BASF Welcome (L. Privalle):

The host company, BASF, welcomed the membership to Research Triangle Park (RTP), NC. RTP is celebrating its 50th anniversary this year and is home to 170 companies. BASF, Bayer CropScience and Syngenta (half of the 6 major biotech companies) are all located in RTP. RTP is the 5th “most internet wired” city and resides in Durham county. The triangle name derives from the fact that it is located between North Carolina State University (Raleigh), University of North Carolina (Chapel Hill) and Duke University (Durham). RTP now consists of 7000 acres with 42,000 full-time workers and 10,000 contract workers. It is a city requirement that no building may be taller than the surrounding trees within RTP which is why most of the buildings are not visible from the streets.

The North Carolina Biotechnology Center was established in 1984. It is funded by the state legislature, however, it is not a government agency. There are five regional offices across the state.

Discoveries that have been made in the RTP area include:
- The UPC symbol
- 3-D ultrasound technology
- Astroturf
- Taxol (cancer drug)
- AZT (AIDS drug)

AEIC Business Meeting (October 14)

Secretary’s Minutes – Spring Meeting 2009 (P. Hunst):

The Secretary’s minutes, posted on the AEIC website, were accepted by the membership via a motion and vote.
**Treasurer’s Report (D. Layton):**

<table>
<thead>
<tr>
<th></th>
<th>Projected ($)</th>
<th>Actual ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning Balance</td>
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<tr>
<td>2008 Membership Dues</td>
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<tr>
<td>TOTAL</td>
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<td>7500</td>
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<td>Expenses</td>
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<tr>
<td>Scientific Paper</td>
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<td>Wire Transfer Fee</td>
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<tr>
<td>DE Franchise Tax</td>
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<td></td>
</tr>
<tr>
<td>ANSI/ISO</td>
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<td>2900</td>
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<td>AEIC Board Meeting</td>
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<td></td>
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<tr>
<td>Spring Meeting</td>
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<tr>
<td>Graphic Design</td>
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<tr>
<td>Pamphlet Reprints</td>
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<tr>
<td>Subscriptions</td>
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<tr>
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<td></td>
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<tr>
<td>CD Interest</td>
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<tr>
<td>GRAND TOTAL Balance</td>
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<td>31461</td>
</tr>
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</table>

The membership approved the Treasurer’s report via a motion and vote.

**Membership Update (D. Layton):**

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Dues Revenue</th>
<th>Unpaid</th>
<th>Unpaid Dues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large Companies</td>
<td>14</td>
<td>$7000</td>
<td>2</td>
<td>Agilent, Cargill</td>
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<td>Small Companies</td>
<td>10</td>
<td>$2500</td>
<td>1</td>
<td>Biogenetic Services</td>
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<td>Associate Members</td>
<td>2</td>
<td>$100</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Individual Members</td>
<td>1</td>
<td>$100</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

The membership discussed fees for small subsidiaries which participate in AEIC (and pay small company fees) but are part of large corporation. For example, Eurofins pays dues as a large company which covers the participation of Eurofins GeneScan and Eurofins STA. After much discussion a motion was made as follows:

    If one site from a large company has less than 100 employees, they may join AEIC as a small company or may choose to join as a large company (under parent corporation) which would allow all their smaller sites to participate.

The motion was seconded and approved by member vote.
It was suggested that possible new members for AEIC might include data handling companies and/or computer systems companies. G. Clapper also indicated that Bunge is interested in AEIC membership.

It was also suggested that the AEIC Board should develop talking points on “what AEIC can do for you” which could be posted on the website and used at other meetings to attract new members.

**AEIC Website Update (P. Hunst/D. Layton):**

The webmaster has raised her hourly rate. The Secretary’s minutes and other documents are now up-to-date on the website.

The webmaster had suggested that AEIC might want to set up a Facebook account to gain more exposure, i.e., contact more potential members. The membership discussed that LinkedIn may be a more appropriate site for AEIC since it is more business-oriented.

It was also suggested that more key words are needed to bring up AEIC website when it is Googled. Another suggestion was to post “updates” to encourage members to visit the site more often.

**Spring Meeting 2010 (F. Spiegelhalter):**

Suggestions for the meeting location were:
- San Diego (hosted by Illumina)
- Texas (hosted by RiceTec)
- Memphis (hosted by Horizon Ag)
- Gastonia, NC (hosted by USDA AMS)
- Indianapolis (hosted by Dow AgroSciences)
- Des Moines (hosted by Pioneer/DuPont; more a possibility for Fall Meeting 2010)

The Board will follow up on the suggestions.

Suggested topics included:
- DNA tests for varietal identification/genetic purity
- One platform for DNA, RNA, protein detection
- Method uncertainty/data
- Measurement of uncertainty
- Margellon’s disease (Agrobacterium)
- Information/data handling
- Certification of LIMS systems
- New Agricultural Research Institute (R. Beachy, head)
- Talk by Chromatin, Inc.
- Variability in expression of proteins
- Plant/animal breeding (maybe topic for Fall Meeting; R. Mum)
- Testing used from gene discovery to commercialization for a biotech product
- Other protein equipment manufacturers (Tecan, MDC, ABI)

**Goals/Activities for AEIC (F. Spiegelhalter):**

Suggestions by membership:
Comparability of protein-based and DNA-based methods for biotech
Training opportunities
   Be involved again in the ILSI workshops
   The upcoming workshops are: Feb. 10 in Japan; Feb. 14 in India; June in Paraguay; August in South Africa.
Travel awards to allow student and/or academics to attend AEIC meetings and also give Presentations
Pay travel to bring in speakers from outside the US for AEIC meetings
Review of available RT-PCR and PCR machines
   Suggestion: a literature review/market overview
Interpretation of PCR results

UPDATES:

USDA GIPSA Proficiency Program (T. Scholberg):

The GIPSA Proficiency Program is a no-fee, voluntary program. Samples are disseminated to participants twice per year. Six (6) corn samples and 3 soybean samples have been sent in the past, however, this year 4 soybean samples will be sent (due to LibertyLink soybean being commercialized). There is also a rice proficiency program with 6 labs (approved by Bayer CropScience) participating.

As of April, 2009, 155 organizations are participants—32 are US-based and 123 are international. The majority of the participants use DNA-based tests. Half of the participants test for all events distributed. The performance of the participants is generally good.

The DNA-based results from the April, 2009 analyses indicate that T25 had a 25% false positive rate whereas the other events were around 10%. Event CBH351 (Cry9C) will be discontinued as a sample in the next round of samples. For the ELISA testing, results were recorded at or below the LOD. The results of the testing may be found at:


R. Jenkins (USDA GIPSA) and T. Scholberg (USDA GIPSA) are co-chairs of the AACC Biotech Division.

Global Conference on GMO Analysis (R. Shillito):

The first global conference was held in 2008 in Como, Italy and was sponsored by the EU. The next global conference is currently being scheduled for June, 2011 with the EU organizing/paying for it. The plenary speaker topics include:
   Detection for environment, biodiversity
   Keeping GMOs away from the food supply
   Gene stacking—complexity/overview of GMOs, multi-target testing

R. Shillito (Bayer CropScience) is representing ILSI on the conference planning meeting. If any of the AEIC members have suggestions for conference topics, please e-mail Ray. The next planning meeting is scheduled for February, 2010 so suggestions would need to be to Ray by then. It was suggested that AEIC should try to have a speaker to possibly give a summary of the PCR validation paper or another topic.
ISO/TC 34/SC 16 (G. Clapper):

The SC 16 is the biomarker sub-committee. Invoices have been sent by AOCS for participation. The participation fees help pay the fees required by ANSI and ISO. Fees are charged under the SC 16 because the US is the Secretariat. No additional fees for participation were charged under TC 34. AOCS solicited funds last year to cover the participation fees, however, no enough funds were pledged, therefore, the invoices had to be sent this year. Without the collection of the fees, AOCS may not be able to continue to administrate.

Nominations (F. Spiegelhalter):

Nominations were opened for the AEIC Board position of Vice President. Denise Theide (BioDiagnostics), Laura Privalle (BASF) and Clara Alarcon (Pioneer/DuPont) were nominated from the floor. All accepted the nomination. Additional nominations will be accepted via e-mail to the AEIC Secretary (P. Hunst) through October 31. Voting via e-mail will commence in November.

Invited Presentations:

Applications of NEAR isothermal DNA amplification (T. Spenlinhauer – EnviroLogix):

NEAR isothermal DNA amplification was developed by Ionian Technologies. EnviroLogix has an exclusive license for the technology.

PCR is the “gold standard” in molecular diagnostics, however, PCR is not practical for field-use. PCR relies on a laboratory equipped with thermocyclers and skilled technicians. The NEAR isothermal technology utilizes isothermal amplification. The technology relies on the ability of DNA polymerases to extend short oligonucleotides along a template at a constant reaction temperature above the Tm of the duplex. The polymerase facilitates and stabilizes the reaction. There are reiterative cycles of extension and DNA nicking. Detection of DNA and RNA is accomplished under 10 minutes. The sensitivity and specificity is equivalent or better than PCR. The reaction is tolerant to sample matrices.

The reaction process is as follows: 1) nicking enzyme nicks the genomic DNA, 2) polymerase attaches to the open 3’ end, 3) the strand is extended and displaced, 4) reverse template anneals and the polymerase extends the strand and then process starts again. There is exponential amplification and the formation of 2 complimentary products 21-28 bases is the result.

The assay can detect any reasonable DNA or RNA sequences and is a true isothermal reaction. The reaction is resistant to exogenous DNA. Reagents for the reaction are stabilized via lyophilization.

To utilize the technology, the following steps are used:

1) Select the target region unique to the organism
2) Design the template sets to amplify the target region
3) Preliminary screening of the templates to define the ability to amplify
4) Template skewing and LOD studies for top template sets
5) Design and test molecular beacons for top template sets
6) Final optimization of tope template sets for molecular beacon detection
7) Molecular beacon-based studies to define assay sensitivity/specificity
8) Development of capture and reporter probes for lateral flow detection
9) Lateral flow-based studies to determine the sensitivity/specificity
10) Sample analysis using molecular beacons and/or LFD
11) Template screening: real-time analysis of the reaction using SYBR green II for continuous monitoring of the reaction to determine the speed of the reaction
12) A shift in the melt curve indicates the amplification of a background product in negative control reactions and the specific product in reactions containing target DNA

The molecular beacon is read on a portable reader via fluorescence detection. This is comparable to PCR detection. It is basically a single heat block and a fluorescence reader. The molecular beacons is dried on the cap of the sample tube which allows for a closed container reaction. The lateral flow device (LFD) is a visual read. The reaction can be run on any heating unit that can maintain 56°C. The LFD is supplied in a hand-held cartridge to allow the release of the amplified product in the closed system. The layout of the LFD is similar to a typical immunoassay LFD but there are no antibodies. The NEAR product is single-stranded which allows for hybridization or sandwiching. The product is sandwiched by single-strand capture, control and conjugate reporters. Reporters are complementary for the NEAR product.

EnviroLogix will initially market the product as an endpoint only assay. Efforts are underway to make the assays real-time. Assay development takes about 6 months. The current scale of the assay is 8 samples. The reaction could be 96, however, the reaction occurs very rapidly.

**Luminex xMAP Technology: Power of multiplexing (M. Hoffmeyer – Luminex):**

Up until 2005, Luminex was primarily an instrument manufacturing company. In 2005, the Bioscience Group was added. Luminex’s Life Science Research works on inflammation, endocrine and cancer. The *In Vitro* Diagnostics works on tissue typing. Molecular Diagnostics works on genetic diseases, infectious diseases and cancer. Bioscience is engaged in newborn screening, immune status and custom assays. Ag Science is primarily engaged in veterinary diagnostics but is expanding into ag biotech.

xMAP is a multi-analyte profiling system based on liquid bead array technology. The beads are 5.6 microns with the assay chemistry on the bead surface. The beads or microspheres are dyed to create about 500 colors. Each has a spectral address in the red/infrared. The beads are suspendable in solution and can be coupled with capture reagent (either oligonucleotides or antibodies). When a sample is added, the analyte is captured onto the beads. The fluorescent reporter is added and the sample is placed into a Luminex analyzer. The analyzer samples each well. Lasers excite the fluorescent labels (red and green). Multiple readings are made for each set. The software reports the results in real-time. Up to 9600 results can be read in one hour.

The benefits include economy, speed, reproducibility, stability, flexible assay formats, and the ability to expand the standard curve.

xTAG is a group of sequence matched for length which do no cross-hybridize. A 5’ tag is incorporated and the 3’ terminates at the SNP. The polymerase extends past the SNP if there is a match at the 3’ end. There is no increase in background when adding strepavidin or erythrin. The Luminex 200 is the workhorse instrument. The Flexmap 3D is a 500 plex, handling 96/384 plates with a 15 minute read time. There is 2-fold improvement in sensitivity and 4.5-5.5 log extended dynamic range. The xPONENT software is used to record results.

More information on Luminex and applications may be obtained at the following links:

[www.luminexpub.com](http://www.luminexpub.com)
[www.luminexcorp.com/support/faqs.html](http://www.luminexcorp.com/support/faqs.html)
Immunoassay applications in expression studies of biotech crops (T. Robinson – Bayer CropScience):

PCR methods are developed by industry during product development. Immunoassays are developed by kit suppliers. Methods are improved as the product comes to market. Different assays are developed depending on the need during development.

ELISA validation process involves 1) sample preparation, 2) extraction (usually 5 extractions-assayed twice to provide 10 data points), 3) analysis, 4) validity criteria (recovery ≥60% and ≤130%), 5) determination of LOD and LOQ. Validation process if performed for all matrices for each type of ELISA. Data analysis is performed via the software supplied with the plate reader. OD values are adjusted for buffer blanks and then converted to protein concentration via the use of the standard curve (run on each plate).

Commercial ELISAs are mostly sold as qualitative assays, however, customers use them as quantitative tests. For best results, it is advisable to try to use one batch of plates per project. Use of commercial ELISAs reduces the labor for production of in-house ELISAs.

New Member Presentation: Illumina (M. Thompson):

Illumina is a leading provider of integrated solutions that advance the understanding of genetics and health. Illumina is advancing the use of genetic variation-based approaches to crops and livestock. Illumina is primarily a provider of instruments and assay systems.

Illumina was founded in 1998 and an IPO offering was made in 2000. The first product was launched in 2002. In 2006, Solexa (sequencing) was acquired. Illumina is now growing their interest in ag products. Illumina is a global organization with its headquarters in San Diego. It employs 1600 people. It is a leader in arrays and genetic sequencing. Illumina was named Forbes Fastest Growing Technology Company in 2009.

The old paradigm for breeding was to find the right animal or plant. The new paradigm is to find the right genotype(s). To meet this new paradigm, Illumina has 3 platforms: sequencing, genotyping and beadXpress. The genome analyzer is the core engine producing 20-25 Gb of high quality data/run.

The sequencing process begins with 1) fragment DNA, 2) repair the ends/add A overhang, 3) ligate adapters, 4) select ligated DNA, 5) hybridize to the flow cell, 6) extend hybridized oligonucleotides, 7) perform bridge amplification, 8) protein sequence on forward strand, 9) regenerate reverse strand and 10) perform sequencing on reverse strand. Applications include sequencing, transcriptome profiling, isolation of mRNA or micro-RNAs, DNA methylation, Tag profiling, histone modification, nucleosome mapping.

Iscan is the bead array technology. Each feature or bead can be independently manufactured and QC’d. Each bead is evenly covered with 800,000 copies. The two assays are Infinium I and Infinium II. Infinium I has oligonucleotides with address for SNP allele. There are two 50-mer probes per assay. The assay is performed on the bead surface and one color readout is produced. In the Infinium II assay, the address stops one nucleotide prior to the SNP allele. The position of every bead type on every chip is verified (800,000 copies of specific oligonucleotides/bead). There are commercial whole genome beadchips available (horse, cattle, sheep, dog, pig, chicken, maize and soy).
The Goldengate assay has 1536 markers in the beadarray or veracode platform. The extension is done in solution and requires low DNA input. The target can be any variant or any species. The Veracode bead is a glass cylinder embedded with a holographic diffractive code. It is functionalized with oligonucleotides or carboxyl groups and is read using the beadXpress reader.

**Marker-assisted breeding (A. Shir – Eurofins STA):**

STA was established in 1987 with the founding technologies as starch gel electrophoresis and plant and seed health/pathology services. STA was acquired by Eurofins in 2008. Today, STA is a full service diagnostic lab for agriculture focusing on inputs (genetics, seeds, plant materials). STA’s mission is to help clients succeed by providing expertise, knowledge, etc. STA has facilities in Colorado and California and three divisions (seed, plant health, genetics).

The purpose of molecular breeding is incorporate/introgress value-added traits into plants via the most effective techniques to utilize all the information and germplasm. The desire is to minimize development time/cost. Areas of application include databases (genotyping/fingerprinting, marker-assisted backcross/selection, trait linked markers, quantitative trait loci (QTL) studies, germplasm organization and protection of IP. Database applications include inbred line development, test cross determination/pedigrees and association/QTL studies. Mapped markers are used in the selection process. The same set of markers are used on all entries. The number of markers used depends on the species, genome size, objectives and cost. Usually 60-100 markers, well distributed along the genome, are used. Markers can be used for germplasm organization to identify heterotic groups, protection of intellectual property and utility in patents/plant variety protection. Markers are useful to assist in the recovery of favorable genotypes during the backcross process. They are also used to identify unknown seed lots.

**Use of P35s and Tnos expression elements in measurement of genetically engineered plant materials (R. Jenkins – USDA GIPSA):**

The project is a collaboration with NIST (Marcia Holden) to have a validated method for the detection of 35s promoter. The results have been published.

The purpose of a method validation is to verify that the analytical method is acceptable for the intended purpose. The validation process looks at parameters such as specificity, linearity, accuracy, precision, range, detection limit, quantitation limit and robustness of the assay.

For validation of quantitative PCR, it is important to determine bias and precision of the method. Material performance assigns a value and uncertainty (or reliability) to a characteristic (usually concentration) of a material. Lab performance permits the evaluation of each participant against preset criteria.

35s is a common promoter in most commercial transgenic crops. Stacked traits contain more than one element. Current quantitative PCR methods lead to an overestimate of GM content (worst case scenario) since for 35s and T-nos there are usually multiple copies in transgenic crops due to multiple events being stacked. Event specific methods cannot distinguish between single events and stacked events.

The objective of the study was to look at the suitability of different 35s screening methods and their applicability for qualitative and quantitative measurements. ILSI conducted a survey on the use of 35s and T-nos in testing labs and whether the use is qualitative or quantitative. Forty-four
(44) labs completed the survey. Forty (40) of the labs use both qualitative and quantitative 35s
tests. Thirty-seven (37) labs use qualitative and quantitative tests for T-nos. The types of
reference material used were powder (predominantly), genomic DNA, plasmid DNA and seeds.
Five public methods on IRMM reference materials were looked at. Method 2 and 5 each
contained a SNP in TC1507 IRMM in the region of the 35s. Method 1 was the FASMAC
method. This SNP caused CT shifts which were initially difficult to explain until the SNP was
identified.

The conclusions of the study were: 1) there is a large variety of methods, 2) 53% of labs were
using their own methods, 3) there is a poor correlation coefficient with larger CT values with the
TC1507 reference material when using methods 2 and 5, and 4) use of one standardized method
would facilitate international trade/harmonization.

New member update: Agri-Diagnostics Manufacturing (S. Miller):
Agri-Diagnostics is a contract manufacturer for immunoassays both in the medical and
agricultural fields. Strict confidentiality is maintained with customers and there is no disclosure
of who the customers are.

For coated ELISA plates, customers supply antibodies. Either the customer’s protocol for coat
plating is used or a standard protocol from Agri-Diagnostics is used. There is in-process quality
control and packaged plates have a moisture indicator to ensure stability. Stabilized ELISA
plates save time and provide consistency in assays. There is a protein blocker (proprietary) with
the stabilizer.

Agri-Diagnostics has a small group of employees with over 20 years of experience. Most orders
are turned around in 2-3 days. Lab space was expanded several years ago so the capacity is 3800
coated plates/week. Agri-Diagnostics also provides technical support and offers special
incentives such as the first 50 plates are no charge in order to try the technology/service. For
more information, contact Suzanne Miller at Agridia2@yahoo.com.

Biotech detection method requirements globally (J. Chen – Syngenta):

Seed, grain and food are traded and move globally. This has fueled the testing of products for
nutritional labeling, seed requirements and GM content. Majority of countries belonging to
OECD have some type of policy for labeling/thresholds for GM products. Only a few non-OECD
countries have similar labeling laws. The scope of the labeling requirements/threshold levels for
GM products differ widely. For example, EU has a threshold of 0.9% GM whereas Japan is 5%
GM content allowed. US does not have any threshold. The majority of the countries have
threshold levels between 1-5%.

The objective of labeling of GM products is to provide consumer information and choice—it is
not about food safety. It is question of preference of consumers for GM vs. non-GM and organic
vs. conventional. Testing is conducted by government agencies and enforcement authorities since
those who are signatories of the Biosafety Protocol must comply with national regulations. Grain
handlers and food/feed companies test to comply with food/feed labeling regulations and policies
on low-level presence (LLP) of unapproved commercial products. Biotech companies use testing
in R&D programs, seed purity and in stewardship programs throughout the life cycle of the
product.

Preferred Detection Methods Along the Value Chain
Biotech companies have developed DNA-based methods (RT-PCR, gel-based PCR), protein-based methods (ELISA, LFD) and herbicide bioassays (glufosinate, glyphosate). All methods are developed for a purpose. Protein methods tend to be cheaper and easier to develop, however, DNA-based methods more appropriate for processed foods. Both sampling and testing have variability and enforcement limits/policies need to reflect test variability/precision. Testing is costly especially at low levels.

In the US, only EPA requires a detection method for plant-incorporated protectants (PIPs). The methods are generally protein-based. EPA does not have jurisdiction over herbicide tolerant or output traits. In the EU, under 1829/2003, registrants must supply a method for detection and information as to the place where reference material is available. Methods are validated by the CRL. Quantitative event-specific PCR and DNA extraction methods are submitted at the time of the dossier submission. The method protocol and analytical data for pre-validation are supplied. Eleven (11) performance characteristics are assessed (applicability, practicability, specificity, dynamic range, accuracy, RSD (precision), LOD, LOQ, robustness and efficiency. For example, the dynamic range needs to be 0.09 – 4.5%, the LOQ must be less than 0.09%. JRC sends the methods out for ring trials. In Korea and China, the PCR product needs to be less than 150 bp, the PCR primer locations on the transgene insert and flanking sequences must be identified and the LOD should be greater than 0.05%.

Reference materials must be highest quality and are provided by the seed registrants to the government agencies. Certified reference materials (CRMs) are made available on a single event basis. This done through a third party source such as AOCS or IRMM. Generally, CRMs are available as seed flour or genomic DNA. In the EU, control samples must also be provided.

**Discovery of novel pesticidal proteins in Bt using de novo sequencing** (D. Tomso – Athenix):

Athenix was established in 2001 in RTP, NC. A strain collection has been isolated which now numbers 39,000 strains. Athenix has focused on trait discovery for transgenic crops to license broadly. Therefore, Athenix has a strong patent portfolio and partners breeding activities with other companies.

Athenix’s proprietary technologies include:

- Microbial strain library
- MiDAS gene discovery platform
- Proteome interaction mapping
- Soybean cyst nematode *in vitro* bioassay.

The key gatekeeper products for the company are glyphosate tolerance and pest resistance traits.

The *Bt* Cry proteins have been used in biocontrol applications for decades and in GM plants for insect control. Cry proteins have a diverse primary structure. Some are produced as protoxins which are activated following cleavage in the alkaline insect gut. Other *Bt* toxin families include...
VIP (vegetative insecticidal proteins), MTX (mosquitocidal proteins) and BIN (binary toxins). The bacterial strains in the collection are screened in insect bioassays to determine insecticidal activity. This is a laborious process. The MiDAS system is used to reduce the process by using sequencing. Sequencing analysis identifies cryptic genes and allows recovery of the activity. This is accomplished by selecting the strain, sequencing the acquisition, analysis of the sequence, cloning/expression of the gene. The SQL database stores all sequences. The MiDAS system has been used since 2001. The SQL database contains over 6 million sequences. Multiple cloning strategies are used and the targets are testing against an activity screen containing 20 insects/nematodes. Candidate genes are then moved into crop plants for field evaluation.

In summary, Athenix has a sequence-based discovery program with 221 genes identified. The genes are evaluated against commercially important pests. There is a substantial dataset to evaluate against.