Cotton fiber properties are the key driving force to get good quality of yarn. Worldwide modern high speed spinning operations require long, strong and fine cotton fibers. The objective of this research was to detect stable fiber quantitative trait loci (QTL) that could be used in cotton breeding through marker assisted selection (MAS). Two cotton lines, MD90ne and MD52ne, are near isogenic with significant difference in fiber properties especially strength. Fiber samples from 734 progeny plants of two F2 populations (A and B) derived from crosses between MD90ne and MD52ne were collected at Stoneville, MS in 2012. Fiber quality traits were measured using a High Volume Instrument 1000. A simple sequence repeat (SSR) genetic linkage map with 165 loci covering 632.53 cM was constructed using the Population 5 consisting of 356 F2 individuals and used for identifying QTLs related to fiber bundle strength (FBS), short fiber index (SFI) and upper half mean length (UHML). One major QTL for FBS originated from the stronger fiber parent MD52ne was identified on chromosome (Chr.) 3. Three QTLs each for SFI and UHML were identified on Chr. 3, 4, 14 and Chr. 3, 11, 24, respectively. The Population 1 consisting of 378 F2 progeny was used to validate these QTLs by analyzing 57 SSR markers mapped on Chr. 3, 14 and 24. Three QTLs, i.e. qFBS-c3, qSFI-c14, and qUHML-c24, were validated and appeared stable. These three QTLs could be potentially used in breeding to improve cotton fiber quality utilizing MAS technique.