

***Allium* CAP: A Plan for Translational Genomics of the Alliums**
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The National Research Initiative (NRI) of USDA-CREES has provided competitive funding for Coordinated Agricultural Projects (CAP) on “translational genomics” of major crop plants. The goal of this program is to move genomic research from the lab to the field to develop value-added cultivars or to solve specific production or processing problems. Dr. Michael J. Havey obtained a USDA-NRI grant to support an “*Allium* CAP” planning conference. The goal of this conference was to identify and prioritize production and processing characteristics important for onion and garlic. These traits will become targets for the identification of beneficial germplasms, tagging by robust molecular markers, and incorporation into competitive cultivars. Marker-facilitated selection offers great promise for the Alliums because of their biennial generation time, effort to harvest and vernalize of bulbs or pseudostems, large cost of doing crosses with insects, and lower seed yields (Havey et al. 1997). During the one-day *Allium* CAP conference, the following four objectives were discussed as critical elements of *Allium* production and processing, as well as areas for which translational genomics could be effectively applied:

I. Biotic stresses:

Development of breeder-friendly molecular markers to identify major genes or quantitative trait loci (QTL) conditioning resistance to a major *Allium* diseases and pests. For pathogens and pests with no genetic resistance, transgenic approaches may have to be considered.

II. Abiotic stresses:

Environmental stresses cause reductions in both yield and quality. Drought, flooding, heat, and cold stresses are potentially key abiotic factors that require research to identify resistant or tolerant germplasms and develop breeder-friendly tools for genetic improvement.

III. Bulb appearance and composition:

Breeder-friendly molecular markers are needed for key bulb characteristics, such as single-centers, desirable skin and flesh colors, pungency, maturity, as well as significant health-enhancing attributes provided by the thiosulfinates, flavonoids, and fructans.

IV. Seed production:

Breeder-friendly markers are needed for traits important in the production of the seed crop, including systems of cytoplasmic-genic male sterility (CMS) and genetic factors affecting premature or poor bolting, heat tolerance, and seed yield.

The economic value of the Alliums is often not appreciated. The Alliaceae is the second most economically important family in the monocots, following only the Poaceae (grasses), and includes such important plants as onion (*A. cepa*), garlic (*A. sativum*), bunching onion (*A. fistulosum*), chive (*A. schoenoprasum*), and leek (*A. ampeloprasum*). Onion is the third most valuable vegetable crop in the US (following only lettuce and tomato) and second most valuable vegetable in the world (following only tomato) (FAO 2005). The annual farm-gate value of onion in the US routinely exceeds \$800 million (greater than peanut or barley), with over \$6 billion in value added after processing (USDA 2005). The annual farm-gate value of garlic is greater than \$160 million. In addition, over 20% of the world’s onion seed is produced in the US.

In order to objectively identify important production constraints for the *Allium* vegetables and processed products in the US, a web-based survey was developed and distributed it to growers,

processors, breeders, productionists, extensionists, etc., through out North America. Survey participants were asked to score the seriousness of onion and garlic diseases, pests, stresses, and quality attributes. A pdf of the survey may be downloaded from <http://haveylab.hort.wisc.edu/cap/index.htm>; however the survey is now closed and no new responses will be accepted. Over 100 respondents completed the survey, from all major *Allium* production regions in North America (Table 1). Most (90%) respondents had acreage planted to onion (90%), followed by garlic (9%) and bunching onion (1%). For onion, production was primarily for yellow (68%), followed by white (17%) and red (15%) cultivars. Table 2 lists the mean scores from the survey for production challenges or quality attributes for the Alliums in North America.

Table 1. US zip and Canadian postal codes for respondents to web-based survey on challenges to production and processing of the Alliums.

13126	93551	99326
14058	93906	99344
14411	95381	99345
15090	95695	99349
16059	97304	99350
53532	97305	99357
78596	97838	99362
80523	97839	LOG IJO
80601	97913	KOA IAO
83606	97914	LOL ILO
83660	98802	KOK 2K0
83672	98371	L32 2A6
87937	98837	N8H 3V4
88012	98857	L3Y 4J9
88030	99037	N7M 5Y8
93230	99320	

Table 2. Results of web-based survey on the seriousness of onion and garlic diseases, pests, stresses, and quality attributes in North America. Survey choices were 1 = Never; 2 = Rarely; 3 = Occasionally; 4 = Often; or 5 = Always a problem. Therefore, higher averages indicate greater seriousness.

<u>Disease, Pest or Attribute</u>	<u>Average Score</u>	<u>10 Most Serious</u>
Insects		
Armyworms/Cutworms (<i>Spodoptera</i> & others)	2.2	
Bulb mites (<i>Rhizoglyphus</i> spp.)	2.0	
Onion maggot (<i>Delia antiqua</i>)	3.0	
Thrips (<i>Thrips</i> and <i>Frankliniella</i> spp.)	4.7	*
Seed corn maggot (<i>Delia platura</i>)	2.7	
Wireworms (<i>Limoniuss</i> spp.)	2.1	
Bacteria		
Bacterial leaf streak (<i>Pseudomonas viridiflava</i>)	2.1	

Bacterial soft rot (<i>Erwinia carotovora</i>)	3.3	*
Xanthomonas blight (<i>X. campestris</i>)	2.0	
Slippery or sour skin (<i>Pseudomonas</i> spp.)	3.3	*
Fungi		
Black mold (<i>Aspergillus niger</i>)	3.3	*
Botrytis leaf blights (<i>B. squamosa</i> or <i>cinerea</i>)	3.5	*
Botrytis neck rot (<i>B. allii</i>)	3.7	*
Damping off (<i>Pythium</i> spp.)	2.8	
Downy mildew (<i>Peronospora destructor</i>)	2.9	
Fusarium basal rot (<i>Fusarium oxysporum</i>)	3.3	*
Pink root (<i>Pyrenochaeta terrestris</i>)	3.5	*
Powdery mildew (<i>Oidiopsis</i> spp.)	2.3	
Purple blotch (<i>Alternaria porri</i>)	2.7	
Rhizoctonia rots (<i>R. solani</i>)	1.9	
Rust (<i>Puccinia porri</i>)	1.6	
Sclerotinia rots (<i>S. sclerotiorum</i> or <i>rolfsii</i>)	1.9	
Smudge (<i>Colletotrichum circinans</i>)	1.8	
Smut (<i>Urocystis magica</i>)	2.4	
Stemphyllium leaf blight (<i>S. vesicarium</i>)	2.2	
White rot (<i>Sclerotium cepivorum</i>)	2.0	
Viruses or Mycoplasmas		
Aster yellows	1.7	
Garlic mosaic	1.5	
Iris yellow spot	2.9	
Onion yellow dwarf	1.6	
Shallot latent virus	1.3	
Nematodes		
Lesion (<i>Pratylenchus penetrans</i>)	1.8	
Northern root-knot (<i>Meloidogyne hapla</i>)	2.1	
Stem or bulb (<i>Ditylenchus dipsaci</i>)	1.8	
Sting (<i>Belonolaimus longicaudatus</i>)	1.5	
Stubby-root (<i>Paratrichodorus minor</i>)	1.8	
Abiotic stresses		
Pre-mature bolting	3.1	*
Ozone	1.8	
Drought	2.7	
Heat	3.5	*
Flooding	2.0	
Cold	2.7	
Quality attributes		
Pungency	2.1	
Single centers	3.0	
Skin color	2.8	
Maturity	2.8	

The *Allium* CAP Conference

The *Allium* CAP conference was held on Wednesday, December 6, 2006, in College Station, Texas, with 66 attendees (Appendix 1) with excellent representation from both the public and private sectors. The goal of this conference was to strengthen contacts among public and private researchers and commodity groups for the *Allium* vegetables, as well as to identify high-impact research goals as targets for translational genomics. The group also discussed and prioritized important research and outreach deliverables of a USDA CAP proposal.

Morning presentations:

- 8:30-8:45: “Welcome and description of conference goals” by Dr. Michael J. Havey, USDA-ARS and University of Wisconsin, Madison WI.
- 8:45-9:00: “USDA Coordinated Agricultural Projects (CAP)” by Dr. Ed Kaleikau, National Program Leader, Competitive Grants, USDA-CSREES, Washington DC.
- 9:00-9:25: “Goals of the funded rice CAP” by Dr. Anna McClung, USDA-ARS, Rice Research Unit, Beaumont TX.
- 9:25-9:50: “The status of North American onion industry in a global economy” by Mr. Wayne Mininger, National Onion Association, Greeley CO.
- 9:50-10:00: “Web-based survey on major challenges to garlic and onion production in North America” by Dr. Michael J. Havey, USDA-ARS and University of Wisconsin, Madison WI.
- 10:30-11:00: “Challenges to US onion and garlic processors in the US and world-wide” by Dr. Daniel Brotslaw, Sensient Dehydrated Flavors, Turlock CA.
- 11:00-11:30: “Challenges to the onion-seed industry in the US and world-wide” by Dr. Rick Watson, Nunhems Seed Company, Brooks OR.
- 11:30-12:00: “Value-added onion and garlic in North America” by Dr. Bill Randle, University of Georgia, Athens GA.
- 12:00-12:30: “Overview of emerging disease and pest challenges in the US and world-wide, especially in light of pesticide losses” by Dr. Howard Schwartz, Colorado State University, Ft. Collins CO.

Afternoon presentations:

- 1:30-2:30: Prioritization of challenges for *Allium* producers and processors. Wayne Mininger, Howard Schwartz, and Michael Havey led an open discussion to prioritize the most important US production and quality challenges for the Alliums.
- 2:30-3:00: “Genomic and bioinformatic resources for the Alliums and other monocots” by Dr. Christopher Town, The Institute for Genomic Research, Rockville MD.
- 3:00-3:15: “Present status of *Allium* breeding and genetics” by Dr. Michael J. Havey.
- 3:15-3:30: “Present status of *Allium* transformation” by Dr. Colin Eady, Crop and Food Research, New Zealand.
- 4:00-5:30: Development of a plan for *Allium* translational genomics to address major production problems or value-added opportunities. An open discussion was chaired by M.J. Havey towards development of a plan entitled

“Research, Education, and Extension Opportunities for *Allium* Translational Genomics”. The discussion addressed:

- Prioritization of target traits.
- Identification of onion populations segregating for target traits.
- Core set of molecular markers for mapping of target traits.
- Strategies for fine mapping and tagging of target traits.
- Technology transfer to breeding programs.

Results of the *Allium* CAP Conference

After the morning presentations, an open discussion was held to prioritize the most important US production challenges, quality attributes, and technologies for the Alliums. The group chose the following pests, characteristics, or technologies (in alphabetical order) as being the most important for North American production:

<u>Pest</u>	<u>Quality Attribute or Technology</u>
Aspergillus black mold	Bioavailability of heath compounds
Aster yellows	Bolting resistance
Bacterial bulb rots	Bulb color
Bacterial leaf blights	Carbohydrate types and concentrations
Botrytis leaf blight	CMS markers
Botrytis neck rot	Flavor/pungency
Cold tolerance	Lacrymatory factor
Downy mildew	Pathogen-derived (transgenic) resistances
Entrobacter rots	Interfering RNA Technology
Fusarium basal rot	Single centers
Garlic rust	Storage ability
Heat tolerance	
Iris Yellow Spot Virus	
Nematodes	
Onion maggot	
Pink root	
Powdery mildew	
Purple blotch	
Smut	
Thrips	
White rot	

After listing the major pests, quality attributes, and technologies, the group discussed and identified those of the highest importance for onion and garlic:

<u>High Priorities For ONION</u>	<u>High Priorities For GARLIC</u>
Bacterial bulb rots	Garlic rust
Black mold	Nematodes
Botrytis leaf spot	Thrips
Botrytis neck rot	Viruses
Downy mildew	White rot
Fusarium basal rot	
Herbicide tolerance	
Iris Yellow Spot Virus	
MAS for carbohydrates ^a	
MAS for CMS ^a	
MAS for Flavor ^a	
Pink root	
Smut	
Thrips	
White rot	

^aMAS = Marker-assisted selection

After presentations on resources available to the *Allium* research community such as molecular markers, maps and mapping resources, and transformation technology, three break-out sessions were held to address diseases and pests, abiotic and other production constraints, and value-added attributes. Each group worked to prioritize traits considering the importance of the trait over years and locations throughout North America and the inheritance of any bona-fide sources of resistances or quality attributes. After about 30 minutes, the entire group re-assembled to make final prioritizations of traits and attributes. The group decided to concentrate on onion traits because of the rudimentary status of sexual reproduction in garlic.

The final prioritizations were made by placing individual traits on the graph shown in Figure 1. The x-axis took into consideration the availability and inheritance of bona-fide germplasm possessing resistances or quality attributes. Placement on the y-axis considered the importance of the trait.

- Traits placed in the upper-right quadrant were highly prioritized; however there are no known sources of genetic resistances and these traits may be targets for transgenic approaches. For example, thrips, IYSV, white rot, and weeds (primarily yellow nutsedge) are serious challenges to onion and garlic production through out North America. There have been reports of resistances to white rot and thrips; however these may be in error or the resistance is not economically viable. For example, resistance to white rot in populations such as Alisa Craig is associated with lower pungencies causing reduced sclerotial germination (Coley-Smith and Esler 1983), representing avoidance rather resistance. Lower waxiness of foliage is associated with reduced susceptibility to thrips (Molenaar 1984). However insect pressure builds quickly on cultivars with reduced leaf waxiness and frequent insecticide applications are still required. It was concluded that this trait does not represent a

viable source of thrips resistance. Although no known sources of resistance exist for black mold, Botrytis neck rot, and bacterial rots, losses can be reduced by proper bulb maturity and management of irrigation regimes. IYSV is a major disease of onion in North America (Gent et al. 2006) and a Western Regional project is working to identify a source of resistance and to assess other control strategies.

- Traits in the lower-left quadrant have bona-fide sources of resistance and effective screening protocols, but were not highly prioritized. In the case of downy-mildew resistance introgressed from *A. roylei*, intellectual property (IP) restrictions affect the availability and use of this resistance.
- Traits or technologies in the upper-left quadrant were prioritized as targets for an eventual CAP proposal. The development of breeder friendly markers tightly linked to target traits would be extremely useful and avoid screening across generations during inbred and hybrid development.
 - Resistance to *Fusarium* basal rot: *Fusarium oxysporum* is responsible for major storage losses of onion and garlic world-wide. Relatively simply inherited resistances have been documented and seedling and field screens are available (Cramer 2000).
 - Resistance to *Botrytis* leaf blight: *Allium roylei* is as the main source of resistance to *Botrytis squamosa* (Walters et al. 1996) and advanced backcrosses to onion have been completed.
 - Restoration of male fertility in S cytoplasm: Hybrid-onion seed is produced using cytoplasmic-genic male sterility (CMS). For the major source of onion CMS, male-sterile plants possess sterile (S) cytoplasm and are homozygous recessive at the restorer (*Ms*) locus. Male-sterile lines are seed propagated by crossing with a maintainer line in normal cytoplasm and homozygous recessive at the restorer locus (N msms). Using classical crosses, it takes at least 4 years to establish if maintainers can be isolated from an uncharacterized population or segregating family. Molecular markers in the organellar DNAs have been developed that distinguish N- and S-cytoplasm (Havey 1993, 1995; Satoh et al. 1993). Although a SNP marker has been identified tightly linked at 0.9 cM to the *Ms* locus (Gokce et al. 2004), these two loci are in linkage equilibrium in open-pollinated populations (Gokce and Havey 2004). Cloning of the *Ms* locus is an imperative goal and, together with the organellar markers, would allow breeders to identify CMS-maintaining genotypes without testcrosses.
 - Soluble solids in onion: Sucrose, glucose, fructose, and fructans are the primary non-structural storage carbohydrates in onion (Sinclair et al. 1995). Fructan consumption is strongly correlated with lower rates of colorectal cancers (Roberfroid and Delzenne 1998) and the Alliums are the second major food source of naturally occurring fructans in the US diet, following only breads and pastas (Moshfegh et al. 1999). Lower solids onions accumulate essentially no fructans and tend to be soft with low pungency. Onions with higher soluble solids tend to be firm and highly pungent. Onion is an excellent system to study carbohydrate accumulation because recurrent selection has produced low and high-fructan accumulating populations. For example, the onion population ‘Southport White Globe’ was subjected to phenotypic recurrent selection for higher fructan content, shifting the population mean from 17% to >23%. Major QTL on chromosomes 5 and 8 are significantly (LOD>3.5) associated with higher fructan concentrations (Galmarini et al. 2001, McCallum et al. 2006).

- **Onion flavor:** Flavor is likely the most economically important characteristic of onion with lower pungency becoming increasingly popular. Enzymatically derived pyruvate is used to estimate pungency (Schwimmer and Weston 1961) and correlates well with taste tests (Schwimmer and Guadagni 1962, Wall and Corgan 1992). Major QTL affecting pungency have been identified (Galmarini et al. 2001, McCallum et al. 2006). However the genetics of the overall flavor profile of onion (pungency, bitterness, sugars, etc.) have not been studied.
- **Flowering:** Onion bulbs flower after a period of vernalization and in response to increasing day lengths (Rabinowitch 1990). Premature flowering during bulb production is a major production problem and occurs when young plants are subjected to temperatures just above freezing. Bulbs from these flowering plants are not acceptable for the fresh or processing markets. Conversely, fall-seeded onions have been strongly selected not to flower after overwintering in the field and seed production can be difficult.
- **RNA Interference (i) RNA:** RNAi is an important technology to assess the phenotypic effects of reduced gene expression (Sen and Blau 2006). Transient expression of RNAi has great potential for reverse genetics of the Alliums. A practical application of RNAi would be to down regulate the expression of genes repressing flowering in fall-seeded onions.

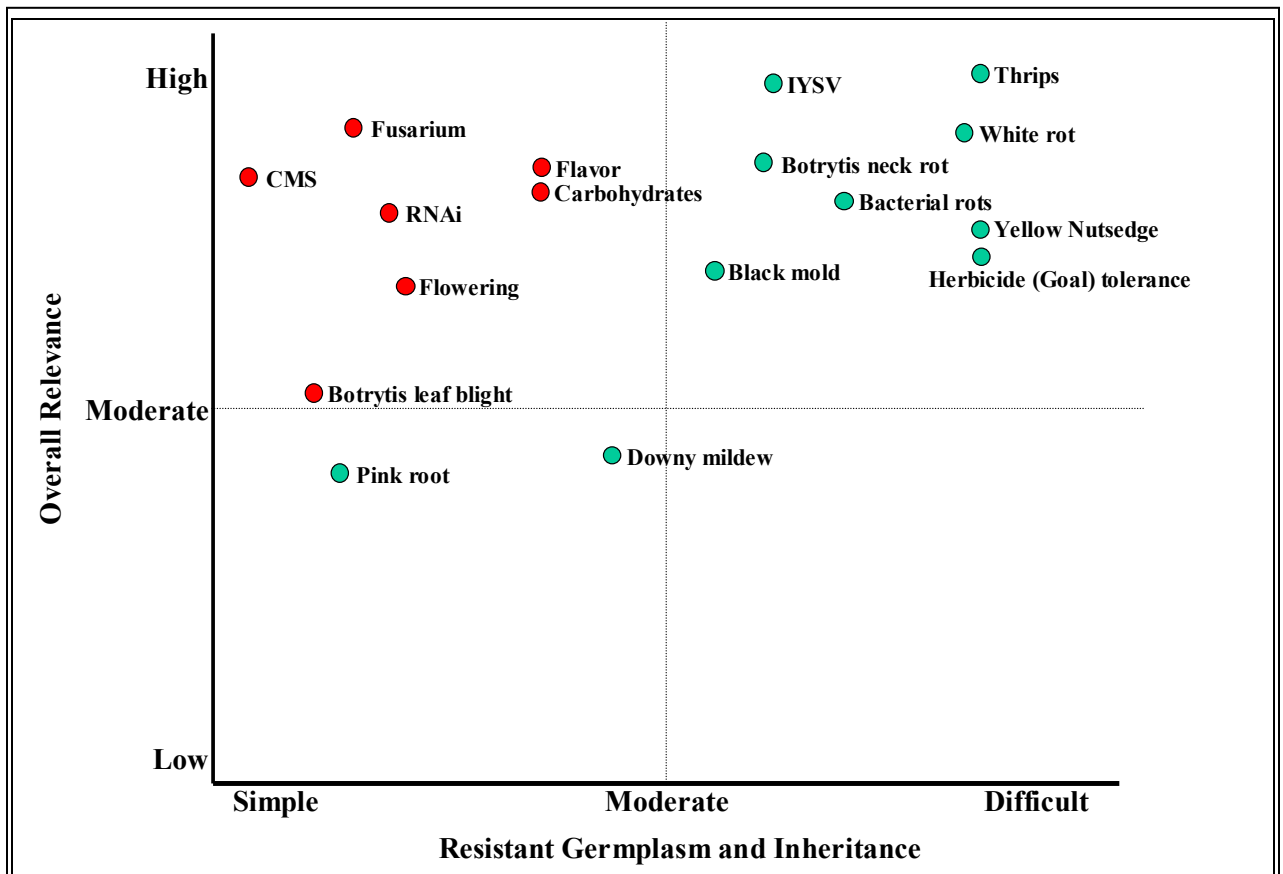


Figure 1. Final placement of production challenges and value-added opportunities for onions in North America. Placement along the x-axis took into consideration the availability of beneficial germplasm and trait inheritance. The y-axis related the overall relevance of traits relative to other priorities. Traits shown in red in the upper left quadrant are priority targets for an eventual *Allium* CAP proposal.

Additional Considerations for the *Allium* CAP Proposal

In addition to establishing research priorities at the *Allium* CAP planning conference, we must also develop plans for information transfer, outreach, and education. A plan for augmenting the genomic resources of the Alliums is also necessary to provide enough sequence information for efficient tagging of prioritized traits.

- **Genomic Resources:** The Alliums have some of the largest genomes among all cultivated plants; for example onion has over 16 times more DNA than rice (Arumuganathan and Earle 1991). In spite of its enormous size, the onion genome is the best studied of all non-grass monocot genomes. The GC content of onion DNA is 32%, the lowest known among angiosperms (Kirk et al. 1970). Cot reassociation kinetics revealed that the onion has a significant component of middle-repetitive sequences that occur in short-period interspersions among single-copy regions (Stack and Comings 1979). Genomic analyses revealed that scant synteny exists between onion and either *Arabidopsis* or rice on the recombinational (Martin et al. 2005) and sequence (Jakse et al. 2006) levels. These results demonstrate that genomic resources developed for the eudicots and grasses are not directly applicable to the Alliums. The *Allium* research community needs to expand the numbers of expressed and genomic sequences and develop comparative maps:
 - Sequencing of random cDNAs is an efficient method to sample expressed regions and enable gene discovery (Rounsley et al. 1996), especially for plants with enormous genomes. Onion ESTs revealed significant differences among expressed sequences in the Alliums and grasses (Kuhl et al. 2004, 2005), as well as provided SSR and SNP markers for comparative mapping (Martin et al. 2005, Zewdie et al. 2005) and cultivar identification (Jakse et al. 2005). The numbers of *Allium* ESTs must be increased by completing single-pass sequencing reactions from directionally cloned cDNAs using a variety of normalized libraries.
 - Reduced-representation sequencing using methyl or Cot-filtered genomic libraries of maize increased the proportion of random shot-gun reads showing significant similarities to expressed sequences (Rabinowicz et al. 1999, Peterson et al. 2002, Palmer et al. 2003, Whitelaw et al. 2003, Yuan et al. 2003). Pilot sequencing from whole-genome shot-gun (WGS) and methyl-filtered genomic libraries revealed that methyl-filtration of onion DNA was very effective in reducing the proportion of both identifiable transposons (from 14% to 3%) and anonymous sequences (from 82% to 55%), as well as increasing non-organellar protein hits over 10-fold. Elimination of more rapidly re-annealing repetitive DNAs (Cot) also increases the proportion of genic sequences as compared to random genomic fragments. For example, Cot filtrations resulted in >13 and 4-fold enrichments for genic regions of wheat (Lamoureux et al. 2005) and maize (Yuan et al. 2003), respectively, and only about 37% overlap with the methyl-filtered maize clones (Springer et al. 2004). The Cot profile of onion is well established (Stack and Comings 1979) and sequencing from a Cot-filtered library of onion should sample different genic regions. Therefore, sequencing of methyl- and Cot-filtered genomic clones should complement EST sequencing as an efficient approach to enrich for genic regions of the onion genome.
 - Processed EST and genomic sequences should be promptly submitted to public data bases and added to the Onion Gene Index (<http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=onion>) with cross-references to putative orthologs in the grass and eudicot genomes. For genomic sequences, clusters of primarily repetitive sequences

- should be searched against curated databases that contain representatives of all known families of transposons and retrotransposons from other organisms.
- Unigenes should be assigned to chromosomes and comparative mapping of major Alliums completed. Onion unigenes can be efficiently assigned to chromosomes using alien addition lines of Japanese bunching onion (*A. fistulosum*) carrying single onion (Shigyo et al. 1996, Martin et al. 2005). Synteny among the Alliums would greatly facilitate marker-facilitated selection in the Alliums, as well as reveal any syntenic regions with other monocots. Comparative mapping of major Alliums can be completed using the following families:
 - Garlic: A S1 family of 84 progenies has been produced from the self of a single plant from PI 540316 and initial mapping completed (Zewdie et al. 2005).
 - Japanese Bunching Onion: Japanese researchers have developed an F2 population of 235 progenies (Song et al. 2004).
 - Onion: An intraspecific segregating family consisting of 59 F2 progenies from the cross of inbreds BYG15-23 and AC43. This family has been used to map over 300 molecular markers (King et al. 1998, Martin et al. 2005). An F2 family has been developed from an interspecific hybrid between onion and *Allium roylei* (Heusden et al. 2000). A new international mapping family is under development using an F1 from the cross of two doubled-haploid (DH) populations.
 - **Information transfer:** A web-based, interactive database must be developed as a center of genomic and genetic data for the Alliums with links to public websites and databases.
 - **Outreach and Extension:** The *Allium* CAP must inform the public, including growers, end-users, and extensionists, about the deliverables of this project. The National Onion Association (NOA) is the main not-for-profit, commodity-based organization in North America with a major focus on the Alliums. The NOA membership represents all steps from seed and vegetable production to the market. The biennial North American *Allium* Research Conference (NARC) is attended by Allium growers, researchers, productionists, and commodity brokers. Workshops on *Allium* Genomics can be developed for the NOA and NARC to introduce the community to sequence information and genomic technologies. Articles can also be written for Onion World magazine (www.onionworld.net), an excellent outreach medium for all sectors of the *Allium* community.
 - **Education:** The project must prepare future scientists for work on the practical application of basic research to applied problems. Educational components could include undergraduate and graduate student training, post-doctoral research opportunities, and workshops at major commodity-specific meetings.
 - **Oversight Committee:** An external review committee must be established to evaluate research progress. Scientists working in the public and private sectors, along with representatives of the *Allium* commodity, processing, and seed industries, will be invited to serve on this committee.

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Appendix 1. Attendees of the *Allium* CAP Conference, Dec. 6, 2006, in College Station, Texas.

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