Application Note

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Arabidopsis thaliana Ecotypes Differentiated by Simple Sequence Repeats

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Abstract

Simple sequence repeats (SSR) or microsatellite regions are essential markers for mapping of quantitative trait loci (QTL). They are the biomarker of choice for many researchers because of their abundance in the genomes of commonly studied plant, animal, and microbial organisms and can employ simple, cost-effective workflows for analysis. Common analysis protocols of SSRs utilize gel electrophoresis workflows which can be time consuming, requiring manual interpretation of data, leading to the possibility of human error. The Agilent ZAG DNA Analyzer system (ZAG) is a versatile tool in molecular biology laboratories and can be used in identifying SSR markers, providing high-resolution fragment analysis with automated size assessment. To demonstrate the utility of the ZAG system for SSR analysis in plant and agricultural research, size analysis of the BSAT1.024 and MSAT3.1 SSR regions were analyzed for three Arabidopsis ecotypes, Col-0, Ler-2, and Nd-1. Characterization of these microsatellites aids in the monitoring of Arabidopsis seed stocks to control for crosses between Arabidopsis ecotypes.

Introduction

QTL mapping has been widely used for decades to identify gene loci controlling phenotypic variation of a trait. QTL mapping requires the identification of molecular markers along the genome that are correlated to polymorphic traits. In the past, QTL mapping occurred through the identification of amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), and single nucleotide polymorphism (SNP) markers. Another molecular marker that has become widely used in genetic analysis of plants are microsatellite markers.

Microsatellite markers, also known as SSRs, are short tandem repeat motifs of 1 to 6 bp. They are relatively abundant in genomes and are flanked by highly conserved regions, allowing them to be easily amplified by PCR regardless of the subspecies or ecotype being analyzed. SSRs occur in plant, microbial, and animal species and are often used for genetic tracing of animal breeds, plant species, species conservation projects linkage mapping, parentage, and more. For example, researchers use microsatellites for mapping locations within a genome in order to identify a gene or mutation responsible for a desirable trait in plants and animals, or diseases such as DNA deletions in cancer. SSRs are present in all types of organisms and are used in many diverse applications.

In this application note, two SSR regions (BSAT1.024 and MSAT3.1) were used to discriminate between the *Arabidopsis thaliana* ecotypes Col-0, Ler-2, and Nd-1 using the ZAG DNA Analyzer system (ZAG). BSAT1.024 represents an AG repeat that can occur up to 25x within the genome. MSAT3.1 represents a GA repeat that can occur up to 28x within the genome. Both of these SSR regions have previously been characterized as targets for the monitoring of Arabidopsis seed stocks to control for crosses between Arabidopsis ecotypes[1].

Experimental

Plant material

Seeds were purchased from The Arabidopsis Information Resource, or TAIR (Col-0: CS1092, Ler-2: CS8581, Nd-1: CS1636). Plants were grown under 16-hour days and harvested at approximately 3 weeks post-germination.

PCR conditions

Genomic DNA (gDNA) was extracted from Arabidopsis young leaves using the traditional phenol:chloroform protocol. PCR was performed in a final volume of 50 µL with approximately 28 ng of gDNA template, 5X Phusion buffer HF (Thermo Fisher Scientific p/n F-520L), 0.2 mM dNTPs (Thermo Fisher Scientific p/n 10297018), 0.5 µM of each primer (Integrated DNA Technologies, IDT), and 1 U Phusion DNA polymerase (Thermo Fisher Scientific p/n F530-L).

Primer sequence for BSAT1.024 Forward: CCATCTGTGTATCAGAATCGC and Reverse: ATGATCCCTCCTCATGCC and for MSAT3.1 Forward: GGACTCGCTGCTGCTACATTC and Reverse: CCACGCACATGCAATTACATA.

Conditions used for PCR amplification were as follows: preincubation 98 °C for 30 s; 35 cycles of denaturation at 98 °C for 10 s, annealing at 62 °C for 30 s, and extension at 72 °C for 30 s; and a final extension step at 72 °C for 10 min.

Polymorphism analysis

Following PCR, amplicons were directly analyzed with the Agilent ZAG DNA Analyzer system (p/n M5320AA) with a FA/ZAG 96-Capillary Array Long (55 cm) (p/n A2300-9650-5580) and the ZAG 105 dsDNA kit (1-500 bp) (p/n ZAG-105-5000). The same samples were analyzed on the Agilent 5300 Fragment Analyzer system (p/n M5311AA) with a FA/ZAG 96-Capillary Array Long (55 cm) (p/n A2300-9650-5580) and the dsDNA Reagent kit (1-500 bp) kit (p/n DNF-905).

Results and discussion

Visualization of DNA polymorphisms is typically performed with agarose or polyacrylamide gels depending on the resolution requirements. Agarose gels provide low resolution separation of nucleic acid fragments, impairing discrimination of some polymorphic bands. While polyacrylamide gels can allow for higher-resolution separations, they are time consuming and use neurotoxic reagents. Both agarose and polyacrylamide gels require constant monitoring and manual data annotation using external software analysis of the gel image.

An alternative to traditional gel electrophoresis workflows is the ZAG system. This instrument provides reproducible separation of DNA fragments with a 3 bp resolution[2]. High-throughput needs are met by analyzing 96 samples simultaneously in as little as 30 minutes.

The ZAG system has the added advantage of the Agilent ProSize data analysis software (ProSize software), which automatically provides sizing with optional Boolean operators for simplified flagging analysis[3], eliminating additional manual annotations required by agarose and polyacrylamide gel electrophoresis protocols. Once flag analysis is set up in ProSize software, a report is automatically generated for all samples assigning presence (+) or absence (-) of the SSR region. This saves time and minimizes misidentification of SSR regions due to misalignment of amplicons on the gel.

SSRs can have varying numbers of tandem repeat motifs from 1 to 6 bp. The ZAG can differentiate between regions with a 3 bp difference when the total fragment size is between 50 to 300 bp[2]. This type of repeat could consist of a single base repeat with a difference of at least 3 bp (for example, AAAA vs AAAAAAA), a double base pair repeat with at least a two repeating motifs (for example, AGAG vs AGAGAGAG), or a 3 bp repeat with a single repeat difference (for example, AGT vs AGTAGT). Achieving 3 bp resolution with agarose or polyacrylamide gel electrophoresis workflows can be difficult, requiring time-consuming optimization of protocols. The ZAG system provides robust and highly reproducible resolution and sizing for all numerous sample types, with the benefits of facile setup, rapid time to results, and automated workflows and data analysis.

Two SSR regions, BSAT1.024 and MSAT3.1, were used to investigate the ability to distinguish between the *Arabidopsis thaliana* ecotypes Col-0, Ler-2, and Nd-1. Analysis with the ZAG and the ZAG 105 dsDNA kit identified sizing differences for the BSAT1.024 (Figure 1) and MSAT3.1 (Figure 2) SSR regions for all three ecotypes analyzed. Both SSR regions are a two base pair repeat. They were easily separated and detected from each other on the ZAG as the difference in sizes between each was a minimum of 10 bp, or a five-repeat difference. The BSAT1.024 SSR region had an average size of 148 bp for Col-0, 164 bp for Ler-2, and 174 bp for Nd-1. The MSAT3.1 SSR region had an average size of 479 bp for Col-0, 383 bp for Ler-2, and 321 bp for Nd-1.

The ZAG demonstrated very high sizing precision for all the SSR regions with less than 1% CV. Analysis of the same samples was also done with the 5300 Fragment Analyzer system, which offers higher-throughput capabilities with the same resolution, accuracy, and automatic data analysis as the ZAG system. The same fragment sizing, precision, and resolution of the BSAT1.024 and MSAT3.1 SSR regions was achieved with the 5300 Fragment Analyzer (Table 1). Discrimination between the three ecotypes of Arabidopsis could be accomplished with either single SSR region. Analysis with both SSR regions would aid in identifying the Col-0, Ler-2, and Nd-1 ecotypes from a larger pool of Arabidopsis ecotypes.

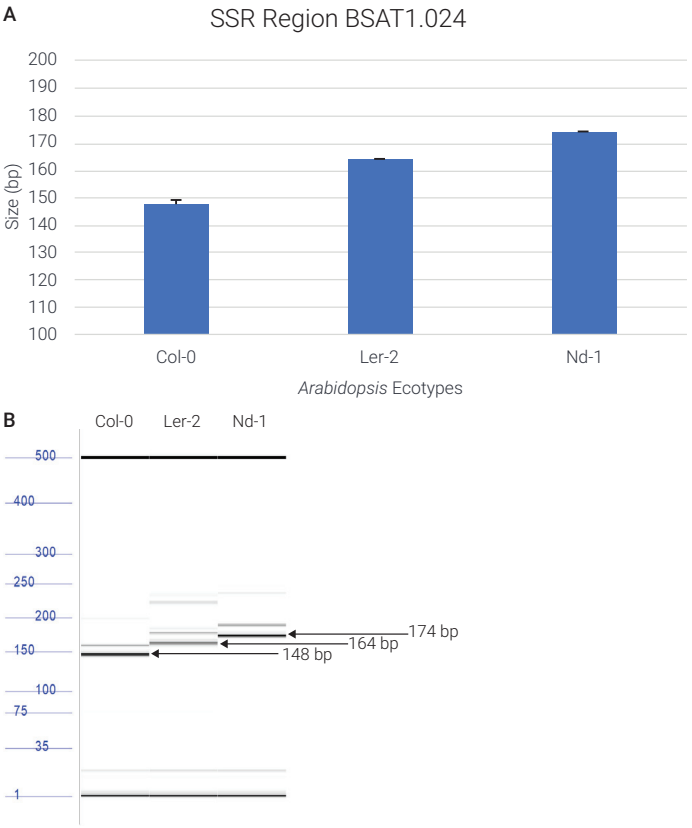

Figure 1. Sizing analysis of the BSAT1.024 SSR region with the Agilent ZAG DNA Analyzer system of Arabidopsis ecotypes Col-0, Ler-2, and Nd-1. (A) Fragment size (B) Digital gel electrophoresis image.

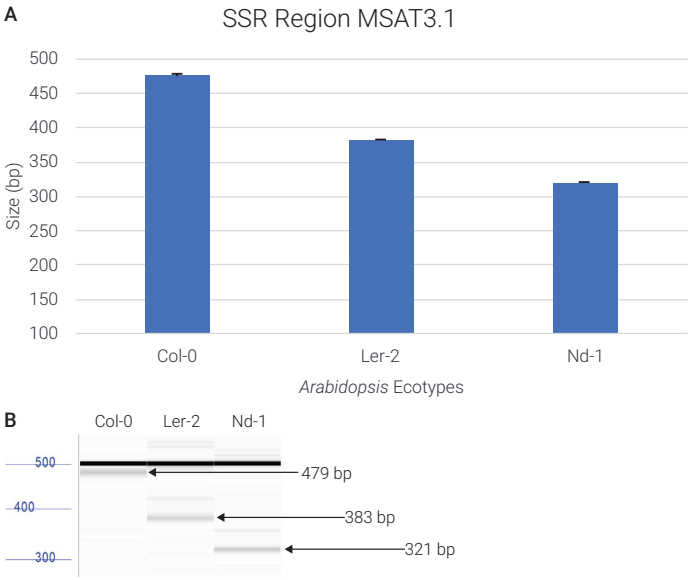

Figure 2. Sizing analysis of the MSAT3.1 SSR region with the Agilent ZAG DNA Analyzer system for Arabidopsis ecotypes Col-0, Ler-2, and Nd-1. (A) Fragment size (B) Digital gel electrophoresis image.

A study by Cosson et al.[1] investigated the sizing differences of the BSAT1.024 and MSAT3.1 SSR regions in 30 different Arabidopsis ecotypes with a 6% polyacrylamide gel. They observed similar sizes for the BSAT1.024 and MSAT3.1 regions for ecotypes Col-0, Ler-2, and Nd-1, as was identified by the ZAG (Table 1). The percent sizing difference between the ZAG and polyacrylamide gel was extremely low for both SSR regions on all three ecotypes, indicating that the sizing was concordant. Corroboration of the region size by two different parties, using two different methods, establishes reliable sizing for these SSR regions.

Table 1. Sizing comparison of the BSAT1.024 and MSAT3.1 SSR regions between the Agilent ZAG DNA Analyzer system, 5300 Fragment Analyzer system, and acrylamide gel for Arabidopsis ecotypes.

	Sizing of SSR Regions (bp)					
	BSAT1.024			MSAT3.1		
Ecotypes	Acrylamide gel*	ZAG	Fragment Analyzer	Acrylamide gel*	ZAG	Fragment Analyzer
Col-0	146	148	146	480	479	479
Ler-2	162	164	162	373	383	383
Nd-1	172	174	174	-	321	320

**Acrylamide gel size taken from supplementary Table from Crosson et al.[1].*

Conclusion

SSRs or microsatellites are commonly used across plant, microbial, and animal species for a variety of applications in genomic analysis laboratories. Analysis of the BSAT1.024 and MSAT3.1 SSR regions with the Agilent ZAG DNA Analyzer system identified sizing differences for three Arabidopsis ecotypes, Col-0, Ler-2, and Nd-1. Evaluation of the SSR regions was streamlined with the ProSize data analysis software and flag analysis feature. These SSR markers constitute a powerful tool to control seed stocks and crosses for the ecotypes studied in this work and possibly others.

The ZAG workflow provides several advantages not available from gel electrophoresis workflows. The ZAG system has the ability to analyze SSR amplicons directly, without requiring an additional, time-consuming purification step prior to fragment sizing, and provides automated data analysis with high-throughput scalability.

Reference

1. Cosson, Patrick; Decroocq, Veronique; and Revers, Frederic, Development and characterization of 96 microsatellite markers suitable for QTL mapping and accession control in an Arabidopsis core collection. *Plant Methods* **2014**, 10:2.
2. Resolution Capabilities of the Agilent ZAG DNA Analyzer System with the Agilent ZAG 105 dsDNA Kit, *Agilent Technologies application note*, publication number 5994-1121EN, **2019**.
3. Genotyping with the Agilent ZAG DNA Analyzer System, *Agilent Technologies application note*, publication number 5994-1186EN, **2019**.

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