Evaluation of a Single-Tube, Long-Read, Two-Mode PCR Technology that Reports the Categorical Range of DMPK CTG and Can Resolve up to 1900 Repeats in Myotonic Dystrophy Type 1

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Summary

- Triplet repeat CTG expansions in the DMPK gene cause Myotonic Dystrophy Type 1.
- Current laboratory workflows require PCR and Southern blot analysis to adjudicate DM1 repeat status.
- Asuragen developed a single-tube, long-read, two-mode PCR/CE assay based on AmpliDx technology that resolves ≥200 CTG repeats and flags larger expanded alleles by both stutter pattern and pile-up peak.

Introduction

Myotonic Dystrophy is the most common adult-onset muscular dystrophy, and the most severe form is type 1 (DM1). The autosomal dominant disease is caused by a pathogenic CTG expansion in the 3' UTR of the DMPK protein kinase gene (DMPK). In the absence of family history, early DM1 symptoms are typically mild, overlapped with other disorders (e.g., baldness, myalgia, cataracts) which leads to under- or misdiagnosis of patients. With the introduction of molecular testing, disease prevalence could be as high as ~1:1000.

In clinical labs, DM1 molecular testing requires both PCR and Southern blot (SB) analysis because most laboratory-developed PCR tests (LDTs) cannot consistently amplify >100 repeats, which is the threshold for the classical disease category. Not only does SB take approximately one week, but due to expansion allele dropout by sizing PCR, expansion detection must often be performed by an independent PCR or other assay. Here, we describe a method was developed for size estimation and categorical genotyping for DM1.

Materials and Methods

Presumed normal specimens (27) were selected (Asuragen, Site 1). Residual clinical specimens (67) were selected with genotypes independently determined using PCR and/or SB at Greenwood Genetic Center (GGC, Site 2). Positive control reference cell lines from Coriell Cell Repository (CCR) (n=7 as described in Kalman et al. and a) and a calibrator were used at both sites. Specimens covered each of the clinical categories defined by the CTG repeat number (Figure 1). Genomic DNA was amplified using primer Ampliseq AmpliDx PCR/CE DMPK Kit (RUO) reactions that were identical to final product formulations. FAM-labeled amplicons were resolved by CE (Thermo Fisher), peaks were called using GeneMapper. Called peaks (bp) were converted to repeat size and category by the DMPK Macro using a calibrator at each site. Expansions larger than 50 repeats were resolved by AGE.

Results

- PCR and CE were >98% concordant within ±10% sizing alleles ≤200 CTGs. The Kit was >98% concordant within ±10% sizing alleles >200 CTGs (60) were expanded in the reference. Four additional mosaic alleles (#2-05, #2-08) with mosaic alleles in the 1000-2000 category were identified.

Conclusions

- We report the first single-tube, long-read PCR technology that can resolve DMPK zygosity and genotype alleles across the categorical range of repeat expansions.
- The PCR/CE assay showed 100% agreement in identifying expansions with >50 CTGs, differentiating normal, mosaic and expanded samples, and flagging larger expansions across all 67 samples. The assay also demonstrated >98% concordance for sizing alleles up to 200 repeats within 10% of reference.
- The PCR/AGE assay demonstrated good categorical agreement to SB analysis up to 1000 repeats (85%) were sized within 10% of reference, however the smeared bands sometimes appeared more diffuse than on SB.
- The largest allele detected and sized by PCR/AGE in this study was 1900 repeats.

This PCR-based workflow could potentially reduce the labor and turnaround time from sample to answer to compare to SB analysis. For samples with >1900 repeats and for any sample with an unresolved AGE smear, SB may be required if sizing is necessary.

References


Figure 1. DMPK Gene Map and Correlation of Phenotype and CTG Repeat Length in DM1 with Expanding Alleles. A) DM1 is caused by an expansion of the trinucleotide repeat CTG in the 3’UTR of DMPK located at Chr 19p13.3. B) The assay consists of two gene-specific (G) primers (FAM-label outer primer) and one triplet repeat primer (TP) (Thermo Fisher), anywhere in the repeat region creating a stutter repeat pattern (RP). The assay produces two complementary data outputs in a single test: CTG amplicon provides sizing of expanded and normal alleles (CE and AGE), whereas short and long alleles are enumerated or flagged by RP (CE only). E) The standardized workflow can be performed within a 7 hr workshift for either PCR/CE or 24 samples with per injection run times) or PCR/AGE (≤1 samples and ≤14 injection run times. Expanded repeat stutter pattern differences are <1 bp. Correlation of phenotype to genotype adapted from Bird et al. A) AGC categorical guidelines.

Figure 2. The AmpliDx PCR/CE DMPK Kit Resolves Zygosity in Normal and Expanded Samples. Phenotypes matched within precision between sites after calibration. Reference information are provided at the top of each track. A) Normal and expanded expanded samples were identified as 3 CTG peaks. Expanded repeat stutter pattern differences expanded. B) Specimens containing >200 CTG repeats also manifested an aggregated “pile-up” peak at ≥500 bp.

Figure 3. The AmpliDx PCR/CE DMPK Kit Identified Sample Genotypes (allele sizes and categorical description) with >98% Concordance Compared to an Orthogonal Method for all 94 Specimens. A) Precision between sites for positive controls were within guidelines described in the Kit Protocol Guide. B) Accuracy of differentiating expanded specimens (>50 CTGs) using each assay compared in a 2:2 contingency table shows 100% agreement. C) The assay was 100% concordant with >1000 CTGs.

Figure 4. PCR/AGE and SB Comparison for 10 Representative Specimens. Sizing of repeat expansions using PCR/AGE were consistent with SB for clinical specimens with ≥100, 500, 1000, 500, 500 in and some cases could not easily be interpreted by AGE (5-0-5, 5-0-5).

Figure 5. PCR/AGE vs SB Agreement with Reference SB Method for 59 specimens with >50 CTGs. Mosaic expanded alleles observed by SB were more pronounced in PCR/AGE results without a blue (PCR/AGE) data point are hidden by the overlapping SB data point. Red dot indicates samples in Figure 4. Category (<1000 CTGs) 2:27 samples (35%) sized by PCR/AGE were within 10% of the reference, 1 was >1000 CTGs. In the 1000-2000 CTGs, 15/15 samples (100%) were similar to the reference. In the 2000-2500 CTGs, 14/15 samples (93%) were similar to the reference. Overall agreement was 124/(0+124) = 100%..

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