

Great Lakes Bioenergy Research Center (GLBRC)

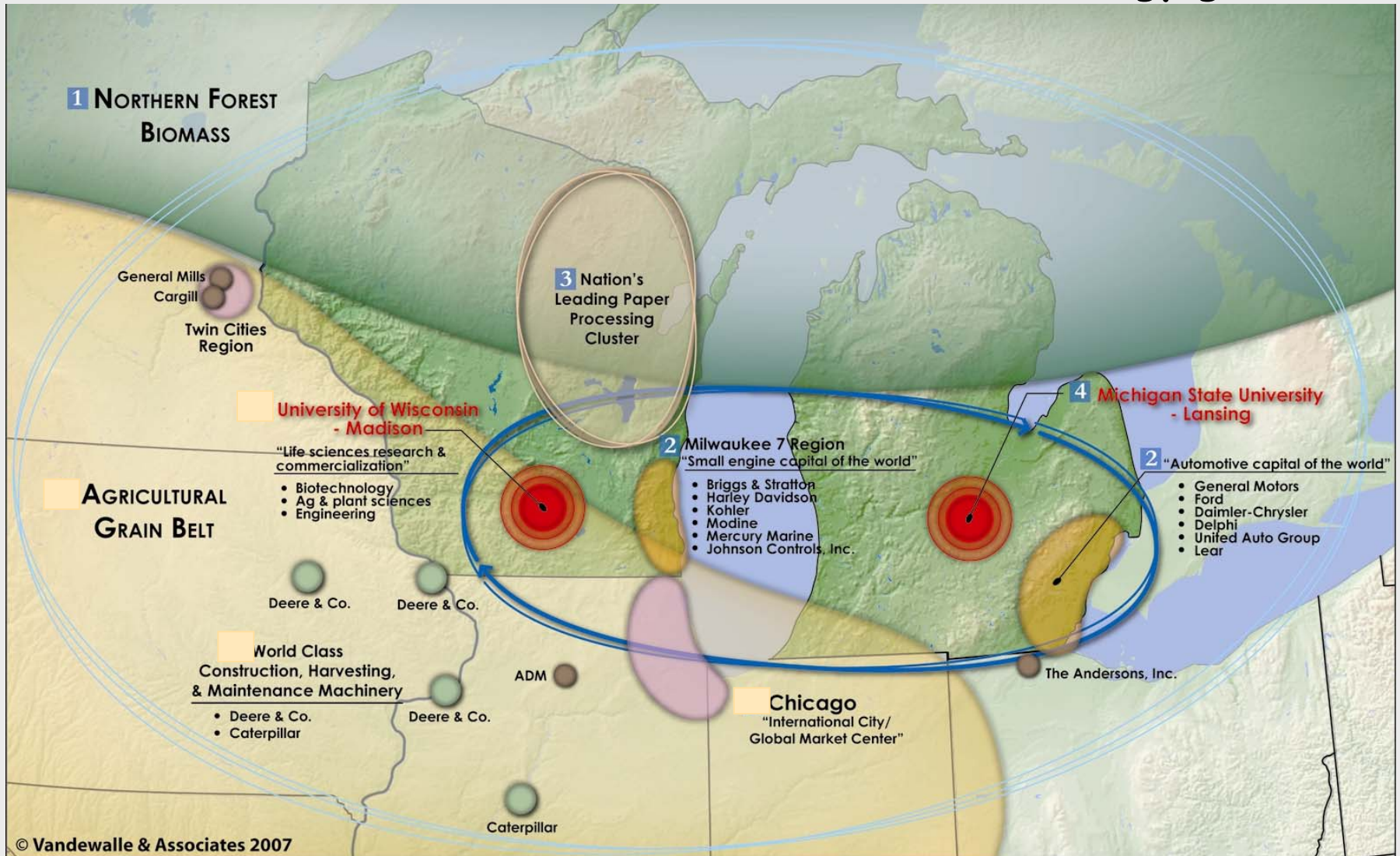


**Tim Donohue (UW-Madison)
BER Advisory Committee
November 29, 2007**

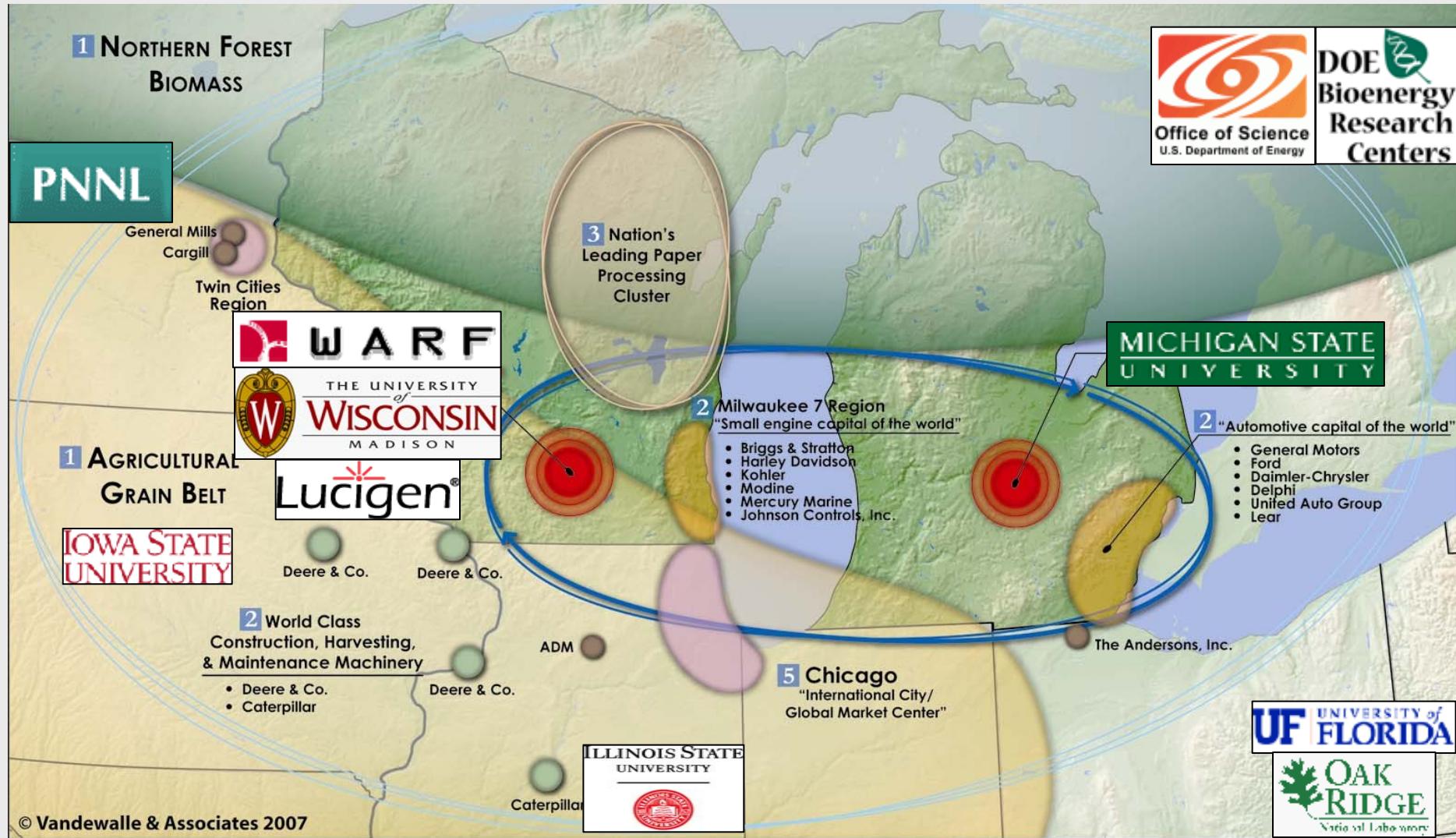
<http://www.greatlakesbioenergy.org>

Great Lakes Bioenergy Research Center (GLBRC)

Vision: Plugging the scientific, agricultural, economic & technological excellence in the Great Lakes Basin into the energy grid



Great Lakes Bioenergy Research Center (GLBRC)



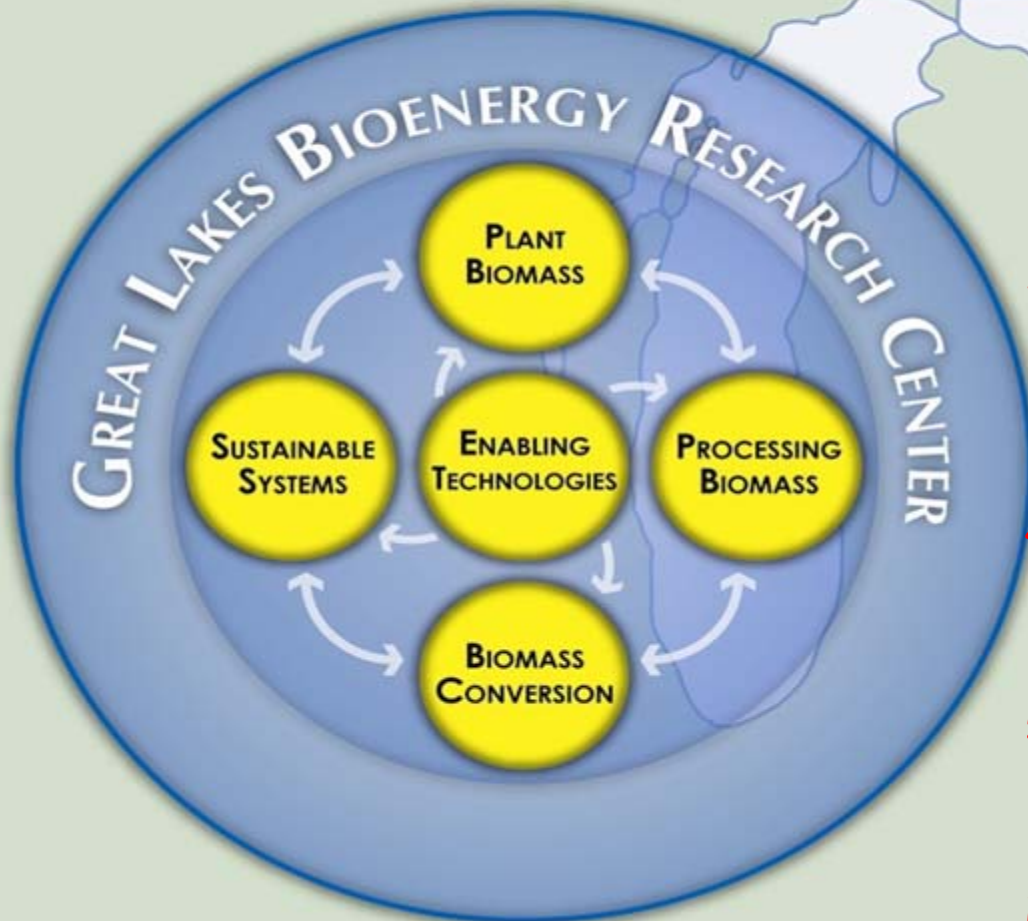
Great Lakes Bioenergy Research Center (GLBRC)

Mission: perform fundamental research aimed at removing bottlenecks in the biomass to bioenergy pipeline

Integrated research goals

1. Improve plant biomass
2. Improve biomass processing
3. Improve conversion of plant biomass to liquid & other fuels
4. Evaluate sustainability of biomass to biofuels pipeline
5. Develop & use enabling genome-based technologies

Education & Outreach



GLBRC Thrust 1: Improved Plant Biomass

Objectives: obtain mechanistic understanding of biochemical & regulatory pathways needed to divert plant carbon into:

- More digestible cell wall polymers
- Starch, fructans and other digestible carbohydrates
- Plant-derived oils

>50% of Thrust One budget devoted to creation of energy crops with novel forms of biomass storage

Discovery of cell wall biosynthetic genes & regulators

Approach: expression profiling of developing seeds

Gene Discovery -Goal: > 1 million ESTs from each system (with JGI)

Fenugreek



Psyllium

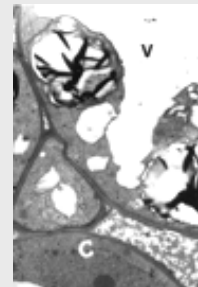


Nasturtium

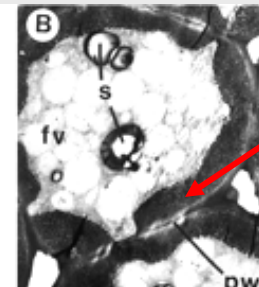


Mucilaginous
layer
60% Xylan

Endosperm
90% Mannan



20 DPA



22 DPA

Cotyledon
30% Xyloglucan

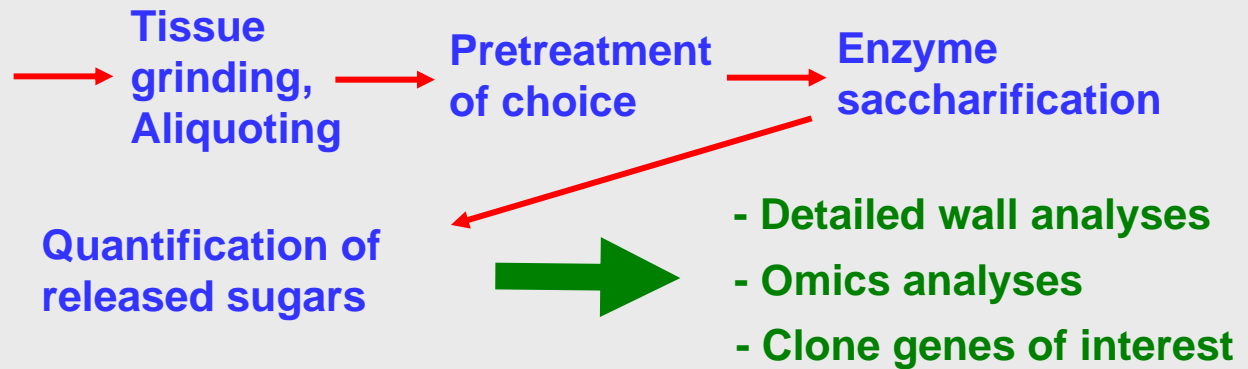
Genes affecting cell wall composition & digestibility

Approach: High throughput mutant analysis (with Thrust 2)

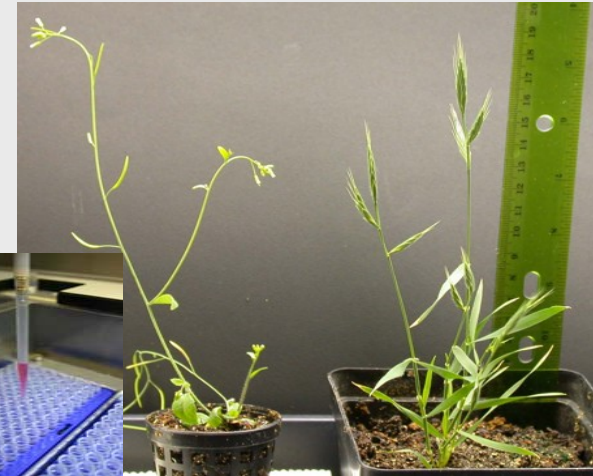
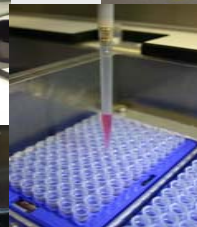
Cell wall analytical platform: Fully automated, 400 samples/day
(WT & mutants)

Collections

Arabidopsis
Silage corn varieties
Brachypodium
Rapid Cycling Maize
Switchgrass



Rapid Cycling
Maize



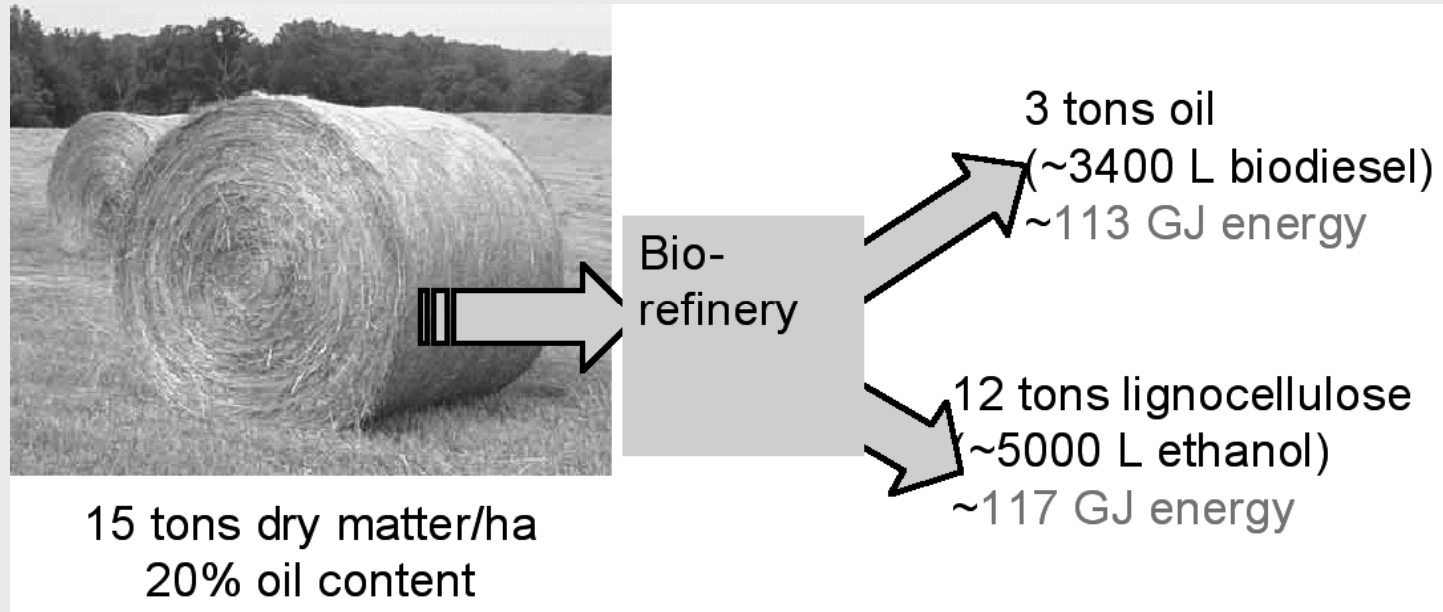
Arabidopsis
Brachypodium

Robotic processing of
cell wall samples

Oil accumulation in biomass plants

Plant oils are the most energy rich, natural form of abundant carbon

Use as energy source does not require fermentation or distillation



Biomass crop with 20% oil content will almost double energy content available for liquid fuel

Similar approaches to dissect metabolic and regulatory circuits that control oil biosynthesis

GLBRC Thrust 2: Improved Biomass Processing

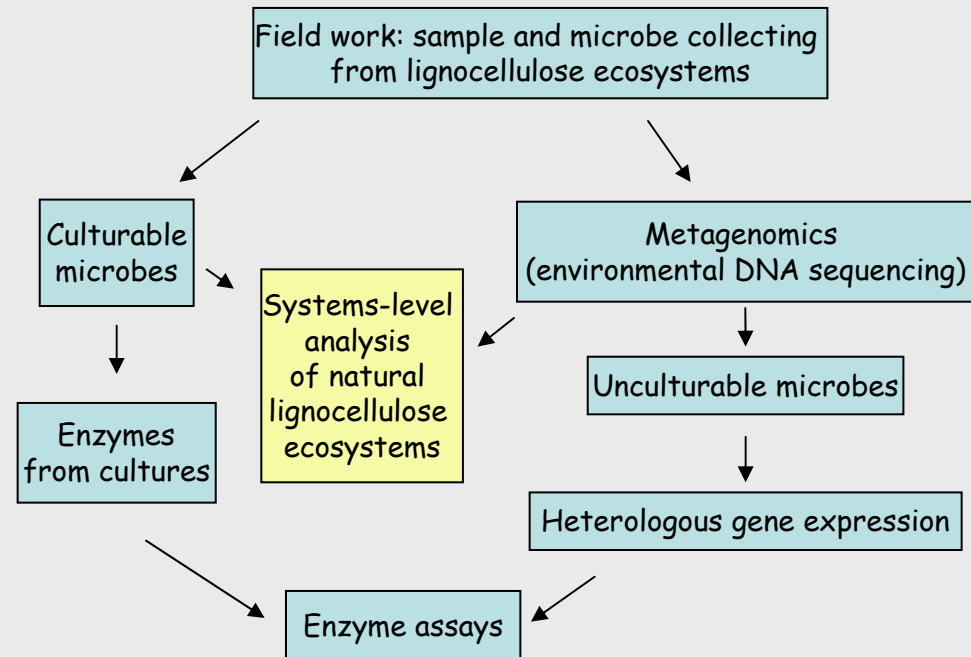
Objective: improve conversion of plant cell walls into fermentable or chemically-convertible materials by

- analyzing a range of plant material & pretreatment conditions
- discovery & application of improved enzymes

Enhanced digestibility



Bioprospecting



GLBRC Thrust 2: Improved Biomass Processing

Goals:

Enhanced biomass conversion potential

- Combinatorial, high throughput, screen of candidate materials with various pretreatments for improved cell wall digestion (with Thrust 1)

Understand pretreatment chemistries

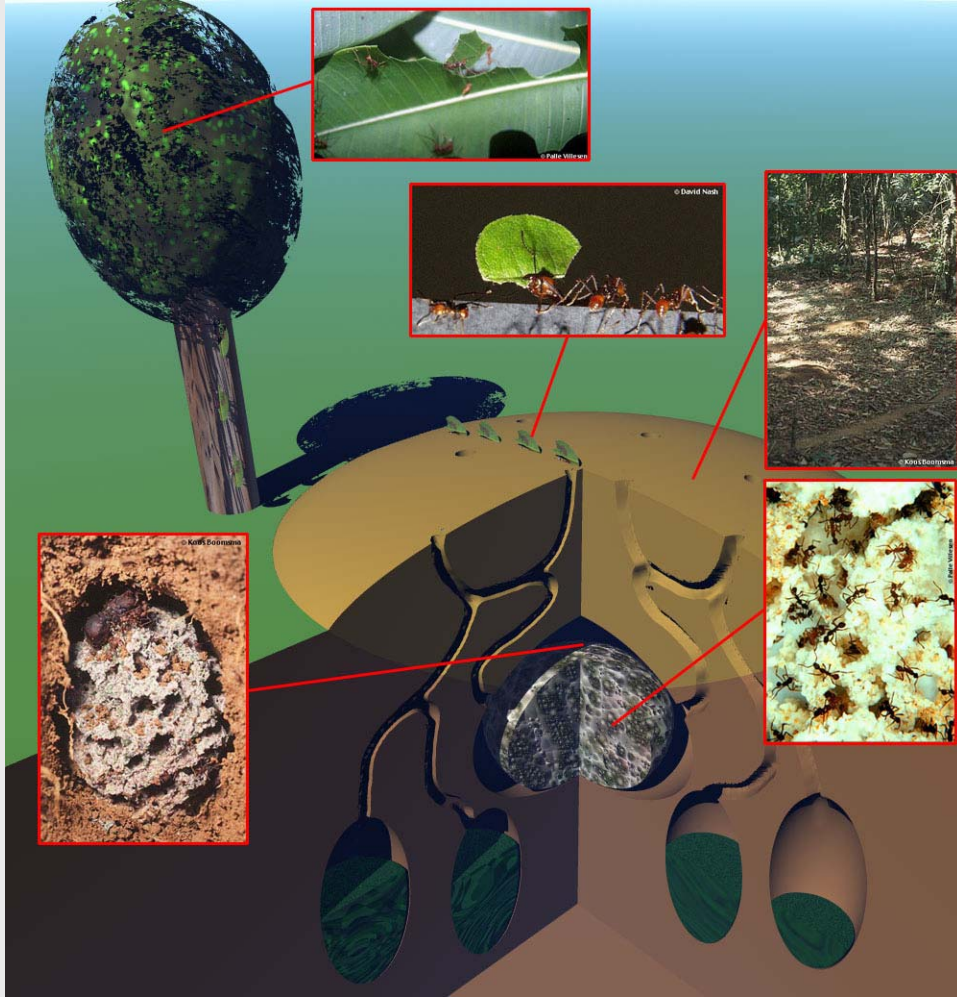
- Identify & quantify relevant small molecules produced by pretreatment chemistries (with Thrust 5)

Screen products for fermentation (microbes) or conversion (chemistry)

- Assess fermentability (with Thrust 3)
- Assess use in catalytic conversion (with Thrust 3)

GLBRC Thrust 2: Improved Biomass Processing

Goal: Discover/improve deconstruction enzymes



- Bioprospecting in cellulose-degrading ecosystems (with JGI)
- High-throughput screening of genetic material from promising niches
- Engineer improved enzymes (with Thrust 5)

GLBRC Thrust 2: Improved Biomass Processing

Goals:

Integrate pretreatments with suitable enzymes for high sugar yield

- Optimize combinations of existing and new enzymes to hydrolyze biomass
- Develop suites of enzymes tailored for specific pretreated biomass samples (with Thrust 5)

Decrease enzyme cost

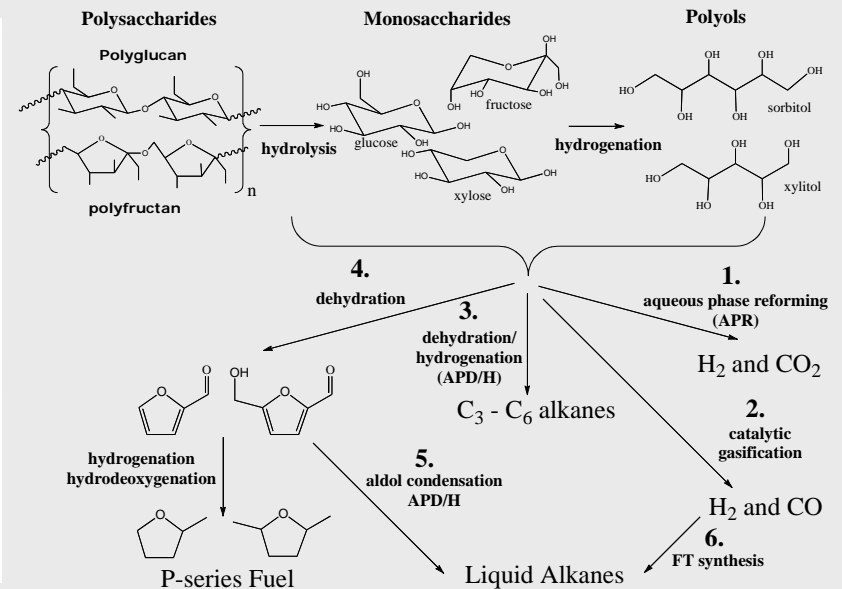
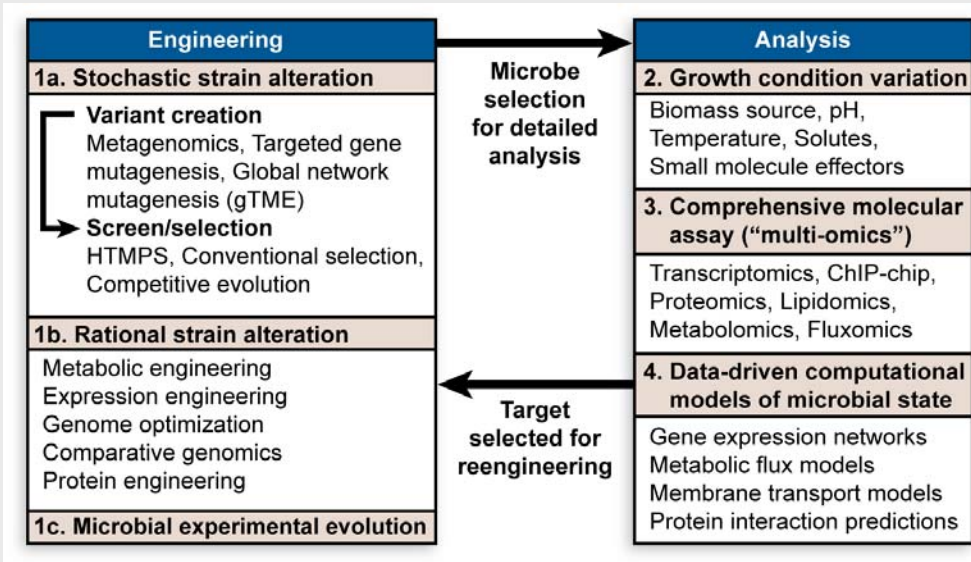
- Test expression of new cellulolytic enzymes in plants (with Thrust 1)
- Improve expression of cellulolytic enzymes in maize, alfalfa and other plants (with Thrust 1)

GLBRC Thrust 3: Improved Biomass Conversion

Conversion of biomass into energy products: improve methods for converting plant biomass into materials that can replace fossil fuels

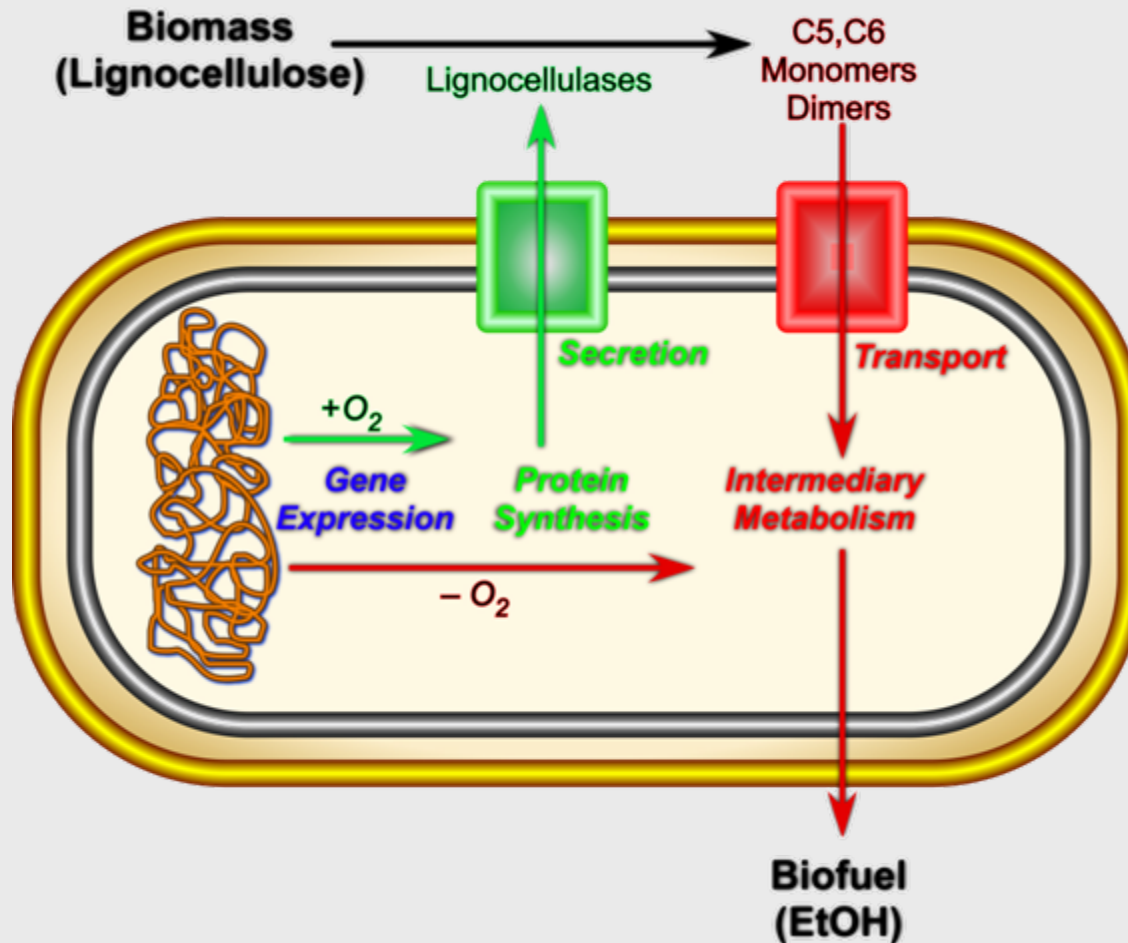
- ethanol
- hydrogen
- chemical feedstocks

Biological & chemical conversion platforms



GLBRC Thrust 3: Improved Biomass Conversion

Long-Term Strategy for Consolidated Ethanologens

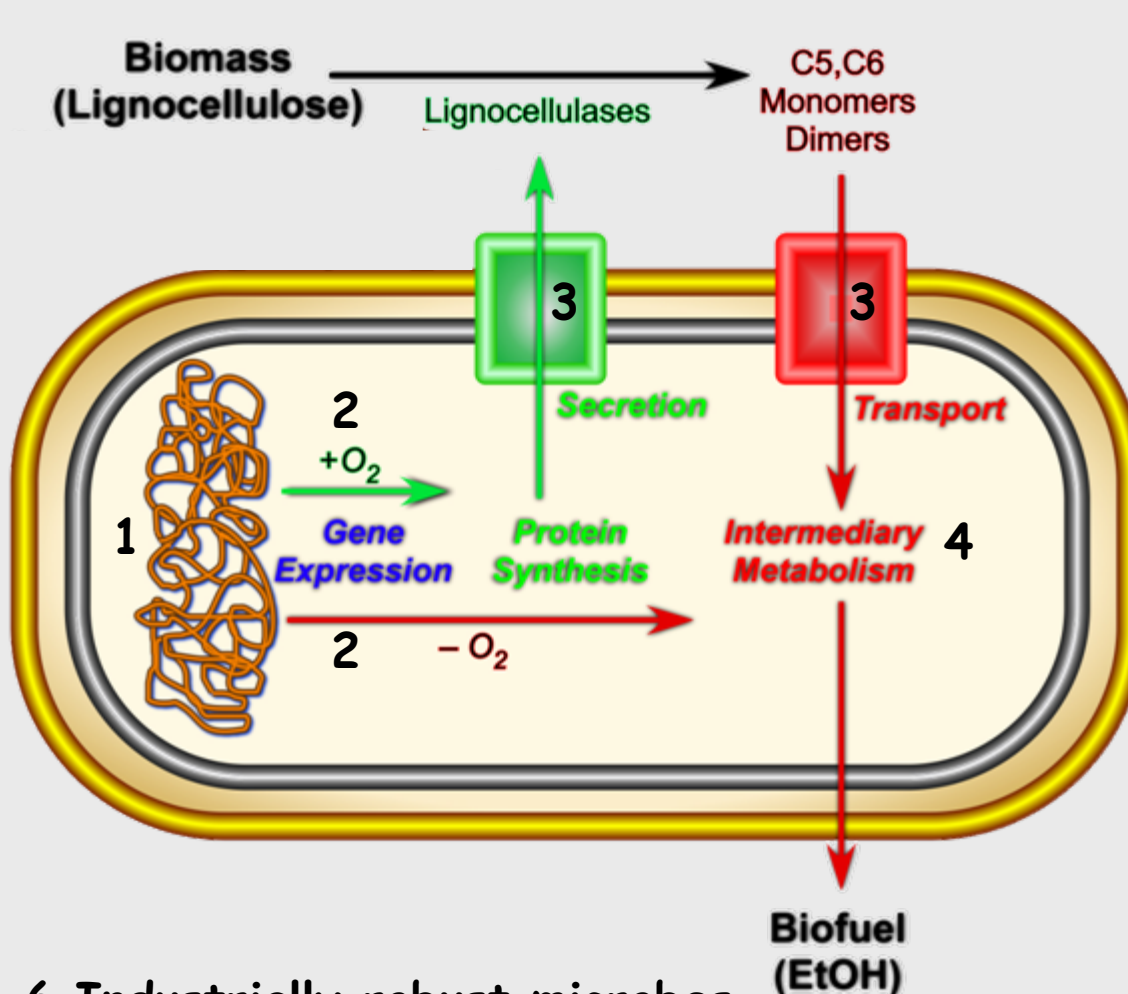


During aerobic growth, optimize production of desired extracellular cellulases & hemicellulases (with Thrusts 2 & 5)

During anaerobic phase, optimize production of enzymes, transporters, & pathways to optimize ethanogenesis (with Thrust 5)

GLBRC Thrust 3: Improved Biomass Conversion

GLBRC Targets for Removal of Bottlenecks in Ethanogenesis



6 Industrially robust microbes

- *product, feedstock, phage, etc. resistance*
- *stress, temperature tolerance*

1 Minimize genome

- *genetic stability*
- *metabolic efficiency*

2 Optimize gene expression

- *enzyme & transporter levels for each phase of refinery (+/- O₂)*

3 Optimize ex- & importers

- *protein secretion*
- *C5 & C6 transport in presence of glucose*

4 Optimize metabolic network

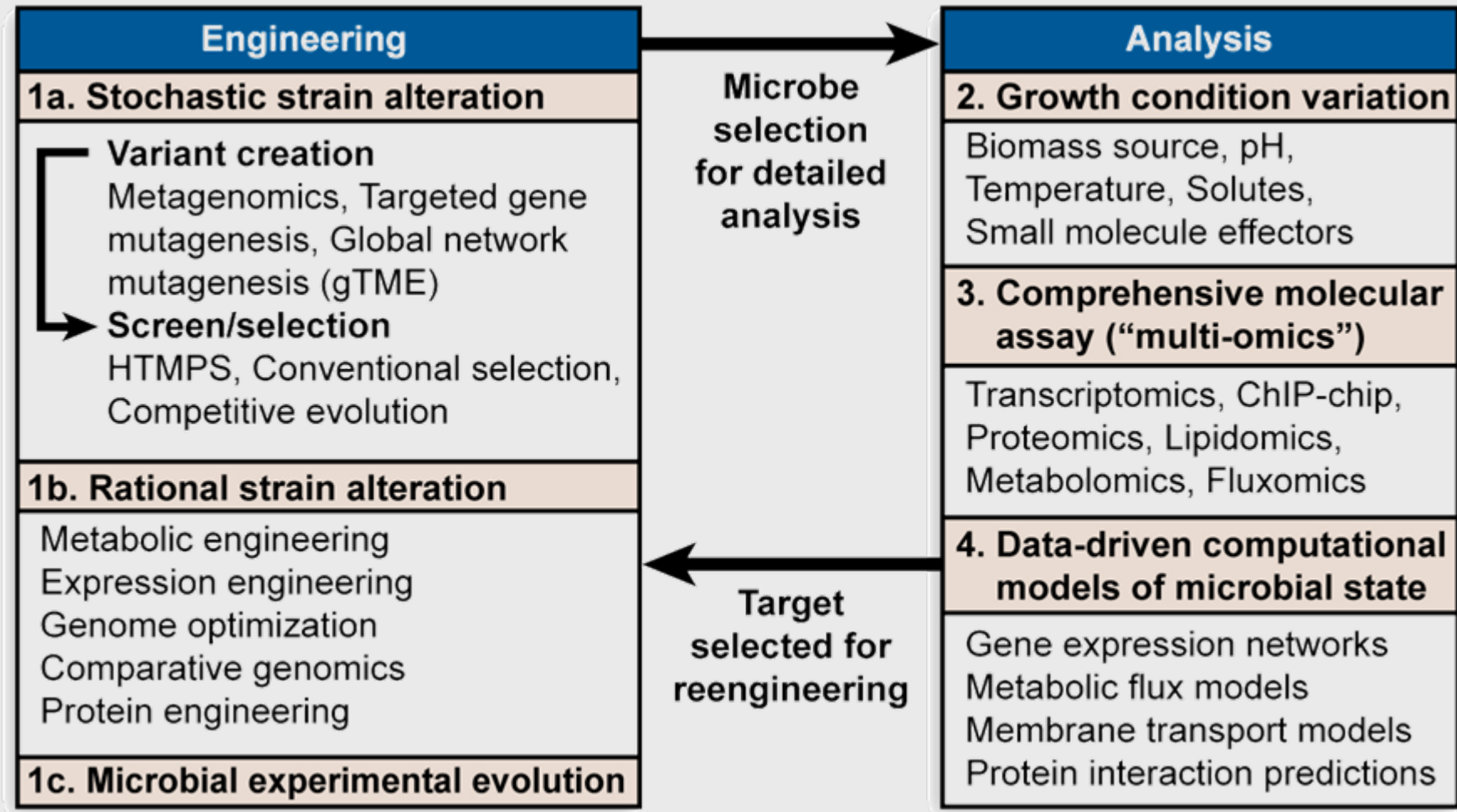
- *increase carbon flow to biofuel (with Thrust 5, JGI, & DOE Jacquard cluster)*

5 Anchor enzymes to microbe

- *efficient depolymerization of biomass (with Thrust 2)*

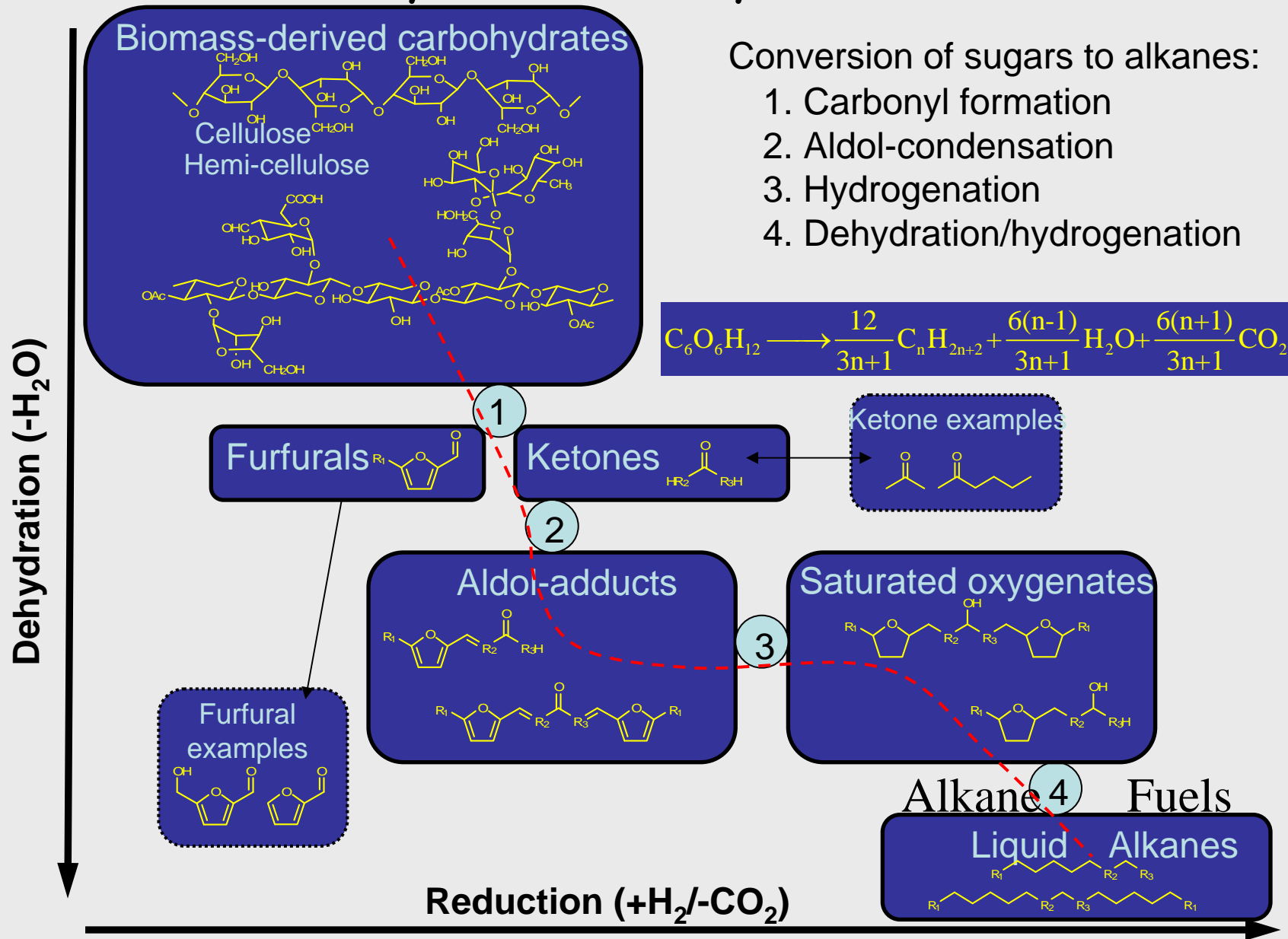
GLBRC Thrust 3: Improved Biomass Conversion

Reiterative Directed Microbial Evolution
(REDIME) to Obtain Improved Bioenergy Microbes
(apply to EtOH, H₂, CO₂ sequestration, other fuels & feedstocks)



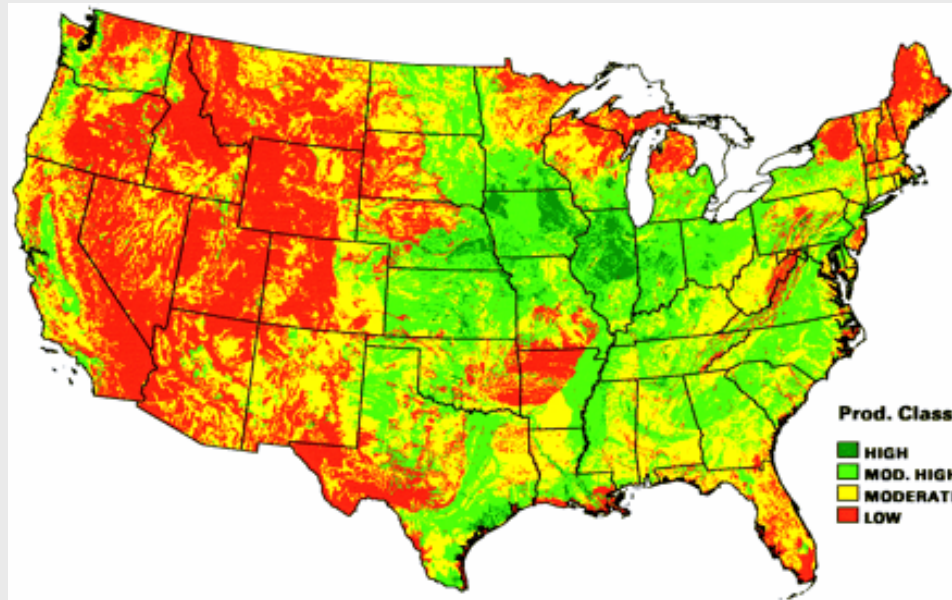
GLBRC Thrust 3: Improved Biomass Conversion

Process & Discovery Chemical Catalysis for Biofuels Production



GLBRC Thrust 4: Sustainable Bioenergy Practices

Development of a sustainable bioenergy economy: support the biomass-to-bioenergy pipeline by developing ecological, agricultural & life cycle practices that are economically viable & environmentally responsive

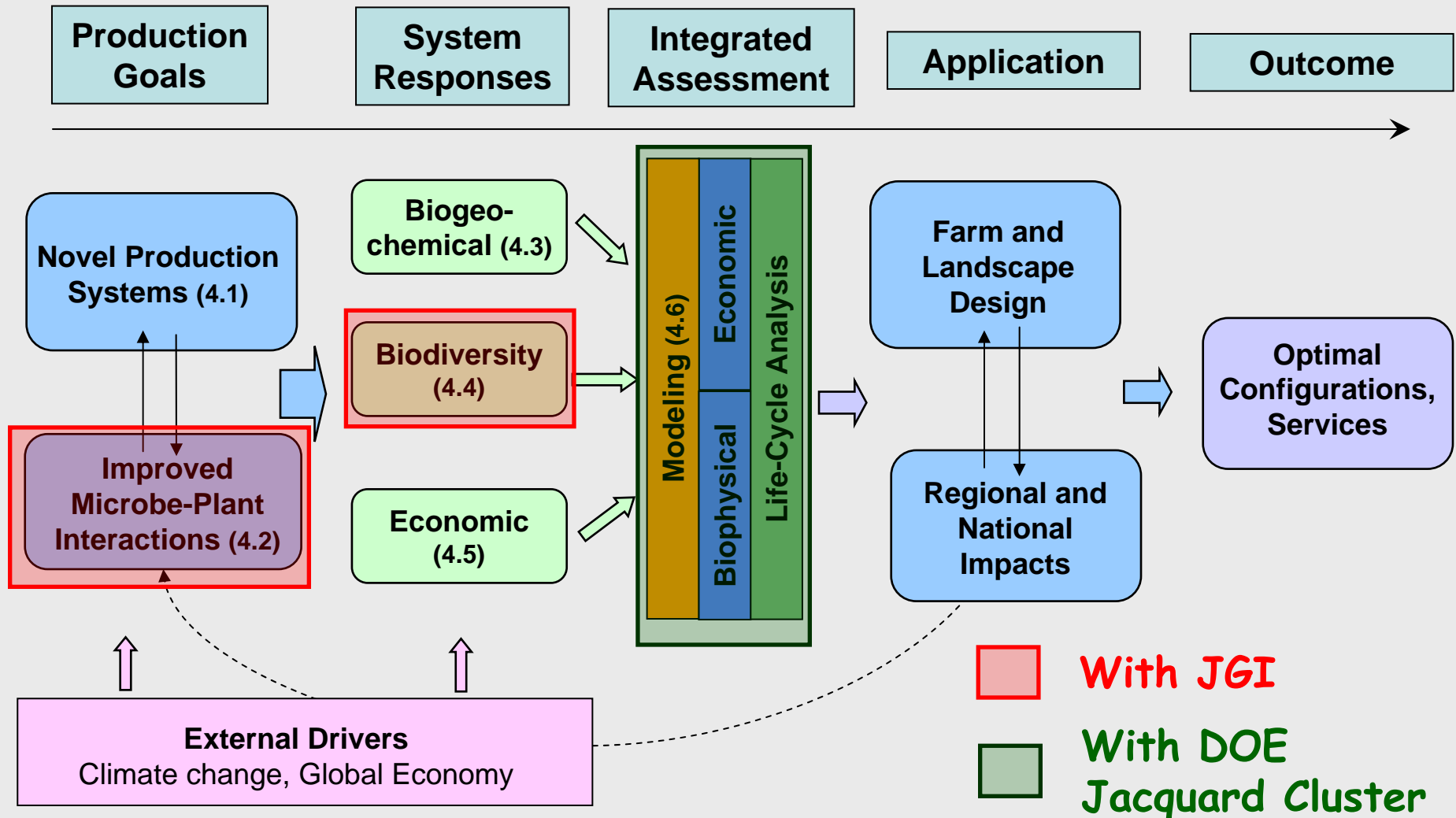


Overcome bottlenecks in agricultural, industrial, & behavioral systems to improve

- **carbon neutrality** and net greenhouse gas mitigation across the entire biofuel life cycle at multiple scales
- ecosystem services in biofuel landscapes (e.g. **water quality, biodiversity, pest suppression**)

GLBRC Thrust 4: Sustainable Bioenergy Practices

Objective: Determine elements of **integrated** biofuel production systems that can be **optimized** to improve **environmental & economic sustainability**



GLBRC Thrust 4: Sustainable Bioenergy Practices

Goal: Predict Behavior of Novel Production Systems

High Input,
Low Diversity
(annuals)

Continuous Corn

Corn-Soybean-Canola



Monoculture switchgrass

Switchgrass - Legumes



Poplars



Low Input,
High Diversity
(perennials)

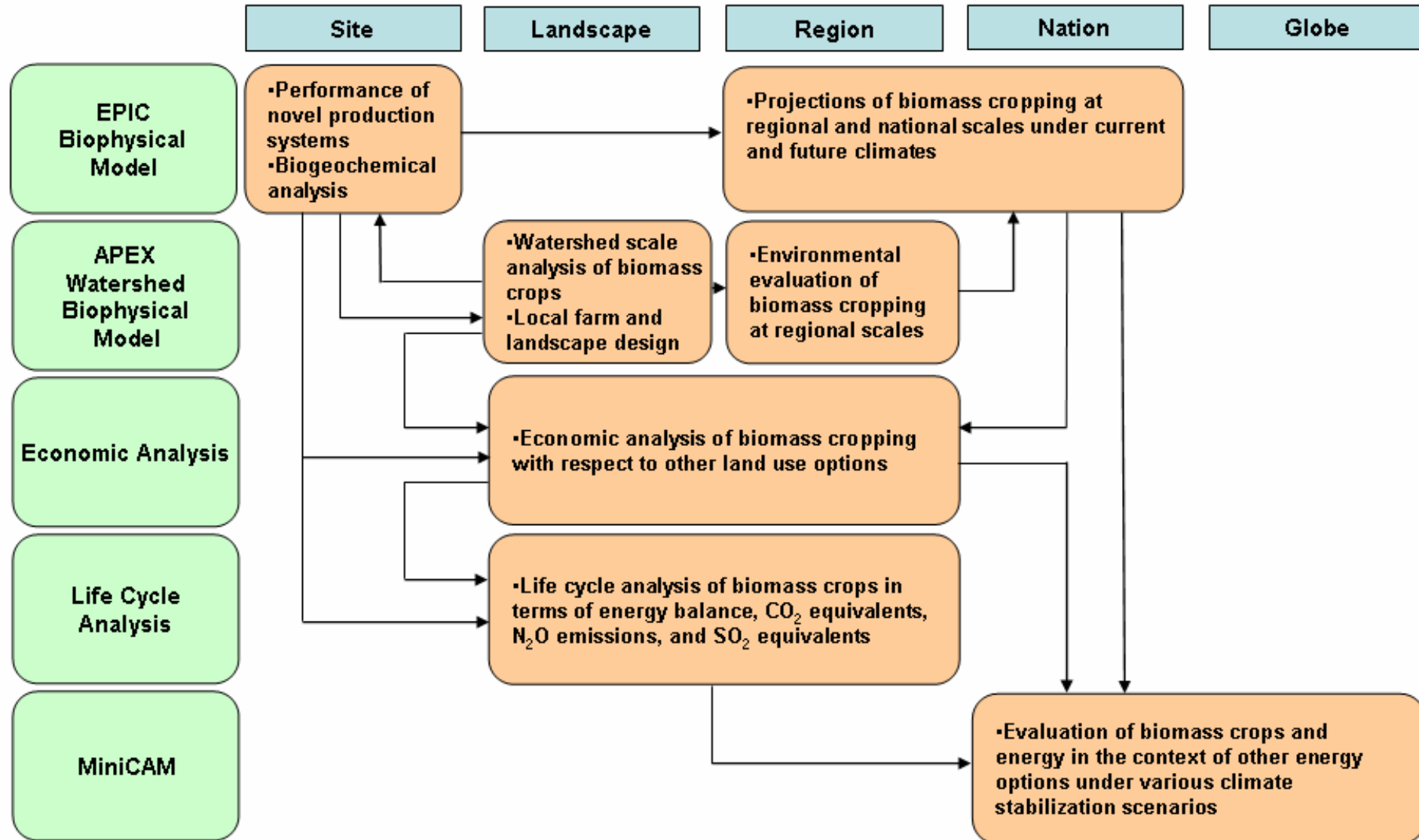
Early successional
Native prairie



➤ replicated plots in MI (KBS)
& elsewhere

GLBRC Thrust 4: Sustainable Bioenergy Practices

Multi-Scale, Modeling Framework (with DOE Jacquard cluster)

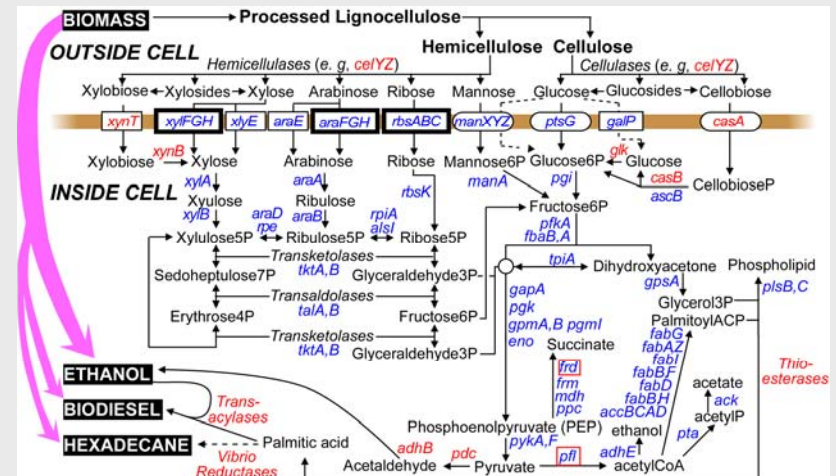
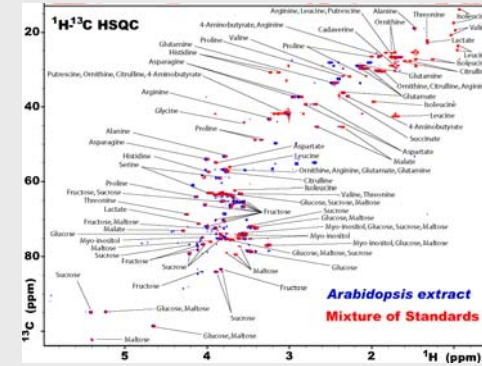
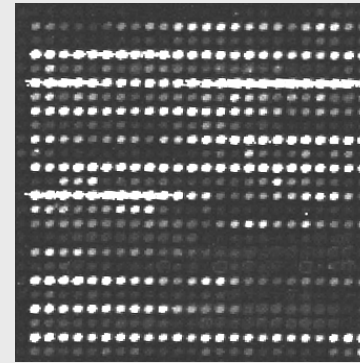


GLBRC Thrust 5: Enabling Bioenergy Technologies

Objective: provide cutting edge genome-based technologies that enable the innovation, discoveries & creative solutions needed to remove biofuels bottlenecks

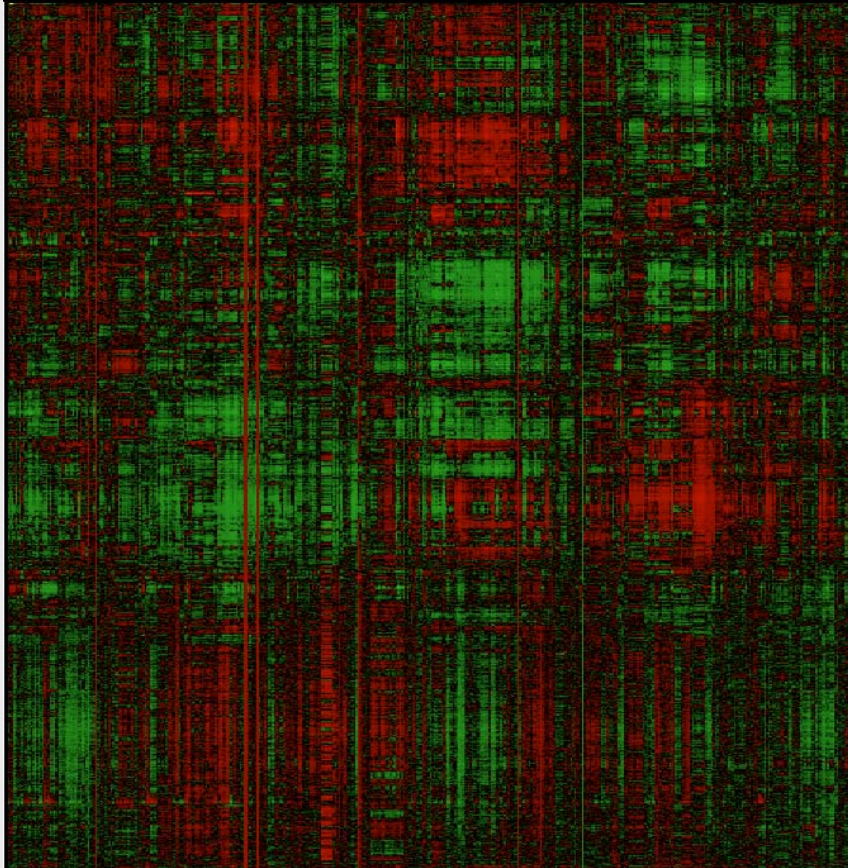
- high-throughput screens
- global analyses
- metabolic flux analysis
- protein & metabolic engineering
- computational modeling

Genome-enabled analyses



GLBRC Thrust 5: Enabling Bioenergy Technologies

Mapping Biofuels Regulatory Networks



- Cluster sets of co-regulated genes to identify **network structure**
- High resolution analysis of **biofuels transcription factor binding sites** (with JGI)
- Predictive **models** for bioenergy **regulators and regulatees** (with DOE Jacquard cluster)

GLBRC Thrust 5: Enabling Bioenergy Technologies

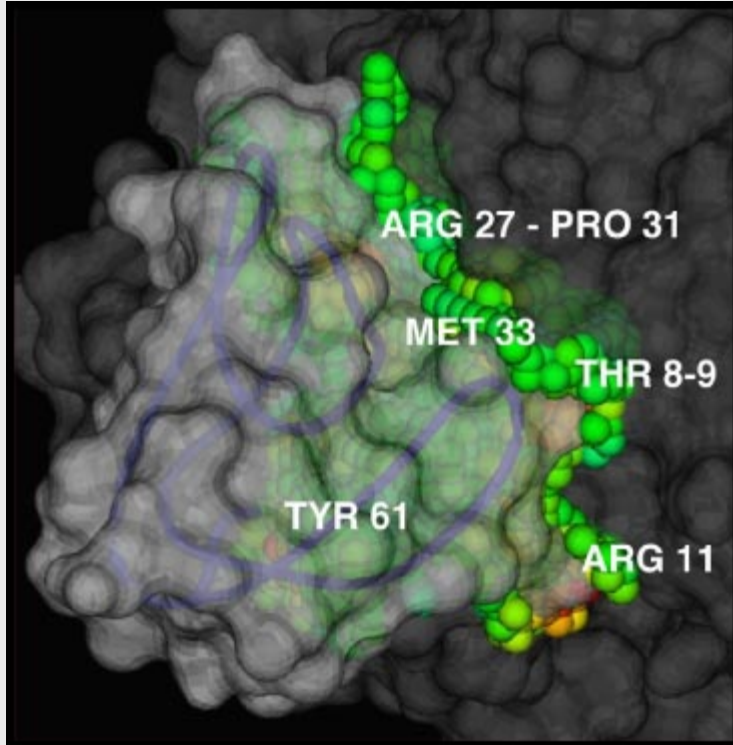
Bioenergy Protein Blueprints



- Isotope-assisted **protein abundance** measurements
- **Localization of proteins** in sub- or extra-cellular fractions
- Monitor and map sites of **covalent modifications** that impact biomass or fuel production

GLBRC Thrust 5: Enabling Bioenergy Technologies

Protein Expression and Engineering



- High throughput pipeline for protein expression and functional analysis
- Multiplex screening for new and improved biofuel enzymes
- Computational predictions of active sites, protein-protein interfaces, or protein stability to engineer new biomass pathways or improve biofuel enzymes (with BACTER & DOE Jacquard cluster)

GLBRC Thrust 6: Education and Outreach

Objectives: Develop a coordinated bioenergy education and outreach program

“.....solve today's bioenergy bottlenecks while training the bioenergy leaders of tomorrow....”

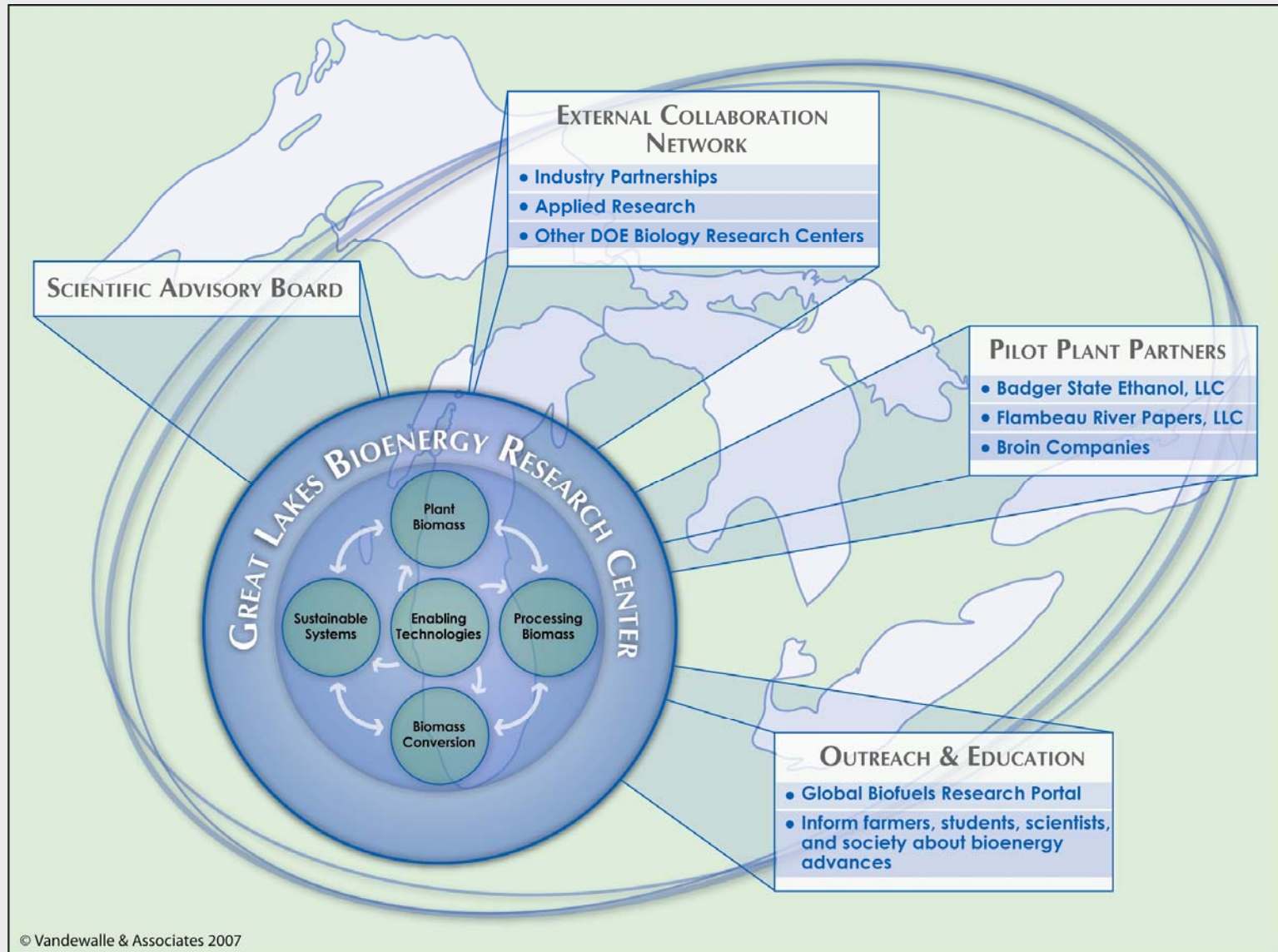
GLBRC Education and Outreach

Goal: Coordinated bioenergy education and outreach program

- **Workshops & educational modules** for K - 12, teachers or public on carbon chemistry, sustainability, biodiversity
- Public talks/workshops/**communications** program
- Develop materials to inform farmers, municipalities and other **members of the community** about bioenergy
- **Exhibits** on biomass & bioenergy (aka- "Bioenergy Discovery Center")
- Bioenergy **seminar** programs and topics in **biology, engineering & computational courses and labs** (partnering with BACTER and others)
- **Summer research programs** for undergraduates from other campuses; including major URM institutions
- **Attract graduate students** from highly rated programs

Integration of GLBRC Functions

Public, private & institutional partnerships to function as a worldwide research Center of Excellence



Great Lakes Bioenergy Research Center

Plugging the scientific, agricultural, economic & technological excellence in the Great Lakes Basin into the energy grid

