Primers for the Amplification of the Circular Chloroplast DNA from the A-genome Group of Cultivated Cotton

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The availability of the plastid genome sequences is one of the bases for comparative, functional, and structural genomic studies of plastid-containing living organisms, in addition to the application of plastid genetic engineering technology. The past efforts to sequence plastid genomes involve complicated preparation protocols. One procedure starts with the isolation of plastids, which was tiresome and time wasting that followed by a second step to extract plastid DNA from the isolated plastids, then finally the build up of plasmid or bacterial artificial chromosome (BAC) library. The second procedure was to mine a ready-made plasmid or BAC library for the target plant and the identification of the appropriate clones that contain the plastid DNA. The third available and most convenient option is the rolling circle amplification (RCA) of the plastid genome from a purified plastid DNA and the use of this amplified DNA to build the library. Here a procedure similar to the third option; the RCA, was followed with the exception of the use of a purified plastid DNA as a template. Instead the total genomic DNA was used as a template, which cuts off the time needed to prepare the purified plastid DNA. This work depends on the reported results that the A-genome group of the diploid cottons and the allotetraploid genome group of cotton contain the same chloroplast genome that originates from the A-genome group of cotton. A set of 25 pairs of primers were designed to amplify overlap fragments range is size between ~1 kb and ~15 kb, which cover the circular form of the chloroplast DNA (cpDNA) from four cultivated species of cotton. The cotton species used in this study were *Gossypium barbadense* L. and *G. hirsutum* L. as the two allotetraploid cultivated species, *G. arboreum* L. as one of the two cultivated diploid species and *G. nanking* Meyen, which is the Chinese synonym of *G. arboreum*. The other diploid cultivated species is *G. herbaceum* L. was not investigated in this study. The results showed some amplified fragment length polymorphism (AFLP), which was found as a result of deletions and insertions accompanied by many direct and inverted repeats. Also, a restriction fragment length polymorphism (RFLP) was detected that was due to the presence of a direct repeat insert of 51 bp in the chloroplast DNA of *G. hirsutum*.