Screening *Medicago truncatula* microsatellite markers for inclusion in a cultivated alfalfa microsatellite map

T. A. Campbell[1], N. Young[2], T. Huguet[3], E. C. Brummer[4] ' G.R. Bauchan[1], Z.L. Xia[1] , and C. He[1]

[1]USDA-ARS, Beltsville, MD; [2]University of Minnesota, St. Paul, MN; [3]CNRS-INRA, Castanet-Tolosan, France; [4]Iowa State University, Ames IA.

The overall objectives of this research are to develop simple sequence repeat (SSR or microsatellite) DNA markers for use in mapping autotetraploid alfalfa (*Medicago sativa* L.), to construct SSR-based genetic maps of alfalfa where markers will be integrated with previously mapped AFLP and RFLP markers, to identify markers associated with agronomically important quantitative and qualitative genes, and to evaluate diversity and phylogenic relationships in *Medicago* sp. germplasm using selected SSR markers. Tri- and tetra-nucleotide SSR markers from alfalfa genomic clones, and from ESTs of the closely related diploid *M. truncatula*, will be mapped initially to an 'ABI 408' purple flowered winter hardy X 'WisFal' yellow flowered population developed by E. C. Brummer. Up to eight SSR alleles can be mapped per locus and there is always the possibility of mappable multiple alleles for a particular locus. The resulting 32 linkage groups should ultimately coalesce into eight. Single nucleotide polymorphisms (SNPs) may also be identified from EST sequencing data during the course of this project and will be integrated into the map. SSR markers (and perhaps SNPs), along with existing AFLP and RFLP markers, will be used to identify quantitative trait loci (QTL) controlling important quantitative and qualitative traits. Subsets of SSR markers will be defined for use in diversity and phylogeny studies in *Medicago* sp. germplasm, and for differentiating among selected alfalfa cultivars and populations.

In the initial phase of this project, 51 and 112 *M. truncatula* SSR markers identified by Huguet and Young, respectively, were screened for mapping potential in the Brummer population as were 44 tri- and/or tetra-nucleotide SSRs from The Institute for Genomic Research's (TIGR) *Medicago truncatula* Gene Index that had not been identified in earlier evaluations by other institutes. Primer pairs were first evaluated using the parents to look for a single dose relationship (marker present in one parent and absent in the other) for at least one allele, in which case primer pairs were reevaluated with the parents and five F1s.

Eleven of the Huguet primer pairs, 24 of the Young primer pairs, and 23 of the TIGR primer pairs yielded single dose responses in at least one allele and several primer pairs yielded four or more alleles with a single dose response. Primer pairs eliciting single dose responses will be retested with the parents and 100 F_1s and analyzed for conformation to a 1:1 ratio based on a χ^2 test. Those conforming to the Mendelian ratio will be mapped to the Brummer population after RFLP and AFLP markers locations are resolved.