# LightScanner® and LightScanner 32 LunaProbes™ Genotyping

Apolipoprotein (ApoE) Multiplexed Assay

Codon 112 (T>C)
Codon 158 (C>T)

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## Assay Background

Apolipoprotein E (ApoE) is a glycoprotein consisting of 299 amino acids and plays a major role in regulating cholesterol levels in the body. Elevated cholesterol is a primary factor in coronary heart disease, which is among the leading causes of death in the United States. ApoE is also linked to Alzheimer's disease, a debilitating neurological condition in which the tissue of the frontal lobe atrophies. Alzheimer's disease is the most common form of dementia and is incurable, degenerative, and terminal. The ApoE gene has two SNPs which are located on codons 112 (c.388 T>C) and 158 (c.526 C>T). The genotype combinations at codons 112 and 158 translate 3 different isoforms of the protein, commonly referred to as: ε3 (normal), ε2 (dysfunctional), and ε4 (dysfunctional).

| Estimated Human Genotype Frequency of ApoE | | | |
|---|---|---|---|
| **Allele** | **ε2** | **ε3** | **ε4** |
| **ε2** | ~1–2% | ~15% | ~1–2% |
| **ε3** | | ~55% | ~25% |
| **ε4** | | | ~1–2% |

Hill JM, Bhattacharjee PS, Neumann DM (May 2007). "Apolipoprotein E alleles can contribute to the pathogenesis of numerous clinical conditions including HSV-1 corneal disease". *Exp Eye Res* **84** (5): 801–811.

- The ε2 allele is associated with the genetic disorder type III hyperlipoproteinemia and with both increased and decreased risk for atherosclerosis.
- The ε3 allele is found in approximately 64 percent of the population. It is considered the "neutral" Apo E genotype.
- The ε4 allele has been implicated in atherosclerosis and Alzheimer's disease, impaired cognitive function, and reduced neurite outgrowth.

## Method Background

Homogenous PCR methods for genotyping usually require fluorescently labeled oligonucleotide probes. We use a 3'-blocked, unlabeled oligonucleotide probe (LunaProbes) and the saturating dye LCGreen® Plus to generate melting curves characteristic of the genotype under the probe. Key to the method is the use of unbalanced PCR primer ratios, which leads to asymmetric PCR or the overproduction of one strand (i.e., the target strand of the reporter probe) essential for generation of sufficient signal.

To set up a successful LightScanner experiment, the following reagents* are required:

| | Vendor | Catalog Number |
|---|---|---|
| LightScanner Master Mix | Idaho Technology | HRLS-ASY-0002 or -0003 |
| Water (PCR Grade) | Various | Various |
| Mineral Oil | Various | Various |

* Please see *LightScanner Operator's Manual* for consumable recommendations.

## Sample Preparation

Hi-Res Melting® compares profiles from independent PCR reactions; therefore, minimizing reaction-to-reaction variability is pivotal. Samples to be analyzed using Hi-Res Melting should be extracted using a common extraction platform. This eliminates the subtle differences in the reagent components between the final elution buffers from multiple extraction platforms. For Hi-Res Melting, 10 to 20 ng of genomic DNA template in a 10-µL reaction is recommended.

## Assay Design

| ApoE Genotyping Using LunaProbes | | |
|---|---|---|
| PCR product | 277 base pairs, 74.7% GC (very high) | |
| | | [Final] |
| Forward Primer | 5'-ACGCGGGCACGGCTGTCCAAGG-3' | 0.1 µM |
| Reverse Primer | 5'-GGCGCTCGCGGATGGCGCTGA-3' | 0.5 µM |
| Probe SNP 112 | 5'-TGGGCGCG**c**ACATGGAGGA**g**GTGTG CG**c**CCGCCTGGTG**g**AGTACC-block 3' | 0.5 µM |
| Probe SNP 158 | 5'-GCGGCTCCTCCGCGATGCCGATGA CCTGCAGAAG**C**GCCTGGC-block 3' | 0.5 µM |

**Note:** Underlined base in RED indicates position of SNP within LunaProbes. "Block" indicates a three-carbon linker used to prevent extension from the 3' end of the LunaProbe. Bases represented as small case characters in the 112 probe are mismatches designed to modify the probe Tm. This allows temperature multiplexing of the 112 and 158 probe in the same reaction.

## Reaction Setup

| Component | Volume per reaction | Final concentration |
|---|---|---|
| 2.5X LightScanner Master Mix | 4 µL | 1X |
| 10X FWD primer | 1 µL | 0.1 µM |
| 10X REV primer | 1 µL | 0.5 µM |
| 10X 112 Probe | 1 µL | 0.5 µM |
| 10X 158 Probe | 1 µL | 0.5 µM |
| DMSO (80% stock) | 1 µL | 8% |
| DNA (10–20 ng/µL) | 1 µL | 10–20 ng |
| | 10 µL Final Volume | |

## Plate Setup (96-well format)

Load the plate as follows:

1. Aliquot 25 µL of mineral of into each well
2. Aliquot template-free PCR mix into each well (9 µL)
3. Add DNA template to each well (1 µL per well)
4. Cover plate with sealing film
5. Centrifuge at 2000–3000 rpm for at least 1 min.

Load the plate into the thermal cycler.

## Amplification Protocol (Thermal Block)

| | Temperature (°C) | Time (s) |
|---|---|---|
| Hold | 95 | 120 |
| 55 cycles | 95 | 30 |
| | 75 | 30 |
| | 77 | 30 |
| 1 cycle | 94 | 30 |
| | 28 | 30 |

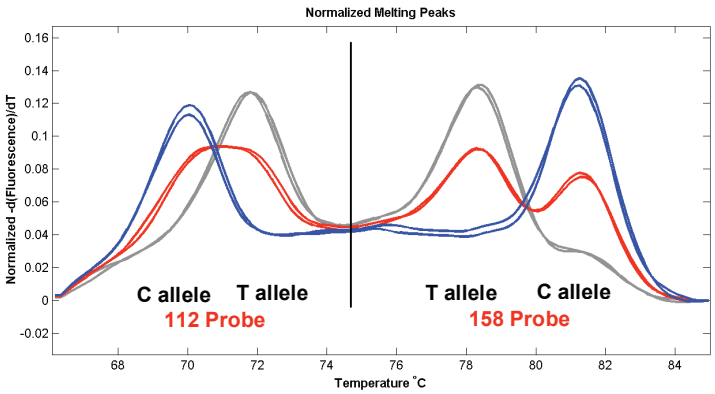
Run the samples on the LightScanner® Instrument using the following melt parameters:

Start Temp: 50°C; End Temp: 97°C; Auto Exp.

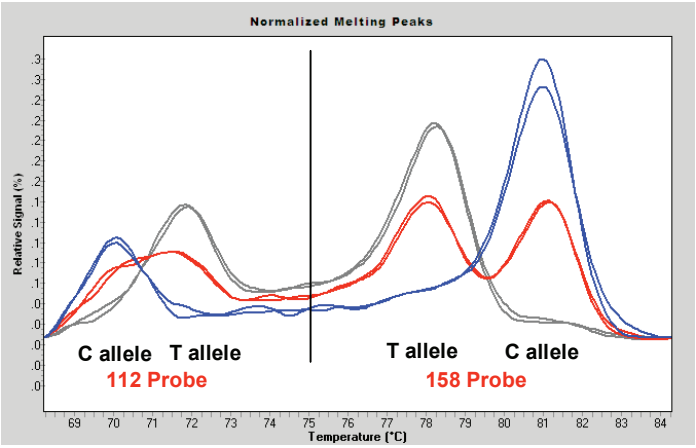
## Amplification Protocol (LS32)

| Programs | Cycles | Analysis Mode | Temperature (°C) | Time (s) | Ramp Rate (°C/s) | Acquisition Mode |
|---|---|---|---|---|---|---|
| Denature | 1 | | 95 | 30 | 20 | |
| Amplification | 55 | Quantification | 95 | 2 | 20 | |
| | | | 68 | 3 | 20 | |
| | | | 81 | 20 | 20 | Single |
| Denature re-nature | 1 | | 95 | 5 | 20 | |
| | | | 45 | 30 | 20 | |
| Air Melt (optional) | 1 | Standard Melt | 55 | | | |
| | | | 97 | | 0.05 | Continuous |
| Hi-Res Melting | 1 | Hi-Res Melting | 53 | | | |
| | | | 58 | | | |
| | | | 97 | 0.3 | | Continuous |

## Genotyping Results (LightScanner)




## Genotyping Results (LightScanner 32)




## References


Poulson MD, Wittwer CT. Closed-tube genotyping of apolipoprotein E by isolated-probe PCR with multiple unlabeled probes and high-resolution DNA melting analysis, *Biotechniques*. 2007;43(1):87–91.



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