DEVELOPMENT OF A STREAMLINED MOLECULAR ASSAY THAT DETERMINES BOTH ALLELE AND EXPANDED REPEAT SIZE IN *DMPK* FOR MYOTONIC DYSTROPHY 1

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SUMMARY

- Triplet repeat CTG expansions in the *DMPK* gene are causative for Myotonic Dystrophy Type 1 (DM1).
- Current laboratory workflows require both PCR and Southern blot (SB) analysis to adjudicate DM1 repeat status.
- We developed a single-tube assay based on AmplideX® PCR technology that resolves up to 200 CTG repeats and flags larger expanded alleles without the need for SB analysis.
- The prototype DM1 PCR/Capillary Electrophoresis (CE) assay unifies gene-specific and repeat-primer designs in a streamlined procedure to generate sensitive and specific results across all categories of expanded alleles.

INTRODUCTION

Myotonic dystrophy type 1 (DM1) is an autosomal dominant disease characterized by >50 CTG repeats in the 3′ UTR of *DMPK*[1]. In the absence of family history, early DM1 symptoms are typically mild, overlapping with other disorders (e.g. baldness, myalgia, cataracts) and leading to under- or misdiagnosis of patients[1]. The introduction of molecular testing suggests a disease prevalence as high as ~1:2000[2]. DM1 testing currently requires a combination of two independent PCR reactions and SB analysis that can take several days. PCR alone typically fails to amplify expansions >100 repeats, and allele drop-outs are indistinguishable from homozygous samples[3]. The method and results described here enlist Asuragen's AmplideX PCR technology to enable accurate sizing of *DMPK* alleles with resolution up to 200 repeats in a single-tube assay.

MATERIALS AND METHODS

Genomic DNA (gDNA) was isolated from 14 matched buccal and whole blood samples of presumed healthy donors (Asuragen) using QIAamp DNA Blood Mini kit (Qiagen). Cell-line materials included samples from the Coriell repository (16 DM1-positive including 10 previously verified genotypes[3] and 2 normals) and 11 additional normal cell-lines (Asuragen). gDNA (40 ng) was amplified using prototype PCR reagents (Asuragen) by two different operators on different days. FAM-labeled amplicons were resolved on a 3500xL Genetic Analyzer (Thermo Fisher Scientific) CE instrument using POP-7™ polymer, with 2.5 kV, 10 sec injection and 40 min run time. Genotyping was achieved following analysis using a ROX 1000 size ladder (Asuragen) and a four-point calibrator.

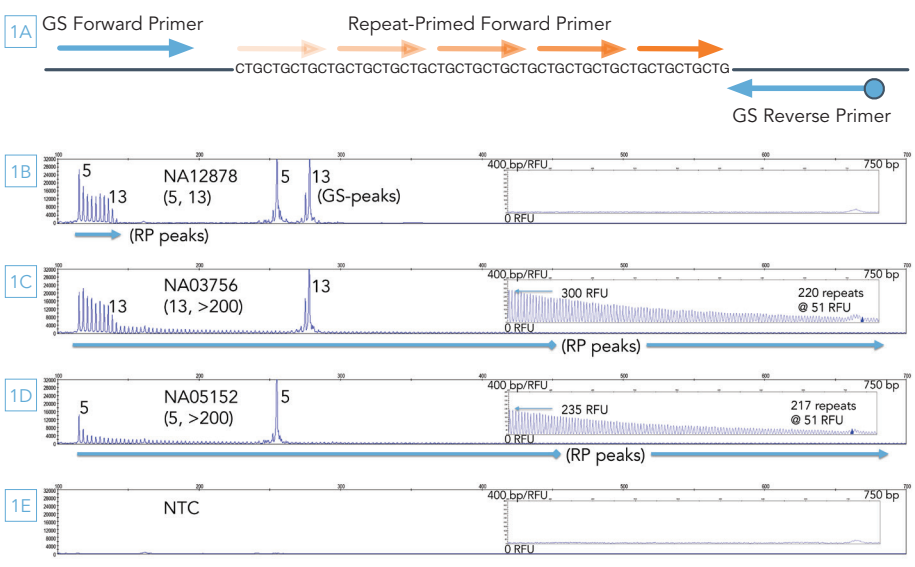

Figure 1. Single-tube, three-primer *DMPK* PCR/CE assay design and representative data. (A) The prototype assay consists of two gene-specific primers where the reverse primer is labeled with a FAM, and one repeat primer that can hybridize and prime anywhere in the repeat region. The assay produces two complementary data outputs in a single-tube: gene-specific (GS) peaks provide sizing of normal and expanded alleles up to 200 CTG, whereas short and long alleles are either enumerated or flagged by repeat-primed (RP) peaks. **(B)** Normal sample where both GS and RP peak profiles agree that the sample contains alleles of 5 and 13 repeats. **(C and D)** Expanded samples display one normal allele with sizing based on GS and RP peak profile agreement, and an expansion with >200 repeats based on the RP peak profile. Also unique to expansions >200 is a peak buildup at ~1030bp (data not shown).

RESULTS

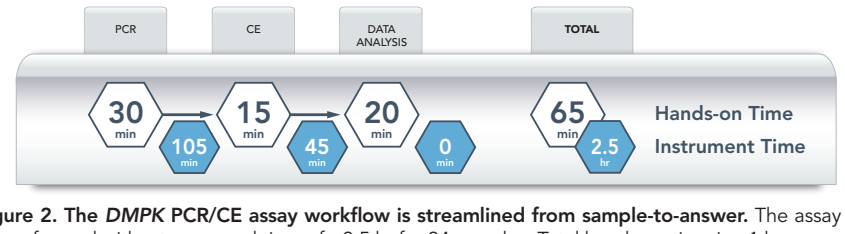

Figure 2. The *DMPK* PCR/CE assay workflow is streamlined from sample-to-answer. The assay can be performed with a turnaround time of ~3.5 hr for 24 samples. Total hands-on time is ~1 hr.

*Research Use Only – Not For Use In Diagnostic Procedures
*Preliminary research data. The full performance characteristics of this assay have not yet been established.
Presented at ESHG 2017 - P10.40D

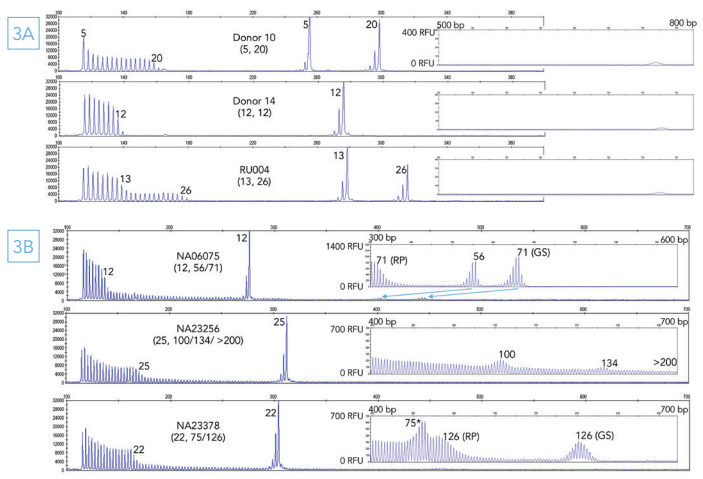

Figure 3. CE traces display clear differentiation of normal and expanded alleles while simultaneously identifying repeat mosaicism. (A) Normal alleles have a characteristic drop in the RP profile whereas **(B)** expanded alleles have a characteristic expansion of the RP profile and identify mosaicism based on the GS profile where previously reported assays could not (*)[3,4]. Repeat counts are provided above the dominant peak with an inset of the zoomed trace.

Table 1. The assay demonstrates repeatability across different operators and days. For Coriell samples, the RP strategy flagged all expanded samples within 1 repeat unit for all samples tested in both runs except NA03991, which agreed within 2 units. Samples were concordant with published literature[3,4] and in two cases identified additional minor alleles not previously observed (*). For whole blood, all samples were successfully genotyped with *DMPK* PCR/CE reagents with identical results between replicates and with buccal samples from the same individual; repeat sizes ranged from 5 to 26 CTGs within an average of 0.02 ±0.20 repeats between runs. There were 5/25 (20%) homozygous samples, consistent with published screening studies[5]. No assay failures were observed.

Coriell DNA			Kalman[3]	Lian[4]	Run 1		Run 2		Concordant
Fibroblast ID	gDNA ID	Genotype	Allele 1, 2	Allele 1, 2	Allele 1	Allele 2	Allele 1	Allele 2	
GM03132	NA03132	<2000	5, 2078 ±217		5	>200	5	>200	Yes
GM03696	NA03696	<1000	12, 697 ±13	12, >165/ >180*	12	>200	12	>200	Yes
GM03697	NA03697	<500	12, 412 ±33		12	>200	12	>200	Yes
GM03756	NA03756	<500			13	>200	13	>200	N/A
GM03759	NA03759	?/2000			14	>200	14	>200	N/A
GM03991	NA03991	?/~50-80			14	110	14	108	N/A
GM04567	NA04567	700	21, 637 ±33	21, >180	21	>200	21	>200	Yes
GM04648	NA04648	1000	5, 1008 ±49	5, >180	5	>200	5	>200	Yes
GM05152	NA05152	1500	5, 1621 ±30		5	>200	5	>200	Yes
GM05164	NA05164	340	22, 377 ±53	21, >180	21	>200	21	>200	Yes
GM06075	NA06075	66	12, 55, 70 ±0.9	12, 55, 71	12	56, 71	12	56, 71	Yes
GM12878	NA12878	Normal			5	13	5	13	N/A
GM23256	NA23256	160-170			25	101, 135, >200	25	100, 134, >200	N/A
GM23265	NA23265	50-60	12, 75	12, 76-77	12	63*, 75	12	63*, 75	Yes+
GM23299	NA23299	90-100			22	>200	22	>200	N/A
GM23300	NA23300	150-160		5, >180	5	>200	5	>200	Yes
GM23376	NA23376	*CNBP* exp.			20	22	20	22	N/A
GM23378	NA23378	80-90	22, 138 ±5	22, 129-132	22	75*, 126	22	75*, 126	Yes+

Blood and Buccal	Run 1		Run 2	
	Allele 1	Allele 2	Allele 1	Allele 2
Donor 1	5	Homozygous	5	Homozygous
Donor 2	5	Homozygous	5	Homozygous
Donor 3	11	22	11	22
Donor 4	12	13	12	13
Donor 5	10	14	10	14
Donor 6	11	12	11	12
Donor 7	13	14	13	14
Donor 8	12	21	12	21
Donor 9	13	14	13	14
Donor 10	5	20	5	20
Donor 11	5	12	5	12
Donor 12	5	11	5	11
Donor 13	5	11	5	11
Donor 14	12	Homozygous	12	Homozygous

Cell Line gDNA	Run 1		Run 2	
	Allele 1	Allele 2	Allele 1	Allele 2
RU001	12	14	12	14
RU002	11	Homozygous	11	Homozygous
RU003	5	Homozygous	5	Homozygous
RU004	13	26	13	26
RU005	11	13	11	13
RU006	5	12	5	12
RU007	6	12	6	12
RU008	5	13	5	13
RU009	5	13	5	13
RU010	12	13	12	13
RU011	5	13	5	13

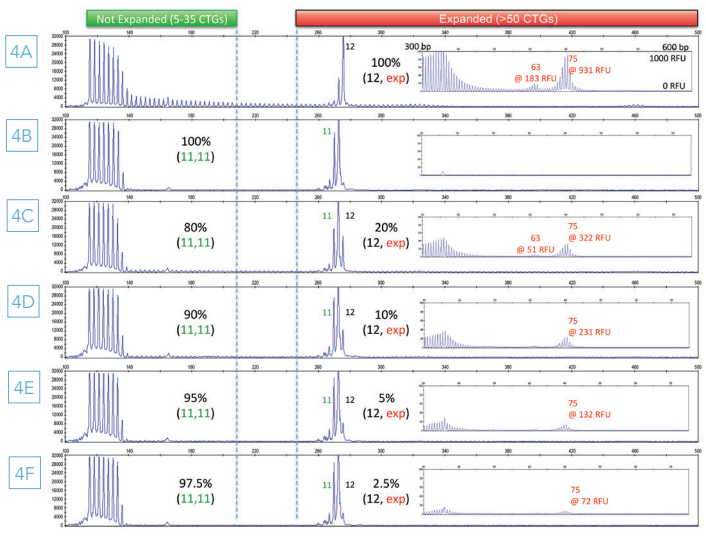

Figure 4. Three-primer *DMPK* PCR report ≥2.5% mass fraction of an expanded sample in the background of 97.5% of an unexpanded sample. A series of admixtures **(C-F)** were created to evaluate the assay's analytical sensitivity. An expanded gDNA (**A**; NA23265; 12, 63/75) was titrated to 2.5% mass fraction of an unexpanded gDNA (**B**; RU002; 11/11). The expanded allele was detected at the 2.5% level (72 RFU).

CONCLUSIONS

- We report the first single-tube, long-read PCR technology that can resolve *DMPK* zygosity, genotype alleles up to 200 repeats, and flag large expansions with ≥2000 repeats.
- The method sizes expanded alleles from the equivalent of 2.5% of cells, shows between run repeatability within 1-2 repeat units, and is applicable to both blood and buccal specimens.
- The PCR/CE assay requires ~1 hr of hands-on time for a 24-sample batch and significantly reduces the need for SB analysis by accurately differentiating unexpanded and expanded alleles, including those that may be associated with DM1 severity.

References

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3. Kalman, L., *et al.* (2013) *Journal of Molecular Diagnostics* 15(4):518–525.
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5. Prior, T. W. (2009) *Genetics in Medicine* 11(7):552–555.

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