Protocols for Pachytene Chromosome and DNA Fiber FISH in Cotton

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Fluorescence in situ hybridization (FISH) has become the most important technique in plant molecular cytogenetics research. FISH-based physical mapping provides a valuable complementary approach in genome sequencing to measure the physical distances between adjacent BAC contigs and delineating the structure and DNA composition of genomic regions of centromere and telomere. The accuracy and precision of the FISH-base physical maps depend on the resolution power of FISH. To enhance the resolution, the FISH on meiotic pachytene chromosomes and extended DNA fibers was developed in some plants. The resolution of pachytene chromosome FISH can reach 100 kb and even can be used to resolve partially overlapped BAC clones. FISH on extended DNA fibers has greatly improved the resolution and sensitivity of FISH with 1 kb and 700 bp, respectively. In cotton, previous FISH studies used chromosome materials generally prepared from mitotic and meiotic metaphases. It is difficult to prepare pachytene chromosomes and extended DNA fibers in cotton mainly because of the thick cytoplasm and hard cell wall. Here we present an efficient method for pachytene FISH and DNA fiber FISH in *Gossypium arboreum* (2n = 2x = 26) with a telomere and 45S rDNA repeat as probes, respectively. The method for preparation of cotton pachytene chromosomes has several key improvements. First, directly digest pollen mother cells rather than buds, to easily get rid of the cell walls. Second, moderately spread the pachytene with acetic acid at 50% to clear the background. There is a set of pachytene chromosomes with 13 extended bivalents, each of which represents fully paired homologous chromosomes. The key to preparing DNA fibers is that liquid nitrogen grinding of leaves is replaced by chopping with a blade in ice-cold nucleus isolation buffer. With the liquid nitrogen method, over-or under-grinding of leaves occurs more frequently, and DNA fibers with the desired quality are not obtained easily. In contrast, it is easier to release nuclei from cells in nucleus isolation buffer by chopping, which results in fewer nuclei being destroyed. Highly extended, intact, and long DNA fibers can be generated readily with this method. In addition, this method is very simple and rapid, requiring only 30 min for the entire process, and is also safe because poisonous mercaptoethanol is replaced by dithiothreitol. DNA fiber preparations were hybridized with 45S rDNA as probes, the signals were observed as fluorescent strings is recognized as linear or near-linear stretches of beads on-a-string fluorescence signals as the typically method. Factors such as loss and inaccessibility of target DNA due to either in situ renaturation or attachment to the glass substrate, as well as suppression of repeat sequences, were proposed as contributing to the origin of the typical discontinuous fiber FISH pattern. In our experiments, the results verified this observation. The reliable preparations of cotton pachytene chromosome spreads and DNA fibers, as well as the primarily success of their FISHs in diploid cotton, will be certainly enhanced, including the recognition of their resolution power and their applications from diploid to tetraploid species in *Gossypium*.