A STREAMLINED PCR ASSAY FOR RAPID AND ACCURATE GENOTYPING OF POLY-T LENGTH POLYMORPHISMS AT RS10524523 OF THE *TOMM40* GENE

Sarah Statt, Julie Krosting, Huiping Zhu, and Gary J Latham
Asuragen, Inc., Austin, TX, United States

SUMMARY

- Alzheimer's disease (AD) is the most common cause of dementia, impacting > 5M Americans and accounting for 60-80% of all dementias in the US.
- An improved risk test may provide insights for clinical research and help guide better patient management for those at-risk for AD.
- We leveraged AmplideX® PCR technology to resolve poly-T polymorphisms at rs10524523 of the *TOMM40* gene.
- The data demonstrate single nucleotide resolution in genotyping poly-T repeats at the *TOMM40* locus using a rapid PCR-based assay.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia. Since AD-related pathologies occur long before symptoms appear, there is an increasing interest in identifying populations that may benefit from early treatment intervention. To achieve this goal, improved assays for genetic risk markers are needed. Poly-T polymorphisms at rs10524523 of the *TOMM40* gene are known to influence the age of onset in late-onset AD (LOAD), with Long (20-29 T) and Very Long (≥ 30 T) alleles associated with an earlier onset of disease.[1] We developed a fast, simple and accurate PCR assay that reports *TOMM40* poly-T length, and demonstrated the performance of this assay across all repeat categories.

MATERIALS AND METHODS

Cell-line gDNA samples were acquired from the Coriell Institute and Asuragen. Whole blood samples, provided by the Blood and Tissue Center of Central Texas, were anonymized and presumed normal donors. For each sample (unless noted), ~20 to 40 ng of gDNA was amplified using *TOMM40* primers and a PCR buffer based on AmplideX chemistry that was optimized for AT-rich regions. PCR products were combined with GeneScan™ 400HD ROX™ dye Size Standard (Thermo Scientific) and HiDi™ Formamide (Thermo Scientific) and analyzed on a 3500xL Genetic Analyzer (Thermo Scientific) CE instrument using POP7 polymer, with a 2.5kV, 5 or 20 sec injection, and 20 min run time. A mobility correction was utilized to adjust the size of the product on CE, as observed with our other repeat PCR assays. Genotypes were determined from the mobility of target amplicon peaks relative to a calibration curve of a three-point synthetic DNA (16T, 24T, 48T) or cell-line DNA (16T, 29T, 36T) of various poly-T lengths.

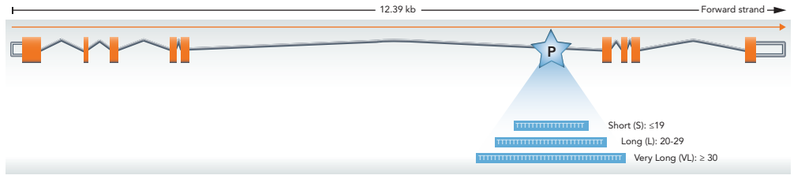


Figure 1. *TOMM40* gene and risk alleles. *TOMM40* (Translocase Of Outer Mitochondrial Membrane 40) is a protein-coding gene and part of the translocase of the outer mitochondrial membrane pore subunit. A variable poly-T tract, rs10524523 ('523), located in intron 6 of the *TOMM40* gene (blue star, figure 1), was found to have a strong relationship with the age of onset, with Long (20-29 T) showing the highest risk, followed by Very Long (≥ 30 T) and then Short (≤ 19 T) with the lowest risk.[1]

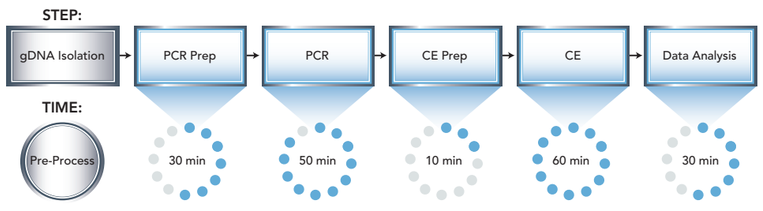


Figure 2. *TOMM40* assay workflow. An overview of the *TOMM40* assay workflow, highlighting the quick turnaround time of ~3 hours from gDNA to answer. Total hands-on time is < 1 hour.

Conflict of Interest Disclosure
All authors have the financial relationship to disclose: Employment by Asuragen.
Research Use Only – Not For Use In Diagnostic Procedures
Presented at AMP 2016 - G25

RESULTS

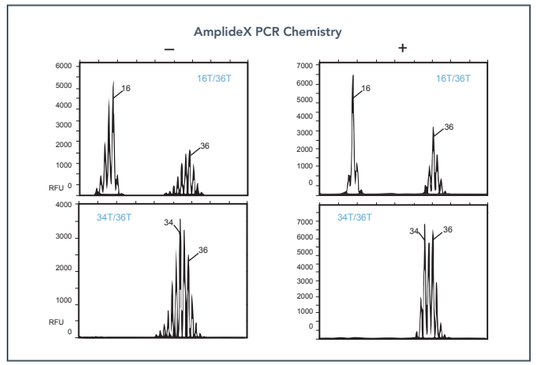


Figure 3. Genotype accuracy and peak resolution for *TOMM40* poly-T repeats is improved using AmplideX PCR chemistry. PCR of the AT-rich *TOMM40* locus and its long stretches of T homopolymers causes promiscuous polymerase slippage and stuttering, as shown in the electropherograms on the left. The incorporation of AmplideX chemistry (right) greatly reduces stuttering, resulting in improved peak-calling accuracy and more clearly resolved poly-T alleles and risk-associated genotypes.

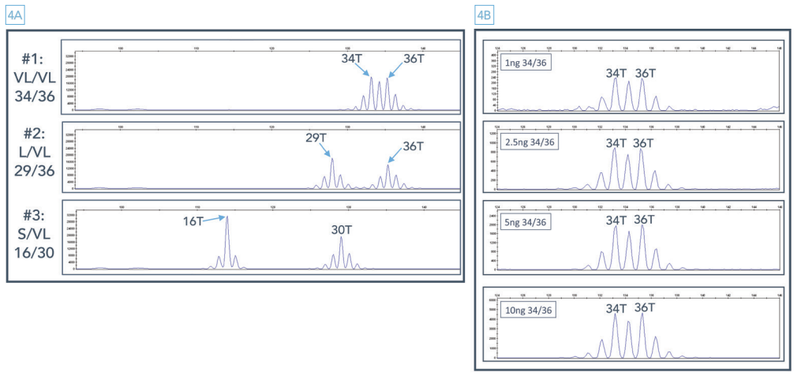


Figure 4. The *TOMM40* PCR assay differentiates a range of poly-T genotypes in cell-line samples, even at low inputs of gDNA. Three characteristic cell-line examples are shown. Figure 4B shows the results of titration of sample #1 to 1 ng gDNA in the assay. Note that the 34T and 36T peaks are resolved even at the lowest input.

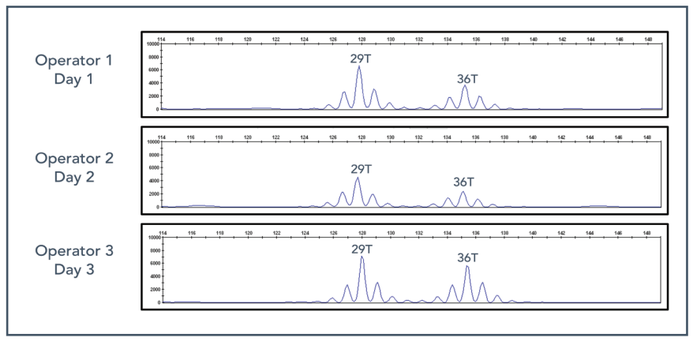
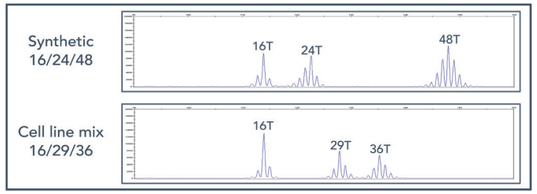


Figure 5. The *TOMM40* PCR assay is highly reproducible across multiple operators and days. Results from assays run by three operators on three different days are shown for a L/VL sample of 29/36 poly-T alleles.



	Genotype		Asuragen Standards		Synthetic 3 pts		Cell Line 3 pts		Concordance	
Name	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
RU_001	S	S	16	16	16	16	16	16	100%	100%
RU_002	S	VL	16	35	16	35	16	35	100%	100%
RU_003	S	L	16	29	16	29	16	29	100%	100%
RU_004	VL	VL	35	36	35	36	35	36	100%	100%
RU_005	VL	VL	34	36	34	36	34	36	100%	100%
RU_006	L	VL	29	36	29	36	29	36	100%	100%
RU_007	S	VL	16	36	16	36	16	36	100%	100%
RU_008	S	VL	16	30	16	30	16	30	100%	100%
RU_009	S	VL	16	35	16	35	16	35	100%	100%
RU_010	S	S	16	16	16	16	16	16	100%	100%
RU_011	S	L	16	29	16	29	16	29	100%	100%

Figure 6. Synthetic or cell-line based mobility correction results in identical genotypes. Synthetic or cell-line based mobility correction is shown for 11 Asuragen cell-line gDNA samples. Both types of mobility correction resulted in the same poly-T allele calls.

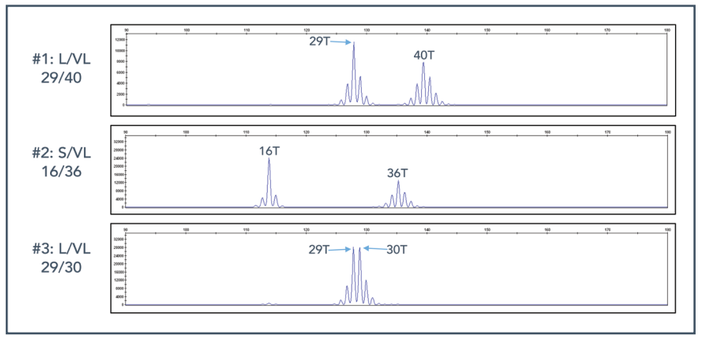


Figure 7. The *TOMM40* PCR assay resolves poly-T genotypes with single nucleotide discrimination in whole blood gDNA samples. The assay resolves a broad range of genotypes; shown are 16T to 40T. Sample #3 highlights the discrimination of alleles at the critical L/VL border.

CONCLUSIONS

- The AT-rich region of the rs10524523 polymorphism poses unique challenges to efficient, high-fidelity PCR amplification and detection.
- The prototype assay described here enables repeatable results, single base resolution, and unambiguous data interpretation with less than one hour of hands-on-time and three hours from DNA to answer.
- This technology may address emerging opportunities and needs for risk assessment in LOAD.

Reference

1. Crenshaw DG, et al. Using genetics to enable studies on the prevention of Alzheimer's disease. Clin Pharmacol Ther. 2013;93(2):177–85.

Asuragen®
www.asuragen.com