COMPARATIVE LOCUS AMPLIFICATION (CLA) FOR DETERMINING COPY NUMBER VARIATION IN COTTON

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Abstract

We have developed a novel PCR-based assay for determining copy number in cotton that is relatively quick, robust, and inexpensive compared to other options currently available. Additionally, it requires little specialized training or equipment and can be adapted to a variety of uses. The Comparative Loci Amplification (CLA) assay makes use of an existing PCR master-mix that had been previously used for SNP genotyping. It contains enzyme, buffers, and fluorochrome dyes that fluoresce in conjunction with target amplification. Copy number is determined by differences in amplification between the suspected monosomic "aneuploid" (lacking a single copy of one chromosome but otherwise normal) and a disomic control as determined by fluorescence. To test this approach, we extracted DNA from leaf tissue of multiple *G. hirsutum* aneuploids lines (monosomic at one chromosome, but otherwise disomic) in our germplasm. The *G. hirsutum* TM-1 line serves as a positive (disomic) control. TM-1 is the line from which these aneuploid were originally derived; so with the exception of CN differences they should have a high degree of sequence similarity. Primers were designed based on CHIP sequence data derived within our lab from TM-1 samples. Most assays were repeated to target multiple loci within the same chromosome being assessed for further verification. Using this method we were able to verify monosomy (1:2 ratio of aneuploid:TM-1 for targeted chromosome) of 10 of these aneuploid lines. Eg; the aneuploid H10 (monosomic for chromosome 10 and disomic for every other chromosome) has a CN ratio of 0.5 for every region on chr. 10 relative to TM-1. The normalized fluorescence ratio produced by the CLA assay targeting a region of chr. 10 was 0.442 relative TM-1; close to expected results. Other aneuploids assessed in this manner gave similar results, ranging from 0.442 – 0.562. It has yet to be determined if this assay can discern differences in CN smaller than 1:2; for instance, 3:4, 5:6, etc for repeated segments. We are currently working to expand our primer library to cover the remaining *G. hirsutum* genome. Future studies will test the efficacy of the CLA assay for determining CNV in non-*Gossypium* species.