

DEVELOPMENT AND USE OF PCR-BASED TECHNOLOGIES FOR COTTON MAPPING

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Abstract

Development of a robust integrated framework map has greatly accelerated molecular genetic research in the model plant *Arabidopsis thaliana*. To efficiently develop a similar set of framework markers for cotton (with markers at approximately 20 cM intervals) we will need to tap multiple sources of DNA polymorphisms including microsatellites, single nucleotide polymorphism (SNPs) and amplified fragment length polymorphism (AFLPs). In our laboratory we have optimized methods for high-throughput retrieval of cotton sequences with simple sequence repeat motifs, primer design, and microsatellite detection (using economical agarose systems). Out of 550 sequences captured using microsatellite repeat oligonucleotides, an initial set of 70 markers was tested in an interspecific F_2 mapping population. In this analysis, 50% of the markers were polymorphic and segregated in a 1:2:1 mendelian ratio. We also have mapped 10 SNPs derived from 20 fiber-subtracted ESTs using the CAPs and dCAPs technologies, thus demonstrating that existing EST sequences are a valuable source of polymorphisms for the development of informative DNA markers. We also utilized a simple methodology for eluting AFLP bands from dried acrylamide gels, direct sequencing, and subsequent conversion into co-dominant markers. Using our interspecific F_2 mapping population, a total of 170 markers (including SSRs developed in our lab, BNL SSRs developed in the laboratory of Dr. Ben Burr, SNPs from fiber ESTs, and AFLPs) were mapped to 30 linkage groups encompassing 85% of the cotton genome.