Improvement of bioethanol production via relocation of sucrose metabolism in *Saccharomyces cerevisiae*

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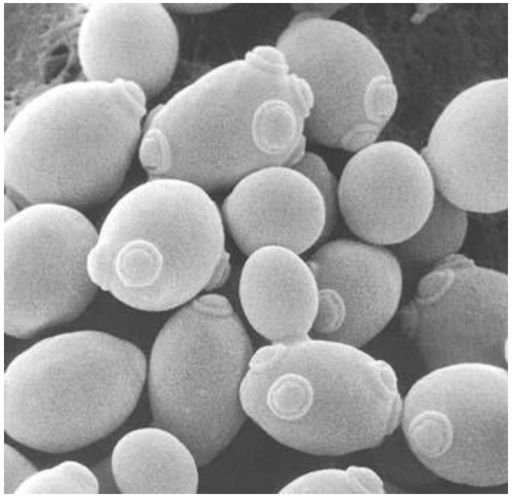
FINANCING

Research project (FAPESP/BIOEN):
Evolutionary engineering of yeast

PROJECT GOAL

To develop improved yeast strains for bioethanol production using metabolic and/or evolutionary engineering



Saccharomyces cerevisiae

First case study

Relocation of sucrose metabolism in *Saccharomyces cerevisiae* to improve ethanol yield

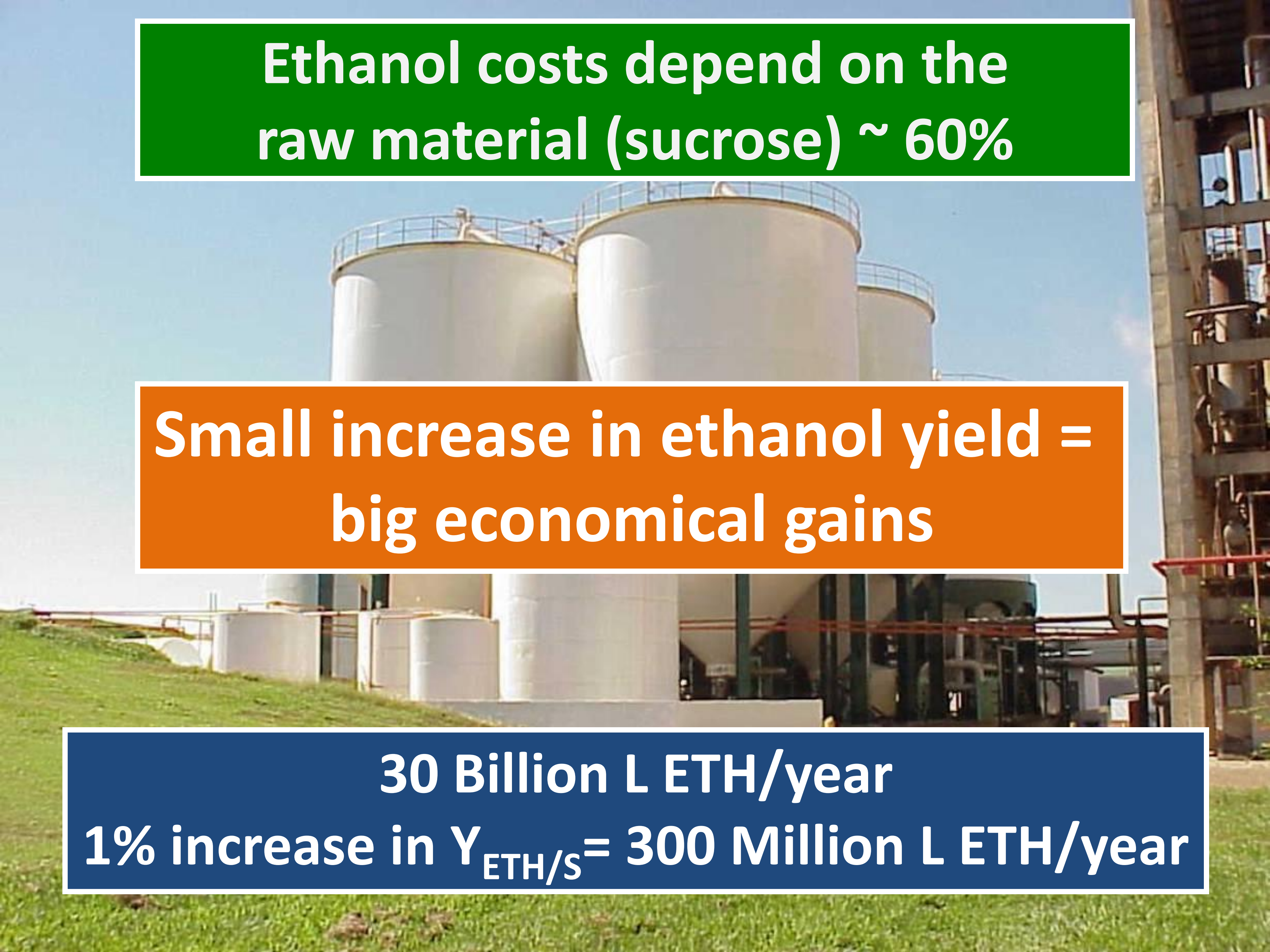
FUEL ETHANOL GLOBAL PRODUCTION = 70 Billion Litres per year

40% - SUCROSE-containing substrates (sugar cane)

Sugar cane - 20% soluble sugars (SUCROSE)

Substrate for fermentation - Sugar cane juice / Molasses

Fermentation step - *Saccharomyces cerevisiae*

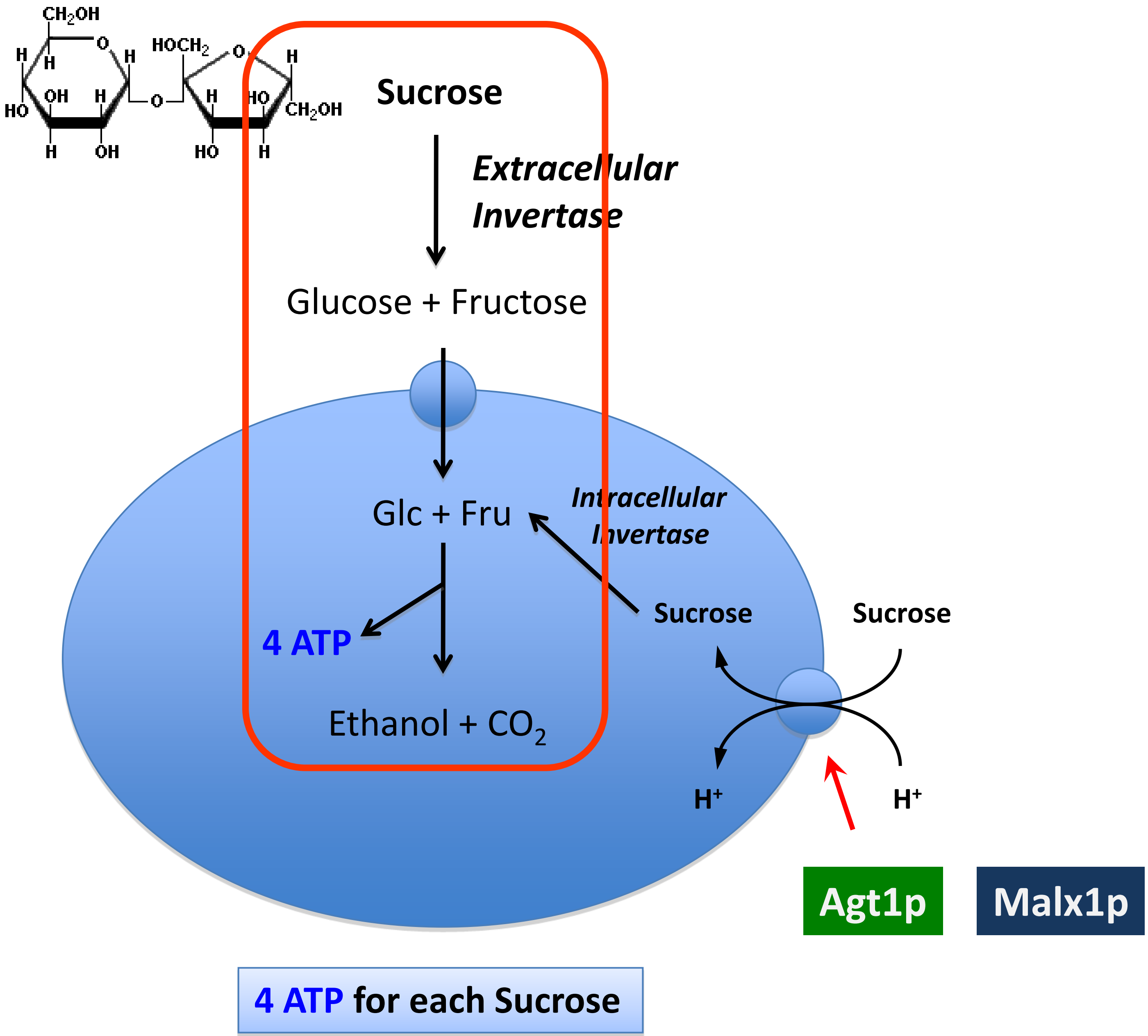


Ethanol costs depend on the raw material (sucrose) ~ 60%

Small increase in ethanol yield = big economical gains

30 Billion L ETH/year

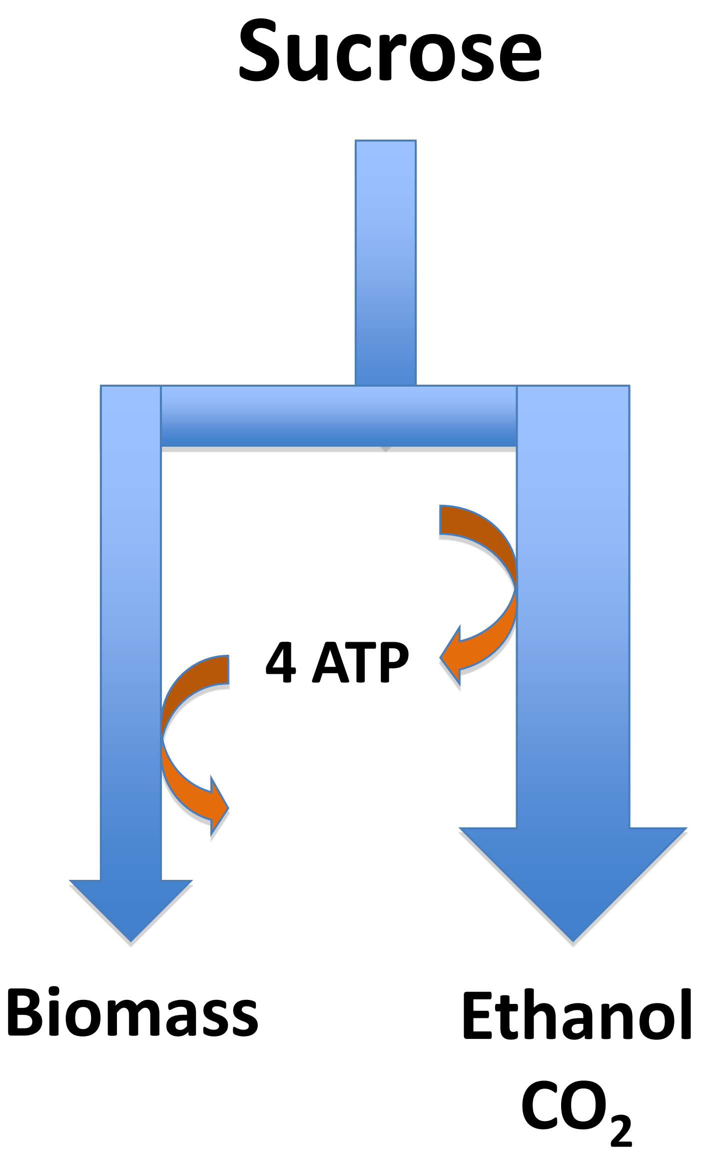
1% increase in $Y_{ETH/S}$ = 300 Million L ETH/year



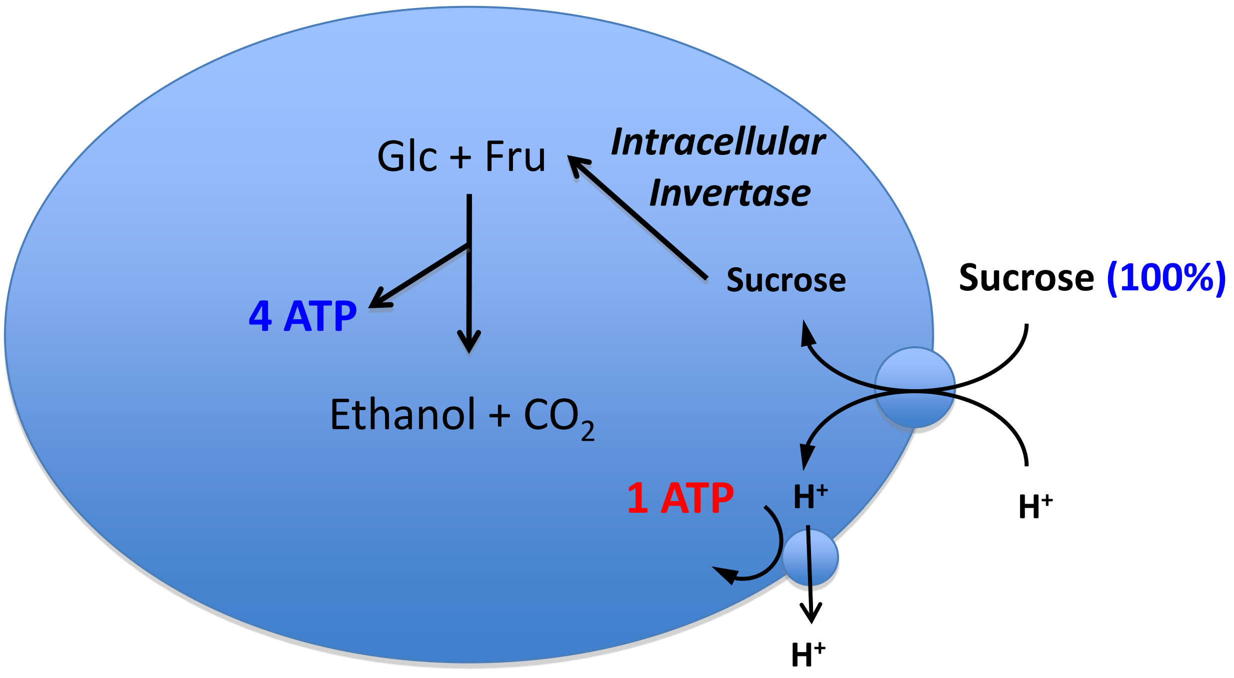
Santos et al. Arch Biochem Biophys 216 (1982)

Stambuk et al. J Biosci Bioeng 89: 212 (2000)

Extracellular invertase

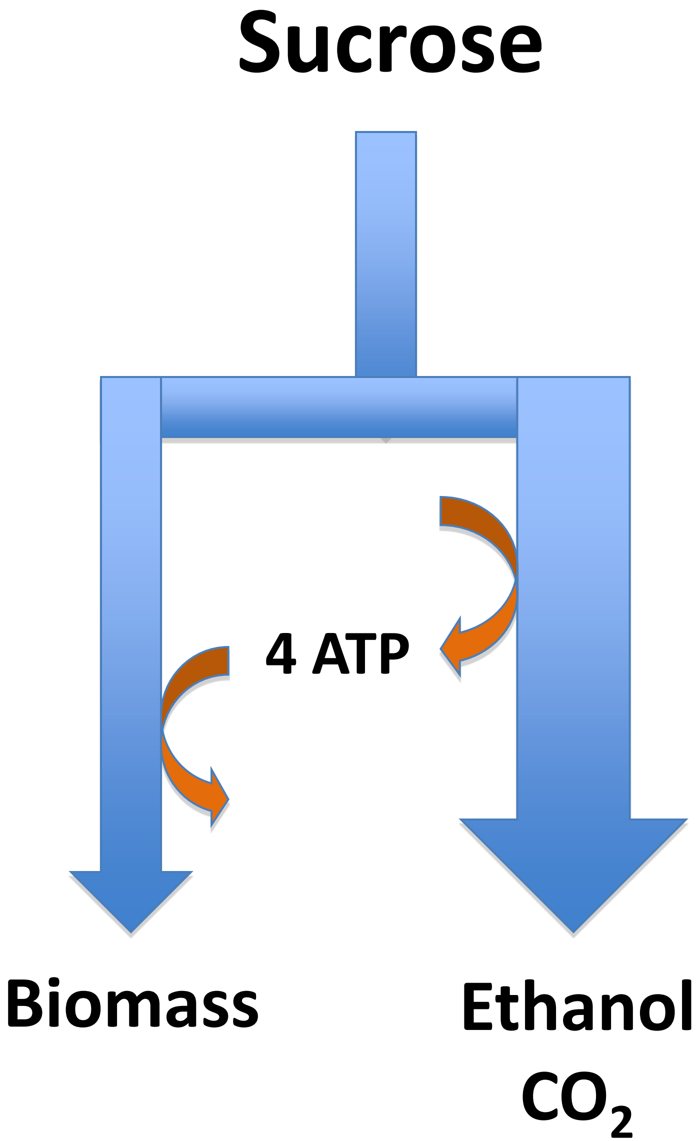


And if…

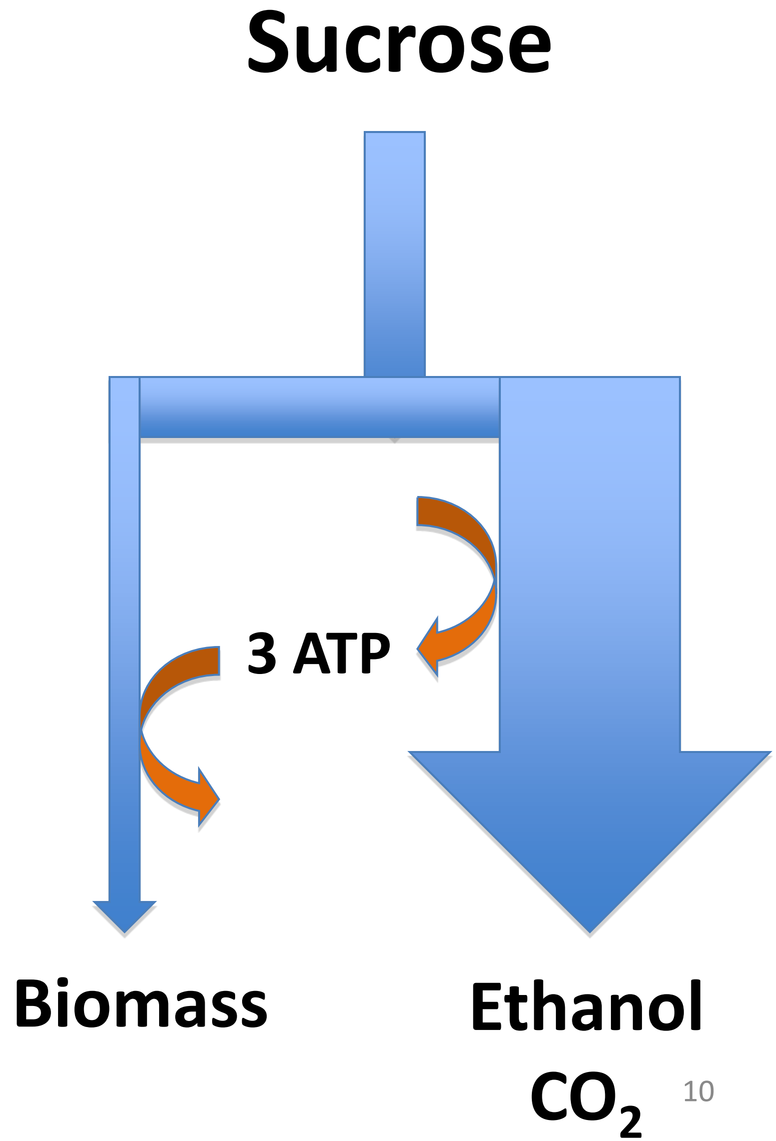


4 ATP - 1 ATP = 3 ATP for each Sucrose

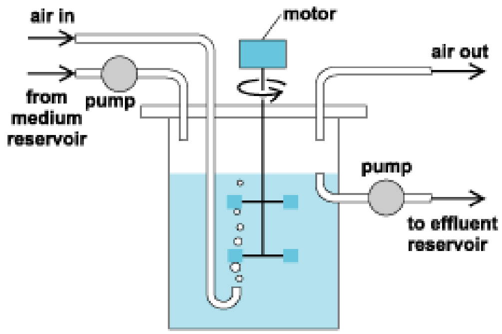
Extracellular invertase



Intracellular invertase



Predicted yields



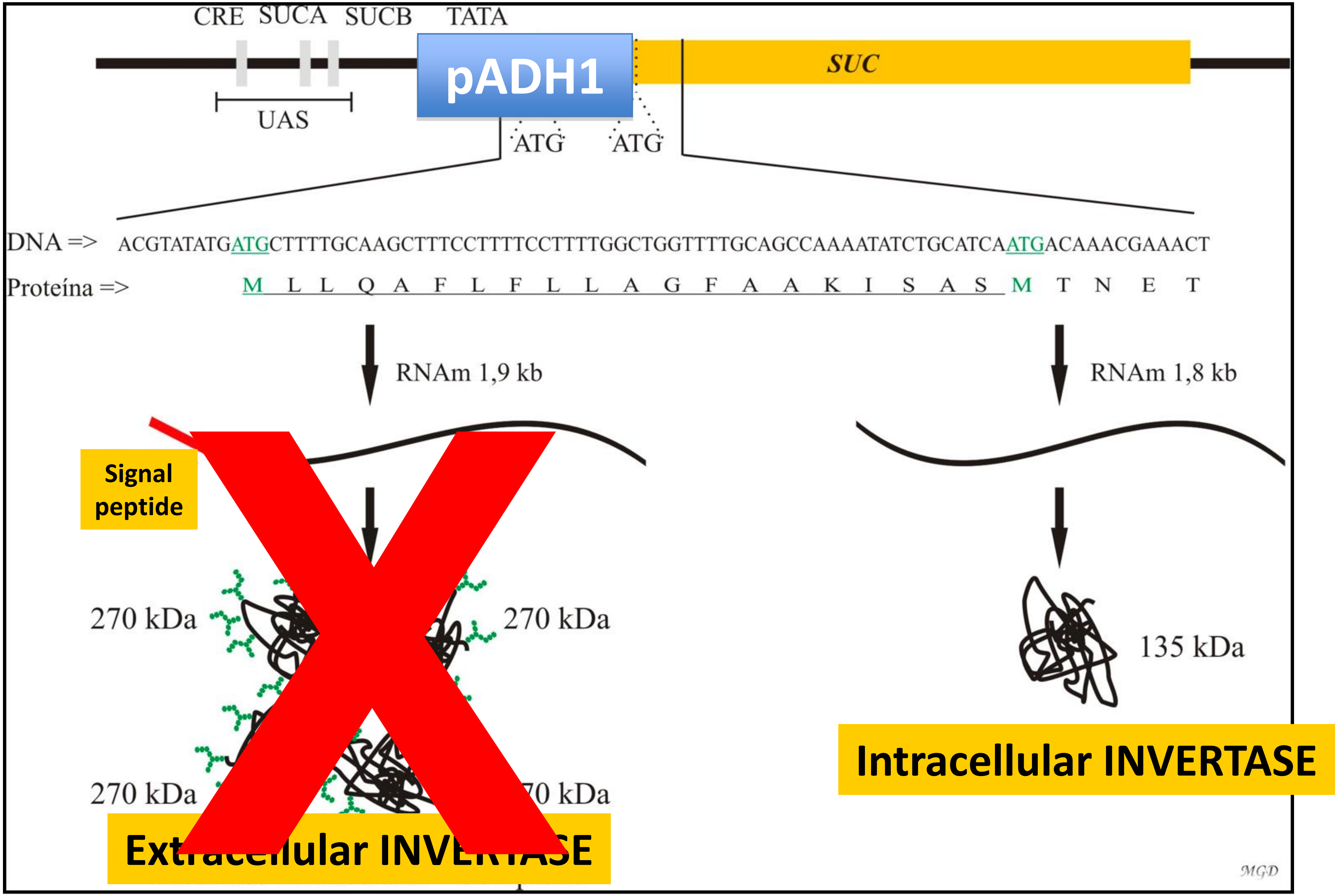
(Verduyn et al., 1991; Weusthuis et al., 1993)

Table: Predicted yields in anaerobic, SUCROSE-limited chemostat cultures of *S. cerevisiae* at ***D*=0.10** h^{-1}, at pH 5 in synthetic media.

Yield	**Mode of sucrose consumption**		**Increase or decrease**
	Extracellular Invertase	**Intracellular Invertase**	
$Y_{X/S}$ (g DW/g hex eq.)	0.103	0.077	- 25 %
$Y_{eth/S}$ (g /g hex eq.)	0.39	0.42	+ 8 %

Stambuk et al. **INPI PI 0901254-0. (2009)**

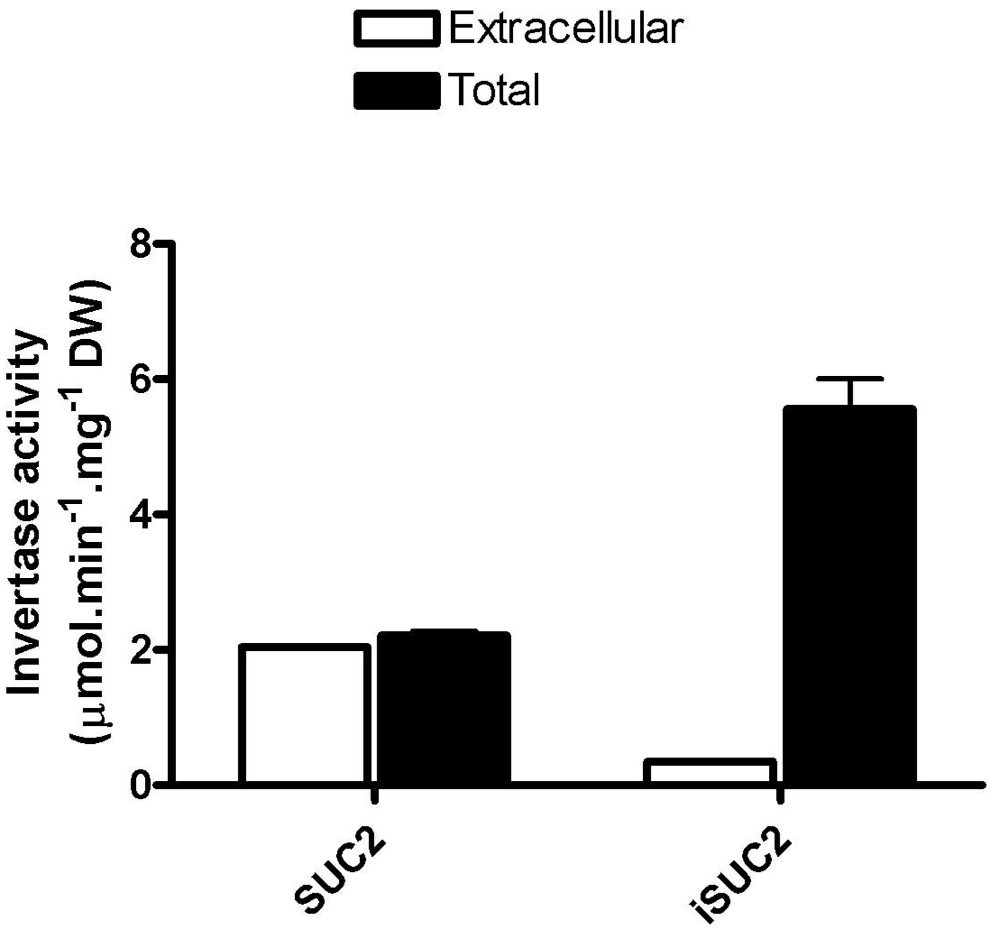
Carlson & Botstein. **Cell** 28 (1982)



0%

100%

Invertase activity



Anaerobic sucrose-limited chemostat $D = 0.10\ h^{-1}$

Parameters	**Strains**		**Change**	
	SUC2	**i*SUC2***	**Observed**	**Theoretical**
$\mathbf{Y_{X/S}}$ (g.g glc eq. $^{-1}$)	0.094 ± 0.001	0.088 ± 0.001	- 6%	- 25 %
$\mathbf{Y_{Ethanol/S}}$ (g.g glc eq. $^{-1}$)	0.378 ± 0.001	0.395 ± 0.007	+ 4%	+ 8 %
Residual sugar (g.l^{-1})	0.05 (glc) 0.11 (fru) 0.00 (suc)	0.09 (glc) 0.16 (fru) 1.79 (suc)	---	---

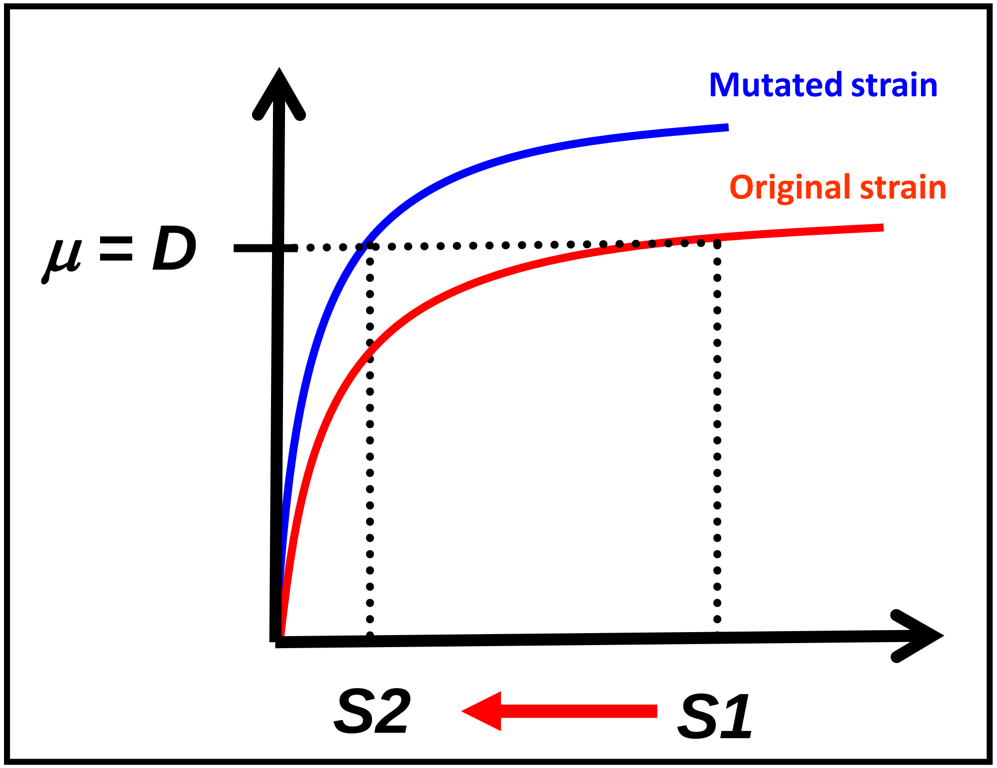
Low affinity for sucrose

Evolutionary Engineering: Chemostat as a tool to increase affinity

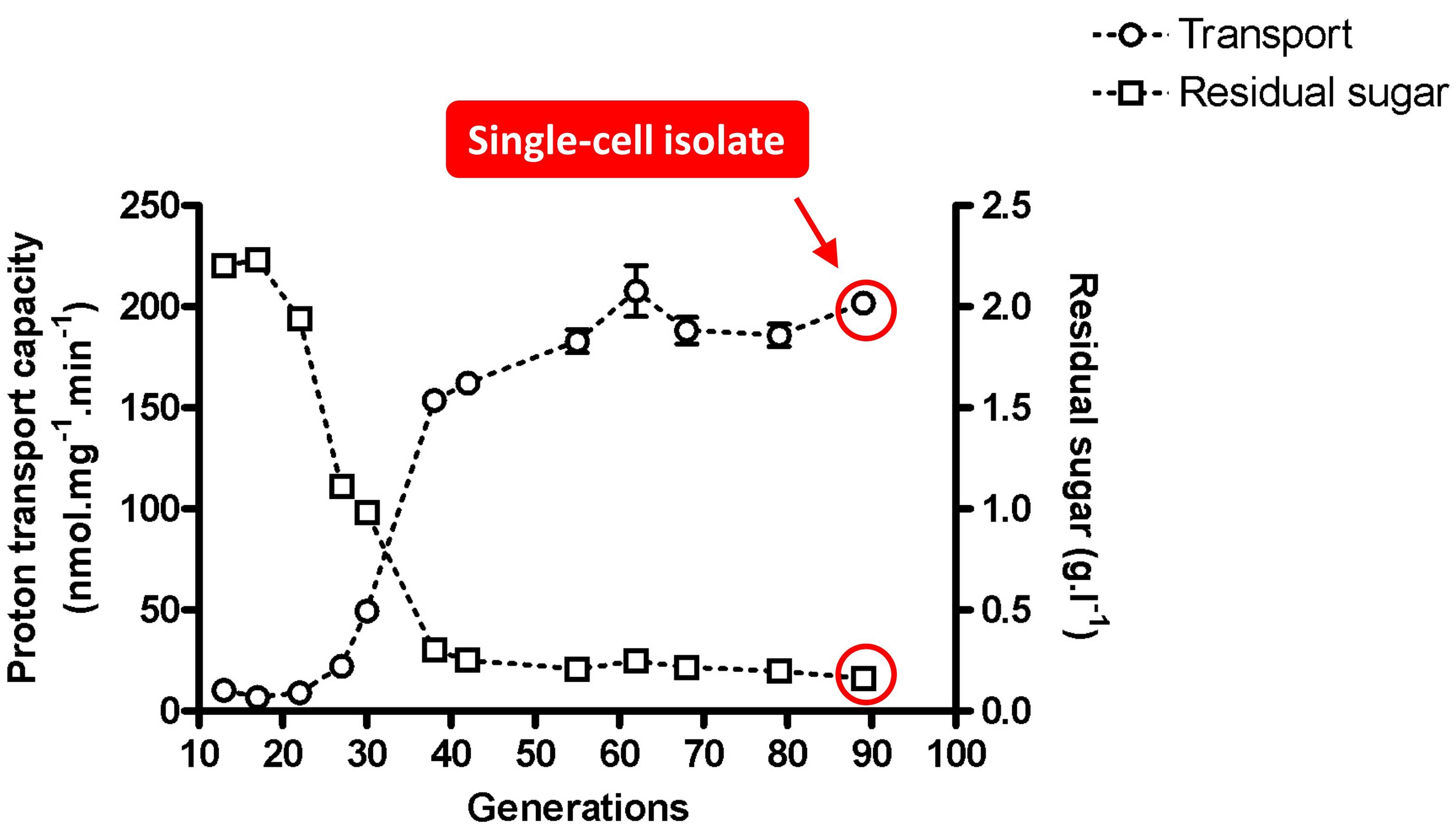
Selective pressure for an IMPROVED AFFINITY/CAPACITY for the growth-limiting substrate (sucrose)

Any adaptation/mutation leading to a higher growth rate at the ambient residual substrate concentration will result in an improved competitiveness

Chemostat setup at LEB/USP



Evolutionary Engineering
(Long-term sucrose limited chemostat culture)



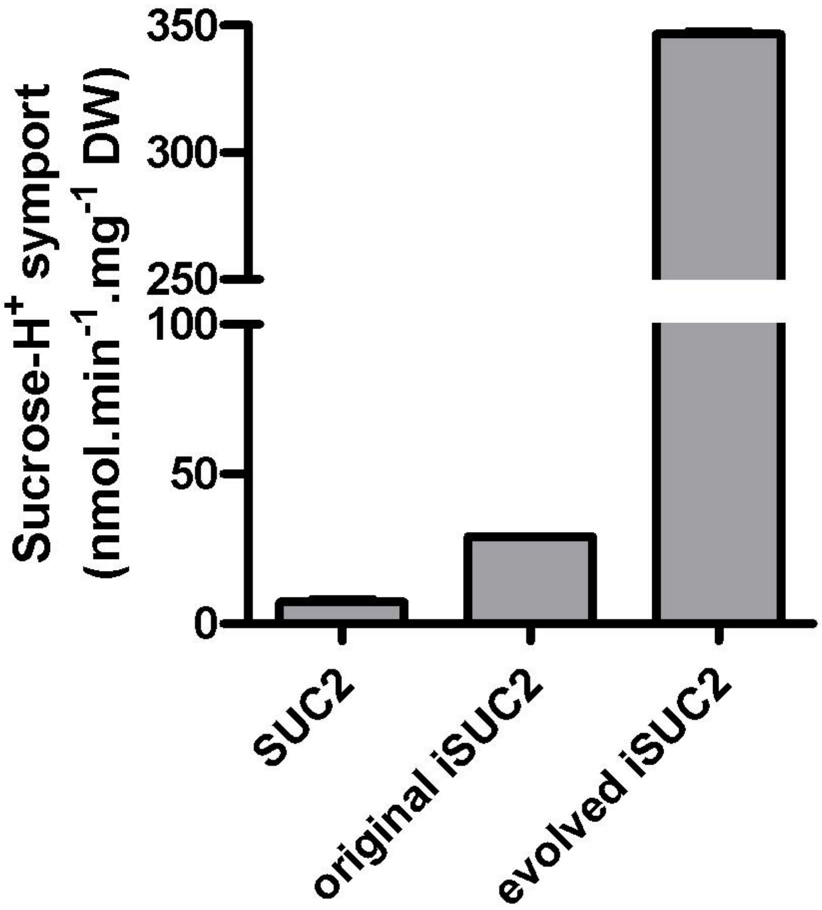
Anaerobic sucrose-limited chemostat $D = 0.10\ h^{-1}$

Parameters	Strains			Change	
	SUC2	i*SUC2* original	i*SUC2* evolved	Observed	Theoretical
$Y_{X/S}$ (g.g glc eq. $^{-1}$)	0.094 ± 0.001	0.088 ± 0.001	0.066 ± 0.002	- 29 %	- 25 %
$Y_{Ethanol/S}$ (g.g glc eq.$^{-1}$)	0.378 ± 0.001	0.395 ± 0.007	0.421 ± 0.006	+ 11 %	+ 8 %
Residual sugars (g.l^{-1})	0.05 (glc) 0.11 (fru) 0.00 (suc)	0.09 (glc) 0.16 (fru) 1.79 (suc)	< 0.01 (glc) 0.03 (fru) 0.08 (suc)	---	---

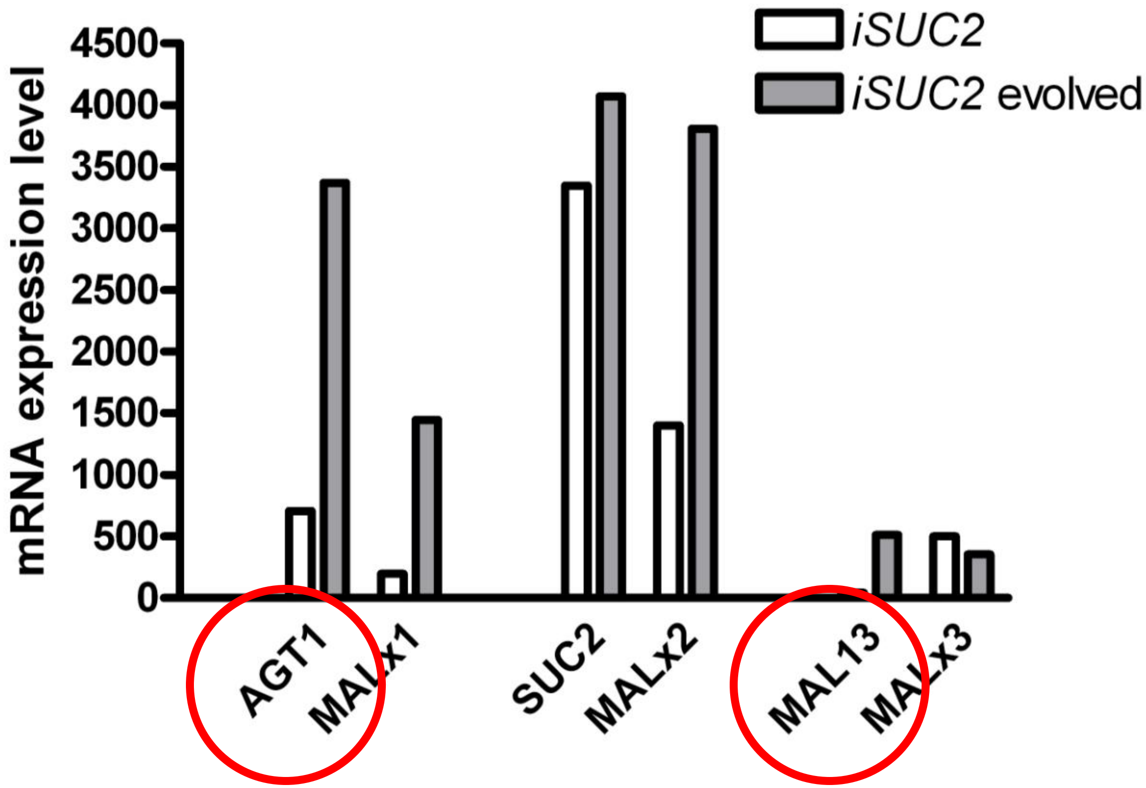
Conclusion

Relocation of sucrose metabolism in yeast, by a combination of metabolic and evolutionary engineering, resulted in an 11 % increase in the ethanol yield on sucrose

What's changed? - transport

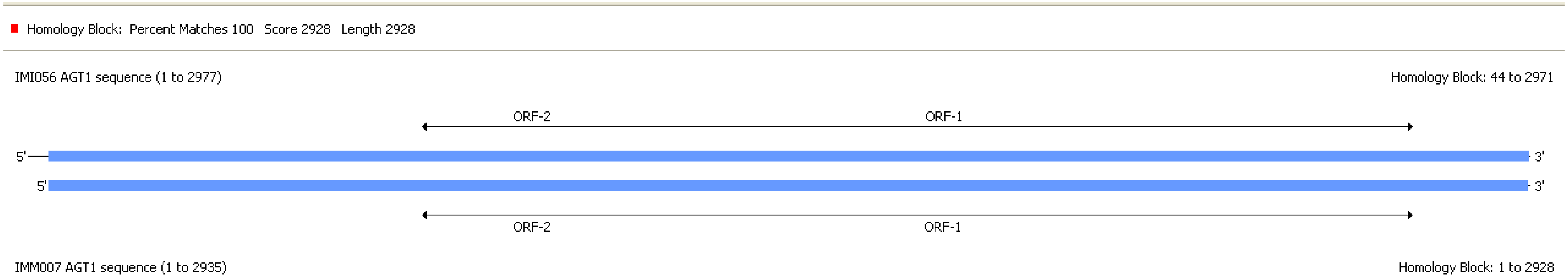


What's changed? - Microarrays



AGT1 sequencing results

Comparing original iSUC2 vs. evolved iSUC2



100% Homology!!!

Deleting the main sucrose transporter (*AGT1*)

Parameters	**Strains**		
	iSUC2	***iSUC2* evolved**	***Δagt1***
$Y_{X/S}$ (g.g glc eq. $^{-1}$)	0.088 ± 0.001	0.066 ± 0.002	0.066
$Y_{Ethanol/S}$ (g.g glc eq.$^{-1}$)	0.395 ± 0.007	0.421 ± 0.006	0.403
Residual sugar (g.l^{-1})	0.09 (glc) 0.16 (fru) 1.79 (suc)	0.01 (glc) 0.03 (fru) 0.08 (suc)	0.01 (glc) 0.01 (fru) 0.09 (suc)

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Thanks for your attention!

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