Locus-specific contig assembly in highly-duplicated genomes, using the BAC-RF method

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Abstract

Polyploidy, the presence of multiple sets of chromosomes that are similar but not identical, complicates both chromosome walking and assembly of sequence-ready contigs for many plant taxa including a large number of economically-significant crops. Traditional ‘dot-blot hybridization’ or PCR-based assays for identifying BAC clones corresponding to a mapped DNA landmark usually do not provide sufficient information to distinguish between allelic and non-allelic loci. A restriction fragment matching method using pools of BAC DNA in combination with dot-blots reveals the locus specificity of individual BACs that correspond to multi-locus DNA probes, in a manner that can efficiently be applied on a large scale. This approach also provides an alternative means of mapping DNA loci that exploits many advantages of ‘radiation hybrid’ mapping in taxa for which such hybrids are not available. The BAC-RF method is a practical and reliable approach for using high-density RFLP maps to anchor sequence-ready BAC contigs in highly-duplicated genomes, provides an alternative to high-density robotic gridding for screening BAC libraries when the necessary equipment is not available, and permits the expedient isolation of individual members of multigene or repetitive DNA families for a wide range of genetic and evolutionary investigations.