Soybean Genomics Research Program Strategic Plan: Implementing Research to Meet 2012-2016 Strategic Milestones

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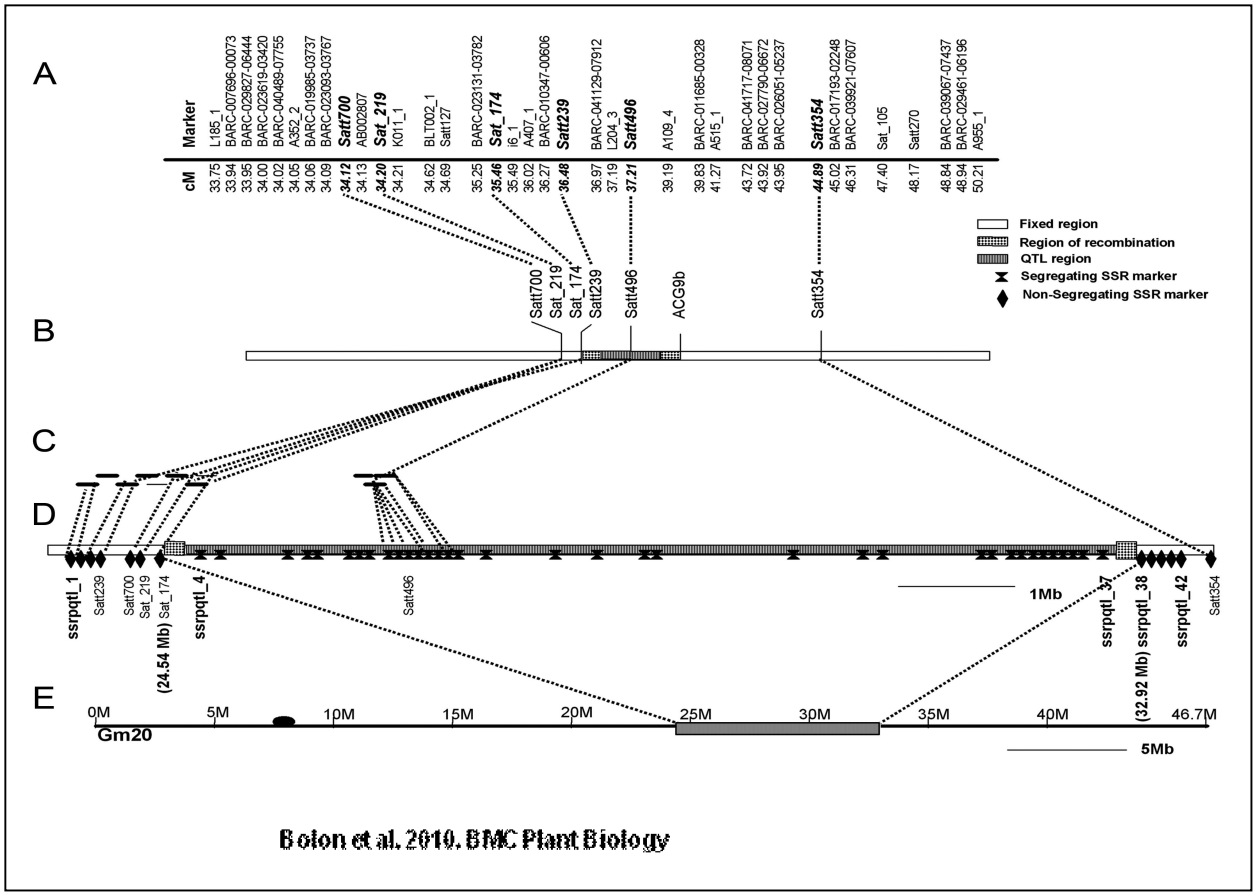

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Executive Summary

This strategic plan builds on the soybean communities' previous efforts (October, 1999; July, 2001; May, 2003; July, 2005; and May, 2007) to review progress on the development and deployment of soybean genomic resources. The results are impressive (see *Soybean Genomics Research Program Accomplishments Report, 2010*, posted on SoyBase). For example, in the last five years the soybean research community has produced a genetic linkage map with over 5,500 mapped markers spanning the entire 2,296 cM soybean genome. A set of 1,536 SNP markers that are evenly distributed across the 20 linkage groups was developed for whole genome analysis of polymorphisms in both elite North American cultivars and breeding lines. In addition, an expanded array of 50,000 SNPs is under development which will be used to create haplotype maps of over 18,000 accessions of the USDA soybean germplasm collection. This research is scheduled for completion in late 2010 and the SNP haplotype map of each accession will be placed on the HapMap Browser on SoyBase.

Large-scale shotgun sequencing of the soybean cultivar Williams 82 was completed late in 2008 by the U.S. Department of Energy Joint Genome Institute (DOE-JGI) and recently reported in the scientific journal *Nature* (Schmutz et al., 2010. *Nature* 463:178-183). The present soybean assembly (Glyma.1.01) captured approximately 975 Mbp of its 1,100 Mbp genome. The gene set integrates ~1.6 million ESTs with homology and predicts 66,153 protein-coding loci available at www.phytozome.net/soybean.

Soybean researchers have developed several microarray technologies for gene expression studies. The GeneChip® Soybean Genome Array is commercially available for studying gene expression (http://www.affymetrix.com/products_services/arrays/specific/soybean.affx#1_1). This GeneChip contains 37,500 *Glycine max* transcripts, 15,800 *Phytophthora sojae* transcripts, and 7,500 *Heterodera glycines* transcripts.

The achievement of milestones in previous strategic plans for soybean genomic research have advanced soybean to its current status as a crop model for translational genomics. Simply stated, soybean genomic resources in hand will accelerate the ability of plant breeders to enhance soybean productivity, pest resistance, and nutritional quality. However, many secrets of the soybean genome have yet to be revealed. In order to continue to make informed decisions it was critical to capture the consensus wisdom of leading soybean researchers on the next logical steps in the development and utilization of soybean's genomic resources.

On 27-28 July 2010 Roger Boerma chaired a workshop sponsored by the United Soybean Board in St. Louis MO that brought together 44 eminent soybean researchers in the areas of genomic sequencing, gene function, transformation/transgenics, and translational genomics. The purpose of the Workshop was to develop a strategy for achieving the critical soybean genomic resources and information required to accelerate the rate of yield gain and addition of value to U.S. soybean cultivars. A consensus was reached on a number of high priority performance measures or research objectives. In addition the anticipated outcomes of successfully achieving these performance measures are included in the final plan.

In summary, two issues emerged as being critically important or overarching issues: i) Provide additional support staff for continued development and population of SoyBase, and ii) Development of a genetic repository/ distribution center for soybean mutants/transgenic lines. The enhancement of SoyBase was deemed important for all four Strategic Goals. The genetic repository/distribution center was broadly supported by Workshop participants. Listed below is an outline of the four Strategic Goals and their respective Performance Measures. Within each Goal, the Performance Measures are listed in order of importance.

Goal 1: Genome Sequence: Improve the quality and utility of the soybean genome sequence

Performance Measure:
1.1: Ensure the accuracy of reference sequence assembly.
1.2: Capturing and leveraging existing genetic diversity in soybean germplasm.

1.3: Improving bioinformatic resources for genomic analysis and practical applications.
1.4: Reveal function of targeted genome sequences to facilitate gene discovery and application.
1.5: Leveraging genomic information from Phaseoloids and other species.
1.6: Determine the role of epigenetics in soybean improvement.

Goal 2: Gene Function: Develop functional genomic technologies to optimize utility of genome sequence information in germplasm enhancement

Performance Measure:
2.1: Develop comprehensive gene expression data for soybean.
2.2: Develop near isogenic lines (NIL) to help reveal genetic mechanisms that mediate useful traits.
2.3: Develop an improved infrastructure to facilitate genome annotation.
2.4: Achieve high-definition genomic characterization of biological mechanisms and regulatory systems in soybean.
2.5: Use functional genomic methods to characterize transcription regulated pathways.
2.6: Advance gene modification technologies to help associate candidate genes with a discrete phenotype.
2.7: Create a saturated transposon insertion population with defined flanking sequences that can be used to identify mutants by BLAST sequence comparison.
2.8: Implement outreach opportunities for education and use of genomic databases.
2.9: Develop an ORFeome library from agronomically important genes and gene families.

Goal 3: Transformation/Transgenics: Optimize and expand transgenic methods and improve understanding of natural genes for modification of trait expression

Performance Measure:
3.1: Establish of a soybean genetic repository and distribution center.
3.2: Develop next-generation transformation and targeting technologies and utilize these transgenic approaches to help elucidate gene function and deploy genes of interest.

Goal 4: Translational Genomics: Optimize breeding efficiency with robust sequence-based resources

Performance Measure:
4.1: Develop analytical approaches to characterize soybean germplasm diversity based on the SoyHapMap 1.0 data to identify parental lines for breeding purposes.
4.2: Discover gene/QTL for qualitative traits and develop tightly linked DNA markers.
4.3: Discover gene/QTL for quantitative traits and develop tightly linked DNA markers.
4.4: Develop and populate a user-friendly database of validated QTL for use in marker assisted breeding applications.
4.5: Define the molecular genetic signatures of selection in 70+ years of U.S. soybean breeding by use of the 50,000 SNP Illumina Infinium Assay
4.6: Define optimum breeding models for different breeding situations using *in silico* analysis.

Our Collective Wisdom: How We Got Where We Are

The advent of a chromosome-scale draft assembly of the soybean (*Glycine max* L. Merr.) genome did not spring up overnight like some magic beanstalk. Rather, it is an outcome of a dynamic, technology driven, and timely strategic process whose origin may be traced formally to the *Soybean Genomics White Paper* [Boerma, H.R., D. Buxton, M. Kelly, K. Van Amburg, Soybean genomics white paper January 2000, http://soybase.org/Genomics/Soybean_Genomics.html (2000)]. That January 2000 document was a product of an October 21-22, 1999 meeting of seventeen experts in plant genomics, DNA markers, plant transformation, and bioinformatics. The workshop was planned by: Dr. Dwayne Buxton, National Program Leader for Oilseeds & Biosciences, USDA Agricultural Research Service; Dr. Roger Boerma, Distinguished Research Professor and Coordinator of the Center for Soybean Improvement, University of Georgia; Maureen Kelly of AgSource, Inc., a subcontractor with the United Soybean Board focusing on Federal Research Coordination; and Kent Van Amburg, Production Committee Manager, United Soybean Board. Elizabeth Vasquez of MCA Consulting facilitated the workshop. A consensus was reached on research priorities in the area of soybean genomics. Milestones included: 1) doubling Simple Sequence Repeat (SSR) markers to 2000 within 3 years; 2) expansion of Single Nucleotide Polymorphism (SNP) markers to 10,000 within three to five years; 3) improving the efficiency of soybean transformation by five- to ten-fold in three years; 4) tagging 80% of the genes in the soybean genome within three to five years; 5) integration of genetic, physical and transcript maps of soybeans within three to five years; and 6) employing comparative genomics to define the structure and attributes of the soybean genome.

However, rising scientific enthusiasm for genomic investigations among living organisms also created an extraordinarily competitive environment for appropriations to finance these rather expensive ventures. Therefore it became mutually beneficial to establish research coalitions to improve the efficiency of genomic investigations among related species. For this reason, the U.S. Legume Crops Genomics Initiative (LCGI) was organized under the auspices of the American Soybean Association, United Soybean Board, National Peanut Foundation, USA Dry Pea and Lentil Council, the National Dry Bean Council, and the Alfalfa Council to facilitate communication and cooperation among scientists with an interest in genomic research on soybean, peanut, pea and lentil, common bean, alfalfa, and model-legume crops. LCGI was founded on the premise that the development of an integrated legume genomics research system would enhance ability to leverage information across legume crops and model species. The first U.S. Legume Crops Genomics Workshop was convened on July 30-31, 2001 at Hunt Valley, Maryland by: H. Roger Boerma, University of Georgia; Judy St. John, Associate Deputy Administrator, USDA Agricultural Research Service; Jennifer Yezak Molen, AgSource, Inc., and was hosted by the USB, the National Peanut Foundation, the USA Dry Pea and Lentil Council, and the USDA-ARS. Twenty-six legume scientists skilled in relevant arts developed a white paper [Boerma, H.R., J. St. John, and J. Yezak Molen, U.S. legume crops genomics workshop white paper, http://www.legumes.org/ (2001)] that outlined high-priority research in the areas of: 1) genome sequencing of strategic legume species; 2) physical map development and refinement; 3) functional analysis: transcriptional and genetic; 4) development of DNA markers for comparative mapping and breeding; 5) characterization and utilization of legume biodiversity; and 6) development of a legume data resource. The nature of this cooperative interaction not only ensured timely research progress in all legume crops associated with the Initiative, but also enhanced the competitive position of the LCGI within the framework of the

National Plant Genome Initiative, which is coordinated by the Interagency Working Group on Plant Genomics, Committee on Science, National Science & Technology Council.

Implementation of a coordinated effort for research & development of genomics across the legume family facilitated progress in the model species *Medicago truncatula* and *Lotus japonicus* and in soybean (*Glycine max*); and accentuated the need to transfer genomic information from the model species to cool-season pulses [pea (*Pisum sativum*), lentil (*Lens culinaris*), chickpea (*Cicer arietinum*), field bean (*Vicia faba*)], and warm-season food legumes [peanut (*Arachis hypogea*), common bean (*Phaseolus vulgaris*)], and forage legumes [alfalfa (*Medicago sativa*), clover (*Trifolium* spp.)]. This mission was codified further by 1) a third white paper, entitled: *Legumes as a Model Plant Family: Genomics for Food and Feed*; and 2) by the publication of the monograph, *Legume Crop Genomics.*

The white paper was an outcome of the CATG (Cross-legume Advances Through Genomics) conference on December 14-15, 2004 in Sante Fe, NM (http://catg.ucdavis.edu) which was organized by the LCGI steering committee: Charlie Brummer (alfalfa), Paul Gepts (beans; chair), Randy Shoemaker (soybean), Tom Stalker (peanut), Norm Weeden (cool-season legumes) and Nevin Young (model legumes). In addition, Bill Beavis served as a bioinformatics resource and funding was provided by the National Science Foundation (Plant Genome Research Program) and the USDA (National Research Initiative). About 50 individuals in attendance represented the respective legume communities as well as various funding agencies. The objectives of the conference were to: (1) identify a unifying goal for an international cross-legume genome project; (2) identify cross-cutting themes to help integrate the different legume crop genomics programs, including a unified legume genomics information system, nutritional and health-related aspects of legumes, and detailed synteny and comparative genomics of legumes; and (3) outline specific components and milestones for the initiative. These deliberations identified four tiers of legume species, each with specific genomic resources to be developed. Based on phylogenetic arguments, and particularly the degree of synteny, two major foci of legumes were identified, the hologaleginoid clade or cool-season legumes and the phaseoloid/millettioid clade or warm-season legumes. In each of these two foci, one or two reference species were identified, *M. truncatula* and *L. japonicus* in the former and soybean in the latter. Development of a full range of genomics resources, including sequencing of the entire genome, was the highest priority for these reference species. For a second group, common bean and peanut, a broad range of genomic resources were recommended, including a physical map, BAC-end sequencing and marker development, anchoring of the genetic and physical map, ESTs of the major organs, chip resources, and sequencing of gene rich regions. A third group consisted of all other legume crops in the two foci, including pea, lentil, chickpea, field bean, clover, cowpea (*Vigna unguiculata*), and pigeon pea (*Cajanus cajan*). For these legumes, translational genomic tools were to be developed, principally for cross-legume markers, species-specific recombinant inbred lines, genetic maps, EST and BAC libraries. A fourth group included other legumes not in the two main foci, such as members of the basal legume clades. An abbreviated version of this white paper was published. (Gepts, P., W.D. Beavis, E.C. Brummer, R.C. Shoemaker, H.T Stalker, N.F. Weeden, and N.D. Young. 2005. Legumes as a model plant family. Genomics for food and feed Report of the Cross-Legume Advances through Genomics Conference. Plant Physiol. 137:1228-1235).

Publication of *Legume Crop Genomics*. (2004. eds. E.C. Brummer, H.T. Stalker and R.F. Wilson, AOCS Press, Champaign) helped the LCGI document in a unified manner the initial research strategies, the development of genomic tools and resources, and future direction of the legume research community. In addition, to establishing a benchmark for then state-of-art technology, this volume presented technical themes in a manner that helped many readers gain a more informed opinion of plant genomics. In that regard, the chapter by Gary Stacey presented

an inventory of the available resources for soybean genomics: 1) Genetic map. The soybean composite genetic map was well developed. The classical map contained 63 loci on 19 linkage groups, while the molecular map encompassed 20 linkage groups and over 2300 cM based on 600 SSRs mapped in 6 populations (http://soybase.org/). In addition, the composite map contained >800 RFLP markers, >600 AFLP markers, and hundreds of RAPDs; 2) BAC libraries. A number of public-sector BAC libraries were available providing >35-fold genome coverage. For example, a 4-6 X Forrest BAC library was used to construct a physical map. Similarly, a 5 X (~130 Kbp, HindIII inserts) library existed for the cultivar Williams 82; 3) Physical map. A double-digest-based map for the cultivar 'Forrest' was constructed (http://soybeangenome.siu.edu/), on which work continued to reduce the number of contigs (presently >3,000 contigs) and to add additional DNA markers (e.g., through BAC-end sequencing); 4) EST sequences. Over 300,000 soybean EST sequences were deposited in Genbank (http://soybase.org/soybeanest.html); 5) Functional Genomics. Lila Vodkin's laboratory (Univ. of Illinois) used ESTs to develop DNA microarrays for functional genomic analysis. Proteomic efforts were underway to apply translated EST sequences to protein identification (e.g., Wan et al., unpublished); 6) Soybean transformation and mutagenesis. Contrary to a NRC report (http://books.nap.edu/books/0309085292/html/R1.html), soybean transformation efficiencies were consistently >5% and, in some labs, efficiencies >12% were common. These improvements in transformation efficiencies led to development of transposon tagging projects for soybean, viral-induced gene silencing systems and TILLING populations for soybean. Soybean also had over 300 phenotypic mutants; some of which were leading to marketable traits (e.g. modified oils, low phytate); 7) Phenotype analysis. Soybean biochemistry, physiology and agronomic knowledge of soybean far exceeded that of any model legume. QTL discovery for production, protection and quality traits were integrated with the genetic and physical maps. These genomic resources firmly underpinned the status of the Soybean research community and helped broker international collaboration in soybean genomics with scientists in Japan, China, Korea, and various countries of South America and Europe. In addition, forums such as the biennial Cellular and Molecular Biology of Soybean Conferences helped improve communication on an interdisciplinary level.

As each of the legume crop communities began to amass critical genomic resources, it became necessary for each community to develop priorities that were crop specific. The strategic foundation for soybean genomics research was laid on May 20-21, 2003, when nineteen researchers participated in a workshop convened by Diane Bellis (AgSource Inc.), Roger Boerma (University of Georgia), Ed Ready (United Soybean Board), Richard F. Wilson (USDA, ARS National Program Staff), and hosted by the United Soybean Board Production Committee in St. Louis, MO. The scientists reviewed the current status of soybean genomic research and reached consensus on a strategic framework for outlining research priorities and significant near-term milestones. This input was captured in the *Strategic Plan for Soybean Genomics 2003-2007*. (http://soybase.org/SoyGenStrat2005/Soy_Genome_Strat_Plan_2005.html) Coordination of plan implementation was delegated through election of the Soybean Genetic Executive Committee (R.C. Shoemaker, P.B. Cregan, W. Parrott, H.R. Boerma, J. Orf). The SoyGEC took action to move forward aggressively on:1) fingerprinting Williams 82 BACs and physical map compilation (Jan Dvorak, Gary Stacey, Henry Ngyuen, David Lightfoot, David Grant, Randy Shoemaker); 2) anchoring ESTs and Williams 82 BACs on the genetic map and enhancing physical map resolution (Perry Cregan, Jim Specht, Randy Shoemaker, Scott Jackson, Brian Scheffler); 3) manual annotation of Williams 82 BAC fingerprints (Gary Stacey, Henry Ngyuen); 4) gene-specific oligonucleotide resources and 3' EST sequences for microarray and bead technology (Lila Vodkin); 5) development of a TILLInG resource (Niels Nielsen, Scott Jackson, David Lightfoot); and 6) RNAi technology for specific gene mutations (David

Sommers, Wang, Tom Clemente, Wayne Parrott, John Finer, Zhang, Gary Stacey, Henry Ngyuen).

The SoyGEC (http://soybase.org/resources/soygec.php) also initiated strategies to proactively communicate soybean research community priorities to representatives of federal granting agencies. Without encumbering individual initiatives, SoyGEC encouraged coordination of dedicated research teams to solve soybean problems of national and international importance. A National Science Foundation-sponsored workshop was held in St. Louis on October 21, 2003 to take an inventory of the current genomic resources in soybean, identify areas where more data were needed, and to set a research a strategy to advance soybean genomics research. Special attention was focused on research opportunities provided by unique aspects of soybean biology. The workshop included academic, governmental, and industrial scientists covering a wide variety of specialties related to both basic and applied research on soybean, along with scientists from outside the soybean field who provided general expertise in genomics and represented a wealth of experience garnered from other genomic projects. Representatives from federal funding agencies and soybean commodity groups observed the meeting. This workshop extended and further defined the findings from earlier workshops, which had surveyed the status and priority goals for soybean & legume genomics (http://soybase.org/Genetic_Resources/Soybean_Genetic_Resources.html; and http://soybase.org/Legume_Initiative/LegGenomicsPaper10Oct01.html). The proceedings for the October 2003 workshop *Genomic Perspectives of Soybean Biology* were published. (Stacey, G., L. Vodkin, W.A. Parrott, and R.C. Shoemaker. 2004. National Science Foundation-Sponsored Workshop Report. Draft Plan for Soybean Genomics. *Plant Physiology* 135:59-70).

Action to address the need for bioinformatic resources was taken by the National Center for Genome Research (NCGR) and USDA, ARS, NPS when the U.S. Congress established the Model Plant Initiative (MPI) to use bioinformatics to leverage genomic information from model plant species. The Legume Information System (LIS), a joint NCGR and ARS effort, was one of the first projects in support of the MPI to demonstrate the integration of genomic information from *Arabidopsis thaliana*, *Medicago truncatula* and *Lotus japonicus* to important agronomic legumes, e.g., soybean, alfalfa, pea, dry beans.

Since its inception, LIS development has been guided by ideas and suggestions from the legume research communities. Based on user needs, USDA-ARS and NCGR developed a plan to amplify the power of this information resource for identification of candidate genes, unique genes, and evolutionary relationships among genes for crop improvement. Bill Beavis, Randy Shoemaker, and Rich Wilson presented details of the *Strategic Plan for Comparative Legume Biology 2005-2010* to NCGR and ARS leadership on January 12, 2004 in San Diego CA. It was agreed to utilize LIS as a foundation for a Comparative Legume Biology (CLB) Program. CLB expanded LIS from a passive data management system to a platform for novel data analysis and visualization tools. With the emergence of high throughput biotechnologies and bioinformatics, comparative biology enabled more sophisticated analyses of genomic sequences, genomic maps, micro-arrays, protein arrays, metabolic arrays, genetic regulatory networks, biochemical and whole organism phenotypes.

The SoyGEC convened the first assessment of research performance against the *Strategic Plan for Soybean Genomics 2003-2007* on July 19-20, 2005 in St. Louis, MO. Jim Specht, SoyGEC chair, along with Randy Shoemaker, Wayne Parrott, and Henry Nguyen organized the meeting that included approximately 50 researchers and administrators. Presentations provided updates on the current status of soybean resources and related genomics technologies. Stakeholder input was generated in facilitated discussion groups to assess the status of soybean genomics, identify needs, and identify milestones to achieve objectives. The discussion groups included the general

areas of Functional Genomics A (Transcriptome and Proteome), Functional Genomic B (Reverse Genetics), Physical and Genetic Maps, and Bioinformatics. Several topics received overwhelming support in all group reports. A high quality physical map in Williams 82 and integration with the physical map of Forrest was a very high priority. A whole-genome sequence was an expectation of the research community. The need for standardization of protocols, terminologies and ontologies was evident as interactions among groups and among research communities expand. Finally, there was urgent need to establish long-term facilities that have the capability to archive, maintain, generate and provide biological resources. Overall, there was consensus that the ongoing research was on target, if not ahead of schedule, and still relevant to the goals and objectives of the *Strategic Plan for Soybean Genomics 2003-2007*.

On January 20, 2006 the USDA and DOE announced plans to share resources and coordinate the study of plant and microbial genomics. The soybean genome was first on the list for sequencing. Dr. Ari Patrinos, DOE Associate Director for SBER, directed the Joint Genome Institute (JGI) to carry out the work with the Stanford Human Genome Center (http://www.energy.gov/news/2979.htm).

A Genome Strategic Planning Workshop to consider research goals and priorities for the next 5-year programmatic cycle was convened by the SoyGEC in St. Louis, MO on 30-31 May 2007 by James Specht (University of Nebraska-Lincoln). This workshop was attended by 48 experts in diverse genomic areas. Stakeholders included the USB, the North Central Soybean Research Program, and one soybean producer. Whereas previous soybean strategic plans had dealt with preparation for the eventual sequencing of the genome, announcement that the soybean genome would be sequenced by DOE-JGI necessitated a reassessment of strategic objectives for the next research plan. Indeed, Jeremy Schmutz, Stanford, the leader of the DOE-JGI soybean sequence assembly effort reported that the work was proceeding exceptionally well, despite the ancient polyploidy of a now well-diploidized soybean. A 4X shotgun genome sequence of the genome had been developed and a draft assembly had been created. This assembly was being evaluated to determine the optimal means for obtaining the final goal of an 8X coverage. The 8X sequence was slated to be completed by the end of 2007, with a final assembly expected to be completed in mid-2008. Given this information, workshop participants adjourned to breakout sessions on: Soybean Genome Sequencing, Soybean Gene Function, or Soybean Germplasm Genomics. Group reports revealed that several objectives identified in the prior plan, such as delivery of large DNA constructs (BAC-sized) for genetic transformation, or a centralized tilling facility, were not deemed as high of a priority. Moreover, some 2005 Plan goals had not met milestones. This included the generation of large numbers of independent Tnt1 and Ds insertions for functional analysis of genes in soybean. Other 2005 Plan targets included methodical characterization of abiotic and biotic stresses using various expression, proteomic, and metabolomic approaches. These approaches had apparently not achieved the desired momentum. (likely due to limited funding). In addition, a gratifying number of objectives and milestones identified in the 2005 Plan had been achieved on schedule. The number of SNPs and STSs proposed for discovery and development was exceeded. Inbred mapping resources (RILs) were developed. Transformation technologies had improved and various gene knock-out systems were working. USB and NSF funding enabled work on physical and transcript maps. Bioinformatic resources and staffing had more than doubled, in time to receive the whole genome shotgun sequence. These technological developments and the diminishing cost of many genomic-based technologies were taken into full account in the development of the *Strategic Plan for Soybean Genomics 2008-2012*.

The SoyGEC reached out to colleagues abroad at the first meeting of the International Soybean Genome Consortium at he NIAS in Tsukuba, Japan on April 20, 2007. This ongoing association

has helped leverage resources for soybean genomic research in Japan, Korea, China, and the USA.

In February 2010, the SoyGEC and USB asked David Grant and Rich Wilson to prepare a report of program performance against objectives of the *Strategic Plan for Soybean Genomics 2008-2012*. The subject report, *Soybean Genomics Research Program Accomplishments Report: Meeting Strategic Milestones for 2008 to 2012 in a Timely Manner, 2010* documented accomplishments relevant to each performance measure of the plan. This information facilitated an assessment of program performance and helped guide the SoyGEC and their designees in developing the next 5-year strategic plan for soybean genomic research.

The current report summarizes the most recent strategic plan for soybean genomics research. This plan was developed at a workshop held on 27-28 July 2010 in St. Louis MO. The workshop was chaired by Roger Boerma and included 44 eminent soybean researchers in the areas of genomic sequencing, gene function, transformation/transgenics, and translational genomics. The purpose of the workshop was to develop a consensus strategy for achieving the critical soybean genomic resources and information required to accelerate the rate of yield gain and addition of value to U.S. soybean cultivars. This plan, *Soybean Genomics Research Program Strategic Plan: Implementing Research to Meet 2012-2016 Strategic Milestones* documents the high priority Performance Measures or research objectives, and anticipated outcomes of successfully achieving Strategic Goals for soybean genomic research in the next 5 years. Within each Goal, the Performance Measures are listed in order of importance.

Strategic Goals for Soybean Genomics Research (2012-2016)

Goal 1: Genome Sequence: Improve the quality and utility of the soybean genome sequence

Performance Measure 1.1: Ensure the accuracy of reference sequence assembly. Quality control is essential for useful reassembly of genome sequences. Accuracy of the reference genome impacts the effectiveness of all subsequent investigations of gene discovery, identification of gene function, comparative genomics, and characterization of the nature of genetic diversity in soybean. The first chromosome scale draft sequence of *Glycine max* is of very high quality. However, there are regions within the genome that remain ambiguous. These regions may contain genes that are important to soybean improvement. Therefore, research is needed to correct portions of the assembly.

Anticipated products:

- Closure of remaining gaps in sequence contigs/scaffolds.
- Correction of the order and orientation of improperly aligned scaffolds/contigs.
- Optical maps to orient contigs/scaffolds and estimate gaps sizes and place unanchored sequence contigs.
- Additional clone libraries targeted to span gaps and/or to help orient contigs/scaffolds.
- New sequencing technologies to generate long-range sequence reads.

Performance Measure 1.2: Capturing and leveraging existing genetic diversity in soybean germplasm. Genotypic diversity is the basis for genetic enhancement of soybean. Linkage disequilibrium and other statistical measures suggest that much of the variation for useful traits within the USDA germplasm collection remain untapped because of the difficulties in effectively identifying genetic differences. However, advances in DNA sequencing technology will enable the high-definition characterization of genotypic differences among germplasm accessions, cultivars, and breeding lines.

Anticipated Products:

- Resequencing a subset of the U.S. germplasm collection (including the ancestral land races) that captures greater than 90% of the genetic variation in *G. max* will access genetic diversity on a base-pair to base-pair level.
- Haplotype maps of the entire USDA *G. max* and *G. soja* collections to identify rare, potentially valuable genes/alleles.
- *De novo* sequencing (near reference level sequencing) of up to 10 selected *G. max* lines to estimate presence-absence variation (PAV), copy number variants (CNV), genes that are not present in the reference genome, and other structural variation.

Performance Measure 1.3: Improving bioinformatic resources for genomic analysis and practical applications. As a consequence of advances in sequencing technology, the amount of the soybean genomic and associated data is growing at exponential rates. Data storage limitations are now eclipsed by problems that limit the curator's ability to compile, analyze, and interpret data in a useful and timely manner. There is a need for expansion of an integrated database for use by all researchers, including breeders, to enhance the utility of genome sequence data.

Anticipated Products:

- Criteria for decision tools for parental selection and distinguishing genotypes during breeding population development. (See Goal 4)
- Software, protocols, and database support for sequence-based genotyping.

- Expansion of SoyBase to completely integrate all relevant information using almost any concept as an entry point. For example starting with a gene name or function, a user should be able to quickly find the position(s) of that gene on the genetic and sequence maps, information about that gene, and links to the scientific literature for more details.

Performance Measure 1.4: Reveal function of targeted genome sequences to facilitate gene discovery and application. High-definition mapping of DNA sequences in quantitative trait loci (QTL) enables the construction of allele specific markers for genetic traits. However, many genes may be present in a QTL region. Association of candidate gene sequences with a specific biological function or response not only facilitates marker development, but also expands knowledge of the biological mechanisms that mediate agricultural traits in soybean. Accurate annotation of the reference sequence is needed to improve the efficiency and utility of gene discovery.

Anticipated Products:

- Definition of entire gene families, 5' to 3', and alternative transcripts.
- Validated gene models with Transcription Start Sites (TSSs) for 20,000 genes.
- Refined accuracy of the transposon element database (TEdb) to recover genes embedded in transposable elements.
- Refined annotation of pseudogenes (e.g. truncated or early stop codons) that may have transcriptional evidence.
- Definition of regulatory elements (transcription factor binding sites, micro RNAs, etc.) that control gene expression.
- Annotation of known promoter elements for all identified motifs, prediction of unknown elements, and identification of co-regulated genes.
- Small RNA sequences from a range of tissues to understand the role of small RNAs in gene regulation.
- Functional annotation of soybean gene sets to facilitate gene discovery and genetic improvement.
- Collection and integration of functional information for all published gene mutant/ descriptions.
- Collection and integration of transcriptome data with genetic and genomic resources in SoyBase, the USDA-ARS soybean genetic database (http://soybase.org).

Performance Measure 1.5: Leveraging genomic information from Phaseoloids and other species. *Phaseolus vulgaris* (dry bean) and *Vigna unguiculata* (cowpea) are diploid species with synteny to soybean. *Phaseolus* and *Vigna* both diverged from *Glycine* about 19-23 MYA, and may be very useful for the discovery of genes for protection against abiotic stresses in soybean. Genome sequencing of both species is underway. The perennial *Glycine* species are also of interest because of the nature of polyploidy in *Glycine* as a whole, and thus for understanding the duplicated nature of the soybean genome. These species diverged from soybean (and its progenitor, *G. soja*) around 5 MYA .The perennial species constitute the secondary germplasm pool for soybean, and may provide traits such as drought tolerance and rust resistance.

Anticipated Products:

- Identification of genetic variability for nutritional traits in common bean and cowpea that may be applicable to soybean.
- Discovery of the genes or quantitative trait loci (QTL) with DNA markers to facilitate transfer to elite cultivars.
- Sets of SNP panels specific to appropriate populations of common bean and cowpea.

- Genetically and chromosomally stable interspecific populations between soybean and *G. tomentella* for resequencing and identification of diploids with resistance to pest and pathogens.

Performance Measure 1.6: Determine the role of epigenetics in soybean improvement. Epigenetics represents non-Mendelian inheritance of phenotypic traits. This area of plant biology is poorly understood. However, a growing body of knowledge suggests a hierarchical stratification in the regulation of gene expression that involves a complex interaction among gene products.

Anticipated Products:

- Enhanced knowledge base for understanding the role of epigenetics in mediating gene expression.
- Understanding the contribution of epigenetic phenomena to phenotypic diversity.

Goal 2: Gene Function: Develop functional genomic technologies to optimize utility of genome sequence information in germplasm enhancement

Performance Measure 2.1: Develop comprehensive gene expression data for soybean. Current annotation of the soybean genome suggests at least 45,000 protein encoding genes. While genome sequencing reveals all of the genes present within an organism, it cannot tell us what specific gene products are needed for different cellular pathways, tissues, or organs. For example, leaves and roots contain the same DNA, yet their very different structures are the result of differences in gene expression. It is laborious to examine the expression of genes on a gene-by-gene basis. Fortunately, new high-through-put sequencing platforms provide a rapid and sensitive means to survey gene expression. Limited soybean gene expression atlases are now available and have already been utilized to study the expression of some genes. However, these resources need to be expanded to include many more specific tissues and, more importantly, environmental treatments, especially those of agronomic importance (e.g., drought or insect predation). The availability of a comprehensive, soybean expression atlas that encompasses all such tissues and treatments would be an invaluable resource for the study of soybean gene function.

Anticipated Products:

- An improved soybean gene atlas developed from RNA-seq approaches, which includes a comprehensive list of all expressed soybean genes, alternative splice products, the identification of co-regulated genes and gene networks.
- RNA-seq data representing 100 different tissues and treatments.
- A standardized methodology for submitting data towards the whole soybean genome annotation effort.

Performance Measure 2.2: Develop near isogenic lines (NIL) to help reveal genetic mechanisms that mediate useful traits. The application of the full repertoire of functional genomic tools to soybean promises to yield new and important insights that can be mined for soybean improvement. However, these tools can be fully utilized only in well controlled experiments that minimize experimental variation, include replication, and provide rigorous statistical analysis. An important variable is genotypic variation. However, this can be reduced by the use of NILs varying only in key alleles that control important agronomic traits. The

availability of such NILs is essential for soybean to fully benefit from the modern molecular tools now available.

Anticipated Products:

- Development of collaborations between functional genomicists and breeders to identify traits.
- Develop 50 sets of NILs in the next two to five years for traits that NILs do not currently exist.

Performance Measure 2.3: Develop an improved infrastructure to facilitate genome annotation. SoyBase is a comprehensive repository for professionally curated genetics, genomics, and related data resources for soybean. SoyBase contains genetic, physical, and genomic sequence maps integrated with qualitative and quantitative traits. SoyBase also contains the "Williams 82" genomic sequence and associated data-mining tools. The genetic and sequence views of soybean chromosomes and the expansive data on traits and phenotypes are extensively interlinked. This allows entry to the database using almost any kind of available information, such as genetic map symbols, soybean gene names, or phenotypic traits. SoyBase is the repository for controlled vocabularies for soybean growth, development, and trait terms, which are also linked to the more general plant ontologies. Annotation of the draft chromosome scale sequence of the soybean genome with gene functions associated with QTL facilitates allele specific marker development. However, the utility of these resources in breeding and other areas of science depends on coordination of data assimilation into bioinformatic systems and training in the practical operation of those resources.

Anticipated Products:

- An improved gene annotation that uses available RNA-seq data and other bioinformatic methods.
- Computational methods to acquire data from existing sequence and expression databases (e.g., Gene Expression Omnibus) for use in future genome annotation releases.
- Improved access to genome annotations through Soybase and Phytozome.
- Updated annotation improvements released every 12 to 18 months.
- A gene expression database management tool populated with internal experimental data.
- Physical map viewer (WebFPC) populated with internal experimental data.
- SoyKB (Soybean Knowledge Base; http://soykb.org/) which integrates all forms of soybean functional data with the genome sequence.

Performance Measure 2.4: Achieve high-definition genomic characterization of biological mechanisms and regulatory systems in soybean. Transcriptomics involves analysis of gene transcription. There is need to build developmental stage-specific catalogs of mRNA expression for key plant organs and biological processes, and improved ways of relating proteomics and transcription products to the annotated soybean genome sequence. Soybean biochemical processes give rise to a plethora of metabolites, each with its own spectrum of activity. The biological regulation of a great majority of these compounds is unknown.

Anticipated Products:

- High throughput methods for soybean such as RNA-seq, CHiP-seq, methylome, sRNAs, and protein covalent modification.
- Advanced knowledge of soybean protein-protein interaction networks with an emphasis on proteomic methods such as TAP-tag approaches.
- Targeted proteomic and metabolomic approaches focused on key soybean traits (e.g., oil and protein).

- Identification and characterization of soybean metabolites expressed in key tissues (e.g. seeds) or in response to environmental stresses and pathogens.

Performance Measure 2.5: Use functional genomic methods to characterize transcription regulated pathways. Information about gene expression does not lead automatically to new biological understanding. It is clear that networks exist for the control of gene expression and can reflect higher order complexity (e.g., coordinate interaction of transcription factor complexes). Elucidating these networks is essential to fully understanding how environment and genotype interactions lead to observable phenotypes (e.g., yield). Fortunately, modern molecular methods provide experimental means to elucidate these networks, their interactions, and outcomes.

Anticipated Products:

- A defined soybean transcriptional regulatory pathway obtained by combining RNA-seq data with ChIP-seq data.
- A defined soybean epigenetic regulatory pathway obtained by integrating sRNA expression and targets, the methylome and histone ChIP-seq.

Performance Measure 2.6: Advance gene modification technologies to help associate candidate genes with a discrete phenotype. Although sequence similarity-based gene annotation may suggest a function, it is necessary to confirm this function through biochemical or genetic studies. It is also expected that the function of the majority of soybean genes will not be easily deduced simply by sequence comparison to other genomes. In these cases, the availability of mutations in each of the soybean genes will be extremely useful to decipher gene function and integrate this function into the context of soybean quality and agronomic performance. For example, TILLING (Targeting Induced Local Lesions IN Genomes) is a PCR-based high-throughput mutation detection system that permits the identification of point mutations and small insertions and deletion "indels" in pre-selected genes. Fast neutron mutagenesis induces small deletions in the genome and is a very effective way to create mutations. Fast-neutron populations can be used for both forward and reverse genetic screens for useful mutations. A variety of new technologies are needed to create robust platforms for the study of soybean gene function.

Anticipated Products:

- Optimized protocols for use of RNAi and VIGS technology high-throughput systems.
- Robust site-directed tools such as zinc finger-based mutagenesis for gene function in soybean.
- Lines derived from fast neutron mutagenesis and a gene deletion database for characterization of gene function.
- High throughput sequencing methods and other analytical tools to identify deletions and characterize deleted genes.
- Cost-effective implementation of resequencing to improve sustainability of TILLING resources.

Performance Measure 2.7: Create a saturated transposon insertion population with defined flanking sequences that can be used to identify mutants by BLAST sequence comparison. Transposons – known as McClintock's 'jumping genes' - are ubiquitous in plant genomes. Transposon insertion has a high likelihood of disrupting gene function. Usually only a few transposon insertions occur in any given individual, making genetic analysis much easier. Recent work has demonstrated that both the maize Ac/Ds and rice mPing transposons are suitable for use in soybean. The Ac/Ds transposon has a strong preference for local transposition, which makes it particularly useful to mutate genes in its vicinity, and may be particularly well suited for

activation tagging. However, a starting population of well dispersed Ds insertions (perhaps 25,000) is necessary before any given soybean gene can be targeted. In contrast, the mPing preferentially targets gene-rich regions on all chromosomes, and germinal insertions occur in the absence of tissue culture. Strategies are available that may increase its frequency of germinal transposition even more.

Anticipated Products:
- Identification of the optimal transposon system for activation tagging.
- Strategies to increase frequency of germinal transposition.
- A transposon-tagged population in which 85% of annotated soybean genes have been tagged.
- Improved methods for high-throughput phenotyping of transposon-tagged lines.
- A comprehensive database of transposon-tagged lines

Performance Measure 2.8: Implement outreach opportunities for education and use of genomic databases. Many valuable tools have been/will be developed for soybean researchers. These tools include genome sequence, genetic, expression, and proteomic databases. However, these databases are useful only if they can easily be used and queried. Given the complexity of the data, training scientists to use and mine these databases will be an asset to the soybean community. These educational and outreach activities need to include multiple formats to reach an ever expanding and diverse audience.

Anticipated Products:
- Standardized syntax and sanctioned methods for data submission to informatic resources.
- Standardized syntax and expanded inclusion of phenotypic descriptors for useful trait informatic resources.
- Leveraged comparative informatics across data sets within and among legume species.
- Web services that allow and encourage communication between databases.
- Training opportunities via meetings and web-based tutorials to familiarize the community with database resources.
- Improved database interactions facilitated by integration and improvement of existing software platforms.

Performance Measure 2.9: Develop an ORFeome library from agronomically important genes (tissue or treatment specific) and gene families. Many molecular methods developed to examine an individual gene's function require the cloning of the gene of interest or its corresponding cDNA. For example, Virus Induced Gene Silencing (VIGS) requires a portion of the targeted cDNA be inserted into a viral construct. When targeting hundreds of individual genes, it is difficult to clone the correct gene or cDNA fragment in a high-throughput manner. An ORF (Open Reading Frame) clone contains all the protein-coding portions of a gene, minus the untranslated regions found in cDNAs that can inhibit some molecular studies. A library of cloned ORFs in Gateway-compatible vectors would facilitate studies of gene localization, protein-protein interactions, epitope tagging, and become a resource to the soybean community.

Anticipated Products:
- Use of cloned genes of interest to evaluate the usefulness of an ORFeome.

Goal 3: Transformation/Transgenics: Optimize and expand transgenic methods and improve understanding of natural genes for modification of trait expression

Performance Measure 3.1: Establish of a soybean genetic repository and distribution center. A permanent repository for community-wide genetic resources is needed for maintenance and distribution of the valuable unique germplasm that the soybean genetics community generates. The public has invested millions of dollars on soybean research for the development of mapping populations, TILLING, and transposon-tagged lines. This investment is in danger of being lost due to the lack of an appropriate infrastructure for long-term storage and distribution. The existence of such a repository is becoming critical in order to leverage additional research funds. In fact, future funding from Federal agencies could be jeopardized if there is no place to store the materials developed with Federal funding. If necessary to attract long-term funding, this genetic repository could be combined with repository for additional crops; legumes and non-legumes.

Anticipated Products

- Identification of a coordinator and advisory board to develop a business plan and find stable long-term financing from public and private sources.
- Construct a sustainable facility and bioinformatics database.
- Determine seed storage conditions and protocols for quality control and resource distribution.
- Consolidation of existing resources into the central facility.
- Web-based resources made available publically, with defined submission and request requirements.

Performance Measure 3.2: Develop next-generation transformation and targeting technologies and utilize these transgenic approaches to help elucidate gene function and deploy genes of interest. Transformation is central to most of the advances that are currently in place in farmers' fields, from introduction of specific genes of interest to determination of gene function. Although these advances were ultimately provided by the private sector, the initial discoveries of genes and gene introduction technologies originated in the public sector. With the availability of the soybean sequence, transformation continues to be an important resource for the soybean community to use to dissect gene function as it relates to the genes of interest to soybean breeders. In addition, improvements in gene introduction efficiencies and quality of transformants, as well as generation of new targeting technologies, are still desperately needed to generate novel germplasm to be utilized by the soybean breeding and genetics community.

Anticipated products:

- Improvements in gene introduction/gene expression efficiencies.
- More rapid and efficient recovery of transgenics, especially homozygous seed identification.
- Established "rules of transgene assembly for desired expression" with promoter/termination identification of 100 events per year.
- RNAi construct toolbox for targeted silencing of 2-5 vectors implemented per year.
- Determine how to assemble multiple stacks (multiple gene constructs) for consistent expression.
- Training opportunities for use of efficient transformation technology.
- Capacity for medium-throughput characterization of gene function.
- Establishment of a Transformation Consortium to coordinate a transformation pipeline for genes of interest to the research community.
- Establishment of a web portal/database for the process of gene identification, submission, and transgenic delivery.

- Genetic modification and insertion of genes that mediate productivity, protection, or quality traits.
- Development of targeting/transposon technologies for site-directed cutting or integration

Goal 4: Translational Genomics: Optimize breeding efficiency with robust sequence-based resources

Performance Measure 4.1: Develop analytical approaches to characterize soybean germplasm diversity based on the SoyHapMap 1.0 data to identify parental lines for breeding purposes. The analysis of the entire USDA Soybean Germplasm Collection of 18,000^{+} cultivated soybean and 1100^{+} wild soybean accessions with 50,000 SNP DNA markers is the only such analysis of a germplasm collection. The resulting development of SoyHapMap 1.0, which will provide the initial definition of soybean genetic diversity based upon the analysis of the 19,000^{+} cultivated and wild accessions in the USDA Soybean Germplasm Collection, will be the basis for the discovery of new genetic diversity and DNA marker resources. In order to maximize the usefulness of this unprecedented crop-haplotype dataset, multivariate approaches and tools are needed to summarize the genomic data into recognizable patterns of diversity.

Anticipated Products:
- A diversity map of in the USDA Soybean Germplasm Collection.
- Genomic analysis tools to maximize the exploitation of soybean haplotype diversity for genetic improvement.
- A maximally diverse Soybean Core Collection.
- Germplasm with enhanced resistance to abiotic stresses.
- Germplasm with enhanced yield potential.
- Public access to all developed SNPs.

Performance Measure 4.2: Discover gene/QTL for qualitative traits and develop tightly linked DNA markers. These are traits that are controlled by one or a few genes and underlie traits such as pest resistance, seed fatty acid and amino acid levels, certain other seed composition traits, and transgenes.

Anticipated Products:
- DNA markers that can be used in breeding for pest resistance including SCN, aphid, Phytophthora root rot, and other important pests.
- DNA markers that can be used in breeding for quality traits including seed fatty acid and amino acid levels and other seed composition traits (phytate, raffinose, etc.).
- DNA markers for mapping and background selection for transgenes: In most cases the developer of the transgenic will provide a perfect marker for the transgene as well as the genome position.

Performance Measure 4.3: Discover gene/QTL for quantitative traits and develop tightly linked DNA markers. These are traits that are controlled by a number of genes/QTL and underlie traits including seed yield, seed protein/oil levels, abiotic stress resistance, and disease tolerance

Anticipated Products:

- DNA markers for seed protein/oil levels that can be potentially be used to stack these seed composition genes/QTL without sacrificing yield potential.
- DNA markers for abiotic stress tolerances including slow wilting, N_2-fixation under water deficit, iron deficiency chlorosis, flooding tolerance, salt tolerance, water use efficiency, root morphology, and response to higher levels of CO_2 and ozone.
- DNA markers for seed yield discovered via traditional QTL mapping and networked association mapping approaches.

Performance Measure 4.4: Develop and populate a user-friendly database of validated QTL for use in marker assisted breeding applications. The successful application of marker-assisted breeding requires access to robust data on marker-trait associations for both perfect and flanking markers associated with genes/QTL conditioning the phenotypic variation in the desired qualitative or quantitative trait.

Anticipated Products:

- A defined format and standards for QTL and marker-trait association data to expedite database entry of marker-trait association data.
- Required submission and compliance that QTL and marker-trait association data are entered to SoyBase for all USB-funded projects.
- Development of training workshops, distance learning, and webinars to introduce and instruct breeders and students on the use of SoyBase (see also Performance Measure 2.8).

Performance Measure 4.5: Define the molecular genetic signatures of selection in 70+ years of U.S. soybean breeding by use of the 50,000 SNP Illumina Infinium Assay. Selection by soybean breeders over the past 70 years for seed yield and other agronomic traits has resulted in allele frequency changes at various places in the soybean genome. The identification of such regions will indicate the positions of genes controlling these agronomic traits.

Anticipated Products:

- Availability of the allele frequency at 50,000 SNP loci based upon analysis with the 50,000 SNP Illumina Infinium Assay of the ancestors and important resistance sources used in elite breeding populations, recently released public cultivars, and the most recent Uniform Test entries.
- Analysis of changes in SNP allele frequency over time will provide information on which portions of the soybean genome have been impacted by continuous breeder selection.
- Identification of key genes/alleles for important agronomic and quality traits.

Performance Measure 4.6: Define optimum breeding models applicable to different breeding situations using *in silico* analysis. To achieve the maximum benefit from haplotype information and marker-trait associations, it is critical for soybean breeders to understand the optimum approaches to efficiently introgress genes/QTL to create elite soybean cultivars. The evaluation of various breeding models using *in silico* analysis will define optimal approaches applicable to different breeding situations and establish soybean as the model for the study of

efficient breeding of a self-pollinated crop. We now have soybean genotypes that will mature in 70 days or less days after emergence. To establish a model soybean system, the soybean community should select 20 diverse plant introductions and/or important land mark cultivars and convert them *via* backcrossing to very early maturity. These converted types will be the basis establishing soybean as a model crop for study.

Anticipated Products:

- Optimum procedure for the introgression of a single gene/QTL into soybean based upon computer modeling.
- Optimum procedure for gene stacking into soybean based upon computer modeling.
- Optimum procedure for the introgression of a polygenic trait into soybean based upon computer modeling.
- Optimum procedure for the introgression genes/QTL into soybean while maintaining background genetic diversity based upon computer modeling.
- A model soybean population for *in silico* studies.

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