# A Single-Assay Diagnostic Workflow for Genotyping and Phasing SNPs with Repeat Expansions for Allele-Selective Therapies in Huntington Disease

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## Summary

- Determination of *HTT* single nucleotide polymorphism (SNP) haplotypes in Huntington Disease (HD) can personalize the use of promising allele-selective gene-silencing therapies that target mutant transcripts and preserve wild-type allele expression.
- We developed a streamlined, prototype reverse transcription polymerase chain reaction (RT-PCR) assay using an *in vitro* diagnostic device ready capillary electrophoresis (IVD-ready CE) platform that phases SNPs with mutant CAG-expanded *HTT* mRNA.
- The AmplideX® HTT SNP/Repeat Phasing assay* accurately genotyped eight HD patient-derived cell-line samples and 27 HD patient whole blood samples using a rapid, single-assay workflow as an alternative to multiple assays and analyzers.
- Wave Life Sciences is currently investigating stereopure antisense oligonucleotides targeting SNP1 (rs362307) and SNP2 (rs362331) in the phase 1b/2a PRECISION-HD1 (NCT03225833) and PRECISION-HD2 (NCT03225846) clinical trials in patients with HD.
- This allele-selective approach has also been applied to a third Wave Life Sciences HD program targeting another SNP which will soon enter clinical development with a clinical trial application submission by the end of 2020.

## Introduction

Huntington Disease (HD) is a neurodegenerative disorder that is caused by expansions of ≥36 CAG repeats in exon 1 of the *HTT* gene. Wave Life Sciences has developed stereopure antisense oligonucleotides (ASOs) designed to selectively bind pathogenic mutant HTT (mHTT) transcripts by targeting SNPs such as rs362307 (SNP1) and rs362331 (SNP2) on the expanded allele. This approach preserves wild-type HTT expression, which may be neuroprotective[1]. Up to 70% of HD patients have SNP1 and/or SNP2 only on the mHTT allele[2] which supports clinical trials, such as NCT03225833 and NCT03225846, for allele-selective ASO therapies[3]. This allele-selective approach has also been applied to a third Wave Life Sciences HD program targeting another SNP which will soon enter clinical development with a clinical trial application submission by the end of 2020. Identifying patients with the target SNP on the mHTT transcript can be complex because it requires phasing assays that can bridge the >7.5 kb gap between SNPs and the CAG repeat. We describe an accurate and streamlined AmplideX HTT SNP/Repeat Phasing assay* that genotypes SNP1, SNP2, and SNP3, quantifies the CAG repeat tract, and determines whether these sequences are on the same allelic transcript.

## Methods

Blood was collected in PAXgene® RNA blood tubes, and RNA was extracted and reverse transcribed. Phased SNP/CAG repeat amplicons were generated in successive PCR reactions and analyzed by capillary electrophoresis (CE) on an Applied Biosystems™ 3500 Genetic Analyzer. SNP zygosity, CAG repeat length and SNP/repeat phasing was determined from the CE electropherogram with a software algorithm developed using machine learning. The assay was evaluated with 78 blood samples from presumed healthy individuals, eight HD patient-derived cell-line samples and 27 HD patient whole blood (WB) samples.


Key: Reverse Transcription (RT), Allele-Specific Long-Range PCR (ASLR PCR), Capillary Electrophoresis (CE)

**Figure 1. Workflow for the AmplideX HTT SNP/Repeat Phasing Assay*.** With less than 3 hours of hands-on time, the Amplidex HTT SNP/Repeat Phasing assay and its assay-specific software will offer a streamlined sample-to-answer workflow.

**This product is under development. Future availability and performance to be determined.*
*Presented at AMP 2020*

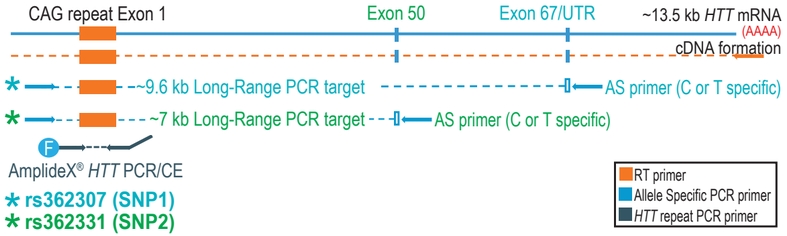
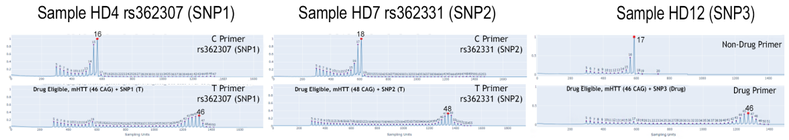


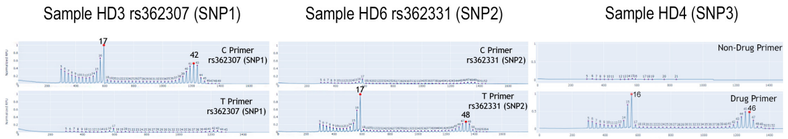

**Figure 2. Assay Design for the AmplideX HTT SNP/Repeat Phasing Assay*.** Starting from RNA, a reverse transcription (RT) reaction is used to form full length *HTT* cDNA (~13.5kb). Allele-specific long-range PCR (ASLR PCR) provides specificity during the allele enrichment process. The PCR product is then used as input into the AmplideX® *HTT* PCR/CE assay. The resulting output is an indirect method for ascertaining phase genotyping; only the allele that is in-phase with the particular SNP location (rs362307 or rs362331) and status (C or T) interrogated in the ASLR PCR reaction will be present in the *HTT* PCR/CE assay output. Results are generated using automated AmplideX Reporter software. Note, SNP3 (not shown) is also performed in a similar fashion.

## Results




Drug Primer = Primer specific for SNP3 targeted by ASO; Non-Drug Primer = Primer specific for alternative SNP3 not targeted by ASO.

**Figure 3. High Specificity for SNP1, SNP2 and SNP3 Ensures Reliable Detection of Investigational Drug Eligible Genotypes.** As shown above in electropherograms for samples HD4, HD7 and HD12, the assay clearly detected the long expanded CAG allele (red dot) in the T Primer and/or Drug Primer trace and the short unexpanded CAG allele (red dot) in the C primer and/or Non-Drug Primer trace, indicating these samples are targetable with allele-selective therapies.




Drug Primer = Primer specific for SNP3 targeted by ASO; Non-Drug Primer = Primer specific for alternative SNP3 not targeted by ASO.

**Figure 4. Reliable Results for SNP1, SNP2 and SNP3 Resolves Genotypes Ineligible for SNP Targeted Allele-Selective Therapy.** Based on the electropherograms, samples HD3, HD6 and HD4 are not targetable with allele-selective therapies due to the presence of two CAG expanded alleles in one trace.

| Sample ID | RIN | rs362307 (SNP1) | | SNP1 CAG Length | | rs362331 (SNP2) | | SNP2 CAG Length | | Drug Eligibility | Concordant with Ref. Method? |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Long | Short | Long | Short | Long | Short | Long | Short | | |
| CL1 | 9.9 | T | C | 44 | 17 | T | C | 43 | 17 | Yes, Both | YES |
| CL2 | 9.6 | T | C | 44 | 21 | T | T | 44 | 21 | Yes, SNP1 | YES |
| CL3 | 9.8 | C | C | 43 | 15 | T | C | 43 | 15 | Yes, SNP2 | YES |
| CL4 | 10.0 | T | C | 59 | 18 | T | C | 59 | 18 | Yes, Both | YES |
| CL5 | 10.0 | C | C | 75 | 17 | T | T | 74 | 17 | No | YES |
| CL6 | 9.7 | C | C | 68 | 15 | T | C | 68 | 15 | Yes, SNP2 | YES |
| CL7 | 10.0 | T | C | 177 | 18 | T | C | 175 | 18 | Yes, Both | YES |
| CL8 | 9.9 | C | C | 38 | 21 | C | T | 38 | 21 | No | YES |
| HD1 | 7.9 | T | T | 40 | 17 | T | T | 40 | 18 | No | YES |
| HD2 | 9.2 | C | C | 48 | 19 | T | C | 47 | 19 | Yes, SNP2 | YES |
| HD3 | 8.3 | C | C | 42 | 17 | T | T | 42 | 17 | No | YES |
| HD4 | 9.3 | T | C | 46 | 16 | T | T | 46 | 16 | Yes, SNP1 | YES |
| HD5 | 9.2 | C | C | 42 | 18 | T | C | 42 | 18 | Yes, SNP2 | YES |
| HD6 | 7.8 | T | C | 48 | 17 | T | T | 48 | 17 | Yes, SNP1 | YES |
| HD7 | 9.3 | T | C | 48 | 18 | T | C | 48 | 18 | Yes, Both | YES |
| HD8 | 8.8 | T | C | 44 | 19 | T | C | 43 | 19 | Yes, Both | YES |
| HD9 | 9.0 | T | T | 56 | 22 | T | T | 55 | 22 | No | YES |
| HD10 | 8.7 | C | C | 42 | 16 | C | C | 42 | 16 | No | YES |
| HD11 | 8.1 | Low Signal (LS) QC Fail | | | | | | | | | |
| HD12 | 8.4 | C | C | 47 | 17 | C | C | 46 | 17 | No | YES |
| HD13 | 8.8 | T | T | 47 | 25 | T | T | 46 | 25 | No | YES |

**Table 1. AmplideX HTT SNP/Repeat Phasing Assay* Results Compared to Reference Results.** All 8 HD cell-line samples (CL1-8) and 12 of 13 clinical HD patient samples (HD1-13) met PCR/CE assay signal criteria. Each sample was correctly phased and genotyped compared to reference results procured from a combination of *HTT* CAG repeat PCR, and Sanger and long-read sequencing analysis[3]. One HD patient sample (HD11) had low RNA yield and resulted in a QC flag due to low signal (LS). SNP3 data was not shown due to incomplete reference results.

| Sample Set | Outcome | SNP1 | SNP2 | SNP3 |
|---|---|---|---|---|
| 27 HD Whole Blood Samples | Samples that passed QC | 25/27 | 26/27 | 25/25 |
| | % Agreement with Reference Method | 100% (25/25) | 100% (25/25) | 100%(16/16) |

**Table 2. Expanded Clinical Cohort Shows High Concordance with Reference Method.** Utilizing our prototype automated, push-button data reporting via AmplideX Reporter software, a total of 27 HD patient samples were analyzed across SNP1, SNP2 and SNP3. Samples that did not meet PCR/CE assay signal criteria (LS QC) were excluded. All samples that passed QC were 100% concordant with available reference results.

## Conclusions

- Current methods for SNP genotyping and phasing with the *HTT* repeat tract rely on multiple work streams and/or exploratory technologies.
- We describe an accurate, unified PCR-based workflow on an IVD-ready CE platform using automated genotyping capabilities with potential to expedite patient selection and improve the efficiency of clinical trials.
- The AmplideX HTT SNP/Repeat Phasing assay* also has implications for diagnostics, including companion diagnostic kits, for other allele-selective, repeat expansion therapies.

## References

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2. Kay C, Skotte NH, Southwell AL, Hayden MR. Clin Genet 2014;86:29–36
3. Claassen DO et al. Neurol Genet. 2020 May 14;6(3):e430

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