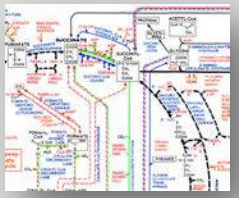
# The metabolic engineering cycle

## Systems Biology

### Modeling & Design

Genome-scale metabolic models
Kinetic models
Thermodynamics
Flux balancing

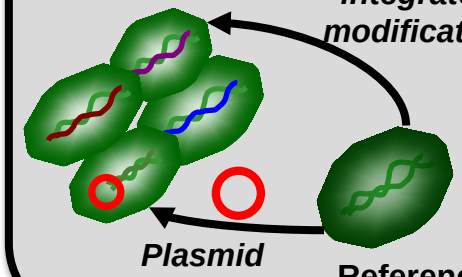


$$\mathbf{Tv} = \mathbf{b}$$

$$\frac{dC^{prod}}{dt} = k_f C_{react} - k_r C_{prod}$$

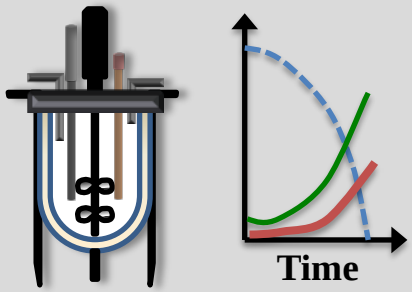
### Strain Construction

*Chromosome integrated modification*



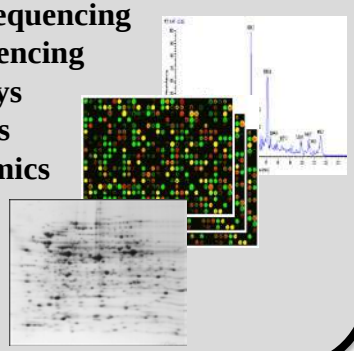

### Fermentation

*Phenotypic characterization*

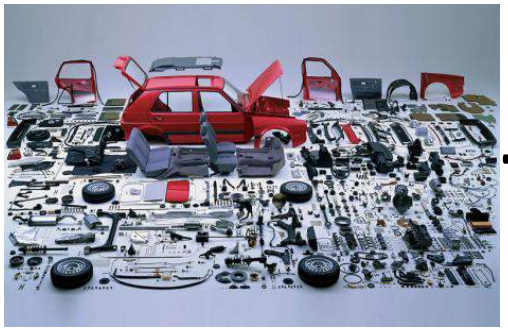
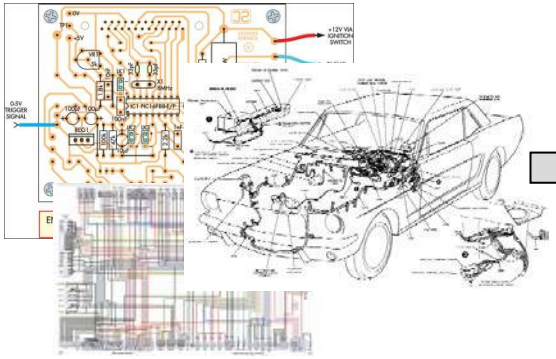
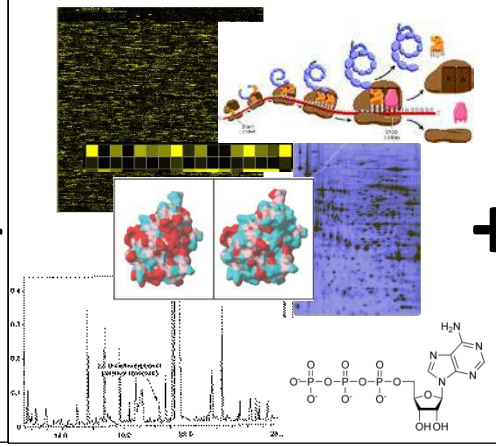
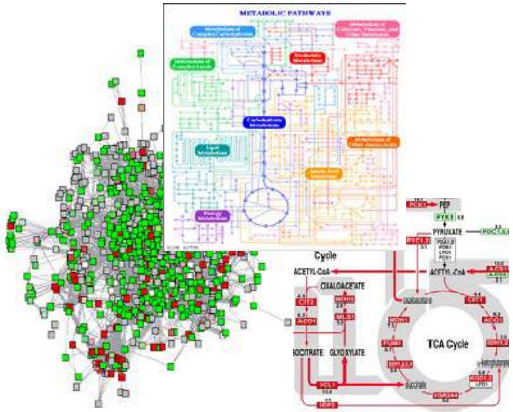



### Omics Analysis

Genome sequencing
RNA sequencing
DNA arrays
Proteomics
Metabolomics
Fluxomics



# Systems Biology

| | Components and their abundance | Interactions and information flow | System |
|---|---|---|---|
| Mechanical system |  + |  |  |
| Biological system |  + |  |  |

FEMS Yeast Research (2008) 8:122-131
Ana Paula Oliveira, PhD thesis (2008) DTU

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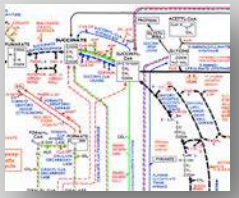

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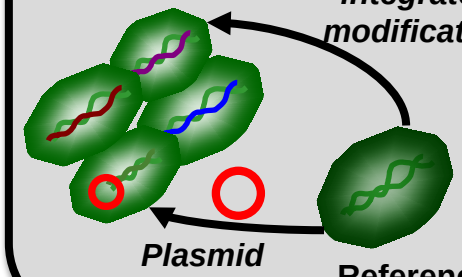
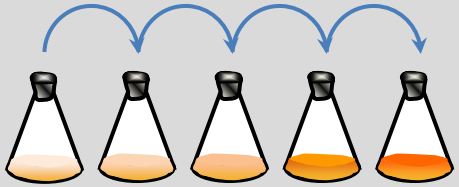


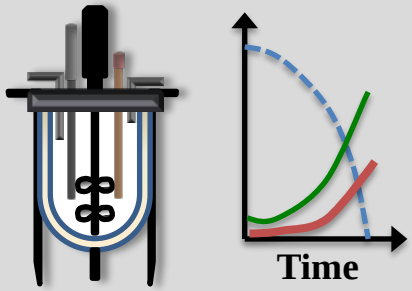

## Directed Evolution



Growth rate selection
Adaption to media
Increasing tolerance

## Fermentation

*Phenotypic characterization*

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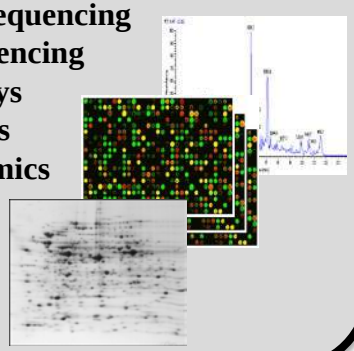
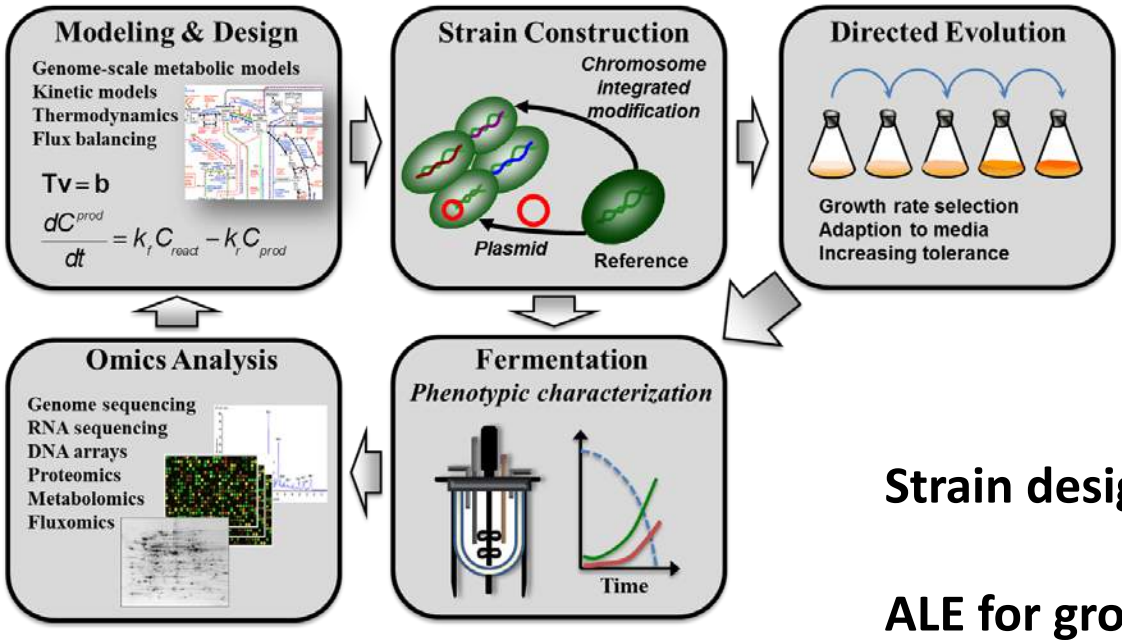


Adaptive Laboratory Evolution (ALE) is today often integrated into metabolic engineering

# Outline

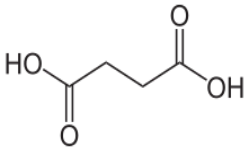



**Strain design for succinic acid production**

**ALE for growth in galactose**

**ALE for temperature tolerance**

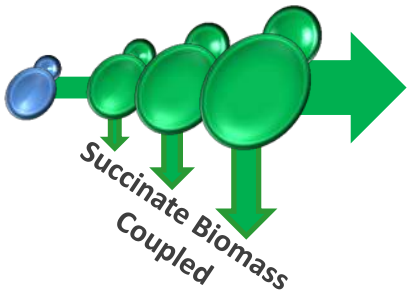
# Succinic acid production

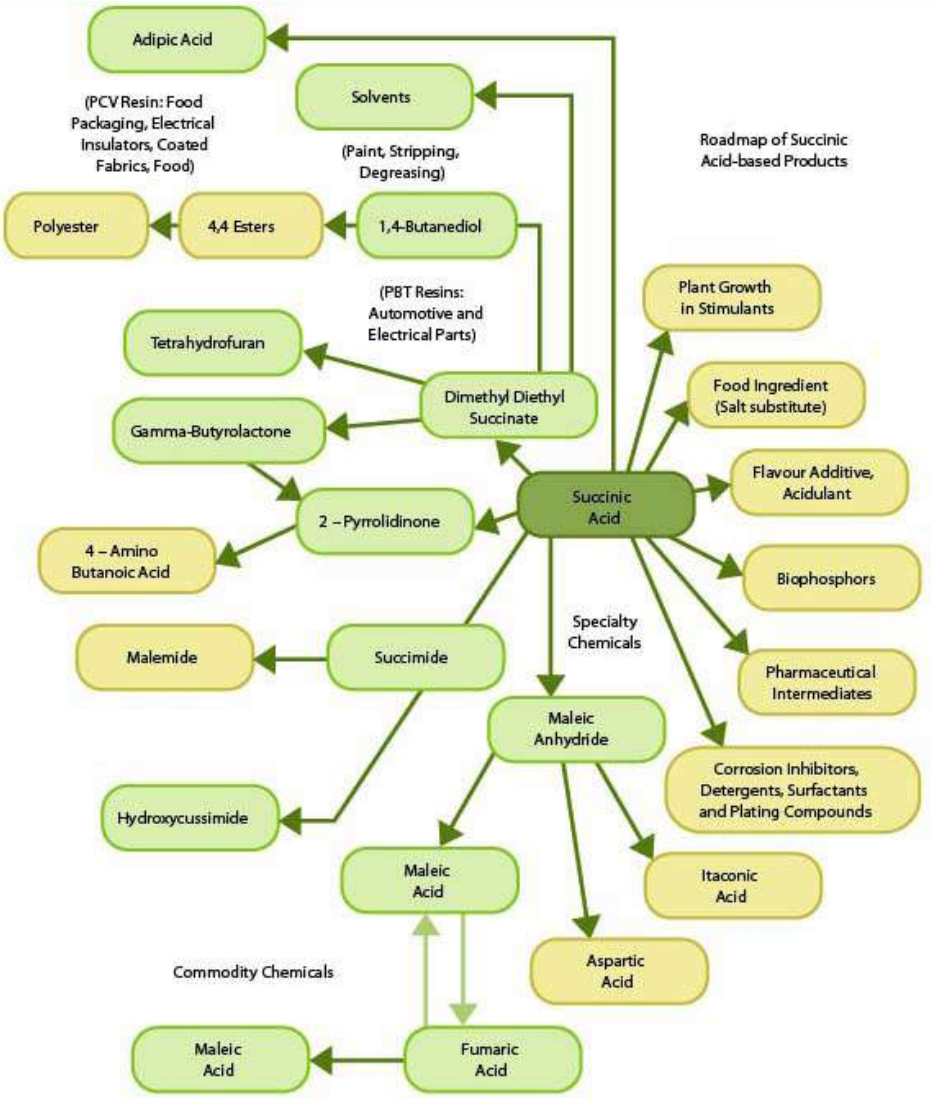



Numerous applications,
direct or as building block

Microbial production platforms:
*Anaerobiospirillium succiniproducens*
*Actinobacillus succinogenes*
*Mannheimia succiniproducens*
*Escherichia coli*

Model guided ME strategy



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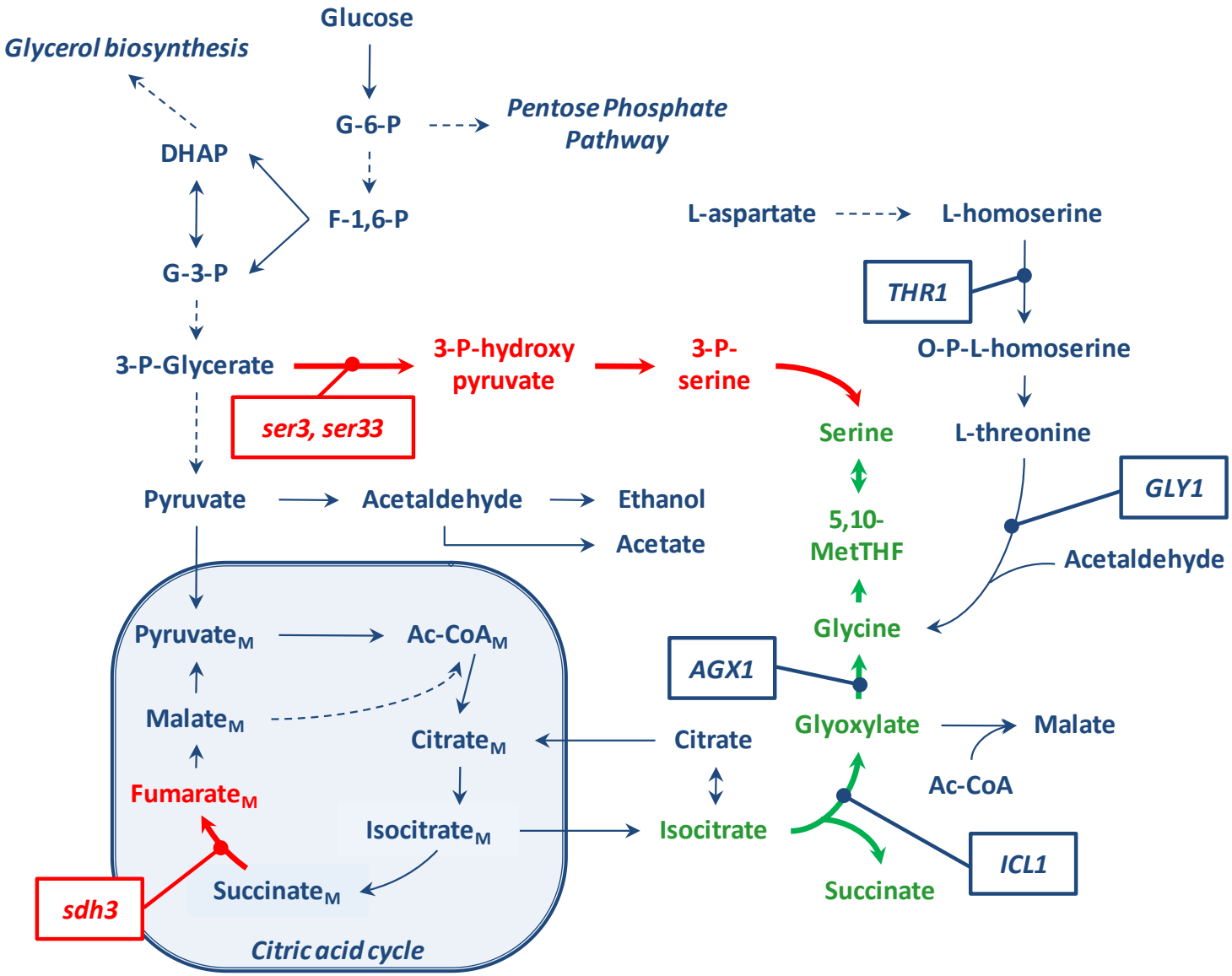


Otero et al., 2013

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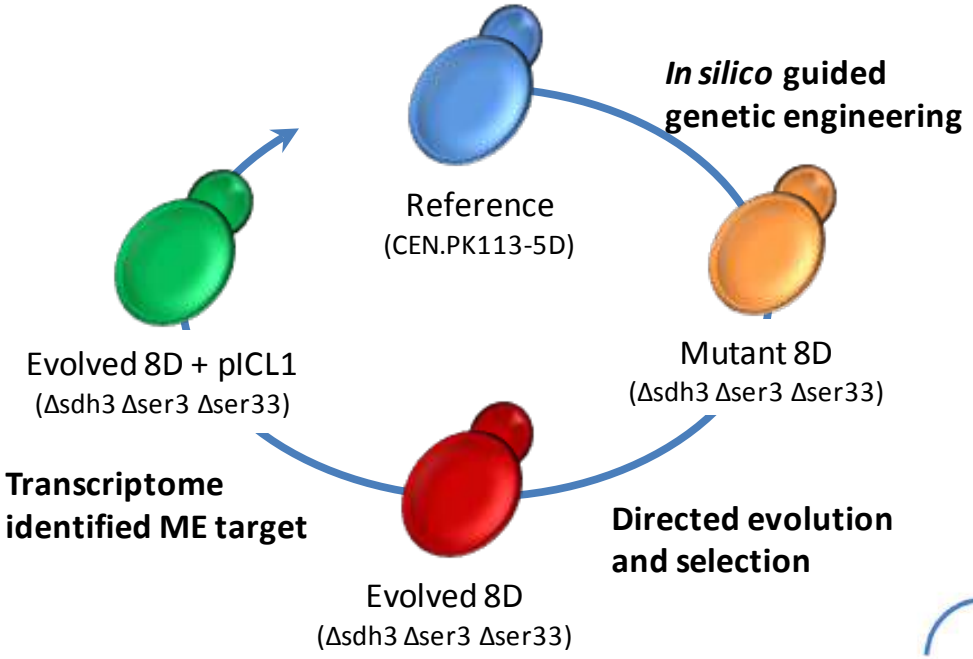


Otero et al., 2013

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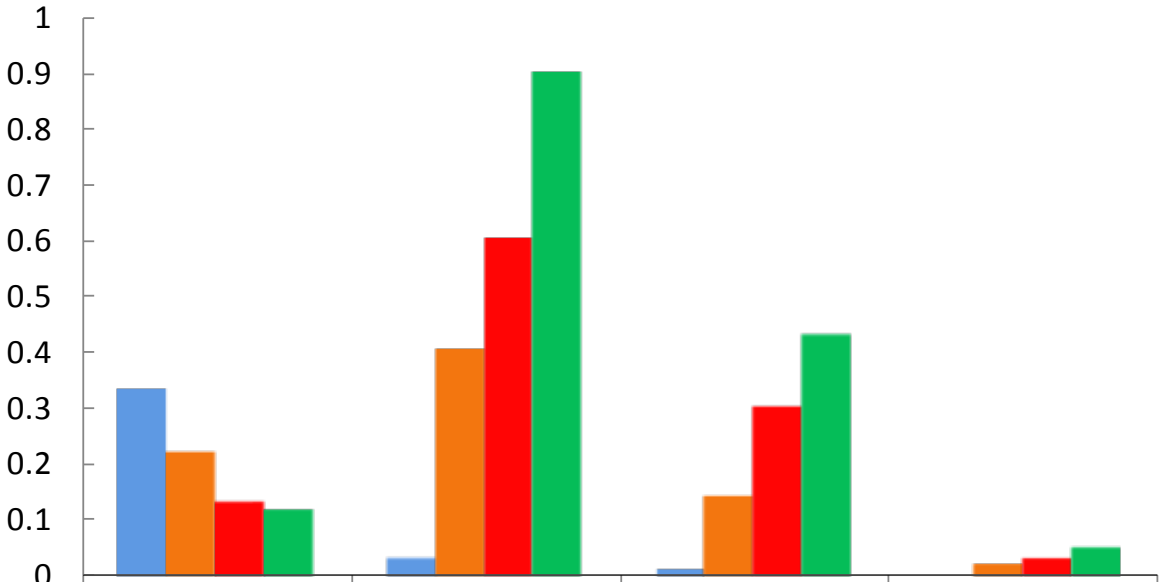
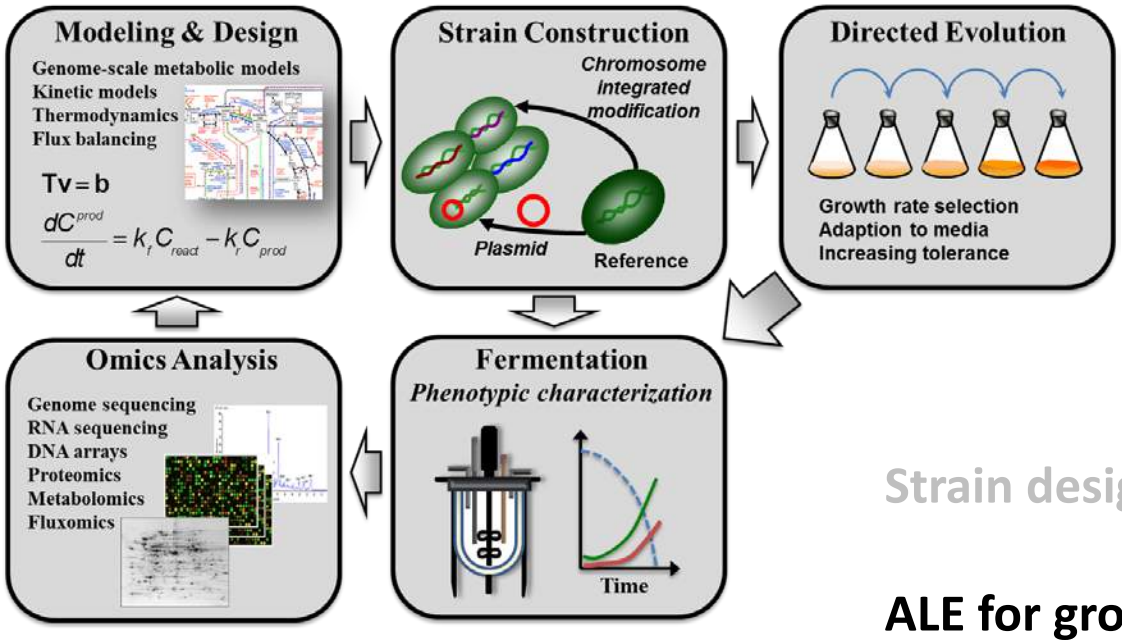



| | Specific Growth Rate (1/h) | Maximum Titer (g/L) | Maximum Yield (g/g-biomass) | Maximum Yield (g/g-glucose) |
|---|---|---|---|---|
| REF | 0.33 | 0.03 | 0.01 | 0.00 |
| 8D | 0.22 | 0.40 | 0.14 | 0.02 |
| 8D Evolved | 0.13 | 0.60 | 0.30 | 0.03 |
| 8D Evolved + pICL1 | 0.12 | 0.90 | 0.43 | 0.05 |

Otero et al., 2013

# Outline




Strain design for succinic acid production

**ALE for growth in galactose**

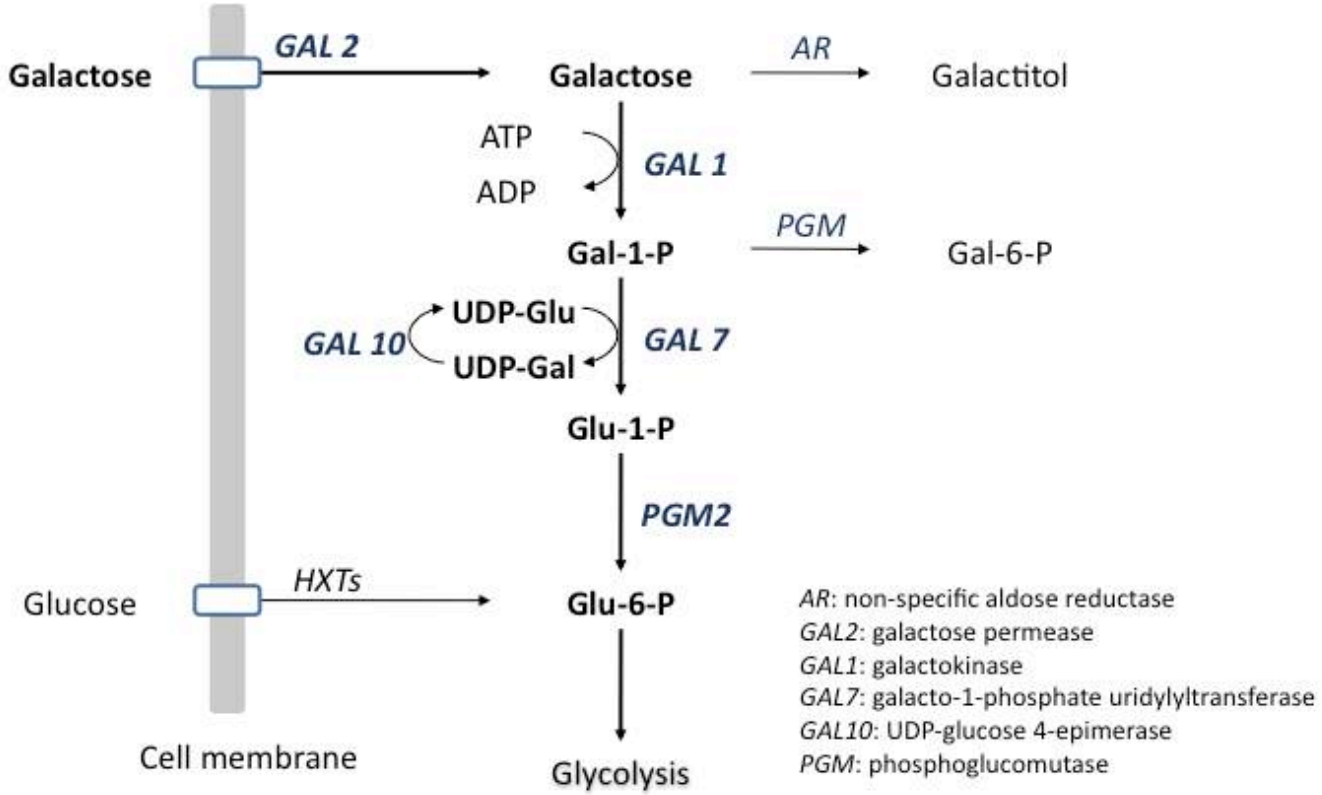
ALE for temperature tolerance

# Galactose utilization

Galactose component of lignocellulosic biomass

Growth rate of *S. cerevisiae* slower on galactose than on glucose

# Galactose utilization

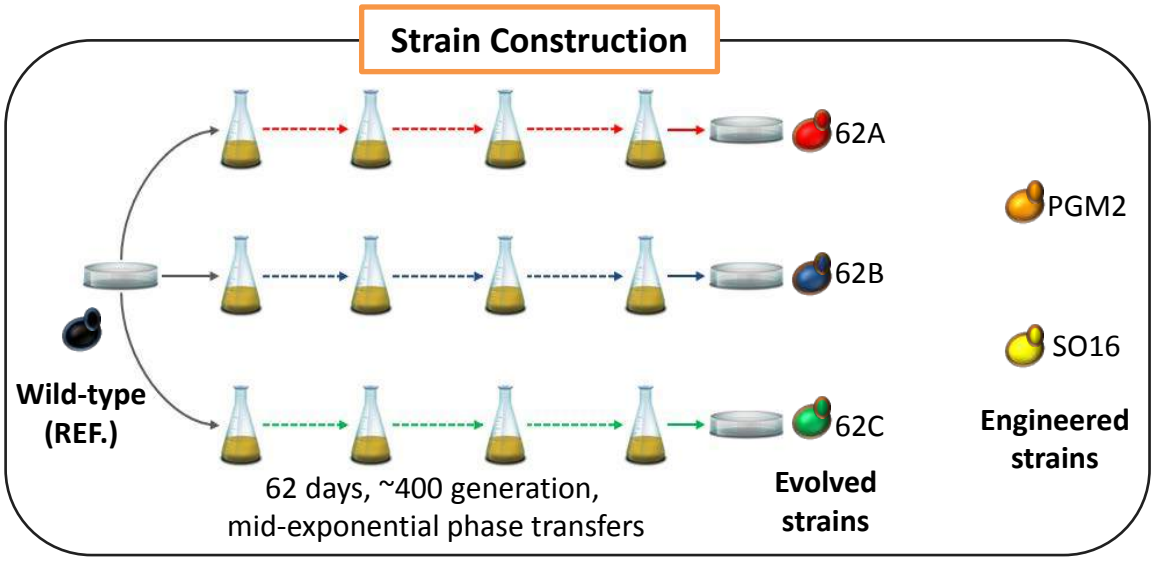



Rational engineering approaches:
Over-expression of *PGM2*; deletion of 3 negative regulators

⇒ Increased galactose uptake rate, but: reduced growth rate

⇒ Evolutionary engineering

# Galactose utilization



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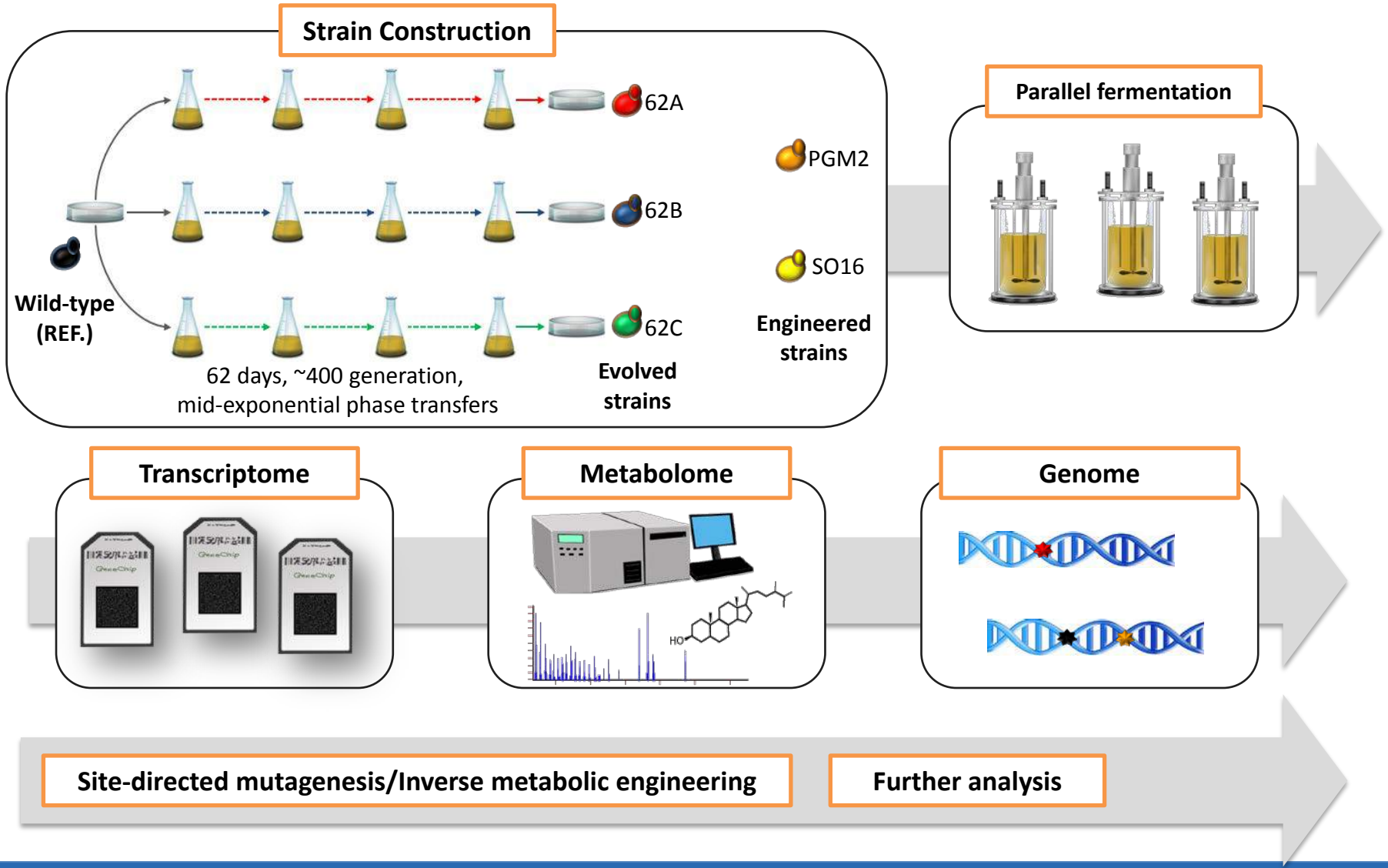


PNAS (2011) 108:12179-12184

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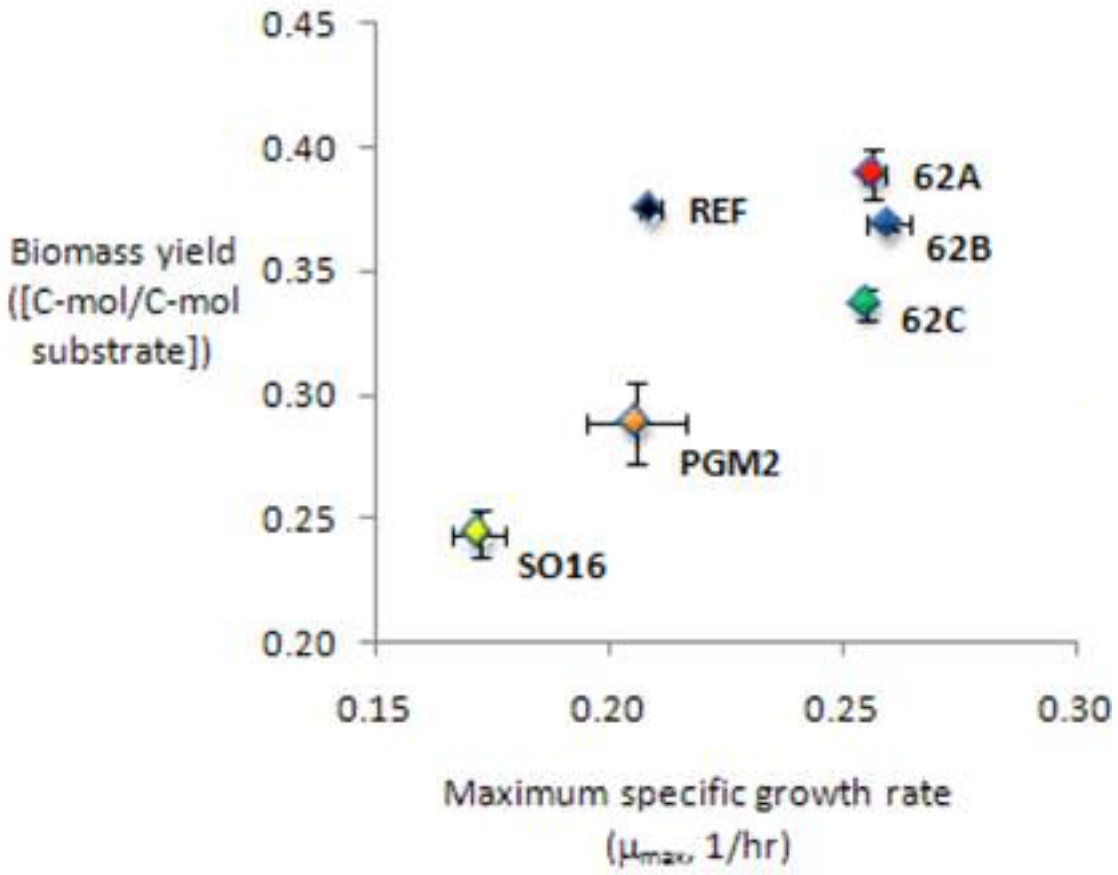


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