

**GENOME-WIDE SPACED SIMPLEX SNP ASSAYS FOR MARKER-BASED INTERSPECIFIC  
GERMPLASM INTROGRESSION AND GENETIC MANIPULATION IN COTTON**

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**Abstract**

The cotton industry is a major contributor to the United States economy amounting to over \$75 billion annually. Thus, even small improvements in cotton quality and yield have significant ramifications. Genomic molecular markers are critical to many types of genetic analysis and manipulation. Single-nucleotide polymorphisms (SNPs) are the most abundant type of marker and most amenable to high-throughput technologies. Marker-assisted selection (MAS) utilizing SNPs is an effective way to manipulate agriculturally and economically important genes and genome regions, e.g., for genes that enhance yield, pest resistance, and drought tolerance. Here, we report on SNPs for efficient introgression (transfer) of wild species germplasm into an Upland cotton genetic background, and subsequent manipulation, analysis, dissection and breeding. We report nearly 400 SNP assays for loci that are approximately evenly spaced across the genome. The SNPs were chosen to distinguish upland cotton, *G. hirsutum*, from other tetraploid cotton species (*Gossypium mustelinum*, *Gossypium tomentosum*, *Gossypium barbadense*), as well as their corresponding F1s). Only biallelic SNPs were used for this project, and most were derived from 18,000 mapped interspecific SNPs on the commercially available high-density CottonSNP63K array that discriminate between *G. hirsutum* and non-*G. hirsutum* cotton species. The SNPs were selected for primer design using BatchPrimer3 v1.0 software and tested to give comprehensive genome coverage. Our initial target was about 15-cM spacing using “Kompetitive Allele Specific” PCR (KASP) or “PCR Allelic Competitive Extension” (PACE) assays. A minimum of 10 markers per chromosome have been successfully assayed. These will be useful for a number of types of breeding and research applications where targeted genotyping is needed and will be especially helpful if large numbers of samples need to be screened using low-cost DNA preps, for which PCR-based assays such as KASP and PACE are generally compatible.