Evaluation of the AmplideX® PCR/CE *HTT* Kit for the Rapid and Accurate Genotyping of CAG Repeat Expansions

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Summary

- Huntington Disease (HD) is a neurodegenerative condition caused by CAG expansions in *HTT* exon 1 and is characterized by a functional decline in motor and cognitive skills.
- We present the performance of the AmplideX® PCR/CE *HTT* Kit*, a single-tube assay that accurately resolves and quantifies CAG repeat expansions in approximately 6 hours or less.
- The data show 100% concordance between the AmplideX PCR/CE *HTT* Kit and a lab-developed test at JHACH, with the precision of the AmplideX assay improving on the recommended ACMG sizing accuracy.

Introduction

Huntington Disease (HD) is an autosomal dominant condition that is caused by an unstable expansion of CAG repeats in exon 1 of the *HTT* gene. HD affects ~1 in 10,000 individuals. The manifestation of this disease leads to a decline of cognitive and motor function through the loss of neuronal cells in certain areas of the brain. Patients in the reduced or full penetrance category may pass on the CAG repeat expansion to offspring. Patients with >70 CAG repeat expansions may show an earlier onset of HD symptoms. For these reasons, accurately determining the number of CAG repeats is of utmost importance.

Herein, we report the analytical performance of the AmplideX® PCR/CE *HTT* Kit, a single-tube PCR reaction assay followed by capillary electrophoresis (CE). This kit reliably genotypes and quantifies CAG repeat expansions in *HTT* while addressing common polymorphisms.

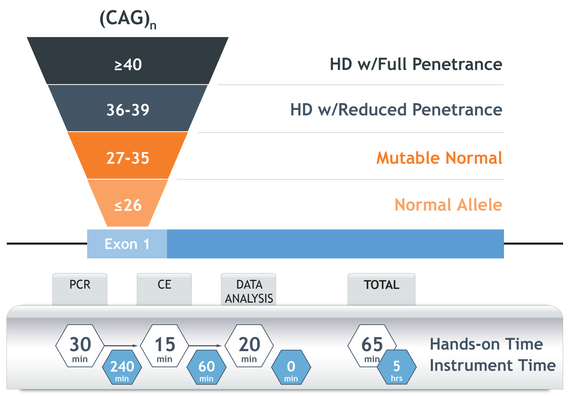

Figure 1. HD Classification and Assay Workflow. Classification of CAG repeat lengths per ACMG 2014 guidelines[1]. The boundary categories include normal (≤26 CAGs), mutable normal (27-35 CAGs), reduced penetrance (36-39 CAGs), and full penetrance (≥40 CAGs). The complete workflow, from sample to answer, is ~6 hours. The hands-on time is ~65 minutes and instrument time is ~5 hours for a 24-sample run on a 3500xL Genetic Analyzer.

Materials and Methods

DNA samples were amplified using the AmplideX® PCR/CE *HTT* Kit and subjected to CE on 3130, 3500, or 3730 series genetic analyzers (Thermo Fisher Scientific). The gene-specific peak calls for each sample were annotated using GeneMapper® or GeneMarker® software and translated to the number of CAG repeats using mobility and correction factors specific to each CE platform type and calibrated to a co-injected ROX ladder. To establish analytical characteristics, more than 30 samples spanning the repeat length categories were tested at various mass inputs using a range of thermal cyclers to produce >1900 unique data points. These samples include cell-line genomic DNA (gDNA) reference samples and whole-blood gDNA isolated using common extraction methods.

*Research Use Only. Not for use in diagnostic procedures.

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The AmplideX PCR/CE *HTT* Kit was also evaluated at Johns Hopkins All Children's Hospital (JHACH) using 51 samples of known genotypes, and the results were compared to those from a PCR-based, laboratory-developed test (LDT) at JHACH.

Results

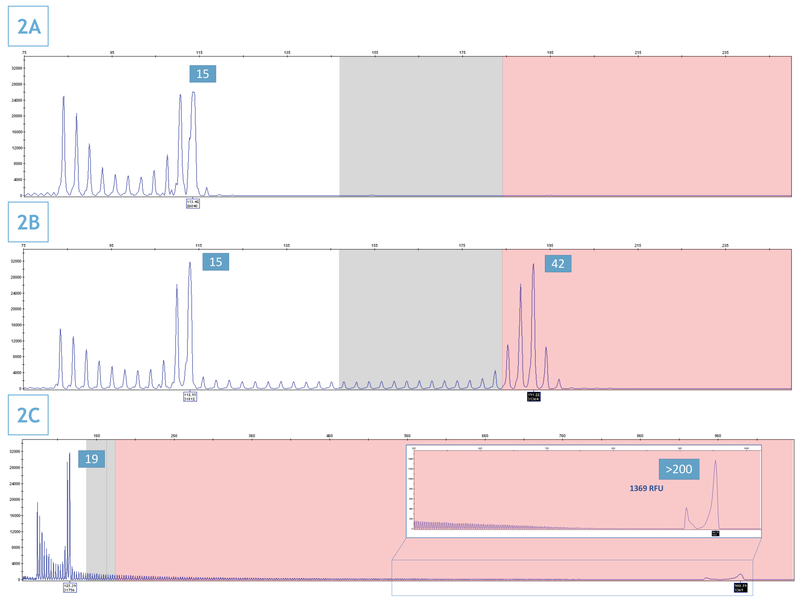

Figure 2. Resolving Zygosity and Large Expansion Detection. Homozygous and heterozygous samples with various CAG expansions were evaluated using the kit. Zygosity was differentiated between **A)** a homozygous, residual clinical sample and **B)** a heterozygous, residual clinical sample by the repeat profile present in each sample. The assay accurately detected large repeat expansions as shown in **C)**, a cell-line with >200 CAG repeats.

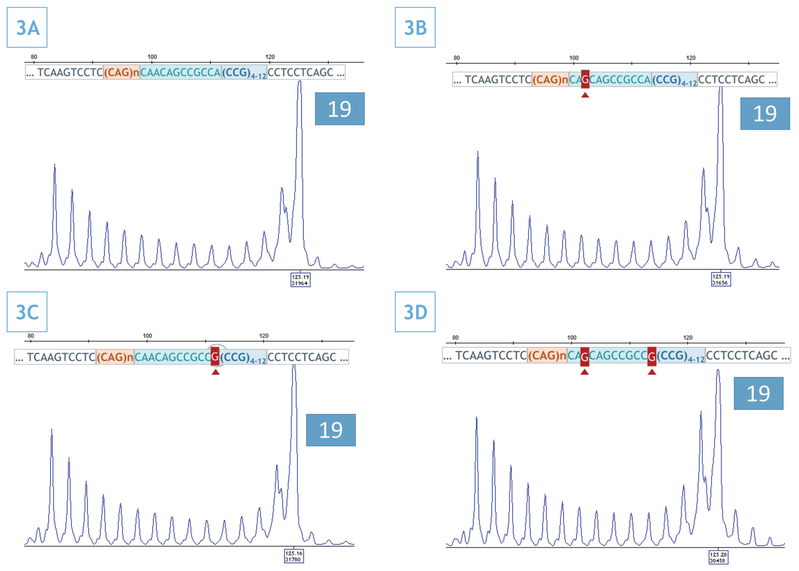

Figure 3. SNP Evaluation. SNP-containing ultramers were used to determine the possibility of allele drop-out. The presence of a SNP in the primer binding region still allowed for gene-specific allele amplification. **A)** shows a normal allele, **B)** contains SNP rs473915, **C)** contains SNP rs76533208, and **D)** contain rs473915 and rs76533208 SNPs.

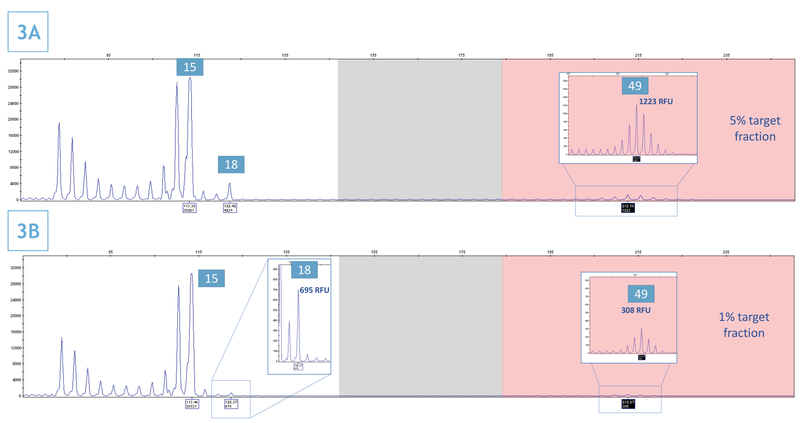

Figure 4. Limit of Detection for a Residual Clinical Sample. The detection of an expanded, minor allele is shown in **A)** a sample with a 5% allele target fraction and **B)** a sample with a 1% allele target fraction. Using Probit Analysis, we found the lowest level of detection of a minor allele in a clinical sample to be 2.3%.

Table 1. Sample Precision. All alleles were detected within 1 CAG of the expected peak. 100% of alleles called were within the target precision range. The study was conducted by 2 operators over five days on multiple CE instruments and types with at least three replicates per sample. The samples include residual clinical sample gDNA as well as cell-line gDNA.

Sample ID	Mode Expected CAG Length (± Target Precision)	Reported CAG Length	Allele Count	Total Alleles	% within Target Precision Range
S0162126	15 ± 1	15	132	132	100
S0162131	15 ± 1	15	132	132	100
S0162141	15 ± 1	15	131	131	100
S0162147	15 ± 1	14 15	1 127	128	100
S0162129	17 ± 1	17	131	131	100
S0162135	17 ± 1	17	132	132	100
S0162139	21 ± 1	21	132	132	100
S0162135	33 ± 1	33	132	132	100
S0162139	36 ± 1	36	132	132	100
S0162129	39 ± 1	39	131	131	100
S0162131	40 ± 1	40	132	132	100
S0162141	42 ± 1	42	131	131	100
S0162129	50 ± 2	49 50	2 129	131	100
S0162129	75± 2	74 75	6 125	131	100

Table 2. Sample Accuracy. Previously characterized Coriell[2] and NIST samples were tested via the AmplideX kit, and all results were in agreement with the reported results. An extra minor allele was detected in samples NA20252 and NA20253 (indicated in red).

Sample ID	Expected Alleles	Expected Genotype	Observed Alleles	Observed Genotype	Concordance (%)
NA20206	17, 18	Normal	18, 18	Normal	100
NA20207	19, 21	Normal	19, 21	Normal	100
NA20245	15, 15	Normal	15, 15	Normal	100
NA20246	15, 24	Normal	15, 24	Normal	100
NA20247	15, 29	Mutable Normal	15, 29	Mutable Normal	100
SRM 2393 A	15, 29	Mutable Normal	15, 29	Mutable Normal	100
NA20248	17, 36	Reduced Penetrance	17, 36	Reduced Penetrance	100
NA20249	22, 39	Reduced Penetrance	22, 39	Reduced Penetrance	100
SRM 2393 B	17, 36	Reduced Penetrance	17, 36	Reduced Penetrance	100
NA20208	35, 45	Full Penetrance	35, 45	Full Penetrance	100
NA20209	45, 47	Full Penetrance	45, 47	Full Penetrance	100
NA20210	17, 74/75	Full Penetrance	17, 75	Full Penetrance	100
NA20250	15, 40	Full Penetrance	15, 40	Full Penetrance	100
NA20251	39, 50	Full Penetrance	39, 50	Full Penetrance	100
NA20252	22, 65/66	Full Penetrance	22, 63, 66	Full Penetrance	100
NA20253	22, 101	Full Penetrance	22, 100, 128	Full Penetrance	100
SRM 2393 C	15, 40	Full Penetrance	15, 40	Full Penetrance	100
SRM 2393 D	35, 45	Full Penetrance	35, 45	Full Penetrance	100
SRM 2393 E	39, 50	Full Penetrance	39, 50	Full Penetrance	100
SRM 2393 F	17, 75	Full Penetrance	17, 75	Full Penetrance	100

Table 3. AmplideX Kit Concordance with the JHACH LDT. 100% categorical and genotype concordance was achieved for all 51 samples tested between the JHACH LDT and the AmplideX kit. Genotype concordance was assessed using precision tolerance, and the maximum deviation in CAG repeat length observed was 2 CAGs at >80 CAG repeats (e.g. 84 vs. 86 CAG repeats).

	Normal	Mutable Normal	Reduced Penetrance	Full Penetrance	Categorical Concordance	Genotype Concordance
Normal	13 Clinical/3 CAP	-	-	-	16/16 (100%)	16/16 (100%)
Mutable Normal	-	2 Clinical/4 CAP	-	-	6/6 (100%)	6/6 (100%)
Reduced Penetrance	-	-	1 Clinical/1 CAP	-	2/2 (100%)	2/2 (100%)
Full Penetrance	-	-	-	23 Clinical/4 CAP	27/27 (100%)	27/27 (100%)

Conclusions

- The AmplideX PCR/CE *HTT* Kit accurately quantified CAG repeat sizes across all four allele length categories, using a single-tube PCR reaction, with a precision of ± 1 repeat.
- 100% CAG repeat allele call concordance was achieved between the AmplideX PCR/CE *HTT* Kit and the JHACH LDT.
- This robust kit allows clear resolution of zygosity and quantification of large CAG expansion repeat sizes while maintaining a streamlined and easy to follow workflow.

References

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

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