AEIC SPRING MEETING 2013 MINUTES
April 17-18, 2013
Madison, WI

P.L. Hunst, AEIC Secretary

The AEIC Spring Meeting was graciously hosted by Covance at their facility in Madison, Wisconsin. There were approximately 75 attendees representing 32 companies and organizations.

AEIC Business Meeting

Fall 2012 Secretary's Minutes: A motion was made, seconded and voted positive to approve the Secretary's minutes of the Fall 2012 meeting which are posted on the AEIC website.

Treasurer Report (D. Layton):

	Planned	**Actual**
As of Jan1, 2012	$35956	$35956
Dues	7200	11550
Interest from Checking/savings/CD	25	150
TOTAL	7225	11700
Expenditures		
Scientific paper	7000	3000
Wire transfer fee		
DE Franchise Tax Rpt	25	25
ANSI/ISO Initiative	2900	2900
Board Meeting	125	136
Spr Mtg Expenses	1500	4090
Website	350	271
Bk Service Chg		10
Fall Mtg Expenses	2500	4785
Graphic design		
Reprints (brochure)	800	
Subscriptions	100	
Misc	100	
TOTAL	15400	15216
Balance	27781	32439

2013 Budget		
As of Jan 1, 2013	$32439	$32439
Dues	7500	2150
Interest on Checking/Savings/CD	125	
TOTAL	7625	2150
Expenditures		
Scientific paper	5000	
Wire transfer fee		
DE Franchise Tax Rpt	25	25
ANSI/ISO	2900	
Spr Mtg	4500	2897
Website	350	240
Bk Service		
Fall	4500	
Reprints	800	
Subs	100	
Misc	100	
TOTAL	18275	3162 YTD
Balance	21789	31427

A motion was made, seconded and voted positive to approve both budgets.

AEIC Member Update (D. Layton):

Members	**Potential Dues**	**Unpaid Dues**
Large (14)	$7000	$3500
Small (13)	3250	2000
Associates (2)	100	100
Individuals (2)	200	50
TOTAL	**$10550**	**$5650**

AEIC Brochure (D. Layton): The supply of brochures is low and a reprinting will need to be done in the near future. A suggestion was made to add the new AEIC paper that was published in 2012. Dean asked for any other suggestions be sent to him soon so that they can be incorporated for the next printing.

AEIC Website (P. Hunst): Members were encouraged to send any suggestions for updates/changes to Penny who will communicate them to the webmaster. It was indicated that the 2012 AEIC paper is not on the website and should be added.

AEIC Fall Meeting 2013 (D. Theide): The Fall Meeting 2013 will be held in the Woodland/Davis area (near Sacramento) of California. It was suggested to not hold it in early October (Oct 9-10 or Oct 16-17) due to a conflict with the Cereal Chem meetings at the end of Sept – early Oct. Monsanto will be hosting the meeting and the focus will be on vegetables. Some topics which were suggested included:

- Disease resistance
- Methods for seed quality/breeding
- Complex genome structure and how markers can work
- Changes in the regulatory frontier for vegetables
- Plant pathogen detection

AEIC Papers (Y. Dudin): The paper on comparison of quantitative vs semi-quantitative PCR is still in progress. B. Kaufman will organize a co-author meeting with the intent to complete the paper by July.

There is a possibility of two papers coming out of the lectin special session (held after this meeting). These would be a methods paper and a paper on collaboration across labs to determine variability of the methods.

AEIC Spring Meeting 2014 (Y. Dudin): Eurofins GeneScan offered to host the meeting in New Orleans. Several suggestions were made for possible topics:

- Quality control in assays: what types of criteria are assessed
- New collaborations for AEIC
- New breeding technologies and detection challenges

USDA GIPSA Certification Program (C. Alarcon):

Updates from USDA were provided to Clara for presentation:

Biotech Proficiency Program: The program will continue. Shipping costs for samples may be charged going forward.

Biotech Rapid Test Kit Program: This program will also continue.

Mycotoxin Rapid Test Kit Program: The program may continue though funding and personnel are continuing challenges. There is always the question as to whether GIPSA will be able to continue to do this work. Currently, funds are appropriated through September, 2013 for the work.

ISO TAG (G. Clapper): The US is the secretary of the Subcommittee on Marker Technology. The 4th Plenary Meeting was held in April with 11 countries attending. The 2nd edition of the protein methods document was published. There is also an overall terms/definitions document being worked on of which the USDA GIPSA is the project leader. There are two new work items. For India, varietal identification for basmati rice. AOCS has a contact for basmati rice certified reference material: Dr. J. Gowrishankar (Shankar), Dir., Staff Scientist, Head Laboratory of Bacterial Genetics, Centre for DNA

Fingerprinting and Diagnostics (autonomous institute of the Dept. of Biotechnology, Ministry of Science & Technology, Government of India); e-mail: director@cdfd.org.in or lbg@cdfd.org.in . There is also a plant pathogen document. The EPPO and IPPC are now engaged on this. The French had dropped out as the lead on this but now would again like to resume that role, however, the project was has been cancelled.

The Business Meeting was then adjourned by the AEIC President.

INVITED TALKS

AEIC: We've Come a Long Way (D. Grothaus, Strategic Regulatory Solutions): AEIC was founded in 1992 based on a recommendation by EPA Las Vegas. The objective was to gain acceptance of immunoassays for chemical detection. The first methods were simple, classic ELISAs utilizing microplate readers. The work then progressed to more rapid methods such as dot blots. Originally, there were representatives at AEIC from about 25 organizations which included research consultants, equipment manufacturers, testing labs, food/mycotoxin companies, agricultural biotech companies, etc. The founding principles are not too different than they are today. The AEIC was renamed in 2005 to Analytical Excellence through Industry Collaboration. The organization has evolved with the technology and the application of the technology. Over the years, AEIC has published key papers on ELISA validation, PCR validation, testing in biotech product development, etc. AEIC is unique in that the organization is based on science and is not political or driven by industry. AEIC has high credibility with organically driven intiatives. AEIC provides a strong networking opportunity for its members. AEIC is always looking to change and as the organization gains new members, the diversity and focus of the group changes. The emphasis is now on rapid, on-site multi-analyte, quantitative detection methods for nucleic acids and proteins.

Power of Microfluidics: Enabling Genetics and Genomics Research (J. McKinney, Fluidigm): Fluidigm technology is focused on the use of nanoliter or picoliter reactions. The BioMark HD system uses chips which allow quantitative PCR, digital PCR, etc. The chips have integrated fluid circuits. The sample and assays are connected by fluid lines. The nanoflex valve contols the mixing. The valve functions much like stepping on a garden hose to restrict water flow. The valve allows opening and closing of the fluid lines multiple times. Dynamic arrays are available in 48/48, 96/96, 912/24. The advantages of the system are: a) open platform (blank chips); b) flexibility from chip-to-chip for sample and assay; c) reagent mixing on the chip is precisely controlled; d) nanoliter reaction volumes; and e) automation capability. In a traditional set-up of 384 samples x 96 genes, this would require 184ml master mix, 18ml primer probe and 96 384-well plates. With the chip, this could be accomplished by 960ul master mix and 1.5 days of work. The standard workflow is 1) pipette (30 min), 2) load (50 min), 3) PCR reaction (30 min to 1.5 hour) and 4) analysis (10-30 min). The PCR chemistry that has been used is TaqMan and DELTAgene.

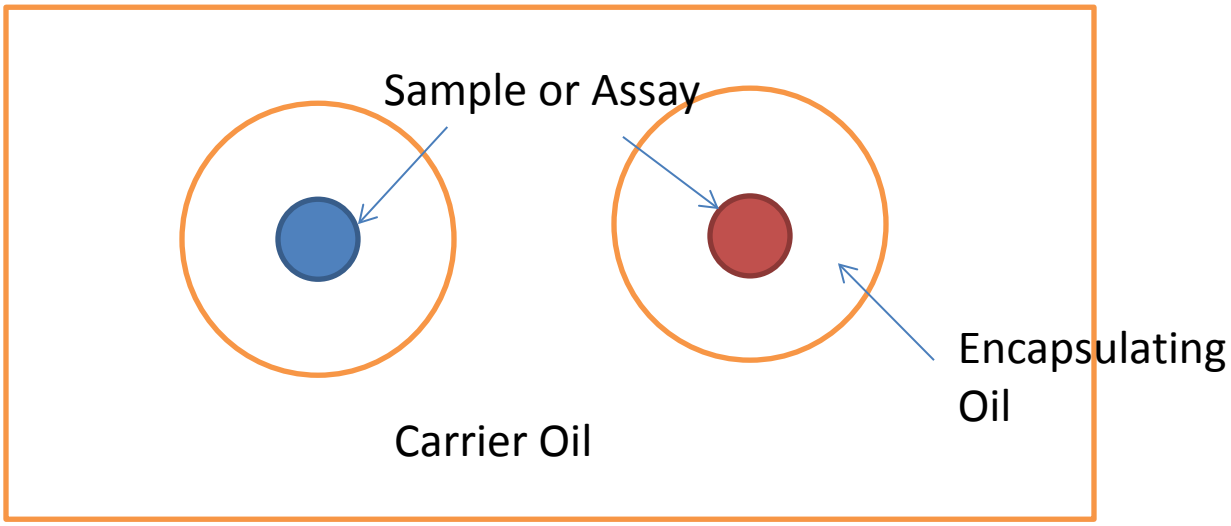
The system is used in Alaska to manage spawning sockeye salmon. The salmon are sampled in tributaries in Bristol Bay for genotyping using SNP panels. The results are reported back to the commercial fishing fleet as to where the salmon are headed. The salmon sampling is accomplished by netting salmon and taking fin clips (1000 – 10,000 samples). The samples are processed in a week utilizing 50 chips and 13ml master mix. There is a 99.3% call rate for genotyping.

Next generation sequencing takes about 4 hours to complete with the Fluidigm system. Approximately 10-50ng/ul DNA is used. Amplicon barcoding is done on the chip. Multiplexing is done by combining 10 primer sets/well. There is 88% sample coverage and 93% exon coverage. The cost is $9250 which is $0.10/amplicon. Fluidigm also has chips that have 6 12x12 arrays on one chip (referred to as "chiplets").

High-throughput Genotyping Using Invader and Composite Liquid Cell Technologies (M. Kaiser, Hologic): Hologic develops and commercializes simple genetic analysis for plants, food animals, microorganisms and companion animals.

The Invader technology detects specific nucleic acid sequences—DNA, RNA or microRNA. It is an isothermal technology using a signal amplifying reaction. Invader can be used for SNP screens, mutations, high-throughput genotyping, infectious diseases, etc. It can also be used for copy number detection up to 4 copies—more than 4 copies becomes difficult to determine.

InvaderPLUS is a one tube reaction—PCR reaction→heat denaturation→detection. The CLC technology is ultra-high-throughput genotyping with no plastic waste. It takes advantage of isothermal Invader technology. It has been developed with prototype instruments with the intent to process thousands samples/hour. The technology utilizes a carrier oil within which is a droplet reactor and encapsulating oil. The encapsulating oil contains the sample or the assay. These are brought together to allow the reaction to occur.



The prototype instrument has a disc with wells for the reaction. As the disc moves, it passes through the opto-fluidics zone which detects the reaction signal and also removes the reaction from the disc well. Within this zone, a new reaction can also be added. It takes 10 min (reaction time) for the disc to rotate around to the detection zone. Tests have shown no cross-contamination using the prototype instrument. The disc has 180 spokes with 8 nodes/spoke. With a 10 min cycle time, 6000-8000 samples can be processed. Currently the disc wells hold a 4ul reaction volume but the intent is reduce this reaction volume further by making the disc nodes smaller. The next steps are to complete the evaluation of the prototype and input specification discussions with customers. The plan is to launch the 2.0 instrument in Q2 or Q3 of 2013.

Better Crops with Arrays and Sequencing from Illumina (C. Soans, Illumina): Illumina was founded in 1998 and went public in 2000. The company is based in San Diego and has 2500 employees with a presence in seven countries. Illumina's vision is to unlock the power of every genome through accelerating genetic gain through predictive breeding, improving human health through agriculture. Illumina also has a program in which they invite proposals to reduce hunger/poverty. Proposals are evaluated and funded. Last year, proposals for work on pidgeonpea and cassava improvement were funded.

The number of publications for sequencing using Illumina technology has dramatically increased. There are now more than 1000 animal genomes and over 700 plant genomes in NCBI. Sequencing has given rise to many applications (marker-assisted breeding/selection, genomic selection, marker-assisted backcrossing, variant discovery, etc).

Illumina has a range of products for sequencing. The HiSeq 2500 can process 50 to 600 Gb of DNA in 2-11 days. In a rapid run (7-40 hours), 20-180 Gb can be sequenced. The sequencer has broad applications utilizing ChIP sequencing, RNA-Sequencing and TruSeq Exome technologies.

MiSeq is a bench top machine for smaller genomes. There has been an upgrade to 2x300 bp read length and 15 Gb output. This will allow more applications such as larger genomes and counting applications.

iScan is microarray platform which fully automated in a bench top unit. The Goldengate (up to 3000 SNPs) and Infinium (>3000 SNPs) assays are used. Up to 1400 samples can be processed per week. There are commercially available chips for maize, bovine, canine, ovine, porcine, etc.

Illumina can also build custom genotyping tools. The process for building the tools is:

discover SNPs→select SNPs→create array→use array→analyze results.

SNPs can be added to the chips. The iSelect is a completely custom chip offering 3,072 – 1,000,000 plex of custom content. The iScan and HiScan instruments are used with the iSelect custom chips.

Illumina also advocates the forming of agriculture consortia to help advance genotyping in agriculture species globally. Consortia formation up the number of samples and thus, decrease the price. Therefore, consortia help agriculture researchers gain access to sequencing technology. Soybean researchers are now forming consortia.

Illumina acquired Moleculo which had the technology to generate long reads via a new sample preparation method which facilitate finishing uncompleted genomes. The workplan is:

Sample→prepare libraries→Moleculo DNA fragmenting and amplification→Illumina sequencing→obtain raw sequence→synthetic long reads prepared→downstream application.

Illumina's NuPCR reagents are probe-based reagents that utilized NuZyme chemistry for analyzing gene expression using qPCR. Oligonucleotides must assemble on the target sequence in order for fluorescence to be generated. The use of a universal substrate provides a cost savings. There are also 4 dyes available to allow for multiplexing.

TruSeq kits provide method for the generation of stranded mRNA from total RNA and helps to reduce chloroplast and ribosomal RNA. The method uses dUTP in the second strand synthesis (instead of dTTP).

Future trends are a) sequence-based genotyping, b) next generation genotyping, c) genotyping by sequencing. Sequence-based genotyping allows the sequencing of an entire genome and is also referred to as "skim sequencing". There is also sequencing by PCR, solid/liquid phase hybridization methods and restriction enzyme sequencing. Restriction enzyme sequencing does not need a reference genome and can be used with uncharacterized species. It allows simultaneous identification/genotyping of SNPs. The high multiplexing also lowers the sequencing costs. However, it has difficulty with complex bioinformatics and missing data requires imputing.

In the future, accessibility to genomic tools will increase. All economically impactful agricultural species, subspecies and their pathogens will be sequenced. There will be field-based genomic diagnostic tools and genomic selection will surpass conventional selection. Arrays will not go away as they are still applicable for many applications.

(Note: Illumina slide presentation available on www.aeicbiotech.org/meetingarchive.html).

Recent Advances in Isothermal Amplification Technology in Ultra-High Throughput System (D. Shaffer, EnviroLogix): Isothermal amplification has been criticized for being

too non-specific and producing too much junk. Non-specific products can consume reactants. Assays have been developed to leverage the background product as an endogenous control. The background product can vary and it eliminates the utility of multiplexing. DNAble is based on NEAR (nicking and amplification reaction). The amplification occurs in 10 min and the target DNA/RNA does not need to be purified. Detection is via a molecular beacon. Crude sample extracts can be used—no need to purify DNA. DNAble also allows a cold start reaction with defined reaction conditions. The method generates a classic sigmoidal amplification curve, is entirely isothermal and allows rapid development of assays.

The DNAble workflow is:

nicking enzyme nicks target DNA→polymerase attaches to nicked strand→polymerase extends/displaces strand→reverse primer anneals/polymerase extends→forward primer anneals/polymerase extends beyond nick site→process repeats/exponential amplification→detection.

DNAble 2.0 is sequence-driven primer specificity. Detection is via fluorescent probe/beacon. DNAble 2.0 provides reaction conditions which allow duplexing/multiplexing and decreases competitive reaction conditions. A cold start reaction is allowed which is conducive for use in robotic liquid handling. A classic sigmoidal amplification curve is generated. Absolute and relative quantitation is achieved. DNAble 2.0 is compatible with all levels of throughput capacity such as tubes and array tapes. The array tape system is an endpoint/isothermal system which is fully automatable. Crude samples can be used. The throughput potential, assuming a continuous run of samples in array tape, it is possible to acquire 200-300,000 data points using 1.6ul DNAble reactions—31,000 reactions/hour. This is equivalent to using 300 384-well plates and Roche LightCycler for 60 days with 10ul reactions. Quantitative PCR typically requires 1-2 hours cycling time whereas DNAble requires <10 min.

(EnviroLogix slides are available at www.aeicbiotech.org/meetingarchive.html)

Improving the Efficiency of AgBiotech Applications with the Douglas Scientific Array Tape Platform (D. Cook, Douglas Scientific and V. Pegadaraju, BioDiagnostics):

(*D. Cook)* Douglas Scientific introduced the global array in 2004 and deployed it within the agbiotech field in 2009. It was first deployed in human diagnostics in 2012. The array tape is embossed with assay wells capable of supporting nanoliter reaction volumes. Douglas also delivers innovative lab automation.

The Douglas system consists of the array tape, Nexar in-line liquid handling,Soellex PCR waterbath, Arraya in-line scanning (3 dye reader) and Intellics software. It provides qualitative isothermal results. In the future, quantitative isothermal system is planned which would be fully integrated and provide a 4-10X gain in throughput using 2ul or less

reaction volume. There are also plans for a quantitative PCR system which would also be fully integrated with a similar output to the isothermal system.

(V. Pegadaraju) BDI has used the array tape system in genomics assisted breeding analysis. For research and production testing, whole genome/transcriptome sequencing, fingerprinting germplasm, forward breeding and backcross breeding are utilized. For large scale testing, the array tape system is used. The array tape provides instant success due to cost savings, low reaction volumes, continuous in-line manufacturing, flexibility and support a wide range of chemistry types. The KASP chemistry is used since provides excellent data quality, flexibility, low cost, minimal DNA needed and is leading SNP to assay conversion rate (>90%).

BDI has optimized the array tape system and validated across multiple array-based platforms. Array tape technology has been used in trait introgression, specifically backcrosses. Genomic selection reduces the cycle time/cost by reducing the frequency of phenotyping needed. Fine mapping has also been accomplished for downy mildew resistance genes in sunflower. Pooled seed testing has also been conducted with array tape. For sunflowers, EU is concerned with the contamination of domestic sunflowers with wild sunflowers. A target SNP was used and converted to the pooled seed approach which allowed detection of 1 wild seed per 10,000 domestic seeds. The array tape has also been used for genetic purity/varietal identification. This allows the determination of all individuals of a line/lot/bag are of the same genetic make-up. Seed producers, regulatory agenices use genetic purity testing to identify outcrosses, selfs, seed mixes and seed swaps.

Exclusion-based Sample Preparation: Simpler, Faster, Better (D. Beebe, Salus Discovery): Salus was established two months ago as a company.

Sample preparation has changed little in 20 years. Improvements in downstream endpoints are shifting the throughput bottleneck to upstream sample preparation. The Salus next generation sample preparation is applicable to purifying molecules and for cell separation. The approach is based on exclusion principles—air-water or oil-water. The method is a single step with no fluid transfers and retention of the sample. The method performs as well or better than standard wash-based methods. The method functionality is high and allows multiple isolations from a single sample. The immiscible filtration by surface tension (IFAST) system consists of mixing the sample with analyte-binding paramagnetic particles (PMP) and loading the mixture into IFAST. A magnet is used draw the PMP-bound analyte through the liquid. A virtual wall is created by the immiscible liquids thus they can be positioned side-by-side. IFAST works with a broad range of common biological reagents. Whole cells, proteins, weakly bound protein complexes have all been separated using the system. It has been successful in separating circulating tumor cells (CTC) from blood. CTC are the “seeds” for metastasis. The IFAST slide was reconfigured to use on its side to avoid pulling unbound cells through.

SLIDE (sliding lid for immobilized droplet extractions) leverages the surface tension of fluid droplets and hydrophilic pinning regions to remove a target analyte from a complex sample. Samples are loaded containing the PMP-bound analyte and a lid with a magnet is moved over the sample carrying the PMP. The analyte is then eluted off the PMP. The SLIDE is particularly good for dense samples.

Exclusion sample preparation is a rapid, high performance sample preparation method that can be used across a range of samples and applications. It provides simple workflows, has added functionality and the costs are comparable or lower than other methods. Salus' strategy is to provide a family of technologies based on the exclusion principle to provide faster sample preparation with fewer steps. Salus is currently working in partnerships and co-development relationships.

Optimizing Extraction Technologies for Efficiency in High Throughput Genotyping Data Generation (F. Schubert, LGC Genomics): The LGC Group was established in 1842 as the scientific testing lab for the UK government. It was privatized in 1996 but still retains its role as the UK National Measurement Instititue as the standard bearer for chemical/bioanalytical measurement. LGC has 30 locations globally with 1800+ staff. LGC specializes in extraction, sequencing and genotyping to deliver accurate, reproducible analytical science solutions. LGC use what they sell to also drive profitable service business. They provide optimized solutions for any application for plants, forensic samples, blood, tissues, food, saliva, plasmids, prokaryotes, etc. The analytical pathway for DNA analysis is:

Sample→isolation/purification of analyte→definition of criteria (quality, concentration, storage, size)→data generation (analysis).

The question is how to cope with millions of samples. Solutions could be parallel processed to increase throughput, decrease scale, implement automation to increase data output, reduce time and save money. Factors affecting these are limitations on throughput, investment, running cost, reliability of chemistry/instruments and flexibility of protocols.

LGC has the sbeadex kits for automated DNA extraction. These are based on the use of magnetic particles which reduce labor, are cost effective and are highly scalable. Magnetic particles also facilitate high throughput extraction and miniaturization. In sbeadex, nucleic acid is bound by polarity followed by a non-covalent crosslinking of the DNA. The beads are washed efficiently and then eluted with a magnet. There are no organic solvents in the wash buffers, no drying required, no chaotrophs for binding required. This results in fast extractions. LGC has validated protocols for certain crops. The sbeadex toolbox contains lysis buffer, binding buffer, sbeadex beads, wash buffers 1 and 2, elution buffer. There can be individual add ons or the addition of tailor made solutions for customers.

The oKtopur is a 96-channel pipetting head optimized for magnetic bead-based extractions. The maximum capacity is 8x96-well plates/run which equals 768 samples. The runt ime is 1 hour 10 min to about 2 hours.

Novel Kompetitive Allele-Specific PCR (KASP) is designed to overcome the high cost of dual-labeled probes. It is a homogeneous fluorescent assay with no separation steps. The results deliver accurate SNP and InDel genotyping data while providing maximum flexibility/cost saving. KASP provides improved genotype clustering, especially with plant and polyploidy organisms. It allows the running of a few or as many assays as needed. It utilizes forward primers (2 alleles), a reverse primer and an enzyme. The workflow is:

Assay assembly→heat/laser seal plate→PCR thermal cycling→fluorescence plate reading→analyze data.

The system uses the SNPline XL: repliKator which is fully automated and processes 24x1536-well plates in 15 min. The repliKator has onboard tip washing and it is integration friendly. The Meridian dispenser is a single or 8-channel dispenser and can dispense 300-1000ul. It has a unique aspirate function which reduces the wasting of expensive reagents. The plate sealers use either heat or a laser and are both automated.

Using the novel primer approach with KASP plus miniaturization to enable 1ul reaction volumes plus the flexibility and scalability of the SNPline can drive the cost per data point to as low as 1-2 cents per data point. This allows customers to do more with their research dollars.

(LCG Genomics slides are available at www.aeicbiotech.org/meetingarchive.html)

Composite Liquid Cell Technology: A Novel Means of Molecular Testing (R. Roeven, GenCell): GenCell is based in Limerick, Ireland and is now opening a US subsidiary. The staff is mainly engineers and scientists. The company is engaged in developing systems for continuous genotyping, automated library preparation, automated sample preparation and live cell screening for compound discovery.

Composite liquid cell (CLC) technology has wide applicability, is automation friendly and uses no plastic consummables. CLC uses synthetic cells which provide an ideal environment for conducting assays without risk of cross-contamination. The droplet reactor can be considered the "nucleus" and the encapsulating oil makes up the "cell". Currently, a prototype machine has been developed which is a disc with nodes. Within each node, the assay can be built using a continuous flow protocol. A video of the droplet reactor can be viewed at http://www.gencellbio.com/technology/.
The CLC Technology provides a reusable system which is simple to use and provides "sample in, answer out" data. It is biocompatible, flexible and has an open architecture. In 2011, proof of concept was achieved and in 2012, functional prototypes were

developed. Ramp up of the prototypes will continue in 2013 and expect to offer commercial models in 2014.

Use of Nextgen Sequencing and Junction Sequence Analysis Bioinformatics (NGS/JSA) to Achieve Molecular Characterization of GM Crops (D. Kovalic, Monsanto): Molecular characterization of GM crops is required by government agencies globally. Characterization is done on the inserted T-DNA and the insertion site *in planta*. Characterization ensures the intended T-DNA is inserted and there is no backbone DNA sequence inserted into the plant genome.

Current characterization methods include Southern blot analysis for T-DNA insertion and copy number and absence of backbone sequences as well as generational stability. Southern analysis has technical challenges, requires the use of unique enzyme combinations and is subject to band interpretation. Currently, directed PCR/sequencing is also used to determine the inserted T-DNA and genomic flank sequences as well as determination of the wild-type insertion site.

Advances in sequencing (especially increased throughput by NexGen class machines) has dramatically dropped the cost of experiments. A whole genome which used to cost $50 million can now be sequenced for about $5000. Bioinformatics has also advanced in that large volumes of short sequences can now be analyzed. Genome sequencing and bioinformatics can now be used to determine T-DNA insertion, copy number, absence of backbone sequences and generational stability. The benefits of using sequencing and bioinformatics is the ability to overcome technical challenges, high reproducibility and easier results interpretation. Basic genome sequencing and bioinformatics methods for molecular characterization are well established. Proof of concept studies have proven the feasibility of utilizing this type of data for molecular characterization. Multiple scientific articles have been published on the topic recently. The method protocol has adequate sensitivity, is validated, GLP compliant and the protocol ensures genome has been sequenced comprehensively.

The method workflow is:

Genomic DNA of test organism→produce sequence fragments→conduct bioinformatics analysis→molecular characterization results.

The test substance is defined as the DNA from crop genome; control substance is genomic DNA from the conventional control line. The reference is spiked in transformation plasmid DNA. Illumina HiSeq instruments are used to produce ~100 nucleotide long DNA fragments. This provides comprehensive genome coverage. For junction sequence bioinformatics analysis developed, public, bioinformatics algorithms are used with optimised selection criteria. There is a low false negative rate (predicted to be $<1x10^{-75}$) 20-30 corn and soybean proof of concept studies completed.

The method is a two step process: Step 1: plant genome→Illumina sequencing→sequence similarity search using the construct sequence→thousands of selected 100-mers
Step 2: fully analyze the selected sequences to determine insert copy number and all sequences characteristic of insertions resulting from the transformation (Junction sequence analysis.

For junction sequence analysis, custom software detects junction sequences with reference to the transformation plasmid. The number of T-DNA/flank sequences detected is correlated to the number of inserts, one junction is detected at each end of each insert. Method sensitivity was determined by assessing coverage and accuracy of spiked-in control—1 copy and 1/10 copy. Genome coverage is determined by sequence depth. Sequence depth is defined as:

Sequence depth = bases sequenced / genome size (bases)

With:

Fractional genome coverage = $1 - e^{\text{sequence depth}}$

Multiple reports have been presented on using sequencing and bioinformatics for molecular characterization. In a paper from the group of Dr. Francis Collins (Dir. of NIH) entitled "Use of microarray hybrid capture and next-generation sequencing to identify the anatomy of a transgene", it was demonstrated that junction sequences may be used in place of Southern data to describe a complex re-arrangement. In another report presented by UC-Davis and JR Simplot on black spot bruise resistant potato, Nextgen sequencing was proposed to replace standard methods for molecular analyses of GE crops considered for de-regulation.

In summary, next generation sequencing affords direct molecular characterization by genome sequencing and bioinformatics. It improves consistency of molecular characterization by the utilization of a consistent, robust method, simplified data interpretation which leads to simplified dossier review. In a GLP environment sequencing uses <50% of the time and resources needed to do Southern analyses.

Southerns-by-Sequencing: Molecular Characterization of Transgenic Events (M. Beatty, DuPont Pioneer): Transgenic event selection is traditionally done by phenotype, expression and molecular characterization of gene cassette. The molecular characterization can be done via quantitative PCR, Southerns and sequencing. Molecular characterization criteria includes copy number, ascertaining if all elements are intact, identification of the location of each insertion and identification of SNPs.

Quantitative PCR provides the copy number of the loci, however, it may not detect SNPs or INDELs. Flanking sequences can be determined by using iPCR, SAFE and LMnPCR. These methods may also not detect SNPs and INDELs. Southern analysis provide copy number and information on the intactness of the insertion. Southerns use restriction enzymes, are subjective in interpretation and identified fragments may need further characterization work. SNPs and INDELs can be detected. Sanger sequencing provides loci sequencing but it is labor intensive for multicopy events. Southern-by-sequencing is performed to sequence the transformation construct and flanking DNA to identify and characterized the location, copy number and flanking junctions. Biotinylated probes are designed for entire construct and optimized for small, low complexity regions. Capture

libraries contain multiple constructs and multiple events are pooled per capture reaction. The workflow is:

capture sequence→filter out endogenous junctions→identify chimeric reads→5' and 3' extension using assembly algorithm→mapp back to construct and genome.

The capture efficiency is 93-97% T read alignment back to construct. The remainder is next-to-construct.

Southerns-by-sequencing determines copy number, identifies location of each insertion, identifies SNPs within features and identifies vector backbone.

New Member Presentation: Strategic Solutions (D. Grothaus): Strategic Solutions was founded in 2012 to provide scientific consulting services to biotechnology and agricultural chemical community. The consultants are David Grothaus and Beryl Packer. Dave formerly worked for Monsanto, EnviroLogix and DuPont Pioneer and has experience through past participation in industry organizations. Dave chaired the CropLife Internationa Detection Methods Committee and was also a course developer/teaching faculty at the ILSI workshops globally. Beryl has experience in diagnostic test development and has worked for IDEXX and in Regulatory Affairs at Monsanto. Strategic Solutions can supply expertise in regulatory compliance, writing/submission of regulatory dossier to US agencies, writing and facilitation of the preparation of white papers, position papers, publications, development/implementation of strategic initiatives, product development and commercialization, innovative technology solutions and regulatory science and regulatory affairs experience. Strategic Solutions sees their potential clients as agbiotech/chemical providers, testing service providers, testing kit providers and equipment/testing technology providers. Dave can be reached at 314-681-2948.

Validation of Enzyme-Linked Lectin Analysis (M. Breeze, Monsanto): Monsanto has been working on a method for soybean lectin analysis called enzyme-linked lectin analysis (ELLA). Soybean lectin is a key anti-nutrient known to inhibit growth in monogastric animals. It is a requirement by most regulatory authorities that it be measured in GM soybean submitted for approval for food/feed. The current method of measurement is based on hemaglutination of red blood cells which is a variable method.

A comparison of methods for soybean lectin has been made:

	Hemaglutination	ELISA	ELLA/ELISA	ELLA
R^2	NA	>0.99	>0.99	>0.99
Precision	>20% CV	NA	9.92% CV	7.62% CV
Incubation	2.5h	1h	2h	2h
Range	0 – 512X	>1 order	1 order	1 order
Sensitivity	+/-	pcg	ng	mcg
Reagents	Source	Polyclonal	Polyclonal	Commercially

	dependent	antibodies	antibodies	available
Cost	$300/sample			<$50/sample

ELLSA is similar to a sandwich ELISA but it uses multivalent monosaccharide-polyacrylamide conjugate. The assay is dependent on the tetrameric nature of soybean lectin. ELLA can deal with a small linear range.

To test efficiency, samples were prepared using the genogrinder. It was found to be 91% efficient on first extraction so multiple extraction is not necessary. Dilutions were tested and it was found that dilution does influence ELLA analysis. To test for matrix effects, samples were spiked in matrix. The average recovery across spike levels was 83%, therefore, other seed components do not influence ELLA. ELLA was found to have a 20% CV across operators, days, etc.

In conclusion, a single extraction is sufficient for ELLA as a sample. Using a tissue to buffer ratio of 1/100 and 1/10 dilution, the LOQ is 0.1mg/g. ELLA analysis demonstrates accuracy and precision similar to other methods. Therefore, ELLA can be used to quantitate soybean lectin in soybean seed.

New Member Presentation: Primera Analytical (D. Jones, Primera): Primera was founded in 2002 and has a strong base of PhD level scientists. Primera is working with international and US companies. Primera staff bring decades of experience in method feasibility, method validations, magnitude of residue, independent laboratory validations, immunoassay development/validation and soil disspation. Primera has a robust system of SOPs, a full GLP/GMP staff training program, secure data back-up. The company was audited by FDA in 2008 and 2012 and will be audited by EPA in 2013.

Primera has the capability for protein isolation, characterize post-translational modification, amino acid composition analysis, cell culture and cell-based assays and a robust program in independent laboratory validation of methods.

New Member Presentation: EPL BioAnalytical Labs (F. Claussen): EPL BioAnalytical was established in 1987 and has had a 25 year focus on agriculture and 14 years of analytical lab experience. EPL has rigorous internal/external technical training, is active in industry training (such as SQA) and their staff is cross-functionally trained.

The EPL facility is a secure facility with state-of-the-art instrumentation such as HPLC, GC, etc. The lab services area is in charge of sample custody, grinding, weighing, etc. There is a coordinator for standards and solutions which provides a central point of control. In the agchem area, residue, product chemistry, method development and validation are performd. For product chemistry, the OPPTS Series 830 guidelines are followed. Group A is the preliminary analysis and Group B is the physical/chemical analysis. Method development/validation group supports other areas within EPL. The nutritional chemistry conducts GM crop analyses such as proximates, fiber, vitamins, minerals, amino acids, anti-nutrients, secondary metabolites and fatty acids. EPL also

has customized analytical support based on a diverse portfolio of analytical testing services.

Future growth for EPL includes the recent acquisition of GC MS/MS and AB SCIEX 6500 Qtrap. A future instrument purchase will be the UPC2 which is for super critical fluid chromatography and further lab automation machines. EPL has also begun some molecular biology work which includes ELISAs, Southerns and PCR.

LIST OF MEETING ATTENDEES

Pearce Smith	Eurofins GeneScan
Gina Clapper	AOCS
Rich Wilson	United Soybean Board
Doug Miller	IL Crop Improvement
Jenny Golbach	EPL Bio Analytical
Fred Claussen	EPL Bio Analytical
William Hernan	EPL Bio Analytical
Jian Zhang	BASF
Denise Thiede	BioDiagnostics
Chong Singsit	OMIC, USA
Rong Guo	Bayer CropScience
Ryan Sizemore	Monsanto
Tom Currier	Bayer CropScience
Chandrasen Soans	Illumina
Dave Jones	Primera
Yelena Dudin	Monsanto
Dean Layton	EnviroLogix
Penny Hunst	Bayer CropScience
Clara Alarcon	DuPont Pioneer
Ryan Johnson	BioDiagnostics
Mariah Benesh	SGS
Hilary Rogers	Eurofins Nutriton Analysis
Kai Liu	Eurofins Nutrition Analysis
David Vaske	Hologic AgBio
Jennifer Darnell	Fluidigm
Matt Breeze	Monsanto
Michele Yarnall	Syngenta
Frank Spiegelhalter	Eurofins GeneScan
John Markin	EnviroLogix
Donna Houchins	Romer Labs
Laura Privalle	BASF
David Levin	Covance
Kathy Miller	Covance
David Kovalic	Monsanto
Ryan Akel	Covance
Ping Song	Dow AgroSciences
Dave Rambow	Agdia
Cathy Zhong	DuPont Pioneer
Jason McKinney	Fluidigm
Mary Beatty	DuPont Pioneer
Hans Mische	Douglas Scientific
Dave Grothaus	Strategic Regulatory Solutions
Robert Roeven	GenCell Biosystems
Scott Berry	Salus Discovery
Michael Kiser	Hologic
Dave Beebe	Salus Discovery
Daniel Shaffer	EnvirLogix
Angela Umthun	Stine Biotechnology

Darren Cook	Douglas Scientific
Jane Sabbatini	Covance
Cecil Dharmasri	Bayer CropScience
Dave McGuire	GenCell
Kieran Curran	GenCell
Walter Iszczyszyn	Hologic
Lisa Ruiz	Eurofins Scientific
Mark Kimble	Romer Labs
Guomin Shan	Dow AgroSciences
Frank Schubert	LGC Genomics (Germany)
Stephanie Pokorny	Monsanto
Edan Hosking	Neogen
Jennifer Helm	Covance
Rhonda Feazel	Monsanto
Venkatramana Pegadaraju	BioDiagnostics
Doug Winters	Covance
Nate Paske	Covance
David Reed	Hologic AgBio
Brian Potts	Covance
Jay Tolley	BioAgilytix
Scott Weigel	LGC Genomics
Amy Pierce	LGC Genomics
Brent Rozema	Covance
Bob Bates	Monsanto
Kaylene Bromenshenkel	Douglas Scientific
Yue Yun	DuPont Pioneer
Randy Arnold	DuPont Pioneer

LECTIN DISCUSSION BREAKOUT SESSION
April 18, 2013
Madison, WI

M. Breeze (Monsanto)

Stakeholder needs analysis Laura Privalle, BASF
Discussion included background on lectins as a class of glycoproteins that specifically interact with different glycotypes. Soybean agglutinin (SBA) interacts with specifically with galactose sugar residues and this binding serves as the basis for the current agglutination methodologies. Wheat Agglutinin (WGA) also has the same specificity, it may be possible to apply the same/similar methodology to detect and quantitate this protein in wheat. Lectins are found ubiquitously in many plants including several legumes. Cooking or heat denaturing processes are in-place for many of the plants that contain these proteins, allowing them to be deactivated prior to human or animal consumption.

SBA is analyzed during the OECD-based characterization of new GM soybean products as part of the substantial equivalence evaluation of new products of biotechnology. As such, stakeholders interested in relative SBA levels and methods of analysis include the Agricultural Biotechnology community, laboratories conducing the analysis, and regulators reviewing data and submissions. All of the stakeholders are interested in developing quality (precise/accurate) information on SBA in soybean seed. In addition, information on natural variability is also a key concern for those generating the data as well as those interpreting the results of the analysis.

Conclusion – Key stakeholders include Agricultural biotech, contract research labs, and data customers including regulators. Data needs include formal method validation data support appropriateness of methodology and natural variability data to better understand past, present, and future commercial soybean products.

Bridging discussion Hilary Rogers, Eurofins
Discussion was conducted in two parts.

Part 1 – Options to develop bridging data in a collaborative environment
Typical method updates include a formal correlation analysis that can be developed as part of a multi-laboratory collaborative study showing developing data from both methods that shows a strong linear correlation.

Part 2 – Limitations of bridging between haemagglutination and other methodology
Data from the haemagglutination procedure conducted with and without external standardization was presented showing there is RBC source dependent results and that traditional correlation analysis is not an option.

Conclusion – Traditional correlation analysis is not appropriate based upon RBC source dependent results and the variability inherent with the haemagglutination procedure. Group agreed that analysis of high and low expressing lines using both the ELLA method and candidate method could allow us to evaluate if there was a similar trend between the analysis at minimum.

Method development effort Ryan Akel, Covance
Covance is currently validating the ELLA methodology and presented their develop and validation data. Highlights of the discussion include the following:

- 5X dilution exhibits significant matrix effects, 10X/20X dilutions are recommended and based upon the expression profile of the varieties evaluated will be sufficient for most samples.
- Precision and accuracy of the analysis completed is similar to other method used to evaluation soybean composition
- Calibration curve is valid
- Future studies needed to evaluate robustness, plate stability, incubation times/temperatures, and automation options

AEIC outreach and deliverables AEIC Board (Denise Thiede)
AEIC has a history of publications on methods used in agricultural biotechnology. Based upon the stakeholder needs, it was discussed that two separate publications will meet the needs of all interested parties.

Publication #1 – Method evaluation and validation
Target Journal for Publication – JAOCS
Target Audience – Laboratories conducting analysis, Data reviewers, and regulators
Proposed Outline:
History of lectin analysis
Proposed method procedure
Validation data
Bridging data
Authorship – Multiple co-authors from multiple stakeholders are desirable. Monsanto can provide a draft manuscript in June, co-authors requested to review, comment, and revise the publication for journal submission. Matt Breeze will coordinate drafting and revision of the Manuscript as well as journal submission.
Action items

- Volunteers to serve as co-authors requested to contact Yelena and/or Matt to volunteer as an author. Yelena.a.dudin@monsanto.com matthew.l.breeze@monsanto.com
- Matt Breeze to forward draft manuscript to volunteers in June of 2013

Publication #2 – Natural Variability of Soybean Lectin Levels
Target Journal for Publication – JAOCS
Target Audience – Data reviewers and regulators
Proposed Outline:
History of lectin analysis including newly validated method

Bridging data
Diverse soybean population data (sourced from multiple germplasm and maturities)
Authorship – Multiple co-authors from multiple stakeholders are desirable. Due to the need for multiple stakeholders to provide germplasm and/or data an individual or organization not associated with the seed industry and laboratory analysis was suggested to help organize and lead this publication effort. Dave Grothaus was suggested as a consultant to help organize and lead the development of the natural variability publication. AOCS also volunteered to help provide germplasm and/or to help coordinate the movement of devitalized materials to laboratories for analysis. National Soybean Board also may be a good external collaborator to help organize and develop a manuscript for publication. Covance, EPL, and Eurofins all expressed interest in helping to develop data as part of the partnership.

Action items

- Volunteers to serve as co-authors requested to contact Yelena and/or Matt to volunteer as an author. Yelena.a.dudin@monsanto.com matthew.l.breeze@monsanto.com
- AEIC board to discuss options to work with a consultant (Dave Grothaus) to organize and draft the publication manuscript.
- Matt Breeze to work with AEIC board, volunteers, and lead author to scope publication and move forward with development of natural variability data

AOCS overview Gina Clapper, AOCS

AOCS offers multiple different laboratory methods and quality programs including Official Methods and Recommended practices, proficiency programs, and certified reference material programs. Minimum requirements to have a method included as an Official Method of Analysis include having the proposed method evaluated by 8 laboratories, in 5 countries, using 5 different levels of analyte. AOCS can manage the proficiency program conduct, funding would be required to cover preparation and shipment of materials the laboratories participating in the method evaluation.

Note: Slide presentations used during the breakout session are available at www.aeicbiotech.org/meetingarchive.html.

Attendee list for the Lectin Session at the Spring AEIC meeting, April 18, 2013.

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