A novel repeat-primed PCR assay to detect the full range of trinucleotide CAG repeats in Huntingtin gene (*HTT*)

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Background and aims: Abnormal expansions of the CAG repeat in the Huntingtin (*HTT*) gene on chromosome 4 are associated with Huntington disease (HD), an autosomal dominant neurodegenerative disorder. Accurate determination of the CAG repeat number is crucial in HD to either confirm the diagnosis in symptomatic patients or to predict the genetic condition in subjects at risk of HD, including prenatal testing. We evaluated the novel tripled repeat primed PCR-based AmplideX PCR/CE HTT Kit for accurate estimation of *HTT* CAG repeats.

Patients and Methods: We assessed the novel AmplideX PCR/CE HTT Kit in 46 HD reference DNA samples, including five samples with alleles in the normal range (6–26 CAGs), two cases with intermediate alleles (27–35 CAGs), five samples with alleles with incomplete penetrance (36–39 CAGs), and 34 samples carrying alleles with full penetrance (> 40 CAGs). Of the samples with alleles with full penetrance, 20 harbored alleles larger than 60 CAG repeats, generally seen in subjects with juvenile onset HD < 20 years and in the rarest pediatric variant. Of these, 41 samples had been previously tested in our laboratory using either flurescent repeat-flanking PCR or tripled-primed PCR method, or both, whereas 5 samples had been tested in other HD reference laboratories.

Results: All samples showed full concordance with the previously verified allele sizes. Identical repeat size or sizing errors within ±1 CAG were obtained for alleles ≤42 CAG repeats, whereas sizing errors within ±3 were obtained for alleles > 43 CAG repeats, conforming to established guidelines. The assay was able to accurately size 18 samples with very large alleles comprised between 60 and 100 CAG repeats, and to detect two exceptionally large alleles with more than 200 CAG repeats (**Table 1**). The HD reference samples ranging from 15 to 101 CAG repeats were also accurately sized with an error within ±1 or ±3 repeats according to what expected based on the repeat size (**Table 2)**. True homozygous normal samples were distinguished from expanded alleles by the absence of a stuttering pattern denoting an expanded allele **(Figure 1)**. Sample #33, which could not be detected by standard fluorescent PCR, was clearly identified by AmplideX PCR/CE HTT Kit, and determined to have an expanded allele of >200 CAG repeats **(Figure 2)**. Nevertheless, only a stuttering pattern with continuous repeat peak pattern and a pileup peak at the end of the run was seen, since the upper limit for accurately size expanded alleles is less of 200 CAG repeats.

Table 1: Categories of 46 HD samples analyzed in this study and their repeat concordance between standard PCR vs TP-PCR

Sample type	No. of correctly sized by both assay	No. of alleles with ±1 repeat concordance	No. of alleles with ±2-3 repeat concordance	No. of alleles within ±>3 repeat concordance	Total No.
Normal allele (6–26 repeats)	4				4
Intermediate allele (27–35 repeats)	2				2
Incomplete penetrance allele (36–39 repeats)	4				4
Complete penetrance allele (40–60 repeats)	12	6			18
Complete penetrance allele (60–200 repeats)	3	5	9	1	18
Total	25	11	9	1	46

Table 2: Reference samples genotyped in other laboratories used for comparison between standard PCR vs TP PCR

Sample Code	Standard PCR	TP-PCR	Ref. Genotype
#11	15R/30R±1	15R/30R	**15R/29R**
#12	22R/63R±3	22R/63R±3	**22R/64R**
#42	15R/24R±1	15R/24R±1	**15R/24R**
#43	15R/40R±1	15R/40R±1	**15R/40R**
#44	22R/100R±3	22R/101R±3	**22R/98R**

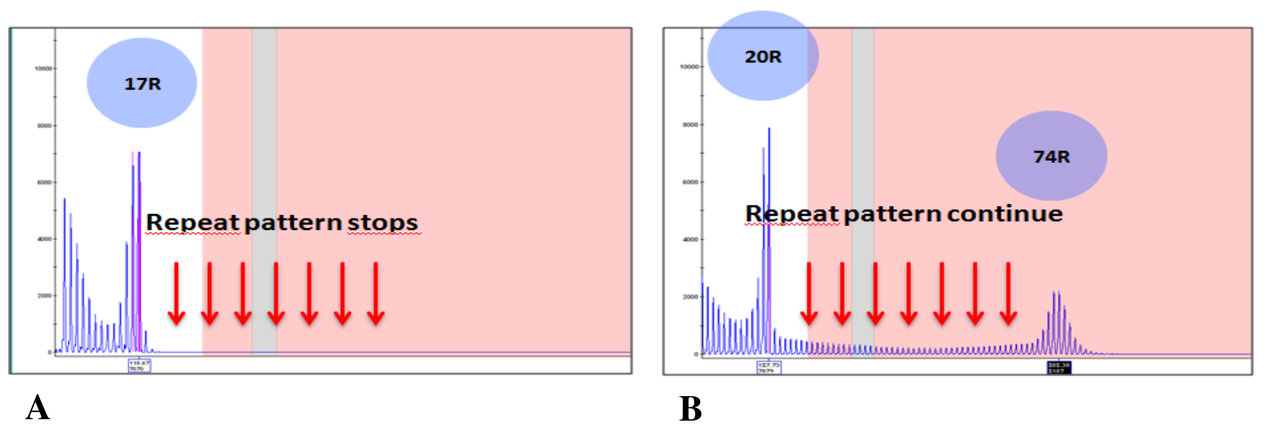

Figure 1: Distinguishing between homo- and heterozygous alleles Electropherogram results of samples. A: Electropherogram results of sample # showing a homozygous allele call of 17 CAG repeats. B: Electropherogram results of sample #25 showing a heterozygous allele call of 20 CAG and an expanded allele of 74 CAG. Note the continuous stuttering after the prominent peak at 20 CAG repeats to peak at 74 CAG repeat in the close-up view, an indication of the presence of an expanded allele.

Figure 2: Detecting highly expanded genotypes
Electropherogram results of samples. **A**: The size of expanded allele > 200 repeats could not be determined by standard PCR. **B**: Electropherogram results of sample #33 showing only a stuttering pattern with continuous repeat peak pattern and a pileup peak at the end of the run. The upper limit for accurately sized expanded alleles is ≤200 CAG.

CONCLUSIONS

This method provides a rapid, sensitive and reliable method to accurately genotype the *HTT* CAG repeat stretches, and extends the detection limit of large expanded alleles to over 200 CAG repeats, thus providing a comprehensive molecular diagnostic evaluation of all HD samples, including those pediatric forms carrying extremely large, hard to detect, alleles.

The authors declare that there is no conflict of interest

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