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Genotyping with the Agilent ZAG DNA Analyzer System

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Abstract

The Agilent ZAG DNA Analyzer system provides high-throughput genotyping
of 96 samples at one time. Genomic DNA extracted from *Arabidopsis* LOH1
knockout seeds were genotyped on the ZAG DNA Analyzer system with the
Agilent ZAG 110 dsDNA kit. Genotyping was easily assessed by a digital gel image
or electropherograms. Agilent ProSize data analysis software provides advanced
settings for establishing criteria to flag samples as wild type, heterozygous,
or homozygous.

Introduction

The introduction of transgenes has been a widely used technique to modify organisms by the addition of a novel protein or knockout of an endogenous gene. Depending on the organism and methodology used, the efficiency of this process can vary widely. To ensure that observed phenotypes of modified organisms are a result of transgene introduction, homozygous lines are usually established through inbreeding. This is especially important during the production of gene knockouts, which require homozygous transgene presence to ensure complete removal of the desired protein. To effectively genotype for transgene knockouts, three primer PCR methods are required that amplify wild type (WT) and transgene sequences. While this method is simple, analyzing large numbers of lines and cataloging the gel electrophoresis analysis data is cumbersome.

The ZAG DNA Analyzer system streamlines the genotyping process by automating the analysis of PCR amplifications using parallel capillary electrophoresis. Outfitted with a 96-capillary array, the ZAG DNA Analyzer system can analyze 96 samples at one time with uninterrupted testing of nine 96-well plates. Ideal for PCR amplicons and other DNA fragments, the four ZAG dsDNA kits cover a sizing range from 35 to 20,000 bp. The ZAG 105 dsDNA kit (1-500) provides sizing for small DNA fragments from 35-500 bp, the ZAG 110 dsDNA kit (35-5000 bp) has a midsize range of 35 to 5,000 bp, and the ZAG

130 dsDNA kit (75-20000 bp) covers the largest sizing range of 75 to 20,000 bp. The ZAG 135 dsDNA kit (1-1,500 bp) is designed for extremely high-throughput labs, with fragments ranging from 100 to 1,500 bp. A higher voltage during sample separation for this kit shortens the run time to approximately 20 minutes, enabling higher sample throughput.

To demonstrate the efficiency of determining genotypes on a high-throughput scale, a segregating knockout line of *Arabidopsis thaliana* was grown and analyzed on the ZAG DNA Analyzer system and the ZAG 110 dsDNA kit. This high-throughput genotyping analysis can be applied to other plants such as maize and model organisms like fruit flies, mice, and zebrafish where gene translocations are being used.

Experimental

Methods

DNA extraction

Arabidopsis LOH1 (At3g25540) knockout (SALK_057546) seeds were obtained from the Arabidopsis Biological Resource Center. gDNA was extracted from approximately three-week-old plants using a modified Edwards extraction protocol. Briefly, Edwards solution was prepared (200 mM Tris-HCl (pH 8.0), 250 mM NaCl, 25 mM EDTA, and 0.5% SDS) and diluted 10-fold in 1X TE (10 mM Tris-HCl (pH 8.0) and 1 mM EDTA) to create the extraction buffer. A small leaf (3 to 5 mg) was ground in 200 µL of extraction buffer using a plastic pestle to obtain DNA.

PCR amplification

PCR was completed using Phusion High-Fidelity DNA Polymerase (Thermo Fisher, p/n F530S) according to manufacturer instructions with 1 µL of extracted DNA used as template. Primer sequences were retrieved from the SALK T-DNA Primer Design tool (LP: CAAGTACAATGATGAGGACGG, RP: GGGTTGGTTGGTGAAACAAC, LB1.3: ATTTTGCCGATTTCGGAAC). The WT product had an estimated size of 1082 bp, with the knockout product having a predicted size of 440 to 770 bp. Amplification conditions followed manufacturer recommendations (initial denature at 98 °C for 30 s; denature at 98 °C for 10 s, annealing at 58 °C for 30 s, extension at 72 °C for 45 s, cycled 35x; final extension at 72 °C for 10 min).

Genotyping

PCR products were analyzed by the Agilent ZAG DNA Analyzer system with the ZAG 110 dsDNA kit (35-5000 bp) (p/n ZAG-110-5000). Samples were prepared by diluting 2 µL of PCR product with 22 µL of 1X TE. Data were analyzed using Agilent ProSize data analysis software. Flag analysis criteria was set to automatically identify WT, heterozygous, and homozygous knockout lines (WT: 1100 bp ± 150 bp AND NOT 500 bp ± 50 bp; Heterozygous knockout: 500 bp ± 50 bp AND 1100 bp ± 150 bp; Homozygous knockout: 500 bp ± 50 bp AND NOT 1100 bp ± 150 bp).

Results and discussion

Genotyping with the ZAG DNA Analyzer system

Genotyping by gel electrophoresis is time-consuming, with long preparation and separation times. In addition, there are limitations on the number of samples that can be run on one gel. This limitation leads to large numbers of gels and issues with analysis and data storage. The ZAG DNA Analyzer system is ideal for high-throughput genotyping applications.

Genomic DNA extracted from *Arabidopsis* LOH1 knockout seeds were genotyped on the ZAG DNA Analyzer system with the ZAG 110 dsDNA kit (35-5000 bp). The entire 96-well plate was analyzed at one time in approximately one hour. A digital gel image of all 96 samples visually displays which seeds are WT, heterozygous, or homozygous (Figure 1). An electropherogram overlay of individual samples A3, A4, and A6 and the corresponding gel image also provides genotyping analysis (Figure 2).

Sample A3 (red) is WT, sample A4 (blue) is heterozygous, and sample A6 (black) is homozygous. The WT peak has an average size of 1,229 bp with a precision of 2.7% CV%, with the homozygous peak having an average size of 518 bp with a
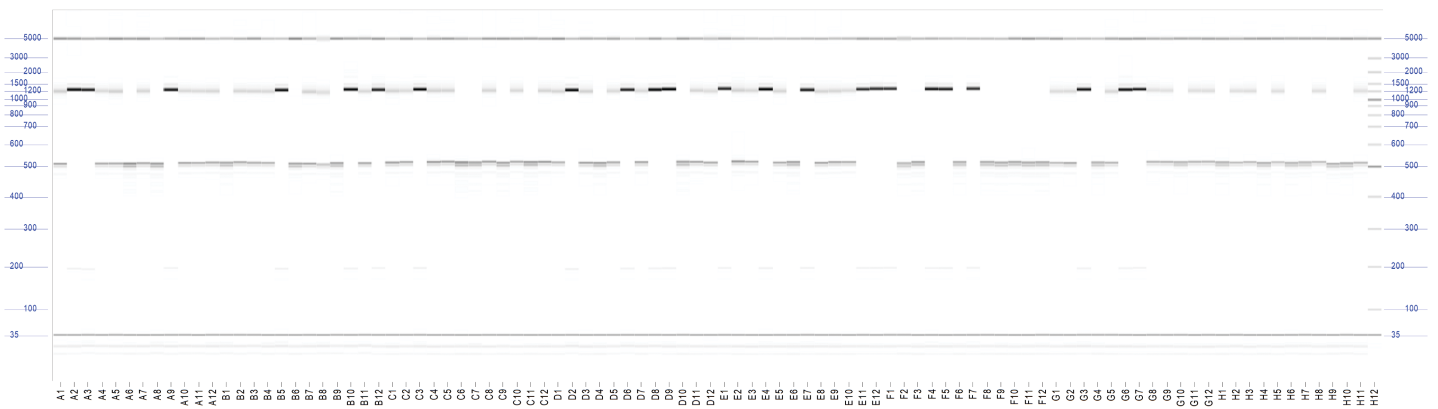

Figure 1. Digital gel image of *Arabidopsis thaliana* on the Agilent ZAG DNA Analyzer system with the Agilent ZAG 110 dsDNA kit (35-5000 bp) and the 96-capillary array.
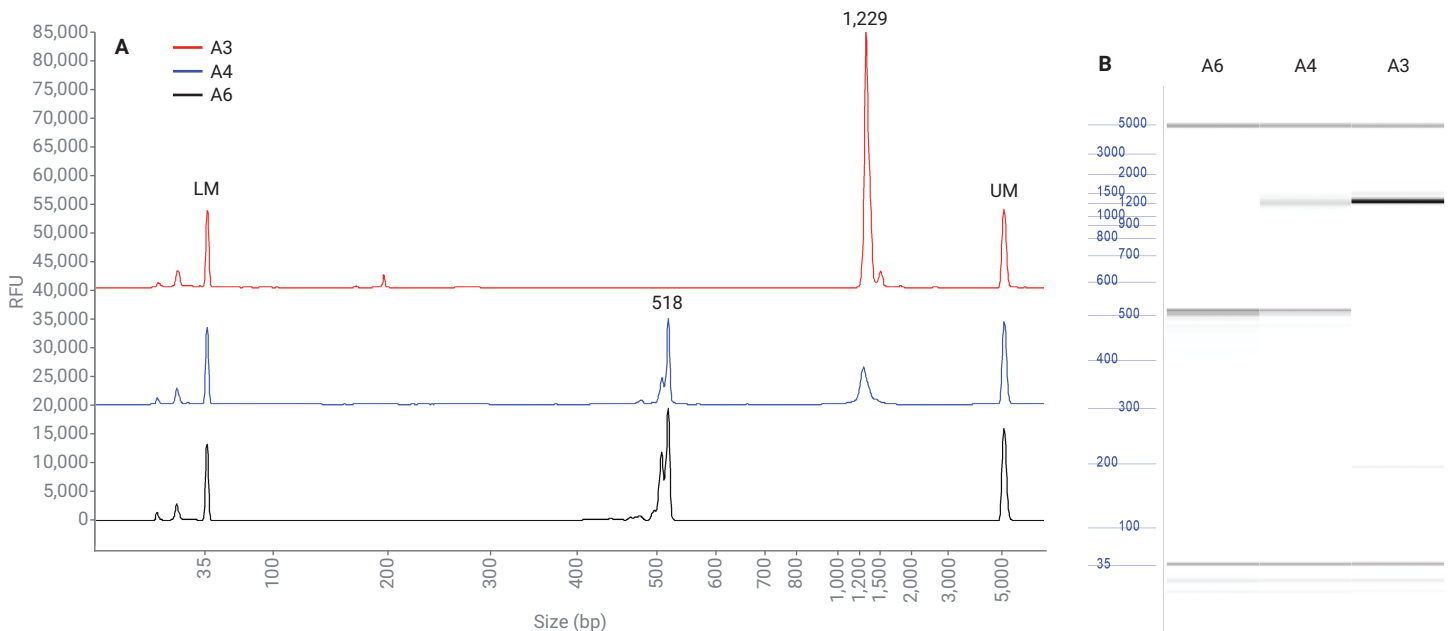

Figure 2. Genotyping of sample A3, A4, and A6 *Arabidopsis thaliana* analyzed on the Agilent ZAG DNA Analyzer system with the Agilent ZAG 110 dsDNA kit (35-5000 bp). (A) Electropherogram overlay. Sample A3 is wild type (red), A4 is heterozygous (blue), and A6 is homozygous (black). Average size displayed. (B) Corresponding digital gel image. LM = lower marker; UM = upper marker.

high precision of 0.6% CV.

ProSize data analysis software and flag analysis

ProSize data analysis software is designed for use with the Agilent Fragment Analyzer systems, the ZAG DNA Analyzer system, and the Agilent Femto Pulse system for the analysis of nucleic acid data. ProSize software has been developed to analyze a wide range of nucleic acids including gDNA, NGS libraries, plasmids, microsatellites, PCR amplicons, restriction enzyme digests, mRNA, small RNA, and total RNA. Results are displayed as both electropherogram traces and digital gel images with a peak table displaying size, concentration, percent concentration, and molarity all on the same screen.

ProSize software includes many additional options in the advanced settings that allow the user to individualize their data analysis and electropherograms to fit their particular needs. The ability to adjust settings and compare runs in one software program saves time and enhances data analysis. The flag analysis tab in advanced settings allows quick determination of fragments of interest. This is very useful for sorting through numerous samples separated with the 96-capillary array on the ZAG DNA Analyzer system.

Flag analysis allows the user to specify specific criteria to be met within the data and generates a binary output where 0 = false and 1 = true. Flag criteria can be based on size, concentration, or peak height with a numerical range and several definable Boolean operations such as AND, OR, AND NOT, and NOR (Figure 3). These Boolean operations can enable exclusion of one or more fragments or inclusion of up to two fragments. This is useful for applications such as genotyping, where the presence or absence of specific fragments must be scored. Results are listed in the Flag

Analysis table, which can be exported (Figure 4). Occasionally, a sample may not meet any of the criteria set and display all zeros, such as in sample A2. After reviewing the electropherogram, it was determined the sample peak size was just outside of the criteria. In such cases, the user may choose to increase the size range criteria to assign a genotype automatically.
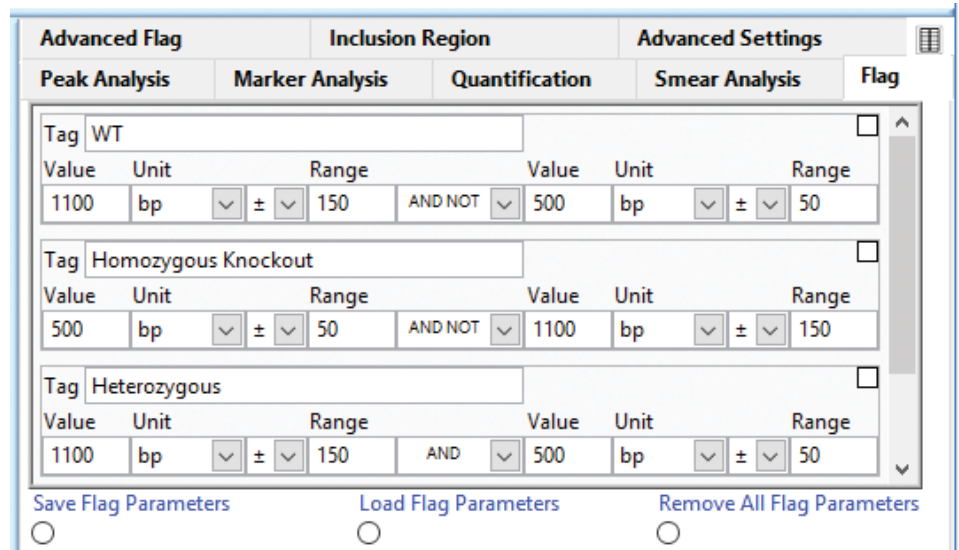

Figure 3. A picture of the advance setting flag analysis criteria set for genotyping.

Figure 4. Picture of the Flag Analysis table with the genotyping criteria. The binary output designations are 0 = false and 1 = true.

The immense amount of data provided by ProSize software is efficiently handled, with the option of creating PDF project reports, exporting data in a generic CSV file format for spreadsheet analysis, or batch data processing. Exporting data and project reports offers the user the option of including all the data or selecting only the information of interest to a file path of choice. Data options include peak table, smear analysis table, quality table, flag criteria analysis table, electropherograms, size calibration data, gel and image format, and all samples or selected samples.

Conclusion

The ZAG DNA Analyzer system streamlines the genotyping process by automating the analysis of PCR amplifications using parallel capillary electrophoresis. High-throughput genotyping of 96 samples at one time is easily visualized through a digital gel image and electropherograms. ProSize data analysis software provides flag analysis, allowing the user to set criteria to determine WT, heterozygous, and homozygous genotyping. The data is easily exported into an Excel spreadsheet for stress-free analysis, or a PDF for project reports.