



Plasma-based APOE Genotyping: Development And Optimization of A Robust Protocol

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BACKGROUND

Apolipoprotein E (APOE) is a well-established genetic risk factor for late-onset Alzheimer's disease (AD) and cardiovascular disease. Blood plasma represents an increasingly attractive, cost-effective matrix for biomarker analysis in both clinical and research settings. Here, we present a robust method for isolating cell-free DNA (cfDNA) from archived plasma specimens to facilitate APOE genotyping, with applications in AD research and diagnostics.

METHODS & MATERIALS

Whole blood from six de-identified donors was centrifuged at 3,000 × g to separate plasma from the cellular fraction; both fractions were aliquoted for downstream processing. Cell-free DNA was extracted from up to 0.25 mL of plasma using the iNA Cell-Free DNA Extraction Kit on the iNA24™ Automated Nucleic Acid Purification Workstation (Instant Nano Biosensors). Nucleic acid yield and purity were assessed by NanoDrop spectrophotometry. Additionally, 25 archived plasma samples from the Global Alzheimer’s Platform Foundation biorepository were similarly processed for APOE genotyping.

Specimen ID	Specimen Type	Input blood-plasma	cfDNA yield (ng)	A260/A280	cfDNA Input for APOE Test (ng)
260218Sample1	Plasma	0.25 mL	330	1.85	25
260218Sample2	Plasma	0.25 mL	840	1.56	63
260218Sample3	Plasma	0.25 mL	396	1.75	30
260218Sample4	Plasma	0.25 mL	720	1.6	58
260218Sample5	Plasma	0.25 mL	492	1.64	37
260218Sample6	Plasma	0.25 mL	384	1.72	29

RESULTS & DISCUSSION

For feasibility studies the APOE genotyping was performed in replicate by two operators on the extracted cfDNA from six plasma specimens using a TaqMan SNP genotyping assay. Genotypes derived from plasma cfDNA were compared against those obtained from matched whole-blood genomic DNA to assess concordance. Genotypes across all six donors were as follows: E3/E3 (n = 4), E3/E4 (n = 1), and E2/E3 (n = 1). Results were fully concordant with genotypes obtained from matched blood-derived gDNA, confirming that plasma cfDNA is a reliable source for APOE genotyping.

Genotyping of 25 archived plasma samples was also carried out in replicate. Results indicated that 4 specimens failed to give a confirmatory APOE genotyping calls. However, all the 21 samples that were called for APOE genotyping gave concordant calls with the known genotyping results from the GAP archive. All the genotyping results are shown in the following Tables.

Specimen ID	Specimen Type	Expected APOE Status	Op1 (n=2)	Op2 (n=2)	Overall Concordance
NG09714	gDNA	E4/E4	E4/E4	E4/E4	Concordant
NG16516	gDNA	E3/E4	E3/E4	E3/E4	Concordant
NG21159	gDNA	E2/E3	E2/E3	E2/E3	Concordant
NG21157	gDNA	E3/E3	E3/E3	E3/E3	Concordant
IF-2	gDNA	E2/E4	E2/E4	E2/E4	Concordant
HM-6	gDNA	E2/E2	E2/E2	E2/E2	Concordant
260218Sample1	Plasma	E3/E3	E3/E3	E3/E3	Concordant
260218Sample2	Plasma	E3/E3	E3/E3	E3/E3	Concordant
260218Sample3	Plasma	E3/E3	E3/E3	E3/E3	Concordant
260218Sample4	Plasma	E3/E3	E3/E3	E3/E3	Concordant
260218Sample5	Plasma	E3/E3	E3/E3	E3/E3	Concordant
260218Sample6	Plasma	E3/E3	E3/E3	E3/E3	Concordant

Barcode Scan ID	APOE Call Rep1	APOE Call Rep 2	Final Call	Genotype: GAP BH1	Concordance
300105-102-48	E3/E4	E3/E4	E3/E4	E3/E4	Concordant
300105-108-43	E4/E4	E4/E4	E4/E4	E4/E4	Concordant
300107-046-10	No amplification	E2/E2	E2/E2	E2/E2	Concordant
300108-011-29	E2/E4	E2/E4	E2/E4	E2/E4	Concordant
300106-070-50	E2/E2	E2/E2	E2/E2	E2/E2	Concordant
300109-128-13	E3/E3	E3/E3	E3/E3	E3/E3	Concordant
300106-091-47	E2/E3	E2/E3	E2/E3	E2/E3	Concordant
300114-021-14	E2/E4	E2/E4	E2/E4	E2/E4	Concordant
300114-025-48	E3/E3	E3/E4	Not called	E3/E4	Not called
300112-056-49	E3/E3	E3/E3	E3/E3	E3/E3	Concordant
100110-028-28	No amplification	No amplification	Not called	E3/E4	Not called
300109-016-22	E3/E3	E3/E4	Not called	E3/E4	Not called
300109-024-06	E3/E3	E3/E3	E3/E3	E3/E3	Concordant
300109-040-39	E2/E3	E2/E3	E2/E3	E2/E3	Concordant
300106-008-49	No amplification	E2/E4	E2/E4	E2/E4	Concordant
300103-012-35	E3/E4	E3/E4	E3/E4	E3/E4	Concordant
300109-008-38	No amplification	No amplification	Not called	E3/E3	Not called
300109-010-30	E3/E4	E3/E4	E3/E4	E3/E4	Concordant
300106-017-31	E3/E4	E3/E4	E3/E4	E3/E4	Concordant
300106-002-49	E2/E3	E2/E3	E2/E3	E2/E3	Concordant
100105-008-30	E2/E4	E2/E4	E2/E4	E2/E4	Concordant
300109-049-41	No amplification	E2/E3	E2/E3	E2/E3	Concordant
100106-043-21	E2/E4	E2/E4	E2/E4	E2/E4	Concordant
100105-021-17	E3/E3	E3/E3	E3/E3	E3/E3	Concordant
300110-006-27	E3/E3	E3/E3	E3/E3	E3/E3	Concordant

Total samples tested	Samples with final calls	Samples with concordant calls	% No amplification or not called	Accuracy of Genotyping
25	21	21	16%	100%

CONCLUSIONS

Collectively, these findings demonstrate that cfDNA extracted from as little as 0.25 mL of plasma using the iNA Cell-Free DNA Extraction Kit on the iNA24™ Automated Nucleic Acid Purification Workstation yields sufficient DNA for accurate and reproducible APOE genotyping from archived specimens.