# Clinical validation and implementation of a highly efficient and sensitive dual molecular diagnostic assay for myotonic dystrophy type 1

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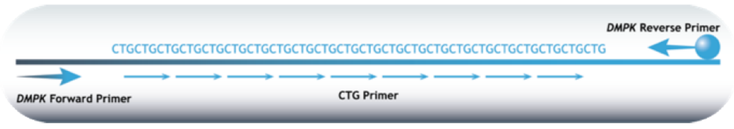
## Abstract

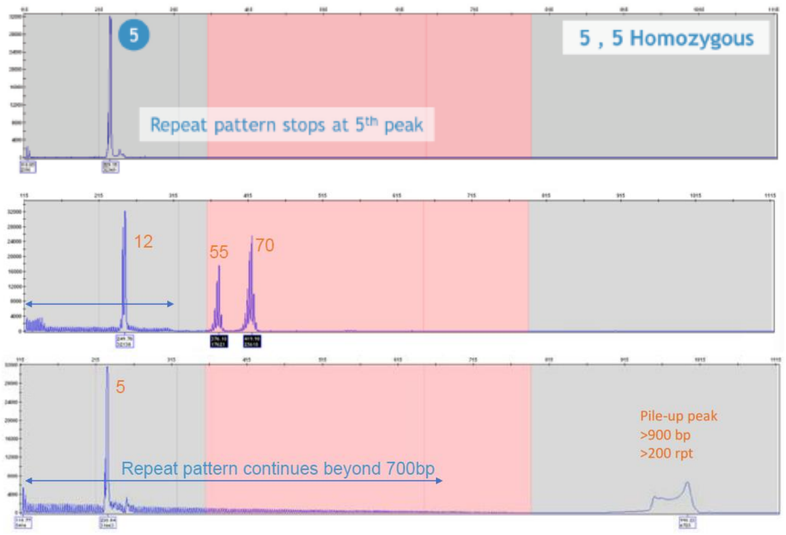
Myotonic dystrophy type 1 (DM1) is caused by a $(CTG)_n$ repeat expansion in the 3' UTR of *DMPK* and is an important and often overlooked consideration in the work up of a hypotonic infant. $(CTG)_n$ repeats are refractory to detection by short read sequencing and therefore, require other specialized methods for accurate quantification including PCR and Southern blot analysis. Here, we describe the clinical validation and implementation of a new commercially available PCR assay that overcomes these specialized methods. Triplet-primed PCR was performed on all samples and resolved using capillary electrophoresis. The assay generated numerical values for alleles up to and including 200 repeats and a categorical value for alleles >200 repeats to facilitate genotyping. Size estimation beyond 200 repeats were flagged by both an expanded stutter pattern and a corresponding pile-up peak. Larger expansions were resolved using an agarose gel electrophoresis method. Samples were selected to provide multiple representatives in each numerical category throughout the dynamic range which included the normal (5-34 repeats), premutation (35-49 repeats), and various disease ranges (>50 repeats). We observed 100% concordance between our results for this sample set and previously reported results with 100% sensitivity, specificity, accuracy, and precision. In addition, we were able to clearly resolve zygosity in all samples. Mosaicism of at least 10% was detectable. Another major advantage of this testing is a total hands-on time requirement of only 60 minutes, a significant improvement over dual PCR / Southern blot methods. Overall, this assay resulted in a faster, accurate, and cost-effective approach for reaching a DM1 molecular diagnosis and highlights the continuing advances in the molecular diagnostic testing space.

## Technique Overview


### Triplet Primed (TP) PCR

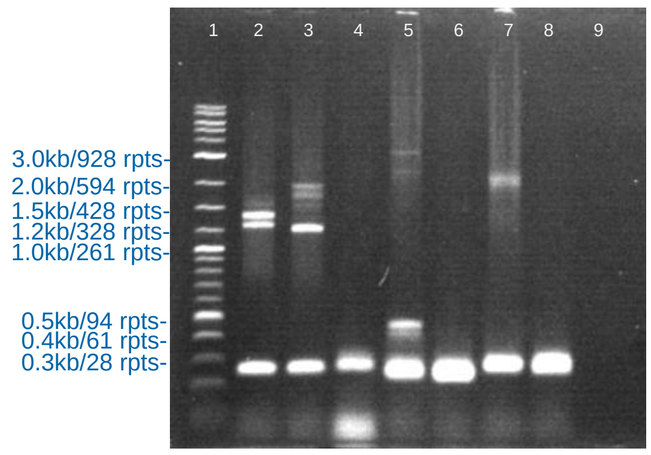



Capillary electrophoresis




### Gene Specific (GS) PCR


Agarose gel electrophoresis




The workflow of the test is streamlined and can be performed within 7 hours with a total hands-on time of 1 hour for each workflow. First samples undergo TP PCR and resolved using capillary electrophoresis. A maximum of 200 repeats can be detected and zygosity can be resolved using this method. In addition, detection of mosaicism can be clearly identified. However, samples with greater than 200 repeats can be resolved by gene specific PCR and agaroase gel electrophoresis. Sample on gel are as followed: Lane 1: ladder, Lane 2: 350 – 428 repeats; Lane 3: 328 – 590 repeats; Lane 4: > 2000 repeats (positive TP PCR, with wild type allele amplification, but no larger band) Lane 5: Mosaic, 61 – 90 repeats and 594 – 928 repeats; Lane 6: homozygous 5 repeats; Lane 7: 594 – 928 repeats; Lane 8: heterozygous 12 and 13 repeats; Lane 9: Blank.

## Sample Summary

| Sample | External Lab PCR + Southern | PCR-CE/AGE (repeats) |
|---|---|---|
| 1 | 12; 396 | 12; >200 / 350-428 |
| 2 | 12; 406 | 12; >200 / 328-590 |
| 3 | 12; 380 | 12;>200 / 350-428 |
| 4 | 12; >2000 | 12; >200 / not visualized |
| 5 | 11; 13 | 11; 13 / not applicable |
| 6 | 28; >150 | 200; >200 / not applicable |
| 7 | 5, 12; 55, 70, >200 | 5, 12; 55, 70, >200 / 60-90, 594-928 |
| 8 | 10; 16 | 10; 16 / not applicable |
| 9 | 5; 21 | 5; 21 / not applicable |
| 10 | 13; 19 | 13; 20 / not applicable |
| 11 | 13; 25 | 13; 25 / not applicable |
| 12 | 5; 5 | 5; 5 / <28 |
| 13 | 5; 5 | 5; 5 / <28 |
| 14 | 14; >600-800 | 14; >200 / 594-928 |
| 15 | 5; >600-800 | 5; >200 / 721 |
| 16 | 11; >150 | 11; >200 / not applicable |
| 17 | 12; 13 | 12;13 / <28 |
| 18 | 5; >150 | 5; >200 / not applicable |
| 19 | 11; 12 | 11; 12 / not applicable |
| 20 | 5; 152 | 5; 124, 151, 185, >200 / 127-268 |
| 21 | 5; 13 | 5; 13 / not applicable |
| 22 | 12; 13 | 12; 13 / not applicable |
| 23 | 12; 14 | 12; 14 / not applicable |

## Validation Summary

| Sample | n | PCR-CE/AGE (n) |
|---|---|---|
| Negative | 12 | 12/3 |
| Positive | 11 | 11/8 |

| Metrics | Concordant PCR-CE | Concordant PCR-AGE | Percent |
|---|---|---|---|
| Sensitivity | 11/11 | 8/8 | 100% |
| Specificity | 12/12 | 3/3 | 100% |
| Accuracy | 23/23 | 11/11 | 100% |
| Precision | 7/7 (3x) | 4/4 (3x) | 100% |
| Analytical Sensitivity | 3/3 | 3/3 | 10% |

## Current Diagnostic Rate

| Sample | n | Percent |
|---|---|---|
| Negative | 3 | 42.9% |
| Positive | 4 | 57.1% |

## Conclusions

- We observed 100% concordance for DMPK status for all of the samples tested with 100% sensitivity, specificity, accuracy, and precision
- We mixed two positive DMPK samples at 50%, 25%, and 10%. All of which were detected by both PCR-CE/AGE indicating a lower limit of detection of at least 10%
- A positive sample from start to finish was completed in 8 hours, which is a significantly reduced turn around time relative to combined PCR and Southern blotting.
- Our laboratory has received a total of 7 samples for DMPK testing (May 2019) and 57.1% have tested positive

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Asuragen

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