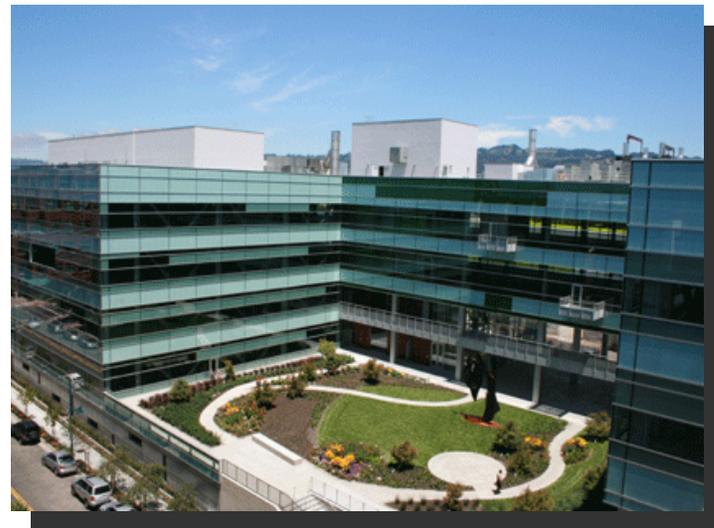


Targeting plant cell walls at JBEI **(advancing cellulosic biofuels)**

Joshua Heazlewood
Feedstocks Division

- Six Partners
 - Lawrence Berkeley National Lab
 - Sandia National Lab
 - Lawrence Livermore National Lab
 - UC Berkeley
 - UC Davis
 - Carnegie Institute
- Four Science and Technology Divisions
 - Feedstocks
 - Deconstruction
 - Fuels Synthesis
 - Technologies
- Single location
 - Emeryville, CA



**Cell Wall
Synthesis**

Director

Henrik Scheller



Team Members

Yuzuki Manabe
Chithra Manisseri
Ai Oikawa
Lan Yin
Ulla Christensen
Michelle Truong
Eva Knoch
Carsten Rautengarten
Yves Verhertbruggen
Anongpat Suttangkakul

4/2/2009

**Cell Wall
Engineering**

Director

Dominique Loque



Team Members

Fan Yang
Parul Tomar
Prajakta Pradhan
Kejian Zheng
Jin-Sun Kim
Aymerick Eudes
Ling Zhang

**Grass
Genetics**

Vice President

Pamela Ronald



Team Members

Laura Bartley
Patrick Canlas
Peijian Cao
Dawn Chinquy
Kihong Jung
Miguel Vega-Sanchez
Brian Conlin
Maw Sheng Chern
Xuwei Chen
Wing Hoi Sze-To

**Systems
Biology**

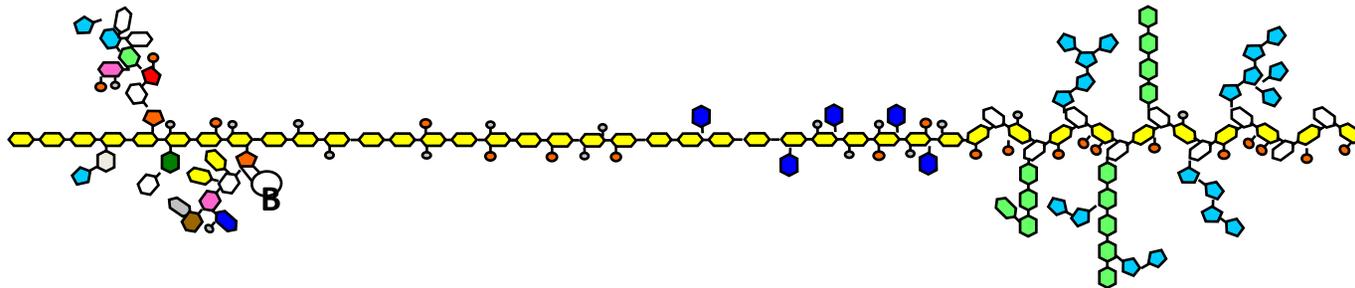
Director

Joshua Heazlewood



Team Members

Katy Christiansen
Jason Ito
Harriet Parsons
Michelle Smith
Sherry Chan

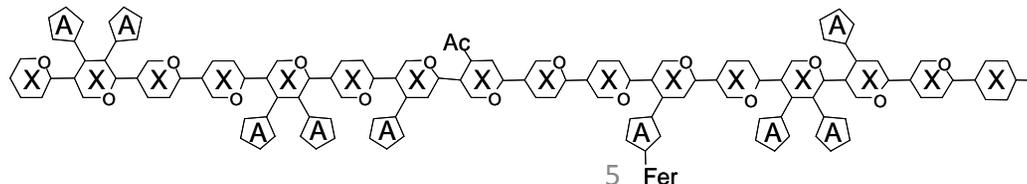


How is hemicellulose synthesized?

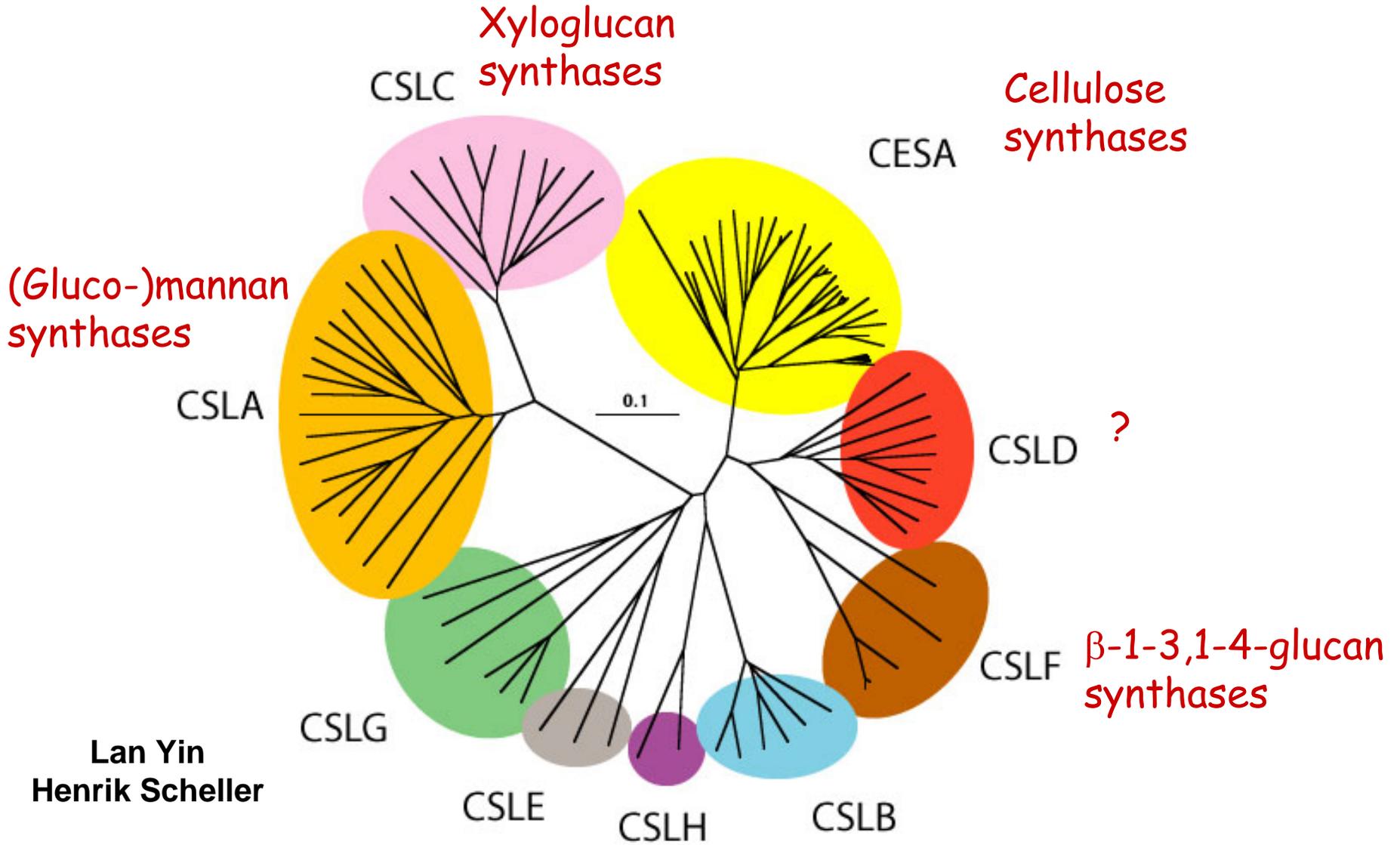
Hemicellulose and pectin (20% of a plant mass)

- Synthesized by large family of redundant proteins (450 to 800)
- highly complex
- contain large fractions of hard-to-ferment sugars (e.g. xylose)
- contain phenolic crosslinks
- contain acetyl esters that inhibit deconstruction and fermentation

Only few of the enzymes involved in their biosynthesis are known!

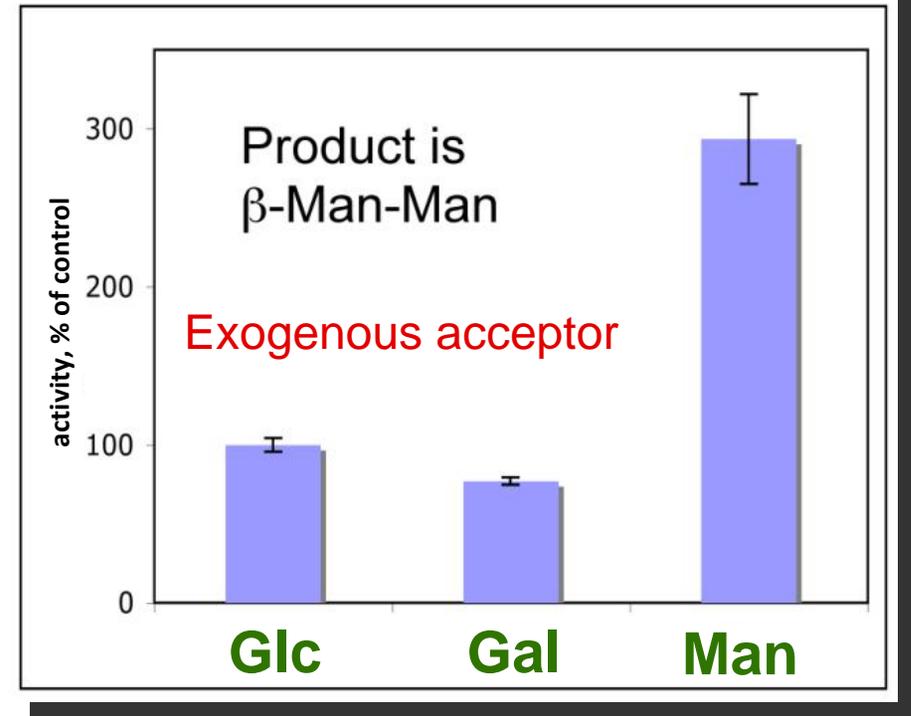
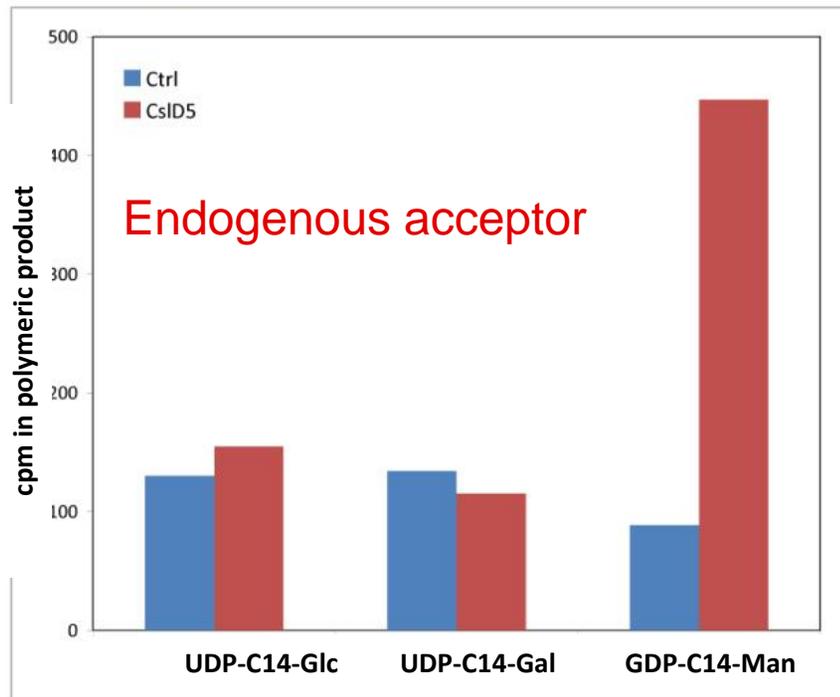
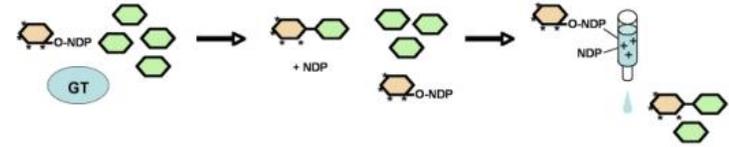


CESA-CSL superfamily responsible for synthesis of cellulose and hemicelluloses



Lan Yin
Henrik Scheller

CsID proteins are mannan synthases



- In two assays, CsID5 shows mannan synthase activity
- Mannan may potentially replace xylan for improved deconstruction and fuel synthesis

Multiple CsID proteins required for one function



csld2/csld3/csld5 triple mutant

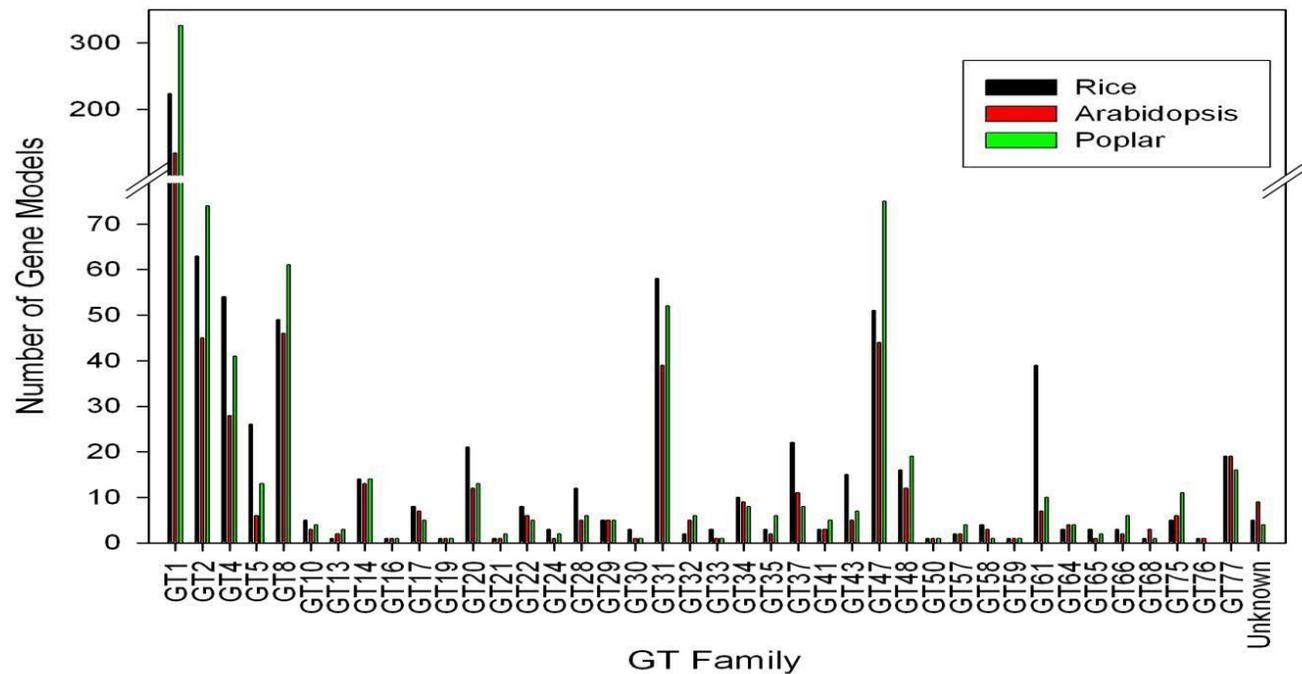
Csld5 + (d3 or d2) required for plant growth

As predicted from genetic analysis and 'Split YFP' analysis, CsID2, CsID3, and CsID5 are redundant

Analysis of double and triple mutants and overexpressors is in progress

Co-immunoprecipitation experiments in progress

769 rice GT gene models were identified and classified into 40 families



282 putative rice-specific GTs in 24 families

(no orthologs in Arabidopsis, Populus, Medicago, Ricinus)

Family	No. (Total)	Family	No. (Total)	Family	No. (Total)
GT1	85(224)	GT24	2(3)	GT47	25(51)
GT2	29(63)	GT28	3(12)	GT48	11(16)
GT4	13(54)	GT29	2(5)	GT58	3(4)
GT5	4(26)	GT31	26(58)	GT61	20(39)
GT8	9(49)	GT34	3(10)	GT64	1(3)
GT10	1(5)	GT37	13(22)	GT66	1(3)
GT14	5(14)	GT41	1(3)	GT75	1(5)
GT20	9(21)	GT43	5(15)	GT77	10(19)

33 genes prioritized based on expression levels in stem/leaf

Peijian Cao
Laura Bartley
Pam Ronald

May 19, 2008

[Home](#) [General information](#) [DB search](#) [Download](#) [Tree viewer](#) [Distribution map](#) [Links](#) [Contact us](#)

Rice GTs Database

Available data for each GT can be mapped to the tree. Select as many fields as you would like to see and click "Submit" to display them on the tree. Click [here](#) to view the tree. Click the [TIGR](#) webpage which

[Sequence Information](#)
[Sequence Quality Information](#)
[Homologs in Dicots](#)
[Knockouts](#)
[Topology](#)
[Conserved Domains](#)
[MPSS mRNA Data](#)
[MPSS smallRNA Data](#)
[Digital Northern Data](#)
[Microarray Data](#)

Instructions

Sequence Information
 Sequence Quality
 Orthologs in Dicots
 Mutants
 Topology
 MPSS mRNA Data
 MPSS smallRNA Data
 Digital Northern Data
 Microarray Data

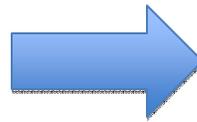
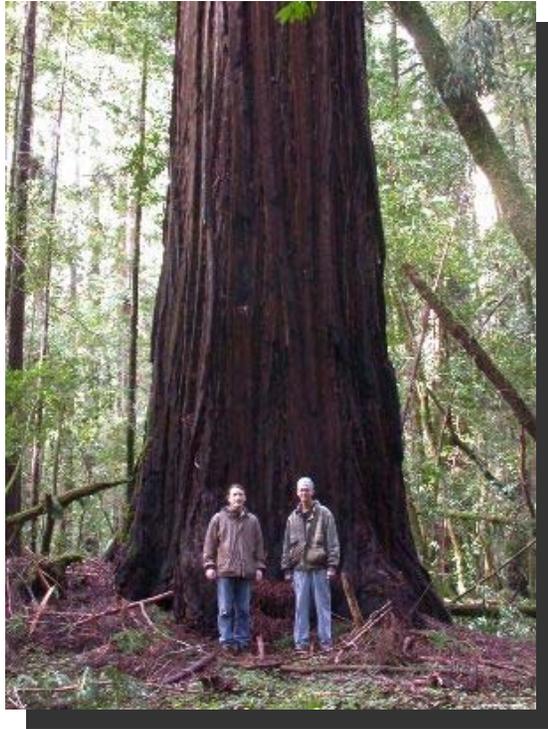
Paralog Family Chromosome
 NCBI Blast

OTL T-DNA FST
 T-DNA FST

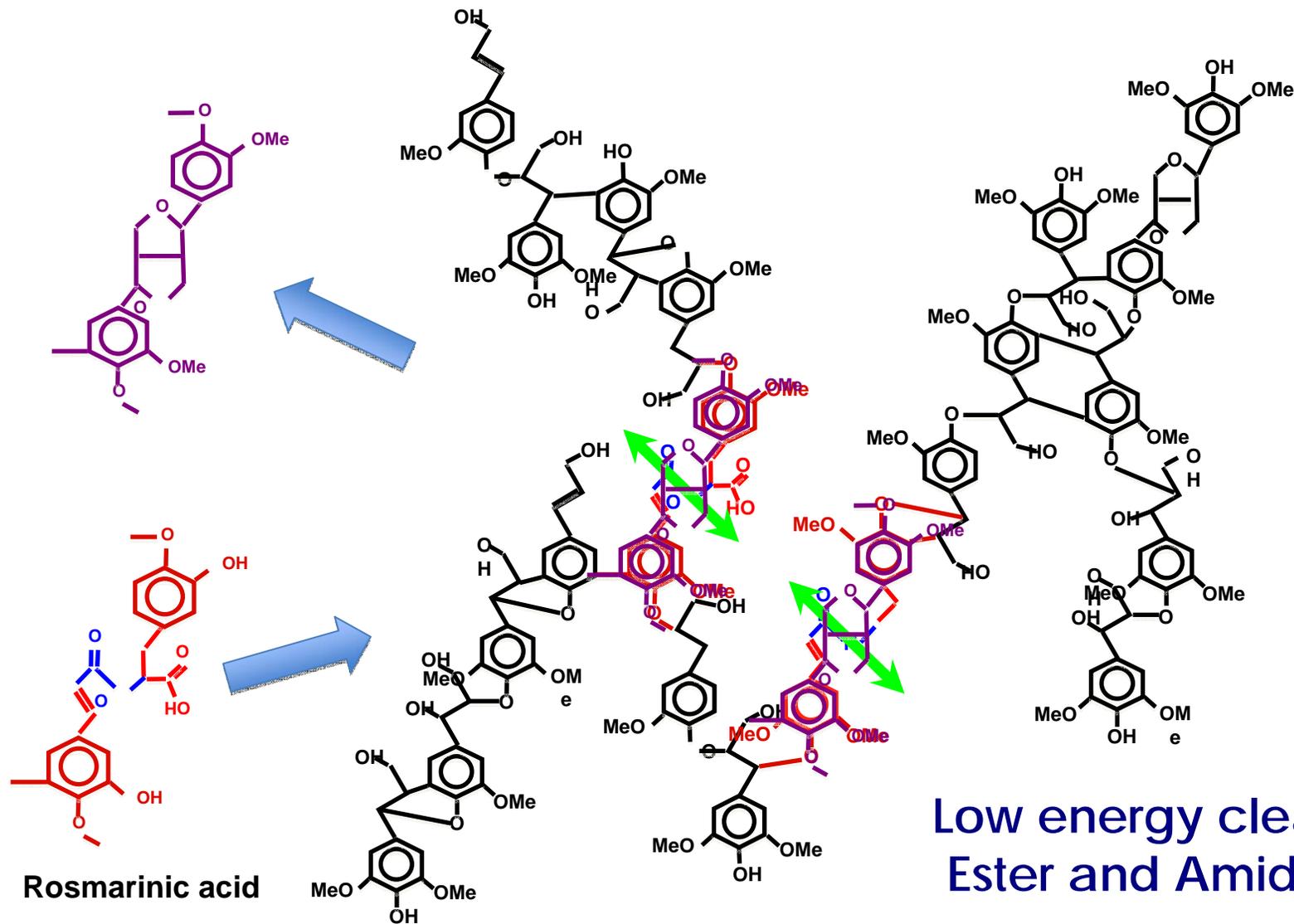
redicted Subcellular Localization
 NYL NYR

stil Root Root tip Seed

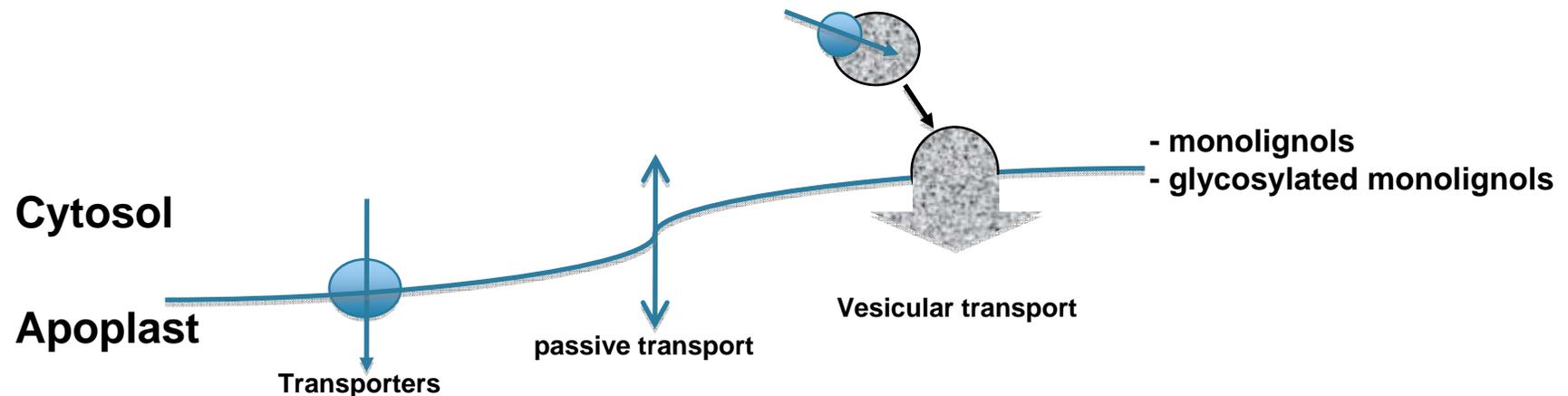
<http://ricephylogenomics.ucdavis.edu/cellwalls/gt/>



Can we overcome lignin recalcitrance?



The elusive monolignol export pathway

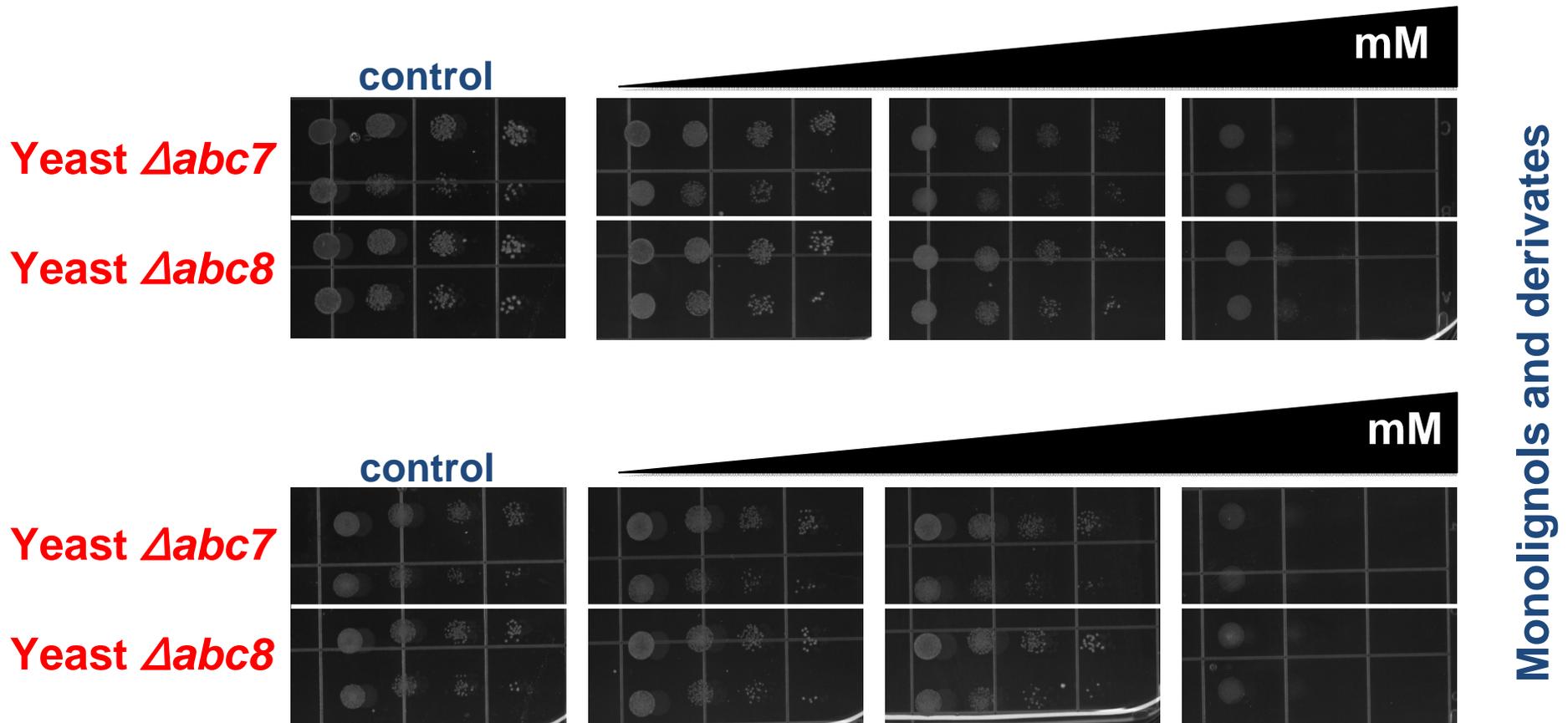


Plasma membrane transport?

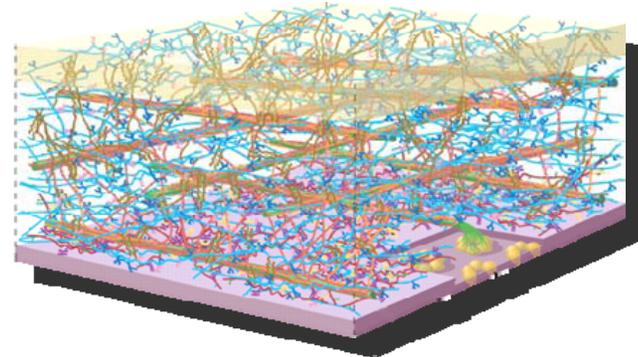
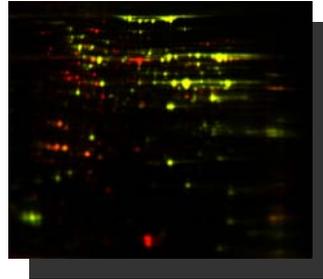
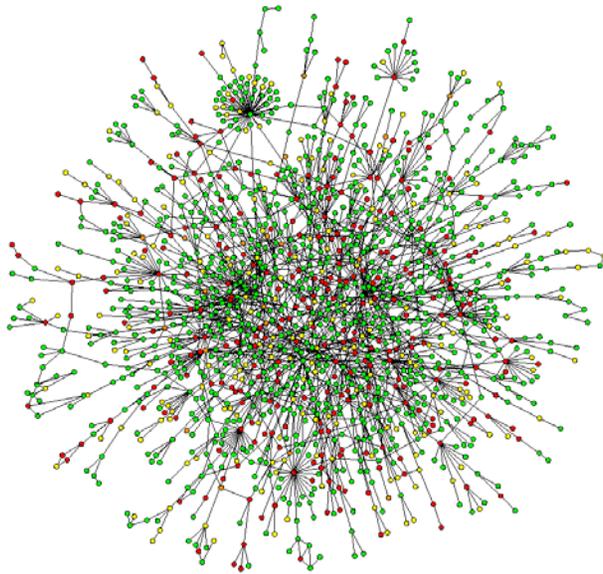
“These data, produced with the best microscopy tools that are available today, support a model where unknown membrane transporters, rather than Golgi vesicles, export monolignols.”

Lacey Samuels (Kaneda et al., 2008 Plant Physiology)

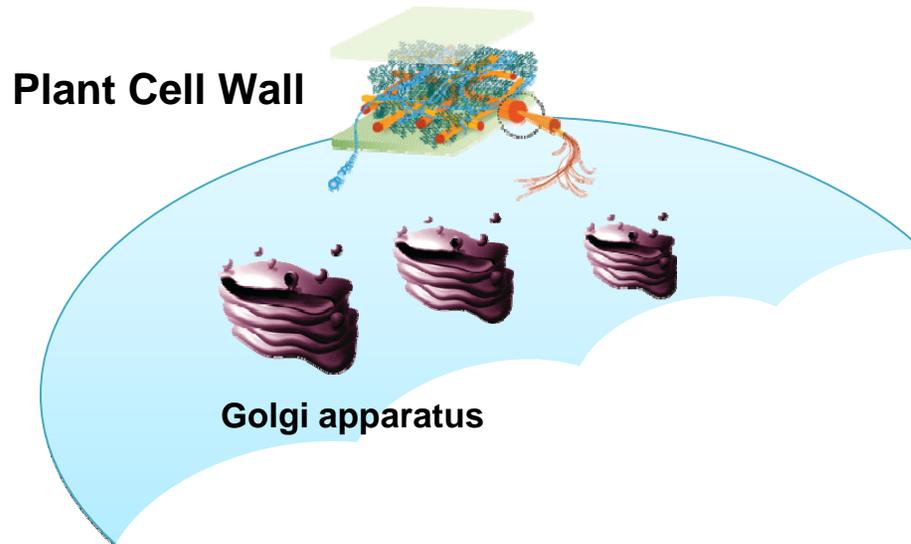
Screening for the lignin transporter



Monolignols and derivatives

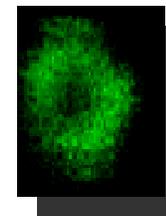


**Can we use 'big biology' to further cell wall biosynthesis
and fast track targets?**

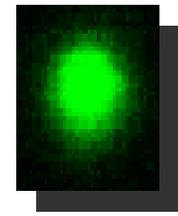


Many complex sugars destined for the plant cell wall are synthesized at the Golgi apparatus

Distinct Golgi sub-compartments by FP



GT2-YFP

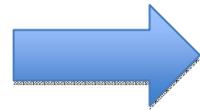


GUT1-YFP

Yumiko Sakuragi
University Copenhagen

The Golgi appears to be sub-compartmentalized, potentially a crucial feature of hemicellulose design

High purity Golgi from plants



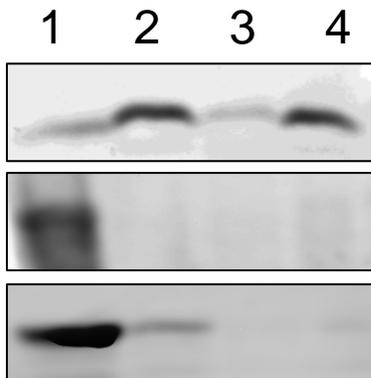
**Isolate Golgi
by density
centrifugation**



**Analyze
proteome**



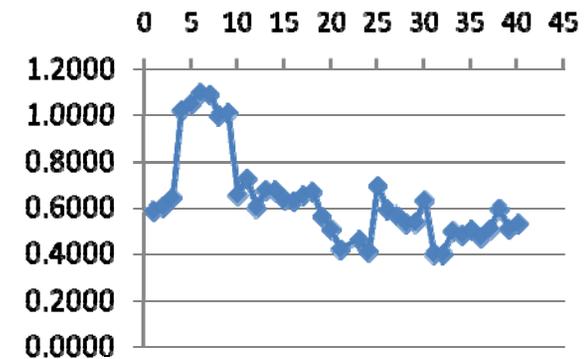
LC-MS/MS



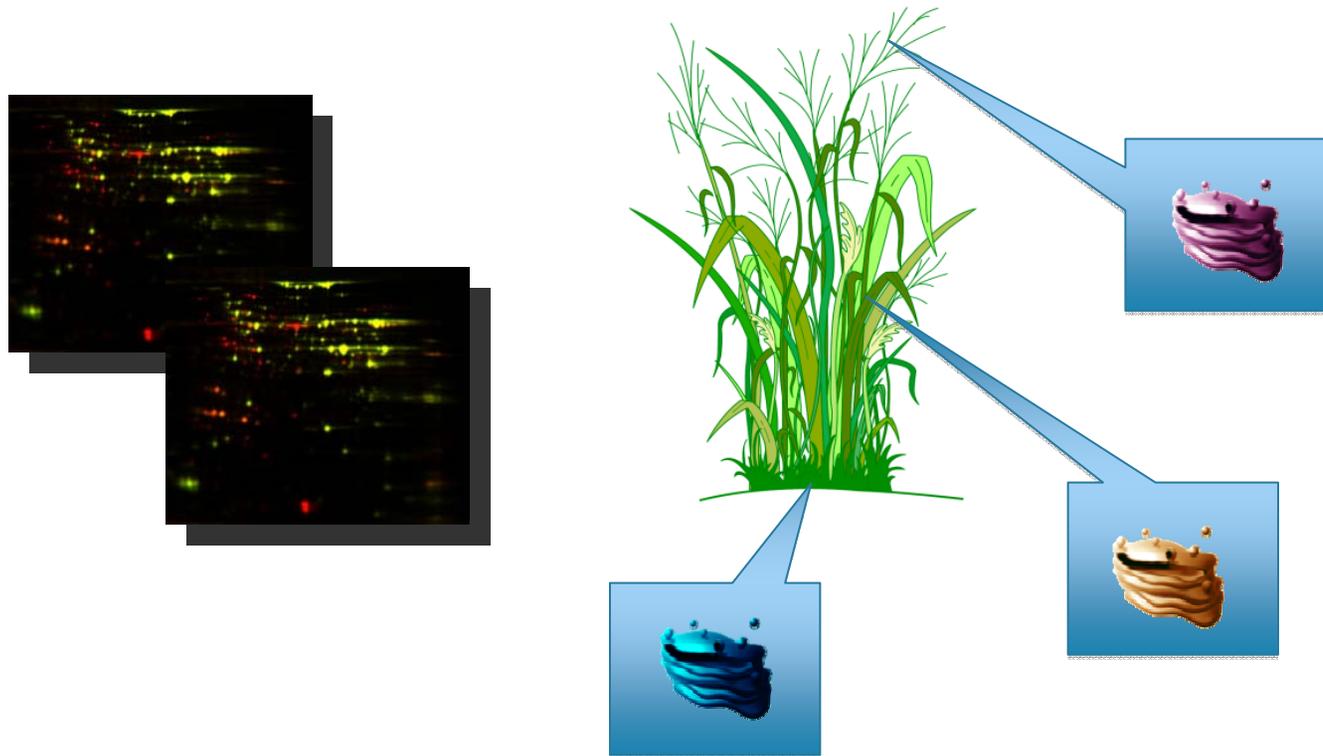
Golgi

Plastid

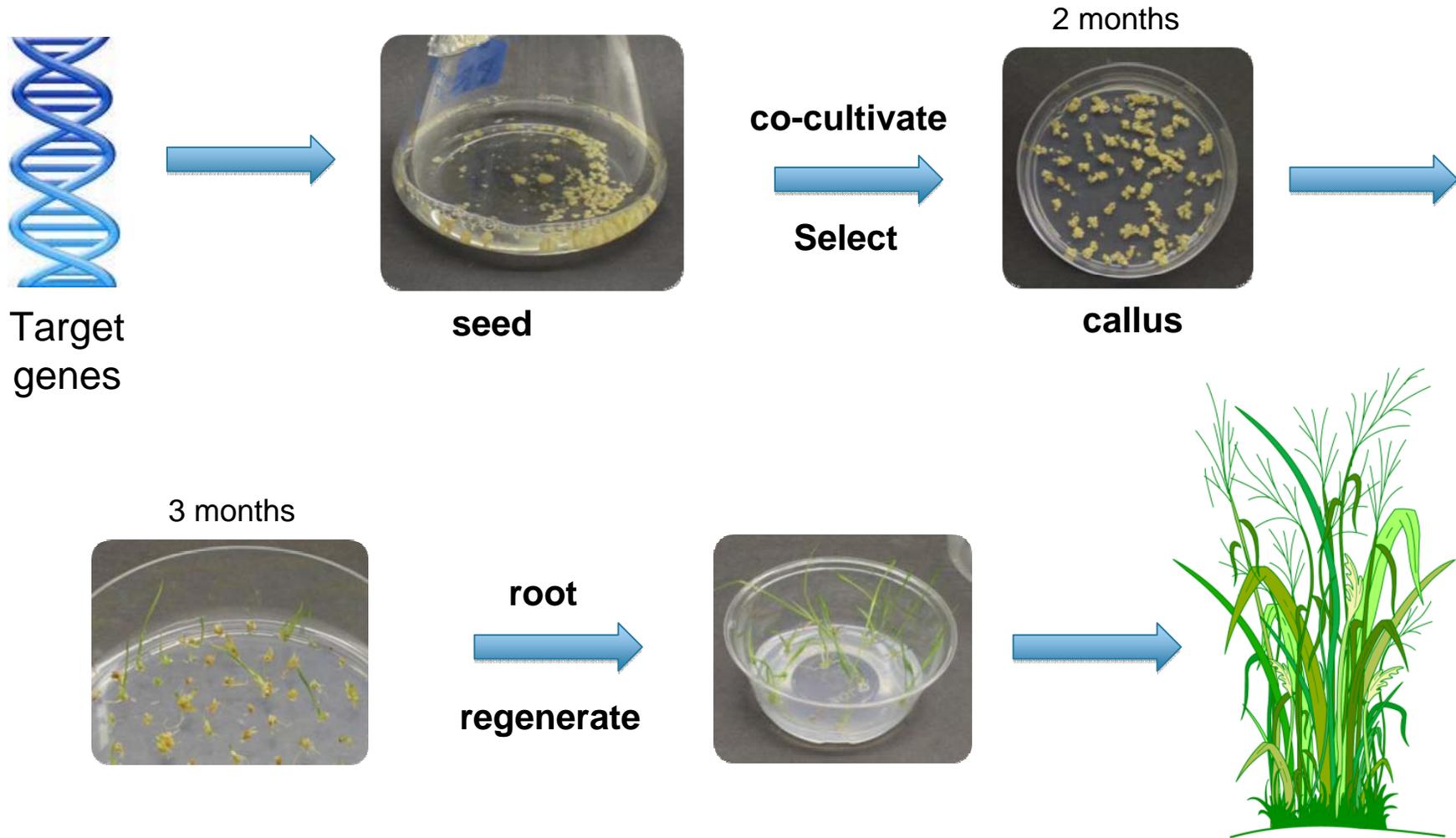
Mito



Mito Golgi



- **Do cell wall biosynthetic compartments from different tissues contain the same protein and sugar content?**
- **Can we correlate proteomics and metabolomics with cell wall structure in tissues, mutants and ecotypes?**



1.1 - 3.6% {50x less efficient compared to rice}

Summary of targets at JBEI

- **Functional characterization of GT's involved in hemicellulose biosynthesis**
- **Targeting monocot (grass) specific GT's involved in hemicellulose biosynthesis**
 - **Golgi proteomics to identify new targets involved in hemicellulose biosynthesis and comparative studies**
- **Attempt lignin replacement for improved deconstruction**
- **Identify lignin transporter to for replacement strategies**

- Understand & modify polysaccharide biosynthesis
 - Focus on hemicellulose
- Modify lignin to aid deconstruction
 - Introduction of cleavable linkages
- Systems approaches to cell wall biology
- Rice and Arabidopsis as model plant systems to apply to switchgrass

