

## **Genotyping of IRlox mice by PCR**

(genotyping of mice for presence of loxp sites in the insulin receptor gene)

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#### Mastermix for one 50 $\mu$ l reaction

H <sub>2</sub> O	39.5 $\mu$ l
10x PCR buffer	5.0 $\mu$ l
MgCl <sub>2</sub> (50 mM)	1.5 $\mu$ l
dNTP (20 mM)	0.5 $\mu$ l
Primer LoxF (100 pM/ $\mu$ l)	1.0 $\mu$ l
Primer LoxR (100 pM/ $\mu$ l)	1.0 $\mu$ l
Taq polymerase	0.5 $\mu$ l

Add 1  $\mu$ l of DNA template to 49  $\mu$ l of mastermix, then cap and vortex.

#### PCR Program

1 Cycle

94°C for 3 minutes

34 Cycles

93°C for 30 seconds

60°C for 30 seconds

72°C for 30 seconds

1 Cycle

72°C for 5 minutes

Electrophoresis on 2 % agarose gel.

Homozygous mice have a band at 300 base pairs.

Wild-type mice have a band at 280 base pairs.

Heterozygous mice have bands at both 300 and 280 base pairs.

#### Primer Sequences

LoxF            5' tgc acc cca tgt ctg gga ccc 3'

LoxR            5' gcc tcc tga ata gct gag acc 3'