A High-throughput System for the Rapid Extraction of Plant Genomic DNA for Genome Mapping and Marker-assisted Breeding Studies

Danhui Wang, Keming Song, Carol Kreader, Scott Weber, Jennifer Van Dinther, and Rafael Valdes-Camin

Sigma-Aldrich Corporation
2303 Laclede Ave., St. Louis, MO 63103

Abstract

Simple Sequence Repeats (SSR) or Simple Sequence Length Polymorphisms (SSLP) are PCR-based molecular markers that have been widely used in genomic mapping and marker-assisted selection. SSLR markers have made it possible to establish a high density genetic map and evaluate genes of interest via tight association between markers and phenotypes. Large-scale marker-assisted breeding studies have created the need for a high-throughput system for plant genomic DNA preparation and analysis. In marker-assisted breeding studies, target genes of interest are identified from a population by genomic markers. Thus, the bottleneck for such breeding studies has shifted from phenotypic analysis to the extraction and purification of genomic DNA from the thousands of plants in a segregating population. Standard methods for purifying DNA from plant tissues can be labor and time-intensive, and not readily amenable to automation. An automated system has been developed for the rapid extraction and subsequent amplification and analysis of plant genomic DNA to facilitate high-throughput marker-assisted breeding studies. This system utilizes Sigma’s Extract-N-Amp™ Plant PCR kit, a novel system for the rapid extraction and subsequent amplification of genomic DNA from plant tissues and the Maize SSR Primer set. This extraction system eliminates time-consuming steps such as organic extractions and mechanical disruption. The extraction treatment releases sufficient genomic DNA from plant tissues for direct use in SSR marker analysis.

Materials

Unless otherwise indicated, all reagents and materials used in this work were obtained from Sigma-Aldrich. Extract-N-Amp Plant PCR Kit (P/N: XNA-R) was used for genomic DNA isolation from plant leaves and PCR reactions setup. Maize SSR Primer Set (P/N: TML-510) was used to isolate genomic DNA from maize leaf as positive controls. Two parental lines (P1, B73, P2, MO17), one hybrid line (F1), and 21 F2 lines of maize population were used for screening.

Genomic DNA Extraction: A 0.5 to 0.7 cm leaf tissue disk was placed into each well of a 96-well PCR plate and kept on ice until use. DNA was extracted with the Extract-N-Amp Plant PCR kit using the automated procedure developed for the Sciclone ALH 2000 workcell.

PCR Reaction Setup: DNA extracts (4 µL) from plant leaf or maize genomic DNA controls (4 µL) were set up in a 20 µL PCR reaction. 6 µL of each PCR reaction was analyzed on a 2% agarose gel.

PCR Analysis of Maize Leaf Sample

Methods

Genomic DNA was extracted from the leaves of two parental lines (P1 and P2) and the resulting hybrid (F1). The SSR fragments were amplified and analyzed as described in Methods. M: PCR marker. (+): maize genomic DNA control. (-): no DNA template control.

Validation of the Automated the Extract-N-Amp Plant PCR Method

Figure 1: Agarose gel analysis of PCR products from 32 Maize SSR primer sets on three Maize populations. A, B, and C are the amplified SSR fragments from four different SSR primer sets identified from different F2 populations.

Genomic Mapping and Marker-assisted Breeding Applications

Figure 4: Agarose gel analysis of F2 products resulting from 32 Maize SSR primer sets on three Maize populations. Genomic DNA was extracted from the leaves of two parental lines (P1 and P2) and the resulting hybrid population. M: PCR marker. (+): maize genomic DNA control. (-): no DNA template control.

Conclusions

- Data demonstrates the effectiveness of Extract-N-Amp Plant Kit in the isolation and subsequent amplification of target genes from a variety of plant types.
- Data presented herein demonstrates that Extract-N-Amp Plant PCR used with the Maize SSR primer set is a powerful solution to facilitate marker-assisted breeding studies.
- The walk-away automated protocol for Extract-N-Amp Plant PCR kit enables high-throughput genomic DNA extractions required for marker-assisted breeding studies.
- The entire process is automated from leaf punch through PCR reaction setup.
- This method a rapid—98 samples can be processed in 30 minutes.