AEIC Spring Meeting 2010
Gastonia, NC
April 28-29, 2010

Secretary: P.L. Hunst (Bayer CropScience)

The AEIC Spring Meeting 2010 was held in Gastonia, NC and was hosted by USDA AMS. The national lab in Gastonia is a testing lab which provides a range of services. The chemistry section is continuing its work on the colony collapse disorder of bees and has begun testing catfish for pesticide residues. The microbiology section is working on pathogen testing in the school lunch program and other projects such as testing MREs (food rations for soldiers) for nutritional value. In a recent audit of the National Organic Program, the Office of Management and Budget has requested that more testing of organic produce be done for pesticide residues. The Gastonia lab will be involved in this. The lab is also houses the lifestock and seed program which enforces the Federal Seed Act. They provide regulatory and service testing (ISTA approved). The lab also administers the OECD seed shipping program.

AEIC Business Meeting

Secretary's Minutes of Fall Meeting 2010: A motion was made, seconded and voted favorably to accept the minutes of the Fall Meeting. The minutes are available on the AEIC website.

Treasurer's Report (D. Layton):
2009 REVIEW

	Planned	**Actual**	Comments
Beginning Balance	$16091	$16091	Balance as of 1/1/09
2009 Membership Dues	8000	8000	
TOTAL Projected Revenue	8000	8000	Actual YTD revenue
Expenditures			
Scientific Paper	4000		
DE Franchise Tax Report	25	25	
ANSI/ISO Initiative (ISO TAG)	2925	2925	
Board Meeting Expenses	100		
Spring Meeting 2009 Expenses	1000	265	
Website updates	500	323	
Bank Service Charges		20	
Wire Transfer Fees		11	
Fall Meeting 2009 Expenses	1000	(16)	
Reprints (Brochure)	300		
Subscriptions	100		
Miscellaneous	100		
TOTAL	10050	3528	
TOTAL Balance (Checking)	14041	20564	
CD Account	11179	11396	Amt rolled into new
CD Interest	235		CD on 2/10/10
TOTAL Accounts Balance	**25455**	**31959**	
2010 REVIEW			
Beginning Balance	$20564	$20564	
2010 Membership Dues	8000	3300	
TOTAL Revenue	8000	3300	
Expenditures			
New Initiative	4000		

Wire Transfer Fees			
DE Franchise Tax Report	25	25	
ANSI/ISO Initiative (ISO TAG)	2900		
Board Meeting	100		
Spring Meeting 2010	1800	179	
Travel Support (Speakers)	1200		
Website	500	128	
Bank Service Charge			
Fall Meeting 2010	1000		
Graphic Design Services			
Reprints (Brochures)	300		
Workshop			
Miscellaneous	100		
TOTAL Expenditures	11925	332	
TOTAL (Checking)	16639	23532	
CD	11396	11396	
CD Interest	150		
TOTAL Accounts Balance	**28184**	**34927**	

A motion was made, seconded and voted favorably to approve the Treasurer's report.

Membership Update (D. Layton): The current membership of AEIC is as follows:

Large Companies	13	
Small Companies	11	
Associates	2	
Individual	1	
TOTAL	27	($9450 projected revenue)

All members are paid except one.

Fall Meeting 2010 Planning (F. Spiegelhalter): GeneSeek, based in Lincoln, NE, graciously extended an invitation to hold the meeting at their facilities. Lincoln is about one hour from Omaha. Both cities have airports. USDA GIPSA also offered to host the meeting again.

Dates for the meeting are not yet set. However, it was suggested to avoid late October since the Cereal Chemistry Meetings occur then and the AOAC Meeting is the week of September 26-29. If there are other dates that should be avoided, the membership is asked to let the Board know as soon as possible.

Possible topics for the meeting were also discussed. The following topics were suggested:

*Sources of methods: where from and how to compare
*Mass spectroscopy for proteins: detection of allergens
*Interpretation of 35S results in stacked event products
*Maybe a half day session on a crops such as canola or flax or soybean or wheat: general uses, where do the products go, where testing is used, etc.
*Challenges of stacked event products for testing
*Update on rapid iso-thermal testing (EnviroLogix)

AEIC members are encouraged to e-mail the Secretary with further suggestions/speakers.

AEIC Goals/Activities (F. Speiglhalter): An idea that has been mentioned for quite a while is whether AEIC should sponsor a study or write a white paper on the comparability of protein-based vs DNA-based methods for biotech traits. There have been numerous questions globally as to how the methods compare. It would be useful to produce a paper with the correct interpretation of how the methods compare. Companies do have some data which could be contributed. The paper would be important for people to understand conceptually the differences/similarities. Generally, there is a poor understanding of detection

methods in the global community. **Ray Shillito will lead a team (Dean Layton, Tara McFadd, Clara Alarcon, Guomin Shan, Tandy Scholdberg, Pearce Smith, Beni Kaufman, Chong Singsit) to discuss and bring a proposal to the Fall Meeting.**

The second idea discussed was AEIC and a contribution to ISO work item for Sub-Sampling Principles for Qualitative Test on a Number of Seed Pools to Give Quantitative Results. The work item has come about due to the confusion when an unapproved event "pops up" and there is the need to sample for it. Beni Kaufman has worked on this and believes that an educational white paper could be done. The method solves the question of units of measurement since it give a range of results comparable to a PCR range. DNA levels in samples make less sense to those in the seed world. It was suggested that the old program in Seedcalc could be used, however, it was mentioned that most do not understand how Seedcalc does the computation so a white paper is needed. The slideset used in the ILSI training workshops could be used as a basis for the paper. It was suggested that a consultant be used to drive the paper. **The Board will take the suggestion under consideration and work on the consultant suggestion to report back at the next meeting.**

A third idea was that AEIC should publish a paper based on L. Privalle's presentation of the steps in commercializing a biotech product and how testing evolves within the steps. There are slides from other organizations that will also be helpful in framing up the paper. **Laura will lead a team (Jingwen, Frank, Cathy, Gina, Penny) to draft a paper for publication.**

Gina Clapper brought to the group's attention an article published in American Laboratory magazine which details the minimum requirements for publishing data on real-time PCR. This type of article may be of interest to AEIC to do similar articles in thefuture. The paper is available online at the magazine's site for members to look at.

Ray Shillito asked for AEIC to again participate in the ILSI Training Workshops. The next workshop will be in Paraguay in August, 2010. Speakers are needed for the workshop and AEIC members are invited to participate.

UPDATES:

USDA GIPSA Proficiency Testing Program (T. Scholdberg): The USDA GIPSA Proficiency Testing Program is for corn and soybean. The mission of GIPSA is to facilitate grain trade and sponsoring the testing program fits into the mission. The Technical Services Dept. had done proficiency testing for ten years. The program was started when StarLink issue hit and the rapid test evaluation program began in September, 2000. The proficiency program was started in 2002.

The proficiency program is meant to improve consistency/reliability of test results. There are no methods specified to be used in the program and no reference materials are specified or provided to the participants. The first sample round was sent out to participants in February, 2002. Results are always posted on the USDA GIPSA website. Participants may be identified if they choose to be. The program is voluntary and free. Currently, there are six corn samples (T25, CBH351, MON810, GA21, Bt176, NK603, TC1507, MON863, DAS-59122-7, MIR604, Event 3272) and 4 soybean samples (RR, LL).

There is global participation in the program. In 2002, there were 22 organizations that participated. As of April, 2010, there are 159 organizations participating. Of the 159 organizations, 32 are US-based and 127 are internationally-based. By continent, there are 2 organizations from Africa, 17 organizations from Asia, 2 organizations from Australia, 81 organizations from the EU, 38 organizations from North America and 19 organizations from South America. In the past sample round, 51 organizations participated and 42 of these provided results to GIPSA. The percent for supplying correct results is running high for the organizations. The results will be posted on the USDA GIPSA website.

Global GMO Conference (R. Shillito): The EU JRC (now the EURL) will be organizing the conference. The conference may be held in June, 2011 and maybe in Como, Italy but this is not yet definite. The

organizing committee wants to have a globally relevant meeting. Speakers for the opening plenary session are being discussed. The two main plenary sessions are Challenges and The Way Ahead. Under challenges, the committee is looking for suggested speakers to address 1) challenges for analytical requirements of analytical tests for impurities (LLP) and 2) challenges for analytical requirements for analytical tests for purity control. For the way ahead, speakers are need for 1) introduction of GMOs in the short, medium and long-terms globally and 2) emerging technologies for transformation of higher organisms, in particular plants, and a forecase of their market introduction.

Other plenary sessions are: 1) methods of analysis (where are methods obtained from; complexity, harmonization, validation, taxon specific reference genes), 2) analytical methods for grain/seed (quality control, approaches to perform tests, sampling, analysis in relation quality assurance/quality management, proficiency testing, assessment of fitness for purpose/assessment of methods, units of measurement), 3) expression of results (measurement of uncertainty, reference materials, resolution), 4) emerging challenges (stacking of traits, microarrays, proliferation of GMOs globally, resources for testing, botanical impurities, fast in-field testing). There will also be a one day symposium/workshop prior to the conference on detection/sampling.

Suggestions for speakers for any of the plenary sessions should be sent to Ray Shillito prior to May 15.

ISO/TC 34/WG 16 (G. Clapper): TC 34 is food products (seed, food, feed) which includes plants, animals and microbes. AOCS is the administrator for the US input into TC 34. The US TAG has had success in getting the GMO sampling document voted down due to its prohibitive sampling methods. SC 16 deals with molecular biomarker analysis and Ray Shillito is the chair of the US TAG for the subcommittee. SC 16 has been operating for almost two years and held the first plenary meeting in November, 2008. The second meeting was held in Japan in February, 2010. The group deals with detection methods, varietal identification, plant pathogen detection. There are four working groups: two are run by France, one is run by Germany and one is run by the US. New work items include the definitions document (Ron Jenkins), validation criteria for qualitative methods (Ray Shillito), semi-quantitative PCR/sub-sampling (Beni Kaufman), endogenous gene detection (Tandy Scholdberg). The ISO 2152172 document on proteins has been commented on and new parts have been added. It is now up to the US to review the comments and consider re-writing or go for another vote. There will be conference call in 2 weeks to discuss. A horizontal working group on sampling which was created by TC 34 at the Rio meeting.

It was suggested that AEIC could support the work item on the endogenous gene SS2B3 work. Tandy needs volunteers to help shape the collaborative study and put together resources, especially in statistics, as well as draft a procedure for the lab study. The document on draft guidelines on validation method of DNA sequences and proteins in food was influenced by AEIC publications.

INVITED TALKS

Excellence Through Stewardship (L. Buckelew – Bayer CropScience):

Excellence Through Stewardship (ETS) is an industry coordinated initiative to promote adoption of stewardship programs and quality management systems. It is focused on establishing/verifying systems and processes are in place. ETS build on the biotech industry commitment to product stewardship and quality management by providing guidance to meet important stewardship objectives. ETS deals with the responsible management of a product from its inception through its ultimate use and discontinuation. Quality management processes are necessary to maintain quality in each phase of the product lifecycle. ETS supports regulatory systems and recognizes that government regulations provide the safety of the product and stewardship program advance the responsible management of the product. ETS verifies that member companies have stewardship programs in place and provides a forum for member companies to share experiences/ideas to improve the processes. Member companies implement stewardship programs/processes, evaluate the effictivenes and continuously improve. ETS complements but does not replace member or industry quality practices and controls for plant product integrity. Regular members of ETS are engaged in discovering, developing and commercializing products. They have voting rights and

must complete third party audits. Associate members are from non-profit organizations, universities or government entities. They have no voting rights and participate in progressing development of products.

The componenets of ETS include stewardship objectives, principles and management practices; guides to understanding and implementing stewardship programs and quality management processes; global stewardship audits which are third party audits to verify appropriate stewardship programs and quality management systems are in place. The objectives for ETS are 1) all members fully comply with regulatory requirements; 2) seek to achieve and maintain plant product integrity through stewardship; 3) work prevent trade disruptions and 4) provide stewardship guidance and information to biotech industry stakeholders globally. The principles are to a) examine and analyze all operations, b) identify potential challenges, c) implement stewardship practices and d) audit to make sure goals are met.

ETS guides have been written on the following topics:

- Stewardship of biotech-derived products (general guide)
- Maintaining plant product integrity (detailed on how to develop and implement processes)
- Product launch (how launch a product stewardship program)
- Discontinuation (prevent new market exposure for discontinued products and utilization of inventories)
- Incidence response management (prompt management of issues)

ETS audits are conducted by third party auditors and they target stewardship and quality management systems. The audits are conducted on-site at global and/or regional headquarters, laboratories and containment facilities. The audits are conducted a the systems level. The auditors examine objective evidence for ETS components according to defined ETS criteria. The documents examined include SOPs, employee training records, policy statements, quality manuals, ISO documents, verbal evidence and GLP documents. The first audits were conducted in 2008 in the U.S. Auditing was extended globally in 2009 and will continue in 2010.

ETS' plans include continuing the audits, a new expanded website (www.excellencethroughstewardship.org), global education and outreach, expansion of membership and translations of outreach materials. ETS' value is 1) provides confidence to stakeholders, 2) provides information, and 3) employs continual improvement process.

Development of a Biotech Product—Testing from Discovery to Commercialization (L. Privalle – BASF Plant Science):

The basic process for development of a biotech products includes the following steps:

Discovery →	Stage 1 →	Stage 2→	Stage 3 →	Stage 4
6 yrs	4 yrs	3 yrs	2 yrs	3 yrs
Genes	gene Optimization	traits	introgression	

To produce a biotech event, in step one the trait of interest, gene must be identified, the plant must be transformed and the plant must be regenerated. The functionality has to then be confirmed and commercial event identified. The process does not work every time. Sometimes the idea is good but it cannot be done in the plant due to: trait being complex; gene is not expressed; harms plant (does not survive through tissue culture); impacts plant metabolism; impacts plant phenotype; impacts plant fertility; not inherited in a Mendelian fashion; and yield drag. It often takes many ideas in order to get the right one and many events have to be evaluated to identify the right one. What happens to those events that do not make it? Some never survive and some never make it out of the greenhouse evaluation. Some do get to the field phase, however, only the best performers with the correct attributes are promoted. Sometimes it is necessary to test for some of these such as Bt10, LLRICE601, triffid flax, etc.
In step two, a complete safety assessment is conducted. The assessment is multi-pronged, looking at the safety of the gene and gene product and the impact on the environment. Biotech is an extension of

traditional plant breeding. Traditional breeding brings many genes together whereas biotech brings one gene. Food/feed safety is assessed for the gene, protein, crop, food/feed and environmental safety.

For molecular assessments, the DNA inserts and their stability is determined. This includes the number of copies, integrity of gene cassettes, presence of additional DNA (backbone DNA), sequencing of genomic flanking DNA, sequencing of inserted DNA and project specific studies. The insertion site is important since it may play a role in efficacy and determines the design of the event-specific test. The insertion site is important to regulatory agencies in Europe, Japan, etc. since they are concerned about interrupted genes and locus organization (deletions, rearrangements, insertions), however, this ignores the performance and plasticity of the plant genome.

For protein assessments, the equivalency of plant-produced and microbially-produced protein is conducted and the level of protein expression in the plant is analyzed. Gram quantity protein production and purification is necessary for conducting toxicology tests. Allergenicity assessments (homology to known allergens/toxins, digestibility, etc.) are also conducted. In general, proteins are not typically a hazard. They are essential to all living organisms and there are more than 2.8 million proteins in 9000 protein families. Biotech proteins are tested at levels equal to a 220 lb man eating 70 tons of corn for lunch.

Agronomic assessments include phenotype, weediness, environmental safety/fate, interactions with target species and non-target species and interactions with abiotic environment and soil. Environmental assessment includes ecotoxicology (avian, fish, Daphnia, earthworm, ladybeetle, honeybee, green lacewing, springtails), non-target assessment, soil fate, and insect resistance management.

Composition and nutrition are also assessed by conducting muli-location, multi-year replicated trials. Nutrients and anti-nutrient levels are assessed as well as grain/forage compositon, fate of novel protein, proximates, vitamins, minerals, fatty acids and amino acids. Animal feeding studies are also conducted. Stewardship studies that are conducted include animal feeding (cattle, pig, poultry, fish, sheep) and processing studies (wet/dry milling).

Many countries are developing their own comprehensive safety assessment guidelines. The whole world is involved and paradigm shifts are occurring. For instance, the USDA is now dealing with submissions for import only for products that will be launched from other countries. Testing is complicated by safety versus politics. All biotech products that have been registered have an exemption from the requirement of tolerancey by EPA. Many countries have labeling laws and threshold guidelines (EU>0.9%; Japan>5%; Korea>3%; Australia/New Zealand>1%; unapproved events>0%). Testing is performed at exit and entry points in countries. The risk is borne by grain handlers and contracts with importers. Different countries require different diagnostic tests.

In summary, safety studies start early in the product process. Regulatory studies require at least 2-3 years to conduct and are comprehensive. Approvals take 2 years or longer and the product must be classified as safe for food/feed/environment. Biotech traits have a high acceptance among growers today.

Whole Genome Sequencing of the Soybean Genome (S. Jackson – Purdue University):

The soybean contains 20 chromosome pairs with a genome size of 1100 Mb. Approximately 40-60% of the genome is repetitive sequences and there have been two rounds of genome wide duplication. The genome was sequenced by shotgun sequencing with the Sanger method using DNA fragments. Genetic markers were used to select BAC clones. The genome does have the level of repeats seen in rice and corn. The hotspots for recombination are at the ends of the chromosomes. There was found to be secondary hybridization on the second set of chromosomes when mapping BAC clones which is possibly due to redundant DNA or polyploidy.

Euchromatin has low copy sequence and epigenetic markers. Heterchromatin has repetitive sequences and epigenetic markers. The soybean chromosome arms are euchromatic and high copy repeats in heterochromatin. This is different from corn and wheat. There have been two rounds of duplication in the genome and each sequence could have 4 sequences in the genome. A high density map is important to

anchor sequences back to chromosomes using SNP maps with markers. 950 Mb have been assemble and anchored. There are 20 chromosome psuedomolecules composed of 397 scaffolds. There are 1148 unanchored repetitive seuqnce scaffolds. There are 46,000 high confidence genes. The two rounds of recombination occurred about 13 million and 59 million years ago. Seventy-five percent (75%) of the genes are duplicated resulting in genetic redundancy. Therefore, it is difficult to knock-out genes for functional assays. The duplication 13 million years ago is believed to be an allopolyploid event. What does duplication mean? In sub-functionalization, both copies complement each other. In neo-functionalization, one gene gets a new function and non-functionalization, the gene is shut off. It has been found that 50% of the genes showed difference in transcription from the 13 million year event. DNA methylation data is easy to generate but difficult to understand. Histone profiling is currently being undertaken to try to understand.

For the genus Glycine, the annuals and perennials diverged about 5 million years ago. G. soja and G. max are mostly in Asia whereas the perennials are in Australia. The perennials are a good source of drought resistant genes and tools are being developed to find these.

In conclusion, polyploidy has been very successful in Glycine with 2-3 rounds of polyploidy. The most recent round was allopolyploidy. Seventy-five percent of the genes in the genome are multiple copy with a great amount of genetic redundancy and evolutionary potential.

Whole Genome SNP Panel Resource for Soybean (D. Hyten – USDA ARS):

The soybean genome has experienced a loss of desirable alleles resulting from domestication, introduction and breeding. Soybean has gone through several genetic bottlenecks. Seventeen (17) landraces make up modern cultivars. There is a lot of diversity in the landraces. G. soja has twice the diversity of landraces. Domestication had the greatest effect on diversity with a 50% loss of nucleotide diversity resulting in elimination of rare alleles and changed the large proportion of allele frequencies. Elite cultivars retained 72% diversity of landraces but lost 79% of rare alleles of landraces.

The soybean genome project objective is to create a next generation map of the soybean genome variation that occurs within soybean breeding to help understand yield. The HapMap Project is the next generation map that charts out genetic variation which can be used for new gene discovery through associative analysis or haplotyping. High throughput SNP discovery is a result of using next generation sequencing. In phase I, genotyping of 96 diverse landraces and 96 elite cultivars will be done. In phase 2, genotyping will be done of the USDA G. soja collection and USDA G. max collection.

SNP discovery was done by creation of reduced representation of the genome library by digestion of the genomic DNA with a combination of 5 blunt-end restriction enzymes. The combination of restriction enzymes was selected such that 5% of the genome is present in 110-140bp fraction. SNP discovery via alignment of short reads to Williams 82 whole genome sequence with 79-92% matching. There were 542 million reads that aligned to Williams 82. High SNP multiplexing technology was accomplished using the Illumina Infinium assay. Twenty-four sample chips can genotype up to 60,800 SNPs in a total of 574 samples. Automatic allele calling easy for exporting to genotype database, however, for soybean it requires more hand annotation since there is duplication in the genome. Ninety-three percent (93%) recombination occurs in 43% of the genome. The chip contains 52,000 SNPs on the 24 DNA chip. Eighty-eight percent of the euchromatin SNPs passed the design phase and 83% of the heterochromatin SNPs passed. There were 39,000 successful euchromatin SNPs and 45,000 successful heterochromatin SNPs.

In summary, 177,000 SNPs have been discovered. Forty-five thousand are polymorphic. All information will be publicly available with phase I information published by the end of 2010 and the entire germplasm by this time in 2011.

New Member Presentation: Bioagilytix (A. Safavi):

Bioagilytix joined AEIC one year ago. Bioagilytix has become a preferred provider to companies that are among the top 5 in both pharma and agricultural industry. The name Bioagilytix is a combination of

biology, agility and analytics. The company was formed to address unment needs and provides expertise in immunoassays, transgenic plant proteins, immunogenicity, biomarkers, cell-based assays and GLP/GMP services. Over the past 12 months, Bioagilytix has worked on over 50 assays, performing developments, feasibilities, optimizations and validations and has provided to clients over 150,000 determinations. This work has been done in support of clients ranging from biotech firms to Fortune 200 pharma companies to agricultural companies. Additionally, Bioagilytix is considered a "Preferred Provider" at companies that are among the top five in both the pharmaceutical and agricultural industries. The name Bioagilytix is a combination of biology, agility and analytics. The company was formed to address unment needs and provides expertise in immunoassays, transgenic plant proteins analysis, immunogenicity, biomarkers, cell-based assays under GLP/GMP regulation. Employees at BioAgilytix Labs have come from major pharma and crop science companies include Merck, Bayer, Glaxo-Smithkline, Syngenta, BMS, Abbott, Lilly and Roche. BioAgilytix works based on three main principles: integrity, sense of urgency and attention to detail. Bioagilytix capabilities include immunoassay, enzymatic/protease/peptidase assay, multiplex assay, biomarker assay, antibody/protein screening, characterization, labeling. The toolbox consists of ELISA RIA, alphascreen, AlphaLISA, MSD-ECL, luminescence, DELFIA, BRET, HTRF, QF, TruPoint, LI-COR. Customer priorities include the need for low backgrounds, wide dynamic range, extreme sensitivity, few quench issues, stable signal, multi-label assay, robust instruments, short incubation time, less matrix interference. Bioagilytix has the equipment and expertise to support the clients on various immunoassay platforms. Since HRP enzymatic activity goes down in approximately 6 months, there is always lot-to-lot variability and lot bridging is required. In addition the sensitivity of the assay is not as good as some of the other platforms in the market. MSD chemiluminescence involves the measurement of light emitted from an excited Ruthenium samples. Ten protein can be measured simultaneously from about 50 mirco-Liter of protein. Dissociation enhanced lanthanide fluorescence (DELFIA) is a plate-based ELISA with lanthanide fluorophore detection. There is a large Stokes shift which results in low background levels. The assay can be multiplexed with up to 4 labels. There are many commercially available labeled antibodies and these tend to be stable for years. DELFIA can be sued for protein, ligand-binding assays, cell function assays, kinase assays, cell proliferation and cytotox assays. LI-COR utilizes near IR fluorescence. As many as twenty molecules can be measured simultaneously per well. For Westerns, two proteins per lane can be measured with IR fluorescence and it can be a quantitative system. Bioagilytix also does immunoassay validation according to the ICH-guideline looking at assay range, intra- and inter-batch precision, specificity, selectivity, duration linearity, LLOQ and Upper LOQ, short-term and long-term stability as well as freeze/thaw stability.

Bioagilytix has a very stringent quality system in place. The facilities are secured with a controlled archive, fire-proof cabinet, archiving/storage of raw and processed lab data for up to 7 years. There is an uninterrupted power supply for lab instruments and temperature monitoring system for refrigerators/freezers. Customers view the company as a virtual lab to their existing bioanlytical laboratory. A bioanalytical project manager is assigned to every project who is a single point of contact that maintains project transparency, is responsible for project phases and keeps the client informed at every step to drive a timely project.

Conventional Processes for Determination of Essential Derivation and Varietal Identification (D. Mesa – Syngenta):

Conventional processes are traditional methods used prior to application of genetic/genomic methods. An essentially derived variety (EDV) is distinct and predominantly bred from an initial variety while retaining its essential characteristics, by backcrossing, transformation or selection of a natural or induced mutant or by other means. The varietal identification is cultivar purity characteristics.

Testing of plants and seeds is not new for new variety registration, commercial seed product or identity/purity. A database with expected characteristics is needed, the seed lot is tested and the results are compared. Conventional testing is important for intellectual property under Plant Variety Protection law and to protect plant breeders rights. It is also important for brands and marketing as well as the quality management and quality standards for saleability of seed lots as well as complying with requirements from the global seed industry.

Traditional methods include growouts and visual checks, chemical/biochemical (peroxidase, phenol, etc.) tests, protein-based tests (isoelectric focusing) and DNA tests (SNPs, SSRs, etc.). The categories do overlap and there are other ways to group (destruct/non-destruct; tissue typing; speed; test is recognized).

Growouts can be done on any crop with the growth stage being important. The disadvantages include a) limited to visual observation, b) environmentally influenced traits, c) require experienced eyes, d) some crops require precise test conditions, and e) costs vary widely. In corn, the winter growout was used widely until the late 1990s to identify off-types, check inbred purity of parent seed lots prior to being used. The advantages of the test include a) most lots pass, b) it is what the grower will see for basic purity. Growouts are a requirement for OECD seed.

Soybean hilum color is a quick check which can be done on many seeds viewed at once. It is an inexpensive, quick test, however, cannot detect colors of off-types and it requires experience and skill. Soybean hypocotyl color requires growing seedlings in a chamber or greenhouse. The predominant color is determined and off-types are reported as a percent of total. Ryegrass fluorescence is another inexpensive test that is required by the National Grass Variety Review Board. The soybean peroxidase test separates soybean varieties in high/low coat peroxidase activity. It is an inexpensive test but there are only two colors.

There are several electrophoretic tests. Starch gel electrophoresis separates multiple forms of an enzyme. It has been widely used since the 1980s and is inexpensive and well accepted. The disadvantages are a) small sample size, b) special skill and equipment needed, c) off-types may not be visible, d) limited by tissue types and presence of isozyme in tissue, e) some bands co-migrate, f) parents may have same banding as selfs, g) difficult to read if lines segregate. Iso-electrofocusing refers to performing electrophoresis of proteins in a pH gradient. Molecules with the molecular weight will separate out by pH. Results are available in 2-3 days and the gels can be stored as permanent records. It is more expensive and not as widely accepted as other methods. Polyacrylamide gel electrophoresis (PAGE) has been used for wheat and small grains. Protein profiles have been obtained of wheat gliadins. Capillary electrophoresis (CE) is performed in long capillary tubes. The method has high resolution but it is not cost effective. Single sequence repeats (SSRs) have been used as markers and are polymorphic and distributed across chromosomes. They are accepted by the International Seed Federation. Single nucleotide polymorphisms (SNPs) are widely accepted for corn and soybean. Trait markers can be added to obtain additional information. They are adaptable to a high throughput platform.

SNP Panels for Essentially Derived Variety of Essential Derivation and Varietal Identification (L. Jones – Pioneer HiBred):

The Plant Variety Protection Act first appeared in the 1960s from UPOV. The intent was for breeders to recoup investments and to encourage new investiments. It was designed specifically to protect plants. Varieties must be distinct, uniform and stable (DUS). There is a research exemption which allows breeding from a variety. The key protection is the essentially derived variety (EDV). It is up to owner of an EDV to determine if there is infringement from another party.

To apply, the variety needs to be described by phenotype and morphology characters (size, flower chraracteristics, silk color, etc.). Characters for corn are not relevant to agronomic performance, are resource demanding to collect and need to compare to all know varieties which is burdensome. In corn, markers have been used instead of characters. Under UPOV, a variety is an expression of characteristics resulting from a given genotype. One concern is that markers from differences in non-coding DNA may not establish distinctness. Also a concern as to what can be defined as "too similar".

There has been some progress in that thresholds will be crop specific. SEPROMA thresholds from the French organization has set out the following: <82% yellow zone: no dependency; >82% - 90%: orange zone: reversal of burden of proof; >90%: red zone: greater proof for dependency. ASTA has established a set of 150 SSR markers. The disadvantages of the SSR markers are that the alleles are resolved by size and they are susceptible to PCR artifacts. Most companies are moving to SNPs for corn markers.

SNPs have lower costs and errors. Also results can be compared between the same alleles with different systems. It was found in the ASTA/SEPROMA studies that only 200-300 SNPs need to be looked at to fall within the previous SSR data. The next steps will be to do the analysis to lay the groundwork with more inbreds and to use more SNPs to test SNP parameters. Another step will be to have ASTA/SEPROMA work together which will start in June, 2010. And the last step is the managing of reference collections, i.e., marker thresholds are being developed by GEVES. There is more acceptance for using marker allele differences to show distinctness in the U.S. The EU will accept markers only if proven to be completely diagnostic for the trait.

A New, Highly Stable, Non-Immuno-Based Detection Technology and Potential Agricultural Applications (B. Carlson – Receptors, LLC):

Receptors LLC is based in Minnesota. It is providing a solution to the need for selective modification of any surface to create "smart materials" via a surface modification chemistry that creates selective sensitive surfaces for application problems and products. The platform technology is combinatorial artificial receptor array (CARA) patented under USPTO No. 7,504,364 publsihed on March 17, 2009. It has also been published in J.Am. Chem. Soc. in the December, 2009 issue. CARA is the basis for diagnostics and sensors or direct capture systems and for fractionation/purification. The facility in Chaska, MN is the focused development environmenta for products and applications. The manufacturing facility is located in Somerset, WI.

The optimized production workflow system utilizes a screen with microarrays. There are 29 molecules on an array which yields 24,000 options. The data is put into a binding map to determine what is the best binding environment. The molecules can then be coupled to any surface.

For diagnostics and sensors, there is self-contained sensing system platform which is similar to a traditional ELISA. It is a competitive sensing system using the binding CARA environment, biosensing material and then detection. The company has worked on a glucose sensor based on the competitive binding for diabetics. They are also working on a biothreat sensor design for viruses such as H1N1. In this case, the microtube has CARA on beads to which the competitor agent is added. This yields a sample signature (flurorescence).

For fractionation and pufirication, there is microtube-based high performance protein fractionation. It allows one step to MALDI analysis. Multiple environments are created which bind peptides. Selective binding and simple efficient on-target fractionation produces proteome windows with minor component detection and enhanced biomarker discovery. A food protein test kit for 2 whey proteins was developed in 4 weeks. The direct capture system captures cells and allows subsequent culture for further research and diagnostic testing. For surfaces (filters,wipes), they system is modified with receptor direct capture for increased sanitation. One-step capture and concentration of target cells provides a clean sample for improved assay performance. For instance, functionalized magnetic beads were used for pulling all bacterial out of a urinary sample. This allows the accumulation of cells which are then lysed, labeled and detected. The system can competitively bind live *E. coli* in the presence of free *E. coli* DNA. In mammalian tissue culture, functionalized surfaces are used for improved mammalian cell products.

Single Lab Validation of Tomato Varietal Identification (D. Sristava – USDA AMS):

Tomato has the smallest diploid genome—12 pairs of chromosomes and is 950 million bp. Seventy-five percent of the genome is densely packed without genes. Ugly ripe tomatoes are grown in the U.S. and Mexico. Ugly ripe tomatoes do not fit the characters for grade 1 tomatoes (smooth skin, round). Therefore, the variety is allowed to be identified based on gene fingerprint. This necessitated a need for a lab validation for high throughput genome analysis for variety identification. The GeneMapper was used to design the multiplex panel.

SSRs are PCR-based markers with 18-25bp primers. SSR polymorphisms based on the number of repeat units/hyper variable. They are stable to amplify and are variable to increased rate of mutation compared to

other neutral regions of DNA. Genotyping was performed on a set of 12 SSRs which provided a genome-wide coverage of chromosomes. These were fluorescently labeled and grouped into multiplex panels. Each pair was assessed in replicated trials for reliability of allele size estimates. SSRs are preferred due to rapid processing, abundant, highly variable, small size, discrete alleles, allelic ladders simplify interpretation and PCR uses small amounts of DNA. Number of repeats may vary between species, varieties and individuals. The sequences flanking the repeats are usually highly conserved. SSRs are randomly distributed and segregate with the chromosomes. The 12 SSR loci can differentiate the 35 tomato varieties.

Customers may send tomato slices from individual fruits. These are frozen in liquid nitrogen and ground. The DNA is extracted using the Qiagen plant DNease or with CTAB/chloroform. PCR is conducted using AmpliTag and the products are run using capillary electrophoresis. The number of peaks is dependent on the size of the repeat. The data is then fed through GeneMapper. A minimum of 15 markers is needed and a one marker difference is not good enough to distinguish varieties.

The AEIC concluded the meeting by traveling to Kannapolis, NC to visit the David H. Murock Research Insitute. The Institute is a non-profit which provide laboratory services to faculty at the North Carolina Research Campus (NCRC) as well as researchers from academia, government and business sectors. The Insitute has adopted a multidisciplinary, integrated approach which includes genomics, proteomics, metabolomics, light microscopy, histochemistry, transgenics, NMR, etc to meet the needs of their partners.