Workshop and Training Program on Sampling and Detection Methods Applied to Transgenic Crops

November 17 – 19, 2011, NIN, Hyderabad, India

Developing New GM Products and Detection Methods

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ILSI International Food Biotechnology Committee

Slides - Thanks to:

- International Life Sciences Institute
- Crop Life International
- Indus try Colleagues
- Hope Hart - Syngenta

Outline

1. How to create a transgenic event
2. How to select the best event
3. What happens after the best event is chosen
4. How detection methods are used to help make these decision?

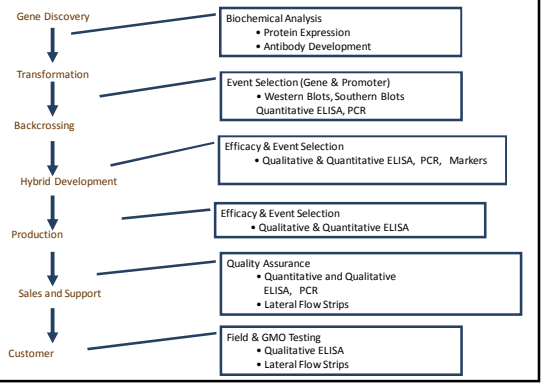
Who Develops Detection Methods and Reference Materials?

- DNA detection methods
  - Ag Biotech Companies during product development
- Protein-based methods
  - Ag Biotech Companies during product development
  - Commercial kits developed by test kit suppliers collaborating with Ag Biotech Companies
- Certified Reference Materials for commercial events (seed/grain) provided by Ag Biotech companies
- Testing Laboratories, and Governments

Who Uses Detection Methods?

- Seed Companies
  - Product discovery and development
  - Seed breeding/ commercial seed quality control laboratory
- Commercial for Profit Testing Companies
- Grain Handlers
- Food Processors and Companies
- Government laboratory testing

Molecular Analysis Applications In Product Development



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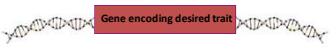
Definitions

Plant Biotechnology –
- Use gene technology to create a plant with novel characteristics

Trait –
- A characteristic that is 1) expressed as a phenotype, e.g. herbicide resistance or insect resistance, and/or 2) can be determined by the detection of a protein or gene.

Event –
- A variety or related varieties that contain the results of a particular insertion of a specific piece of DNA – can usually be determined by the presence of specific border sequences



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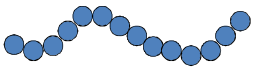
DNA: Target of Modern Biotechnology

DNA is like a strand of pearls:



Each pearl represents a gene that encodes a protein.

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How to create a transgenic event

Prepare gene for plant expression

The "instructions": DNA sequence that encodes a protein

Serves as the "switch" and the "engine", turning the gene on and off and driving expression of the gene.

The transcription "stop sign"

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How to create a transgenic event

Transformation steps

1. Target tissue and DNA preparation
2. DNA delivery
   a. Biological (Agrobacterium)
   b. Physical (Biolistics - gene gun)
3. Selecting transformed tissue
4. Regeneration

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How to Choose the Best Event

- Event selection is used to determine the transgenic plant with the best performance and ideal genotypic and phenotypic characteristics

The best event

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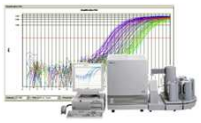
How to Select the Best Event: Genotypic Analysis



- Real-time PCR assay and Southern blot are the techniques used to genotype transgenic events by:
  – Determining the number of transgenic inserts
  – Determining the structure of the inserts

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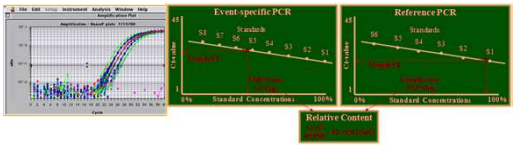
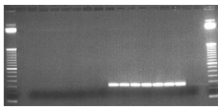
Quantitative Real-Time PCR Method

- Initial driver: Global Food/Feed Regulations, e.g.: EU Food/Feed regulation Articles 5 (3) (i) and 17 (3) (i) of Regulation (EC) No. 1829/2003
- Currently supplied to: EU (EURL); Japan (MAFF); Korea (KFDA, NAQS); China (MOA); Taiwan (BFDA); Mexico (COFEPRIS, SAGARPA); India (GEAC); Singapore (AVA)



Qualitative Gel-Based PCR Method

- Initial driver: Global Food/Feed Regulations, e.g.: Safety Evaluation Guidelines by Notification 1999-46 of the KFDA
- Currently supplied to: Korea (KFDA, NAQS); China (MOA); India (GEAC); Russia (Russia's Institute of Nutrition of RAMS)



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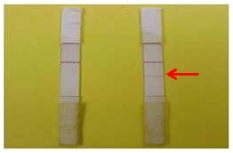
Sample	U1	U2	U3	U4	U5	U6	U7	U8	U9	U10
Result	Neg	Neg	Neg	Neg	Neg	Pos	Pos	Pos	Pos	Pos

How to Select the Best Event: Phenotypic Analysis

- Presence of a specific protein: Immunostrips



- Amount of a specific protein: ELISA

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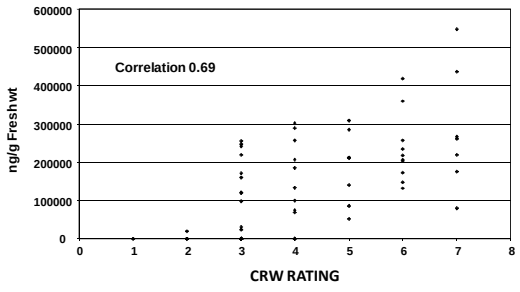
Promoter Evaluation Using Quantitative ELISA

CONSTRUCT	# SAMPLES ASSAYED	# ELISA POSITIVE SAMPLES(>50 ppm)	AVERAGE ELISA SIGNAL (ppm)	Bioassay Results: % EFF. EVENTS
a	115	82 (71.3%)	312	9.6%
b	123	116 (94.3%)	6374	86.2%
c	14	10 (71.4%)	5399	71.4%

- Promoter a
- Promoter b
- Promoter c

CRW TARGET PROTEIN EXPRESSION VS. CRW RATING



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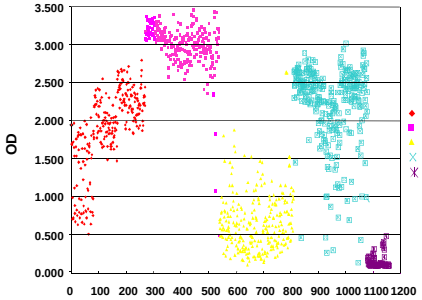
Distribution Of ELISA OD's Within Events



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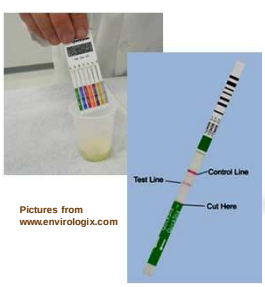
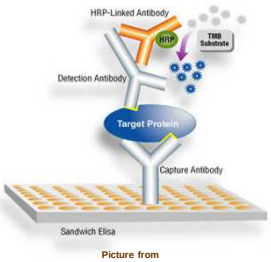
Protein Based Tests

Driver for commercial kits is largely stewardship and consumer choice

Field: Lateral Flow Devices (strips) **Lab: ELISA**



Pictures from www.envirologix.com



Picture from www.synchronium.net

How to Select the Best Event: Phenotypic Analysis

Performance/Efficacy

insect-resistance traits

herbicide-tolerance traits

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How to Select the Best Event: Phenotypic Analysis

Physical Characteristics

- stand establishment
- leaf orientation
- plant height
- silk date
- leaf color
- root strength (lodging)
- tassel size
- stalk rating
- early plant vigor
- silk color
- ear shape
- silk color
- tassel color
- reaction to fungicides/herbicides
- late season staygreen/appearance
- susceptibility to pathogen/pests
- ear height
- ear tipfill
- above ear intactness
- yield

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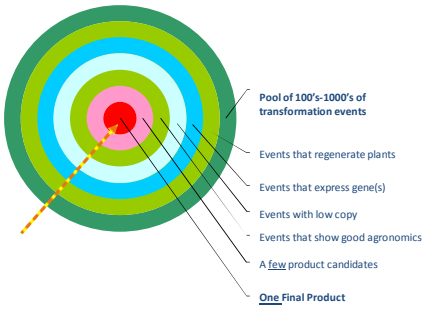
Many events are produced, but few are chosen



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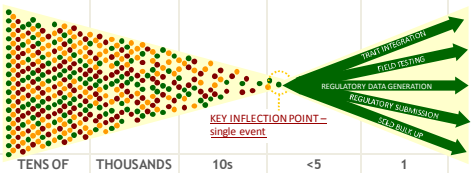
Extensive Selection in Choosing a Lead Event Eliminates Off-Types

	DISCOVERY Gene/Trait Identification	PHASE I Proof Of Concept	PHASE II Early Development	PHASE III Advanced Development	PHASE IV Pre-launch
AVERAGE DURATION	24 to 48 MONTHS	12 to 24 MONTHS	12 to 24 MONTHS	12 to 24 MONTHS	12 to 36 MONTHS
GENES IN TESTING	TENS OF THOUSANDS	THOUSANDS	10s	<5	1



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After Best Event Is Chosen: Trait Introgression and Conversion

- Backcross conversion is a breeding method in which the donor parent is the line that contains a desired trait that is integrated into the recurrent parent line by repeated crossing
- The final transgenic product will have a genetic makeup that is mostly made up of the genome of the recurrent parent (commercial line) but will have the added benefit of the transgenic trait

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After Best Event Is Chosen: Regulatory Assessment of Biotechnology Products

Gene / Protein Safety — Food, Feed & Environmental Safety — Crop Safety

Gene(s)
- Source(s)
- Molecular characterization of event
- Insert stability /copy number /gene integrity

Protein(s)
- Function / specificity / mode of action
- Expression Levels
- Analytical methods used
- Protein safe use history
- Allergencity/toxicity assessment

Equivalency of Proteins
- Bacterial-produced vs crop-produced

Crop Characteristics
- History of safe use and consumption
- Morphology, Yield
- Toxicology / allergenicity

Environmental Safety
- Non-target organisms
- Outcrossing
- Invasiveness, weediness
- How to deal with volunteer plants

Food/Feed Composition
- History of crop food/feed use
- Anti-nutrient composition
- Nutritional equivalence
- Nutritional assessment

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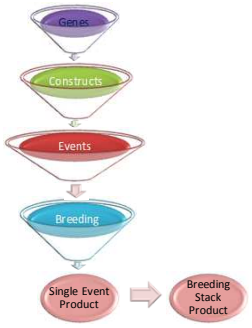
Steps to Create a Plant Biotechnology Product

1. Choose a gene that will give the desired phenotype
2. Proof of concept experiments testing many promoter-gene-terminator combinations for best plant expression
2. Put the DNA into the plant genome
4. Select the best plant
5. Breed - Elite line conversion
6. Regulatory assessment



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How to create a transgenic event

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