Application Note

QIAxcel® system — linkage analysis of zebrafish mutants

Takashi Akanuma, Okazaki Institute for Integrative Biosciences, National Institutes of Natural Sciences, Okazaki, Japan

In this application note, we describe the transfer of methods based on agarose gel electrophoresis for linkage analysis of zebrafish mutants to the QIAxcel system. The simple sequence length polymorphisms (SSLP) marker 'a' was analyzed. Using the QIAxcel system, we were able to resolve size differences of DNA fragment s down to a few base pairs, a resolution that was not attainable using conventional agarose gel electrophoresis. The QIAxcel system provides significant advantages in determination of genotype for gene linkage studies.

Introduction

To understand the roles genes play in development, there is a need to identify as many of the genes involved in development as possible. Identification can be problematic, since vertebrates are not as amenable to genetic analysis as invertebrates. However, the zebrafish is an excellent genetic system for the study of vertebrate development and disease.

Materials and methods

Linkage studies of zebrafish mutants were performed by using the SSLP marker 'a'.

Genomic DNA was isolated from a zebrafish recessive mutant X heterozygote, wild-type control, TL/India heterozygous mutant, plus normal individuals and homozygous mutants obtained by crossing 2 TL/India heterozygous mutants.

PCR products were separated by electrophoresis on a 3% agarose gel and by electrophoresis using the QIAxcel system together with the QIAxcel DNA High Resolution Kit, and the OM700 method. Genotypes were determined based on the sizes of the amplified products.

Results

PCR products of SSLP marker 'a' for the TL line resolve at 242 bp (Figures 1 and 2; lane 1) and for the India line at 233 bp (Figures 1 and 2; lane 2). In the 3% agarose gel, separation of PCR products derived from the TL line and India line was incomplete (Figure 1; lane 3), and as a result, it was very difficult to assess the genotypes in the F_2 population (Figure 1; lanes 4–9).

By comparison, using the QIAxcel system, PCR products derived from the TL line and India line were clearly separated into 2 bands (Figure 2; lane 3), enabling the simple determination of genotypes in the F_2 population (Figure 2; lanes 4–9).

Based on these results, it is clear that the gene responsible for mutant X is linked to SSLP marker 'a'.These results demonstrated that the QIAxcel capillary electrophoresis system resolves DNA fragments that are very close in size much more clearly than conventional agarose gel electrophoresis.

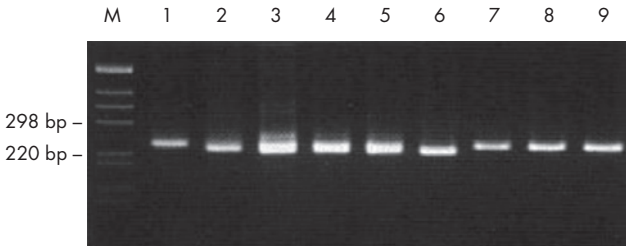

Figure 1. Results using 3% agarose gel. M: Marker; **Lane 1**: TL line (P_0); **Lane 2**: India line (P_0); **Lane 3**: Individual (F_1) obtained from TL x India cross; **Lanes 4, 5, 6**: Normal individual (F_2) of brood; **Lanes 7, 8, 9**: Homozygous mutant (F_2) of brood.

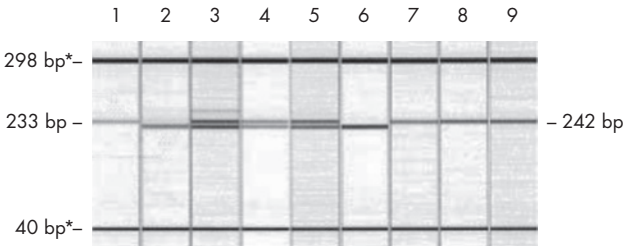

Figure 2. Results using the QIAxcel system with the QIAxcel DNA High Resolution Kit. Lane 1: TL line (P_0); **Lane 2**: India line (P_0); **Lane 3**: Individual (F_1) obtained from TL x India cross; **Lanes 4, 5, 6**: Normal individual (F_2) of brood; **Lanes 7, 8, 9**: Homozygous mutant (F_2) of brood. * Alignment marker.

Conclusions

These results demonstrated that the QIAxcel system can resolve the size difference of DNA fragments down to just a few base pairs, a resolution that was not achievable using conventional agarose gel electrophoresis. The QIAxcel system provides significant advantages in determination of genotype for gene linkage studies.

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

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Visit **www.qiagen.com/linkage-analysis** and find out how automated gel electrophoresis can benefit your lab!