## MULTI-SITE EVALUATION OF A SINGLE-TUBE LONG-READ PCR ASSAY FOR THE RELIABLE DETECTION

## AND CHARACTERI7ATION OF C9orf72 HEXANUCIEOTIDF REPEAT EXPANSIONS

EunRan Suh ${ }^{1 *}$, Eran Bram ${ }^{2 *}$, Kamyab Javanmardi ${ }^{2}$, Kimberly Nicholson ${ }^{2}$, Kristen Culp ${ }^{2}$, Julie Krosting ${ }^{2}$, Jon Kemppainen ${ }^{2}$, Kaitlyn Grando ${ }^{1}$, Vivianna M Van Deerlin ${ }^{1}$, and Gary J Latham ${ }^{2}$ ${ }^{1}$ Dept. of Pathology and Lab Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA; ${ }^{2}$ Asuragen, Inc., Austin, TX

## SUMMARY

- $\mathrm{A}\left(\mathrm{G}_{4} \mathrm{C}_{2}\right)_{n}$ hexanucleotide repeat expansion in the noncoding region of the C9orf72 gene represents the first genetic link between amyotrophic lateral sclerosis (ALS) and

We developed sensitive and robust single-tube, 3-primer C9orf72 PCR reagents that can flag expanded samples irrespective of length and provide accurate sizing up to $\sim 145$ repeat units when resolved by capillary electrophoresis.
A gene-specific, 2 -primer configuration of this PCR can detect low-level mosaicism and reproducibly generate products with up to $\sim 800$ repeats from expanded samples. Assay performance was evaluated at two sites (Asuragen, Inc. and the University of Pennsylvania) using a large set of ALS cell-line derived DNA samples from the Coriell repository $(\mathbb{N}=174)$ and a diverse set of residual clinical samples, including brain, blood, and saliva.

## INTRODUCTION

An intronic $\left(\mathrm{G}_{4} \mathrm{C}_{2}\right)_{n}$ hexanucleotide repeat expansion in the C9or72 gene has been observed in the general population with a frequency of $\sim 1 / 600$ and is present in $\sim 10 \%$ of all amyotrophic lateral scleros ALS) and frontotemporal dementia (FTD) cases. Fewer than 30 repeats are considered normal whereas
pathogenic C9orf72 expansions have 100's to 1000 's of repeats. The GC-rich repeat poses formidable challenges to routine PCR-based fragment sizing methods, and currently requires analysis using multiple short-range PCR reactions for each sample, followed by Southern blot analysis of expanded samples Here we describe a two-site evaluation of a single-tube, highly streamlined PCR assay that both detec

MATERIALS AND METHODS
enomic DNA was extracted from blood, saliva, fresh-frozen or formalin-fixed parafin-embedded (FFPE) brain tissues of patients with FTD and/or ALS by using the QuickGene 610L (Autogen), OIAamp DNA mini kit (Qiagen), or Q|Aamp DSP DNA FFPE Tissue Kit (Qiagen), respectively, using the manufacturer's
protocols. The AmplideX exanucleotide repeats. Amplicons were sized on a 3500xL or 3130xl Genetic Analyzer (Thermo Fisher), and/or SeaKem LE Plus agarose gel electrophoresis (AGE; Lonza) after amplification with only the flanking gene-specific primers and modified PCR conditions. The assay was evaluated at both the University of Pennsylvania (UPenn; Site 1 ) and Asuragen (Site 2). Site 1 evaluated a subset of NINDS ALS
samples ( $n=50$ ) and an independent set of 166 ALS and FTD patient-derived samples from peripheral blood ( $n=114$ ), saliva ( $n=28$ ), and brain ( $n=24$ ) and compared results to both Southern blot analysis and a CR lab-developed test (LDT)'. Site 2 assessed a broader set of NINDS ALS samples ( $n=774$ ), including hose in common



Equal contibution authors



RESULTS
Across both evaluation sites, C9orf72 repeat numbers were resolved in agreement with previous annotations ${ }^{2}$ for all but a single ambiguous sample. Fragment sizing using capillary electrophoresis
(CE) was limited to 145 repeats by the sizing constraints of the CE liauid polymer, and not by PCR amplification. Compatibility with blood, saliva, fresh-frozen- and FFPE- brain DNA at 40 ng inputs wa demonstrated at Site 1 , and samples with expansions, size mosaicism and/or indels could be identified from the repeat-primed CE traces. Site 2 further demonstrated sensitivity to as few as 1 ng input o expansions with up to 800 repeats.



 [3A


Figure 3 . A 2 .site comparison of the 2 -primer C9orr72 PCR/AGE assay for detection of expanded alleses up to -800 repeats. Modified





## CONCIUSIONS

- AmplideX PCR technology can amplify C9orf72 repeat expansions that are more than 10-fold larger than conventional assays.
A 3-primer RP-PCR design provides robust performance across multiple sample types (including challenging FFPEs), high sensitivity and accuracy up to 145 repeats on CE, and clear indication of both size mosaicism and $3^{\prime}$ sequence variations.
A gene-specific, 2-primer PCR format identified low-level size mosaicism and produced amplicons consistent with up to at least $\sim 800$ hexanucleotide repeats in expanded samples when resolved on agarose gel.
This simple, single-tube PCR technology has potential to advance clinical research and emerging diagnostic, therapeutic, and screening applications for the C9orf7 marker in the context of ALS, FTD and other late-onset neurodegenerative disorders.


## Reference

1. Suh E ., et al., Semi-automated quantification of C9orf72 expansion size reveals inverse correlation between hexanucleotid

 4. Van der Zee J., et all, A. Pan-European Study of the C9orf2 Repeat Associated with FTLD: Geographic Prevalence, Genomic 5 Rutherford $\mathrm{N} . \mathrm{J}$., et al. C9orf72 hexanuleotide reneat expansions in patients with AIS from the Corill Cell Repositor Rutherford N.J., et al al., $9: 9$ orf72
Neurology, 2012. $31 ; 795 ; 482-3$.
