Biotech Club Fall Welcome
Bio-Rad Event

Moderator:
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UC Davis Biotechnology Program
Lecturer, Plant Biology

http://www.biotech.ucdavis.edu
Event Agenda

- **5:30-6pm** Introductions & Basic Biotech
- **6pm-7pm** Bio-Rad “Genes in a Bottle” Ag Biotech Lecture & Discussion
- **7pm-7:30pm** Pizza and “Goodies”

Note: The Bio-Rad Photo Team will be staging photos throughout the evening—student participation is voluntary, but encouraged. If you would like to participate, please sign a photo release form before you leave.
Biotech Education at UC Davis

- BA/BS in Biological Sciences (various majors)
- BS in Biotechnology
- MS/PhD in Biological Sciences (various majors)
- Designated Emphasis in Biotechnology (for PhD students)
Current Progress in Biotechnology Seminar

- 11am-noon, Fridays, 1022 LSA
  - Open to the public/all students
  - Speakers from the biotech industry and academia
    - Biofuels and Clean Energy
    - Stem Cells and Tissue Regeneration
    - Genomics
    - Plant-based Products
    - Personalized Medicine and Diagnostics
    - Many other cutting edge topics…
“Genes in a Bottle”

Bio-Rad

Biotechnology Explorer

DNA Extraction Activity
Ag Biotech

Plant Genetic Engineering

The GMO Controversy
People use plants for:

- Food & Feed
- Fuel
- Fiber
- “Pharming”
- Pharmaceuticals
- Bioremediation

http://calphotos.berkeley.edu/
Active Areas of Ag Biotech Research

- **GMO Crops** → Plants with enhanced agronomic & quality traits
  - Reduce pesticide/herbicide use
  - Increase crop yields via engineered disease resistance & stress tolerance
  - Increase nutritional properties of staple crops

- **“Pharming”** → Plants used as bioreactors, producing useful biomolecules.
  - Specialized oils for nutritional and industrial uses
  - Vaccines and other pharmaceuticals
  - Nutraceuticals (ex: human lysozyme & lactoferrin)

- **Bioremediation** → Plants are used to clean up contaminated soils, waters, etc..
  - Salt-tolerant tomatoes (Dr. Blumwald, UCD)
  - Poplar trees that remove heavy metals from soil

- **Biofuels & Bioenergy** → Plants are used as a source of fuels and to provide renewable energy
  - Cellulosic ethanol
  - Biodiesel
We need Factual Information about Ag Biotech
Plant Traits are the Result of Gene x Environment Interactions

Environmental Inputs
- Light
  - Diff wavelengths
  - Day vs. Night Length
- Soil Water
- Soil Salinity/Minerals
- Temperature
- Biotic stresses
  - Herbivores
  - Pathogens

Genetic Contributions

Proteins act alone or in complexes to perform many cellular functions
Genetic Engineering =

- Cutting and moving snippets of DNA (genes for specific desirable traits) from one plant, animal or microbe to another.
- Unlike traditional crossbreeding, only one or a few genes are introduced into the host species. Therefore, unwanted traits are usually avoided.
Plant Biotechnology Generations

Value

1st Wave
Agronomic Traits

2nd Wave
Quality Traits

3rd Wave
Plants as Factories

4th Wave
Renewable Resources
Genetic Modification of Crops

1. Choose desirable trait
2. Clone the gene
3. Engineer the gene
4. Transform gene into plant
5. Backcross GM plant into high yield crops
Choose Desirable Trait

• Pest Resistance: Bt crops
  • *Bacillus thuringiensis* protein is a delta endotoxin that kills corn borers

• Herbicide Tolerance: Round Up Ready crops
  • *Agrobacterium tumifaciens* protein with resistance to Round Up herbicide (glyphosate)
Clone the Gene of Interest into a Ti Plasmid from *Agrobacterium tumifaciens*.

**Bacillus thuringiensis**

Delta endotoxin crystal—encoded by the “Bt” gene that is taken from the bacteria and cloned into a plasmid vector.
Scientists have genetically modified the bacterium’s Ti plasmid to deliver genes that we are interested in to the plant genome, rather than the bacterial genes for making a crown gall tumor.
A Modified Agrobacterium Delivering a Gene of Interest to a Plant Cell

- T-DNA flanking sequences mediate integration into the plant genome
  - Nopaline left: TGTGGCAGGATATATTGTGGTGTAAACAA
  - Nopaline right: TTTGACAGGATATATTGGCGGGTAAACCT
  - Consensus nopaline...tGCAGGATATAT tg...gCTA aac...

Diagram showing Agrobacterium tumefaciens delivering a gene of interest to a plant cell.
Engineer the Gene of Interest

May need to modify promoter, terminator and other regulatory gene sequences

Gene may be subcloned from original Ti vector into a Ti vector with different selectable markers

Slide courtesy of Bio-Rad
Screening Techniques: Use of Reporter Gene to Detect rDNA

- **Antibiotic Resistance**
  - Screen for recombinants on growth medium containing the chosen antibiotic (one that is not commonly used in medicine, such as kanamycin)

- **Herbicide Resistance**
  - similar to above but use herbicide such as glyphosate ("Round-up") in the growth medium

- **Fluorescence [Luciferase & GFP system]**:
  - insert gene for luciferase (firefly) or GFP gene (jelly fish) into rDNA insert. “Glows in the Dark”
Transform Gene into the Plant

1) Isolate plant cells
2) Grow undifferentiated callus
3) Transform cells
4) Redifferentiate callus
5) Grow transgenic plant

Slide - courtesy of Bio-Rad
The Whole Transformation Process on one plate: Steps 1-4

1. Wounded plant tissue
2. Formation of a Callus after treatment with *Agrobacterium*
3. Shoot development from callus (unlike animal cells, plants are pluripotent and any plant cell can develop into a new plant in the right conditions)
4. Shoots are rooting and growing into new plants (hopefully with new transgene)
The “Gene Gun” Can Blast Plant Cells with Particles Coated in DNA

This is a good method for introducing genes into monocot crops, such as maize and rice.
Ralph M. Parsons Foundation Plant Transformation Facility (RMPFPTF)

College of Agriculture and Environmental Science

Manager: David Tricoli
Staff: Kim Carney
Director: Abhaya Dandekar

Scientific Advisory Board:
- John Bowman
- Kent Bradford
- George Bruening
- Dave Burger
- Doug Cook
- Chuck Gasser
- Dave Gilchrist
- Carole Meredith
- Richard Michelmore

Transformation Service:
Tomato, Tobacco, Rice, Cannola, Lettuce, Alfalfa, Melon, Carrizo, Lemon

Protocol Development:
Alfalfa, Wheat, Walnut, Almond

Provide service to the research community
Backcross GM Plant to High Yielding Crop Variety

**GM plant =** $yyGG$

**High yield plant =** $YYgg$

**PARENTS**

- $YYgg \times yyGG \rightarrow YyGg$
  
  **BC1**
  - $YYgG$
  - $YygG$
  - $YYgg$
  - $Yygg$

- $YYgg \times YyGg \rightarrow$
  
  **BC2**
  - $YYgG$
  - $YYgg$
  - $YYGg$
  - $YYGG$

Want to get a “pure-breeding” line that has both traits—high-yielding and gene of interest
Current GM Food Crops

US Approval for GM food crops
• Corn
• Soy
• Papaya
• Canola
• Potato
• Chicory
• Rice
• Squash
• Sugarbeet
• Tomatoes

Approval does not necessarily mean these crops are distributed

Database of GM crops: www.agbios.com
Global Area (Million Acres) of Biotech Crops, 1996 to 2005: by Crop

Source: Clive James, 2005

Source: ISAAA
Rice - The Miracle Food

- Rice - the most important food crop
- Eaten by 3.8 billion people
- Average farm <2 acres
- Only 6% traded in the world market
- 60% more rice needed by 2020 - with less land, water, labor and chemicals
- Yield losses - 220 million tons

How can Biotech Help????
Field evaluation of insect resistant transgenic MH63 with Bt gene against stem borer (Datta, IRRI)

Transgenic Bt Rice—increased yield of grain

Wild Type—reduced yield due to insect herbivory

Bt Insect Resistance = Agronomic Trait
“Golden Rice”

High Provitamin A (β-carotene) rice is a major advance for plant biotechnology and focuses international attention on the metabolic engineering of output traits.

Increased Nutrition = Quality Trait
Over 120 million children worldwide are deficient in vitamin A. Rice has been engineered to accumulate β-carotene, which is converted to vitamin A in the body. Incorporation of this trait into rice cultivars and widespread distribution could prevent 1 to 2 million deaths each year.

Cotton

- Major crop for China, South Africa...India, Egypt, Indonesia..
- 60 million people in India financially dependent on cotton
- Annual losses due to bollworm were ~$1.5 billion in India and China
- Bt Cotton - yield increases up to 40%!
  - Million+ Chinese farmers now grow it
  - Savings up to $182 per hectare

Impact on Pesticide Usage:
- Cotton is responsible for ~50% of pesticides sprayed
- Bt Cotton reduces pesticide application by ~90%
- ~2 million gallons of pesticide saved annually in US alone using Bt Cotton
Opponents of Agricultural Biotechnology...

Argue that plant biotech may lead to:

- Creation of super pests
- Creation of super weeds
- Loss of biodiversity
- Biotechnology companies controlling agriculture
- Health concerns

Greenpeace Demonstration
Safety Data Requirements for Registration of Biotech Crops

Product description (7 items)
Molecular characterization (17 items)
Toxicity studies (as necessary) (5 items)
Antibiotic resistance marker genes (4 items)
Nutritional content (7+ items)
Substantial equivalence with parent variety
Literature review and background
Allergenicity potential
Similarity to natural toxicants
Anti-nutritional effects
Protein digestibility
Environmental aspects (5 items)
Germination, growth, flowering studies (8 items)
Ecological impact (5 items)

Total regulatory and testing costs: $2,000,000 to $10,000,000.
None of this is required for traditionally bred crops.
Education is the Key to Public Acceptance of Biotech Foods

- Survey in Seed Trade News (Dec 1999) by Thomas Hoban

**Question?** Ordinary Tomatoes do not contain genes, while genetically modified ones do?

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<th>False</th>
<th>Don’t Know</th>
<th>True</th>
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<td>Canada</td>
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<td>Germany</td>
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<tr>
<td>Sweden</td>
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<td>24</td>
<td>30</td>
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Why Test for GMO’s?

- **Legislation**
  - US: food labeled “GM-Free” <5% GM
  - EU: food labeled “GM” if >1% GM
  - Japan: food labeled “GM” if >5%

- **Export**

- **What about unlabeled food?**
  - BioRad Biotechnology Explorer “GMO Investigator Kit”
  - PCR-based screen that uses the same primers as regulatory agencies
How to Test for GMOs:

**ELISA:**
Test for presence of proteins expressed from genetic modifications
Pro: Quick, cheap, low tech
Con: Crop specific, protein stability

**PCR:**
Test for presence of inserted foreign DNA
Pro: ID different GM crops, DNA stability
Con: Expensive, timely
GMO Investigator Kit Contents

- Bio-Rad certified Non-GMO food
- InstaGene
- Master Mix
- GMO primers
- Plant PSII primers
- GMO & PSII positive control DNA
- PCR MW Ruler
- DPTPs, microtubes, PCR tubes, foam floats
- Manual

Not Included but required:
- Thermal cycler
- Water bath/heat block
- Electrophoresis Module (agarose, TAE buffer & Fast Blast DNA stain)
- Electrophoresis equipment & power supply
  - 2-20 ul pipettes & barrier tips
PCR Test for GMOs

Test for GMOs by PCR:
1. Grind food
2. Extract DNA from sample
3. Test sample DNA for viable plant DNA
4. Test sample DNA for genetic modifications
PCR Controls in the Kit

- Bio-Rad certified non-GMO food
  - Verify PCR is not contaminated
- GMO positive control DNA
  - Verify GMO-negative result is not due to PCR reaction not working properly
- Primers to universal plant gene (Photosystem II)
  - Verify viable DNA was extracted
Why amplify a plant gene?

To confirm that viable DNA was extracted and that negative GM result isn’t due to a non-viable template.

Use highly conserved chloroplast gene from Photosystem II – part of the light reaction of photosynthesis.
Why use CaMV 35S and NOS?

**CaMV 35S** – Sequence for the promoter of 35S transcript of the Cauliflower mosaic virus. Used because it functions in every plant cell.

**NOS** - Sequence for nopaline synthase terminator from soil bacterium *Agrobacterium tumefaciens* Used because it evolved to be recognized in most plants.
Extract DNA from Food

1. Grind food samples
2. Add food sample to InstaGene matrix
3. Incubate at 100°C for 5 minutes
4. Centrifuge samples for 5 minutes to pellet matrix

DNA template preparation

Slide courtesy of Bio-Rad
Why these steps?

- Grinding food to release DNA
- InstaGene chelates divalent ions (e.g. \( \text{Mg}^{2+} \)) necessary for DNA degrading enzymes (e.g. DNases)
- Only 50 \( \mu l \) of food transferred otherwise InstaGene is overwhelmed (~ 5 mg of original material)
- Boiling releases DNA from food into the InstaGene solution
- Pellet InstaGene and food debris because InstaGene inhibits PCR reaction (Taq needs \( \text{Mg}^{++} \))
<table>
<thead>
<tr>
<th>Very Reliable</th>
<th>Reliable</th>
<th>Less Reliable</th>
<th>Very Difficult / Not Possible</th>
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<tr>
<td>Fresh corn</td>
<td>Veggie sausages</td>
<td>Veggie burgers</td>
<td>Oil</td>
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<tr>
<td>Fresh papaya</td>
<td>Tortilla chips</td>
<td>Fried corn snacks</td>
<td>Salad dressing</td>
</tr>
<tr>
<td>Corn bread mix</td>
<td>Flavored tortilla chips</td>
<td>Popcorn</td>
<td>Cereal (e.g. cornflakes)</td>
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<tr>
<td>Corn meal</td>
<td>Puffed corn snacks</td>
<td>Fries</td>
<td>Wheat flour</td>
</tr>
<tr>
<td>Soy flour</td>
<td>Meatballs and burgers containing soy protein</td>
<td>Potato chips</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Soy-based protein drinks/powders</td>
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</tr>
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</table>

Which foods yield viable plant DNA?

Slide courtesy of Bio-Rad
The PCR Reaction
What do you need?

- **Template** - the DNA to be amplified
- **Primers** - 2 short specific pieces of DNA whose sequence flanks the target sequence
  - Forward
  - Reverse
- **Nucleotides** - dATP, dCTP, dGTP, dTTP
- **Magnesium chloride** - enzyme cofactor
- **Buffer** - maintains pH & contains salt
- **Taq DNA polymerase** – thermophillic enzyme from hot springs
Set Up PCR Reactions

Red master mix with GMO primers

Green master mix with plant primers

Sample

Add master mix to sample and control DNA templates

Place tubes in thermal cycler and amplify target DNA sequences

Set up polymerase chain reaction and amplify DNA samples

LAB 2
PCR Cycling Conditions

Heat (94°C) to denature DNA strands

Cool (59°C) to anneal primers to template

Warm (72°C) to activate Taq polymerase, which extends primers and replicates DNA

Repeat 40 cycles
Analysis of PCR Results

**Gel Lanes:**
1: non-GMO food with plant primers
2: non-GMO food with GMO primers
3. Test food with plant primers
4: Test food with GMO primers
5: GMO positive template with plant primers
6: GMO positive template with GMO primers
7: PCR MW Ruler

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**GMO positive**

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**GMO negative**
Trouble shooting PCR

- **False Positives**
  - Contamination-sterile technique; 10% bleach to clean pipette barrels, mortars & pestles, bench tops; barrier tips for all steps.

- **False Negatives**
  - No DNA extracted
  - Possible food type or possibly primers do not work on that plant species
  - InstaGene matrix transferred to PCR reactions
“Real Time” or RTi-PCR Allows Quantification of Amplified Nucleic Acid Fragments

- PCR products are fluorescently labeled
- Amt of fluorescence is measured after each PCR cycle
- Theoretically, fluorescence intensity should increase exponentially (assuming 100% efficient PCR)
## Bio-Rad Real Time PCR Detection Systems

<table>
<thead>
<tr>
<th>Feature</th>
<th>MiniOpticon</th>
<th>MyiQ</th>
<th>Opticon 2</th>
<th>Chromo4</th>
<th>iQ5</th>
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<tbody>
<tr>
<td>Excitation range</td>
<td>470–500 nm</td>
<td>475–495 nm</td>
<td>470–505 nm</td>
<td>450–650 nm</td>
<td>475–645 nm</td>
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<tr>
<td>Light source</td>
<td>Light-emitting diode (LED) array (48 blue-green LEDs)</td>
<td>Tungsten-halogen lamp</td>
<td>LED array (96 blue-green LEDs)</td>
<td>4 LEDs in photonics shuttle</td>
<td>Tungsten-halogen lamp</td>
</tr>
</tbody>
</table>