DEVELOPMENT OF INTEGRATIVE SSR MARKERS FROM THE TM-1 BAC

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Abstract

The utilization of DNA markers associated with agronomically important traits increases the efficacy of breeders in developing improved cotton cultivars. Simple sequence repeat (SSR) markers have been proven to be most desirable markers for marker-assisted selection and germplasm evaluation in cotton. Generation of SSR markers from a large-insert BAC library is unusual in contrast to small-insert genomic DNA clones enriched for SSR loci. However, BAC-derived SSR markers provide numerous advantages over those developed from small-insert DNA clones. First, the SSR-containing BACs provide bridges that integrate physical maps with genetic maps. Secondly, the SSR-containing BACs provide or can be used to develop many different types of polymorphic DNA markers for a locus of interest, which is significant for marker-assisted breeding. Thirdly, the SSR-containing BACs streamline high-resolution mapping and map-based cloning of genes and QTLs of interest. We report the first isolation of SSR markers from one (TM-1 *Hin*dIII BAC library) of three large-insert BAC libraries of the Upland cotton genetic standard line TM-1 that we developed recently for the construction of a whole-genome BAC/BIBAC-based integrated genetic/physical map of Upland cotton. The TM-1 *Hin*dIII BAC library was screened with four SSR oligo sequences (CA, GA, TA, and GAA), the SSR-positive BACs were subcloned, and initially 303 SSR-positive subclones were identified and sequenced. Each of these subclones was found to contain one or more of the four SSR oligo sequences that were used as probes. Sequence analysis by BLAST search against the cotton SSR database showed that 254 of the 303 SSR subclones are new, indicating that the cotton genome is abundant in SSR loci. Primer pairs were designed from the unique flanking sequences of the new SSR loci and they were used to estimate the polymorphic information content (PIC) among a panel of diverse Upland cottons. Of the primer pairs surveyed thus far, an average of 39.4% were found to be polymorphic among these cotton lines and 60.6% was shown to produce a single PCR fragment, indicating that they are locus-specific in tetraploid cottons. We are now in the process of completing an initial set of 1,000 such SSR markers for cotton research.