

Poplar functional genomics

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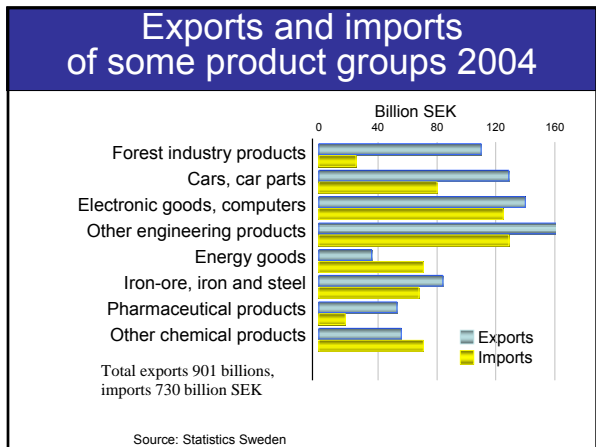
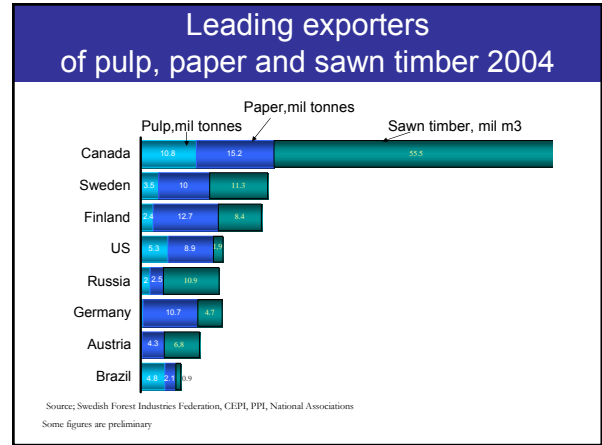
Acknowledgements

Wood and fibre Rishi Bhalarao Eva Mellerowitz Björn Sundberg Hannele Tuominen Alan Marchant Markus Grebe	Nutrition and seedlings Torgny Nasholm Anita Sellstedt Arsenio Vilarejo Stanislaw Karpinski Barbara Karpinska	Enzymology Kristina Blomqvist Alwyn Jones Harry Brumer Tuula Teeri Jerry Ståhlberg
Biomass Stefan Jansson Thomas Moritz Göran Samuelsson Göran Sandberg Maria Eriksson Per Ingvarsson Vaughn Hurry	Stress and seedlings Per Gardeström Petter Gustafsson Jan Karlsson Leszek Kleskowski Cristiane Funk Jan Erik Hallgren Åsa Strand	Genomics technology Joakim Lundeberg Peter Nilsson Fredrik Sterky Mathias Uhlén Gunnar Wingsle
Flowering Ove Nilsson Peter Engström	Flowering Ove Nilsson Peter Engström	Fibre and pulp chemistry Ulf Edlund Lars Wallbäck Staffan Sjöberg Michael Sjöström
Clonal forestry Sara von Arnold David Clapham	Clonal forestry Sara von Arnold David Clapham	Material sciences Marc Rytland Lars Berglund Eva Malmström

Why work on trees?

Trees present unique biological questions

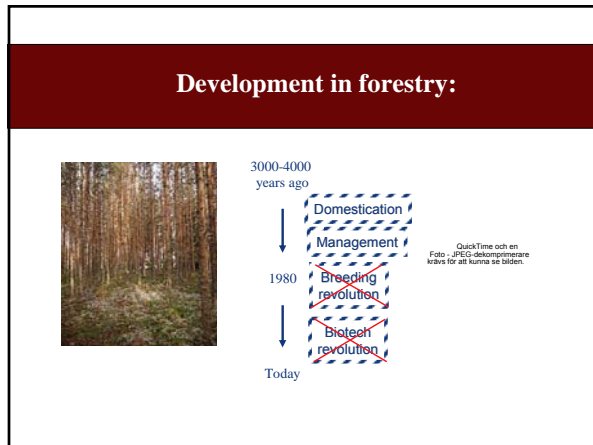
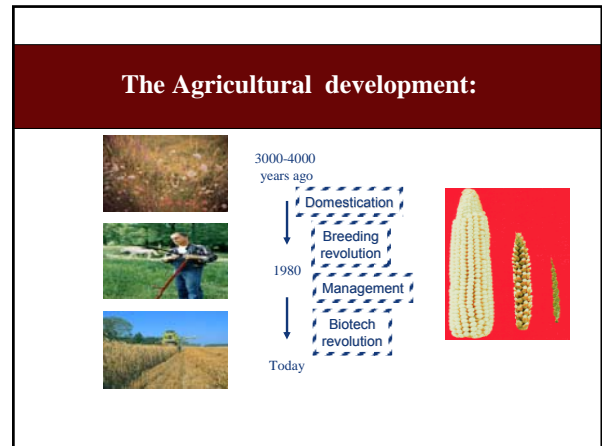
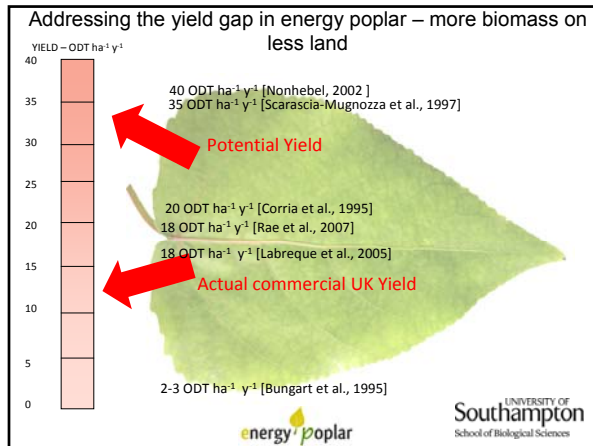
- Perennial
- Late flowering
- Juvenile to maturity transitions
- wood formation



Why work on trees?

- An important source for biomass
- Environmentally friendly source of biomass

However trees need to be domesticated and improved!!!!



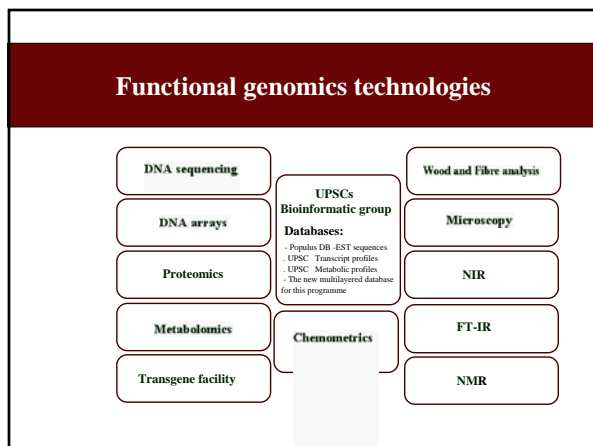
Poplar-A tree model

Rapid growth

Genetic maps available

Facile transformation

UPSC



Sequencing

EST sequencing

Our effort on *Populus tremula x tremuloides*

We have produced 158 000 EST sequences from 20 cDNA libraries

5000 full length inserts

Our effort in other species: *Salix*: 5000 ESTs

Construction of EST database

UPSC
SLU
KTH

Home Project Search Blast Results

Welcome to PopulusDB

This open resource for tree genomics is the result of a collaboration between UPSC (Department of Plant Physiology, Umeå University and Department of Forest Genetics and Plant Physiology, SLU Umeå) and Department of Biotechnology, KTH Stockholm, with financial contribution from Knut and Alice Wallenberg Foundation, Kempestiftelsen, the Foundation for Strategic Research, the Swedish Research Council and the Swedish Research Council for the Environment, Agricultural Sciences and Spatial Planning.

Latest changes

- 2004-07-04: Chose web ID numbers over contig link to the TIGR necessary database.
- 2004-06-15: Gene headers updated.
- 2004-02-19: Gene ontology updated (based on TIGR)
- 2004-02-27: New release
- 2004-02-27: Library distribution is now based on clone rather than sequences

Evaluation of annotation programs for *Populus* sequences

Eugene, Genemark.hmm, GlimmerA, GlimmerR,
Fgenes.h.dicot, Fgenes.monocot
-> Annotate poplar genes from GenBank ->Fgenes.monocot

Development of microarrays

DNA micro arrays

Poplar array 1	3 000 sequences
Poplar array 2	13 000 sequences
Poplar array 3	25 000 sequences (currently used)
CATMA Arabidopsis array	25 000 sequences

Transcript profiling database

- Storage of microarray data
- Cross experiment comparisons
- Genetical-genomics type comparisons

BASE BASE details

- Web-based, i.e. everything is controlled using your favorite web-browser
- Creates MIAME-compliant data and can export MAGB-ML objects for easy deposition to (other) public databases
- Can handle 2-channel data as well as Affymetrix data but is primarily designed for the former
- Runs entirely on free and open-source software.

BASE The UPSC installation

- Experiment data can be loaded batch-wise instead of per-slide import
- Search tool that searches all analyzed data matching certain value (M, A or R/G) criteria and reporter criteria
- Create 'analysis packages' which run a pre-defined set of plug-ins (such as array visualization followed by normalization, etc.)

UPSC-BASE

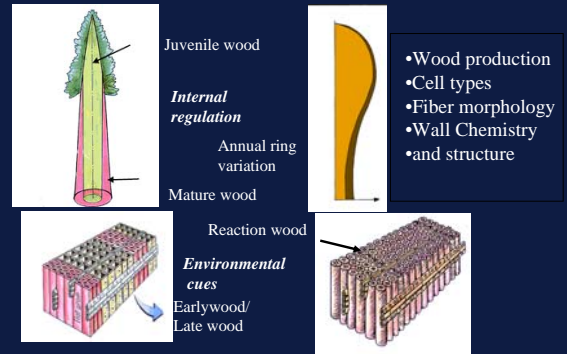
Transcript profiling data from:

- Poplar-cDNA arrays
- Arabidopsis-GST arrays (CATMA)

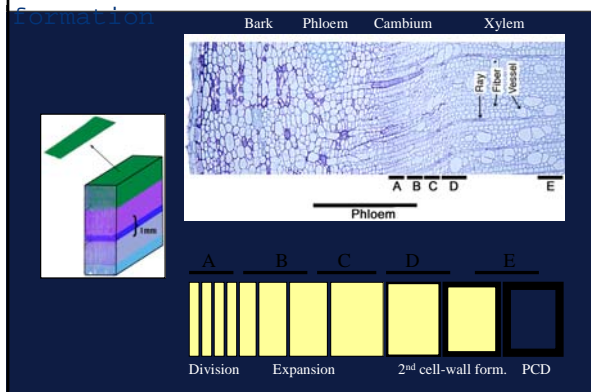
More than 100 users

Data from more than 3000 hybridisations

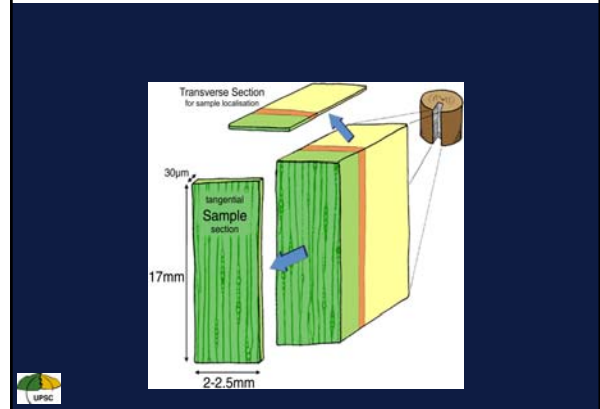
Understanding the molecular basis of vascular development



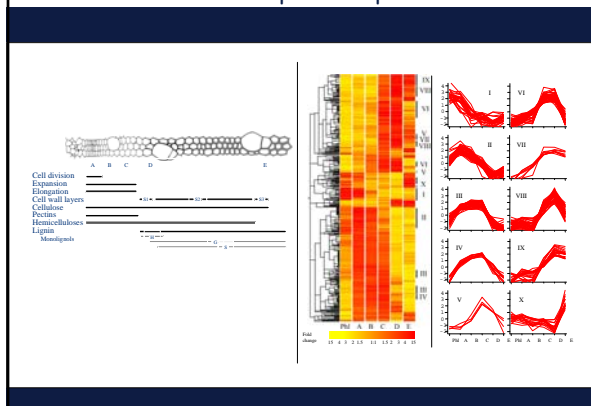
A Expression Roadmap to Wood Formation



Sampling procedure for obtaining single cell layers



Differential expression patterns



Functional analysis of candidate genes

-Use of Arabidopsis as a test bed

-Large scale RNAi based knockdown

-Large scale overexpression of transcription factors

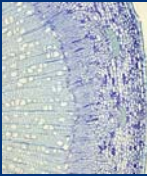
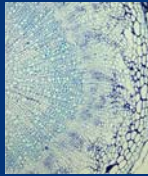
Collaboration with industrial partner: SweTree Technologies
www.swetree.com

Advantages of using *Arabidopsis* as a model system

- Rapid lifecycle
- Easy to transform
- Fully sequenced
- Genetic analysis
- Characterised mutants

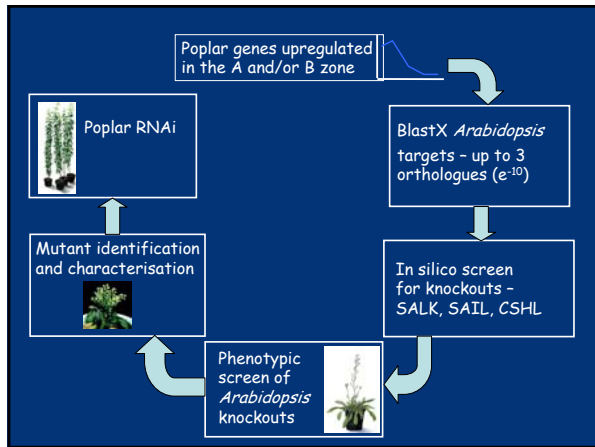
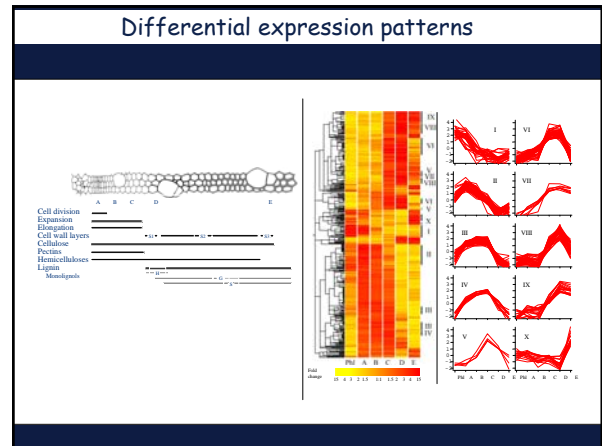
Orthology between *Arabidopsis* and Poplar genes

Arabidopsis as a tree model?

Poplar *Arabidopsis*

Arabidopsis grown under short day conditions exhibits secondary thickening and fiber formation. Chaffey *et al* (2002)

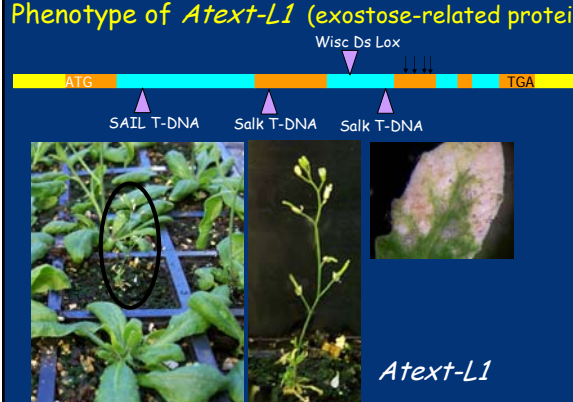


Phenotype of *Atext-L1* (exostose-related protein)

Wisc Ds Lox

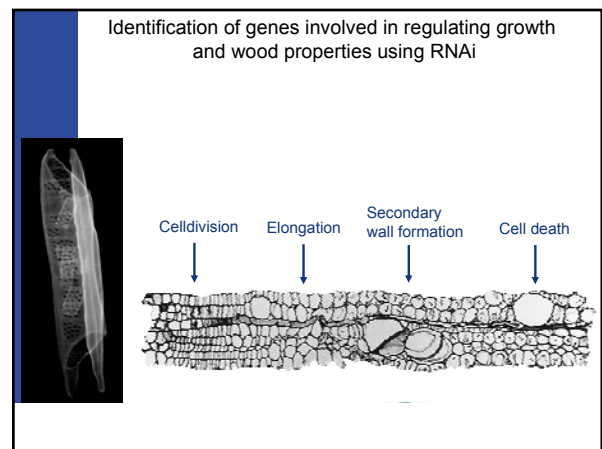
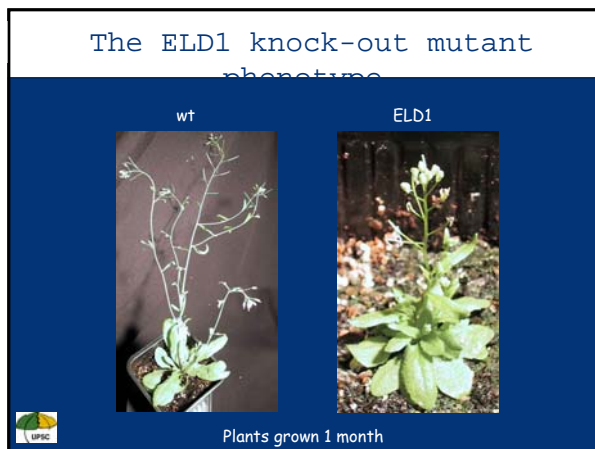
ATG TGA

SAIL T-DNA Salk T-DNA Salk T-DNA



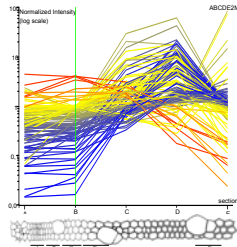
Atext-L1

Alan Marchant
Southampton University

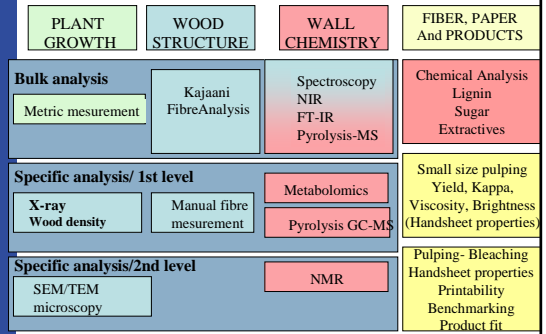


Gene selection

- Genes that have a clearly differential expression during wood development.
- Genes involved in
 - Cell wall chemistry
 - Fiber dimensions
 - Growth



Analysis of transgenic poplar plants -Funcwood Explorer package

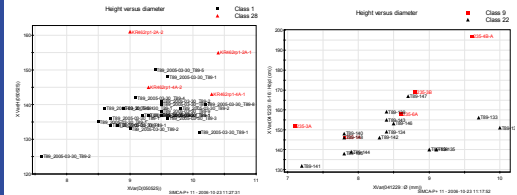


Results

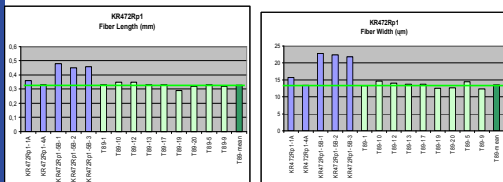
- 40% Of the total number of genes analysed have phenotypes
- This can be due to our pre selection of the genes to be tested
- the Knockdown technology (RNAi) can knock down close gene family members.



Growth increase of 26-33% obtained

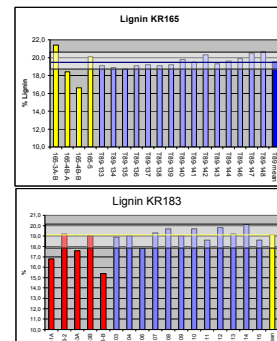


Fiber length increase of 40-48%

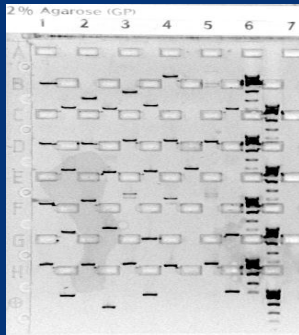


Plants with high growth not necessarily have longer fibres

Chemistry -Lignin levels reduced by 15-20%



Amplification of insert fragments



Over 95% success rate
in first pass

Confirmation of insert
via sequencing

[POPTFdb: popf.db.umu.se](http://POPTFdb:popf.db.umu.se)

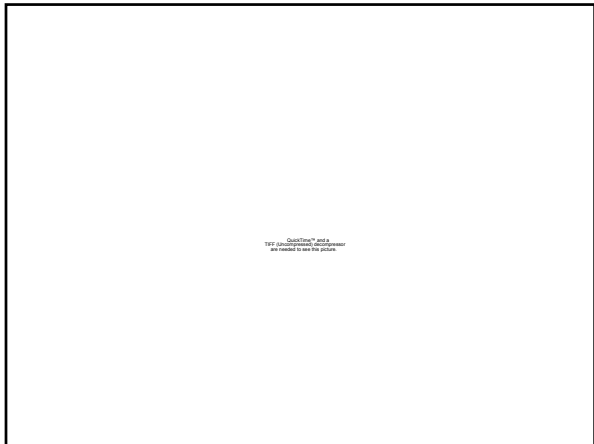
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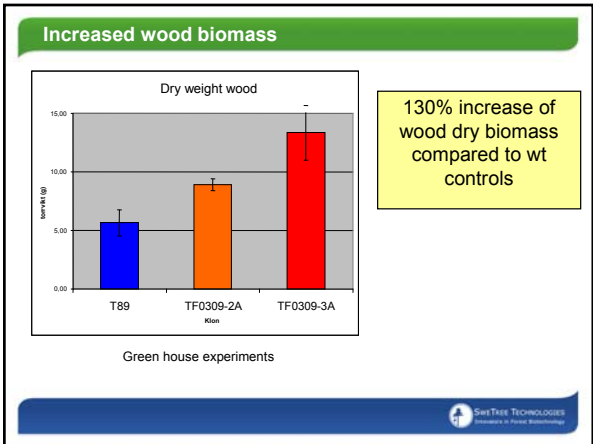
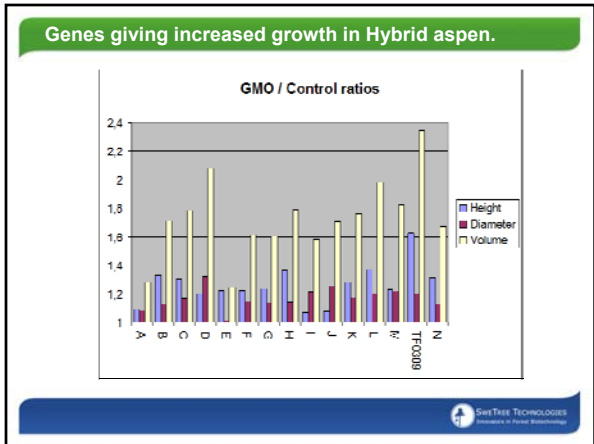
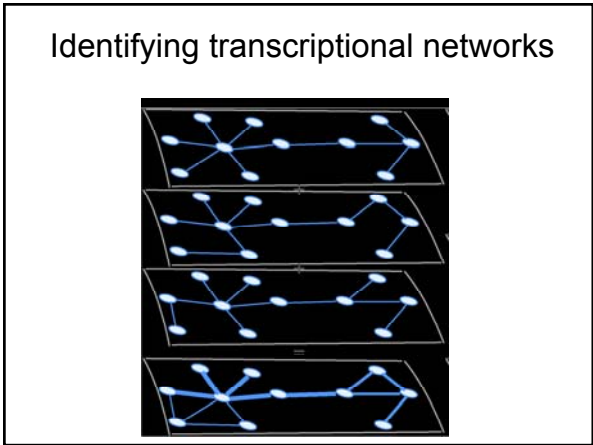
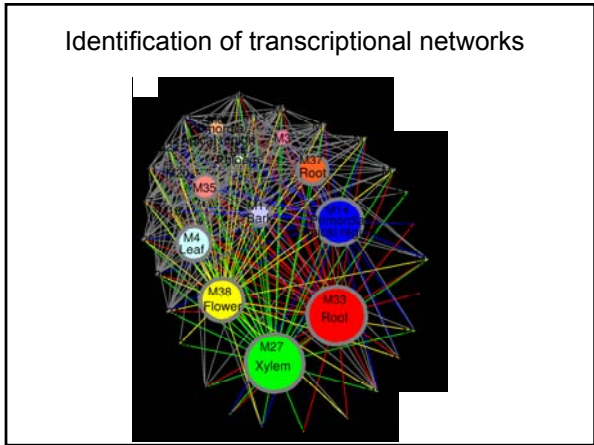
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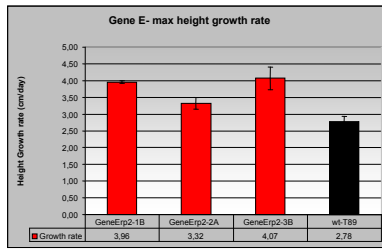


How do you select genes for functional analysis?

- 45-55 thousand open reading frames
- c. a. 4000 transcription factors



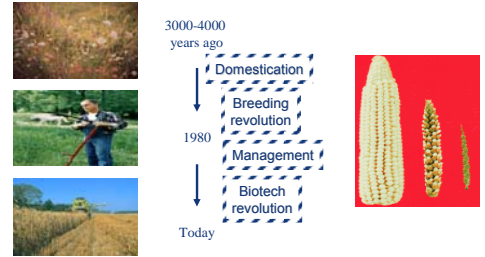
Increased height growth rates (cm/day)



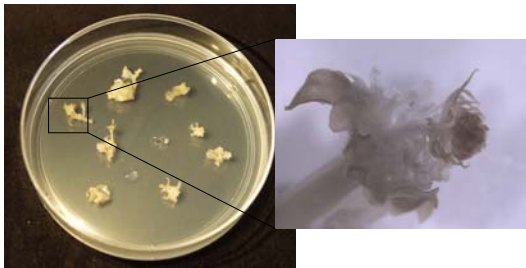
This gene gives an 50% increased growth rate when over expressed



The Agricultural development:



35S::FT transgenic aspens are extremely early flowering!



Flowers 4 weeks after transformation!!!

35S::FT transgenic aspens produce catkins



6 month old plants

35S::FT transgenic aspens

