**Appendix 4**

**Methods**

This appendix represents a full version of the methods that are proposed.

Objective 1: Improving Yield Potential

Subobjectives 1a. Resistance and pathogen variability for biotic stresses:

Bacterial Diseases:

Common bacterial blight. (CA, NE, ND, PR, WA). The differential reaction between resistance genes/QTL and Xap strains collected across the US (CO, ND, NE, UPR-Zapata, WI) and worldwide will continue to be studied. Diverse strains will be employed to identify sources of CBB resistance in pod tissue which is currently lacking. Better markers for MAS of SAP-6 (Xap-1) will be generated. Numerous RIL populations, with a VAX line as a parent, exist among W-2150 members and will be used collectively to identify markers useful for MAS of the new Xa11.4 QTL. Development of cultivars with improved resistance to CBB using MAS and traditional breeding will continue for the major common bean market classes. The complete genome sequences of strains of CBB representing the known genetic diversity will be determined using whole-genome sequencing via Illumina Hiseq and Pacific Biosciences sequencing. The strains to be sequenced have been selected for having the maximum genetic and pathogenic variability (Mkandawire et al., 2004; Duncan et al., 2011) and will include the globally predominant Xap strains, East African Xap strains and Xaf strains from a number of locations. In addition, the complete genome sequences of a number of nonpathogenic xanthomonad strains associated with common bean will be determined. Whole genome sequences will be analyzed to identify common virulence factors (e.g., type III secretion system and associated effectors) as well as strain specific virulence factors.

Halo blight/Brown Spot. (CA, NE, ND, WA). The races represented by Psp isolates sampled across regions will be determined primarily by inoculation of host differentials. Isolates will be assessed for phaseolotoxin production using a bacterial growth inhibition assay and for the presence of the phaseolotoxin gene with gene-based PCR primers. Subsequently, they will be characterized by repetitive element PCR to assess whether Psp races have distinct DNA fingerprints.

Approximately 140 RILs developed from Rojo/CAL 143, 60 RILs from Canadian Wonder/PI150414, and other crosses involving the five PI landraces with putative resistance to race 6 will be evaluated for halo blight reactions in the greenhouse and under field conditions. These populations will be used to identify novel QTL conferring resistance to race 6. Markers with utility for MAS of the QTL conditioning resistance to race 6 will be developed. The pinto breeding line US14HBR6 will be crossed with susceptible lines from the same gene pool to develop RIL populations for validating and mapping the two recessive resistance genes conditioning resistance to race 6. Phenotypic data obtained from the screening of the USDA core collection will be used for association mapping to identify genomic regions associated with resistance to Psp.

Strains of the brown spot pathogen (Pss), will be characterized in a similar manner to that described above for Psp in response to the resurgence of brown spot disease in some Midwestern states. Additionally, NDSU will lead screening of the Mesoamerican and Andean Diversity Panels (MDP and ADP, respectively) in the greenhouse for reaction to Pss.

Bacterial Wilt. (CA, NE, ND, WA). RILs in the process of development will be screened for reaction separately to yellow, purple, and orange bacterial wilt isolates. The RILs will be screened using the cotyledonary node inoculation method. In addition, there will be efforts to develop insight into the molecular basis of host-pathogen interaction and conduct QTL analysis of new bacterial wilt resistant sources to complement other genomic approaches within the W-3150 project.

Fungal diseases:

Anthracnose. (MI, ND). The bean research community continues to actively screen, characterize and map all new resistance sources following suggested protocols for race characterization and allelism testing. Improved genomic tools will be used to fine map genes with widespread resistance spectrum and identify more robust markers to breeding efforts. Determining the genetic and allelic relationship between many of these resistance genes that reside on adjacent genomic regions will be invaluable to identify the more durable resistance alleles for breeders. The information gained from these efforts will be most valuable to bean breeders and provide tools for effective gene pyramiding and gene deployment.

To date, there is limited data available on the anthracnose resistance of lines within the ADP, but there are plans to validate some resistance present in the Mesoamerican Diversity Panel (MDP), along with the development of more reliable markers for some of the resistance genes. Phenotypic data would be compiled on reaction of these lines to multiple and virulent races of anthracnose and a genome wide association mapping analysis (GWAS) would be conducted as the ADP and MDP have been genotyped with the BARCBean6K\_3 Beadchip and is currently being genotyped using GBS. This information on resistance sources and their location in the genome would complement other studies carried out within international programs such as the Legume Innovation Lab dealing with traits such as yield potential, drought resistance, and enhanced biological fixation within the ADP and be useful to the bean breeding community in the U.S.

In addition, monitoring the pathogen will continue in order to detect changes in race structure of the pathogen to ensure that the most effective resistance genes are being deployed in breeding programs and that currently deployed genes are still effective. Research also is being conducted to improve molecular quantification of C. lindemuthianum as a tool for the seed industry as well as to evaluate the host pathogen interaction.

Root rots. (CO, MI, NE, ND, OR, PR, WA). Fusarium species: Disease surveys will be conducted and root samples collected from grower fields across production regions (MI, MN, ND, NE, OR, PR, WA). Pathogen isolation and identification will be conducted using standard microbiological methods followed by molecular confirmation using PCR and sequencing. To minimize the resources needed, a multiplex PCR assay will be developed to identify Fusarium species in planta. Breeding lines of several market classes from resistant x susceptible crosses will be screened in the greenhouse and field for Fusarium root rot and Fusarium wilt pathogen isolates from the Central High Plains and Michigan. Also, PCR-based MAS methods for Fusarium wilt resistance genes/QTL exist (Singh and Schwartz, 2010). Promising breeding lines will be tested in adaptation nurseries and yield trials across the country; including collaborator states that host root rot nurseries with various soil-borne fungi maintained for germplasm evaluation and other studies. These nurseries should be characterized for the composition of major pathogens.

For Rhizoctonia species, a pot test with inoculum consisting of mycelia applied to the seeds of the host plant or added to a soil similar to the local soil type will test both virulence on differential bean lines and resistance of breeding lines (Pena et al., 2013). Subgroups will be identified by morphology, mycelial compatibility (anastomosis groups), and use of informative primers (Singh and Schwartz, 2010). Pythium spp. have been encountered in the High Plains during wet early seasons and will be characterized and used to screen for resistance. Findings from various national and international research efforts including those involved with USDA-NIFA Root Rot projects will facilitate identification of resistant germplasm, elucidation of the genetics of specific pathogens, identify novel molecular markers and supply advanced breeding material for further use within W-3150 efforts.

Rust. (CO, MD, MI, NE, ND, PR, WA). The common bean scientists working in different research aspects of the rust disease continue to collaborate closely particularly in the introgression of rust resistance into cultivars of various markets classed in the US. Combinations of effective Andean (new gene in PI 260418, Ur-9, Ur-12, and even Ur-4) and Mesoamerican (Ur-11, the new gene in PI 310762, Ur-5, and even Ur-7) rust resistant genes will continue to be developed in cultivars in all US market classes. Identification of new sources and genes for resistance to rust in the greenhouse as well as under field conditions will continue. The ADP screened under field conditions in Beltsville and South and East Africa during 2013 and 2014, revealed new landraces identified as having resistance to rust. These lines will be evaluated under greenhouse conditions with various specific races of the rust pathogen to identify Andean beans with broad resistance to rust with the objective of identifying new Andean sources or genes with broad rust resistance. Collaborations among W-3150 researchers led by ARS-Beltsville will continue the search for markers linked to new and existing rust resistance genes in common bean so these genes could be easily identified using MAS. Interaction between several rust resistance genes including Ur-4 and Ur-5, Ur-4 and Ur-11, Ur-3 and Ur-11, and Ur-4 and the gene in PI 260418 will be examined for efficacy of resistance to specific races of the bean rust pathogen.

The identification of new sources and genes for resistance to rust in the ARS-Bean Project at Beltsville continues in the greenhouse as well as under field conditions. In addition, the reaction of 400 bean accessions of Andean Diversity panel (ADP) nursery was evaluated for their reaction to rust under field conditions in Beltsville, Maryland and in South Africa. This is an activity of the Grain Legumes Project in Africa supported by the Norman Borlaug commemorative Research Initiative between USAID Feed the Future and ARS-USDA to identify common beans with resistance to diseases and with resistance or tolerance to other biotic and abiotic constraints in Eastern and Southern Africa (Pastor-Corrales, et al., 2014). The introgression of rust resistance genes into cultivars with seed of the US market classes, including pinto, great northern, black, light and dark red kidney, pink, and other others, continues as collaboration between the ARS Bean Project in Beltsville, MD and bean projects in state universities including the Universities of Nebraska, Puerto Rico at Mayaguez, Cornell, North Dakota State, Colorado State, Michigan State, as well with bean scientist in other ARS locations and with various scientists in private seed companies is ongoing.

White mold. (CO, MI, ND, NY, NE, OR, WA). Knowledge of genomic locations of QTL conditioning avoidance and partial physiological resistance to WM through fine mapping will allow pyramiding of small effect QTL and generate bean lines for release with higher levels of resistance. Association mapping, next generation sequencing, and RNA expression will be used for fine mapping and candidate gene analysis. Association mapping diversity panels and RIL populations will continue to be shared by the W-3150 research community for characterization of QTL for various traits including resistance to WM. A MAGIC population (Multi-parent Advanced Generation Inter-Cross) to study white mold resistance within the Mesoamerican gene pool is under development at NDSU. In addition, a new technology, metabolic profiling, will be deployed for identification of novel WM resistance mechanisms. Avoidance and physiological resistance from multiple and independent sources will be combined for increased WM resistance. Levels of resistance incorporated into preferred seed types with high agronomic performance will continue to be tested in a coordinated uniform BWMN. Characterization of isolates will involve collection of more grower field isolates in the Great Lakes, Red River Valley and High Plains bean production areas. Pathogen haplotypes and their relationship to MCGs and aggressiveness relationships as well as isolate comparison studies between grower fields and screening nurseries will be studied. In addition, another important characteristic of S. sclerotiorum isolates, fungicide sensitivity, will be determined for selected isolates maintained by W2150 researchers. Ascospores will be used in addition to mycelium to test for fungicide sensitivity. The experimental design allows statistical comparison of isolates within locations as well as between locations and over years (Otto-Hanson et al., 2011).

Subobjective 1b. Abiotic stresses (MI, NE, NY, PR, WA):

Drought tolerance. Putative sources of drought tolerance will be evaluated in a new annual trial, the Dry Bean Drought Nursery (DBDN), with drought trials planted in WA, NE, PR, CO and MI and shuttle breeding between NE and PR will continue. Segregating populations and association mapping (AM) panels will be evaluated in drought stress (DS) and non-stress (NS) environments through a collaborative effort in several states. The characterization of drought tolerance will be conducted on the Durango Diversity Panel (DDP), the MA96 Mesoamerican panel, and the Andean Diversity Panel using AM, as well as in bi-parental populations using QTL analysis. These panels will be genotyped using GBS, followed by AM or QTL analysis, after replicated field trial data has been collected. Through collaboration with other funded projects, e.g. Climate Resilient Bean Project, Legume Innovation Lab Projects, and FtF-ARS, which also have a component of abiotic stress research, some of these trials will be conducted and the data and results shared. The mechanistic studies conducted by these projects can also feed into the breeding objectives of this project when key traits are identified. The goal will be the rapid identification of promising drought tolerant germplasm for breeding, and the development of tools, such as molecular markers and key phenotypic traits, associated with drought tolerance for selection of this critical trait.

Heat tolerance. Collaborative breeding for high ambient temperature tolerance in the dry bean and snap bean market classes will continue under hot summer field conditions (33C/24°C) in Puerto Rico. Thus, tolerance to both high day and high night temperature conditions will be effectively tested in this environment. Greenhouse evaluation will also be conducted at several sites, including NE, where high ambient temperatures can be achieved. Phenotypic selection of advanced lines will be based on yield components, including seed yield, pod number, and 100 seed weight, and on reproductive traits such as pollen shed. Additional phenotypic traits facilitating rapid evaluation and associated with heat tolerance will be implemented as they are developed in this or other projects. Improved heat tolerant germplasm is being developed in the snap bean and in the black, kidney, cranberry, pinto, and small red market classes using pedigree and recurrent selection. Advanced lines selected for drought tolerance through a shuttle breeding program between PR and NE will be tested for heat tolerance and superior lines selected. A RIL population (RCB 593 x INB 841) will be tested in collaboration with the Climate Resilent Bean Project and AM analysis will be conducted on existing panels, including the MA96 and the ADP, for the development of markers for heat tolerance (reviewed in Miklas et al., 2006a).

Subobjective 1c. Characterization/Utilization of Exotic Germplasm (CA, CO, MD, MI, NE, PR, WA):

Characterization and introgression of key traits for U.S. agriculture from exotic germplasm will continue to be the focus of this effort. Evaluation of exotic common bean germplasm for root rot, common bacterial blight, rust, and angular leaf spot disease resistance; for Empoasca and bruchid insect resistance; for drought, heat, and low fertility stress tolerance; and for cooking time, nutrition traits, and efficient biological nitrogen fixation will continue. Selected exotic germplasm and sister species will continue to be used to introgress these specific traits through long-term breeding efforts, for example use of interspecific P. vulgaris x P. acutifolius lines for introgression into commercial common bean market classes. General adaptation and the introgression of photoperiod insensitivity (ppd) will be achieved through the use of recurrent parents representing major market classes in the U.S. or through using selected compatible common bean parents, in the case of crosses with sister species. Cycles of hybridization and selection for the target traits will be coordinated with W-3150 collaborators using methods previously developed for wide crosses (e.g. Beaver and Kelly, 1994) and cutting edge genotyping methods. Attempts will be made to recombine potentially different mechanisms of drought tolerance by multiple rounds of crosses (conical crosses) and evaluating the progenies as fixed lines at the genotypic and phenotypic levels. Furthermore, the hypothesis will be tested that the environment of origin of wild germplasm is a preliminary indication of its potential to contribute useful genetic diversity, e.g. wild beans growing in the driest areas are the most likely to provide genes for drought tolerance. Conservation of and preservation of wild species and their genetic identities is an important task of the National Plant Germplasm System, while habitat for these wild species is diminishing, and changes in the environment are changing the genetic makeup of these species in situ. A specific effort will involve the collection of the North American wild kidney bean or thicket bean (Phaseolus polystachios (L.) Britton, Sterns, & Poggenb.), a perennial vine found in the eastern U.S. from Texas to Connecticut. Although the main focus of the W-3150 is on common bean, other domesticated Phaseolus species, such as lima bean (P. lunatus) or tepary bean (P. acutifolius), can provide alternative crops that, in turn, need to be improved, such as lima bean in California. In the latter situation, resistances to Lygus insect and nematodes are prime objectives. Genetic and molecular information of P. vulgaris can – to a certain extent – be transferred to these alternative species. In other cases, species-specific information will have to be developed.

Subobjective 1d. Genomics/Marker Assisted Selection (CA, MD, MI, ND, NE, OR, PR, WA):

SNP Chip and SNP Calling Parameters. Genome resequencing efforts will be initiated in both the Middle American and Andean gene pools. An adequate sequencing depth of 6x would be suitable for ~200 lines from each gene pool. This data will be augmented with 25x sequence coverage from a set of genotypes that are ancestral to the modern varieties currently used in pedigree breeding programs (~25 lines each). These data will be complemented with SNP data from current genotyping-by-sequencing (GBS) efforts including a 300 wild bean panel at UC Davis, funded by USDA NIFA, a 380 snap bean association mapping panel (SnAP), a Durango Diversity Panel (DDP) with 170 genotypes, the AM96 Mesoamerican drought panel, and a 384 Andean diversity panel (ADP), that will be expanded further during the project. Wild beans include representatives from both Andean and Mesoamerican gene pools. The unique ancestral demography of common bean (two gene pools with multiple races within each gene pool) poses specific challenges when mapping the resequencing reads with the BWA algorithm. The major challenge is faced by genotypes with Middle American ancestry because the reference sequence is from the Andean gene pool. This is an important consideration because the two gene pools are highly divergent. Thus, appropriate mapping parameters (percent mismatches per read, number of gaps) will need to be calculated for each gene pool. Similar issues are of concern when making SNP calls with VarScan, the preferred SNP software. Here we will be concerned with the number of genotypes per putative SNP and the number of variants at the SNP location. Once these issues are resolved, these mapping parameters will be shared by any group using sequenced-based methods to evaluate diversity.

Once these parameters are established, the resequencing data can be mapped to discover SNPs from throughout the genome. It is anticipated that ~5 million SNPs will be discovered from the 6x and 25x resequencing efforts. From the SNP discovery platform, individual SNPs can be selected that are 1) highly variable within each gene pool and 2) have a high minor allele frequency. The community must determine the desired number of SNPs for the next generation chip at a cost point that all programs can afford. The current minimum target should be at least 50,000 SNPs.

The community will strategize regarding the placement of the SNPs. Several strategies are possible. Since most relevant mutations are within a specific gene, it might be appropriate to place one SNP within each exon. The common bean genome contains ~27,000 genes, and each gene contains ~4 exons. This approach would require ~110,000 SNPs. While this seems a reasonable approach, there will be large gaps within the pericentromeric region of the genome that will lack SNPs because only 25% of the genes are located in this region. Therefore, it may be necessary to couple an exonic-focused chip development approach with a requirement of a minimum distance between each SNP. This would increase the marker density within the pericentromeric region and would push the number of SNPs for the chip to ~150,000. At this point the community will need to decide the value of a chip of this density versus the cost of each assay. If a select set of genotypes will be a target for long term phenotyping, then a SNP chip of 150,000 would be useful. If alternatively, the community would rather evaluate a large number of genotypes, it will be most cost effective to develop a 50,000 SNP chip. The final size of the chip will be determined by a community discussion over the next year as the resequencing efforts progress.

Genotyping-by-sequencing. Future work during this project will build on results from both the single-enzyme and two-enzyme reduced representation methods previously described. The advantage of using both methods is that imputation methods can be applied such that SNP calling data can be merged from sequencing multiple GBS libraries. The advantage of this approach is that the genotype data from multiple populations will provide deeper SNP coverage. This will allow the community to leverage the results from multiple labs into a much larger genotypic dataset that will be critical for association mapping approaches discussed in the next section.

Association mapping. This project involves multiple investigators performing intensive phenotyping and trait testing with individual populations at multiple locations. The availability of the trait data, along with genotypic data generated by either of the two approaches described above, enables genetic mapping at a greater depth. Data will be collated from these trials and analyzed using association mapping (AM) techniques. A critical question is environmental variability that will affect the trait value at each location. To account for this, all data will be adjusted using a Z transformation that places the multiple location data on an equivalent value. Efforts will also be made to define a standard set of genotypes that will be grown at each location. These standards will provide another correction that will provide a better estimate of the phenotypic performance across the many locations. The adjusted phenotypic data will be coupled with the imputed genotypic data, and an AM analysis will be performed using statistical corrections that account for population structure and/or relatedness. Those AM results will inform the project with regards to 1) the regions of the common bean genome that affect the trait value, 2) the magnitude of the effect on phenotypic variance, and 3) candidate genes that may affect the trait. The much larger density of SNP will greatly improve the candidate gene selection.

PhaseolusGenes marker database. The PhaseolusGenes molecular marker database will be expanded. Given the increasing importance of DNA sequencing as both a way to generate new markers (SNPs, SSRs, and Indels) and to determine segregation and linkage (for example, by genotyping-by-sequencing, GBS), the PhaseolusGenes database needs to be expanded to accommodate sequence diversity data. These will be obtained from (re-)sequencing of multiple genotypes, such as germplasm collections for association mapping (e.g., Huang and Han, 2014; Morrison and Linder, 2014) or segregating populations for biparental linkage mapping (e.g., Gardner et al., 2014; Li et al., 2014). Alternatively, selected targeted regions of the genome will be sequenced because they contain genes of agronomic interest. A third application will be the use of the common bean genome sequence as a reference for sequences of other Phaseolus genomes, like the lima and tepary bean genomes, in addition to the current synteny efforts with other members of the clade (e.g. soybean and cowpea).

Hence, we propose to develop a workflow/pipeline (e.g., Huang et al., 2014) to 1) filter, trim, assemble, and upload sequence data into the database; 2) establish a list of polymorphisms according to chromosome and position on chromosomes in different genotypes; and 3) display sequence polymorphisms among different genotypes across the bean genome.

Epigenomics. We propose a common bean histone DNA interaction project that captures differential histone DNA interactions in key abiotic and biotic stresses (example bacterial blight, rust, anthracnose etc., or drought, heat, salinity etc.,) with a limited number of histone modification marks known to be important to these stresses in plant and mammalian systems. This will lay the groundwork for future large scale projects in common bean and other legumes (similar to the mammalian NIH ENCODE and Epigenomics Roadmap projects) and will allow us to understand the effect of and inheritance of specific marks during the presence and absence of these stresses.

Crampton and Kalavacharla (unpublished) are also comparing the standard sodium bisulfite treatment and next generation sequencing (Methyl-seq) with a modified immunoprecipation method called methylated DNA immunoprecipation (MeDIP-seq) to identify methylation patterns in the Mesoamerican common bean genotype Sierra. Since methylation of DNA can render these regions inaccessible to regulatory proteins and thus provide another layer of control of gene expression, this is another area where we propose that reference methylation maps of common bean be developed in selected Andean and Mesoamerican genotypes, and subsequently the effects of selected abiotic and biotic stresses on genome-wide DNA methylation be analyzed by MeDIP-seq (as this will be a cheaper method compared to whole genome sodium bisulfite treatment and sequencing) if this proves to be as effective and efficient in identifying methylated regions.

Subobjective 1e. National/Regional Nurseries (CA, CO, MD, MI, ND, NE, NY, OR, PR, WA):

The Bean Rust Nursery (BRN), national Cooperative Dry Bean Nursery (CDBN), Midwest Regional Performance Nursery (MRPN), BeanWhite Mold Nursery (BWMN), Western Regional Bean Trial (WRBT), and the Dry Bean Drought Nursery (DBDN) will continue to be conducted. In addition to these six nurseries, the winter nurseries in Puerto Rico allow W-3150 breeding programs to rapidly advance generations and multiply seed of breeding lines during the winter months (especially in early generations). Winter nurseries also permit breeders to evaluate bean lines for relative maturity and other agronomic traits, in order to ensure broader adaptation of selected lines. Dry bean breeding programs on the mainland U.S. can screen bean breeding lines in the greenhouse for reaction to diseases or can use marker-assisted selection in the laboratory while the winter nursery is growing in Puerto Rico. Collaborators will also have an opportunity to evaluate the performance of bean breeding lines in a low N nursery that will be planted at the same time the winter nursery is planted in Puerto Rico. In the same way, a new Winter Cooperative Dry Bean Nursery (WCDBN) will be conducted in Puerto Rico. We propose to compare the performance of bean lines in Puerto Rico with their performance in different bean production regions of the U.S. A multi-location analysis approach will be used to analyze the results from the CDBN and the WCDBN. Results from the analysis should help to determine how well the performance of bean breeding lines in Puerto Rico predicts the performance of bean lines in different bean production regions of the U.S. This information will provide breeders with a better idea of the degree of selection pressure that can be applied on bean lines in Puerto Rico. A similar approach will be used for other nurseries such as the MRPN and the WRBT in an attempt to estimate genetic gains across years and regions. The Bean Rust and Bean White Mold multi-state nurseries will give breeders a good evaluation of broad resistance to these two diseases.

Objective 2. Analyze, document, and utilize genomic resources to enhance nutritional qualities and identify diversity within Phaseolus to facilitate development of nutritious food products to promote human health and well-being (CA, CO, IA, IL, MD, MI, MO, ND, NE, TX, WA):

Nutritional Value. Seed mineral concentrations will be evaluated using material grown in various regional trials, and as part of the Feed the Future Grain Legumes Project. Emphasis will be given to genotyped diversity panels (ADP, DDP, MDP, etc.), such that these data can be analyzed by association mapping to identify nutrient-related molecular markers. All data will be combined with previous year’s data from the Bean CAP and FtF Grain Legumes projects and will be made publically available. Additional whole-plant mineral investigations will be conducted to determine the spatial and temporal dynamics of mineral transport through bean plants, such that bottlenecks can be identified with respect to mineral flow to seeds. This information will enable the development of strategies to increase seed mineral concentrations.

Processing Quality/Flavor. Flavor is a very important consideration for consumers. Research is planned to gain knowledge regarding variation in sugar and flavor content among a sample of dry bean and green pod-type PI accessions from the USDA Phaseolus Germplasm Core Collection, Pullman, WA. Knowledge of the variation will allow better utilization of germplasm resources in the development of new bean cultivars with more desirable sugar and flavor profiles. The results of this project could be used to market product quality and offer unique opportunities to expand market share to an increasingly health conscious population. From the USDA Phaseolus Core collection containing 423 accessions, a diverse sub-core of 94 Plant Introductions (PI) characterized as snap beans, Romano-types, and other beans eaten as edible immature pods, and 20 dry bean PI accessions will be developed. A replicated field trial consisting of the 94 PI accessions and 14 checks will be planted at West Madison Agricultural Research Station (ARS) the first week of June and will be maintained following standard agricultural practices (Bussan et al., 2012). Experiment design and sampling procedures will be based on preliminary research (Vandenlangenberg et al., 2012a). The stage of maturity of tissue is known to affect sugar accumulation and composition of flavor components in vegetables (Bianco and Pratt, 1977; Hughes and Yamaguchi, 1983; Lester and Dunlap, 1985; McCollum et al., 1988; Villanueva et al., 2004). Measuring snap bean pod maturity using days after flowering or seed size as indirect criteria are problematic among the PIs in this experiment due to different rates of pod and seed development. In addition, small diameter immature pods do not have measurable seeds. To provide a consumer-based evaluation of sugar content relative to pod maturity, pods corresponding to sieve size no. 4 (8.33 to 9.52 cm) or optimal sieve size for each cultivar and PIs will be harvested. Sieve size determination will be done 90° off the suture at the center of the pod. Dry bean checks and PIs will be harvested based on seed maturity (e.g. days after anthesis). A sample of 10 pods will be harvested from each plot at random and bulked. All pods will be harvested in the early morning and immediately placed in a cooler containing ice. The 5 pods intended for sugar extraction will be stored at –80 °C until the pods can be freeze-dried. The remaining 5 pods to be analyzed for 1-octen-3-ol will be frozen at -18 °C until extraction. Breeding for temperate popping bean for production in Washington will be conducted. In addition, the populations produced in crossing will be used for genotyping by sequence (GBS) to elucidate the genes responsible for the popping characteristic.

Canning quality is also a trait that influences bean consumption. Canning quality will be measured on the CDBN from two locations annually. Following harvest, two samples of each variety will be canned according to the methods of Hosfield et al. (1984) with a 90 g seed sample per can based on dry matter. Approximately one month after the beans are canned, visual appeal will be evaluated by 18-20 trained panelists on a hedonic scale of 1 to 7, 1 being least desirable and 7 being most desirable. This scale takes into account whole bean integrity, uniformity of size, and brine color (Wright and Kelly, 2011). Following the visual rating beans will be evaluated for color with a Hunter Lab Colorimeter Lab Scan XE (Reston, VA). The colorimeter uses the Hunter L, a, b color scale, where the “L” value (luminosity) measures white/black on a scale of 1-100, the “a” value measures blue/yellow, and the “b” value measures green/red. Texture will also be measured with a standard shear-compression cell of a Kramer Shear Press. The Kramer Shear press uses a dynamic hydraulic system that reports the peak force needed for loss of total bean integrity. In addition, a machine vision system specifically designed for bean evaluation will be used to automatically measure color, appearance, and texture of beans based on the best image features extracted from the drained seeds and brine. The extracted image information and quality ratings from the sensory panel will be modeled using multivariate and pattern recognition methods for automatic prediction and classification of beans. Seed nutritional composition will be measured in each of the canned bean samples. Specifically minerals and protein will be quantified.

Color retention is one aspect of canning quality that will be genetically dissected. Two QTL have been identified for color retention and canning quality appearance in the Shiny Crow x Black Magic population and one of the QTL for color retention on chromosome 5 overlaps with a QTL for anthocyanin content in the same population (Cichy et al., 2014). These QTL will be tested and validated. A modified bulk segregant approach with bulks of the best and worst black beans for color retention and canning quality will be employed. Polyphenolic amount and composition will also be measured in the black bean genotypes used to develop the bulks for best and worst color. HPLC will be used for the analyses according to the method of Lin et al. (2008). The QTL and markers identified here would be made available publically so breeders can use them for marker assisted selection.

Nutritional Databases. Data bases will be developed as repositories for phytochemical compositional information on beans grown throughout the multistate region. Such compositional information will include, but not limited to, proximates (total protein, moisture, ash, lipids, and carbohydrates), phenolic content (phenolic acids, flavonoids, anthocyanins, tannins), minerals, amino acids, prebiotics (stachyose and raffinose), dietary fiber, resistant starch, phytic acids, saponins, carotenoids, total lignin, starch, vitamins (A, B, and C), etc. This information will then be linked to regional production, environmental conditions, farming practices, and processing operations described throughout this proposal, as it expected that each will affect the composition profiles of beans. The databases will be made available to the different researchers for input and review by means of a web site.

Health Effects. Chronic inflammation is an insidious self-perpetuating cellular stress that is strongly prevalent in obese individuals but also afflicting the general population of western cultures. If left unchecked, chronic inflammation can lead to cardiovascular disease, inflammatory bowel syndrome, cancer, and insulin resistance, to name a few (Cassetta et al., 2011; Giuseppe et al., 2003). Indeed, chronic inflammation has been cited as the secret killer of industrialized nations (Gibbons, 2012). Although various phytochemicals present in dry beans have been shown to prevent chronic inflammation (González-Gallego et al., 2007; Middleton et al., 2000), research that directly links dry bean as protectors against this serious condition is virtually non-existent. Therefore, the long term goal of this research is to establish dry beans as a food system able to prevent or remediate chronic inflammation and its associated disease risk factors. Our hypothesis is that beans will protect against chronic inflammation and/or its disease risks; however, certain bean classes will be more effective than others due to their diverse chemical composition. This hypothesis will be tested by completing the following studies.

Study 1: The effects of individual components present in dry beans and combinations thereof (particularly the phenols, flavonoids and their metabolites) on chronic inflammation will initially be studied using murine RAW 264.7 macrophages. These immortal cells were selected because they are able to express both a pro-inflammatory and anti-inflammatory phenotype. Considering the large number of treatments anticipated for this first phase screening process, these cells are also less costly to prepare and maintain compared to primary cell systems. Briefly, the cells will be grown and maintained as described by Park et al. (2008). After determining non-toxic treatment levels (Zbasnik et al., 2009), the cells will be co-incubated for 24 hours with different concentrations of the bean based phenols and lipopolysaccharide (LPS), a pro-inflammatory inducer, and then will be subjected to LPS for 24 hours followed by the bean based components for another 24 hours. Nitric oxide, which is produced by the pro-inflammatory macrophage, and arginase, which is produced by the anti-inflammatory phenotype, will be monitored to determine the inflammatory state in response to the bean based components (Zhongshi et al., 2011). The treatment results will be compared to controls, i.e., comparing the i.e., cells treated only inducers of a pro-inflammatory state (LPS) or an anti-inflammatory state (interleukin 4). These screening experiments will thus provide information on the ability of the components present in beans 1.) to induce a pro-inflammatory state, 2.) to maintain or remediate to an inactive state, or 3.) to evoke an anti-inflammatory state. Pro-inflammatory cytokines, (tumor necrosis alpha and interleukin 2) and anti-inflammatory cytokines (interleukin 4 or 10) will also be monitored for the most effective bean treatments (Park et al., 2008) to confirm their bioactivity.

Similar studies will then be applied to extracts prepared from dry beans collected throughout the multistate region. For these experiments, the most effective bean extracts will be characterized in terms of their phytochemical compositions using various techniques, such as GC-MS, LC-MS, etc. (Junio et al., 2001). Moreover, the compounds in the bean extracts that are responsible for the anti-inflammatory response will be determined by applying the synergy-direct fractionation method as described by Junio et al. (2001).

As a means to understand the mechanism in which beans exert their expected anti-inflammatory benefits, several cellular transcription factors (hypoxia inducible factor and nitric oxide synthase 2, and carbohydrate kinase-like), and intermediates of glycolysis, pentose phosphate pathway, and citric acid cycle will be measured (Hamalainen et al., 2007; Haschemi et al., 2012; Taylor, 2008). These cellular proteins / metabolites were selected due to their involvement in glucose metabolism, which plays a major role in polarizing macrophages from the anti- to pro-inflammatory states and vice versa (Blagih and Jones, 2012).

While RAW 264.7 cells are ideal model systems for screening a large number of treatments, translational feasibility is best conducted with primary cells derived from bone marrow. As such, we will apply the most effective bean extracts to standard CtrBL/6 mice bone marrow derived macrophages to verify the effects observed in the RAW 264.7 macrophages. These cells will be prepared and maintained as described by Stout et al. (2005). Mechanistic studies will also be completed on these cells as cited previously.

Study 2: In vivo studies will be completed to determine the effects of select bean market classes, cultivars, and/or the potent extracts determined from Study 1on cholesterol levels and intestinal inflammation caused by a fatty diet. A hamster model will be used for this purpose as their lipid metabolic profiles are similar to humans. Additionally, the two cited targets were selected because both are linked to chronic inflammation (Dominic et al., 2013) and can be evaluated in a single animal study. Moreover, it has been shown that fatty diets induce inflammation of the bowel (Ding et al., 2010). Briefly, hamsters will be fed an atherogenic diet supplemented with and without beans or bean extracts at different doses for 4 weeks according to Lee et al. (2014). After four weeks, the animals will be euthanized, and evaluated for liver / plasma cholesterol markers (Lee et al., 2014) and intestinal inflammation markers, as described in Studies 1 and 2. Lastly, the effects that beans have on the microbiome of these hamsters will be evaluated as described by Martinez et al. (2009).

Objective 3. Implement sustainable and profitable agricultural systems that improve bean seed yield, conserve natural resources and protect the environment (CO, MD, MI, ND, NE, OR, PA, PR, WA):

Sub-objective 3a: Integrated Pest and Disease Management:

The proceedings from the annual W-3150 meetings will be documented and distributed to participants and stakeholders. A concerted effort will be made to inform the bean community about online resources and upcoming W-3150 meetings via trade magazines, email and internet promotions.

A more integrated point of exchange between researchers, growers and other interested parties will be developed and expanded using web sites (e.g., the Bean Improvement Cooperative or BIC) while encouraging a more open dialogue of W-3150 findings, concerns, questions and comments. The goal is to provide the most current research results and industry needs that can stand as a platform for further progress. We will support and initiate strategic plans that will identify priorities of the bean industry. Efforts will continue to hold joint biennial meetings of the W-3150 and the BIC to maximize interactions among researchers, extension specialists, bean growers and allied industry representatives.

W-3150 members will continue to share results from this project and learn from colleagues involved with various research and extension projects (e.g., CAP, translational genomics, pathogen diagnostics, Root rot, Climate resilient beans, Legume innovation lab) funded in recent years by the USDA-NIFA, USAID and Specialty Crop Research Initiative (SCRI) regarding issues of relevance to the national bean industry.

Annual meetings for the W-3150 project will be held to share information, update participants on current research and extension, identify potential sources of support for research and extension needs, prioritize research and extension needs, establish cooperative approaches to research and extension needs, and assign committees to address specific disease, insect and related issues as needed. Participants will include scientists and Extension professionals from California, Colorado, Idaho, Michigan, Nebraska, New York, North Dakota, Oregon, Puerto Rico, Washington, Wisconsin and USDA-ARS.

An annual report, including summaries and impact statements from each participant will be generated along with the minutes of the annual meeting and will be sent to committee members and archived on the BIC and NIMSS web sites. The annual report will also be sent to appropriate University Deans, Agricultural Experiment Station Directors, USDA-ARS administrators, key legislators, and other identified stakeholders.

Research results from each sub-project will be published in refereed and non-refereed journals, extension bulletins, the Bean Improvement Cooperative, and posted on the web sites of individual institutions or programs, NIMSS, and other media outlets. In-field research trials and demonstrations will be viewed by the bean industry where appropriate, such as at annual field days, grower educational meetings and workshops, professional society meetings and at the BIC.

All of the major bean grower associations and committees, and seed and chemical companies will continue to be engaged by the W-3150 (see attachment).

Sub-objective 3b: Improved fixation, acquisition and utilization of nitrogen:

Puerto Rico has ideal sites for screening the performance of beans in low N soils (Dorcinvil et al., 2010). The small red breeding line TARS SR05, which was selected in Puerto Rico for adaptation to edaphic stress (Smith et al., 2007), produced among the highest seed yield and had the highest rates of N accumulation and BNF in organic field trials conducted in Michigan (Heilig and Kelly, 2012). The University of Puerto Rico will plant the Cooperative Dry Bean Nursery (CDBN) and other bean breeding lines developed by W-3150 collaborators nursery at the Isabela Substation in a low N environment. The nurseries will be planted in Puerto Rico during the winter months so that collaborators who visit Puerto Rico to evaluate lines in the winter nursery can also evaluate lines in the low soil N nursery. The cooperative nursery will not be fertilized. The trial will be inoculated with Rhizobium strains CIAT 899 and CIAT 632 to promote nodulation. The cooperative nursery will also be planted at sites on the mainland of the U.S. to evaluate the adaptation of lines to temperate climatic conditions and soils. Recently-released bean cultivars selected for adaptation to conventional production systems will be included as check cultivars. In addition to seed yield and agronomic traits, a ‘shovelomic’ method (Trachsel et al., 2014) developed at Penn State University for common beans will be used to measure root traits and nodulation. This approach should help to identify root traits associated with adaptation to low N soils. The Middle American and Andean Diversity Panels will be used to evaluate the genetics of these traits using cutting edge SNP chip and GBS technologies and methods of association mapping.

Finally, ground peat inoculants have been largely used for seed inoculation. Source of peat for inoculants is limiting and evaluation of large number of genotypes for BNF can be challenging at the time of planting. Liquid inoculants are widely used for Bradyrhizobium in soybeans. Evaluation of Rhizobium liquid inoculants with different polymeric additives polyethylene glycol (PEG), polyvinyl alcohol (PVA), gum arabic, cassava starch, and sodium alginate will be evaluated in Puerto Rico (Tittabutra et al., 2006). Different inoculant formulations (liquid and granular) will be applied to the seed and compared with seed inoculation with peat based inoculants under greenhouse and field conditions. This study will contribute to the release of liquid inoculants for common beans.