New Technologies for New Markets:

What is Modern Genetics Telling Us About Cell Walls

Gerald Tuskan[1,4]

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4 Joint Genome Institute, Walnut Creek, CA
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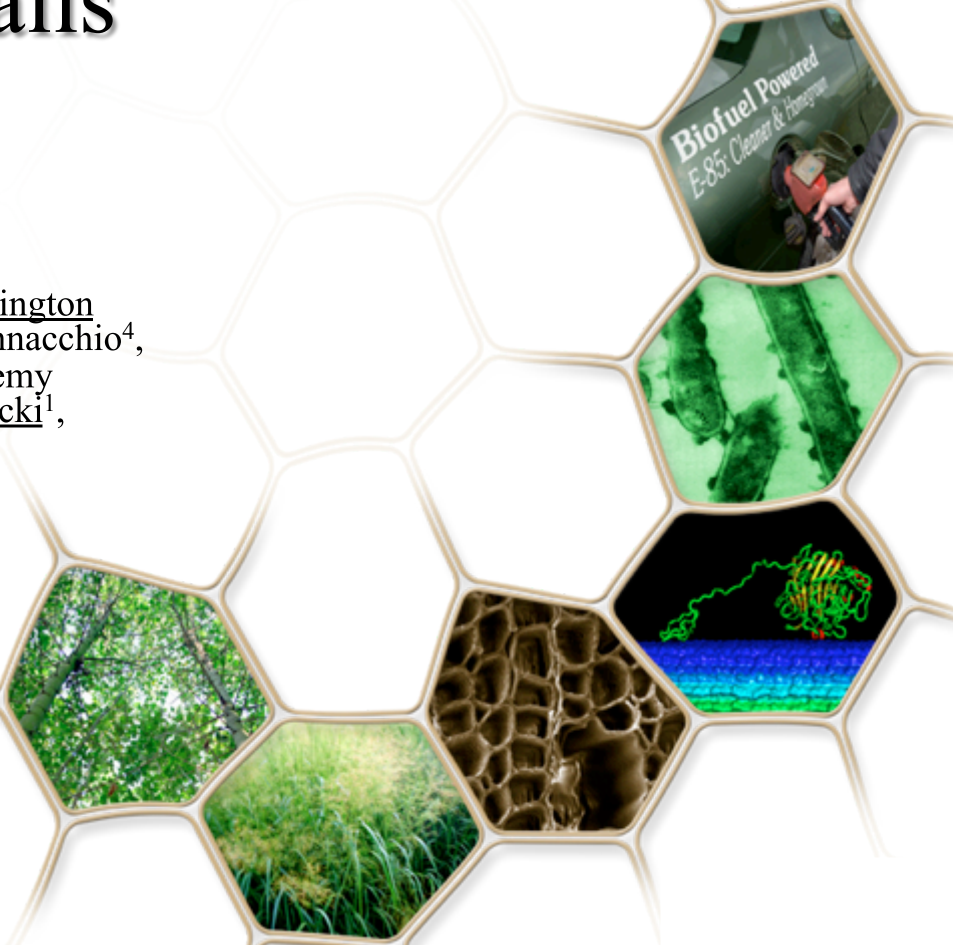
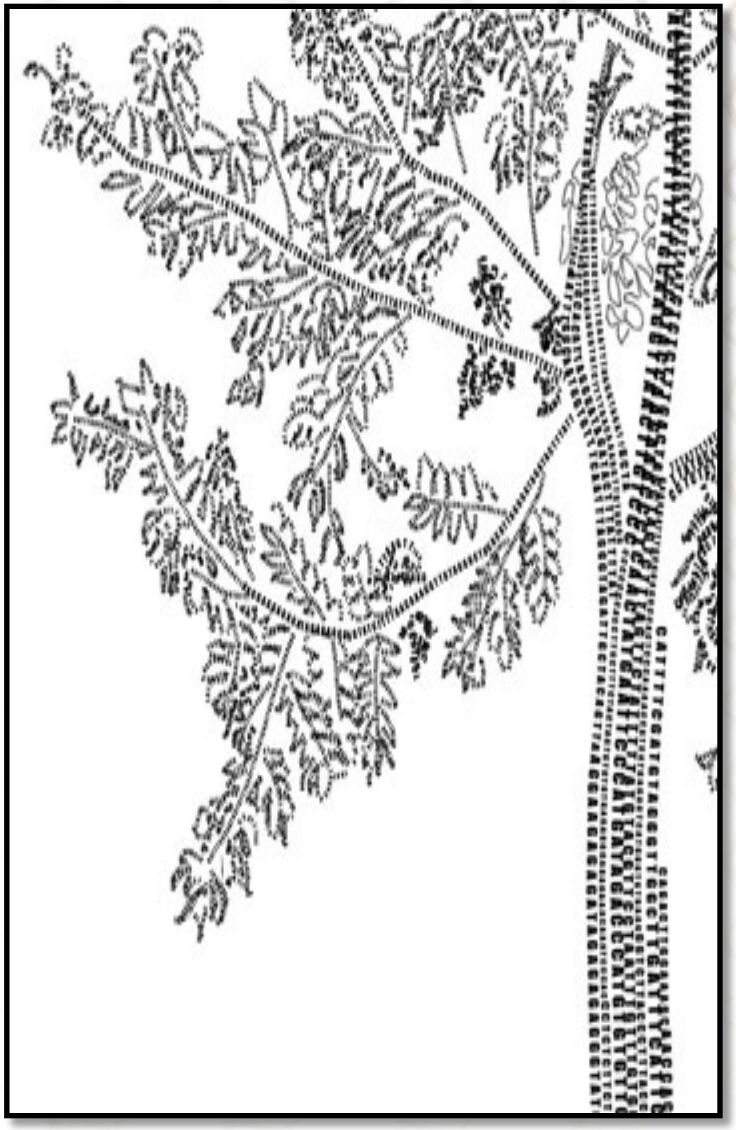


Scope of the Talk

I. Association Mapping
  - SNP detection and distribution
  - Genome-wide Association Genetics

II. Activation Tagging
  - TAIL PCR Identification
  - Arabidopsis Confirmation and Recapitulation

III. Stable Transformation
  - Projected Transgenics
  - Early Phenotypes

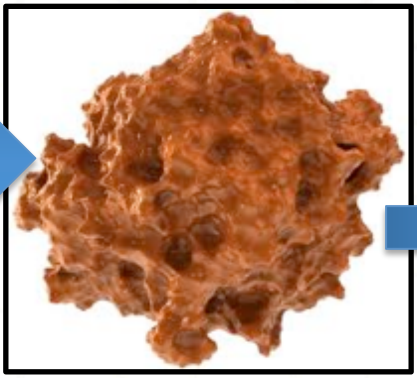


Lignocellulose by any other name . . .

Cell Wall Deconstruction and Product Output

Dedicated Feedstock
Wood Chips

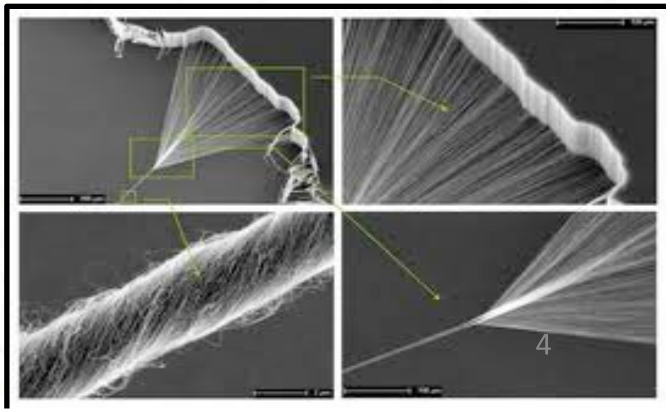
Cellulose



Lignin

Ethanol,
Isobutanol,
etc.

Spun
Carbon
Fibers

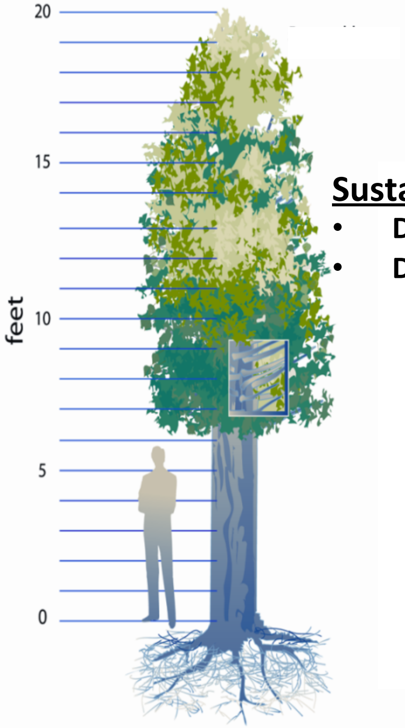


Populus as a Model for Cell Wall Formation

Enhancing Traits for Energy Applications

Capture and Allocation of Carbon

- Increased photosynthetic efficiency
- Optimized crown and leaf architecture

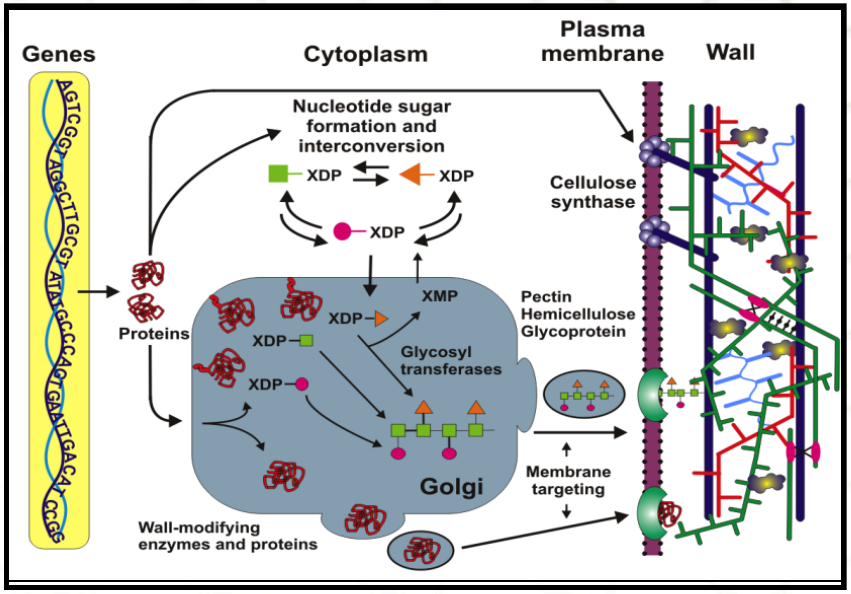


Biomass

- Tailored biomass
- Enhanced production

Sustainability

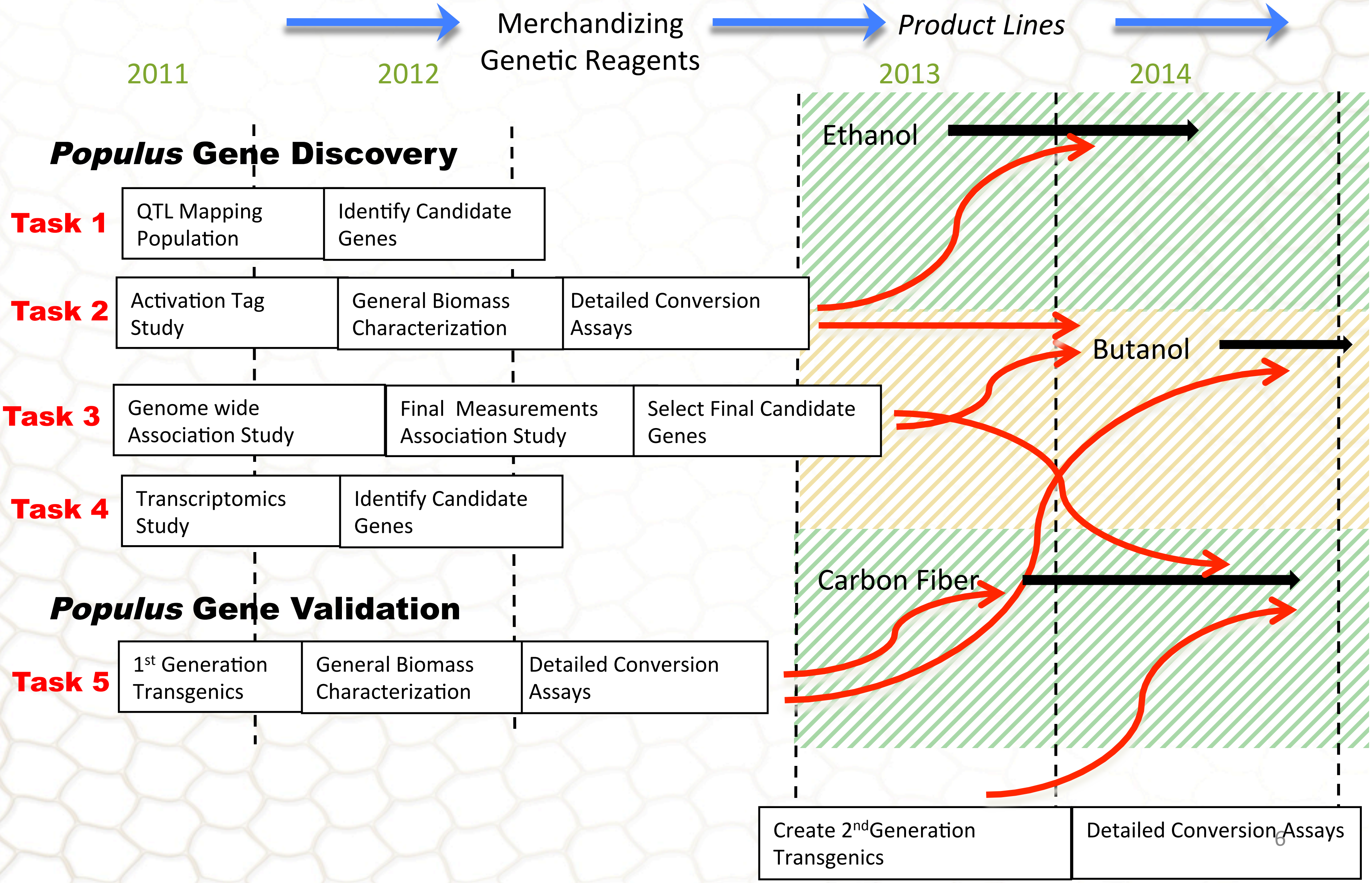
- Disease resistance
- Drought tolerance



Goal: To reduce the recalcitrance of lignocellulosic biomass for economic production of biofuels

Focus: To understanding basic mechanisms of cell wall formation in *Populus*

De Novo Gene Discovery – BioEnergy Science Center



Merchandizing Genetic Reagents

Product Lines

2011

2012

2013

2014

Populus Gene Discovery

Task 1
- QTL Mapping Population
- Identify Candidate Genes

Task 2
- Activation Tag Study
- General Biomass Characterization
- Detailed Conversion Assays

Task 3
- Genome wide Association Study
- Final Measurements Association Study
- Select Final Candidate Genes

Task 4
- Transcriptomics Study
- Identify Candidate Genes

Populus Gene Validation

Task 5
- 1st Generation Transgenics
- General Biomass Characterization
- Detailed Conversion Assays

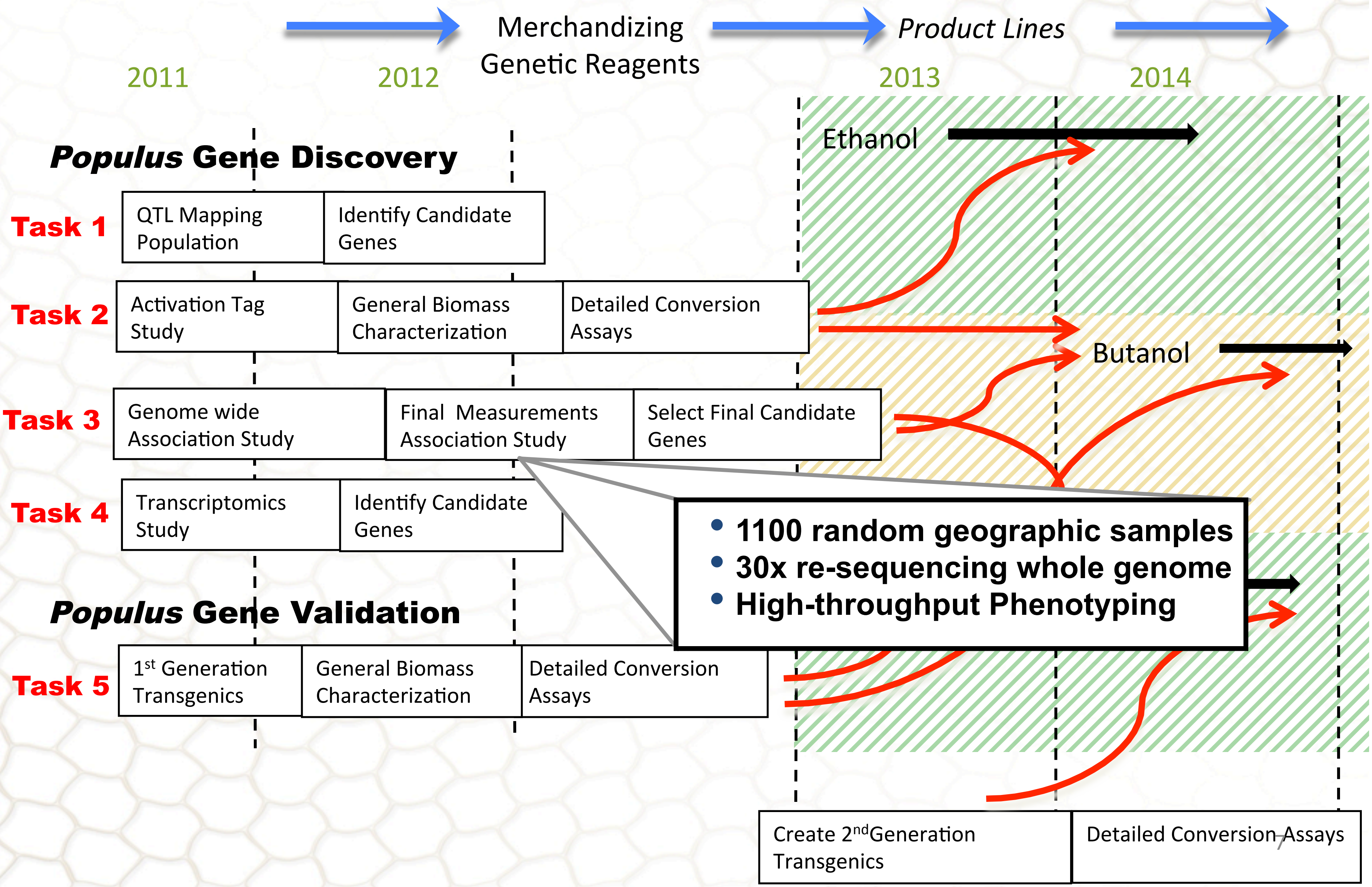
- Create 2nd Generation Transgenics
- Detailed Conversion Assays

Ethanol

Butanol

Carbon Fiber

De Novo Gene Discovery – Association Genetics



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Ethanol

Butanol

***Populus* Gene Discovery**

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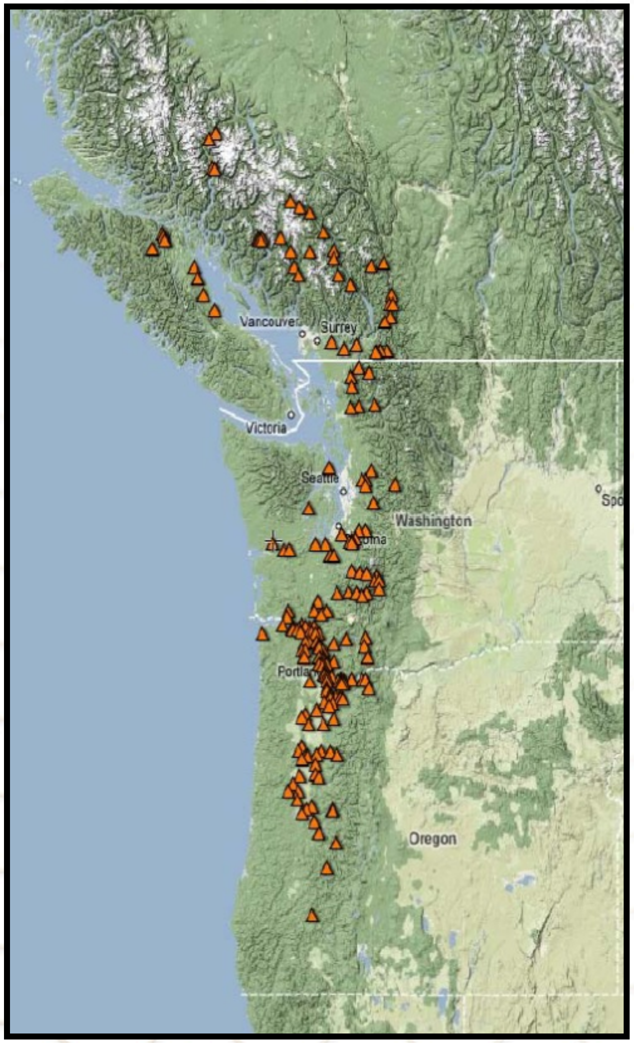
- **1100 random geographic samples**
- **30x re-sequencing whole genome**
- **High-throughput Phenotyping**

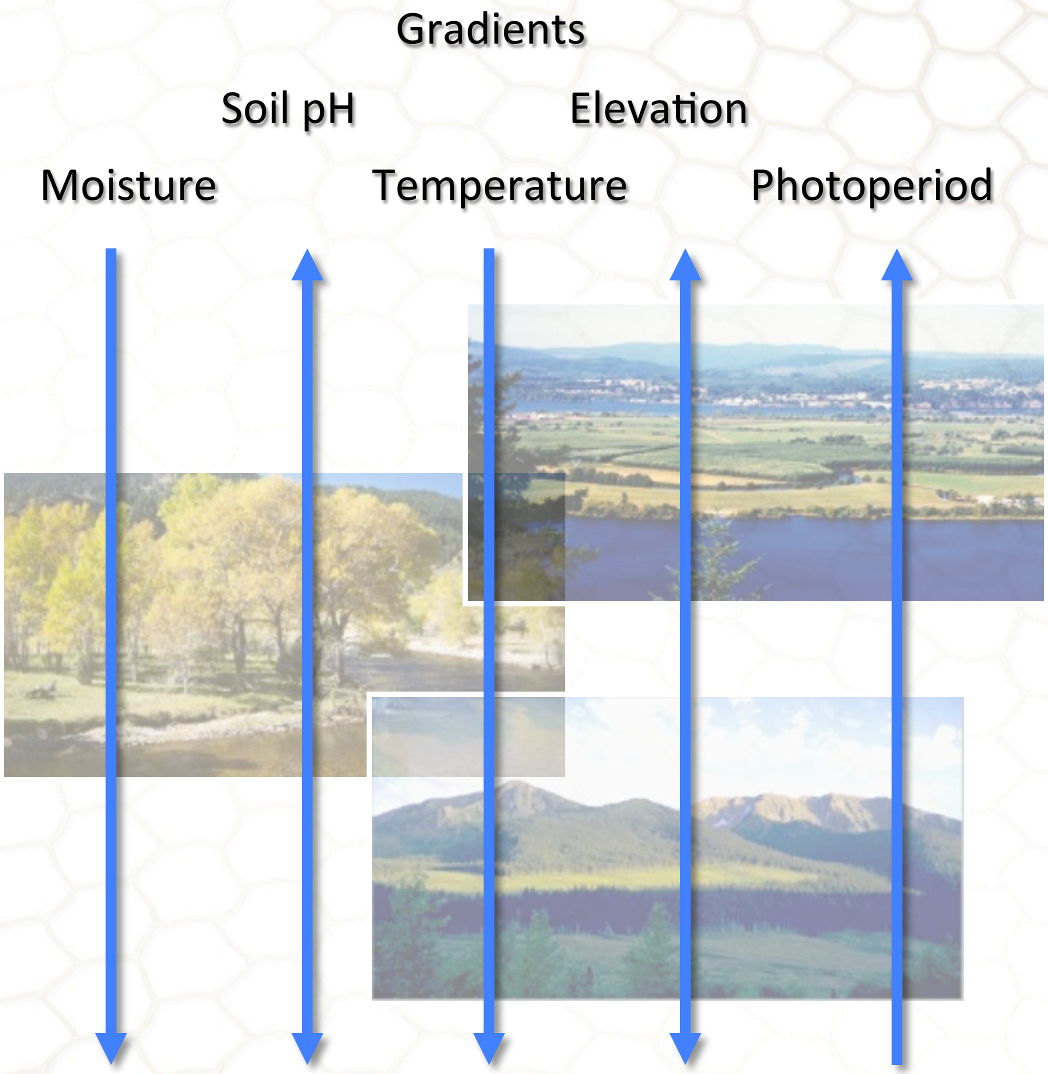
***Populus* Gene Validation**

Task 5
- 1st Generation Transgenics
- General Biomass Characterization
- Detailed Conversion Assays

- Create 2nd Generation Transgenics
- Detailed Conversion Assays

GWAS -- Mining Adaptive Allelic Variation





Gradients

Soil pH

Elevation

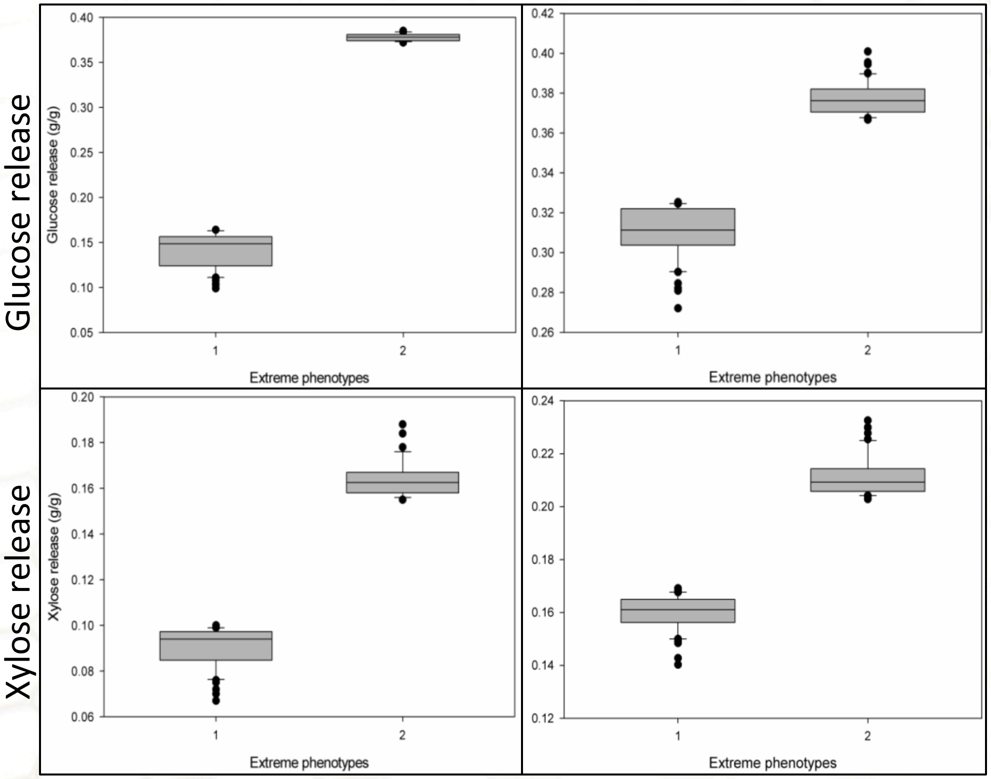
Moisture

Temperature

Photoperiod

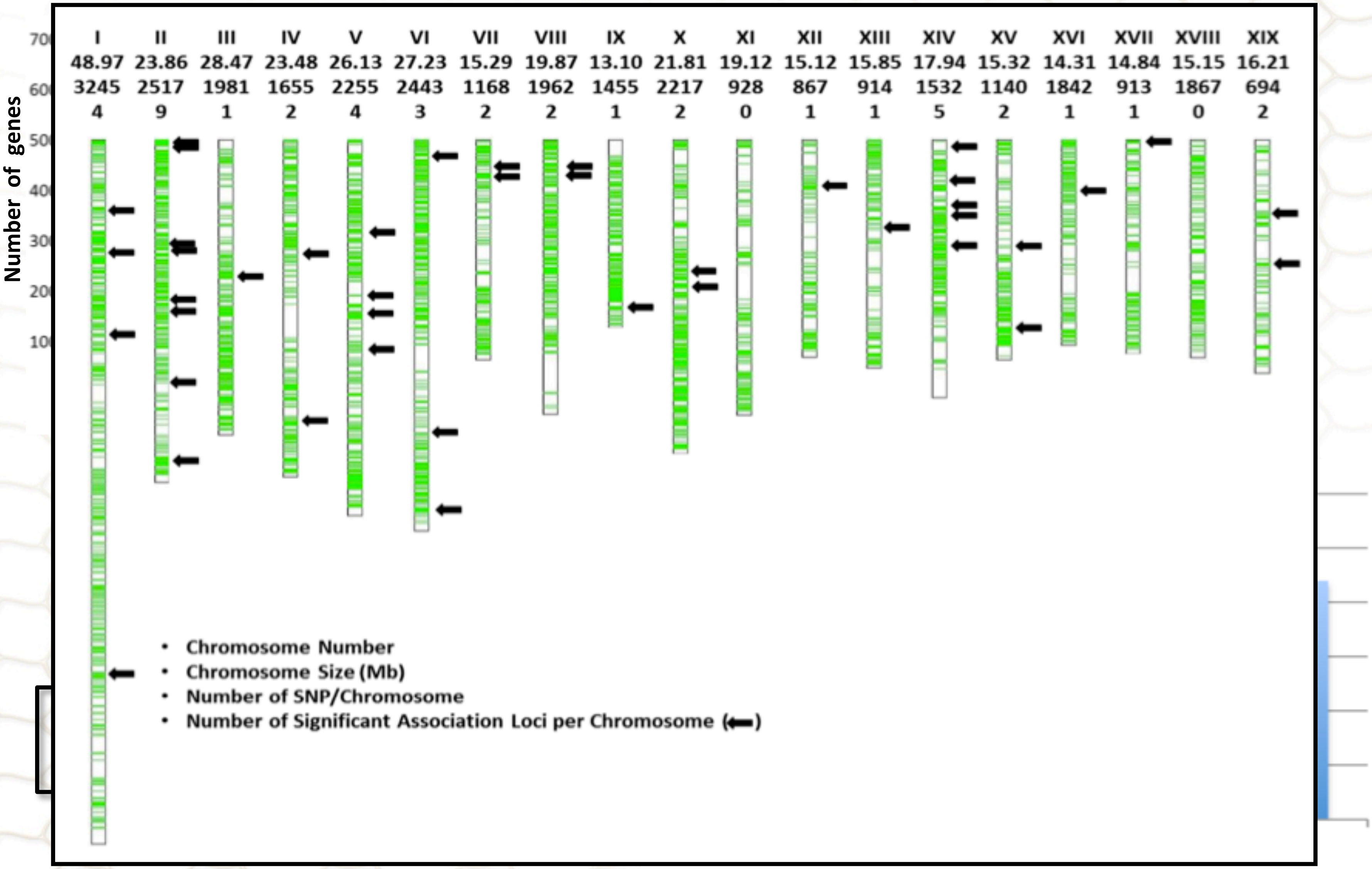
Natural Variation in Sugar Release

Native environment   Corvallis, OR field site



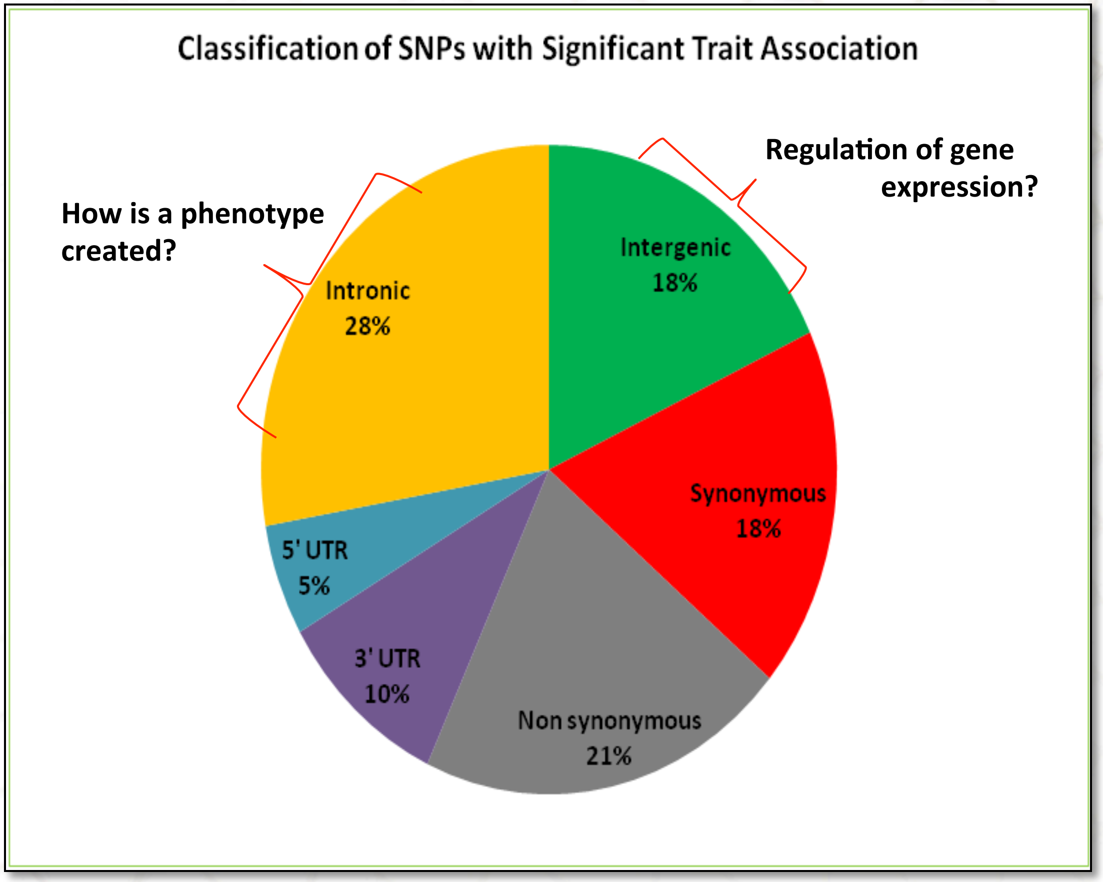
Phenotypic extremes

GWAS Analysis -- SNP distribution by genic region



Association Mapping: Results

- **42** SNPs had significant associations with cell wall chemistry and/or sugar release
- **6** were intergenic
- **34** SNPs mapped in **15** genes **– 18** were non-synonymous
- Of the 15 genes, **7** were **transcription factors**

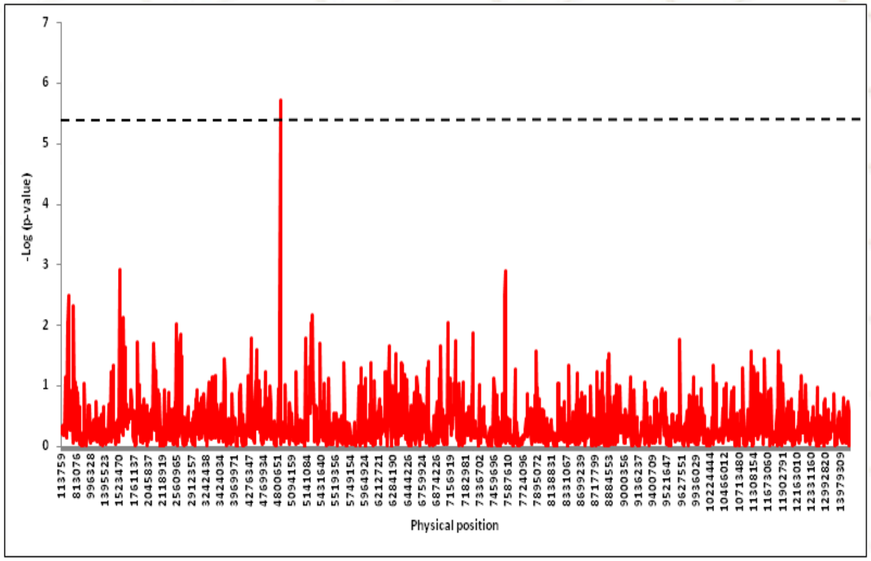

Classification of SNPs with Significant Trait Association

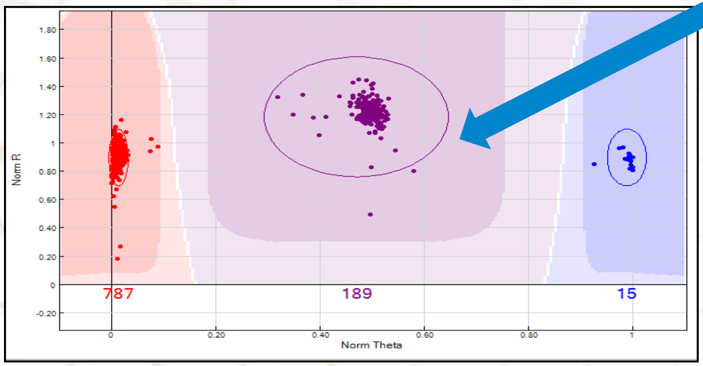
How is a phenotype created?

Regulation of gene expression?

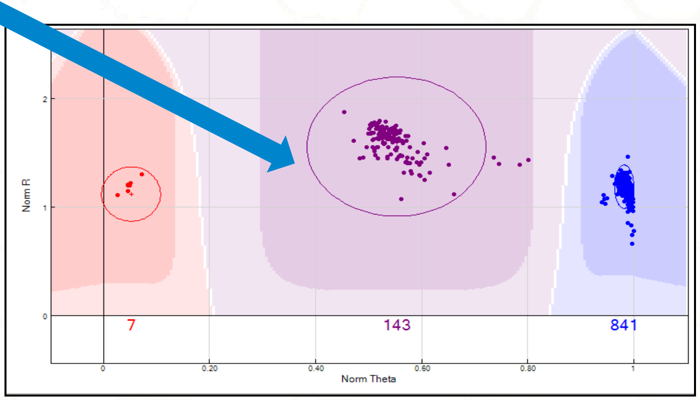
Association Mapping: Results

- *Populus* has 2 copies of a CW Synthase gene as a result of a whole-genome duplication event
- The two paralogs are highly identical (90% similarity at the sequence level)
- Preliminary evidence suggests that paralogs cannot complement each other
- Rare natural variants exhibit profound metabolite changes



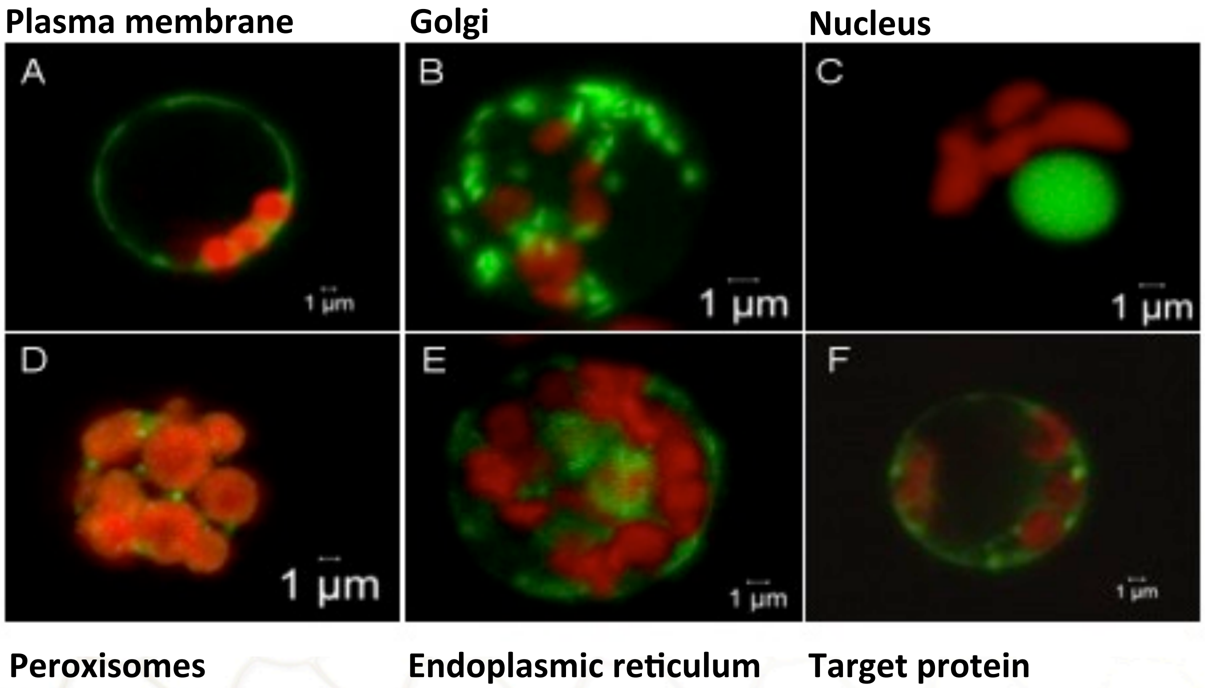


Scaffold_14_CW Synthase



Scaffold_2_CW Synthase

Transient Expression – Protoplast Reporter Genes



<u>Lignin</u>
-- 6 reporter genes
<u>Cellulose</u>
-- 5 reporter genes
<u>Hemicellulose</u>
-- 3 reporter genes

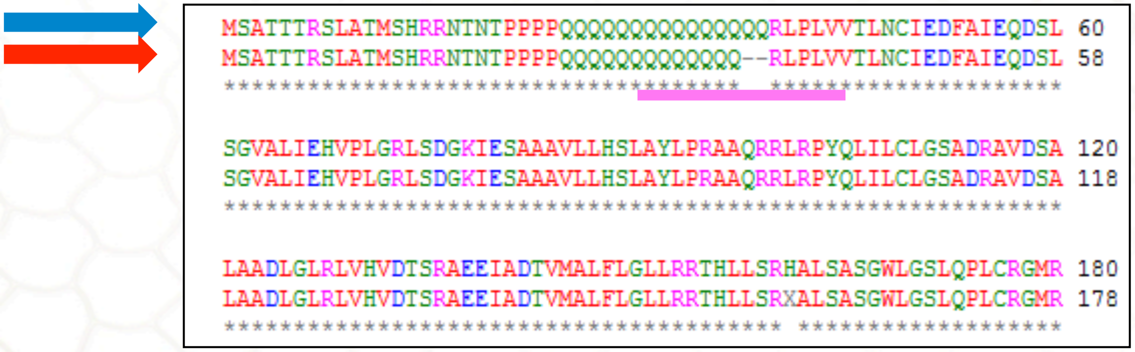
Transient transformation + GFP (or RT PCR)
= Initial verification of gene effect on targeted phenotype

Jay Chen, Jim Guo

Association Mapping: Transcription Factor CtBP

- cDNA cloning and sequencing revealed a **polyglutamine (PolyQ)** length polymorphism
- **PolyQ polymorphisms have been associated with numerous human degenerative disease**

Alternate allele amino acid alignment



```
MSATTTRSLATMSHRRNTNTPPPPQQQQQQQQQQQQQQQRLPLVVTLNCIEDFAIEQDSL 60
MSATTTRSLATMSHRRNTNTPPPPQQQQQQQQQQQQQ--RLPLVVTLNCIEDFAIEQDSL 58
*************************************  *********************

SGVALIEHVPLGRLSDGKIESAAAVLLHSLAYLPRAAQRRLRPYQLILCLGSADRAVDSA 120
SGVALIEHVPLGRLSDGKIESAAAVLLHSLAYLPRAAQRRLRPYQLILCLGSADRAVDSA 118
************************************************************

LAADLGLRLVHVDTSRAEEIADTVMALFLGLLRRTHLLSRHALSASGWLGSLQPLCRGMR 180
LAADLGLRLVHVDTSRAEEIADTVMALFLGLLRRTHLLSRXALSASGWLGSLQPLCRGMR 178
**************************************** *******************
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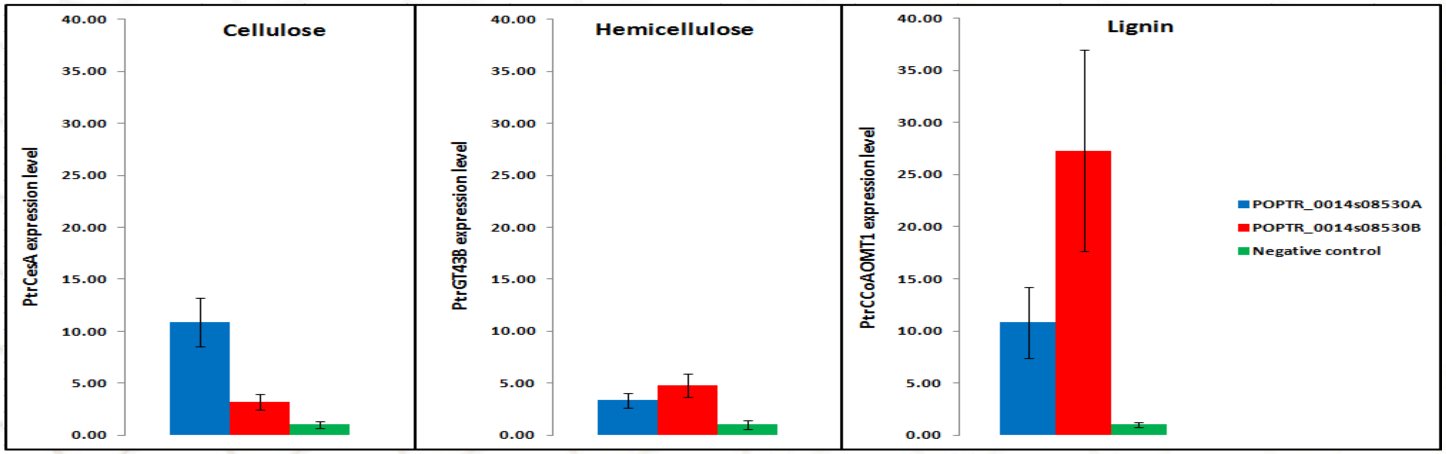
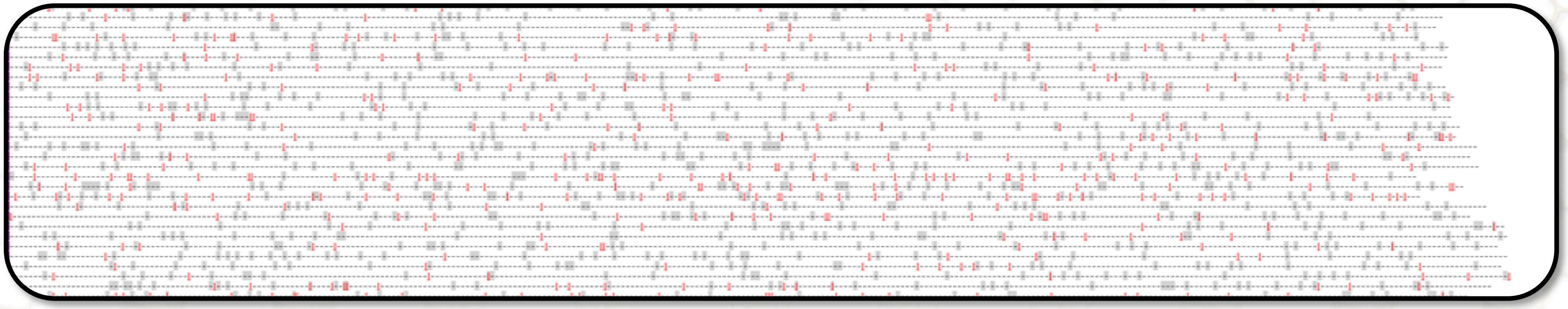
Side-by-side transfection of *Populus* protoplasts using alternate alleles

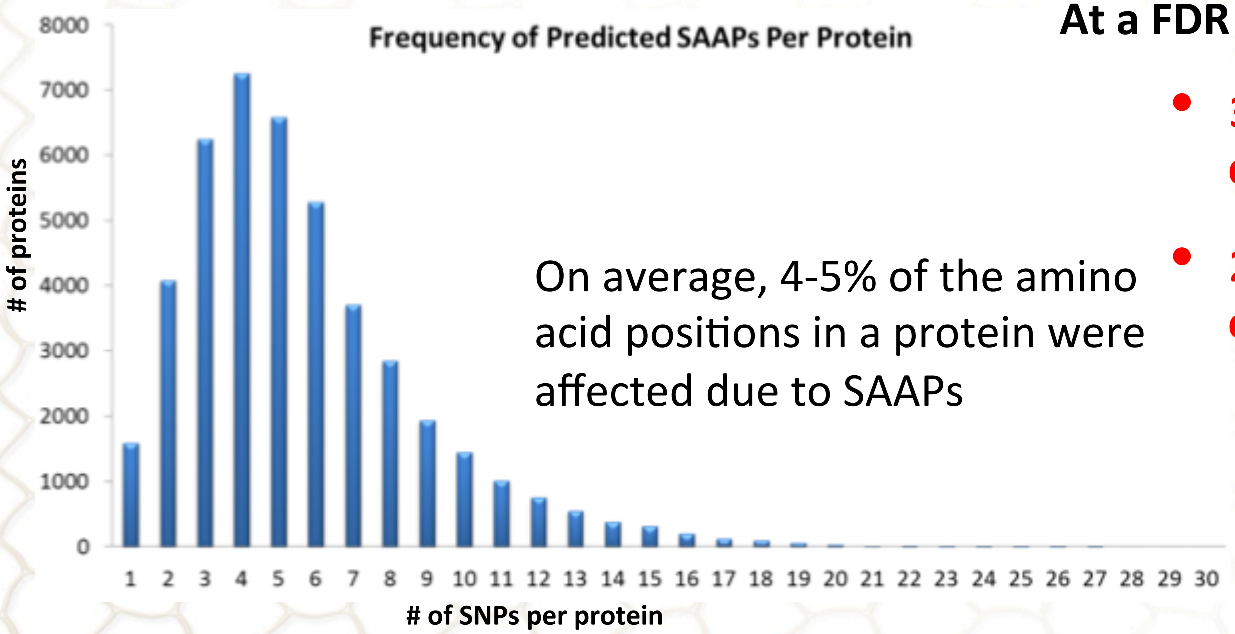


Association Mapping: Single Amino Acid Polymorphisms

Database includes:

- Over 400,000 SAAP positions
- Over 7.2 million SAAP peptides





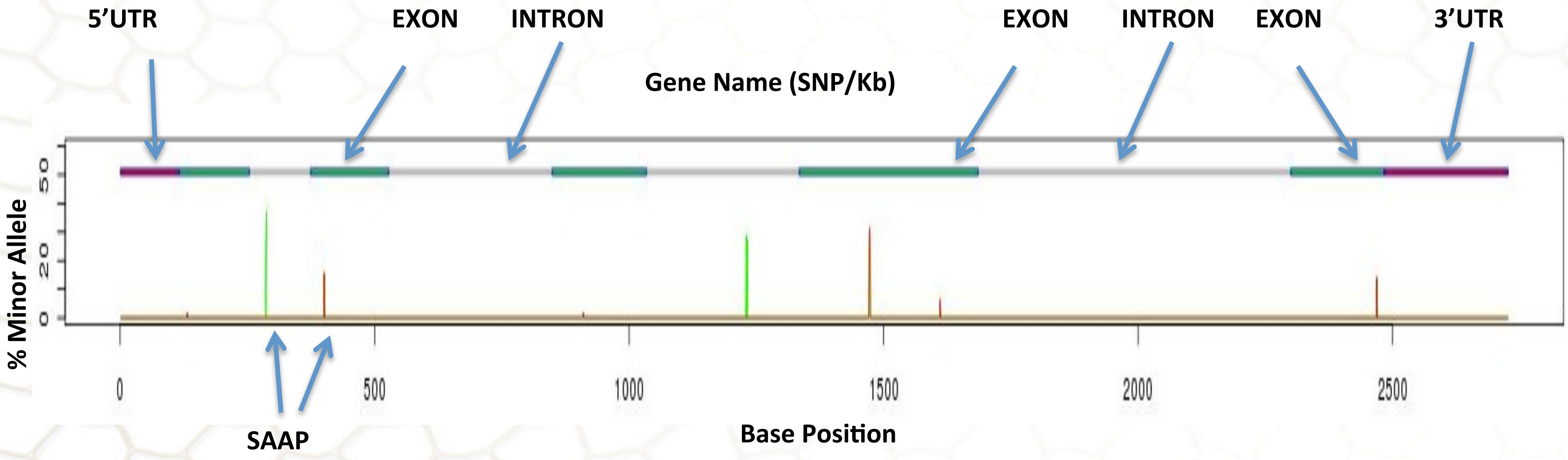
On average, 4-5% of the amino acid positions in a protein were affected due to SAAPs

At a FDR of <1%:

- 3% increase in detected proteins
- 25% increase in peptide coverage per protein

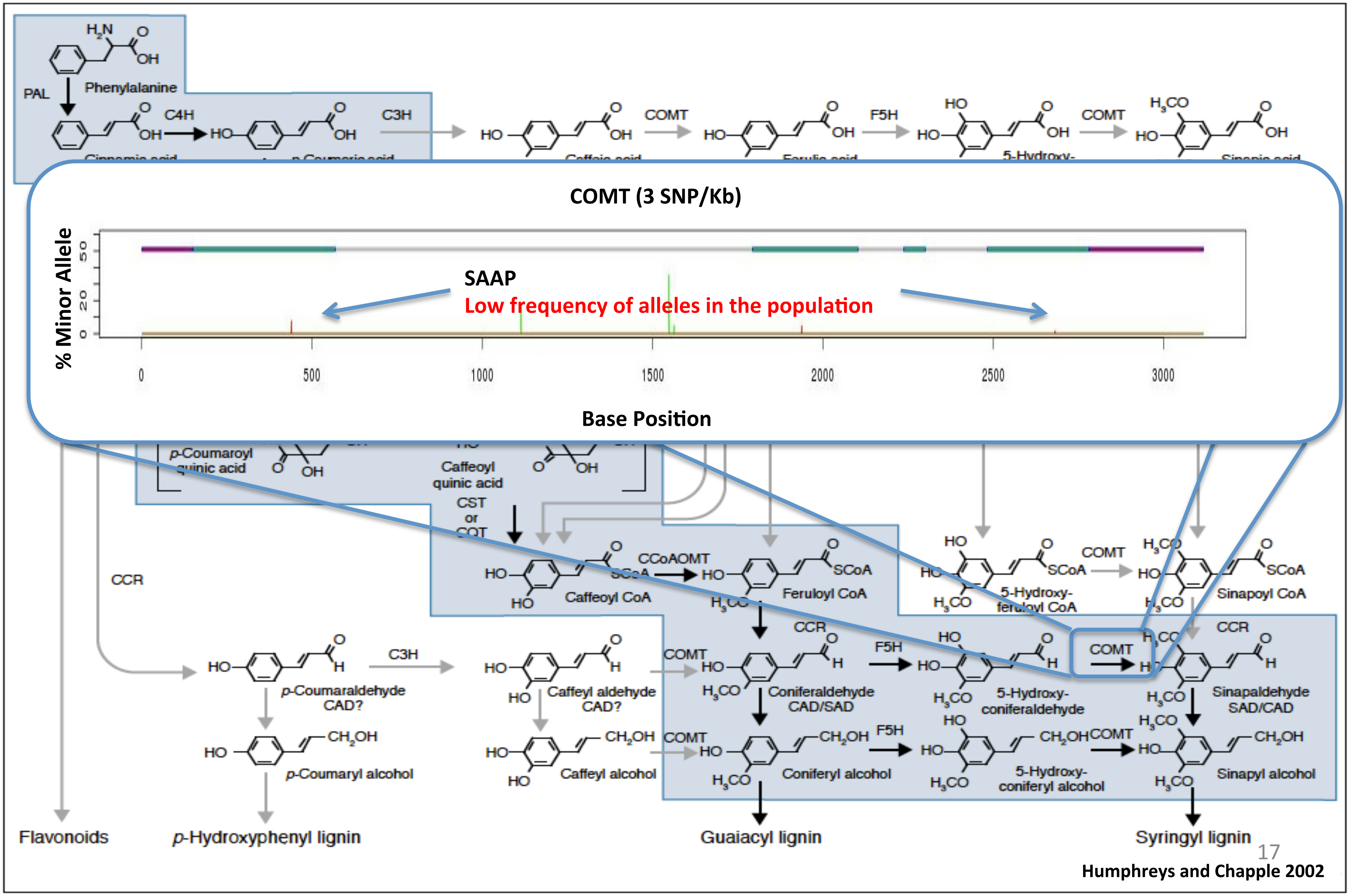
SAAP-based Functional Protein Modifications

- Non-synonymous substitutions
- Splice site mutants
- Premature stop codons
- Domain disruptions
- Conformational changes in protein folding
- Post-Translational Modifications



Ranjan Priya, Paul Abraham, Rachel Adams, Bob Hettich

Lignin Biosynthetic Pathway -- COMT



COMT (3 SNP/Kb)

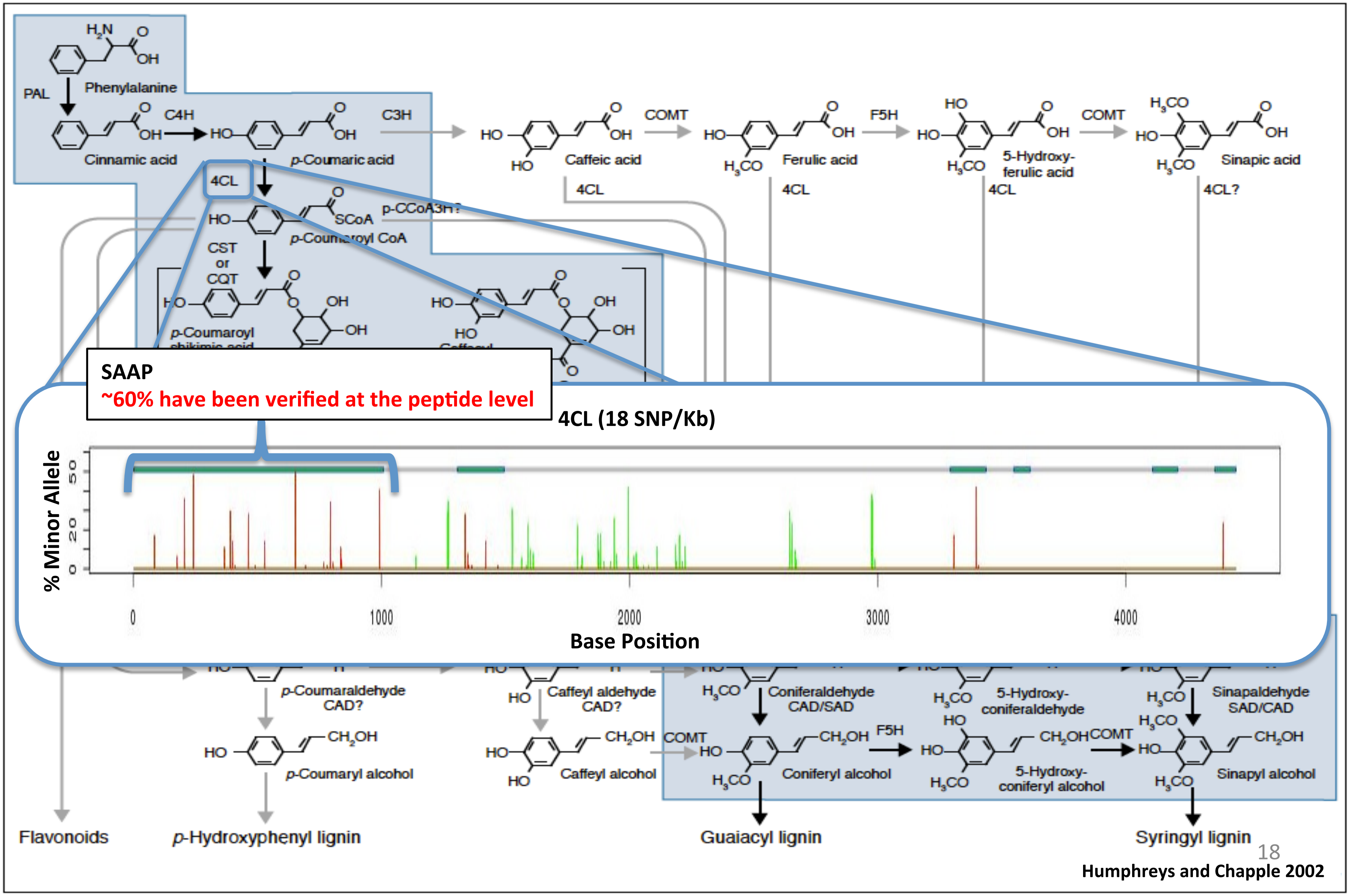
SAAP

Low frequency of alleles in the population

Base Position

Humphreys and Chapple 2002

Lignin Biosynthetic Pathway – 4CL



SAAP

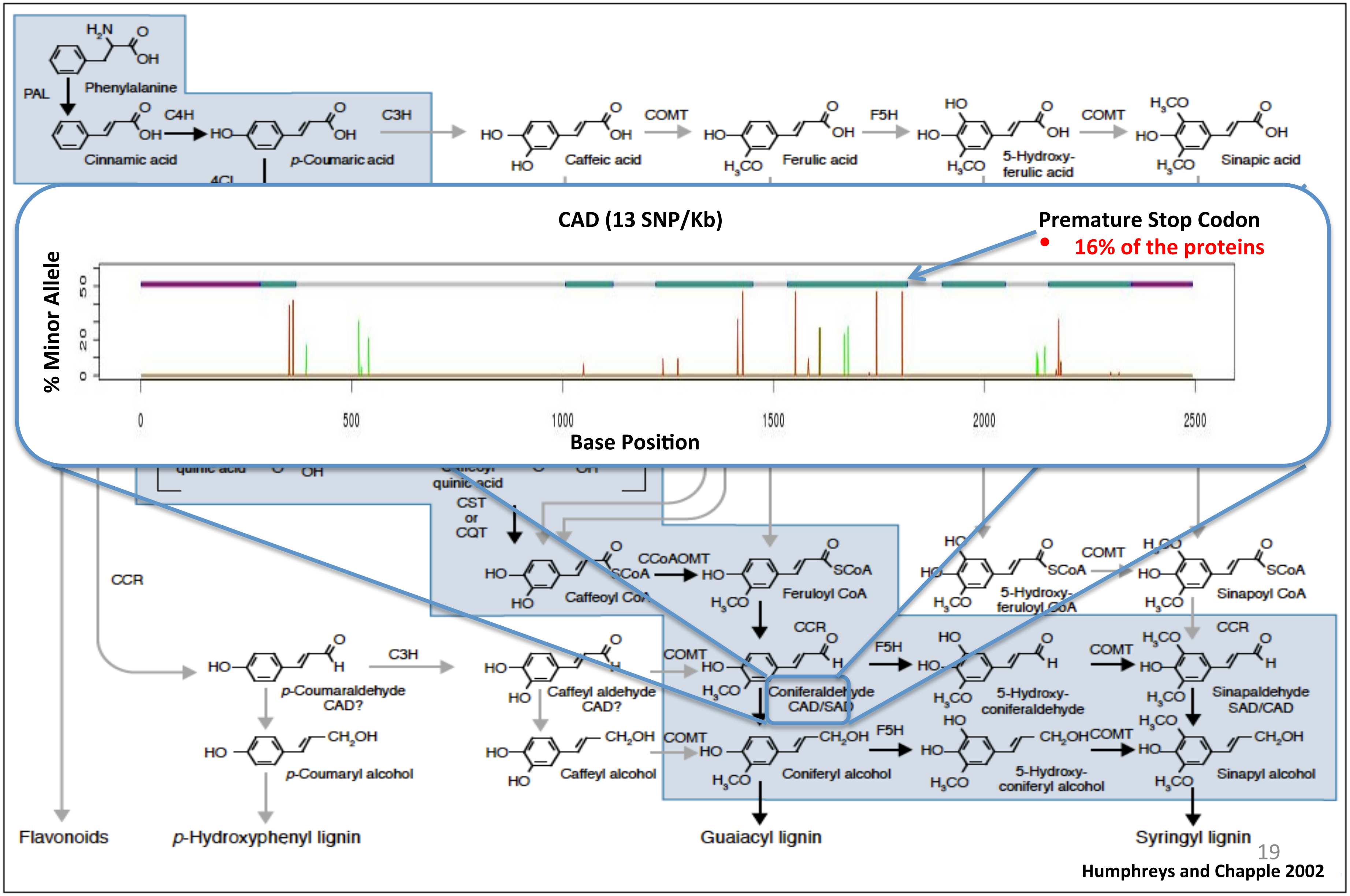
~60% have been verified at the peptide level

4CL (18 SNP/Kb)

Base Position

Humphreys and Chapple 2002

Lignin Biosynthetic Pathway -- CAD



CAD (13 SNP/Kb)

Premature Stop Codon

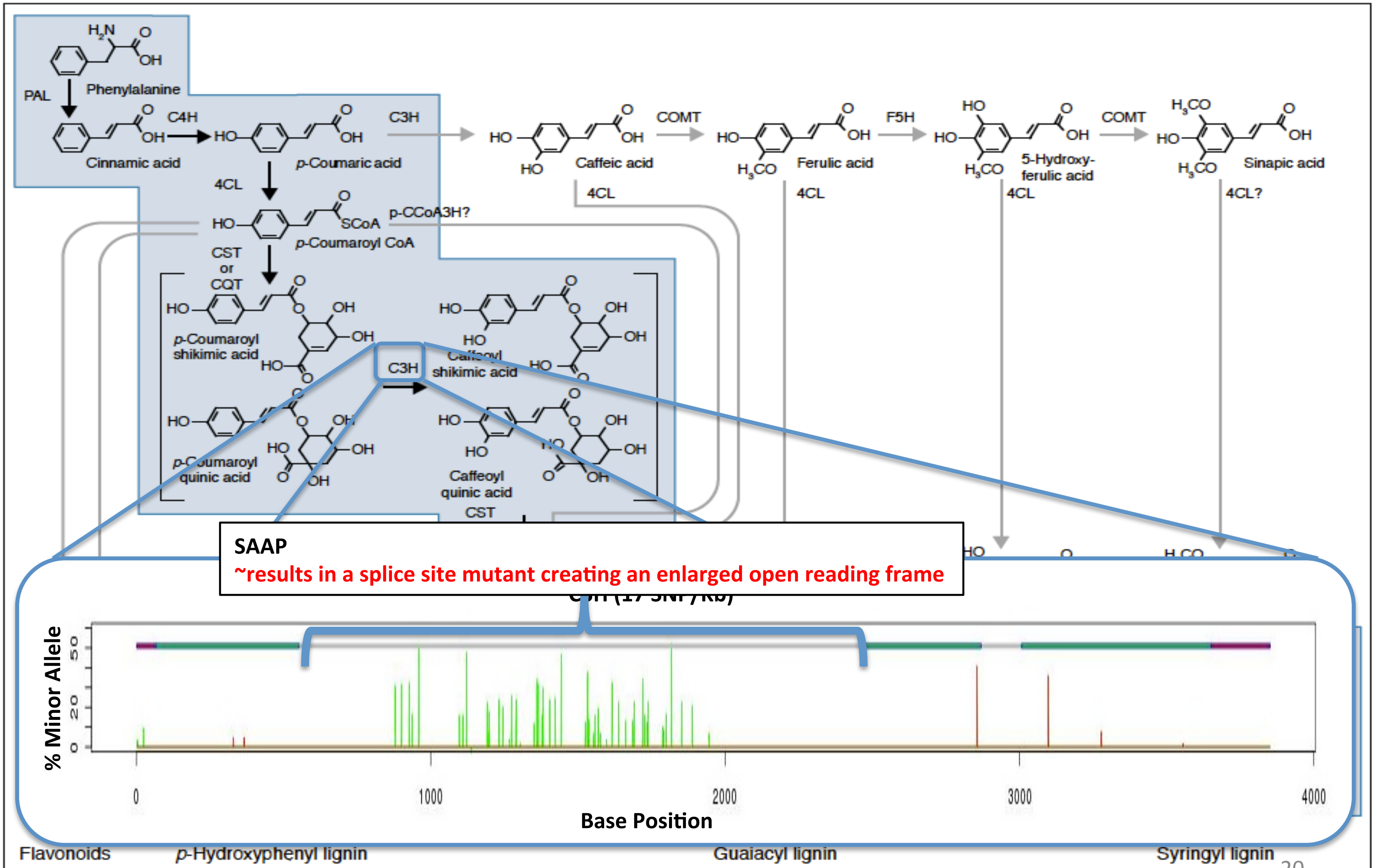
- **16% of the proteins**

% Minor Allele

Base Position

Humphreys and Chapple 2002

Lignin Biosynthetic Pathway – C3H

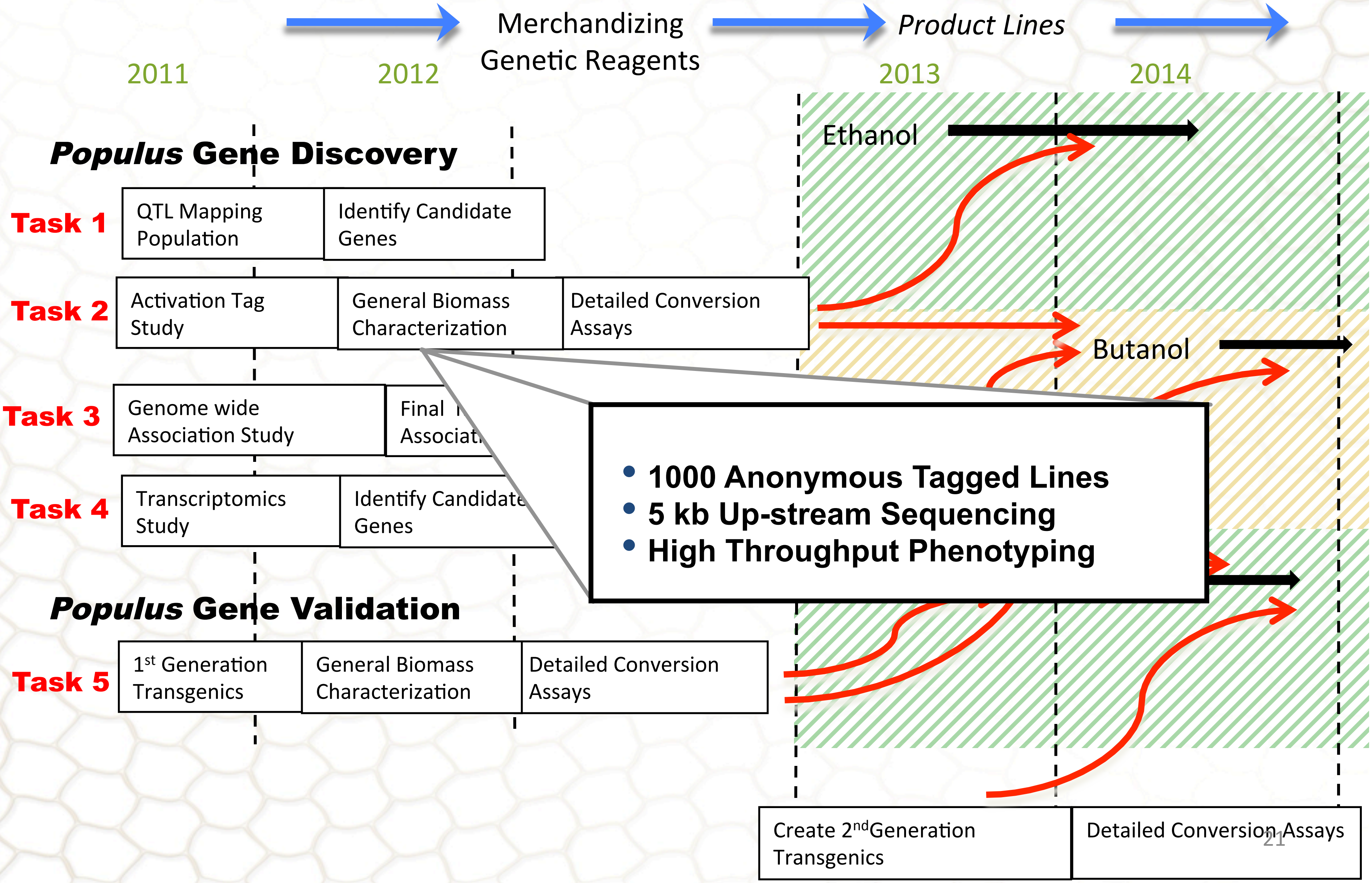


SAAP

~results in a splice site mutant creating an enlarged open reading frame

Humphreys and Chapple 2002

De Novo Gene Discovery – Activation Tagging



Merchandizing Genetic Reagents

Product Lines

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***Populus* Gene Discovery**

Task 1
QTL Mapping Population
Identify Candidate Genes

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General Biomass Characterization
Detailed Conversion Assays

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Genome wide Association Study
Final
Associat

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***Populus* Gene Validation**

Task 5
1st Generation Transgenics
General Biomass Characterization
Detailed Conversion Assays

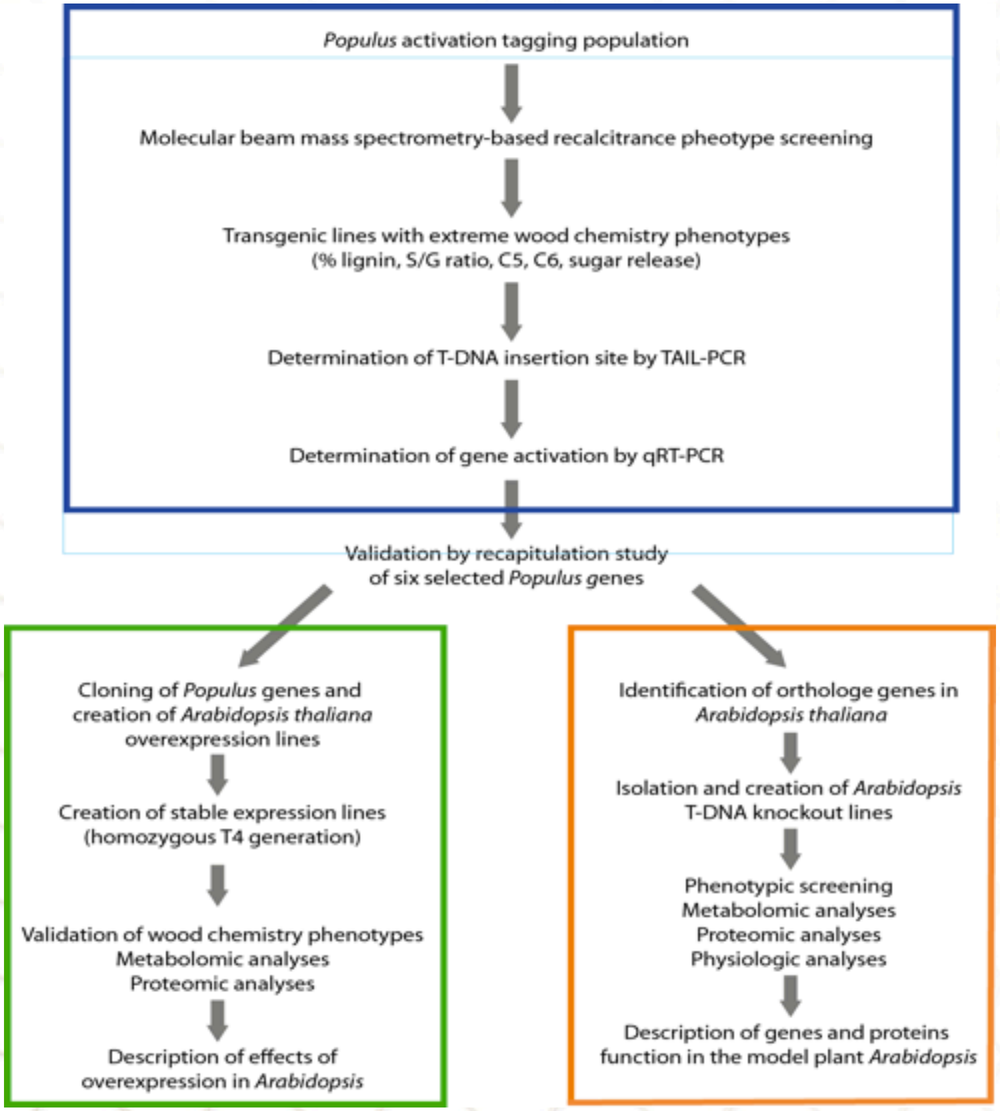
Create 2nd Generation Transgenics
Detailed Conversion Assays

Ethanol

Butanol

- **1000 Anonymous Tagged Lines**
- **5 kb Up-stream Sequencing**
- **High Throughput Phenotyping**

Activation Tagging: Results



Populus activation tagging population

Molecular beam mass spectrometry-based recalcitrance pheotype screening

Transgenic lines with extreme wood chemistry phenotypes
(% lignin, S/G ratio, C5, C6, sugar release)

Determination of T-DNA insertion site by TAIL-PCR

Determination of gene activation by qRT-PCR

Validation by recapitulation study
of six selected *Populus* genes

Cloning of *Populus* genes and
creation of *Arabidopsis thaliana*
overexpression lines

Creation of stable expression lines
(homozygous T4 generation)

Validation of wood chemistry phenotypes
Metabolomic analyses
Proteomic analyses

Description of effects of
overexpression in *Arabidopsis*

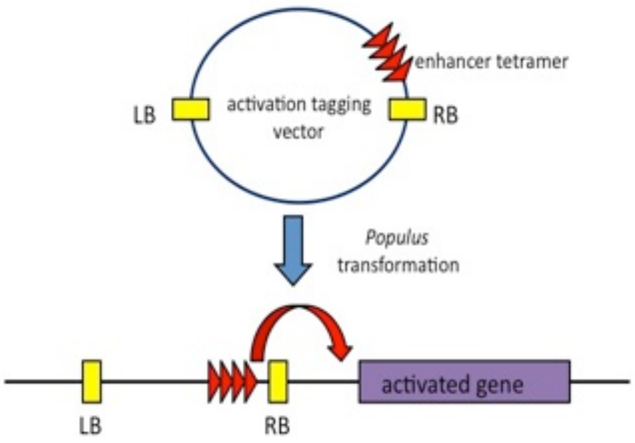
Identification of orthologe genes in
Arabidopsis thaliana

Isolation and creation of *Arabidopsis*
T-DNA knockout lines

Phenotypic screening
Metabolomic analyses
Proteomic analyses
Physiologic analyses

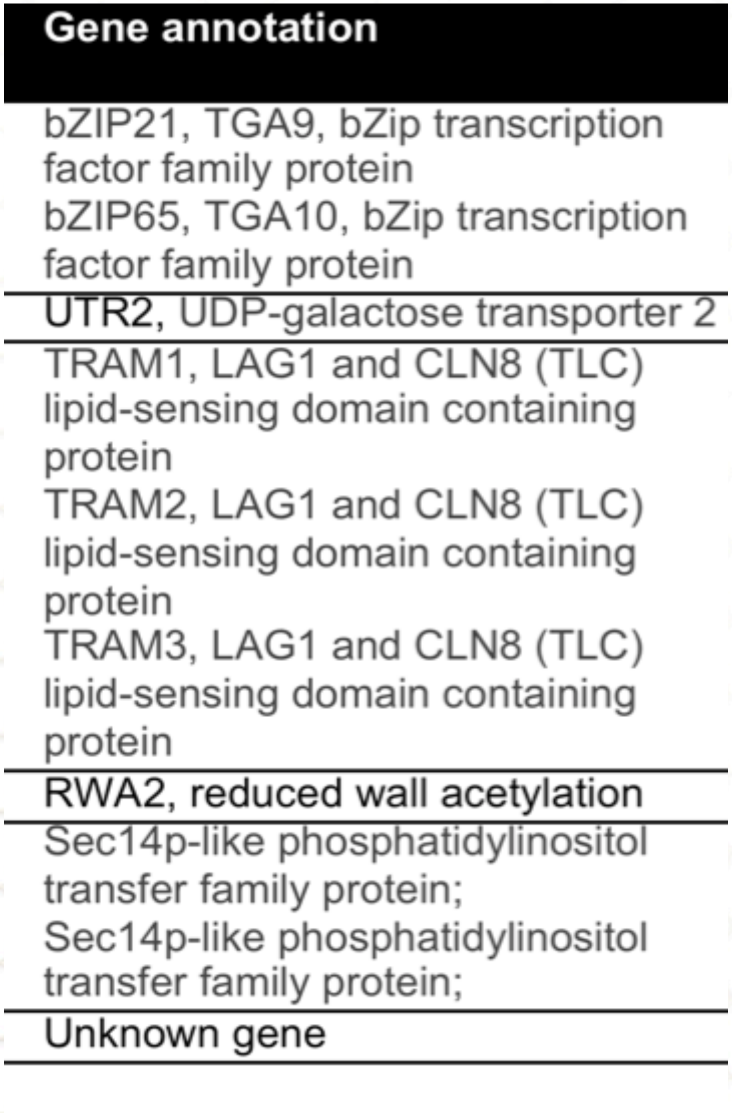
Description of genes and proteins
function in the model plant *Arabidopsis*

Research strategy to discover genes regulating cell wall biosynthesis and biomass recalcitrance



Activation tagging: an insertional mutagenesis technique that results in the dominant up-regulation of an endogenous gene.

Activation Tagging: Results



Gene annotation
bZIP21, TGA9, bZip transcription factor family protein bZIP65, TGA10, bZip transcription factor family protein
UTR2, UDP-galactose transporter 2
TRAM1, LAG1 and CLN8 (TLC) lipid-sensing domain containing protein TRAM2, LAG1 and CLN8 (TLC) lipid-sensing domain containing protein TRAM3, LAG1 and CLN8 (TLC) lipid-sensing domain containing protein
RWA2, reduced wall acetylation
Sec14p-like phosphatidylinositol transfer family protein; Sec14p-like phosphatidylinositol transfer family protein;
Unknown gene

Arabidopsis genes putatively involved in cell wall biosynthesis and biomass recalcitrance

Six *Populus* genes, Ten *Arabidopsis* homologues, 28 *Arabidopsis* T-DNA insertion lines . . .

Jay Chen, Olaf Czarnecki

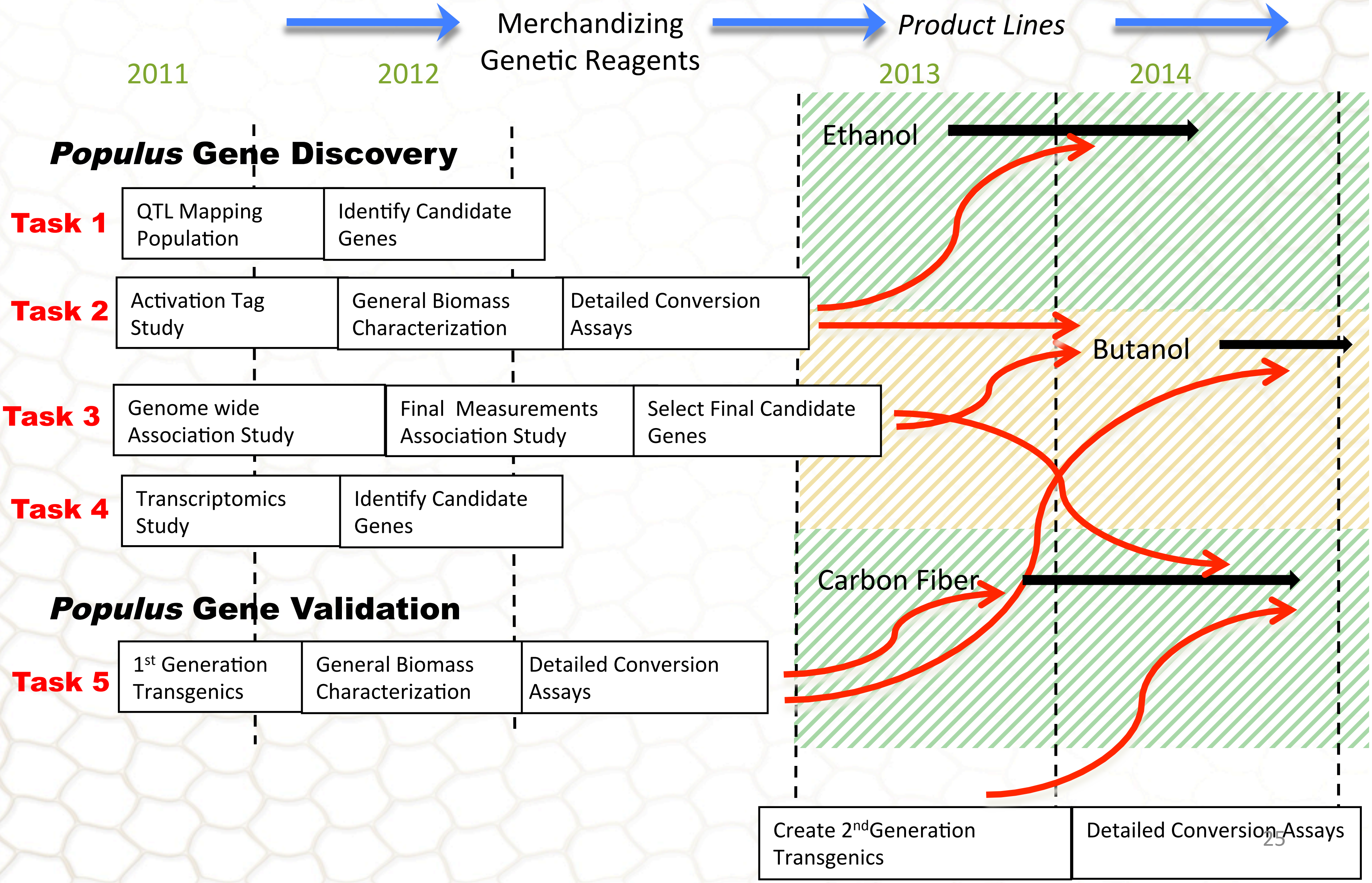
Activation Tagging: Results

One clear morphological phenotype so far…

2-week-old
Arabidopsis
mutant

4-week-old
Arabidopsis
mutant

De Novo Gene Discovery – Transformation Efforts



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Task 5

1st Generation Transgenics

General Biomass Characterization

Detailed Conversion Assays

Ethanol

Butanol

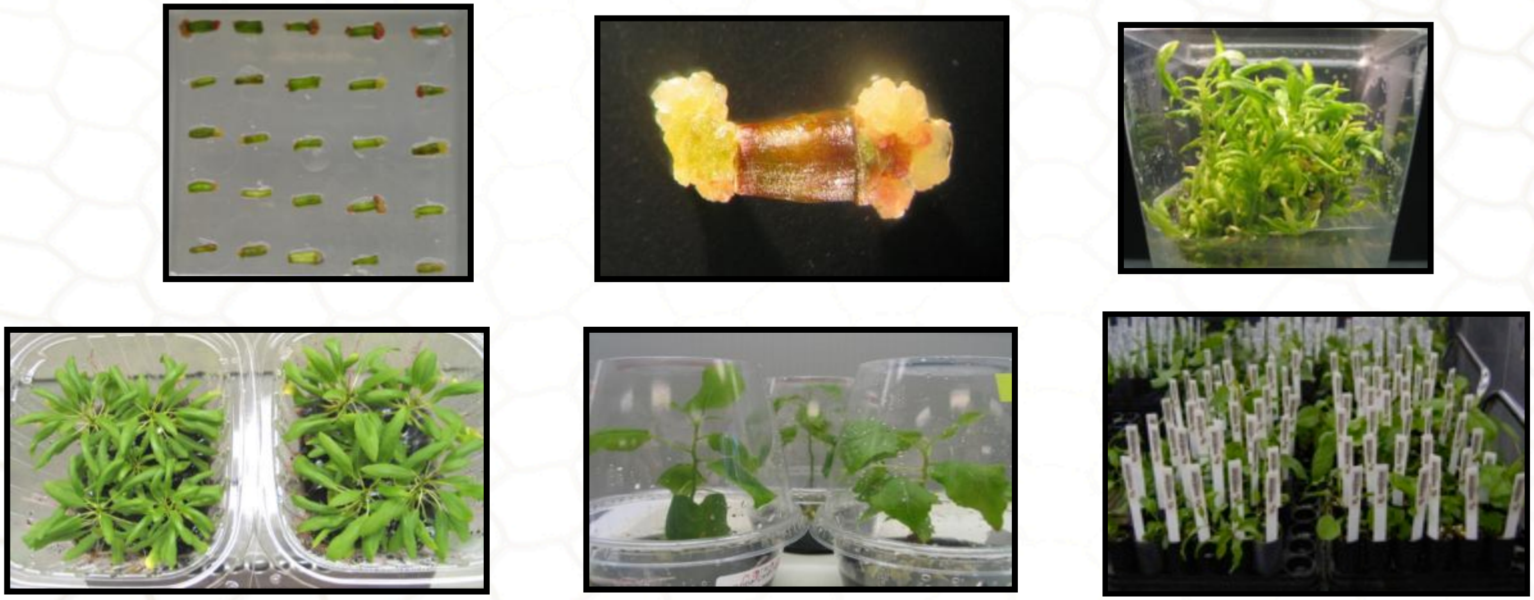
Carbon Fiber

Create 2nd Generation Transgenics

Detailed Conversion Assays

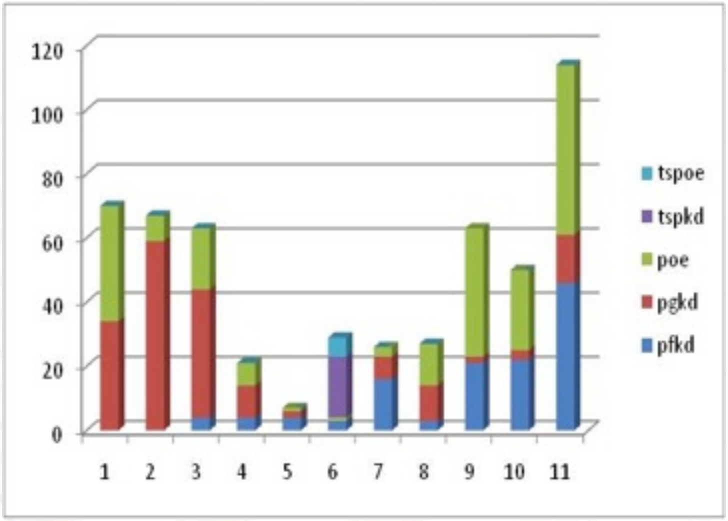
Stable Transformation Results

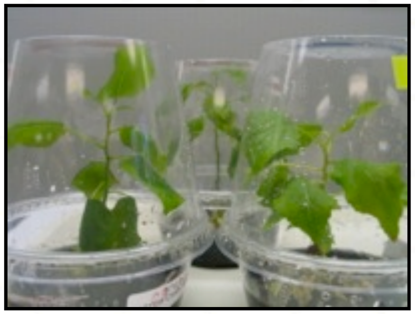
Constitutive Over-Expression and RNAi Knockdown lines have been created



Maud Hinchee, Will Rottmann, Lee Gunter

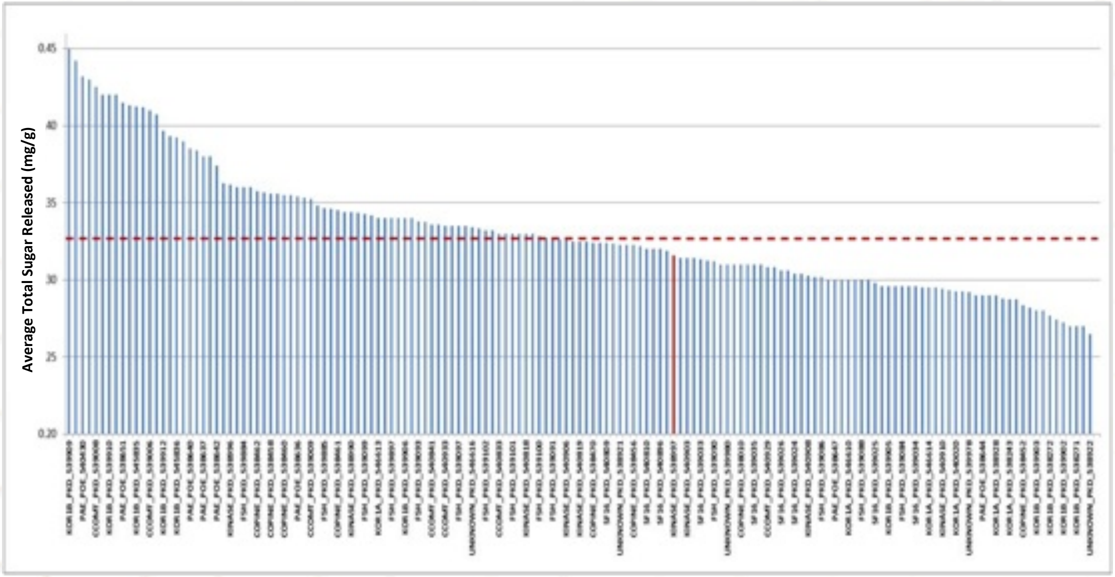
Stable Transformation Results



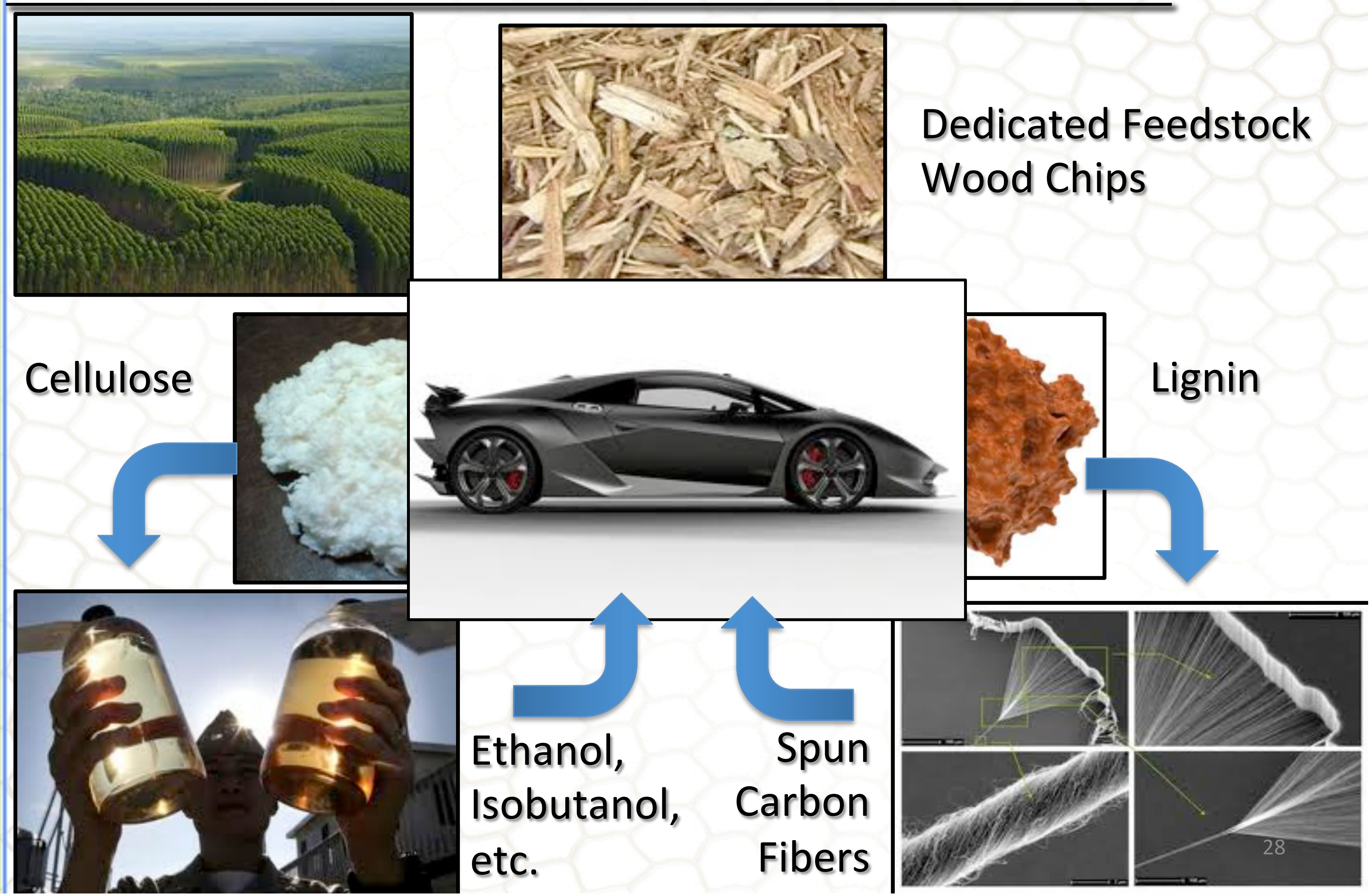


Candidate gene submission and analysis

- **Primer design for 537 gene expression constructs**
- Sequence verification for **273** constructs
- Ramets/constructs received (**24,508/127**)
- Analyzed for wood chemistry (**3,717**) and sugar release (**1,654**)
- **42** elite lines selected for qPCR expression and metabolite analysis
- Propagated **98** constructs in stool beds



Cell Wall Deconstruction and Product Output



Dedicated Feedstock
Wood Chips

Cellulose

Lignin

Ethanol,
Isobutanol,
etc.

Spun
Carbon
Fibers

Acknowledgments – Thank You

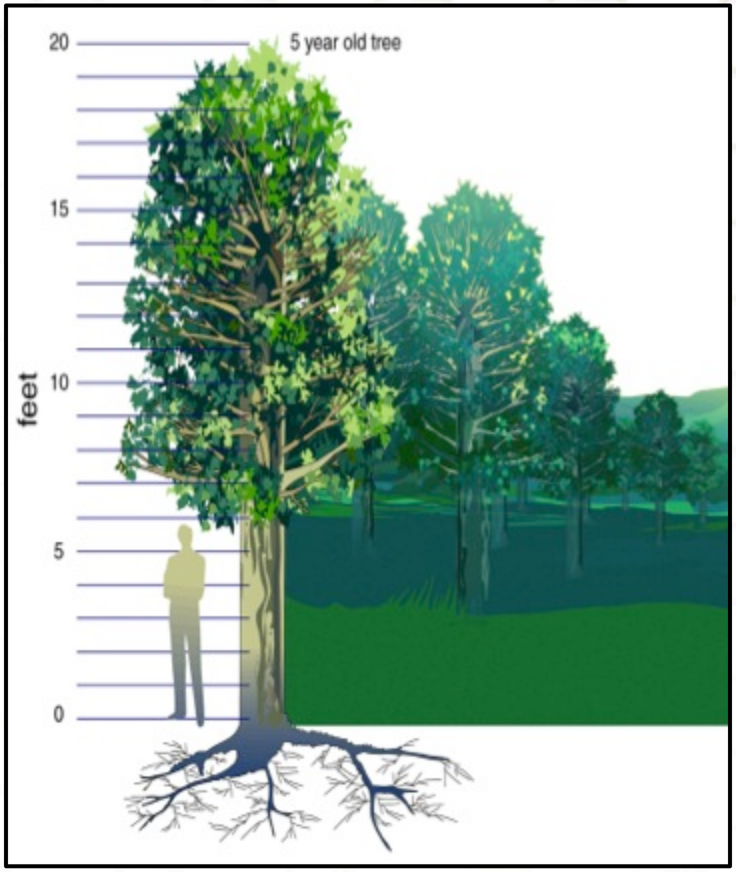
SNP Detection -- Wendy Schackwitz, Joel Martin, Len Pennacchio

SNP Array -- Stephen DiFazio, Gancho Slavov

Association Genetics -- Priya Ranjan, Wellington Muchero

Proteomics -- Paul Abraham, Rachel Adams, Robert Hettich

Genome Assembly & Annotation – Dan Rokshar, Jeremy Schmutz



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ARBORGEN

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BESC BioEnergy Science Center