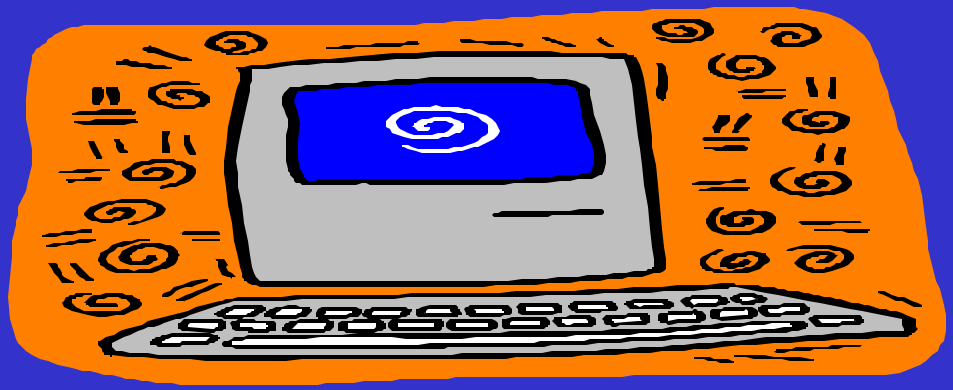
Metabolic Designs to Maximize Ethanol Production from Lignocellulose

Lonnie Ingram and Colleagues
University of Florida

USDA & DOE

Application of Functional Genomics to The Development of Biocatalysts for Fuels and Chemicals (Initial Results)

Han Tao, Alfredo Martinez, Maria Rodriguez,
K.T. Shanmugam, J.F. Preston,
Ramon Gonzales and L.O. Ingram



Fuel Ethanol in the United States

Corn starch+Enzymes > Glucose + Yeast -->Ethanol

1,200,000,000 gallons of fuel ethanol per year
= 1% of US automotive fuel
E10, E95 Trials, Ethanol-based fuel cells

US Auto Fuel = 120 Billion gallons per year = 50% oil

Photosynthesis

CO_2-->Plant Biomass -->Fuel Ethanol-->CO_2

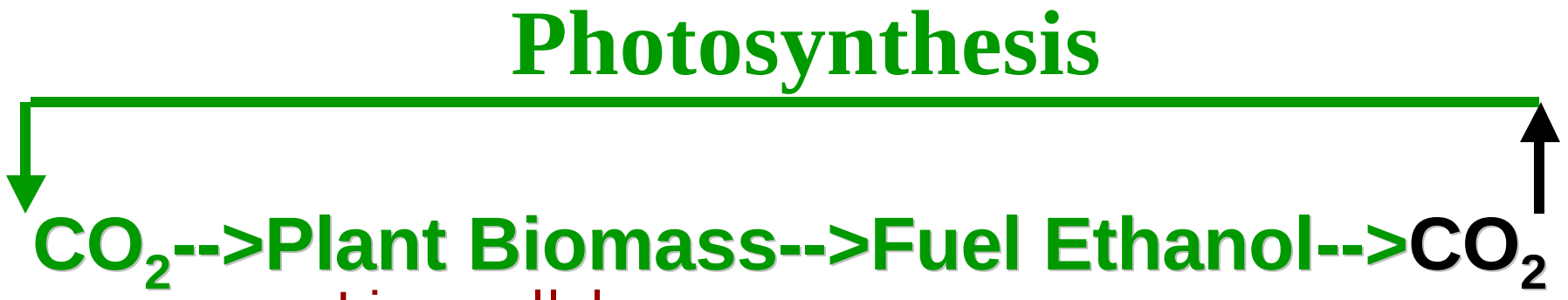
Fuel Ethanol in Brazil: 25-yr Experiment

Sugar Cane Sucrose + Yeast -->Ethanol

Over 4 billion gallons of fuel ethanol per year

E95, E70, E30, E15

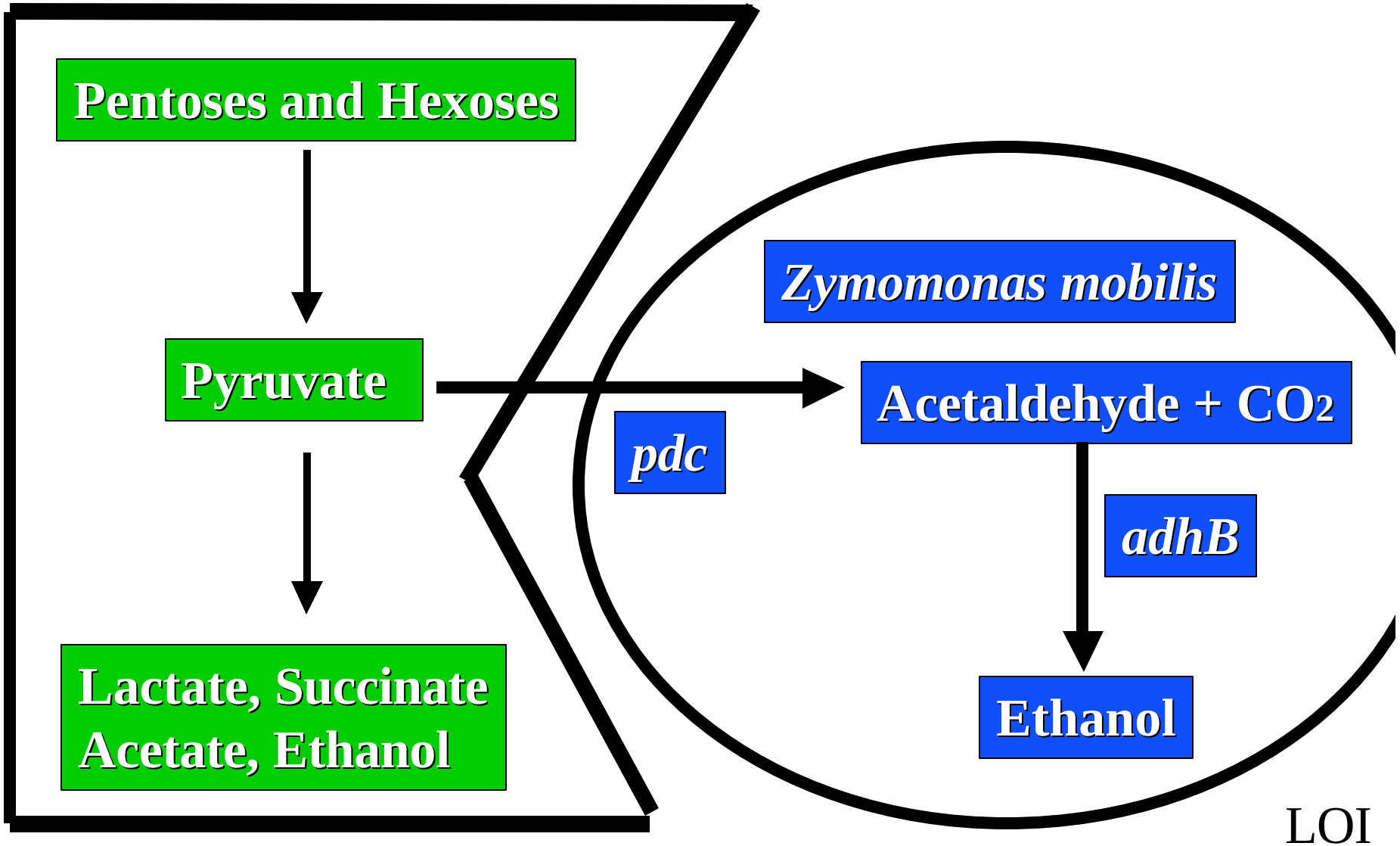
Ford, GM, Volkswagen, etc.



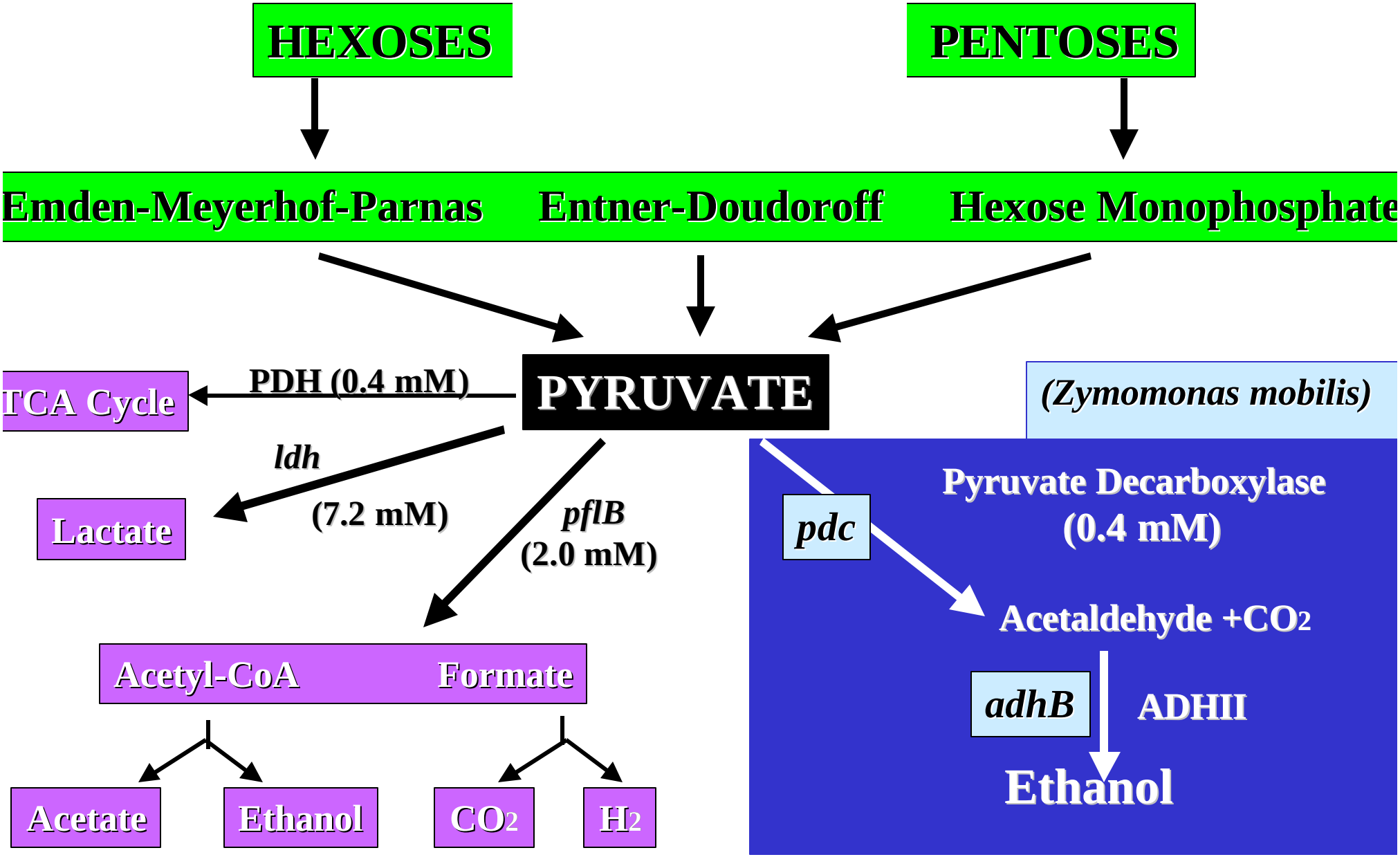
Lignocellulose Composition

20-30% Lignin	30-50% Cellullose	20-40% Hemicellulose
Phenolic Polymer	Glucose Homopolymer	Xylose & Arabinose (C5) Mannose, Glucose (C6) Galactose, acetyl esters
Fuel or Plastic	Enzyme or Conc. Acid Hydrolysis	Dilute Acid Hydrolysis
Problems:	**Costly Enzymes & Acid Recovery**	**Lack of suitable Biocatalyst for C5 & C6**

Engineering *Escherichia coli* for Hemicellulose Fermentation



LOI


HEXOSES
PENTOSES
Emden-Meyerhof-Parnas
Entner-Doudoroff
Hexose Monophosphate
PYRUVATE
TCA Cycle
PDH (0.4 mM)
ldh
(7.2 mM)
Lactate
pflB
(2.0 mM)
Acetyl-CoA
Formate
Acetate
Ethanol
CO2
H2
(Zymomonas mobilis)
pdc
Pyruvate Decarboxylase
(0.4 mM)
Acetaldehyde +CO2
adhB
ADHII
Ethanol

Sugars Fermented by Ethanologenic *E. coli*

Hexoses

glucose
fructose
mannose
galactose
sorbose
fucose
rhamnose

Pentoses

xylose
arabinose
ribose

Alcohols

sorbitol
mannitol
glucitol
galactitol
arabitol
ribitol

Saccharides

cellobiose
cellotriose
lactose
sucrose
maltose
raffinose
stachyose
melibiose

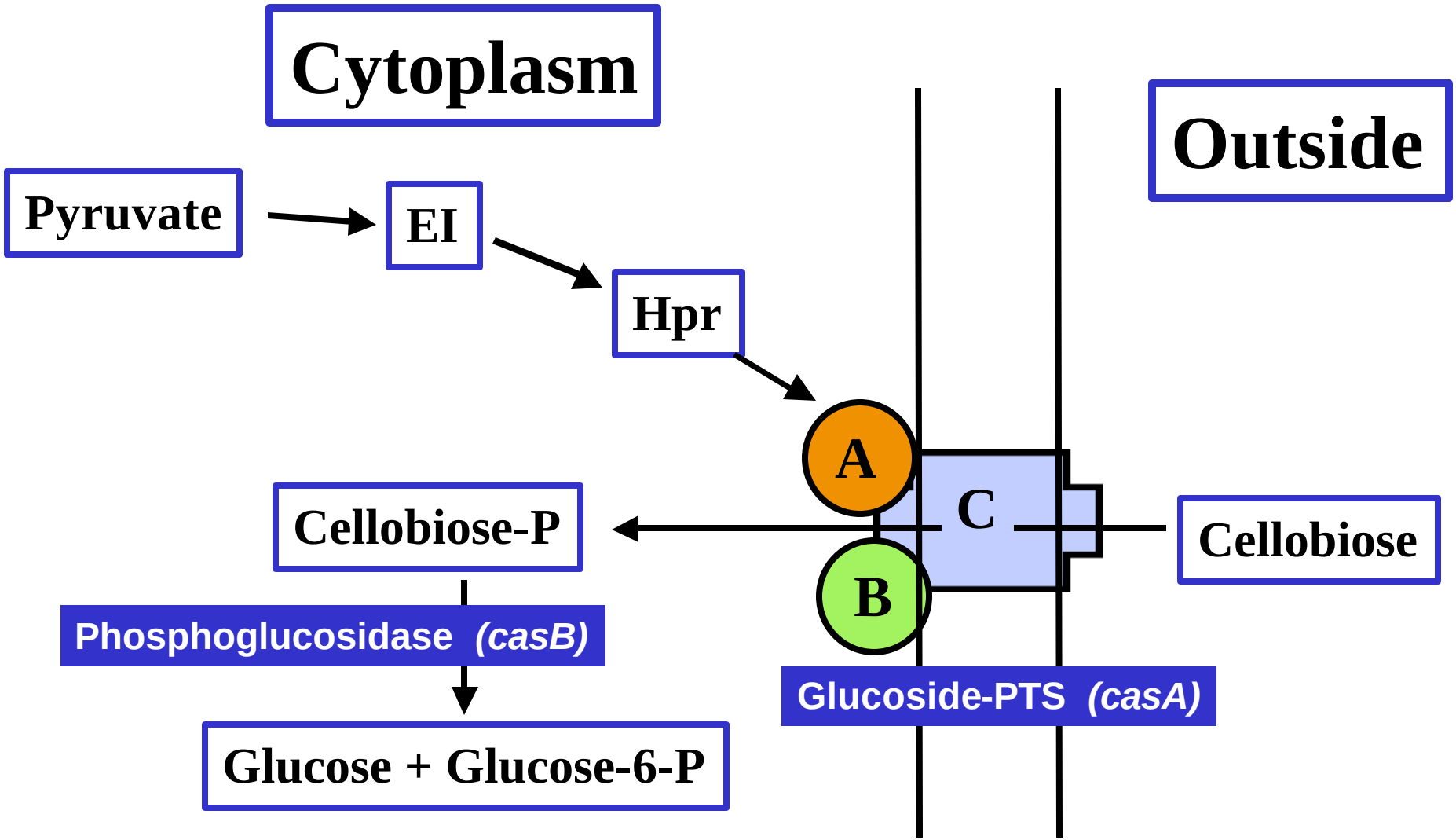
Amino Sugars

glucosamine
N-acetyl glucosamine

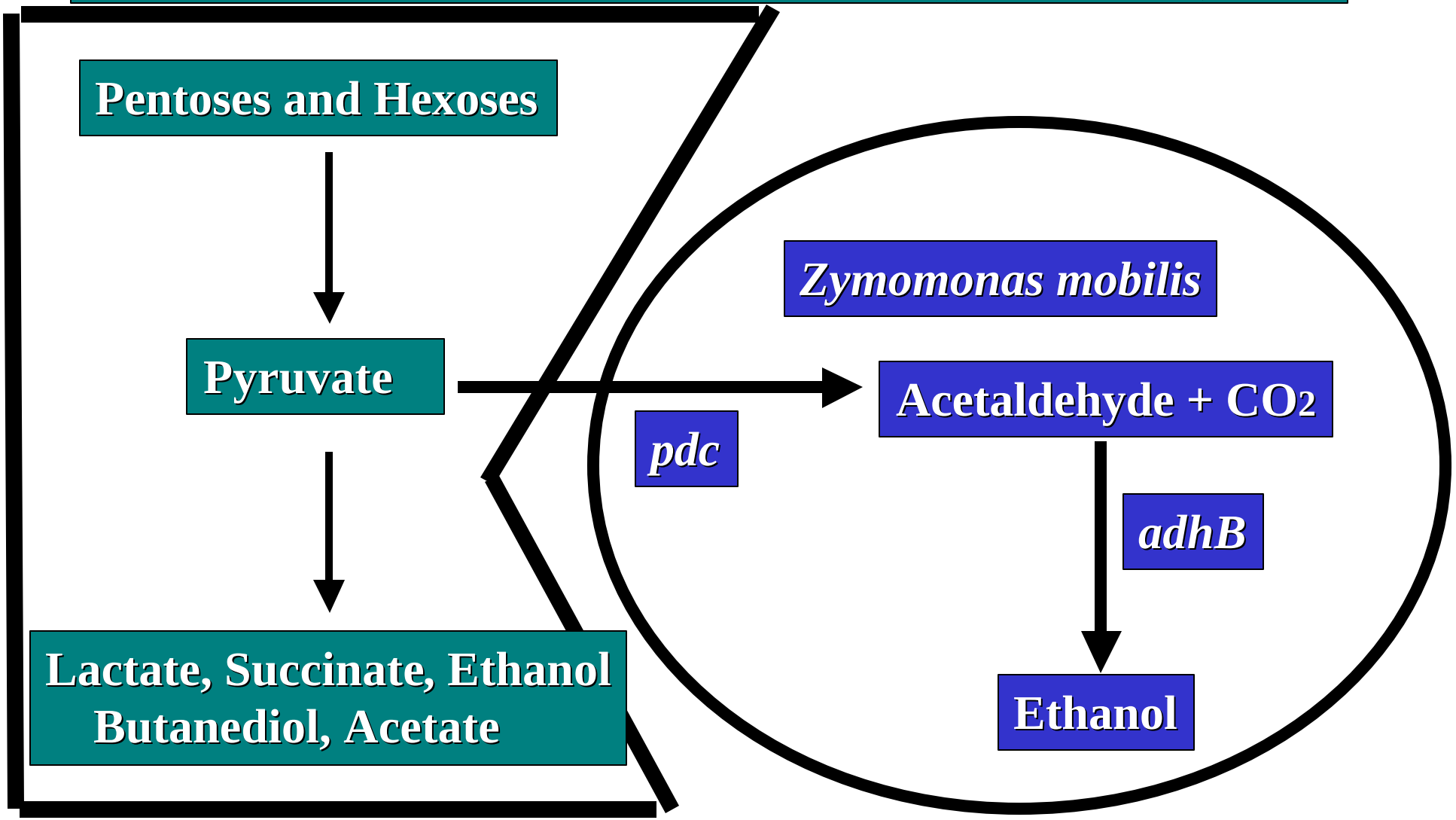
Uronic Acids

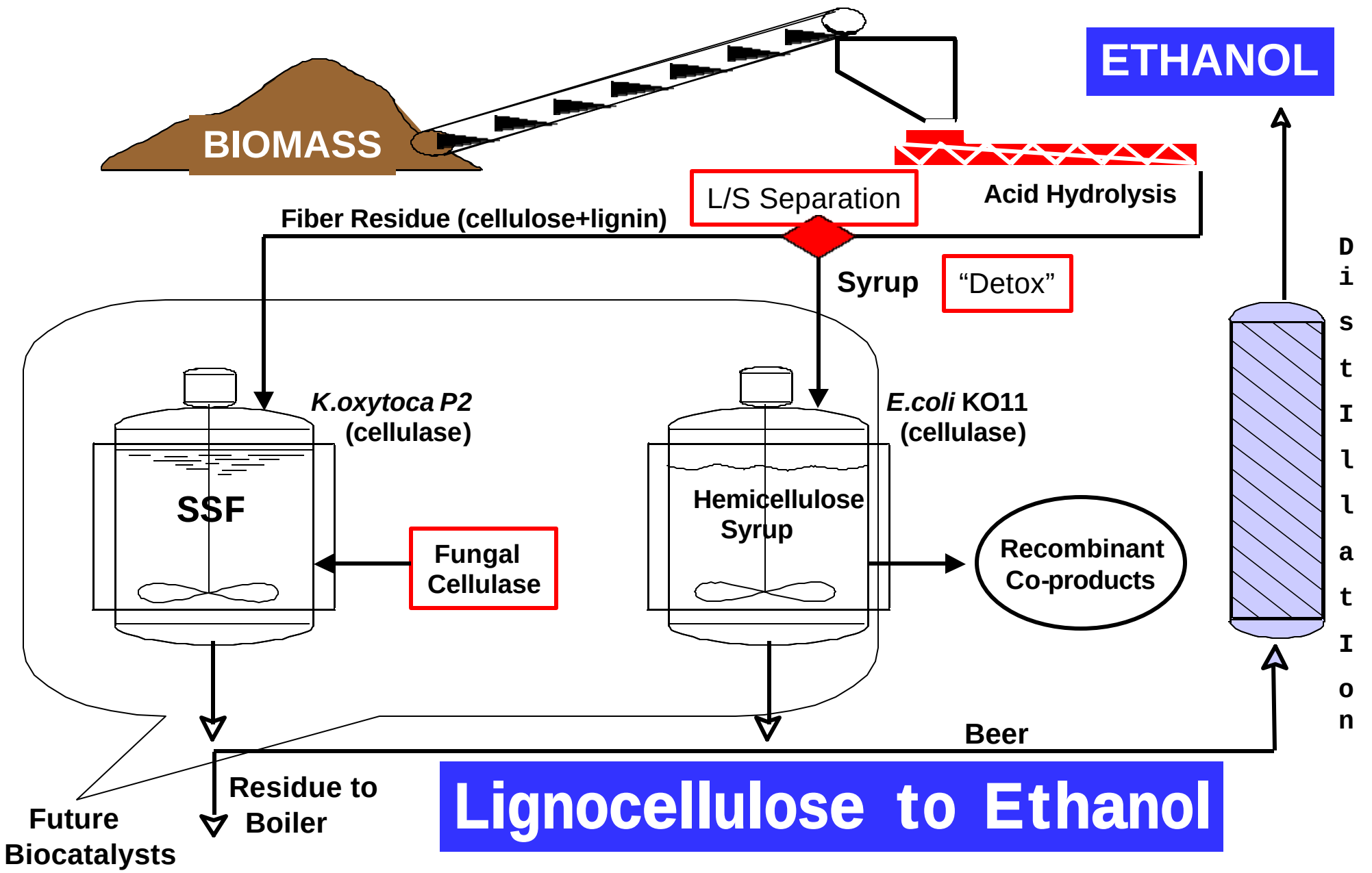
galacturonic acid
glucuronic acid

Cellobiose transport in *Klebsiella oxytoca*



Engineering *Klebsiella oxytoca* for Cellulose Fermentation (PTS-cel)

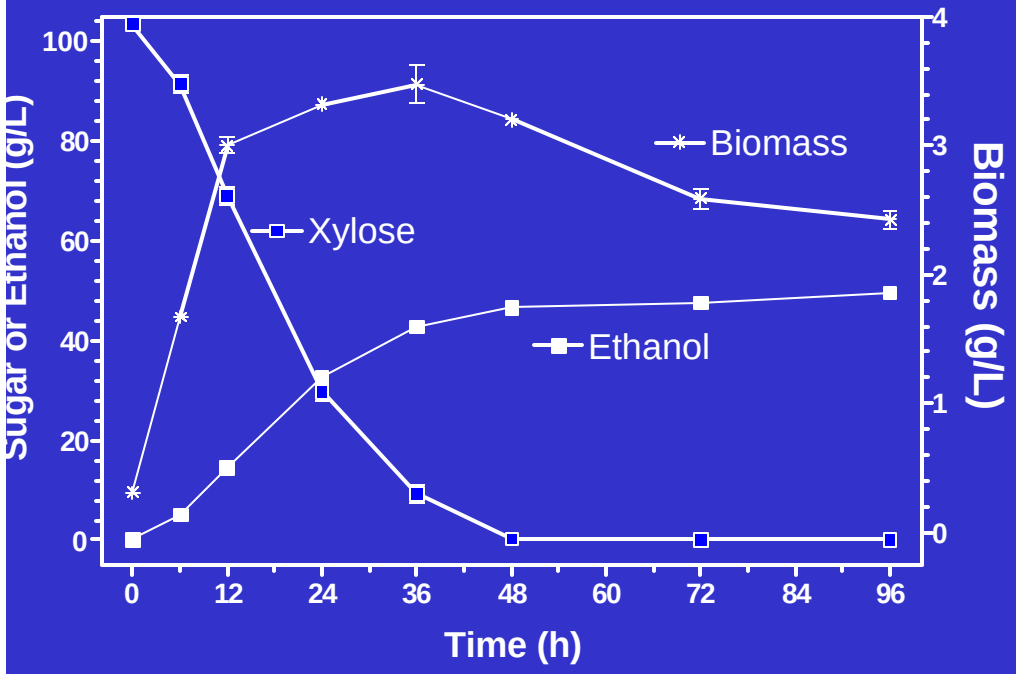



ETHANOL
BIOMASS
L/S Separation
Acid Hydrolysis
Fiber Residue (cellulose+lignin)
Syrup
"Detox"
K.oxytoca P2
(cellulase)
E.coli KO11
(cellulase)
SSF
Hemicellulose
Syrup
Fungal
Cellulase
Recombinant
Co-products
Distillation
Beer
Residue to
Boiler
Future
Biocatalysts
Lignocellulose to Ethanol

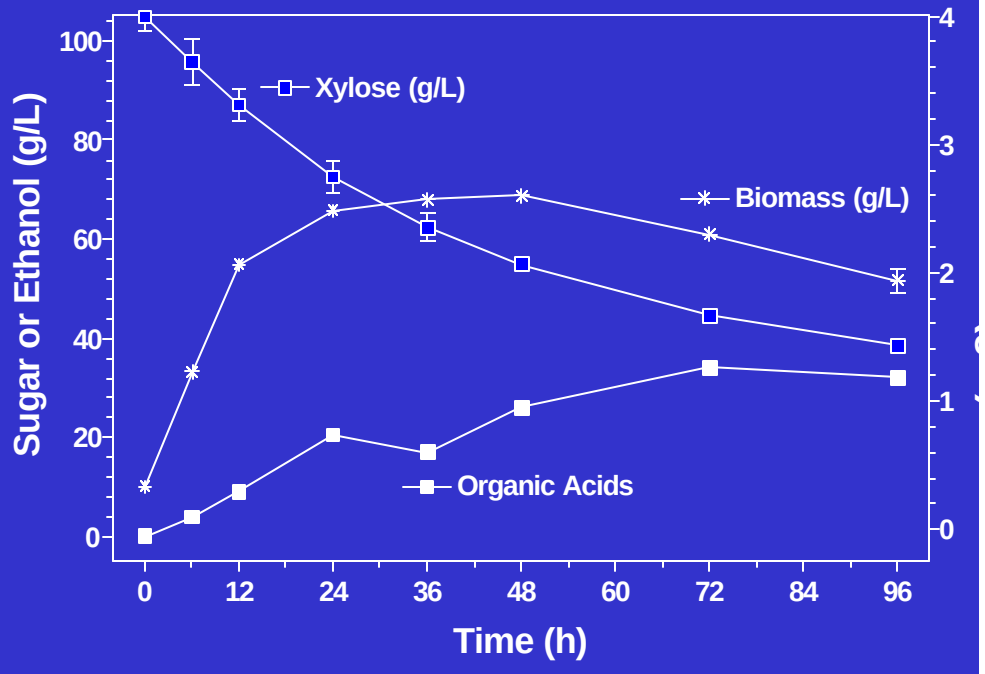
Anaerobic Batch Fermentations

100 g xylose/L, Luria Broth, pH 6.5, 35°C, 100 rpm

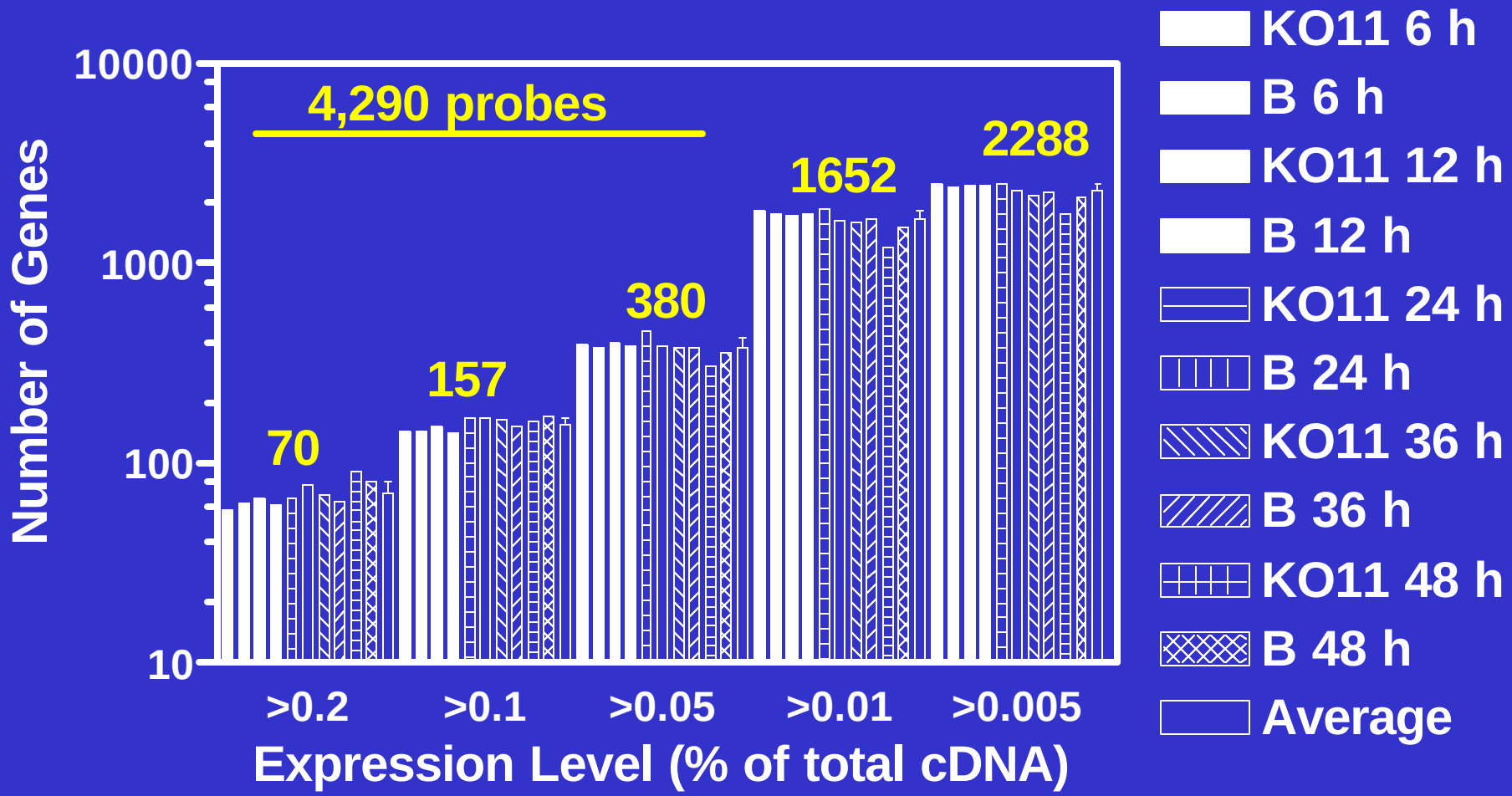
E. coli KO11 Fermentation



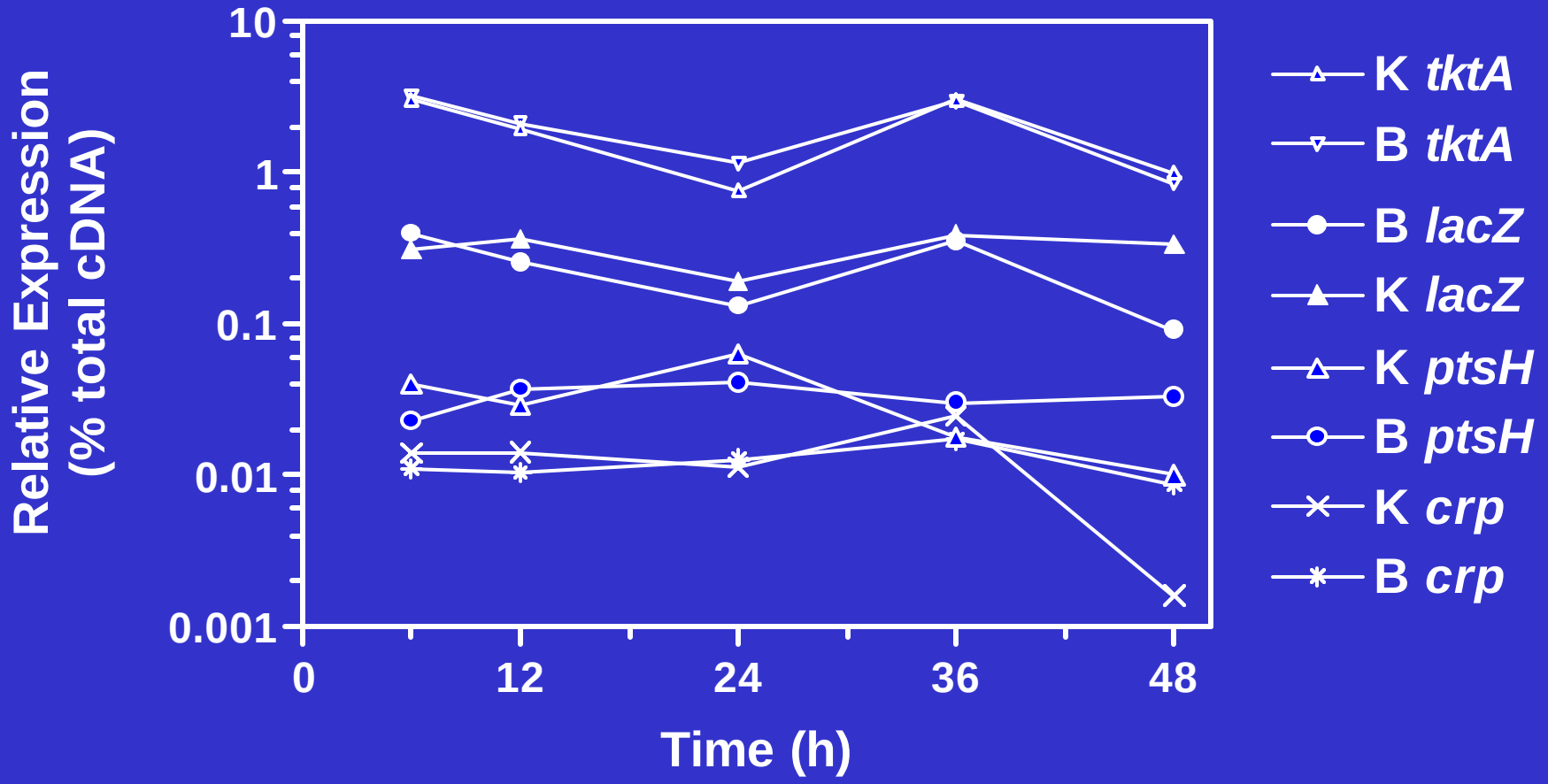
E. coli Strain B Fermentation



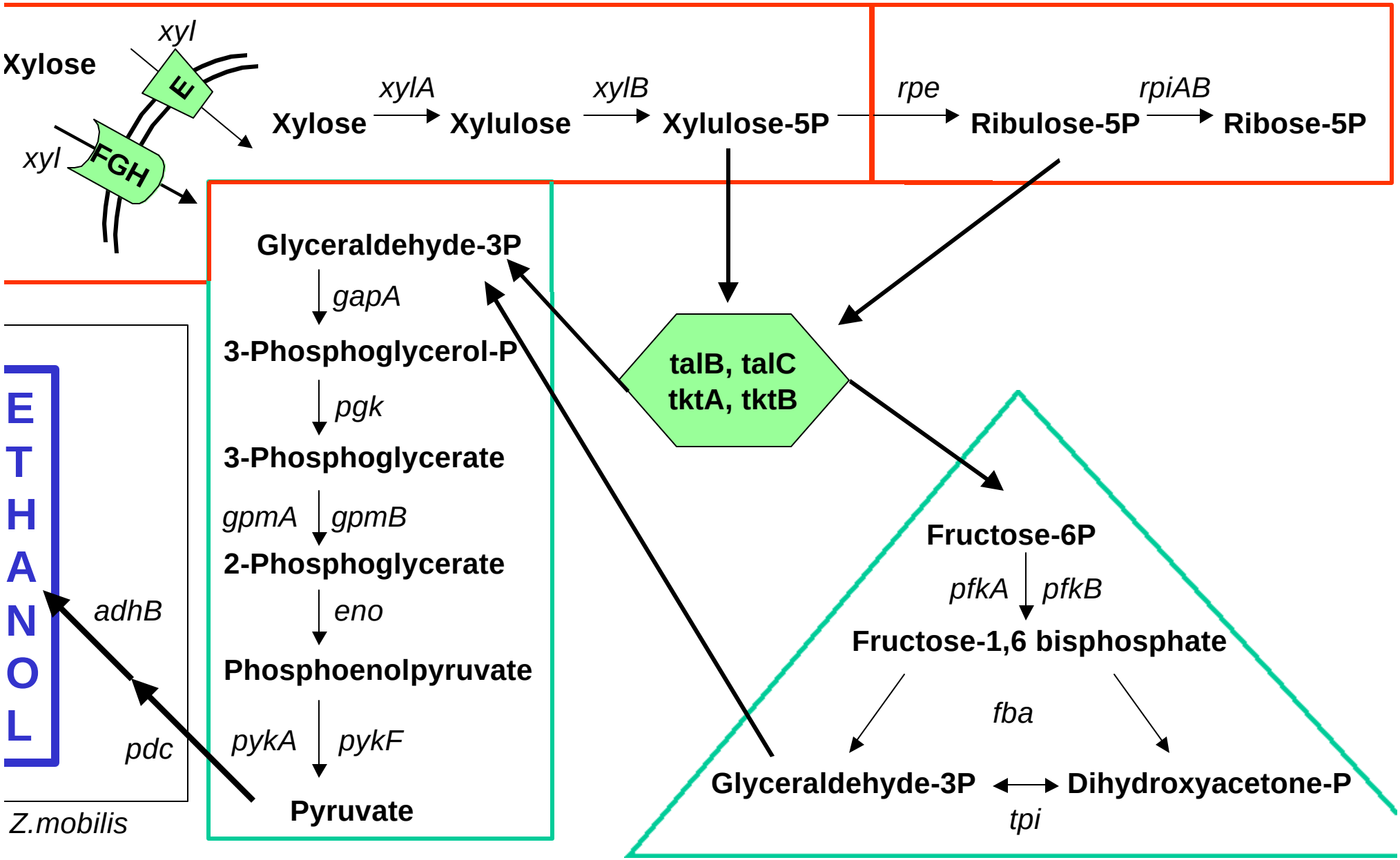
Gene Expression during Fermentation



Expression of Selected Genes (300-fold range)



Xylose Metabolism in Ethanologenic *E. coli*



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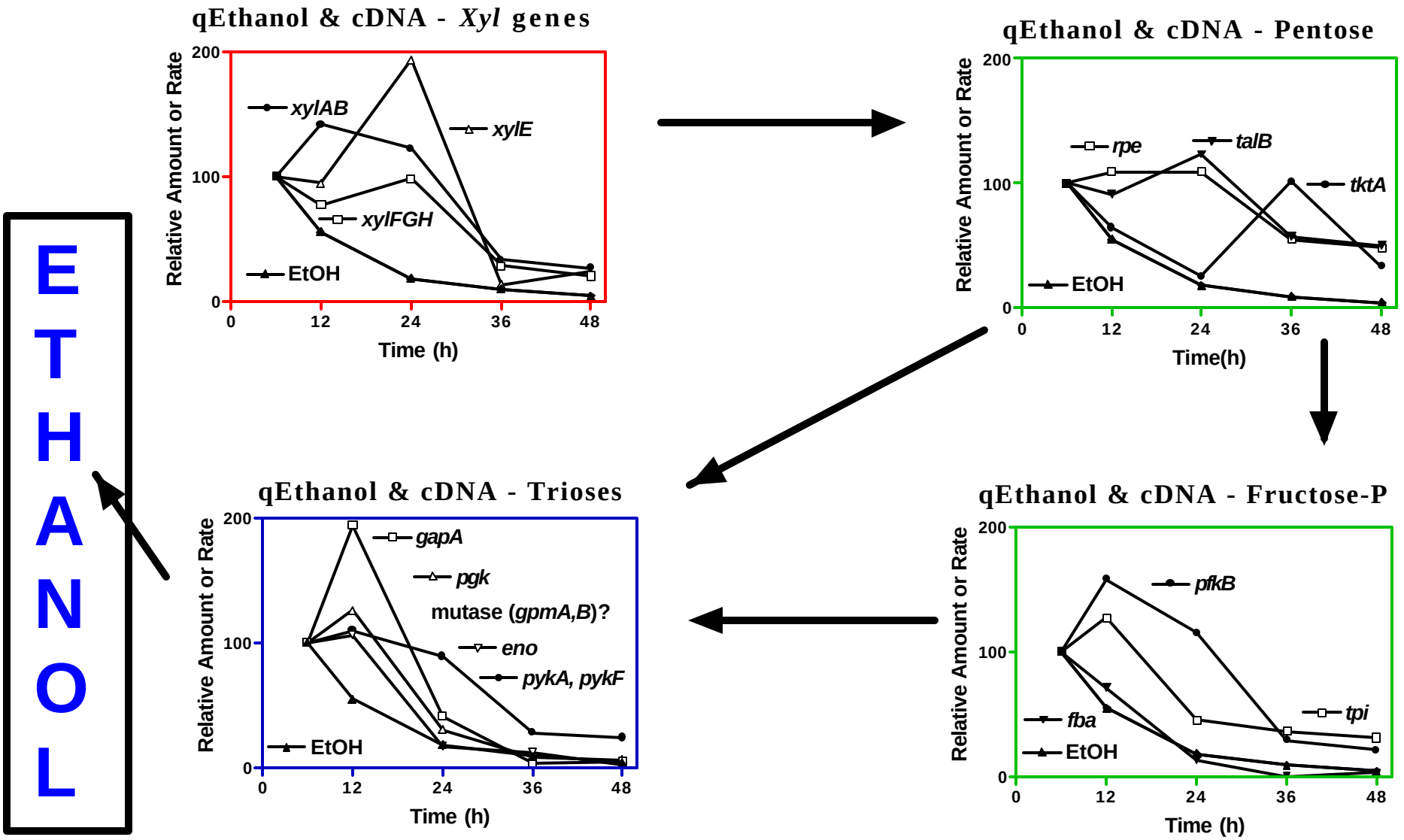

Transcriptome Analysis:

- **Relative Expression - isoenzymes?**

  pfkA, pfkB **= 10:1** *pykA, pykF* **= 1:1**
  talB, talC **= 10:1** *tktA, tktB* **= 20:1**
  rpiA, rpiB **= 1:2**
  gapA, gapC1, gapC2 **= 10:1:1**

  6 h *rpoD, E, H, N, S* **= 10:05:01:07:01**
  24 h ----------> 10:16:12:04:01

- **Patterns of Expression? Regulation?**

  xylAB, xylFGH, xylE **(***xylR* **+)**
  pfkB, tpi, pykA *talB, rpe*
  gapA, pgk, eno *gmpA, gmpB?*

Ethanol Genes alter Transcription

Increased in ethanologenic KO11

xylA, xylE, xylF - xylose metabolism (7x)
cspE - cold shock (26x)
caiD - carnitine racemase (10x)
dcuB - dicarboxylate uptake (4x)
cycA - D-ala uptake (4x) *cmtB* - pts mannitol (3x)

Decreased in ethanologenic KO11

dps, aceE, ynaF, cbpA, slp - (1/10 x)
gadA, gadB - glutamate decarboxylase (1/5 x)
dnaK, uspA - stress responsive proteins (1/4 x)

Transcriptome Analysis:Cellular Needs?

- **Amino acid biosynthesis (24 h)**

  5 *arg genes* **3** *met genes*
  2 *ser genes* **2** *ilv genes*
  1 each *lys, thr, cys, trp, tyr, & ala-racemase*

- **Transport genes (24 h)**

  2 nucleoside permease genes
  N acetyl-glucosamine

  oligopeptide leucine arginine
  glutamate/aspartate high affinitiy amino acid

  xylose (*xylE*) potassium molybdate

Gene Arrays (Transcriptome):

A new tool to study complex processes & develop testable hypotheses

- Relative Expression - genes, isoenzymes
- Patterns of Expression – Regulation
- Genetic Basis of Complex Mutants

Secrets of life - Growth rate, culture density, cell division, differentiation, etc.

Physiological limitations - Nutrients? Flux?
Functions of unknown genes
Global consequences of metabolic engineering
Optimization of biocatalysts for renewable chemicals
Global effects of environmental stresses

Fuel Ethanol and Renewable Chemicals

Department of Microbiology and Cell Science
Institute of Food & Agricultural Sciences, University of Florida

Ana Borges
Fernando Morales
Sean York
Arne Heggestad
K.T. Shanmugam

J.F. Preston
John Rice
H.C. Aldrich
Julie Maupin
F.C. Davis

Brice Causey
Stuart Underwood
Jesus Zaldivar
Melisa Wells
Frank Healey
Adnon Hasona
Jason Hurlbert
Tony Guiterrez
Sylvia Coleman
Kwang Myung Cho
Jack Sheldon

Alfredo Martinez
Lorraine Yomano
Shengde Zhou
Ramon Gonzales
Han Tao
Chandra Raj
Maria Rodriguez
Lorraine McDowell