Application Note

QIAxcel® system — mapping mutant gene loci in *Arabidopsis thaliana*

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In this application note, we describe the assessment of simple sequence length polymorphism (SSLP) and cleaved amplified polymorphisms (CAPS) markers in the mapping of mutant gene loci and the homo/hetero examination of known mutant gene loci using the mutated CAPS or derived CAPS (dCAPS) markers. Both applications utilize the QIAxcel system and the QIAxcel DNA High Resolution Kit, with the OM500 method.

The QIAxcel system demonstrated superior separation capability, enabling the resolution of markers differing by only 4 base pairs in size. It is predicted that the QIAxcel system will be a powerful tool for increasing the speed of mapping and genotyping in the future.

Introduction

There are several methods used in the identification of gene loci responsible for the mutation in *Arabidopsis thaliana*. Of these, mapping of the F_2 populations from crosses between different ecotypes has been a highly effective technique. However, as the entire genomic sequence became available, the increasing numbers of DNA markers made gene cloning much easier and faster using mapping and chromosomal walking. In addition, these DNA markers have often been used to detect polymorphisms of *Arabidopsis thaliana*. For polymorphism studies, we have routinely performed the following experiment types:

- Assessment of SSLP and CAPS markers in the mapping of mutant gene loci
- Homo/hetero examination of known mutant gene loci using the mutated CAPS or dCAPS markers
- Homo/hetero examination of T-DNA insertion in T-DNA insertion mutations

Design of PCR primers for the SSLP and dCAPS markers was extremely challenging, due to the sequence limitation, and the resulting PCR products were small (less than 200 bp). Consequently, the size differences among PCR products or restriction-enzyme–digested fragments are often just a few base pairs. Until recently, polyacrylamide gels or high concentration (2–4%) agarose gels were used to resolve such small size differences between DNA fragments. The introduction of the QIAxcel system has enabled the generation of rapid, reproducible results for these types of analysis.

Materials and methods

Assessment of Col and Ler using the SSLP marker NGA707

The sizes of SSLP marker NGA707 for two ecotypes of Col and Ler are 132 bp and 128 bp, respectively, meaning a difference in size of only 4 bp. Genomic DNA samples from Col, Ler, and a hybrid of Col and Ler cross were extracted using a miniprep system and PCR was performed on a 10 µl scale. PCR products were analyzed using the QIAxcel system together with the QIAxcel DNA High Resolution Kit and the OM500 method.

Hetero/homozygote examination of mutants using dCAPS markers

When base sequence polymorphisms, derived from the mutations of substitution, insertion, or deletion do not generate or modify a restriction enzyme recognition sequence, the PCR primers can be designed to introduce a new restriction enzyme recognition sequence to enable the easy assessment of polymorphisms (dCAPS method). The *gun5-1* mutation is shown as an example (Figure 1 shows the sequence) and has a base substitution (from G to A). PCR products amplified using specially designed, mismatching primers will include the *Eco*T14 I recognition site in the wild type, but not in the *gun5-1* mutant (Figure 1).

Figure 1. Sequence of the *gun5-1* mutant.

Results and Discussion

Assessment of Col and Ler using the SSLP marker NGA707

Figure 2 shows the analysis of the PCR products using the QIAxcel system in conjunction with the QIAxcel DNA High Resolution Kit. In the hybrid (H) sample, the two DNA bands were easily distinguished and the identification of hybrid was clear with no ambiguity. Presence of additional 2 heteroduplex bands confirmed heterozygosity.

Hetero/homozygote examination of mutants using dCAPS markers

The predicted sizes of *Eco*T14 I digested fragments from wild-type and mutant are shown in Figure 3. The *Eco*T14 I digested samples were analyzed on the QIAxcel system after a 5- to 10-fold dilution with water (Figure 4). The gel view of the QIAxcel BioCalculator Software enables easy assessment of wild-type, heterozygote, and mutant — with no ambiguity.

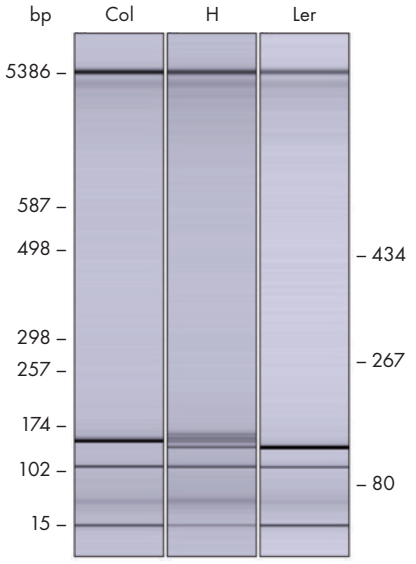

Figure 2. Assessment of ecotypes based on the NGA707 marker.

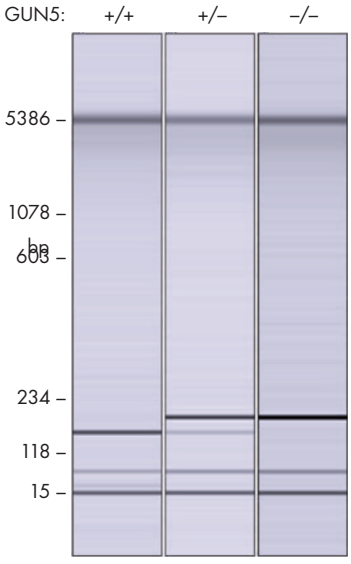

Figure 4. Examination of mutations based on dCAPS.

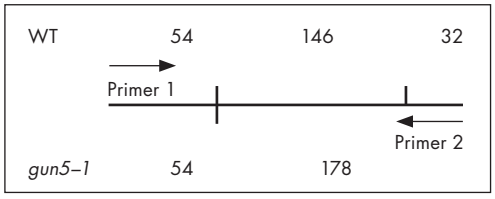

Figure 3. Predicted sizes of restriction digested wild-type and mutant fragments.

Conclusions

Although many DNA markers are recorded in the *Arabidopsis thaliana* database, markers with differences of only several bases pairs may be difficult to analyze. We anticipate that the use of the QIAxcel system, which demonstrates superior separation capability and simplicity, will enable the effective use of these markers and further increase the speed of mapping and genotyping.

References

1. Bell, C.J., Ecker, J.R. (1994) Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. Genomics, **1**, 137.
2. Konieczny, A., Ausubel, F.M. (1993) A procedure for mapping Arabidopsis mutations using co-dominant ecotype-specific PCR-based markers. Plant J. **4**, 403.
3. Neff, M.M., Turk, E., Kalishman, M. (2002) Web-based primer design for single nucleotide polymorphism analysis. Trends Genet, **18**, 6135.

Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced system	Capillary electrophoresis device, including computer, and ScreenGel Software; 1-year warranty on parts and labor	9001941
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002

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