

C9orf72 testing in a diagnostic laboratory using the Asuragen AmplideX® PCR/CE C9orf72 kit



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Introduction

The pathogenic expanded hexanucleotide repeat element (G⁴C²) in intron 1 of the Chromosome 9 open reading frame 72 gene (C9orf72; NM_001256054.2) is the most prevalent genetic cause of the neurodegenerative disorders frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). Here we describe our experiences in using the newly introduced Asuragen AmplideX® PCR/CE C9orf72 assay compared to our home-made long range and repeat-primed PCR tests for the detection of C9orf72 repeats. Our home-made tests are limited to detection of up to ~60 C9orf72 repeats.

Methods

DNA samples isolated from EDTA blood (n=23) were tested for C9orf72 using the Asuragen AmplideX® PCR/CE C9orf72 kit. Amplicons were sized on an ABI 3730xl Genetic Analyzer and analyzed using GeneMarker software. All samples were previously tested using long range and repeat-primed PCR tests. For purposes of this study, normal repeats were categorized as <30 repeats and pathogenic repeats >30 repeats.

Results

All samples previously scored as pathogenic using the home-made tests also showed a pathogenic repeat expansion of at least 145 repeats using the new Asuragen test (for examples see Figure 1). No repeats were sized in the range 60-145. Repeats in the normal range were sized exactly the same. DNA concentrations of 50ng/μl (our standard lab dilution) and 25ng/μl showed similar results. A dilution (including a second 30s injection on the ABI) of the PCR products was needed to overcome the saturated normal allele peaks in the undiluted PCR products.

Conclusions

The Asuragen AmplideX® PCR/CE C9orf72 kit is an effective method for the detection of C9orf72 repeats.

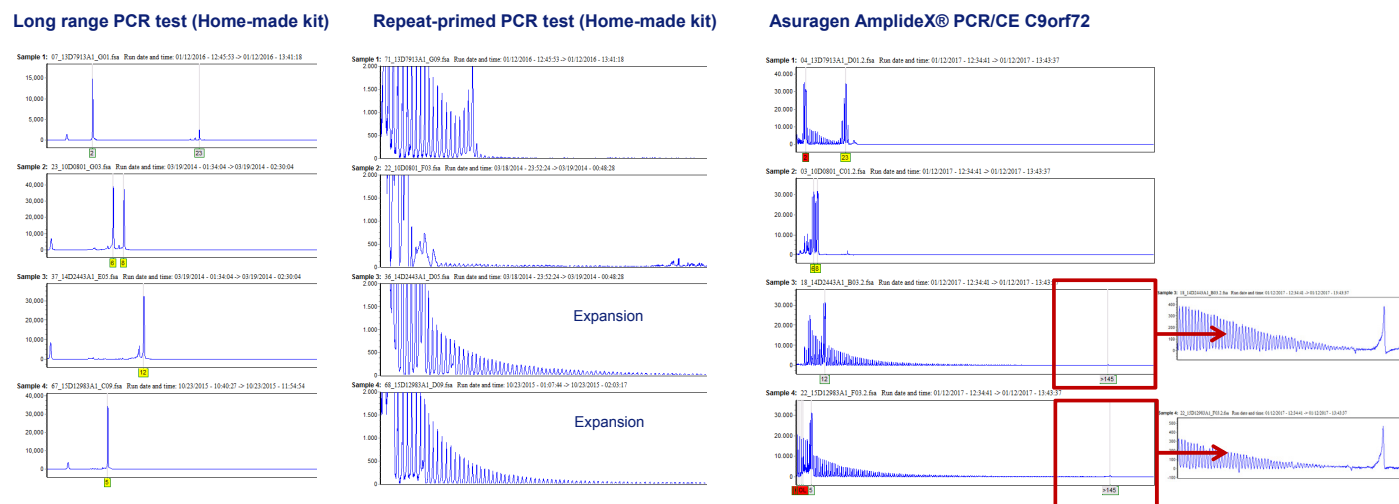


Figure 1: Comparison of several samples runned with home-made kit versus Asuragen AmplideX® PCR/CE C9orf72 kit

Funding: Sanbio B.V. provided financial support for presenting this poster.



Conflicts of interest: Saskia Theunisse and Jody Salomon are employees of Sanbio B.V.. Kristen Culp is an employee of Asuragen, Inc. The remaining authors disclose no conflicts.