advanced biofuels development summit

April 20-21, 2009
Marriott at Metro Center
Washington, DC

accelerating innovation to commercialization

WELCOME

TO CAMBRIDGE HEALTHTECH INSTITUTES

2nd INTERNATIONAL

Advanced Biofuels Development Summit

Accelerating Innovation to Commercialization

April 20-21, 2009

OPENING REMARKS

THANK YOU

- CHI ORGANIZERS
- SCIENTIFIC ADVISORS
- WORKSHOP LEADERS
- CORPORATE SPONSORS

Corporate Sponsors

ICM, Inc,.
McKenna Long and Aldridge

Media Parteners

Advanced Biofuels
Argus Media
Biofuels Digest
Bio-IT World
BioMedCentral
Biotechnology for Biofuels
Emerging Markets Online
Science AAAS
The Scientist

advanced biofuels development summit

accelerating innovation
to commercialization

Corn Futures (C)
Delayed 10 minute data as of April 02, 2009 16:53 CDT

Soybeans Futures (S)
Delayed 10 minute data as of April 02, 2009 16:53 CDT

USDA NASS: USDA EXPECTS TOTAL CORN, SOYBEAN ACRES ON PAR WITH LAST YEAR

USDA National Agricultural Statistics Service (NASS)

EXPECTS TOTAL CORN, SOYBEAN ACRES ON PAR WITH LAST YEAR

Principal Crop Area Expected to Decline Nearly 8 Million Acres

8 billion gallons of ethanol production per year, approximately 800 million gallons of corn oil is potentially available for biodiesel production

US Energy Independence and Security Act of Jan 2008

SUMMARY OF BIOFUELS ELEMENTS

1. The Renewable Fuels Mandate 500 percent increase to 36 billion gallons of renewable by year 2022.

Stimulus Package changes?

2. The Vehicle Fuel Economy Mandate specifies a national mandatory fuel economy standard of 35 miles per gallon by 2020.

FACTS

Ethanol production has increased from 1.6 billion gallons 2000 to and estimated 7.2 billion gallons end 2008

Next generation biofuels such as cellulosic ethanol being tested in new format pilot refineries.

U.S. produced about 450 million gallons of biodiesel – up 80 percent from 2006.

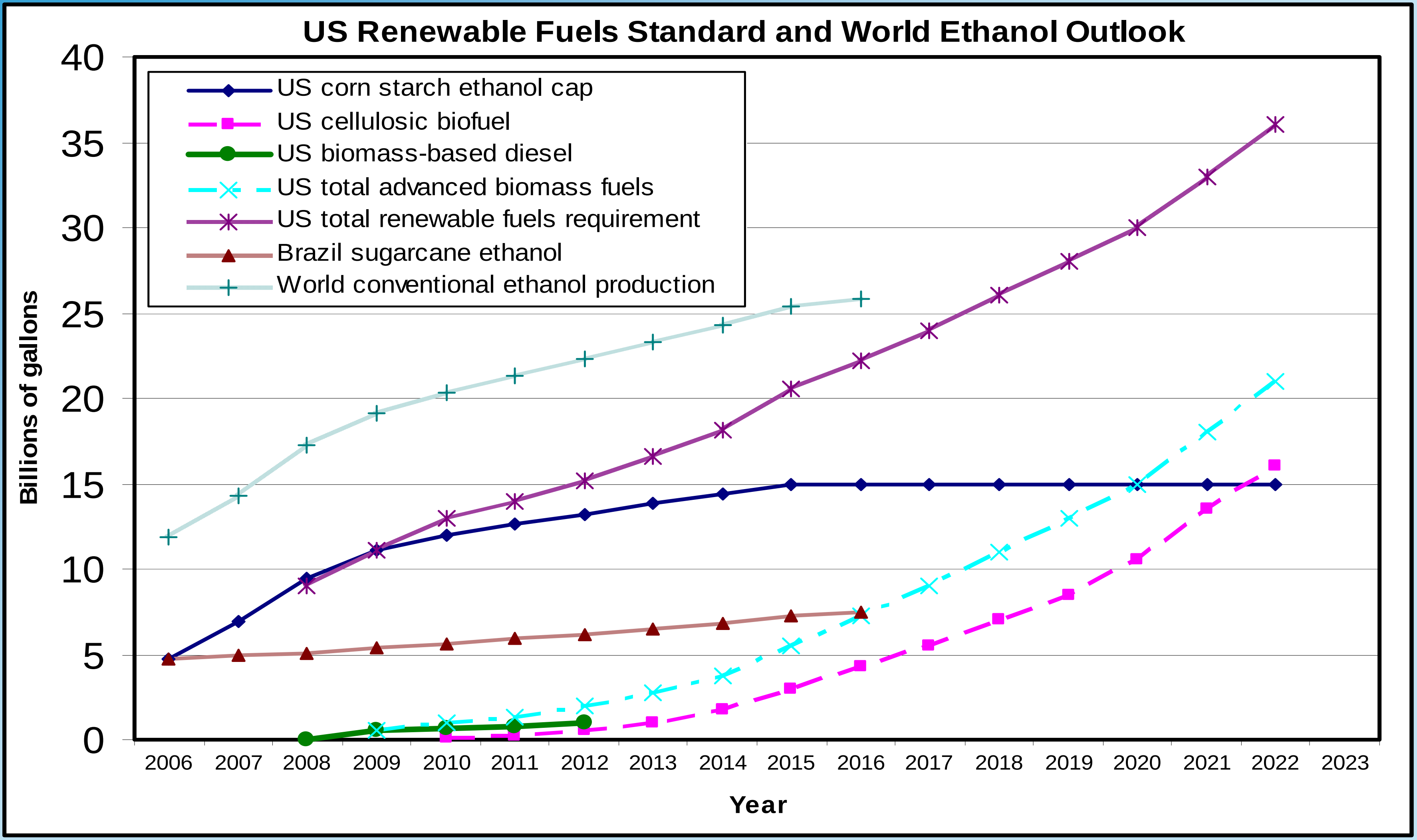
Over the last five years, the U.S. invested about $1.2 billion in hydrogen research

Net Energy: US energy needed to produce ethanol

Table 2--Total energy requirements of farm inputs for nine State and nine-State weighted average, 2001

	IL	IN	IA	MN	NE	OH	MI	SD	WI	9-State Weighted average
					BTU/bushel					
Seed	525	557	451	512	804	780	827	623	548	603
Fertilizer:										
Nitrogen	25876	25446	20147	19305	24146	32764	26792	25257	19864	23477
Potash	2395	2798	1356	1285	474	2670	2669	907	1278	1899
Phosphate	2211	1897	1508	1283	1053	2142	1745	1721	1139	1631
Lime	76	79	73	0	0	89	97	0	255	63
Energy:										
Diesel	3853	4941	4609	5700	14136	5207	9558	6336	8576	7491
Gasoline	1478	2135	1138	1698	2266	1834	3141	2044	1536	3519
LPG	1644	1938	4067	5058	2635	3823	2694	406	1241	2108
Electricity	614	1868	1035	1739	10685	744	2081	2425	470	2258
Natural Gas	550	1063	0	332	7544	1363	2033	69	986	1846
Custom work	2001	1197	1417	1294	1291	1434	1859	1913	2526	1581
Chemicals	3453	3464	2877	2134	2501	4530	4227	2684	2542	2941
Purchased water	0	0	0	0	946	0	0	0	0	136
Input hauling	143	167	178	176	242	209	254	121	251	202
Total	44821	47551	38856	40516	68723	57590	57977	44486	41212	49753

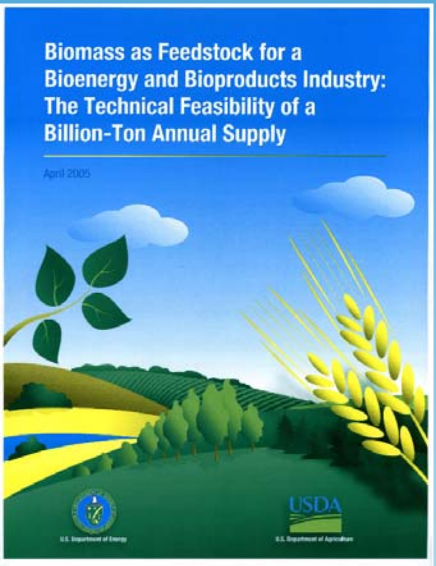
Global Ethanol Production

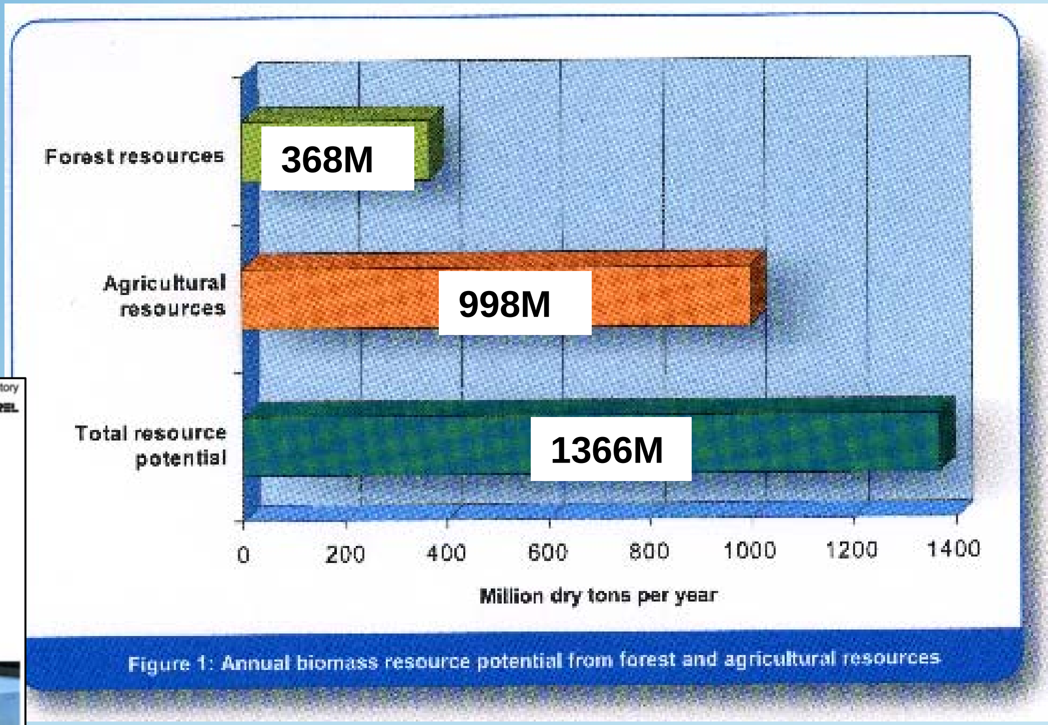


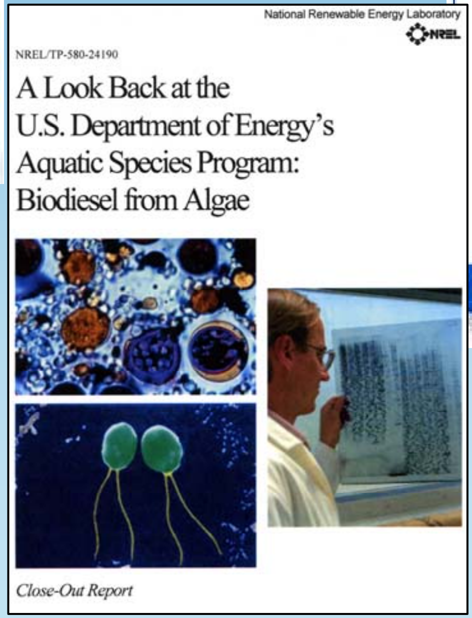
editor Nasib Qureshi, authors Hughes, S., Gibbons, W., Kohl, S. 2008 Wiley Books . *Biofuels: Chapter 4*

BIOMASS TO FUEL

http://www1.eere.energy.gov/biomass/pdfs/final_billionton_vision_report2.pdf







Fischer- Tropsch reactor fed by syngas

coal into ethanol

http://www1.eere.energy.gov/biomass/pdfs/biodiesel_from_algae.pdf advanced biofuels development summit

April 20-21, 2009
Marriott at Metro Center
Washington, DC

accelerating innovation
to commercialization

Date: **April 21, 2009 at 11:35pm**

Seminar Title: Anaerobic Conversion of Pretreated Lignocellulosic Sugars to Ethanol and Biodiesel: The GMAX-L Saccharomyces cerevisiae strain concept

Authors:

Stephen R. Hughes, PhD	*USDA, ARS, NCAUR, BBC*
Ken Tasaki, PhD	*Mitsubishi Chemical Corporation*
Bryan Moser, PhD	*USDA, ARS, NCAUR, FIO*
Ken Doll, PhD	*USDA, ARS, NCAUR, FIO*
Marge Jones, PhD	*llinois State Univrsity*
Amanda Harmsen	*Illinois Stsate I|University*

USDA ARS
NCAUR Where the future is Created

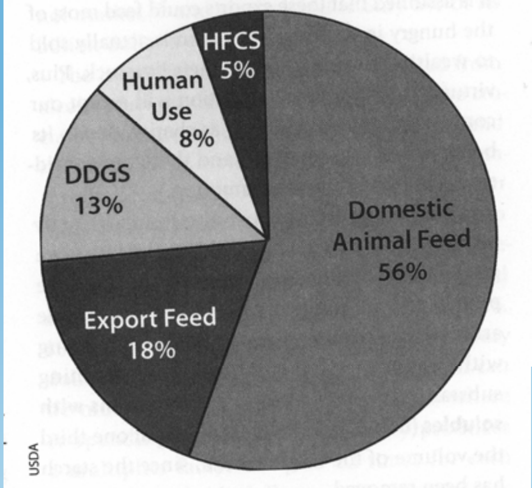
USDA ARS to boldly go…………

USDA ars

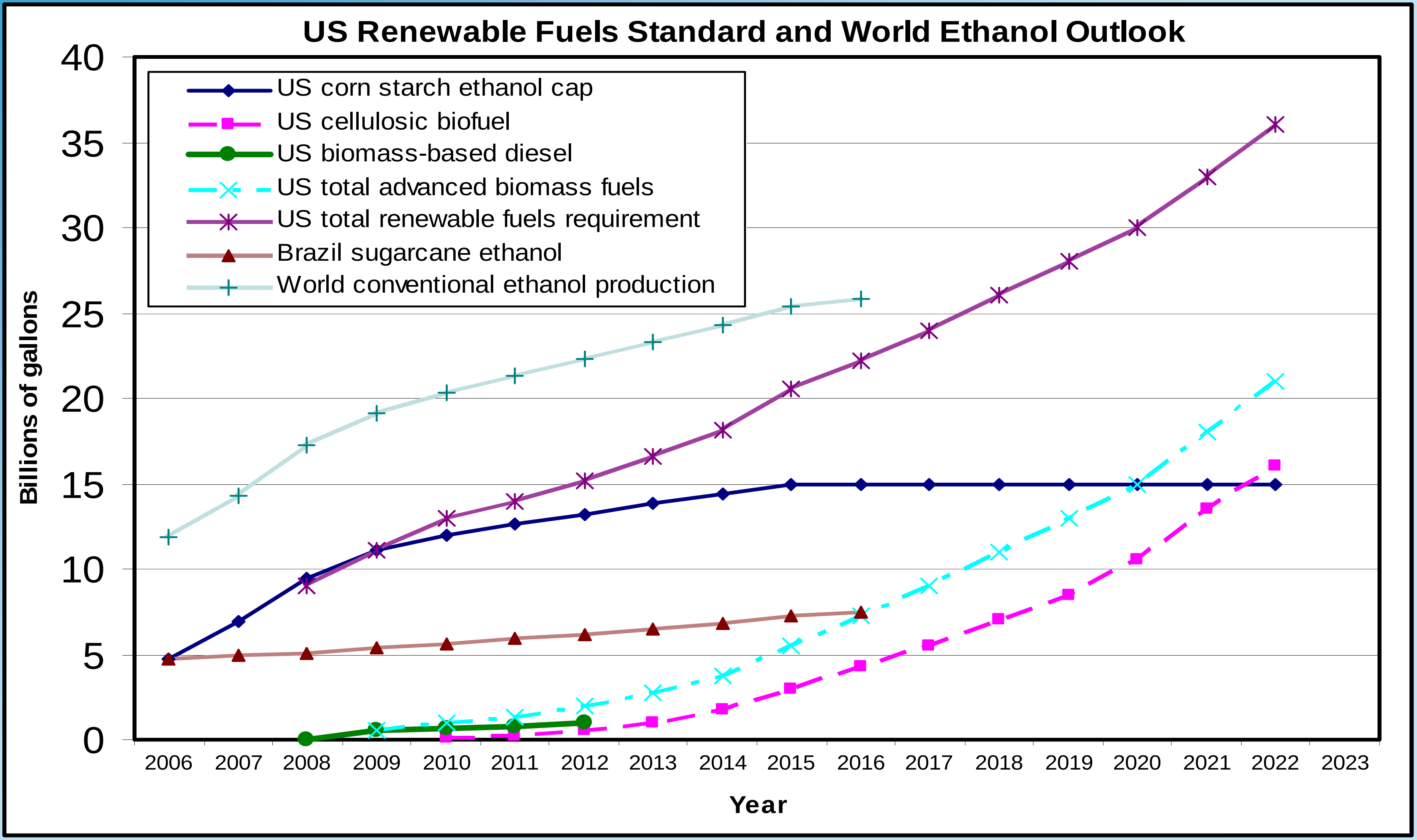
NCAUR
Where the future is Created

US Ethanol

- Corn starch
- Sugarcane
- Sugar Beets
- Potato starch
- Biomass
- Sweet Sorghum



Global Ethanol Production



editor Nasib Qureshi, authors Hughes, S., Gibbons, W., Kohl, S. 2008 Wiley Books . *Biofuels: Chapter 4*

Max Corn Use

- 2005 (present use)

Corn used 7.5%

- 2010-2015 (corn cap)

Corn use 13%

World Ethanol Production Statistics and Costs

Refinery Statistics Operations in 2007	Wet Mill	Dry Mill / Grind	Sugarcane	Lignocellulose	Combined Refinery [H]
AVG. COST OF PLANT [A]	$233.84 million	$115.5 million	$62.5 million	>$375.00 million	>$200.00 million
LIFESPAN OF PLANT [B]	>60 years	30-60 years	40-60 years	continuous	continuous
PRICE FEEDSTOCK [C]	$188.46 mt	$188.46 mt	$42.00 mt	$95.00 mt	<$50.00 mt
PRODUCTION COSTS [D]	$1.03/gallon	$0.85/gallon	$0.81/gallon	$2.25/gallon	<$1.07/gallon
COST ENZYMES [E]	$0.06/gallon	$0.06/gallon	<$0.01/gallon	$0.30/gallon	potentially $0
TOTAL ETHANOL PROFIT [F]	$2.56 billion	$16.77 billion	$46.65 billion	$0.051 billion	> $70 billion
TOTAL COPRODUCT PROFIT [G]	$5.05 billion	$9.80 billion	$6.64 billion	experimental	> $100 billion

A Based on first quarter 2008 average prices in US dollars.
B Time projections made at time of refinery construction.
C Average prices based on USDA ERS 2008 first quarter values in US dollars.
D Cost in US dollars for plant operation in 2007 using sucrose refinery operation in Brazil or for wet and dry mill refinery operation in US Midwest.
E Based on 2007 Novozyme and Danisco price levels.
F Values represent world production levels for 2007 in US dollars based on Chicago Board of Trade ethanol average price in first quarter 2008.
G Value of combined coproducts for 2007 in US dollars.
H For concept plant using shared utilities and operational staff (crossover model from Center for Bioresearch and Development, South Dakota State Univ.)

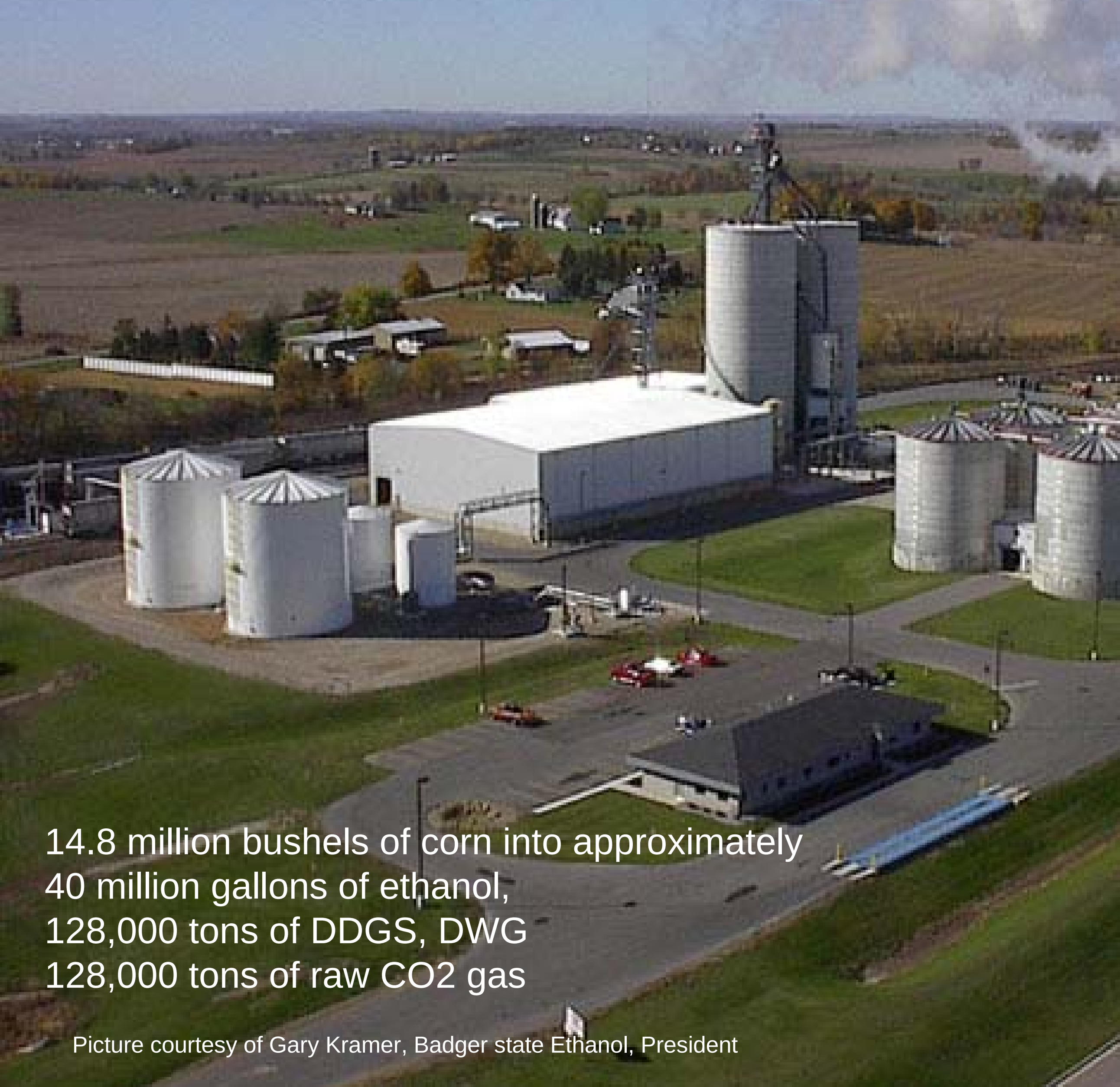
USDA ARS

SUGARCANE ETHANOL PLANT

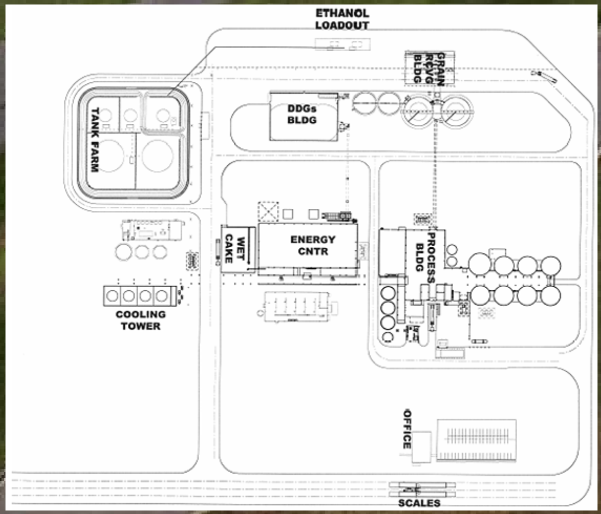
Courtesy of Edward Richard,
RL for sugarcane studies at ARS SRRC New Orleans and Houma, LA USDA sugarcane outstation

Badger State Ethanol dry grind ethanol plant
designed by ICM/Fagan joint design
Located: at 820 W. 17th Street, Monroe, WI 53566.

BADGER STATE ETHANOL



14.8 million bushels of corn into approximately
40 million gallons of ethanol,
128,000 tons of DDGS, DWG
128,000 tons of raw CO_2 gas



Picture courtesy of Gary Kramer, Badger state Ethanol, President

Aventine Renewable Energy 110 mgy Wet Mill

Courtesy G. Welch, Aventine Renewable Energy, Inc.

LIGNOCELLULOSIC ETHANOL PRODUCTION

- USDA Automation to Screen for Cellulosic Ethanol Yeast Transformed With XI and XKS
- Screened by Addition of Whole Fungal and Bacterial FLEXGene Libraries
- Screened Mated Library for Anaerobic Growth on Xylose

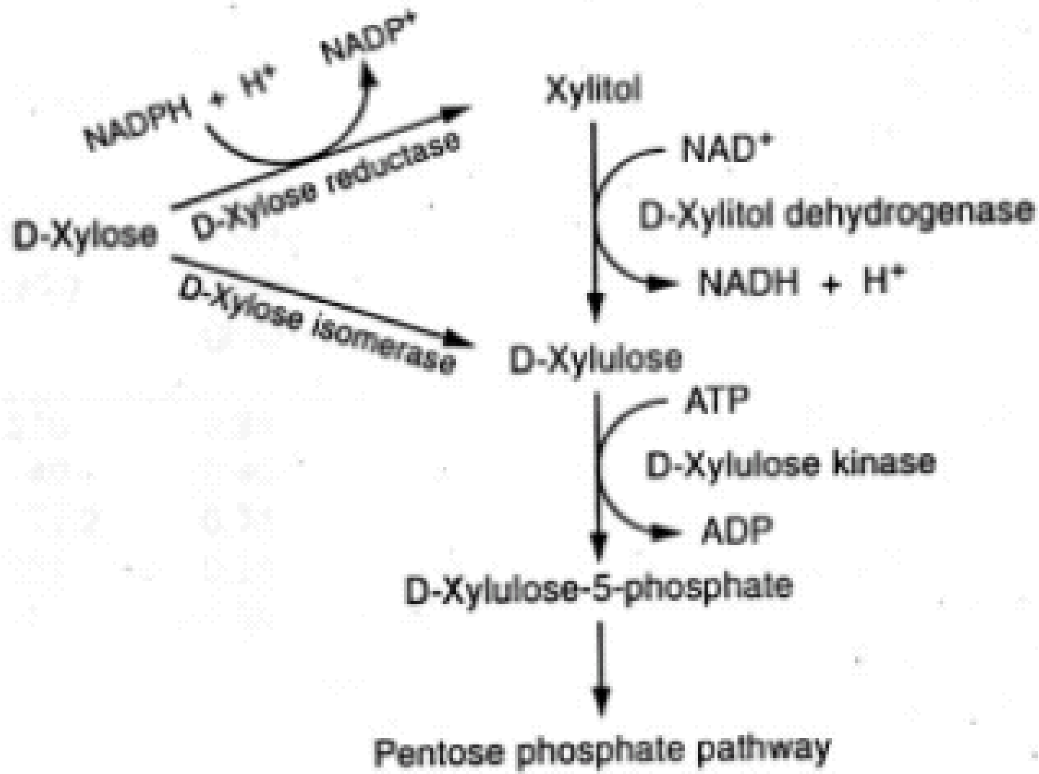
WHICH ETHANOLOGEN ?

ETHANOLOGEN TRAIT TABLE	*Saccharomyces cerevisiae*	*Scheffersomyces stipitis* (formerly *Pichia stipitis*)	*Candida Shahatae or Pachysolen tannophilus*	*Kluyveromyces marxianus*	*Escherichia coli* (FBR2)	*Zymomonas mobilis* (Zm4)
SUGARS METABOLIZED	glucose, sucrose, maltose, galactose, fructose,trehalose, isomaltose, raffinose, maltotriose, ribose, glucuronic acid and have been engineered to use lactose, xylose, arabinose	glucose, sucrose, maltose, galactose, fructose, trehalose, isomaltose, raffinose, maltotriose, ribose, glucuronic acid, lactose, xylose, arabinose, cellobiose, rhamnose, fucose, sorbose and maltotetrose	glucose, sucrose, maltose, galactose, fructose, raffinose, xylose, arabinose,	glucose, sucrose, maltose, galactose, fructose,trehalose, isomaltose, raffinose, maltotriose, xylose, arabinose, lactose	glucose, sucrose, maltose, galactose, fructose, glucuronic acid, galacturonic acid, xylose, arabinose, mannose	glucose, sucrose, maltose, galactose, lactose, fructose, xylose, arabinose, mellibiose, raffinose, mannose
SUGARS FERMENTED	glucose, sucrose, maltose, galactose, fructose,trehalose, isomaltose, raffinose, maltotriose,	glucose, sucrose, maltose, galactose, fructose, trehalose, isomaltose, raffinose, maltotriose, xylose, arabinose	glucose, sucrose, maltose, galactose, fructose,trehalose, isomaltose, raffinose, maltotriose, xylose, arabinose	glucose, sucrose, maltose, fructose, xylose, arabinose	glucose, sucrose, maltose, galactose, fructose, xylose, arabinose, mannose	glucose, sucrose, maltose, galactose, lactose, fructose, xylose, arabinose, mellibiose, raffinose, mannose
MAXIMUM TEMPERATURE GROWTH	<44⁰C	26-35⁰C	10-40⁰C	<40⁰C	<49⁰C	27-37.5⁰C
pH RANGE	3.0-8.0	4.0-7.5	3.0-7.5	4.8-6.3	4.8-6.3	5.5-6.8
ETHANOL PRODUCTION /TOLERANCE	15-21% / <22-23%	4.4-6.0% / <10%	3.5-3.8% / <4.6-4.8%	6.0-11.1 / <22.5%	4.38% / <5%	8.1-10.5% / <15%
CRABTREE TYPE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	N/A	N/A
GENOME SEQUENCED	YES	YES	NO	NO	YES	NO
FDA-CVM STATUS	GRAS	Possible GRAS	NOT GRAS	GRAS	NOT GRAS	NOT GRAS

W. Gibbons and S. Hughes, Springer Verlag In Vitro Plant Journal : Section on Cellulosic Biorefinery 2009

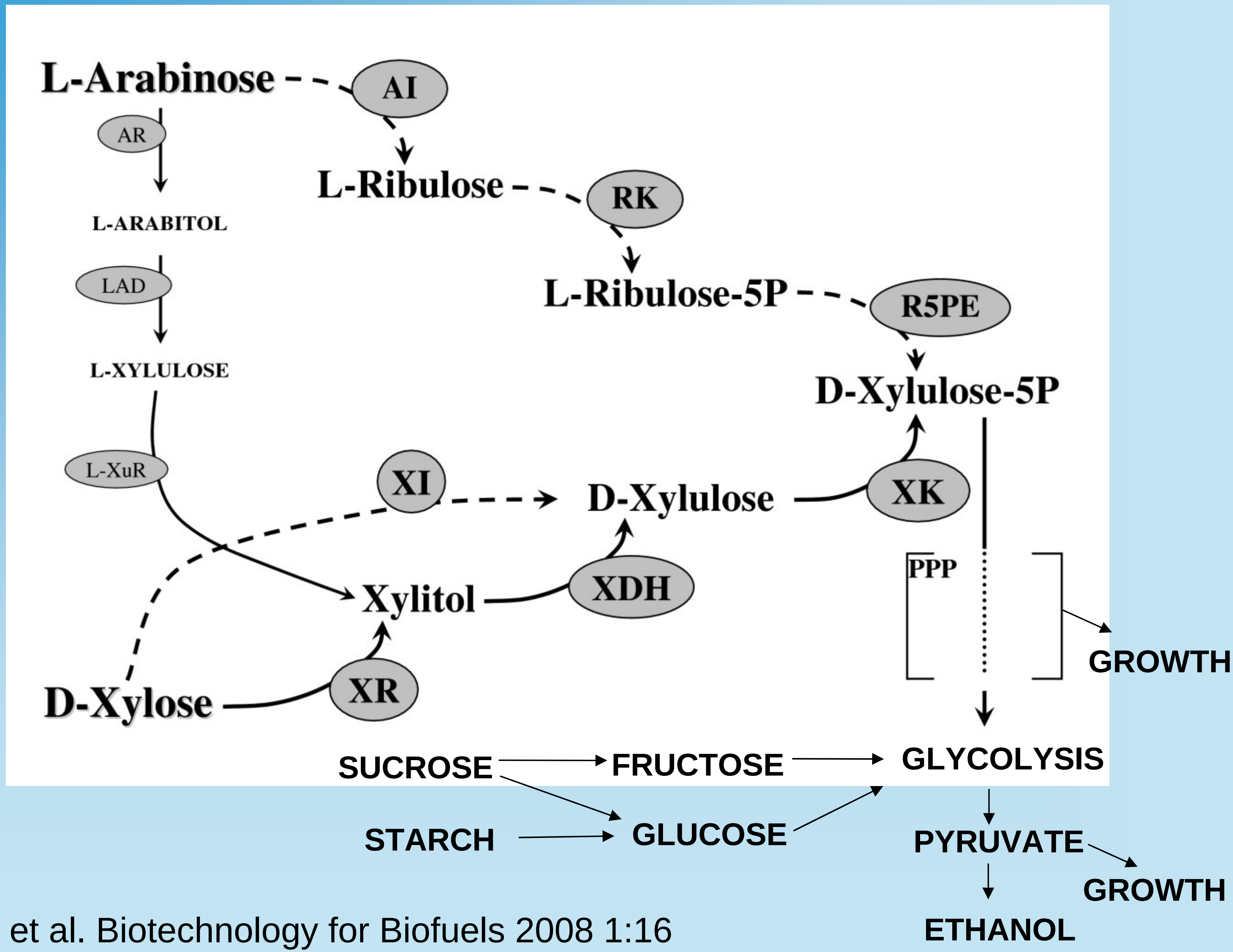
CELLULOSIC ETHANOL YEAST

(Saha, 1997)



NEED AN ENGINEERED YEAST

A GMAX-YEAST FOR CELLULOSIC ETHANOL FERMENTATION IN ADDITION TO OTHER ETHANOL FEEDSTOCKS?



Bettiga et al. Biotechnology for Biofuels 2008 1:16

Lignocellulosic Hydrolysate: Corn Plant Sugars

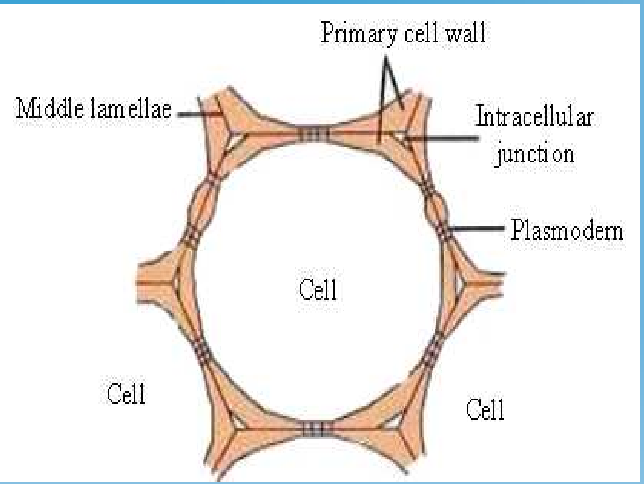
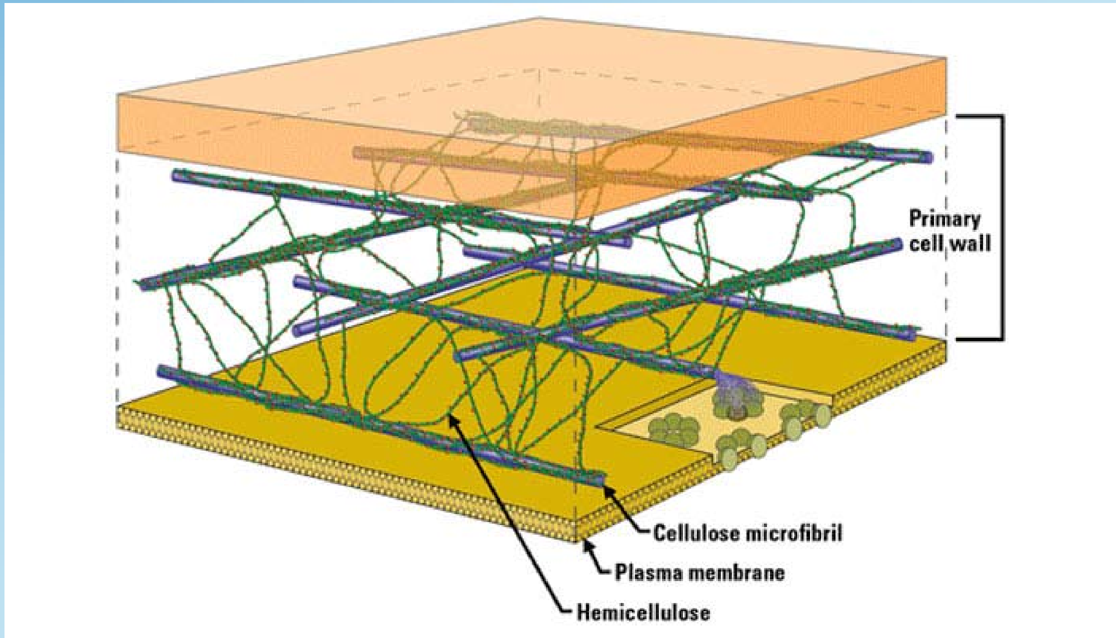
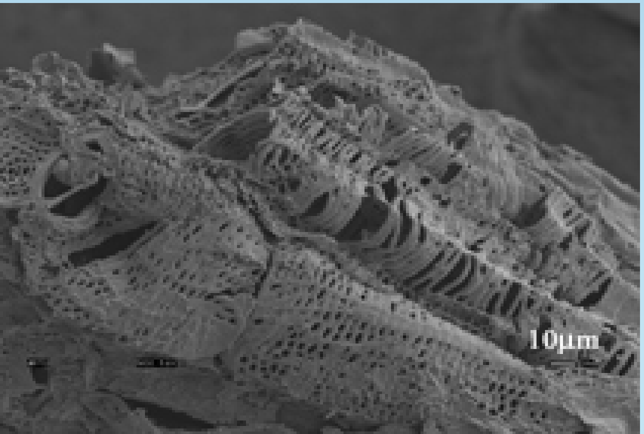


Table. Composition of defatted corn germ. From Timothy Leathers USDA ARS

	Wet mill	Dry mill
Glucose from glucoamylase digestion[a] (mg g^{-1} dry wt)	148 ± 7	227 ± 4
Protein (mg g^{-1} dry wt)	251 ± 2	180 ± 2
Neutral sugars in trifluoroacetic acid hydrolysates[c]		
Glucose (mg g^{-1} dry wt)	164 ± 9	285 ± 4
Xylose (mg g^{-1} dry wt)	101 ± 7	87± 2
Arabinose (mg g^{-1} dry wt)	110 ± 6	81± 15
Total (mg g^{-1} dry wt)	375 ± 13	453 ± 16

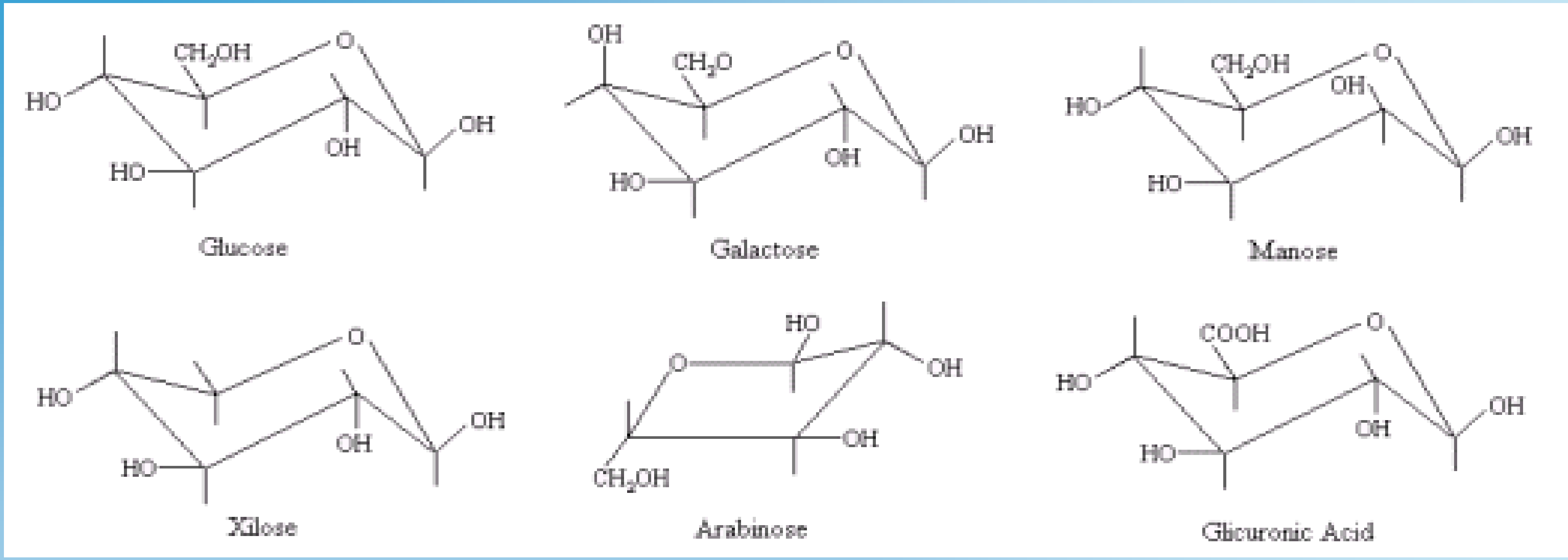
30% Glucose
20% Xylose
11% Arabinose
5% Galactose
3% Mannose
21% Lignin
10% Protein
(T. Leathers, USDA, ARS, NCAUR, BBC 1997)



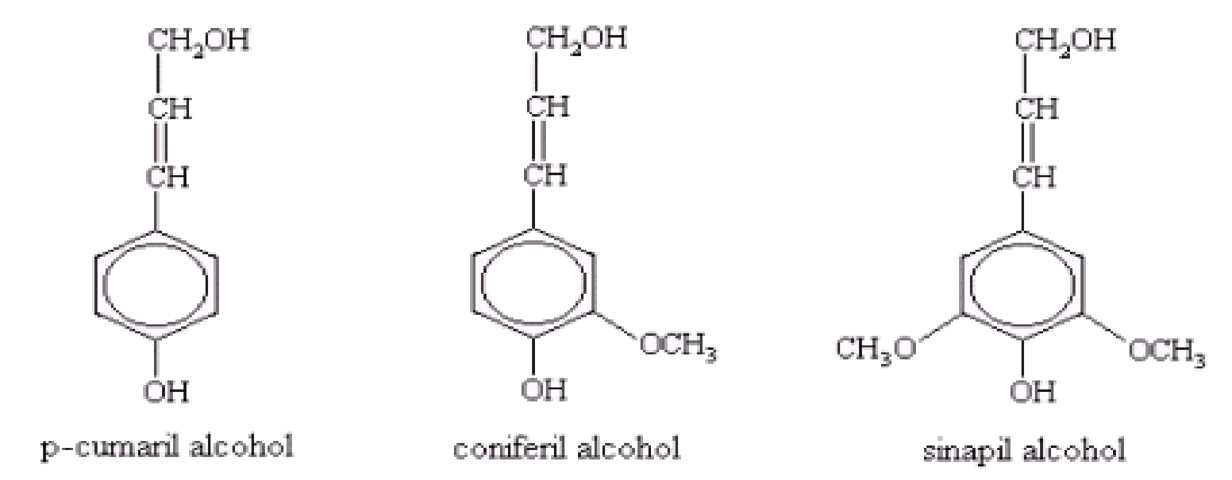


From Rose and Bennett (1999) Trends in Plant Sci. 4:176-183

ULTIMATELY: MONOMER SUGARS AND LIGNOL MOIETIES

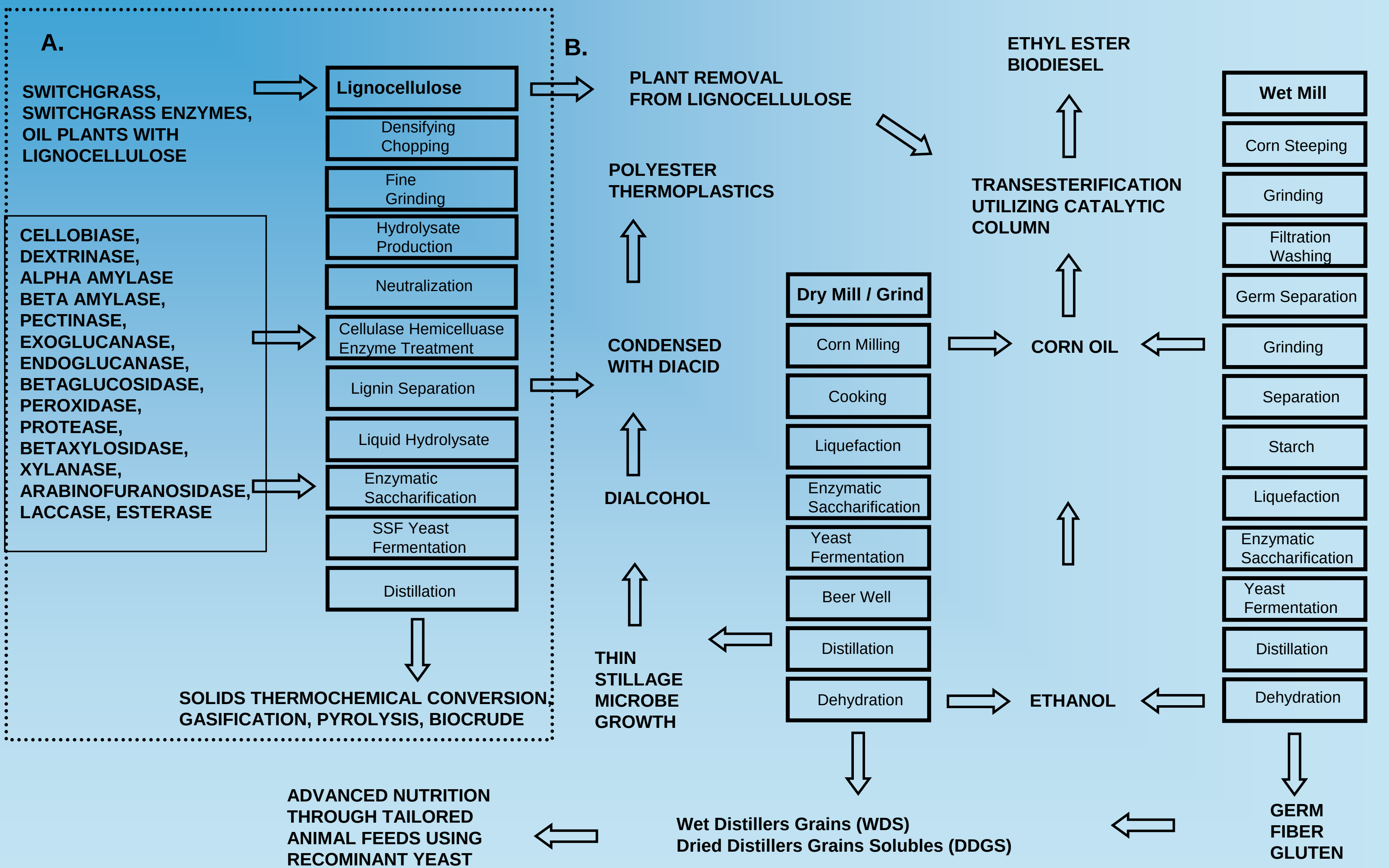


Chemical structures of the main components of cellulose and hemicellulose



Chemical structure of the main components of lignin

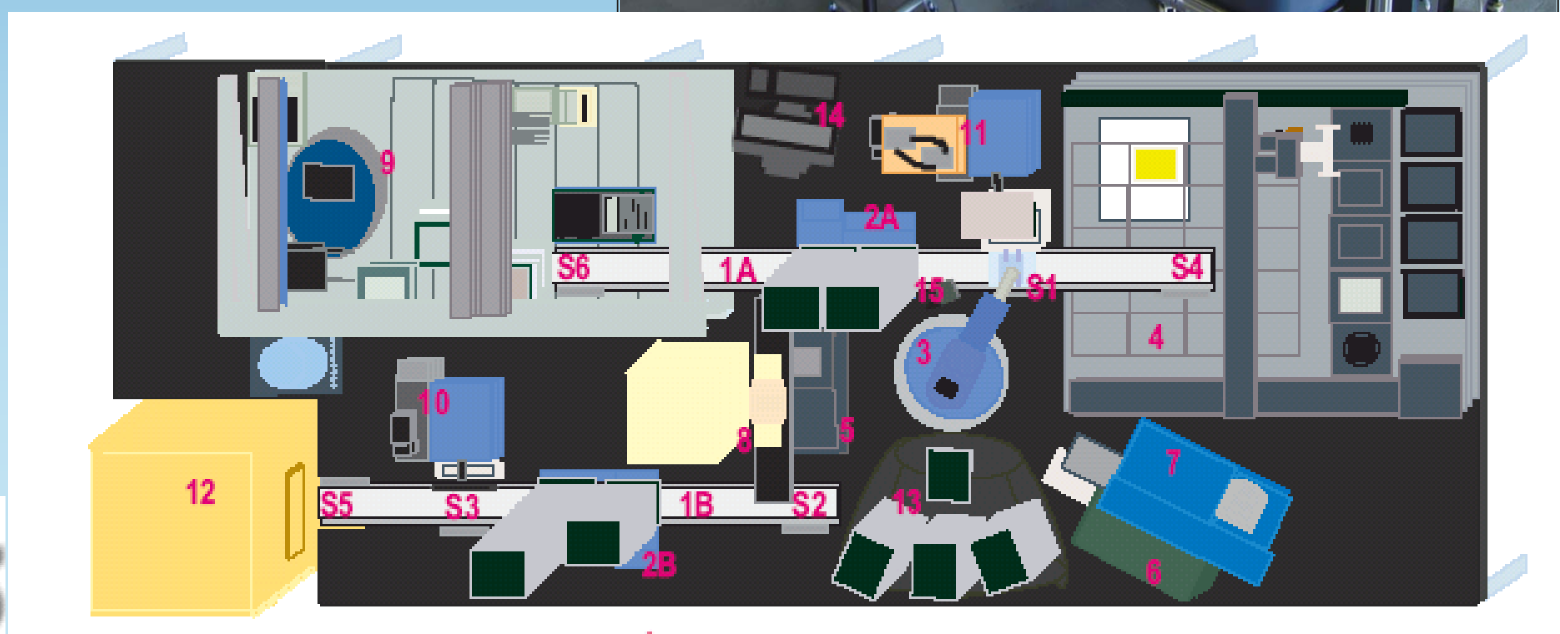
Future Crossover Refinery



S. Hutchinson, K. Tasaki, J. Thomas, S. Hughes, W. Gibbons, S. Kohl, South Dakota Biofuels Consortium Section of CBRD

USDA NCAUR Automated Integrated Plasmid-Based Functional Proteomic Workcell

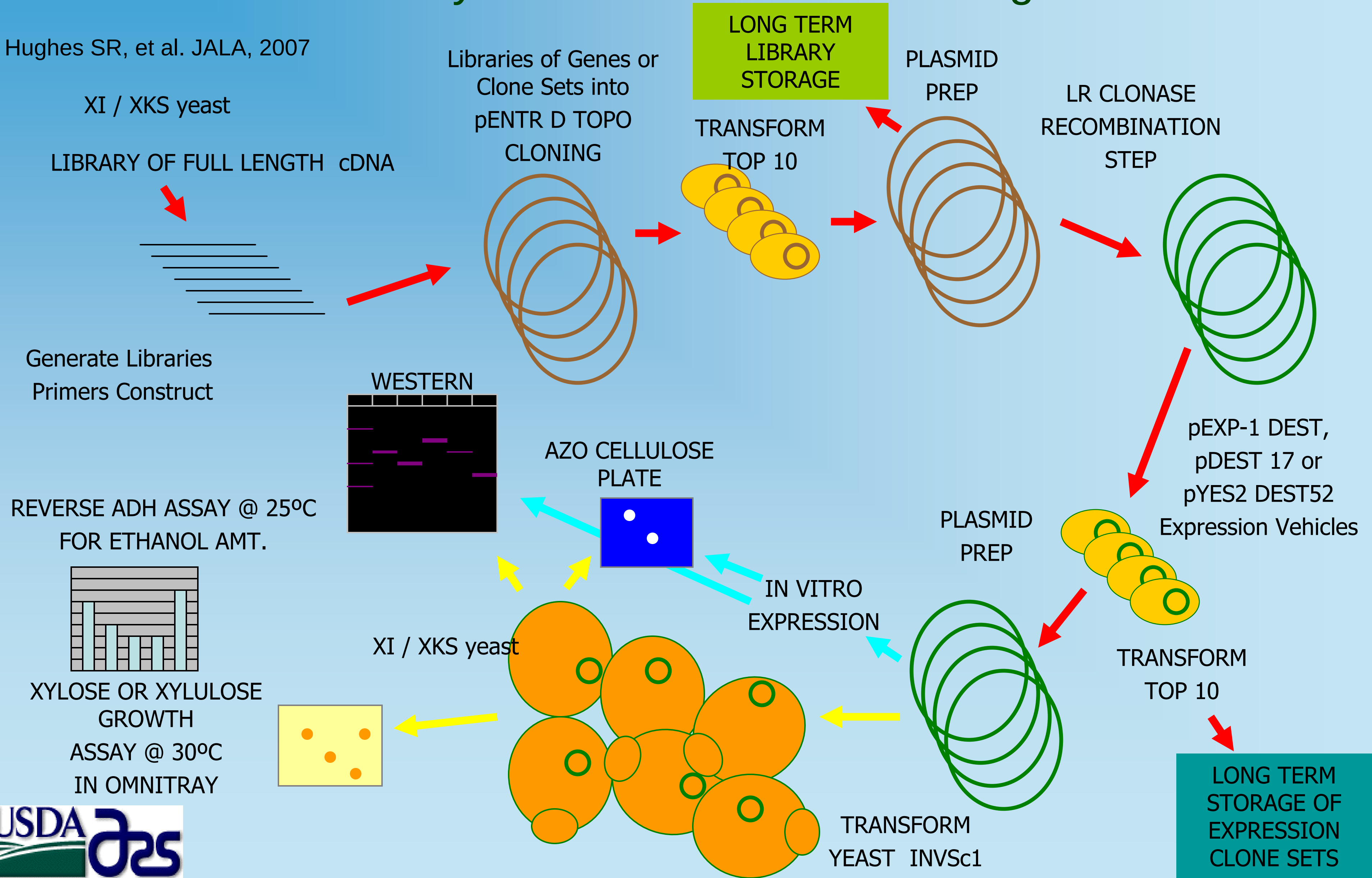
- **Genes Assembly**
- **Amino Acid Scanning Mutagenesis**
- **Mass Transformations**



USDA ars

Workcell Adapted Molecular Biology Paradigm Using Combination of TOPO ENTR Cloning of Libraries and Invitrogen Gateway® Recombinational Cloning

Hughes SR, et al. JALA, 2007



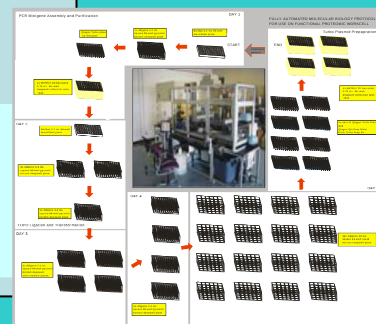
Engineer Yeast For Ethanol Production from Respective Feedstocks and Value-Added Products

Core Automated
HTP-Molecular Biology

Xylose Isomerase
Xylulokinase

Hemicellulases
Cellulases

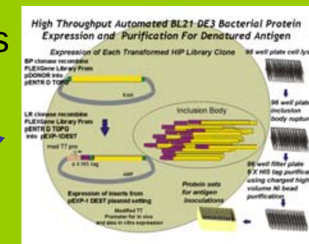
Fungal & Bacterial
cDNA Libraries



S cerevisiae
K Marxianus

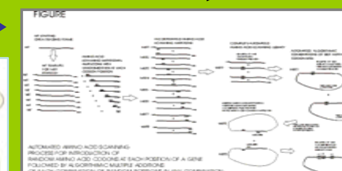
Plasmid-Based
Functional Proteomics

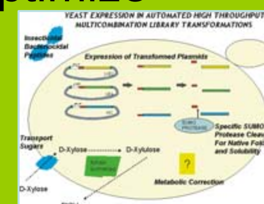
soluble



active, stable

Assemble
Express
ORF
Optimize





indirect gene analysis

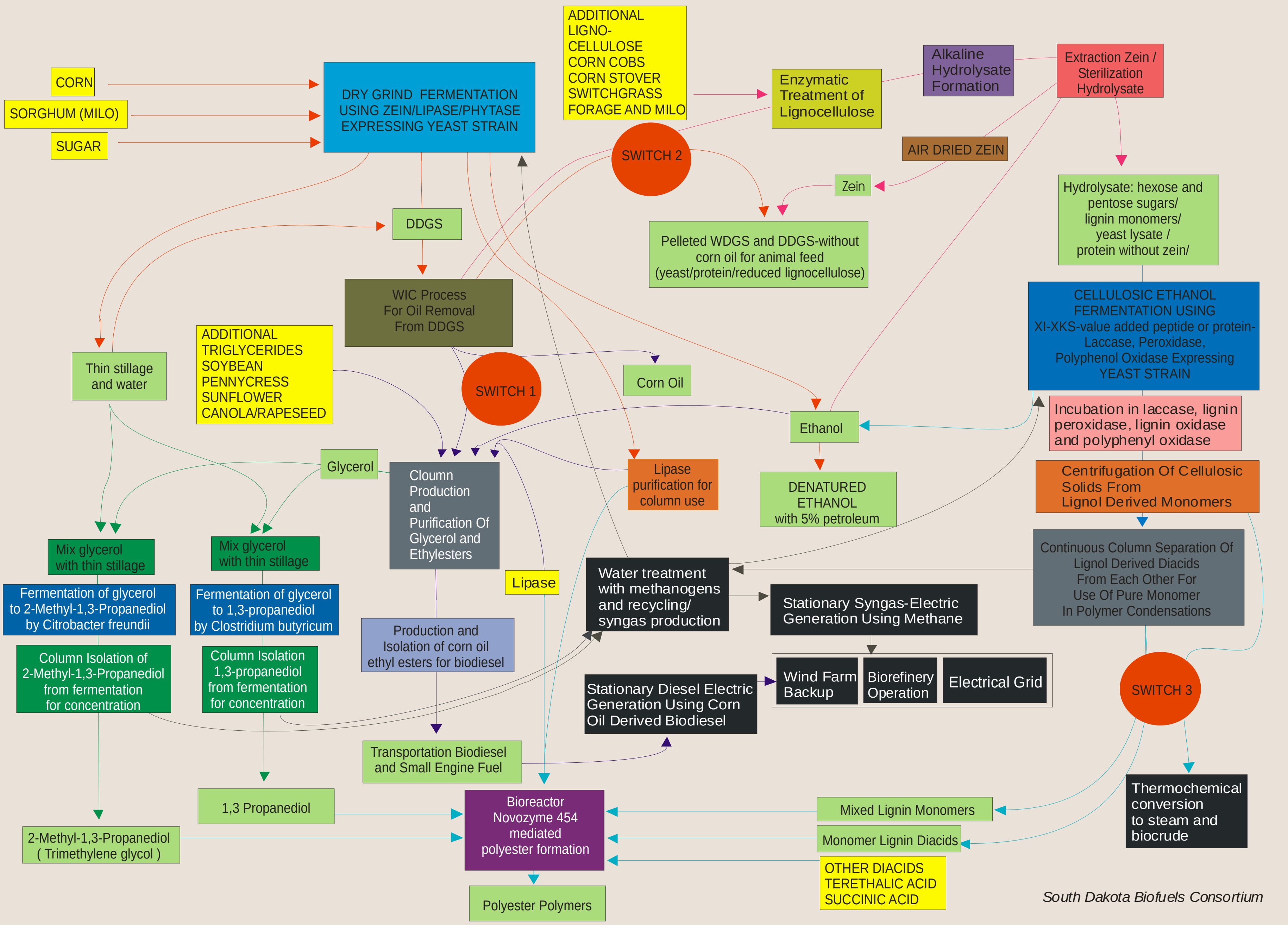
direct gene insertion
strain production

Mass Transform
Strain Improvements
Proteomic Arrays

★ Need Process: USDA proposal for cellulosic ethanologen According to National Program 213 (formerly NP307):
http://arsserv0.tamu.edu/research/programs/programs.htm?NP_CODE=307

USDA

CROSSOVER BIOREFINERY DESIGN FOR COMBINED STARCH ETHANOL/ CELLULOSIC ETHANOL OPERATION CONCURRENT WITH BIODIESEL PRODUCTION AND POLYESTER PRODUCTION



AEROBIC YEAST GROWTH ON XYLOSE USING XX-STRAIN

- 6 Hr. Doubling Time on Xylose
- 2 Hr. Doubling Time on Glucose

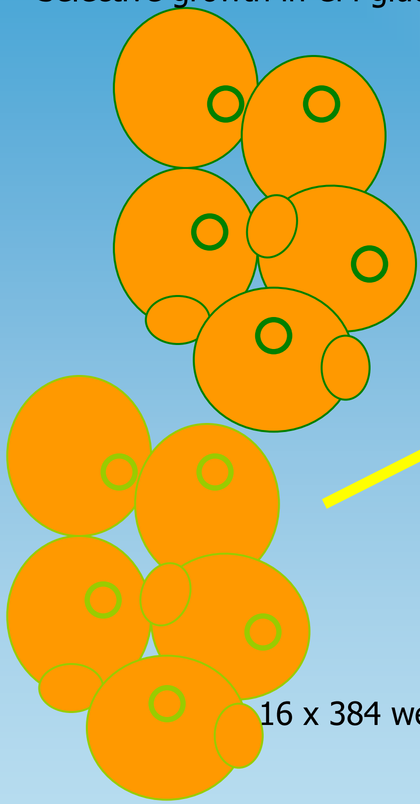
ANAEROBIC GROWTH OF *Saccharomyces cerevisiae*

- What gene is needed?
- What strategy can find this gene?
- Can this be added to the fast growing xylose utilizing S. cerevisiae XI-XKS-INVSc1?

Robotic Screening Using Yeast Mating Paradigm To Isolate Anaerobic Diploid Yeast

XI expressing pJ694 MATa haploid

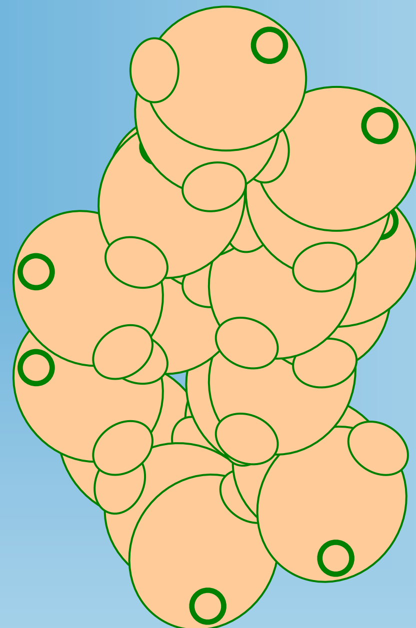
Selective growth in CM glucose - TRP



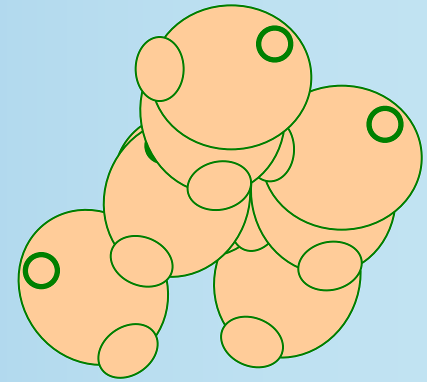
16 x 384 well plates

5632 Full Length Yeast Clone Expression Library pJ694 MATalpha haploid

Selective growth in CM glucose - LEU



Diploid Mated pJ694 with XI and Library Clone in YPD



Diploid Mated pJ694

Selective growth in CM glucose -TRP-LEU

Diploid Mated pJ694

Selective growth in CM xylose -TRP-LEU

Incubation at 30C

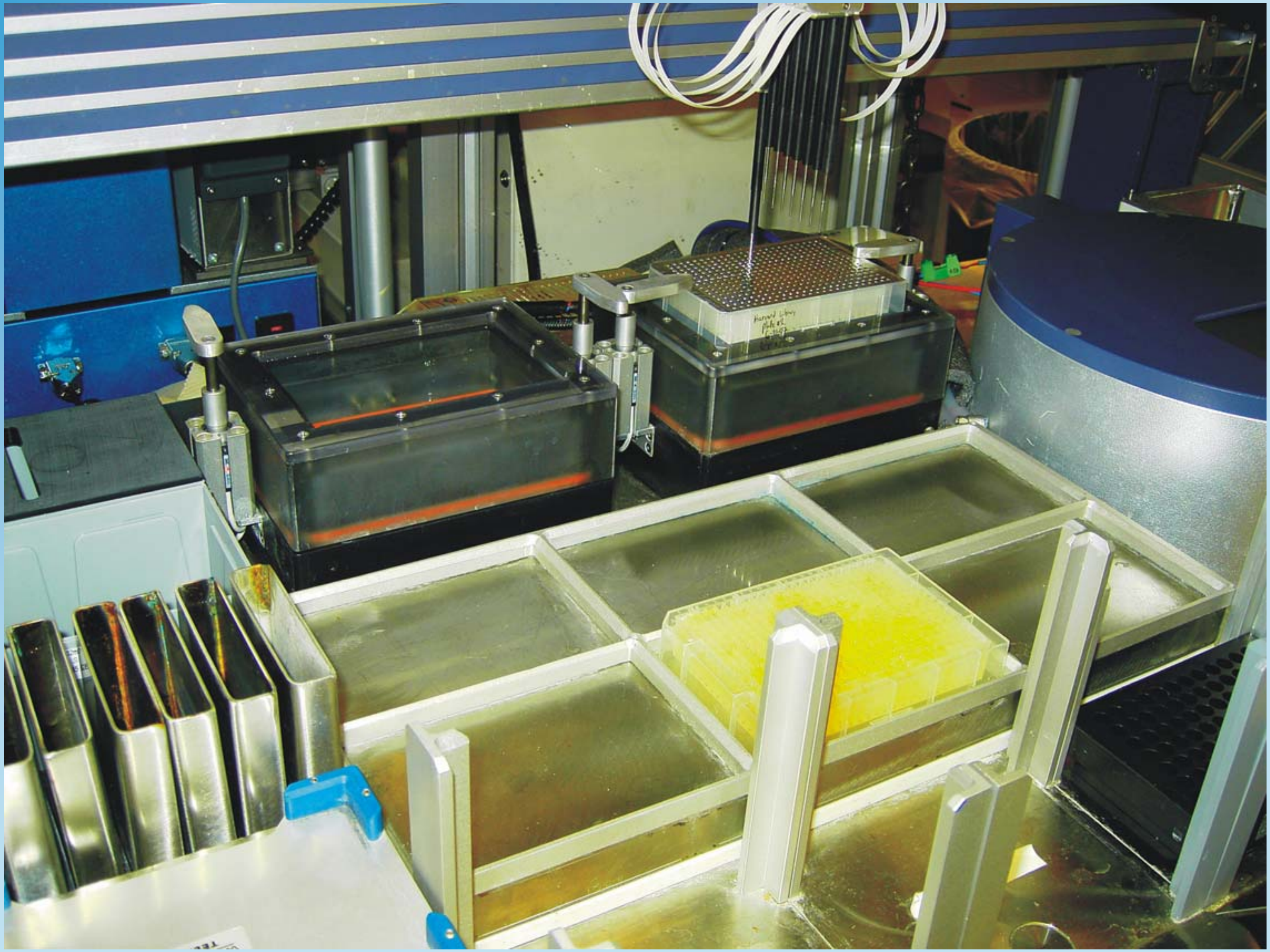
Fully Anaerobic Conditions

Isolate Colonies that grow Anaerobically on Xylose

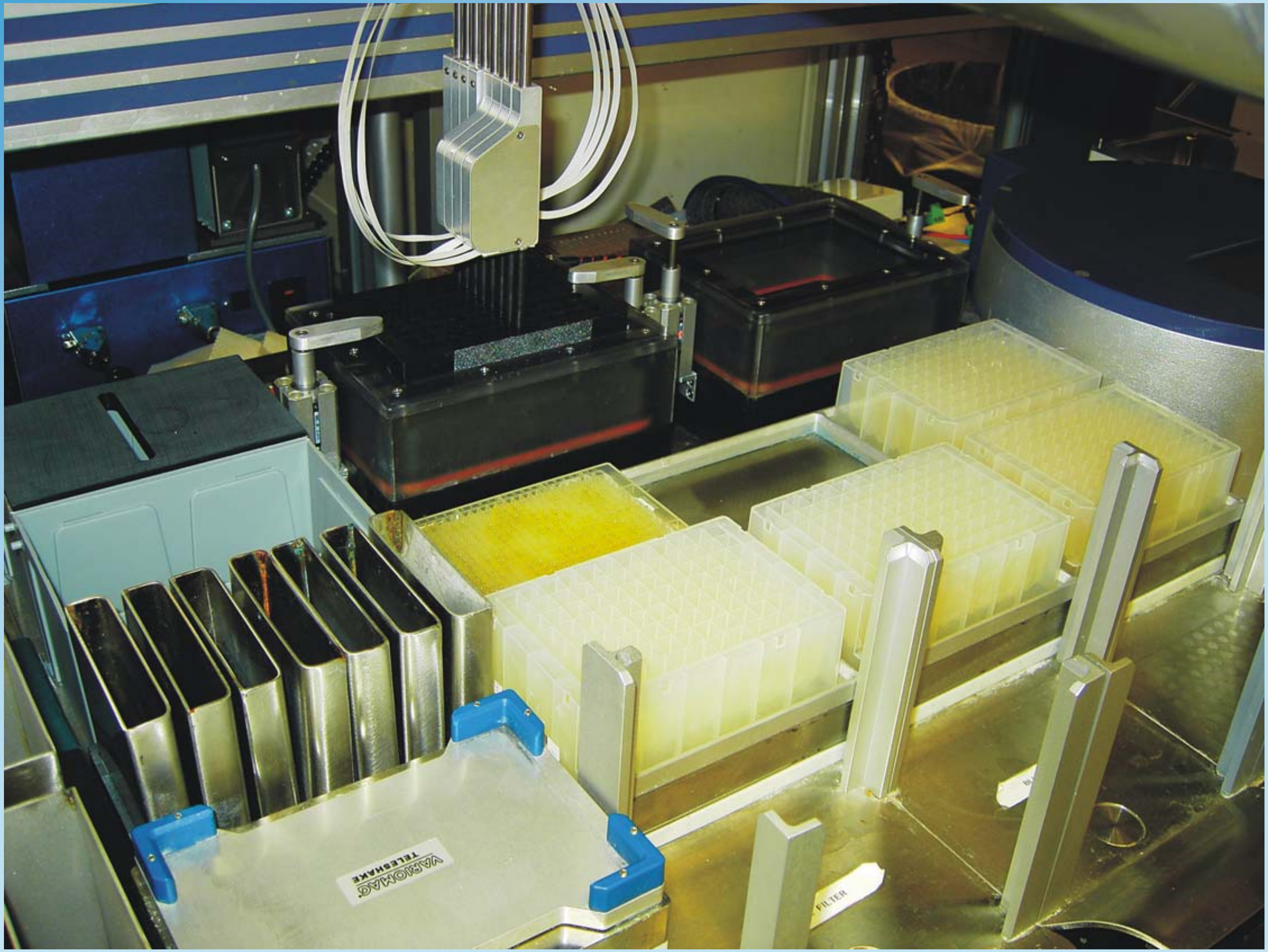
Glycerol Stocks Made Microaerophilic Glucose

Glycerol Stocks Used to Start 300 mL Microaerophilic Xylose Growth

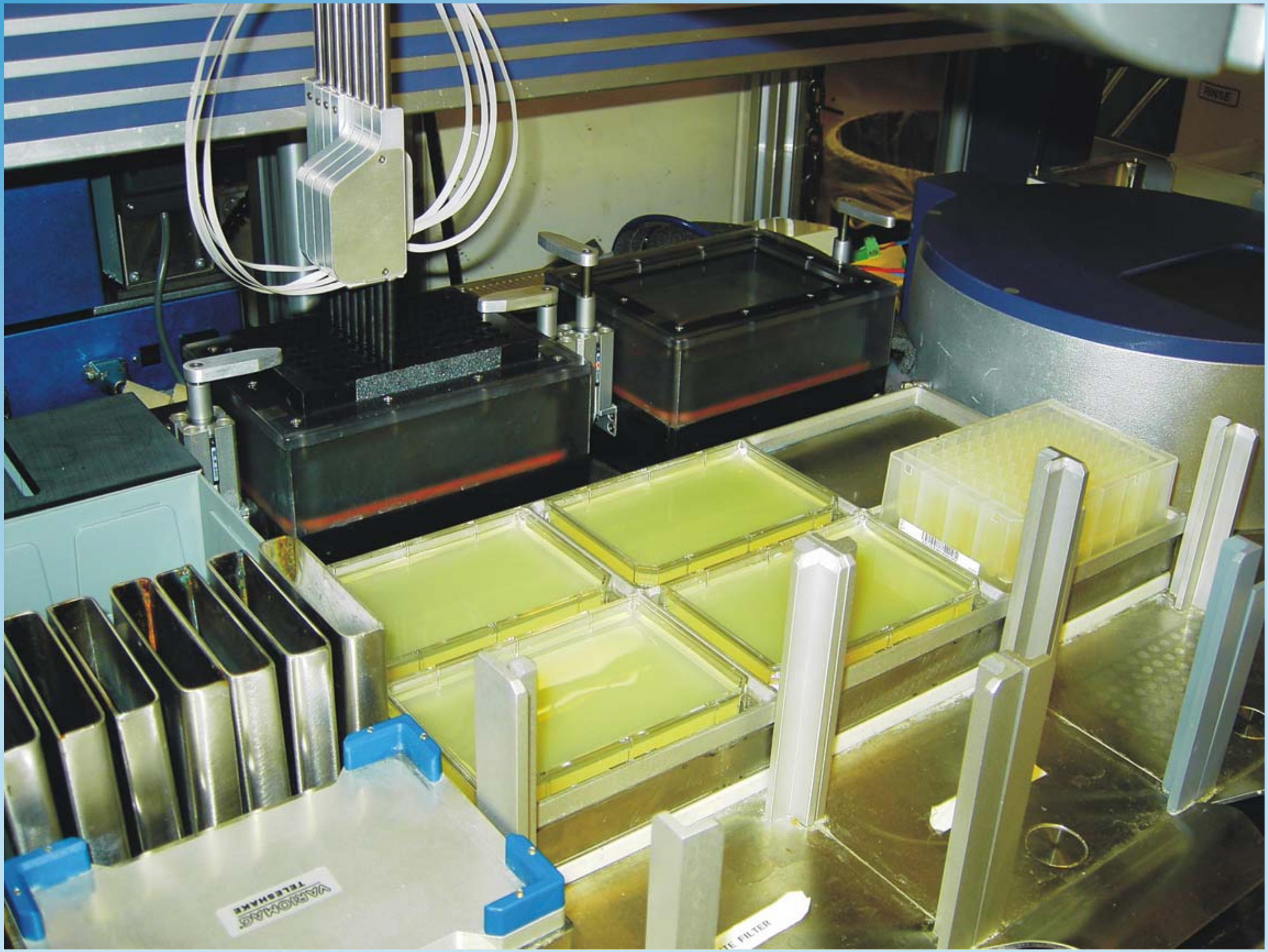
Yeast Mating

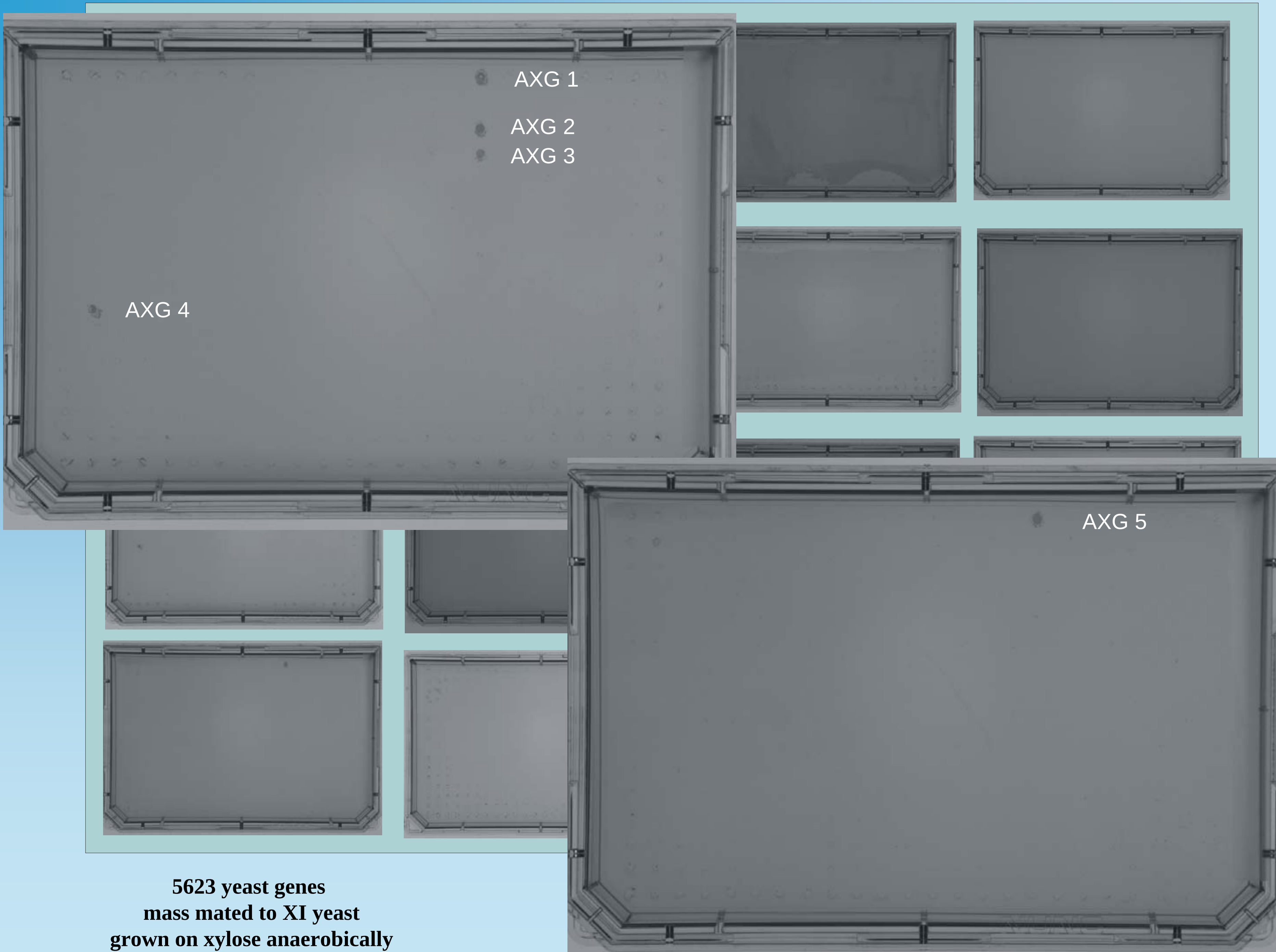


Broadcast Matings Into Yeast Selective Medium and Make Storage Glycerol Stocks



Spot Onto Xylose Anaerobic Plates

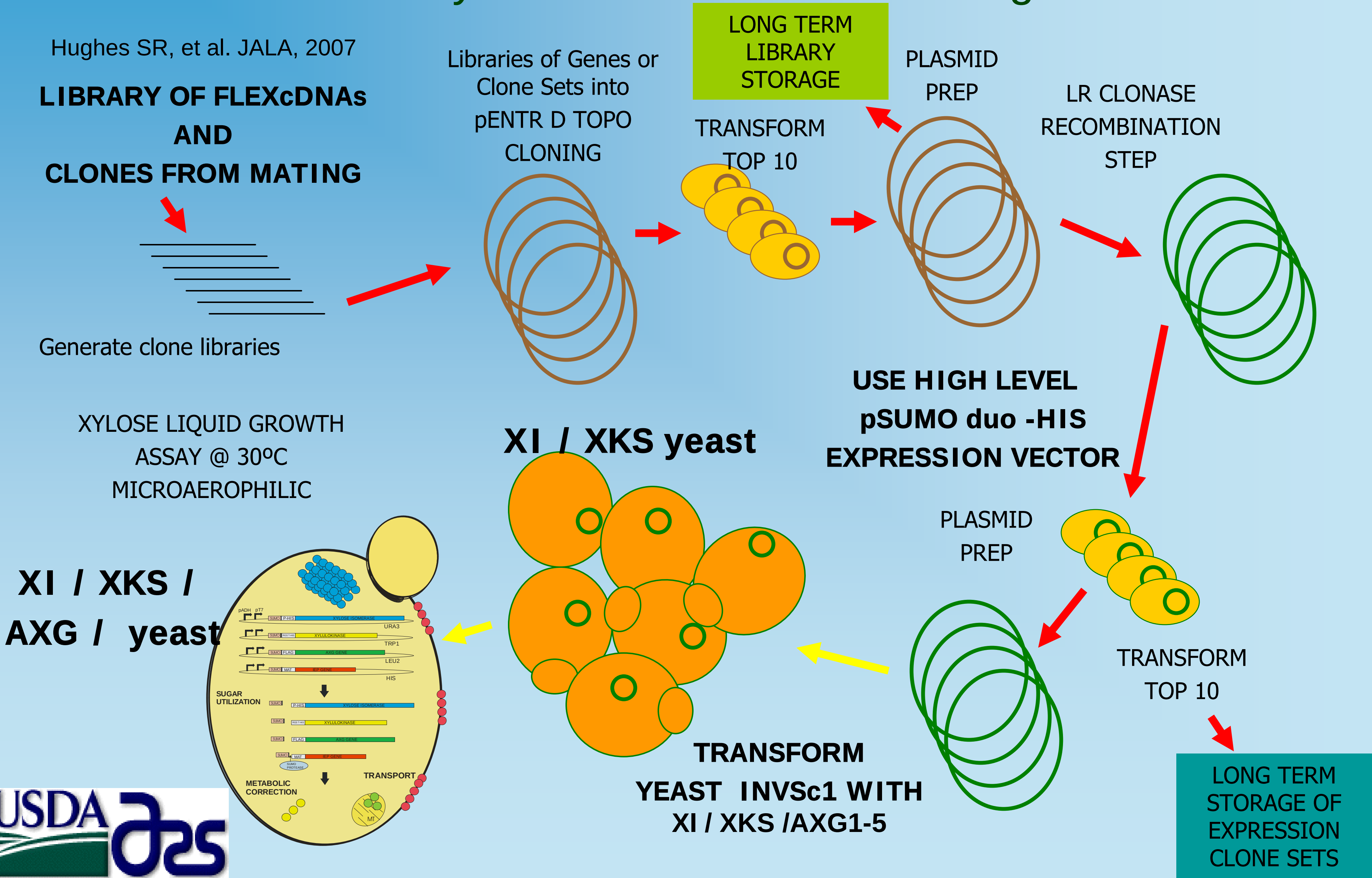




5623 yeast genes
mass mated to XI yeast
grown on xylose anaerobically

Workcell Adapted Molecular Biology Paradigm Using Combination of TOPO ENTR Cloning of Libraries and Invitrogen Gateway® Recombinational Cloning

Hughes SR, et al. JALA, 2007



Added
Saccharomyces cerevisiae

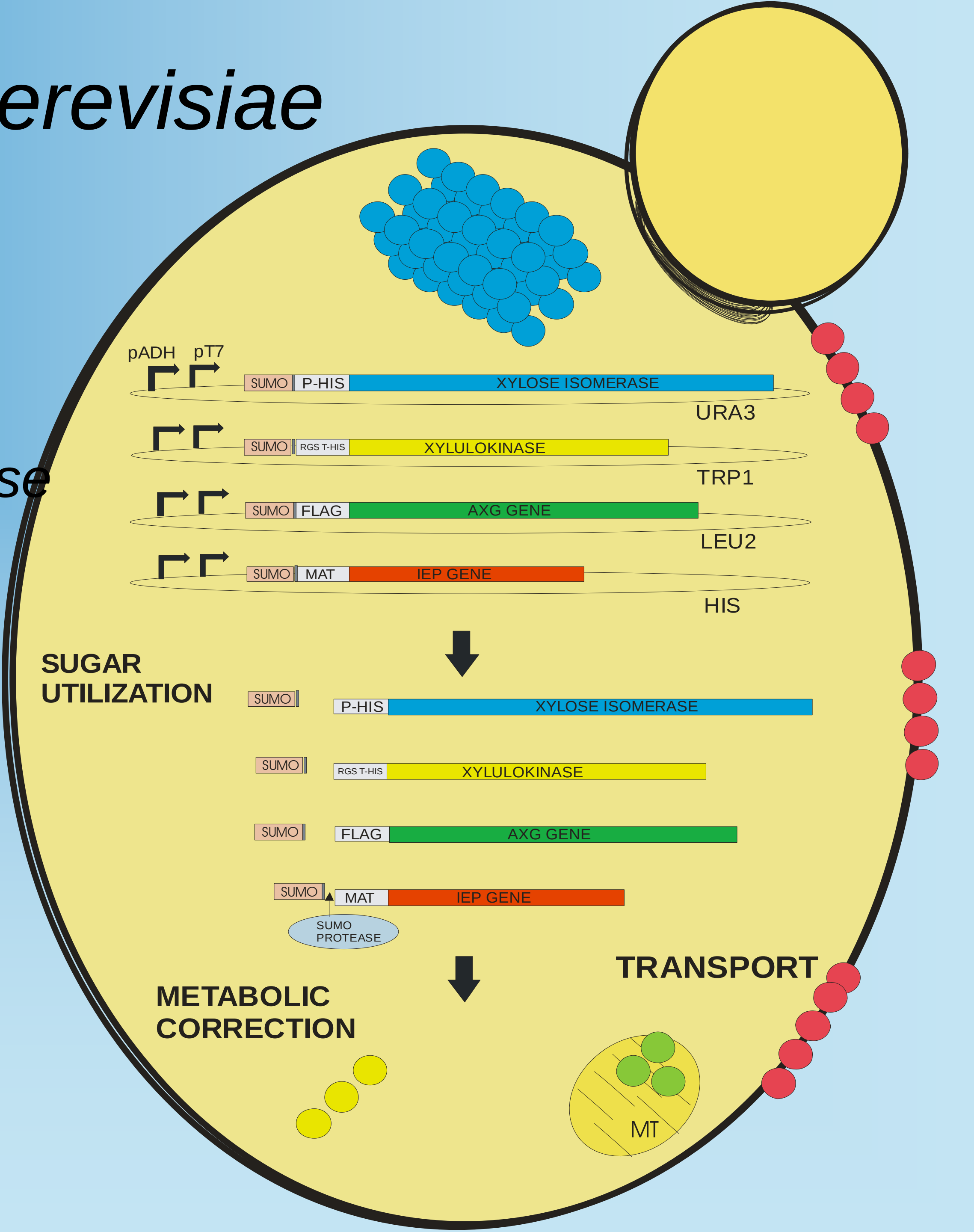
The GMAX yeast

Glucose Mannose Arabinose
Xylose Utilization

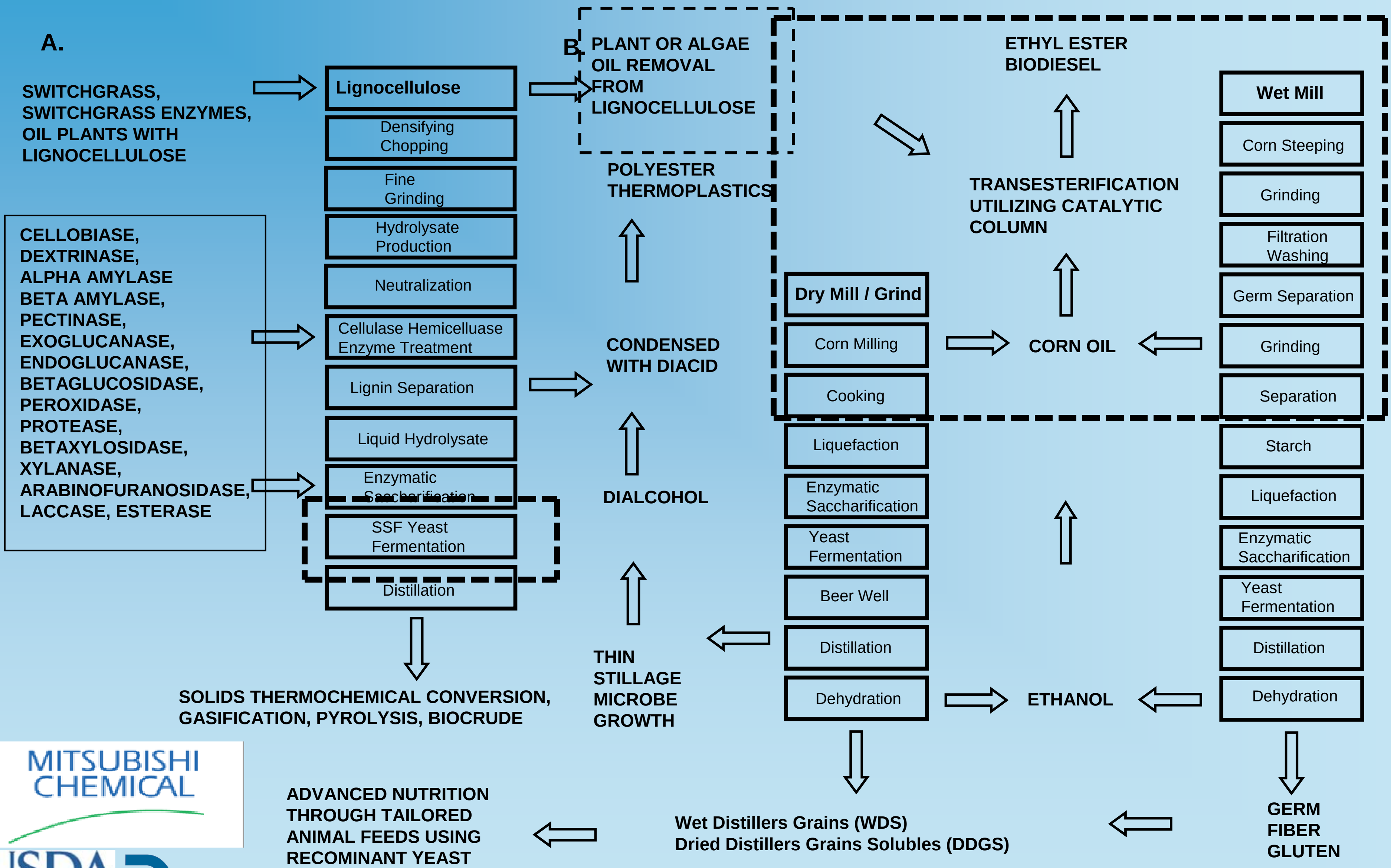
Low xylitol production

Fastest anaerobic growth

Ethanol Production



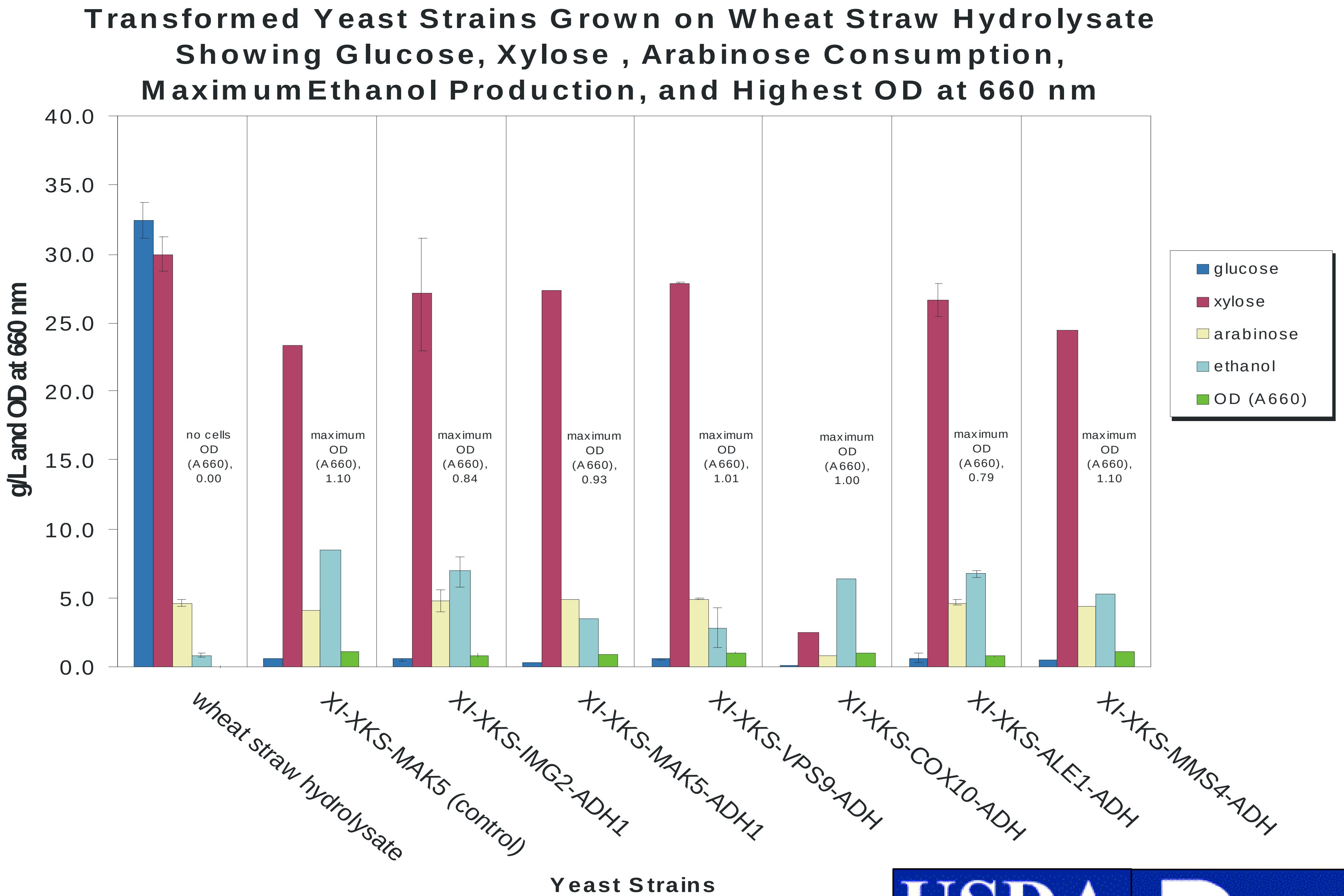
GMAX-L Yeast Crossover Refinery



MITSUBISHI CHEMICAL

USDA ars

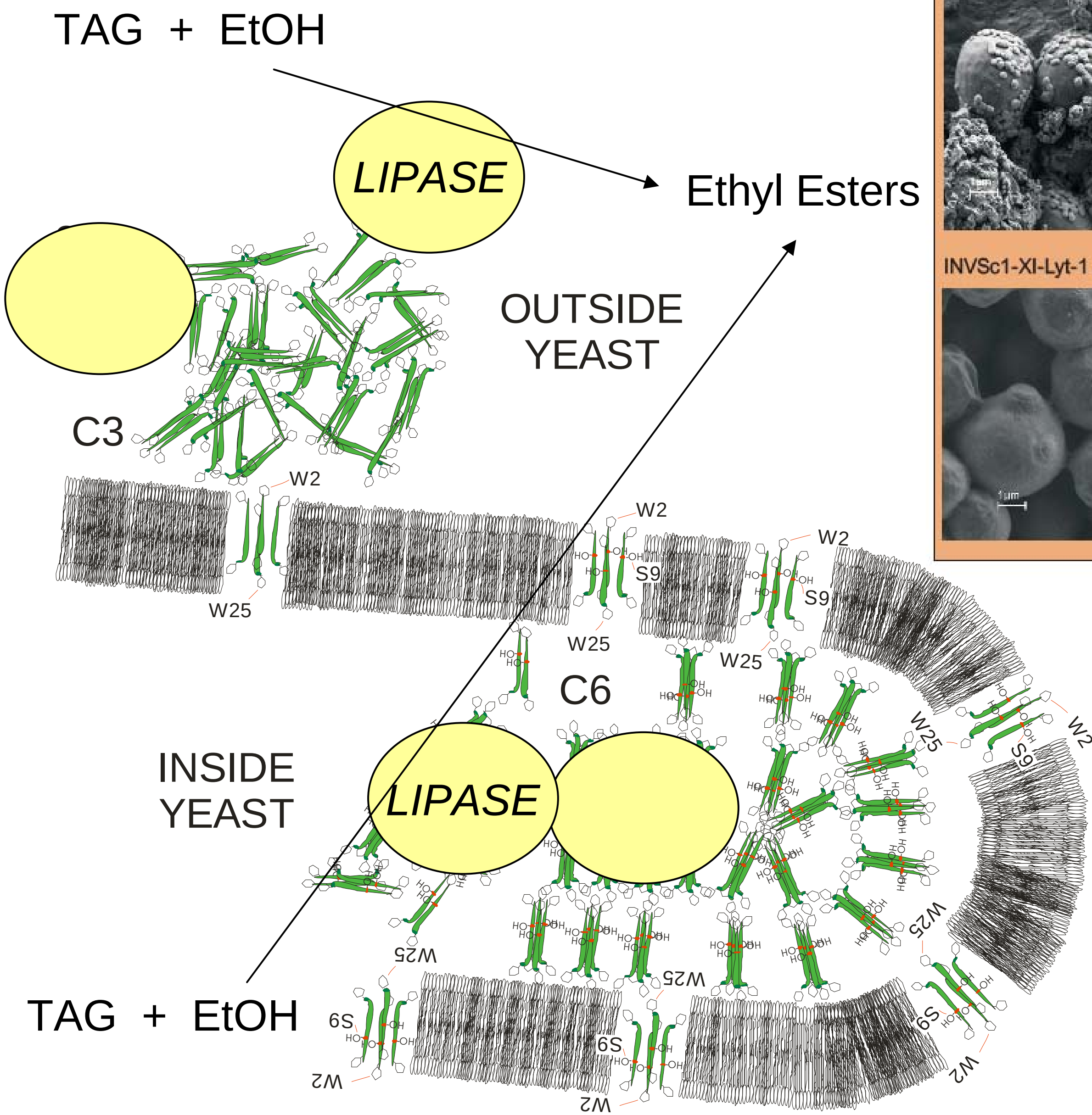
S. Hutchinson, K. Tasaki, J. Thomas, S. Hughes, W. Gibbons, S. Kohl, South Dakota Biofuels Consortium Section of CBRD

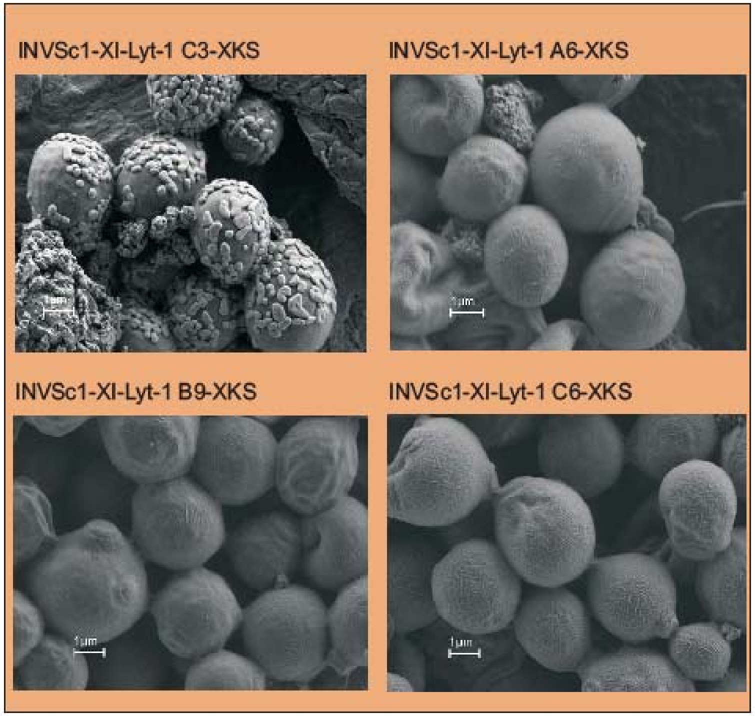


true cellulosic ethanol strain

Hughes et al. JALA 2009 b

LIPASE-LYT-1 Yeast Model and SEM of Lyt-1 Yeast





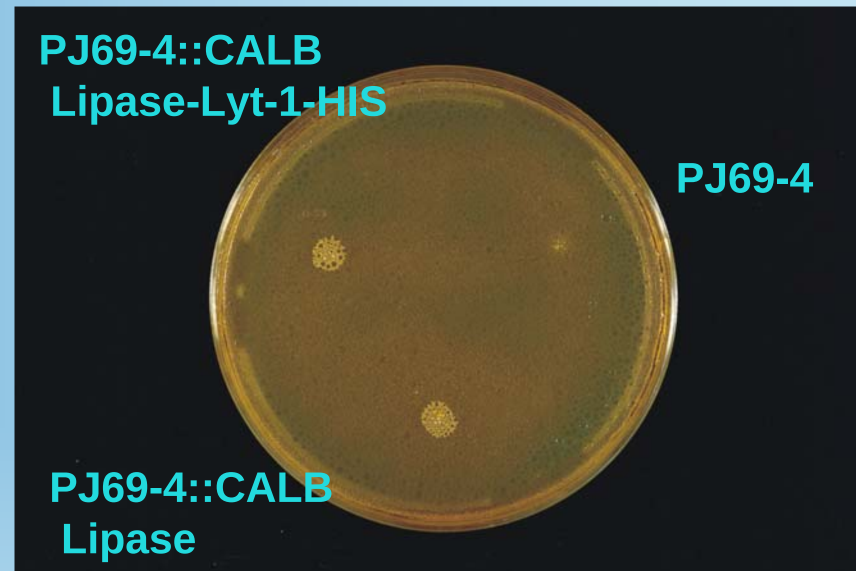
MITSUBISHI CHEMICAL

USDA

External CALB Lipase-Lyt-1-HIS Yeast Crawls On Dry Grind Corn Oil and Internal CALB Lipase Yeast Does Not

CM galactose plate all amino acids

CM glucose plate -URA

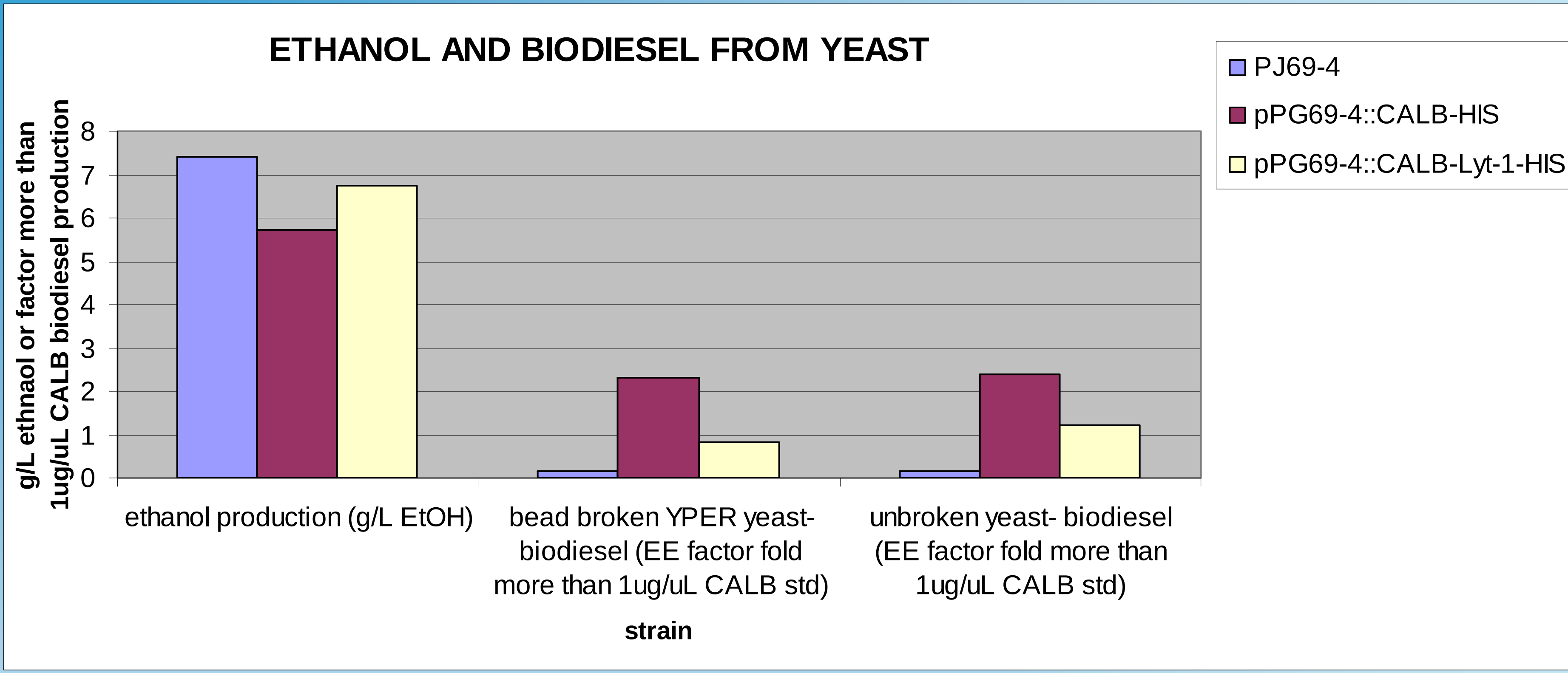


INCUBATION 30ºC 5 DAYS

MITSUBISHI CHEMICAL

USDA ars

Coproduction of Ethanol and Biodiesel From Corn Oil and Ethanol Using *Saccharomyces cerevisiae* Biocatalyst



MITSUBISHI CHEMICAL

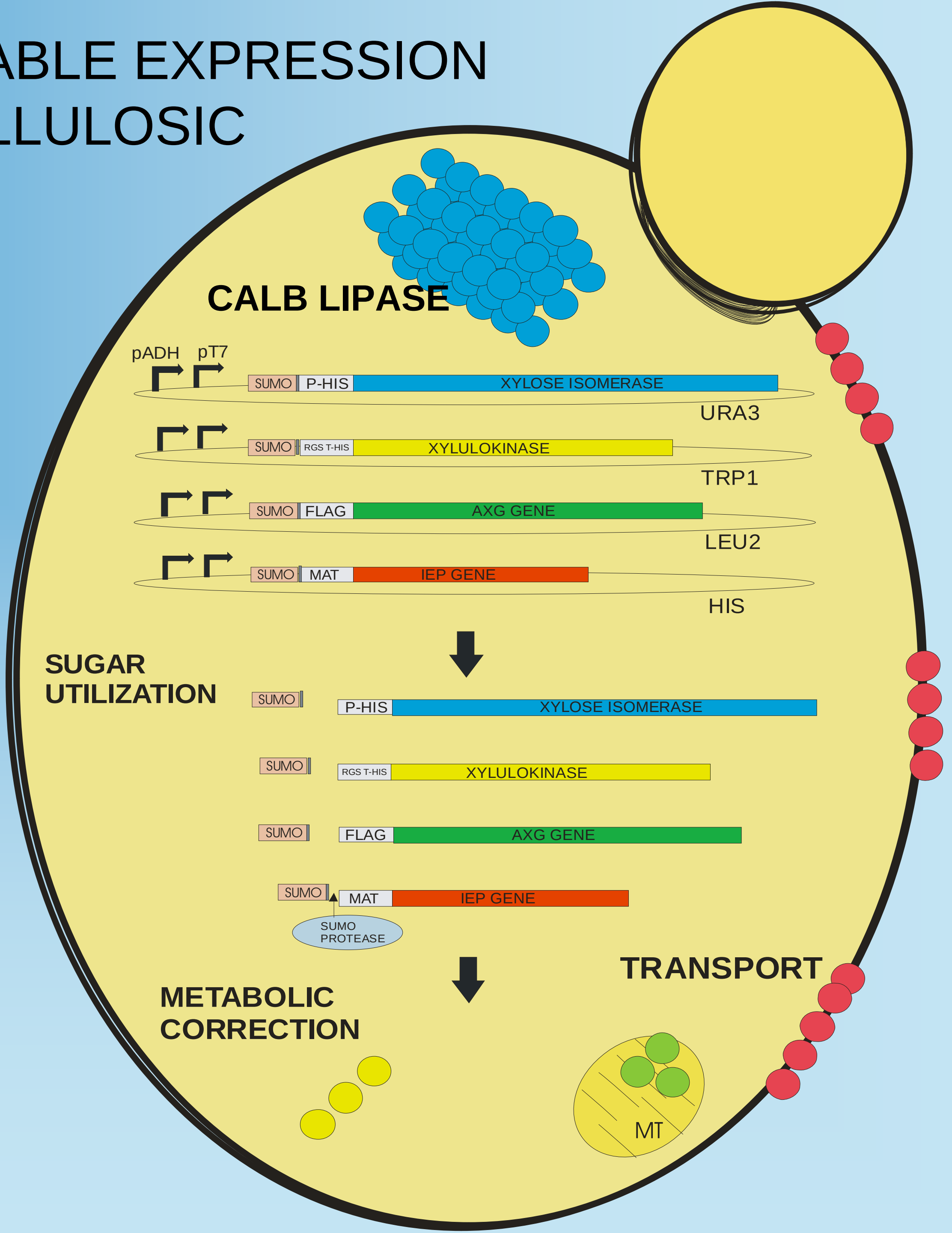
NCAUR
Where the future is Created

USDA ARS

MITSUBISHI ETHANOL AND BIODIESEL REFINERY USING COLUMN PROCESS

GMAX-L: ALLOWS STABLE EXPRESSION OF CALB LIPASE IN CELLULOSIC YEAST BACKGROUND AFTER ETHANOL PRODUCTION HAS COMPLETED

HIGH LEVEL EXPRESSION INSIDE CALB LIPASE



PLANT COSTS COULD BE LOW

Ethanol Refinery Cost Comparison	Wet Mill	Dry Mill / Grind	Sugarcane	Lignocellulose	Crossover Refinery
AVG. COST OF PLANT	$233.84 million	$115.5 million	$62.5 million	>$375.00 million	>$200.00 million
LIFESPAN OF PLANT	>60 years	30-60 years	40-60 years	continuous	continuous
PRICE FEEDSTOCK	$188.46 mt	$188.46 mt	$42.00 mt	$95.00 mt	$<50.00 mt
PRODUCTION COSTS	$0.88/gallon	$0.71/gallon	$0.54/gallon	experimental	experimental
COST ENZYMES	$0.06/gallon	$0.06/gallon	<$0.01/gallon	$0.30/gallon	potentially $0
TOTAL ETHANOL PROFIT	$2.56 billion	$16.77 billion	$46.65 billion	$0.051 billion	> $70 billion
TOTAL COPRODUCT PROFIT	$5.05 billion	$9.80 billion	$6.64 billion	experimental	> $100 billion

264 billion barrels

US security

US dependant

Short term amounts (2052)

Replace petroleum products:

oil
diesel
tar
plastics
olefins
alkanes
C3-C8
alkenes

DEPENDENCY=SECURITY/SUSTAINABILITY THE BIOFUEL BENEFITS

Henry Ford designed the famed Model T Ford to run on alcohol – he said it was "the fuel of the future".

Powered by a 2.9-litre, four-cylinder engine with a two-speed transmission, the Model T was simple and reliable, but surprisingly fast for its day. Top speed was around 45mph, with fuel economy of around 40mpg depending on how the car was driven. It was originally designed to run on bio ethanol, but the decreasing cost of oil (and US prohibition) meant that most were run on oil-derived petrol. The standard 4-seat open tourer of 1909 cost US$850

Conclusion

-World Ethanol Production Will Climb Dramatically. With World Production Reaching 25 Billion Gallons By 2015

-US Energy Independence and Security Act Will Require Large Amounts of Biofuels To be Produced From Cellulosic, Starch, and Sucrose Feedstocks

-Ethanol alone is not profitable and valuable coproducts will make biorefineries sustainable

-Better strains needed quickly. Advanced Biorefineries Could Produce Fuels, Plastic, Chemicals, and Animal Feed Simultaneously Using Engineered *Saccahromyces cerevisiae*

Acknowledgements

USDA, ARS, NCAUR, Peoria
Fermentation Biotechnology Group
Michael Cotta
Xin-Liang Li
Jeff Mertens
Doug Jordan
Ronald Hector
Bruce Dien

USDA, ARS, NCAUR, Peoria
Molecular Genetics and Bioprocessing
Clete Kurtzman
Jody Robinson
Jennifer Steele

Mitsubishi Chemical Corp.Inc.
LifeSensors, Inc.
Hudson Control Group, Inc.
VeraSun Bioenergy, Inc.
ICM, Inc.
Harvard Institute of Proteomics
Washington University
Arryx, Inc

USDA, ARS, NCAUR, Peoria
Crop Bioprotection Group
Patricia Slininger
Patrick Dowd
Eric Johnson

USDA, ARS, NCAUR, Peoria
Food and Industrial Oils
Ken Doll
Bryan Moser

USDA, ARS, NCAUR, Peoria
Bioproducts and Biocatalysts
Joseph Rich
Timothy Leathers
Ken Bischoff
Siqing Liu
John Jackson
John Lagazo
Watson Chau
Amanda Harmsen
Paige Pearson
Elby Joe Cox

Thank you

MITSUBISHI
CHEMICAL

USDA ars