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Chromosome walking and jumping

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A new method to improve the efficiency of flanking sequence identification by genome walking was developed based on an expanded, sequential list of criteria for selecting candidate enzymes, plus several other optimization steps. These criteria include: step (1) initially choosing the most appropriate restriction enzyme according to the average fragment size produced by each enzyme determined using *in silico* digestion of genomic DNA, step (2) evaluating the *in silico* frequency of fragment size distribution between individual chromosomes, step (3) selecting those enzymes that generate fragments with the majority between 100 bp and 3,000 bp, step (4) weighing the advantages and disadvantages of blunt end sites vs. cohesive end sites, step (5) elimination of methylation sensitive enzymes with methylation-insensitive isoschizomers, and step (6) elimination of enzymes with recognition sites within the binary vector sequence (T-DNA and plasmid backbone). Step (7) includes the selection of a second restriction enzyme with highest number of recognition sites within regions not covered by the first restriction enzyme. Step (8) considers primer and adapter sequence optimization, selecting the best adapter-primer pairs according to their hairpin/dimers and secondary structure. In step (9), the efficiency of genomic library development was improved by column-filtration of digested DNA to remove restriction enzyme and phosphatase enzyme, and most important, to remove small genomic fragments (<100 bp) lacking the T-DNA insertion, hence improving the chance of ligation between adapters and fragments harbouring a T-DNA. Two enzymes, *NsiI* and *NdeI*, fit these criteria for the *Arabidopsis thaliana* genome. Their efficiency was assessed using 54 T3 lines from an *Arabidopsis* SK enhancer population. Over 70% success rate was achieved in amplifying the flanking sequences of these lines. This strategy was also tested with *Brachypodium distachyon* to demonstrate its applicability to other larger genomes.

Biography

Ali Taheri has completed his Ph.D. in 2009 from the University of Guelph, Ontario and is doing his postdoctoral studies at Agriculture and Agri-Food Canada. He is currently working on Brassica flea beetle resistance via next generation sequencing and metabolomics techniques.

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