Application Note

QIAxcel® analysis of CTG trinucleotide repeats

Natasa Teran[1] and Mirjana Kozulic[2]

[1] Division of Medical Genetics, University Medical Centre Ljubljana, Ljubljana, Slovenia

[2] QIAGEN Instruments AG, Hombrechtikon, Switzerland

The QIAxcel system was used to identify the number of CTG trinucleotide repeats present in the myotonin protein kinase (DMPK) gene in a group of individuals with cataracts.

Introduction

Myotonic dystrophy (DM1) is a multisystemic disease associated with myotonia, disabling distal weakness, severe cognitive problems, and other symptoms. Severity and range of symptoms vary according to the type of gene defect and the number of affected alleles (1). DM1 is inherited as an autosomal dominant trait and occurs at a rate of 5 in 100,000 worldwide. The affected gene, *DMPK*, encodes myotonin protein kinase (1). DM1 patients were shown to carry an increased number of CTG trinucleotide repeats in the *DMPK* gene (2). An increase of 50–80 repeats in the *DMPK* gene is designated "protomutation". Persons carrying protomutations exhibit only minor muscular symptoms and often have cataracts (3). A further increase in the number of repeats correlates roughly with the severity of DM1 symptoms (4). Since DM1 reduces reproductive fitness severely and no de novo mutations appear to occur, the disease should, in principle, disappear from the population. However, phenotypically healthy individuals may carry a *DMPK* protomutation and, thus, comprise a genetic pool for DM1 defects. To test this hypothesis, DNA samples from 270 individuals with cataracts (and no other DM1 symptoms) were evaluated for the number of CTG repeats present in *DMPK* alleles.

Materials and methods

PCR analysis was performed as described in (5). The amplification mixture contained 5 ng of genomic DNA, primers 101 and 102 (1), and AmpliTaq Gold® *Taq* DNA Polymerase (Roche MolecularSystems). Fragments were amplified using the "touchdown PCR" method described in (6).

Samples were analysed using the QIAxcel DNA High Resolution Kit on the QIAxcel system with the 0M700 method. The QX Alignment Marker 50 bp/500 bp and the QX DNA Size Marker 25–450 bp were included in the run.

Results

The QIAxcel system processes samples in batches of 12 and allows analysis of up to 96 samples without manual intervention. Results can be displayed as gel-like and electropherogram images as well as a table listing up to 12 parameters. PCR-amplified *DMPK* fragments were resolved using the QIAxcel DNA High Resolution Kit. Precise determination of repeat number was simplified using the BioCalculator software. ▷

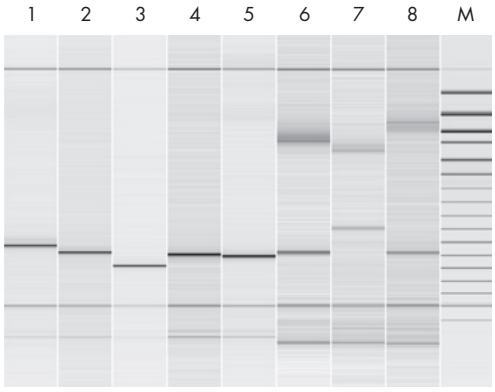

Figure 1. Determining the number of CTG trinucleotide repeats in the *DMPK* gene. Sequences containing CTG-repeats were amplified and PCR products were resolved using the QIAxcel DNA High Resolution Kit. Numbers of repeats present were **1**: 17, **2**: 13, **3**: 5, **4**: 12, **5**: 11, **6**: 13 and 19, **7**: 29 and 74, **8**: 13 and 93. **M**: QX DNA Size Marker 25–450 bp.

References

1. Brook, J.D. et al. (1992) Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3' end of a transcript encoding a protein kinase family member. Cell **68**, 799.
2. Monckton, D.G. et al. (1995) Somatic mosaicism, germline expansions, germline reversions and intergenerational reductions in myotonic dystrophy males: small pool PCR analyses. Hum. Mol. Genet. **1995**, 1.
3. Barcelo, J.M. et al. (1993) Intergenerational stability of the myotonic dystrophy protomutation. Hum. Mol. Genet. **2**, 705.
4. Harley, H.G. et al. (1993) Size of the unstable CTG repeat sequence in relation to phenotype and parental transmission in myontonic dystrophy. Am. J. Hum. Genet. **52**, 1164.
5. Kunej, T. et al. (2004) CTG amplification in the DM1PK gene is not associated with idiopathic male subfertility. Hum. Reprod. **19**, 2084.
6. Medica, I. et al. (2007) Patients with primary cataract as a genetic pool of DMPK protomutation. J. Hum. Genet. **52**, 123.

Size differences due to different numbers of trinucleotide repeats were easily distinguished for fragments in the desired size range (Figure 1). Normal control homozygous samples with 5–37 CTG repeats (lanes 1–5) were analyzed with heterozygous samples exhibiting protomutations (50–80 CTG repeats, lanes 6 and 7) and a heterozygous sample with a mutation (>80 CTG repeats, lane 8).

Conclusions

- Accurate determination of the number of CTG-repeats in the *DMPK* gene is crucial for DM1 biomedical research. Since the number of repeats increases during gametogenesis (6), the identification of aberrant CTG repeats also enables molecular genetic research of DM1.
- Our results support the initial hypothesis that individuals with primary cataracts constitute a genetic pool in which the *DMPK* protomutation is present.
- The QIAxcel DNA High Resolution Kit enabled the resolution of amplified DNA fragments more effectively than agarose or polyacrylamide gels (data not shown). The QIAxcel system outperforms these traditional methods with greater resolution, improved sensitivity, and increased speed. In addition, more detailed information was provided by the QIAxcel system.
- Results acquired using the QIAxcel system are fully reproducible due to controlled running conditions. Automatic sample analysis and automated data recording ensures reliability and data safety.

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/qiaxcel-genotyping** and find out how automated gel electrophoresis can benefit your lab!