

## B6;129S7-Apob<sup>tm2Sgy</sup>/J

Stock No: 002877

Protocol 550: Restriction Enzyme Digest Assay - Apob<tm2Sgy>

Version 1.1

### Notes

This assay does not work well without the use of a Hotstart (We are using Taq Start Antibody mixed 1:1 with Taq polymerase).

The genotyping protocol(s) presented here have been optimized for reagents and conditions used by The Jackson Laboratory (JAX). To genotype animals, JAX recommends researchers validate the assay independently upon receipt of animals into their facility. Reaction cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

### Expected Results

Mutant = 2500 bp and 300 bp

Heterozygotes = 2800 bp and 2500 bp and 300 bp

Wild type = 2800 bp (uncut)

Separated by gel electrophoresis on a 1.5% agarose gel.\*\*

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR1745		TCC TGA GCT GCT GTA GTT TTT C		Common		
oIMR1776		CCG CAT GAA GAC TGA CTC TG		Common		

#### Reaction A

COMPONENT	FINAL CONCENTRATION
ddH <sub>2</sub> O	
AB PCR BufferII	1.00 X
MgCl <sub>2</sub>	2.50 mM
dNTP	0.20 mM
oIMR1776	0.50 uM
oIMR1745	0.50 uM
Taq DNA Polymerase +	0.02 U/ul
DNA	

#### Cycling

STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	
4	72.0	--	repeat steps 2-4 for 35 cycles
5	72.0	--	
6	10.0	--	hold***

JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

#### Reaction B

COMPONENT	FINAL CONCENTRATION
AB PCR BufferII	X
Hind III	

