AEIC 2021 Fall Meeting Minutes

P.L. Hunst, AEIC Secretary

Virtual Meeting

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AEIC Spring 2021 Meeting Minutes

April 13-14, 2021

Virtual Meeting

P.L. Hunst (BASF), Secretary

The AEIC Fall 2021 Meeting was held virtually on October 5-6. Kristen Kouba (Corteva), AEIC VP, welcomed everyone to the virtual meeting and presided over the round table introductions.

AEIC BUSINESS MEETING

Approval of 2021 Spring Meeting Minutes: A motion was made and seconded to approve the minutes posted on the website. Motion was approved by member vote.

Treasurer Report (L. Muschinske): The Treasurer presented the 2021 budget as follows:

ITEM	PROJECTED	ACTUAL
Beginning Balance	34393	34393
2020 dues	6800	7675
Mtg registration	2250	
TOTAL REVENUE	**9050**	**7675**
Expenses		
Scientific paper	2000	--
DE Franchise Tax Report	25	25
ANSI/ISO Initiative	2900	2900
Board Meeting Expenses	300	--
Spring Meeting 2020 Expenses	1500	--
Website Expenses	500	240
Credit card proc	400	111
Fall Meeting 2021	6000	--
Graphic design		--
Marketing (brochure)	500	--
Subscriptions		--
Miscellaneous	600	600
TOTAL	14125	3875
BALANCE	31318	40193

The Miscellaneous funds were used to re-instate the 501(c)(3) designation (non-profit) for AEIC. A motion was made and seconded to approve the Treasure's report. A member vote approved the motion.

Membership Update (L. Muschinske): The following table depicts the current membership composition of AEIC:

Category	*Number*	*Projected Dues (Paid)*	*Unpaid*	*Unpaid amount ($)*
Large Companies	7	3500	0	0
Medium Companies	12	3000	1	250
Small Companies	9	1125	1	125
Associate Members	3	75	0	0
Individual Members	2	100	0	0
TOTAL	**33**	**$7800**	**2**	**$375**

John Jackson, representing LGC, gave a brief new member introduction. LGC does high throughput genotyping and supplies the reagents. They also have NGS service capability and products such as SNPs, BHQ primers and the array tape.

Vice President Nominations (M. Cheever): A request was put to the membership for nominations for Vice President. Donna Houchins, Romer Labs, and John Zheng, Indiana Crop Improvement, were nominated. Both accepted the nomination. Nominees will supply short bios by November 1 for inclusion in the ballot. Additional nominations will be taken via email to the Secretary by October 22.

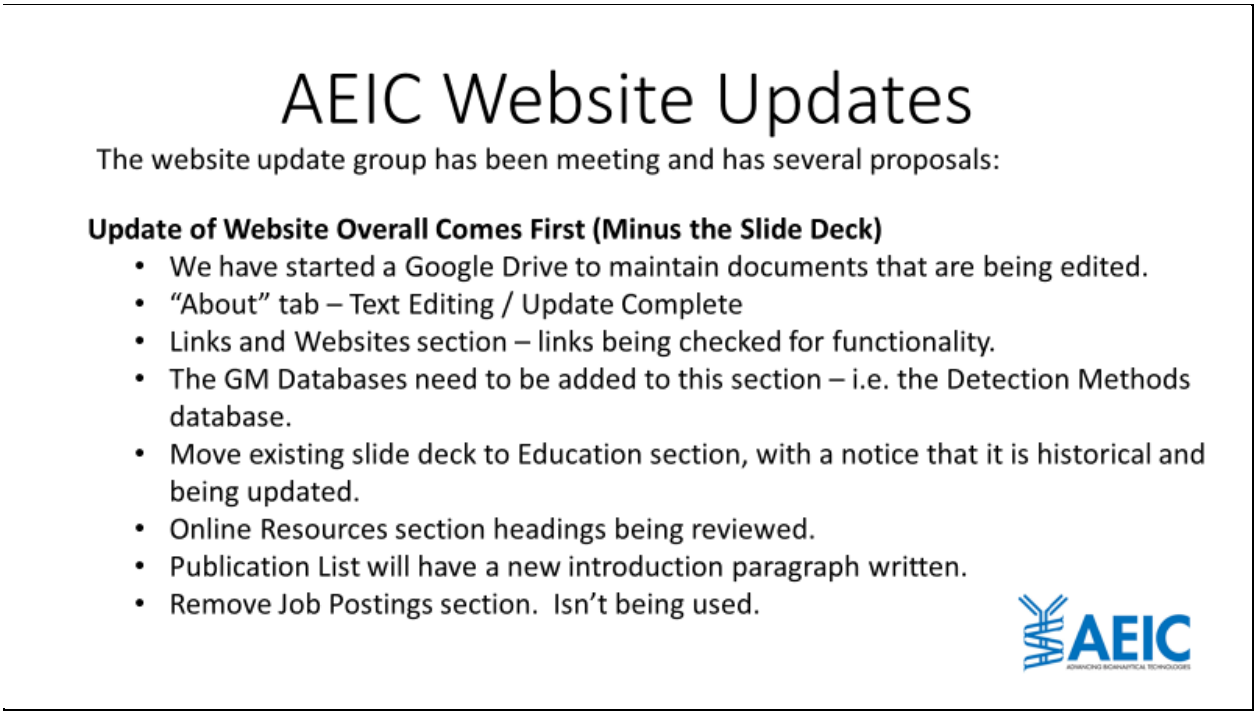
Protein Working Group Updates (T. Gao, Bayer): The Protein Working Group (PWG) currently has 5 active work streams. These include a) multiplex validation; b) MS for protein expression; c) allergen analysis; d) extraction efficiency an e) intractable proteins. The **allergen analysis** work stream is working on standardization of the pepsin digestion assay. The plan is for a publication and designation as an ISO method. A question was raised as to why industry keeps doing the pepsin digestion assay when it has no correlation with allergenicity. It was pointed out that the assay does give information about stability which is a component of allergenicity. It was also pointed out that regulatory agencies will not drop this data until there is an appropriate allergenicity assay to replace it. The **multiplex validation** work stream is working to publish guidance for validation. The **MS for protein quantitation** is publishing a paper reviewing/summarizing ELISA and MS. The group is currently in round 2 of the drafting process. The **extraction efficiency** group is discussing methodologies and plan to

publish a paper on these. They expect to draft the paper by the end of 2021. The **intractable protein** group has 14 members and is currently drafting a manuscript.
Composition Working Group (M. Bedair, Bayer): The group is working on ways to support acceptance of combustion vs the Kjeldahl method in the biotech industry for estimation of crude protein levels. The literature review is done. The group will map out the way forward to provide the necessary support for the combustion method (Dumas). The Dumas method uses non-corrosive chemicals and is capable of high throughput. The group is considering a lab round among the member companies using crop samples. This would help determine how environment and crop condition affect crude protein levels. Forage samples from conventional varieties will be used. Syngenta has used both Kjeldahl and Dumas on grain and found that both methods correlate.

Next Generation Sequencing Group (F. Ghavami, Eurofins BioDiagnostics): The group has met twice and discussed updating the AEIC website with NGS information such as information on NGS methods, digital PCR, RT PCR, endpoint PCR, isothermal methods. The group is also thinking on harmonization of nucleic acid analytical tests standards (ISO).

Ambiguous Results Working Group (R. Shillito, BASF): The group was formed after the Spring Meeting but has not yet met to discuss objectives.

Website Updates (D. Houchins, Romer Labs): The following slides were presented by the team:



AEIC Website Updates

Update of Slide Deck:

- The slide deck will be the second phase of the project.
- Need to find out whether AEIC has the rights to the graphics used in this presentation.
- Many of the slides are not in an editable format as we have them now.
- Need to work through issues surrounding copyright of the slide deck.
- Graphic style – match rest of website?
- Format – one large presentation or broken down into several, like it is now?

AEIC Website Updates

Questions for the Group:

- On the publication section of the website, what do we want to show here? Should the list only include AEIC sponsored publications or those that came out of AEIC working groups? Only publications from AEIC members? Or publications of interest from others, possibly in a separate section?
- On the "AEIC Accomplishments" section, there is a bullet point that states "AEIC worked to establish voluntary performance standards for immunoassay kits." What does this refer to? GIPSA standards at the time? Something else?
- Does the AEIC have a master slide deck, or can one be created, for the update of the slides? It would look nice to have the slide deck graphics, colors, and text be in the same style as the rest of the website.

For the update of the slide deck, it was discussed to have slide graphics that match website. The question was asked whether the webmaster could assist. The AEIC Secretary will ask the webmaster and if she can, the Secretary will work with the team

to connect with the webmaster. For the question concerning what the statement under the "Accomplishments" section ("AEIC worked to establish voluntary performance standards for immunoassay kits"), the Secretary clarified that this goes back to why AEIC was founded in 1992, i.e., during the EPA summit meetings a need was identified for ELISA kits for chemicals that they lacked harmonized performance standards. This was one of the earliest projects of AEIC to develop a publication on performance standards (Mihaliak, CA and Berberich, SA. 1994. ***Guidelines for the Validation and Use of Immunochemical Methods for Generating Data in Support of Pesticide Registration. In: Immunoanalysis of Agrochemicals: Emerging Technologies.*** Eds. Nelson, JO, Karu, AE and Wong, RB. ACS Symposium Series 586: 288-300) and to harmonize information in the kit inserts. Another question from the membership pertained to how much traffic, i.e., clicks, the website has had in the last 6-12 months. This would be useful for determining how much updating is needed.

Spring Meeting 2022 (M. Cheever): The Spring Meeting may be a face-to-face meeting depending on company travel policies. BASF would host the meeting in RTP, NC. Matt will reach out to Merieux to see if they may be interested in hosting the Fall 2022 meeting (depending on travel policies). Suggested topics to consider for the Spring 2022 meeting were:

- Calyxt and genome editing
- Metagenomics and consensus genomes
- Diversity/inclusion in agriculture
- How to improve meetings—getting away from Powerpoints
- New technologies: mRNA vaccines produced in plants; CRISPR-based detection methods; proteomics analyses

ISO TC 34/SC 16 Update (R. Shillito, Bayer): SC 16 is the Biomarker group working on standardization of biomolecular testing methods applied to foods, feeds, seeds and other propagules of food and feed crops. Ray is the Chairperson and Mike Sussman (USDA) is the Committee Manager. The group has published 30 standards and have 7 standards under development. SeedCalc now has an ISO method number (ISO 22753). There is a vocabulary document being worked on (ISO 16577) and one for cotton biomarkers (ISO 5354-2). A new document is being drafted for PCR which will be for everything, including GMOs. Sherry Whitt (BASF) is working on this document. There is also an IEC Standardization Evaluation Group (IEC/SEC 12) working on BioDigital Convergence. BioDigital Convergence is a complex combination of new conceptual and practical connections between engineering, biology, physics, nanotechnology and information science. The group will investigate current research and technology activities, identify critical challenges and propose a roadmap for standardization in the area of biodigital convergence. There are 7 working groups under IEC/SEG 12.

AEIC Mission (M. Cheever): The AEIC Mission was briefly discussed. A new group on outreach and recruiting will be formed. For education, R. Shillito informed the group that Colombia has requested a workshop on detection methods which he will coordinate with AEIC and Cereals & Grains.

A motion was made and seconded to adjourn the Business Meeting. The Working Group Meetings were then in session until 1:30pm EDT.

INVITED TALKS

Towards NIST standards for biology and genome editing (S. Maragh, NIST): NIST is the National Institute of Standards and Technology which works with industry and science to advance innovation and improve the quality of life. NIST's portfolio of services for measurements, standards and metrology provides solutions that ensure measurement traceability, enable quality assurance and harmonize documentary standards and regulatory practices. Standards provide value by validity of data, confidence in data and rapid, integrated technology development. They also provide common understanding (vocabulary), common practices, traceable materials and common operational and management systems. Reproducibility does not always mean there is high confidence in results, i.e., how confident can one be that the answer is correct? NIST's advanced biometrology provides measurement assurance and standards. NIST works closely with communities to meet needs, either through one-on-one or through consortia. NIST works closely with US FDA and provides an avenue for FDA to participate in industry discussions. NIST's platforms include PCR, NGS, transcriptomes and advanced biological reference materials (living materials such as cells, yeast, etc.). NIST also runs the Genome Editing Consortium to fulfill the issue of compiling and organizing all the genome editing research materials into one place and to increase confidence and lower the risk of utilizing genome editing technologies in research and commercial products. The goals of the consortium are a) evaluate genome editing assay pipelines; b) develop benchmark materials; c) generate benchmark data; d) develop suggested minimal information reporting for public studies and e) generate a common lexicon for genome editing studies. The standard needs identified by the consortium include off-target activity, genome variants generated, genome editing components needed for manufacturing, evaluate and compare delivery system. Genome editing outcomes are complex. FDA requires reporting off-target genomic positions as well as the frequency of off-target and on-target occurrences. Assays have been developed for off-target activity as well as targeted assays, control samples and the use of inter-lab studies. Issues around collection of data and metadata are also being discussed. The file format for data should be human readable, database ready, can be validated and used by NIST. The lexicon working group is identifying terms to facilitate the conversation. This includes genome editing concepts, tools and outcomes. There is an ISO standard being worked on. It was started in 2018 and proposed to be a standard in 2019. More information about the consortium may be found at: The Genome Editing Consortium | NIST.

Detection of genome edits (R. Shillito, BASF): A paper has been published entitled "Detection of genome edits in plants—from editing to seed" by R. Shillito, et al., 2021, In Vitro Cellular & Developmental Biology – Plant (Detection of genome edits in plants—from editing to seed (springer.com)). Genome editing is just another breeding tool. The challenge is just getting the changes into plant cells. The regulatory environment for genome edited products is variable globally. The UK recently declared some new

guidance. Since grain is imported/exported around the world, regulatory systems have to respond and one of the criteria is how to detect the edits. Large insertions can be detected by PCR. Small insertions/deletions are more complicated to detect, especially in bulk samples. PCR detects DNA so the question is what does a single bp edit look like to a PCR primer, i.e., how to predict if the primer will recognize? There are very few publications on this. Initial publications indicate that the initial PCR reaction informs about base pair change. Reactions beyond this look all the same. Modified nucleic acids can be used to increase base differentiation in PCR by binding specific sequence. Detecting large edits is similar to detecting DNA inserts. Small edits are difficult to detect, especially in bulk samples. Sequencing is useful for individual plants. Amplicon-based sequencing is used for smaller regions. Ligation mediated sequencing is used for larger indels. Sequencing also has signal to noise issues and is not sufficient for bulk samples. Methods are being developed using isothermal technology, CRISPR detection and targeting by Cas enzymes. Isothermal methods can be used in non-lab (field) situations. The choice of method depends on the edit. Off-target edits are a low concern in plants since off-type plants can be discarded. Cannot do this with humans. Also plant breeding, via backcrossing, is used to remove off-types in a plant population. DNA sequence changes occur in nature. Single base deletions and rearrangements and additions are constantly occurring. Transposons are extremely active in some species such as maize. The ENGL (European Network of GMO Laboratories) report concluded that changes can be detected but these changes cannot be discriminated as to whether they are natural or genome edits. Detection does not equal identification. Challenges for detection of genome edits: capacity limitations, costs; several approaches used to detect and most employ PCR steps; preferred detection tool depends on specific context to be used; small edits cannot e distinguished.

Selecting molecular marker variety identification panels and sub-sets for quality assurance and intellectual property enforcement (B. Nelson, Corteva): Single nucleotide polymorphisms (SNPs) are useful to characterize germplasm, manage costs, increase throughput of samples, manage quality assurance and support intellectual property rights (IP). SNPs can be selected by variety identification applications and genetic similarity applications. As an example for soy, use variety identification for selection of SNPs and use similarity comparisons in soy to select a minimum number of SNPs. For variety identification, the data set was BARCSOY6K SNP and 276 varieties. The algorithm used is for "uniqueness" or better known as the "traveling salesman algorithm" (select most efficient route while visiting each city one time). For markers, this means selecting the fewest number of markers to differentiate the most varieties. In the variety identification process, either generate or access fingerprint data. Breeder input to select varieties is important for elite varieties. Data is put into program to select the SNP panel to differentiate the 276 varieties. The number of markers is increased to differentiate a variety each time. The program was also run with missing data levels (up to 40%). The SNP markers were still identified. The computer program is available as open-source software at Corteva Agriscience · GitHub. For the SNP selection for similarity comparisons, a 6000 SNP data subset from the 50,000 soybase data set and 322 soy varieties were used. The 6000 genetic map positions were maintained even with SNP distribution across the genome. The expected heterozygosity was also

calculated. The expected heterozygosity was maintained at 0.357 across all marker sets. The genome coverage was maintained at >99%. Pairwise comparisons yielded a good correlation with the full data set. In summary, 6000 SNPs work well as a starting point to subset SNPs or variety identification and similarity comparisons in soy. Uniqueness is effective for selecting variety identification SNPs and is robust in the case of missing data. Maintaining the expected heterozygosity and even genome coverage is an effective method to select more efficient marker sets for similarity comparisons.

Next generation sequencing in next generation agriculture (F. Ghavami, Eurofins BioDiagnostics): Multi-omics technologies are being used to move crop breeding and management to next generation agriculture. The international phenotyping network synchronizes activities and creates databases. There is a need for artificial intelligence (AI) to make sense of the data captured by drones, field observation and greenhouse observation. Next generation sequencing (NGS) was commercially available in 2005 and produced a lot of data. The third generation of NGS is focused on single molecule long reads or reducing the cost of sequencing. NGS is used for whole genome sequencing, either de novo or resequencing uses. It can also find sequence variants to be used for marker discovery, gene discovery and genome structure variations. Transcriptomic sequencing can use SAGE, microarrays, DD-AFLPs. RNA sequencing is a new tool. NGS is also used in genotyping by sequencing. Genotyping by sequencing can be restriction enzyme mediated in which the enzyme fragments are amplified by primers. Another method is hybridization based targeted sequencing where probes are attached to beads and then sequenced. The genome fragments are generated by physically or enzymatically fragmenting the genomic DNA to small fragments. Amplicon-based targeted sequencing results in hundreds of regions in the genome being amplified using multiplex PCR. The PCR fragments are then sequenced. Skim sequencing is a substantial reduction in cost. It is shallow sequencing by a coverage of less than 1X or less. Enough fragments provide the markers. It is an alternative to array-based genotyping when >50,000 markers are needed. NGS applications include whole genome sequencing, 16S/18S/ITS sequencing, metagenomics (shotgun sequencing) and single cell sequencing. In summary, NGS has changed agriculture. The cost reduction in NGS has made it a proper tool for different applications. Genotyping by sequencing is now the main tool for ultra-high throughput screening of >50,000 markers. Microbiome studies using whole genome or targeted sequencing shed light into a new definition of plant and animal health management and host environment interactions.

Development and commercialization of AquAdvantage salmon (M. Walton, AquaBounty): AquaBounty is located in Maynard, MA and has farms in Albany, IN and Prince Edward Island, Canada. The AquAdvantage salmon are triploid females having a single copy of GH-1 gene from Chinook salmon and a promoter from Ocean Pout AFP gene. AquaBounty farms salmon on land in controlled disease-free tanks which is efficient production since it is near the consumption (local delivery of fresh salmon). The tanks are recirculating aquaculture systems (similar system to water treatment plants). Other salmon is farmed in ocean tanks (significant amount comes from Chile). Ocean tanks have environmental and safety issues and an inefficient supply chain. The salmon

have to be exported and air-freighted to consumers and they also have a reduced shelf life. AquaBounty is planning to expand to a farm in Pioneer, OH in 2023 with additional sites in the US and Canada in the future. The company is targeting Israel for international expansion. The AquAdvantage salmon is approved in Brazil for consumption (no production in country). AquAdvantage salmon were first submitted to FDA in 2003. In 2010, the salmon were found to be no different from other salmon and in 2015, the salmon were approved for consumption in the US. In 2016, approvals for consumption and production were obtained from Canada. In 2019, salmon eggs were introduced in the US from Canada. The salmon carry the Chinook salmon gene and the Ocean Pout promoter which is believed to keep the fish feeding which promotes their growth. The salmon grow faster, not larger and are feed efficient. Broodstock is masculinized transgenic females. PCR is used to confirm triploidy and the genotype of the broodstock. In the US, the salmon is regulated by FDA as a new animal drug. Data submitted included human food safety, animal health and safety and environmental risk. In Canada, the salmon are regulated under Environment and Climate Change Canada, Health Canada and Canada Food Inspection. Only fillets can be sold in Canada for consumption at this time. Regulators asked questions about direct and indirect toxicity to the animal, whether salmon had hazardous phenotype, risks to user (those who rear the salmon), risks to animal from the biological containment, durability (genotype/phenotype, lifespan) and any effects on human health (equivalency to other salmon, changes to composition, endogenous allergens, survival if escape). The risk assessment finds were that the salmon are the same as other salmon, no risk to health or well-being due to the presence of the transgene, genotype and phenotype are stable, composition, hormones, endogenous allergens no different and risk of escape is low. The conditions of use include land-based culture, must be all female fish, must be cultured from egg to harvest in fresh water. New farms all need approvals. Initial filings have been done in China and Israel.

Revisiting a cold case: Devitalization of germinated seedlings by freezing (M. Gillen, Eurofins): Seedling devitalization is important when discussing a process for regulated or stewarded material. Compliance packets have a typical list of approved methods for devitalization which includes grinding, heat or steam treatment, incineration, composting, bleach treatment or deep burial. The challenges include demonstration of devitalization, necessary equipment and calibration, routine verification and certification, time and resources to conduct devitalization and capacity to do it. The freezing method reduces the challenges with capacity and equipment and allows for high throughput for disposal of germinated seedling such as corn. The type of sample testing often determines the method of devitalization. PCR poot testing requires the grinding of seeds into powder. For germination testing, canola seed is steam treated to devitalize whereas the evaluated corn germinated material is put into bags to devitalize. Ungerminated seeds are physically devitalized. In ELISA, the extraction of the protein devitalizes the seed. In trait purity testing, ungerminated seed is destroyed physically and germinated is put into bags for devitalization. A validation method was set up for the freeze method for devitalization. The design included corn, cotton and soy which were found to be devitalized after 3 days in -20C. All regulated material handling procedures were adhered to. Defrosted samples (after 72 h in freezer) were

placed in buckets and given water to facilitate growth and then placed in a light chamber. No sign of vitality was observed. Thus, there was no seedling survival in the 3 crops. Hard or ungerminated seed will need to be removed and devitalized in a grinder or another method. A proposal is moving through ASTA in support of submission to USDA/Seed Science Foundation.

Seed quality testing: Seed Science Foundation objectives (D. Miller, Illinois Crop Association): The Seed Science Foundation is a new organization which was formed by two legacy organizations came together. The mission of SSF is to address seed and plant science challenges and encourage plant breeding education and seed research. It is a proactive and integrated source for seed and recommends solutions. The Board has 13 members serving 3-year terms. There are also 4 ex-officio members from the ASTA staff. SSF has six subject matter areas that have been designated as significant importance to SSF members: breeding systems, seed quality, seed production and technology, seed health and pathology, digital agriculture and seed applied technology. For seed quality and testing, an effective decision tool for seed testing must deliver accurate assessment of seed viability based on a representative sample. SSF projects include seminars sponsored at UC Davis, APHIS funded studies for weedy hosts of cucumber green mottle mosaic virus, seed coat permeability and now considering a project on freezing validation study. Also supplying outreach to convey the importance of seed quality testing. More information about SSF can be found at Seed Science Foundation.

AEIC Fall 2021 Attendees:

Name	Organization
Ament, Chris	ECT
Atkinson, Tara	Corteva
Balvin, Kevin	SGS
Bedair, Mohamed	Bayer
Bednarcik, Mark	Syngenta
Benatti, Matheus	IN Crop Imp Assn
Birukou,Ivan	Syngenta
Bohnker, Laura	EBDI
Boico, Irina	Syngenta
Brix, Kalyn	SoDak Labs Inc
Brune, Phil	Syngenta
Brustkern, Sarah	Corteva
Bryenton, Matt	AquaBounty Canada
Cheever, Matt	BASF
Corea-Gomez, Christian	IN Crop Imp Assn
Culkin, Chris	Agdia
Cummings, Simone	Syngenta
Dharmasri, Cecil	BASF
Dreesen, Rozemarijn	BASF
Duray, Zach	IL Crop Imp Assn
Fast, Brandon	Corteva
Fendley, Ann	BASF
Fisher, Ashley	Simplot
Fu, Huihua	BASF
Fung, James	IN Crop Imp Assn
Gabriel, Adam	EFCT
Gadola, Mary	Neogen
Geng, Tao	Bayer
Ghavami, Farhad	EBDI
Ghoshal, Durba	BASF
Gillen, Maranda	EBDI
Gillikin, Nancy	BASF
Goddard, Terry	EnviroLogix
Haudenshield, James	Merieux
Helm, Jennifer	Eurofins
Herrero, Sonia	Syngenta
Houchins, Donna	Romer Labs
Houston, Norma	Corteva
Hunst, Penny	BASF

Islam, Shofiqul	IN Crop Imp Assn
Jackson, John	LGC, Biosearch Tech
Johnson, Brenda	EBDI
Komorek, Jessica	Bayer
Kouba, Kristen	Corteva
Kumar, Santosh	BASF
Lang, Tieming	Bayer
Larue, Dustin	Eurofins
Lawal, Remi	Bayer
Liu, Lucy	Bayer
Makani, Mildred	Syngenta
March, Chantal	AquaBounty
Muschinske, Luke	EML
Nelson, Barry	Corteva
O'Grady, John	Corteva
Poe, Martha	BASF
Rambow, Dave	Agdia
Salazar, Melissa	IL Crop Imp Assn
Sathischandra, Sashi	BASF
Scaife, Ann	EFII
Schafer, Barry	Schafer Scientific Solns
Secrist, Heather	FoodChain ID
Shippar, Jeffrey	Eurofins
Smith, Dan	FoodChain ID
Supekar, Nitin	Bayer
Temple, Stephen	Forage Genetics
Tetteh, Afua	BASF
Trombley, Arthur	EnviroLogix
Walton, Mark	AquaBounty Technologies
Wang, Kelin	Bayer
Wang, Rong	Bayer
Wang, Yanfei	Bayer
Wang, Yongcheng	Bayer
Waxdahl, Heather	SGS
Whitt, Sherry	BASF
Williams, Denise	AOCS
Wu, Pei-Ying	BASF
Xia, Min	BASF
Zhang, John	Corteva
Zheng, John	IN Crop Imp Assn