

A Systematic Approach for Functional Analysis of Cotton Genes

-Cotton Full-length cDNA Over-expressing gene hunting system, cFOX

ICGI 2012

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Supervised by Dr. Z. Jeffrey Chen

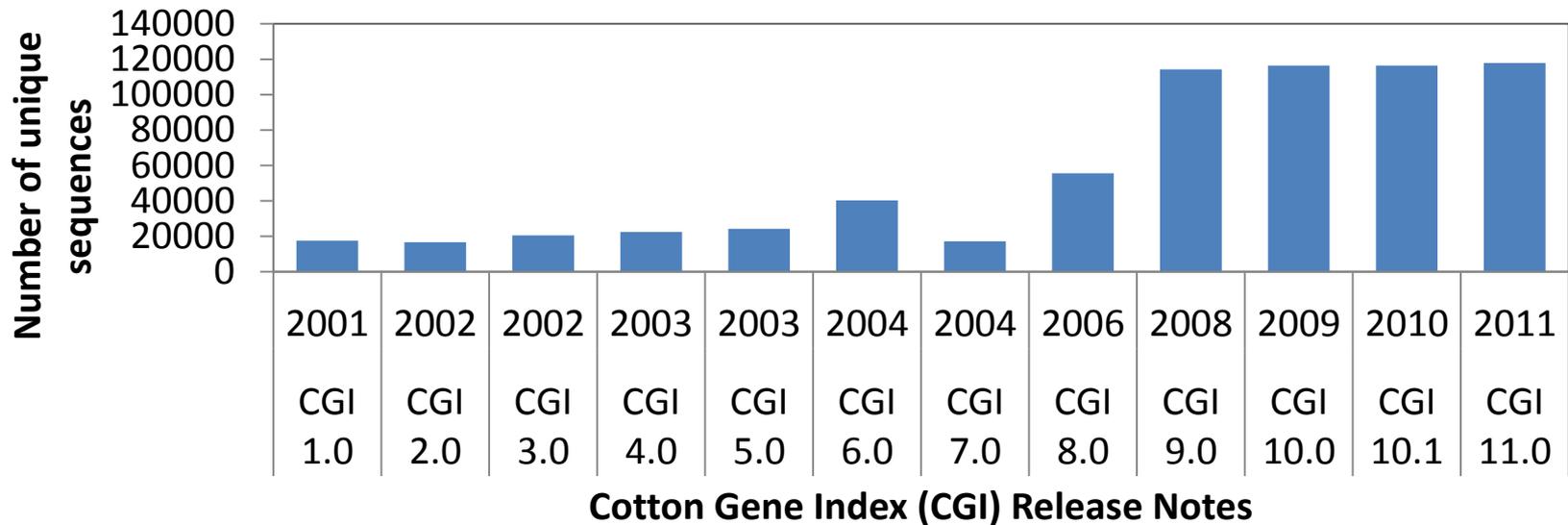
Poster #



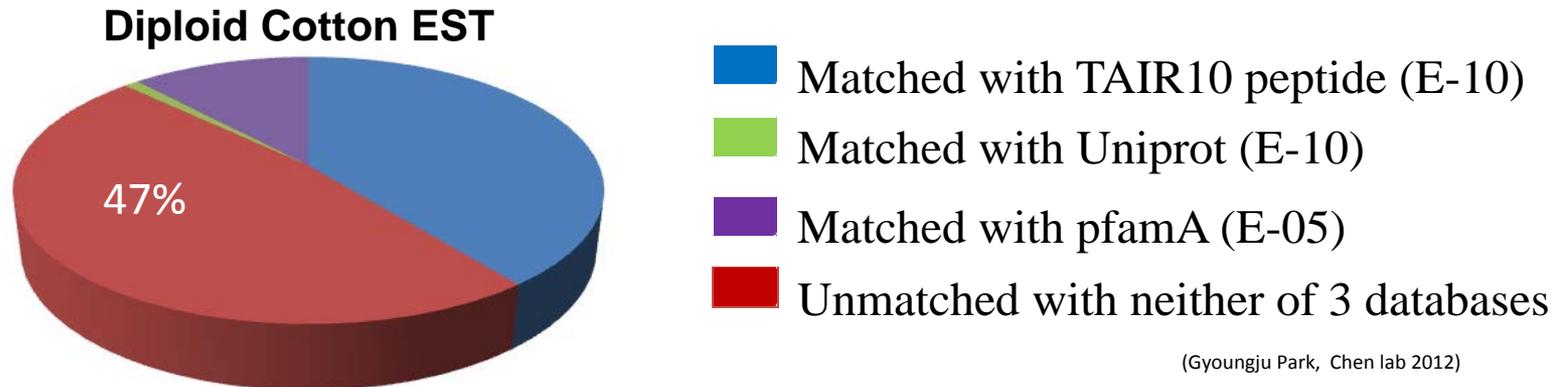
WHAT STARTS HERE CHANGES THE WORLD

THE UNIVERSITY OF TEXAS AT AUSTIN

Challenge: Assignment of gene function in postgenomic era.

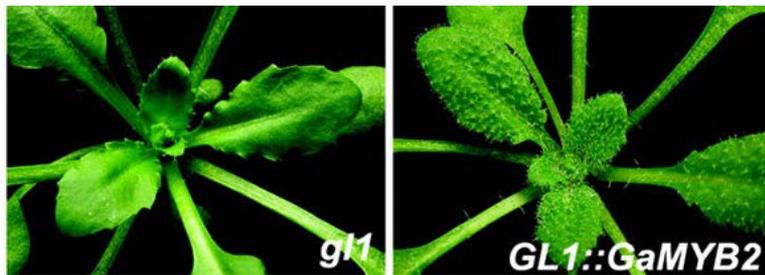


(http://compbio.dfci.harvard.edu/cgi-bin/tgi/T_release.pl?gudb=cotton)



(Gyoungju Park, Chen lab 2012)

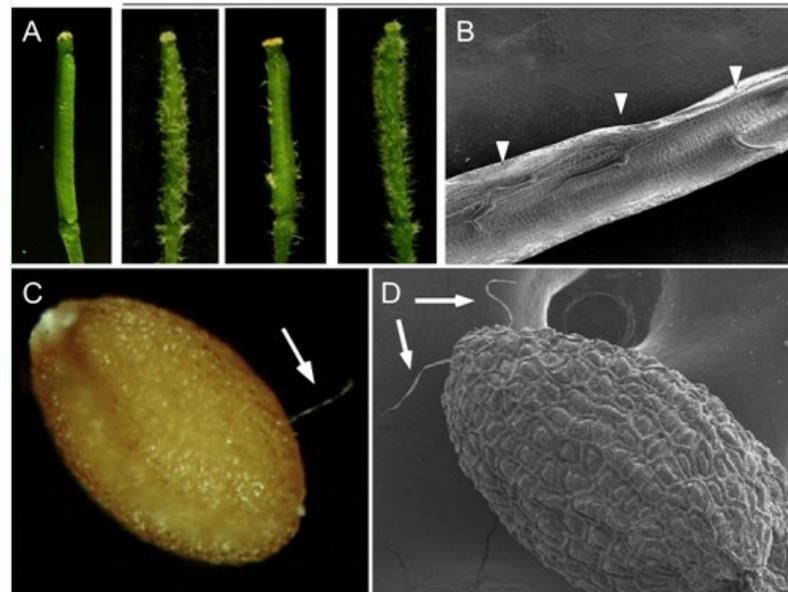
Arabidopsis served as a promising system for testing cotton fiber gene function.



(Wang et al., 2004 Plant Cell)



(Guan et al., 2008, *Physiologia Plantarum*)

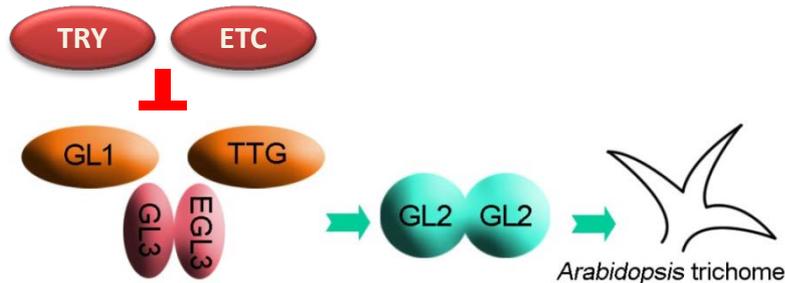
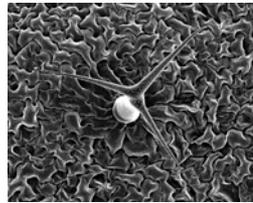


(Guan et al., 2011, PloS One)

Comparison of Arabidopsis trichome vs cotton fiber

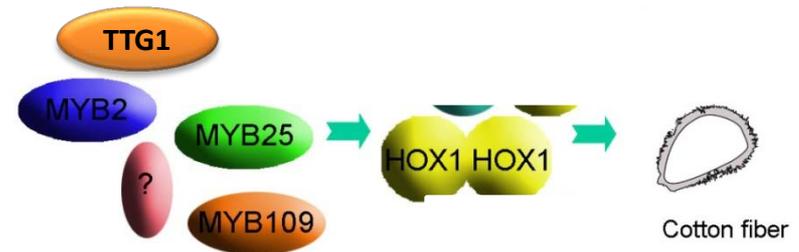
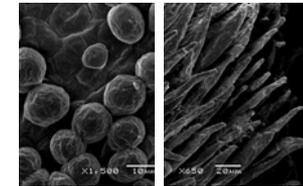
Arabidopsis **leaf/stem** trichome

Single cell
Cellulose
0-3 branches
On leaf/stem epidermis
Development regulation:

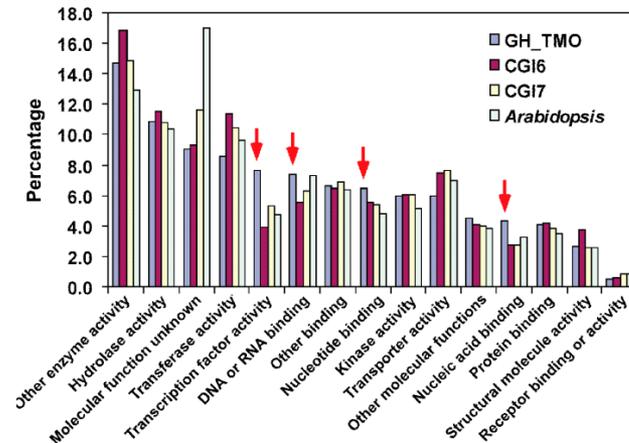


Cotton **ovule** fiber

Single cell
Cellulose
0 branches
On ovule epidermis
Development regulation:



32,789 high-quality ESTs derived from *G. hirsutum* L. Texas Marker-1 (TM-1) ovules (GH_TMO).

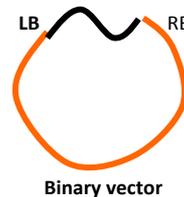


Yang S, et al., 2006, Plant Journal

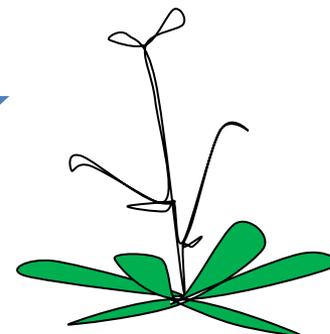
Cotton Full-length cDNA Overexpressing (cFOX) gene hunting system



TMO cDNA library



TMO_Agrobacterium



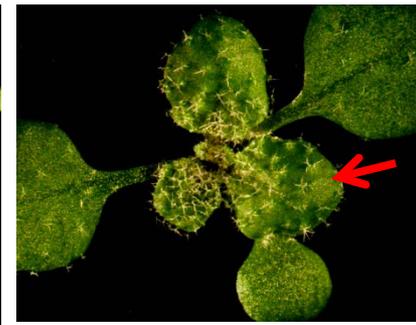
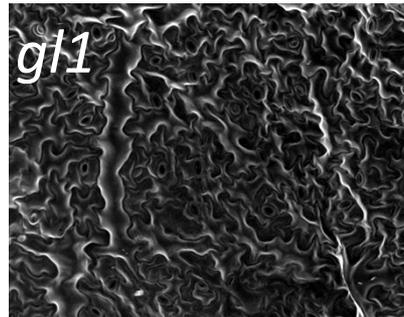
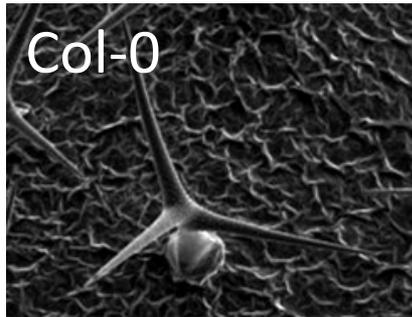
TMO_Arabidopsis

Arabidopsis transgenic background

Col-0: wildtype

gl1: trichomeless, hairless

try : hairy, multiple branched and clustered trichome



Screening of functional cotton genes

| Background | Total Lines | Phenotypic Lines | Frequency |
|------------|-------------|------------------|-----------|
| Col-0 | 453 | 14 | 3.1% |
| <i>gl1</i> | 872 | 16 | 1.8% |
| <i>try</i> | 844 | 43 | 5.1% |
| Total | 2169 | 73 | 3.3% |



Screening of functional cotton genes

| Significant Phenotype | Total Lines* | Background | | |
|------------------------------|--------------|------------|------------|------------|
| | | Col-0 | <i>gl1</i> | <i>try</i> |
| Trichome development related | 13 | 2 | 4 | 7 |
| Biomass related | 20 | 6 | 12 | 2 |
| Stressed | 9 | 1 | 1 | 7 |
| Bushy stem | 5 | NA | 1 | 4 |
| Later flowering time | 14 | 1 | 1 | 11 |
| Pin-head stem | 2 | NA | 1 | 1 |

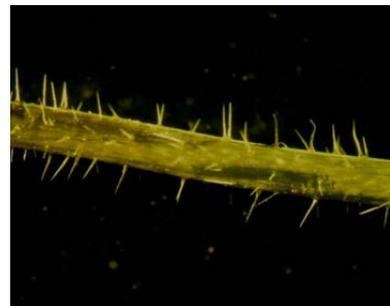
*: Out of 2,169 lines



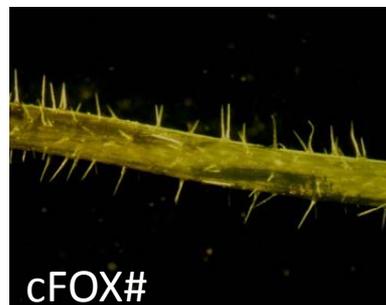
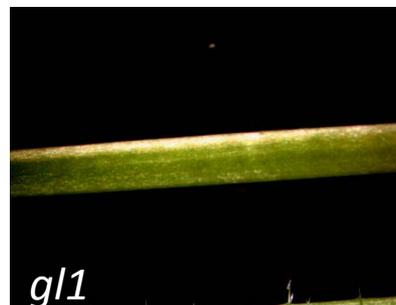
Plant fiber/trichome development related Phenotypic lines

Background

cFOX lines



Trichome/fiber development related Phenotypic lines

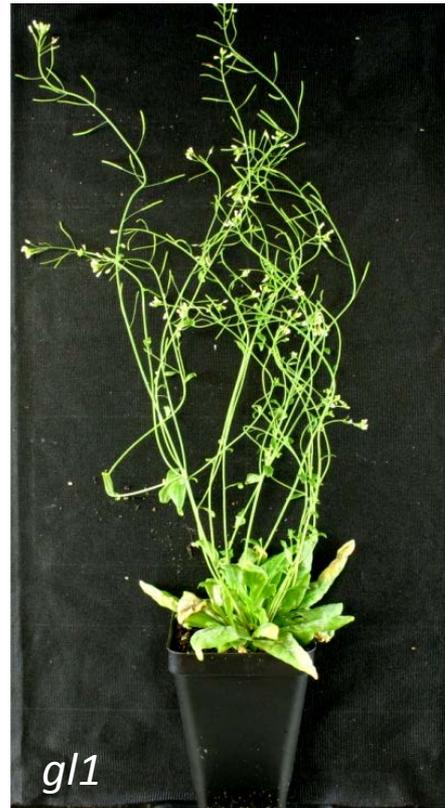


| Code | Background | Phenotype Description | Annotation of Inserted Cotton Sequence |
|----------|------------|--|--|
| 15020102 | cFOX-col0 | no trichome on bolt | Unknown |
| 15040401 | cFOX-col0 | no trichome on bolt | Unknown |
| 02011101 | cFOX-gl1 | rosette leaf hairy, dominant | DELLA protein GAI, putative [Arabidopsis thaliana] |
| 02020101 | cFOX-gl1 | late trichome on stem | Unknown |
| 02021402 | cFOX-gl1 | recover the trichome development | Unknown |
| 02020301 | cFOX-gl1 | late bolting time, trichome on rosette leaf and stem | Mitochondrial processing peptidase alpha subunit, putative |
| 10010409 | cFOX-try | unbranched trichome | Unknown |
| 06020308 | cFOX-try | unbranched trichomes | Phospholipid/glycylthalianaerol acyltransferase family protein [Arabidopsis] |

➤ A series of cotton fiber regulators are unveiled through cFOX screening.

Biomass/late flowering related mutant

Background



cFOX lines



cFOX#: non-specific lipid-transfer protein-like protein (LTP).

Pin-head stem mutant

Background



cFOX lines



cFOX#: Transcription factor bHLH121 , a potential regulator in auxin pathway.

Screening of functional annotated cotton genes

| Code | Background | Phenotype Description | Annotation |
|----------|------------|--|--|
| 13030401 | cFOX-Col0 | unusual development of flowers dominant | Predicted protein, homeobox-leucine zipper protein HDG2 [Arabidopsis thaliana] |
| 13030608 | cFOX-Col0 | unusual development of flower | Protein RarD [Streptococcus vestibularis F0396]; major facilitator superfamily protein [Haliangium DSM 14365] |
| 13030704 | cFOX-Col0 | unusual development of flower | DELLA protein GAI, putative [Arabidopsis thaliana] |
| 13040208 | cFOX-Col0 | late bolting | Mitogen-activated protein kinase kinase kinase 13 [Arabidopsis thaliana] |
| 12020707 | cFOX-gl1 | late bolting time, recessive | Mitochondrial processing peptidase alpha subunit, putative [Ricinus communis] |
| 02011101 | cFOX-gl1 | rosette leaf hairy, dominant | DELLA protein GAI, putative [Arabidopsis thaliana] |
| 02020301 | cFOX-gl1 | late bolting time, trichome on rosette leaf and stem | Mitochondrial processing peptidase alpha subunit, putative [Ricinus communis] |
| 02021301 | cFOX-gl1 | pin head stem | Transcription factor bHLH121 [Arabidopsis thaliana] |
| 12010407 | cFOX-gl1 | two primary bolts, merged stem | C2H2L domain class transcription factor, zinc finger transcription factor ZF1 [Malus x domestica]; [Populus trichocarpa] |
| 12010606 | cFOX-gl1 | only one stem. No secondary bolting, very late bolting, dominant | non-specific lipid-transfer protein-like protein [Tamarix hispida]; lipid binding protein, putative [Ricinus communis] |
| 06020308 | cFOX-try | unbranched trichomes | Phospholipid/glycerol acyltransferase family protein [Arabidopsis thaliana] |
| 06020401 | cFOX-try | overall slow growth | NHL domain-containing protein [Arabidopsis thaliana] |
| 10010106 | cFOX-try | 5 rosette leaves. Slow rosette leaf growth and late bolting | Cytochrome P450 76A2 [Vitis vinifera] |
| 10010707 | cFOX-try | very slow growth in leaves and late bolting | Rossmann-fold NAD(P)-binding domain-containing protein [Arabidopsis thaliana]; putative dihydroflavonol-4-reductase [Vitis vinifera] |

Cotton Full-length cDNA Overexpressing (cFOX) gene hunting system

➤ Advantages

Short transgenic period

Economic on usage of space and effort

High frequency for identifying new gene function

➤ Further application

Screen under abiotic or biotic stress condition

Improving functional annotation of cotton genes

Select the gene candidates to conduct transgenic cotton



COTTON FIBER GENOMICS PROJECT

FUNCTIONAL AND SEQUENCE ANALYSIS OF FIBER DEVELOPMENT IN TETRAPLOID COTTON

NSF Plant Genome Research Program (IOS-1025947)

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