Overall Safety Assessment of GM Crops- Allergenicity

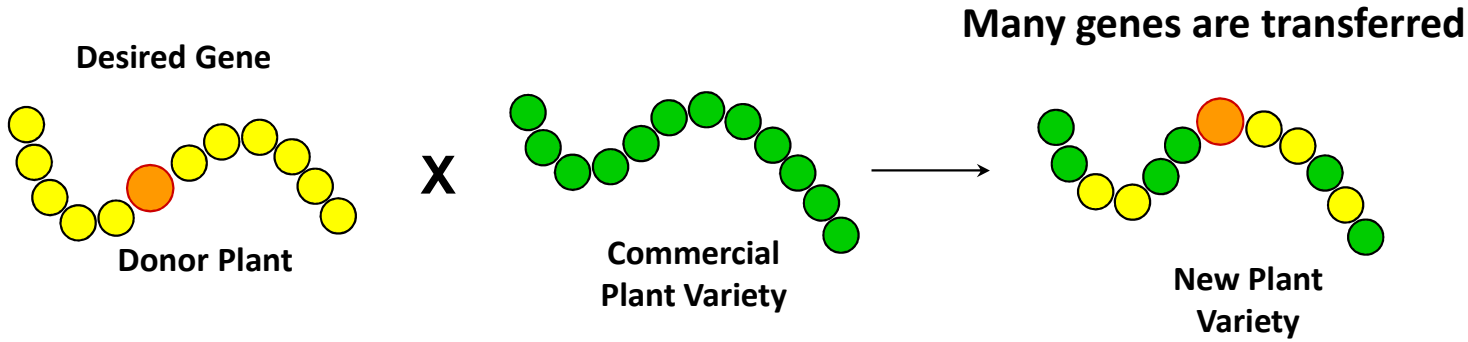
Gregory S. Ladics, Ph.D., DABT, Fellow ATS

DuPont Co.

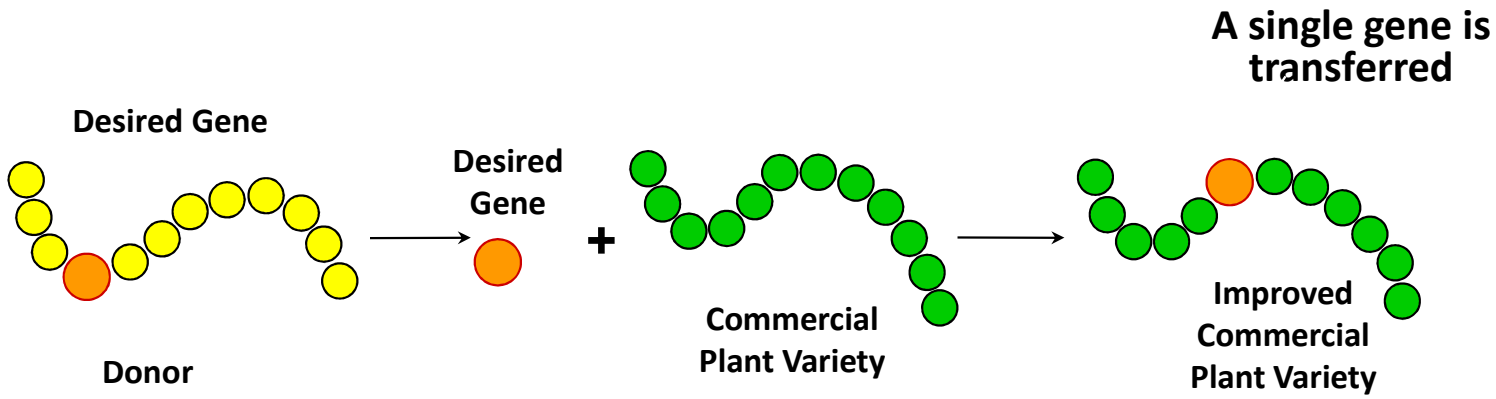
Newark, DE USA

Biotechnology is an Extension of Traditional Plant Breeding

TRADITIONAL PLANT BREEDING



PLANT BIOTECHNOLOGY



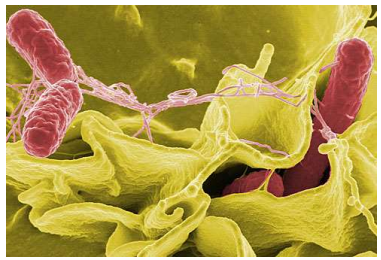
Safety for humans, animals and the environment

- Safety assessment for plant biotech products is mandatory worldwide
- Considers human + animal health as well as environmental safety
- Approval only if authorities conclude:
  - ➔ Genetically optimized plant is as safe as a conventionally bred plant
  - ➔ Safety assessment starts early in the development process

Food Safety is a Global Concern :

Consumers need assurance that food will not cause harm and covers contamination by chemical and biological agents and concerns about inherent food nature.

Key Global Concerns (http://www.who.int/features/factfiles/food_safety/en/) include:



Spread of microbiological hazards

1,500,000 deaths/yr

Chemical Food Contaminants

>300,000 affected by melamine and urea in milk in 2008

Genetic Modification

0 deaths or illnesses resulting from GM foods

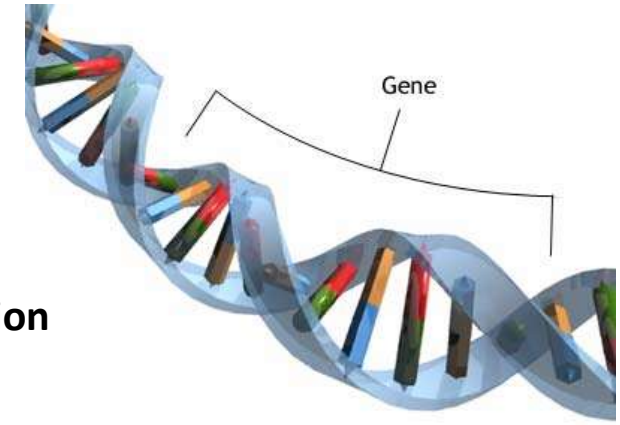
Safety Assessment is a multi-pronged undertaking

Crop Safety

Gene / Protein

Food / Feed and Environmental Safety

- **Gene(s)**
- **Protein(s)**
- **Crop Characteristics**
- **Food/Feed Composition**
- **Environmental Safety**



Regulatory Studies are grouped in four categories:

- **Molecular characterization**
- **Protein Characterization/Food/feed safety**
- **Agronomic and Compositional Equivalence**
- **Environmental safety**

Protein characterization, Food / Feed safety

- Gram quantity protein production and purification
- Establishing protein level in plant tissues
- Protein characterization and equivalence
- ***Allergenicity assessment***
- Toxicity assessment

Why proteins do not typically represent a hazard

- Proteins are relatively large and labile.
- Proteins are an essential part of the diet (avg. consumption 100 g/day).
- Digestive systems have evolved to convert the protein to its building blocks for incorporation. (very efficient only 6 – 12 g protein lost/day)
- The human body synthesizes approx. 300 g protein/day.

Protein Hazards

- Pathogenic bacterial toxins- botulinum, diptherium, active <100 mg/kg body weight.
- Plant toxins - ricin (0.5 mg MLD)
- Animal toxins - prions
- Allergens – Ara H2, b-lactoglobulin, glutens
- Antinutrients - trypsin inhibitors, some lectins

Categories of Potential Health Risks of Agricultural Biotechnology Relative to Allergenicity

- Transfer an existing allergen or cross-reactive protein into another crop.
- Creation of food allergens *de novo*
  (*i.e.,* potential to become a new allergen.)
- Alteration or quantitative increase of endogenous (existing) allergens
  (*i.e.,* increasing the hazard of currently allergenic foods)

Safety Assessment Approach- IgE-Mediated Allergy

- Avoid transfer of known allergens
  - Assume genes from allergenic sources encode an allergen until proven otherwise
- All introduced proteins evaluated
- Endpoints
  - gene source, sequence homology with known allergens, immunologic analyses and physicochemical properties
- 'Weight-of-the-evidence' provides reasonable assurance that foods will not become more allergenic

CODEX Guidelines (2003; 2009)

- CODEX recommended allergy assessment includes:
  - Source of the introduced protein
  - Similarity of the introduced protein to known allergens
  - Susceptibility to enzymatic digestion (pepsin)

Currently, no single test can predict food allergy for humans

CODEX Guidelines (2003; 2009)

- ***If introduced protein from a non-allergenic source***
  - ✓Assess amino acid sequence similarity to known allergens
  - ✓Assess pepsin resistance
- ***If introduced protein from an allergenic source***
  - ✓Assess amino acid sequence similarity
  - ✓Assess in vitro pepsin resistance
  - ✓Assess specific IgE binding
  - ✓Assess skin prick testing

CODEX Guidelines (2003; 2009)

- CODEX recommended allergy assessment
  - ***Other considerations***
    - ✓Exposure level of the introduced protein
    - ✓As science and technology evolves other methods may be considered
      - ➢T-cell epitopes and structural motifs associated with allergens (glutens)
      - ➢Animal models

Non-IgE Mediated Immune Reactions to Foods

Limited information in CODEX regarding the evaluation for the potential of non-IgE mediated reactions:

- “The transfer of genes from organisms known to elicit gluten-sensitive enteropathy in sensitive individuals should be avoided unless it is documented that the transferred gene does not code for an allergen or for a protein involved in gluten-sensitive enteropathy.”

- “This assessment strategy is not applicable for assessing whether newly expressed proteins are capable of inducing gluten-sensitive or other enteropathies.”

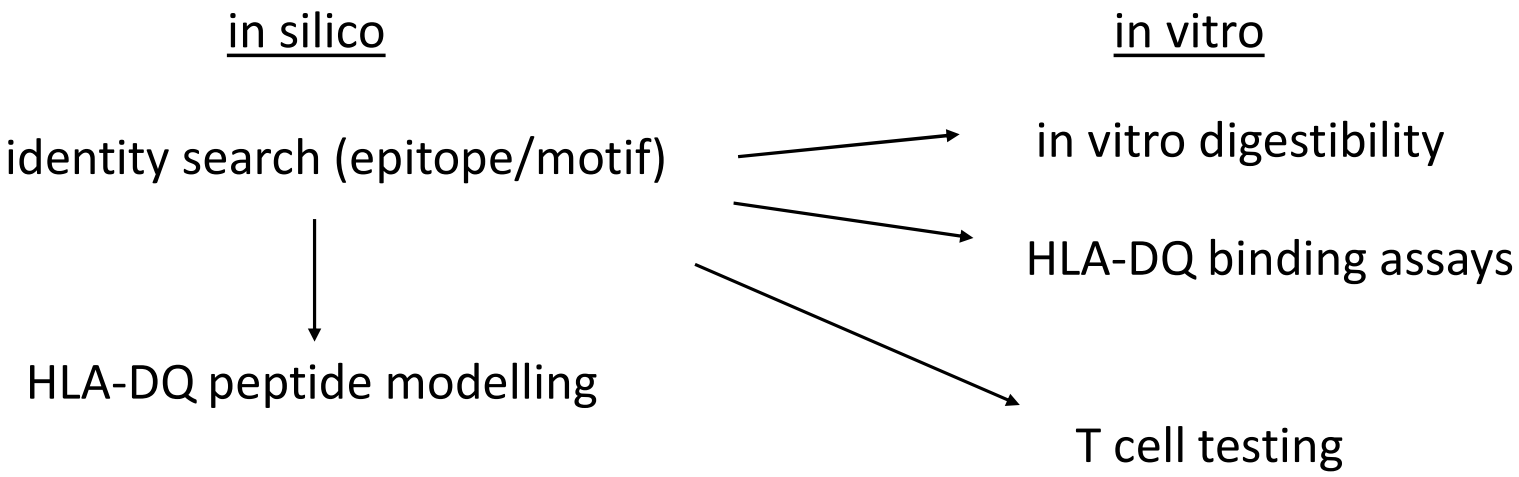
Safety Assessment Screening: Focus on Celiac Disease

- Gene Source: avoid genes from wheat, barley, rye and oats
- In silico exact peptide mapping of novel protein to those peptides identified in the peer reviewed literature as inducing celiac disease
- Evaluation occurs early in product development- (i.e., before constructs are made).

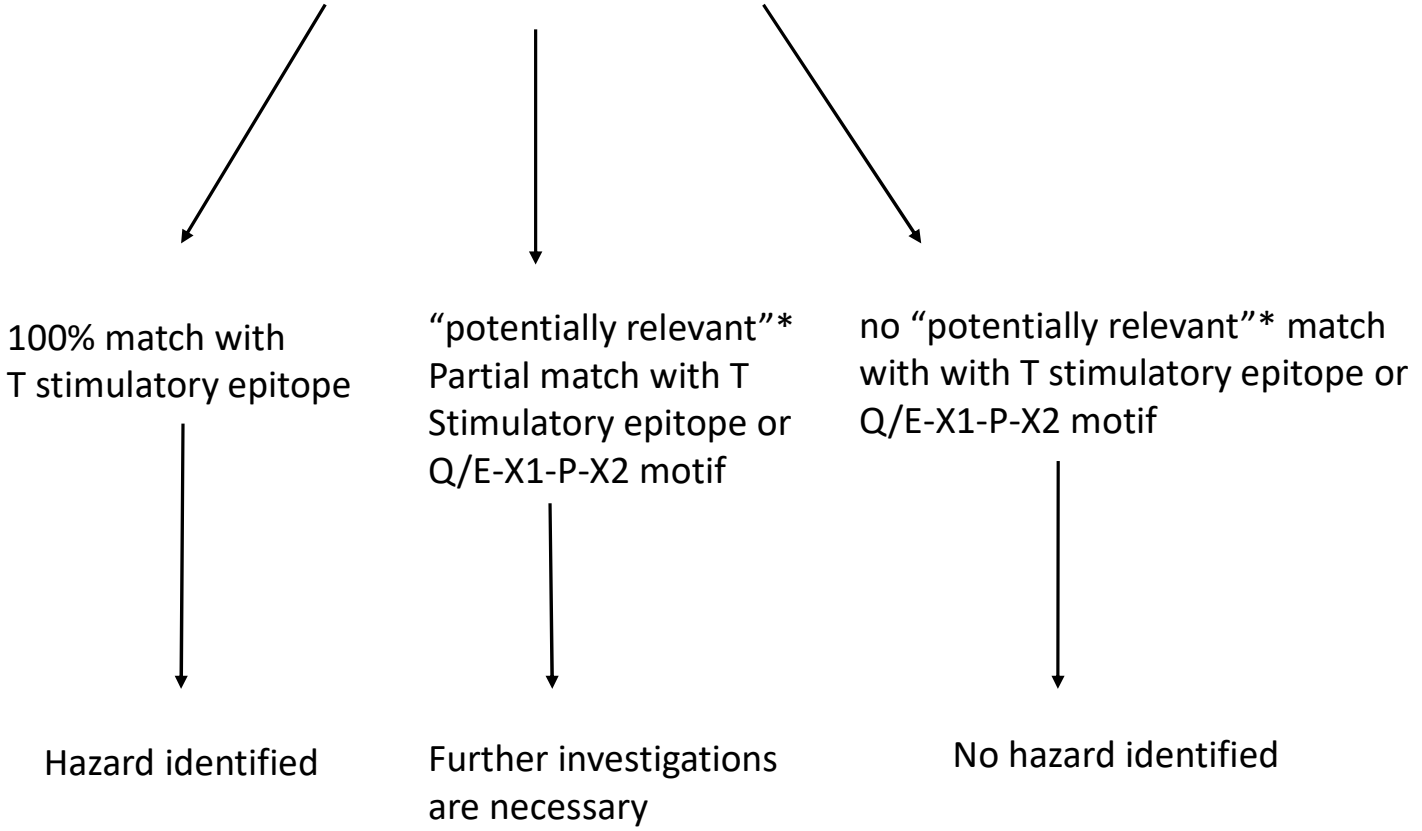
EFSA Draft Guidance on Allergenicity Assessment of GM Plants

Non-IgE-Mediated Immune Adverse Reactions to Foods

Stepwise Approach for Risk Assessment



Identity Searches(epitope/motif)



*Match with a known T cell-stimulatory peptide which raises concern due to the position and nature of the identical amino acids.

Questions/Comments for Discussion

- Stepwise approach for risk assessment versus a weight-of-the-evidence approach is suggested in the draft guidance.
- If the source of the gene is not from a gluten-containing cereal or taxonomically related species and has no homology to a gluten protein, is there a need for further assessment?
- If the source of the gene is from a gluten-containing cereal and/or has homology to a gluten protein, suggest to search for exact match with T-cell epitopes.
- Is there any data to otherwise suggest that only very specific peptides from glutens are responsible for eliciting celiac disease (i.e., mismatched peptides concept)?
- What is the threshold for similarity? How similar should a peptide be for cross-reactivity to occur? How many amino acid mismatches are allowed before a peptide sequence is indicated to be a potential T-cell epitope (biologically significant)?
- What is the relevance of the Q/E-X1-P-X2 motif search? Preliminary data suggest that it is relatively common in proteins from organisms not associated with celiac disease (high level of false positives).
- If there are no stable fragments following pepsin digestion, are additional in vitro studies still needed with positive bioinformatic findings?