SCAMing: an efficient high throughput approach to discovery and analysis of SNPs for genotyping of tropical crop species

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SCAMinG: An Efficient High Throughput Approach to Discovery and Analysis of SNPs for Genotyping of Tropical Crop Species

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Model organisms have well established genetic marker tools. However, genotyping of the wide range of agricultural and food species requires the discovery of genetic variations in each species that can be used to distinguish genotypes. Single Nucleotide Polymorphisms (SNPs) are the basis of most high throughput genetic analysis technologies. We have developed a generic approach to the discovery and analysis of SNPs in non-model organisms. SNP Characterisation and Mapping in Genomes (SCAMinG) has been developed from mutation detection based on enzymatic cleavage of DNA heteroduplexes as applied to naturally occurring genetic variation (ecoTILLinG). Our protocol involves application of high throughput capillary electrophoresis to provide efficient discovery of SNPs. SNPs discovered in this way are then available for analysis by other high throughput methods such as mass spectrometry using the mass array platform (Sequenom). These methods can be combined to map SNPs in the genome and to associate SNPs with key traits. The combined approach provides an efficient strategy for SNP genotyping in agricultural species.

Step One: High throughput SNP Discovery Analysis of fragments from nuclease digestion, using capillary electrophoresis, for identification of multiple SNP sites.

Step Two: Chromatogram from capillary electrophoresis (Cordeiro et al, 2006a). Peaks on chromatogram illustrate ability of the method to show location of multiple SNPs in nuclease digested amplified target.

Step Three: Primer extension assay for SNP analysis by Mass Spectrometry.

Step Four: Mass spectrum obtained from Mass Spectrometer Marker 10813-1726 is a GA polymorphism. The blue dotted line is the expected mass and the peak represents the actual mass of the extended primer.

Suggested References:

www.scu.edu.au/research/cpcg

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