U.S. Peanut Industry Genomics Initiative

History & Next Steps

June 17, 2012

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Executive Summary

THE INDUSTRY CHALLENGE: *One of the biggest challenges for the U.S. peanut industry is the ability to compete with other crops for production. Most growers today are focused naturally on dollar value per acre and peanuts have often been uncompetitive in regards to yield and production costs as compared to crops such as cotton and corn. As an industry, the best way to compete is to enhance our peanut varieties for disease resistance and yield potential. This can best be done through genomics. We have to maximize yield while minimizing inputs in order to sustain and compete with other crops. The industry is committed to peanut consumption growth through marketing efforts to promote the nutritional aspects of peanuts. As we grow consumption, we must grow our yield potential to sustain our industry. Genomics is the key to a sustainable future for peanuts*.

On March 24, 2004 the American Peanut Council's Board of Directors authorized The Peanut Foundation to organize and coordinate peanut genomic research with the goals of reducing the cost of production and improving yields and quality. It was apparent that the need for this work was urgent. Our peanut industry was at least 6 to 10 years behind the technology for improved variety development compared to corn, cotton, soybeans, and other major competing crops. As a result, U.S. peanut production was becoming less competitive with these other major crops. To become more competitive, the Peanut Foundation challenged scientists to engage in peanut research to:

1. Take 8-10 years off the normal time (12-15 years) for variety development
2. Develop varieties with multiple resistances to TSWV, nematodes, leaf spot, sclerotina, CBR and other diseases
3. Increase peanut yielding ability
4. Achieve drought tolerance, and improve efficiency of water use to preserve natural resources
5. Develop early maturing varieties to avoid off-flavors, reduce growing time and production costs
6. Enhance oil quality (i.e. high oleic acid) and essential nutrients (i.e. increased folate)
7. Enhance resistance to pre-harvest aflatoxin

The successful outcome of that research mandate would certainly reduce production costs to growers, which also will benefit shellers and manufacturers. Our University and USDA economists estimated in 2011 that the lack of varieties with superior disease resistance and other improved traits typically costs growers as a whole over $200 million a year or close to $200/acre. These estimated grower losses are a huge target for the whole industry to overcome, but we must work together because shellers and manufacturers also suffer annual losses due to inferior peanuts in the food stream.

The Peanut Foundation considered three different genomic approaches to develop improved peanut breeding technologies: 1) genetic modification (GMO), 2) marker assisted selection (MAS), and 3) mutation breeding. Research in each of those approaches revealed that there were significant drawbacks to GMO and mutation breeding. Therefore on June 21, 2010, The Peanut Foundation and the American Peanut Council concluded that Marker Assisted Selection (MAS) would be the best means to deliver the new varieties. MAS is a breeding method that relies on the use of DNA-markers found in plants to identify hybrids from a cross that have a desired trait before the hybrids are grown in the field, thereby giving breeders a time advantage in variety development. However, by 2010 peanut breeders only had about 6,000 DNA-markers and few were associated with selectable or measurable traits; whereas soybean and corn scientists had over 100,000 very useful DNA-makers. The reason soybean and corn had so many markers is because DNA-markers were easier to discover after the DNA sequence

of the soybean and corn genome was known. The same is true for cotton. Now nearly every agricultural crop has a genomic research effort aimed at determining the structure and order of genes in its genome. It is generally recognized that the information gained from a crop genome sequence enables quantum leaps in ability to develop and deliver improved varieties in a timely manner.

Thus, it became clear that sequencing the peanut genome was necessary to find a larger inventory of useful DNA-markers to move forward with MAS breeding. The framework for organizing those efforts was published in a Strategic Plan covering research goals, priorities, and expected deliverables between 2008 and 2012. With funding from growers, shellers, manufacturers, allies, USDA and universities the work done under that Plan made it possible to develop the building blocks that had to be in place before we were ready to tackle the genome sequence. A few of those corner stones include:

1. Development of germplasm (starting material) necessary for sequencing the peanut genome
    a. Replacement of lost germplasm in the USDA peanut germplasm collection at Griffin GA.
    b. Discovery of new germplasm resources with resistance to leaf spot and other diseases
    c. Development of special breeding populations to find genes for TSWV, leaf spot, pre-harvest aflatoxin contamination (PAC), CBR, root-knot nematode and sclerotinia/white mold resistance; high-oleic peanut oil; and drought tolerance.
    d. Discovery of drought tolerant peanuts by researches in the U.S. and India.
2. Use of DNA markers for high-oleic acid peanut oil and nematode resistance to develop the high O/L variety Tifguard. This achievement was a major breakthrough in peanut genetics because it convinced breeders that MAS could shorten the time for developing a new variety from 12-15 years to about 5 years.
3. Ability to superimpose DNA-marker maps on individual chromosomes to better locate genes.

In addition, collaborative research expanded among about 135 members of the International Peanut Genome Initiative (IPGI) at 79 institutions in 20 countries; and strong collaborative alliances were cemented with international research agencies like ICRISAT (India), EMPRAPA (Brazil) and MOST (China). Because of extensive cooperative research among U.S. and international collaborators, research progress advanced so well the IPGI moved ahead to publish a Strategic Plan covering 2012 to 2016. In conjunction with this new overall plan, a workgroup (The Peanut Genome Consortium) was formed to organize a research framework (the Peanut Genome Project, PGP) for the steps needed to achieve a useful genome sequence for peanut.

The Peanut Genome Project is composed of six research components that are necessary for generating useful tools from the genome sequence for the researcher's and breeder's Toolbox. U.S. breeders and researchers were assigned lead roles in each research Component. A brief description follows:

Research Component 1: This work involves sequencing the genome to help breeders find genes on each peanut chromosome.

Research Component 2: This work enables the identification of thousands of DNA-markers in wild and cultivated peanuts.

Research Component 3: This work identifies all genes (many genes may govern a trait) in the genetic networks for resistance to diseases like TSWV, tolerance to stresses like drought; and seed quality traits like higher levels of folate.

Research Component 4: This work explores new options in genome sequencing technology that may help reduce the PGP budget and still ensure high quality genomic data. New technology already has reduced the budget for research Component 1 from $5.6 million to $2.8 million.

Research Component 5: This work goes hand in hand with identification of DNA-markers by showing what traits the markers mark. These associations are critical to the identification of genes.

Research Component 6: This work provides a home for storage and a caretaker for web-based genomic libraries and the Breeder's Toolbox in Santa Fe, NM at the National Center for Genome Resources (NCGR) and other U.S. locations.

The Peanut Genome Project budget is $6,000,000 over five years [$1,460,000 (2012); $1,790,000 (2013); $1,510,000 (2014); $620,000 (2015); $620,000 (2016)]. The cost must be measured against the anticipated returns on investment. Obviously all desired traits will not be discovered or available at the beginning of the project. They will be discovered and developed over time. But, assuming that all varieties in commercial production had superior disease resistance and desired traits, the savings and increased revenue are estimated to be over $200 million, recurring each year. With complete and timely funding of the Peanut Genome Project budget, the Consortium of peanut researchers is confident that all Peanut Genome Project Research Components will be achieved by the end of 2016. As in all research nothing is a certainty. However, as discoveries are made throughout the project, the results will be disclosed and available to all breeders whether they use conventional, MAS or GMO technology. In summary, all breeding methods will be helped by the discovery of more useful DNA-markers.

The Peanut Genome Project is now moving forward with authorization from The Peanut Foundation. The first critical step was to select a partner do the sequencing and assembly work in Research Component 1. After an exhaustive search, the U.S. and international experts of the Peanut Genome Consortium were convinced that BGI in Beijing, China was by far the best institution to sequence and initially assemble the peanut genome. Accordingly, a contract for $2,800,000 to perform work under Research Component 1 was signed on March 29, 2012 between The Peanut Foundation and BGI, and this sequencing work has begun. The work will be conduced in two phases. The initial down-payment of $840,000 for Phase 1 has been made. Additional payments are subject to satisfactory performance of the BGI during Phase 1.

Since the completion of the original Strategic Plan for 2008-2012, several companies in the U.S. peanut industry who have been heavily involved in this effort have made initial commitments toward the Peanut Genome Project (PGP) 2012-2016 budget. MARS has committed $1,290,000; J.M. Smuckers ($230,000); and Birdsong Peanuts ($200,000). In addition, several Chinese Agricultural Academies have committed $480,000 toward the Peanut Genome Project budget. These commitments now total $2,200,000. It is a great start but far from the total $6 million that is needed over the next five years.

To accomplish this project we will need broad support from growers, shellers, manufacturers and allied industry sectors who are the ultimate beneficiaries of the Peanut Genome Project. The benefits include:

- Effective low cost crop yield protection against losses to diseases and plant stress
- Improved food safety through quality control of aflatoxin contamination
- Potential health claims on peanut food products; and
- Enhanced profitability for the entire peanut industry

The Peanut Foundation and other industry representatives will be contacting industry groups over the next few months to explain this plan and solicit support.

This "White Paper" on the U.S. Peanut Industry Genomics Initiative is written to convey specific details of this complex program in understandable terms. Please read it carefully. You will find that our U.S. peanut researchers are heavily involved in this work and you will see from reading their comments in the report that they are excited about using the full capabilities of Marker Assisted Selection. Efforts to get help from the major private seed research companies have been unsuccessful because our peanut crop acreage is not large enough. Therefore, to help reduce the cost of production, improve yields, and enhance quality of U.S. peanuts, we will have to primarily fund this work ourselves.

The First Phase of the Peanut Genome Initiative, 2004-2012

What is the History of the Peanut Genome Initiative (PGI)?

The Peanut Foundation (TPF) was charged by the American Peanut Council (APC) on March 24, 2004 with the goals of reducing the costs of production and improving the quality of U.S. peanuts. With the approval of the American Peanut Council's Board of Directors, The Peanut Foundation established the Peanut Genome Initiative (PGI) on March 25, 2004 to organize and coordinate peanut genomic research to reach the goals set by the industry.

At the time, research on DNA-markers in all major legume crops was lagging behind corn and cotton. The legume community guided by the USDA-Agricultural Research Service took steps to overcome this disadvantage by forming a coalition, the Legume Crop Genome Initiative (LCGI). PGI leaders represented the peanut industry on the LCGI, and together with leaders from the soybean, dry bean, peas & lentils, and alfalfa (Medicago) industries published the research status and goals for developing a genome sequence for each legume crop in the book *Legume Crop Genomics* (Reference 1). Because of substantial funding, soybean, dry bean, and alfalfa (even pigeon pea) now know the sequence for all chromosomes in their respective genomes and now are using that information to develop as many DNA-markers as corn and cotton. Because of lesser resources, research to sequence the peanut genome has lagged behind soybean, corn and cotton at least 6 to 10 years. Due to the effort of many industry leaders, the Peanut Genome Initiative (PGI) and TPF, peanut research is starting to catch up.

The Peanut Foundation recommended to the American Peanut Council on December 7, 2006 that they formally begin a program to determine if genomics was the best way to move forward. The American Peanut Council asked The Peanut Foundation to coordinate this effort. In 2007 The Peanut Foundation held the first meeting of the International Peanut Genomic Initiative (IPGI) and developed the International Strategic Plan for the PGI 2008-2012 (Reference 2) to guide the industry genomics research effort. That Plan consisted of six goals. Among those were three primary genomic research approaches: 1) to develop improved transformation systems and GMO peanuts, 2) to develop DNA-markers for resistance genes for the primary peanut diseases as well as genes for quality attributes, and 3) to create a seed collection using chemical knockouts for mutation breeding. This Strategic Plan (Reference 2) was used for determining the research budget for 2008-2012 and to prioritize research to be funded. A lot of work was accomplished given the limited funds available. However, after four years of focusing primarily on developing, licensing, and registering a GMO peanut, it became clear to The Peanut Foundation Board that developing a GMO peanut would be too expensive and would take too long to release, and that a GMO peanut could negatively impact peanut trade. Likewise, although mutation breeding succeeded in creating thousands of plants, it became clear that known sequences for the mutated genes were still needed to make use of those plants. Therefore, it was determined by The Peanut Foundation Board that providing breeders with genomic tools that speed up variety development was the most cost effective and most expeditious way to achieve the industry goals of reduced production costs and improved quality. This technology is known as Marker Assisted Selection (MAS). This recommendation was made to the American Peanut Council Board on June 21, 2010 and the new focus of MAS was approved.

How were U.S. industry funds invested from 2007-2011 through The Peanut Foundation?
A total of $2,503,016 was contributed by the U.S. peanut industry from 2007–2011 to fund all peanut research projects through The Peanut Foundation. Contributions by sector were: Allied ($109,996), State Growers ($848,600), National Peanut Board ($600,000), Shellers ($282,500), Manufacturers ($662,000). Of those contributions between 2007-2011, $1,580,069 was spent on genomic research as indicated below and in Exhibit A.

- In 2007, The Peanut Foundation funded 11 projects were funded totaling $162,188.
- In 2008, The Peanut Foundation funded 20 projects totaling $408,395. These collaborative projects explored the types of genes that are expressed during growth of peanuts, developed germplasm for mutation breeding, and attempted to develop genetically modified peanuts.
- In 2009, funds were spent on 14 projects totaling $310,230. DNA-markers for nematode resistance and the high oleic trait were important initial findings. Work also continued on building genetic road maps, describing how the peanut genome was put together, and getting approval for the genetically engineered peanuts.
- Funding in 2010 included 20 projects totaling $331,727. Building on earlier work at Texas A&M, DNA-markers for nematode resistance and the high-oleic acid trait were published by a team led by Peggy Ozias-Akins. Meetings were held with the U.S. breeders to decide how to incorporate this technology in their breeding programs.
- In 2011, funding of 14 projects totaled $367,529 and with much assistance from our global partners, we completed the Strategic Plan for 2008-2012, and began to think about the key next steps.

What are the most significant accomplishments of the Peanut Genome Initiative from 2007-2011?
Work toward the goals of the PGI Strategic Plan 2008-2012 (Reference 2) produced the following most significant accomplishments:

Crop Management & Productivity

1. *Optimized the use of new varieties and farming practices to improve productivity***.** This work helped establish best management practices for peanut production.

2. *Identified genotypic differences in water-use and drought/temperature tolerance.* This work revealed that some peanuts had the ability to use water more efficiently and were more tolerant to heat stress than most peanuts. Breeding programs were started to develop varieties for dry-land peanut production.

Genetics & Germplasm Enhancement

1. *Enhanced availability of genetic diversity and genomic variation for important traits in peanut.* This work began the process of replacing lost or poor germinating germplasm in the USDA peanut collection at Griffin GA by upgrading long-term cold storage facilities; and also helped save the wild germplasm collection at Stephenville TX.

2. *Use of gene markers in peanut breeding.* This work demonstrated the time-savings that could be gained in breeding programs through Marker-assisted selection (MAS). The first example was use of DNA-markers to select a high yielding high-oleic version of Tifguard in only 26 months, and develop the commercial variety in about five years.
3. *Development of mapping populations and resources for genome sequencing.* This work created *g*enetic road maps for gene locations (called Quantitative Trait Loci, QTL) in wild and cultivated peanuts. Use of DNA-markers and special breeding populations helped put road signs on the maps for genes that control oleic acid content, CBR, TSWV, early/late leaf spot, and white mold resistance.
4. *Integrating genetic information.* This work imprinted genetic road maps with the location of genes on specific chromosomes to present a high-definition picture of the genome.
5. *Ability to produce several generations of breeding lines per year.* This work helped accelerate breeding progress through support of a winter nursery in Puerto Rico.

<u>Gene Discovery & Genome Analysis:</u>

1. *DNA sequence resources for characterization of peanut genome structure.* This work created a library of over 10,000 partial sequences of genes that are known to be translated into specific proteins. Half of the peanut genome comes from one wild species, half from another. This library of partial gene sequences helped show which proteins (and their genes) came from each of the ancestral wild species.
2. *Characterization of wild peanut genomes*. This work involved making crosses with wild ancestors and cultivated peanut to develop ways to transfer useful genes from wild to peanut varieties.
3. *Identification of the basis for genetic diversity in peanut genomes*. This work generated about 6000 DNA-markers for peanut. There are several types of DNA-markers, such as simple-sequence repeats (SSR) and single nucleotide polymorphisms (SNP). All are valuable genetic tools for the identification of useful genes in peanut. Soybean has over 100,000 DNA-markers, so thousands more are yet to be discovered in peanut.
4. *Development of tools and technologies for characterizing gene function*. This work was facilitated by ability to create mutations or recessive genes within the peanut genome by technologies, such as: TILLInG (Targeting Induced Local Lesions in Genomes), RNA interference (RNAi) and Zn-finger proteins. This work relates to mutation breeding.

<u>Disease & Pest Management</u>

1. *New sources of disease resistance.* This work led to discovery of a wild peanut that was immune to TSWV. Researchers found DNA-markers that helped detect the general location of genes for TSWV and also early leaf spot in wild peanuts.
2. *Discovery of QTL for resistance to pests*. This work led to the development of Tifguard a new variety with resistance to root knot nematode. Marker Assisted Selection (MAS) was used later to improve Tifguard varieties with the high oleic trait.

Product Quality

1. *Progress toward elimination of pre-harvest aflatoxin contamination in peanut***.** This work identified field-grown peanuts that were more resistant to infection by *Aspergillis* specie. This was an important step toward reducing aflatoxin contamination in the field.
2. *Enhanced peanut flavor.* This work led to the development of early maturing varieties as a means to avoid off-notes in flavor due to immature peanuts and also helped reduce production costs.

Highlights of major accomplishments attributed to the PGI up to 2012 are summarized in Exhibit B. Additional information on PGI research accomplishments is posted at http://www.peanutbioscience.com/.

How will these accomplishments help breeders to develop new varieties?

Many new peanut varieties have been released over the last five years with moderate resistance to several key diseases, but with proper DNA-markers, plants can be bred to have much better immunity to those diseases. This technology also enables breeders to more easily build resistance to three or more diseases and/or quality traits in the same plant and in a shorter length of time. Conventional breeding techniques take 12-15 years to release new varieties for commercial production. With MAS the high oleic Tifguard was developed in about 2 years with an additional 3 years for seed increases and release. This demonstrates that MAS could potentially cut 8-10 years from the breeding cycle for new varieties, saving time and saving the breeders and industry money in confronting new diseases as they occur.

At this point one new variety has been developed using new DNA-markers: the high oleic Tifguard. The high oleic trait is also being included in most breeding programs, by using MAS, with those releases coming over the next 3-5 years. The rapid development of the high oleic version of Tifguard was the major breakthrough that convinced most breeders to use MAS in their programs in the future. Several other new varieties with Nematode resistance are in various stages of development and will be released over the next five years. Global work also continues, especially in India, with the identification of the late leafspot resistant gene markers. These markers will be adopted for the U.S. crops and distributed to all breeders during 2012-2013.

The Next Phase of the Peanut Genome Initiative 2012 - 2016

What are the next steps for the Peanut Genome Initiative?

Since the organization of the IPGI by a coalition of scientists from the U.S., China, India and Brazil, membership in this initiative has grown to 135 representing 79 institutions in 20 countries. At the IPGI meeting in Brazil on June 16, 2011 the International Peanut Genomic Research Initiative Strategic Plan 2012-2016 (Reference 3) was developed, and an international governing board to guide the final major steps was organized. This organization is known as the Peanut Genome Consortium or PGC (Exhibit C). Working with other international groups, The Peanut Foundation was able to start the first steps in the current Strategic Plan. The key research thrust was to sequence and assemble the peanut genome. A search was begun for organizations that could do this work. Many companies and agencies were considered, and their qualifications were examined. BGI (previously known as Beijing Genomic Institute) was selected based on their history of sequencing other plant and animal genomes, the state-of-the-art facilities they have in China, and their vast experience with sequencing and assembly. Members of the PGC also had worked with BGI on sequencing the cacao, lettuce, and various legume genomes. Other sequencing groups confirmed that previous work by BGI on sequencing crop genomes had proven to be both timely, cost effective, and of the highest quality. For these and other good reasons, the PGC selected BGI as the best partner to do the work for the IPGI. A contract between The Peanut Foundation and BGI to sequence and assemble the peanut genome was signed on March 29, 2012 and the initial payment of $840,000 was made. This collaboration will enable us to develop a road map to find DNA-markers for all the genes that control key peanut diseases and quality traits.

Without the organization and planning of the Peanut Genome Corsortium, it would take 20 plus years at current funding levels to reach the goals and milestones (Exhibit D) of the International Peanut Genomic Research Initiative Strategic Plan 2012-2016 (Reference 3). With proper funding, those milestones can be substantially completed by the end of 2016. U.S. researchers are heavily involved in this complex project. Key aspects of the work are highlighted below:

Genome Sequencing & Assembly: This work involves advanced sequencing technology and library construction at BGI in Beijing, China. The genome will be sequenced in two phases. Phase 1 involves cutting peanut DNA up into a variety of fragment sizes and then sequencing those fragments. Work in Phase 2 will put the sequenced DNA fragments back together in proper order to create a fully sequenced genome. Assembly of all the genome fragments also will be done at BGI using their high powered computer program called SOAP *de novo,* a tool for rapid assembly of many small fragments (sequences). Knowing the order of genes on a chromosome will help researchers and breeders pick better parents for variety development because of the ability to locate desired genes in breeding lines.

DNA-marker Discovery: Sequencing work also will be conducted at UC Davis, the University of Georgia, Texas A&M, USDA and other university locations on germplasm collections and other lines to generate thousands of DNA-markers. Some of these markers will help anchor the fragments together as they should occur in each chromosome. The rest will help locate specific genes on those chromosomes.

Phenotyping: Corley Holbrook (USDA at Tifton, GA) is coordinating a team of U.S. researchers that include: Peggy Ozias-Akins (University of Georgia), Tom Isleib (NCSU), Barry Tillman (University of Florida), Mark Burow (Texas A&M), and others who will develop special breeding populations. These populations will be used to identify genetic differences in the way DNA-markers react with the genome of each segregating line (genotyping), and then associate those genotypes with observed differences in the plant traits (phenotyping). This work is critical to Marker-Assisted-Selection. This team also will spearhead an effort to standardize the terminology and methods by which phenotypes are measured and recorded by all breeders in the world.

What are the future projects that will help us accomplish our goals?

The Peanut Genome Consortium (Exhibit C) was established in 2011. Working with the other researchers in the IPGI, they developed the IPGI Strategic Plan 2012-2016 (Reference 3). The Peanut Genome Consortium (PGC) also published Policies and Procedures (Reference 4) to establish operational guidelines for the Peanut Genome Project (PGP). Information from the genome sequence generated by the PGP will be used to accelerate accomplishment of the goals and priorities set in the IPGI Strategic Plan.

The PGP will use the newest and best sequencing technologies available and is guided by expert researchers who have a great deal of experience in sequencing and assembly of other crop genomes. Still, the successful achievement of PGP goals depends on research collaboration within and among each research component.

In very technical terms, specific PGP goals include: 1) a high quality chromosome scale draft of a tetraploid (cultivated species) as the reference genome sequence, plus high density maps of both progenitor and synthetic amphidiploids genomes; 2) characterization of gene space in amphidiploids and diploid (progenitor species) germplasm, 3) high throughput transcriptome characterization of the reference tetraploid cultivar; 4) evaluation and potential application of advances in DNA sequencing technology in the PGP; 5) phenotypic association with mapped genetic markers, and 6) interactive bioinformatic resources for data storage and application in a breeder's toolbox to enable molecular breeding approaches for enhancing peanut yield, optimizing resistance to diseases and insects, tolerance to environmental stresses, and improved quality traits. This technology will promote peanut crop competitiveness and enhance growers' profitability in an environmentally sustainable manner.

Research Components & Teams

The Peanut Genome Consortium (Exhibit C) created teams to cover key identified areas (components) of research. Team members who are the best in their fields were selected for each research component of the Peanut Genome Project as follows:

Research Component 1. Sequencing & Assembly of the Peanut Genome.

Team: BGI. & PGC Technical Steering Group: Scott Jackson, Chairman (University of Georgia - Athens, GA), Peggy Ozias-Akins (University of Georgia - Tifton), Brian Scheffler (USDA-ARS-Stoneville), Richard Michelmore (University of California-Davis), David Bertioli (University of Brasilia), Rajeev Varshney (ICRISAT-India), Richard Wilson (The Peanut Foundation)

This work reveals the basic nucleotide structure of the peanut genome, with reference to individual chromosomes. The reference genome will come from the cultivar Tifrunner. All

the DNA fragments will be compiled in high-density genetic road maps, and will be assembled with the aid of genetic landmarks from research Components 2, 3 & 5. Tools from research Component 6 will help make certain the assemblies are correct.

Research Component 2. Discovery of Useful DNA-markers.

Team: Richard Michelmore (University of California-Davis), David Bertioli (University of Brasilia), Rajeev Varshney (ICRISAT-India), Lutz Froenicke (University of California-Davis), Nicole Barkley (USDA-ARS, Griffin), Boshou Liao (Chinese Academy of Agricultural Sciences-Wuhan).

This work identifies gene clusters in each chromosome and reveals how different peanut lines differ in gene structure. Gene clusters in wild peanut genomes also will be characterized, and in core-collections of germplasm from the U.S., India, and China. This work will reveal thousands of new DNA-markers.

Research Component 3. Discovery of all Genes that Control Targeted Traits.

Team: Peggy Ozias-Akins (University of Georgia-Tifton), Brian Scheffler (USDA-ARS-Stoneville), Scott Jackson (University of Georgia-Athens), Baozhu Guo (USDA-ARS, Tifton), Xingjun Wang (Shandong Academy of Agricultural Sciences), Weijian Zhuang (Fujian Agriculture and Forestry University).

This work helps identify genes in the pathways that control production, protection and quality traits. Work will focus on networks or families of genes for: 1) protection against TSWV, leaf spot (early & late), rust, white mold, pre-harvest aflatoxin contamination, and additional markers for nematodes; 2) tolerance to abiotic stresses, such as: drought, temperature and nutrient deficiency; and 3) genes for oil, fatty acid, protein, flavor, nutraceuticals and other quality traits. A peanut gene atlas will catalog all these genes. This information will provide genetic landmarks for genome assembly, and lead to gene (allele) specific DNA-markers for Marker-Assisted breeding.

Research Component 4. Evaluating New DNA Sequencing Methods.

Team: Richard Michelmore (University of California-Davis), Lutz Froenicke (University of California-Davis), Xun Xu (BGI).

This work explores new genome sequencing technologies that may help reduce the PGP budget and ensure high quality genomic data. Genome sequencing technology is rapidly changing. The PGP will use the most effective, efficient and accurate sequencing technology that is available.

Research Component 5. Matching up Genes and Traits.

Team: Corley Holbrook (USDA-ARS, Tifton), Mark Burow (Texas A&M, Lubbock), Soraya Bertioli (Embrapa-Brasilia), Ignacio Godoy (Instituto Agronomico de Campinas), Xingjun Wang (Shandong Academy of Agricultural Sciences), Tom Stalker (NCSU), Barry Tillman (University of Florida-Marianna), Tom Isleib (NCSU), Charles Chen (Auburn University), Vincent Vadez (ICRISAT – India).

This work provides the connective link between DNA markers for a gene and the change that variations in that gene create in the plant. Special breeding populations will be developed and used to identify genetic differences in the way DNA-markers react with segregating genes in a breeding population (genotyping), and then associate those genotypes with observed differences in the plant response (phenotyping) to TSWV, early & late leaf spot, CBR, nematodes, PAC, drought, seed fatty acid composition, flavor quality, nutritional benefits, peanut yielding ability and other traits. This work is critical to Marker-Assisted-Selection. This team also will spearhead an effort to standardize the terminology and methods by which phenotypes are measured and recorded by all breeders in the world.

Research Component 6. Web-based Genome Libraries and the Breeder's Toolbox.
Team: Steven Cannon (Iowa State), John Crow (NCGR-Santa Fe), Liu Xun (BGI-Davis, CA)
This work provides a home for storage and a caretaker for the massive web-based libraries or datasets that are generated from genome analyses, and develops tools that make this information useful to breeders. These tools will: 1) facilitates navigation from maps to genes to traits; 2) identify genetic diversity in gene sequences among accessions in germplasm collections and in breeding populations, and 3) provide ability to combine all data sets to get a clear picture of the genome structure.

What are the benefits of the U.S. Peanut Industry's Genomic Initiative?

The information derived from this research will provide each sector of the entire peanut industry with the ability to achieve:

- Security: assurance that useful genetic diversity is available for improving yield, protecting crops and enhancing quality such as sources of resistance to leaf spot and other diseases.
- Enabling technology: Knowledge of gene networks that govern key traits and ability to meld multiple traits in the same breeding population such as a reference genome sequence of the variety Tifrunner and ancestral diploid species.
- Solutions to Complex Problems: DNA-markers & road maps that accelerate discovery of key genes in breeding varieties with traits like resistance to pre-harvest aflatoxin contamination.
- Quality control: Elimination of mycotoxin threats and the means to ensure adequate supply of high quality, safe peanuts such as varieties with improved folate and essential nutrients.
- Enhanced profitability: Timely and cost effective development of high yielding commercial cultivars with traits that reduce production costs and enhance product quality such as varieties with resistance to multiple diseases and with high quality oil.

Why is it possible and important to move forward now?

It is important that this work be done now to prevent research institutions and seed companies from patenting peanut genes and making it difficult for the peanut industry to use them in MAS. Sequencing technology has advanced by quantum leaps, making it possible to overcome the inherent challenges posed by peanut. In addition, the cost of legume genome projects has plummeted making it easier to assemble genomes as complex as peanut.

Based on the International Peanut Genome Consortium's research and past experiences in mapping other crop genomes, it was determined that the best and most affordable way to get this genomic sequencing done was through an agreement with BGI in Beijing, China. BGI has the capability to complete the work and has done many other genome maps for various crops. A gold-standard reference genome sequence of cultivated peanut will be the cornerstone for all future advances in peanut improvement.

The data will be housed in the U.S. at locations such as National Crop Genome Resources-Santa Fe, NM; Iowa State University-Ames, Iowa; and Phytozome (DOE)-Walnut Creek, CA. This will include sequences of 96 different peanuts (i.e., two parents, cross, 93 segregating populations). These population sequences will be fully assembled from end to end and can be used in the

Legume Information System to compare to other legume species already sequenced and with identified markers (soy, Medicago, dry bean, alfalfa).

One of the biggest challenges for the U.S. peanut industry is the ability to compete with other crops for production. Most growers today are focused on dollar value per acre and peanuts have often been uncompetitive in regards to yield and production costs as compared to crops such as cotton and corn. As an industry, the best way to compete is to enhance our peanut varieties for disease resistance and yield potential. This can best be done through genomics. We have to maximize yield while minimizing inputs in order to sustain and compete with other crops. The industry is committed to peanut consumption growth through marketing efforts to promote the nutritional aspects of peanuts. As we grow consumption, we must grow our yield potential to sustain our industry. Genomics is the key to a sustainable future for peanuts.

How much money is needed to fund this research?

This Peanut Genome Project will cost more than the U.S. Peanut industry has ever invested in any one research project. The Peanut Genome Consortium has projected the Peanut Genome Project 2012-2016 budget is $6,000,000 over five years [$1,460,000 (2012); $1,790,000 (2013); $1,510,000 (2014); $620,000 (2015); $620,000 (2016)]. See Exhibit E for more details. The budget will be spent mainly on U.S. researchers and for projects that will use data from the PGP. These funds will need to be raised primarily from the U.S. Peanut Industry.

Since completion of the Strategic Plan for 2008-2012, several companies in the U.S. have been heavily involved in this project and have made major initial commitments toward the new PGP 2012-2016 budget. Mars has committed $1,290,000; J.M. Smuckers ($230,000); and, Birdsong Peanuts ($200,000). Chinese Agricultural Academies also have committed $480,000 toward sequencing work at BGI. This current commitment of $2,200,000 is a great start but far from the needed $6,000,000 to fund this project. We must have support from growers, shellers, manufacturers, and the allied segments of the U.S. peanut industry to fully fund this project. No other private seed research company will do this work for us. Therefore, we must fund this work ourselves.

With complete and timely funding of the Peanut Genome Project budget, the Consortium is confident that all Peanut Genome Project milestones (Exhibit D) will be achieved by the end of 2016. Any DNA-markers or other data found by research in the Peanut Genome Project will be available for any researchers or breeders to use as they are discovered.

What is the value of these new varieties to the peanut industry?

Dr. Marshall Lamb, USDA-ARS Economist, and other researchers have estimated cost savings/ gains to the U.S peanut industry if all breeders used all available genomic tools for key diseases and quality traits in variety development would be over $200 million per year. For details see the following Table. This assumes that all varieties in commercial production would have superior disease resistance and other desired traits. The amount of savings each year of course is subject to planted acreage and weather. Still, an investment of $6 million will not only net a substantial return on investment, but also pay large dividends in future years.

Estimated Savings & Revenue per year if All Available Genomic Tools are Used
(based on estimates done in 2011)

Information supplied by Dr Marshall Lamb, Dr Tim Brenneman, Dr Bob Kermirant, Dr David Jordan, Dr John Damecone, Dr Barbara Shew, Dr Jason Woodward

Trait	Savings	Increased Revenue	Total[1]
Drought Tolerance[2]	$8.87 million	$60.32 million	$69.19 million
Early Leafspot	$31.75 million		$31.75 million
Late Leafspot	$21.31 million		$21.31 million
TSWV[3]		$24.99 million	$24.99 million
White Mold Rhizoctonia	$45.15 million		$45.15 million
Sclerotinia	$2.18 million	$6.75 million	$8.93 million
Nematode	$12.24 million	$43.62 million	$55.86 million
Subtotal	$121.50 million	$135.68 million	$257.18 million
A Flavus/A Paraciticus	$25.00 million		
Increase Folate & High Oleic		$100.00 million[4]	
Subtotal	$25.00 million	$100.00 million	$125.00 million
TOTAL	$146.50 million	$235.68 million	$382.18 million

[1]Savings and revenues will vary year to year based on acreage and weather conditions. This chart is based on 1.34 million acres.

[2]Savings estimate based on 36,086,044,968 gal/year in water use efficiency; revenue estimate based on improved drought tolerance

[3]Reflects losses only to TSWV. No estimates were included for expenses related to condensed planting window and potential losses due to later harvest.

[4]Revenue for each new health claim per JM Smuckers marketing groups

What do key peanut researchers and breeders have to say about The Peanut Genomic Initiative (PGI)?

The following are a few testimonials of the peanut researchers on the benefits peanut genome sequencing will bring.

William D. Branch, Professor/Peanut Breeder, The University of Georgia

"In a nutshell, Marker Assisted Selection (MAS) would be the most beneficial aspect of this whole research effort."

Mark Burow, Associate Professor, Peanut Breeding and Genetics Texas AgriLife Research Texas A&M System and Texas Tech University Department of Plant and Soil Science

"The genome sequence will allow alignment of transcriptome sequence data of germplasm accessions and parents of breeding populations to the peanut genome sequence. This is needed because of the complex (tetraploid) nature of the peanut genome which would make use of transcriptome sequences by themselves problematic. It is expected that the genome sequence will help in providing the tools for rapid genotyping of peanut, and accelerate the breeding program. We expect to use this for development of peanut varieties with tolerance to drought and heat stresses, improved flavor and quality traits, and resistance to diseases including TSVW, Sclerotinia blight, and leaf spots. Genomics will also assist with use of the AgriLife wild species collection, accelerating introgression of useful alleles from wild species, while selecting against undesirable traits."

Charles Chen, Breeder-Auburn University

"More than twenty years after the advent of DNA markers, marker-assisted selection (MAS) has become a routine procedure for some major crop breeding programs, such as maize, soybean, and tomato and others. Adding new traits to existing commercial released cultivars has been one of the most productive applications of MAS in breeding programs, reducing time to market and resulting in countless numbers of commercial products. Recently, applications of MAS for forward breeding have been shown to increase significantly the rate of genetic gain when compared with conventional breeding. Costs associated with MAS are still very high, but with improvements in marker technologies, data handling and analysis, phenotyping, MAS will eventually become a feasible tool to realize the full benefits of MAS for breeding programs and to allow the transfer of proven approaches and protocols from other crop breeding program to peanut breeding programs. The potential benefits of using markers linked to genes of interest in peanut breeding programs, thus moving from phenotype based towards genotype-based selection, have been obvious for many years. However, realization of this potential has been limited by the lack of markers, genetic map construction, and QTL identification in peanuts."

Albert K. Culbreath, Plant Pathologist, The University of Georgia

"Diseases of peanut represent major yield- and/or profit-limiting factors in peanut production in all peanut producing regions of the world. The use of field resistant cultivars is absolutely critical for management of Tomato spotted wilt. However, the mechanism and genetic control of that resistance is poorly understood. Sequencing the peanut genome would provide a huge information resource that would aid in determining which genes are involved in the resistance that we have, whether resistance from diverse peanut germplasm

is governed by the same genes, and potentially in identification of new sources of resistance. Although the leaf spot diseases and peanut rust can be controlled effectively with available fungicides, such control represents a significant portion of the cost of production in peanut producing regions where fungicide use is an option. In both developed and developing countries improved resistance to leaf spot and/or rust pathogens would be highly desirable to minimize direct losses and/or cost of disease control. Better understanding of gene linkages, interactions, and gene regulation would be helpful in developing cultivars with resistance to both of the leaf spot pathogens and in finding ways to develop leaf spot resistant cultivars that are more acceptable based on other characteristics. A sequence of the peanut genome would be a major resource for developing that understanding. Development of markers for resistance to these and other pathogens would help make breeding programs more efficient in production of resistant cultivars and help ensure that genes for resistance to other pathogens are carried forward even when selection pressure from a particular pathogen is not present. In addition, as progress is made in the characterization of the genomes of the various pathogens of interest, the sequenced peanut genome should provide an excellent resource for examining the interactions of peanut with the pathogens that limit peanut production."

Baozhu Guo, Research Plant Pathologist, USDA-ARS, Crop Protection & Management Unit

"Despite the agricultural and biological importance of peanuts, knowledge of their genetics and genome is very limited. The sequences of the peanut (both tetraploid and diploid) genomes and high density linked map will provide invaluable new genomic resources and detailed map of genes for genetic research and molecular breeding of peanuts. Given the important role of peanut in world food/protein/oil production and security, the peanut genome provides a new resource for use in breeding for specific trait, such as disease resistance, high yield and high oil content. Many traits of interest to breeders are quantitative in nature and the genome sequence will simplify both their characterization and deployment in cultivars with gene-specific marker-assisted breeding."

Corley Holbrook, Breeder-USDA, ARS, Tifton GA

"Molecular markers are widely used in other crops to improve breeding efficiency and effectiveness. Use of marker assisted selection (MAS) in peanut breeding has lagged other crops because of a lack of molecular markers for important traits. Recent advances in peanut genomics research have resulted in the development of a few markers that can be used in peanut breeding. Several breeding programs are using these markers to speed the development of new cultivars Thanks to Peggy Ozias-Akins and Juliet Chu, my program is the largest MAS effort. An excellent example of the potential benefits of MAS is the development of high oleic Tifguard. This project which would have taken 10 to 12 years using conventional technique will be completed in 24 months. In order for the peanut industry to reap the maximum benefits from MAS, additional markers need to be developed for important traits. This should improve the efficiency and effectiveness of all U.S. peanut breeding programs."

Thomas G. Isleib, Professor/Breeder, Crop Science Department, NC State University

"I have never been more sanguine about the value of the genomics work supported by The Peanut Foundation. Because there has been very limited federal funding of such work, and because we have had no federally funded lab with which breeders could collaborate (wheat alone has four), peanut has lagged behind other crops in developing genomic tools for breeders' use. It was long thought that peanut did not exhibit much genetic variation for DNA markers when the standard was RFLPs, RAPDs, and AFLPs, but we are rapidly acquiring a broad array of variable SSR DNA markers that we hope to associate with economically important traits in the crop, traits such as resistance to major diseases, fatty acid balance, and perhaps even folates content or flavor. Some of this type of work has already been accomplished at ICRISAT using peanut rust, a disease of globally importance but currently not much so in the USA. We hope to make similar associations using traits that are important to the US producer, sheller, processor, and consumer. We breeders look forward to incorporating DNA technologies in our peanut improvement efforts."

Peggy Ozias-Akins, Department of Horticulture and NESPAL, The University of Georgia Tifton Campus

"Advancements in molecular breeding of peanut have been slow due to low levels of polymorphism among cultivated genotypes. Discovery of polymorphisms and analysis of gene expression can now be most effective with high-throughput sequencing technologies whose strength is sequence depth rather than length. Polymorphic markers enable tagging of genomic regions associated with traits of interest, which for my lab include disease resistance, reproductive development and seed quality. Quantitative gene expression data assist with the identification of candidate genes and novel alleles for traits of interest. Gene discovery and allele mining facilitate either marker-assisted breeding or transgenic approaches, both of which can accelerate the genetic improvement of peanut."

Tom Stalker, Breeder-Crop Science Department, NC State University

"Development of molecular markers has great potential for increasing breeding efficiency. For example, genotypes can be accurately identified by using any plant tissue at any developmental stage, which can greatly reduce the time and space necessary to evaluate plant populations only present at plant maturity. Molecular markers will allow breeders to follow gene introgression from related species into the cultivated genome, and thus preserve the desired genetic trait(s) vs. routinely losing them during semi-sterile plant generations. Traits that are extremely difficult to select in the field (such as leaf spot resistance, seed quality, or drought resistance) should be significantly easier; and combining high levels of plant resistance and other desired traits into one cultivar will be possible. Importantly, marker assisted selection gives peanut breeders an important tool which is greatly needed to develop improved cultivars for the producer, processor, and consumer."

Barry Tillman, Breeder-University of Florida

"Investment in peanut genomics is important for the future of peanut improvement. From the standpoint of peanut breeding, I believe that these items are a high priority for investment:

- Marker Assisted Selection

- Developing a large group of polymorphic genetic markers
- Developing populations that are genetically divergent for key traits like disease resistance
- Measuring (phenotyping) key traits and associating markers with those traits
- Transgenics

In cases where genotypic variability in natural peanut germplasm is lacking, transgenic approaches are attractive. The costs associated with conducting research in those two areas and in actually implementing those practices in a breeding program on a routine basis are in addition to the costs of running the breeding program as is it now. Certainly, we hold hope that Marker Assisted Selection will improve efficiency and thereby reduce some of the current costs in peanut breeding. However, the majority of the costs to run a breeding program are for labor and that is not likely to be offset by efficiencies gained by implementing MAS, in the near term."

What does the peanut industry expect from The Peanut Genomic Initiative?

George Y. Birdsong, CEO, Birdsong Peanuts

"Based on conversations and comments from breeders, we at Birdsong Peanuts believe Marker Assisted Selection is the most cost effective, efficient and fastest way for the industry to develop new varieties with enhanced disease resistance, drought resistance and improved traits and yields. Marker Assisted Selection also avoids the objection by some users to genetically modified peanuts. Therefore, we have and will continue to support this effort with our financial resources and commitment of time and energy."

Victor Nwosu, Program Manager, Mars Chocolate North America

"The Manufacturing sector of the Peanut Industry relies on supply of quality of peanuts from U.S. to make our products. This supply is being threatened by competition for acreage by other crops such as cotton, soybean, corn, and other future new crops like camelina. Additionally, rising input cost, lack of major improvement in yield, and drought are limiting competitiveness, sustainability and profitability of peanuts. We see the genome project as a means to develop tools to improve yield, water use efficiency, and disease resistance to reduce input cost and make peanut competitive, profitable for growers and sustainable. Therefore Mars Chocolate fully supports the peanut genome project."

Jim Leek, Founder, JLA Global

"As of January 1, 2012, there is a world-wide shortage of peanuts for both the kernel and the crushing markets. I do not see the shortage becoming balanced in the foreseeable future. The shortage is largely due to both a spiraling increase in consumer demand for foods including peanuts worldwide and due to the lack of economic competitiveness of peanuts at the farm level versus other crops. Perhaps more than any other food, peanuts has the potential to alleviate much of the world hunger. Yet we cannot meet the current demand for peanut products among the well-fed population much less the hungry. Completing the peanut genome and identifying genetic trait markers provides us with the tools to improve peanut economic and nutritional characteristics. Equally important, identifying genetic markers allows us to accomplish the improvements by conventional seed breeding. As I see the situation, everyone wins with successful completion of such a project. "

Bill Brown, Logistics Manager, J.M. Smucker Co, LLC

"The peanut genome project is an important strategic initiative with benefits for all segments of the peanut industry. Our challenges for the future are varied and many (i.e., water, disease, pests, land, farm economics, legislation, food safety, consumer acceptance etc). It is imperative that we support efforts like this that will allow us to overcome potential future threats to our viability. Developing tools which can address our long term needs is critical. I fully support the peanut genome project!"

Exhibits

Exhibit A	Funds Spent Through The Peanut Foundation on Genomic Research 2007-2011
Exhibit B	Timeline & Milestones of Success of the International Strategic Plan for the PGI 2008-2012
Exhibit C	Organization of the Peanut Genome Consortium and the Peanut Genome Project
Exhibit D	Milestones for the Peanut Genome Project
Exhibit E	Budget for the Peanut Genome Project

Exhibit A. FUNDS SPENT THROUGH THE PEANUT FOUNDATION ON GENOMIC RESEARCH 2007-2011

SOURCE	ACCOUNT	FUND HOLDER	PROJECT TITLE	APPROVED FUNDS	ANNUAL TOTAL
The Peanut Foundation	04-803-07	Nielsen	Genetic Modification of peanut seeds; development of tools and procedures	$15,000	
	04-805-07	Ozias-Akins	Tilling for Peanut Mutants with Reduced Allergen or Aflatoxin Contents	$15,000	
	04-806-07	Isleib	A Winter Nursery for Peanut Breeding Programs	$20,000	
	04-812-07	Simpson	Maintaining and Evaluating a Backup Collection of Wild Species of Arachis	$10,000	
	06-905-07	Rowland	Physiological responses and functional genomic of cultivated peanut for improved TSWV resistance	$14,000	
	06-906-07	Holbrook	Evaluation of production systems using breeding lines with resistance to leafspot and TSWV to improve Farm net profit	$9,000	
	06-907-07	Holbrook	Developing & evaluation of cultivars with resistance to peanut root-knot nematode & TSWV	$5,000	
	Genomic travel & Consultant fee's 2007		Meetings in Atlanta, Dallas, Washington	$24,188	
National Peanut Board [1]	2007	Stalker	Genomics Databases for Peanut EST's and Proteins	$20,000	2007 TOTAL
	2007	Knapp	Genomic Approaches for Accelerating and Enhancing the Development of Superior Peanut Cultivars	$20,000	
	2007	Grabau	Gaining Regulatory Approval for Bioengineered Peanuts	$10,000	$162,188

SOURCE	ACCOUNT	FUND HOLDER	PROJECT TITLE	APPROVED FUNDS	ANNUAL TOTAL
The Peanut Foundation	04-806-08	Isleib	A winter nursery for peanut breeding programs	$25,000	
	04-810-08	Burrow	Identification of SNP's for genotyping the peanut minicore collection, and for genetic mapping of cultivated crosses	$15,000	
	04-812-08	Simpson	Maintaining and evaluating a backup collection of wild species of Arachis	$10,000	
	04-811-08	Grabou	Folate biofortification of peanuts for domestic and international markets	$5,000	
	04-814-08	Knapp	The development & genetic mapping of a critical mass of DNA markers in peanuts	$60,000	
	04-828-08	Stalker	Genomics databases for peanut EST's and proteins	$30,000	
	06-901-08	Maleki	Antibody production and development of immuno-assays to screen peanut cultivars for allergen levels	$3,000	
	06-902-08	Pittman	Increasing diversity of cultivated peanuts with new resistant germplasm sources from the wild species	$12,000	
	06-905-08	Rowland	Physiological responses and functional genomic of cultivated peanut for improved TSWV resistance	$16,000	
	06-906-08	Holbrook	Evaluation of production systems using breeding lines with resistance to leafspot and TSWV to improve Farm net profit	$9,000	
	06-907-08	Holbrook	Developing & evaluation of cultivars with resistance to peanut root-knot nematode & TSWV	$9,000	
	06-908-08	Culbreath/ Guo	Characterization of two peanut oxalate oxidase genes and development of peanut cultivars with resistance to white mold	$6,000	
	06-909-08	Culbreath/ Guo	Identification and cloning of TSWV resistance genes in cultivated peanuts and development of molecular markers for breeding selection	$9,000	
	Genomic travel & Consultant fee's 2008		IPGI meeting in Atlanta	$99,395	
National Peanut Board [1]	2008	Weissinger	Development of an efficient Agrobacterium-mediated transformation method for peanut	$5,000	
	2008	Grabau	Expanding transformation tools for peanut genomics	$10,000	
	2008	Stalker	Genomic databases for peanut ESTs	$15,000	
	2008	Nielsen	Genetic modification of peanut seeds; development of tools and procedures 2	$20,000	
	2008	Knapp	Genomic resources and approaches for accelerating and enhancing the peanut cultivar development	$25,000	2008 TOTAL
	2008	Burow	Identification of SNPs for genotyping the peanut minicore collection, and for genetic mapping of cultivated crosses	$20,000	
	2008	Ozias-Akins	Enhancing a TILLInG resource for peanut	$5,000	$408,395

SOURCE	ACCOUNT	FUND HOLDER	PROJECT TITLE	APPROVED FUNDS	ANNUAL TOTAL
The Peanut Foundation	04-806-09	Isleib	A winter nursery for peanut breeding programs	$25,000	
	04-814-09	Holbrook	Development and use of molecular markers to accelerate peanut cultivar development; and breeding peanut with resistance to preharvest aflatoxin contamination	$12,500	
	06-901-09	Maleki	Antibody production & development of immuno-assays to screen cultivars for allergen levels	$3,000	
	06-903-09	Culbreath/ Guo	Characterization & genetic mapping of disease resistance genes to TSWV & white mold: Develop molecular markers for breeding selection	$16,000	
	06-906-09	Holbrook	Development & evaluation of cultivars with disease resistance	$20,000	
	Genomic travel & Consultant fee's 2009		Meetings in India, Clearwater, Washington	$33,730	
National Peanut Board [1]	2009	Stalker	Genomics databases for peanut EST's	$15,000	
	2009	Nielsen	Genetic modification of peanut seeds	$20,000	
	2009	Stalker	Sequencing the B genome of peanut	$5,000	
	2009	Grabau	Biotechnology approaches to peanut improvement	$5,000	
	2009	Cook	Genetic markers for the full suite of disease resistance	$90,000	
	2009	Stalker	Transformation of peanut for imporved resistance to fungal diseases	$5,000	2009 TOTAL
	2009	Ozias-Akins	Peanut mutant collections to target reduced allergenicity	$15,000	
	2009	Knapp	The development and genetic mapping of a critical area of the peanut genome	$45,000	$310,230

SOURCE	ACCOUNT	FUND HOLDER	PROJECT TITLE	APPROVED FUNDS	ANNUAL TOTAL
The Peanut Foundation	04-801-10	Stalker	Peanut genomics strategic planning Workshop	$5,000	
	04-802-10	Dang	Creating a bioengineered peanut	$10,000	
	04-803-10	Stalker	Establishing a backup collection of wild Arachis species at North Carolina State University	$6,000	
	04-804-10	Tallury	Using wild species derived lines to develop high oleic peanut cultivars with multiple disease resistance	$3,900	
	04-806-10	Isleib	A winter Nursery for peanut breeding programs	$25,000	
	04-814-10	Holbrook	Development and use of molecular markers to accelerate peanut cultivar development; and breeding peanut with resistance to preharvest aflatoxin contamination	$10,000	
	06-901-10	Maleki	Antibody production & development of immuno-assays to screen cultivars for allergen levels	$2,000	
	06-902-10	Holbrook	Breeding peanuts for disease resistance	$20,000	
	06-903-10	Culbreath/ Guo	Identification of molecular markers associated with resistance to TSWV & leafspot through genetic mapping & selection of high oleic cultivars with high resistance to TSWV	$15,000	
	06-905-10	Ozias-Akins	Mutagenesis of peanut for functional genomics and gene targeting	$15,000	
	06-906-10	Culbreath/ Guo	Building a bridge to future peanut sustainable production; genetic map and marker assisted breeding for healthy high oleic peanuts	$10,000	
	Genomic travel & Consultant fee's 2010		Meetings in Mali, Raleigh, Washington	$84,827	
National Peanut Board [1]	2010	Isleib	A winter Nursery for Peanut Genomics Population	$20,000	
	2010	Stalker/Guo	Peanut Genomics Strategic Planning Workshop	$5,000	
	2010	Stlaker/ Holbrook	Marker Assisted Breeding for Tomato Spotted Wilt Virus	$17,000	
	2010	Holbrook	Development and Phenotypic Evaluation of Recombinant Inbred Line	$15,000	
	2010	Stalker/ Burow	Assessing Allelic Diversity in wild species of Peanut through SSR fingerprinting	$14,000	2010 TOTAL
	2010	Stalker/ Bertioli	Production of Peanut Lines with Controlled Chromosome segment introgressions	$20,000	
	2010	Cook	Integrating Genetic Markers for the Full Site of disease resistance	$34,000	$331,727

SOURCE	ACCOUNT	FUND HOLDER	PROJECT TITLE	APPROVED FUNDS	ANNUAL TOTAL
The Peanut Foundation	04-801-11	Ozias Akins	SNP Genotyping of Cultivated Peanut Populations Segragating for Disease Resistance	$40,000	
	04-802-11	Guo	Construction of a genetic linkage map for cultivated peanut and development of QTL's/Markers for marker assisted breeding	$20,000	
	04-803-11	Stalker	Establishing a Backup Collectgion of Wild Arachis species at North Carolina State University	$10,000	
	04-806-11	Isleib	A winter nursery for peanut breeding programs	$25,000	
	06-901-11	Maleki	Screen peanut cultivars for allergen levels	$2,000	
	06-902-11	Holbrook	Development and evaluation of cultivars with disease resistances to increase on-farm profitability	$18,000	
	06-903-11	Culbreath/ Guo	Identification of molecular markers associated with resistance to TSWV and Leafspot through genetic mapping and selection of high oleic lines with high resistance to TSWV	$16,000	
	06-906-11	Ozias Akins	Molecular markers for leafspot	$13,000	
	Genomic travel & Consultant fee's 2011		Meetings in Brazil, San Antonio, Washington	$98,529	
National Peanut Board [1]	2011	Guo/ Culbreath	Development of a low-cost & high throughput system for peanut genotyping & molecular markers associated with resistance to TSWV, Leafspot, and other traits	$15,000	
	2011	Holbrook/ Ozias-Akins..	Development of Peanut Cultivars with Improved Efficiency & Development & Use of Molecular Markers for Selection Penotyping the RIL population	$32,000	
	2011	Holbrook	Genetic Mapping of Leafspot resistance, Zinc Finger mutation	$45,000	2011 TOTAL
	2011	Patterson	Leveraging Genomic Data & Tools from Botanical Models in Peanut Improvement	$13,000	
	2011	Rowland/ Faircloth/ Ferrell..	Enhancing the Water-Use Efficiency & Drought Sustainability of Peanut Production Through Crop Management & Cultivar Development	$20,000	$367,529
Grand Total 2007-2011					$1,580,069

[1] **National Peanut Board. The projects shown were chosen by The Peanut Foundation and funded by the National Peanut Board. The National Peanut Board spent additional funds on genomics research that did not pass through The Peanut Foundation**

Exhibit B

Timeline & Milestones of Success of the International Strategic Plan for PGI 2008-2012

Peanut Genome Initiative Research Progress Chart (updated April 2012)

Priority	2007	2008	2009	2010	2011	2012
Molecular Markers	Library of 10,000 expressed peanut genes	Gene chip with 8400 genes markers; 5500 gene markers for A genome genetic map	6000 new DNA markers positioned on genetic map	Customized microarrays with genes for disease resistance & peg development	Customized microarrays for food safety,	Adoption of marker assisted selection of traits in all breeding programs
Key Traits	Conventional breeding & inheritance studies	Developed germplasm with PAC and RKN resistance	Early maturing, high O/L varieties for improved flavor characteristics in yield tests	Varieties with stacked traits (PAC, RKN, O/L) in yield test	Agronomic varieties with improved water use efficiency	
Genomic Maps	Useful mapping populations for QTL discovery	Discovery of QTL for Key Traits	First SSR-based genetic map of the peanut genome	SNP-based genetic reference map of the peanut genome	Genetic maps of A, B, AB genomes with sufficient DNA markers for anchoring sequences and QTL discovery	16 CAP populations for mapping high-oleic CBR, TSWV, Early & Late leaf spots, white mold & Sclerotinia blight genes
Germplasm Collection	Flavor & quality analysis of UPPT entries	Initial evaluation of oil; tocopherol, folate, amino acid in core collection	Entry of seed composition descriptors in the USDA GRIN database	Ensure seed viability in germplasm collection	Phenotypic analysis of germplasm collection	High throughput capacity for association of genotypes with disease resistance traits
GM Methods	Gene gun used to transform plants with the desired foreign DNA sequences	Breakthough protocol for efficient transformation frequency	Ability to create stable transformations and reduce time to regenerate fertile GM plants	Ability to transform any peanut genotype	Methods that target specific genes or regions of chromosomes	Ability to insert stacked genes for multiple traits
Biotech Peanut	Transform peanut with resistance to Sclerotinia blight (OK)	Transform peanuts with resistance to Sclerotinia (VA), Stem Rot	Transform peanuts with elevated folate	Transform peanuts with modified protein composition (reduced allergenic potential)	Transformed peanuts with drought tolerance	Yield assessment of current GM peanuts
Regulatory Approval	Initiated regulatory approval process for GM Sclerotinia resistance	Demonstrated little problem with pollen transfer between field grown peanuts	Operative agreements for freedom to operate with GM technology	Regulatory approval for field testing of transgenic material	Methods to control volunteer GM peanuts in commercial production systems	Deregulation of GM peanuts
Gene Discovery	Ability to create gene markers in chemically mutated peanuts	Discovered 3 DNA markers for ara-h2 (allergen protein genes)	Characterize the alleles for the high O/L trait	Discovery of TSWV resistance genes & appropriate gene markers	Amphidiploids to facilitate transfer of genes from wild to cultivated peanut	Routine use of bridge species for development of interspecific hybrids
Gene Characterization	Biotechniques to silence genes in peanut	Silenced the expression of a major allergen, ara-h2	Eliminate genes for specific peanut proteins, oil and fatty acids	Abilty to identify mechanism for genetic resistance to PAC	Ability to identify mechanism for genetic resistance for TSWV & leaf spot resistance	Ability to identify mechanism for genetic resistance for drought tolerance
Animal Test Model	Inbred pigs (F2) selected for hypersensitivity to peanut, monoclonal antibodies against swine IgE	Inbred pigs (F3) selected for hypersensitivity to peanut, monoclonal antibodies against swine IgE	Inbred pigs (F4) selected for hypersensitivity to peanut, ELISA assay for swine IgE	Inbred pigs (F5) selected for hypersensitivity to peanut, histology, immunology	Distribute uniform pig lines on a fee basis for clinical, animal & plant research on food allergy	
Peanut Information System	Alignment with the National Legume Information System	Transcript assembly & EST database for advanced DNA marker discovery	Interactive access to portions (gene clusters) in the peanut genome sequence	State of art interactive genomic database for peanut	Advanced software for comparing genome sequences among species	Access to all sequenced plant genomes through LIS affiliation in the Virtual Plant Information System
Genome Sequencing	Estimated A genome size at 1.7Gb with many repeting sequences; BAC libraries for A, B, AB genomes; Diploid & Tetraploid cDNA librares with 1.1 and 1.4 million bp; 4000DNA markers anchored to BAC sequences; Peanut Genome Index with 35,000 contiguous EST sequences			Established Peanut Genome Consortium for peanut genome mapping	Genomics planning workshop in Brazil; Launched Peant Genome Sequencing Project	Implementation of genome sequencing and reassembly, diploid and tetraploid species

Highlighted areas represent completed work

Exhibit C

Organization of the Peanut Genome Consortium and the Peanut Genome Project

The central thrust of the IPGI Strategic Plan for 2012-2016 will be implemented by the Peanut Genome Consortium (PGC). The PGC is a coalition of international scientists and stakeholders engaged in the Peanut Genome Project (PGP). The PGC is governed by published *Policies & Procedures.*

Contributing members are: Howard Valentine, The Peanut Foundation (***Administrator***); Howard Shapiro & Victor Nwosu, MARS, Inc; Richard Michelmore, University California-Davis (***Co-Chairperson***); Lutz Froenicke, University California-Davis; Scott Jackson, University Georgia (***Chairperson***); Peggy Ozias-Akins, University Georgia (***Co-Chairperson***); Baozhu Guo (***liaison to China***), Corley Holbrook & Brian Scheffler, USDA ARS; Greg May, National Center Genome Resources; David Bertioli, University Brasilia (***liaison to South America***); Soraya Bertioli, EMBRAPA; Rajeev Varshney, ICRISAT (***liaison to India***); Xingyou Zhang, Henan Academy of Agricultural Sciences; Xun Xu, The Beijing Genome Institute; Xingjun Wang, Shandong Academy of Agricultural Sciences; Mark Burow, Texas A&M University; Farid Waliyar, ICRISAT (***liaison to W. Africa***); Graeme Wright, Peanut Corporation of Australia (***liaison to Australia***); Sachiko Isobe, Kazusa DNA Research Institute (***liaison to Japan***); Ran Hovav, Agricultural Research Organization of the Volcani Center (***liaison to Israel***); Tom Stalker, North Carolina State University; and Richard Wilson, Oilseeds & Biosciences Consulting. **Ex Officio** members are: Roy Scott, USDA, ARS, ONP; Pedro Arraes, President, EMBRAPA; Jean-Marcel Ribaut, Director, The GENERATION Challenge Grant Program; Chairman Luo Fuhe, Vice-Chairman CPPCC and Executive Vice-Chairman CAPD Central Committee, Beijing; and David Hoisington, Deputy Director General for Research, ICRISAT; Steve Cannon, Iowa State; John Crow (NCGR); Arvind Bharti (NCGR).

The chart below demonstrates how the PGC is organized, and how it relates to the IPGI and the PGP.

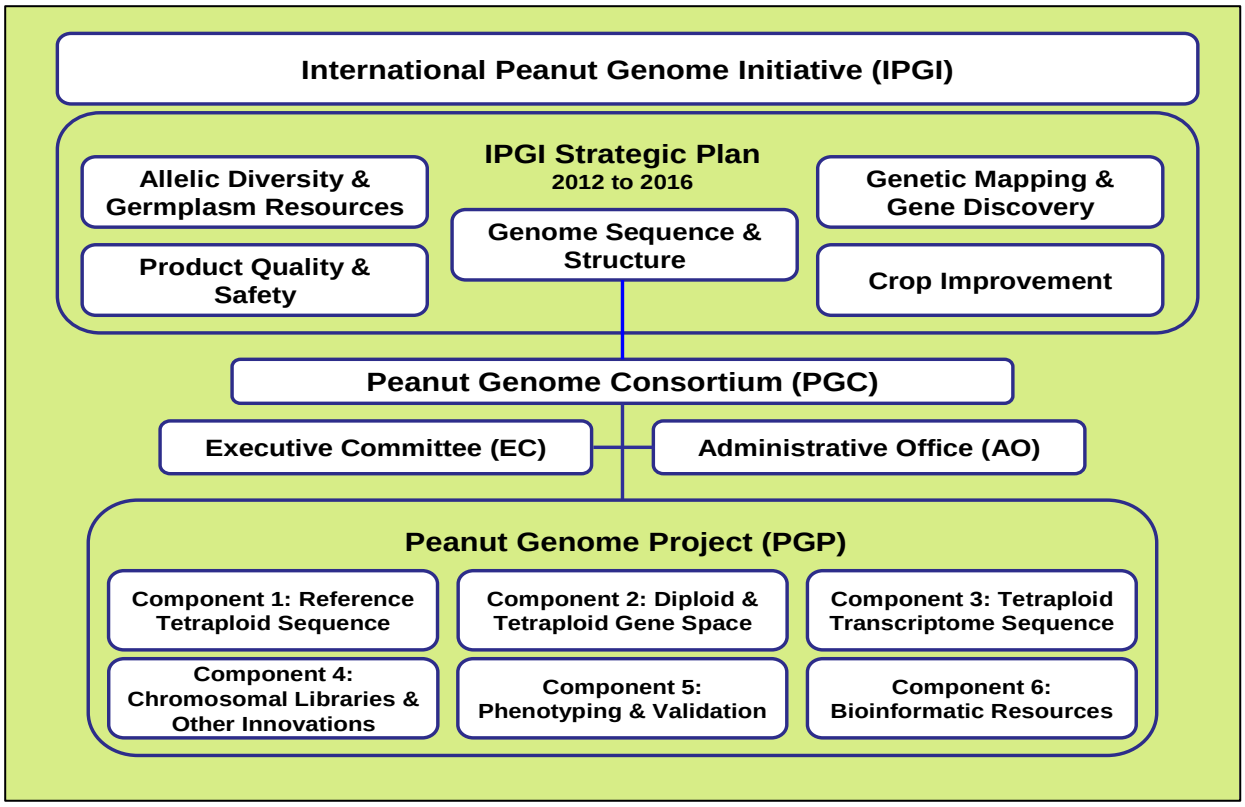

Exhibit D

Milestones for the Peanut Genome Project					
PGP Component	2012	2013	2014	2015	2016
1 Reference Tetraploid & Progenitor Diploid Genome Sequencing & Assembly	Accept proposal & negotiate contract for sequencing & assembly of peanut genomes	High-density genetic maps based on whole-genome shotgun sequencing of cultivated peanut populations	Initial chromosome scale assembly of the reference genome for the cultivated variiety Tifrunner	Genome annotation and quality control protocols	Final report and comparative genome analyses
2 Characterization of Gene-Rich Regions & Genetic Diversity in Cultivated & Wild Peanut Genomes	Genome-wide association studies of gene rich regions in the diploid A genome	Genome-wide association studies of gene rich regions in the diploid B genome	Genome-wide association studies of gene rich regions in the cultivated peanut genome	Genome-wide association studies of genetic diversity in core germplasm collections from the US, India & China	Validated assembly of A and B genomes from progenitor species and synthetic tetraploids derived from diploid specie matings
3 Characterization of Gene Expression & Function in cultivated peanuts	Establish protocol for gene expression profiles of developing organs of the cv Tifurnner	Gene expression profiles for TSWV, Early & Late Leaf Spot, rust, white mold, nematode, & PAC resistance	High-throughput systems for association of trait genes with an expressed phenotype	An atlas for the genotypic origin of genes at the chromosome level for cultivated and wild peanut genomes	Analytical resources to facilitate implementation of DNA-sequence assisted selection in breeding programs
4 Evaluation of Emerging Genomic and Sequencing Technologies	Experimental tests of Pacific-Bio sequencing technology	Experimental tests of NanoPore sequencing technology	Application of new technologies that reduce cost & improve genome sequencig	To be determined as new technologies emerge	Molecular technologies for modification of trait expression in gene networks
5 Phenotyping and Validation of Gene Function	International workshop for training young scientists in the implementation of phenotyping guidelines	DNA marker-assisted selection tools for oleic acid concentrtion and root-knot nematode resistance	DNA marker-assisted selection tools for TSWV, Early & Late Leaf Spot, CBR resistance	DNA marker selection tools for pre-harvest aflatoxin resistance, drought tolerance, folate & flavor	DNA marker-assisted selection tools for peanut yielding ability and stacked traits
6 Bioinformatic Resources and the Breeder's Toolbox	A peanut genome data base that facilitates navigation from maps to genes to traits	A brower that identifies sequence diversity among germplasm and breeding lines	Ability to overlay data from Components 2 & 3 on QTL in the reference genome	Methods to validate and maintain Quality Control of the reference genome	A plan for long-term curation & quality control of all peanut genome sequences

Exhibit E

Budget for the Peanut Genome Project (PGP)

PGP Component	2012	2013	2014	2015	2016	Total
1 Reference Tetraploid &Progenitor Diploid Genome Sequencing & Assembly	$840,000	$1,120,000	$840,000	$0	$0	$2,800,000
2 Characterization of Gene Rich Regions & Genetic Diversity in Cultivated & Wild Peanut Genomes	$100,000	$100,000	$100,000	$100,000	$100,000	$500,000
3 Characterization of Gene Expression & Function in cultivated peanuts	$100,000	$100,000	$100,000	$100,000	$100,000	$500,000
4 Evaluation of Emerging Genomic and Sequencing Technologies	$20,000	$20,000	$20,000	$20,000	$20,000	$100,000
5 Phenotyping and Validation of Gene Function	$300,000	$300,000	$300,000	$300,000	$300,000	$1,500,000
6 Bioinformatic Resources and the Breeder's Toolbox	$100,000	$150,000	$150,000	$100,000	$100,000	$600,000
Grand Total	$1,460,000	$1,790,000	$1,510,000	$620,000	$620,000	$6,000,000

Glossary

Allele
An allele is a viable DNA (deoxyribonucleic acid) coding that occupies a given locus (position) on a chromosome

BAC based sequence
A bacterial artificial chromosome (BAC) is a DNA construct, based on a functional fertility plasmid (or F-plasmid), used for transforming and cloning in bacteria, usually E. coli. BAC's are often used to sequence the genome of organisms in genome projects. A short piece of the organism's DNA is amplified as an insert in BAC's, and then sequenced. Finally, the sequenced parts are rearranged in silico, resulting in the genomic sequence of the organism

BGI
Previously known as Beijing Genomic Institute

Bioinformatic
The application of computer science and information technology to the field of biology and medicine. Bioinformatics deals with algorithms, databases, and information systems, web technologies, artificial intelligence and soft computing, information and computation theory, software engineering, data mining, image processing, modeling and simulation, signal processing, discrete mathematics, control and system complementary DNA

CBR
Cylindrocladium black rot, a disease that continues to slowly spread across the Southeastern peanut production belt and is a particularly big threat to Virginia type peanuts

cDNA
Complementary DNA, derived from the sequence of an expressed gene or EST

Chromosomes
A molecular "package" for carrying DNA in cells, organized as two double-helical DNA molecules that encode many genes. Some simple organisms have only one chromosome made of circular DNA, while most eukaryotes have multiple chromosomes made of linear DNA

Consensus maps
Consensus genetic linkage maps provide a genomic framework for quantitative trait loci identification, map-based cloning, assessment of genetic diversity, association mapping, and applied breeding in marker-assisted selection schemes.

Cultivar
A cultivar is a plant or group of plants selected for desirable characteristics that can be maintained by propagation.

Curator
Administrator who maintains a collection of plant germplasm as either seed or plants in an environment that allows saving the genetic properties for extended periods of time

Diploid
Having two sets of chromosomes

DNA
A polymeric molecule made of deoxyribonucleotides, hence the name deoxyribonucleic acid. Most often has the form of a "double helix," which consists of two paired DNA molecules and resembles a ladder

that has been twisted. The “rungs” of the ladder are made of base pairs or nucleotides with complementary hydrogen bonding patterns.

EMBRAPA
An agricultural research corporation in Brazil; similar in mission to the USDA Agricultural Research Service

EST
An expressed sequence tag or EST is a short sub-sequence of a cDNA sequence.

Folates
Forms of the water-soluble vitamin B9. Vitamin B9 (folic acid and folate inclusive) is essential to numerous bodily functions.

Genotyping
Use of DNA-markers to locate differences in genes among or within a plant

Germplasm
A germplasm is a collection of genetic resources for an organism. For peanuts, the germplasm may be stored as a seed collection or in a nursery as plants.

GMO
Genetically modified organism, typically generated by biotechnology

GWAS
Genome wide association study, the detection of all DNA-markers by comparison of genome sequences among plants of the same species

High Oleic Acid
An oil quality trait that improves the oxidative stability of the oil and reduces trans-fat in food ingredients

ICRISAT
The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is a non-profit, non-political organization that conducts agricultural research for development in Asia and sub-Saharan Africa with a wide array of partners throughout the world

IPGI
International Peanut Genome Initiative

LCGI
A coalition of researchers and stakeholders representing the U.S. peanut, soybean, dry bean, pea & lentil, chickpea & pigeon pea, and alfalfa industries who organized to attain genome sequences of cool and warm season legumes.

MAS
Marker Assisted Selection is a process whereby a marker (morphological, biochemical, or one based on DNA/RNA variation) is used for indirect selection of a genetic determinant or determinants of a trait of interest (e.g. productivity, disease resistance, abiotic stress tolerance, and/or quality).

MOST
Ministry of Science and Technology, a governmental body in China that is similar to the United States Department of Agriculture

Mycotoxins
The toxic chemical products produced by fungi that readily colonize crops

NanoPore platform
This technology may reduce the cost of whole-genome DNA sequencing by more than four orders of magnitude. The technology uses single strand DNA that is hybridized and passed through a nanopore that is electrically charged. This creates an electrical pulse that can be translated to a short DNA sequence.

Nutraceuticals
Combination of the words “nutrition” and “pharmaceutical.” Nutraceuticals are foods or food products that reportedly provide health and medical benefits.

PAC
Pre-harvest aflatoxin contamination, the outcome of crop infection by Aspergillus fungal species

PGC
Peanut Genome Consortium

PGI
Peanut Genome Initiative, (U.S. only)

PGP
Peanut Genome Project

Phenotyping
Identifying the traits that you see on the surface of the plant

Progenitors
Ancestors of a hybridized peanut

RIL
Recombinant Inbred Lines, a highly inbred population of plants from a cross between two parental lines

RNA
Ribonucleic Acid (RNA) is made up of a long chain of nucleotides. Each nucleotide consists of a nucleobase, a ribose sugar, and a phosphate group. The sequence of nucleotides allows RNA to control gene expression for processes like protein synthesis. RNA is similar to that of DNA, with two differences: (a) RNA contains the sugar ribose, while DNA contains deoxyribose, and (b) RNA has the nucleobase uracil while DNA contains thymine.

QTL
Quantitative trait loci (QTL’s) are stretches of DNA containing or linked to the genes that underlie a quantitative trait

Reference sequence
Genomic sequences to be used as reference standards for well-characterized genes

SOAP
A novel short-read assembly method that can build a de nova draft assembly for the human-sized genomes

SNP
Single-nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single nucleotide — A, T, C or G — in the genome (or other shared sequence) differs between members of a biological species or paired chromosomes in an individual. These variations are a predominant form of DNA-markers

SSR
Single sequence repeat, a form of DNA-marker based on variations in nucleotides sequences that are frequently repeated in the genome

Synthetic amphidiploids
To introduce variability from diploid wild species into tetraploid cultivated Arachis hypogaes, a synthetic amphidiploids is used as donor parent to generate a backcross population. Treating an A and B genome cross with colchicin treatment is the method used to create a synthetic diploid

Tetraploid
The main difference between diploid and tetraploid is the number of chromosomes per cell. Diploid plants have two sets of chromosomes per cell while tetraploids have four.

TILLinG
TILLING (Targeting Induced Local Lesions in Genomes) is a method in molecular biology that allows directed identification of mutations in a specific gene. TILLING is a form of mutation breeding

Transcriptomes
The transcriptome is the set of all RNA molecules, including mRNA, rRNA, tRNA, and other non-coding RNA produced in one or a population of cells. The term can be applied to the total set of transcripts in a given organism or to the specific subset of transcripts present in a particular cell type.

TSWV
Tomato spotted wilt virus

References

Reference 1

Legume Crop Genomics, H . Thomas Stalker , E . Charles Brummer, and Richard F. Wilson , AOCS Press, 2004, ISBN: 978-1-893997-48-6
eBook ISBN: 978-1-4398-2226-5

Reference 2

International Strategic Plan for the PGI 2008-2012
http://www.peanutbioscience.com/images/Peanut_Genome_Intitative-StratPlan_DRAFT_v1.2_Mar08.pdf

Reference 3

International Peanut Genomic Research Initiative Strategic Plan 2012-2016
http://www.peanutbioscience.com/images/IPGRI_StratPlan_v4_3_Sep11b.pdf

Reference 4

Peanut Genome Consortium Policies & Procedures
http://www.peanutbioscience.com/images/Peanut_Genome_Consortium-bylaws_v5.7.pdf