PROJECT NARRATIVE

(iii) Executive summary and table of contents.

1. Project title - Accelerating grape cultivar improvement via phenotyping centers and next generation markers

2. Project type - Coordinated Agricultural Project

3. Legislatively mandated focus areas being addressed (as numbered and described in the RFA) with percent effort:

1. Research in plant breeding, genetics, and genomics to improve crop characteristics (60%).
    a. product, taste, quality, and appearance;
    b. environmental responses and tolerances;
    d. pest and disease management, including resistance to pests and diseases resulting in reduced application management strategies; and
    e. enhanced phytonutrient content.
2. Efforts to identify and address threats from pests and diseases (15%).
3. Efforts to improve production efficiency, productivity, and profitability over the long term (including specialty crop marketing) (15%).
4. New innovations and technology (10%).

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5. Critical stakeholder need addressed by the project and the project's long-term goals. Grape industry stakeholders have repeatedly emphasized the need to improve powdery mildew resistance and cold tolerance while maintaining and enhancing fruit quality for environmentally and economically sustainable grape production (p.2-3, Table 1). By removing financial, time, and validation barriers to the development and application of molecular markers, this project aims to accelerate the improvement of these critical traits for the direct release of new cultivars and improved efficiency of selection in subsequent generations (p.1, 4-5, 8-9). Augmenting our current participatory breeding approach, economic assessments of the value of particular traits to producers and consumers will guide breeding strategies to promote adoption of improved cultivars (p.4, 6, 8, 14-16, Fig.1).

6. Outreach plan. This project will include a significant outreach and extension component to communicate its progress and results. The outreach plan will focus on developing materials and programming for three audiences: 1) Grape growers and processors who bring grape products to consumers, 2) grape breeders and geneticists, and 3) the general consuming public (p.1-2, 10, Fig. 1) The extension effort will provide information to all of these audiences on traits that are being incorporated into new varieties, explaining new advances in technology that accelerate the development of new and improved grape cultivars, and the importance of these new traits and characteristics with regard to farming practices, product development and enhancement, and market acceptance. These materials will be disseminated through trade journals, short videos, regular internet postings including the eXtension Grape Community of Practice, regional extension newsletters and listservs, and extension presentations at industry technical meetings. Information regarding the progress and impact of the outreach plan will be supported with systematic evaluation by the Project Manager with input from the Industry Advisory Panel (p.22-23, Appendix Tables A1-A4). In addition, we will train geneticists from other specialty crops in the genotyping-by-sequencing (GBS), a cutting-edge genotyping platform (p.17)

7. Potential economic, social, and environmental benefits. The biotic and abiotic stresses identified for marker development cost the industry an estimated $250M dollars each year. This includes the application of 18 million pounds of pesticides http://is.gd/jR0W5, lost yield and replanting of vines due to cold injury. Marker application will encompass traits well beyond these, including diseases that can destroy entire crops. With a total farm gate value of 3.5 billion dollars annually http://is.gd/5y3Xf, even a 1% crop reduction reduces farm income by 35 million dollars. This project will develop regionally adapted cultivars that produce high quality fruit with fewer inputs (p.7, 11, 12, Tables 2-4), providing tangible benefits to grape growers (reduced inputs; reliable production), neighboring communities (reduced environmental impacts), other users of water (increased water quality and availability), and consumers (healthy foods / desirable traits).

8. Stakeholder engagement throughout the project. Industry members were active collaborators in crafting and refining this proposal (p.2-3). An Industry Advisory Panel will meet semi-annually to discuss this SCRI project, providing broad representation in stakeholder feedback and guidance in response to research and extension progress (p.10, 23, Figs. 1-2, Appendix Table A1). Participation of Grape Genetics Focus Group members will ensure alignment with USDA-ARS grape genetic research, facilitating continuity beyond the conclusion of SCRI funding (Table A4). In addition, two small panels will actively participate in experimental design, interpretation of results, and decision-making related to: 1) Traits and Populations and 2) Economics (p.4, 10, 23, Figs 1-2, Appendix I).

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(iv) Introduction.

1. Critical Needs and Goals

A. Long-term goals of the project. Within the scope of the current project, we aim to:

I. Develop breeding strategies for grape genetic improvement driven by consumer, industry, and broader market preferences, and documented economic, environmental, and social benefits.
II. Develop 35 new molecular markers associated with biotic and abiotic stress tolerance, fruit quality, and additional traits in germplasm relevant to U.S. grape breeding programs.
III. Apply new and existing markers across 40,000 breeding lines to accelerate the breeding and selection of improved cultivars combining biotic and abiotic stress tolerance with excellent fruit quality, directly resulting in new cultivars adapted to diverse regions of the U.S.;
IV. Optimize genotyping-by-sequencing (GBS) to increase to 50,000 the standard number of Single Nucleotide Polymorphism (SNP) markers for linkage mapping, decrease the per sample cost to $30, and train up to 30 geneticists from other specialty crops in GBS protocols and data analysis.
V. Enhance communication between grant participants and the industry and public and develop education resources on breeding and genetics.

B. Addressing the critical needs of the grape industry. Grape production is a high-input, expensive endeavor, largely due to the widespread planting of unimproved cultivars, developed 150-2000 years ago, that are highly susceptible to biotic and abiotic stresses. In marker development, we will address several problems that are routinely listed among the highest priority factors in grape production and are genetically tractable:

1) Powdery mildew – U.S. grape growers apply 18 million pounds of pesticides annually to manage powdery mildew, totaling 13% of the total pesticides applied on <u>all</u> crops in CA.
2) Cold tolerance – Cold winters and autumn and spring freezes are limiting factors in the expansion of grape production, destroying entire harvests and/or killing grapevines, causing short or long term losses of grower income.
3) Fruit qualities – The end product is the most important trait. While desirable fruit qualities will vary depending on market type and end use (table, juice, wine, raisins), flavor is important for all markets. Introgression of stress tolerance often brings a linkage drag of negative flavor attributes that this project seeks to break early in selection.

In addition, traits that are regionally-important or specific to an end use (e.g. berry size) will be mapped after phenotyping with standardized protocols. While addressing current critical needs of the grape industry, we will partner with industry leaders to better understand consumer preferences and the economic impacts of improved traits and improved cultivars. Obviously, the most environmentally friendly cultivar would be worthless in the absence of consumer interest or if growers did not find it profitable to adopt for other reasons.

C. Supporting outreach objectives and research questions. The outreach objectives for this project will vary depending on the audience that is being targeted. The objectives will include:

Objectives for grape and affiliated industry members:

- The grape industry has demonstrated a continuing support for disease- and pest-resistant cultivars to overcome weaknesses (e.g. production costs) in those that are currently available. In addition to their continued involvement in participatory evaluation of breeding lines, primary emphasis will be placed on: a) developing a common vocabulary between the grape industry and scientists regarding the processes and applications of genomic technologies and b) traits that can be incorporated into new grape varieties to reduce environmental impacts from grape farming and improve profitability, while maintaining or improving positive fruit and production characteristics.
- Our studies of consumer attitudes, preferences, and economic analyses related to new varieties and products will be communicated to the industry.

Objectives for general public, consumers:

- The project will support the development of materials for distribution in the general media, specialized consumer publications (e.g., wine-focused newsletters and magazines, nursery catalogs), and video-sharing sites about the development of new grape varieties, the potential environmental and business sustainability benefits for both growers and the public, and the techniques used to develop them.

Objectives for plant breeders, geneticists:

- The development and application of markers will have immediate impact in breeding programs. New tools and technologies, including the Genotyping-by-Sequencing (GBS) pipeline, will be of interest to breeders and geneticists who work on grapes and other specialty crops. We will host GBS workshops and online seminars in order to actively transfer technologies to other specialty crops, and have budgeted to use workshop participants' populations as examples.

2. Stakeholder participation

A. How stakeholders were engaged to identify project goals and objectives. Industry priorities were surveyed, discussed, and summarized at six grape stakeholder meetings from 2005-2010, organized by USDA-ARS and held across the country in St. Louis, MO; Davis, CA; Kennewick, WA; Charlottesville, VA; and two in Geneva, NY (Table 1). Participants were invited from each segment of the grape industry, (raisins, table grapes, juice, wine, and nurseries) from different regions of the U.S., and from different sized companies. Formats varied; however, the meetings encompassed presentations by stakeholders representing each segment and/or geographical region of the industry, by leaders of national efforts including the National Grape and Wine Initiative (NGWI) and the American Vineyard Foundation, and by breeders, geneticists, and other scientists from universities and ARS. At each meeting, input from the grape industry was summarized during breakout groups or large discussions, and the priorities and meeting notes disseminated among participants.

Further, two meetings held in Geneva, NY, involved a stakeholder group that is specifically focused on grape genetic research at ARS-GGRU. The GGRU Focus Group mission is to: Promote, enhance, and support GGRU research programs, improve their relevance to and impact on the US grape and grape product industries, and accelerate knowledge and technology transfer of GGRU research to key stakeholders and customers. The focus group membership diversity is based on market sector, geography, and company size. University and ARS administrators, grape scientists, breeders, and extension specialists are active stakeholders in the Focus Group.

During the 16-month preparation of this proposal, more than 20 conference calls and in person meetings were held among NGWI and project leaders to develop a draft proposal and then substantially revise and optimize it. Strong positive feedback was received from NGWI on the community approach to developing and applying molecular markers, the strength of the team, the cutting-edge marker technology, and most importantly the strong connection to deliverables. However, a number of significant revisions were made at the request of NGWI or specific industry collaborators to narrow the focus and shift to scientifically stronger, more immediate, and more achievable impacts in the scope and objectives: 1) Downy mildew and drought lack the knowledge base to meet the scientific rigor of the rest of the proposal and were eliminated from the centralized phenotyping and marker development portion. 2) To focus the proposal, muscadine- and rootstock-related objectives were eliminated since they were very different from bunch grape scion improvement objectives. 3) A component of the consumer perceptions study comparing traditional hybridization vs. cisgenics was troublesome for the industry, and the project was revised to focus instead on trait desirability, consumer and stakeholder education, and economic value of traits added by hybridization vs. chemical additives. 4) The socioeconomic component was expanded to encompass not only consumer preferences relevant to trait selection but also economic analyses relevant to producers. 5) Using project data to inform marketing strategies was seen by one industry board as indicating government preference

for one variety over another. Therefore, data on consumer education and preferences will be available with no mention of a value in marketing. 6) Our many discussions identified the need for a common genomics vocabulary for the industry and scientists to discuss research and examples of existing and potential contributions of genomics apart from GMOs.

In addition to these activities by scientists in partnership with the grape industry, several industry driven reports are available outlining top priorities (Table 1). At the national level, a well-organized strategy has been developed under the leadership of NGWI (www.ngwi.org), with priorities listed at several levels of specificity and detail. The American Vineyard Foundation (AVF) conducted surveys in 2003, 2006, and 2009 (www.avf.org/survey.html) asking growers and vintners (primarily on the West Coast) to identify and rank their top research priorities "to channel [AVF's] limited research dollars where they are most needed." In the eastern U.S., four broad priorities provide direction for Viticulture Consortium-East research, with 8 states individually contributing additional priorities (www.nysaes.cornell.edu/adm/rfpgrapersch/).

Table 1. Grape industry priorities most relevant to the current proposal as identified in stakeholder workshops and surveys.

	ARS Stakeholder St. Louis 2005	ARS Stakeholder Tricities 2007[a]	USDA CSREES CAP Davis 2007	ARS Stakeholder Geneva 2009	ARS Stakeholder Charlottesville 2010	American Vineyard Foundation[b]	National Grape & Wine Initiative[c]	Viticulture Consortium East[d]
Powdery Mildew	✓	✓	✓	✓	✓	✓	✓	✓
Fruit Quality	✓	✓	✓	✓	✓	✓	✓	✓
Cold Hardiness	✓	✓		✓	✓		✓	
New Varieties	✓	✓	✓	✓	✓	✓	✓	✓
Consumers & Economics	✓	✓		✓	✓		✓	

[a] www.ars.usda.gov/research/programs/programs.htm?np_code=305&docid=16731
[b] www.avf.org/results.html
[c] http://ngwi.org/index.php?page_id=212
[d] http://www.nysaes.cornell.edu/adm/rfpgrapersch/

To document industry commitment to molecular breeding, four industry grants are currently funding application of existing markers in grape breeding programs, including selection for seedlessness and for resistance to Pierce's Disease, powdery mildew, and downy mildew. Together, these projects screened in excess of 10,000 seedlings in 2010 and demonstrated that a core set of markers are validated for application and *immediate impact.*

B. How stakeholder involvement will continue. For this project, an Industry Advisory (IA) Panel (Appendix Tables A1, A4), would provide long-term vision and cohesion through overlapping membership with the ARS-GGRU Focus Group to assure continued support and guidance for marker-assisted breeding beyond the conclusion of the project. Semi-annual meetings of the IA Panel will include one in-person meeting and one conference call per year to communicate results of the project and receive input on how to enhance project deliverables. As described above, this approach would encourage participation from each segment of the grape industry, from different regions of the U.S., and from different sizes of companies, as well as major organizations like NGWI and AVF, and university and ARS scientists and administrators.

Engaged participation will occur at frequent decision points (outlined in Table A1) through the Populations & Traits Advisory (PTA) Panel and the Economics Advisory (EA) Panel. These are small, focused groups of industry participants, breeders, and relevant co-PIs (Table A4) that will as a team guide data analysis, experimental design, and prioritization. The engagement of stakeholders is exemplified by NGWI and Gallo, who will maintain five acres of replicated populations and phenotype fruit chemistry for 1600 fruit samples.

3. The body of knowledge and past activities that substantiate the need for the proposed project. While industry priorities are communicated frequently, the basis of those priorities is not always supported by economics and typically focuses on production efficiency over value. Breeding programs would benefit from more rigorous data upon which to plan long-term product development strategies. Building on economics, plant breeding requires expertise in diverse scientific arenas – genetics, horticulture, physiology, statistics, plant pathology, entomology, tissue culture, microscopy, molecular biology, genomics, and computational biology to name a few. Breeding grapes has additional complexity due to long generation times; plant size and training requirements; and complexity of end use (i.e. table, raisin, wine, juice). Still, every grape breeder in the U.S. focuses some attention on the few key traits outlined below and would benefit from access to specialized facilities and expertise for more rigorous phenotyping.

For example, ***powdery mildew*** resistance, while genetically tractable, requires knowledge of both host and pathogen biology and genetics. Given that it takes decades to develop and commercialize grape cultivars, we need to be cognizant of the durability of individual resistance genes and design strategies to combine, or pyramid, multiple resistance genes when appropriate. Powdery mildew gene-for-gene resistances that are effective after formation of the haustorium are common in grapevine (Cadle-Davidson 2008, Gadoury and Pearson 1991) and are rapidly overcome by genetically diverse powdery mildews (Cadle-Davidson and Ramming, unpublished data). We and our colleagues overseas have documented the value of molecular markers in selection for several disease resistance loci (Table 4). Individuals carrying multiple resistance alleles are fully resistant, even with the possibility of combining resistance to multiple diseases (Reisch et al. 2010; Eibach 2007). However, since 2001, only about one powdery mildew marker per year and one downy mildew marker per year have been discovered in spite of strong international efforts (Table 4). To pyramid resistance genes, U.S. breeders need more molecular markers specifically relevant to the breeding germplasm being used.

Various quantitative traits underlying ***cold tolerance*** are even more difficult to phenotype. Winter survival varies in different vine tissues and is influenced by the timing of cold acclimation, rate of cold acclimation, the degree of cold acclimation, retention of sub-zero temperature tolerance developed during acclimation, timing of de-acclimation, rate of de-acclimation and ability to re-acclimate (Stushnoff 1972). The complexity of the plant and environment interactions and the lack of information available on the molecular basis of these traits results in reliance on long-term evaluations in multiple locations and extensive cultural studies after cultivars are released to identify optimum management practices. Although these are well-established techniques, they add considerable land use requirements and expense to the breeding process and delay the release and adoption of improved varieties. These factors plus climate change, which intensifies the need to identify chilling requirements and timing of budbreak traits for changing environments, make it essential to identify markers for these core physiological processes in grapevines.

While selection for biotic and abiotic stress tolerance can be complicated or time consuming, selection for ***fruit quality traits*** is even more costly because it requires mature vines and the expense of maintenance from germination, to greenhouse, to trellising in a vineyard. Evaluation of grapes destined for wine and other value-added products may be further complicated if the trait of interest is only apparent after processing. Marker-assisted selection has already been implemented by U.S. table grape breeders for selection of seedless individuals, allowing them to discard seeded individuals years before they would bear fruit, but this approach has not been extended to flavor quality. Flavor quality is critical to all breeders, and excessive concentrations

of several undesirable compounds have been identified in wild species, including methyl anthranilate (MA) and o-aminoacetophenone (o-AAP), which contribute to the frequently undesirable 'foxy, Concord grape' aroma of *V. labrusca* (Jackson 2000; Acree et al 1990). Other fruit quality traits that are typically negative and frequently occur in introgressions from wild species include: methoxypyrazines (IBMP, IPMP), which are nearly an order of magnitude above sensory threshold in *V. riparia* and *V. cinerea* wines (Sun, et.al. 2010); six-carbon (C6) compounds, namely *cis*-3-hexenol, and the 'eucalyptus'-smelling 1,8-cineole; and excessive titratable acidity (Elias and Dykeman 1990) that in many wild grapes is 2-3X that of vinifera varieties (Sacks, personal comm.). Identifying markers associated with these wild-species derived off-flavors will allow for early selection against undesirable traits at the seedling stage.

The time, expense, and validation required for initiating a marker-based selection program, either for developing new markers or even for applying existing markers, are significant barriers to most grape breeding programs. Since the late 1990s, the costs of developing predictive markers have declined from $500,000 and several years of effort diverted from other tasks to just over $100,000 and one year, with additional costs to applying markers. However, this is still a significant barrier to most breeders who are stretched too thin with too few resources. If we are going to enhance cultivar improvement through molecular breeding, we need to think *bigger, faster, and freely available.*

4. Ongoing or recently completed activities related to the proposed project. Introgression of traits from wild *Vitis* species is routinely used as a cultivar improvement strategy in every U.S. grape breeding program. Some mapping has been conducted in *Vitis*, and a number of markers are available to track specific traits in certain crosses (Table 4). Two genome sequences have been completed and thoroughly annotated for *V. vinifera* (Jaillon et al. 2007, Velasco et al. 2007), integrated with RNA-Seq to define coding regions, further manual curation, and molecular network analysis (Bellin et al. 2009, Grimplet et al. 2009). Building on these resources, the USDA-ARS recently completed a SNP genotyping project across thousands of accessions in the National Plant Germplasm System's (NPGS) *Vitis* species collection using a custom Illumina Infinium genotyping array for 6500 reliable SNPs (Myles et al. 2010). In preparing for this proposal, we assessed the utility of this array for linkage mapping in six grapevine mapping populations. In BC_2 and BC_3 populations with predominately *V. vinifera* DNA, we observed 1000-1200 segregating SNPs primarily tracking vinifera DNA (Cadle-Davidson, Owens and Myles, unpublished data). However, the physical distribution of SNPs across the genome was highly uneven: for half of the populations, fewer than 5 segregating markers were found on chromosome 10, for example. Coupled with a *V. vinifera* bias, the uneven physical distribution of SNPs across the genome makes this technology unsuitable for the purposes of the proposed project.

How duplication of effort with similar activities by others will be avoided. All public U.S. bunch grape breeders are participating in this project. Private and international grape breeders will benefit from the public knowledge and markers developed, but will not be provided public funds for marker application. Markers that are developed and published outside of the project will be applied within the scope of this project, when relevant.

5. Additional preliminary data. Several of the co-PIs on this proposal are actively mapping traits, and several mapping populations with previously mapped traits will be included in this study as positive controls and to initiate fine mapping (Dalbo et al. 2000, Garris et al. 2009, Lowe et al. 2009). Marker-assisted breeding is actively being applied for seedlessness, fruit color, cold tolerance, powdery and downy mildew, and Pierce's disease resistance (Walker, Ramming, Reisch, Owens, and Cadle-Davidson). For this project, we propose to use a SNP discovery and genotyping strategy based on Illumina sequencing adjacent to restriction sites, as used previously to discover SNPs in *Vitis* (Myles et al. 2010) and to genotype maize (Gore et al. 2009). Our 2010 preliminary *Vitis* data suggests that we will map at least 50,000 loci in each population, regardless of genetic background. By discovering and genotyping SNPs in each mapping population, we will avoid the limitations of the Vitis9KSNP array described above.

(v) Rationale and Significance. Currently, improved grape cultivars are developed to satisfy the expressed demands of grape growers, packers, and processors, and other market intermediaries, based on priorities that are frequently communicated, as described above. Consumers, however, are not directly linked to development of grape breeding priorities, and we have no concrete estimates of the annual economic impacts of improved cultivars or the value of those impacts. As summarized in Figure 1 and Table 2, this project's primary goals are: I.A) to characterize and incorporate consumer preferences into long-term genetic improvement strategies; I.B) to understand and incorporate the unique needs and preferences of various industry sectors; I.C) to document the environmental, social, and economic impacts of traits under selection in grape breeding programs; II-III) to intensively pursue key industry and consumer priorities in cultivar improvement to promote sustainable production of high quality grapes; IV) to reduce genotyping costs and train other specialty crop scientists in our genotyping technology to accelerate cultivar improvement across commodities; and V) to enhance communication between grant participants and the industry and public, with a common vocabulary on breeding and genetics and improved understanding of industry and consumer needs. To aid the simultaneous pursuit of complex breeding priorities (biotic and abiotic stress tolerance while maintaining fruit quality), this project will launch centers for genotyping and phenotyping to provide specialized support and access to cutting-edge technologies for grape breeders, thereby accelerating cultivar improvement specifically targeted to meet industry and consumer priorities.

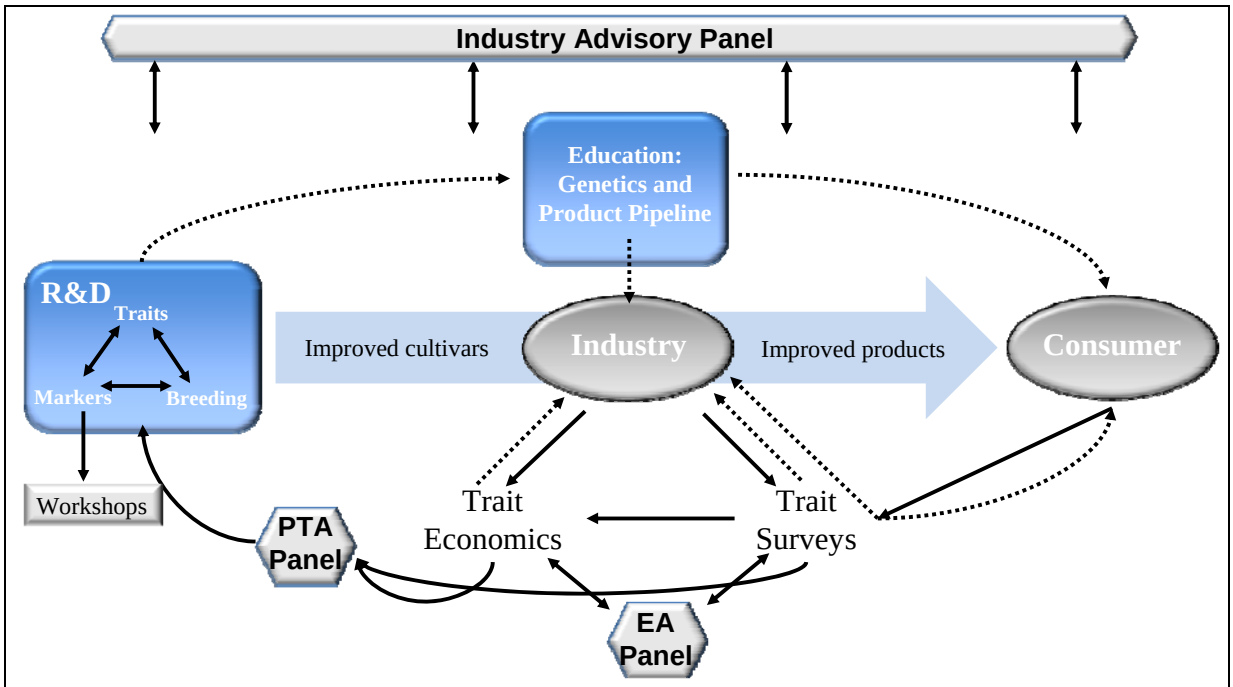

Fig. 1. Project relationships and the integrated role of Extension (E) in results communication and education. Solid lines represent information flow; dotted lines are specific to Extension information flow. R&D = Breeding Research and Development; PTA Panel = Populations and Traits Advisory Panel; EA Panel = Economics Advisory Panel.

Rationale for integrating socioeconomics in grape breeding strategies. The alignment of crop breeding goals and methodologies with consumer preferences is critical for eventual consumer acceptance of new cultivars. In contrast to many food crops, grapes are identified by their varietal name in certain market sectors, e.g. 'Concord' grape juice and 'Chardonnay' wine. Thus, consumers may have an unusually strong, positive association with familiar varietal names. For this reason, a dialogue with grape consumers about new cultivars is necessary to link plant breeding with the eventual marketplace.

Table 2. Relationship of Objectives and Deliverables to SCRI Focus Areas and Stakeholder Priorities.

Objective/Sub-objective	Deliverables
I. Develop consumer-driven breeding strategies for grape genetic improvement with documented impacts A. Survey consumer and industry perceptions of trait desirability and identify marketing barriers B. Quantify the economic, social, and environmental benefits of cultivars with improved traits	***Data to inform funding, breeding, and business strategies*** A. Inform new cultivar development to be more in line with the market, via improved understanding of public views on new grape cultivars B. Ability to document the impacts of improved cultivars and make informed, strategic decisions about priority traits.
II. Develop and apply new molecular markers A. Powdery mildew B. Cold tolerance C. Negative fruit qualities D. Locally-phenotyped traits e.g., berry shape, DOV, other pest/disease	***35 new molecular markers (estimates)*** A. Ten markers for major or minor resistance genes B. Five markers for genes with moderate effects on components of cold tolerance C. Ten markers for genes that negatively impact fruit quality D. Ten markers for genes affecting traits phenotyped by breeders
III. Marker application across 40,000 breeding lines A. Apply new and existing markers, including e.g., downy mildew and nematode resistance; color, seedlessness, sex, berry size	***Accelerated selection*** A. Pyramid genes for stable resistance and prescreen seedlings for abiotic stress tolerance and fruit traits in elite selections leading to new cultivars
IV. Train in GBS for 50,000 markers per population	***Provide cross-commodity experience with GBS mapping technologies***
V. Communicate goals and results clearly	***Common vocabulary for genetics; understanding industry and consumer needs***

Relevant Focus Areas and High-Priority Stakeholder Needs from SCRI Announcement

1. Research in plant breeding, genetics, and genomics to improve crop characteristics ***(Objectives II-IV)*** -Develop innovative breeding systems that improve flexibility and speed for delivering unique specialty crop cultivars to meet future challenges **(Objectives II-IV)**. -Describe and develop systems to remove non-technical barriers to adoption of new cultivars— such as economic or marketing barriers—that inhibit full exploitation of the genetic potential of specialty crops **(Objectives I, V)**. -Preserve and utilize national grape germplasm stocks to develop and expand commercial accessions that meet regional industry cost and quality requirements, and eliminate production hurdles relating to pests/diseases and environmental stress **(Objectives II, III)**. -Facilitate and improve connections between genomic projects and specialty crop breeding programs, including phenotypic characterization, marker development, transgenic research and risk assessment, and cloning of key genes of economic importance for vegetable crops **(Objectives II-IV)**. -Develop new cultivars co-designed to be amenable to increased mechanization **(Objective II.D)**. -Utilize genetic approaches to enhance the nutritional value of specialty crops **(Objective II.D)**.
2. Efforts to identify and address threats from pests and diseases ***(Objective II.ABD)*** -Create new scientific developments and tools that will help reduce the incidence and impact of industry-critical insect and disease problems **(Objective II.ABD)**.
3. Efforts to improve production efficiency, productivity, and profitability over the long term (including specialty crop policy and marketing) ***(Objectives I-V)*** -Improve understanding and application of nutrition, economic, social science, and marketing factors influencing the consumption of specialty crops: develop and evaluate the impact of strategies to improve knowledge, attitudes, beliefs, and behaviors related to consumption; test or develop new methods to measure the outcomes of strategies to increase consumption of specialty crops **(Objective I)**.
4. New innovations and technology ***(Objective II,IV)****, including improved mechanization* ***(Objective II.D)***. -Remove key barriers to achieving labor savings and product quality enhancement in production and processing through research, development, application, and adoption of technologies that are both robust and economical **(Objective II.A-D)**.

Most grape breeding programs use traditional, interspecific hybridization to produce cultivars with adaptive traits from wild grape species, particularly increased tolerance to biotic and abiotic stress which will improve sustainability through reduced inputs, local production, and sustaining rural economies. We propose to develop molecular markers linked to important traits, which will increase the efficiency of production of new, traditionally-bred interspecific hybrid cultivars.

However, even if researchers or industry perceive new cultivars to be beneficial, the prospects for consumer acceptance of new cultivars, let alone their willingness to pay a premium for particular attributes of a particular grape product or the process by which it is produced, remain largely unknown and likely vary among market sectors. The cost and time commitment is large for both developing (grape breeder) and adopting (e.g., grape grower and processor) new cultivars. Thus, *the decisions underlying grape breeding and commercial planting strategies must be based on solid market information.*

Among the many factors that may affect new cultivar perceptions are concerns about human health and safety, economic advantages or disadvantages, social or ethical considerations, potential environmental impacts, perceptions about fruit/product quality, and trust in scientists or governmental regulatory agencies. Furthermore, numerous studies have shown that consumers do not have a clear understanding of plant breeding methods and may equate traditional hybridization with genetically modified organisms or chemical growth enhancers (Levy and Derby 2000, Teisl et al. 2002). The objective of this study is to determine both consumer and industry perceptions of new cultivars and the desirability of traits that can be improved. By gaining better knowledge of consumer and industry preferences for grape traits, we will provide the critical information needed for grape breeders to translate advances in genomics into marketable cultivars.

In an ideal world, consumer preferences might be sufficient to drive adoption of new varieties. However, the added value that a consumer places on a new variety is only one component informing decisions along the production chain. For new traits, we need to understand their economic, environmental, and social benefits. These benefits include reduced costs, increased revenues, reduced use of pesticides (including reduced risks to the environment, farm worker safety, and consumers), and reduced demands placed on the natural resource base more generally. The market information obtained from consumer attitude studies will feed directly into this economic analysis.

By targeting the traits of greatest value to industry and consumers and by providing grape breeders with no-cost access to specialized phenotyping and molecular breeding expertise and resources, we will accelerate cultivar improvement and adoption. Economic analysis has shown that reducing such "innovation lags" by even one year can have substantial effects on the benefit-cost ratio for the investment.

Rationale for marker development for negative fruit qualities. While we focus on marker development for abiotic and biotic stresses that impose chronic costs on growers, in seeking solutions to these problems we necessarily look to wild species, which have notoriously poor flavor for most industry purposes (Jackson 2000). As is the case elsewhere in flavor science, off-flavors often arise from excessive concentrations of only a few compounds, sometimes only a single compound (Reineccius 2006). As a result, several off-flavors arising from wild-species have been characterized, and thus phenotyping and marker development for negative quality is expected to be straightforward. By comparison, generating a reasonable facsimile of positive aromas of most foods and beverages often requires a dozen or more compounds, and increasing the concentration of any one compound does not invariably lead to better consumer acceptance. Thus, defining off-flavors and negative quality traits is invariably more straightforward than defining positive quality traits.

But if phenotyping of negative qualities is straightforward, why bother with markers? Phenotyping is in fact the current approach of all U.S. grape scion breeding programs. However, the long generation time of grapes demands that breeding lines be maintained, transplanted, and pruned for

up to 3-4 years before preliminary assessments of fruit quality can be made, requiring valuable time, materials, and greenhouse and vineyard space. Furthermore, detecting known off-flavors by sensory panel, and even instrumentally, can be more costly and lower throughput than genetic marker analysis. By developing markers for negative fruit qualities, grape breeders can eliminate 50% or more of the progeny as greenhouse seedlings.

Relationship of Objectives and Deliverables to Focus Areas and Stakeholder Priorities. The grape industry has identified several high-priority traits for genetic improvement, and in partnership with grape breeders, recognizes the need for consumer input and an economic analysis of desirable traits to inform long-term breeding strategies. These project goals mesh well with the USDA-NIFA SCRI Focus Areas and Stakeholder Priorities, impacting priority topics in four SCRI Focus Areas and nine High-Priority Stakeholder Needs. The relationship of Objectives and Deliverables to Focus Areas and Stakeholder Priorities is shown above in Table 2, with color-coded shading to relate Focus Areas and Stakeholder Priorities back to Objectives I (blue), II and III (green), and IV including other specialty crops (purple).

Novel ideas and contributions. With the development of the Vitis9KSNP array, USDA-ARS increased 10-fold the number of informative markers easily mapped in a segregating population (from 100 to 1000) and reduced the time spent in genotyping from years to months. However, this array was not fully appropriate for use on wild *Vitis* species. Here, we propose to apply a recently developed genotyping platform for an additional 50-fold increase in marker information, to 50,000 SNPs, at a lower per sample cost ($70 plus data analysis) than custom arrays ($140 plus processing and data analysis). This approach will provide unprecedented access to SNP marker development for all public U.S. grape breeding programs. The primary benefit will be marker-assisted breeding; however, the secondary benefit - an average of 25 SNPs per cM (1 SNP every 10 kb) - will facilitate fine mapping projects to identify genes and alleles contributing to desirable traits at a cost that we hope to reduce to $30 per DNA sample.

An equally exciting novel contribution will come from the consumer perceptions portion of the project. Grape consumers may have a strong, positive association with familiar varietal names (e.g. ‘Chardonnay’ wine and ‘Concord’ grape juice). For this reason, a dialogue with grape consumers about new varieties is necessary to link breeding with the eventual marketplace. To our knowledge this will be the first example of consumer preferences *directly* guiding grape breeding programs. For example, in market sectors where new grape cultivars are of mutual interest to the industry and the breeding programs but where large percentages of consumers have a negative perception of new cultivars, breeding efforts could be focused on integrating the traits of greatest consumer preference and appropriately target those new cultivars to encourage adoption.

(vi) Approach:

1. Activities proposed, key personnel roles in those activities, and the sequence in which the activities are to be performed. Project activities, responsibilities, and evaluation are outlined in Appendix I and under the collaborative agreements section. Specifically these appendices include activities and time sequence proposed (Table A1); key project personnel roles in those activities and their inputs, outputs, impacts, and evaluation (Table A2, Logic Model); program evaluation rubric (Table A3) and advisory panels (Table A4). The project workflows are described here.

The present project significantly ***complements*** existing programs. We will build upon the resources available in public breeding programs and provide an obvious synergy and added efficiency to those efforts. Our project will capitalize on research programs in grapevine pathology, physiology, genetics, and genomics, as well as efforts to study pathogen genomics, race-specificity, and related effector proteins. Project efforts will be well integrated with viticulture/enology extension via direct inclusion and/or interaction with leaders of those efforts.

Advisory panels and project evaluation. Three advisory panels are in place for this project, with current membership outlined in Table A4. The Industry Advisory (IA) Panel was designed to ensure membership diversity by industry segment, geography, and company size. This group will meet semi-annually, annually in person and six months later by conference call. Membership is essentially open to any interested stakeholders and to all project participants. Two smaller panels, the Populations & Traits Advisory (PTA) Panel and the Economics Advisory (EA) Panel, will meet at specific decision points for experimental design, data analysis, and prioritization, as shown in the timeline, Table A1. Team leaders will communicate by quarterly conference calls to report and assess progress and potential issues. The PD will communicate quarterly with all co-PIs concerning activities, progress and findings. The size and complexity of the project necessitates a PhD-level project manager to regularly evaluate progress and impact (Tables A2-A3), to provide objective assessment of individual and group progress, and to inform project management decisions.

Consumer and industry surveys. Surveys will be designed annually in a collaborative effort between the EA Panel and the OIKOS undergraduate program (Davidson, Lusk, and Alston). Results of the survey will be analyzed by Davidson, Lusk, and Alston and communicated to the EA Panel before publication and content development for extension. Over the course of the project 40 undergraduate students will facilitate the activities of the project, interacting with grape geneticists, economists, and extension faculty.

Economic, social and environmental analyses. Analyses will be conducted by Alston using input from surveys and active guidance on prioritization, design, and analysis by the EA Panel.

Extension and outreach. A partnership between extension specialists and a geneticist will develop content for genetic improvement extension and outreach (Walter-Peterson, Wolpert, Stafne, and Project Manager). Content will be published in trade journals, regional internet postings, video-sharing sites, newsletters, and presentations (Walter-Peterson, Wolpert), and eXtension Grape Community of Practice (GCoP) (Stafne) in a manner that protects the ability to patent cultivars. Extension impacts will be measured (Project Manager, IA Panel).

Mapping Populations. Twenty mapping populations have been selected for this project (Table 3) following NGWI input. These are based on ongoing importance to each breeding program, expected biotic and abiotic tolerances, likely commercial value, and contribution to the project in diversifying the regional adaptation, species sources, and market types (Table 3). Each breeder (Walker, Fennell, Reisch, Owens, Ramming, Lu, Luby, Cousins, and Hwang) will maintain these populations through the duration of the project and provide plant material as outlined below and under the Intellectual Property Policy (Appendix II). At-risk populations will be safeguarded in a replicate planting at Gallo property in California.

Centralized Genotyping and Mapping. For marker discovery, just after budbreak in Year 1, each breeder will collect one leaf (dime-sized) from each of 94 progeny and two parents into a 96-well plate for each mapping population and ship to the genotyping center (Schweitzer) for DNA isolation, whole genome amplification (WGA), and processing the 20 plates of 96 DNA samples for genotyping as described below, increasing the number of samples in Year 3 for populations selected by the PTA Panel. For marker application, existing markers can be immediately applied for marker-assisted breeding. DNA from additional populations will be sampled each spring and existing markers (with some examples provided in Table 4) relevant to any breeding population will be screened for segregation and then across the entire population and the resulting data provided to the breeder for validation and/or selection (Schweitzer and Postdoctoral scientist).

Computational analysis. The genotyping center will apply established restriction sequencing (Schweitzer) and computational analysis protocols (Buckler, Sun, and Cornell Postdoctoral Research Associate) to each mapping population. A linkage map will be developed for each population;

Table 3. Populations and traits selected for this project.

Mapping Population/ *Vitis* Species Involved (a-l key below)	**Grape Breeder**	**Market Type**	**Expected to segregate for:**			
			Powdery Mildew Resistance	**Cold Tolerance**	**Negative Fruit Quality**	**Other**
-Riesling x Cab. Sauvignon /a	Andy Walker	Wine				*cluster traits, fruit and juice quality, phenolics, anthocyanins, phenological traits
V. vinifera Malaga Rosada x *V. cinerea* B9/a,e		Wine	✓		✓	*Powdery mildew resistance in a *cinerea* background without other resistant species
V. vinifera F2-35 x *V. arizonica/girdiana* b42-26/a,l		Wine			✓	**Xiphinema index* resistance
-*V. riparia* '37' x Seyval F_2 /c,d,h	Anne Fennell	Wine		✓	✓	*critical photoperiod, dormancy, budbreak, chilling requirement
-*V. rupestris* x Horizon/a,d,h	Bruce Reisch	Wine	✓	✓	✓	*black rot, Phomopsis
-Horizon x Ill. 547-1 /a,d,e		Wine	✓	✓	✓	*antioxidants, secondary metabolites, flower type
-Horizon x *V. cinerea* /a,e,h		Wine	✓	✓	✓	*black rot, Phomopsis
-St. Pepin x Cabernet Franc /a,c,f	Chris Owens	Wine		✓	✓	*budbreak, fruit phenology, pigments and phenolics, berry size, cluster size, secondary metabolites
C87-41 x B82-43 BC2 /a,g	David Ramming	Raisin	✓			*dry on vine raisin, seedless, berry size, skin color, muscat flavor
B37-28 x C56-11 BC1 /a,h		Table	✓			*seedless, berry size, skin color
C81-213 x C74-56 BC2 /a,e		Table	✓			*markers to eliminate off-flavors, seedless, berry size
A90-37 x C45-64 BC1 /a,j		Table	✓			*seedless, berry size, skin color
-N18-6 x Flame Seedless /a,h,j	Jiang Lu	Table	✓		✓	* flower type, berry color, seedlessness, cluster structure, berry size, firmness, productivity, vine vigor
-C30-5-1 x Chardonnay /a,b		Wine	✓		✓	*all major grape diseases, flower type, berry color, berry size, firmness
-Shuangfeng x Chardonnay /a,k		Wine	✓	✓	✓	
-MN 1264 x MN 1214 /a,c,d,f,h	Jim Luby	Wine	✓	✓	✓	*black rot, anthracnose, foliar phylloxera, pigments and phenolics, acids
-Marquette x Regent /a,c,d,f,h		Wine	✓	✓	✓	*black rot, anthracnose, foliar phylloxera, pigments and phenolics, acids
-MN 1276 x Marquette /a,c,d,f,h		Wine	✓	✓	✓	*black rot, anthracnose, foliar phylloxera, pigments and phenolics, acids
-*V. rupestris* 'Wichita Refuge' x (*V. riparia* x *V. cinerea*) /c,d,e	Peter Cousins	Rootstock	✓	✓	✓	**Meloidogyne* nematode resistance, *Xiphinema index* dagger nematode, and phylloxera
-Norton x Cabernet Sauvignon /a,h	Chin-Feng Huang	Wine	✓	✓	✓	*berry rot, berry color, secondary metabolites

a=V. vinifera
b=V. champinii
c=V. riparia
d=V. rupestris
e=V. cinerea
f=V. labrusca
g=V. romanetii
h=V. aestivalis
j= V. shuttleworthii
k= V. amurensis
l= V. arizonica/girdiana

Table 4. Examples of known markers available and relevant for application in marker-assisted breeding.

Symbol	Trait/Allele	Chromosome	Origin	Selected references
SdI	seedlessness	18	*V. vinifera*	Doligez et al. 2002; Cabezas et al. 2006
Sex	hermaphroditism	2	*V. vinifera*	Dalbó et al. 2000; Lowe and Walker 2006; Riaz et al. 2006
Ufgt	berry color	16		Fischer et al. 2004
Mtc	monoterpene content	5	*V. vinifera*	Battilana et al. 2009; Duchene et al. 2009
Lin	Linalool content	10	*V. vinifera*	Battilana et al. 2009; Duchene et al. 2009
5-gt	anthocyanin 3,5-diglucosides	9	*V. labrusca*	Janvary et al. 2009
MybA	berry skin color	2	*V. vinifera*	
Be size	berry size (berry weight)	18	*V. vinifera*	Doligez et al. 2002; Mejia et al. 2007
Ver	véraison	16		Fischer et al. 2004; Costantini et al. 2008
Pdr1	Pierce's disease	14	*V. arizonica*	Riaz et al. 2006, 2008
Rpv1	Downy mildew	12	*V. rotundifolia*	Merdinoglu et al. 2003
Rpv2	Downy mildew	18	*V. rotundifolia*	Bellin et al. 2009
Rpv3	Downy mildew	18		Welter et al. 2007
Rpv4	Downy mildew	4		Welter et al. 2007
Rpv5	Downy mildew	9	*V. riparia*	Marguerit et al. 2009
Rpv6	Downy mildew	12	*V. riparia*	Marguerit et al. 2009
Rpv7	Downy mildew	7		Bellin et al. 2009
Rpv8	Downy mildew		*V. amurensis*	Schwander et al., in preparation
Ren1	Powdery mildew	13	*V. vinifera*	Hoffmann et al. 2008
Ren2	Powdery mildew	14	*V. cinerea*	Dalbo et al. 2001
Ren3	Powdery mildew	15	*V. hybrid*	Welter et al. 2007
Ren4	Powdery mildew	18	*V. romanetii*	Mahanil et al 2011
Run1	Powdery mildew	12	*V. rotundifolia*	Barker et al. 2005
Run2	Powdery mildew	18	*V. rotundifolia*	Riaz et al. 2011
Rdv1	Phylloxera	13	*V. cinerea*	Zhang et al. 2009
Xir1	Nematode: *Xiphinema index*	19	*V. arizonica*	Xu et al. 2008

marker-trait associations identified by QTL mapping; and SNP assays designed for marker-assisted breeding and interval mapping (Owens, Sun, Myles, and Cornell Postdoctoral Research Associate).

Centralized, Standardized Phenotyping. Abiotic stress phenotyping of freezing tolerance by differential thermal analysis (Mills et al. 2006) will be initiated using canes collected at key timepoints during the dormant season. Eight of the eleven populations expected to segregate for cold will be prioritized by age, vigor and diversity of native species by the PTA panel. Three populations per year will be phenotyped, and each population will be sampled in 2 consecutive years, one set in years 1 and 2 and a second set in years 3 and 4.In November, December, January and February each breeder will collect and overnight ship replicate cuttings of the parents and progeny of the selected mapping populations to Fennell. An experienced technician will coordinate shipping and processing schedules with individual breeders. The Fennell lab can, with addition of second recorder and four sample trays to current freezing equipment, phenotype three populations (100 genotypes per population) per year for freezing tolerance and dormancy status.

Powdery mildew phenotyping will be initiated using detached leaves. Every summer of the project, each breeder will sample the parents and genotyped progeny, overnight-shipping leaves to the plant disease phenotyping center (Cadle-Davidson, Gadoury, Seem, and Wilcox). Phenotyping capacity will allow for screening one mapping population per week. An experienced technician will coordinate shipping and processing schedules with individual breeders. Powdery mildew populations will be prioritized by the PTA panel based on expected strength of resistance and mean 50% bloom date as an approximation of foliar development. Cuttings of parents and 20 progeny will be propagated and grown in Geneva, NY for race-specificity and mechanism experiments outlined in the methods.

Chemical quality phenotyping will be initiated in Year 1 to discover populations that segregate for quality traits. In Year 1, on two occasions during the growing season (~40 days post-bloom, and ~100 days post-bloom), breeders will sample fruit (100 g) from 20 progeny in each population. The rationale for early sampling is that many negative fruit qualities are formed early in berry development, and thus will be less sensitive to changes in harvest date or ripening conditions. Furthermore, high concentrations at harvest could arise from either excessive accumulation of the compound early in the season or slow degradation later, and thus selecting early and late fruit may reveal two different QTLs. The fruit will be frozen at -20°C, and overnight-shipped to the chemical quality phenotyping center (Sacks and Mansfield). A technician (Matthew Gates, Cornell) will be responsible for coordinating shipping dates with the breeders and receiving and cataloguing samples. With the assistance of an experienced enology support specialist (Ben Gavitt, Cornell), Mr. Gates will process a portion of the samples for basic juice chemistry analyses, and the remainder frozen for future use. Following receipt of the last samples, Mr. Gates will analyze samples for trace volatiles and prepare samples for submission to the NYS Wine Analysis Lab for analysis of organic acids and nitrogen. *Gallo and NGWI have generously offered to process 400 additional samples each year, doubling our phenotyping throughput.* Populations and traits will be prioritized by the breeders and PTA panel based on the phenotypic data obtained in Year 1, and 5-6 selected populations will be phenotyped in each of the last three years, with preference to populations segregating for multiple traits.

Population improvement. Results from the biotic and abiotic stress phenotyping and from marker application will be communicated to the relevant breeder within three months of sampling, to help inform decisions about future crosses. The PTA Panel will also receive phenotypic data summaries and provide input on prioritization of genotyping and phenotyping efforts to maximize commercial impact. Breeders will be able to take advantage of newly identified markers by having additional selections from their programs screened for markers of

interest. Such results will allow breeders to use genotype-based information in the selection of parents for crosses in the following year.

2. Methods to be used in carrying out the proposed project, including the feasibility of the methods. Figure 2 depicts the systems thinking and contribution of trans-disciplinary approaches in the project.

Consumer and Industry Surveys. Given the strong experience of grape industry participants on the EA Panel in assessing consumer preferences, the panel will interactively develop the annual design of consumer perceptions surveys. The following outlines some critical concepts involved in design and analysis.

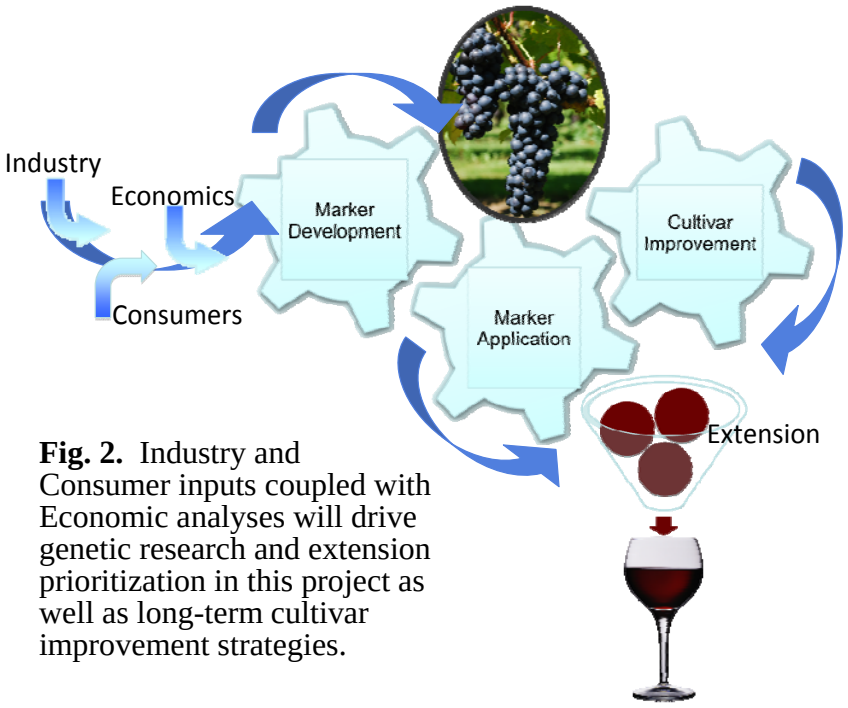

Fig. 2. Industry and Consumer inputs coupled with Economic analyses will drive genetic research and extension prioritization in this project as well as long-term cultivar improvement strategies.

For example, in Year 1, a nationwide survey of a random sample of primary shoppers could assess consumer perceptions about the desirability of traits that can be improved and whether the perceptions differ among market sectors. Exploring general concepts such as improved environmental sustainability (e.g. powdery mildew resistance), improved health compounds (e.g. resveratrol), improved or novel quality characteristics (e.g. fruit shape), and locally produced (e.g. abiotic tolerance), traditional breeding can be compared with treatments generally perceived as being more artificial, such as chemical additives or treatments. To measure the relative importance of three traits, one question could be "how desirable is environmental sustainability provided in a traditional hybrid grape on a scale of 1 to 5, where 1 is very undesirable and 5 is very desirable?" This basic approach would lead to the testing of several hypotheses about consumer preferences over desired traits and the methods of acquiring them: H1: Are gains in environmental sustainability more desirable than gains in flavor? H2: Does the value of mildew resistance (sustainability) depend on technology?

In addition to gathering this information about consumer preferences, we will gather basic demographic information about the respondents. These demographics will be analyzed to identify associations between demographic factors and consumer perceptions, such as proximity to production regions or frequency of grape product consumption. We can also determine whether consumer perceptions vary with respect to the type of grape product; for instance, does consumer perception of a new cultivar used in a luxury product such as wine, for adult consumption, differ from their perception of a new cultivar used in grape juice, which is strongly marketed for children's consumption, or from table grapes that are consumed in over 95 percent of US households on a regular basis?

In conjunction with the Year 1 consumer survey, a second survey will be conducted to understand perceptions about existing and new cultivars among stakeholders who directly interface with consumers of grape products, including: grocery produce purchasers, juice

processors, table grape and raisin wholesalers, nurserymen, wine retailers, sommeliers, restaurant owners, and wine brokers. Ultimately, the decisions made by these professionals will affect the adoption level of new cultivars, from decisions about planting to recommendations about wine pairings for a meal. Data regarding their perceptions will thus provide valuable information about the level of desirability of the development of new traits within the industry.

Annual Survey Revision. The results of the Year 1 survey will be rigorously evaluated by the EA Panel. With their input, the surveys for subsequent years will be developed to focus on specific market sectors where new cultivars are of mutual interest to the industry and the breeding programs, and will focus on understanding which benefits and risks resonate with consumers of grape products. Some possible directions are as follows:

Effects of consumer education. Consumers provided with information about new cultivars with obvious consumer benefits have a more positive perception of them than those who have no information (Brown and Ping 2003). Frewer et al. (1998) also showed a role for education or marketing in consumer preferences - that if the perceived benefit is high, the perceived negative associated with the unknown is lower, indicating acceptance (Frewer et al. 1998). To gauge the effect of information on consumer perception, one survey group would read only descriptions of the three types of cultivars (Info 1), followed by questions about their perceptions (positive, negative, or neutral). The other survey group would be provided with specific descriptions of benefits, such as increased nutrition, lower price, and decreased environmental footprint (Info 2), and then asked about their perceptions. By comparing the data from the two surveys, we will be able to test two hypotheses: H1: With increased consumer education, is an improvement in each trait perceived as a significant improvement to the consumer? H2: With increased consumer education, does an improvement in each trait depend on technology? Further, with demographic information, we will assess whether core consumers who have strong existing preferences are less influenced by information about benefits.

What potential benefits are most likely to affect a change to positive consumer perceptions: sustainability, economics, health, or novelty? New cultivars developed as a result of this project are expected to have several advantages, including increased disease resistance, cold tolerance, yield, health components, and novel shapes or flavors. New cultivars may also decrease the labor, energy, pesticides and other inputs required to grow grapes, resulting in a lower price for consumers and/or greater environmental and economic sustainability. New cultivars will be more likely to be grown in geographically diverse areas, enabling grape production more proximal to grape consumption. Knowledge of which of these benefits are most valued by consumers will help to focus breeding efforts on traits with impact.

While the above approaches would provide general guidance on the relative importance of breeding traits, to directly inform the economic analysis, we will need to obtain information about consumers' willingness to pay for one product over another. Using conjoint analysis, we could measure the economic value that consumers put on the development of traits in various types of grape products (Lusk et al. 2008). Such an analysis provides an economic measure of how valuable the development of new traits is for consumers and can directly inform the economic analysis. The *feasibility of success is high* for some questions, but will be *difficult* for others, such as targeting specific market classes. Some respondents may not be consumers of each market sector, requiring us to increase our number of surveys for statistical significance.

Economic Evaluation of Improved Grape Cultivars. In this phase of the work we will estimate the value of cultivars improved for each of the traits under consideration: powdery mildew resistance, cold tolerance, and improved fruit quality. The benefits to be assessed include (a) the benefits to producers from improved profits (reduced costs, increased revenues, or both), (b) the benefits to consumers from improved quality, cheaper and more-abundant fruit and fruit products, or both, and (c) other benefits associated with reduced use of pesticides, including reduced risks to the environment, farm worker safety, and consumers, and reduced demands

placed on the natural resource base more generally. These benefits will not be realized unless producers adopt the new cultivars, so to some extent producers' expectations of benefits are revealed by their technology choices.

To measure the benefits to producers and others requires information on economic outcomes in scenarios with and without the varietal technology in question, for the range of environments in which they might be adopted, as well as estimates of the actual adoption pattern (in a backward-looking evaluation) or likely future adoption pattern (in a forward-looking evaluation) over the years for which the technology is to be evaluated. In each case it is necessary to define and quantify an appropriate alternative (counterfactual) technology to compare with the one being evaluated (e.g., powdery mildew resistant cultivars versus current non-resistant cultivars), in some cases envisioning different scenarios (e.g. a frost killing 1%, 10%, or 50% of grape buds). Information on costs of production and consumer willingness to pay for the product under each scenario can be used to parameterize a supply and demand model to be used to evaluate the impact of the innovation on production, consumption, prices, and returns to producers. Additional information on environmental impacts of the alternative technologies can be used in conjunction with the market simulation model to evaluate the market-wide environmental benefits from the adoption of the innovation.

The general methodology for such evaluations has been laid out in the book by Alston, Norton, and Pardey (1995, 1998) titled *Science under Scarcity: Principles and Practice for Agricultural Research Evaluation and Priority Setting*. Every particular application of these methods entails some adaptation of the general procedures, to account for the nature of the industry being modeled, the nature of the innovations, and the available data, along with the specific focus of the analysis and the questions to be addressed. The general methods are particularly well suited to the evaluation of varietal innovations, and have found most of their applications there—in particular to yield improvements for annual crops. A good example is the study by Pardey et al. (2006). Varietal innovations for perennial crops have been studied less often and entail some additional complications in modeling, because of the capital nature of the plants themselves. In addition, pesticide technologies bring with them some complications, as discussed by Mullen et al. (2003). A significant part of the work in this phase will be spent developing practicable methods for the present application, hand-in-hand-with the estimation work, which will be the main task, in particular in terms of gathering data on impacts of alternatives at the unit level, and on adoption patterns. The *feasibility of success is <u>high</u>* for successfully documenting the economic benefits, though the estimates will necessarily require the use of forward-looking assumptions about markets and model parameters, and therefore will be subject to measurement error. This aspect of the estimates can be explored through sensitivity analysis to obtain measures of precision to be presented along with the best estimates.

Centralized Genotyping and Mapping. <u>Receiving and processing DNA.</u> Each spring, the genotyping center will receive leaf samples (dime-sized leaves) from breeders in 96-well plates for marker development or for marker application. DNA isolation by modified Qiagen DNeasy and whole genome amplification (WGA) will be completed using protocols optimized for *Vitis*. WGA will ensure sufficient quantity and quality of DNA for use at the genotyping center and for returning an aliquot to the breeder if requested. The *feasibility of success is <u>extremely high</u>* given that the genotyping center has been operating as a Cornell core facility for decades and routinely processes samples for hundreds of clients.

<u>Genotyping-by-Sequencing (GBS).</u> We have completed preliminary analysis of GBS in *Vitis* F_1 and modified BC_2 mapping populations. GBS combines multiplexing (currently 48 samples with unique barcoded adapter sequences) and the generation of reduced representation libraries (RRLs) to provide the read-depth necessary for confidence in SNP calls. We successfully identified over 75,000 SNP loci that map to the *V. vinifera* reference genome and will identify additional SNPs that are novel in these crosses. Thus, we are confident that we will be able to map at least 50,000 SNPs in each population in this project, with well over 1000 markers per

chromosome. To be clear, this approach is not an array based approach and will avoid the weaknesses described above for the Infinium array.

The specific challenge with GBS in grape is calling heterozygotes, phasing the data afterwards and then imputing missing genotypes. These are statistical problems that are a focus of the Sun/Buckler/Myles groups, and we have already identified one marker-trait association in *Vitis* with this approach. Briefly, the 8 bp multiplexing tag of each Illumina sequencing read will be used to sort the data into one FASTQ file per line, and the tags will be trimmed from the reads. The SLIM alignment tool (Real Time Genomics) will be used to align the sequencing reads from each line to the grape reference genome. The candidate polymorphic sites (SNP and small indels) between the reference genome and individual sequenced lines will be called by using the pileup software from the SAMtools (Li et al. 2009) package and scripts developed at Cornell Bioinformatic Service Unit (CBSU). The pileup results from all lines will be integrated using a pipeline created at CBSU. A chi-square test will be used to filter out low confidence SNPs/INDELs (Gore et al. 2009).

All raw data files will be deposited in the NCBI Short Reads Archive database. A project web site will be created to make the processed genotyping results available to the research and breeding community. The web site for downloading data will be maintained at Cornell CBSU after the project is finished. One component of this objective is to continue to reduce the cost to $30 per sample while obtaining more than 50,000 informative SNPs. With improvements in the number of reads per Illumina lane, we anticipate increasing from 48 to 96 the number of barcoded samples per lane, cutting current costs in half. The *feasibility of success for genotyping and mapping is high* given that the methods have been published by several labs, and we have documented the number of markers that can be genotyped with this approach in *Vitis*. The *feasibility of success for reducing costs is high*, but may occur at a slight reduction in marker number if sequencing technologies stop improving.

GBS Workshops. Unlike genotyping arrays, SSR primers, and most SNP assays that are essentially crop-specific, GBS can readily be applied in any organism (though genomes larger than 2Gb may require significant additional cost). Twice during the project, we will host GBS workshops to train scientists from other specialty crops (up to 15 scientists per workshop), advertized by email to relevant academic departments and USDA locations, by announcements at scientific conferences and newsletters, and by posting on the project website. At each workshop, attendees will have the opportunity to construct libraries and analyze data for DNA provided by three of the attendees, so they can learn at least part of the techniques hands-on. The *feasibility of success for the workshops is high*, as it is with GBS in grapes. The number of markers in each crop will be proportional to the number of restriction sites (approximately genome size). Larger genomes may need more sequencing to have sufficient read depth.

QTL Identification. Once the parameters for genotyping are established, the 20 plates of 96 DNA samples will be processed (a total of 1920 samples), and a linkage map developed for each population with marker-trait associations identified by QTL mapping. High density maps for these populations will be constructed using the total number of SNPs identified through the SNP discovery phase, at least 50,000. Since this procedure will provide an excess of co-segregating molecular markers for populations with progeny sizes of approximately 94 individuals, only a subset of 400 SNPs (approximately 20 evenly spaced per linkage group) will be utilized for subsequent QTL analysis. Framework parental maps for QTL detection will be obtained with JoinMap 4.0 (Van Ooijen and Voorrips 2001) by dropping markers within clusters until marker order is supported by LOD 1.0 or more.

QTL will be identified using the double pseudo testcross strategy that has been used extensively in *Vitis* and other highly heterozygous species (Dalbo et al. 2001, Doligez et al. 2006, Doligez et al. 2002, Fischer et al. 2004). Composite Interval Mapping will be conducted using MapQTL 6.0 (Zeng 1994; http://kyazma.nl/index.php/mc.MapQTL/sc.General/). Permutation tests with

1000 permutations will be conducted to determine the experiment-wise significance threshold (Churchill and Doerge 1994, Doerge and Churchill 1996). For each trait, an experiment-wise type-I error rate of 5% will be chosen as the threshold to declare QTL significant. The maximum LOD score will be used to estimate the individual QTL positions. Because 94 progeny may not be sufficient to map some traits in each of the 20 populations (1920 samples including parents), we will reserve 960 slots to be assigned by the PTA Panel after Year 2.

Considering the large number of diverse, segregating populations analyzed in this project, direct comparisons will be made of marker order to identify genomic rearrangements within and between crosses. Physical locations of SNP markers will be referenced relative to the publicly available *V. vinifera* genome. Additionally, regions of elevated and suppressed recombination rate will be determined and compared across the diverse populations to determine if there are species level patterns of recombination hot-spots. Variable segregation distortion is often observed in *Vitis* crosses and will be compared between the different populations to determine if there are coherent patterns within inter- and intra-specific crosses. Characterization of genome rearrangements and meiotic irregularities with respect to *V. vinifera* will inform continuing population development and breeding efforts in the targeting of marker application and determination of population size and crossing strategies for trait introgression. Assuming quality genotypic and phenotypic data, the *feasibility of success is extremely high*, given the routine nature of QTL analysis.

Marker application. Existing markers can be immediately applied for marker-assisted breeding, with 40,000 samples prioritized by the PTA Panel based on equitability, potential impact, and after year 1, results of consumer and industry surveys. Greater emphasis will be placed on major genes that are more likely to be reproducible across environments and populations. DNA from breeding populations will be sampled, and relevant existing markers (Table 4) will be screened for segregation and then across the entire population, if appropriate. The resulting data will be provided to the breeder for validation and/or selection. For new SNP markers developed during this project, a redundant, SNP haplotype signature will be developed using a multi-SNP technology such as Sequenom iPlex. By genotyping 24 markers at a single resistance locus, for example, we have found that multiple markers associated with resistance will be segregating, and can be used for selection, regardless of the susceptible parent selected for the cross. The *feasibility of success is dependent on the marker and source of the trait*; we have documented success with several resistance genes and seedlessness (Cadle-Davidson, Walker, Reisch, and Ramming, pers. communication), but will need to validate other markers before application.

Centralized, Standardized Phenotyping. Cold tolerance: Fennell lab has been screening freezing tolerance, dormancy status, and chilling fulfillment in collaboration with cooperative growers from several locations throughout winter for the last three years and has developed appropriate sample processing protocols. Bud freezing tolerance will be determined in selected populations by differential thermal analysis (DTA) as described by Mills et al. (2006). Cane sections (nodes 3 to 10 from the base of the cane, 3 replicate canes /time point) from selected populations will be collected monthly in early to mid-November, December, January and February by breeder of the panel selected populations and overnight shipped to Fennell lab for freezing tolerance phenotyping. Each population will be scheduled in advance to achieve the same sampling time points in 2 consecutive years without overlapping population arrival. A total of six to seven populations (depending on population size and PTA panel prioritization) will be tested in the four year period.

Samples will be equilibrated at 4C overnight and DTA analysis (70 genotypes/day) will be conducted. Nodes 3-6 will be used for DTA analysis, nodes 7-9 will be placed in forcing conditions to monitor dormancy status/chilling fulfillment (Mathiason et al. 2008) and node 10 will be sectioned and visually examined for preexisting freezing injury. Dormancy status/and level of chilling fulfillment can be acquired with minimal additional assay time (3 hr/week for

each population) once samples are processed in placed in forcing conditions. Collection of first samples in November will provide a uniform freezing tolerance status before most northern populations encounter severe temperature plunges and provide early acclimation response uniform testing point for chilling requirement fulfillment studies. However, differences in population vine size and availability of sufficient numbers of buds for testing may require that some populations are tested at fewer time points, in such cases the number of critical time points will be determined in cooperation with breeder and remain constant for the 2 years of testing. These studies are designed to provide a uniform measure of freezing tolerance differences and dormancy status between genotypes within a population rather than testing genotype by environment interactions. Although beyond the scope of this proposal, longer term studies of individual populations will be able to use the genotyping information in the future to develop genotype x environment information. The *feasibility of success is high* for successfully identifying variation within populations, given the expertise at the center and breeders knowledge about the mapping populations targeting cold tolerance.

Powdery mildew resistance: Located in the center of origin for grape powdery mildew (Brewer and Milgroom, 2010), the ARS and Cornell have been screening breeding germplasm as a collaborative research service for the past five years, resulting in improved knowledge of the mechanisms, inheritance, and race-specificity of resistance (eg, Ramming et al., 2010). We propose to expand our current phenotyping pipeline to accommodate 15 sources of documented resistance to powdery mildew caused by *Erysiphe necator*. The pipeline will include:

(1) Phenotype the parents and 20 progeny (maintained in Geneva greenhouse) for resistance segregation using a single isolate of *E. necator*. If resistance is not observed, screen with a panel of 4 isolates to identify avirulent isolates.

(2) Challenge resistant individuals with a panel of 8 diverse *E. necator* isolates to identify virulent and avirulent isolates. If no virulent isolates are found, repeat with more diverse panel and with natural infection in *E. necator* center of origin.

(3) For each uniquely avirulent isolate in (2), phenotype entire mapping population for resistance, with 10 conidial droplets on 4 leaves per progeny.

(4) With virulent and avirulent isolates on 2 susceptible and 4 resistant progeny of each population, quantify penetration success rate, host necrosis, microcolony success rate, and hyphal length at 72 hours post-inoculation for standardized phenotyping of resistance mechanism and strength in the presence and absence of virulence.

Steps (1), (2), and (4) will be completed using potted vines of five populations each year during the dormant season and Step (3) will be completed twice for each of five populations during the growing season. A fourth year will allow us to phenotype any populations that were not available when needed or failed quality controls (susceptible checks and differential lines). The *feasibility of success is high* for successfully identifying variation within populations, given the experience of the center in providing these services and our knowledge about the mapping populations targeting powdery mildew resistance.

Negative fruit qualities: In collaboration with Gallo and NGWI, phenotyping will be performed on 20 samples from 20 populations at two maturities in the Year 1, with phenotyping of selected populations in later years. Phenotyping of negative fruit qualities will include targeted measurements of volatile and non-volatile compounds previously associated with undesirable characteristics of wild species (IBMP, IPMP, MA, o-AAP, C6 compounds, 1,8-cineole) as described in the Introduction. Volatiles will be extracted by headspace solid phase microextraction (HS-SPME) coupled to gas chromatography time of flight mass spectrometry (GC-TOF-MS). In addition to targeted measurements, we also plan to collect and store full mass spectral data from our volatile analyses. This non-targeted, "omics"-like approach will allow for

post hoc mapping or trait segregation studies. Finally, we will attempt to identify other flavor compounds responsible for negative fruit qualities in wild species using modern flavor chemistry techniques.

For volatile analysis, replicate 25 g berry subsamples will be thawed and homogenized. After a regular, pre-determined time the homogenate will be combined with an equal weight of buffer, the internal standards, and $CaCl_2$ to interrupt further enzymatic activity, particularly of C6 compounds (Tikunov et al 2005, Bezman et al 2003). HS-SPME-GC-MS of the target analytes have been previously reported (e.g. Ryona et al 2009, Flamini, et al 2009, Canuti et al 2009) and we do not expect any difficulty in merging these into a single method. A recent publication by our group on IBMP/IPMP quantification will be used as a starting point (Ryona, et.al. 2009). During phenotyping, qualifier ion ratios will be used to confirm that interfering compounds are not present, and problematic samples can be re-run on the same system in comprehensive 2-D GC mode (GCxGC) to resolve co-elutions by simply turning on the modulator (Herrero et al 2009). Two types of internal standards will be used: i) Deuterated standards will be used for quantification of targeted volatiles, to provide the best compensation for matrix effects (Giovannini 1991). The Sacks lab has the capability and expertise to synthesize standards not available commercially. ii) For non-targeted studies, a cocktail of five non-native, unlabeled standards to account for the diverse chemical properties of volatiles (Lopez, 2002). All ion traces will be detected and stored. Due to the time and expense necessary for data analysis, we do not immediately plan to use the non-targeted raw data set, but it will be available for retroactive data mining. For target analyte quantification, calibration curves will be generated daily. Method stability for the non-targeted analytes will be evaluated by running a "composite sample" generated by homogenizing subsamples from all populations (Tikunov 2005) once every other day. An alkane standard will also be run every other day to correct for retention time shifts. For analysis of organic acids (malic, tartaric, citric), replicate 25 g grape subsamples will be crushed by hand, stirred, and heated to redissolve potassium bitartrate crystals. Samples will be pressed through cheesecloth. A portion of the juice will be retained for analysis of basic chemical parameters (pH, Brix, TA) using common protocols, and the other portion submitted to the NYS Wine Analysis Lab for organic acids analysis by HPLC.

Identification of novel compounds responsible for off-aromas in wild species will be performed at Cornell. In Years 1-2, accessions of wild species important to breeding (e.g., *riparia*, *rupestris*) will be obtained from the USDA Cold Hardy Grape Collection. At least 2L of each juice will be prepared, with accessions combined if necessary. The fruit will be macerated, pressed, and stabilized with DMDC and stored cold to prevent spontaneous fermentation. Control juices from *V. vinifera* will also be produced. Within one week of production, the juices will be submitted to quantitative descriptive sensory analysis (QDSA) using a trained sensory panel (Lawless and Heymann 1998). Juices that demonstrate undesirable sensory characteristics (i.e. bitterness, off-aromas) will be dereplicated, and 1-2 juices selected for detailed flavor analysis. Samples will be extracted and fractionated, and submitted to a bio-assay (LC-Taste, GC-Olfactometry) to detect the most potent and relevant fractions/components (Ferreira and Cacho 2009). Compounds will be identified by MS, NMR, and comparison with authentic standards. The importance of compounds discovered will be evaluated by spiking/reconstitution experiments into neutral *vinifera* juices. During Years 2-3, we will quantify newly discovered traits in the USDA accessions and survey 10 breeding populations to determine appropriate populations for further study. In Years 3-4, we will phenotype the trait(s) among 2-4 selected populations for molecular marker discovery.

The *feasibility of success is <u>high</u>* for successfully identifying variation within populations for known target analytes, given the expertise at the center in chemical analyses and our knowledge about the mapping populations targeting negative flavor components. Additionally, several pathways and genes associated with the off-flavors are known which will facilitate selection of appropriate SNPs for QTL analyses. The *feasibility of success is <u>moderate</u>* for identifying novel

compounds responsible for off flavors in wild species. While off-flavors are often the result of single compounds, some of the off-flavors in wild species may arise from the combination of sub-threshold concentrations of multiple compounds, which would be recalcitrant to bio-assay based flavor chemistry techniques.

3. Expected outcomes, including how the project expects to contribute to long-term profitability and sustainability of specialty crops. **Consumer perceptions and economic benefits.** The analysis will be designed to quantify the advantages and disadvantages and economic value of various traits from the perspectives of consumers and market intermediaries such as retailers, processors, and wholesalers, and to determine under what circumstances and for what traits consumer education can affect consumer perceptions. A further outcome of this work will be the calculation of the total economic value of traits to producers and the economy as a whole, which may justify additional support for targeted genetic improvement projects. The data, along with existing industry priorities, will be used to guide long-term breeding strategies and the development of new cultivars targeted to increase the quantity, quality and/or value of grapes consumed.

Phenotype-based selection in breeding populations. In addition to the use of phenotypic data for marker development, these data can be directly used for selection of parents or of cultivars for commercial release. These decisions will be supported by standardized abiotic and biotic stress phenotyping. The cultivars released as a direct result of this project will increase the spectrum of resistant cultivars available and the environments where they can be grown, thereby enhancing the profitability and sustainability of specialty crops.

Mapping traits. QTL analyses in grapevine have proven highly effective in identifying broad genomic regions underlying important traits. The proposed research will greatly assist in improving marker-density by up to 500-fold above existing *Vitis* linkage maps, making population size the sole limiting factor. Most populations in the present project include more than 200 seedlings and are available if needed to increase mapping resolution. Considering the small genome size of grape, combining improved marker density and available whole genome sequence will lead to a greatly improved ability to identify genes underlying important traits. It is expected that QTL of variable effect will be identified in all populations for the phenotypes assayed. It is unknown if QTL will be identified in the same genomic regions across populations. These results should prove directly useful in marker-assisted breeding of grape and would allow for further research into the high-resolution mapping of important QTL. Further, by training other specialty crop scientists in GBS data analysis, we can enhance molecular breeding, cultivar improvement, and profitability and sustainability across commodities.

Applying markers. Breeding programs operate on limited budgets, though the expense of maintaining plantings, pruning, training and trait evaluation is high. No public breeder has the financial resources to apply molecular marker-based selection without grant funding specifically targeted toward such work. This project will provide a no-cost service to breeders seeking to validate and apply marker technology for grapevine cultivar improvement.

4. Means by which results will be analyzed, assessed, or interpreted. Analysis of consumer perceptions data will include routine statistical analyses to detect significant differences of means, or more complex analyses, as described in the Pitfalls section. For the PTA Panel to compare relative strength of phenotype expression, the average response will be calculated for each seedling and plotted from one extreme (eg, most tolerant) to the other (eg, most sensitive). This simple data will inform prioritization for phenotyping additional seedlings or replicates and for increasing population sizes. As importantly, statistical analysis of experimental error in phenotyping in Year 1 will allow calculation of the number of replicates needed in Years 2-4 for each population. Methods for linkage mapping and QTL analysis are well established and should provide no obstacles. The key will be whether markers discovered in the project can be confirmed to predict phenotypes in related populations. For many of the populations, related

crosses are already available for marker validation. Breeders will be encouraged to provided DNA from related populations to confirm marker efficacy.

5. How results or products will be used. The results of the economic analysis will guide long-term breeding strategies and the development of new cultivars targeted to increase the quantity and/or value of grapes consumed. New and existing markers will be used for marker application. The results of marker application will be used to confirm marker-trait associations and apply marker data for marker-assisted selection. Application of these markers will accelerate selection for abiotic stress tolerance, disease resistance, and/or fruit attributes, as well as the pyramiding of resistance genes for durable resistance. The development of new cultivars with these traits targeted to meet consumer and industry priorities will result in increased sustainability by reduced fungicide application and by grape products being grown locally; increased acreage in climates that are currently marginal for grape production; and increased value for grape products that have value-added labels, such as organic or heart healthy.

6. Outreach plan: including, where appropriate, science-based tools disseminated, participants involved in delivery, and how impacts will be measured. The outreach plan anticipates that work done in the first two years will focus primarily on raising awareness of the project and its goals, and the importance of breeding in the advancement of industry goals such as reducing the environmental impacts of grape growing and improving the sustainability and profitability of vineyards through the introduction of improved grape varieties. These efforts will primarily be targeted to members of the grape and grape products industries, and the general public.

Later, the focus of our outreach efforts will shift towards the dissemination of research results as relevant. Project members will work with the extension team to create content and develop materials describing these results for distribution to industry members, as well as breeders and geneticists who work with grapes and other specialty crops. Outreach to the general public and interested consumers will not stop altogether in the final years of the project, but more effort will be focused on distributing results to the industry and fellow scientists.

A critical extension component of this project will be to interact with the Grape Community of Practice (GCoP) within eXtension. The project members of this proposal will act as a content team under the umbrella of the GCoP. The plan to develop a Grape Community of Practice was generated by the Extension and Outreach Education Committee of NGWI. The community of interest to be served by the GCoP is a wide-ranging group of individuals from seasoned viticulturists at well-established vineyards, to small, family farming operations in rural areas of the country, to the hobby grower, to enologists. The driving force behind the eXtension GCoP is the demand for basic, intermediate, and advanced viticulture education geared at commercial producers (large and small) hosted in a single location and accessible to all interested.

Some of the specific deliverables that will be created over the duration of the project include:

- A printed brochure that will introduce people to the project, the personnel involved and its goals. This will be distributed to viticulture and enology faculty and extension personnel, state industry associations and others for distribution. Digital versions will be available for websites and other online resources.

- The extension team and other leaders of the project will work together to develop written articles for distribution in industry trade publications about the purpose of the project, the specific traits that are being targeted, and other information demonstrating the importance of the project to the long-term sustainability of the industry. A similar article will be developed for general media and consumer publications. In addition to specifics about the project, these articles will educate their audiences on basic genetic concepts and techniques, including a description of the differences between using modern tools to enhance traditional breeding techniques (this project) and using transgenic techniques, about which some consumers have

significant concerns and which may make them less open to the products of genetic research. We will also produce a short series of videos and computer generated animations, available through YouTube and other video-sharing sites, which explain these ideas and concepts in a manner accessible for people who not familiar with genetics and breeding.

- An annual newsletter will be written which will summarize the previous year's work, highlight different members of the project team, and communicate the potential impacts of the project on industry practices. The newsletter will be distributed primarily online through the project's website (see below).

- The team will develop a website that will be used to document the progress of the project, highlight the work of the various participants, and communicate results. The website will be modeled to an extent after the website developed for a similar project on marker-assisted breeding for Rosaceae species (http://www.rosbreed.org). Project members will be encouraged to write blog entries, participate in online interviews and/or web videos, and other activities which will be hosted on the site. Many elements of the website will also be accessible through the eXtension GCoP's site (http://www.extension.org/grapes).
- One activity that will conducted directly through the eXtension GCoP would be to provide continuing education webinars over the course of the project. These webinars will focus on communicating the project's results to Extension professionals nationally as well as grape industry professionals, including producers and those in allied industries. The PIs will not only make presentations using the eXtension Adobe Connect technologies, but they will also provide their teaching materials in PowerPoint format for download. The presentations will be recorded for those who cannot attend the session live. The GCoP will promote the webinars through coordinated publicity to traditional grape media outlets, through the viticulture extension listserv, and through other appropriate methods. Current members of the GCoP are also responsible for hosting the viticulture extension listserv virtual community, which is separate from the GCoP. The members of the project's Extension team are all members of the GCoP.

Outreach to the public will be accomplished primarily through written articles that can be placed in general media outlets such as newspapers and targeted consumer publications such as Wine Spectator or Wine Enthusiast. These materials will also direct consumers to further sources of information if they are interested, such as our online resources including the project website and the eXtension GCoP website. At the request of industry partners, extension materials will be subject to IA Panel review and will be released only as results warrant rather than on a pre-determined timeline.

Project evaluation. This five-year project will be supported with systematic evaluation efforts by the Project Manager and overseen by the IA Panel. The logic model (Table A2) references projected Research and Extension roles, inputs, outputs, and impacts providing a basis to evaluate the overall project goal: to *accelerate grape cultivar improvement via phenotyping centers and next generation markers for the purpose of improving production efficiency, productivity, and profitability*. The evaluation will adhere to a goals-oriented approach and collect data to document the degree of goal attainment in addition to capturing unintended consequences of the project. The evaluation process will be guided by two objectives: 1) to provide formative evaluation throughout the implementation of the program to help the project leaders address areas in need of improvement; and 2) to systematically evaluate the impacts of the project in terms of measuring short and medium term outcomes.

Data will be collected each year and will be fed back to project collaborators for program improvement purposes (Table A3). In the final year of the project, summative evaluation will be conducted to determine the overall impact of the project and whether project goals have been achieved. The evaluation will adhere to the American Evaluation Association standards for program evaluation including accuracy, feasibility, usability, and propriety (Sanders 1994).

***7. Pitfalls that may be encountered*. Consumer perceptions and trade-offs.** Although surveys such as those described above are common in marketing and psychology, they have a number of well-known drawbacks, most importantly, that they do not require respondents to make trade-offs. To address this problem, we will include a "best-worst" scale (Finn and Louviere 1992) and willingness to pay in order to link consumer perceptions with economic analysis.

Population size and genotype x environment interactions. We are committing to a minimum of mapping with 94 progeny, which may be too few for some QTL. In *Vitis*, it is common to map traits with 100 individuals (Dalbo et al. 2000, Doligez et al. 2006, Doucleff et al. 2004, Fischer et al. 2004), although frequently these studies have been conducted in only one location and have not been validated in additional populations. However, one reason for reserving 960 samples to be assigned mid-project is recognition that some populations and traits may require improved mapping resolution attainable by mapping with additional progeny. We also recognize that many novel QTL discovered as part of this project, as well as previously reported QTL, will need validation in relevant environments and germplasm before wide-scale marker-assisted breeding is adopted in *Vitis*. Therefore, we aim to identify top-priority markers early in the project and validate them rigorously to support continuity such that each grape breeder is fully confident in the quality of selection provided and technical protocols needed.

Phenotype estimation. A more significant barrier to mapping traits could be limited precision of phenotype estimation due to insufficient replication to account for variation caused by experimental error or genotype x environment interactions. We have significant experience in characterizing powdery mildew resistance for grape breeders (Walker, Ramming, Reisch, Luby, Lu) using the approaches outlined above; we have documented success in mapping resistance (Mahanil et al., 2011). In a worst-case scenario, we may need to increase phenotyping replication and/or population sizes and decrease the number of populations.

In most cases, only a single vine is available for each seedling in a population, therefore abiotic stress phenotyping will be conducted in each of 2 years. Even so this limits the ability to account for spatial variation in mapping some locally phenotyped traits. Consequently, only relatively large differences among phenotypes may be detected which may correspond to only a few (or single) loci with large effects. Precision can be improved in instances where evaluated families are related and will have some alleles identical by descent.

Fruit chemistry is dynamic over the growing season, and also dependent on environmental conditions. The timing of sampling will therefore affect our ability to detect some differences. Furthermore, since sampling will be performed at the same time within a population for practical reasons, differences in chemistry may in some cases reflect differences in bloom time or veraison and thus maturity. To overcome this, we will measure basic fruit chemistry and berry size and eliminate any gross outliers from QTL analyses. We also plan to sample both immature and mature fruit, which will give us further opportunity to detect lines that are developmentally delayed or precocious – yet another valuable trait to map.

There is also a risk of vine loss reducing population size. To overcome this risk, NGWI and Gallo have offered to maintain populations on five acres of vineyard, which would also enable replicated local phenotyping of additional traits.

Cutting edge marker technology. The SNP discovery protocol we propose has been used to discover hundreds of thousands of SNPs in maize and in *Vitis*. The detailed steps of our proposed protocol have been successfully optimized in *Vitis*. Any unanticipated hurdles would be in bioinformatics and could require several additional months to overcome (as when the protocols were originally developed by our team in maize) but we have the expertise from maize to adapt the procedures early in the project. In the worst-case scenario, we could use or re-design the Infinium array that has already been used for linkage mapping in *Vitis*.

SNP sampling. Reduced representation library (RRL) projects in *Vitis* (Myles et al. 2010) and maize have documented an approximately 1% false-positive SNP rate from sequencing, errors that can be identified and removed by existing computational pipelines when dozens of samples are genotyped (Buckler and Sun, personal communication) with essentially the same computational pipeline for SNP calling as used in Gore et al. (2009). Because the Illumina sequencing technology employs random sampling of DNA pools, not all progeny are genotyped at all SNP loci, which is not as big of a problem in small genome species like grapevine as it is in large genomes like maize. However, the majority of the missing data can be reliably imputed by considering haplotype structure, using novel algorithms and methods that will be highly valuable to the other heterozygous crops for which mapping populations have been generated. (Buckler, Myles, and Sun, personal communication).

8. Limitations to proposed procedures. There is some debate between the academics and a wine industry stakeholder about the limitations of the consumer perceptions study. The question is: can consumer preferences for existing cultivars (e.g. Cabernet Sauvignon) be overcome by any new traits introduced by breeding? This is an exciting question and exactly the type of information that grape breeders need to develop new cultivars. Perhaps we will find that Cabernet Sauvignon is irreplaceable, but that the entrenched Thompson Seedless market, for example, can be outmatched by a new cultivar that can be locally grown without powdery mildew sprays. Complicating these analyses is that fact that attitudes can change quickly, especially in response to crises, such as an invasive species that might make controversial approaches like GMOs suddenly desirable. This underscores our approach to supporting diversity in regional adaptation and locally-phenotyped traits, while expanding the genetic resources and tools available for cultivar development.

The biggest limitation of the molecular marker work, yet one of the main arguments in favor of the project, is that grapevines require a great deal of space and time to evaluate traits. This is a limitation because with the current project design we will only be able to map traits that have high heritability or many years of locally-phenotyped data. However, in order to overcome these limitations, a seed must be planted. Data must be obtained to identify the traits with significant genotype x environment interaction and therefore requiring multi-location, replicated plantings, and the populations best suited to identification of minor QTL. An initial investment must be made to justify the expenditure for propagation, planting, maintenance and characterization. NGWI and Gallo recognize the value of multilocation, replicated plantings and are investing generously in the establishment and maintenance of replicated populations in California for this project.

9. A full explanation of any materials, procedures, situations, or activities related to the project that may be hazardous to personnel, along with an outline or precautions to be exercised to avoid or mitigate the effects of such hazards. The project introduces no new hazards, and each participating institution has existing precautions to avoid and mitigate the hazards these existing materials and procedures introduce. For example, pesticides are applied to existing breeding populations to maintain healthy vines, and the EPA's Worker Protection Standard Training is required for all personnel handling plant material that has been sprayed with pesticides, whether at a sampling or phenotyping location. Shipment of plant material between states will take place in strict accordance with regulations adopted by each state, and permits will be obtained when necessary. The pathogen phenotyping center enables the standardized screening of all mapping populations with the same New York isolates and populations from the center of diversity, without the need to ship the isolates under quarantine to other sites, eliminating the risk of unintentional introduction. All infected leaves and cuttings will be maintained under permit in quarantine and autoclaved after screening to prevent introduction of new pests and pathogens. At the genotyping center, Cornell requires all personnel to complete laboratory safety training.