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

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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. SSR 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 segregating population by genetic 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: M4193, see **Table 1**) was used for the screening of SSR primer polymorphism in maize population. GenElute™ Plant Genomic DNA Miniprep Kit (P/N: G2N10) 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 utilized for screening.

Methods

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 utilizing the automated procedure developed for the Sciclone ALH 3000 workstation.

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 incorporating the 2x PCR Ready Mix and universal chloroplast primers or SSR primers utilizing the automated Extract-N-Amp Plant PCR procedure developed on the Sciclone ALH 3000 workstation.

Agarose Gel Analysis: 6 µL of each PCR reaction was analyzed on a 2% agarose gel.

SSR Name	Lanes on Gel Image	Linkage group (chrom.)	Map location (IBMn)
p-bnlg1444	A1-3	4	462.5
p-umc2281*	A4-6	4	158.6
p-umc2039	A7-9	4	196.4
p-umc1117	A10-12	4	218.5
p-umc1854	B1-3	4	595.7
p-umc1328	B4-6	4	618.1
p-umc1573	B7-9	4	644.3
p-bnlg589	B10-12	4	670.2
p-phi036	C1-3	3	159
p-umc2158	C4-6	3	176.6
p-umc1655	C7-9	3	191.1
p-mmc0132	C10-12	3	208.6
p-umc1008	D1-3	4	2.9
p-umc2279	D4-6	4	22.9
p-umc1682	D7-9	4	47.6
p-umc1973	D10-12	3	371.4
p-bnlg490	E1-3	4	237.8
p-umc2282*	E4-6	4	254.9
p-umc2282	E7-9	4	271.4
p-umc1175	E10-12	4	287.3
p-umc1109	F1-3	4	687.8
p-umc2289*	F4-6	4	707.8
p-umc1738	F7-9	4	720.48
p-umc2290	F10-12	4	736.7
p-umc1968	G1-3	3	250.4
p-umc1683	G4-6	3	266
p-umc2049	G7-9	3	38.7
p-umc2377	G10-12	3	47.91
p-umc1702	H1-3	4	305.2
p-mmc0371*	H4-6	4	331.3
p-mmc0371	H7-9	4	349.8
p-umc2152	H10-12	3	738.7

Table I: Maize SSR primer sets (Please see http://www.sigmaaldrich.com/Area_of_Interest/Life_Science/Plant_Biotechnology/Plant_Molecular_Biology/maizessrprimer.html for the entire sets)

* : primer sets used to study co-segregation patterns of F2 population (see **figure 5**)

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

PCR Analysis of Maize Leaf Samples

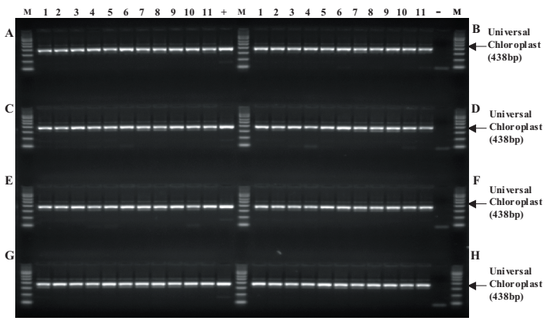

Figure 1: Agarose gel analysis of 96 PCR samples. DNA was extracted from 88 maize leaf samples. Amplification of the 438 bp fragment of universal chloroplast genomic DNA is indicated by the arrow. M: PCR marker. (+): maize genomic DNA control. (-): no DNA template control.

Cross-contamination Anaylsis

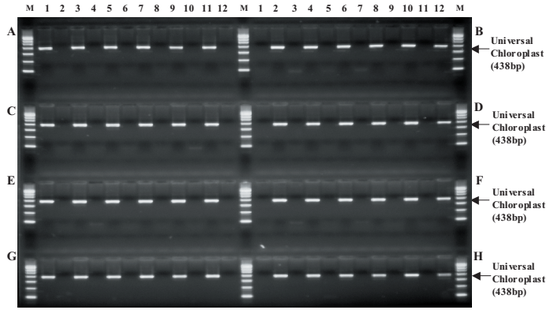

Figure 2: Agarose gel analysis of cross-contamination test. Maize leaf punches and blanks were placed in alternating wells of a 96-well plate. DNA extraction, amplification, and analysis was carried out as described in Methods. No PCR products were detected in the wells containing no plant tissue samples.

PCR Analysis of Different Plant Types

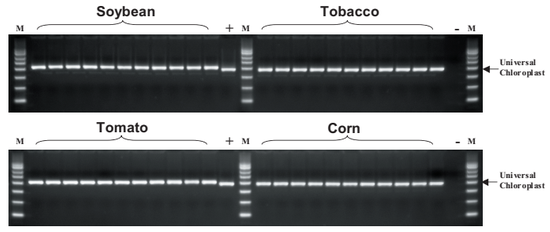

Figure 3: Agarose gel analysis of 48 PCR samples from different tissue extracts. DNA was extracted from soybean, tobacco, tomato, and maize leaves using the automated Extract-N-Amp Plant PCR procedure on the Sciclone ALH 3000. Amplification and analysis of 400–500bp fragment of the universal chloroplast genomic DNA was performed as described in Methods. M: PCR marker. (+): maize genomic DNA control. (-): no DNA template control.

Genomic Mapping and Marker-assisted Breeding Applications

Workflow for Genomic Mapping and Marker-assisted Breeding Applications

Reconstitute and dilute the Maize SSR primer set using the automated Sciclone method

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Collect leaf disks from two parental lines (P1 and P2) and their offspring (F1)

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Isolate genomic DNA using the automated Extract-N-Amp Plant method

↓

Set up PCR reactions with DNA extracts, SSR primers, and PCR ReadyMix using Sciclone method

↓

Amplify the SSR marker regions, load the PCR products onto agarose gels, and identify the primer sets that yield different sized PCR products amongst P1 and P2 lines

↓

Collect leaf disks from segregating F2 population

↓

Isolate genomic DNA using the automated Extract-N-Amp Plant method

↓

Set up PCR reactions using the automated Sciclone method. Reactions include DNA extracts, PCR ReadyMix, and selected SSR primers that resulted in differences between P1 and P2 lines

↓

Amplify the SSR marker regions, load the PCR products onto agarose gels, and identify the co-segregation patterns within the population for a specific phenotype

Screening of Polymorphic Primers for Maize Population

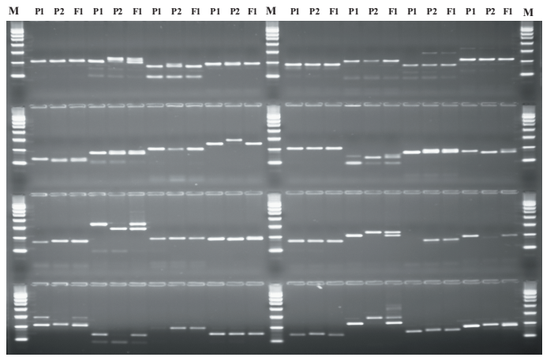

Figure 4: Agarose gel analysis of PCR products resulting from 32 Maize SSR primer sets on three Maize populations. Genomic DNA was extracted from the leaves of two parental lines (P1 and P2) and the resulting hybrid line (F1). The SSR fragments were amplified and analyzed as described in the Method. M: PCR marker.

Segregation Pattern Study within an F2 Population with Selected SSR Primer Sets

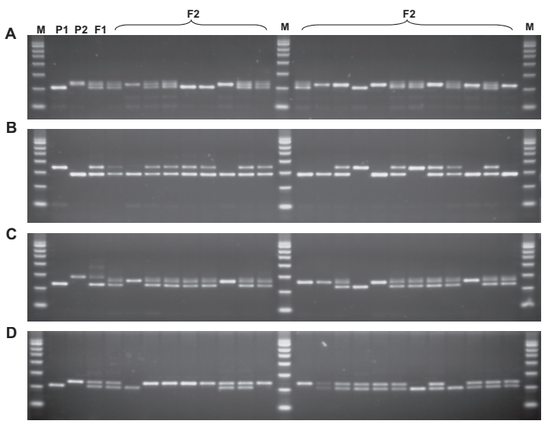

Figure 5: Agarose gel analysis of PCR products resulting from the use of four Maize SSR primer sets on an F2 population. Genomic DNA was extracted from the leaves of two parental lines (P1 and P2), one hybrid line (F1), and 21 samples from the F2 line. The SSR fragments were amplified and analyzed as described in the Method. Panel A, B, C, and D are the amplified SSR fragments from four different SSR primer sets identified from **figure 4.**

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 here 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 is rapid—96 samples can be processed in 30 minutes

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