Two-site Evaluation of a One-tube PCR/CE Assay that Resolves CAG Length Polymorphisms in Exon 1 of the *HTT* Gene

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Summary

- Huntington disease (HD) is a slowly progressive, hereditary fatal brain disease caused by a CAG expansion in *HTT* Exon 1; *HTT* genotyping is challenged by known rare polymorphisms surrounding the CAG repeat region that can cause allelic dropout.
- We present the evaluation of a streamlined, single-tube AmplideX® PCR/CE assay† for the amplification of CAG repeat polymorphisms in Exon 1 of the *HTT* gene across two sites (Site 1: Asuragen, Site 2: University of Pennsylvania).
- Both sites successfully genotyped all repeat alleles in all samples. Expansions with <200 repeats were resolved well within recommended precision limits of CAG repeat sizing accuracy, and alleles with >200 repeats were reliably flagged.
- In a cohort of 37 samples, Site 2 achieved 100% concordance with Site 1 and an external reference method (University of Iowa) across all repeat categories and genotypes.

Introduction

Huntington disease (HD) is a progressive brain disorder caused by expansion of an unstable, trinucleotide CAG or poly-Q repeat in exon 1 of the huntingtin (*HTT*) gene. Accurate determination of CAG repeats is critical for molecular diagnosis of HD. HD CAG expansion is typically identified using PCR-based methods. Polymorphisms near the CAG tract can lead to mis-priming and inaccuracies in allele sizing. Here we describe a robust, rapid and accurate PCR assay† evaluated at two sites that can resolve *HTT* zygosity and enable accurate repeat quantification even in the presence of known gene polymorphisms.

Materials and Methods

A prototype assay† was evaluated at Asuragen (Site 1) and the University of Pennsylvania (Site 2). Both sites evaluated a common set of cell-line samples and NIST controls[1] that covered a range from 15 to 250 CAGs. Each site also assessed peripheral blood samples independently. Site 2 evaluated an additional 19 expanded samples obtained from the University of Iowa previously genotyped using a reference method comparing the labeled CAG-containing PCR products to ddA and ddT sequencing products utilizing 7% denaturing acrylamide sequencing gels. Additionally, Ultramer® DNA Oligonucleotides (IDT) were synthesized as controls containing well-characterized *HTT* SNPs between the polymorphic CAG and CCG regions associated with allele dropout in other PCR-based detection methods. Sample gDNA and/or Ultramers were PCR amplified using AmplideX PCR technology and amplicons were resolved by capillary electrophoresis (CE) on either a 3130*xl* or 3500xL Genetic Analyzer (Thermo Fisher). Genotypes were determined from the mobility of target peaks relative to a calibration curve.

Figure 1. *HTT* Gene and Risk Alleles. A) CAG repeat length and associated HD boundary categories: Normal allele (≤26 CAGs), Intermediate (27-35 CAGs), Reduced Penetrance (36-39 CAGs), Expanded (≥40 CAGs)[2]. **B)** Diagram of the *HTT* gene located in exon 1 with the outlined location of the polymorphic CAG and CCG regions with the interjecting 12-bp spacer sequence.

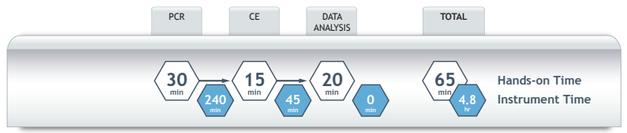

Figure 2. Time-motion Analysis of the AmplideX PCR/CE *HTT* Assay† Workflow. Total hands on time is ~65 min for 24 samples with one CE injection on the 3500xL Genetic Analyzer.

*Equal contribution authors

†This product is under development. Specifications are not finalized.

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Results

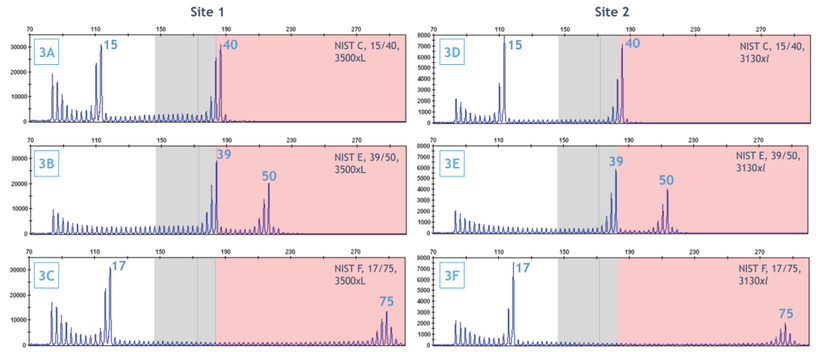

Figure 3. Accurate Genotyping of CAG Repeat Length in NIST *HTT* Disease Controls Across 2 Sites. 6 NIST controls (SRM 2393) at 16-24 ng per reaction were used in the prototype *HTT* PCR/CE assay† across both sites. Representative electropherograms are presented for **A)** Control C 15/40 [site 1], **B)** Control E 39/50 [site 1], **C)** Control F 17/75 [site 1], **D)** Control C 15/40 [site 2], **E)** Control E 39/50 [site 2] and **F)** Control F 17/75 [site 2].

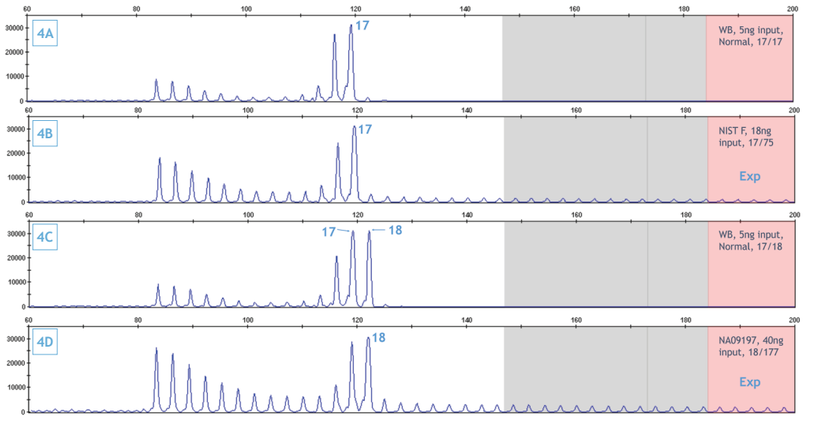

Figure 4. Zygosity Resolution. The prototype assay can determine allele sizing across a variety of sample types, inputs and zygosity: **A)** Whole Blood (WB) homozygous sample 17/17, **B)** cell line NIST F 17/75 sample or 17/exp, **C)** WB heterozygous sample 17/18, and **D)** cell line NA09197 (Coriell) sample 18/177 or 18/exp. 3500xL Site 1 data is shown.

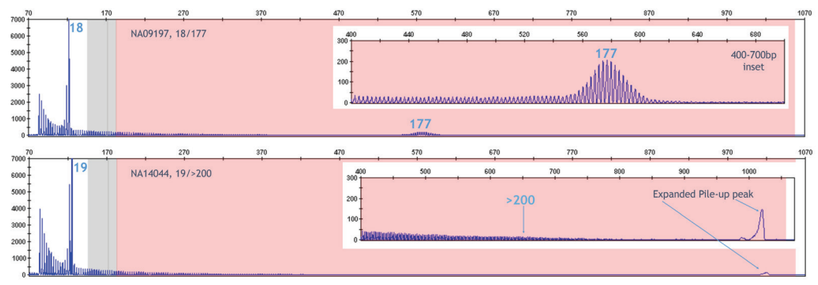

Figure 5. Resolution of Large Repeat Expansions. NA09197 and NA14044 (Coriell), highly expanded samples, can easily be resolved (NA09197) or flagged as > 200 repeats (NA14044) across both sites. Site 2 chromatograms on 3130*xl* are shown.

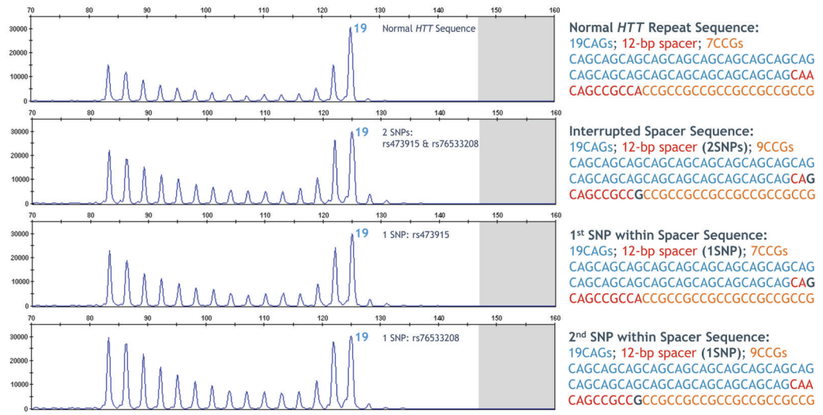

Figure 6. Accurate Genotyping of Synthetic Sequences Containing Well-characterized *HTT* SNPs. Ultramer DNA Oligonucleotides (IDT) were synthesized with well-characterized *HTT* SNPs located between the polymorphic CAG and the non-clinically relevant CCG repeat regions as outlined in the sequences to the right of the corresponding electropherograms. Site 1 data run on the 3500xL is shown.

		Normal	Intermediate	Reduced Penetrance	Expanded	Categorical Concordance	Allele Genotype Concordance
HTT Genotype, Prototype Assay†	Normal	10	-	-	-	10/10 (100%)	20/20 (100%)
	Intermediate	-	2	-	-	2/2 (100%)	4/4 (100%)
	Reduced Penetrance	-	-	2	-	2/2 (100%)	4/4 (100%)
	Expanded	-	-	-	23	23/23 (100%)	46/46 (100%)

Table 1. Call Concordance Across 37 Samples. Site 2 achieved 100% concordance with Site 1 results within ACMG precision guidelines[2] using the prototype *HTT* PCR/CE assay†. Genotypes were compared to an external reference method (University of Iowa) across both repeat length category and genotype.

Conclusions

- A rapid, single-tube *HTT* PCR/CE assay† was successfully evaluated at two laboratories.
- A total of 37 Normal, Intermediate, Reduced Penetrance and Expanded samples were accurately genotyped at high resolution at both sites.
- The assay flagged very large expansions (>200 CAGs), resolved heterozygous and homozygous samples, and accommodated polymorphic regions and known SNPs.
- This assay has potential to remedy concerns of false-negative results with some existing *HTT* technologies, and streamline clinical research in Huntington's disease.

References

1. Kalman, L. et al. Development of genomic reference materials for Huntington disease genetic testing. Genetics in Medicine; 9, 719-723 (2007).
2. Bean, L. et al. American College of Medical Genetics and Genomics Standards and Guidelines for Clinical Genetics Laboratories, 2014 editions: technical standards and guidelines for Huntington disease. Genetics in Medicine; 16, e2 (2014).

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