A SIMPLE AND HIGH-THROUGHPUT ASSAY FOR DETECTING CNV IN *GOSSYPIUM* GERMPLASM

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Abstract

Here we describe a method of CNV detection that we have developed for screening cotton germplasm. This PCR-based assay measures copy number (measured by amplification-induced fluorescence) of a sequence of interest relative to an endogenous reference sequence. We have used it to recover sexually transmitted hypoaneuploids that lack a specific chromosome or segment, as part of breeding research, and also to identify novel aneuploids, as part of efforts to identify hypoaneuploids of all chromosomes. While tests to date have targeted endogenous sequences, we will report on ongoing assessment of transgene copy number, too. There are several advantages to this method over other methods currently being used for CNV detection. Results can be obtained rapidly (within a few hours) at a relatively low cost. It is adaptable to high-throughput applications (96-well plate based) but requires no specialized equipment beyond a thermocycler and plate-based fluorescence spectrophotometric scanner and no training beyond that necessary to perform any PCR assay. Because this assay involves no probe, primer design is relatively facile, even for difficult, GC-rich, or very short regions, and the cost of testing new primers is relatively low. We believe this assay will benefit breeders and researchers in advancing their own cotton cultivars by providing an assay for rapid, large scale CNV detection that can be performed in-house, at a reasonable cost, and can be readily adapted to their specific requirements.