PCR: APPyen

Amplifluor: No

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
APPyen Fwd	GAATTCCGACATGACTCAGG
APPyen Rev	CTAGTTCTGCATCTGCTCAAAGAAC

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	Volume (µL)
DNA Sample			1.0
10x Buffer		20.0 mM MgCl2	2.0
dNTPs		2.0 mM	0.4
Primer: **APPyen Fwd**		10.0 µM	
Primer: **APPyen Rev**		10.0 µM	
Water			15.6
Taq Polymerase			0.2

Comments on protocol: Founders were identified by PCR analysis of lysates from tail biopsies using primers APPyen_for 5'-GAATTCCGACATGACTCAGG-3' and APPyen_rev: 5'-CTAGTTCTGCATCTGCTCAAAGAAC-3' which yields an amplification product of 294 base pairs. A restriction digest of the amplification product by AluI confirms the presence of the APP23yen transgene. Mutation of the YENPTY motif introduces an AluI site which enables digestion of the PCR product generated with primers APPyen_for and APPyen_rev (294bp) to differentiate between APP23yen (digested to yield 256bp and 38bp fragments) and APP23 mice (undigested 294bp band).
All reagents used (Taq, dNTPs and Buffer) are
from Roche

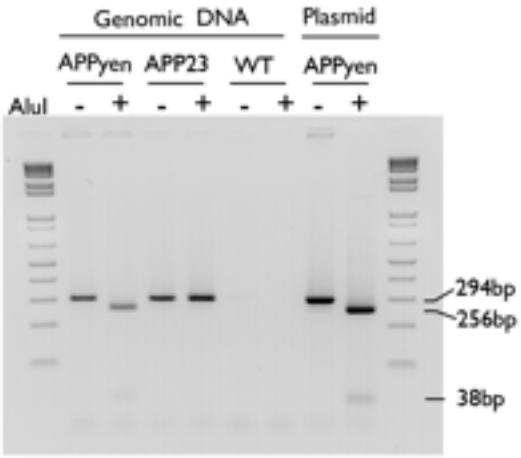
Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: **No**		
Initiation/Melting	95.0	120
Denaturation	95.0	30
Annealing	56.0	30
Elongation	72.0	30
Number of cycles (repeat denature, anneal & elgonate): **30**		
Strand completion (i.e. 72°C, 10 min)	72.0	600
Finish (i.e. 4°C, indefinite)		

Electrophoresis

Running buffer:	
% Agarose:	2.0
Volt:	
Estimated running time (min):	60.0

Image


Genomic DNA
Plasmid
APPyen
APP23
WT
APPyen
AluI
- + - + - + - +
294bp
256bp
38bp