

IRS-1 GENOTYPING BY PCR

(Genotyping of mice for knockout of Insulin Substrate 1)

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Mastermix for one 25 μ l reaction

H ₂ O	15.76 μ l
DMSO	2.0 μ l
10x PCR buffer	2.5 μ l
dNTP (2.5 mM)	2.0 μ l
MgCl ₂ (50mM)	0.75 μ l
Primer K5F (20 pmol/ μ l)	0.25 μ l
Primer Neo New (20 pmol/ μ l)	0.25 μ l
Primer K5R (20 pmol/ μ l)	0.35 μ l
Taq polymerase	0.125 μ l

Add 1 μ l of DNA template to 24 μ l of mastermix, then cap and vortex.

PCR Program

1 cycle

94°C 2 minutes

32 cycles

94°C 30 seconds

59°C 30 seconds

72°C 30 seconds

1 cycle

72°C 5 minutes

Electrophoresis on a 2% gel.

Knockout mice have a band at 560 base pairs.

Wild-type mice have a band at 438 base pairs.

Heterozygous mice have bands at both 560 and 438 base pairs.

Primer Sequences

K5F: 5' ccc cag ctc cag gaa cag cg 3'

K5R: 5' ctc gtc atc cgc ggc aat gg 3'

Neo New 5' cga gga tct cgt gac cc 3'