A Streamlined PCR-based Fragment Analysis Assay that Resolves Both Single Nucleotide and Poly-T Length Polymorphisms at APOE and TOMM40 Susceptibility Loci in Alzheimer’s Disease

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Introduction
Alzheimer’s disease (AD) accounts for 60-80% of all dementia in the US. Previous studies have identified AD susceptibility loci in a region that includes Apolipoprotein E (APOE) and Translocase Of Outer Mitochondrial Membrane 40 (TOMM40). Of the three common APOE alleles, ε4 is the most informative but only ~25% of Caucasians’ independently, poly-T polymorphisms within the TOMM40 gene have been reported to influence both age of onset in late-onset AD (LOAD) and measures of cognitive decline elevating informative risk data for LOAD to ~97% of individuals. The ApoE (APOE) and TOMM40 (TOMM40) is a commercially available option for this marker. Here we describe the first PCR-based workflow that unifies APOE and TOMM40 genotyping.

Materials and Methods
Cell-line gDNA samples (14 AD, 104 other) were acquired from the Coriell Institute. Whole blood (WB), CD45+ and matched WB-Buccal gDNA samples (77) were isolated from presumed healthy donors (Asuragen, Equitech Enterprises). Sample gDNA was PCR amplified using prototype AmplideX reagents (Asuragen) by different operators on different days. Amplicons tagged with FAM (APOE) or HEX (TOMM40) were resolved on a 3500xL Genetic Analyzer (Thermo Fisher Scientific) CE instrument using POP-7™ polymer with 2.5kV, 20 sec injection and 20 min run time. Genotype analysis utilized their AT- and GC-rich character.

Results
Figure 1. Genotyping APOE and TOMM40 in a streamlined workflow from sample to answer. As is found in human Chromosome 19 (figure adapted from Liu et al. [1]). Three common alleles designated α2, α3, and α4 (corresponding to 2 SNP variants in Exon 4 were associated with AD in HapMap). Sequence variants in TOMM40 and Translocase Of Outer Mitochondrial Membrane 40 (TOMM40) are two genetic risk predictors of AD. Sequence variants in TOMM40 are located in intron 6 of the gene (blue star). This length polymorphism was binned into the following genotypes: short (5 – 14 Ts), long (15 – 29 T), and very long (30+ Ts). For the prototype assay, PCR and thermocycling conditions were independent but similar for the two targets. Dye-labelled amplicons were combined in a single CE injection after PCR. The unified assay has a turnaround time of ~4.5 hrs for 24 samples and a total hands-on time of ~1.5 hrs.

Figure 2. All six possible APOE and TOMM40 genotypes were resolved by the prototype assay agreed with reference calls for all 292 cell-line, blood and Buccal cell samples. A subset of samples and datasets were duplicated across different operators and days. All results were repeatable. Each gDNA concentration was measured by qPCR and the feasibility of each PCR condition was used as a reference method to determine agreement. AmplideX kit (1) and each aCD45 cell-line genotype as per ROC 21.0 were shown. By Representative kit and Buccal PCR electropherograms demonstrate -6% of the 8 possible APOE genotypes.

Figure 3. APOE genotypes resolved by the prototype assay agreed with reference calls for all 242 cell-line, blood and Buccal cell samples. A subset of samples and datasets were duplicated across different operators and days. All results were repeatable. Each gDNA concentration was measured by qPCR and the feasibility of each PCR condition was used as a reference method to determine agreement. AmplideX kit (1) and each aCD45 cell-line genotype as per ROC 21.0 were shown. By Representative kit and Buccal PCR electropherograms demonstrate -6% of the 8 possible APOE genotypes.

Figure 4. Assayed APOE and TOMM40 genotypes reflect known allele variant associations. As distribution of APOE and TOMM40 genotypes matched for all 292 samples from WB (80%) and WB-Buccal cell line over 4502, 85–94.

Figure 5. APOE and TOMM40 PCR amplicons could be resolved from a single-extractor RLUE workflow in a single-tube reaction. As this objective and process were previously published — TOMM40 (p)T length was found to have a strong relationship with age of onset, further refining the APOE association with AD (with a showing the highest r2). As a single reaction of all couples simplified both the workflow and data analysis, and stratified the largest subset of APOE genotypes (ε2-ε2 and ε4-ε4) into high and low risk groups.

Conclusions
• A single PCR/CE technology resolved SNP and length polymorphisms in APOE and TOMM40, respectively, and produced consistent genotypes from 292 samples across three gDNA sources, multiple operators and different days.
• This streamlined workflow assays purified DNA with a turn-around time of 4.5 hrs.
• This multimodal approach has the potential to advance clinical research using a standardized assay that can harmonize results across laboratories.

References