# MULTI-SITE EVALUATION OF A SINGLE-TUBE LONG-READ PCR ASSAY FOR THE RELIABLE DETECTION AND CHARACTERIZATION OF *C9orf72* HEXANUCLEOTIDE REPEAT EXPANSIONS

EunRan Suh<sup>1\*</sup>, Eran Bram<sup>2\*</sup>, Kamyab Javanmardi<sup>2</sup>, Kimberly Nicholson<sup>2</sup>, Kristen Culp<sup>2</sup>, Julie Krosting<sup>2</sup>, Jon Kemppainen<sup>2</sup>, Kaitlyn Grando<sup>1</sup>, Vivianna M Van Deerlin<sup>1</sup>, and Gary J Latham<sup>2</sup>

<sup>1</sup>Dept. of Pathology and Lab Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA; <sup>2</sup>Asuragen, Inc., Austin, TX

## **SUMMARY**

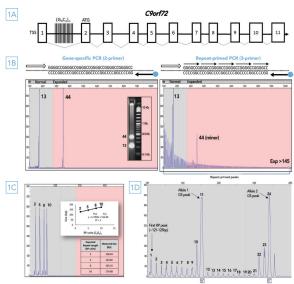
- A  $(G_4C_2)_n$  hexanucleotide repeat expansion in the noncoding region of the *C9orf72* gene represents the first genetic link between amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD).
- We developed sensitive and robust single-tube, 3-primer C9orf72 PCR reagents that can flag expanded samples irrespective of length and provide accurate sizing up to ~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 ~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 (N=774) and a diverse set of residual clinical samples, including brain, blood, and saliva.

#### INTRODUCTION

An intronic  $(G_4C_2)_n$  hexanucleotide repeat expansion in the *C9orf72* gene has been observed in the general population with a frequency of ~1/600 and is present in ~10% of all amyotrophic lateral sclerosis (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 detects *C9orf72* expansions and sizes expanded alleles with 100's of repeats.

## **MATERIALS AND METHODS**

Genomic DNA was extracted from blood, saliva, fresh-frozen or formalin-fixed paraffin-embedded (FFPE) brain tissues of patients with FTD and/or ALS by using the QuickGene 610L (Autogen), QIAamp DNA mini kit (Qiagen), or QIAamp DSP DNA FFPE Tissue Kit (Qiagen), respectively, using the manufacturer's protocols. The AmplideX® PCR/CE *C9orf72* Kit\*\* (Asuragen) was used to amplify and detect *C9orf72* hexanucleotide 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 PCR lab-developed test (LDT)¹. Site 2 assessed a broader set of NINDS ALS samples (n=774), including those in common with Site 1, as part of a larger study to analyze the full collection (n=2100).



<sup>\*</sup>Equal contribution authors

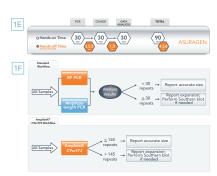


Figure 1. A novel PCR technology for the amplification of the hexanucleotide (G, C<sub>2</sub>), repeat element in the C9orf72 gene. A) Schematic representation of the C9orf72 gene structure showing the predicted 11 exons (boxes) and location of the intronic hexanucleotide repeat expansion (vertical lines). B) A 2-primer (left) or 3-primer (right) FAM-labeled PCR design, with representative CE profiles with either gene-specific (GS) peaks only or GS peaks overlaid with a consistent repeat (RP) profile (Coriell sample ND06769). The 2-primer PCR products can also be resolved by AGE (left inset). C) A mix of cell-line DNAs can be used to generate a size calibration curve (inset) that converts size (in bp) to repeat number and corrects for the differential mobility of GC-rich DNA on CE. D) Repeat sizing by direct counting of repeat peaks. E) A modular design can accommodate both PCR/CE and PCR/AGE assays, requiring ~6 hr or ~9.5 hr to complete, respectively, with up to 1.5 hours of hands-on-time per 24 sample batch. F) The described PCR approach offers a simpler workflow and more informative C9orf72 molecular analysis compared to a standard PCR workflow.

#### **RESULTS**

Across both evaluation sites, *C9orf72* repeat numbers were resolved in agreement with previous annotations<sup>2</sup> 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 liquid polymer, and not by PCR amplification. Compatibility with blood, saliva, fresh-frozen- and FFPE- brain DNA at 40 ng inputs was 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 of cell-line DNA. Both sites also showed proof-of-concept for agarose gel sizing of PCR amplicons from expansions with up to 800 repeats.

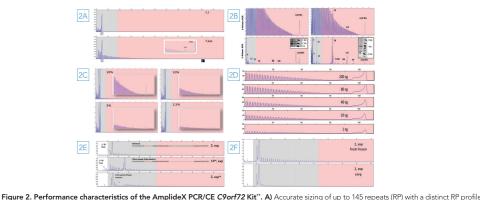


Figure 2. Performance characteristics or the Ampliada PCR/CE C90772 Nt. 2, Accurate sizing of up to 143 repeats (Rr) with a distinct, Rr profile for expanded samples is achieved using 3-primer PCR. The multiplicity of peaks generated using the repeat primer enables zygosity resolution. B) Sample-specific low-level minor alleles are detected in the 2-primer assay configuration on both CE and AGE (inset). C) A 2.5% mass fraction of an expanded sample can be flagged in the background of an unexpanded sample. D) The 3-primer PCR demonstrates robust performance across a 200-fold gDNA input range down to 1 ng/reaction, and consistent with previous reports<sup>3,4</sup> also provides clear indication for the presence of 3' indels in representative patient samples E). P) C90rf72 repeat expansions were consistently detected in matched fresh-frozen and FFPE brain specimens.

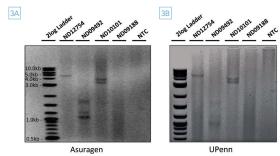


Figure 3. A 2-site comparison of the 2-primer C9orf72 PCR/AGE assay for detection of expanded alleles up to ~800 repeats. Modified thermocycling conditions were used to achieve PCR of up to ~5 kb amplicons from Coriell ALS cell-line gDNA samples at both Asuragen A) and UPenn B). Note the commonality in banding patterns indicating the consistent detection of size mosaicism in ND09492 and ND10101. ND09188 is a normal (non-expanded) sample. Low MW bands (<0.5 kb) consistent with normal allele sizes were observed under low exposure conditions (data not shown).

4	Assay Method		Asuragen C9orf72 PCR/CE (n=166)					Assay Meth- od													
			WB	S	В	WB	S	В	WB	S	В	WB	S	В			# cases with indels	Detected in normal allele	Detected in expansion allele	Inconclusive	None
	UPenn	c9orf72 allele	No	Normal (2-19)			Intermediate (20-29)			Expansion (≥30)			Inconclusive			UPenn	Detected in normal allele	5	0	1	0
	LDT (n=166)	Normal (2-19)	51	2	10	0	0	0	0	0	0	0	0	0			Detected in expansion allele	0	2	0	0
	(–100)	Intermediate (20-29)	0	0	0	1	0	3	0	0	0	0	0	0		(n=151)	Inconclusive	0	0	0	0
		Expansion (≥30)	0	0	0	0	0	0	61	26	11	1	0	0			None	0	11	0	132

Table I. Genotype concordance of C9orf72 alleles in FTD and ALS residual samples between the AmplideX PCR/CE C9orf72 Kit" and UPenn's LDT'. A) Genomic DNA from a diverse set of 166 residual clinical samples from FTD and ALS patients was extracted followed by PCR amplification using Asuragen's C9orf72 PCR/CE assay and a previously established LDT'. Samples included 114 whole blood (WB), 28 saliva (S) and 24 brain (B) samples. Genotype assignments were further confirmed using Southern blot analysis. All sample results, save a single ambiguous expanded sample, agreed across the assays. B) Detection of 3'- insertions/deletions (indels) in C9orf72 alleles using 151 unique residual clinical samples.



40				
			Genatype	e Minor
AND ALL THE MAN AND ALL SHEET SHEET STATE OF THE STATE OF	2,2	<b>66177</b> , <u>16627</u> (1256), (2379, 1256), (3545, 1277), (3555, 1551), (5615, 1576).	6, 0	
12102, 12416, 12038	2, 3	10715 12078	4,10	
37730, 08878, 09487, 09858, 09859, 90209, 11258, 11912, 12343, 12541, 12587, 12594, 12970, 13488, 13522, 14942, 15135, 21444	2,4	15633	6,16	$\overline{}$
0865 000 0874 0000 0775 0001 0817 0070 0975 0071 0715 0715 0715 0715 0715 0715 07	2,5	08419, 13274	7, 7	
26122, 0472-00076, 06983, 09781, 09794, 10078, 10074, 10029, 10032, 10069, 10077, 10078, 10082, 10083, 10084, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 10094, 100944, 100944, 100944, 100944, 100944, 100944, 100944, 100944, 100	2,6	<u>00886</u> , 12694, 14360, 16115	7, 0	
DADSS DASSA DESTI 09253, 10802, 10802, 10805, 10926, 11087, 11736, 11736, 12081, 12487, 12517, 12808, 12828, 12972, 13047, 12241, 12513, 13686, 14412, 14483, 14787, 15815, 20267	2,7	1168c 14243, 14647	7,10	
MMSS (1812) 04130 (1812) 04130 (1812) 04130 (1814) 04140 (1814) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150 (1812) 05150	2, 8	11526	7,11	
<u>19960</u> 15172, 20657	2,9	16025	7,13	
09947, 09134, 09210, 09466, 10441, 10686, 10741, 10928, 10949, 11048, 11129, 11253, 11432, 11875, 13023, 12341, 13825, 14117, 14497, 14795, 14889, 15239	2, 10	1292	7,17	$\overline{}$
56050 09605, <u>09710, 10246,</u> 10314, <u>10912, 11925,</u> 11407, 11764, <b>12994</b> , 12221, 12347, 12364, 12892, 14413, 16088	2,11	<u>06187</u> , 10623, <u>11030</u> , 11134, 11699, 13882, 16265	0,0	$\overline{}$
2006 10445 10401 10844 14252 14591, 19940	2, 12	12612	0,9	T
3323 <u>4</u> , 11858	2, 13	12747, 13512	8,10	$\overline{}$
PIEE	2,14	1170, 12131, 14741	8,11	$\overline{}$
12054	2, 15	11926, 12120	0,12	$\overline{}$
10710-10743	2.16	12048 13659 13827	0.12	-
22076	2, 17	1070, 12426	8,14	-
9191	2, 19	14185	9,10	
115501. 19315	2.10	13754-15227	10.10	(1)/12
12172-11121	2.20	2041	10.11	_
1202.003	2.21	12995	10.12	(2)23
955271	2.24	16714	10.14	(2)/19
1132	2.25	1607	11, 11	1000
125560	2,56	2012	11, 14	(4)19, 25
11079	2.2	Gallack   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982   1982	11, 24	(4)17, 22
15425	2.6	2008	11, 29	-
72510		THE STATE OF THE S	12, 14	-
10952 12786 NSSB 14653 15750	4.5	7000	14, 15	-
99713, 11773, 11937, 14389, 14559	4.6	10518 10518	14, 22	-
11111, <u>11725</u> , 12114, 14619	4,0	00486, 00486, 00490, 00527/02, 0554/042, 00204, 00275, 00475, 02402, 00503, 00534, 00657, 00402, 00004, 00004, 002175, 00475, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00485, 00	2, Esp	(5)40, 52, 5
14126	4, 20	100052, 10070, 100000, 100000, 00000, 00000, 000000	4, Exp	(6)8
07104, <u>07154</u> , 07993, <u>09992</u> 09943, 10379, <u>10538</u> , 11542, 11543, 12128, 12441, 14293, 20081	5,5	10023-, <u>10489</u> 7), <b>10808</b> (8), 11480, 11917, 12205-, 13026-, <u>13244</u> , 14339-, 14753-, <b>14911</b>	5, Exp	(7)65 (8)66
27475 08459 (0014: 10557 11037, 11750, 12393 13144, 14123, 14682, 19949	5, 6	08078, 10221, 12009, 12161, 12199(9, 12904 15365	6, Exp	(9)46
29494, 10157, 14224, 22914	5,7	12(02, 13442/10)	7, Exp	(10)2
0000 08430 00421 08440 10474 10732 10907 10945 11841 11827 11845 11779 11971 12984 12987 12984 13183 13842 13770 18074 18792 15179 25084	5.0	04751 (1790), 19101, 10778, 11374, 11494, 11824, 12894, 14768, 15362, 29448	8. Exp	-
10294 10734 1093Z 11389, 14235, 1483Z, 22053,	5.10	10946, 10973	9. Exp	-
12430	5.11	09373, 10000, 12526, 12815, 14395, 14846	10. Exp	_
19257	5.12	ONLIS, 11345, 22575	11. Exp	
22471	5, 13	2008	12, Exp	+
11606	5, 14	06749(11)	12, Exp	(11)44
901	5.17	1904	16. Exp	
				+
9252, 11488, 26194	6,6	12947-	17, Exp	

Figure 4. Genotyping of 774 Coriell NINDS ALS samples. A) The normal and intermediate C9off72 repeat size distribution (by allele) within the tested Coriell NINDS ALS sample set is consistent with previous reports<sup>2,4</sup>. B) Categorical distribution of tested samples. C) C9off72 genotypes (in repeat units) for all 774 samples that were genotyped from the collection. Shared sample genotypes were concordant across test sites (Asuragen and UPenn; overlapping samples in bold) and consistent with previous reports<sup>5</sup> (overlapping samples underlined). Samples bearing 3'- deletions/insertions are denoted with -4+, respectively. Minor allele information corresponds to sample IDs with matching parenthesized numbering.

# **CONCLUSIONS**

- 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' sequence variations.
- A gene-specific, 2-primer PCR format identified low-level size mosaicism and produced amplicons consistent with up to at least ~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 *C9orf72* 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 hexanucleotide repeat number and disease duration in frontotemporal degeneration. Acta Neuropathol. 2015 Sep;130(3):363-72.
- 2. Rutherford N.J., et al., Length of normal alleles of C9orf72 GGGGCC repeat do not influence disease phenotype. Neurobiol Aging. 2012 Dec; 33(12): 2950.e5–2950.e7.
- 3. Rollinson S., et al., A small deletion in C9orf72 hides a proportion of expansion carriers in FTLD. Neurobiol Aging. 2015 Mar; 36(3): 1601.e1–1601.e5.
- 4. Van der Zee J., et al., A Pan-European Study of the *C9orf72* Repeat Associated with FTLD: Geographic Prevalence, Genomic Instability, and Intermediate Repeats. Human Mutation, 2013. 34(2):363-73.
- 5. Rutherford N.J., et al., *C9orf/2* hexanucleotide repeat expansions in patients with ALS from the Coriell Cell Repository. Neurology, 2012. 31;79(5):482-3.





<sup>\*\*</sup>For Research Use Only – Not For Use In Diagnostic Procedures.

Preliminary research data. The performance characteristics of this assay have not yet been established. Presented at AMP 2016 - TT43