Application Note

A. thaliana genotyping with a CAPS marker for a pks3 mutant allele

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The QIAxcel® system was used for genotyping *A. thaliana* with CAPS markers, following mutations in the PKS3 gene (At1g18810), and to identify mutants in various crosses.

Introduction

Arabidopsis thaliana is a small flowering plant that is widely used as a model organism in plant cellular and molecular biology. Its genome sequence is known and is available through the Arabidopsis Information Resource (TAIR), as well as other sources, including seed stocks and collections of genetic and physical markers.

The short life cycle (approximately 6 weeks from germination to seed maturation), enables highly efficient preparation of mutants for in-depth analysis of gene function. Once a mutant of interest has been identified, cleaved amplified polymorphic sequences (CAPS) are used to map the mutation (1). CAPS markers are also used to genotype known mutations.

Previously, no mutations were known for the phytochrome kinase substrate protein 3 (PKS3) gene (At1g18810), a member of a small gene family in A. thaliana (2). The "targeting induced local lesions in genomes" ("tilling") approach was used to identify a mutant in the PKS3 gene, pks3-*7*. Subsequently CAPS markers were used for genotyping, allowing the mutant to be followed in various genetic crosses.

Materials and Methods

Small arabidopsis leaves were homogenized by grinding in an Eppendorf® tube in 500 µl of 200 mM Tris (pH 7.5), 250 mM NaCl, 25 mM EDTA, and 0.5% SDS. After centrifugation, DNA was precipitated by adding equal amounts of isopropanol to the supernatant. After an additional centrifugation at 12,000 x g for 10 minutes, the DNA pellet was washed with 70% ethanol, air dried, and resuspended in 100–200 µl 10mM Tris, 1 mM EDTA (pH 8.0).

PCR amplification was performed with Taq DNA polymerase (proprietary preparation) understandard reaction conditions in a 20 µl volume. PCR amplification using the CF523 (AAACA AGCCG ACATG GAACG) and CF524 (TCGTT ATGTT CTCAA TCTCG) primers yielded a prominent 518 bp fragment.

PCR product (10 µl) was digested in a total volume of 40 µl with 10 U MboI (New England BioLabs) by incubating for 70 minutes at 37°C. The 518 bp wild-type fragment was digested into 3 fragments: 29 bp, 182 bp, and 307 bp. The pks3-7 mutant sequence is missing an MboI restriction site, and digestion of the mutant 518 bp fragment yielded 2 fragments: 211 bp and 307 bp.

Digested samples were analyzed using the QIAxcel capillary electrophoresis system with the QIAxcel DNA Screening Kit and the AM320 method. The QX Alignment Marker 50 bp/500 bp and QX DNA Size Marker 50 bp–800 bp were included in the analysis.

The QIAxcel capillary electrophoresis system processes samples in batches of 12 and allows analysis of up to 96 samples without manual intervention. The results can be displayed as an electropherogram as well as a gel-like image.

Results and Discussion

CAPS analyses were performed to screen the progeny of a backcross of pks3-7 against its isogenic wild-type control. By analyzing the F2 generation for the presence of the pks3-7 mutation using a specific CAPS marker, it was possible to follow the mutant allele through various crosses.

The results of a CAPS analysis using the QIAxcel system are shown in Figure 1. In lanes 2, 4, and 6, DNA fragments from the wild type PKS3 gene are present (29 bp, 182 bp, and 307 bp). The fragments present in lane 3 (211 bp and 307 bp) indicate a homozygote for the pks3-7 mutation. The fragments in lanes 5, 7, and 8 (29 bp, 182 bp, 211 bp, and 307 bp) indicate heterozygosity for the pks3-7 mutation. The sizes of the DNA fragments estimated by BioCalculator Software are given in Table 1.

Although the size differences between the fragments are small, the sharp banding patterns achieved using the QIAxcel system allowed more accurate size estimation than is possible with agarose gel electrophoresis (data not shown).

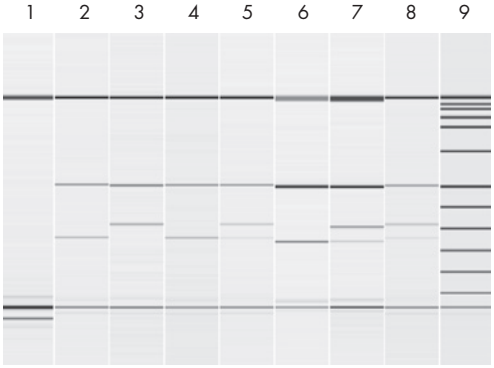

Figure 1. CAPS analysis for the *A. thaliana* PKS3 gene. CAPS of the PKS3 gene were prepared from individual *A. thaliana* plants and resolved on the QIAxcel system using the QIAxcel DNA Screening Kit.
1: negative control;
2, 4, 6: wild type;
3: homozygous pks3-7 mutant;
5, 7, 8: heterozygote pks3-7 mutant;
9: QX DNA Size Marker 50 bp/800 bp.

Table 1. BioCalculator analysis of the gel image in Figure 1

Lane	Estimated fragment size (bp)										
1	41										
2	180	306									
3	210	303									
4	179	304									
5	179	210	304								
6	29	170	300								
7	34	171	204	299							
8	178	210	303								
9	50	100	150	200	250	300	400	500	600	700	800

Conclusions

- The sharp banding patterns achieved with the QIAxcel capillary electrophoresis system simplified and accelerated the routine sizing of wild type and mutant DNA fragments. Due to the accurate sizing of DNA fragments compared to conventional agarose gel electrophoresis (data not shown), the QIAxcel system enabled unambiguous size estimation in significantly shorter time.
- Up to 96 samples can be analyzed in a single run without manual intervention using the QIAxcel system. In addition, the QIAxcel system provides more information from CAPS analyses than traditional methods, saving time and effort. Controlled running conditions and automated data acquisition ensure data safety, reliability, and reproducibility.
- QIAxcel capillary electrophoresis uses only minute quantities of DNA for electrokinetic injection, allowing the samples to be used for downstream procedures, such as sequencing or cloning.

References

1. Konieczny, A., and Ausubel, F.M. (1993) A procedure for mapping Arabidopsis mutations using co-dominant ecotypespecific PCR-based markers. Plant J. **4**, 403.
2. De Carbonnel, M. et al. (2010) The arabidopsis PHYTOCHROME KINASE SUBSTRATE2 protein is a phototropin signaling element that regulates leaf flattening and leaf positioning. Plant Physiol. **152**, 1391.

Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced System	Capillary electrophoresis device, including computer, and QIAxcel ScreenGel Software; 1-year warranty on parts and labor	9001941
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QIAxcel DNA Screening Kit (2400)	QIAxcel DNA Screening Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004
QIAxcel DNA Fast Analysis Kit (3000)	QIAxcel DNA Fast Analysis Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX DNA Size Marker 50 bp – 1.5 kb, QX Alignment Marker 15 bp/3 kb, 12-Tube Strips	929008
QIAxcel RNA QC Kit v2.0 (1200)	For 100 runs of 12 samples: QIAxcel RNA Quality Control Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX RNA Alignment Marker, QX RNA Size Marker 200–6000 nt, QX RNA Denaturation Buffer, 12-Tube Strips	929104

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