

Primers:

ApoE neo 19: 5' -GCC GCC CCG ACT GCA TCT-3' 18-mer
 ApoE Anti-S 20: 5' -TGT GAC TTG GGA GCT CTG CAG C-3' 22-mer
 ApoE Sense 21: 5' -GCC TAG CCG AGG GAG AGC CG-3' 20-mer

Reaction Components:

DNA template 1.0 µl
 ApoE Sense primer 0.1 µl
 ApoE Anti-Sense primer 0.1 µl
 ApoE Neo primer 0.1 µl
 Promega Master Mix 10.0 µl
 Taq polymerase 0.1 µl
 H₂O 8.6 µl
 20.0 µl total

PCR Program:

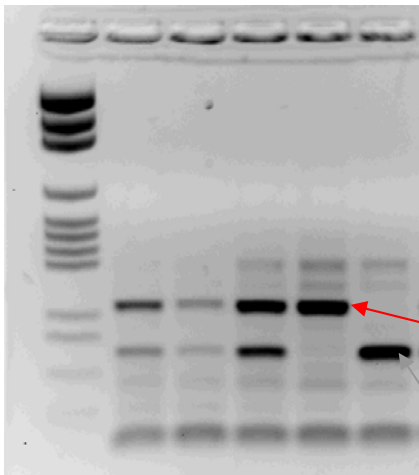
94°C for 30 Sec.
 94°C for 1 Min. }
 55°C for 1 Min. } 35 cycles
 72°C for 1 Min. }
 72°C for 5 Minutes

Expected bands:

245bp for apoE -/
 155bp for apoE +/+

Electrophoresis Conditions:

Run on a 2% agarose gel at 100 Volts for ~1 hour to achieve a clear separation.



Targeted locus will produce a 245 bp product
 Wild Type locus will produce a 155 bp product

Reference: Piedrahita *et al.* Proc Natl Acad Sci (1992) 89:4471-4475.

Filename: Z:\AAA PPG\Rateri Core\PCR protocols\ApoE_PCR updated.doc