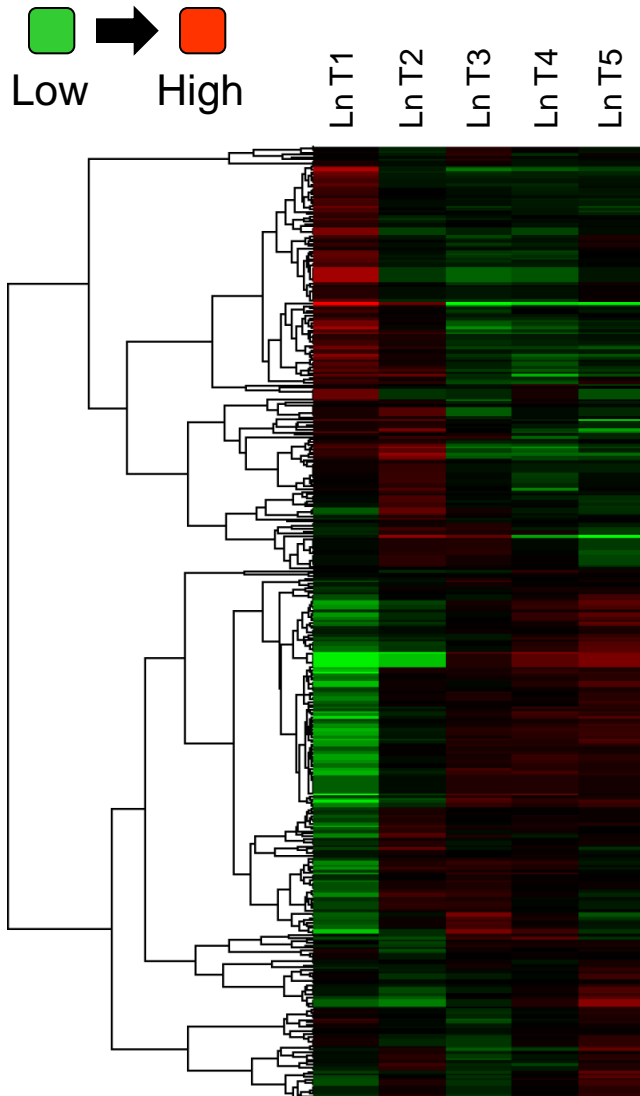
### *Oxidoreductase*

Stress response and vitamin biosynthesis

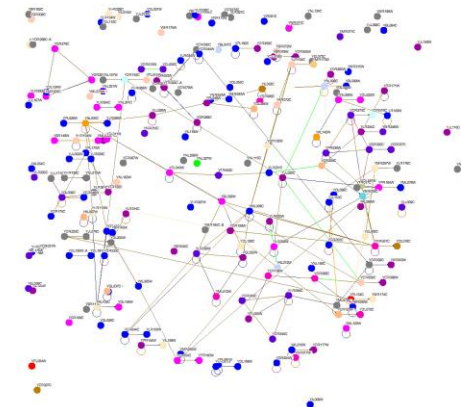
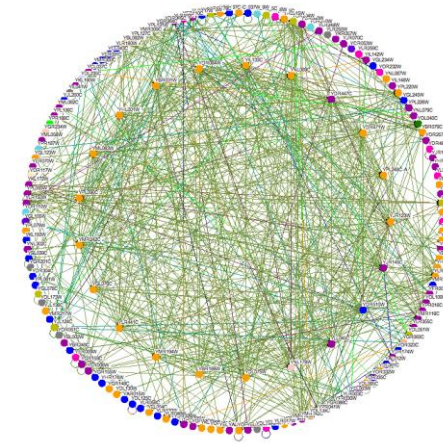
# Protein abundance



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Interactions of proteins with **decreasing** abundance

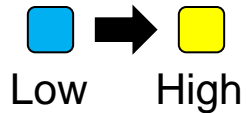


Interactions of proteins with **increasing** abundance

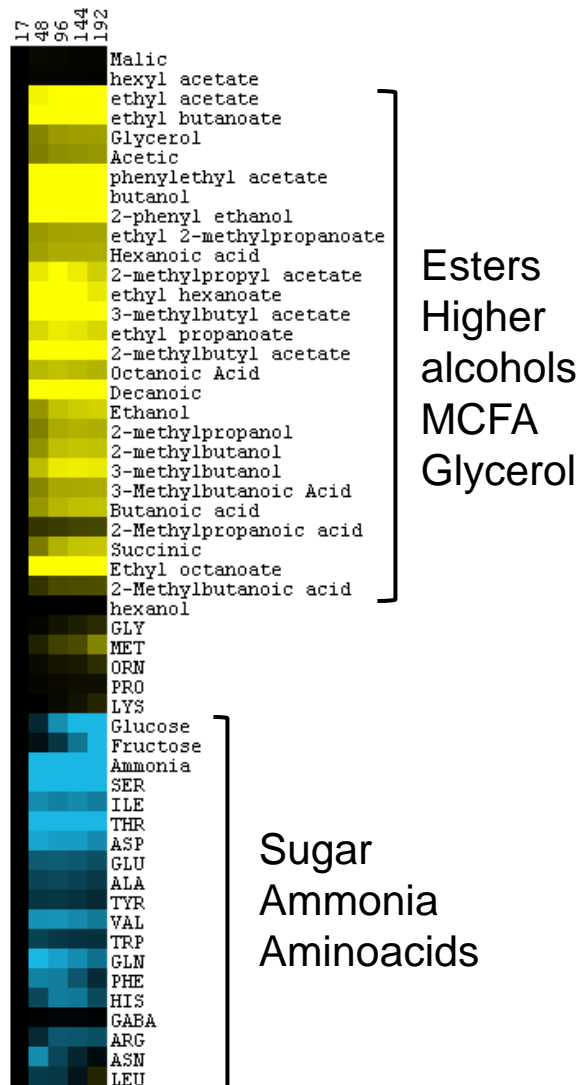
# Metabolome – targeted analysis



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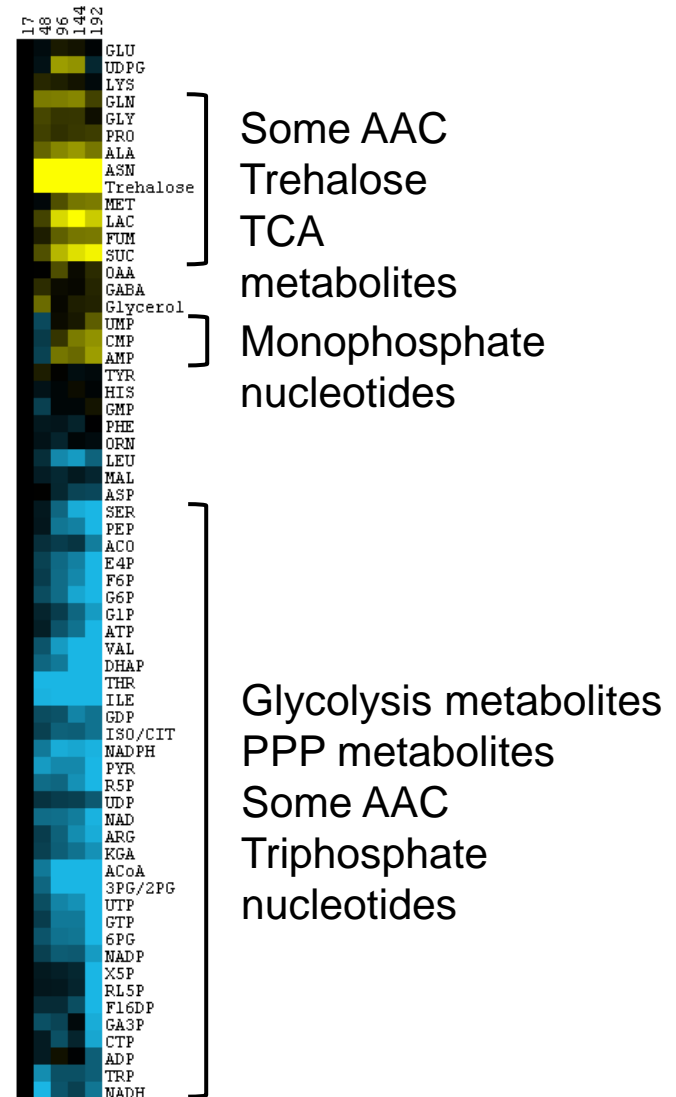
## Exometabolome



Esters  
Higher  
alcohols  
MCFA  
Glycerol

Sugar  
Ammonia  
Aminoacids

## Endometabolome



Some AAC  
Trehalose  
TCA  
metabolites

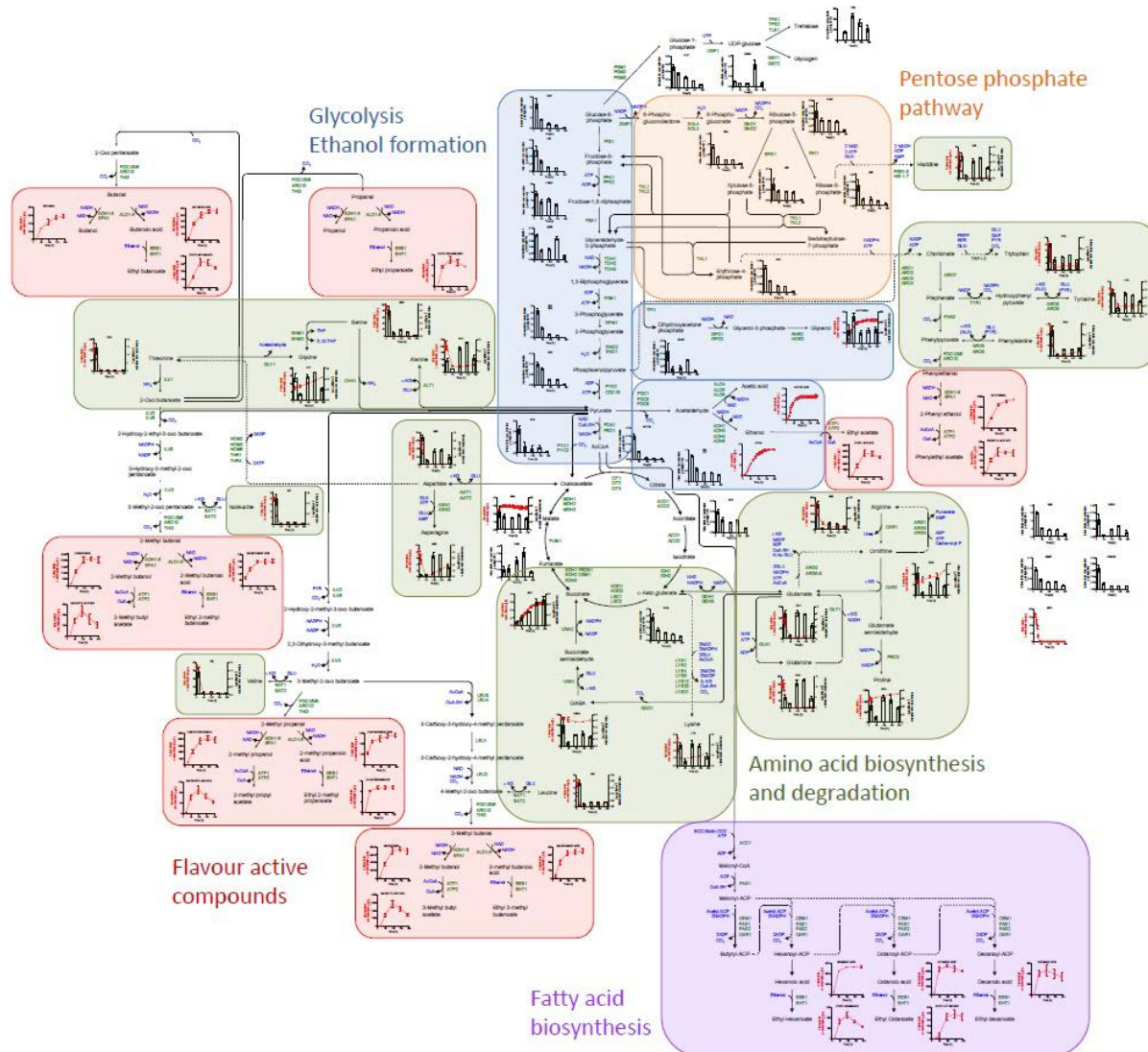
Monophosphate  
nucleotides

Glycolysis metabolites  
PPP metabolites  
Some AAC  
Triphosphate  
nucleotides

# Metabolic network



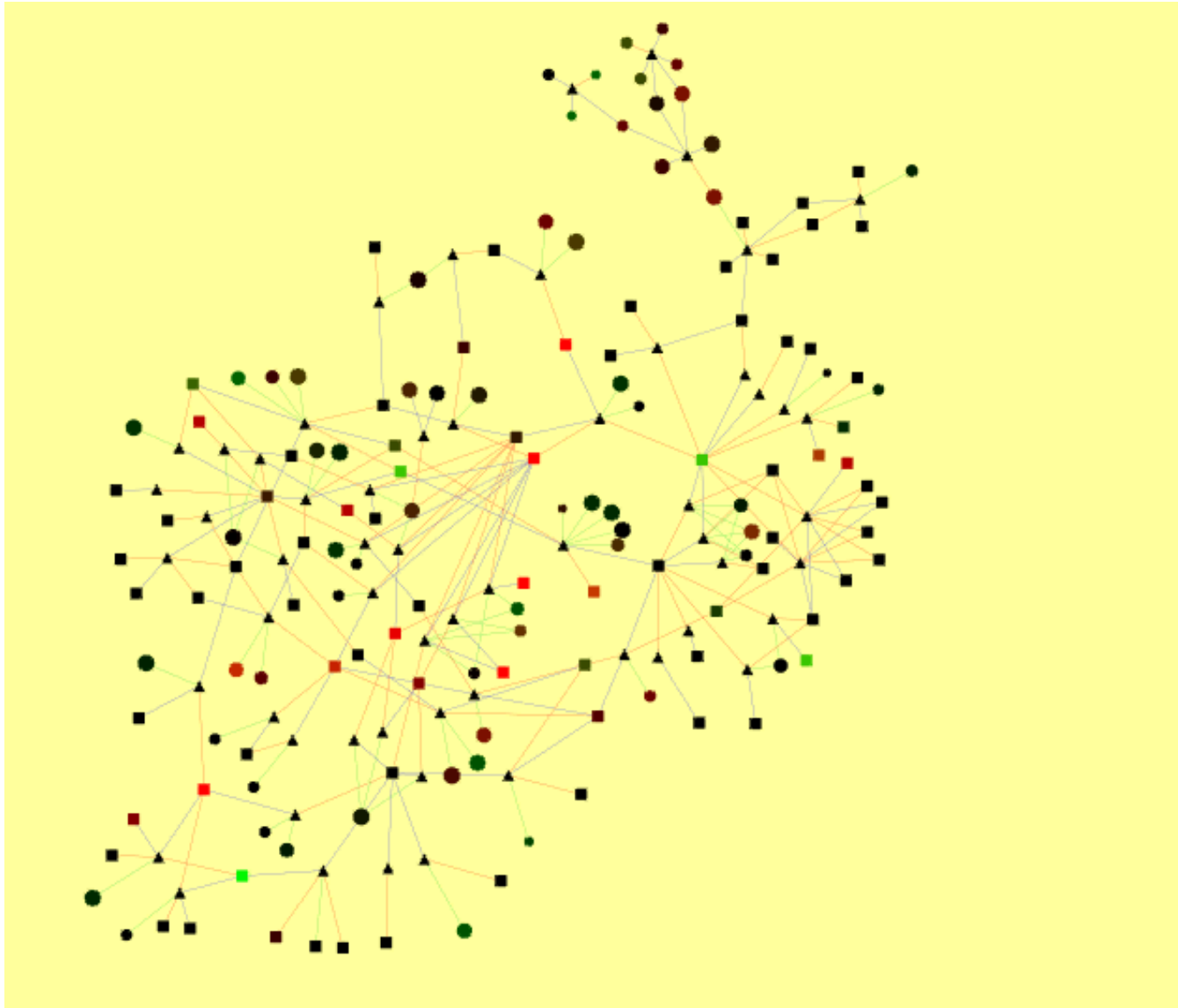
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# Can we rebuild yeast metabolism?



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- Different 'omics platforms technologies provide different, but complementary, information
- Correlations, and differences, between transcriptional, protein and metabolite profiles across the metabolic network
- Transcriptional profiles very dynamic throughout wine fermentation
- Protein profiles more subtle with the majority of variation occurring before 48h

# Acknowledgments



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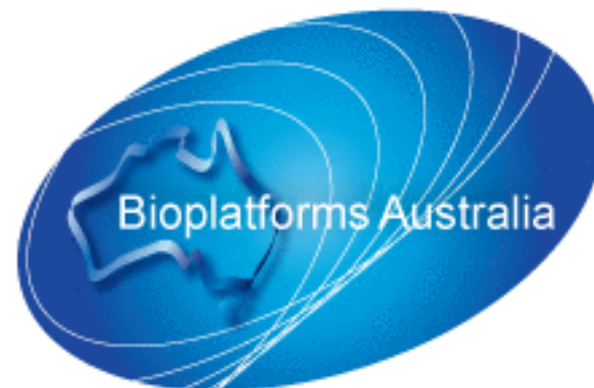
## Ramaciotti center

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