Draft comprehensive outline for

ARS plant transgene procedures and practices

This document is a draft comprehensive outline for Agency-wide procedures and practices for handling transgenes and genetically-engineering materials in USDA/ARS crop breeding stock and genebank collections. The procedures and practices will focus initially on the five major crops with widely cultivated varieties that incorporate deregulated transgenes: cotton, maize, soybeans, alfalfa, and sugarbeets. A team of USDA/ARS researchers, genebank curators, line managers, and National Program Leaders developed the outline, focusing on five major elements, identified by boldface type:

- Well-documented, reviewed, and accessible best management practices for maintaining seed quality in both breeding and genebank programs.
- Testing for quality at critical control points.
- Mandatory quality testing of new varieties or enhanced germplasm prior to formal release.
- Plans for mitigating the effects of inadvertent presence of transgenes in non-transgenic breeding stocks and germplasm samples.
- Communication strategies for disseminating information about Agency policies and for handling future occurrences of inadvertent presence of transgenes.

1) Well-documented, reviewed, and accessible best management practices for maintaining seed quality in both breeding and genebank programs.

Best Management Practices (BMPs) must address both adventitious presence (AP) of transgenes in non-transgenic/genetically-engineered seed stocks and the management of transgenic/genetically-engineered germplasm in NPGS genebank collections, USDA/ARS breeding programs, and other USDA/ARS research programs. Practices for preventing, detecting and addressing AP in non-transgenic/genetically-engineered germplasm might be different from those designed for managing transgenic/genetically-engineered germplasm.

Researchers, breeders, and curators are obliged to follow any best management practices stipulated by the regulatory agencies with responsibilities for particular transgenic/genetically-engineered traits. This document therefore focuses on BMPs for dealing with (AP) in non-transgenic/genetically-engineered seed stocks and NPGS genebank collections.

Examples for BMPs are available for breeding of many major crops, and can be adopted or modified to address USDA/ARS crop research and genebank needs. Procedures are available for AP detection and monitoring methods, and genebanks have BMPs as well. Current BMPs should be evaluated to determine whether they fully address USDA/ARSs needs, and can be strengthened as necessary.

The BMP aspects listed below are broadly based (organizationally) on Hazard Analysis and Critical Point system of Food Safety (HAACP) Principles, described in the Excellence through Stewardship document.

- BMPs must be developed (or refined) and adopted to address the following needs of genebanks and breeding programs, as relevant:
    - Product integrity (define purity and tolerance standards, nature of AP) based on
        - reproductive biology of the crop;
        - whether transgenic/genetic-engineering events are regulated or deregulated, proprietary or non-proprietary;
        - specific national regulations or phytosanitary requirements of international germplasm recipients.
    - Risk analysis for AP from external sources
    - Likelihood of AP due to various factors
    - QA processes that encompass sampling and testing as required (See information under "Testing" section).
    - QA standards for a transgenic/genetically-engineered accessions or breeding stocks to be maintained, including an understanding of the differences in processes required for maintaining transgenic/genetically-engineered and non- transgenic/genetically-engineered germplasm.
    - Critical control points, or potential sources of AP identified for critical aspects of operations and workflows during:
        - Acquisition of germplasm
        - Storage and maintenance of germplasm
        - Propagation
        - Harvest and post-harvest processing
        - Exchange of germplasm for research operations
        - Distribution of germplasm (releases, collaborative research, national and especially international).

- Potential corrective actions if BMPs are not followed (see information in "Mitigation" and "Testing" sections) if AP occurs and genetic integrity is compromised.

- Documentation requirements and procedures established to assure that:

    - Crop-specific BMPs are in place and followed for each breeding program and genebank
    - Specific methods/tools/actions for all critical control points and workflows are available to ensure genetic integrity, which might encompass:
        - Protocols to separate transgenic/genetically-engineered from non-transgenic/genetically-engineered accessions spatially or temporally
            - Clear, distinct labeling of transgenic/genetically-engineered from non-transgenic/genetically-engineered accessions in databases, on all packets, containers, tags, etc. for regeneration or research activity workflows
            - Separate fields or growing environments, appropriate storage regimens

        - Separate harvest and seed processing activities for transgenic/ genetically-engineered from non- transgenic/genetically-engineered accessions;
        - Different cleaning equipment and times;
        - Effective vacuum systems in seed cleaning labs;
        - Separate equipment when feasible
        - Protocols to devitalize seeds with AP
    - Personnel have been trained in BMPs
    - BMPs/methods/tools to ensure genetic integrity and purity of samples are maintained and/or produced and are accurately identified (see information in "Mitigation" and "Testing" sections).
    - Monitoring to determine if BMPs are followed and to evaluate their effectiveness
    - Potential corrective actions when AP or misidentification occurs (see information in "Mitigation" section)
    - Actions when recipients indicate germplasm with AP were received(see information in "Mitigation" and "Communication" sections)
    - Statement regarding degree of confidence that BMPs limit probabilities of AP in USDA/ARS programs. Guidance similar to seed certification documents as provided by NMSU for example: "The word none or zero tolerance should not be construed as expressed or warranty that the field is completely free of a given factor, rather, that none were observed during the inspection."

2) <u>Testing</u> for quality at critical control points.

- Recommendations and Guidelines for Threshold or Acceptable Tolerance Levels of Adventitious Presence (AP)
    - Plant breeding programs and genebank programs have different acceptable tolerance levels because of their different outputs and/or products.
        - Recommendation for genebank accessions.
            - Delivery of accessions that are true-to-type: aspiring to zero tolerance of AP, but testing for X level of AP. Testing requirements and protocols must balance level of required probability of detection with resources available.
            - If a 0% tolerance is sought, statistically the actual level of AP can range from 0.01% - 0.03% AP, depending on the crop.
        - Recommendation for plant breeding programs which both release varieties and operate breeding nurseries
            - Continuing process for removal of AP (see information in "Mitigation" section).
            - Follow seed certification testing protocols
                - They are also varied depending on crop and AP
                - AP levels of 0.1%-5% are expected, depending on crop and information available

- Statement regarding degree of confidence that BMPs limit probabilities of AP in USDA/ARS programs. Guidance similar to seed certification documents as provided by NMSU for example: "The word none or zero tolerance should not be construed as expressed or warranty that the field is completely free of a given factor, rather, that none were observed during the inspection."

- Critical Control Points for Testing for AP in Genebanks and Plant Breeding Programs
  - Development of Decision Tree to assess for probability of AP in genebanks and plant breeding programs
    - Reporting accurate levels of AP through approved standard methods
    - Probabilities for potential AP will vary depending on crop/trait, and the degree of confidence that BMPs have been followed (see information in "BMP" section). Personnel should be able to identify when potential AP is at an increased level. Decisions for testing can be based on increased potential for AP.
      - Consideration of testing occurs at critical control points in the breeding process, determined by decision tree. This will vary with breeding methodology and crop, and may not be confined to those factors listed below:
        - Introduction of new germplasm into breeding program
        - Identification of key parents for crosses
        - F1 seed prior to creation of segregating F2 population
        - Line selection of elite progeny before entry into replicated trials
        - Before shipping seed to international nurseries
        - Harvests from seed increases
      - Consideration of testing occurs at key points in genebank management process, determined by decision tree.
        - Introduction to collection of new germplasm with potential risk due to lack of BMP documentation, circumstances of collection in the field, etc.
        - Before shipping seed internationally; shipping to domestic (FL, HI, PR) winter nurseries potentially subject to special scrutiny – to be discussed further
        - Harvests from seed increases
          - Establish potential requirement for testing seed increases incoming from external cooperator
          - Establish potential requirement for testing genebank seed increases to monitor effectiveness of BMPs for regenerating accessions true-to-type.

- Testing Standards
  - Recommendations and guidelines for tests available to detect AP (e.g. PCR, phenotyping, and screening for promoters, immunostrips) and

recommended/approved laboratories with validated testing protocols. This will be developed and provided for each known crop/trait, and must be updated periodically.
- Requirements for samples to be tested. (see below Section **3 Mandatory Quality Testing…**)

3) Mandatory quality testing of new varieties or enhanced germplasm prior to formal release.

- The extent of mandatory testing of new USDA/ARS cultivars and enhanced germplasm prior to formal release will be determined by the permissible level of AP, which will in turn depend on several interacting factors:
  - Certification standards for the crop
  - Crop breeding system
  - Degree of genetic improvement (enhanced population segregating genetically vs. synthetic population vs. "inbred" line)
  - Resources available for testing
- Information about remedial actions to be taken with breeding stock appears in the "Mitigation" section.
- The release notice, registration article, etc. for released transgenic USDA/ARS cultivars or enhanced germplasm should include a full description of the transgenes or genetic-engineering constructs (e.g., promoters) present, plus details regarding the extent and nature of testing (types of test, genes tested, sample sizes, etc.; see "Testing" section for more information)
- The release notice, registration article, etc. for released non-transgenic USDA/ARS cultivars or enhanced germplasm should include a full description of the transgenes or genetic-engineering constructs (e.g., promoters) present, an estimate of their frequency, plus details regarding the extent and nature of testing (types of test, genes testing, sample sizes, etc.; see "Testing" section for more information).
- Testing of relevant genebank accessions before distribution would become mandatory primarily when documentation that curatorial BMPs have been followed is lacking, or a breach of such practices is known. The extent of such mandatory testing would be determined primarily by the interacting factors listed above and in the "Testing" section. An estimate of their frequency, plus details regarding the extent and nature of testing (types of test, genes testing, sample sizes, etc.) should be documented for curatorial records. See information about remedial actions in the "Mitigation section.
- Mandatory testing of materials to be incorporated into genebanks has been covered under the "Testing" section.

4) Plans for mitigating the effects of adventitious presence of transgenes in non-transgenic breeding stocks and genebank samples

GERMPLASM COLLECTIONS:

- Stop distribution, mark source of AP accessions as unavailable, and determine if clean-up or destruction is appropriate.
    - Modify GRIN records so that the accession is categorized as "unavailable".
    - If an external germplasm source reported it as containing AP, request their testing results and methodology/source, and determine if independent testing is needed.
- Notify any recipients of seed from AP seed lot (see information in "Communication Strategies" section).
- Identify seed lots from previous propagations of the accession in question and test for AP (immunostrip, PCR, or other).
    - Seed lots identified as having AP should be sequestered temporarily, with intent of eventual destruction or return to donor.
    - Use the most recently-increased seed lot that has been identified as AP-free to propagate seed and re-establish accession, through standard increase methods.
    - After propagation of a "true-to-type" seed source, all seed lots with AP should be destroyed (heat or other method).
    - If no "true-to-type" seed source is found within the collection
        - Purify a recent seed lot or…
        - Request donor provides a "true-to-type" source.
- "Purifying" a seed source with AP.
    - Plant a plot or multiple pots of the source with AP in the field or greenhouse.
    - Divide the plot or the pots into quarters or other appropriate fractions, and harvest fresh tissue from each fraction, bulking tissue by fraction.
    - Use appropriate immunostrips or other appropriate method to test for the previously identified AP off-types in the fractions.
    - Harvesting tissue, testing, and plant destruction should occur prior to plant flowering following defined protocols.
    - If fractions with AP cannot be tested and destroyed prior to flowering, then forced self-pollination in an environment that precludes crossing with other accessions should be practiced until destruction can occur.
    - Destroy fractions with AP; grow the remaining fractions to maturity and harvest.
    - If no "true-to-type" fractions are found, test individual plants within a chosen fraction with appropriate immunostrips or other method, destroy transgenic plants, and grow remaining plants to maturity and harvest.
    - Subsequent to re-establishment of an AP free source, destroy all seed lots with AP.

BREEDING PROGRAM

- Test for AP in incoming germplasm and at appropriate critical control points in the breeding program. (see information in the "Testing" section)
- If AP is detected in incoming germplasm, inform donor of presence in their germplasm and return seed to sender. Determine if alternative, AP-free sources can be provided.

- If AP is found in a seed lot at critical control points in breeding program (parents, F1, etc):
    - Do not plant the seed lot in a breeding block or increase nursery.
    - Plant a plot or multiple pots of the source with AP in the field or greenhouse.
    - Divide the plot or the pots into quarters or some other appropriate fractions, and harvest fresh tissue from each fraction, bulking tissue by fraction.
    - Use appropriate immunostrips or other method to test for previously identified AP in the fractions.
    - Harvesting tissue, testing, and plant destruction should occur prior to plant flowering, according to defined protocols.
    - If fractions with AP cannot be tested and destroyed prior to flowering, then forced self-pollination should be practiced until destruction can occur.
    - Destroy fractions with AP; grow the remaining fractions to maturity and harvest.
    - If no "true-to-type" fractions are found, test individual plants within a chosen fraction with appropriate immunostrips or other method, destroy off-type plants, and grow remaining plants to maturity and harvest.
- If breeding materials with AP have been distributed from the breeding program, inform recipients according to procedures in "Communication Strategies" section.

5) <u>Communication strategies</u> for disseminating information about Agency policies and procedures and for handling future occurrences of adventitious presence of transgenes.

- Standard information statements and/or responses to queries about non-transgenic-GE/transgenic-GE status of NPGS accessions. These statements might serve many purposes, including replying to more general queries about the nature of USDA/ARS's plant genetic resource, genomic, and genetic improvement research. Several different messages are required to cover different crop species and/or situations.
    - "Available information about trueness-to-type or quality for each accession is provided through GRIN-Global or by the crop curator. No transgenic varieties of this crop have been approved for cultivation in the United States; therefore the NPGS does not contain any transgenic accessions for this crop."
    - "Available information about trueness-to-type or quality for each accession is provided through GRIN-Global or by the crop curator. The accessions you requested were acquired by the NPGS before any transgenic varieties of this crop were approved for cultivation in the United States, and the seed supplies for these accessions have not been increased since then."
    - "Available information about trueness-to-type or quality for each accession is provided through GRIN-Global or by the crop curator. Any available information about trueness-to-type or quality for each accession is provided through GRIN-Global. The accessions you requested were acquired by the NPGS from geographical regions where transgenic varieties of this crop have not been approved for cultivation and the seed supplies for these accessions have not been increased since then."

- "Available information about trueness-to-type or quality for each accession is provided through GRIN-Global or by the crop curator. The accessions you requested were acquired by the NPGS before any transgenic varieties of this crop were approved for cultivation in the United States, and the seed supplies for these accessions were increased with standard genebank procedures (controlled pollination, individual plot harvesting) designed to minimize the chance of cross-pollination or seed admixture."
- "Available information about trueness-to-type or quality for each accession is provided through GRIN-Global or by the crop curator. The seed supplies for the accessions you requested have been increased with standard genebank procedures (controlled pollination, individual plot harvesting) designed to minimize the chance of cross-pollination or seed admixture."

- Dissemination of information about Agency policies and procedures.
  - Posted on web site: USDA/ARS and possibly USDA sites
  - Regulatory agencies (see contact list)
  - Collaborators, partners, and customers/stakeholders (see contact list)
  - Communicated on request

- Procedures for communicating information about AP <u>of unauthorized and/or regulated</u> transgenes in USDA/ARS genebank accessions or breeding stock to regulatory agencies, governmental officials, seed recipients and/or donors, collaborators, partners, and customers/stakeholders.
  - Following the initial report of the AP to USDA/ARS line management and ONP, specific USDA/ARS personnel will be designated by the Agency to manage the communication of information within the Agency and the Department, and external to the latter.
  - The telephone is usually the preferred medium for contacts.
  - The initial contacts are within USDA/ARS, and include the Administrator, Associate Administrators, relevant Area Directors, Director of Information Staff (Sandy Miller Hayes or Sean Adams).
  - Briefing papers and/or talking points will likely then be prepared at USDA/ARS HQ for USDA/ARS and USDA audiences.
  - ARS Administration and/or Information Staff will inform USDA Office of Communications, REE Office, and Office of the Secretary.
  - Key contacts for further notifications and/or for information dissemination are listed below.
  - Seed recipients and/or donors are notified as soon as is practicable, usually first by telephone, and then by e-mail message. They are provided with as much relevant information as possible, preferably in a standard format.
    - Seed donors are asked for information about how the seeds were managed prior to their receipt by the USDA/ARS genebank or breeding program.

  - Seed recipients are asked for information about how the seeds were used, and to destroy or to return remnant seeds to the USDA/ARS genebank or breeding project.

- Procedures for communicating information about AP of deregulated transgenes in USDA/ARS genebank accessions or breeding stock to donors and recipients.
  - Seed recipients and/or donors are notified as soon as is practicable, usually first by telephone, and then by e-mail message. They are provided with as much relevant information as possible, preferably in a standard format.
  - Seed donors are asked for alternative, AP-free seed sources, if extant. Seed recipients are asked to destroy, or to return, remnant seeds to the USDA/ARS genebank or breeding project that distributed them.

US Government Agency Contacts (also see attached spreadsheet)

- APHIS: Ed Jhee, Natalia Weinsetel
- EPA: John Kough, Chris Wozniak
- FDA: Jason Dietz, Kathleen Jones
- GIPSA: John Pitchford, Tandace Bell
- FAS: Fan-Li Chou, Ed Porter (if international partners are involved)
- Relevant US Embassies and Agricultural Attachés (if international partners are involved)

Seed and Biotech Industry Contacts (will vary according to crop and situation)

- ASTA: Andy LaVigne, Jane DeMarchi, Bernice Slutsky
- CropLife: Denise Dewar
- BIO: Matt O'Mara, Kate Hall

Commodity Groups Contacts (will vary according to crop and situation)

North American Export Grain Association: Gary Martin, Paul Green

- National Corn Growers Association: Sarah Gallo
- American Soybean Association: Steve Censky
- US Grain Council: Floyd Gaibler, Andrew Conner
- North American Wheat Growers Association: Burleson Smith
- National Cotton Council: Bill Norman, Keith Menchey
- Cotton Inc.: Kater Hake, Don Jones

Specific contact information for key U. S. government offices

Institution	First Name	Last Name	E-mail address	
USDA/APHIS				
	Natalia	Weinsetel	Natalia.A.Weinsetel@aphis.usda.gov	301.851.3894
	Ed	Jhee	Edward.M.Jhee@aphis.usda.gov	301.851.3948
	Compliance	Hotline	BRSCompliance@aphis.usda.gov	301.851.3935
EPA				
	John	Kough	Kough.John@epa.gov	703.308.8267
	Chris	Wozniak	Wozniak.Chris@epa.gov	703.308.4043
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