

UC Davis CREATE-IGERT Participants at the 18th Annual Biotechnology Retreat in Napa, CA April 4, 2009



Back Row (L->R): Tim Butterfield, Chris Simmons, Mark Wolf, Lucas Arzola, Denneal Jamison-McClung (Program Coordinator)

Front (L->R): Rachel Kerwin, Dawn Chiniquy, Tiffany Glavan, Karen McDonald (PI), Ben Lindenmuth and Mitch Elmore

UC Davis & Tuskegee University CREATE-IGERT Participants Annual Biotechnology Retreat in Napa, CA - April 4, 2009



Back Row (L->R): Dr. Karen McDonald (PI), Sharina Richard, Steven Samuels, Tim Butterfield, Dr. Ramble Ankumah (Tuskegee Faculty Trainer), Mark Wolf, Lucas Arzola, Dr. Rebecca Parales (UCD Faculty Trainer)
Front (L->R): Tiffany Glavan, Elenor Castillo, Mitch Elmore, Dr. Denneal Jamison-McClung (Program Coordinator) and Ben Lindenmuth

UC Davis CREATE-IGERT Welcomes Tuskegee University Colleagues to the 18th Annual Biotech Retreat on April 4, 2009



Sharina Richard, Dr. Ankumah & Steven Samuels

Dr. Ankumah and
Tuskegee University
colleagues conduct
research on a variety of
GM crop applications
(eg. engineering disease
resistance to Cotton
Seedling Disease).



CREATE-IGERT Participants at the 18th Annual Biotechnology Retreat, Christian Brothers Retreat Center, Napa, CA - April 4, 2009



CREATE-IGERT PI Karen McDonald & Lab (L->R): Kittypong Rattanaoporn, Nate Kingsbury, Dr. Karen McDonald, Lucas Arzola, Ben Lindenmuth and Sany-Kyu Jung

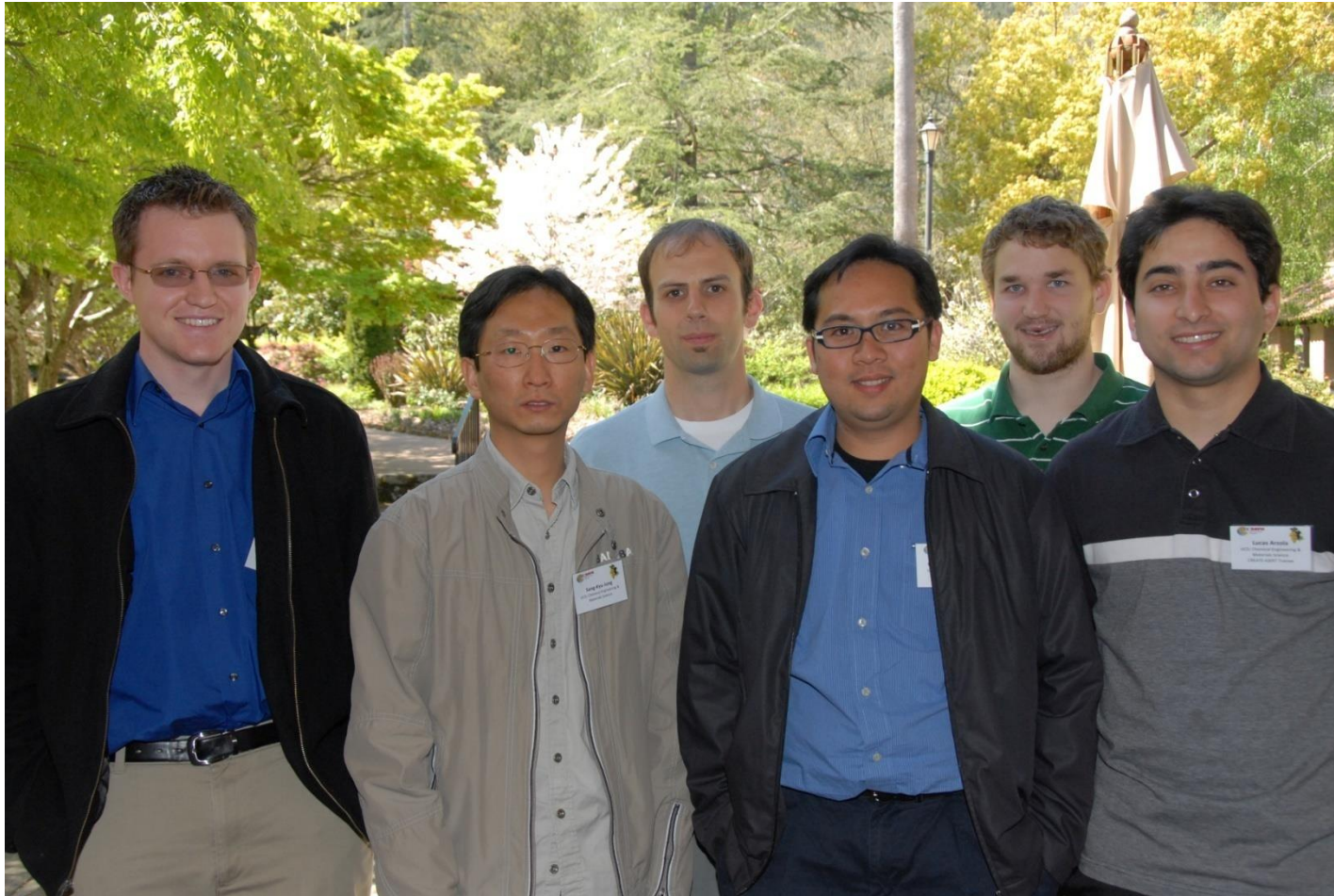


Dr. Larry Joh (CREATE-IGERT Development Engineer & Erstwhile Photographer)



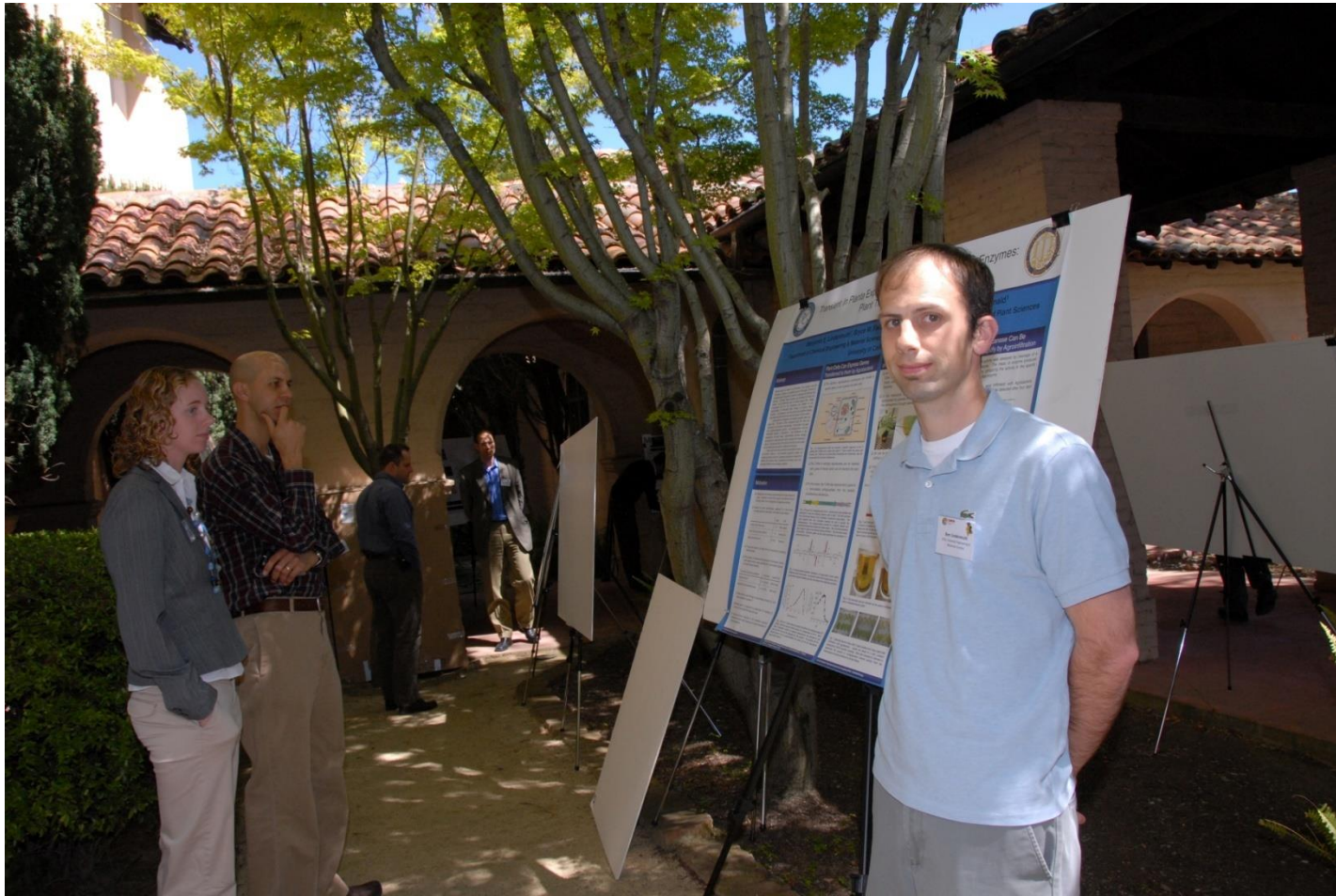
Elenor Castillo & Dr. Denneal Jamison-McClung (CREATE-IGERT Program Coordinator)

When CREATE-IGERT Biological Systems and Chemical Engineers Gather... Watch Out World!

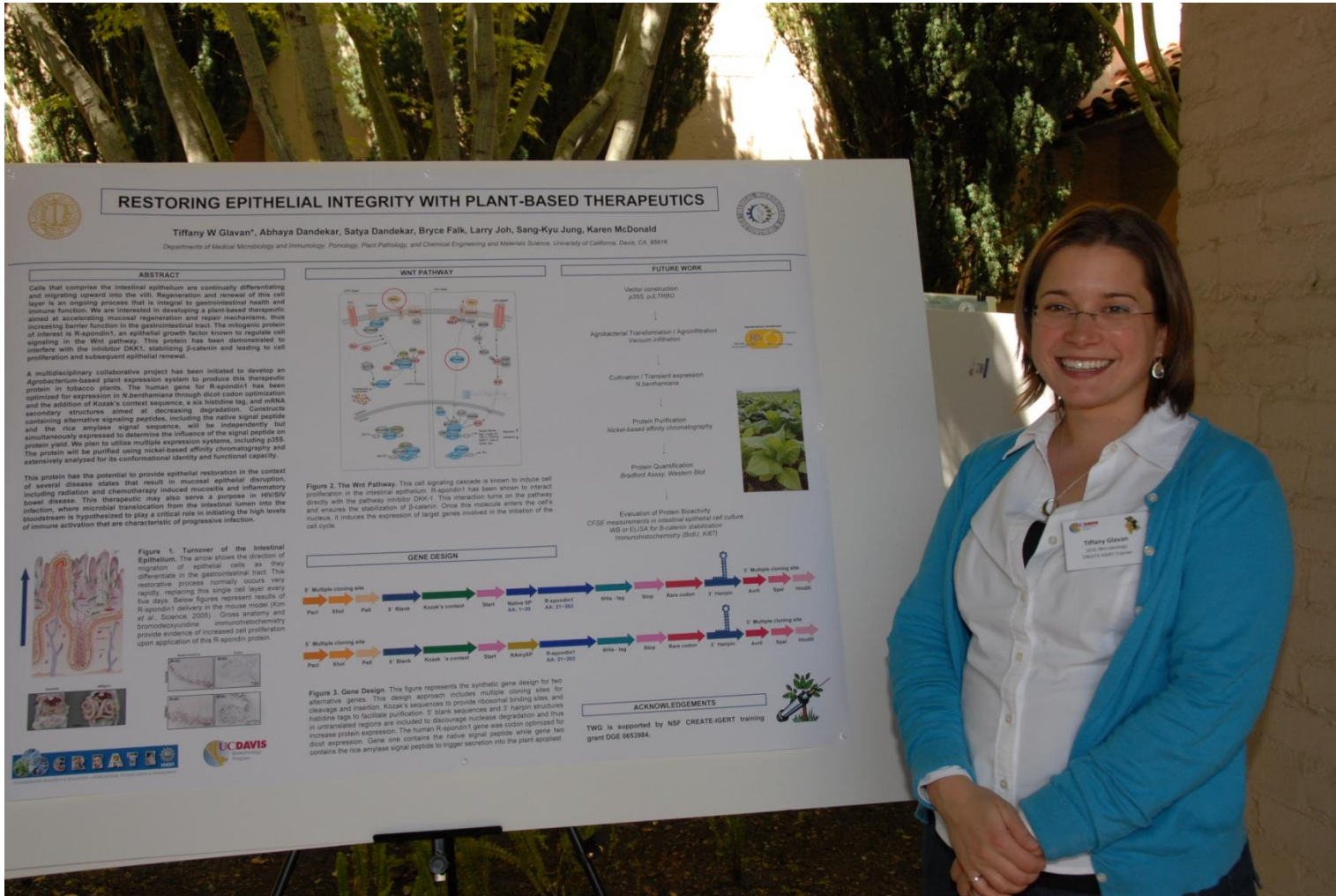


(L->R)Chris Simmons (trainee), Sang-Kyu Jung (affiliate), Ben Lindenmuth (trainee), Kittypong Rattanaorn (affiliate), Nate Kingsbury (affiliate) and Lucas Arzola (trainee).

CREATE-IGERT Trainee Ben Lindenmuth Exhibits His Work on *In Planta* Expression of Cellulose-Degrading Enzymes



Tiffany Glavan is Conducting Interdisciplinary Research on Plant-Made Therapeutics



RESTORING EPITHELIAL INTEGRITY WITH PLANT-BASED THERAPEUTICS

Tiffany W Glavan*, Abhya Dandekar, Satya Dandekar, Bryce Falk, Larry Joh, Sang-Kyu Jung, Karen McDonald
Departments of Medical Microbiology and Immunology, Pomology, Plant Pathology, and Chemical Engineering and Materials Science, University of California, Davis, CA, 95616

ABSTRACT

Cells that comprise the intestinal epithelium are continually differentiating and migrating upward into the villi. Regeneration and renewal of this cell layer is an ongoing process that is integral to gastrointestinal health and immune function. We are interested in developing a plant-based therapeutic aimed at accelerating mucosal regeneration and repair mechanisms, thus increasing barrier function in the gastrointestinal tract. The endogenous protein of interest is R-spondin1, an epithelial growth factor known to regulate cell signaling in the Wnt pathway. This protein has been demonstrated to interfere with the inhibitor DKK1, stabilizing β -catenin and leading to cell proliferation and subsequent epithelial renewal.

A multidisciplinary collaborative project has been initiated to develop an Agrobacterium-based plant expression system to produce this therapeutic protein in tobacco plants. The human gene for R-spondin1 has been optimized for expression in *R. solanaceum* through codon optimization and the addition of Kozak's context sequence, a six histidine tag, and mRNA secondary structures aimed at decreasing degradation. Constructs containing alternative signaling peptides, including the native signal peptide and the rice amylose signal sequence, will be independently, but simultaneously expressed to determine the influence of the signal peptide on protein yield. We plan to utilize multiple expression systems, including pSS protein yield. The protein will be purified using nickel-based affinity chromatography and extensively analyzed for its conformational identity and functional capacity.

This protein has the potential to provide epithelial restoration in the context of several disease states that result in mucosal epithelial disruption, including radiation and chemotherapy induced mucositis and inflammatory bowel disease. This therapeutic may also serve a purpose in HIV/SIV bowel disease. This therapeutic may also serve a purpose in HIV/SIV bowel disease. This therapeutic may also serve a purpose in HIV/SIV bowel disease. This therapeutic may also serve a purpose in HIV/SIV bowel disease.

WNT PATHWAY

FUTURE WORK

Vector construction
pJ01, pJ1700

Agrobacterial Transformation / Agrobacterium
Vacuum infiltration

Cultivation / Transient expression
N. benthamiana

Protein Purification
Nickel-based affinity chromatography

Protein Quantification
Bradford Assay, Western Blot

Evaluation of Protein Bioactivity
CFSE measurements of intestinal epithelial cell culture
WB or ELISA for β -catenin stabilization
Immunohistochemistry (IHC), IRT

GENE DESIGN

Figure 1. Turnover of the Intestinal Epithelium. The arrow shows the direction of migration of epithelial cells as they differentiate in the gastrointestinal tract. This restorative process normally occurs very rapidly, replacing this single cell layer every five days. Below figures represent results of R-spondin1 delivery in the mouse model (Kim et al., Science, 2005). Gross anatomy and bromodeoxyuridine immunohistochemistry provide evidence of increased cell proliferation upon application of the R-spondin protein.

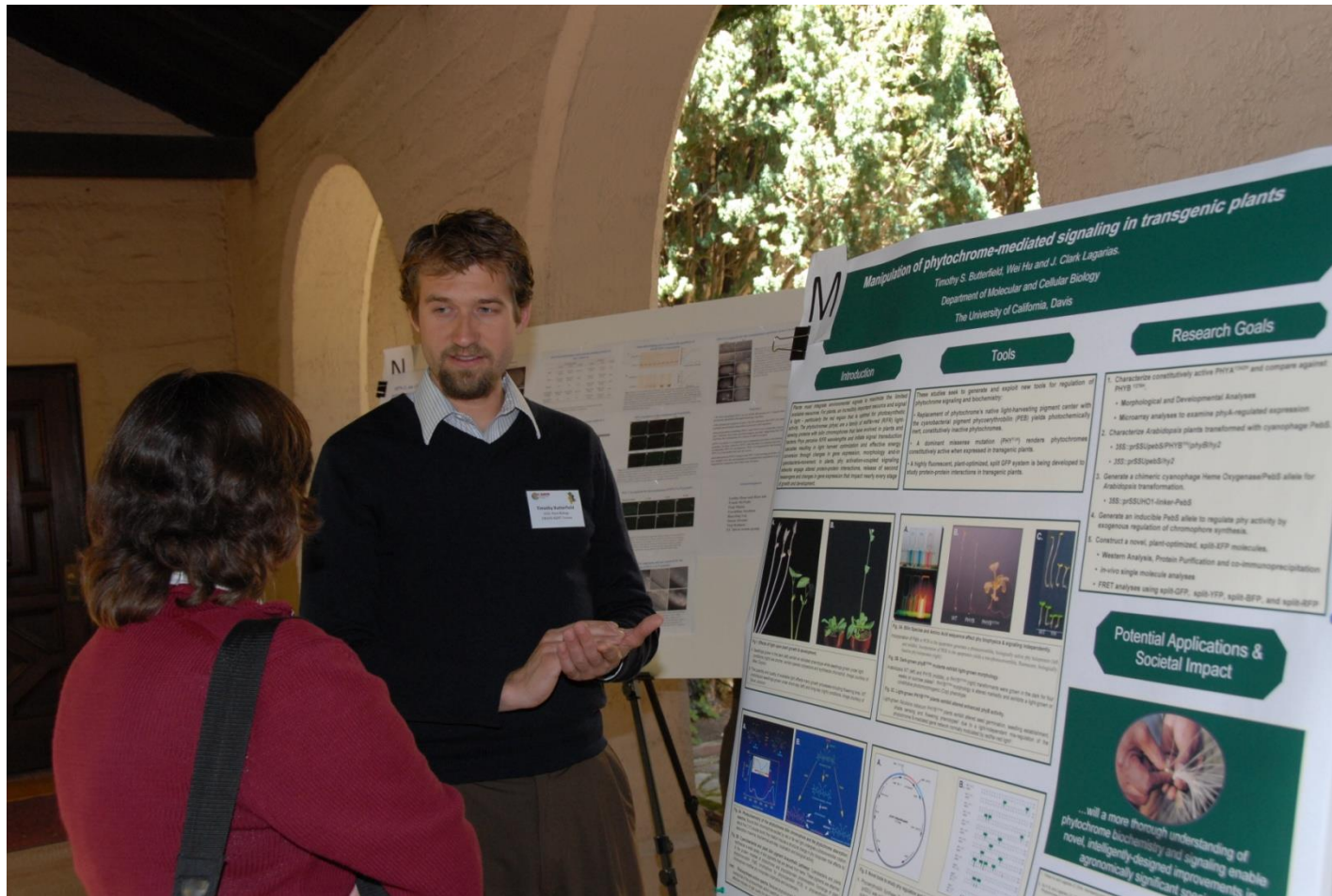
Figure 2. The Wnt Pathway. This cell signaling cascade is known to induce cell proliferation in the intestinal epithelium. R-spondin1 has been shown to interact directly with the pathway inhibitor DKK1. This interaction turns on the pathway and ensures the stabilization of β -catenin. Once this molecule enters the cell's nucleus, it induces the expression of target genes involved in the initiation of the cell cycle.

Figure 3. Gene Design. This figure represents the synthetic gene design for two alternative genes. This design approach includes multiple cloning sites for cleavage and insertion. Kozak's sequences to provide ribosomal binding sites, and histidine tags to facilitate purification. 5' flanking sequences and 3' flanking structures in untranslated regions are included to discourage nuclease degradation and thus increase protein expression. The human R-spondin1 gene was codon optimized for direct expression. Gene one contains the native signal peptide while gene two contains the rice amylose signal peptide to trigger secretion into the plant apoplast.

ACKNOWLEDGEMENTS

TWG is supported by NSF CREATE-IGERT training grant DGE 0653884.

Tim Butterfield Explains Manipulation of Phytochrome-Mediated Signaling in Transgenic Plants



Faculty Trainer/PI Karen McDonald and 2009 CREATE-IGERT Trainees Ben Lindenmuth and Lucas Arzola



Plant-based expression systems coming right up!

Faculty Trainer J. Clark Lagarias and 2009 CREATE-IGERT Trainee Timothy Butterfield



Phytochrome engineers extraordinaire!

Faculty Trainer Rebecca Parales and 2009 CREATE-IGERT Trainee Mark Wolf



Using *Acidothermus cellulolyticus* to identify thermostable enzymes for use in the production of biofuels and industrial chemicals.

2009 UCD CREATE-IGERT Trainees

Rachel Kerwin, Dawn Chiniquy and Elenor Castillo



Engineering crops for maximum biomass and environmental sustainability!