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SNP discovery and linkage mapping in Nicotiana

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We are developing a genetic linkage map in F2 populations descended from a cross between *Nicotiana plumbaginafolia* and *N. longiflora* as well as the reciprocal cross. We are using an interspecific cross because is an outbreeder and is a self-pollinator. Combining our linkage map with estimates of self-fertilization in the F2 will allow us to make a Quantitative Trait Locus (QTL) map that may allow us to identify chromosome segments that are associated with self-pollination. We are concentrating on discovering new genetic markers. We are seeking SNPs (Single Nucleotide Polymorphisms) because they are co-dominant markers, i.e., both kinds of homozygotes and the heterozygote can be identified (e.g., AA, Aa, aa). In contrast, dominant markers can only identify the recessive homozygote unambiguously (aa); the dominant homozygote and the heterozygote are indistinguishable (AA = Aa). Therefore, co-dominant genetic markers are much more informative than dominant markers. We are seeking to identify genetic polymorphisms from known COS (Conserved Ortholog Set) loci because COS markers have been used to map many other species. Therefore, our linkage map will allow us to compare the chromosomal locations of these loci in our cross, versus other COS-mapped plants.In order to determine SNPs in the genome of Nicotiana, there are two possible approaches. We can sequence both parents of the F2 population and look for the differences between the two species as well as sequence the heterozygotes and look for differences within the F2. Thus far, we have tried 3 COS loci and found 1 polymorphism. We are using the CAPS procedure to assay the polymorphisms. We use restriction enzymes to cut at specific locations within the primer region and determine if each individual is homozygous dominant, homozygous recessive, or heterozygous.